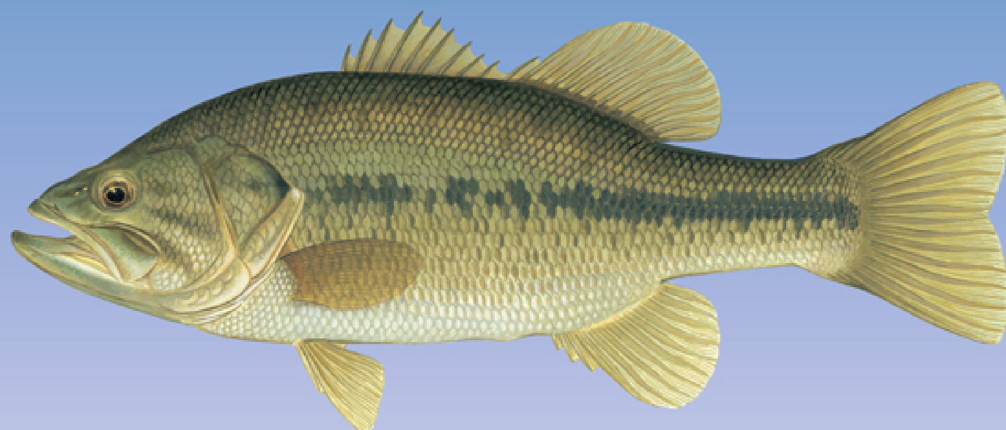
LARGEMOUTH BASS AQUACULTURE



EDITED BY
JAMES H. TIDWELL, SHAWN D. COYLE, & LEIGH ANNE BRIGHT



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Contributors

Phillip W. Bettoli, Department of Biology, Tennessee Technological University, Cookeville, Tennessee, USA

Leigh Anne Bright Kentucky State University, Division of Aquaculture, Frankfort, Kentucky, USA

Angela Caporelli, Kentucky Department of Agriculture, Frankfort, Kentucky, USA

Shawn D. Coyle Kentucky State University, Division of Aquaculture, Frankfort, Kentucky, USA

Robert M. Durborow, Kentucky State University, Aquaculture Research Center, Frankfort, Kentucky, USA

Carole R. Engle, Engle-Stone Aquatic$, LLC, Adjunct Faculty, Virginia Seafood AREC, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA

Boris Gomelsky, Kentucky State University, Division of Aquaculture, Frankfort, Kentucky, USA

Todd S. Harmon, Walt Disney World, Animal Programs, Lake Buena Vista, Florida, USA

Habte-Michael Habte-Tsion, Aquaculture Research Center, Kentucky State University, Frankfort, Kentucky, USA

Chengyue Liu, Fisheries College, Ocean University of China, Qingdao, China

Michael D. Matthews, A.E. Wood Fish Hatchery, San Marcos, Texas, USA

Leandro E. Miranda, US Geological Survey, Mississippi Cooperative Fish and Wildlife Research Unit, Mississippi State University, Mississippi, USA

Kathryn M. Mitchell, Kentucky State University, Aquaculture Research Center, Frankfort, Kentucky, USA

John G. Nickum, Retired, National Aquaculture Coordinator, Department of the Interior, U.S Fish and Wildlife Service, Washington D.C., USA

Eric Peatman, Auburn University, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn, Alabama, USA

Waldemar Rossi Jr., Aquaculture Research Center, Kentucky State University, Frankfort, Kentucky, USA

Kenneth J. Semmens, Kentucky State University, Division of Aquaculture, Frankfort, Kentucky, USA

James H. Tidwell Kentucky State University, Aquaculture Research Center, Frankfort, Kentucky, USA

Joseph R. Tomasso, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Auburn, Alabama, USA

Yangen Zhou, Key Laboratory of Mariculture (Ocean University of China), Ministry of Education, China

Chapter 1
Largemouth bass natural history

Leandro E. Miranda[1] *and Phillip W. Bettoli*[2]

[1] US Geological Survey, Mississippi Cooperative Fish and Wildlife Research Unit, Mississippi State, Mississippi, USA

[2] Department of Biology, Tennessee Technological University, Cookeville, Tennessee, USA

1.1 Introduction

The largemouth bass (*Micropterus salmoides*) stands out as one of North America's most popular sport-fish. Its common name, and other vernacular names (Table 1.1, Cloutman and Olmsted, 1983), reflect the striking size of its jaws, with the lower jaw projecting very strongly, and the maxilla in the adult extending beyond the hind margin of the eye. It is a member of the family Centrarchidae, a diverse and prominent family endemic to North America. The family consists of seven genera, among them the black basses, genus *Micropterus*, which includes multiple species. Evidence suggests that the ancestral *Micropterus* began allopatric speciation about 10 million years ago, driven by vicariance caused by sea-level fluctuations (Near et al., 2003; Smith et al., 2015). It is estimated that the largemouth bass separated from the Florida bass *M. floridanus* less than 5 million years ago (Smith et al., 2015).

The largemouth bass was first described by French naturalist Bernard Germain de Lacépède in his 1800 book *Histoire Naturelle des Poissons, Volume III*. The description was made in France based on a drawing and accompanying manuscript notes sent from the vicinity of Charleston, South Carolina (Henshall, 1881). The local name of the fish was "trout," as it still is in some southern states. Lacépède named it *Labrus salmoides*, trout-like wrasse, in accordance with its general appearance and vernacular name. The genus name *Micropterus* is also attributed to Lacépède and his 1801 book *Histoire Naturelle des Poissons, Volume IV*, but he associated the name with the smallmouth bass *Micropterus dolomieu. Micropterus* means "small fin," named after a specimen that happened to have a deformed (trimmed) dorsal fin (Henshall, 1881). The largemouth bass was not associated with the genus *Micropterus* until the 1870s, having some bewildering classifications in the interval that included genus names such as *Bodianus, Calliurus, Lepomis, Etheostoma, Cichla, Huro, Grystes, Centrarchus*, and *Dioplites.*

Whereas the genus classification was settled, ichthyologists have not always agreed on the number of *Micropterus* species. Ramsey (1975) commented that when compared with the speciose North American genus *Notropis* (more than 100 valid named species) and genus *Etheostoma*

Table 1.1 Selected attributes of largemouth bass natural history.

Attribute	Description
Scientific name	*Micropterus salmoides*
Common name (English)	Largemouth bass
Common name (French)	Achigan à grande bouche
Common name (Spanish)	Lobina negra
Other vernacular names	Bigmouth, widemouth, bucketmouth, green bass, green trout, pond trout, Oswego bass, Welshman
Age of the species	<5 million years
Native range	Eastern North America
Native range area	3,297,900 km^2

Attribute	Description
Naturalized range	>50 countries in all continents but Antarctica and Australia
Environment	Warm, eutrophic, mostly lentic, and shallow
Habitat	Submerged plants and underwater structures
Sexuality	Monomorphic, mostly monogamous
Nesting	Excavated substrate in shallow water
Age at sexual maturity	1–4 years old depending on growth rate
Size at maturity	Minimum length near 200–250 mm TL
Contribution of gonads to body weight during spawning season	As high as 10–13% female; <2% males
Fecundity	$F = 0.00003*TL^{3.407}$
Diameter of mature eggs	0.75–1.5 mm
Diameter of water–hardened eggs	1.5–2 mm
Spawning temperature	Start 14–16℃; peaks 18–20℃
Length of spawning season	30–120 days, depending on latitude
Nest spacing	3–6 m apart
Nest guarding	Males, possibly females
Egg hatching	3–4 days
Total length of larvae at hatch	4 mmTL
Total length of larvae at swim–up	6 mm TL
Average length at age 1,2, and 3	102, 202, 273 mm TL
Maximum size and age	787 mm TL, 10.1 kg, 24 years

Attribute	Description
Weight–length relationships for fish >150mmTL	W = 0.000002965*L^{3}.^{273}
Weight–length relationships for fish 25–150 mm TL	W = 0.000020216*L^{2}.^{858}
Age 0 mortality	10–20% per week
Age 1+ mortality	35–37% per year
Diet	Invertebrates to about 35 mm TL; switch to fish diet at 35–160 mm TL; mostly fish afterwards
Daily ration	2–6% of body weight
Gape width	Gape width = −5.59 + 0.14*TL (all units in mm)
Optimum temperature for growth	25–30℃
Temperature tolerance	Juveniles stop feeding below 6℃; can tolerate spells up to 40℃
Dissolved oxygen tolerance	Growth reduced below 4 mg/l; prolonged exposure below 1 mg/l is lethal
Salinity tolerance	up to 8–12 ppt

(over 80 species), the half-dozen or so species of *Micropterus* would seem to have presented a relatively straightforward problem in taxonomic definition. Nevertheless, over 40 years later the number of extant species has increased to nine (Near and Koppelman, 2009), and as many as eight additional species are being considered (Baker et al., 2013; Long et al., 2015). Until recently the Florida bass had been considered a subspecies of largemouth bass, but has now been given species status (Kassler et al., 2002); therefore, facts about largemouth bass assembled during the 20th century and reported herein reflect these two species as one.

1.2 Geography

1.2.1 Native range

Figure 1.1 Native range of largemouth bass in North America (modified from Lee et al., 1980).

Encompassing an area of 3,297,900 km^2 over the eastern half of North America, the largemouth bass has the widest native distribution of the black basses (MacCrimmon and Robbins, 1975; Lee et al., 1980; Pyron, 1999). The eastern limit of its native range is the Atlantic seaboard extending northward from the St. Mary's River in Georgia to the James River in Virginia in coastal watersheds (Figure 1.1). The southern limits of the native range are the Gulf of Mexico drainages from the Suwanee River in Florida to northern Mexico. The exact southwestern limit is obscured by early transplants, but would appear to have been the Rio Conchos, a tributary of the Rio Grande in north central Mexico, to the Rio Soto La Marina, a tributary of the Gulf of Mexico in northern Mexico. The western limit includes much of the western Mississippi River Basin extending into the Great Plains region where the precise boundary is variable depending on annual precipitation. It likely extends from the confluence of the Rio Conchos and Rio Grande in a northeasterly direction through central Texas, western Oklahoma, central Kansas, extreme eastern Nebraska, western Iowa, and most of Minnesota and Wisconsin south of the Rainy River and Lake Superior drainages. The northern boundary includes much of the Great Lakes basin, exclusive of Lake Superior but including the upper St. Lawrence River. The northeastern limit is the Ottawa River system southward into the St. Lawrence River and the Chaudiere River south of Quebec City. This northeastern limit extends south into the upper Ohio River drainage in western New York, western Pennsylvania, Ohio, West Virginia, and possibly western Maryland. The species is not native to the New England states or the Atlantic seaboard north of the James River in Virginia, and was absent, except peripherally, from much of the Appalachian and Ozark Mountains.

1.2.2 Naturalized range

The native range has been extended through intentional and unintentional transfers in North America, and to every continent but Antarctica and Australia. Extensions of self-sustaining populations of largemouth bass beyond its native range have been mostly the result of intentional transfers but, occasionally, have resulted from natural movements through manmade waterways or other connections. The widespread construction of reservoirs throughout most of the 20th century created favorable habitats in regions otherwise unsuited for the species (Moyle, 1986; Gido and Brown, 1999). Largemouth bass stockings were sanctioned by US government actions beginning in 1871, and by 1900 largemouth bass were found in all conterminous states of the USA (MacCrimmon and Robbins, 1975; Moyle, 1976). The species has also become established across southern regions of

Canada warm enough to support it, and most of Mexico and Central America (Lee et al., 1980). Beginning in the late 1800s, the species was introduced into Europe, southern Africa, South America, parts of Asia and many oceanic islands, and by the end of the 20th century it was established in over 50 countries (MacCrimmon and Robbins, 1975; Welcomme, 1992; Rahel, 2007). The goals of introductions have included improving recreational fishing, improving local fish assemblages, and aquaculture. However, in some countries the largemouth bass is listed as a nuisance species because of its effects on native fish communities (Jackson, 2002), and is currently targeted for eradication in Japan (Tsunoda et al., 2010).

1.3 Habitat

The largemouth bass is a warmwater generalist that inhabits a wide variety of habitats, partially accounting for its broad spatial distribution (Pyron, 1999). Generally, it seems to be better adapted to warm and eutrophic waters than most other *Micropterus* species, excluding Florida bass, although throughout its range it co-occurs with many of its congeners. The species is often common in natural lakes, wetlands, backwaters, slow moving streams, and coastal marshes, and has thrived in artificial impoundments small and large (Warren, 2009). In streams, they occupy various lotic habitats but are most common in low-gradient streams and slow-moving pools; avoiding low-order streams. The species is often found along shallow littoral areas associated with submerged aquatic vegetation; with underwater structures including woody debris, rocks, and flooded terrestrial vegetation; and with steep bank slopes or undercut banks (Annett et al., 1996; Raibley et al., 1997).

Largemouth bass can tolerate a fairly broad range of conditions (Chapter 4). Although they can tolerate the high temperatures (near 40℃; Beitinger et al., 2000) associated with shallow waters in their southern range, the duration and severity of winters has been associated with the northern limits of their geographical distribution (Miranda, 2014). Prolonged stints with temperatures <6℃ reportedly limit food intake and cause increased mortality of juveniles (Garvey et al., 1998). Although largemouth bass can tolerate a wide range of dissolved oxygen concentrations, their growth is reduced substantially at levels below 4 mg/l (Stewart et al., 1967) and prolonged exposures to levels below 1.0 mg/l are considered lethal (Moss and Scott, 1961). Although activity levels and foraging success may vary with water clarity (Reid et al., 1999; Shoup and Wahl, 2009), largemouth bass can thrive in both clear and turbid waters. The pH level affects juveniles more than adults; juvenile mortality has been reported at pH levels lower than 5 due to increased energy demand and reduced feeding (Orsatti and Colgan, 1987; McCormick and Jensen, 1992). Largemouth bass inhabiting coastal marshes can tolerate, at least temporarily, salinities up to 8–12 ppt, with juveniles being more tolerant than adults (Meador and Kelso, 1990).

1.4 Feeding

1.4.1 Adult diets

It could be argued that few freshwater fishes have a broader diet than adult largemouth bass, which is partly related to their wide geographic distribution. In addition to fish prey, the literature has documented adult largemouth bass consuming terrestrial insects, aquatic insects, mollusks, crayfish, reptiles, amphibians, birds, and even small mammals (Hodgson and Hansen, 2005; Purdom et al., 2015). That said, the diet of adult largemouth bass in all but the least productive aquatic systems consists primarily of fish. In southern US waters, important fish prey includes gizzard shad (*Dorosoma cepedianum*) and threadfin shad (*D. petenense*); other centrarchids, especially bluegills (*Lepomis macrochirus*); and various minnow (Cyprinidae) species.

In less productive aquatic systems at higher latitudes, largemouth bass will rely heavily on zooplankton, and aquatic and terrestrial insects (Hodgson and Kitchell, 1987), in addition to whatever fishes are present (for example, yellow perch [*Perca flavescens*]), including juvenile largemouth bass (Clady, 1974). In coastal lakes in New England with access to the Atlantic Ocean, introduced largemouth bass readily prey upon anadromous river herring (alewife [*Alosa pseudoharengus*] and blueback herring [*A. aestivalis*]; Yako et al., 2000). On the Pacific coast, introduced largemouth bass prey upon Pacific salmon (Tabor et al., 2007), as well as other native and introduced fish species.

When transplanted abroad, adult largemouth bass prey upon whatever suitably size fish prey are available. For instance, introduced largemouth bass in Cuba preyed heavily upon several species of topminnows (Cyprinodontidae; Rivero, 1937). In Puerto Rico, introduced adult largemouth bass preyed on other introduced species, such as threadfin shad, sunfish, tilapia (*Oreochromis mossambicus* and *Coptodon rendalli*), and peacock bass (*Cichla ocellaris*), as well as native species, such as the bigmouth sleeper (*Gobiomorus dormitor*) (Bacheler et al., 2004). In a South African river, introduced largemouth bass adults relied primarily on dragonflies (Odonata) and river crabs (*Potamonautes sidneyi*) during low-flow seasons but availed themselves of juvenile estuarine fishes entering the river during periods of high flows (Wasserman et al., 2011). As these and numerous other articles will attest, the largemouth bass in its native and introduced range is an extremely opportunistic feeder that will prey on fish when present, but readily switch to other food resources when necessary.

Perhaps the only factor curbing what a largemouth bass will consume, be it fish or some other prey item, is its gape limitation. First described in detail by Lawrence (1958), the size of prey fishes of various species that can be consumed by largemouth bass is gape limited, although the mouth morphology of largemouth bass allows them to consume larger prey than other predatory species of similar length, such as smallmouth bass (*M. dolomieu*), muskellunge (*Esox masquinongy*), and walleye (*Sander vitreus*). The gape width of largemouth bass increased rapidly from about 3% to 10%

in largemouth bass 50 to 140 mm total length (TL), and reached 13% by about 550 mm TL (Hill et al., 2004). Although largemouth bass are physically capable of ingesting very large prey, they typically prey upon fish with body depths well below the maximum size that could be ingested (Hambright, 1991).

1.4.2 Ontogenetic dietary shifts

Like most other fish species, at hatching a larval largemouth bass must rely on its yolk sac for nutrition for several days. Once yolk-sac absorption is complete, largemouth bass fry that are about 6 mm TL leave the nest and become swim-up fry. At this critical period, the fry must switch to exogenous feeding. They may feed initially on small zooplankton such as rotifers (Wickstrom and Applegate, 1989), but fry quickly switch as they grow in length to larger microcrustacean zooplankton (copepod nauplii, adult copepods, and cladocerans) and immature aquatic insects (Parmley et al., 1986). If zooplankton are abundant and a largemouth bass hatches earlier in the growing season than other competitors for zooplankton prey (for example, juvenile clupeids and other centrarchids), growth can be rapid (1.0–1.4 mm per day; Goodgame and Miranda, 1993; Dorsey, 1997).

At a length of about 25 mm TL, largemouth bass will start to prey on fish if suitably sized prey are abundant and readily available and they will stop preying on zooplankton at about 35 mm TL (Huskey and Turnigan, 2001). In southern US waters, age 0 bluegills and shads are typical fish prey of fingerling largemouth bass. The onset of piscivory, which has been the subject of intense study for decades (Keast and Eadie, 1985; Bettoli et al., 1992; Mittelbach and Persson, 1998; Yasuno et al., 2012), has profound ramifications for individuals as well as the entire cohort of largemouth bass hatched in a given year. Specifically, a fingerling that switches to piscivory early (that is, at a small size) will grow much faster than a bass feeding on insects or zooplankton and it will likely maintain and increase that growth advantage over the course of the first growing season and beyond (Olson, 1996; Ludsin and DeVries, 1997).

As noted in the previous section, piscivores, such as largemouth bass, face gape limitations when consuming fish prey (Mittelbach and Persson, 1998; Nowlin et al., 2006) and faster growth by piscivorous fingerlings allows them to consume a broader suite of potential prey fishes throughout the growing season than smaller nonpiscivorous fingerlings. Conversely, if largemouth bass hatch late in the spring their potential fish prey (for example, age 0 shads and sunfishes) may be too large and grow too fast to be vulnerable to predation (Olson, 1996). Faster growth and larger size often equate to better survival of juvenile largemouth bass through their first growing season (Shelton et al., 1979; Maceina et al., 1988). Switching early to piscivory and achieving larger sizes also promotes the deposition of lipids and higher lipid reserves can increase overwinter survival in many species, including age 0 largemouth bass (Miranda and Hubbard, 1994; Ludsin and DeVries, 1997; Biro et al., 2004).

Hatching early in systems with abundant fish prey does not guarantee early onset of piscivory by largemouth bass, with all of its attendant advantages. Savino and Stein (1982) demonstrated in a laboratory study that the predatory success of largemouth bass feeding on bluegills declined with increases in structural complexity. Bettoli et al. (1992) and others subsequently demonstrated in field studies that structural complexity in the form of aquatic vegetation delays the onset of piscivory in largemouth bass. For instance, largemouth bass collected from densely vegetated habitats in Lake Conroe, Texas, did not become piscivorous (that is, at least 60% of fish with some stomach contents had fish remains in the gut) until reaching lengths of 160 mm; whereas, largemouth bass collected from vegetation-free habitats were piscivorous at 80 mm. Concomitant with the earlier onset of piscivory were significantly greater mean lengths at age 1 (~200 mm versus ~130 mm). As a final note, early onset of piscivory does not necessarily guarantee that a largemouth bass does not revert back to relying on invertebrate food resources. For instance, Huskey and Turnigan (2001) observed that following the onset of piscivory 122–317 mm TL largemouth bass relied more on crayfish prey than fish in their diet in a lake in Florida.

In the context of aquaculture, piscivory by largemouth bass has long received attention because largemouth bass (like most piscivorous species) will readily resort to cannibalism (Stranahan, 1906). If given the opportunity, largemouth bass 10–14 mm long in laboratory aquaria will cannibalize 6 mm TL individuals (Johnson and Post, 1996). In hatcheries, cannibalism will arise to problematic levels unless the fingerlings are routinely graded to achieve homogenous size groups (Nelson et al., 1974; Bondari, 1983) and sufficient quantities of natural forage (or pelleted feed) are provided.

1.4.3 Feeding behavior

Unlike a classic ambush predator, such as northern pike (*E. lucius*; Harper and Blake, 1991), largemouth bass are opportunistic, mobile predators that rely mostly on visual cues when stalking their prey. Suction feeding (that is, rapid expansion of the buccal cavity) is employed by many piscivorous fishes and other species rely primarily on ram feeding (that is, forward motion of the body or jaws to engulf prey); most species, including largemouth bass, can employ a combination of these two modalities (Gardiner and Motta, 2012). Largemouth bass can successfully forage in a variety of habitats with diverse levels of structural complexity, at diverse depths and water clarities, and at diverse light levels. Their ubiquitous distribution and the wide array of prey they consume reflect their plasticity in feeding behavior (Almeida et al., 2012). Although they rely mostly on visual cues, largemouth bass are capable of feeding at very low light levels (McMahon and Holanov, 1995) and when deprived of sight, they can locate and successfully ingest fish prey using their lateral line system (Gardiner and Motta, 2012). When largemouth bass grow large enough to reduce their risk of being preyed upon by other piscivores (including their own kind), they are able to utilize a broader range of habitats and prey to support their diets (Ward and Neumann, 1998; Huskey and Turnigan, 2001).

In their native habitats, largemouth bass have coevolved with their principal prey species and prey fish have developed behavioral strategies to reduce their predation risk. Perhaps the best studied largemouth bass predator–prey system involves bluegill as the prey. For instance, bluegill will inhabit beds of vegetation and exhibit distinct swimming behaviors to reduce their predation risk when in the presence of largemouth bass (Savino and Stein, 1989). Potential prey species that did not coevolve with largemouth bass lack the behavioral traits necessary to reduce their predation risk. For instance, early efforts to control nuisance aquatic vegetation by stocking grass carp *Ctenopharyngodon idella* consistently failed when small (< 200 mm TL) fish were stocked because of heavy predation by largemouth bass (Shireman et al., 1978). Many other accounts exist of naive fish species lacking an appropriate repertoire of behaviors to reduce largemouth bass predation, to the detriment of local populations (Rivero, 1937; Azuma and Motomura, 1998; Jang et al., 2006).

1.5 Bioenergetics

The bioenergetics of largemouth bass have not been as well-studied as their food habits and foraging behavior; nevertheless, a sizeable literature on the thermal ecology and bioenergetics of largemouth bass extends back at least four decades (reviewed by Bevelhimer and Breck, 2009). One of the earliest largemouth bass bioenergetics models was produced by Rice et al. (1983) and subsequently validated by Rice and Cochran (1984). Other bioenergetics models for largemouth bass have been produced and evaluated by Carline (1987) and Whitledge and Hayward (1997). In those and other studies of bioenergetics, the goal often is to model growth and consumption as a function of fish size, temperature, and perhaps the genetic traits of the fish stock (Venables et al., 1977). Of particular interest to aquaculturists is the temperature at which daily ration, food conversion, and growth are maximized for fish at different life history stages. The temperature at which growth is optimized can be measured directly, in a hatchery setting, or indirectly by estimating the final temperature preference. The temperature that a fish will ultimately gravitate towards when given a choice is termed the final preferendum; that thermal endpoint can also be defined as the temperature at which the acclimation temperature of a fish equals the temperature selected acutely in a laboratory gradient. The final preferendum is usually a good predictor of the temperature that maximizes growth and fitness of individuals (Jobling, 1981; Kelsch, 1996).

Temperatures that optimized growth were reported to be 27℃ for largemouth bass fry (Strawn, 1961) and ~25℃ for juveniles and subadults (Niimi and Beamish, 1974). Tidwell et al. (2003) reported that feed conversion and growth by fingerling largemouth bass were similar at 26 and 32℃ and both were higher than at 20℃. Díaz et al. (2007) stated the final preferendum of fingerling largemouth bass was similar to the range of temperatures that optimized growth (28–29℃). The final preferendum for largemouth bass has also been reported to be ~30℃ (juvenile and subadults in field; Neill and Magnuson, 1974), 27–32℃ (adults in laboratory; Venables et al., 1978), 32.2℃ (juveniles in laboratory; Cincotta and Stauffer, 1984), and 27.1–30.5℃ (juveniles in laboratory; Koppelman et al., 1988).

Neal and Noble (2006) applied a bioenergetics model to ascertain why non-native largemouth bass in Puerto Rico had short lifespans (few fish lived beyond age 3) and slow growth. They concluded that multiple spawning events each year directed more energy to reproduction than somatic growth; water temperatures that never cooled below 23℃ were also implicated. They reported that largemouth bass across the globe achieved their maximum size at a latitude of about 28°N (peninsular Florida; northern Mexico); shorter growing seasons at higher latitudes decreased the maximum size that largemouth bass achieved.

Bioenergetics models require information on (or predict) daily rations. Although daily rations of largemouth bass fed *ad libitum* can reach 5 or 6% of body weight$\cdot d^{-1}$ (Cochran and Adelman, 1982; Carline, 1987), daily rations in field and hatchery settings are usually closer to 2% (Whitledge and Hayward, 1997).

1.6 Reproduction

1.6.1 Sexual dimorphism and monogamy

Dimorphism evident in many fish taxa is commonly manifested as size, color, or shape variations (Hedrick and Temeles, 1989; Parker, 1992). In *Micropterus*, sexual dimorphism is not evident as it is in other centrarchid genera, particularly *Lepomis* (Ehlinger et al., 1997). In fact, outside of the breeding season when gametes may be extruding, the only positive way to determine the sex of largemouth bass is through meticulous examination of the vent (Benz and Jacobs, 1986).

The monomorphism exhibited by largemouth bass has usually been associated with monogamous species (Clutton-Brock, 1989). Evidence suggests that *Micropterus* males are only rarely bigamous (Ridgway et al., 1989; Wiegmann et al., 1992). However, males may have more than one female mate as a result of extended or disrupted breeding seasons. In a largemouth bass population in South Carolina studied through genetic analyses, eggs in nearly 90% of nests were almost exclusively the product of one male and one female. The other 10% of the nests included eggs from 1 male and 2–3 females (DeWoody et al., 2000).

1.6.2 Nesting habitat

Largemouth bass nest on the substrate of shallow areas of lakes, rivers, and backwaters (Breder, 1936; Annett et al., 1996). Nests are excavated over a variety of substrates but coarse gravel and sand and the roots and stems of aquatic vegetation tend to be used most often (Allan and Romero, 1975; Annett et al., 1996). Although nests have been reported in water deeper than 5 m, most nests are constructed in shallower water and average 0.5–2 m deep (Kramer and Smith, 1962; Heidinger, 1975). Most males select nest sites near cover where they are less exposed to nest incursion by egg predators

(Vogele and Rainwater, 1975; Annett et al., 1996). Average nest spacing ranged from 6 to 9 m in an Arkansas reservoir or about 15 nests/100 m transect (Hunt and Annett, 2002). Other studies have reported lower densities of 1–3 nests/100 m of shoreline (Vogele and Rainwater, 1975).

1.6.3 Age and size at maturity

Largemouth bass typically reach sexual maturity at ages 1–5 and at minimum sizes near 200–250 mm TL. In fast-growing populations in the lower latitudes of their native range, large-mouth bass can reach 250 mm and become sexually mature by age 1 (Clugston, 1964; Nieman et al., 1979). In higher latitudes fish grow slower and maturity is often delayed to ages 3–5 for males and ages 4–5 for females (Kelley, 1962; Scott and Crossman, 1973).

1.6.4 Gametogenesis

The ovaries of largemouth bass are bilobed, elongated nearly circular in cross section, and can make up as much as 10–15% of the body weight during the spawning season (Timmons et al., 1980; Miranda and Muncy, 1987). Male testes make up a smaller fraction of the body weight, peaking at 1.5–2% (Bennett and Gibbons, 1975; Brown and Murphy, 2004). Fecundity increases with female size exponentially, but is highly variable, even for fish of the same age or size (Kelley, 1962; Heidinger, 1975; Timmons et al., 1980). Fecundity estimates compiled by Bishop (1968) and Carlander (1977) from various water bodies in the USA indicated ovaries of female bass contained 2,000–145,000 eggs. Warren (2009) used published data to derive an equation that estimated fecundity (f) from total length as:

$$f = 0.00003 * TL^{3.407}$$

This equation predicts that females can produce an average of about 2,000 eggs at 200 mm TL, 8,000 at 300 mm, and 47,000 at 500 mm.

Mature eggs in the ovaries range 0.75–1.5 mm in diameter; after they are fertilized and water-hardened they increase to 1.5–2 mm (Kelley, 1962; Meyer, 1970; Heidinger, 1975). After they are fertilized, the eggs settle to the bottom of the nest and adhere to the substrate. The eggs lie with the oil globule up, and water-harden within 15 min. Sperms have an ovoid head approximately 0.002 mm long, with a tail 0.02 mm long (Carr, 1942). Sperms are viable for only a minute or so after they are released.

1.6.5 Spawning season

Once they reach maturity, largemouth bass spawn annually. Spawning typically occurs from early spring to mid-summer, extending for less than 1 month to several months depending on latitude (Conover, 1992; Waters and Noble, 2004; Rogers et al., 2006). Spawning begins when water temperature rises to 14–16°C, peaks at 18–20°C, and dwindles as water warms up to and exceeds 25°C. Large adults reportedly spawn before smaller adults (Miranda and Muncy, 1987). The male guards the eggs and continues to guard the young fish for several weeks after they hatch (Heidinger, 1976). During this stage, male largemouth bass show heightened aggression and demonstrate a high susceptibility to angling (Suski and Philipp, 2004).

Duration and timing of spawning by largemouth bass at the population and individual levels depend on many interacting factors. Duration, as estimated from the range of number of daily growth rings in otoliths of fish from the same cohort, exhibits a latitudinal gradient: 26 days in New York (Schmidt and Fabrizio, 1980), 36–51 days in Illinois (Miller and Storck, 1982; Kohler et al., 1993), 60–71 days in Mississippi (Goodgame and Miranda, 1993), 68–75 days in Texas (Isely and Noble, 1987; Maceina et al., 1988), and over 120 days in Puerto Rico (Ozen and Noble, 2002). This gradient may represent an adaptation to length of the growing season (Conover and Present, 1990).

In theory, a cohort may follow one of two primary sequences of events during the first year of life, depending on how early in the reproductive season they hatch (Ludsin and DeVries, 1997). An early hatched cohort should begin feeding on invertebrates and make the transition to piscivory during summer, allowing for rapid growth the remainder of the year. Alternatively, a late-hatched cohort is less prone to make the transition to piscivory because fish prey may be too large, leading to slower growth, higher exposure to predation, and comparatively lower contribution to the new year class (Ludsin and DeVries, 1997).

1.6.6 Nesting, spawning behavior, and parental care

Largemouth bass are solitary nesters. Males use caudal sweeping to excavate circular, depression nests 0.5–1.0 m diameter (Kramer and Smith, 1962; Cooke et al., 2001). Nests are usually spaced out 3–6 m apart. The males normally guard an area about 2 m diameter around the nest (Annett et al., 1996). If there is a visual obstruction or barrier, nests tend to be excavated closer together (Vogele and Rainwater, 1975).

When a female joins the male, they slowly circle the nest, side by side. Male courtship behaviors include opercle flaring, hovering, flashing, and herding potential female partners with gentle nudges and nipping (Allan and Romero, 1975; Cooke et al., 2001). Spawning occurs with both fish tilted laterally so their vents are close together. Both parents shudder as eggs and sperm are released simultaneously (Heidinger, 1975).

The male then takes a position over the eggs and continuously fans them with his fins until they hatch (Cooke et al., 2001). The current created by the fanning keeps the eggs free of silt and ensures that they are always in contact with freshwater. Depending on water temperature, in the southern US eggs generally hatch in 3–4 days (Heidinger, 1975). The male will continue to guard the fry until they disperse from the nest. Nesting success may be reduced if the temperature drops below 14–15°C. This temperature usually causes the male to leave the nest (Kelley, 1968; Allan and Romero, 1975). Without a guard the eggs will not be fanned or protected and the likelihood of suffocation and predation increases. During the spawning season the males reportedly do not eat and many males may die during this period likely due to poor body condition (Heidinger, 1975).

Whereas most accounts indicate that parental care in largemouth bass is provided exclusively by males (Breder, 1936; Heidinger, 1975; Cooke et al., 2006), DeWoody et al., (2000) documented biparental care. Most of the nests examined by DeWoody et al. (2000) in a South Carolina reservoir were attended by a female and a guardian male. The attendant female generally faced the nest while 1–2 m away and the attendant male positioned over the nest, but these positions were occasionally reversed. The guardian male showed no aggression towards the female, and the attendant female actively chased away conspecific nest intruders and predators. Nests with attendant females occurred across several stages of brood development, indicating that nest guarding by the female extended well past spawning, and into incubation of eggs to the free-swimming fry stage of the brood. A few nests that lacked parental males were guarded exclusively by females. Biparental care may not be of general occurrence across largemouth bass populations, but the existence of biparental care in largemouth bass is consistent with several reproductive life history traits including large body size, large eggs, sexual monomorphism, monogamy, and extended parental care (DeWoody et al., 2000).

1.6.7 Early larvae

Newly hatched larvae measure about 4 mm TL (Heidinger, 1975). Depending on temperature, the larvae remain in the nest for about 5–10 more days until they become free-swimming fry, schooling above the nest. After another day or two, parents move the fry to allow them to forage while still providing parental care (Cooke et al., 2002). Initially the fry form tight schools or “fry balls” that begin to forage on zooplankton, but as they grow, schools become broader and looser. Schools break up 20–30 days after hatching when fish are about 20–25 mm TL. Largemouth bass may be one of the most attentive parents in the centrarchid family, often remaining with schooling fry for several days or weeks after hatching (Breder, 1936). On average, largemouth bass may invest 3–6 weeks in parental care from spawning to fry dispersal (Kramer and Smith, 1962; Cooke et al., 2006).

1.7 Growth and Mortality

1.7.1 Maximum size and age

The largest largemouth bass on record was caught by angling in a pond in the state of Georgia, USA, in 1932. It weighted 10.1 kg with an estimated length of 787 mm TL (IGFA, 2015). This record was recently tied by a largemouth bass caught in Lake Biwa, Japan (IGFA, 2015). Even bigger largemouth bass may have once lived. For instance, Smith (1907) reported that "examples weighing as much as 20–25 lbs [9.1–11.3 kg] have been taken in lakes" in Florida. In 1907 only two species of *Micropterus* were recognized – largemouth bass and smallmouth bass. The oldest largemouth bass aged was a 23- or 24-year-old individual measuring 584 mm TL that was collected in the state of New York (Green and Heidinger, 1994). The largest largemouth bass in Canada was caught in Ontario in 1948 and weighed 4.7 kg (Scott and Crossman, 1973).

The maximum age of largemouth bass also follows a latitudinal distribution. In northern latitudes including Minnesota, Wisconsin, and Ontario longevity can be 13–16 years (Carlander, 1977), but in Puerto Rico few largemouth bass live beyond age 3 (Neal and Noble, 2006). There is evidence that at least in some populations older individuals are mostly females (Heidinger, 1975; Webb and Reeves, 1975; Carlander, 1977).

1.7.2 Growth rate

Growth rates of largemouth bass vary greatly across their range, with populations in southern latitudes having some of the highest growth rates (Beamesderfer and North, 1995). The variability in growth rates is due mostly to the longer growing season and higher temperatures in southern latitudes (Clugston, 1964; Coutant and DeAngelis, 1983), although local forage availability can account for much of the variability (Keast and Eadie, 1985; Hoyle and Keast, 1987; Garvey and Stein, 1996). Largemouth bass generally do not grow in temperatures less than 5–10°C and reach maximum growth rates near 25–30°C depending on fish size (see Bioenergetics section). Growth rates generally decrease in temperatures above 30°C, and growth ceases at temperatures > 36°C (Stuber et al., 1982).

Beamesderfer and North (1995) surveyed the literature on largemouth bass in North America searching for estimates of mean length at age. Median length at age of 698 populations ranged from 102 mm at age 1 to 514 mm at age 15 (Figure 1.2). Lengths were inversely correlated with latitude and elevation and directly correlated with air temperature and degree-days. However, these variables accounted for only part of the observed variability in growth rates. Other research suggests that the unexplained variability may also be related to trophic state of the water body, water quality, habitat availability, prey abundance, and fish community composition (Miranda and Durocher, 1986; McCauley and Kilgour, 1990; Meador and Kelso, 1990; Bettoli et al., 1992; Putman et al., 1995). Considering these sources of variability, Helser and Lai (2004) reported that growth across North America varied by more than 120% in terms of average maximum attainable size (L_∞; 360–800 mm) and by more than 88% in terms of the Brody growth coefficient (K; 0.091 to 0.670 per year).

1.7.3 Weight–length relationship

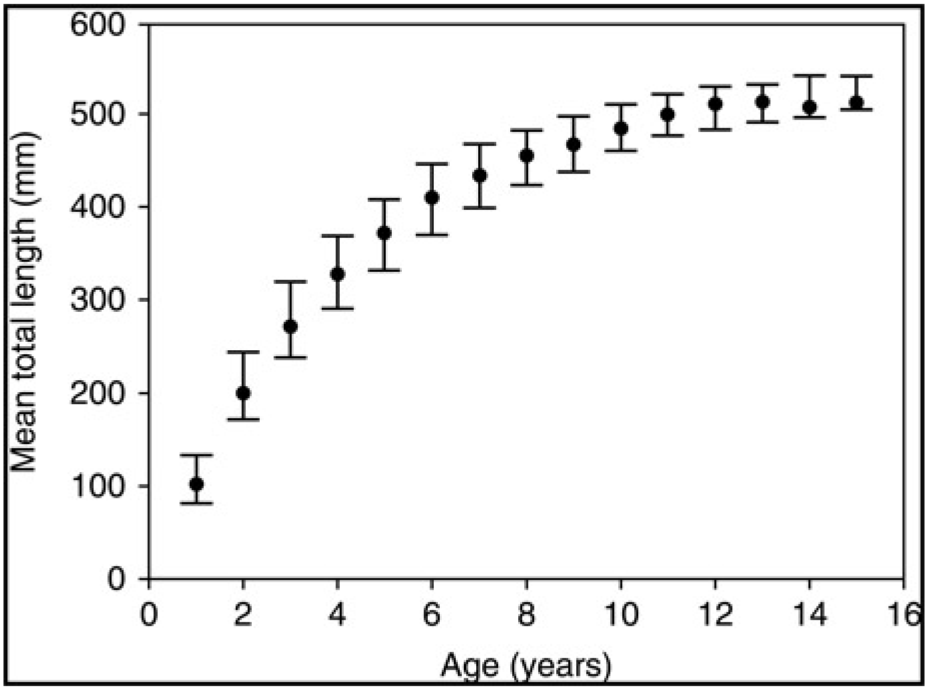

Figure 1.2 Relationship between total length and age of largemouth bass in North America. Values represent median length at age and whiskers indicate 25–75 percentiles. Estimates represent 698 populations compiled by Beamesderfer and North (1995).

In many cases, size is more relevant than age because physiological and ecological factors are more size-dependent than age-dependent. Weight–length relationships have several applications including estimation of weight from length, estimation of body condition, and morphological comparisons between populations (Froese, 2006). The weight (W) of largemouth bass is exponentially related to their length (L) according to the equation $W = aL^b$, where a is the intercept and b is the slope. The slope is an indicator of condition and growth pattern. When $b = 3$, the growth is considered isometric (that is, all fish dimensions increase at the same rate as fish get longer), hypoallometric if $b < 3$ (that is, the fish increases less in weight than predicted by its increase in length, becoming relatively more elongated as it grows), and hyperallometric if $b > 3$ (that is, the fish increases more in weight than predicted by its increase in length, becoming more roundish as it grows). Based on the assumption that heavier fish of a given length are in better condition, when comparing two populations, the one with the larger b

may be considered to be in better condition. A standard weight–length equation commonly used for largemouth bass is $W = 0.000002965*L^{3.273}$ where weight is in grams and total length in millimeters (Neumann et al., 2012). This equation is suitable for fish > 150 mm TL, and because it was developed to score body condition, it yields the 75th percentile weight. For fish 25–150 mm TL, we derived the 75th percentile equation $W = 0.000020216*L^{2.858}$ with fish collected in Alabama, Georgia, Iowa, and Mississippi (unpublished data).

1.7.4 Age 0 mortality

Juveniles of highly fecund organisms such as fish can experience high mortality rates. Small size and high abundance often render these organisms more susceptible to high rates of mortality during early life stages than during later stages (Houde, 1989). Miller and Kramer (1971) estimated a 9–23% per week embryo mortality, and Miranda et al. (1984) estimated a 13–21% weekly mortality of juveniles in the months after hatching. Length differences within a cohort often result from interactions between time of hatching and subsequent growth. Largemouth bass hatched earlier in the season obtain an initial length advantage over those hatched later, an advantage that may be maintained and increased through the first year of life (Shelton et al., 1979; Miller and Storck, 1984; Isely and Noble, 1987). Time of hatching can affect the ability of young largemouth bass to switch to a fish diet early in the growing season and eventually affect their survival to age 1. Bass that achieve a large length during their first growing season may be less vulnerable to overwinter predation and starvation than smaller members of their cohort (Gutreuter and Anderson, 1985; Goodgame and Miranda, 1993). Post et al. (1998) reported that overwinter mortality was size dependent, strongly affecting bass entering the winter at < 50–60 mm TL.

1.7.5 Age 1+ mortality

Most mortality estimates for largemouth bass available in the literature have been derived through catch-curve analysis, which estimates overall natural and fishing mortality for fish age 1 or older (Miranda and Bettoli, 2007). This analysis assumes constant recruitment and mortality over all age groups. Fewer studies have estimated fishing mortality, which along with overall mortality can be used to derive conditional natural mortality. Conditional natural mortality reflects potential deaths during an interval, say 1 year, had natural mortality been the only acting force of mortality (Miranda and Bettoli, 2007). Beamesderfer and North (1995) reported that conditional annual natural mortality in 40 largemouth bass populations in North America averaged 37% (25–75 percentiles: 21–47). In a separate review, Allen et al. (2008) reported total and fishing mortalities for over 30 largemouth bass populations and based on those estimates conditional annual natural mortality averaged 35% (25–75 percentiles: 27–42).

1.8 Role of stocking

Hatchery-reared largemouth bass have been stocked to mitigate for projects that drastically alter natural habitats and their native fish communities and sport-fisheries. Perhaps one of the best-known examples of mitigation stocking has been the decades-long stocking of largemouth bass into Lake Mead in Nevada–Arizona, which commenced in the 1930s and continued through the 1980s (McCall, 1980). However, at the present time most largemouth bass and Florida bass in North America are stocked to either increase fishable stocks, influence the genetic characteristics of the receiving stock, or to meet both objectives.

A rich literature exists on the efficacy of stocking largemouth bass and Florida bass (hereafter referred to simply as “largemouth bass”) at different life-history stages to increase the number and standing crop of largemouth bass in receiving systems. In some instances, stocking adult (~300 mm TL) largemouth bass provided an immediate, albeit short-term, boost to fishing success (Buynak et al., 1999). In other studies, stocking adult largemouth bass had no effect on the resident population (Alford et al., 2009). Most hatchery-reared largemouth bass are stocked as fingerlings or advanced fingerlings and the verdict on those efforts is checkered, at best. For every report of a reasonable contribution of stocked largemouth bass to year-class strength or the fishery (for example, Buynak and Mitchell, 1999; Colvin et al., 2008; Mesing et al., 2008), the literature is replete with observations that persistence of stocked fingerlings after a year or two was negligible (for example, Buckmeier and Betsill, 2002; Hoffman and Bettoli, 2005; Hartman and Janney, 2006; Diana and Wahl, 2008; Ashe et al., 2016).

Several factors can contribute to low contributions of stocked fish. Fingerling largemouth bass harvested from culture ponds or raceways and transported to stocking sites are invariably stressed and will suffer high immediate mortality if remedial measures are not undertaken such as anesthetizing fish before and during hauling, transporting at cool temperatures, and adding salt to the water (Carmichael, 1984; Carmichael et al., 1984). Diana and Wahl (2009) estimated that stress associated with hauling and stocking accounted for up to 50% of the mortality of the fingerling largemouth bass they stocked, especially at water temperatures above 24℃. Porak et al. (2002) also noted highly variable, and occasionally very high (83%), stocking mortality of stocked fingerling largemouth bass.

Once stocked, inexperienced largemouth bass inevitably face a suite of potential predators that will readily prey upon them and high predation is usually cited as the reason for failed stocking programs (Schlechte et al., 2005). For instance, Buckmeier et al. (2005) estimated that nearly 28% of stocked fingerling (30–46 mm TL) largemouth bass were lost to predation within 12 h of stocking. Resident juvenile and adult largemouth bass, in particular, will readily prey upon stocked fingerlings, as will other piscivores (for example, white bass [*Morone chrysops*]) and zooplanktivores/insectivores such as bluegill (Buckmeier et al., 2005; Diana and Wahl, 2009).

To reduce predation losses, researchers and managers have investigated stocking larger, advanced (> 75 mm TL) fingerlings (Buynak and Mitchell, 1999; Mesing et al., 2008; Diana and Wahl, 2009), as well as stocking fish into structurally complex habitats (Buckmeier et al., 2005; Ashe et al., 2016) or at times coinciding with adequate prey resources (Hoxmeier and Wahl, 2002; Mesing et al., 2008). In a novel approach to produce advanced (~100 mm TL) fingerlings for stocking early in the growing season when suitable prey may be more abundant, Matthews and Stout (2013) manipulated water temperatures and photoperiods to induce captive broodstock to spawn in the fall. Stocking fish that are reared on or trained to consume live forage (instead of just pelleted-food) has also been investigated because pellet-reared largemouth bass may delay their transition to feeding on natural prey (Porak et al., 2002) and suffer higher mortality (Heidinger and Brooks, 2002; Pouder et al., 2010; Rachels et al., 2012). Allowing stocked fish to briefly recover and habituate to their new environment in predator-exclusion pens has also been examined, especially in systems with low habitat complexity and high predator densities (Schlechte et al., 2005; Schlechte and Buckmeier, 2006).

Despite the plethora of largemouth bass stocking programs that experienced poor survival and low contributions to year-class strength, the ability of hatchery fish to alter the genetic composition of receiving populations is indisputable. Specifically, the production and stocking of vast numbers of Florida bass, which began in the early 1970s and continues to this day, are an important component of the fisheries management programs in several states, such as Texas, Oklahoma, and Tennessee. The introgression of Florida bass genes into northern and intergrade largemouth bass populations has increased the maximum size that largemouth bass achieve in states outside the native range of Florida bass. For instance, the Tennessee state record large-mouth bass caught in 1954 (6.6 kg) was eclipsed in 2015 by a 6.9 kg F1 hybrid largemouth bass caught in a reservoir that had been stocked with more than 2 million Florida bass and F1 hybrid fry and fingerlings since 2000. The Texas state record fish caught in 1943 (6.1 kg) was bested by a 6.4 kg fish in 1980 and the current state record (8.3 kg) was caught in 1992. The 50 heaviest largemouth bass caught in Texas have all been caught after Texas initiated its Florida bass stocking program in the

1970s and the catches of trophy (> 5.9 kg) largemouth bass were greater in reservoirs stocked with fingerling Florida bass (Myers and Allen, 2005). Oklahoma experienced a similar phenomenon after its Florida bass stocking program commenced; a forty-year old record was broken in 1983 and by the 1990s most of the largest fish caught possessed some Florida bass genes.

Given the preponderance of evidence that survival of stocked fingerling largemouth bass is often poor, how have agencies managed to produce significant introgression of the Florida bass genome into resident stocks of northern or intergrade largemouth bass? As Dunham et al. (1992) and others have reported, introgression is promoted by long-term stocking programs (6–15 years) consisting of many stocking events and high stocking numbers (up to 100,000 per stocking site [10–41 per hectare]; Buckmeier et al., 2003). The exception to that rule is when newly impounded waters are stocked for a few years with Florida bass as the reservoir basin fills (Maceina et al., 1988). It is indisputable that the maximum size and catches of trophy largemouth bass have increased in the tier of states in the southern USA with large Florida bass stocking programs; however, introduced non-native genes outside of their native range will persist through many generations (Johnson and Fulton, 2004; Naninni et al., 2014). As such, many authors have cautioned against the introduction of possible maladaptive genes (Philipp et al., 2002). To address those concerns in part, many US fisheries agencies have policies that either prohibit the introduction of Florida bass into their waters, or restrict their introductions into waters deemed suitable from a thermal standpoint. For instance, the Oklahoma Department of Wildlife Conservation determines which waters are stocked based on heating degree days; Florida bass will not thrive in cooler waters that support native largemouth bass populations; therefore, they are stocked where the thermal environment is more conducive to their growth and survival. The Tennessee Wildlife Resources Agency follows a similar strategy (that is, relying on heating degree days to determine which waters are and are not stocked).

1.9 Conclusion

A few years ago, the first author was pulling a seine in Represa Mourão, a small hydropower reservoir in Parana State in southern Brazil. Filled with the anticipation of catching a rich and diverse assemblage of unfamiliar taxa, he was extremely disappointed, but surprised, and yet even comforted by the catch of only two species, both familiar friends and invitees from other continents: largemouth bass and Nile tilapia (*Oreochromis niloticus*). This collection in a remote reservoir in Brazil illustrates the adaptive capacities of largemouth bass and tilapia, two of the most introduced fish taxa on the planet (Welcomme, 1992). Both species have demonstrated their resilience in diverse abiotic and biotic environments. The versatility of largemouth bass can be attributed to a merely average fecundity that is reinforced by parental care, to a diverse diet, to rapid early growth, and to flexible habitat utilization. Success in spreading largemouth bass outside their native range can also be linked to the relative ease with which juveniles are cultured in earthen ponds and transported to release sites (Chapter 7 and 11). The impetuses for spreading the species include its aesthetic qualities, taste, and gameness. The largemouth bass is an aesthetically pleasing fish considering its desirable shades of bronze, black, and greenish colors over an attractive trout-like shape. Relative to taste, the meat is white, flaky, and low in oil content making largemouth bass a good culinary fare if properly cleaned soon after being caught. And as to gameness, we leave you with a quote from Henshall (1881, emphasis added) “I consider him, *inch for inch* and *pound for pound*, the gamest fish that swims. The royal Salmon and the lordly Trout must yield the palm to a [largemouth bass] of *equal weight*.”

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Chapter 2
History of largemouth bass production

James H. Tidwell[1] *and John G. Nickum*[2]

[1] Kentucky State University, Aquaculture Research Center, Frankfort, Kentucky, USA

[2] Retired, National Aquaculture Coordinator, Department of the Interior, U.S. Fish and Wildlife Service, Washington, DC, USA

2.1 Introduction

The art of aquaculture, in general, likely began in China and dates back 4,000–5,000 years (Stickney, 1979). These early efforts focused primarily on common carp (*Cyprinus carpio*). There is evidence of pond aquaculture in Egypt dating back over 2,000 years and their culture included tilapia. For a comprehensive review of the history of aquaculture see Stickney and Treece (2012). The general focus of this historical account is the development of the aquaculture of the large-mouth bass including its production as a food-fish. However, this industry largely evolved from procedures developed for producing juvenile largemouth bass for pond and reservoir stocking as sport-fish. In fact, sport-fish stocking has been the main driver for the development of culture methods for many species of fishes.

While we know that efforts to reproduce largemouth bass extend back to at least the mid-1800s, written reports are difficult to find. What was documented was often in hatchery records or organizational reports, not in publications available to the general public. There are many gaps in the records of fish culture in general, including the history of largemouth bass culture. Techniques that individual culturists developed for their own use were hopefully passed along orally, or went with them to their graves.

Especially in the era of immediate access to information, it is easy to miss or minimize the contributions of those who have come before us, especially if their work is not available in electronic form. This would be a serious mistake. Let this chapter pay tribute to the culturist, hatchery managers, fishery managers, and researchers who have contributed to the knowledge base for the largemouth bass, now extending back over 150 years.

2.2 Background

The largemouth bass is a member of the sunfish family, Centrarchidae, which also includes the "bream" species like bluegill as well as rock bass and crappies. All are native to North America. The largemouth bass is a member of the genus Micropterus whose members are collectively known as the black bass. The largemouth bass is *Micropterus salmoides*, meaning small fin and a salmon-like body. The largemouth bass was described in 1802 by Lacépède. While native to the drainages of North America east of the Rocky Mountains, the largemouth bass has been widely transplanted. Heidinger (1976) listed 37 countries where the largemouth bass could now be found. Within the USA, intentional stocking programs were initiated in 1871 or 1872, and by 1900 largemouth bass were found in all 48 of the contiguous states.

In the 19th century largemouth bass were harvested recreationally as a sport-fish but were also commercially harvested as a food-fish. In the 1800s all fisheries were widely considered to be inexhaustible resources (Clepper, 1966). However, by the mid to late 1800s it began to be realized that this was a fallacy and efforts were initiated to control harvests and increase stocking programs. Declines in fish populations supporting sport-fisheries led managers to focus on fish propagation and stocking as a primary management approach for maintaining "fishable" populations. This pattern was applied first to brook trout, American shad, and Atlantic salmon. Depletion of largemouth bass and smallmouth bass populations later led managers and culturists to attempt similar practices for bass.

2.3 Early culture

Exactly when American fish culturists first tried to propagate largemouth bass is unknown. Efforts of these early hatchery workers were not recorded. We can safely conclude that largemouth bass culture began sometime in the latter half of the 19th century. American fish culture for any species was essentially non-existent prior to 1850, although a presentation about propagating brook trout has been reported to have been made in South Carolina in 1804. A publication titled *A Treatise on the Artificial Propagation of Certain Kinds of Fish, with the Description and Habits of Such Kinds as are Most Suitable for Pisciculture* was produced by Dr. Theodatus Garlick in 1857, but focused primarily on his experiments with brook trout (Davis, 1961). Information on salmonid propagation efforts in Europe dated back to the 14th century and came to North America with European immigrants. Although salmonids were the focus of early fish culture efforts, the popularity of black basses as warmwater sport-fishes stimulated the curiosity of anglers and encouraged scientists to conduct practical experiments on the culture of bass and other species of value.

2.4 Hatchery scale-up

In 1870, the American Fish Culturist Association was formed (later to become the American Fisheries Society). Its primary function was to serve as a way to share information on artificial fish propagation and one of the first topics addressed was black bass culture (Moffitt, 2001). The journal *The*

Progressive Fish-Culturist was their primary conduit for information dissemination and many of the fundamental papers on LMB propagation and culture were published there.

In 1871, the federal government became directly involved. The Office of the Commissioner of Fish and Fisheries (commonly known as the U.S. Fish Commission) was established with a mandate of enquiring into the causes in the decrease of food-fishes and to recommend remedies. In 1872, the Commission undertook the propagation of food-fish. Millions of eggs were hatched and fingerlings stocked. Much of the focus was still on salmonids, such as Atlantic salmon, Pacific coho, and Chinook salmon. However, largemouth bass were also produced (along with many others). For the fiscal year 1897, the U.S. Fish Commission reported that 95,358 largemouth bass eggs, fry, and juveniles were distributed. That same year, the U.S. Commission of Fish and Fisheries published *A Manual of Fish-Culture*. Methods for propagating "black basses, crappies, and rock bass" were of sufficient interest to warrant a 25-page chapter in that manual. Many of the pond-based culture methods described in that chapter are still used in present day hatcheries.

In 1898, the state of New York reduced the number of black bass a fisherman could catch per day and also recognized a need to devise a means "for the hatching of bass artificially" (Long et al., 2015). State fish agencies in New York, New Jersey, and the New England states developed state fish culture and management programs and also fostered early private fish farms. The emphasis, however, was on brook trout, the native freshwater salmonid in northeastern America, and to a lesser extent, the American shad. Old government reports also indicate an interest in the common carp.

2.5 Legacy of overfishing

While overfishing was recognized as having a negative impact on fish populations, during the late 1800s it also began to be recognized that man's impact on the environment could also negatively impact fish populations. Logging and mining increased sediment run-off, manufacturing produced chemical effluents, and dam construction changed flowing water environments (lotic ecosystem) to more of a still water environment (lentic ecosystem). Black bass were proposed as suitable species for stocking into these new stillwater conditions. Unlike trout and shad, bass could establish self-reproducing populations (Long et al., 2015). Supplying juvenile fish to stock into these systems, and other impoundments developed during the country's expansion west, created a need for black bass hatchery programs. By the 1900s, black bass had been stocked into 26 states outside of their native range (Robbins and MacCrimmon, 1974).

Although the average citizen and fisherman primarily still saw recreational fishing as a source of food, certain individuals and groups began to call for a halt to the commercial harvest of black bass and to push for their status as a recreational only (game fish) species. The Izaak Walton League was formed in 1922 and worked with the states to pass laws protecting black bass from commercial harvest for food-fish markets.

As these state laws were promulgated, a new set of issues developed. Black bass harvested illegally in one state began to be "bootlegged" across state lines and sold in another state where sales were still legal. This led to a federal statute known as the Black Bass Act 1926, which made it a federal offense for fish caught illegally in one state to be sold into a different state. For many years this basically ended the status of black bass as a commercial food-fish species.

2.6 Government intervention and expansion

As the country moved from the 1920s into the depression era of the 1930s, President Franklin Roosevelt launched his New Deal programs. Several of the programs included water development projects, including the Tennessee Valley Authority (TVA) Act 1933 and the Flood Control Act 1936. Both of these supported the construction of large dams, which impounded large reservoirs. However, it was feared that the rapid filling off these large water bodies would result in biological deserts (Miranda, 1996). To prevent this, fish hatcheries were often constructed on site as part of the overall project.

These New Deal programs not only supported the construction of huge reservoirs but also the building of a large number of small farm ponds. Farm pond construction was promoted to control soil erosion, as well as a self-sustaining source of food, by the Soil Conservation Service in 1954. The number of farm ponds estimated to exist in 1934 was 20,000. By 1965, this number had increase to 2,000,000 (Swingle, 1970). The largemouth bass was the species most often stocked into these ponds (Swingle, 1970). The number of state and federal hatcheries producing largemouth bass grew from fewer than 100 in 1900 to 99 federal hatcheries and over 500 state hatcheries by 1950. The number of private bass hatcheries has not been documented, but they could be found in most states across the South and the Midwest.

Research on fisheries management, and the biology of fish that supported recreational fisheries, was stimulated by the establishment of the US Fish and Wildlife Service Cooperative Fishery Research Units beginning in the early 1960s. Co-op Units were located at land-grant universities across the USA and provided research, graduate level training, and extension on matters of interest to state and federal agencies. Research on largemouth bass issues was a major part of the programs in Missouri, New York, Texas, Oklahoma, and several other states. Much of the research focused on strategies to use the enormous reproductive potential of natural reproduction, rather than hatchery propagation, to increase recruitment.

2.7 Modern culture of the largemouth bass

However, the demand for fingerling largemouth bass to stock ponds and reservoirs continued to increase. Robbins and MacCrimmon (1974) reported that > 35 million largemouth bass were stocked annually between 1966 and

1970. Despite increased demand, culture methods remained essentially those in use during the late 1800s. If a production system works predictably and produces fish relatively efficiently, "Why change?" Hatchery managers and fish farmers saw no compelling reason to change when the old system worked.

While managers agreed that stocking new water was beneficial, the positive effects of continued supplemental stocking came to be considered as questionable (Long et al., 2015). Most of the fingerlings stocked in these early stocking programs were small (< 25–50 mm). However, researchers indicated that the best success came when stocking large size largemouth bass fingerlings (178–229 mm). This created an increased demand for the production of these advanced fingerlings.

As discussed previously, first attempts at largemouth bass culture mimicked the techniques that had been developed for trout culture. However, there are fundamental differences between these fishes in large-scale reproduction and fry rearing techniques. Stripping eggs and milt from bass that appeared ready to spawn revealed a major problem immediately: bass anatomy does not lend itself to egg or milt stripping procedures. It did not matter how "ripe" the bass were, they could not be stripped. Although "ripe" female bass release eggs and attentive males would rush in to fertilize them in a pond or lake environment, attempts to strip milt from mature bass, or other centrarchid fishes, simply did not work.

A second difference between trout and bass involved larval and fry feeding. Trout have large eggs and larval yolk sacs. With these stored reserves, trout fry can survive for weeks without exogenous feeding. In contrast, bass eggs are small, and fry have very little stored energy and nutrient reserves in their yoke sacs; therefore, they must begin feeding soon after hatching, typically in no more than 3–4 days. Largemouth bass are primarily reared in nursery ponds, relying on natural or enhanced zooplankton populations. Brine shrimp, rotifers, copepods, and other forms of zooplankton have been used in tank or trough systems and shown to be technically feasible for first-feeding bass, but have not yet proven practical at production scale. Bass have to undergo a feed-training regime at 3.5–5 cm, while trout can be trained to take artificial feeds at swim-up (first-feeding).

Figure 2.1 Jack Snow of the Marion National Fish Hatchery in Marion, Alabama. Photo courtesy of the Auburn University Fisheries and Allied Aquacultures Image Gallery.

In review, there was a steady increase in the demand for hatchery production of largemouth bass dating back to the 1800s. By the 1900s, black bass had been artificially propagated and stocked into scores of states, including > 25 outside of their natural range. In the 1930s the federal government supported the construction of both small impoundments on farms as well as large reservoirs, requiring millions of largemouth bass fingerlings for stocking. Most of these were small fingerlings (5–8 cm), but fisheries managers found that larger fingerlings (18–22 cm) were much more successful after stocking. In the 1960s and 1970s, the work of Jack Snow (Figure 2.1) and colleagues at the Marion National Fish Hatchery in Alabama resulted in the development of the "Marion Method" (Snow 1960, 1963, 1968, 1975) with which large numbers of advanced and large largemouth bass fingerlings could be produced per unit of pond space.

2.8 Largemouth bass as a food-fish

So, when did largemouth bass begin to be grown for food-fish markets? While federal and state hatcheries sometimes document their production through reports and technical advancements through publications, private hatcheries largely do not. We know that private hatcheries have also been producing largemouth bass likely back to the 1800s. They have utilized the technologies developed and shared by the public hatcheries while also making and refining their own technologies and innovations. As discussed previously, there has been resistance to the sale of largemouth bass in the USA back to at least the 1920s. However, what is actually legal and what is actually illegal is not always well defined and differs between states. Producers might choose to not ask and raise the issue. This is the fisheries version of "don't ask, don't tell." If the questions were asked, the assumption is they would be ruled illegal due the legislation from the 1920s banning the harvest and sales of black bass as commercial fisheries. It is likely that large markets for largemouth bass developed early in Toronto, Canada not only because it is a large population center of ethnic Asians, but also because they were in close proximity to, but still outside of, the US border. Very little of the history of the production of largemouth bass as a food-fish in the USA has been captured in written form. So, the question is, when did largemouth bass begin being raised as a food-fish?

Bardach et al. (1972) in their early classical work on aquaculture, reported that in the 1950s, several centrachids were evaluated for commercial production by Auburn University. The blue-gill (*Lepomis macrochirus*) and the flier (*Centrarchus macropterus*) were rejected as unsuited for culture, but they found that the crappies and largemouth bass showed potential. The authors reported that the LMB in the 1970s was much more common in commercial fish culture in Europe than in its native land. They indicated that the LMB had been stocked in Latin America and Africa and in 1965 experimental culture was initiated in Tunisia. In Europe, they were used as a predator in polyculture to control unwanted trash fish and the excessive reproduction of the other polyculture species. The commercial international seafood supplier Sea Port (2018) indicates that the largemouth bass was introduced in to Taiwan in the 1970s. They have been raised there since that time and currently are the source of fish for their commercial export of whole frozen largemouth bass. In the USA, Bardach et al. (1972) reported that in the early 1970s, a few commercial fish hatcheries, particularly in Arkansas, were already raising edible size centrarchids, including the largemouth bass. However, they were being marketed to and through fee fishing ponds. Major limitations noted were that the production rate of food size largemouth bass was only 44 kg/ha (Bardach et al., 1972) and their sale in some areas was forbidden.

Because of a lack of written information, the authors of this chapter conducted a number of interviews with both private producers and government officials to try to establish some aqua-culture dates and timelines. A longtime fish producer in northern Ohio indicates that he sold his first load of largemouth bass food-fish in the mid-1980s. The fish were sold into an Asian market that was already in existence, but was still relatively small. To "break into" this market required traveling to New York or Toronto for face-to-face meetings with the buyer. Only then could any fish be sold. His first loads of live largemouth bass went to Toronto, Canada.

An Ohio grower also indicated that he had started feed-training largemouth bass fingerlings back in 1979, using moist cat food. Around 1985, he sold some third-generation feed-trained fish to producers in Arkansas. As documented in Chapter 5, the use of domesticated feed-trained brooders makes for rapid progress in the feed-training success of offspring and adaptation to production conditions. The producer reported that initially, there were a lot of problems with "liver-disease" (this was probably glycogen deposition from using high carbohydrate feeds, see Chapter 7). By 1995, there was a lot of interest in the state (Ohio) from producers requesting help getting into largemouth bass production. He indicated though that these efforts were all "grass roots" with little or no support from state agencies or universities.

In another interview, a large fish hatchery in Arkansas reported that they began selling largemouth bass into the Asian food-fish market before 1998. Prior to that they had already been raising feed-trained largemouth bass fingerlings. The first fish they sold into the food-fish market were retired broodfish. Their business had concentrated on sport-fish as their selling price was more than double what food-fish sold for. As the largemouth bass became more domesticated, and their techniques improved, the fish performed better on feed and survivals increased. With that, the farm moved into producing large quantities of largemouth bass for the food-fish market in 1998. Initial fish for these markets were sold at 225–340 g. However, markets later expressed a desire for larger sizes. The producer indicated that at the time of interview (2018), there is still unfulfilled demand for food-fish largemouth bass. Limitations on expansion include high production costs, primarily due to high feed costs, poor labor availability, and government regulations. He indicated that he was not sure if consumers would continue to pay the high selling prices imposed by these high production costs. This farm not only sells fish into Toronto markets, but also reports significant markets on the west coast, including San Francisco, Oakland, San Diego, and San Jose (California).

To understand the history of the development of the largemouth bass as a food-fish, we need to understand some aspects of the ethnic Asian market which is by far the primary outlet. The market is covered more thoroughly in Chapter 12. While these markets may be relatively new to us, they are actually part of a culture whose desire for live seafood goes back millennia. In an interview with food writer Wendy Goodfriend, Lisa Li, who grew up in Guanghou China explained "in Chinese culture, we like to get our protein as close to live as possible" (Goodfriend, 2018). What could be fresher than a fish that was swimming less than an hour before you eat it? The cultural context is captured even more when they discuss a Chinese New Year's Eve feast where a whole fish is the traditional last course. The word fish (*yu*) is also the Chinese word for abundance, making a simply dressed, steamed whole fish a symbolic way to end the meal. Serving the fish whole represents "completeness." It is essential that the fish be served with head and tail still attached to make sure the coming year has both a good beginning and a good end. At the New Year's Eve dinner, the head of the fish is always pointed toward the oldest or most honored guest. It is also a tradition to not finish the fish course on New Year's Eve, but to leave some uneaten for the next day so that the abundance of *yu* will continue into the New Year (Goodfriend, 2018).

A significant change in the live largemouth bass market occurred when, at the beginning of 2013, the New York State Department of Environmental Conservation opened the market in New York City for hatchery-raised largemouth bass. They required that bass be in labeled containers, purchase and sales records must be maintained by distributors, and fish can be sold live but must be killed before being transferred to the retail customers. This change was opposed by sport-fishing advocates who argued that the rule change would create a black market that could damage the states recreational fisheries (ActivistAngler, 2013). The counter argument was made by aquaculturists in New York that the ready availability of legal farm-raised LMB would actually lessen the demand for black market fish and relieve pressures on the natural fisheries (Figura, 2012).

2.9 Conclusion

The history of largemouth bass aquaculture dates back to the mid-1850s. Techniques were adapted from those developed in Europe for salmonids. Expansion of hatchery production occurred as the country expanded westward, the farm pond program of the 1930s led to the construction of thousands of small ponds, and New Deal programs constructed large reservoirs. Most of these activities were based on small fingerlings. However, larger fingerlings were shown to be more effective in stocking programs. Snow and associates at the Marion Lab in Alabama established feed-training procedures that allowed larger sizes to be produced at higher densities. Larger fish began to be marketed into ethnic Asian markets in the 1970s. In 2013, the New York market was allowed to sell farm-raised largemouth bass. The market for live largemouth bass is likely approaching 1,000,000 kg/year.

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Chapter 3
Largemouth bass production in China

Yangen Zhou[1] *and Chengyue Liu*[2]

[1] Key Laboratory of Mariculture (Ocean University of China), Ministry of Education

[2] Fisheries College, Ocean University of China, Qingdao, China

3.1 History of largemouth bass production in China

The largemouth bass (*Micropterus salmoides*) is a freshwater fish native to North America. It has been widely stocked into a number of other countries, primarily as a sport-fish. Around 1970, it was introduced into Taiwan from North America. The first successful artificial spawning in Taiwan occurred in 1983 (Liao, 2000). It was then introduced into Guangdong Province, China, in 1983, and has since become a popular culture species across the whole country (Bai et al., 2008; Bai and Li, 2013b; Ma et al., 2003; Lou, 2000; Zhang and He, 1994).

While largemouth bass has become an economically important freshwater species in many parts of China when compared to some other cultured species, it has a slower growth rate, advanced age at sexual maturity, a high feed conversion ratio, and low resistance to stress. For a time, these issues restricted the further development of the species in China. However, in 2010, a new breeding variety "Youlu No. 1" was produced by the Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, China. The culture of this new genetic variety has become popular across large areas of Guangdong, Jiangsu, and Hunan provinces (Liang et al., 2007). The Youlu No. 1 variety has demonstrated three advantages over other varieties: (1) a desirable body shape, with a low rate of deformities; (2) faster growth and high production rates (kg/ha); and (3) minimal growth differences among individuals, producing high yields while maintaining size uniformity (Bai and Li, 2013a).

3.2 Largemouth bass production areas in China

With the continuous development of improved farming technologies, this species can now be cultured in most provinces of China, including those in the north, such as Liaoning Province, Heilongjiang Province, and Xinjiang Uygur Autonomous Region (Zhan, 2001; Zhang et al., 2017). However, the main largemouth bass culturing areas are Foshan city in Guangdong Province, Huzhou city in Zhejiang Province, Wujiang district and Nanjing in Jiangsu Province, and Mianyang city in Sichuan Province (Figure 3.1) (Bai et al., 2009; Li et al., 2012). Table 3.1 shows that the production of largemouth bass in China increased 23.7% between 2007 to 2013, going from 125,500 metric tons (MT) to 152,200 MT. If we compare the data in Tables 3.1 and 3.2, they indicate that in 2007 and 2013 largemouth bass production accounted for 80% and 45% of total freshwater bass production, respectively. In these data other fish included in the freshwater bass category included the roughskin sculpin (*Trachidemus fasciatus*), the Asian seabass (*Lates calcarifer*), and the European perch (*Perca fluviatilis*).

Table 3.1 Area of production and yields of largemouth bass across China in 2007 and 2013.

Province	City	Culture method	2007[1]		2013[2]		Diet
			Area (km^2)	Annual yield (10^3t)	Area (km^2)	Annual yield (10^3t)	
Guangdong	Foshan	Pond culture	40	90	47	100	Frozen fish
Zhejiang	Huzhou	Pond culture	14	15	14	18	Frozen fish
Jiangsu	Wujiang	Cage and pond culture	7	11	17	14	Frozen fish
Jiangsu	Nanjing	Pond culture	5	6	13	8	Frozen fish
Jiangsu	Suzhou	Cage and pond culture	–	–	10	8	Frozen fish
Sichuan	Mianyang	Cage and pond culture	3	3	4	4	Frozen fish with feed
Sichuan	Jianyang	Cage culture	0.1	0.1	0.1	0.1	Frozen fish with feed
Sichuan	Guangyuan	Cage culture	0.1	0.1	0.1	0.1	Frozen fish with feed
Total				125.5		152.2	

Sources: [1]Bai *et al.* (2009), [2]Bai and Li (2013).

3.3 Largemouth fry and fingerling rearing

Reproduction of largemouth bass in China begins with broodfish stocked into spawning ponds. Resulting fry can then be cultured to sizes suitable for feed training (~2 cm) either in nursery ponds or in concrete tanks.

3.3.1 Spawning ponds

For fry production, the selected broodfish are placed in the spawning pond. The density of broodfish is 0.45–0.60 fish/m^2 at about 300 kg/ha. The ratio of males to females is 4: 6. The reason for fewer male fish in the pond, is to prevent unnecessary energy loss caused by male to male territoriality and aggressive interactions. Largemouth bass spawning ponds should have an area of 2,000–3,300 m^2 (0.2–0.3 ha), water depths ranging 1.3–1.5 m, and an adequate supply of high-quality water. A pond of this size and depth has three advantages: (1) it is easy to seine; (2) the temperature at the bottom remains suitable; and (3) the dissolved oxygen (DO) is sufficient, which is beneficial to the spawning of the fish.

Generally, the spawning ponds have 2–3 aerators, and aerators are turned on in the evening. With rainy and cloudy weather, aerators may be turned on during the day to maintain

Table 3.2 Annual outputs of bass (freshwater) in different provinces of China (10^3t).

Province	Year											
	2003	2004	2006	2007	2008	2009	2010	2011	2013	2014	2015	2016
Beijing	0.28	0.15	0.13	0.03	0.06	0.02	0.05	0.05	0.19	0.19	0.00	0.01
Tianjing	0.15	0.00	0.17	0.09	0.00	0.00	0.00	0.00	0.76	0.00	0.80	0.92
Hebei	0.02	0.03	0.02	0.01	0.02	0.03	0.01	0.04	0.03	0.04	0.19	0.25
Shanxi	0.01	0.00	0.00	0.01	0.02	0.02	0.04	0.03	0.04	0.04	0.02	0.02
Liaoning	0.09	0.07	0.19	0.05	0.07	0.18	0.22	0.11	0.14	0.17	0.10	0.09
Jilin	0.01	0.00	0.01	0.03	0.04	0.26	0.16	0.15	0.05	0.04	0.03	0.04
Heilongjiang	0.00	0.00	0.03	0.01	0.01	0.01	0.04	0.01	0.01	0.01	0.01	0.01
Shanhai	0.00	0.69	0.70	0.49	0.28	0.37	0.36	0.20	0.25	0.34	0.37	0.01
Jiangsu	10.77	12.83	14.23	14.23	20.15	19.65	22.68	27.18	34.86	34.97	36.21	36.88
Zhejiang	8.86	9.36	11.49	10.29	11.43	14.24	15.01	18.07	18.16	18.58	21.34	29.06
Anhui	1.24	0.82	1.31	1.27	0.99	2.55	2.49	4.11	0.82	4.97	5.85	5.93
Fujian	6.38	4.03	6.44	5.40	4.39	6.44	7.63	7.98	10.18	10.41	11.08	11.27
Jiangxi	2.18	6.55	11.37	12.70	13.92	15.14	16.00	17.22	20.19	21.01	21.78	22.70
Shandong	0.80	0.77	0.86	0.79	0.29	0.39	0.74	0.54	0.71	0.73	0.60	0.59
Henan	0.02	0.06	0.25	0.28	0.25	0.28	0.13	0.27	0.38	0.41	0.57	0.83
Hubei	0.96	0.47	0.90	1.18	1.61	2.04	2.94	2.42	3.34	3.56	3.84	4.39
Hunan	1.03	1.30	1.14	0.94	1.01	0.99	1.29	1.68	1.54	1.43	1.51	3.09
Guangdong	89.22	94.81	104.54	101.36	102.72	101.47	104.71	115.57	231.84	237.70	227.94	234.69
Guangxi	3.20	3.22	0.64	0.51	0.55	0.57	0.61	0.80	0.74	0.75	0.77	0.72
Chongqing	0.03	0.05	0.11	0.14	0.17	0.33	0.33	0.74	0.94	1.08	1.61	1.96
Sichuan	1.03	1.37	5.42	7.06	8.38	8.31	8.78	9.40	10.95	11.43	12.23	12.39
Guizhou	0.03	0.11	0.79	0.45	0.00	0.88	1.10	1.34	2.48	2.75	4.79	6.44
Yunan	0.00	0.01	0.02	0.01	0.03	0.04	0.01	0.01	0.51	0.12	0.19	0.18
Shaanxi	0.01	0.13	0.10	0.06	0.08	0.08	0.08	0.05	0.10	0.00	0.11	0.11
Gansu	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00
Ningxia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.25	0.31
Xijiang	0.06	0.04	0.07	0.12	0.15	0.20	0.58	0.36	0.66	0.87	0.89	1.10
Total	**126.37**	**136.87**	**160.96**	**157.49**	**166.60**	**174.47**	**185.94**	**208.33**	**339.84**	**351.77**	**353.08**	**374.06**

Source: China Fisheries Statistics Yearbook.

DO concentrations. The water in the pond is kept clear, with a transparency of ≥ 30 cm. The water quality of the pond should be maintained in the range of: pH 7.0–7.8, ammonia-nitrogen < 0.4 mg/l, and nitrite concentration < 0.3 mg/l.

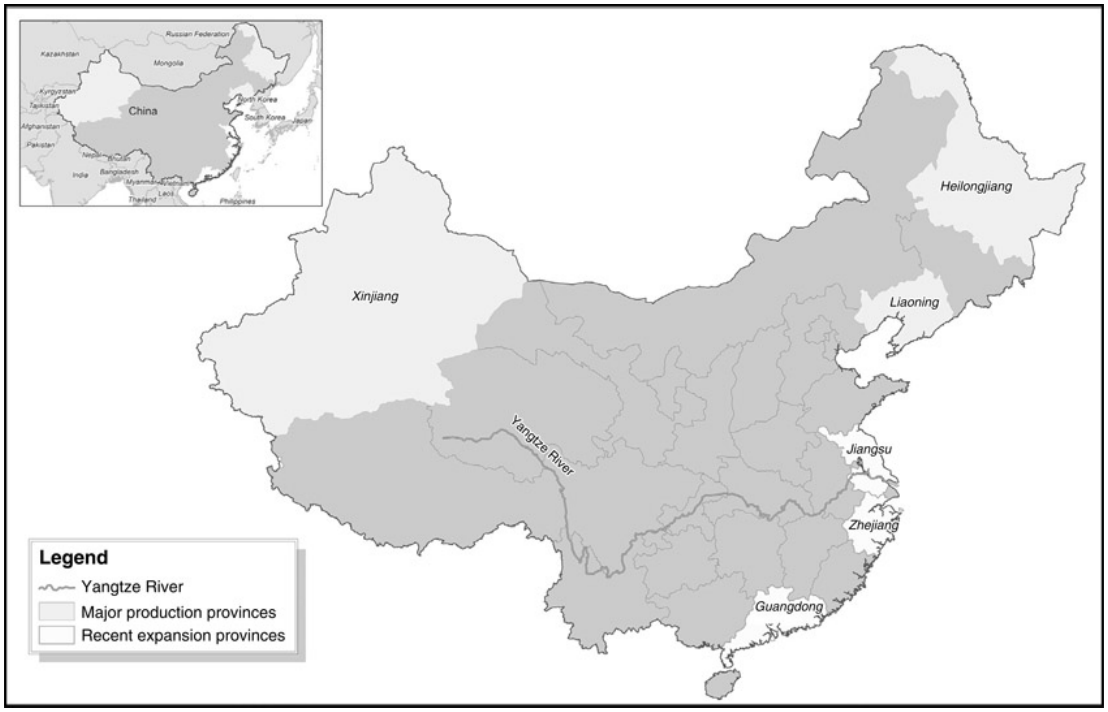

Figure 3.1 Main Chinese largemouth bass production regions and northern regions of recent expansion.

After spawning and with fry-swim up, the fry deplete their yolk-sac storage of endogenous nutrition and switch to exogenous feeding. However, in this species the fry cannot be immediately trained to accept artificial feeds and must be cultured on natural foods until they reach a size when they can be acclimated to artificial diets. This period of natural foods is considered the nursery phase. In China, there are two main nursey methods used to rear largemouth bass fry. These methods are concrete tanks and nursery ponds. In general, the fry survival rate is greater when reared in concrete tanks (> 80%) compared with ponds (~50%), but the production costs are higher (Li, 2008; Wu, 1993).

3.3.2 Fry rearing in concrete tanks

The size of the concrete tanks used to rear largemouth bass fry in China ranges from 20 to 30 m^2. Walls should be smooth to prevent skin abrasions.

Initially, the water depth is 20–25 cm, and then increases with new water input to 50–70 cm. Fry density at stocking is approximately 600 fish/m^2. Fry initially are fed small zooplankton, such as rotifers and caudate (salamander) larvae that have been harvested from ponds, and can be fed 2–3 times daily. Later, when fish fry reach 1.5–2.0 cm in length, larger zooplankton such as cladocerans, copepods, or rotifer should be provided. Wickstrom and Applegate (1989) reported that six genera of invertebrates served as feed sources for largemouth bass fry during a 25 d period. In that trial, the fry preferred to eat the cladoceran *Moina brachiate*. When fish length exceeds 2 cm, they can be fed with water earthworm (Oligochaeta), and then begin acclimation to frozen fish or commercial food. However, in 2017 the Chinese government started to prohibit the feeding of frozen fish for aquaculture, so fingerlings are now all acclimated to artificial feeds (Li, 2008; Wu, 1993). If the fingerlings are reared over the winter in tanks, they should be in a greenhouse to maintain warm temperature and reduce the impact of seasonal weather changes.

3.3.3 Fry rearing in nursery ponds

The stocking density of nursery ponds ranges from 40,000 to 70,000 fish/1,000 m^2 (400,000–700,000 fish/ha). Fry stocked together should be derived from the same batch to ensure a consistent size and prevent cannibalism. Since largemouth bass fry mainly feed on plankton, a certain degree of water fertilization must be maintained. Fish reaching a mean body length of 1.5 cm are ready to be trained to accept commercial feeds (Li, 2008; Wu, 1993).

As noted previously, the largemouth bass requires a training period for acclimation to commercial feed. In China, the feed-training procedure usually takes place in the concrete tanks previously described for use in the nursery phase of fry rearing or in large plastic tanks. On some small farms with limited resources a corner of the fry pond is cordoned off with netting or a simple net-pen is constructed. These enclosures are shaded by a roof. The feed-training procedure is as follows: The water should be rich by using fertilizer to promote natural productivity in pond. The fish fry (about 1.32 cm) are fed red insect powder (dried chironomids) along with commercial fry feed powder or fish paste. If the fish fry eats the fish paste well, you can add the fry feed powder to the fish paste, and increase the amount of feed powder in the mix day by day.

After about 1 week, the mix comprises approximately 60–70% feed powder. The fish are usually then ready to transition to commercial feed. After 7–10 d, the amount of commercial feed is slowly increased until the fish are completely acclimated to it. In general, fry at sizes ranging from 1.98 cm to 2.64 cm should be completely acclimated to commercial feed. It should be noted that in China the fish are then size graded and the small fish fry are put through feed-training again. After a second feed acclimation period of 7–10 d, these fish should also be trained and can be transferred to be cultured in ponds or cages. There the fish are fed three times per day (morning, noon, and night), using the feeding rule of "slow, quick, and slow" until most fish are not coming to surface for more feed. Since this species has the characteristic of avoiding strong light, it is better to offer most of the feed in the morning or at night (Bai and Li, 2013a).

3.4 Pond and cage culture

Pond and cage culture (Figures 3.2 and 3.3) are the main production systems for the rearing of largemouth bass in most culturing provinces in China. Pond culture mostly occurs in southern China, such as Guangdong Province (Li et al., 2012). Cage culture is distributed across the Yangtze River regions (Figure 3.1) which contain lakes and reservoirs, such as Sichuan Province (Bai et al., 2009; Li and Li, 2000; Zhan, 2001).

Figure 3.2 Largemouth bass pond aquaculture in Jiangmen, Guangdong province.

Figure 3.3 Largemouth bass cage aquaculture in Nanning, Guangxi province.

3.4.1 Pond aquaculture

Aquaculture ponds used in largemouth bass production must maintain good water quality. The pond bottom is usually comprised of fine sand and gravel, with low levels of silt. The pond size is usually 2,000–3,500 m^2 (0.2–0.35 ha) and water depth is 1.5–2.0 m. Ponds are routinely disinfected before use.

The stocking densities of largemouth bass in ponds are: if stocking 8–10 cm juveniles 1,500–2,300 per 1,000 m^2 (15,000–23,000/ha); or if stocking 15 cm juveniles 1,000–1,500 per 1,000 m^2 (10,000–15,000/ha). To help maintain water quality, approximately 200–250 large-size silver carp (*Hypophthalmichthys molitrix*), crucian carp (*Carassius auratus*), and bighead carp (*Aristichthys nobilis*) can be placed in the pond (a total of 600–1,000 carp/ha).

3.5 Pond polyculture

Ponds for extensive polyculture production should exceed 2,500 m^2 (0.25 ha) in surface area to ensure adequate space for polyculture. The ponds should not contain snakehead (*Ophiocephalus argus*), catfish (*Parasilurus asotus*), or other aggressive and carnivorous fish. Approximately 100–150 juvenile largemouth bass 5–6 cm in size are stocked per 1,000 m^2 (1,000–1,500/ha) on an annual basis in April or May. The size (average individual weight) of other fish in the polyculture pond, such as grass carp (*Ctenopharyngodon idella*), silver carp, bighead carp, carp (*Cyprinus carpio*), and bighead carp should not exceed 150 g each (Li et al., 2012; Zhan, 2001).

3.6 Cage culture

Cages are constructed of polyethylene no-knot mesh and are generally 2 m × 2 m × 2 m or 3 m × 3 m × 2.5 m in size. The mesh size used is dependent on the size of fingerlings being stocked. A cage with 1 cm mesh is suitable for 8 cm fish, while 2.5–3.0 cm mesh should be used when fish exceed 15 cm. The cage mesh is suspended within a floating closed frame that provides some shaded area with good air flow. Cages should be located in open water, in an area with good air movement, a water depth > 3.5 m, and limited water flow (< 0.1 m/s) (Bai et al., 2009).

Largemouth bass density in culture cages is determined by the size of fish being stocked, with densities decreasing as fish size increases. The cages are initially stocked with 8–10 cm juveniles at approximately 300 fish/m^2. When the juveniles reach 30–50 g average weight, the density is decreased to 150–200 fish/m^2. When fish weight reaches 150 g the density is reduced to 100 fish/m^2 (Bai et al., 2009; Li and Li, 2000; Zhan, 2001).

3.7 Feed types and sources

Until 2016, largemouth bass culturing in China was still dominated by frozen trash fish feed, or a mix of frozen trash fish and formulated diets (Zhu et al., 2006). However, with the development of intensive high-density largemouth bass culture, formulated diets have become more popular (Li et al., 2012; Zhou et al., 2015).

Currently there are more than a hundred commercial aquaculture feed companies in China. Commercial largemouth bass feeds are produced by both domestic and international feed companies. Currently largemouth bass are primarily fed floating feed. The domestic feed producer TECH-BANK Food Co. Ltd. has produced the extruded floating pellets for largemouth bass since 2015. The protein content of commercial feed for largemouth bass feeds ranges from 44% to 48%. The main protein source in the feeds is still fish meal.

3.7.1 Frozen fish feed

As stated, in the past largemouth bass in China were largely fed frozen trash fish during grow out. When feeding frozen fish, the feed conversion ratio (FCR) exceeded 4.0 but was still reasonably profitable. However, since 2017 the Chinese government has prohibited the feeding of frozen fish in aquaculture for several reasons. First, it is difficult to guarantee the quality and quantity of frozen fish. Second, feeding frozen fish can transfer diseases. Finally, when feeding frozen fish, water quality can easily be negatively impacted.

3.7.2 Formulated diets

In recent years, commercial feeds for largemouth bass have been more fully developed. Using commercial pelleted feeds, feed conversion ratios (FCR) of 1.1–1.3 have been achieved. The three primary advantages associated with the use of commercial feeds are: (1) reduced labor requirements for feeding; (2) maintenance of better water quality; and (3) reductions in disease (Yang et al., 1995).

3.8 Markets and product forms

Largemouth bass as a food-fish is rich in protein, lipids, and essential amino acids. The market demand for largemouth bass in China has increased considerably in recent years. Live largemouth bass are primarily transported in refrigerated containers. Long-distance transport using refrigerated containers involves three phases: live fish holding, packaging, and transportation.

Of the largemouth bass cultured in the Pearl River Delta, 5–8% are sold into the local aquatic product markets in the cities of Beijing, Xi'an, Zhengzhou, and Shanghai. In the provinces of Jiangsu and Zhejiang, production has increased in recent years. Most of these fish are sold into the growing nearby markets of Nanjing, Hangzhou, and Shanghai, with a small amount being sold in Beijing and Xi'an (Bai and Li, 2013b).

The size of fish desired by the different regions and markets varies. In northern markets, such as Beijing and Zhengzhou, the primary demand is for large fish (> 500 g), and they are often used in restaurants. However, in Shanghai, Jiangsu, Zhejiang, and Xi'an, smaller fish (< 400 g) are more popular (Zhou et al., 2015). The fish are transported live by specialized transport companies and marketed live for preparation at home.

Different regions also have different cooking preferences for largemouth bass. In Guangdong province, people prefer to steam them or eat them raw (sashimi). In other provinces, varied preparations, such as steaming or dry frying are utilized.

3.9 Conclusion

With improvements in culture technologies, genetics, and changes in the modes of transportation and marketing, the economics of largemouth bass aquaculture in China has continuously improved. This has supported a steady and stable development of the largemouth bass aquaculture industry. However, some challenges remain for the largemouth bass aquaculture industry in China (Bai and Li, 2013b).

3.9.1 Genetic challenges

The foundation population originally introduced from North American was very small. Since that time there has been little attention paid to broodstock management or breeding and genetics. The rate of survival has decreased, the age at sexual maturity has increased, and disease resistance has decreased.

The development and dissemination of improved genetic varieties is required. Even if a new breeding variety, such as Youlu No. 1, is developed, currently the hatchery production of large-mouth bass is on small farms. National and provincial level hatcheries are needed for this species.

3.9.2 Feed challenges

Currently, largemouth bass feeds in China still contain about 30% fish meal. Commercial feeds for largemouth have improved. However, the diet formulations for largemouth still need to be optimized for satisfying their nutrient requirements (Chen et al., 2012; Li et al., 2017).

The development and optimization of commercial feeds is required. Research and development of feeds for this species have progressed rapidly among some Chinese companies in recent years. However, there is still a need to improve and optimize feed formulations specific for this species (Li and Chen, 2011). Also, from a genetic breeding standpoint, the adaptability of largemouth bass individuals to accept commercial feed varies greatly. Therefore, there is a need to combine improved diet formulation, improved feed processing technology, and improved genetics to build a new largemouth bass aquaculture model (Bai and Li, 2013b).

3.9.3 Disease challenges

To increase yields and profits, the culture density of largemouth bass has been continuously increasing. With the genetic degradation of the species, disease issues are a frequent problem. Currently there are several common diseases, including parasites, viral and bacterial diseases, as well as a "multi-pathogen comprehensive disease phenomenon" (Bai and Li, 2013b). Disease susceptibility and its interaction with nutrition and genetics need to be better understood.

Better disease control is required. Appropriate stocking densities need to be established for this species. Overstocking at unsustainable densities is a problem for Chinese aquaculture farmers. More sustainable stocking densities would reduce the use of chemicals and drugs. Increased use of ecological control techniques such as probiotics and prebiotics should be evaluated. Moreover, the Chinese government should strengthen its support of research on disease control of this species, establish rapid detection technologies, and speed up the research and development of vaccines, especially those targeting viral disease (Bai and Li, 2013b).

3.9.4 Industrial management challenges

There are no large companies or enterprises rearing this species. Cultivation of largemouth bass is almost entirely by small farmers. Therefore, there is little supply chain management and the industrial chain is short and the level of technology development is low. This also contributes to the market price fluctuations which lead to fluctuations in production (Bai and Li, 2013b).

Industrial management and brand establishment should be improved. There is a need to establish the model of "company + farmer." Larger companies could provide high-quality fingerlings and high-quality feeds. Larger companies could also support advanced breeding technologies and provide market consultation to farmers. This model has been put into operation in Pearl River Delta of China. Branding and marketing of the largemouth bass could help to avoid large fluctuations in market price. In addition, there is a need to study the food science aspects of the species, including processing and cooking techniques. Also, there is a need to promote the use of the largemouth bass in traditional dishes, to develop unique dishes and preparations featuring the culinary attributes of the largemouth bass (Bai and Li, 2013b).

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Chapter 4
Environmental requirements for the culture of largemouth bass

Joseph R. Tomasso

School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Auburn, Alabama, USA

4.1 Introduction

Largemouth bass are adapted to temperate, freshwater environments. Since temperate aquatic environments characteristically exhibit extensive variability in physiochemical characteristics, it is not surprising that largemouth bass are tolerant to wide ranges of specific characteristics. In this chapter, the preferences and limits of largemouth bass to water quality characteristics important to aquaculture will be summarized.

4.2 Temperature

Temperature is the predominant physiochemical characteristic in aquaculture operations. It affects the amount of oxygen a given amount of water will hold, the rate of photosynthesis and decomposition, the ionization of ammonia, and the metabolic rate of the fish. Changing temperatures are also major cues for reproduction. Fry (1947, 1971) characterized the role of temperature as an environmental controller, and as such, should be given primary consideration when siting an aquaculture operation.

Largemouth bass eggs spawned at 17–21℃ exhibited good hatching (≥ 90%) when incubated between 13 and 24℃. By slowly (0.2℃/h) changing from spawning to incubation temperature, the range of good hatching was extended to 10–27℃ (Kelley, 1968). When tested under constant-temperature conditions ranging from 15 to 36℃, fry grew best in 25–27℃ water (Coutant and DeAngelis, 1983).

Largemouth bass fingerlings (mean 9.1 g) were cultured in tanks for 97 days on a commercial salmonid diet at either 20, 26, or 32℃ (Tidwell et al., 2003). Survival averaged 94% and was not affected by water temperature. Weight gain in the 26 and 32℃ treatments was similar and averaged 647% while weight gain in the 20℃ treatment averaged 325%. Across the three treatments, feed conversion ratio ranged from 1.0 to 1.2 and was lowest in the 26℃ treatment. Condition factor (K) ranged from 1.3 to 1.4 and was lowest in the 20℃ treatment. In aggregate, these results indicate that 26℃ is the best temperature of the three tested in which to grow largemouth bass fingerlings. A study by Díaz et al. (2007) using a temperature preference approach estimated the optimum temperature for growth to be between 28.1 and 28.6℃.

Table 4.1 Temperatures of importance to the culture of largemouth bass *Micropterus salmoides.*

Temperature (℃)	Action	References
2.0	Temperature at which 48% of Florida subspecies died after 5 days exposure	Carmichael et al., 1988
10.0–27.0	Good (≥ 90%) hatching success when acclimated to hatching temperature at 0.2℃/h	Kelley, 1968
25.0–27.0	Best temperature range for fry growth	Coutant and DeAngelis, 1983

Temperature (℃)	Action	References
26.0	Best temperature for fingerling growth based on a 97–day growth trial	Tidwell et al., 2003
28.1–28.6	Best temperature for growth based on temperature preference	Díaz et al., 2007
37.3	Chronic thermal maximum for northern subspecies	Fields et al., 1987
39.2	Chronic thermal maximum for Florida subspecies	Fields et al., 1987

Two subspecies of largemouth bass, the northern largemouth bass (*Micropterus salmoides salmoides*) and the Florida largemouth bass (*Micropterus salmoides floridanus*), are recognized and cultured in the USA (Philipp et al., 1985). As the names imply, one subspecies is native to more northern areas while the other is native to more southern areas. The two subspecies do differ from each other in characteristics that may be important to aquaculture. For instance, when water temperature is reduced to 2℃ (at a rate of 1℃/day) and then held for 5 days, about one-half of the exposed Florida largemouth bass fingerlings died while only 4–5% of northern fish died (Carmichael et al., 1988). Interestingly, hybrids of the subspecies (both originals and reciprocals) exhibited low-temperature tolerance similar to the northern subspecies. When water temperature was increased at a rate of 1℃/day, the northern subspecies was the most susceptible to high temperature while the Florida subspecies was most resistant and the hybrids were intermediate (Fields et al., 1987). Table 4.1 summarizes temperatures of importance to the culture of largemouth bass.

4.3 Dissolved oxygen

Fish, like all animals, require oxygen to transfer energy efficiently from molecules making up food to molecules that can transfer the energy to the host of physiological processes within the organism. Most oxygen in culture water comes from two sources. First, oxygen diffuses from the atmosphere down a pressure gradient into the water (mechanical aerators simply enhance this process). Second, oxygen is produced within the water by photosynthetic activity of algae. Some intensive aquaculture systems may also add pure oxygen. Oxygen dissolved in the water will enter a fish by diffusing down a pressure gradient across the gills into the blood of the fish. Once in the fish, the oxygen is transported to the tissues where it is needed, at which point it further diffuses down a pressure gradient into the tissue that will consume it. It should be noted that the driving force for delivering oxygen to the tissues is passive (diffusion down a pressure gradient). The pressure gradient is maintained by a high concentration of oxygen in the water (because of constant inputs) and a low concentration of oxygen in the tissues (because of constant consumption). There is no active uptake system for oxygen. Hence, acceptable environmental oxygen levels must be maintained at all times or the fish will not have adequate oxygen to function to its potential (Colt and Tomasso, 2001).

When discussing oxygen concentrations required for fishes, temperature must be an integral part of the discussion for two reasons. First, increasing temperature increases the metabolic rate of fishes, which is reflected in increased oxygen needs (Fry, 1947, 1971). Second, warmer water holds less oxygen than cooler water (Table 4.2 this volume, discussed in Colt and Tomasso, 2001). Taken together, fish in warmwater have a higher oxygen requirement in an environment with a decreased pressure gradient with which to deliver the oxygen to the tissues. For example, oxygen consumption of largemouth bass fingerlings acclimated for 30 days to 20, 23, 26, 29, and 32°C demonstrated a linear increase in oxygen consumption (from 46 to 86 mg O_2/kg/h) over the range of acclimation temperatures, an 87% increase (Díaz et al., 2007).

Largemouth bass embryo development and egg hatching as a function of temperature and dissolved oxygen concentrations were investigated in a comprehensive study by Dudley and Eipper (1975). Their results indicated that dissolved oxygen concentrations should be above 2.0, 2.1, and 2.8 mg/l for incubation temperatures of 15, 20 and 25℃, respectively, for successful hatching. However, the investigators observed many abnormally developed larvae. A further analysis of the results suggested that dissolved oxygen concentrations of 2.0, 2.5, and 3.5 mg/l for incubation temperatures of 15, 20, and 25℃, respectively, are adequate to reduce the incidence of abnormally developed larvae to the level found in incubation water containing 90% oxygen saturation. The Dudley and Eipper study also indicated that vertical movement of the hatching chamber in the water column was detrimental to hatching.

Critical oxygen levels (the lowest dissolved oxygen level at which fish consistently survived for 24 h) for largemouth bass fingerlings were determined by Moss and Scott (1961). Fish acclimated to 25, 30, and 35℃ demonstrated critical oxygen levels of 0.92, 1.19, and 1.40 mg/l, respectively. These values fit well with residual oxygen concentrations (dissolved oxygen concentrations at time of death from hypoxia) of ~0.7 mg/l for largemouth bass fingerlings acclimated to 22℃ (Carmichael et al., 1988).

Table 4.2 Dissolved oxygen saturation in pure water at selected temperatures under an air atmosphere at 760 mm Hg pressure (modified from Boyd, 1979).

Temperature (℃)	Dissolved oxygen (mg/l)	Temperature (℃)	Dissolved oxygen (mg/l)
8	11.5	22	8.5
10	10.9	24	8.3
12	10.4	26	8.0
14	10.0	28	7.8
16	9.6	30	7.5
18	9.2	32	7.3
20	8.8	34	7.1

Table 4.3 Dissolved oxygen concentrations of importance to the culture of largemouth bass *Micropterus salmoides.*

Dissolved oxygen (mg/l)	Action	Reference
2.0, 2.5, 3.5	Minimum oxygen levels in 15, 20, and 25℃ water, respectively, for good hatching success and normally developed larvae	Dudley and Eipper, 1975
0.92, 1.19, 1.40	Critical levels of dissolved oxygen (the lowest level at which fish consistently survived for 24 h) at 25, 30, and 35℃	Moss and Scott, 1961
0.7	Residual oxygen concentration (oxygen concentration at time of death) at 22℃	Carmichael et al., 1988
1.5	Residual oxygen concentration at 22℃ in the presence of 0.7 mg/l un–ionized ammonia	Carmichael et al., 1988
1.6	Reduced growth and feed conversion at 26℃	Stewart et al., 1967
4.0	Concentration at which fingerling growth and feed conversion did not differ from fish in higher oxygen concentrations at 26℃	Stewart et al., 1967

In a short-term (11–15 day) laboratory study at 26℃ (Stewart et al., 1967), largemouth bass fingerling growth was shown to increase with increasing dissolved oxygen levels in a near-linear fashion up to air saturation levels (~8.2 mg/l dissolved oxygen). Feed conversion also improved as dissolved oxygen levels increased. Dissolved oxygen levels as low as 1.6 mg/l did not affect survival but did affect growth and feed conversion. The authors indicated 4 mg/l as an oxygen level above which growth and feed conversions did not differ markedly. Interestingly, dissolved oxygen levels above air saturation levels (achieved using pure oxygen) had a detrimental effect on growth and feed conversion. The high pressure gradient in the supersaturated water may have suppressed ventilation (breathing) activity of the fish because oxygen was flowing quickly into the blood. However, a consequence of reduced ventilation is reduced release of carbon dioxide which can lead to metabolic pH disturbances in the fish (Wood, 1991) and perhaps have an impact on growth and feed conversion.

Table 4.3 summarizes dissolved oxygen levels of importance to largemouth bass. It appears that largemouth bass are fairly tolerant of low dissolved oxygen concentrations. However, oxygen levels affect fish health and immune systems as well as survival, growth and feed conversion. It is advised that culturists design production systems to maintain dissolved oxygen levels well above minimums discussed here.

4.4 Dissolved solids

Surface and ground waters contain dissolved substances that are derived from the environment as the water passes through, on and below the surface of the Earth. When speaking of freshwater, the term "total dissolved solids" is used to denote all dissolved solids, regardless of the specific nature, found in a sample. When speaking of sea water, the term "salinity" is used to describe the total concentration of a very specific set of ions and complex ions that exist in specific ratios to each other (Spotte, 1979). The terms salinity and total dissolved solids are often used interchangeably, but doing so can be misleading.

Table 4.4 Major ions, their chemical forms, and concentrations found in full–strength sea water (adapted from Bowen, 1966, and Spotte, 1979).

Ion	Major form	mg/l	Ion	Major form	mg/l
Chloride	Cl^-	19,000	Potassium	K^+	380
Sodium	Na^+	10,500	Bromide	Br^-	65
Magnesium	Mg^{++}	1,350	Carbon	CO_3H^-	28
Sulfur	$SO4^{--}$	885	Strontium	Sr^{++}	8
Calcium	Ca^{++}	400	Silicon	$Si(OH)_4$	3

Within freshwater, two important ions are calcium and magnesium. Collectively, they make up the hardness of the water. Hardness is expressed as milligram per liter of equivalent calcium carbonate (Boyd, 1979). The environmental calcium is especially important in fish culture because it helps reduce water and ion flux across the gills, reducing the energy requirements to maintain homeostasis in the fish (Weirich et al., 1992; Grizzle et al., 1993). Water hardness requirements for the culture of freshwater fishes are not well defined. The recommended hardness for the culture of striped bass *Morone saxatilis* juveniles is 150 mg/l as calcium carbonate (Hall, 1991). Largemouth bass appear to do well in a range of hardnesses.

Seawater contains 35 g/l sea salt (Spotte, 1979). Sea salt contains over 60 elements. Table 4.4 lists some of the elements and their major forms found in seawater. In the tidal areas of rivers entering estuaries, it is common to find an extended continuum of sea water to freshwater. Largemouth bass are often found in the continuum (Meador and Kelso, 1989, 1990; DeVries et al., 2015). Freshwater fishes do have a limit to the salinity they can tolerate for extended periods of time. This limit corresponds to 10–12 g/l salinity, the point where water begins leaving the fish osmotically across the gills. Long-term survival above 10–12 g/l requires physiological mechanisms characteristic of estuarine and seawater-adapted fishes (Colt and Tomasso, 2001). Salinity is expressed as parts per thousand, grams per liter, or per mille (‰). For practical purposes, all mean the same thing (gram of sea salt per liter of solution).

In a laboratory study by Meador and Kelso (1990), largemouth bass (185–289 mm total length) were collected from a freshwater lake and a 2 g/l salinity marsh. The fish were then cultured for 120 days at 0, 4, 8, or 12 g/l salinity (22°C). Fish from the freshwater lake grew equally well in 0 and 8 g/l salinity. Growth was lower in 8 g/l, and no fish survived the study in 12 g/l. Fish from the marsh grew equally well in 0, 4, and 8 g/l salinity, while no fish survived the study in 12 g/l. These results indicate the potential to grow largemouth bass in low-salinity water.

4.5 pH, carbon dioxide, and alkalinity

The negative logarithm of the hydrogen ion concentration of a solution is defined as pH. It presents on a scale of 1 to 14. Solutions with a pH below 7 are acidic, solutions with a pH above 7 are basic (alkaline), and solutions with a pH of 7 are neutral. The further the number is from seven, the stronger the acid or base. The sensitivity of largemouth bass to pH is not well defined. However, anecdotal evidence indicates that they do well in the range of 6.5 to 9.0 recommended for hatchery operations (Piper et al., 1982).

When discussing pH and culture conditions, it is important to distinguish between chronic and transient exposure. During pond culture, fish are typically exposed to a daily cycle of pH with lowest pH just after sunrise and highest pH just before sunset (see below in the discussion of alkalinity). In raceway culture, using well or stream water, or recirculating culture, the fish may be exposed to constant pH values. Largemouth bass appear to do well in pond culture and can tolerate a wide range of pH values, including short term exposures of up to 10 (characteristic of low alkalinity ponds). However, continuous exposure to high or low pH water may have detrimental effects.

Two major detrimental effects can be attributed to extreme culture pH values. At the low end, bicarbonate (H_2CO_3) in solution will dehydrate to form free carbon dioxide (CO_2). This process increases in rate as pH falls below 7.0 (Boyd, 1979). As the partial pressure of carbon dioxide increases in the culture water, it inhibits the excretion of carbon dioxide from the fish by way of the gills. As carbon dioxide builds up in the blood, it interferes with oxygen transport. At the high end, pH will increase the fraction of ammonia in its toxic form (see below discussion on ammonia). Consequently, extreme pH situations, especially during continuous exposure, need to be monitored and managed. Little work has been conducted to understand the sensitivity of largemouth bass to carbon dioxide. However, one study using corticosteroid hormones as indicators of stress reported that largemouth bass appeared unstressed (pH ~7.3) when exposed to 35 mg/l carbon dioxide but slightly stressed when exposed to 135 mg/l (Carmichael et al., 1984).

An example of a management strategy is one used in the author's lab for recirculating systems. In this case, we set pH limits of 7.5 and 8.0. The range is applied to both freshwater and seawater systems and considers three aspects of pH and culture – low pH increases free carbon dioxide concentrations, high pH increases the fraction of ammonia in its toxic form, and the pH range that supports efficient biofilter function (Spotte, 1979). We monitor pH daily, or every few days. Functioning biofilters will suppress pH as hydrogen ions are released in the nitrification process. Buffering capacity is less in freshwater than saltwater, so pH will fall faster in freshwater systems. When pH reaches 7.5, we add adequate sodium bicarbonate to raise the pH to near 8.0.

The total titratable base in an aqueous solution is referred to as total alkalinity (Boyd, 1979). It is expressed as equivalent calcium carbonate. In ponds, alkalinity interacts with algal photo-synthesis in that periods of high net carbon dioxide production by algae (evening) tend to reduce pond pH while periods of high net algal carbon dioxide consumption (daytime with sunshine) tend to allow pH to rise. The degree of the daily pH shift is partly a function of the alkalinity – high alkalinity ponds have a higher buffering capacity and lower pH shift, lower alkalinity ponds have a lower buffering capacity and broader pH shift (Boyd and Tucker, 2014). The alkalinity of culture water has no direct impact on the culture animals. It is the pH that is important, so managing alkalinity is an indirect way of managing pH. Managing alkalinity by adding lime to ponds is discussed in Boyd (1979) and Boyd and Tucker (2014).

4.6 Nitrogenous wastes

Fish feed contains protein to support fish development, growth and health. When feed is introduced into a pond or other culture unit, most (hopefully) of the feed is consumed by the fish. The remainder is consumed by the decomposer community in the pond. By either route, part of the protein is retained in additional biomass (fish or decomposer growth) and part is used as an energy source. Largemouth bass, being carnivores, will use more protein for energy than omnivores, such as catfish. Hence, catfish feeds have a lower level of protein than feed for carnivorous fishes. When protein is metabolized for energy, it is first reduced to its component amino acids which, in turn, are deaminated. The deamination step produces ammonia, which is very toxic to animals and must be excreted. Once in the culture water, ammonia is normally removed by the processes of the nitrogen cycle in ponds, which includes the process of nitrification, or removed simply by nitrification in recirculating systems (Spotte, 1979; Boyd and Tucker, 2014). If the nitrogen cycle and nitrification are working correctly, the concentration of ammonia in the culture water remains low. However, if feed inputs exceed the capacity of the ammonia removal systems or if some other factors affect the systems (for example, rapid temperature change), a buildup of ammonia may occur in the culture system.

In aqueous solution, ammonia exists in two forms – ammonia (NH_3) and ammonium (NH^+_4). Together, these two chemical species are referred to as total ammonia. NH_3 is the toxic form of ammonia because it is capable of diffusing across the gill membrane (Tomasso, 1994; Wright and Wood, 2009). The toxicity of ammonia to fishes depends largely on the pH of the culture water because the pH controls the proportion of the total ammonia that is in the toxic form (Table 4.5). Temperature has a lesser effect on ionization. As can be seen in Table 4.5, the percent of ammonia in the toxic form rises sharply as pH rises above 8, so ammonia levels should be carefully monitored when culturing fish at constant pH values above 8. Transient pH values above 8 such as during daily pH cycling in ponds may not be detrimental because the animals have an opportunity to excrete ammonia during the periods of lower pH.

Limited studies (unpublished, Carmichael and Tomasso) indicate that largemouth bass have a 72 h median-lethal concentration (the concentration of a poison that will kill one-half of exposed animals in 72 h) of 15.3 mg/l total ammonia-nitrogen at pH 8.0 and temperature 25℃. Of this total, 0.82 mg/l is in the un-ionized, or toxic, form (see Table 4.5). Recommendations for "safe" levels vary, but a rule of thumb is to keep ambient levels no higher than one-tenth and preferably below one-twentieth of lethal levels (0.082 and 0.041 mg/l, respectively).

Table 4.5 The percent of total ammonia in aqueous solution that is in the un–ionized (NH_3)form at five pH values and three temperatures (modified from Emerson et al., 1975)

pH	Percent in un-ionized form				
	10°C	15°C	20°C	25°C	30°C
6	00.019	00.027	00.040	00.057	00.081
7	00.186	00.273	00.396	00.566	00.799
8	01.830	02.670	03.820	05.380	07.460
9	15.700	21.500	28.400	36.300	44.600
10	65.100	73.300	79.900	85.100	89.000

The process of nitrification is mediated by nitrifying bacteria (Spotte, 1979; Boyd and Tucker, 2014). During this process, ammonia is oxidized to nitrite (NO^{-}_2) and then to nitrate (NO^{-}_3). In a balanced pond or recirculating system, ammonia and nitrite concentrations are low and nitrate either accumulates or is removed by assimilation into aquatic plants. As noted above, perturbations of the nitrification process may lead to increases in ammonia concentrations. It may also lead to increases in nitrite concentrations. Nitrite, like ammonia, may be toxic to fish. Conversely, nitrate is not very toxic.

Nitrite is highly toxic to many species of fishes (Tomasso, 1994). However, largemouth bass is not one of them. Palachek and Tomasso (1984) reported a 96 h median-lethal concentration to largemouth bass fingerlings of 140 mg/l nitrite-N, compared to 7.1 mg/l for channel catfish (*Ictalurus punctatus*). The basis for the large interspecies difference in toxicity was later shown to be related to differences in chloride-uptake mechanisms (Tomasso and Grosell, 2005). It is doubtful that nitrite will reach detrimental levels in largemouth bass culture systems. The most obvious clinical sign of nitrite toxicity is the presence of brown blood brought about by nitrite-induced oxidation of the iron in hemoglobin (Tomasso, 1994). If a culturist suspects nitrite toxicity, a brownish coloration to the blood or gills would serve as a presumptive diagnosis.

No work has focused on nitrate toxicity to largemouth bass. However, work on the closely related Guadalupe bass (*Micropterus treculi*) showed a 96 h median-lethal concentration of 1,261 mg/l nitrate-nitrogen (Tomasso and Carmichael, 1986). This value fits well with other toxicity values for freshwater fishes reviewed in Camargo et al. (2005). Nitrate does not appear to be a consideration from a toxicity perspective; however, nitrate is a fundamental plant nutrient, so high environmental nitrate levels can be expected to affect phytoplankton growth in any culture system receiving sunlight.

4.7 Gas supersaturation

Atmospheric gases over water tend toward an equilibrium with gases dissolved in the water. Biological and chemical activity in the water consume dissolved gases (particularly oxygen), usually keeping the total gas pressure in the water below that of the air above it and providing a pressure gradient for further diffusion from the atmosphere. Occasionally, culture water may become gas supersaturated – the total gas pressure in the water is higher than in the atmosphere above it. Gas supersaturation is an unstable condition in which the excess gas in the water is prone to form bubbles (much like the bubbles that form when a carbonated drink is opened). If these dissolved gases diffuse across the gills into the fish, and then bubbles form, capillary and small vessel circulation will be inhibited (termed gas bubble disease) resulting in death of the culture animals.

Gas bubble disease is not usually a problem in ponds given the large surface area that is the interface between the water and atmosphere. Also, mechanical and wind aeration promote degas-sing from water to atmosphere down the pressure gradient. Problems with supersaturation tend to occur in hatcheries, holding facilities and recirculating systems where water is pumped or heated. Heating reduces the ability of water to hold dissolved gases, so increasing the temperature of saturated water puts it in a supersaturated condition (this is why bubbles form on the sides of a saucepan when heating water on a stove). Pumps with air leaks on the low-pressure side will draw in air and then force it into the water on the high-pressure side resulting in supersaturation. Another common way water is supersaturated in fish production facilities is filling empty pipes with water with the end valves closed, pressurizing the air in the pipes. Colt and Tomasso (2001) and Hargreaves and Tomasso (2004) discuss supersaturation in more detail.

No controlled studies of the effects of gas supersaturation on largemouth bass were found. However, anecdotal reports from hatcheries clearly indicate that largemouth bass are susceptible to gas supersaturation and exposure should be avoided.

Largemouth bass are also susceptible to swim bladder stress syndrome (Carmichael and Tomasso, 1984), a malady that may be mistaken for gas bubble disease. This syndrome is characterized by overinflated swim bladders that may cause the fish to broach. It is distinct from gas bubble disease in that no emboli form in the blood. The etiology of swim bladder stress syndrome is not clear; however, it appears to be more related to handling stress than gas supersaturation.

A final consideration when discussing gas supersaturation is oxygen supersaturation, which can occur when pure oxygen is being used to maintain proper oxygen levels in water. This often occurs in transport tanks when oxygen is being metered into the tanks at too high a rate. Measured oxygen levels can reach two or more times the saturation level for aeration systems; however, the total gas pressure does not exceed atmospheric pressure though because oxygen is displacing nitrogen in the water. Oxygen supersaturation can cause developmental problems if transporting eggs, embryos or fry; however, it is generally not a problem when transporting fingerlings.

4.8 Hydrogen sulfide

Hydrogen sulfide (H_2S) is a toxic gas produced by way of anaerobic activity of bacteria. It is sometimes found in well water and in anaerobic pond bottoms where organic material has collected. A common problem in warmwater pond culture is the release of hydrogen sulfide from the sediments when fingerling ponds are drained to collect fish. Raking pond bottoms and allowing the ponds to remain empty between production cycles reduces the accumulation of organic material and incidence of hydrogen sulfide release.

Table 4.6 Effect of pH on the percentage of total hydrogen sulfide and total chlorine in the toxic forms (H_2S and HOCl, respectively).

pH	Percent in toxic forms	
	H_2S	HOCl
5	99	100
6	91	97
7	51	*76*
8	9	23
9	1	3

Source: Hydrogen sulfide information adapted from Wedemeyer (1996) at 25°C. Chlorine information adapted from Boyd and Tucker (2014) at an unreported temperature.

Maintaining aerobic conditions will promote the oxidation of sulfides to sulfates, which are not toxic. Hydrogen sulfide exists in a pH- and temperature-dependent equilibrium with the HS^- ion. The non-ionic species is the toxic form because it is gill permeable (similar to ammonia). At pH 7 and temperature 25°C, about one-half of the sulfide is in the toxic form (Table 4.6). At a pH of 9, only about 1% is (Wedemeyer, 1996). The USEPA (1986) recommends a maximum safe exposure level for aquatic animals of 0.002 mg/l H_2S-S. Tucker (1993) suggests half the USEPA maximum.

4.9 Chlorine

Chlorine is a very effective disinfectant. However, it is also very toxic to aquatic animals. Chlorine can be applied as a liquid, gas, or solid. Regardless of the form of application, an equilibrium forms in the water between the highly toxic hypochlorous acid (HOCl) and less toxic OCl^-. The pH of the water determines the nature of the equilibrium with more HOCl present at high pH values (Table 4.6). The equilibrium is at molar equality at a pH of about 7.5 (Boyd and Tucker, 2014).

Recommended maximum exposure levels for aquatic animals range from 0.001 to 0.10 mg/l total chlorine (summarized in Colt and Tomasso, 2001). Chlorine can be removed by activated carbon, aeration, or sunlight. Simple-to-use test kits are commercially available and should be used after any process in which chlorine is used.

4.10 Copper sulfate

Copper sulfate is commonly added to aquaculture ponds for the control of algae and external parasites. Caution must be exercised when using copper sulfate because copper is very toxic to fish and water chemistry has a large impact on both toxicity and effective concentrations. For example, the 96 h median-lethal concentration of copper (from copper sulfate) to channel catfish increases 20-fold (0.05 to 0.98 mg/l) as the alkalinity is increased from 16 to 240 mg/l as $CaCO_3$ (Straus and Tucker, 1993). The Biotic Ligand Model (DiToro et al., 2000) was developed to integrate the effects of multiple water quality characteristics on both toxicity and effectiveness. Calculating effective and safe levels of copper sulfate for pond application is complex. Inexperienced culturists are encouraged to contact their local extension office for guidance.

4.11 Formalin

Formalin is a water solution containing formaldehyde at saturation level (about 37%). Since malachite green was banned from use, formalin has become the standard antifungal treatment and preventative for fish eggs. It is also used for the treatment of ectoparasites on fish. The 24 h median-lethal concentration for formalin to largemouth bass fingerlings is about 140 µl/l. For ectoparasite control, fish are usually exposed to high concentrations (150–250 µl/l) for about 1 h.

An interesting aspect of formalin use with largemouth bass is that fingerlings are about twice as resistant to formalin as are tadpoles (*Rana berlandieri*). A common problem during largemouth bass fingerling production is that large numbers of tadpoles are harvested from ponds along with the fish – at times the biomass of tadpoles exceeds the biomass of fingerlings. Separating the fish and tadpoles is time-consuming and stressful to the fish. Carmichael and Tomasso (1983) demonstrated that separation could be accomplished by exposing mixed groups of largemouth bass fingerlings and tadpoles to 200 µl/l formalin for one hour followed by flushing with 6.9 turnovers per hour. By the end of 24 h, all of the tadpoles were dead and washed toward the drain end of the tank. As with copper sulfate, inexperienced culturists are advised to contact their local extension office for guidance and the latest regulations regarding the use of formalin on food-fish.

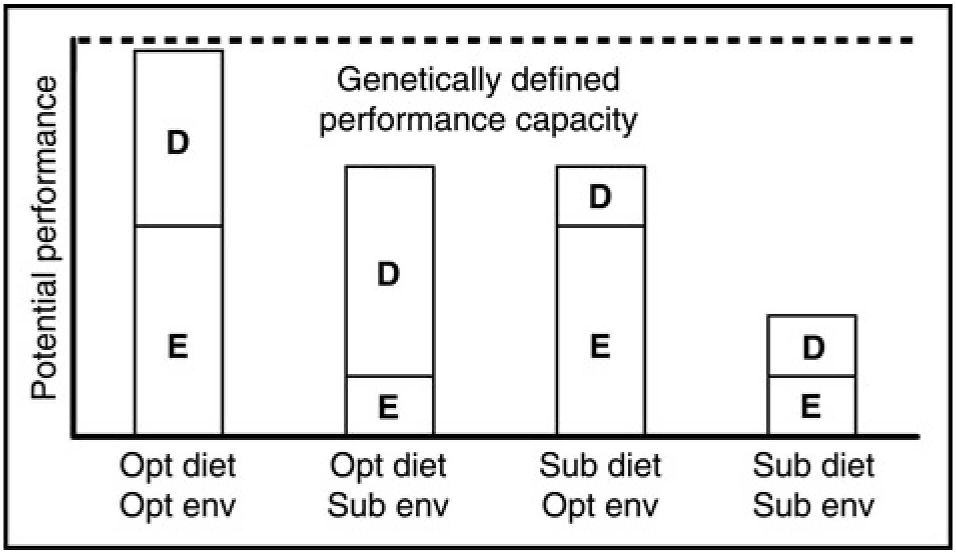

Figure 4.1 Conceptualization of the relationship among genetically defined performance capacity (dashed line), diet, and environment. Suboptimal diets (sub diet) and suboptimal environments (sub env) can reduce performance compared to optimal diets (opt diet) and optimal environments (opt env). The relative contributions of diet and environment shown here are for illustration and not based on experimentation.

4.12 Conclusion

The largemouth bass is a hardy temperate species that is tolerant of a range of water quality. This chapter has highlighted several aspects of the environmental requirements of the species. Several other resources (Williamson et al., 1993; Colt and Tomasso, 2001; Hargreaves and Tomasso, 2004; Boyd and Tucker, 2014) provide additional details and insight.

This chapter has focused on the environment for producing largemouth bass. However, it is important to put environment into perspective. Schreck (1981) used the concept of performance capacity to describe the relationship of a fish to its environment. Essentially, an animal has a genetically defined maximum capacity to perform (survive, grow, reproduce, and so on). Maximum performance capacity is reduced by suboptimal environments or stress. This concept was later put into an aquaculture context (Tomasso, 1995). Figure 4.1 shows the performance capacity concept and how two broad categories (environment and nutrition) can have an impact on performance. In summary, maintaining environments (and diets) as close to optimum as possible will result in better performance (survival, growth, reproduction, feed conversion).

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Chapter 5
Reproduction and genetics

Boris Gomelsky,[1] Kenneth J. Semmens,[1] Eric Peatman,[2] Shawn D. Coyle,[1] and Michael D. Matthews[3]

[1] Kentucky State University, Division of Aquaculture, Frankfort, KY, USA

[2] Auburn University, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn, AL, USA

[3] A.E. Wood Fish Hatchery, San Marcos, TX, USA

5.1 Reproduction

5.1.1 Natural spawning

5.1.1.1 Introduction

Natural spawning (allowing broodfish to select a mate) is recommended for largemouth bass. This section focuses on natural spawning techniques in ponds and raceways. Largemouth bass fingerlings are rarely reared to a size > 5 cm in the same ponds in which they are spawned because competition for food and cannibalism limit production numbers. Instead, fry are produced in several ways and then stocked into nursery ponds. Using a separate spawning phase allows fry production intensification so that adequate fry can be produced for the nursery phase. Advanced fry (10–20 mm) may be produced through the spawning/rearing method by allowing broodfish to spawn naturally in ponds and harvesting large fry and broodfish after 40–50 d (Hutson, 1990). Alternatively, pond or raceway spawning methods are used to produce eggs or swim-up fry that are transferred to incubation tanks or stocked directly to nursery ponds. Commonly, fry are collected from the spawning ponds and stocked into fingerling production ponds (Piper et al., 1982; Simco et al., 1986). More controlled raceway spawning paired with indoor incubation to swim-up allows for more consistency in fry size and accuracy in numbers stocked.

5.1.1.2 Broodstock selection, care and maintenance

If pond space allows, broodfish should be separated into ponds by year class and gender. This allows the allocation of forage based on broodfish needs, prevents unexpected spawning and minimizes handling during harvest and pairing of broodfish. Older fish generally ripen and spawn earlier than younger fish which, when different age groups are mixed in spawning ponds, can increase size disparity in fry populations promoting cannibalism (Piper et al., 1982). It has been recommended that largemouth bass broodfish be stocked at 200–350 kg/ha with equal numbers of males and females (Hutson, 1990). Glenewinkel et al. (2011) recommended stocking 140 kg females/ha and equal numbers of males to females regardless of the weight of males.

Secondary sex characteristics are poorly defined in largemouth bass. Several methods have been suggested for distinguishing between male and female fish (Snow, 1963). Mature fish can be sexed with reasonable accuracy just prior to spawning. At other times, morphological differences are not distinct enough for this to be done. Broodfish are selected for spawning in the early spring when water temperatures are consistently above 15°C. In spawning condition, females are easily distinguishable from males or unripe females by examining them side-by-side belly up. Ripe females exhibit distended, soft, pendulous ovarian region, and a swollen, red, protruding vent. Ripe males usually release a small amount of milt when palpated. To palpate fish, turn the fish belly up and slowly but firmly apply pressure along the sides or middle of the abdominal region. For spawning, only use males that freely express milt when palpated.

In the southern half of the USA, largemouth bass can attain sexual maturity at 1 year of age if a minimum size of 180 g is reached (Swingle, 1950). Since growth is slower in the northern part of its range, 2 years may be required. Snow (1965) reported that good results can be obtained from 1-year-old fish which have reached a minimum size of 320 g. A key factor in producing bass which will spawn at 1 year of age is supplying an ample quantity of forage fish of a suitable size when water temperatures are > 15°C. As much as 20% of the body weight of the fish may be consumed per day under optimal conditions. It is suggested that 1 kg of gain in bass weight requires about 5.1 kg of forage consumption (Piper et al. 1982). When producing broodstock on forage, stocking densities of 250–370 handpicked fingerlings per hectare have been used successfully (Snow, 1961).

Eggs are formed in the late summer and fall prior to spawning the following spring. Adequate nutrition during the period of egg formation is necessary for normal development. While artificial food can be used, growth is slower and egg quality generally not as good as from fish fed a natural diet (Snow, 1970). However, there are several advantages of using broodstock habituated to artificial diets. Artificial diets can be fed in the spawning ponds without contaminating the environment with unwanted fish. Feeding artificial diets also greatly reduces the possibility of bass tapeworm infestation and bacterial disease can be treated by feeding medicated feed (Snow, 1970). Some management initiatives may necessitate the use of forage fish for maintaining genetically "wild type" broodstock; although bass produced for food-fish purposes are generally the progeny of several generations of feed trained or "domesticated" broodstock.

Species of forage fish that appear to be best for propagation as bass food include the gold-fish (*Carassius auratus* [Linnaeus]), common carp (*Cyprinus carpio*), fathead minnow (*Pimphales promelas*), and golden shiner (*Notemigomis crysoleueas*). Goldfish are preferred by some managers because of ease of spawning, high production rates, and rapid growth. Carp have similar characteristics although they tend to outgrow bass to a greater degree and do not spawn over as long a period as goldfish. Fathead minnows and golden shiners seldom get too large for bass to swallow but do not yield as much weight per unit area. Bluegill (*Lepomis macrochirus*) spawn at higher water temperatures where offspring are not large enough for forage until late in the summer. Where legally allowed, tilapia (*Oreochromis* spp.) are an excellent forage species, being prolific, fast growing, and disease resistant. The primary disadvantage being low tolerance to water temperatures below 12℃. Goldfish, carp, and tilapia also inhibit the development of aquatic vegetation when present at densities of > 224 kg/ha (Snow, 1961).

The use of anesthetics is recommended when handling largemouth bass broodfish. Handling large broodfish by hand can increase the chance of stress, injury, and disease, which will delay and likely reduce spawning success and fry production. Skaggs et al. (2017) showed that handling and holding largemouth bass broodfish out of water for 60 s increased the stress response significantly to impact spawning. MS-222 is an approved sedative for warmwater fish. Dosing concentrations of 15–330 mg/l are permitted and has a 21 d withdraw period. Dosage required to sedate bass broodfish for handling is 25 mg/l and complete sedation requires 50 mg/l. Aqui-S®E (AquaTactics Fish Health, Kirkland, WA) is a relatively new anesthetic for fish which currently can only be used under the Investigational New Animal Drug (INAD) program. Dosing concentrations of 10–100 mg/l are permitted and requires a zero withdraw period if the fish are sedated less than 15 min or requires a 3 d withdraw period in all other applications. Aqui-S concentrations diminish over time in water so is not recommended for longer sedation periods. A dose of 30 mg/l on largemouth bass will effectively sedate fish for capture, measurement, and distribution for spawning.

5.1.1.3 Spawning behavior

Spawning activity of largemouth bass is closely related to rising water temperatures following winter. Largemouth bass are relatively synchronous regionally in their reproductive cycle. Bass typically spawn from early spring into the summer, displaying latitude variation between northern and southern climates with spawning times ranging from February to July within the USA (Heidinger, 1975). A water temperature of 18°C is generally recognized as the spawning temperature of this species. Often, spawning will not take place until the second or third time the temperature warms to this level. Spawning activity tends to peak when a majority of the fish are stimulated to spawn as temperature rises but spawning may continue for weeks after the "main spawn" has occurred. The length of the spawning season is generally 3–4 weeks (Waters and Noble, 2004).

Largemouth bass are classified as fractional, batch or "multiple" spawners. Only a portion of the eggs are released during a spawning event and the females spawn more than once during the spawning season. The eggs are released at intervals, usually over several days or weeks. This allows more, smaller, and immature eggs to be carried in a limited abdominal cavity space as the intervals allow the smaller eggs time to mature. Eggs mature at different times, thus avoiding complete loss of a season's spawning to predators or weather perturbations. Females normally release about one-half of their total eggs during the first spawn and one-half of the remaining eggs during the second spawn. A third spawn up to a month later to release the remaining eggs is common (Davis and Lock, 1997). This behavior reduces the efficacy of using hormone injection for artificial spawning, as only a portion of the oocytes respond and as a result is generally not practiced in commercial production (Mayes et al., 1993).

The eggs of largemouth bass require a relatively long period for development beginning in the fall and continuing through the winter and spring. Largemouth are seasonal spawners, where after spawning no more eggs will develop until water temperatures begin to decrease during the following fall. Largemouth bass eggs are demersal and adhesive. Fertilized eggs range in diameter from 1.2 to 1.8 mm. Smaller and younger fish produce smaller eggs. The number of fertilized eggs per milliliter averaged

571 for 1-year-old fish as compared to 374/ml for fish 3 years old (Snow, 1970). Egg counts of samples from ovaries of unspawned fish indicate a potential to yield 30,000 to 45,000 per 454 grams of body weight. Even higher numbers have been reported in some instances (Davis and Lock, 1997).

Largemouth bass are nest builders and guarders. The male selects and prepares the nest site guarding it against intruders. Preferred sites are protected locations in shallow water over firm bottom or a fibrous substrate such as tree roots. Largemouth bass will preferentially use artificial spawning substrates such as Blocksom or Spawntex™ mats (Isaac and Staats, 1992). These substrates are generally placed 1.8 m apart in raceways to reduce male territory aggressive behavior and 3–6 m apart in ponds at water depths of 0.5–1.0 m and close to the pond bank to allow easy observation. Mats can then be transferred indoors or directly to other ponds for hatching.

When broodstock are first stocked into ponds or raceways for spawning typically they school together initially. After a couple of days, males begin to select and guard spawning sites. Once the nest is established the male becomes territorial and stays in close proximity to the nest attempting to entice a female to spawn. Following a short courtship, a female lays eggs on the nest and the guarding male fertilizes them. After spawning is completed, the male continues to guard the nest. Spawning can occur at any time of day. Maintaining clear water and using a flashlight helps to locate spawns. If water is stained or murky it may be necessary to use spawning mats and gently lift up each mat to examine for eggs. If a guarding male does not readily abandon a nest being examined a spawn is likely present.

Spawns should be collected as early in the morning as possible so that subsequent spawning activities can resume quickly. Spawning mats can be collected by one person by lifting each mat out of the water and quickly submerging it in a tub of the same water. If two people are available, it may be preferable not to lift the mats out of the water. This can be accomplished by one person lifting the mat and another sinking a tub underneath the mat to retrieve it. Both people then raise the tub to the water surface and slowly pour off the excess water before transport.

Incubation time varies with temperature. Heidinger (1975) summarized several studies which give incubation times ranging from 317 h (13 d) at 10°C to 49 h (4 d) at 28°C. Bass eggs incubated at 17.7°C required 3–4 d to hatch and only 2 d to hatch at 22.2°C (Matthews et al., 2012). Newly hatched fry swim-up in 7–9 d at 18–20°C and in 5–7 d at 22–24°C (Matthews and Stout, 2013). Maintaining consistent water temperatures during egg incubation is recommended and incubating bass eggs at 22–23°C increases hatch rates over lower incubation temperatures in intensive culture programs and reduces dependence on chemical treatments (Matthews et al., 2012).

Ponds should be stocked with spawns (eggs or fry) from three consecutive days or fewer to reduce size variability and subsequent cannibalism before fingerlings are harvested. This 3 day "rule" can be increased to ~ seven days if the eggs and fry are manipulated by reducing temperatures of one group to elongate time to swim-up and increasing temperature on another group to shorten time to swim-up. At hatching, the fry are quite small ranging from 3 to 5.5 mm. Growth and development continue during the yolk sac absorption period which may require 120 h or longer. When ready for first feeding a size of 5.5 to 6.5 mm has been attained and color has changed from amber or cream color to some shade of brown.

5.1.1.4 Spawning methods

Spawning methods used for largemouth bass fry production are based on the available facilities infrastructure and production goals of the hatchery. The scale of hatchery operations varies from small private producers with only a small pond or two and production goals of a few thousand fingerlings to large state and federal hatcheries with highly sophisticated indoor and outdoor facilities and production goals of several million. In recent years, new technologies have led to refinement of culture methods and improvements in bass fingerling production. The appropriate technology is a site based decision largely determined by resource availability. Hatchery production of largemouth bass involves three main culture methods: spawn and rear, egg or fry transfer and intensive culture.

For best results, spawning ponds should be prepared each year. Earthen ponds should be drained and allowed to dry and the bottoms disked and packed. Bottoms and sides of ponds should be sprayed with an approved herbicide to control unwanted vegetation. If lined ponds are used, they should be cleaned of bottom sediments including removal of sediments from drain boxes or kettles. Pond filling should ideally begin 1–7 d before broodfish are stocked. Incoming water should be filtered through a 1,000 μm sock filter to prevent unwanted organisms from entering the pond. In plastic-lined ponds spawning substrate should be provided. The number of pans or mats placed in each pond should equal the number of males.

The spawn-and-rear method is the oldest and simplest approach to largemouth bass culture. Broodfish are stocked into spawning ponds when water temperatures approach 18°C and are allowed to pair-up and spawn freely. Stocking densities are typically 25–100 broodfish/ha (White, 1981). Broodfish are left in the pond until the fingerlings reach a size large enough for harvest and either distribution or subsequent feed training which is generally 30–45 d after broodfish have been stocked (Hutson, 1990). The spawning/rearing method offers some advantages over spawning-only ponds as the larger fry (> 15 mm) produced are easier to count accurately providing more control over stocking densities and fry survival in rearing ponds increases with stocking size (Kurten, 2001). The spawning/rearing method typically yields mixed-age fry that should be graded before the next rearing stage. Owing to cannibalism of fingerlings by the broodstock, this method is only effective for fingerlings up to 25 mm. Ponds are typically fertilized to stimulate primary productivity and subsequent production of zooplankton and larval insects to provide food for the developing fry and fingerlings.

For the egg transfer method, ponds are stocked with broodstock as described for the spawn-and-rear method although the stocking densities of broodstock are generally 100–200 broodfish/ha. Ponds are managed for water clarity and are not fertilized. Fibrous spawning mats such as Blocksom or Spawntex™ are used as a removable substrate for bass to spawn for egg collection (Isaac and Staats, 1992). Spawning mats are checked daily for eggs.

Nests with spawns are transferred from spawning ponds into fertilized nursery ponds where the eggs hatch and the fry remain and grow to the target size (Hutson, 1983). The disadvantage of this method is that the number of fry to hatch successfully is unknown and may be too high or too low relative to the carrying capacity of the pond. This makes management difficult, production unpredictable and could require significantly more pond hectares than other methods.

For the fry transfer method, ponds are managed and stocked as described above for the egg transfer method, but the swim-up fry are transferred from spawning ponds to fertilized nursery ponds. Where eggs or fry are harvested directly from spawning ponds, these ponds are usually managed for water clarity as it is important to be able to observe fry and spawning activity. These ponds are unlikely to require fertilization, since fry will be removed before they begin feeding. To visually identify schooling swim-up fry, managers often use a tool to silhouette the fry. Examples include: white boat paddles or bucket lids attached to a long handle. These are pulled under the water surface near the shoreline. Free-swimming schools of fry remain near the nest for several days following swim-up where they can be collected by dip netting, seining, trapping or pond draining (Snow, 1975; White, 1981; Hutson, 1990). Largemouth bass swim-up fry can be enumerated using a standard weight index of 275 fry/g (Glenewinkel et al., 2011).

The fry transfer method allows for stocking densities to be tailored for specific production goals, such as fingerling size (White, 1981). Also, ponds can be more easily stocked with fry of similar size and age to minimize cannibalism, which can significantly reduce fish yield. Unfortunately, spawns can be missed in the fry transfer method resulting in fry size differences and cohort cannibalism. Mixed fry sizes become more pronounced as the spawning season progresses due to the mixing of fry from subsequent spawns, as a result most hatcheries using this method only transfer fry to nursery ponds from the earliest fish to spawn within a few days of each other. Another disadvantage is reduced spawning by males protecting broods where the removal of spawning substrates containing eggs and replacement stimulates continued spawning of males.

Methods for indoor incubation of largemouth bass eggs were developed to improve efficiency and ameliorate inconsistent production results associated with other methods (Snow, 1972). Broodfish stocking densities in ponds are similar to those of the egg transfer method while densities in raceways varies by facility. Broodfish are spawned in ponds or tanks and the eggs are collected and incubated under controlled conditions in either vertical-tray incubators (Snow, 1972), hatching jars (Sarti, 1986), or on spawning mats suspended in raceways (Matthews and Stout, 2013). Hatching in tanks is preferred as fry of similar age and known number can be stocked into a nursery pond. If eggs are to be incubated in trays or hatching jars the mats are typically dipped in a solution of 45 g/l sodium sulfite to remove the adhesiveness of the eggs followed by a light water spray to remove the eggs from spawning mats. However, Matthews and Stout (2013) attributed a poor hatch rate of eggs (50%) to egg damage caused by the removal process and discouraged this process. Swim-up fry are either stocked into prepared nursery ponds or cultured in tanks on a combination of natural and artificial foods (Snow, 1965; Skudlarek, et al. 2013).

5.1.1.5 Preliminary experiments on tank spawning largemouth bass

Developing technologies for intensive fry production, such as controlled indoor spawning, egg incubation, and fry rearing, could improve the reliability of bass fingerling production. Currently the majority of commercial largemouth bass production in the USA occurs in the southeastern states. Bass producers in more northerly locations possess marketing advantages in proximity to large urban markets; although, spawning dates are later compared to more southern states. Early spawning allows for the production of larger first-year fingerlings by the end of the first summer growing season (Pine et al., 2000). The delayed temperature-dependent spawning of largemouth bass in northern areas can “domino” through the production cycle, causing fish to not achieve market size (0.50–0.75 kg) by the end of the second year (Tidwell et al., 1998).

Preliminary trials were conducted to examine the feasibility of a simple method to manipulate water temperature to induce early spawning of largemouth bass and subsequent egg incubation in temperature controlled tanks (unpublished data, Coyle and Tidwell). Nine 19,000 l fiberglass tanks located outdoors were filled to a depth of 0.7 m. Each of the tanks contained four spawning mats (Spawntex^{TM}) and four pairs of largemouth bass broodfish were stocked into each tank. Three treatments were evaluated in triplicate tanks. The control treatment was uncovered tanks to simulate natural temperature conditions. For the second treatment, tanks were covered with clear plastic stretched over PVC pipe to create a passive "greenhouse." In the third treatment, the water surface was covered with a blue solar swimming pool cover. Spawning mats were examined each morning for eggs then transferred indoors for incubation.

The use of passive solar heating warmed water to suitable spawning temperatures for large-mouth bass approximately 2–3 weeks earlier than natural conditions. The greenhouse treatment was more effective at reducing spawning time than the solar cover treatment, but due to algae growth in the greenhouse tanks it may be more practical to use solar covers, which did not allow algae growth. Collecting eggs on mats allowed for easy transfer indoors and subsequently provided increased control over water temperature and disease.

5.1.1.6 Controlled indoor spawning

Where suitable facilities exist, spawning largemouth bass in raceways offers several advantages to spawning ponds for fry production. Broodfish can be used more intensively in a smaller culture space freeing up pond space for additional fingerling growout. In ponds, females may only spawn one time due to the large variability of conditions possible in a natural environment. Spawning in controlled environments increase the likelihood of females spawning multiple times, and as a result, more fry can be produced per kilogram of broodfish (Isaac and Staats, 1992). Controlled spawning reduces the spawning season duration and number of broodfish required (Mayes et al., 1993; Matthews and Stout, 2013), so nursery ponds can be stocked within a shorter time frame, yielding more uniform size and number of fry.

Presently, the maximum number of spawns per female is unknown and likely varies widely among females. In one trial (personal communication, Matthews), 14 female Florida largemouth bass were allowed to spawn over a 115 d period. During this time, an average of seven spawns per female were collected from in a single 24 m raceway. These females, averaging 2 kg each, collectively produced 1.13 million eggs, 40,464 eggs/kg female, 11,000 eggs/spawn, and 80,929 eggs/female. These results are averages, meaning an individual female could have spawned more than seven times. However, in production typical spawning periods are much shorter (21–28 d) and the number of spawns per female are less than two (Matthews and Stout, 2013).

Broodstock can be stocked at a 1: 1 female to male sex ratio in indoor raceways with the number of spawning mats, with their proper separation, dictating the number of males. Stocking with a 3: 2 female to male sex ratio increases the number of spawns collected (personal communication, Matthews). In a 26 × 4 m raceway, 20 mats, 10 per side, can be evenly spaced (~2 m apart) along the length of each long wall. Broodstock need time to acclimate to the artificial environment. This could take 3 to 8 weeks for fish the first time they are spawned indoors. Broodfish used again in subsequent years become habituated to spawning in raceways and, if kept indoors all year, can begin spawning within 1 to 3 d. If kept in gender segregated ponds outside, broodstock may still require 1 to 2 weeks of acclimation and courtship behavior before spawning. Co-mingling bass broodstock, prior to the addition of spawning structures, reduced spawning latency by approximately 2 weeks (Jackson, 1979; Matthews and Stout, 2013). Bass can be paired and placed in a clean-bottomed spawning raceway without mats with minimal risk of spawning on the bare raceway floor.

Broodstock should not be fed during spawning as it is difficult to clean spawning raceways and can increase the numbers of bacteria and parasites present, which could reduce hatch rates and fry survival. During a prolonged spawning period, spawning mats can be removed and the broodfish fed. After feeding broodfish in spawning tanks the raceway should be cleaned (preferably with a vacuum or siphon as not to drastically alter the water level or crowd and stress the bass) before spawning resumes. Bass should be given 4 d to feed and 3 to 4 d to clear their digestive tracts before replacing mats.

The raceways should be checked each morning and mats containing spawns removed and hung vertically in an "incubation" tank or raceway. Spawns are transferred in water and remain in the transfer tubs less than 15 min. Each incubation tank should be filled with enough water to completely immerse the spawning mat. Incoming water is screened through 500 μm mesh to exclude unwanted organisms. The drain pipe should include a saran-screen guard to prevent fry escapement and an air ring at the base to help prevent clogging of the screen. A divider screen at the end of the tank is also recommended. Low pressure air is gently bubbled in front of the divider screen to help prevent clogging. Air is also bubbled along one side, down the length of the trough, to keep the water moving around the mats and to mix therapeutic chemicals if required. A supported wire is stretched along the length of the trough allowing the spawning mats to be vertically suspended in the water. A 4.5 m trough supplied with 15 to 40 l/min can hold 20–30 spawning mats without overcrowding hatched fry. An example of spawns in concrete raceways is shown in Figure 5.1.

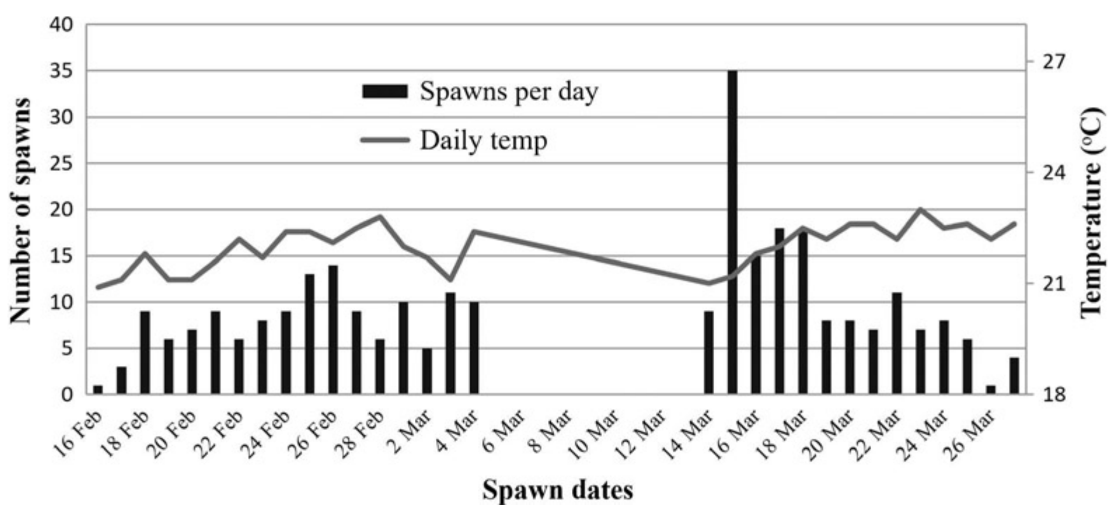

Figure 5.1 Results from spawning 101 females using a 3: 2 female to male ratio in four indoor 24 m concrete raceways for 17 days producing 136 spawns. Mats were removed, bass were fed koi, and mats

replaced after seven days. Spawning resumed two days after mats were replaced for 14 d producing 155 more spawns from the same broodfish. Water temperature was recorded each morning from 16 February to 4 March and 14 to 28 March.

Spawns from up to 3 d old apart can be incubated together in the same trough depending on temperature and required chemical treatments. Spawns can be treated with approved chemicals to control "fungus" water mold from growing. Typically, the eggs hatch in 2–4 d after spawning depending on water temperature and swim-up fry are typically ready for harvest 7–10 d post-stock. Take caution incubating bass eggs at water temperatures above 26℃. The increased temperature promotes accelerated hatch and fry growth, quickly reducing yolk sac volume and reducing the time frame for "first feeding." The yolk-sac may be depleted before body structures required to capture and utilize live prey items are developed. The occurrence of physical deformities also increases. The hatched fry initially appear as small golden globules along the sides of the troughs and edges of the mats. The color of the fry darkens over time and they become dark-gray. Mats are removed from troughs when the fry begin to swim freely in the water column. Mats should be examined for trapped fry during removal; this is especially important when different aged spawns are incubated together. Avoid vigorous rinsing of mats to prevent injury to fry and releasing unhatched eggs which may foul the tanks.

5.1.1.7 Out of season spawning

Out of season spawning of largemouth bass using photoperiod and temperature manipulation was first described by Carlson (1973). It is relatively common to delay spawning of bass by holding at water temperatures below 17℃ in order to avoid unpredictable spring temperature fluctuations and/or maximize sequential hatchery production of other species (Brauhn et al., 1972). A limiting factor in delayed spawning is suitable pond water temperatures for adequate zooplankton quantities and sizes during the subsequent nursery phase. Since zooplankton population dynamics are dependent on water temperature, out-of-season spawning of bass is limited by seasonal zooplankton abundance. Zooplankton abundance is highest during the spring "bloom" which corresponds with natural spawning. In regions having sufficient periods of suitable water temperature zooplankton blooms also occur in the fall proving an opportunity for out-of-season spawning and subsequent fingerling production of largemouth bass.

The development of a technique that successfully produces bass spawns in September and October is described in detail by Matthews and Stout (2013). Spawning and rearing bass in the fall allowed for production of 100 mm bass by the following March and better utilized pond space in the fall. Protocols, stocking ratios, and stocking rates are the same as described earlier in this chapter in the controlled indoor spawning section, but requires the addition of temperature and daylight manipulation to naturally induce spawning. Water temperatures were systematically lowered to 12℃ over a 4 week period, held there for 3 weeks, then increased over a 4 week period back to ambient.

The amount of daylight was manipulated using black plastic to completely block light and simulate the dark period of winter. Raceways had 10 h of light for the first four weeks, 8 h for 3 weeks (held at 10 to 12℃), increased to 10 h for 2 weeks and then increased to 14 h for the last 2 weeks of conditioning. The 14 h of light were maintained throughout spawning. An example of spawns collected over a single out of season spawning period is shown in Figure 5.2.

Observations between multiple seasons of spring spawning and out of season spawning showed no obvious differences in fry size (300 fry/g), health, or behavior. The only obvious difference was the average number of fry/spawn. Spring production averaged 6,000 to 12,000 fry/spawn while out of season produced 4,000 to 7,000 fry/spawn. Florida largemouth bass production at the A.E. Wood State Fish Hatchery in San Marcos, Texas, and Texas Freshwater Fisheries Center, Athens, Texas, similarly average 8,000 to 12,000 fry/spawn during spring production. The reduced fry/spawn numbers from the out of season spawning remains unknown but could possibly be a result of regressed gonad development typically observed during July–

October (Brauhn et al., 1972; Rosenblum et al., 1994; Gross et al., 2002). Comparisons are further complicated by differing production requests (numbers required) and removing mats after production numbers were achieved. The data in Table 5.1 represent production totals achieved in a specific short time frame and should not be considered maximum values.

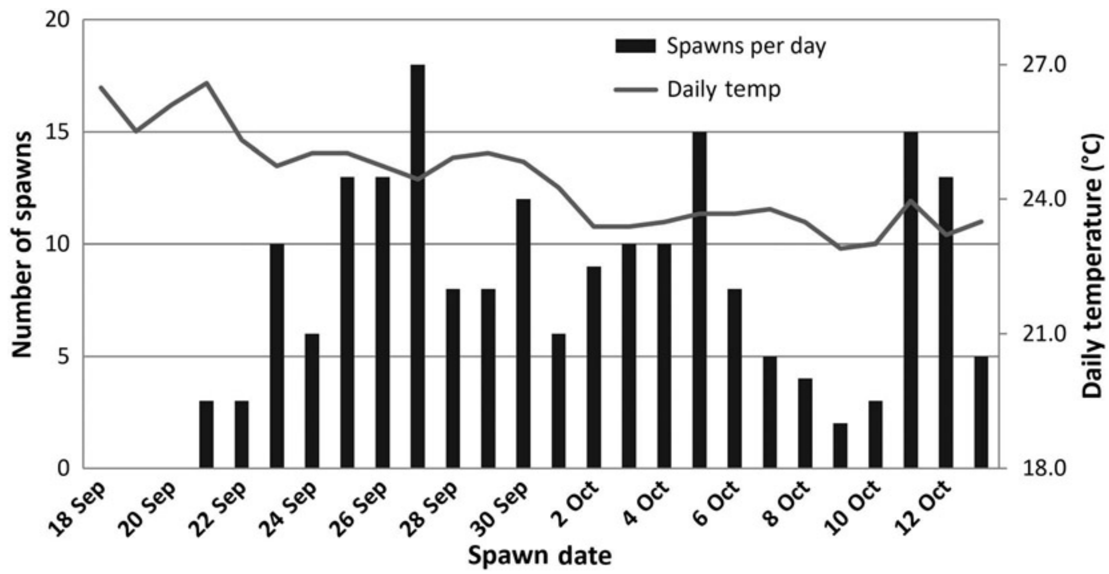

Figure 5.2 Results of spawning female LMB "out of season" in indoor raceways with morning water temperatures shown on the right-hand y axis.

5.1.1.8 Disease control methods for eggs and fry

Although possible, newly hatched fry kept in clean tanks with adequate water quality are not typically infested with parasites or have bacterial infections. However, eggs are very susceptible to fungus which, if left untreated, can cause up to 100% mortality. Water mold, commonly referred to as fungus due to visual characteristics, is caused by eight species of mold from the family Saprolegniaceae, but only *Saprolegnia* sp., *Achlya* sp., and *Aphanomyces* sp. are of concern in freshwater aquaculture (Tucker and Robinson, 1990; Woo and Bruno, 1999). There are several options for treatment depending on location and water quality, which can be found at https://www.fws.gov/fisheries/aadap/home.htm. Temperature, hydrogen peroxide, and formalin are effective at controlling Saprolegnia infestations (Marking et al., 1994). Water mold can grow at water temperatures of 5–30°C (Koeypudsa et al., 2005). However, by incubating bass eggs at water temperatures above 20°C the eggs hatch in ~48 h, greatly reducing water mold-induced mortality and the need for chemical treatment.

One experiment evaluated the effects of water temperature and hydrogen peroxide on hatch rates. Florida largemouth bass eggs were incubated under four treatments; ambient temperature (17.9–19.2℃), ambient temperature plus hydrogen peroxide (100 mg/l), heated water (22.1–23.6℃), and heated water plus hydrogen peroxide. The four treatments yielded hatch rates of 49, 79, 79, and 91% hatch, respectively (Matthews et al., 2012). Hydrogen Peroxide (35% active) is approved for use on bass eggs at 750 to 1,000 mg/l, but may be toxic to newly hatched fry (personal communication, Matthews).

Table 5.1 Example of actual spring and fall production totals and corresponding averages for Florida largemouth bass spawned in indoor 24 m raceways. Requests for larger amounts of fry in the fall account for the greater total fry and fry/female averages. The spawning data listed represents multiple years, but all fall within a 21 to 36 d spawning duration.

Raceway unit	Total spawns	Spawns/ female	Fry/g	Fry/ spawn	Total fry	Fry/ female	Spawning females
Spring spawn							
R	34	1.1	300	12,010	372,324	11,635	32
R	34	1	300	12,426	410,042	11,715	35
R	41	1.4	300	11,047	408,726	13,624	30
R	15	0.4	300	10,819	162,284	4,271	38
R	37	1.0	300	11,205	414,586	11,205	37
R	32	0.9	300	10,821	346,268	10,184	34
Average				11,388	352,372	10,439	
Fall spawn							
R	36	1.2	300	4,807	173,049	5,768	30
R	68	2.7	300	6,545	314,153	12,566	25
R	81	3.7	300	5,189	254,274	11,558	22
R	86	2.3	300	7,065	586,380	15,431	38
R	106	2.9	300	7,367	744,063	20,110	37
R	94	2.8	300	8,112	697,621	20,518	34
Average				6,514	461,590	14,325	

Another trial evaluated the use of salt to control water mold (*Saprolegnia*) during the incubation of largemouth bass eggs. Northern largemouth bass eggs were collected from a newly transferred spawn mat for the experiment. Twenty eggs were placed into each of eighteen 2 l containers to be hatched at different salinities. Salinity concentrations of 0–10 ppt, in increments of 2 ppt, were evaluated with three replicates per treatment. Eggs were initially stocked into 1 l of water and then the salinity was gradually increased, to prevent osmotic shock, until the desired concentration was reached. The eggs were monitored daily for hatching and presence of swim-up of fry. The salt treatments found to be most effective for control of water mold (*Saprolegnia*) were 4 and 6 ppt, resulting in 9.2 and 5.0% greater swim-up rates, respectively (personal communication, Coyle and Tidwell).

5.1.2 Artificial spawning

Largemouth bass is a difficult fish species to reproduce artificially for a number of reasons. While spawning hormones have been utilized, it is difficult to predict reaction of fish to hormonal stimulation. This reaction cannot be standardized and depends on many factors; such as preparedness of fish to spawning and conditions of fish rearing (temperature and photoperiod, presence of brooders of opposite sex, density, and others). This difficulty is rooted in a complex spawning behavior of largemouth bass, which includes building of nests by males and a period of courtship. Hormonal injection results in final maturation and ovulation of only a small portion of oocytes in the ovaries. This reaction to hormone injection is consistent with the fact that largemouth bass females are intermittent (partial) spawners. During the spawning season, a female may spawn with several males in several locations over a period of time. Even if the fish culturist is able to induce ovulation in female bass, and collect adequate numbers of viable eggs, obtaining suitable volume of viable milt is a problem. Hormonal injection results in release of very small amount of sperm. In many cases there is no apparent release of milt at all after hormonal injection. As a result, sperm is not stripped from males, and must be obtained with other methods.

Artificial spawning of largemouth bass has been used almost exclusively in cases when it was essential for the purposes of research. These cases included distant hybridization or application of some chromosome set manipulation methods (induced triploidy and gynogenesis) when there was a need for influence on gametes or early embryos. Below, the experience of artificial reproduction of largemouth bass is summarized. (In this section only the methods of artificial breeding are only described. Results of experiments on distant hybridization, triploidy and gynogenesis are presented in section 5.2.)

Stevens (1970) and Wilbur and Langford (1975) (cited in Heidinger, 1976) were the first to show that injection of human chorionic gonadotropin (HCG) with dose of 4,000 IU per one kg of fish weight can result in final oocyte maturation and ovulation in females and spermiation in males of the largemouth bass.

Beaty and Childers (1980) injected largemouth bass females with 4,000 IU/kg for induction of eggs ovulation in order to produce interspecies hybrids (female largemouth bass × male smallmouth bass). Gomelsky et al. (2004) also reported production of the same type of hybrids (largemouth bass × smallmouth bass) by artificial spawning. Largemouth bass females were injected with HCG with dose 4,000 IU/kg; largemouth bass and smallmouth bass males were injected with HCG at dose 2,000 IU/kg. Eggs were stripped 30–36 h after injection (Figure 5.3). Largemouth bass and smallmouth bass males did not release sperm after injection. Therefore, males were dissected and removed testes were macerated and washed with 0.85% saline solution. In further studies on largemouth bass × smallmouth bass hybridization (Stilwell, 2008) largemouth bass females were also injected with HCG with a dose of 4,000 IU/kg; eggs were stripped 30–36 h after injection. In order to investigate influence different types of hormones on spermiation in males, injections with HCG (2,000 IU/kg), LHRHa (100 μg/kg) and carp pituitary extract (CPE, 3 mg/kg) were tested. Largemouth bass and smallmouth bass males did not produce sperm by stripping after injection of any of these hormones. Therefore, males were sacrificed and their testes were dissected out and macerated through a 500 μm screen using 0.85% saline solution. In total, approximately 7,000 actively feeding largemouth fry and 2,000 largemouth bass × smallmouth bass fry were produced in this study (Stilwell, 2008).

In a study on triploidy induction in largemouth bass, Garrett et al. (1992) injected females and males with HCG at dose of 4,000 IU/kg. Sperm was stripped from males, collected by pipette and used for fertilization of eggs stripped from females. Injection of HCG at the same dose was also used by Fries et al. (2002) in another study on induced triploidy in largemouth bass. It was estimated that 200–2,000 eggs were obtained from one female; about 40–60% of stripped eggs were not viable. The average number of viable fry per female obtained by hand-stripping after HCG injections was less than 12. Fry production increased up to about 1,000 in further trials when instead of hormone injections fish were allowed to enter into spawning behavior in a natural manner prior to hand-stripping.

Figure 5.3 Stripping eggs from a largemouth bass female after hormonal injection.

Neal et al. (2004) also used artificial spawning in studies on induced triploidy in largemouth bass. Both largemouth bass males and females were injected intramuscularly with 5 mg/kg of CPE and with 50 μg/kg of LHRH in the dorsal lymphatic node. Injection of these hormones induced production of free-flowing gametes in more than half of fish. Peak gamete production occurred within 21–24 h following injection at water temperatures ranging 23–28°C. Males produced very low volume of sperm which was collected by pipette. Before fertilization of eggs, sperm was mixed with about 10 ml of 0.3% NaCl to increase the volume. In a further study on induced triploidy Neal (2014) induced ovulation and spermiation in largemouth bass females and males by injection of Ovaprim with initial dose of 0.1 ml/kg and resolving dose of 0.5 ml/kg with 8 h between two injections. The testes were removed and macerated with approximately 10 ml of 0.3 NaCl solution. For fertilization, macerated testes were mixed with water poured through a fine mesh over the eggs.

In a study on induced gynogenesis in largemouth bass, Glennon et al. (2012) injected females with HCG at a dose of 4,000 IU/kg to induce ovulation. The time between hormonal injection and ovulation was 36–40 h. For induction of gynogenetic development, largemouth eggs were inseminated with UV-irradiated white bass or striped bass sperm.

A recent study (personal communication, Semmens and Gomelsky) at Kentucky State University compared HCG and Ovaprim® (Western Chemical, Inc.) as inducing agents for artificial spawning of largemouth bass. Ovaprim® is a commercial product containing salmon gonadotropin releasing hormone analog (20 μg/ml) and dopamine antagonist (10 mg/ml). In late April of 2017, largemouth bass (36 females and 36 males) were injected with the two inducing agents in two trials spaced a week apart. Mean weight of females was 1,139 g while mean weight of males was 979 g. All fish were given a single intramuscular injection early in the morning (approximately 7 am) and examined for a response the following day. HCG was applied at a rate of 4000 IU/kg for females and 2000 IU/kg for males while Ovaprim® was applied at a rate of 0.5 ml/kg to both males and females. The response rate for HCG and Ovaprim® was 89% and 78% with a mean weight of eggs 31.2 and 26.8 g per female, respectively (Table 5.2). The number of eggs per female was similar for both treatments and ranged from 5,600 to 40,500 and from 5,000 to 44,000 for HCG and Ovaprim®, respectively. The mean latent period for egg ovulation was also similar for both treatments being 33.4 and 34.4 h for HCG and Ovaprim®, respectively.

In this study, regardless of inducing agent, it was not possible to strip sperm from the males. Therefore, males were euthanized, dissected and the testes were extracted as a source of sperm (Gomelsky et al., 2004; Neal, 2014). Testicular tissue was screened through a fine mesh (high density polyethylene filter cloth 540 µm mesh) to create a suspension containing sperm. The mesh retaining tissue was rinsed with 6 ml saline (0.85%) per testes of one male. Average weight of testes was 4.9 g with a range of 2.8 to 6.5 g among both treatments. Testes from one male was used to fertilize ≤ 75 g of eggs; testes from two males were used for larger spawns. For artificial fertilization of eggs, ~200 ml of water was added to suspension of sperm in saline solution and then this mixture was added to the eggs and gently stirred with a feather for 1 min (Figure 5.4).

Table 5.2 Egg ovulation response of largemouth bass females to two hormonal treatments and results of eggs artificial fertilization and incubation.

Parameter	Hormonal treatment	
	HCG	Ovaprim®
Mean weight of females (g)	1132±55*	1146±62
Number of injected females	18	18
Number of females releasing eggs	16	14
Response rate (%)	88.9	77.8
Mean latent period (h)	33.4±0.55	34.4±0.74
Range of latent period (h)	30.2−36.4	27.1−37.8

Parameter	Hormonal treatment	
	HCG	Ovaprim®
Total weight of eggs released (g)	499	375
Mean weight of eggs per female (g)	31.2±4.6	26.8±5.2
Number of eggs in 1 g (before fertilization)	572±28	658±21
Total number of eggs collected	295,506	240,532
Number of eggs collected per female (mean)	18,469±3,191	17,181±3,316
Number of eggs collected per female (range)	5,600−40,500	5,000−44,000
Number of eggs taken for fertilization	292,887	222,761
Number of incubation jars	**5**	3
Mean number of eggs incubated per jar	58,577±18,080	74,254±50,123
Total number of collected swim−up larvae	13,047	14,286
Number of swim−up larvae per female (mean)	815	1,020
Yield of swim−up larvae (%)**	4.5	6.4

Notes: * Mean values ± SE are presented in table. ** From the number of eggs taken for fertilization.

Subsequently, 700 ml of a fuller's earth slurry (25 g/l) was added to prevent egg adhesiveness and clump forming. The mixture was stirred for approximately 20 min and loaded into a McDonald hatching jar for incubation. The yield of swim-up larvae was 4.5 and 6.4% for HCG and Ovaprim® treatments, respectively, with corresponding numbers of swim-up larvae per female of 815 and 1,020. There was no possibility to determine fertilization rate at cleavage stage since eggs at that time were heavily coated with fuller's earth particles. However, it can be suggested that low yield of larvae was caused by low fertilization rate. Inability to strip sperm from males can be considered as one of the main obstacles for practical application of induced spawning for production of mass quantities of fry in largemouth bass.

Figure 5.4 Fertilization of eggs with suspension of sperm.

5.2 Genetics

5.2.1 Genetics and domestication

It is estimated that only about 15% of all aquaculture production is based on genetically improved stocks. However, when fish are cultivated in captivity even for a relatively short period, they are under strong and permanent “domestication” pressure, even if unintentional. Domestication can be defined as “a process by which a population of animals becomes adapted to man and the captive environment by some combination of genetic changes occurring over generations and environmentally induced developmental events recurring during each generation” (Price, 1984). Selection of fish for fitness under new conditions (ability to survive under high density, ability to consume artificial diet and so on) occurs permanently.

In 1960s–1970s, the intensive method of largemouth bass production had been developed. This method includes training of largemouth fingerlings to take artificial feed (for details see Chapter 6). Apparently, the changes in outcomes in training largemouth bass to artificial feed can be a good example of selection of fish for fitness under new conditions. Heidinger (2000) noted that one can expect in excess of 60% of the fingerlings to learn to accept the prepared diet while those fingerlings that have been produced from first- or second-generation hatchery-reared broodfish tend to have training rates in excess of 90%. Currently, broodstocks of largemouth bass have been raised on artificial diets for more than 7–8 generations at about ten different fish hatcheries in the US southern region. During this time, not only the percentage of fish accepting prepared diet has increased but the training process of fingerlings to accept artificial diet became much easier. Possibly, to some extent these changes can be attributed to some progress in training techniques (for example, by development of high-quality floating pellets). However, undoubtedly the underlying genetic composition of the fish themselves has changed as a result of domestication.

There are several examples in the literature, when the effects of animal domestication have been extremely rapid, and within even just a few generations of selection large changes occurred (Jensen and Wright, 2014). For example, selection of silver foxes for tameness for only several (4–6) generations resulted in profound changes in animal behavior and physiology (Trut, 1999; Trut et al., 2009). Along with progress in selection, animals rapidly acquired morphological traits, which are typical for some breeds of domesticated dog (for example, floppy ears and rolled tails). The level of corticosteroids (stress indicators) in the blood was significantly lower than in unselected animals. This research demonstrated that domesticated animals were less stressed by being kept in captivity. It would be incorrect to make direct analogies from domestication of mammals to domestication of largemouth bass. However, it can be suggested that adaptation of largemouth bass to new conditions has also caused many relatively rapid changes in fish behavior and physiology.

5.2.2 Florida and northern largemouth bass

In bass, the most common and best studied hybridization event occurs between the subspecies/species of largemouth bass, *Micropterus salmoides salmoides* (northern largemouth [NLMB]) and *Micropterus salmoides floridanus* (Florida largemouth bass [FLMB]). These two fish are now commonly recognized to represent separate species (Near et al., 2003; Barthel et al., 2015), although variable classifications are still seen in the literature. First described by Bailey and Hubbs (1949), the ranges of NLMB and FLMB bass were originally delineated based on morphometric and meristic techniques. They restricted FLMB to peninsular Florida, and NLMB to the majority of the midwestern, eastern, and southeastern US regions, outside of Alabama, Georgia, and South Carolina where a natural intergrade between the two was identified. Interestingly, Bailey and Hubbs (1949) refer to the already well-established larger maximum size of the FLMB (Hubbs, 1932), referring to it as a “giant” and commenting on differences in physiology between northern and Florida forms. They also comment on the difficulty of establishing the original boundaries between the subspecies given “extensive stocking.” These themes, touched on in brief almost 70 years ago, have dominated the genetics of largemouth bass in intervening years and have continuing importance for genetic improvement and selection of largemouth bass as an aquaculture species.

5.2.2.1 Stocking of Florida and northern largemouth bass: controversy and consequences

Transport and stocking of largemouth bass (whether FLMB or NLMB) in non-native waters has been something of a long-standing tradition in the USA, sometimes directed by angling enthusiasts themselves and often as part of widespread state agency-directed efforts to improve recreational fishing. By the time Philipp et al. (1983) revisited the distribution of Florida/Northern alleles in 1983 using allozyme markers, stocking had led to hybridization between *M. salmoides* and *M. floridanus* far beyond the natural integrade zone. FLMB have been stocked most extensively throughout the southeastern USA, particularly in manmade reservoirs. Stocking has also occurred in the US Midwest, California, Japan, China, the Caribbean islands, South America, and elsewhere. One consequence of such widespread stocking is that geographical region can no longer be safely used as an indicator for determining the type of resident largemouth bass, status of introgression, degree of inbreeding, domestication and so on. Genetic testing is a pre-requisite before sourcing largemouth bass for a hatchery or aquaculture facility (see marker discussion below).

Much of the evidence for differential performance of FLMB and NLMB comes not from carefully controlled pond or tank studies, but rather from reservoir assessment reports following stocking. The ideological divide over stocking FLMB is best encapsulated in Maceina and Murphy (1992) where the authors spar with Philipp and Whitt over potential positive and negative effects of stocking outside the native range. While not advocating indiscriminate stocking, Maceina and Murphy argue that the reservoirs created by impoundment are unnatural habitats for native NLMB and, in some cases, more ideally suited for FLMB, using the success of FLMB and FLMB × NLMB hybrids in Texas reservoirs as evidence (Maceina et al., 1988). Philipp working with several coauthors has proposed vigorously that introducing FLMB in waters outside peninsular Florida has deleterious fitness consequences for recipient populations (Philipp, 1991) due to swamping of native populations through hybridization, outbreeding depression, lowered reproductive success, heightened susceptibility to disease, and so on (Fields et al., 1987; Cooke et al., 2001; Grant et al., 2003; Goldberg et al., 2005). However, Maceina and Murphy (1992) point out that many of these experiments involve stocking FLMB outside their thermal optima (for example, Central Illinois, Wisconsin), potentially biasing against the success of FL alleles. While this ongoing debate may seem to reside primarily in the realm of conservation and fisheries management, there are definite spill-over effects as state fisheries biologists weigh potential consequences of escapement from largemouth bass hatcheries and farms during permitting processes and regulate the movement of NLMB and FLMB. For example, the state of Florida, as part of a genetics conservation program, has declared it illegal to possess NLMB or their hybrids within the native range of FLMB and is restricting transport of bass among different genetic regions within the state (Porak et al., 2015). Other states, when faced with permitting largemouth bass aquaculture facilities, are likely to be heavily influenced by the "outbreeding depression" literature regarding non-native bass hybridization.

5.2.3 Differential performance of largemouth bass genotypes

Studies in both reservoir and aquaculture settings have highlighted differences in behavioral and production-important traits in FLMB, NLMB, and their hybrids. Results in these studies have been conflicting, and, oftentimes, a sufficient body of literature is not available to resolve differing findings. Differences in thermal optima for NLMB and FLMB are expected to impact performance and must be accounted for in comparing genetics stocks. Fields et al. (1987) reported that NLMB had chronic and critical thermal maxima significantly lower than NLMB × FLMB hybrids, FLMB ×

NLMB hybrids (note female in cross is listed first), and FLMB. Even outside these maxima, suboptimal temperatures undoubtedly affect performance. Studies also often fail to provide the domestication history of genetic strains/species they compared. A comparison between a NLMB under domestication for 10 generations with wild spawned FLMB and their hybrids is not an accurate reflection of genetic potential, but rather a testament to the importance of domestication.

With these caveats, the literature does provide importance insights into the comparative growth and performance of bass genetic stocks. The higher catchability and trainability of NLMB have long been noted. Zolczynski and Davies (1976) found faster growth in NLMB than FLMB or their hybrid in the first summer of growth. Many studies, both reservoir and aquaculture, point out the faster initial growth of NLMB, a favorable aspect for food production where the larger eventual size of FLMB over 10+ years is of little consequence. Similarly, Williamson and Carmichael (1990) found that NLMB had faster growth, 40% lower feed conversion ratio, lower mortality during net confinement, better feed training success, and other benefits in concluding that they were superior to FLMB and F_1 hybrids as potential aquaculture candidates. Here, more detail about source of stocks would have been helpful. They state that fish used in their trials came from wild populations. Given the large natural range of NLMB, for example, one wonders the aquaculture suitability of broodstock from Louisiana for a Texas growout facility versus broodstock sourced from northern Minnesota. Both are technically NLMB. Recent genetic analyses (Li et al., 2015) have revealed (not unexpectedly) that southern NLMB share a greater proportion of alleles with FLMB than do NLMB. Further studies comparing performance of various NLMB sourced across their natural range would be helpful in evaluating aquaculture broodstock candidates.

A 1990 study in Texas ponds (Kleinsasser et al., 1990) found that the FLMB × NLMB cross was superior for growth in its second year (641.1 g), having a significantly higher ending weight than NLMB (462.4 g), the reciprocal hybrid NLMB × FLMB (506.8 g), or FLMB (403.3 g). The wary, skittish nature of FLMB was noted as a cause for its poor showing (403 g average ending weight). Kubitza and Lovshin (1997) examined the impact of strain on feed training success. They utilized Fx hybrid crosses (roughly 70/30 FL/N or 70/30 N/FL contributions). They found comparable acceptance of a pelleted diet by both crosses. While the literature remains fairly sparse and confusing on this subject, the general consensus is that the faster initial growth, superior feed trainability, and more aggressive nature of the NLMB make it the preferred large-mouth bass candidate for aquaculture. However, as described above, further intra-NLMB studies are needed and the geographic location and configuration of a grow-out facility may ultimately dictate the optimal genotype. Within the right thermal window, FLMB × NLMB hybrids deserve additional research and scrutiny as well (Bonvechio and Rydell, 2016).

5.2.4 Genetic markers: ancestry, parentage and beyond

The potential for success in selective breeding is governed in large part by the presence of sufficient genetic variation in founding populations. Loss of genetic variation is often attributable to a lack of knowledge of base populations or hatchery practices that introduce artificial genetic bottlenecks (Bai et al., 2008). The long history of movement and stocking of largemouth bass (as documented above) makes uninformed assumptions about broodstock or fingerling origin or levels of diversity fraught with peril. The propensity of NLMB and FLMB to hybridize, the presence of natural intergrades, and lack of clear morphological features distinguishing largemouth bass forms, has long necessitated chemical and molecular testing to clearly delineate ancestry. Beginning with the work of Philipp et al. (1983), where 28 enzyme loci were examined, largemouth bass genetic testing has followed the progression of the advance of molecular genetic techniques from allozymes, to microsatellites and then to single-nucleotide polymorphism (SNP) markers.

In their 1983 work, Philipp et al. screened 28 enzymes, but identified only two, isocitrate dehydrogenase-B and aspartate aminotransferase-B, with fixed allelic differences useful for determining the contribution of the subspecies to the gene pool of various populations throughout the USA. Other enzymes were useful in demonstrating genetic distinctness among populations, although not necessarily correlated with NLMB/FLMB composition. Allozyme analyses became the gold standard for population genetics research and for testing commercial populations for the next two decades. However, with only two diagnostic loci, clear detection of hybridity across the largemouth bass genome was inconsistent at best. As sequencing technologies allowed the identification of simple sequence repeats, or microsatellites, these markers were developed for largemouth bass. Lutz-Carrillo (2008) developed 52 microsatellite markers for FLMB and demonstrated their utility in other largemouth bass. Seyoum et al. (2013) developed an additional 18 microsatellites, focusing on development of multiplex panels with similar annealing temperatures and cycling parameters. These microsatellites included no fixed loci, but several loci possessed clear allelic frequency distribution differences between FLMB and NLMB (and other black basses). Microsatellites have well-established pros and cons as a marker technology. Their allelic richness makes each locus highly informative. However, they suffer from null alleles, expense of reagents, difficulty in multiplexing, and the high labor cost associated with careful scoring of loci. Furthermore, changes in technology for sequencing have made it more difficult to maintain microsatellite running capabilities in many labs.

The dramatic decline in sequencing costs associated with next generation sequencing has increased the accessibility of SNP markers for population genetics (Hohenlohe et al., 2011). SNPs are valued for their even genome-wide distribution, abundance, ease of multiplexing, and low genotyping error rate. Li et al. (2015) described the work conducted to develop diagnostic SNP marker panels for largemouth bass at Auburn University. Two panels of 25 and 38 fixed SNP loci can clearly distinguish purity and hybridity (including multi-generation backcrosses) across the genome. In daily running, the single 38-plex panel is sufficient for these purposes. Over 5,000 largemouth bass from rivers, reservoirs, hatcheries, and ponds across the USA, China, and Japan have been genotyped to date with these markers. The short, discrete nature of SNP loci also allow use of highly degraded samples, allowing DNA to be collected by swab sampling, use of cruder (and cheaper) DNA extraction techniques, and even for genotypes to be obtained from long term formalin-fixed museum samples.

Once questions of FLMB/NLMB purity and hybridity have been answered, genetic markers are often needed to determine parentage. As hatchery configuration and space restraints often preclude 1: 1 mating schemes, so raceways, tanks, or ponds are used for communal spawning. Identification of superior broodstock (for example, walk-back selection) based on progeny performance in a common-garden growout necessitates the use of markers to establish parentage. Parentage analyses are also critical in determining effective numbers of breeders contributing to offspring to avoid inbreeding and ensuring broodstock maintain essential genetic diversity. Austin et al. (2012) screened broodstock and fingerlings at the Richloam Fish Hatchery in Florida with microsatellite loci to assess hatchery effects on genetic diversity and to determine sibling relationships. While they concluded that their current raceway breeding practices were sufficient to maintain diversity, they found that the use of nine microsatellite loci was insufficient to allow parentage assignment for individual fingerlings. A subsequent study, Hargrove and Austin (2017) used a different set of microsatellites to examine parentage, demonstrating that most broodfish contributed to reproduction, and that most spawns could be attributed to one pair of parents. Similarly, SNP markers recently have been developed and demonstrated for parentage analyses in FLMB (Zhao et al., 2018), with downstream applications in hatchery management, selection, and parentage-based tagging in reservoirs.

Further aquaculture applications for molecular tools in largemouth bass lie in the realm of marker-assisted selection for trait performance. In this, Chinese aquaculturists appear to be leading the way. NLMB were introduced into China in 1983 and now account for 100,000 tons of annual aquaculture production. Genetic analyses show a distinct signature of genetic bottlenecking due to the founder effect (Bai et al., 2008). Recent research has identified SNPs and other polymorphisms linked to growth traits and early efforts are underway to use these as a basis for selective breeding (Li et al., 2009, 2017). Other more cryptic literature from China references long-term selection of largemouth bass for growth.

5.2.5 Potential for selective breeding and continued genetic improvement

Selective breeding for largemouth bass has been sporadic and poorly reported, often residing in "grey" literature of state hatchery reports or conference communications. While selection for pelleted feed acceptance has a long history (Snow, 1960; Kubitza and Lovshin, 1997), foundational stocks were often not rigorously evaluated, record keeping was sporadic, and the current genetic integrity of these lines is often difficult to ascertain. Private hatcheries have also carried out selection, but often their goals have been aimed primarily at trophy bass production for recreational angling rather than shorter-term aquaculture growout potential, and their success is difficult to rigorously evaluate. Clearly, the need and potential remains for a well-controlled, multi-generation selective breeding program for largemouth bass and/or the FLMB × NLMB hybrid aimed at food-fish production.

As noted, a comparison of the growth potential of NLMB sourced from different regions and river systems is needed. Studies examining the heritability of traits important for aquaculture production are also needed (growth, disease resistance, fillet yield, and so forth). The ongoing ShareLunker program in the state of Texas, has recently reported that angler return of trophy fish caught in Texas reservoirs for use as hatchery broodstock has yielded offspring significantly heavier than resident cohorts (1.19 kg versus 0.99 kg at age 4) within several reservoirs (Baird et al., 2016), indicating that, even in large, highly variable environments, genetic contributions to growth can be realized. As with much of aquaculture, the development of a long-term, well-structured selection program for largemouth bass, is hindered by economic constraints. Investment in additional hatchery and grow-out facilities, record keeping, and molecular marker analysis must be offset by a return from a higher priced fingerling, lowered costs of production, or higher yields. The small size of the largemouth bass market, at least in the USA at this writing, likely makes it difficult to realize an economic gain on large-scale investments in genetic selection. Development and release of improved lines may be better achieved through developing partnerships with existing university programs or U.S. Department of Agriculture research labs focused on aquaculture. Once a stable breeding program is developed with well-established lines or strains, additional gains may be realized through all female bass production (Bonvechio and Rydell, 2016), potentially augmented by use of molecular markers to develop monosex all-female breeding populations as described below (gynogenesis). Towards that end, efforts are ongoing to develop high density genetic maps for largemouth bass with markers linked to sex determination (Peatman lab at Auburn University).

5.2.6 Production and evaluation of triploids

Studies on induced triploidy in largemouth bass were initiated based on the suggestion that sterile triploids could have better growth parameters than normal fertile diploids. Also, stocking of sterile triploids into water bodies outside native range could minimize impacts on genetic pools of local populations. Garrett et al. (1992) produced triploid progenies of largemouth bass using hydrostatic pressure for retention of second polar body in freshly fertilized eggs. Pressure levels from 4,000 pounds per square inch (psi) to 8,000 psi (281–563 kg/cm^2) were tested; pressure shock durations were 3 min for pressure levels from 4,000 to 6,500 psi (457 kg/cm^2) and 1 min for 8,000 psi, respectively. In all experimental variants pressure shock was applied 5 min after fertilization; pre-shock water temperature was 21–25℃. Ploidy of 1- or 2-month-old fish was determined by measurement of DNA content by flow cytometric analysis. All applied treatments produced triploids in experimental groups. A treatment of 8,000 psi (or 563 kg/cm^2) was considered as the best; it yielded 100% triploids and a relatively low mortality. Parameters of pressure determined by Garrett et al. (1992) were usually used in later studies on inducement of triploidy in largemouth bass. Fries et al. (2002) used pressure shock with these parameters (initiation: 5 min after fertilization, duration: 1 min, intensity – 563 kg/cm^2) for production triploids. Results of flow cytometric analysis showed that triploidy induction technique was effective; overall success rate was 95.5%.

Neal et al. (2004) used the same parameters of pressure shock (5 min after insemination, duration 1 min, 563 kg/cm^2 for production triploids in largemouth bass in Puerto Rico. Flow cytometry analysis revealed 100% triploid fingerlings in pressure-treated group. Also, a method of fish ploidy determination based on erythrocyte lengths on blood smears was developed. Neal and Noble (2008) have presented comparative data on growth and maturation of 1-year-old diploid and triploid largemouth bass raised in a reservoir in Puerto Rico. No difference in growth rate between 1-year-old diploid and triploid fish was detected. Diploid females had much better developed ovaries than triploid females and had mean gonadosomatic indices of 1.98 and 0.29%, respectively. In a further study, Neal (2014) had not detected differences in growth rate between diploid and triploid largemouth bass at fish age 2 years and older. Maximum sizes, which were attained by diploid and triploid fish, were similar. Most of females with total length 250 mm and larger were mature while no triploid females displayed maturing ovaries. Diploid males had significantly larger mean gonadosomatic index than triploid males. These data showed that retardation in gonad development in triploid largemouth bass did not result in increased growth rate compared to normal diploids.

5.2.7 Sex control

Largemouth bass are sexually dimorphic. Females of largemouth bass demonstrate faster growth rate and attain larger size than males. Therefore, it would be attractive to produce and raise all-female progenies. Also, raising monosex (either all-female or all-male progenies) would restrict unwanted reproduction.

Artificial sex regulation in fish is connected mainly with elaboration and application of the hormonal sex reversal method. Hormonal sex reversal is a change from the normal process of sex differentiation under the influence of steroid sex hormones (or some other substances) so that genotypic females develop testes or genotypic males develop ovaries. Sex reversal changes only fish phenotype while the genotypic formula of sex chromosomes remains the same. Hormonal sex reversal may be used in two different ways for sex regulation. The direct method for producing monosex (all-female or all-male) populations involves hormonal treatment of all reared fish during the period of sex differentiation. The indirect method, or genetic sex regulation, involves crossing normal fish with previously obtained sex-reversed fish. Most studies performed in large-mouth bass until now were aimed at direct feminization or masculinization of fish by treatment of estrogens or androgens, respectively. These studies performed are briefly reviewed below.

Garrett (1989) performed a series of experiments on hormonal sex control of largemouth bass. In one experiment, largemouth bass fry were fed with hormone-enriched live brine shrimp (Artemia) from beginning of active feeding for 10 weeks. Two estrogens (17a-estradiol and estrone) and two androgens (17a-methyltestosterone and androsterone) were used for sex reversal. For enrichment, each hormone was dissolved in 95% ethanol and added to brine shrimp culture media to concentration of 5 mg/l; in the control variant only ethanol was added without any hormone. Feeding largemouth bass fry with hormone administration through brine shrimp was highly effective for sex reversal. Experimental groups, which consumed androgen-treated (with both methyltestosterone and androsterone) brine shrimp, consisted of males only while groups fed with estrogen-treated (with both estradiol and estrone) brine shrimp consisted only of females. The sex ratio in control group was significantly skewed towards males (89% males). It was suggested that skewed sex ratio in control group has resulted from androgen influence via water vapor since the control jar was adjacent to the jars with hormone-treated brine shrimp. In a later similar experiment, which used hormone concentration of 10 mg/l for Artemia enrichment, the control container was placed approximately 2 m away from hormone-treated containers and resulting sex ratio in control group was close to expected 1: 1; hormonal influence in that experiment was again 100% effective in all experimental groups. In another experiment performed by Garrett (1989) 20–30 mm 5-week-old largemouth bass fry nursed in a pond were fed with hormone-containing food for 6 weeks. The same two estrogens (17a-estradiol and estrone) and two androgens (17a-methyltestosterone and androsterone) were used for sex reversal. Two doses of 17a-methyltestosterone (50 and 100 mg/kg of food) were evaluated while for the other three hormones only one dose of 100 mg/kg was used. Treatments of androgens resulted in sex reversal of most of the genotypic females (90–98% of males in androgen-treated groups). In the control group the sex ratio did not differ significantly from 1: 1. Estrogens appeared to be ineffective for sex reversal in genotypic males; fish sex ratios in groups receiving estradiol or estrone with diet did not differ significantly from 1: 1.

Porter (1996) studied effectiveness of feeding largemouth bass fry with 17a-methyltestosterone (MT) with doses of 30 and 50 mg/kg for periods of 4, 6, or 10 weeks. Hormonal treatment started when fish had mean total length about 25 mm. Sex of fish in hormonally treated and control groups was determined based on two criteria: gonad morphology (structure) and histology. Morphologically, “V-shaped” testes were distinguished from “Y-shaped” ovaries. Histologically, testes were identified by the absence of oogonia. In control variants about 50% of fish were identified as males based both on gonad morphology and histology. In experimental groups receiving MT in their food, 96–100% of fish were identified as males based on gonad histology; however, about 50% of these fish had gonads with the morphological structure of ovaries.

Al-Ablani and Phelps (2001) indicated that 17a-estradiol and diethylstilbestrol were both effective in producing 100% feminization in 40 d-old largemouth bass fry when fed at concentrations of 100, 200, or 400 mg/kg of diet for 40 d. Arslan et al. (2009) reported that feeding of 40 d-old largemouth fry with 17a-estradiol at a concentration of 200 mg/kg for 30, 45, or 60 d resulted only in slight increases in the frequency of females (up to 59.8–70.5%) in experimental groups. Feeding of fry of the same age with diets containing MT at a concentration 60 mg/kg for 30, 45, or 60 d did not result in a shift in sex ratios. The three- or six-time immersions of largemouth fry in a MT solution of 1 mg/l for 5 h between 40 and 46 or 54 d post-hatching were also not effective in inducing sex reversal.

As mentioned above, the indirect method of sex control, or genetic sex regulation, involves crossing normal fish with previously obtained sex-reversed fish. Genetic sex regulation is regarded as the preferred method since there is no need to treat all reared fish with a hormone and hormonally treated fish are not used for human consumption. The scheme of crosses used to produce all-female progenies depends on the type of heterogamety in given fish species. In fishes, both male (XY males, XX females) and female (WZ females, ZZ males) heterogamety are described; sometimes different types of heterogamety are revealed in closely related species. In the case of female homogamety, all-female progenies may be produced by crossing normal females XX with sex-reversed XX males (neomales) obtained by hormonal sex reversal of genotypic females. In the case of female heterogamety the scheme for production of all-female progenies is more complex. In this case it needs to identify WW females either in progenies obtained by crossing sex-reversed WZ males with normal WZ females or in meiotic gynogenetic progenies. Cross of WW females with normal ZZ males should produce females (WZ) only.

One of the basic methods for determining the type of heterogamety in fish is based on sex composition of gynogenetic progenies. In the case of male heterogamety (XY/XX), gynogenetic progenies usually consist of females only; the presence of both females and males indicates female heterogamety in a given species (WZ/ZZ). Gynogenetic development in fish is usually induced by insemination of eggs with genetically inactivated (by UV-irradiation) sperm while restoration of diploidy is caused by suppression either second meiotic division in eggs (meiotic gynogenesis) or first mitotic (cleavage) division in haploid embryos (mitotic gynogenesis).

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Figure 5.5 Petri dishes with diluted white bass sperm are placed into Crosslinker for UV-irradiation.

Only one study on induced meiotic gynogenesis in largemouth bass has been performed so far (Glennon et al., 2012). In this study, gynogenetic development was induced by insemination of largemouth bass eggs with genetically inactivated (by UV-irradiation) sperm from white bass or striped bass. Sperm was irradiated at a dose of 1,000 J/m^2 with a FisherBiotech UV microprocessor-controlled Crosslinker (FB-UVXL-1000). For irradiation, 2 ml of sperm diluted 10-fold with 0.85% NaCl solution was placed in 6 cm-diameter glass Petri dishes (Figure 5.5).

Suppression of the second meiotic division in largemouth bass eggs was achieved by hydro-static pressure; pressure shock of 8,000 psi for 1 min was initiated 5 min after the eggs were inseminated with irradiated sperm (Figure 5.6).

Fifteen 1 year old fish of gynogenetic origin were dissected for sex determination; seven fish were male and eight fish were female. Another six gynogenetic fish were sexed live using ultra-sound investigation and were identified as females. In total, of the 21 gynogenetic fish analyzed, 7 fish (33.3%) were male and 14 fish (66.7%) were female. Based on presence of males in meiotic gynogenetic progeny the existence of female heterogamety (WZ/ZZ) in largemouth bass was suggested (Glennon et al., 2012). In meiotic gynogenetic progeny obtained later all seven analyzed fish were female (Robert Glennon, personal communication). Based on this information, it may be stated that existence of female heterogamety in largemouth bass suggested earlier (Glennon et al., 2012) needs further confirmation.

5.2.8 Interspecies hybridization

Childers (1975) noted that hybridization of largemouth bass with fish of other species is a rare phenomenon under natural conditions. However, many different interspecies hybrids of largemouth bass with fishes of genus *Lepomis* and other fish of genus *Micropterus* have been obtained artificially. In many cross combinations, the obtained hybrids had low viability and poor growth rate. The only hybrids, which appeared to have some superior characteristics, were those obtained by cross of largemouth bass females with smallmouth bass (*Micropterus dolomieui*) males. Hybrids obtained by reciprocal cross (females smallmouth bass × males largemouth bass) were inviable. Childers (1975) and Beaty and Childers (1980) described that largemouth bass × smallmouth bass hybrids demonstrated better growth rate than pure largemouth bass. However, further studies (Buck and Hooe, 1986; Stilwell, 2008) have not confirmed these observations. In these studies, growth rate of hybrids was similar to or slower than that of largemouth bass. There are other factors which make production and raising of these hybrids impractical. Largemouth bass × small-mouth bass hybrids can be produced only by artificial spawning. Also, Gomelsky et al. (2004) showed these hybrids are fertile and can backcross with parental species. Thus, possible escapement of artificially obtained hybrids could result in genetic contamination of natural populations.

Figure 5.6 Largemouth bass eggs inseminated with irradiated white bass sperm are placed into pressure chamber for applying of hydrostatic shock.

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Chapter 6
Production of feed trained largemouth bass fingerlings: nursery phase through feed training

Shawn D. Coyle[1] *and Michael D. Matthews*[2]

[1] Kentucky State University, Aquaculture Research Program, Frankfort, Kentucky, USA

[2] A.E. Wood Fish Hatchery, San Marcos, Texas, USA

6.1 Introduction

Largemouth bass fingerlings have been produced by state and federal hatcheries since the 1890s for stocking programs (Lydell, 1903; Turner and Kraatz, 1921). Before the 1960s, hatcheries relied solely on live feeds, beginning with zooplankton and then switching to forage fish until the desired stocking size was reached. Unlike channel catfish (*Ictalurus punctatus*) and rainbow trout (*Oncorhynchus mykiss*), newly hatched largemouth bass fry (swim-up stage) cannot be easily trained to accept prepared diets (Brandt et al., 1987; Williamson et al., 1993). Therefore, bass fry are usually raised in fertilized nursery ponds until they reach 25–50 mm TL (1–2 g) (Tidwell et al., 2000). Fry stocking densities in ponds are commonly 125,000–500,000 fry/ha to produce 25–50 mm fingerlings. In well managed ponds, growth rates of 1.0–1.5 mm/d and target lengths of 38 mm can be achieved in 30–45 d after fry stocking (Hutson, 1990). Previously, fingerlings were then transferred to growout ponds where they were raised to larger sizes on forage fish. However, this extensive production method requires many ponds for both the bass and the forage fish, as it requires approximately 5–10 kg forage fish to produce a 1 kg bass (Nelson et al., 1974).

Studies have examined largemouth bass production on artificial feeds since the 1960s (Snow, 1968a, 1970a; Snow and Maxwell, 1970; McCraren, 1975; Snow and Wright, 1975; Sloane, 1993). Today many federal, state, and private hatcheries produce 10–15 cm fingerlings on feed for stock enhancement programs and for further growout. To economically produce large numbers of fish of this size requires that fingerlings be feed-trained to accept artificial feeds. This allows much higher stocking densities, which produces more advanced fingerlings from finite pond resources especially if forage fish production is considered. Feed training is the production phase where pond-reared juveniles are crowded into tanks at high densities and presented highly palatable food items at frequent intervals. Juveniles are gradually transitioned to a 100% artificial feed over 10–14 d. Several studies have tested different initial food items, such as ground fish, fish eggs, moist pellets, and freeze-dried krill (FDK) for feed-training largemouth bass fingerlings (Snow, 1965; Anderson, 1974; Kubitza and Lovshin, 1997a).

Advances in hatchery design have also contributed to the intensification of largemouth bass production. For example, spawning bass in raceways has reduced dependence on spawning ponds for fry production (Isaac and Staats, 1994; Mayes et al., 1993; Lyon et al., 2002) and allowed production intensification of 25–50 mm fingerlings. Techniques to intensively spawn bass “outof-season,” enabling managers to stock 100 mm fingerling in early spring, have been developed (Matthews and Stout, 2013). Lined ponds have reduced water and maintenance requirements compared to typical earthen hatchery ponds and advances in pond aeration systems, better harvest structures, and research into pond fertilization and management have improved outcome predictability for rearing fry and fingerlings. Approved chemical treatments, antibiotics, and vaccines increase egg, fry, and fingerling survival. In this chapter, we summarize some of these advances in knowledge about largemouth bass nursery and feed training and simultaneously point out those areas where improvements are still needed.

6.2 Nursery phase

The nursery phase is the most vulnerable time in production as largemouth bass have relatively small larvae that do not accept artificial diets at first feeding. Developing and sustaining desirable phytoplankton and zooplankton populations is essential for high survival and fast growth of largemouth bass fry in nursery ponds, because these fish rely completely on naturally occurring foods (Kurten, 1995). Largemouth bass fry are small to medium size (6–7 mm TL at swim-up) for a warmwater, freshwater fish, compared to 10–12 mm TL for channel catfish and 2–6 mm TL for sunshine bass (*Morone chrysops* × *M. saxatilis*) fry (Ludwig, 1999). Fry size at first feeding determines what size zooplankton are required, which is the basis for species-specific nursery pond management. Larval stages are critical times for fish survival because of higher predation and starvation risks (Hubbs and Blaxter, 1986; Dabrowski, 1989; Timmerman et al., 2000). Ready access to a suitable food source is especially critical to larval fish survival, since they have a relatively short time to feed before succumbing to an energy deficit incurred from unsuccessful attempts to capture food (Laurence, 1969).

Production methods during the nursery phase are now designed to produce 25–50 mm TL fingerlings, which can be successfully habituated to commercial diets. Purely pond-based nursery culture generally has been limited to smaller fingerlings, because when fish near 50 mm TL zooplankton abundance becomes insufficient (grazing pressure plus seasonal changes) and cannibalism increases. Production procedures described by Piper et al. (1982) are still used today and are summarized in the following paragraphs.

Fry are stocked in fertilized nursery ponds at rates varying from 100,000–200,000/ha. Production rates for small largemouth bass in fertilized nursery ponds ranges from 34–168 kg/ha depending on fish size, nursery pond productivity, and natural food availability. Some producers have successfully feed-trained largemouth bass fingerlings ≤ 25 mm, which increases acceptable stocking densities and potentially decreases required time in nursery ponds, but also increases time required to feed train (Matthews, 2009). If a fingerling size > 50 mm is desired, the fry stocking density should be reduced proportionally.

Bass fry numbers are estimated in various ways. One method used at the Peter W. Pfeifer Fish Hatchery in Frankfort, Kentucky, consists of counting a known number (usually 1,000) into a "dish pan" containing water and then placing fry in other dish pans until the densities appear to be equal. This method allows fry to be transferred in water using small pitchers or cups. Largemouth bass fry can also be enumerated volumetrically (Swanson, 1982) or gravimetrically. Lyon et al. (2002) reported an average weight of 0.0036 g (275 fry/g) and an average length of 7 mm at swim-up for Florida largemouth bass (FLMB). A prepared pond should be completely stocked in one day to ensure early growth uniformity and to reduce cannibalism.

The amount of time required for bass fry to grow to a target size depends mainly on stocking density, water temperature, food supply, and initial stocking size. Normally, 30–45 d are required at 18–24°C. Survival rates are usually 75–90%. Largemouth bass must be harvested once zoo-plankton are depleted as bass become very cannibalistic and many fish can be lost in just a couple of days (Heidinger, 2000a). Typical yields from stocking 80,000 fry/0.4 ha earthen pond are 48,000, 25–40 mm fingerlings after a 25–35 d growout period in central Florida (Matthews and Stout, 2013) and stocking ~200,000 fry/0.4 ha lined pond are 132,000, 25–42 mm fingerlings after a 27–38 d growth period (Matthews, 2015).

Figure 6.1 Grading feed-trained largemouth bass with a floating adjustable box grader.

Variable growth among bass fingerlings is common, but if size difference become too great cannibalism can cause heavy losses. If this occurs, the pond must be harvested, and fingerlings size graded and separated into size groups (Figure 6.1). It is therefore important to stock equal-sized largemouth bass, ideally cohorts within 2–3 d of swim-up. Size variation among cohabitating predator species often results in smaller fish becoming prey (Cooper, 1937; Wright, 1970; Snow, 1971). Cooper (1937) found cannibalism among age 0 largemouth bass in ponds occurred when fish lengths differed by a factor of 1.6. Factors encouraging size differences, such as stocking densities disproportional to prey availability, and size variation at stocking, accentuate differences in survival probabilities for faster and slower growing individuals.

Herbicide application to nursery ponds is sometimes used to prevent fingerling entrapment within filamentous algae mats during pond draining and harvest. However, avoid herbicide use if possible, as applications typically increase production costs and reduce dissolved oxygen and zooplankton densities (Summerfelt et al., 1994). If herbicides are applied, use them near the end of production when fingerlings have more likely converted to consuming insect larvae (for example, chironomid) and negative effects may not be as significant (Fairchild, 1982; Piper et al., 1982; Parmley et al., 1986).

Largemouth bass fingerlings are cultured throughout the USA and across the world using vastly different water sources and culture facilities. As a result, many culture practices are site-specific, and universal management techniques have not been developed. Consequently, most culture practices for largemouth bass have developed from trial and error rather than experimental design. Heidinger (2000b) suggested each largemouth bass fingerling producer needs to develop a fertilization regime that works well for his or her own unit.

6.3 Pre-season pond management and selection

If using earthen ponds, proper management begins before water is added. With older impermeable ponds, it has been suggested to dry and disk pond bottoms during winter to promote aerobic breakdown of nutrient-rich sediments (Piper et al., 1982). Drying ponds in the fall has also been suggested to eliminate predaceous insects, fish, and diseases (Davis and Lock, 1997). Relatively new ponds with little organic material buildup are less likely to require drying and disking. Some nutrients are desirable for fingerling culture because they promote phytoplankton growth on which zooplankton graze, but an overabundance tends to produce growth of undesirable blue-green and filamentous algae. Additionally, by excess nutrient removal through oxidation, the pond manager has more control over fertilization timing and zooplankton succession.

Piper et al. (1982) suggested seeding the edges of ponds with ryegrass (9–11 kg/ha) if it is to remain dry for several months. Snow (1975) also recommended ryegrass or wheat be sowed in largemouth bass nursery ponds to produce a green cover crop before filling and stocking. This cover prevents pond dike erosion and provides an organic fertilizer source when the pond is flooded in spring (Davis and Lock, 1997). Grass should be cut and partially dried before pond filling, or rapid decay may deplete dissolved oxygen (Piper et al., 1982). Bowling et al. (1984) evaluated largemouth bass fingerling production in similarly fertilized ponds with and without a green ryegrass cover crop. Ponds with cover crop were dried, disked, and then seeded with 9.0 kg seed/ha in fall. At harvest, there were no differences in fingerling production or zooplankton numbers between ponds seeded with ryegrass and control ponds. The authors concluded the added expense of a cover crop was not justified. However, erosion control benefits may outweigh costs for hatcheries with erodible levees.

Agricultural lime application (1,120–3,363 kg/ha) during the fallow time may be necessary to improve buffering capacity of ponds with soft-water. Boyd (1990) recommended freshwater ponds be limed to enhance fertility where water supplies have a total alkalinity < 20 mg/l. Wyatt et al. (2000) compared using hydrated lime and agricultural lime in earthen largemouth bass rearing ponds. Three treatments were evaluated: agricultural lime broadcast at 30.5 kg/ha, hydrated lime broadcast at 30.5 kg/ha, and agricultural lime applied at 2,245 kg/ha to the corners of ponds. Mean water quality and fish production variables were statistically similar among treatments. Zooplankton densities were lower in the hydrated lime treatment compared to agricultural lime treatments, and the hydrated lime elevated pH for several days after application. The effects of hydrated lime use on fish production were unclear due to high within-treatment variations in fish survival. However, all three methods of liming successfully elevated pond water alkalinity from 12 mg/l (incoming) to > 20 mg/l.

Fish hatchery ponds are increasingly being lined with a variety of impervious materials, usually plastics. Lining ponds offers several advantages over earthen ponds. Seepage is reduced or eliminated, erosion is substantially reduced, and herbicide needs are largely eliminated. The need to dry and disk pond bottoms is eliminated, disinfection is easier and harvest is simplified. However, accumulated sediments in lined ponds require periodic removal to prevent difficulties at fingerling harvest. Although there are no side-by-side comparisons of lined vs. un-lined ponds, generally, lined ponds reduce fertilization requirements and produce more uniform results. Well-managed lined ponds can have exceptional yields of 32 mm bass fingerlings, as high as 450,000 fish/ha and 190 kg/ha (Smith et al., 2000).

Ponds to be fertilized should be carefully selected. Fertilization may be economically impractical if a pond is too large or too small. Do not fertilize muddy or turbid ponds with poor light penetration, those containing filamentous algae, or rooted macrophytes, or those with a history of low dissolved oxygen. Ponds which have a high water-exchange-rate or low water temperature from shading or spring source water may yield poorly relative to amount of fertilizer applied. Some largemouth bass fingerling producers do not use fertilizer in nursery ponds as off-cycle or rotational growout ponds provide enough fertility to support objectives. Most managers select ponds for fertilization based on past performance, overall condition, water color, and the absence of filamentous algae.

6.4 Nursery pond fertilization

Appropriate pond fertilization programs depend mainly on local conditions. The level of intensification depends on the level of hatchery modernization. Earthen ponds are inherently less predictable in response to fertilization than lined ponds and seem more vulnerable to predacious insects and nuisance organisms. Conversely, earthen ponds may be better buffered against water quality changes and nutrient pulses. Pond size and shape uniformity within a hatchery may dictate whether routine fertilization regimens can be applied to all ponds. Local costs and fertilizer availability may drive the choice of which to use.

Fertilization promotes fish production by increasing the quantity and quality of naturally occurring food organisms. Numerous factors affect fertilizer use, and responses are not predicable under all conditions. Physical and biological factors influencing appropriate fertilizer use are: pond depth, shoreline amount, water exchange rate, turbidity, water temperature, plant and animal types already present, and food habits of the cultured fish (Piper et al., 1982). Chemical factors to consider are water and bottom mud composition such as: nitrogen, phosphorus, pH, alkalinity, calcium, and magnesium (Boyd, 1979). Another factor to consider is pond age: older ponds are generally more fertile than new ponds (Mischke and Zimba, 2004). Application rates and frequencies vary depending on region and natural pond productivity, therefore, universal rates applicable to all hatcheries are difficult to recommend.

Where facilities, budget, and trained staff exists, fertilization program dependability can be enhanced by basing application rates on target nutrient levels and periodically monitoring and validating those levels (Anderson and Tave, 1993). In some cases, cost savings may be made by reducing or eliminating unnecessary fertilization or critical supplements may be added that dramatically improve production. Owing to the vast differences in natural pond fertility, research results should be considered with respect to local conditions, and results may only be applicable where they were observed.

6.4.1 Organic fertilizers

Organic fertilizers are widely used for fertilizing largemouth bass nursery ponds (Piper et al., 1982; Parmley et al., 1986; Young and Flickinger, 1989; Hutson, 1990). The rationale for organic fertilization is to stimulate autotrophic production by supplying nutrients for phytoplankton uptake and to stimulate heterotrophic production of bacteria, fungi, and invertebrates, which serve as food for zooplankton and fish (Geiger, 1983a; Boyd, 1990; Harding and Summerfelt, 1994).

Organic fertilizers may accelerate zooplankton production by supplying a direct food source for the zooplankton even before phytoplankton bloom development, and provide a carbon source for potentially carbon-limited phytoplankton and heterotrophic pathways (Snow, 1975; Piper et al., 1982; Boyd, 1990). Organic fertilizers have been recommended particularly to enhance zooplankton production in new or sterile ponds (Piper et al., 1982).

Simco et al. (1986) indicated an initial application of 1,500 kg/ha alfalfa hay produces a more immediate zooplankton response than inorganic fertilizer. The rationale being that zooplankton feed primarily on detritus while inorganic fertilizer must be incorporated into phytoplankton pathways before becoming available to zooplankton. Boyd (1990) recommended using organic fertilizers to reduce occurrences of high pH in ponds by providing a reserve of carbon dioxide for phytoplankton. Chironomid larvae are known to use organic matter in the pond substrate (Oliver, 1971); therefore, organic fertilizers may be especially important late in culture when largemouth bass shift to a diet dominated by insect larvae (Fairchild, 1982; Parmley et al., 1986).

Organic fertilizers used in largemouth bass fingerling ponds are typically locally available agricultural grains or by-products such as soybean meal, rice bran, cottonseed meal (CSM), alfalfa meal (AFM), chopped hay, or animal manures. Generally, choice of organic fertilizer is based on local availability, cost, and what has worked best previously. Organic fertilizer carbon, nitrogen, and phosphorus content and decay rates can vary considerably (Boyd, 1990; Anderson and Tave, 1993; Wedemeyer, 2001).

Barkoh (1996) conducted a side-by-side comparison of AFM and CSM in plastic-lined largemouth bass fingerling rearing ponds which were also fertilized with inorganic nitrogen and phosphorus. CSM provided better water quality and fish production in these ponds compared to AFM. These results agreed with similar findings for two fertilizers in palmetto bass *Morone saxatilis* × *M. chrysops* fingerling growout in plastic-lined ponds (Buurma et al., 1996). Additionally, CSM is generally cheaper than AFM.

Kurten et al. (1999) reported earthen largemouth bass nursery ponds fertilized with either CSM, rice bran, or AFM, produced similar fish yield, growth, survival, and zooplankton densities. In another trial, CSM and inorganic fertilizer, AFM and inorganic fertilizer, and inorganic fertilizer alone were compared, and no significant difference in fish production was found (Kurten et al., 1995). The authors hypothesized eliminating organic fertilizers may reduce production cost and improve oxygen levels without adversely affecting production.

A subsequent trial evaluated the effect of fertilizing earthen largemouth bass nursery ponds with either inorganic fertilizer alone or inorganic fertilizer plus CSM on plankton population dynamics, water quality dynamics, and fingerling production. Supplementing ponds with CSM enhanced largemouth bass fingerling production by increasing fish harvest weight, reducing pH, and increasing desirable zooplankton densities but also reduced overall oxygen (O_2) levels compared to ponds fertilized with only inorganic fertilizer (Kurten et al., 1999). This study also validated that CSM can provide a significant source of carbon dioxide and can ameliorate high pH. Fertilization cost was 260% higher with added CSM. The authors concluded higher production cost and potential low dissolved oxygen should be weighed against the gain in fish production when deciding whether to use organic fertilizer.

Organic fertilizers are seldom applied based on estimated nutrient composition, and application rates are commonly reported on weight per surface area (kg/ha) making comparisons of fertilizers and application rates difficult, especially between ponds of variable depth but similar surface area. However, some studies have calculated the nutrient composition of organic fertilizers and based applications on weight per volume. Barkoh et al. (2005) found best water quality, phytoplankton standing crop, and zooplankton densities are achieved when organic meal application rates were based on nitrogen content.

Barkoh et al. (1994) compared pond productivity and largemouth bass fingerling production in ponds fertilized with either chicken manure or plant meals (consisting of equal parts by weight of CSM, AFM, and wheat shorts). Liquid inorganic fertilizer (10–34–0; N–P_2O_5–K_2O) was also applied to all ponds at a rate and ratio of N: P = 470: 750 µg/l/week. Moist chicken manure (75% moisture) was applied at N: P = 219: 67 µg/l the first week, 158: 49 µg/l the second week, and 105: 32 µg/l/week in subsequent weeks. Plant meals were added at a weekly rate of N: P = 2,442: 149 µg/l/week for the first two weeks and at 1,221: 75 µg/l/week in subsequent weeks. Largemouth bass fingerling (mean size 51–55 mm) yield was significantly greater in plant meal fertilized ponds (133.8 kg/ha) compared to ponds fertilized with chicken manure (84 kg/ha). Ponds fertilized with plant meals had higher chlorophyll-*a* concentrations and lower dissolved oxygen concentrations than ponds fertilized with chicken manure. Phytoplankton standing crop was also consistently higher in plant meal fertilized ponds, likely from higher nitrogen content of the plant meal regime compared to chicken manure. Zooplankton densities were generally adequate (717–923 organisms/l) in all ponds and did not follow a consistent pattern over time. With no difference in zooplankton food base between treatments, the authors suggested that observed differences in yield may have been from differences in heterotrophic production. Food habit studies have established immature insects, such as chironomids and chaoborins, are important food items for post-larval largemouth bass (Hodson and Strawn, 1968; Noriega-Curtis, 1979; Parmley et al., 1986). Benthic invertebrate numbers were not measured in this study, although they may have contributed to reported yield differences.

Combining organic and inorganic fertilizers is a common practice, particularly in hatchery ponds where draining is frequent and time for suitable food supply development is limited. It has been reported a combination of organic meal and superphosphate in a 3: 1 ratio provides higher fish production than organic material alone (Piper et al., 1982). While costs associated with organic fertilizer application are higher, the high value of a largemouth bass crop justifies the expense. Simco et al. (1986) recommended a combination of organic and inorganic fertilizers in largemouth bass nursery ponds: begin fertilization 2–3 week before stocking by initially applying 168 kg/ha peanut hay; 10 d later, add 100 kg/ha 16–20–0 inorganic fertilizer followed by a second application 10 d later at 50 kg/ha. Kurten (2001a) reported high production rates of 32 mm TL largemouth bass fingerlings (400,000 fish/ha; 140 kg/ha) was achieved by an initial application of 57 kg/ha CSM, 0.08 mg P/l, and 0.3 mg N/l at pond filling (8 d before stocking fry) followed by a large fertilizer dose consisting of 227 kg/ha CSM, 0.16 mg P/l, and 0.6 mg N/l 2 d before stocking fry. Thereafter, ponds were fertilized twice weekly with 57 kg/ha CSM, 0.08 mg P/l and 0.3 mg/l N until 1 week before harvest.

Disadvantages of organic fertilizers are they are more expensive than inorganic fertilizers, may deplete oxygen during decomposition, may stimulate filamentous algae growth, and require more labor to apply (Piper et al., 1982; Culver et al., 1993; Middleton and Reeder, 2003). Owing to these concerns, there has been an increase in research emphasis and use of liquid inorganic fertilizers to supply nitrogen and phosphorus directly to phytoplankton (Boyd, 1979, 1990).

6.4.2 Inorganic fertilizers

Inorganic fertilizers are relatively inexpensive sources of nitrogen, phosphorus, and, sometimes, potassium that stimulate phytoplankton growth directly. Their contributions to ambient pond nutrient concentrations are easier to calculate and trace than organic fertilizers based on their known composition. Once in solution, inorganic nutrients stimulate phytoplankton growth and reproduction, which subsequently support populations of zooplankton (Piper et al., 1982). They may also accelerate organic material breakdown, including organic fertilizers (Boyd, 1990). Excessive use of inorganic fertilizers can lead to high pH and ammonia levels which may negatively impact fish survival (Boyd, 1990; Bergerhouse, 1994; Barkoh, 1996).

The most important limiting nutrients in freshwater are nitrogen and phosphorus, and typically aquatic algae contain a N: P mass ratio of 7: 1 (Wetzel, 2001). Absolute concentrations and ratios of nitrogen and phosphorus can influence phytoplankton succession and dominance patterns (Welch, 1980; Wetzel, 2001). This consideration should drive the choice of inorganic fertilizer and application rates. However, inorganic fertilization programs should be site specific, as soil and water chemical composition differs widely across different geographical areas.

Phosphorus is generally considered to be the most essential limiting element in freshwater pond fertilization for phytoplankton growth (Boyd, 1990). Phytoplankton growth can be limited at phosphorus concentrations < 20–35 μg P/l (Sommer, 1985; Wetzel, 2001). Phosphorus is an active chemical which cannot exist alone except under very specialized conditions (Boyd, 1990). Most applied phosphorus eventually collects in sediments in the form of insoluble compounds that become unavailable to the water column. Calcium may precipitate phosphorus in hard water, resulting in greater daily decline rates (Boyd, 1990). Phosphoric acid has been recommended as the best phosphorus source for pond fertilization (Anderson and Tave, 1993) because of its high solubility.

Phosphorus fertilizers are commonly used to stimulate phytoplankton production in large-mouth bass nursery ponds (Parmley et al., 1986; Young and Flickinger, 1989; Kurten, 1995). Applying the appropriate phosphorus regime to your culture strategy can be challenging. Too little phosphorus will not sustain adequate phytoplankton production and limits zooplankton production (Kurten, 1995). Adding too much phosphorus can allow high levels to be discharged into receiving waters during pond drainage or can promote excessive blooms of unwanted blue-green and filamentous algae (Seymour, 1980; Culver et al., 1993).

Snow (1970a) suggested fertilization of largemouth bass nursery ponds, after filling, at 8 kg/ha superphosphate (35 μg P/l for an average pond depth of 1 m) and 9 kg/ha nitrogen at weekly intervals until abundant zooplankton is established, typically requiring three or four applications. After the zooplankton bloom becomes established, Snow (1970a) recommended biweekly fertilizer applications to sustain the bloom until about 10 d before anticipated harvest. Several workers have recommended applications of 1 mg/l phosphorous pentoxide (P_2O_5; 0.22 mg P/l) periodically during production (Piper et al., 1982; Geiger, 1983a, 1983b).

Parmley et al. (1986) used 1 mg P_2O_5/l (0.22 mg P/l) two to three times weekly for largemouth bass fingerling production. Young and Flickinger (1989) reported applying 1.0 mg H_3PO_4/l (0.32 mg P/l) three times weekly pre-stocking and twice weekly post-stocking to largemouth bass nursery ponds. Hutson (1990) recommended phosphorus application rates for largemouth bass nursery ponds of 1.00 mg P/l (from phosphoric acid) for initial fertilization and 0.33 mg P/l three times weekly for follow-up applications. Culver et al. (1993) and Anderson and Tave (1993) recommended lower phosphorus fertilization rates, suggesting 30 μg P/l as a target concentration for phosphorus, to avoid high pH and problems with excessive filamentous algae.

Culver et al. (1993) reported increased production and decreased production costs by eliminating organic fertilizers and relying solely on inorganic fertilizer in percid production ponds. This work was revolutionary in that the authors suggested applying fertilizer on an as needed basis according to chemical analysis of nutrient concentration. Although phosphorus is generally the most limiting nutrient in ponds, Mischke and Zimba (2004) reported ponds in the Yazoo-Mississippi River flood plain were more nitrogen limited than phosphorus limited. They recommended applying inorganic fertilizer at an initial rate of 20 kg/ha N and 2 kg/ha P (0.5 mg N/l and 0.05 mg P/l assuming a pond depth of 1 m) followed by subsequent weekly applications of one-half the initial rate for 3–4 weeks in nursery ponds for channel catfish.

Boyd (1990) recommended applying 0.5–1.0 mg N/l in ponds with low nitrogen concentrations to stimulate phytoplankton growth and to reapply at this rate at weekly intervals to sustain the bloom. If the hatchery pond is 1 m deep, apply nitrogen at 2.8–11.8 kg/ha (0.28–1.18 mg N/l) (Boyd, 1990). Using a typical N: P phytoplankton mass ratio of 7: 1 (Wetzel, 2001) and a minimum algal requirement of 35 μg P/l (Sommer, 1985) supports a rate of 245 μg N/l as an appropriate target for nitrogen maintenance concentrations in fertilized ponds to stimulate algal productivity. These rates are lower in nitrogen than the weekly restoration targets 600 μg N/l and 30 μg/l phosphorus suggested by Culver et al. (1993) for percid fingerling growout ponds. Urea has been recommended as the best nitrogen source (Anderson and Tave, 1993; Boyd and Daniels, 1994).

Kurten (1995) presented a case study where phosphoric acid (1.0 mg P/l) and ammonium nitrate (0.5 mg N/l) were applied weekly to largemouth bass spawning/rearing ponds over several years. An excess of filamentous algae (*Hydrodictyon* and *Anabaena* spp.) was a recurrent problem in these ponds and hindered fry harvest. While monitoring nutrient levels in spawning and rearing ponds, unexpectedly high phosphorus levels were found. Five weeks after initial fertilization, phosphorus concentrations up to 4.0 mg P/l were measured. Filamentous algae also appeared 4–5 week after initial fertilization following the clear-water phase associated with increased zooplankton densities and grazing (Parmley and Geiger, 1985; Lampert et al., 1986). Kurten (1995) reported phosphorus concentrations in those ponds rarely declined < 0.5 mg P/l between applications. The following year, weekly phosphorus application rates were reduced to 0.5 mg P/l and applied only when individual pond concentrations fell below 0.25 mg P/l. This change alleviated filamentous algae problems although fry production was low.

The following year, an experiment was conducted to determine if lower phosphorus concentration would prevent filamentous algae problems without reducing phytoplankton and zooplankton populations and subsequent largemouth bass fingerling production. Target phosphorus concentrations of 0.5 and 1.0 mg P/l were maintained based on weekly additions following water quality analysis. All ponds received the same nitrogen and organic fertilization regime. Both treatments provided adequate phosphorus for phytoplankton and zooplankton development and were low enough to avoid excessive filamentous algae growth. Total lengths, weight, and number of fingerling largemouth bass at harvest were similar between the two treatments. However, zooplankton succession differed. Phytoplankton biomass and zooplankton densities increased more rapidly and reached higher maxima in high phosphorus ponds, although they were sustained longer in low phosphorus ponds. In high phosphorus ponds, initial rotifer and cladoceran blooms rapidly overgrazed phytoplankton and limited food for latter copepod populations. These results suggest, in this particular situation, the lower phosphorus application rate was beneficial from the provision of a more stable zooplankton population over the longer culture periods typically used for largemouth bass fingerling production.

As most waters have an ample potassium supply, it is generally considered to be less important than nitrogen or phosphorus for plankton growth. However, in waters deficient, or where heavy fertilization with N and P is employed, it may be beneficial to add potassium. The ratio of 4–4–1, N_2–P_2O_5–K_2O, has been reported to produce favorable plankton growth for fingerling production ponds (Piper et al., 1982). The fertilizer grade most commonly used providing this ratio is 20–20–5.

6.4.3 Timing of filling ponds

The size and quantity of zooplankton available must be appropriate to the size and quantity of bass fry present, necessitating precise timing of pond filling and fertilization (Bishop, 1968; Simco et al., 1986). Filling time likely differentiates management strategies used for culturing different species of warmwater fish more than any other variable. Filling time directly affects zooplankton succession patterns in rearing-ponds. This is important because prey size largely determines consumption by fish (Geiger, 1983a).

Previous work has shown that phytoplankton and zooplankton abundance in ponds declines sharply 4–5 weeks after filling (Geiger et al., 1985; Culver, 1988). Zooplankton egg-to-egg generation times, and time to peak reproduction, are therefore important given the short time to maximize zooplankton populations in rearing ponds (Geiger, 1983b). Rotifers have a short eggto-egg generation time of 2–3 d, while cladocerans and copepods have egg-to-egg generation times of 7 d and 14 d, respectively (Allan, 1976). At 20°C, time of peak reproduction is 3.5 d for rotifers, 14–15 d for cladocerans, and about 24 d for copepods (Allan, 1976). Accordingly, if copepod nauplii abundance is desired at stocking, it may be beneficial to fill and fertilize ponds 14–20 d in advance.

Recommended time intervals between filling and stocking for largemouth bass nursery ponds vary widely. Parmley et al. (1986), based on a detailed evaluation of largemouth bass food habits over 33 d, suggested stocking fry 8–14 d following filling to provide appropriate zooplankton succession in terms of prey type, size, and overall abundance. Heidinger (2000a) suggested large-mouth bass nursery ponds should be filled with water 10–20 d before stocking. Davis and Lock (1997) suggested ponds should be filled no more than 14 d before stocking to reduce predaceous insect build up. As there are many variables affecting fertilization response and zooplankton population progression beyond filling time, this too should be a site-based management decision considering all available information.

Filling time also depends on the water source, as surface waters from reservoirs or streams usually contain nutrients and may contain zooplankton; thus, allowing ponds to be filled closer to stocking compared to ponds filled with well water (Brewer and Rees, 1990). Filling time also depends on management strategy for dealing with filamentous algae and predaceous insects. Fairy shrimp (Anostraca) and predaceous insects can become troublesome soon after ponds are filled. Fairy shrimp compete with desirable crustacean zooplankton for available food resources and predaceous insects may feed directly on larval fish (Geiger, 1983a). Filling ponds immediately before stocking allows less time for establishment of unwanted organisms but also does not allow time for desirable zooplankton populations to develop properly. If possible, filtering incoming water through a filter sock can be an effective method of excluding unwanted and predaceous insects.

Some producers fill ponds slowly, a process known as "puddle culture," to more quickly establish a bloom. The concept being water temperatures may increase faster and sunlight can penetrate more completely through the water column in shallow water. Barkoh et al. (1994) evaluated pond volume manipulation effects in producing largemouth bass fingerlings. Stage-filled ponds filled to one-third volume the first week, to two-third volume the second week, and then completely filled the fourth week were compared to ponds fully filled initially. Largemouth bass fry (0.0025 g; 7 mm TL) were stocked at 88,462–94,392 fry/ha at 9–13 d after initiation of pond filling. During the first 18 d, temperature and dissolved oxygen in stage-filled ponds were 22–24℃ and 5.0–8.0 mg/l, respectively compared to 19–20℃ and 2.3–5.0 mg/l in full ponds. Yield (mean size 53–55 mm) was significantly greater in stage filled ponds (133.8 kg/ha) compared to full ponds (99.0 kg/ha). Additionally, since fertilization rates were based on water volume, stage filled ponds reduced the fertilizer cost per kilogram largemouth bass by 50%. With puddle culture, it is important to watch upcoming weather patterns, as unseasonably cold weather could cause shallow ponds to become colder than full ponds.

6.4.4 Monitoring and managing zooplankton densities

Availability of zooplankton in sufficient abundance and appropriate size is necessary for successful nursery culture of largemouth bass fingerlings. This can be challenging considering the dynamic nature of the sequential changes in feeding habits of the fish and prey availability during nursery culture. Fortunately, much of this timing is predetermined based on water temperature, as fish spawning temperatures coincide with natural cyclic increases in prey availability. Also, as zooplankton population dynamics typically follow predictable succession patterns, species composition can be manipulated with management practices tailored to the nutritional needs and preferences of the cultured species.

Several authors have indicated rotifers are target prey for largemouth bass fry for only a short time (Applegate et al., 1967; Parmley et al., 1986; Wickstrom and Applegate, 1989). Wickstrom and Applegate (1989) reported largemouth bass swim-up fry initially consume rotifers, switching to copepods and cladocerans soon thereafter. Copepod nauplii are reported to be major prey items for first-feeding largemouth bass fry (Parmley et al., 1986). At 2 weeks post swim-up, moderate cladoceran concentrations of > 100/l are required to sustain good numbers of largemouth bass fry. Cladoceran densities typically increase quickly, attain their maximum density, and then decline rapidly over only a few days (Parmley and Geiger, 1985; Kurten, 1995).

When fish predation is sufficient to control zooplankton populations, rotifers usually displace cladocerans over time (Geiger, 1983a). Bass fry then switch to larger invertebrates (for example, insect larvae; Morris and Clayton, 2009). Insect larvae become increasingly important in small-mouth bass *Micropterus dolomieu* diets once they reach 20 mm TL (Farquhar and Guest, 1991). This is likely also the case for largemouth bass fry (Parmley et al., 1986; Kurten, 2001b).

Parmley et al. (1986) described feeding habits of largemouth bass fry as they grew from an initial size of 5 mm to 25 mm TL in nursery ponds and provide a detailed examination of the relationship between composition and abundance of available crustacean zooplankton prey and predation by young hatchery reared largemouth bass. Fish 4.5–5.5 mm TL collected from nest sites contained no food items in their guts. No rotifers were found in fish gut contents at any sampling even though they were present in high numbers in ponds. Fish began feeding at a mean TL of 6.7 mm and exhibited positive selection for copepod nauplii. Sequentially, selection shifted to copepod adults for 7.5–10.5 mm fish, to adult copepods and cladocerans for 10.9–20.4 mm fish, and lastly, to predominantly immature insects for fish > 22.8 mm. Although selection values were negative for 7.5–10.5 mm fish feeding on cladocerans, 30% more cladocerans than copepod adults were consumed. Copepods may provide more energy on a dry weight basis than cladocerans, but copepods are also known to be faster moving and better able to avoid attack (O'Brien, 1979; Parmley et al., 1986). Young bass fry are opportunistic sight-based strike-feeders and are likely better adapted to feeding on slower constantly moving cladocerans than swifter, intermittently moving copepods (O'Brien, 1979; Parmley et al., 1986).

Elliot (1976) examined stomach contents of a school of largemouth bass fry (25.3 mm; mean weight 0.18 g) over 24 h. Fry fed continuously on limnetic zooplankton throughout the day and did not feed at night. Although rotifers and copepod nauplii were more abundant than any of the other prey groups, they were not consumed. Daphnia and calanoid copepods were the most abundant and were eaten nearly proportional to their abundance. The fry fed selectively on the largest prey (*Leptodora* spp. and cyclopoids) and selected against the smallest (*Bosmina* spp.). Largemouth bass fry appear to be opportunistic predators feeding predominantly on the most available prey of a suitable size, yet actively select larger, more visible prey. Based on feeding habit studies, it appears first feeding largemouth bass fry prefer copepod nauplii for about 1 week, then transition to copepod adults and cladocerans for the next 2–3 weeks. By week 4, they should be 25 mm, and insect larvae such as chironomids become more important.

Kurten (2001a) examined the relationships between fingerling harvest variables and fingerling stocking variables and zooplankton densities for 197 largemouth bass rearing ponds over a 6 year period with a production goal of 38 mm fingerlings. Harvest biomass and harvest length appeared to be improved by lengthening the interval between pond filling and fish stocking. Interestingly, zooplankton densities at stocking and 7 d after stocking were weak indicators of harvest variables. Low correlation of fish production and zooplankton variables and the indication that lengthening the pond filling to stocking interval improves production and size indicate managing for larger pond invertebrates may improve fish production above managing for zooplankton alone (Kurten, 2001a).

Successful culture of planktivorous fry depends heavily on zooplankton composition and density established in rearing ponds during the 4–6 week culture period (Geiger, 1983a). Fish number and age affect the zooplankton number needed for successful culture. In general, 100–500 zooplankton/l of suitable size and species should be present throughout culture (Geiger and Turner, 1990). Generally, planktivorous fish preferentially consume the largest zooplankton they can capture and ingest. This creates a succession pattern where there is an increase in the proportion of smaller zooplankton over time as larger zooplankton are selectively removed. An increase in numbers of smaller zooplankton usually indicates predation pressure by fish is too great, and it is time to harvest (Morris and Mischke, 1999). Further fertilization is usually not warranted by this point.

The culturist should periodically check zooplankton population densities in fertilized nursery ponds. A practical method for visually assessing zooplankton densities is to examine ponds at night using a spot light where a trained eye can observe and estimate density, type and can distinguish the presence of undesirables, such as clam and fairy shrimp. Traditional plankton nets with detachable collecting containers are often used for collecting plankton from ponds to determine species composition (Hoff and Snell, 2004). Sampling zooplankton populations using towable nets provides good qualitative data, but it may be difficult to estimate density (number of zooplankton per liter).

Techniques showing promise for quantitative analyses are using pumps and tube-samplers (Morris and Mischke, 1999). Farquhar and Geiger (1984) described a pump system and Graves and Morrow (1988) evaluated a simple tube sampler and reported good quantitative estimates. Pump systems are more elaborate and therefore more expensive. Tube samplers can be made of 25–50 mm polyvinyl chloride pipe equipped with a check valve on the bottom end of the pipe. The tube is then lowered into the water column and either filtered through a plankton net or collected in a bucket for further sampling, enumeration, and identification of zooplankton taxa. Consistency in sampling is important to obtaining good quantitative samples. As zooplankton may not be uniformly distributed throughout the pond, it is important to obtain samples from several locations (Morris and Mischke, 1999). Zooplankton are known to migrate vertically within the water column throughout the day, so data should be collected and compared over time (Morris and Mischke, 1999).

Hatcheries frequently inoculate or supplement largemouth bass rearing ponds with zooplankton from dedicated, heavily fertilized zooplankton ponds. This strategy is based on the belief this practice will enhance zooplankton populations or provide significant amounts of additional food. Martinez et al. (2005) confirmed this practice enhanced the initial and later zooplankton density in largemouth bass ponds. However, the expense and time required to inoculate ponds was not warranted, because fish production was not significantly enhanced.

6.4.5 Clam shrimp and fairy shrimp

Clam shrimp (Conchostraca) and fairy shrimp can reduce fish production in large-mouth bass nursery ponds. Fairy shrimp are known to compete with desirable crustacean zooplankton for available food resources thus limiting zooplankton production (Geiger, 1983b). Large numbers of clam shrimp can also greatly reduce zooplankton densities and impede fish harvest by clogging screens and suspending sediment (McCraren et al., 1977; Czarnezki et al., 1994). High clam shrimp densities cause excessive turbidity, which interferes with photosyn-thesis (McCraren and Phillips, 1977). Clam shrimp and fairy shrimp can be controlled with chemicals, such as Dylox; however, these chemicals are not approved as pesticides in ponds and also kill *Cladocera* (McCraren and Phillips, 1977) which are important food for largemouth bass fry.

Fairy shrimp and clam shrimp characteristically inhabit temporary ponds and pools and are most plentiful in spring and early summer (Pennak, 1978; Czarnezki et al., 1994). Drying and freezing are reported as a stimulus for hatching their eggs in spring (Weaver, 1943; Czarnezki et al., 1994). Czarnezki et al. (1994) successfully controlled clam shrimp in walleye nursery ponds by refilling ponds either immediately after harvest or during summer and keeping ponds full through winter, thus interrupting the clam shrimp reproductive cycle. However, the authors cautioned this practice may reduce zooplankton numbers, as winter drying also promotes hatching of zooplankton eggs (Pennak, 1978; Geiger, 1983a).

Kurten et al. (1996, 1997, 1998) examined several strategies for controlling clam shrimp and fairy shrimp in largemouth bass rearing ponds. In the first study, ponds were filled and fertilized either 7 or 14 d before stocking fish. Shortening the pre-stocking interval from 14 to 7 d allowed largemouth bass fingerlings to prey on and reduce densities of smaller clam and fairy shrimp, but did not eliminate them completely and did not improve fish production (Kurten et al., 1996). In a second study, ponds were flooded at 28 d and 14 d before stocking fish and fertilized at 14 d before stocking (Kurten et al., 1997). The 14 d pre-fertilization interval (incubated ponds) allowed shrimp to complete their life cycle, and shrimp were nearly absent by fish stocking. Zooplankton densities were higher, and shrimp were absent in incubated ponds at fish harvest which alleviated enumeration and screen clogging problems, but fish production was not improved. In a third study, the pre-fertilization interval was reduced to 7 d and was not superior to the 14 d pre-fertilization interval (Kurten et al., 1998). The authors concluded filling ponds and allowing the clam and fairy shrimp to complete their life cycle before pond fertilization improved ability to harvest and enumerate largemouth bass, and such a strategy was not detrimental to production.

6.5 Feed training

As in the propagation of other widely cultured species an evolution to more intensive culture methods for largemouth bass has slowly occurred. Demand for largemouth bass has stimulated the development of intensive production practices at both private and public hatcheries. Primary obstacles to intensive production of piscivorous fish are cannibalism and the failure of fry and fingerlings to voluntarily accept commercially available formulated feeds. To overcome this problem, feed training techniques have been developed over time that have become standard practice in largemouth bass production.

Prior to the 1960s, state and federal hatchery programs stocked fingerling largemouth bass (25–50 mm) directly from nursery ponds into managed lakes and reservoirs for stock enhancement (Snow, 1965). Small fish were used due to poor acceptance of formulated feeds and increased cannibalism of cohorts in pond reared populations larger than 50 mm. Subsequently, in the 1970s, fisheries managers determined that survival of stocked largemouth bass fingerlings was low when stocking small fish and requested hatchery managers produce larger fingerlings (100 mm) for stocking programs (Snow, 1975).

The transition to stocking larger bass fingerlings was to improve sportfish recruitment based on the supposition that larger fish would be less vulnerable to predation and would benefit from the ability to utilize larger prey items. However, the food preferences of largemouth bass > 50 mm shifts from primarily insects to small fish, requiring the use of forage fish which greatly increased production costs (Snow, 1971). The desire to produce larger largemouth bass fingerlings for stock enhancement programs prompted a considerable amount of research effort on production techniques to produce bigger (≥ 100 mm) largemouth bass cost effectively (Snow and Maxwell, 1970; Snow, 1973). One of the primary areas of research was developing technologies to improve success during the feed-training phase of largemouth bass production.

Early researchers wrote “how to” papers to achieve the crucial step in getting fish to recognize and accept non-living food items (Snow, 1968a; Snow, 1970a; Snow and Maxwell, 1970). Weaning fry from live feeds to dry diets is one of the most expensive processes with farming predatory fishes (Curnow et al., 2006). The costs associated with this process are mainly associated with labor, the high prices of live food, and mortality during habituation (Baskerville-Bridges and Kling, 2000; Callan et al., 2003). Seed stock costs are a relatively large percentage of operating cost and minimizing mortality is an important goal for increasing farming profitability (Fletcher et al., 2007). Engle and Southworth (2008) estimated that the cost of raising 100 mm largemouth bass fingerlings on forage results in a more than 100-fold increase in the cost per fingerling compared to feed-training and raising them on feed. Production efficiency is also greatly improved by feed-training largemouth bass fingerlings compared to raising them on forage fish. For example, it is possible to produce up to 123 kg/ha of largemouth bass when they are pond-reared together with forage fish (Snow, 1970b; Sloane and Lovshin, 1995). However, when trained to accept dry, pelleted commercial feeds, largemouth bass productivity ranges from 2,700 to 7,281 kg/ha in a 117 to 153 growing period (Kubitza, 1995; Sloane and Lovshin, 1995).

Owing to increased production efficiency, feed-training largemouth bass fingerlings has become a standard practice among hatchery managers with goals of producing fish > 50 mm. However, the procurement cost of feed-trained largemouth bass fingerlings for commercial food-fish producers remains relatively high (approximately US$0.25 per each 50 mm fish; personal communication, Robert Mayer, Mayer's Fish Farm, Bardstown, Kentucky), suggesting productivity and success during feed-training likely have a major impact on production costs of fingerlings.

6.5.1 Feed training diets

Feed consumption is influenced by several factors including: flavor, taste, and smell. Many natural ingredients, such as meals, hydrolysates, and oils, derived from fish and crustaceans are highly palatable and can increase feeding response and feed intake (Barrows, 2000). Several studies have described and tested different initial food items, such as using ground fish, fish eggs, moist pellets, and freeze-dried krill (FDK) for feed training largemouth bass fingerlings (Snow, 1965; Anderson, 1974; Willis and Flickinger, 1981; Brandenburg et al., 1979; Kubitza and Lovshin, 1997a, 1997b).

Krill (*Euphausia* sp.) are a group of oceanic planktonic crustaceans having commercial fishery value primarily for use as food for aquaculture. There are two primary species of commercial importance: the Antarctic krill (*E. superba*) and the Northern Pacific krill (*E. pacifica*). The latter is smaller and better sized for use as a feed training diet for largemouth bass. Sloane (1993) was one of the first to utilize FDK as a training diet for largemouth bass; however, only 13% of the fish were successfully transitioned to the dry diet (feed trained). Kubitza and Lovshin (1997a) reported 27% feed training success when using only FDK to transition largemouth fingerlings to a dry diet. In another experiment, Kubitza and Lovshin (1997b) reported 77% success rate when they incorporated FDK into specially formulated training diets using "gradual feed ingredient transition."

Primarily because of the effectiveness and ease of use, FDK has become one of the most popular feed training diets for largemouth bass. However, the cost of FDK (≥ 40 $US/kg; Argent Chemical Laboratories, Redmond, Washington) can be a significant expense in the feed training phase. Engle and Southworth (2008) evaluated the costs of raising largemouth bass fingerlings and determined that the cost of krill was the greatest cost (77% of total costs) followed by labor for the feed training phase of production. Campbell and Phelps (2002) evaluated the use of dried shrimp (*Penaeus* sp.) to replace krill as a feed training diet for largemouth bass. The motivation for this work was that FDK was considerably more expensive than dried shrimp. The authors reported that the feed training success was 81% for fish fed FDK and 78% for those fed dried shrimp. The authors concluded that replacing FDK with dried shrimp reduced the cost of feed training diets by 82%.

Kubitza et al. (1997) compared the feed training success of largemouth bass fed with ground fish and reported percent feeders was 75% when ground fish was the starter diet compared to 41% for FDK. An unpublished study (Coyle and Tidwell), evaluated ground fathead minnows (*Pimephales promelas*) as an alternative to FDK for feed training largemouth bass. The minnows were ground in a blender and used to top coat and soften 1.5 mm floating pellets for one hour prior to feeding. The relative proportion of ground minnows to dry feed was reduced by 25% every three days from the initial 20% by wet weight ground minnows to 80% dry diet. This technique was compared to using FDK in decreasing proportions as described by Kubitza and Lovshin (1997a). Feed training success was greater for the fish fed the pellets top coated with minnows (75%) than for those receiving the krill (41%).

Skudlarek et al. (2007) evaluated the modification of a commercial dry pellet to see if its acceptance as an initial training diet could be improved. In the control (CTL), fingerlings were initially fed FDK then gradually transitioned to a 1.5 mm commercial diet (Steelhead; Skretting USA, Murray, Utah, USA). For days 1–3, fish were fed only FDK (10% initial body weight/day [BW/d] divided equally between three feedings), for days 4–6 they were fed a mixed ration containing 75% FDK and 25% commercial diet (75: 25 ration), for days 7–9 a 50: 50 ration, for days 10–12 a 25: 75 ration, and then for days 13–18 they were fed only the commercial diet. In the second treatment (DRY), only the dry commercial pellet was fed throughout the experiment. In Treatment 3 (MST), the commercial pellets were moistened with water prior to feeding. In Treatment 4 (OIL), pellets were top dressed with menhaden fish oil (Rangen Inc., Buhl, Idaho, USA), to soften the pellet and potentially increase palatability. The DRY treatment resulted in a decrease in feed training success (82%) compared to other treatments which averaged 95%. The MST and OIL treatments may be advantageous due to reduced feed cost. The OIL treatment may be preferable to the MST as it would reduce the likelihood of nutrients leaching out of the pellets during water softening. Previous studies showed that semi-moist or soft diets perform better than dry/hard diets during feed training largemouth bass (Snow, 1965; Lovshin and Rushing, 1989; Sloane, 1993).

FDK is one of the most popular training diets used for habituating largemouth bass to artificial diets. Several alternatives to FDK have proven successful as feed training stimulants for largemouth bass. Ground fish and dried shrimp were both found to be more effective than FDK. Use of a floating diet rather than a sinking pellet may be advantageous and softening or oil coating pellets appears to be beneficial. A summary of some studies evaluating feed training diets and protocols is presented in Table 6.1.

6.5.2 Other factors affecting success during feed training

Training success can be related to many variables such as initial fish size and condition, the acceptability of the training diet, the suitability of the training system, and the genetic potential of the particular strain (Williamson, 1983). The size of fingerlings at the onset of feed-training is known to be an important factor in successful-feed training (Snow, 1965). Largemouth bass fry are approximately 3–4 mm TL at hatch and 5–6 mm TL at swim-up. The size of the fry harvested from nursery ponds for feed-training is dependent on the food availability and duration of the nursery period. Most producers feed-train largemouth bass fingerlings at sizes ranging from 25 to 50 mm when feed-training is more successful.

However, sometimes it may be advantageous to wean fry onto artificial diets as early as possible and may be necessary to harvest nursery ponds before bass fry reach sizes > 25 mm due to natural food depletion or other management considerations. Early reports documented attempts to feed-train smaller bass fingerlings using common carp, (*Cyprinus carpio*) eggs. Brandenburg et al. (1979) cultured 20 mm largemouth bass on common carp eggs with a mean training success of 52%. In another report (Willis and Flickinger, 1981), largemouth bass fry > 10 mm were trained to accept carp eggs with a success rates of 49–97% which increased as the initial fry size increased from 10 to 30 mm. An initial size of 10 mm was determined to be the minimum threshold for successful training to carp eggs, likely due to the inability of smaller fish to ingest the 1.0–1.3 mm diameter eggs. In an unpublished study (Coyle and Tidwell), a feed training trial evaluated two size groups of pond-reared largemouth bass fingerlings harvested from the same nursery pond and size-graded into two groups with initial weights of 0.9 g (21 mm) and 1.4 g (28 mm). The percent training success was greater for the larger size fish (88%) compared to the smaller group (59%).

Table 6.1 Select studies showing different training diets and protocols for feed training largemouth bass fingerlings.

Citation	Initial size	Water temp. (°C)	Stocking density	Training diet	Times fed per day	Disease treatment	Feed trained (%)
Campbell & Phelps (2002)	40 mm (1.0 g)	22	3.5 g/l	Dried shrimp	5	Prophylactic; 2 mg/l potassium permanganate daily for the first 7 days	78.0
Willis & Flickinger (1981)	20 mm	22	13 fish/l	Carp eggs	12	None	79.0
Williamson (1983)	0.45 g	23	7.0 g/l	Biodiet	Cont. for 6 h/d (15% SB/d)	Flexibacter columnaris; 5 mg/l copper sulfate once daily for a week	43.1
Williamson & Carmichael (1990)	0.27 g	23	3.3 g/l	Biodiet	Cont. for 12 h/d (20% SB/d)	None reported	96.0
Brandenburg et al. (1979)	20 mm	27	4 fish/l	Carp eggs	4 (15% BW/d)	None reported	52.0
Kubitza & Lovshin (1997a, 1997b)	1.4 g			FDK			58.0

Notes: SB, stocked biomass of fish; BW/d, body weight per day; FDK, freeze-dried krill.

Kubitza and Lovshin (1997b) compared the feed training success of two genetic strains and different initial sizes of largemouth bass. Both strains were northern × Florida hybrids. Fish of 0.2, 0.4, 0.6, 0.9, and 1.4 g initial weight were fed using gradual feed ingredient transition (GFIT) with diets containing 80, 60, 40, 20, and 0% ground fish or 70, 50, 30, 10, and 0% krill meal. Feed-training success of the two strains was similar. Average percent feeders were 58% and 53% starting bass on FDK and ground fish, respectively. Percent feeders increased from 7% to 52% as initial fish weight increased from 0.2 to 1.4 g for fish started on FDK. Percent feeders increased from 59% to 88% as initial fish weight increased from 0.9 to 1.4 g among fish started on ground fish. In this study the feed-training diet and the initial size of the fish had more to do with success than the strain; however, domestication is thought to be important to feed-training success and is discussed later in this chapter. As a rule of thumb, pond-reared fingerlings should be a minimum of 20 mm TL or around 1 g to optimize feed-training success.

Williamson and Carmichael (1990) evaluated two wild (undomesticated) strains of FLMB and northern largemouth bass (NLMB) subspecies and first-generation hybrids under intensive conditions to determine their respective suitability for commercial aquaculture. Feed-training trials used a semi-moist diet fed via electronically controlled automatic feeders set to deliver approximately 20% of the fish biomass daily over 12 h at 15 min intervals. The northern strain had the highest percent feed-trained (96%), with the hybrids intermediate (91%) and worst performance in the Florida strain (80%). In addition, the northern strain grew faster, converted food to biomass more efficiently, resisted net-stress better and tolerated high ammonia, low temperature and low oxygen better. The northern strain largemouth bass is generally considered to be the better subspecies for commercial food-fish production in the USA.

Willis and Flickinger (1981) conducted a series of trials to evaluate factors influencing feed-training success in largemouth bass and made several important observations. The authors reported improved feed-training success with red rearing containers over white containers and reported that partially covering the containers greatly improved feed-training success. Improved success in the covered tanks was thought to be due to the lower light intensities of 6–12 lux compared to uncovered tanks (62 lux). Deeper tanks were found to be preferable over shallow tanks and rectangular tanks were better than round tanks in these trials. It was suggested that because largemouth bass do not eat food from the tank bottom the deeper tanks increased the time that food remained in the water column as the training diets were sinking pellets. The initial size of pond reared fry ranged from 9–30 mm, with improvements in feed-training success as the size increased to 20 mm. Training success was generally > 90% (on carp eggs) for fry > 20 mm.

Most early published reports used sinking pellets (Biodiet™ or Oregon Moist Pellet) during feed-training of largemouth bass because that was all that was commercially available at the time.

Manufacturing methods used to produce fish feeds have improved greatly over the past 50 years. Most fish feed manufactures now offer extruded floating feeds in sizes as small as 0.8–1.2 mm where previously high protein floating feeds for carnivorous species were limited to sizes ≥ 2.5 mm. Young largemouth bass generally only strike at pellets on the surface or as they slowly sink, and do not readily eat pellets on the tank bottom (McCraren, 1975; Flickinger et al., 1975; Willis and Flickinger, 1981). Kubitza and Lovshin (1997a) indicated that the tendency to float was one of the positive attributes of FDK as a training diet. Skudlarek et al. (2007) reported feed training success of 97% using FDK as the initial training diet and transitioning to a commercial 1.5 mm floating diet. Fish were observed to ingest and reject pellets many times before they would consume them. Floating pellets remain accessible for an extended time and may increase the success of feed-training largemouth bass.

Temperature is the single most pervasive environmental factor for poikilothermic animals (Stickney, 1979). Temperature significantly affects the growth and body composition of fish by controlling feed consumption and food retention time (Coutant, 1975). In a study performed by Tidwell et al. (2003), it was shown that higher culture temperatures (26 and 30℃) significantly increased growth and production of juvenile largemouth bass compared to those reared at 20℃. However, feed training is typically practiced at ambient pond and/or well water temperatures of < 24℃. The ground water temperature for the Mississippi River valley alluvial aquifer in eastern Arkansas, one of the major commercial production areas in the USA for juvenile largemouth bass, averages 22.1℃. Water temperature is important during the feed training phase as it influences appetite and starvation time.

Coyle et al. (2009) evaluated the effect of water temperatures on feed training success and growth of largemouth bass juveniles. Bass fingerlings (2 g) were stocked at 700 fish/m^3. Three water temperatures were evaluated (20, 24, and 28℃). Fingerlings were initially fed FDK then gradually transitioned to a 1.5 mm commercial floating pellet containing 45% protein and 12% fat according to a schedule modified by Kubitza and Lovshin (1997a). For days 1–3, fish were fed only FDK, for days 4–6 they were fed 75% FDK and 25% commercial diet (75: 25 ratio), for days 7–9 a 50: 50 ratio, for days 10–12 a 25: 75 ratio, and then for days 13–24 they were fed only the commercial diet.

One week of receiving only the formulated diet was long enough to ensure that feeders and non-feeders could be easily differentiated. Initially bass were fed a daily ration of 10% initial BW/d, divided into two equal feedings at 0900 and 1600 hours. On day 8, owing to an increase in feed consumption, the daily ration was increased to 15% initial BW/d in all treatments.

Average weight and feed trained percentage increased with increasing water temperature. The percentage of fish successfully feed trained averaged 70, 82, and 90% for the 21, 24, and 28°C treatments, respectively. Individual weights averaged 5.4, 6.8, and 7.8 g for fish feed trained at 21, 24, and 28°C, respectively. The results of this experiment indicate that water temperature has a significant impact on feed training success and growth rate of largemouth bass fingerlings during the feed training phase. Given that increasing water temperature increases the energy demand for maintenance and activity of fish (Weatherley and Gill, 1987), at some point further increases would not be productive. It is unknown if higher temperatures than those evaluated in this study would be beneficial or detrimental during feed training largemouth bass. Training success in this study was 70–90%, which is higher than many published reports, but is comparable to previous feed training experiments at this laboratory using the same strain, similar size fish and a floating pellet as the training diet (Skudlarek et al., 2007). According to Heidinger (2000b), most established producers of largemouth bass are approximately 75% successful in training pond reared fingerlings to feed on a prepared diet. It is not known if the increased percentage of feed trained fingerlings that could be produced by increasing water temperatures would justify the increased cost associated with heating water.

6.5.3 Feed training improvements over time may be related to domestication

Reported feed-training success of largemouth bass in the published literature is highly variable, ranging from 13–90% (Lovshin and Rushing, 1989; Sloane, 1993; Kubitza and Lovshin, 1997b; Skudlarek et al., 2007). Many early publications (1970–1990) reported feed-training success of only 25–50% whereas today most private and public hatcheries achieve 70–90%. These improvements are partially due to the development of better technologies through applied research, but non-systematic selection and domestication have also likely contributed.

Little research has been conducted on the genetic aspects of largemouth bass culture traits. One possible explanation for the 90% feed-training success reported by Coyle et al. (2009) and Skudlarek et al. (2007) could be domestication. Snow (1965) reported that offspring from pellet-trained broodfish survived better under hatchery conditions. He recommended a "genetic approach" to developing a strain of largemouth bass more readily adapted to the controlled, intensive culture environment. Subsequently, Williamson (1981) reviewed several reports from researchers and federal fish hatcheries during the 1970s, indicating better survivals of largemouth bass fingerlings from feed-trained parents and suggested that adaptability to eat artificial food was a heritable trait. It was also suggested that first generation largemouth bass from the wild did not feed-train as readily as those reared under hatchery conditions for several generations.

Williamson (1983) compared the feed-training success of two genetic strains of largemouth bass from different National Fish Hatcheries in Texas. The San Marcos strain (San Marcos National Fish Hatchery, Texas) were descendants of the Marion strain (Marion National Fish Hatchery, Alabama) developed by J.R. Snow in 1966 from a single pair of broodfish in response to a request by the U.S. Bureau of Sport Fisheries and Wildlife for the development of a national reference strain. Offspring of the single mating were maintained as the only source of bass at the Marion hatchery and were the progeny used in Snow's pioneering work in intensive largemouth bass culture (Snow, 1968a, 1968b, 1968c, 1970a, 1970b, 1973, 1975; Snow and Maxwell, 1970). The ancestors of the pairing are thought to have originated from waters near the hatchery in 1950. From 1950 to 1966, Marion broodfish were selected based on appealing body conformation (short, deep bodies and small heads). The Marion strain was later determined to be from a naturally occurring intergrade or hybrid population of the NLMB and FLMB subspecies (Harvey et al., 1980). The second strain evaluated was the Inks Dam strain (Inks Dam National Fish Hatchery, Texas). Broodfish from the Inks Dam strain were of northern origin and had always been maintained on forage.

The fingerlings of both strains were pond reared to 0.48 and 0.45 g for the Inks Dam and San Marcos strains, respectively. Fingerlings were fed Biodiet (Bioproducts, Warrenton, Oregon, USA) continuously during daylight hours with automatic feeders at 15% of the initial fish bio-mass daily. The training success for the San Marcos strain was 43% and for the Inks Dam strain was 23%. This represents an 84% difference in feed training success between the two strains, supporting the hypothesis that genetics may play a major role in determining hatchery performance between different strains of largemouth bass.

An unpublished study (Coyle and Tidwell), compared feed-training success of two strains of largemouth bass, "wild" and "domestic," and found survival to be greater in the domestic strain (60%) than the wild strain (34%). Conversely, the average weights were greater for the wild strain (1.9 g) than the domestic strain (1.6 g) at the end of the training period (both groups averaged 1.1 g at stocking). The wild strain were sourced from Barren River reservoir in Kentucky and are known to be 100% northern subspecies *Micropterus salmoides salmoides*. The domestic strain were sourced from the Pfeiffer Fish Hatchery, Frankfort, Kentucky, and were the same strain as used in Coyle et al. (2009) and Skudlarek et al. (2007). The domestic strain was progeny from a line of largemouth bass broodfish that had been pellet-raised in Kentucky for over 15 generations and were hybrids of the original Marion strain (natural northern-Florida hybrid) and wild broodfish sourced from Kentucky waters (personal communication, Mike Larimore, Hatchery Manager, Pfeiffer Fish Hatchery, Frankfort, Kentucky). Although little is known about the selection process involved in creating the Pfeiffer line, it is apparent that approximately 40 years of non-systematic selection have occurred for attributes that allow for survival in a semi-intensive aquaculture environment. It seems likely that domestication may, at least in part, explain the high feed-training success in Coyle et al. (2009) and Skudlarek et al. (2007).

To further evaluate the effect of domestication on feed-training success in largemouth bass a second study was conducted to compare growth and survival of wild, domestic, and both hybrid crosses during the feed-training phases. The domestic strain was the same as described above having been raised on pelleted feeds for more than five generations. Wild largemouth bass brood-fish were collected from Barren River reservoir in Kentucky, which has been impounded over 60 years and has not received supplemental stocking. Broodfish from both strains were paired and pond-spawned. Swim-up fry from each group were transferred to fertilized nursery ponds for 6 weeks, then harvested by seine, and stocked into tanks for feed-training.

Fingerlings of similar size (average individual weight 0.9 g) were stocked in 190 l tanks at a rate of 150 fish per tank. Dechlorinated municipal water, maintained at 27°C, was supplied at 1.5 l/min to each tank. Each of the four genetic groups (Domestic♀ × Domestic♂, Domestic♀ × Wild♂, Wild♀ × Wild♂, and Wild♀ × Domestic♂) were randomly stocked into four replicate tanks. Fingerlings were fed twice daily at a rate of 10% of initial biomass per day. They were fed FDK for the first 3 d, then gradually weaned to a floating commercial diet (45% protein, 16% fat), according to previously established procedures. After 21 d, all fish in each tank were removed, individually weighed, and measured for total length.

Final average individual weight (g) was significantly greater for the Wild♀ × Wild♂ strain (2.95 g) compared to other groups: Domestic♀ × Domestic♂ (2.70 g), Domestic♀ × Wild♂ (2.53 g), and Wild♀ × Domestic♂ (2.55 g). Percent survival was significantly lower for the Domestic♀ × Domestic♂ group (86.9%) compared to the other groups, which averaged 97–100%. Reduced survival in the Domestic♀ × Domestic♂ group was primarily due to *Aeromonas hydrophila* infection which was isolated.

The faster growth of the Wild × Wild fish supports results from a previous study that compared fish of Barren River origin with domestic fish from the same commercial strain. However, in that previous study survival of the wild strain fish was lower than in the Domestic strain fish, in contrast to the superior survival of wild strain fish in the present study. Previous studies have reported improvements in feed-training success in subsequent generations of feed-trained large-mouth bass. However, in the present study, feed-training success for the wild group was very high (99%). Based on these studies, the effect of non-selective domestication on feed training success is not clear. These data indicate it may be beneficial to evaluate the culture potential of various wild strains of largemouth bass geographically for selective breeding aimed at commercial aquaculture.

Domestication of largemouth bass may be beneficial for food-fish production but could be detrimental to genetic diversity of natural populations for stock enhancement programs (Lorenzen et al., 2012). The goal of domesticating bass for better and more consistent feed-training relates to food-fish production and generally not for fisheries management strategies.

6.5.3.1 Effect of diet on health

Low survival of pellet-reared Florida bass stocked in lakes (Porak et al., 2002) lead to the discovery of "pale liver syndrome" caused by inadequate nutrition in artificial diets used for production. The bass were fed commercial diets designed for trout and striped bass that contained unsuitable carbohydrate and fatty acid profiles leading to bass-glycogen storage disease and bass-fatty liver disease (Cardeilhac, 2009).

Seven years of testing a largemouth bass diet found that dietary carbohydrate content reduced to 12%, high protein content ~60% and reduction of dietary linoleic acid concentrations in a palatable dry pellet corrected "pale liver syndrome" and produced healthy 100 mm FLMB (Cardeilhac et al., 2004; Cardeilhac, 2009). The two diets resulting from this work are known as Diet 11 and Diet 12 or "Richloam bass diets" and are commercially available from Skretting USA, Tooele, Utah, USA. Richloam bass diets are milled once a year, typically in the fall.

Feed selection (brand) for feed training and growout needs to consider production purpose, intensity, water quality conditions, and longevity of "on feed" duration. Price is also a factor to consider but should be carefully weighed against final product health. Nutritional requirements should dictate feed selection, but once bass are feed trained they will eat many brands of feed. Csargo et al. (2013a) compared Otohime, Bio-Oregon, and Purina's Aquamax as initial feeds for feed training 3 g bass fingerlings. All three diets achieved > 90% survival, but the Otohime and Bio-Oregon feed produced significant increases in weight and condition factor (K).

The duration the bass remain on artificial feed and the nutrient content of the feed require consideration for best results. More research of long-term effects on differing feeds is needed. Florida bass reared on the Richloam bass diet for up to six months remained healthy, but the 3 mm maximum pellet size limited further usage (personal communication, Matthews).

6.5.3.2 Feed training in large-scale intensive culture systems

Small to moderate numbers of largemouth bass are feed trained annually at hatcheries producing warmwater sport-fish and several of the previously proposed feed training protocols involve hand feeding in combination with small battery-operated belt feeders. Feed training several hundred thousand bass fingerlings in large modern hatcheries requires a modified approach to be successful using minimal labor. It is important to start with a known number of robust, healthy fingerlings that are of similar length. *Artemia* (brine shrimp, a freshwater invertebrate similar in size to the zooplankton found in nursery ponds) are used to assist in transitioning bass feeding on microscopic invertebrates to artificial diets.

During feed training, bass fingerlings should be fed 24 h a day, and uneaten feed must be removed regularly to ensure water quality remains adequate. Successful feed training starts with the correct stocking density, ranging from 11 to 16 fingerlings/l of water. By nature, bass are very aggressive and survival depends of how successful they are at capturing prey. By stocking training tanks at high densities, competition helps stimulate bass to take prepared diets. Besides density, temperature can impact feed training success with optimal temperatures ≥ 22°C. Bass not transitioning to the artificial feed will get weak and aggregate to the surface and sides of the tank eventually moving to the back of the tank and clogging the outlet screens. These fish should be manually removed as they are vectors for bacterial diseases such as *Flavobacterium columnare*.

Typical success of FLMB subjected to feed training using the following protocol is about 60% and ranged from 35 to 97% over 8 years and more than 70 trials (Matthews, unpublished data). The fingerlings in these trials were all F1 generation spawned from wild caught FLMB broodfish. Higher success rates are expected in hatchery produced broodfish specifically selected for feed trainability for the use in food-fish propagation. Although larger fish (35 mm) commonly have greater training success than smaller fish, fish as small as 25 mm can be successfully trained. The following protocol can be modified to fit site specific conditions and is given to serve as a guide that has worked successfully in an intensive culture hatchery. The time frames listed are flexible and the progression and acceptance of feed items is more important to monitor as no two groups of fish train alike.

Before pond harvest

1. Start one to two heated vats of *Artemia* (45 g/vat) 24 to 36 h prior to harvest. Need to be ready when the bass are stocked into the feed training tank.
2. Set up feed training schedule for the next 14 d. Bass need to be fed every 30 min for the first to 4 d (longer if smaller than 0.45 g or if the water is consistently less than 20°C).
3. Make sure all equipment; vacuum hoses, feed cart, buckets, small plastic beakers, graduated cylinders, mixing spoons and screen/feeder brush are ready.

Feed training

1. The bass start out on *Artemia* and a diet of Otohime C-1 (Marubeni Nisshin Feed Co., Tokyo, Japan). Amount depends on the number of fish and volume in the tank. There are ~275,000 cysts per gram. At 85% hatch a 15 l vat yields 701,000 *Artemia*/l. Sample counts (*Artemia*/ml) of each batch of *Artemia* is recommended. An *Artemia* hatching vat stirred by aeration filled with 15 l of water and 45 g of *Artemia* cysts feeds 75,000 bass fry for 2 d depending on tank volume. The larger the tank, the more *Artemia* required. If bass are held at an 11 to 16 fish/l density, calculate and add enough *Artemia* to the tank to reach > 100 *Artemia*/l. *Artemia* incubated at 26.7°C hatch in 18 to 24 h.
2. Otohime C-1 is fed every half-hour and *Artemia* every hour. The *Artemia* are mixed in with the feed every hour for the first 48 h. Feed only Otohime every hour on day 3 and 4. Bass should be given 10% of their BW/d. This allows maximum exposure to the feed as it slowly falls to the bottom of the tank at which time the bass lose interest. After 4 d start mixing in 1 mm Richloam bass diet (Skretting USA, Tooele, Utah, USA). The ratio of these feeds and duration used is dependent on initial fish size and to a lesser degree water temp. Larger fish (> 2 g) need 6 to 8 d to feed train while smaller bass (< 2 g) need to stay on the C-1 feed longer requiring 8–10 d. Temperatures below 20°C may extend these intervals. Start mixing with 75% C-1 and 25% Diet 11. Slowly start decreasing the amount of C-1 and increasing the amount of Diet 11 until a 0% C-1 and 100% Diet 11 ratio is reached.
3. The bass need to be hand fed for the first 6 to 8 d (preferably for the first 14 d) or until all fish are actively feeding. The feed needs to be placed "in front of the fish's nose" by taking a small amount of feed in a plastic beaker mix it with 1 l of water and "slurry feed" the corners, sides, middle, and in front of the screen if fish have their nose in the screen.
4. After the first 24 h, the feed can be offered dry (not slurried in water) in the corners, sides, middle, and in front of the screen. It is best to slurry one time then dry feed the next.

5. The bass need to be fed every 60 min during the transition from Otohime to their final diet.
6. Once the bass are actively feeding the feed can be placed in an automatic feeder. Still hand feed several times a day to observe the fish.
7. The goal is to get the bass on the 1 mm bass diet as soon as possible without sacrificing feed training percentage. This protocol typically yields a 60% feed training percentage.

Gut contents should be examined under a dissecting scope 40 h from the start of feed training. Length and weight should be recorded as well. Gut contents should be examined every 24 h from this point. Size somewhat dictates this but usually at 112 h full guts can be found in 80% or more of the surviving bass. *Artemia* and small amounts of food are usually found as early as 40 h from the onset of feeding. It is best to measure gut contents 10 min after a good feeding. Feed trained bass get thick from head to tail and can easily be distinguished from non-feed trained bass. Once trained and actively feeding, spread the bass out (decrease density) and feed them often. Growth rates of 1 to 2 mm/d are possible if water quality remains acceptable. The Richloam Diet is over 60% digestible protein, so ammonia can spike to unacceptable levels quickly if left unchecked (Csargo et al., 2013b).

6.5.3.3 Intensive indoor production of largemouth bass swim-up fry on artificial feed

The intensification of larval rearing techniques for largemouth bass has been identified as a priority area of research for commercialization of the species (Dupree and Huner, 1984). Developing technologies for intensive fry production, such as controlled indoor spawning, egg incubation, and fry rearing, could improve the reliability of bass fingerling production. Out of season spawning in indoor raceways allows for large-scale fry production (Matthews and Stout, 2013) and recent research at Kentucky State University was successful in developing a protocol for raising bass fry entirely indoors, eliminating the need for nursery ponds, by habituating largemouth bass fry to dry diets at smaller sizes than previously considered to be possible (Skudlarek et al., 2013). When combined, these technologies could enable largemouth bass fingerling production to occur at any time of the year.

In pond-based fingerling production systems, largemouth bass swim-up fry normally begin to feed on naturally occurring zooplankton in ponds. In intensive indoor hatchery systems, there is no nursery pond phase and bass fry are fed newly hatched brine shrimp nauplii (*Artemia* sp.) at the time of swim-up. Preliminary trials indicated that first feeding largemouth bass fry are large enough to consume *Artemia* nauplii, thus eliminating the need to culture live rotifers, as is the case with striped bass *Morone saxatilis* (Webster and Lovell, 1990). Live brine shrimp nauplii (*Artemia* sp.) comprise approximately 85% or more of the prepared live foods fed to larval fish worldwide (Sorgeloos, 1980). Determination of the proper combination of live food and larval diets for the first 2–3 weeks after hatch allows the nursery pond stage to be eliminated, thereby increasing control and predictability. This combination has proven effective in the other freshwater predators such as striped bass (Webster and Lovell, 1990) and walleye *Stizostedion vitreum* (Nickum, 1986).

A series of experiments were conducted to investigate live foods, natural foods, prepared diets, and optimal feeding schedules required to raise largemouth bass fry from first feeding until fully habituated to a commercial dry diet (Skudlarek et al., 2013). In each experiment, large-mouth bass eggs were hatched under controlled conditions and swim-up fry were transferred to a recirculating system and stocked at 50 fry/l into either 3 l (Trials 1 and 2) or 10 l (Trial 3) acrylic aquaria. Trial 1 was a preliminary trial to screen six candidate diets to evaluate whether largemouth bass fry could be transitioned directly to prepared diets or if they required live foods. In Trial 2 the necessary duration for feeding live *Artemia* (1, 2, or 3 weeks) and the appropriate size of commercial diets (> 200 or 200–360 μm) were determined. Based on the results of Trials 1 and 2, largemouth bass fry in Trial 3 were fed *Artemia* for two weeks then transitioned either to a commercial marine finfish starter (Otohime; 200–360 μm), decapsulated *Artemia* cysts (Decap), a commercial trout starter, or freeze-dried copepods for one week. Fish were then fed a commercial trout starter for 2 weeks.

In Trial 1, Otohime and decapsulated *Artemia* cysts performed better than other diets tested. However, survivals even in these treatments were relatively low (6–8%). In Trial 2, fry fed *Artemia* for 2 weeks then transitioned to diet sizes of 200–360 μm performed better than other combinations tested. In Trial 3, survival was significantly better for fry fed Decap and Otohime. Average individual weight was significantly higher for fry fed Decap and trout starter. Use of decapsulated *Artemia* or Otohime fry diet as transitional feeds produced the best combination of high survival and average weight. These data indicate that live food is likely necessary for the first two weeks followed by a gradual transition to either decapsulated *Artemia* cysts or Otohime (200–360 μm) for one week then transition to trout starter. This protocol yielded survival rates of approximately 70% and may have significant potential for improving the reliability of largemouth bass fingerling production.

In Skudlarek et al. (2013), *Artemia* were fed to excess and optimal bass stocking and prey densities were not determined. Subsequently two independent feeding trials were conducted to determine optimal fry and *Artemia* densities for the first 2 weeks of largemouth bass larviculture. In the first trial, largemouth bass swim-up fry (9 mg) were stocked in a factorial arrangement at densities of 20, 40, and 80 fry/l and *Artemia* nauplii feed rates of 5, 10, and 20 nauplii/ml were administered twice daily. The results for Trial 1 indicated that after 14 days the lowest fish densities and the highest *Artemia* densities (20 fry/l fed 20 *Artemia*/ml and 40 fry/l fed 20 *Artemia*/ml) resulted in higher average weights (39 and 40 mg, respectively). There were no statistical differences in survival between treatments, which averaged 51–83%.

Based on these results, Trial 2 evaluated lower fish densities and higher *Artemia* densities. The second trial evaluated fry stocking densities of 10, 20, and 40 fry/l and *Artemia* nauplii feed rates of 20, 30, and 40 nauplii/ml administered twice daily. Results for Trial 2 again indicated that the lowest fish densities and the highest *Artemia* densities (10 fry/l fed 30 *Artemia*/ml, 10 fry/l fed 40 *Artemia*/ml, and 20 fry/l fed 40 *Artemia*/ml) resulted in significantly higher average weights (40, 43, and 40 mg, respectively). There were no significant differences in survival, which averaged 81–94%. Based on these data, fry stocking densities of 10–20/l in combination with *Artemia* feed rates of 20–40 nauplii/ml twice daily appear suitable for rearing large-mouth bass fry for the first two weeks of feeding. This protocol yielded survivals of 60–80% in experimental units.

The last trial in this series (Coyle and Tidwell, unpublished data) evaluated two strains of large-mouth bass for their adaptability to these intensive protocols. The two strains were followed from hatch through to the transition to 1.5 mm floating pelleted feeds at which point they would be considered commercially marketable fingerlings to food-fish producers. The domestic strain was the one cultivated by fish farmers in the region. Wild largemouth bass broodfish were collected from Barren River reservoir, Kentucky, USA. Broodfish from both strains were pond-spawned in separate outdoor ponds provided with Spawntex™ spawning mats. Fertilized eggs from the two strains were collected on mats from outdoor tanks on the same day, transferred indoors and incubated in separate 380 l tanks maintained at 26°C and supplied with dechlorinated municipal water in a flow-through system. Swim-up fry were fed live *Artemia* nauplii twice daily for two weeks and then restocked into eight separate 150 l tanks with four replicate tanks per strain. Fry were then fed decapsulated *Artemia* cysts for one week, transitioned to a commercial trout starter (no. 0) for 2 weeks, then fed the same formulation in a no. 1 crumble (1 week), and finally transitioned to a 1.5 mm floating trout diet (1 week). At this point, all fish in each tank were removed, counted, and individually weighted to determine survival and average weight.

After 5 weeks of receiving only dry feed, survival of the domestic strain (60%) was higher than the wild strain (40%). However, the wild strain (1.9 g) had higher average individual weight than the domestic (1.6 g). In general, the results of this study show that the described technique for feeding largemouth bass swim-up fry may have potential for scale up. Once habituated to trout starter (400 μm) and fed for 2 weeks, fry appeared to retain this learned feeding behavior and are easily transitioned to first a no. 1 crumble or a 0.8 mm floating pellet (1 week), and finally transitioned to a 1.5 mm floating diet (1 week).

A commercial scale trial of this protocol was conducted which identified some constraints to scale-up. Current bottleneck issues appear to be synchronization of the spawn needed to maintain appropriate fry densities in larger tanks. Labor associated with waste removal (siphoning) is demanding because of the need to feed to excess, particularly when transitioning to fry starter. The lack of a commercially available fish grader of the appropriate size (< 8/64") is also a problem as size variability inevitably leads to reduced survival due to cannibalism. These constraints appear to be researchable questions. Further development may lead to the intensive commercial production of feed-trained largemouth bass juveniles entirely under controlled conditions.

6.5.3.4 Disease control

Stress or injury to largemouth bass fingerlings during pond harvest, transportation, size grading, and routine husbandry practices, such as tank cleaning, can result in mortality during feed-training. In addition to directly causing mortality, stressors caused by poor handling combined with the stress associated with the feed-training process can result in disease outbreaks. Also, the better the condition the fingerlings are in, the more successfully they will adapt to formulated feeds and intensive culture conditions. Poor condition at nursery pond harvest can result from declining food availability or overpopulated ponds. Fingerlings in poor condition seldom have the energy reserves needed to tolerate harvest stresses, and adapt to a new culture environment, and transition to a different food source.

The most prevalent diseases during the feed-training processes in largemouth bass are columnaris disease and hemorrhagic septicemia caused by the bacteria *Flavobacterium columnare* and *Aeromonas hydrophila*, respectively. These bacteria are ubiquitous and are considered opportunistic pathogens meaning that they occur at increased frequency when one or more stressors occur. These can include environmental changes, behavioral or physiological stressors or changes in water temperature. Additionally, because of high fish densities and high nutrient loads, fungal infections (*Saprolegnia* sp.) and parasitic protozoans such as *Trichodina* sp. and *Ichthyophthirius multiphiliis* can cause disease-related mortality in largemouth bass during feed-training.

In the past, a number of compounds were used to alleviate stress, prophylactically prevent, or therapeutically treat disease in largemouth bass fingerlings. However, the use of many of these compounds is no longer permitted. Accordingly, refer to Chapter 10 in this book that discusses disease and chemotherapeutic use in largemouth bass. Disease outbreaks can be minimized by implementing least-stress harvesting and husbandry procedures, combined with prophylactic static salt baths (0.7% for 4 h) administered after unavoidable stressful procedures such as weighing and measuring (Malison and Held, 1996).

Lange (2003) conducted a series of experiments evaluating preventative treatments to reduce the mortality associated with columnaris disease during feed-training largemouth bass. The three treatments evaluated were a one-time attenuated immersion vaccine for *F. columnare* (VAC; Intervet/Schering-Plough, Keniworth, New Jersey, USA), a 20 mg/l Oxytetracycline bath (OTC bath) for 10 min every 3 d or feeding Oxytetracycline top-coated on the training diets at a rate of 5.5 g active ingredient (AI) per 100 kg fish (OTC fed). A low level of columnaris disease occurred during feed-training, and survivals ranged from 87.1 to 96.6% with no differences between the treatments. Following feed-training, a *F. columnare* challenge was performed where fish were intentionally exposed to live cultures. Following the 20 d challenge, survivals were—OTC bath treated fish (92%) > OTC fed fish (72%) > VAC fish (55%) > control fish (38%).

Bass intensively reared in indoor raceways require grading to keep size ranges small to avoid predation-induced disease outbreaks. This additional handing can also increase the risk of disease. Bebak et al. (2009) followed Florida bass fry 7–9 d post-hatch immersion vaccinated with AQUAVAC-

COL (Intervet/Schering-Plough, Kenilworth, New Jersey, USA), reared in nursery ponds for ~33 d, feed trained in 4,914 l raceways, and reared for an additional 44 d in cohabitation with sham-vaccinated bass had significantly less mortality from *F. columnare* than the sham-vaccinated fish. However, producers will need to weigh the cost of the vaccine against possible additional fish production and labor costs.

Chemical bath treatments for fingerlings may also be effective. Bowker et al. (2013) reported results from four separate chloramine-T (20 mg/l) trials and three separate hydrogen peroxide (150 mg/l and two at 50 mg/l) trials on bass and Bluegill (*Lepomis macrochirus*). Both chemicals were effective in controlling *F. columnare* disease when used as 60 min bath treatments on three consecutive or alternate days. Identifying and beginning treatment early greatly increases the chances of survival. No withdrawal period is required with either of these chemicals.

In large volume tanks, it may be necessary to use an antibiotic feed additive to treat *F. columnare*. Matthews et al. (2013) reported Aquaflor (florfenicol, 50%) top-coated feed at 10 mg/kg of fish body weight effectively controlled columnaris disease in both feed-trained bass and bluegill (10 d treatment). The use of Aquaflor requires a Veterinarian Feed Directive (VFD) which can be obtain through a licensed vet. There is a 15 d withdraw period after treatment meaning fish require 15 d post-treatment before they can be processed, sold live for food, or stocked in public waters.

6.5.3.5 Cannibalism and size grading

Cannibalism has been reported in many species of cultured fish (Nishimura and Hoshino, 1999). Cannibalism can cause significant losses especially during intensive culture of carnivorous fishes and can be affected by several factors including fish age and size uniformity, food availability and stocking density (Baras and Jobling, 2002). Not all fish start to exhibit cannibalism at the same size nor is cannibalism equally intense in different species or life stages. The impact of intracohort cannibalism is dictated by the time when it emerges. Intracohort cannibalism in largemouth bass has been reported to begin as early as 9 d after swim-up (Johnson and Post, 1996).

As discussed earlier, in largemouth bass there is a natural dietary conversion from consuming invertebrates to consuming fish that occurs sometime in their first year usually at around 40 mm TL (Olson, 1996). It is during this natural transition that feed training typically occurs. It is also during this transition that cannibalism can become pronounced. Increased size variability between cohorts is a normal occurrence, due to differential growth among individuals within the population (Jensen, 1990). Early cannibalism may promote growth heterogeneity and accentuate size variability because digestible nutrients are often higher in fish than in plankton prey (Kubitza and Lovshin, 1997b). Due in part to their increased tendency for cannibalism at sizes > 40 mm; largemouth bass nursery ponds are typically harvested at this size and the fish are brought into the hatchery for feed training.

Predator to prey size ratios vary substantially between fish species and life stages. Cannibalism is limited by mouth gape size and allometric growth of mouth parts. It is well known that piscivorous fish are size selective predators and seek out as large a prey item as they can efficiently consume (Werner and Hall, 1974; Mittlebach, 1981; Hambright, 1991). This size selectivity is based on the optimal allocation of time spent searching for and handling food items related to energy return. This allows them to take in the maximum nutrition with least expenditure of energy in feeding. As the name implies, largemouth bass are capable of consuming large food items relative to their size. Largemouth bass swallow prey whole, therefore mouth diameter (gape) limits prey size. Johnson and Post (1996) used morphological measurements to predict a threshold predator-prey size relationship. According to these calculations largemouth bass can swallow prey items with a length approximately 60% of their total length.

Timmerman et al. (2000) evaluated factors limiting prey size swallowed by juvenile large-mouth bass (> 31 mm TL) and determined there was a large individual variation in gape size which may contribute to improved feeding success and growth potential for some cohorts within a population which may contribute to eventual cannibalism. Empirical evidence suggests that largemouth bass juvenile populations should have no more than a 20% variability in TL to reduce the occurrence of cannibalism (Timmerman et al., 2000).

Size variability is common among many species of same-age farmed fish and cannibalistic species need to be mechanically size graded more frequently to maintain survival rates (Kelly and Heikes, 2013). Fish graders consist of smooth steel bars equally spaced to allow a portion of the population to pass through while retaining the larger fraction. Several commercial graders are available, although many producers make their own graders to fit their specific needs. Mixed sizes of fish may require grading through more than one size of grader.

The two types of graders that are most commonly used for grading largemouth bass in tanks prior to and during the feed training phase are floating box graders and panel or drag graders. Box graders are designed to float in a tank. The frame typically holds interchangeable inserts made of 4.8 mm aluminum bars with 0.4 mm spacing between the bars. Fish are placed into the floating box grader where large fish are retained and smaller fish pass down through the grader. The process can be repeated with a smaller size grader. Panel graders are often used in vats or trough type race-way tanks and consist of evenly spaced bars welded or bolted to a rectangular frame with rubber flanges attached to the outside of the panels to allow them to fit tightly against the tank walls. Like box graders the larger fish are retained as the panel is pulled from one end of the tank to the other. Panel graders typically require less handling of the fish and cause less stress than box graders.

A series of sizes of graders are necessary as pond-reared nursery-phase largemouth bass populations may consist of several size groupings of fish possibly from several spawns. It is also important to size grade again after the feed training phase to ensure size uniformity before stocking back out into ponds for first year growth and again after harvest from first year ponds prior to stocking second year ponds for food-fish production. Table 6.2 provides bar grader sizes for different length largemouth bass modified from

Kelly and Heikes (2013). These should be considered estimates as individual populations of fish may be different as passing through the grader depends on the individual condition or body width of the fish not the length. Prior to and after feed training most food-fish producers grade off the largest 1–2% and either discard them as cannibals or use them for recreational sales. Next, they typically attempt to separate the remaining population into two groups, the larger fraction and the smaller, by experimenting with different size grader bars.

Table 6.2 Bar grader size to retain largemouth bass fingerlings of different total length.

Bar width in inches	Size retained in inches (cm)
10/64-11/64	1.00(2.5)
12/64-13/64	1.50(3.75)
14/64-15/64	2.00 (5.0)
18/64	2.75 (6.875)
21/64	3.00 (7.5)
44/64	6.00 (15.0)
60/64	8.00 (20.0)

Factors other than size variability are also likely to affect cannibalism in largemouth bass but have not been fully studied. In better studied species, it has been determined that cannibalism can be triggered or enhanced by a wide range of biotic and abiotic factors that influence food requirements and access. The influence of genetics on cannibalism is somewhat controversial. One idea is that some fish may have a greater genetic propensity toward cannibalism (‘natural born killer’ hypothesis), and the other that cannibals are the winners of an intense initial competition (‘lottery winner’ hypothesis) (Baras and Jobling, 2002).

It has been shown that some strains of fish are more prone to cannibalism than others of the same species and that domestication may reduce or exacerbate the condition depending upon the selection process criteria (Hecht and Pienaar, 1993). Selection for or against cannibalism in largemouth bass may be based on the ultimate goals of the producers. Food-fish producers may desire uniform growth with little competition and discard cannibals when found, whereas producers supplying managed trophy fisheries may wish to select for the most aggressive fish within a population.

Cannibalism can also be influenced by population density. Generally, under conditions where food is adequately provided, increased stocking densities result in a lower incidence of dominance hierarchies and reduced cannibalism in predatory fish (Petit et al., 2001). However, factors that induce changes in spatial distribution, such as water flow and light intensity, may affect cannibalism by increasing the chances of encounter between cannibals and prey. Also, environmental factors that increase feed intake in largemouth bass such as increased water temperature and 24 h light may have an effect (Petit et al., 2001). Any restriction of food availability may enhance cannibalism through both food shortage and the resulting increase in differential growth.

6.5.3.6 Post-feed training

After the 25–50 mm largemouth bass fingerlings are trained to accept a prepared diet, they are size graded. This allows the separation of cannibals (large) and non-feeders (small). The uniform trained fish are then typically stocked back into ponds at first year growout densities of 33,000–44,000 fingerlings/ha. However, sometimes only 50% of the fingerlings will continue to feed on the prepared diet when stocked at these pond densities after only a 2 week feed-training period (Heidinger, 2000a). This problem is thought to be related to when fingerling producers use primarily automatic feeders during feed training. It does not seem to be as big of a problem when bass are trained by hand feeding. To ameliorate this problem, some producers confine fish to one area of the pond with a net or concentrate the fingerlings at much higher densities of 110,000–220,000/ha in small ponds, for a few weeks or until the fingerlings reach > 75 mm. During this period, the fingerlings are fed several times per day as reinforcement training. If well water or ample surface water to supply flow-through tanks is available, concentrating the feed-trained fingerlings indoors in tanks for an additional two weeks transition at densities of 1–3 fish/l provides the producer with better disease control options, such as daily formalin (25 ppm) and/or salt (5 ppt) treatments, and reduces handling stress when ultimately moving the fish back out into ponds at the reduced density for further growout.

During this phase, it is advisable to feed by hand only two or three times per day. Many commercial producers of feed-trained bass use automatic feeders to deliver small amounts of feed at frequent intervals throughout the daylight hours. This approach conditions the fish to respond to the equipment as their food stimulus and not directly with hatchery personnel, and frequent feeding in a small area allows a small number of more aggressive fish or “bullies” to patrol the feeding area. Hand feeding helps the fish to identify with people as their food providers and feeding less frequently with greater amounts of food spread more uniformly over the water surface promotes equal distribution of feeding opportunity. At this stage they should already be feed-trained; the goal here is to strengthen the learned behavior without promoting size disparity.

6.6 Conclusion

Meeting the challenges of intensifying largemouth bass culture will require further research into all facets of existing culture techniques for the species. Much can be learned by reviewing the techniques developed, investigated, and highlighted in this chapter; however, incorporating and applying these methods will depend largely on the level of hatchery design and infrastructure development. Aquaculture facilities with good water supplies, large raceways, many troughs and tanks, and control over environmental conditions can intensify production at every step along the way to producing market-size largemouth bass. Currently, the highest level of intensification consists of spawning broodfish in raceways, harvesting eggs and hatching them in troughs, stocking lined ponds with fry for fingerling production, and then transitioning fingerlings to commercial diets for growout to market or stocking-size. Aquaculturists should realize that each of these steps poses challenges and opportunities for optimization. Timing and understanding the environmental conditions and variability unique to each hatchery are crucial to developing a successful program. Managing natural food production and pond fertility will always be a significant aspect and challenge of production because fry do not readily take to artificial diets.

We suggest the following research priorities for intensifying largemouth bass production. These are provided in order of production sequence and with the previous caveat on hatchery site specificity. These questions plague all hatcheries, but their degree of importance must be prioritized individually. What is the optimum density of broodfish for spawning ponds and raceways to produce adequate numbers of high quality fry in the least amount of space? Are there suitable feed substitutes to managing natural food for initial fry and fingerling production? What are the optimum timing and fertility levels in ponds to best product the most desirable food organisms? What is the optimum and acceptable water quality for managing each life stage? How can nuisance organisms be managed effectively? What is the optimum production size for stock enhancement or the commercial market?

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Chapter 7
Culture methods

James H. Tidwell

Kentucky State University, Aquaculture Research Center, Frankfort, Kentucky, USA

7.1 First-year ponds

As noted previously, the primary focus of this book is a review of the culture of the large-mouth bass as a food-fish. However, food-fish culture evolved out of production of sport-fish for stocking. Because of this, production methods so far remain largely the same during the early stages. Largemouth bass require two growing seasons to reach food-fish target sizes (≥ 0.5 kg). Production of largemouth bass as food-fish can be broken up into five phases including a hatching phase, a nursery phase (when fingerlings feed on zooplankton), a feed-training phase, then first-year growth and second-year growth. The first phase (fry) is covered in Chapter 5. Nursery and feed-training are the subject of Chapter 6. This chapter will review production procedures after the fish are trained to accept artificial diets.

After the initial feed-training period, fingerlings are usually graded to ensure non-feeders are separated out and that fingerlings stocked together are as uniform in size as possible. This helps to reduce the likelihood of cannibalism and possibly improves overall growth, as larger fish can dominate feeding areas. Research has suggested that feed-trained fingerlings of 4–5 cm can be stocked into prepared ponds at 50,000–75,000/ha (Tidwell et al., 2000). Some producers add an intermediate step to “reinforce” feed-training during the transition from feed-training to tanks to ponds (Heidinger, 2000). One option for this is a short period (2–4 weeks) in small ponds (0.01 ha) at very high densities (250,000 fish/ha) with frequent feedings. Another method is to cordon off a section of the first-year production pond using a seine as a block-net. All of the fish are confined within this area for several weeks until actively feeding. Water quality, especially dissolved oxygen, must be closely monitored during these high-density periods.

Once fish are released into the open pond for first-year growth, they are fed high-protein, low-carbohydrate, floating diets two to three times per day (see Chapters 8 and 9). Fish are fed to apparent satiation at each feeding. Feed response will vary based on environmental changes such as sunlight, water temperature, and/or changes in other water quality variables. If feeding response is reduced for several consecutive days without obvious external factors such as weather changes, the producer should suspect and monitor for water quality problems and/or disease onset.

Fish spawned in the spring, feed-trained in tanks, then cultured in ponds should reach 15–20 cm by the fall of this first year. Fish that do not reach a minimum size of 20 cm can be marketed for sport-fish stocking at this stage and this is a significant market outlet. If fish are to be grown on to food-fish sizes (> 0.5 kg), a second growing season will be required.

Kubitza and Lovshin (1997) evaluated various stocking densities for the production of advanced juvenile (100–200 g) largemouth bass during the first summer growing season. large-mouth bass fingerlings were feed trained from May to June and stocked into ponds at various densities as they became available. The study evaluated a range of stocking densities from 17,000 fish/ha to 67,136 fish/ha. At harvest, average weights were generally higher (202–225 g) for largemouth bass fingerlings stocked at densities of 17,000 to 18,680 fish/ha. Medium densities of 20,533 to 34,124 fish/ha resulted in 184 to 189 g fish, while higher densities of 39,016 to 67,136 fish/ha resulted in smaller fish ranging from 85 to 130 g. Fish stocked at the lower densities also grew considerably faster (1.36–1.56 g/d) than the medium (0.87–1.18 g/d) and the high density (0.68–0.95 g/d). Gross yield was generally less for fish stocked at the lower densities (2,743–3,219 kg/ha) than for the medium (4,050–5,007 kg/ha) and high densities (3,832–7,281 kg/ha). The authors suggested stocking densities of 15,000 to 20,000 fish/ha to maximize average weight gain of first-year largemouth bass and achieve fingerling sizes needed for second-year growth to food-fish sizes. The authors reported that the feeding response of first-year largemouth bass during summer was noticeably higher in the early morning compared to evening feedings, especially when evening water temperatures exceeded 30℃.

Experience and collaboration with commercial producers suggests that lower stocking densities produce more predictable outcomes with fewer incidences of water-quality problems and disease outbreaks (personal communication, Robert Mayer, Kentucky). Following a 15–20 d period of high-density (110,000 fish/ha) feed-training-reinforcement, producers typically stock first year ponds at 26,000–34,000 fish/ha based on the availability of pond space and the number of surviving feed-trained fingerlings. These stocking densities are considerably lower than recommended in much of the research literature. This is largely based on the need to produce marketsize fish of > 568 g by the end of the second summer growing season. This minimum market size is not based on the average weight of the group, but on the smallest fish in the group. Buyers do not want fish in the load that are smaller than what they consider marketable size. Owing to inevitable size variability, the average harvest size may be > 600 g to ensure that the smallest fish in a group are > 568 g at the end of the second growing season. This requires that largemouth bass fingerlings attain a minimum size of 20 cm (approximately 150 g) and preferably 25 cm (> 200 g) by the end of first-year growth.

Stocking densities can also be based on assumed maximum carrying capacities, which are dependent on farm infrastructure, acceptable risk, and producer experience. If management determines that 5,600 kg/ha is the targeted maximum carrying capacity for that farm, then stocking densities can be back calculated. For example, for a 5,600 kg/ha carrying capacity, a first-year fingerling target size of 250 g, and an assumed survival of 100%, then the appropriate stocking density would be 22,400 fish/ha. If a 90% survival is assumed, the density would be 24,800 fish/ha.

Another variable that needs to be considered is the length of the local growing season, or the number of days in which the water temperature is above 18℃. Largemouth bass are considered warmwater fish, with optimal water temperatures for growth of 26–28℃. However, largemouth bass will actively feed at water temperatures > 18℃. In Kentucky, these conditions (water temperatures > 18℃) exist May through October, or approximately 160 d. The spawning season for largemouth bass in the region is usually early May. This means that the locally produced feed-trained fingerlings are not available until the second week in July (based on one week of egg incubation, a 4–6 week nursery period, and 2 weeks of feed training). As a result, most largemouth bass producers in the central and north central USA purchase feed-trained fingerlings from more southerly states with earlier spawning seasons. This functionally extends the local growing season, producing a larger first-year fingerling.

7.2 Second-year ponds

As discussed, to reach a final target size of > 0.5 kg largemouth bass in temperate zone ponds requires at least a second year of growth. First year fish can be thinned to grow-out densities either in the fall or spring. For second-year growout densities, Heidinger (2000) recommended a density of 4,950 fish/ha. However, Tidwell et al. (1998a) reported no significant differences in the average harvest weight or survival during second-year growout of largemouth bass fingerlings stocked at either 6,175 or 12,350 fish/ha. In that trial, Tidwell et al. (1998a) followed fish stocked in their second summer at two densities for a full 13 month period (May–May). Fish were fed a 44% protein diet once daily to satiation. In terms of water quality, overall means for total ammonia-nitrogen, nitrite-nitrogen, and un-ionized ammonia did not differ between ponds stocked at the different densities.

Survival also did not differ among fish stocked at the two densities and averaged 93%, overall. At harvest, there were no significant differences in weight gain or average weight (Table 7.1). In other better studied culture species, a higher stocking density usually results in smaller harvest weights (Tucker and Robinson, 1991).

Table 7.1 Initial individual weight (mean ± SE), final individual weight, final lengths, percentage, survival, average individual gain, total yield, and feed conversion ratio (FCR) for largemouth bass fed prepared diets for one year in ponds. Means within a row followed by the different letters are significantly different ($P \leq 0.05$).

	Stocking rate (fish/ha)	
	6,175/ha	12,350/ha
Stocking weight (g)	124 ± 4	122 ± 2
Final weight (g)	406 ± 32	406 ± 22
Weight gain (%)	282 ± 29	283 ± 23
Final length (cm)	29.3 ± 0.8	29.1 ±0.5
Survival (%)	93.9 ±8.5	91.7 ±6.7
Total yield (kg/ha)	2,354 ± 242b	4,598 ± 418a
FCR	3.3 ± 0.3a	2.3 ± 0.2b

Total yield was greater for largemouth bass stocked at 12,350/ha than at 6,175/ha (4,598 kg/ha and 2,354 kg/ha, respectively). Also, at the higher stocking density feed was more efficiently utilized, as indicated by a lower feed conversion ratio (FCR) (2.3) compared to the low stocking density (3.3) (Table 7.1) Again, these results differ from the better studied channel catfish, where higher stocking densities usually result in higher FCRs due to reduced feed intake, reduced availability of natural food items, and decreased efficiency of feed conversion (Tucker and Robinson, 1991).

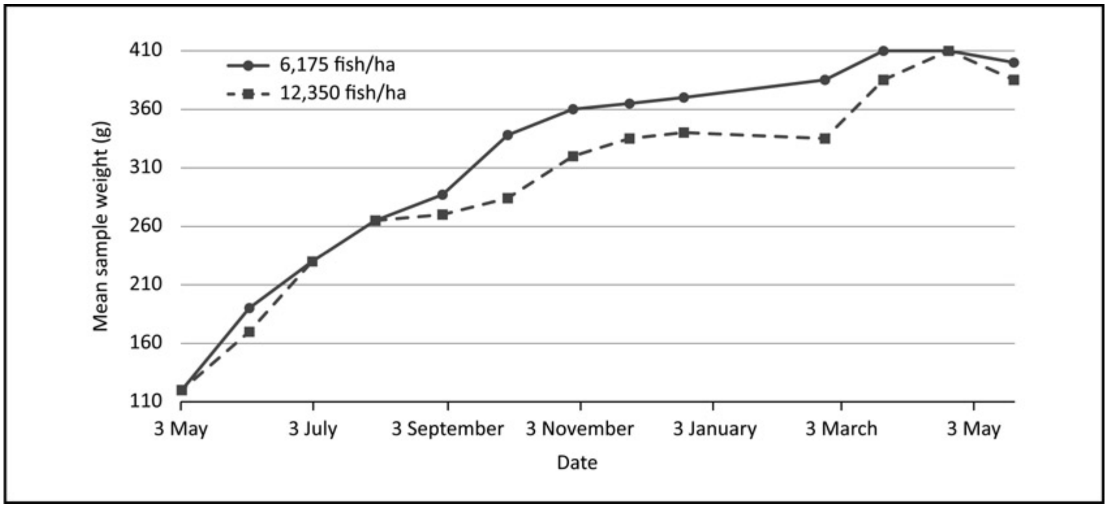

Figure 7.1 Mean sample weights of largemouth bass stocked at 6,175 or 12,350 fish/ha during second-year growth in ponds. There were no significant differences ($P > 0.05$) in average weights at any sampling date.

Tidwell et al. (1996) had previously reported that the most active feeding period for large-mouth bass during second-year growth was June–July (water temperatures > 25℃). However, in Tidwell et al. (1998b) the fish stocked at the lower density gained weight more rapidly in fall months during the period of declining water temperatures (September–November), while those stocked at the higher density gained weight more rapidly during the rising water temperatures of spring (March–May) (Figure 7.1). However, at final harvest, average weights of fish in the two treatments was similar.

Stocking largemouth bass juveniles at the higher density (12,350/ha) resulted in higher net and gross yields, and more efficient feed conversion than stocking at the low density. The average standing crop at harvest of largemouth bass stocked at 12,350/ha (4,600 kg/ha) in Tidwell et al. (1998b) was comparable to yields reported in channel catfish culture (Busch, 1985) (4,000–6,000 kg/ha). Optimal stocking density is normally a compromise between higher total unit production rates (kilograms per hectare), offset by reduced individual growth of higher densities, and deterioration of water quality at higher densities (Tucker and Robinson, 1991). In Tidwell et al. (1998a) measured water quality variables were not significantly impacted, and average fish size and survival at harvest were not reduced at the higher stocking density and the FCR was actually improved. These factors may indicate that the optimal stocking density for largemouth bass growout may be greater than 12,350/ha.

Engle et al. (2013) evaluated three largemouth bass stocking densities over a 2 year period. These included the same stocking densities of 6,175 and 12,350 fish/ha as Tidwell et al. (1998a) plus a higher density of 18,525 fish/ha. The study was stocked in late October with first-year largemouth bass fingerlings averaging 57 g. Mean weight after the second summer of production ranged from 426 to 515 g. Largemouth bass growth differed significantly among densities, with the low density having the highest growth rate and the high density the lowest growth. The medium density (12,350 fish/ha) was similar in production to the low-density treatment (6,175 fish/ha), supporting the findings of Tidwell et al. (1998a). FCR was significantly higher for the low density as compared to the medium and high densities. The optimum temperature for maximum feeding of largemouth bass ranged from 27 to 30°C in Engle et al. (2013), which was higher than the optimal temperatures of 25 to 28°C previously reported (Kubitza and Lovshin, 1997; Nelson et al., 1974).

In an earlier study, Kubitza and Lovshin (1997) evaluated largemouth bass (mean = 62.4 g) stocked at densities of 2,470 and 7,410 fish/ha during the second summer growing season. Growth rate and final weight for second-year largemouth bass were 1.47 g/d and 370 g, respectively, at the low stocking density, and 1.16 g/d and 307 g at the high stocking density. Gross yield was 738 kg/ha at the low stocking density and 1,742 kg/ha at the higher density. FCR and survival rates were similar and averaged 1.5 and 78% overall. The authors reported daily feed consumption increased rapidly during April and May as the water temperatures reached > 18°C, but dropped slightly and remained relatively constant during periods when afternoon water temperatures exceeded 29°C. The authors reported an increase in daily feed consumption in September in response to decreasing water temperatures followed by a decline as water temperatures dropped below 25°C. These results differ from Tidwell et al. (1998b) and Engle et al. (2013), suggesting a density effect on growth rates of largemouth bass even at relatively low stocking densities.

When discussing appropriate stocking densities, it is also important to consider the desired target weight at harvest. It is particularly problematic to compare and make suggestions related to stocking density if the fish do not reach market size. Even though there was no significant difference in average weight between largemouth bass stocked at the two densities of 6,175 of 12,350 fish/ha by Tidwell et al. (1998a) and Engle et al. (2013); the fish in neither study reached the current target market size for food-fish (568 g) after two summer growing seasons. Second-year largemouth bass in Kubitza and Lovshin (1997) study only reached 307–370 g. The reason the fish in these studies did not attain market size is likely because the 1 year-old largemouth bass stocked were too small (Kubitza and Lovshin, 1997, 62.4 g; Tidwell et al., 1998a, 122.1 g; Engle et al., 2013, 57 g). Cochran et al. (2009) achieved average weights of 516 to 546 g for second-year using fingerlings stocked at 210 g.

Under commercial culture conditions it is common for a small percentage of the fish (10–20%) to not reach the target size of 568 g following the second summer growout. Producers have to grade off those smaller fish and either stock them into a separate pond for continued growth during the third year or sell them into the recreational sport-fish market. This situation creates an inherent advantage for large farms which supply largemouth bass into both the food-fish and sport-fish markets.

Commercial producers commonly stock second-year largemouth bass at densities of 6,600 to 9,600 fish/ha to achieve a final weight of 600 g, reduce the numbers of undersized fish, and lower biomass densities (personal communication, Robert Mayer, Kentucky). For example, using an assumed 90% survival and a 600 g average weight this equates to biomass densities at harvest of 3,565 kg/ha and 5,185 kg/ha, respectively, for fish stocked at 6,600 and 9,600 fish/ha, respectively. These are reasonable biomass densities for largemouth bass produced in aerated ponds.

7.3 Third-year ponds

While largemouth bass can achieve target sizes of 0.7–1.1 kg in a second year of pond growout, there are markets that desire even larger fish. These include food-fish markets but also corrective pond stocking and trophy sport-fish ponds. Tidwell et al. (1998b) evaluated the effect of stocking density on third-year growth of largemouth bass in ponds.

The pattern of weight gain during third year growth (Figure 7.2) differed from that reported during second-year growth. The primary difference was during the period of August to November. In second-year fish, those stocked at the lower density gained more weight, while in third-year fish those stocked at the higher density gained more weight. Since increased density did not reduce average fish size, treatment densities resulted in large differences in total biomass densities with the high-density treatment producing significantly greater total yields.

The differences in feed conversions in ponds stocked at different densities were large (9.6 and 3.8 in low- and high-density ponds, respectively). The FCR for third-year bass at high density (3.8) was much higher than reported for second-year bass at high density (2.3; Tidwell et al., 1998a). This agrees with Lovell (1989) who stated that growth rate and feed conversion efficiencies generally decrease as fish sizes increase. In terms of fish growth, while density did not have a significant impact, overall growth was slow averaging 154 g of absolute growth and only 41% weight gain over the period of third-year growth.

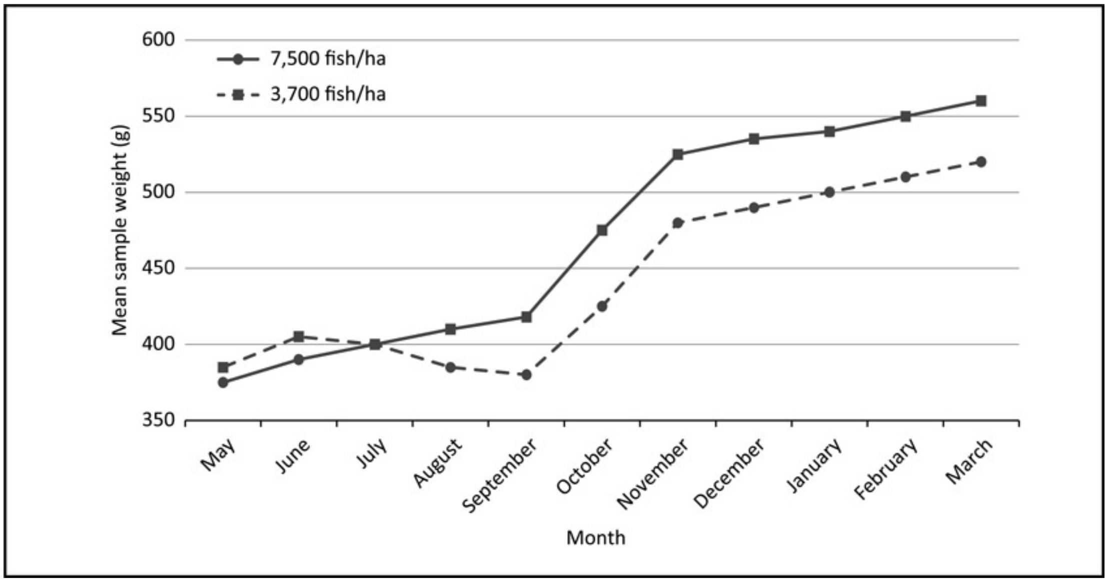

Figure 7.2 Mean sample weights of largemouth bass stocked at 3,750 and 7,500 fish/ha during third-year growth in ponds. There were no significant differences ($P > 0.05$) in average weights at any sampling date.

Engle et al. (2013) evaluated the production performance of largemouth bass stocked at three stocking densities during their third year of growth. Stocking densities of 6,175, 12,350 and 18,525 fish/ha were evaluated over a period of 2 years. Gross yields were significantly greater at higher densities, achieving 9,096 kg/ha at the highest density. Average harvest weight was lower at the high stocking density and ranged from 744 to 896 g. Third-year growth rates in Engle et al. (2013) ranged from 0.8 to 1.0 g/d, which were considerably better than growth rates reported for third-year largemouth bass by Tidwell et al. (1998a) of 0.40 and 0.52 g/d for large-mouth bass stocked at densities of 3,750 and 7,500 fish/ha, respectively. There was no significant difference in survival rates among the three densities which averaged 70% overall. The FCR was significantly greater for the low density (3.6) as compared to the medium and high densities, which both averaged 2.5.

Engle et al. (2013) reported gross yields of 9,096 kg/ha and 65% survival for largemouth bass stocked at 18,525 fish/ha after three growing seasons. This reported yield and stocking densities are higher than other previous reports (Tidwell et al., 1998b) and are higher than those commonly used on commercial farms. The focus of the research of Engle et al. (2013) was to evaluate the potential for largemouth bass to be produced for a fillet market and the authors reported the lowest breakeven price was at the medium stocking density (US$7.26/kg) due to better feed conversion efficiency compared to the lowest density (US$9.34/kg) and larger average weights and slightly better survival compared to the highest density (US$7.61/kg).

It is important to evaluate the economic risks associated with intensive pond culture of large-mouth bass, as the production costs and value are both high compared to many other commonly cultured food-fish. Ultimately management decisions such as intensification level are site based decisions related to acceptable risk as determined by the operator.

7.4 Recycle systems

Pond culture of finfish, although generally considered the most economical approach to fish production, requires significant land, labor, and water resources, has a greater range of potential environmental impacts and harvest is highly vulnerable to weather (Tucker and Hargreaves, 2012). Considering the relative high value of largemouth bass it might be possible and economically advantageous to intensify and better control largemouth bass production by culturing the fish indoors in temperature-controlled tanks; however, very little research has been conducted on rearing largemouth bass indoors. Indoor controlled environment production using recirculating aquaculture system (RAS) technologies could potentially reduce the growout period by maintaining ideal temperatures year-round and “eliminating” winter.

Widespread usage of RAS technology has not yet been adopted. In the 2013, USDA census of aquaculture, farmers reported that 35.8% of the total value of US aquaculture products was raised in ponds compared to only 8.7% for RAS systems (USDA, 2014). However, RAS systems allow for year-round production with consistent volumes of product throughout the year as compared to seasonal availability and slow winter growth, which are a characteristic of pond production. Recirculating technologies can also allow for greater economies of scale with higher production per unit area, production per unit water volume, and per unit worker than ponds (Ebeling and Timmons, 2012).

To achieve financial feasibility, RAS systems require high stocking densities per unit of volume. In pond culture of largemouth bass the maximum harvest density reported is equivalent to approximately 0.5–0.91 kg/m^3 (5,000–9,100 kg/ha, Tidwell et al., 1996; Engle et al., 2013) whereas reported maximum densities for species well suited to high-density culture in RAS, such as tilapia (*Oreochromis* sp.), are 50–100 kg/m^3 (Ebeling and Timmons, 2012). High population densities are known to affect fish health, food intake, and growth (Petit et al., 2001). Fish living in crowded conditions can become stressed from altered social interactions, restrictions of their ability to move freely or to otherwise behave normally (Wedemeyer, 1996). Baker and Ayles (1990) reported that the level of antagonistic interaction between cohorts of arctic charr (*Salvelinus alpines*) and rainbow trout (*Oncorhynchus mykiss*) changed with changes in stocking density.

No previous published studies were found evaluating growout of largemouth bass to food-fish sizes in RAS. The determination of appropriate stocking densities is the logical first step in determining the species suitability to RAS production. Watts et al. (2016) conducted a study to compare the growth, feed conversion, and survival of 1 year-old largemouth bass during second-year growth stocked at three different densities in an RAS. Largemouth bass fingerlings (112.0 ± 38.0 g) were randomly stocked into nine 900 l tanks to achieve densities of 30, 60, or 120 fish/m^3 with three replicate tanks per density. The RAS consisted of a 3,000 l sump, 0.25 hp pump, bead filter for solids removal, mixed-moving-bed biofilter for nitrification and a 400 W UV light for sterilization. Fish were fed a commercially available floating diet (45% protein and 16% lipid) once daily to apparent satiation. At harvest all fish were counted, individually weighed, and measured.

Harvest data indicated that biomass densities significantly increased with stocking rate achieving 6.2 kg/m^3 at 30 fish/m^3, 13.2 kg/m^3 at 60 fish/m^3, and 22.9 kg/m^3 at 120 fish/m^3 (Table 7.2). The stocking densities evaluated in this trial had no significant impact ($P > 0.05$) on survival, average harvest weight, uniformity index (UI_{10}, Bell, 2002) or FCR, which averaged 93%, 294 g, 26.2 and 1.8, respectively (Table 7.2).

Table 7.2 Means (± SD) of average harvest weight (AHW) (g), minimum/maximum of individual harvest weight (g), total production (kg/m^3), survival (%), feed conversion ratio (FCR), percent weight gain (% wt gn), and uniformity index$_{10}$ (%) of largemouth bass raised in tanks at three densities and fed a commercially available extruded pellet diet.[a]

	30/m^3	60/m^3	120/m^3
AHW (g)	301.3 ±31.9[a]	298.7 ± 20.1[a]	283.5 ± 17.5[a]
Min/max (g)	151/492	124/556	108/598
Total production (kg/m^3)	7.6 ± 1.0[c]	16.4 ± 1.1[b]	28.2 ± 3.9[a]
Surv (%)	90.7 ± 4.6[a]	98.7 ± 2.3[a]	89.3±7.1[a]
FCR[b]	2.0 ± 0.3[a]	1.6 ± 0.1[a]	1.7±0.3[a]
% wt gn[c]	269.0 ± 28.5[a] :	266.7 ± 17.9[a]	253.1 ± 15.6[a]
UI_{10} (%)[d]	25.1 ±5.9 :	27.1 ± 2.7	26.5 ± 8.8

Notes: [a] Values within columns within trials followed by different superscripts are significantly different ($P \leq 0.05$). [b] FCR = g dry feed fed/g wet weight gain. [c] Percentage weight gain (% wt gn) = (average harvest weight (g)/ average stock weight (g)) × 100. [d] Ul_{10} (%) = $(n_{10}/N) \times 100$, where n_{10} is fish number (frequency) between "mean × 0.9" and "mean × 1.1" and N is total fish number measured.

Although survival and feed conversion were acceptable, after 6 months of culture the large-mouth bass did not achieve target sizes of 500 g, which is considered a marketable-size in most food-fish marketplace. It appears that intraspecific competition within tanks resulted in increased size variation as densities increased. The UI for body weight was 25 at 30/m^3, 27 at 60/m^3 10, and 27 at 120/m^3. Observations indicate that each tank had one or two fish that did achieve market size, but the remainder of the population did not. The size ranges are illustrated by minimum and maximum sizes (min/max) (Table 7.2) and again increase as densities increased, although average weights did not differ significantly. Smaller fish were observed to often show signs of tail biting and bullying from cohorts.

Petit et al. (2001) compared juvenile largemouth bass growth and size variation in aquaria stocked at different densities. The authors evaluated initial biomass densities of 1, 2, and 3 kg/m^3 compared to the initial biomass densities of 2.6, 5.1, and 10.2 kg/m^3 evaluated in this trial. In agreement with the current study, they reported no differences in average weight although the coefficients of variation (CV) of weight increased with stocking densities. Increases in the CV for weight within a population are known to be indicative of the establishment of hierarchies which can suppress the growth of cohorts (Brett, 1979). Petit et al. (2001) also observed antagonism during feeding, reporting that one or a few fish ate first and that dominant fish ate more than the others. The authors fed a sinking feed once per day and reported that their feeding method may have influenced antagonism and weight variability.

Extruded floating pellets are typically used in commercial production of largemouth bass in ponds as it allows the farmer to see how much, and how actively, the fish eat. However, it may be beneficial to feed a slow sinking pellet to largemouth bass in RAS to reduce the ability of a few fish to prevent others from access to feed on the water surface. Perhaps multiple daily feedings would allow more aggressive individuals to become satiated reducing antagonism during subsequent feedings.

Development of genetic strains of largemouth bass better suited for high-density tank culture would likely also be beneficial. Production in RAS would allow the largemouth bass to be produced in closer proximity to their primary markets (Toronto, New York) reducing transport stress and the subsequent negative impacts on survival in these live markets.

Other production systems are successfully utilized in China, such as net-pens in lakes and reservoirs. These are reviewed in Chapter 3.

For fish cultured in high-density environments such as tanks and raceways, different species have different tolerances in terms of crowding. Fish densities can be based on water quality and water flow. In raceway culture, a "flow index" is determined based on species and size and flow rate (Fornshell et al., 2012). However, some species require a certain amount of "personal space", independent of water quality. In raceway systems an independent index determined for different species termed a density index looks at the effect of the physical proximity of individual fish. The largemouth bass, as a top-level carnivore, may be a species which does not tolerate physical crowding well.

As part of evaluating largemouth bass for culture in tank-based systems, Park et al. (2015) conducted two trials. In the first trial, experimental treatments were based on initial biomass densities of 15, 20, 25, 30, 35, and 40 kg/m^3. Tanks were 190 l (18 tanks), 9 g fish were stocked, and these densities equated to 300, 400, 500, 600, 700, and 800 fish per tank. Fish were fed a diet containing 45% protein and 15% lipid twice daily for 60 days. Results were based on survival, growth, and feed conversion. However, size variability was also important as cannibalism on small cohorts could be a problem.

At harvest, survival was good in all treatments (95–97%) as were feed conversions (0.96–1.13). CV for individual growth measures did not indicate greater size variability among the different density treatments.

A subsequent study was conducted by the same research group to approximate the maximum yield and final size distribution patterns in stocker size largemouth bass (Park et al., 2017). Fingerling largemouth bass (37 g) were stocked into 165 l tanks (18 tanks) at 4.5, 9.1, 18.8, 36.5, 54.6, and 73 kg/m^3 (three replicates of each). This equated to 22, 44, 88, 176, 264, and 352 fish per tank. Fish were fed the same diet (46/16) twice daily for 60 d.

At harvest, survivals ranged from 82–97%, specific growth rate from 1.16 to 1.45%/day, final weights of 64–75 g, and FCR from 1.00 to 1.45. Best results were achieved in tanks with initial stocking rates of 18–36 kg/m^3. It was determined that a maximum yield of approximately 70 kg/m^3 was achievable for largemouth bass of this size.

There is some commercial production of largemouth bass advanced fingerlings in RAS to supply growout ponds with one year old fish. A producer in Kentucky is using reconditioned hog barns for largemouth bass advanced fingerling production and growing largemouth bass food-fish in renovated manure or lagoon ponds. This system has the advantage of requiring fewer ponds and provides the advantage of year-round feeding of first-year fish ensuring that they reach > 200 g during the first year.

7.5 Conclusion

Although the largemouth bass has been cultured for over 100 years, most of the production methods used today in the USA are derived from pond methods used to raise small fingerlings for sport-fish stocking. These systems have not changed radically and neither have the fish. There is increasing interest and activity in producing the largemouth bass in other types of production systems. This will likely require that genetic lines of largemouth bass be developed which are selected and adapted for more intensive culture conditions. In China (Chapter 3) the fish is widely produced in cages and net-pen systems and there are genetically improved strains being developed and utilized.

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Chapter 8
Largemouth bass nutrition

Waldemar Rossi Jr. and Habte-Michael Habte-Tsion

Aquaculture Research Center, Kentucky State University, Frankfort, Kentucky, USA

8.1 Introduction

Perhaps the first study on the nutritional requirements of largemouth bass (*Micropterus salmoides* L.), using formulated diets, was published by Anderson et al. (1981). These authors used semi-purified diets to evaluate the protein requirements of largemouth bass at ages 0 (~2 g) and one (~6 g). Although the importance of the knowledge on nutritional requirements for the expansion of largemouth bass production in the United States has been recognized since the 1980s (JSA, 1984), the first summary report on the subject was not published until almost 20 years later (Tidwell et al., 2002). Even then, only limited information was available regarding the nutritional requirements of the species. Today, because of increased interest on the production of food-size fish in North America and Asia, and the global need to optimize cost-effective feed formulations for maximum production performance and minimum nutrient output to the environment, more is known about the nutritional requirements of largemouth bass. However, much work lies ahead.

This chapter will summarize information on known nutritional requirements of largemouth bass, as well as raw material utilization and nutritional value, and feed additives. A brief discussion on recent findings from immunonutrition and nutrigenomics studies is also included.

8.2 Digestive tract

The digestive tract of largemouth bass can be divided into mouth, buccal cavity, esophagus, stomach, anterior and posterior intestine, and rectum. Auxiliary digestive organs include the liver, gallbladder, and distinct pancreas. Pyloric caeca are also present and can contain more than 20 digitiform tubules opening into the anterior intestine immediately posterior to the pyloric constriction of the stomach (Sarbahi, 1951) (Figure 8.1). As a general characteristic of predatory fish species, the digestive tract of largemouth bass is short, extending only slightly beyond its total length (Figure 8.2). The activities of digestive enzymes including pepsin, trypsin (gastric and pancreatic), lipase and amylase have long been reported in largemouth bass (Sarbahi, 1951; McGeachin and Debnam, 1960).

Figure 8.1 Dissection of largemouth bass intraperitoneal cavity showing the gallbladder, liver, and caeca.

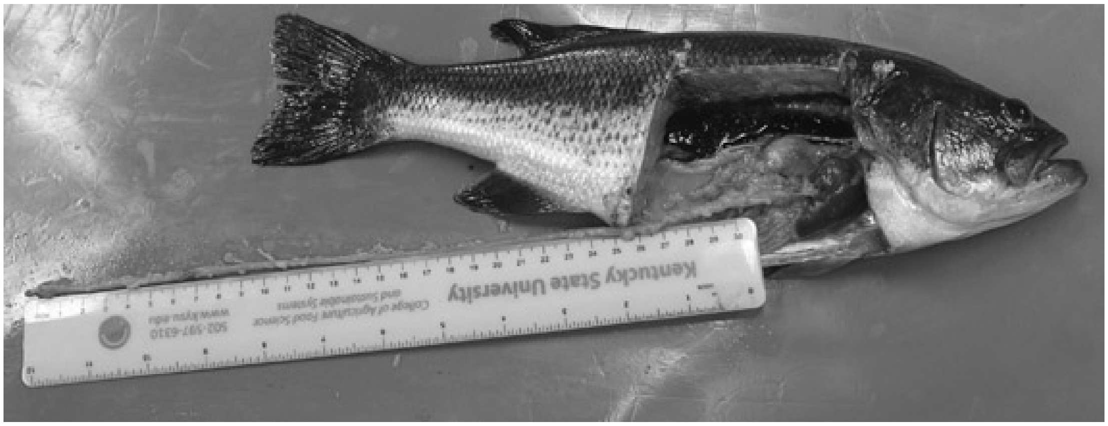

Figure 8.2 Dissection of largemouth bass intraperitoneal cavity showing the relative length of the intestine.

8.3 Nutritional requirements

8.3.1 Protein and essential amino acids

As a carnivorous species, largemouth bass appears to be highly responsive to dietary protein, even at late growout phases near market sizes (400–700 g). In general, the available literature indicates that largemouth bass require between 40 to 50% dietary crude protein (CP) for maximum production performance. Most requirement studies were conducted using juvenile fish, and the observed differences in requirement values are likely due to factors including initial fish size, ingredient composition and digestibility of experimental diets, feeding rate, dietary non-protein energy, and possibly genetics.

Brecka et al. (1996) evaluated four semi-purified diets formulated to contain 31, 34, 37, or 40% CP and an estimated 3.27 kcal/g digestible energy (DE) in largemouth bass (12.2 g MIW). Diets containing 37 or 40% CP and energy-to-protein ratios of 8.8 and 8.2 kcal DE/g, respectively, were found to support maximum growth of the fish. Portz et al. (2001) assessed the dietary protein and energy requirements of largemouth bass (14.5 g MIW) and concluded that a minimum of 43.6 and 44.8% dietary CP was required to maximize growth and minimize feed conversion ratio (FCR). The authors also indicated that DE: CP ratios ranging from 8.9 to 9.6 kcal/g would support FCR values between 0.96 and 1.10.

Slightly higher dietary CP levels have been recommended. In the study of Huang et al. (2017), largemouth bass (8.7 g MIW) were fed eight diets containing 42, 45, 48, and 51% CP and two lipid levels (8 or 12%). The authors recommended 48–51% CP and 12% lipid as most suitable levels. Likewise, in a study evaluating different dietary lipid-to-protein ratios in largemouth bass (10.1 g MIW), Chen et al. (2012a) concluded that the lipid-to-protein ratio supporting the best growth performance was 0.23, corresponding to a diet containing 46.3% digestible protein (DP), 10.1 of digestible lipid, 4.1 kcal/g DE, and 8.7 kcal DE/g DP.

Information on the dietary protein requirement of largemouth bass at larger sizes (> 100 g) might be limited to the study of Tidwell et al. (1996). In a 12 month pond feeding trial, large-mouth bass (122.1 g MIW) were fed diets containing 42, 44, or 47% CP. The highest weight gain and lowest FCR were observed in fish fed the 47% CP diet, indicating that diets formulated to contain over 45% CP can support relatively faster growth rates and higher feed efficiency during growout.

Based on the essential amino acid (EAA) requirements of aquatic species reported to date, it can be assumed that largemouth bass require all ten classic EAAs (that is, arginine [Arg], histidine [His], isoleucine [Ile], leucine [Leu], lysine [Lys], methionine [Met], phenylalanine [Phe], threo-nine [Thr], tryptophan [Trp], and valine [Val]). However, to date, quantitative requirement values have only been determined for Arg, Lys, and Met. In general, amino acid requirement studies have been carried out for 8 to 10 weeks utilizing semi-purified diets containing both peptide-bound and crystalline sources of amino acids that, together, follow the relative ratios found in largemouth bass muscle.

Zhou et al. (2012) determined the Arg requirement of largemouth bass (~25 g mean initial weight [MIW]) using 46% CP and 12% lipid diets containing Arg from 1.7 to 3.0%. The available dietary Arg requirement for maximum weight gain was found to be 1.91% (4.16% of CP).

In Dairiki et al.'s (2007) study, largemouth bass (1.29 g MIW) were fed diets containing 43% CP, 9% lipid, and Lys ranging from 1.2 to 3.5%. The dietary Lys requirement for maximum growth was found to be 2.10% (4.9% of CP), while 1.69% dietary Lys (3.9% of CP) was required to minimize FCR. Using larger fish (14.1 g MIW) and diets formulated to contain 40% CP, 12% lipid, and Lys from 1.0 to 3.0%, Woodward et al. (personal communication) estimated the dietary Lys requirement for maximum growth and feed efficiency of largemouth bass to be 1.6 and 1.7% (4.0 and 4.3% of CP), respectively.

The Met requirement of largemouth bass was first reported by Chen et al. (2010) who fed largemouth bass (37.9 g MIW) 44% CP and 9.2% lipid diets containing Met from 0.6 to 1.6% and a constant level of 0.3% cysteine (Cys). The dietary Met maximizing the performance of the fish was found to be 1.2% (2.8% of CP), corresponding to a total sulfur amino acid (TSAA) requirement of 1.5% (3.4% of CP). A second assessment of the Met requirement of largemouth bass (6.0 g MIW) was conducted by Rossi et al. (2018) using 42% CP, 13% lipid diets containing Met from 0.5 to 1.3% and a constant level of 0.26% Cys. The dietary Met supporting maximum weight gain and feed efficiency was found to be 0.85% (2.0% of CP), corresponding to a dietary TSAA requirement of 1.1% (2.6% of CP). Results from the same study also suggested that dietary Cys could spare around 40% of the Met requirement of largemouth bass.

The study of Frederick et al. (2016) indicated that taurine (Tau) is not essential in the diet of largemouth bass and that the species is capable of synthesizing it. Although not strictly an amino acid due to the lack of a carboxyl group, Tau has been found to be dietary indispensable for an increasing number of fish species (reviewed by Salze and Davis, 2015), or to spare dietary Met in Nile tilapia (*Oreochromis Niloticus*) fed marginally deficient diets (Michelato et al., 2018). Hypothetically, any species capable of synthesizing Tau through the transsulfuration pathway might spare Met with dietary Tau to some extent, but has not yet been assessed in largemouth bass.

8.3.2 Lipids and essential fatty acids

Lipids comprise the most concentrated source of chemical energy and supply essential fatty acids, phospholipids, and sterols in feeds. In general, total lipid content found in isolipidic formulations for largemouth bass have ranged from 8.5 to 14% (Coyle et al., 2000; Subhadra et al., 2006; Tidwell et al., 2007; Yun et al., 2013; Yadav et al., 2018). However, findings from a limited number of studies evaluating diets containing ≥ 40% CP suggest dietary lipid in the higher end of that range for maximum production performance of largemouth bass (Chen et al., 2012a; Huang et al., 2017).

Bright et al. (2005) fed 43% CP diets containing lipid from 8.2 to 24% (dry matter basis) to largemouth bass (16.3 g MIW) and found no effects on production performance – although the reported FCR and protein efficiency ratio (PER) appeared to be correlated (negatively and positively, respectively) to dietary lipid. The authors also observed higher whole-body fat in fish fed diets containing ≥ 21% lipid.

Considering the upper-tolerance limit for total digestible carbohydrates in largemouth bass (see Section 8.3.4), total digestible lipid content in formulations for this species should be based on digestible carbohydrate and protein contents, and energy-to-protein ratio. For instance, based on the energy of combustion of 5.65, 4.20, and 9.50 kcal/g of protein, carbohydrate, and lipid, respectively (Merrill and Watt, 1955), diets formulated to contain 35 to 45% DP, 10% digestible carbohydrates, and a DE: DP ratio of 8.7 (Chen et al., 2012a), would require digestible lipid ranging from 7 to 10%.

Most studies evaluating essential fatty acid (EFA) requirements in largemouth bass have indicated that highly unsaturated fatty acids (HUFA) of both n-6 (arachidonic acid, ARA) and n-3 (eicosapentaenoic acid, EPA; docosahexaenoic acid, DHA) series are dietary dispensable. These findings support the notion that as a freshwater species, largemouth bass EFA requirements can be satisfied by the dietary supply of 18-carbon HUFA precursors, namely linoleic acid (LA, 18:2 n-6) and a-linolenic acid (ALA, 18:3 n-3).

Subhadra et al. (2006) fed largemouth bass (5.0 g MIW) with 47% CP and 14% lipid diets containing 30% solvent-extracted fish meal and supplemented with canola oil, chicken oil, or menhaden oil. The authors found no significant differences in production performance of the fish fed the experimental diets containing different levels of ARA (0–2.0%), EPA (0–13.8%), and DHA (0–5.3% of total fatty acids). Likewise, Tidwell et al. (2007) fed largemouth bass (15.7 g MIW) diets containing ARA, EPA, and DHA ranging from 0.2, 0.9, and 0.9% to 28.9, 11.0, and 3.9% of total fatty acids, respectively, and observed no dietary effects on production performance.

Notwithstanding the above, positive responses to supplemental EPA and DHA rich oils have been observed in largemouth bass. In the study of Yadav et al. (2018), largemouth bass (13.8 g MIW) fed 45% CP and 12% lipid diets containing 1 or 2% EPA+DHA displayed higher weight gain and feed efficiency compared to fish fed a control diet devoid of n-3 HUFA. These results indicate that the supplementation of ~1% EPA+DHA to nutrient-dense feeds may maximize production performance of largemouth bass.

Since the utilization of high-performance feeds is a common practice in the rearing of large-mouth bass fingerlings and early grow-out of juveniles, more studies on the potential benefits of supplemental n-3 HUFA in feeds are recommended. Additionally, from a consumer perspective and in view of the health benefits associated with the consumption of n-3 HUFA, the evaluation of finishing feeds designed to modulate the concentrations of these fatty acids in largemouth bass muscle is also warranted.

8.3.3 Vitamins and minerals

Information on the dietary vitamin and mineral requirements of largemouth bass is limited although supplementation in commercial feeds is a common practice to ensure adequate supply. The available information on dietary vitamin requirements of largemouth bass is summarized in Table 8.1. Despite discrepancies among different studies, the vitamins A, E, and C might be the only vitamins with more than a single requirement estimate for largemouth bass.

Table 8.1 Summary of dietary vitamin requirements of largemouth bass.

Vitamin	Recommended level (unit/kg)	Reference
A	6,000 IU	Qi (2014)
	2,600-3,550 IU	Lianetal. (2017)
D	2,200 IU	Qi (2014)
E	150 IU	Qi (2014)
	73-108 mg	Lietal. (2018)
K	10 mg	Qi (2014)
Ascorbic acid	780 mg	Qi (2014)
	≥ 1,000 mg	Xie et al. (2006)
	148 mg	Chenetal. (2015)
	≥ 700 mg*	Yuanetal. (2015)
Biotin	2.0 mg	Qi (2014)
Choline	2,500 mg	Qi (2014)
Cyanocobalamin (B_{12})	0.01 mg	Qi (2014)
Folic acid	5.0 mg	Qi (2014)
Niacin	200 mg	Qi (2014)
Pantothenic acid	60 mg	Qi (2014)
Pyridoxine (B_6)	20 mg	Qi (2014)
Riboflavin	20 mg	Qi (2014)
Thiamine	30 mg	Qi (2014)

Note: * Diets containing oxidized fish oil.

The production performance of largemouth bass was significantly enhanced in response to dietary vitamin A in the range of 415 to 2,755 IU/kg, tending to plateau at higher levels (3,000–4,000 IU/kg diet) and the optimum requirement was determined to range between 2,600 and 3,550 IU of vitamin A/kg (Lian et al., 2017). However, Qi (2014) reported higher dietary vitamin A requirement (6,000 IU/kg diet) for largemouth bass.

Reported signs of vitamin C deficiency in largemouth bass include anorexia, poor survival and growth, lordosis, and skin darkening along with sluggish behavior and exophthalmia (Chen et al., 2015). Chronic mortality in ponds has been observed in largemouth bass fed diets containing vitamin C at 100 mg/kg (Porak et al., 2002). Reported dietary requirement values for vitamin C in largemouth bass are quite variable and range from 148 mg/kg (Chen et al., 2015) to over 1,000 mg/kg (Xie et al., 2006). However, it appears that a level of 1,000 mg/kg of vitamin C in more stable forms (for example, Stay C, 35%) can be recommended to support adequate health and growth of largemouth bass even when semi-purified diets are used. The vitamin E requirement for largemouth bass was reported to range from 73 to 108 mg by Li et al. (2018). The review of Qi (2014) gathered most of the known vitamin requirements for largemouth bass. The dietary levels (dry matter basis) of supplemental vitamins used in practical or semi-purified diets in the Kentucky State University Aquatic Animal Nutrition Laboratory are presented in Table 8.2.

Largemouth bass dietary requirements (mg/kg, dry matter basis) for manganese (180.0), copper (8.0), cobalt (1.5), iodine (6.0), iron (66.0), zinc (150.0), and selenium (0.3) have been reported (Qi, 2014). Except for the relatively higher dietary requirement for manganese and zinc, values for the other microminerals are within requirement ranges reported for other fish species (Watanabe et al., 1997; NRC, 2011). To date, it appears that dietary requirements for macrominerals in largemouth bass are still lacking in the literature. Results from a recent study indicated that soybean meal-based diets containing $\geq$ 0.60% non-phytate phosphorus can support adequate production performance and health of largemouth bass, but ~0.8% non-phytate phosphorus was required to maximize whole-body phosphorus and ash contents (Miller et al., personal communication).

Table 8.2 Dietary levels (dry matter basis) of supplemental vitamins in practical diets for largemouth bass (Kentucky State University Aquatic Animal Nutrition Laboratory).

Vitamin	Unit	Unit/kg of diet
A	IU	9,920
D	IU	661
E	IU	529
K	IU	5.5
Ascorbic acid	mg	1,050
Biotin	mg	0.5
Choline	mg	1,800
Cyanocobalamin (B_{12})	mg	0.04
Folic acid	mg	13.2
Niacin	mg	331
Pantothenic acid	mg	159
Pyridoxine (B_6)	mg	38
Riboflavin	mg	79
Thiamine	mg	49

8.3.4 Carbohydrates

No dietary requirement for carbohydrates (CHO) has been reported for aquatic animals. However, digestible CHO are widely utilized in aquaculture feeds for two main reasons: (1) they comprise the least expensive sources of dietary energy; (2) starch gelatinization during processing increases water stability and adds buoyancy to finished feeds.

Carnivorous fish species generally utilize digestible CHO poorly as a source of energy. At dietary levels exceeding tolerance limits, digestible CHO can also be detrimental to the health and production performance of fish. Goodwin et al. (2002) reported a case of unexplained mortality of largemouth bass in the farm of a major producer in the USA. After thorough examination, the cause of the escalated mortality was attributed to the increased accumulation of glycogen in the liver and subsequent necrosis of hepatic tissue. In a follow-up feeding trial with diets containing 21, 27, and 35% nitrogen-free extract (NFE), the authors reported higher liver glycogen levels in fish fed diets containing > 21% NFE. After the producer switched to a commercial feed containing approximately 45% CP, 25% lipid, and 22% CHO no signs of liver pathology were found in 16 month-old fish.

Perhaps, the study of Goodwin et al. (2002) was the first indicating an upper tolerance limit for dietary CHO in largemouth bass and their findings were corroborated by later studies. Tan et al. (2005) fed largemouth bass (8.0 g MIW) with diets ranging from 15 to 23% CHO and found the best growth and feed efficiency at the dietary CHO level of 19%. Similar responses were found by Amoah et al. (2008) after feeding largemouth bass (128.5 g MIW) with diets containing 13 to 25% NFE. Lower growth rates and higher FCR were observed in fish fed the diet containing 25% NFE; and a positive correlation between liver vacuolization and dietary NFE was found. The authors recommended < 20% NFE in feeds for largemouth bass.

Although most studies evaluating dietary CHO in largemouth bass have recommended NFE ≤ 20%, good production performances of largemouth bass have been observed with diets containing as much as 35% NFE (Tidwell et al., 1996; Hulefeld et al., 2017, unpublished). Discrepancies in dietary CHO tolerance of largemouth bass may be attributed to the variable chemical composition and digestibility of the NFE fraction of feeds (Morales et al., 1994; Sanz et al., 1994; Rahman et al., 2016). Factors such as processing temperature have been found to influence NFE digestibility (Hernández et al., 2010). Therefore, caution should be exercised when limiting CHO in largemouth bass feeds solely based on NFE.

Formulations based on digestible CHO rather than NFE values may help in optimizing CHO inclusion in largemouth bass feeds. For instance, when largemouth bass (28.36 g MIW) were fed diets containing digestible starch from 5.9 to 21.7%, growth and PER of the fish was maximized at the digestible starch levels of 9.2 and 10.1%, respectively (Gou et al., 2015). Similarly, Lin et al. (2018) fed largemouth bass (16.9 g MIW) with semi-purified diets containing 5, 10, or 20% wheat starch and observed better production performance in fish fed the diets containing 5 and 10% starch. Overall, these findings suggest that best production performance of largemouth bass can be achieved when dietary digestible CHO is ≤10%.

Literature information on the effects of dietary fiber and other non-starch polysaccharides in largemouth bass is extremely scarce. Qian (2000) indicated that crude fiber should be kept below 3.5% in diets for largemouth bass.

8.3.5 Energy

Based on the levels of dietary protein, carbohydrate (measured as nitrogen free extract [NFE]), and lipid reported by different studies (Coyle et al., 2000; Subhadra et al., 2006; Tidwell et al., 2007; Csargo et al., 2013; Yun et al., 2013; Hulefeld et al., 2017, unpublished; Yadav et al., 2018) and the respective physiological fuel values (4, 4, and 9 kcal/g) of these components, DE in practical diets for largemouth bass has ranged from 3.5 to 4.6 kcal/g. Considering the DE: DP ratio of 8.7 kcal/g found to maximize the production performance of largemouth bass in the study of Chen et al. (2012a), diets formulated to contain DP in the range of 35 to 45% should contain DE ranging from 3.1 to 4.0 kcal/g. This range of DE appears to be in close agreement with that estimated from practical diets.

8.4 Raw materials utilization

Digestibility and availability coefficients of practical ingredients in largemouth bass have been reported (Portz and Cyrino, 2004; Masagounder et al., 2009) and are summarized in Table 8.3. Irrespective of all potential sources of variation including raw material origin and quality, fecal collection method and experimental protocol, the reported coefficients for the same raw material-type are fairly similar and within ranges found in the literature for other fish species (NRC, 2011).

Several plant-protein feedstuffs have been utilized in diet formulations for largemouth bass with soybean meal (SBM) being the most widely used and often constitutes the main source of dietary protein. Contrary to some carnivorous species such as Atlantic salmon (*Salmo salar*), largemouth bass appear to tolerate high dietary levels of SBM. For instance, fish meal (FM) levels in SBM-based diets for largemouth bass have been reduced to as low as 8% without detrimental effects on production performance (Cochran et al., 2009; Kolimadu et al., 2018; Hulefeld et al., 2017, unpublished).

The utilization of protein feedstuffs of animal origin has been proven effective in diets for largemouth bass. The inclusion of poultry by-product meal (PBM) in low FM, plant-based formulations appears to sustain adequate palatability and nutritional value for the species (Tidwell et al., 2005; Cochran et al., 2009). Tidwell et al. (2005) demonstrated that PBM could replace all dietary FM (included at 30%) in a SBM-based diet fed to largemouth bass without negatively affecting production performance. Insect meals are regarded as promising feed ingredients and are becoming increasingly more available for use in aquaculture feeds. A high nutritional value of defatted black soldier fly meal to largemouth bass was demonstrated in the study of Walling et al. (2017), as nearly 100% of dietary FM (included at 40%) was successfully replaced with the insect meal.

Literature information on lipid utilization shows that a wide range of practical lipid sources can be utilized in diets for largemouth bass as long as adequate amounts of n-6 and n-3 fatty acids are present. Lipids sources evaluated in studies have included marine oils derived from fish and algae, vegetable oils derived from canola, soybean, palm, linseed, and sunflower, and oils and fats derived from the rendering of terrestrial animals (Coyle et al., 2000; Bright et al., 2005; Subhadra et al., 2006; Tidwell et al., 2007; Laporte and Trushenski, 2011; Yun et al., 2013; Kolimadu et al., 2018; Yadav et al., 2018). More recently, hydrogenated soybean oil was found to be a suitable alternative lipid source in the diet of largemouth bass (Laporte and Trushenski, 2011).

Table 8.3 Apparent digestibility and availability coefficients (%) of practical feedstuffs in largemouth bass.

Ingredient	FM	PBM	MBM	SBM	CGM	Corn	Wheat
Energy	78.3–87.4	85.2–87.0	72.3	75.4–79.8	76.5	53.0	55.3
Dry matter	70.0–77.6	82.6–83.4	58.2	70.4–79.0	75.3	50.0	55.5
Protein	87.7	81.5	–	94.3	93.6	–	–
Lipid	98.2	98.2	–	93.3	83.4	–	–
Calcium	62.8	86.7	–	81.1	74.5	–	–
Phosphorus	72.3	93.9	–	88.0	82.8	–	–
Arginine	92.5–93.9	91.2–94.7	83.6	97.0–97.8	98.4	91.8	92.7
Histidine	85.8–91.9	93.1–93.5	86.0	91.0–96.4	91.9	90.5	91.9
Isoleucine	88.9–92.6	85.8–90.6	83.4	94.2–96.5	94.9	81.9	85.4
Leucine	85.7–93.1	88.6–91.0	84.3	94.1–97.6	88.9	91.9	90.8
Lysine	94.8–95.8	90.8–95.4	85.8	95.7–96.1	95.9	80.1	82.7
Methionine	82.7–91.8	71.3–92.7	85.3	80.3–94.3	83.1	85.1	90.4
Phenylalanine	91.1–91.7	87.5–90.1	83.5	94.5–94.7	93.1	89.0	91.5
Threonine	88.0–93.5	86.1–92.6	82.6	93.6–96.3	95.5	78.4	85.2
Tryptophan	82.2–92.5	51.5–92.8	89.0	86.6–96.9	80.0	–	83.8
Valine	91.7–92.0	83.0–89.0	82.1	93.7–98.6	97.1	90.6	89.3
Alanine	87.5–91.7	87.6–90.7	83.3	92.6–99.4	97.0	87.9	83.6
Aspartate	85.0–91.0	82.0–91.9	82.3	96.0–99.6	93.8	86.0	87.8
Cysteine	41.5–82.9	50.4–82.3	53.6	51.2–94.7	56.2	80.1	90.4
Glutamate	91.1–93.2	87.5–92.9	83.3	96.8–99.8	93.1	94.2	97.6
Glycine	88.2–91.4	91.0–93.1	80.6	93.0–99.9	99.4	81.2	85.7
Proline	90.8–92.8	89.2–91.6	80.3	95.3–99.9	97.9	90.2	95.6
Serine	87.3–92.1	77.0–91.7	81.1	95.5–99.7	91.8	88.5	92.9
Tyrosine	91.7–95.9	92.5–96.2	79.5	95.7–98.2	94.7	80.6	86.3

Note: FM = fish meal; PBM = poultry by-product meal; MBM = meat and bone meal; SBM = soybean meal; CGM = corn gluten meal.

Source: Summarized from Portz and Cyrino (2004) and Masagounder et al. (2009).

8.5 Feed additives

A limited number of studies have evaluated different feed additives in the diet of largemouth bass. Kubitza et al. (1997) concluded that the dietary inclusion of inosine-5-monophosphate at 2,800 mg/kg enhanced feed intake in juvenile fish by 23%. Liu et al. (2015) included ethoxyquin from 0 to 1,500 mg/kg in the diet of largemouth bass and observed higher survival and antioxidant protection at the ethoxyquin level of 300 mg/kg. Likewise, Yu et al. (2018) found that the inclusion of butylated hydroxytoluene (BHT) at a level of 150 mg/kg in the diet improved lipid metabolism and antioxidant response of the fish. Li et al. (2017) found that diets supplemented with selenium-yeast enhanced lipid metabolism and antioxidant response of largemouth bass and recommended 1.29 mg Se/kg. When fed diets supplemented with hydrolyzed yeast at 30g/kg, largemouth bass displayed significant improvements in growth, feed intake and intestinal health (Zheng et al., 2015). Similarly, Zhou et al. (2018a) concluded that dietary brewer's yeast hydrolysate might beneficially modulate the gut microbiota of largemouth bass. Niu et al. (2010) found that the supplementation of phytase at 1,000 FU/kg improved growth, FCR and protease activity in both the stomach and pyloric caeca of largemouth bass.

8.6 Immunonutrition and nutrigenomics

Based on our comprehension of the linkage between nutrition, immunology and genetics (specific signaling molecules), appropriate feeds and feeding regimes give optimum fish health. Immunomodulatory nutrients supply the basic requirements for the fish immune system to realize its function as well as to protect tissues from collateral damage (Figure 8.3). In addition, to prevent diseases, nutrition research is investigating how nutrition can optimize and maintain cellular, tissue, organ and whole-body homeostasis. This requires understanding how nutrients act at the molecular level and, to this end, the use of nutrigenomics is becoming increasingly more common in fish nutrition research to characterize gene products, their physiological functions, and their interactions with nutrients.

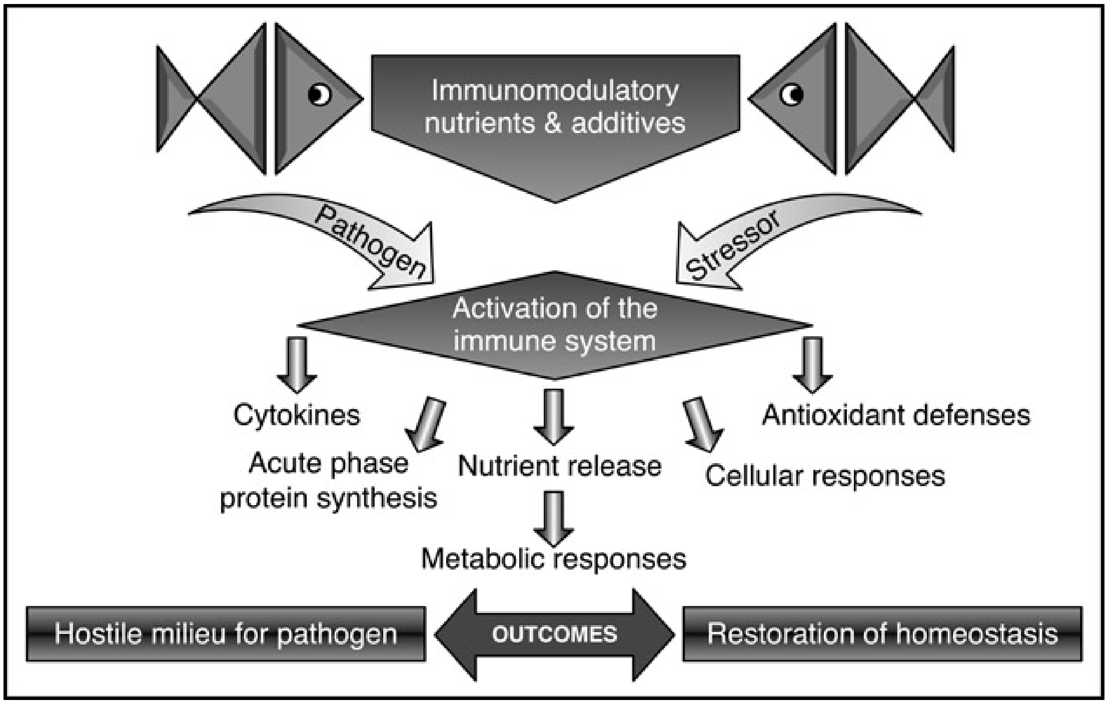

Figure 8.3 Concept of fish immunonutrition in preventive health

Source: Fish immune system and its nutritional modulation for preventive health care; Viswanath Kiron; Animal Feed Science and Technology; Volume 173 Issue 1–2 (2012)

The role of dietary EAA on fish immune response and signaling molecules related to immune and metabolic functions has received little attention (Habte-Tsion et al., 2015, 2016). The exception is research on the role of Arg (Liang et al., 2016, 2018; Figure 8.4). In a study on largemouth bass, dietary Arg improved serum protein content, serum lysozyme and respiratory burst activities of head kidney leucocytes (Zhou et al., 2012), indicating the immunomodula-tory function of this amino acid in this species. Moreover, dietary Arg and carbohydrate/lipid ratios differentially regulated the mRNA expression of growth-related genes such as growth hormone (GH), insulin-like growth factor-I (IGF-I), and insulin in largemouth bass (Chen et al., 2012b). Chen et al. (2012b) suggested that dietary Arg level might exert an independent and positive effect on the GH and IGF-I transcription, which confirms the fundamental role of the endocrine system in the regulation of the nutrient metabolism and somatic growth of largemouth bass.

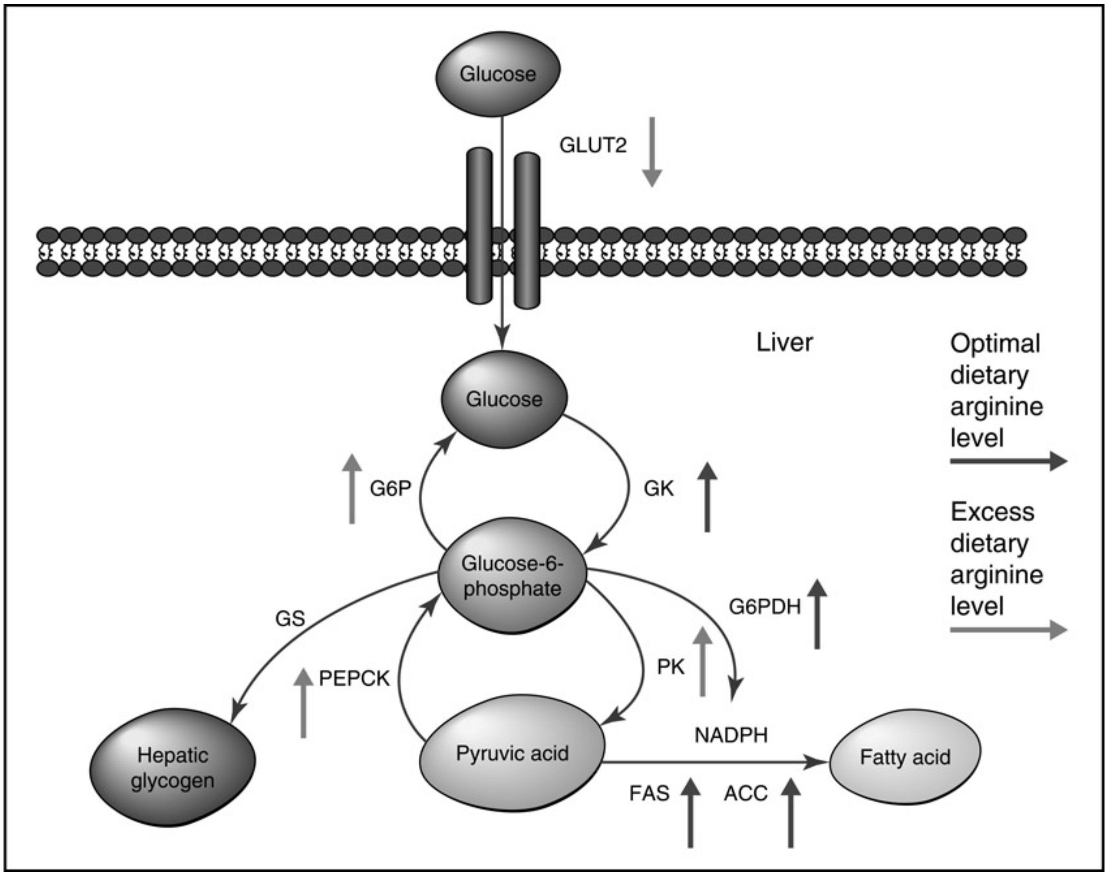

Figure 8.4 The concept of nutrigenomics: glucose and lipid metabolism signaling pathway, adopted from Liang et al. (2017). Optimal dietary arginine level (1.62%) elevated the relative expression of glucokinase (GK), glucose-6-phosphate dehydrogenase (G6PDH), fatty acid synthase (FAS), and acetyl CoA carboxylase (ACC) in fish. High dietary arginine level (2.70%) increased the relative gene expressions of phosphoenolpyruvate carboxykinase (PEPCK), glucose 6-phosphatase (G6P) and pyruvate kinase (PK), and lowered glucose transporter 2 (GLUT 2) in fish.
Source: Dietary arginine affects the insulin signaling pathway, glucose metabolism and lipogenesis in juvenile blunt snout bream Megalobrama amblycephala: Hualiang Liang, Habte-Michael Habte-Tsion, et al. Scientific Reports, volume 7, Article number: 7864 (2017) – Creative Commons.

Lipids are the energy dense macronutrients in feeds that fulfil both energy and EFA requirements of fish. A number of studies have been conducted with largemouth bass to evaluate alternative lipid sources (terrestrial plant and animal oils/fats) and EFA requirements (Coyle et al., 2000; Bright et al., 2005; Subhadra et al., 2006; Tidwell et al., 2007; Yun et al., 2013; González-

Félix et al., 2016; Huang et al., 2017; Liu et al., 2017; Yadav et al., 2018), but only few reports are available regarding immunomodulatory effects (Subhadra et al., 2006; Chen et al., 2012a; Zhu et al., 2018). Subhadra et al. (2006) used diets supplemented with 10% lipid as canola (CAN), chicken (CHK), menhaden fish oil (MFO) or CHK + MFO (50/50%), and reported no significant differences in immune parameters (hematocrit, hemoglobin, mean corpuscular hemoglobin content and serum lysozyme activity) of largemouth bass fed the test diets, but lysozyme activity was lower in fish fed a commercial trout diet compared to fish fed the CHK + MFO (50/50%) diet. The report also added that largemouth bass fed diets with 10% MFO or CAN had higher alternative complement activity than fish fed diets with CHK. In addition, an increased oxidative stress was reported in largemouth bass fed oxidized lipid that stimulated hepatic antioxidant defenses (Chen et al., 2012c). Zhu et al. (2018) found no significant effects of dietary lipids on the peroxide (malondialdehyde, MDA) and antioxidant responses (superoxide dismutase and glutathione peroxidase) except catalase activity. However, there is no report regarding the molecular effects of dietary lipid and fatty acids on largemouth bass.

Microminerals are supplemented to aquaculture feeds due to their essential roles, and their low and/or variable levels in practical feedstuffs (Hunt, 2003). Elements including iron (Lim et al., 2001), zinc (Sanchez-Dardon et al., 1999) and selenium (Fontagné-Dicharry et al., 2015) have been shown to have immunomodulatory functions in other fish species. However, currently there is no report regarding the effects of microminerals on the immunity and target genes of largemouth bass.

Reports of vitamin deficiency in fish farms are often linked to vitamins C and E (Hardy, 2001), and these two vitamins are frequently studied in relation to their effects on immune responses. Chen et al. (2015) reported that optimum dietary vitamin C supplementation not only increased antioxidant capacities of the liver and muscle of largemouth bass, but also exerted a sparing effect on vitamin E utilization in plasma and liver, thereby decreasing lipid peroxidation (MDA concentration). Vitamins A (Lian et al., 2017) and C (Xie et al., 2006) improved the growth, antioxidant capacity and nonspecific immunity of largemouth bass; while dietary vitamin E and selenium were found to protect largemouth bass from oxidative damage in the study of Chen et al. (2013).

Plant-protein feedstuffs are promising sources of amino acids for aquaculture feeds mainly due to their quality, competitive price and availability (Collins et al., 2012; Slawski et al., 2013; Zhou et al., 2018b). However, a major concern is the presence of antinutritional factors (ANFs) in these ingredients, including protease inhibitors, non-starch polysaccharides and phytic acid, which can adversely affect the growth and health of fish. Hence, besides novel processing technologies designed to reduce ANF levels and improve the nutritional value of traditional ingredients, nutrigenomic tools are very important to predict long-term detrimental effects (for example, subtle physiological changes) of dietary modifications on farmed aquatic animals, including largemouth bass.

8.7 Conclusion

Culture of largemouth bass as food-fish continues to expand and knowledge on nutritional needs of the species is a premise for efficient and profitable production of nutritious fish to consumers. As for any farmed fish, the optimization of feeds for largemouth bass relies upon accurate information on nutritional requirements, quality and nutritional value of feed ingredients, proper use of key feed additives that aid in supporting optimum production performance and health, and precise formulation of feeds for different life stages.

Research on EAA requirements of largemouth bass must be intensified as only three out of ten EAA have been studied to date. As aquaculture feeds continue to depart from the use of traditional marine-based proteins to the use of terrestrial plant and animal-based protein sources, proper supplementation of EAA based on quantitative requirements is critical for supporting growth and feed efficiency, and to minimize nutrient load to receiving waters.

Information on mycotoxin effects on health and production performance of largemouth bass, as well as upper limits thereof in feeds for this species is lacking. This is of particular importance considering that mycotoxin occurrences in feeds are increasingly more common, primarily due to the extensive use of plant-based ingredients prone to contamination.

Finally, yet importantly, information on the immunonutrition and nutrigenomics of large-mouth bass also needs to be expanded. It is reasonable to assume that, as for other species, both approaches represent new tools allowing nutritionists to prevent or attenuate challenges related to health and production of largemouth bass.

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Chapter 9
Practical feeds and feeding

James H. Tidwell

Kentucky State University, Aquaculture Research Center, Frankfort, Kentucky, USA

9.1 Evaluation of largemouth bass on feed

The largemouth bass is highly regarded as a sport-fish in the USA and around the world. The species has been cultured since the late 1800s (Heidinger, 2000). Federal hatcheries began producing the fish in the early 1900s, but all early work was in support of stocking programs. Because of the Farm Pond Program of the 1930s there was a greater impetus for increased production of stocker largemouth bass (Simco et al., 1986). Most of this production targeted large numbers of small juvenile fish (2.5–5.0 cm) to establish new sport fisheries. Later, to give management options for sport-fish stocking, larger fingerlings (> 10 cm) were needed (Simco et al., 1986).

This greater need for larger sizes presented a problem for hatchery managers. The largemouth bass is known for its aggressive, predacious nature. During early life, the fry are raised in fertilized ponds where they feed on zooplankton, microcrustaceans, and small insects. However, at about 3.8–5.0 cm the fingerlings switch to becoming more piscivorous. If fingerlings are left in the ponds, small sized natural foods become depleted and losses to cannibalism significantly reduce production (Simco et al., 1986).

Different approaches to largemouth bass fingerling production have been utilized. If the number of required fingerlings is small the Spawning-Rearing Pond System can be utilized. Broodfish are stocked at 25–100 broodfish/ha and the pond is fertilized to promote zooplankton blooms. Fingerlings are harvested at ≤ 5.0 cm and ≤12,000 fingerlings/ha are produced. However, predation on fry by the broodfish can be severe. Another method is the Fry Transfer Method. To maintain water clarity, ponds are not fertilized. Schools of fry are captured and transferred to separate fingerling rearing ponds which are fertilized to establish and maintain zooplankton blooms. If target sizes are 2.5–5.0 cm rearing ponds can be stocked at 120,000–240,000 fry/ha. However, if the target size is 10 cm fingerlings, stocking sizes as low as 24,000/ha may be required. Properly managed rearing ponds yield 30–150 kg/ha of fingerlings at 3.8–5.0 cm total length (TL). To produce 10 cm fingerlings 50% survival is considered acceptable, yielding < 5,000 10 cm fish from a 1 ha pond.

As long as the food provided is limited to natural foods, production will be low. In species feeding low on the food chain, organic and inorganic fertilizers can increase primary productivity. The target fish species still relies on natural foods but the carrying capacity of the pond can be increased. As a top predator, largemouth bass may not respond to pond fertilization as well as species feeding lower on the food chain. Because of this, production of largemouth bass fingerlings, especially of advanced sizes (≥ 10 cm), requires a very large amount of pond resources to produce even relatively low numbers of fish.

In other species, a shift to prepared (pelleted) feeds is the most effective way to intensify production (that is, produce more kilograms of fish per hectare of pond). In some species, such as rainbow trout and channel catfish, first feeding fry will readily accept artificial diets. For the largemouth bass, a shift to a pelleted feed requires that the fish first be "trained" to accepted non-living food. This can be done during a critical period of development when in nature the fish are going through a changeover from consuming zooplankton and small insects to being a piscivore and feeding on other fish. This natural "reprogramming" phase can be hijacked to now program the fish to accept artificial diets. This process is very important to the modern intensive production of largemouth bass and it has an entire chapter devoted to it (see Chapter 6). Once trained to accept artificial feeds, largemouth bass retain the learned behavior indefinitely (McCraren, 1974). The remainder of this chapter will focus on feeding fish after feed training and the development of practical diets.

As previously stated, to increase the number of advanced fingerlings (≥ 10 cm) that could be produced per unit of hatchery pond space, an Intensive Culture System for largemouth bass was needed. As early as the 1930s, Langlois (1932, 1933) conditioned largemouth bass fingerlings to consume ground fish. In a series of studies J.R. (Jack) Snow at the Marion National Fish Hatchery in Alabama developed the "Marion Program" which established the basic procedures used today to produce large numbers of advanced fingerlings (15–20 cm) using artificial diets (Simco et al., 1986).

Early feeding trials by Snow (1963, 1968) first utilized a manufactured artificial diet developed for salmon, the Oregon Moist Pellet (OMP). It was felt that the soft texture was more acceptable to the largemouth bass than the hard-dry pellets used for trout and catfish. Snow and Maxwell (1970) reported that they produced 3,000 kg/ha of 10–23 cm fish feeding this diet. This represents a 1,900% increase in production per unit of pond area over the 150 kg/ha reported for fertilized ponds (Simco et al., 1986). However, diets with > 10% moisture levels tend to be expensive and require refrigerated storage. As largemouth bass have become more adapted to intensive culture conditions, succeeding generations have become more amenable to hard-dry pellets.

Growout to sizes for advanced stockers (15–20 cm) for corrective stocking can be achieved in the first summer of growth. However, to achieve sizes desired for food-fish (600–900 g) requires a second summer of growth in temperate ponds (see Chapter 7). Current procedures are variations of the following steps. After feed training fish can be maintained in tanks actively feeding on pellets for up to 2 weeks. Fingerlings can then be stocked into ponds for first-year growth at 50,000–75,000/ha. They are fed two to three times per day using a 2.3 mm diameter pellet containing 40 to 48% protein diet with 8 to 10% fat. Most producers currently use commercial diets formulated for salmonids. However, diets formulated specifically for largemouth bass are becoming commercially available. Feed the fish all they will actively consume at each feeding. Largemouth bass will feed voraciously on some days and not so actively on others. Differences are likely associated with water temperature, sunlight, and or water quality changes (Tidwell et al., 2000).

9.2 Feeding rates

In terms of feeding rates, a number of different factors come into play. Small fish need high protein levels in small pellets, and a feed volume at a high percentage of their body weight (BW) per day. As they grow the protein requirement decreases, the pellets get larger, and their food consumption as a percentage of BW decreases. Davis and Lock (1997) indicated that after being stocked into ponds the fish should be consuming about 15% of total biomass daily and that rate will gradually drop to about 5% of biomass per day. However, the total amount of feed being fed (kg/ha pond/d) will increase as the fish get larger. According to Boyd (1990) when feeding rates exceed about 30 kg feed/ha/d episodes of low oxygen can become a problem. Simco et al. (1986) reported that standing crops of 1,684 to 2,245 kg/ha of largemouth bass might be raised before low oxygen levels become a limiting factor. That equates to a feeding rate of approximately 20 kg/ha/d in unaerated ponds.

Under conditions where aeration is available and oxygen levels are monitored and managed, total ammonia-nitrogen becomes the limiting factor to acceptable feed rates and biomass densities. Kubitza and Lovshin (1997) reported maximum daily feed rates of 87 kg/ha for first-year largemouth bass stocked at 67,136 fish/ha and having a gross yield of 7,281 kg/ha at harvest. The authors reported having total ammonia-nitrogen (TAN) values of 4.0 mg/l in the same pond in October; demonstrating the risks associated with high stocking densities and associated high feed rates. Tidwell et al. (1996) also reported elevated levels of TAN during cooler water temperatures and lower feeding rates of fall and winter in intensively fed largemouth bass ponds receiving high protein diets (47%) and having total gross yields at harvest of 5,330 kg/ha. The authors suggested that high ammonia readings in the fall was likely due to reduced assimilation of ammonia by phytoplankton and reduced bacterial nitrification at cool temperatures (Tucker and Robinson, 1990). Cochran et al. (2009) reported maximum TAN readings of < 0.5 mg/l in intensively fed second-year largemouth bass ponds achieving gross yields at harvest of < 3,020 kg/ha. More recently much higher feeding and production rates have been achieved (Chapter 7).

Efficient feeding practices can be as important as having a diet properly formulated for the species being fed. A perfect diet that is not fed efficiently decreases feed conversion efficiency and increases the cost of production. In largemouth bass feed costs were estimated at 51% of total operating costs. (Engle et al., 2013). Under commercial culture conditions largemouth bass are usually fed floating feeds to satiation and feeding rates are limited by stocking densities which are limited by targeted biomass densities of usually > 5,600 kg/ha. During the early fall when water temperatures drop to 20°C, largemouth bass will normally consume approximately 1% of their BW which can be used to estimate total standing crop.

9.3 Feeding frequency

Feeding frequency refers to the number of times a day that fish are fed in culture systems. Feeding frequency is an essential consideration in fish feed management as it affects growth, feed conversion, water quality as well as profit maximization. The optimal interval between meals is related to environmental factors, such as water temperature and water quality, as well as the diet fed, the size of the fish and the species. Other factors that can affect optimization of the feeding regime are the nutrient density of the feed, the maximum voluntary food intake in one meal, and the evacuation rate of the stomach. These parameters have not been studied in largemouth bass, but have for better studied finfish species from which inferences can be made.

Riche et al. (2004) determined that gastric evacuation rate and return of appetite following a satiation meal for Nile tilapia (*Oreochromis niloticus*) to be 4 h at 28°C suggesting that tilapia should be fed three or four times per day. Ruohonen et al. (1998) determined that in order to achieve maximum growth rainbow trout (*Oncorhynchus mykiss*) should be fed at least three times per day and that feeding higher nutrient dense diets reduces the feeding frequency required to achieve maximum growth. Jobling (1983) reported that dominance hierarchy effects increased as access to food became more limiting in Arctic charr (*Salvelinus alpinus*). The authors observed that feeding by the majority of the fish was inhibited by the presence of larger individuals. Thia-Eng and Seng-Keh (1978) determined the optimal feeding frequency for food intake, weight gain and feed conversion efficiency of estuary grouper (*Epinephelus tauvina*) to be every 48 h or every other day.

Clearly, there are species specific differences related to appropriate feeding frequencies in fish. However, there are also some generalities that seem to be common to all species. Generally, younger fish perform better when fed more often than do older, larger fish. Smaller fish have higher energy demands than larger fish and usually their stomachs are too small to hold all the feed they require during one feeding a day. Herbivorous species and grazers (tilapia) require more frequent feedings than predatory species (grouper) due to inherent differences in gut length associated with the breakdown of lower nutrient dense feedstuffs.

As a robust predator largemouth bass have relatively large mouths and stomachs designed for swallowing large prey items and consequently do not need to be fed as often as some other species. Additionally, largemouth bass are often fed highly nutrient dense diets, such as Skretting's Classic Bass formulation with 48% protein and 18% fat (Skretting, Utah, USA), further reducing the necessity of multiple daily feedings. Under commercial culture conditions, fry and small largemouth bass fingerlings (< 7.5 cm) are fed up to eight times a day or as few as two times a day provided they are thoroughly satiated. During the feed training stage largemouth bass are often fed several times per day; although the feed is poorly utilized until the fish are habituated. Larger fingerlings (5.0–25 cm) seem to benefit from two feedings per day (morning and evening) during the first summer growing season. Largemouth bass >25 cm are typically fed only one time per day for further growout to food-fish size.

To avoid the effect of dominance hierarchy on feeding, some largemouth bass producers use feed blowers mounted to a truck (Figure 9.1) or utility vehicle to quickly spread feed over the entire length of the pond levee (personal communication, Robert Mayer, Bardstown, Kentucky, USA). The most aggressive fish in the pond tend to swim alongside the vehicle as the feed is being spread allowing for subordinate fish to feed freely. This is difficult to achieve when feeding by hand. Additionally, largemouth bass feed best early in the morning (30 min after sunrise) and late in the evening (30 min before sunset). During the hottest months of the summer it may be preferable to feed in the morning when the water temperatures are cooler.

Figure 9.1 A truck mounted feed blower. It allows feed pellets to be uniformly spread over the pond surface.

9.4 Types of pellets

A single type of feed is not fed all the way through a fish's growout. As a fish grows, usually the pellet size increases and the protein content decreases. Also, as a fish grows, the feeding rate as a percentage of bodyweight decreases while the actual amount of feed fed increases. Other factors involved in efficient feeding include the correct choice of pellets with the proper physical characteristics. There are a number of pellet forms and manufacturing options to produce them.

9.5 Fry and fingerling feeds

Meal type feeds are fine particles used to feed first feeding fry in species that go directly from endogenous feeding (relying on nutrition in the yolk sac) to accepting artificial diets immediately upon switching to exogenous feeding (utilizing external sources of food). Largemouth bass fry do not readily accept artificial feeds at these early stages, so meal type feeds are not currently utilized in their production. During the feed-training process for largemouth bass, the artificial diets that the fish are transitioned to are usually pellets of 1.0–2.5 mm. These may be sinking pellets (steam pelleted) or sometimes floating pellets (extruded pellets). As the fish train to the feed and grow, pellet sizes are increased gradually to 5.5 mm during the first year of growth.

9.6 Development of practical growout/production diets

In early work on feed-trained largemouth bass at the Marion Fish Hatchery, Snow (1968) utilized a ration composed of 80% ground frozen fish, 10% dry commercial trout feed, and 10% ground beef liver. Snow (1968) fed this mixture at rates up to 100 kg/ha/d and estimated this to be 5.3 to 11.2% of total BW (biomass) per day. The author also reported that artificially fed bass readily adjusted back to consuming forage fish if needed and that artificially fed largemouth bass achieved spawning success similar to broodfish raised on natural foods.

As noted previously, Snow and colleagues at the Marion, Alabama, National Fish Hatchery in 1968 began trials evaluating the OMP for use in largemouth bass production. The OMP was based on 10 years of research and was successfully tested in several salmonids including chinook, coho, and sockeye salmon and steelhead trout. The OMP proved to be more economical (34% savings compared to ground fish diets) and relatively efficient (food conversion ratio [FCR] 2.3). They also reported less disease problems with largemouth bass raised on OMP. Snow and Maxwell (1970) reported that largemouth bass in ponds fed the OMP "were more consistent" with respect to yield, feed conversion, survival, and rate of gain than ponds receiving other diets. Consistency of production is very important to both hatchery managers and commercial producers.

However, the OMP had some disadvantages, a primary one being it had to be stored under refrigeration (Hublou, 1963). This is due to moisture contents of 30–35%. To achieve longer shelf life dry pelleted feeds usually contain less than 10% moisture. Reducing moisture also reduces weight, resulting in substantial savings in shipping and handling costs. Piper et al. (1982) recommended a moist feed, such as OMP for largemouth bass, but also said a quality dry feed such as W-7 coolwater fish feed could be used. They also presented a feeding rate chart for largemouth bass on dry feeds (Table 9.1).

Table 9.1 Bass feed chart: percent body weight fed per day in raceway culture for formulated dry feeds (Piper etal., 1982).

	Size (inches)				
	1–2	2–3	3–4	4–5	5+
	Weight (pounds)				
Water temperature (°F)	0.002	0.002–0.015	0.015–0.03	0.03–0.06	0.06
65	4.4%	4.0%	3.2%	2.4%	1.6%
70	5.5%	4.7%	2.5%	2.2%	2.0%
75	6.0%	5.0%	4.0%	3.0%	2.0%
80	6.5%	5.4%	4.3%	3.3%	2.2%
85	7.1%	5.9%	4.7%	3.5%	2.4%
90	7.5%	6.3%	5.1%	3.9%	2.7%
Feedings per hour	4	4	2	1	1

Note: Winter feeding rate: 1% body weight per day.

Much of the work referenced up to this point was still focused on producing fish for stocking programs and sizes (≤ 100 g). However, demand for larger fish increased during the 1980s (Brandt, 1991), based largely on increasing utilization of larger largemouth bass in fee-fishing (JSA, 1983), managed trophy fisheries (Dupree and Huner, 1984), and as live food products in ethnic markets. In the USA in 1983, the Joint Subcommittee on Aquaculture (JSA) listed research priorities for developing largemouth bass aquaculture. These priorities included determination of efficient growout procedures, evaluation of effects of water quality (metabolic wastes) under intensive culture conditions, and development of species-specific, cost-efficient, diets utilizing practical feed ingredients (JSA, 1983). The Joint Sub-Committee proposed that if this information can be generated, there appears to be a favorable financial potential for commercial production of this species.

Beginning in 1995, researchers at Kentucky State University (KSU) began a series of studies focused on (1) developing species specific-feeds for largemouth bass and (2) culture methods to produce large harvest sizes (≥ 500 g) intensively in ponds. Tidwell et al. (1996) stocked advanced juvenile (122 g) largemouth bass at 12,350/ha and fed three dietary protein levels (42, 44, and 47%) once daily for a full 12 months. This allowed feed consumption, water quality, and fish growth to all be monitored monthly over four seasons of growth.

In terms of water quality, overall means (over the full 12 months) for total ammonia-nitrogen (TAN), were significantly different among ponds in which fish were fed the 42, 44, and 47% protein diets, averaging 0.62, 0.83, and 1.3 mg/1, respectively (Figure 9.2). Differences in concentrations of TAN at individual sampling dates occurred primarily during periods of low water temperatures, especially December, January, and February. Concentrations of TAN during those periods reflected the same relationships to dietary protein levels as did overall TAN means (47% > 44% > 42% diets).

Overall nitrite concentrations for the 12 month culture period also reflected dietary protein levels (47% > 44% > 42%), though these differences were not statistically significant. There were significant treatment differences in nitrite concentrations at some individual sampling dates (Figure 9.3), primarily during periods of rapidly decreasing (November–December), and increasing (February–March) water temperatures, with ponds in which fish were fed the 47% protein feed having higher levels.

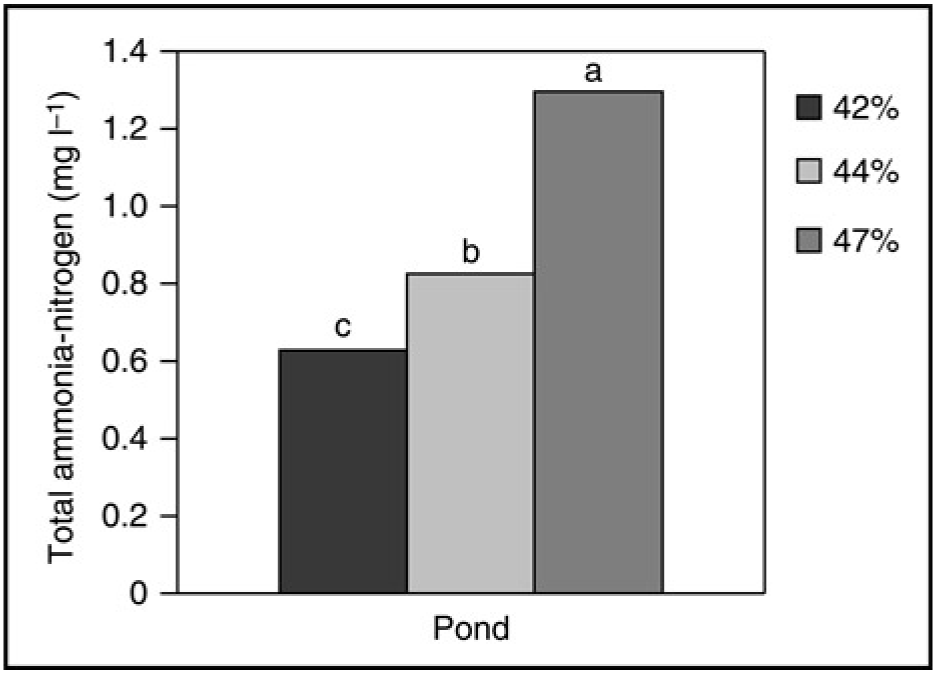

Figure 9.2 Overall means of total ammonia-nitrogen (mg l^{-1}) in ponds in which juvenile largemouth bass were fed diets containing 42, 44, or 47% protein. Each bar represents a mean of 52 weekly samples per pond in three replicate ponds per diet. Different letters above bars indicate significant differences among treatment means ($P < 0.05$).

In terms of growth and production, at final harvest largemouth bass fed the 47% protein diet had significantly higher average weights (436 g), gains (351 g), survival (99%), and total biomass (4,252 kg/ha), and significantly lower feed conversion ratios (FCR; 2.0), than bass fed the 42% protein diet (Table 9.2). Bass fed the 44% protein diet were not significantly different from those fed 42–47% protein diets in any of these variables. Significant differences in average weights developed by 2 months post-stocking. Bass fed 47% protein gained approximately 202% during

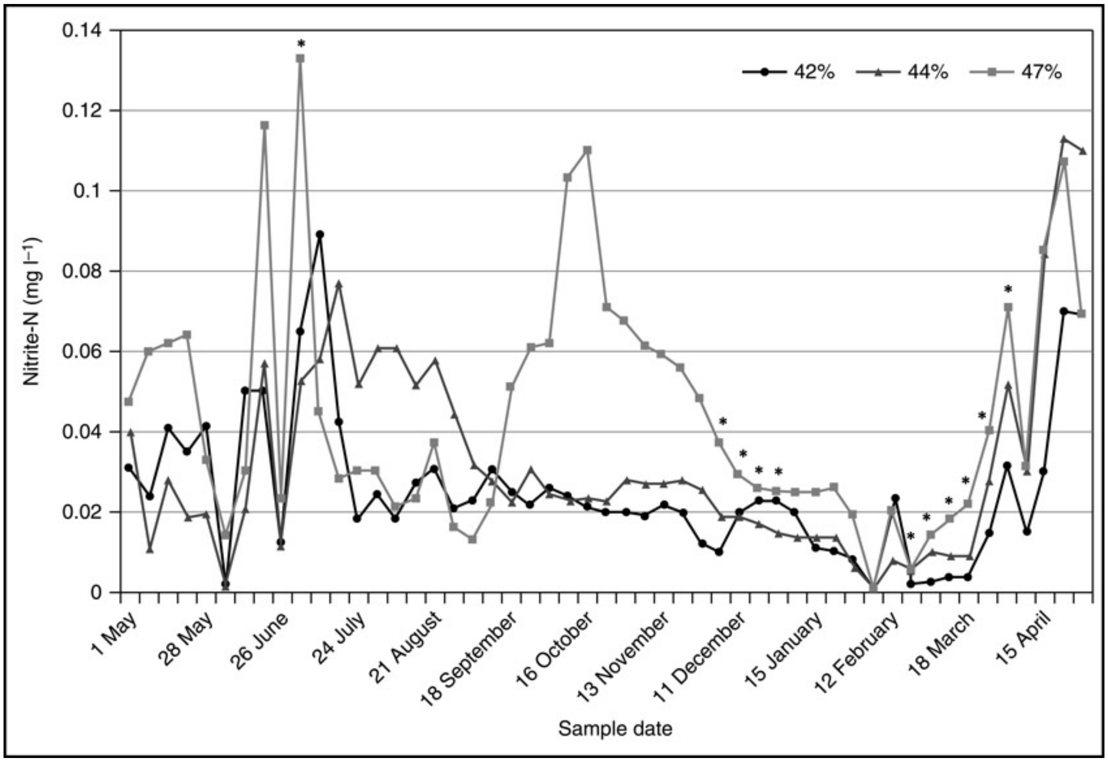

Figure 9.3 Weekly means of nitrite-nitrogen (mg l^{-1}) in ponds in which juvenile largemouth bass were fed diets containing 42, 44, or 47% protein. Each point represents four weekly samples per pond in three replicate ponds. An asterisk indicates a difference among treatments ($P < 0.05$).

Table 9.2 Individual weight, weight gain, percentage survival, feed conversion ratio (FCR), and unit production of largemouth bass fed diets containing three protein percentages.

Production variable	Dietary protein		
	42%	44%	47%
Harvest weight (g)	374± 19b	406 ± 23ab	436 ±38a
Total individual gain (g)	313±22b	331 ± 18ab	351 ±25a
Survival (%)	86 ± 6b	92 ± 7ab	99 ± la
Total FCR	2.6 ± 0.3a	2.3 ± 0.2ab	2.0 ± 0.2b
Gross yield (kg ha^{-1})	3972 ± 325b	4613 ± 469ab	5330 ±505a
Summer individual gain (%)	162.5 ± 14.8b	157 ± 7.0b	201.7 ± 6.2a
Summer specific growth rate (%)	0.54 ± 0.04b	0.53 ± 0.02a	0.62 ± 0.01a

Production variable	Dietary protein		
	42%	44%	47%
Summer FCR	2.2 ± 0.1ab	2.4 ± 0.2a	1.9 ± 0.0b
Winter individual gain (%)	19.6 ± 11.0	28.5 ± 5.4	17.3 ±5.1
Winter specific growth rate (%)	0.09 ±0.6	0.12 ±0.03	0.09 ± 0.03
Winter FCR	4.2 ± 1.9	2.4 ± 0.4	3.9 ± 1.0

Notes: Values are means of ± s.e. of three replicate ponds. Means in the same row with different letters are significantly different ($P < 0.05$).

the summer period and 17% during the winter (Table 9.2). There were no significant differences between fish in the three treatments in winter individual gains, winter specific growth rate (SGR), or winter FCR.

Dressout percentages, and head, fillet, and gut weights (as a percentage of BW) did not differ significantly among bass fed the three diets. Moisture, lipid, protein, and ash averaged 71.2, 6.0, 18.9, and 3.8% in the whole body and 76.9, 1.3, 20.6, and 1.1% in the fillet.

In terms of feeding patterns, the bass were active feeders, especially in early summer (June) and slowed later in the summer (Figure 9.4). Feeding continued until temperatures dropped below approximately 8°C. In the spring, bass began to feed actively again as temperatures rose above 8°C. This response is similar to those reported for the hybrid bluegill (*Lepomis cyanellus* × *L. macrochirus)* (Tidwell et al., 1994).

Largemouth bass feeding and growth slowed during the winter months, but winter gains (October–March) averaged approximately 22% over all three diets. When compared with another centrarchids, winter gains were similar to results for small (40 g) hybrid bluegill (28%) (Tidwell et al., 1992), and higher than winter gains of larger (100 g) hybrid bluegill (17%) (Tidwell et al., 1994). Total weight gains over the 12 month period (257%) were higher than those reported for hybrid bluegill (167%) stocked at the same density for a similar culture period (Tidwell et al., 1994). Feed conversion ratios were superior to those for hybrid bluegill, averaging 2.0 for largemouth bass, while hybrid bluegill averaged 5.7 (Tidwell et al., 1994).

Currently several fish feed manufacturers produce formulations designed specifically for large-mouth bass. Commercially available largemouth bass diets typically contain relatively high levels of protein (45–48%) and lipids (16–22%) and low levels of carbohydrate (<21%) compared to diets for other commercially important fish species. These highly nutrient dense diets are considered to be necessary for health maintenance of largemouth bass and are typically formulated using high levels of animal source proteins, primarily fish meal.

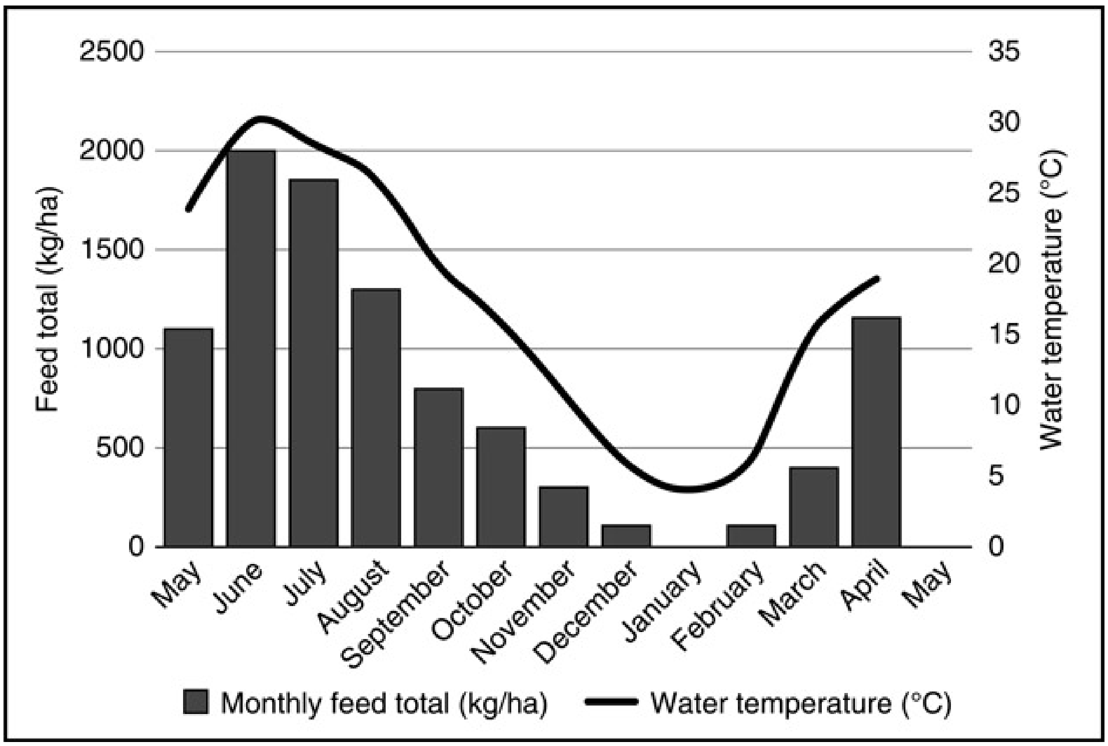

Figure 9.4 Overall monthly feed totals per pond for all diets at different water temperatures over a 1 year culture period.

9.7 Effects of stocking density on feed response and feed conversion

In an associated study, Tidwell et al. (1998a) also evaluated the impact of stocking density on growth and feed conversion of advanced largemouth bass fingerlings. Feed-trained fingerlings (122 g) were stocked in May at either 6,175 or 12,350/ha and fed a 44% protein diet once daily to satiation for a full 12 months. At harvest, there was no significant difference in weight gain or average weight of largemouth bass stocked at 6,175 or 12,350 fish/ha (Table 9.3). These data differ from those reported by Tidwell et al. (1994) for hybrid bluegill (also a centrarchid) for whom increasing stocking density from 6,175 to 12,350/ha significantly decreased weight gain and average weight at harvest. Tucker and Robinson (1990) stated that for channel catfish increasing fish density normally decreases average fish size. Total yield was greater for largemouth bass stocked at 12,350/ha than at 6,175/ha (4,598 and 2,354 kg/ha, respectively). At the higher stocking density, feed was more efficiently utilized, as indicated by a significantly lower FCR (2.3) compared to the low stocking density (3.3). Results differ from those for catfish, where higher stocking densities usually result in higher feed conversion ratios due to reduced feed intake and decreased efficiency of feed conversion (Tucker and Robinson, 1990).

Tidwell et al. (1996) reported that the most active feeding period for largemouth bass was June–July (> 25°C). However, in this study largemouth bass stocked at the lower density consumed more feed and gained weight more rapidly during the fall period of declining water temperatures (September–November), while those stocked at high density consumed more feed and gained weight more rapidly during the rising water temperatures of spring (March–May) (Figure 9.5). At final harvest, average weights of fish in the two treatments were similar. Stocking largemouth bass juveniles at the higher density (12,350/ha) resulted in higher net and gross yields and more efficient feed conversion than stocking at low density, without decreasing average individual weights or reducing survival.

Table 9.3 Initial individual weight (mean ± SE), final individual weight, final lengths, percentage, survival, average individual gain, total yield, and feed conversion ratio (FCR) for largemouth bass fed prepared diets for one year in ponds. Means within a row followed by the different letters are significantly different ($P < 0.05$).

	Stocking rate (fish/ha)	
	6,175/ha	12,350/ha
Stocking weight (g)	124 ± 4	122 ± 2
Final weight (g)	406 ± 32	406 ± 22
Weight gain (%)	282 ± 29	283 ± 23
Final length (cm)	29.3 ± 0.8	29.1 ± 0.5
Survival (%)	93.9 ±8.5	91.7 ±6.7
Total yield (kg/ha)	2354 ± 242b	4598 ± 418a
FCR	3.3 ± 0.3a	2.3 ± 0.2b

There is demand for large (> 500 g) largemouth bass for corrective stocking in sport-fish ponds, fee-fishing, and managed trophy fisheries. A study was conducted at KSU to evaluate the effects of stocking density on third-year growth of feed-trained largemouth bass raised in ponds (Tidwell et al., 1998b). Lovell (1989) observed that feed consumption, growth rate, and feed conversion efficiencies generally decrease as fish size increases. Busch (1985) demonstrated in channel catfish that growth slowed and feed conversion ratios increased during third-year growth. Tidwell et al. (1998b) reported that during second-year growth largemouth stocked at higher density had significantly lower FCRs than those stocked at low density. The objective of this study was to evaluate the effects of stocking density on growth and feed conversion of largemouth bass during third-year growth.

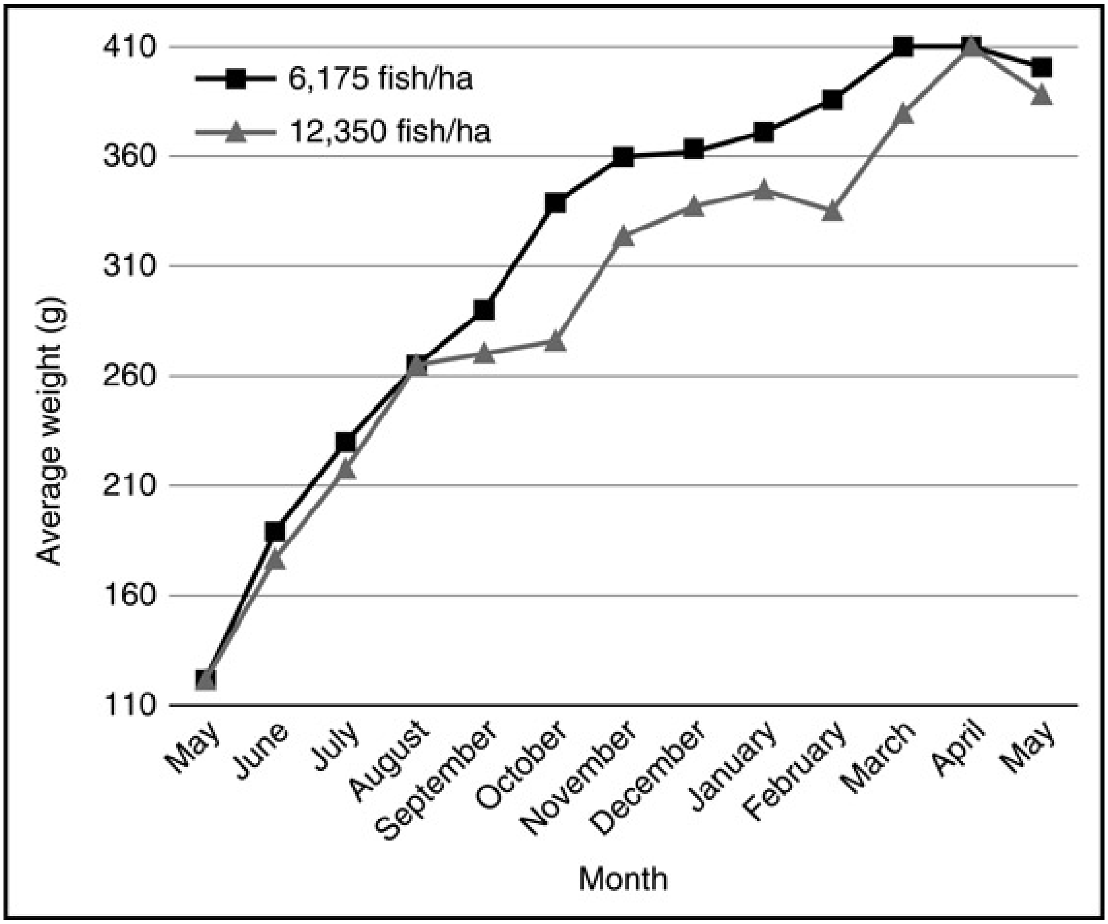

Figure 9.5 Mean sample weights of largemouth bass stocked at 6,175 or 12,350 fish/ha. There were no significant differences ($P \geq 0.05$) in average weight at any sampling date.

Juvenile largemouth bass (383 g) were stocked in May at either 3,750/ha or 7,500/ha and fed a 44% protein diet once daily to satiation for 334 days. In terms of weight gain the pattern of weight gain in this third-year study (Figure 9.6) differs from that reported in Tidwell et al. (1998a) for second-year largemouth bass. The primary difference was during the period of August to November in second year fish those stocked at low density gained more weight, while in third-year fish those stocked at high density gained more weight. Production data are presented in Table 9.4. There was no significant difference in individual weight gain, with an overall average harvest weight of 536 g and average individual gain of 40%. This compares to 113% individual weight gain for channel catfish during third-year growth

(Busch, 1986). Since increased density did not reduce average fish size, treatment densities resulted in large differences in pond production data, with the high-density treatment producing significantly greater total yields and pond unit production rates than the low-density treatment (Table 9.4).

When we look at feeding activity and efficiency of feed utilization, the differences in feed conversion ratios were large (9.6 and 3.8 in low- and high-density third-year ponds, respectively) but not statistically significant due to high within-treatment variation. Feed conversion ratios for third-year bass at high densities (3.8) were much higher than those reported for second-year bass at high densities (2.3; Tidwell et al., 1998a, 1998b). These data agree with Lovell (1984) who stated that growth rate and feed conversion efficiencies generally decrease as fish size increases. Busch (1986) reported an FCR of 2.2 for third-year channel catfish. Average individual gains and FCRs averaged over both treatments (58% and 7.3, respectively), were similar to those reported for third-year walleye production in ponds (45% and 9.1, respectively) (Coyle and Tidwell, 1997). High FCRs are usually indicative of overfeeding, inefficient utilization of consumed feed, or both. In this study feeding rates were based on fish response. This required that some feed be presented to fish in each pond to allow the opportunity to feed. On days when fish did not respond, this feed was wasted. This was especially true during the winter. During summer months, fish normally consumed feed but apparently did not convert efficiently.

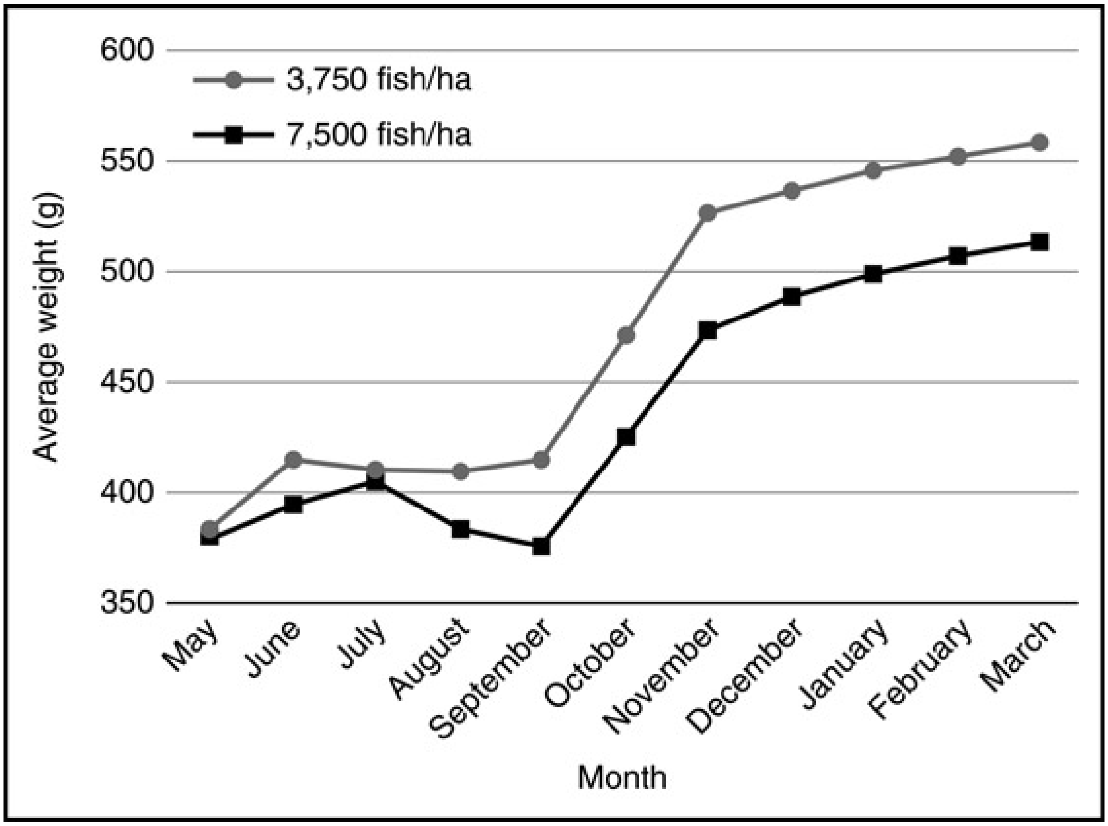

Figure 9.6 Mean sample weights of largemouth bass stocked at 3,750 and 7,500 fish/ha. There were no significant differences ($P \geq 0.05$) in average weight at any sampling date.

Table 9.4 Initial individual weight (mean ± SE), final individual weight, final lengths, percentage, percent survival, average individual gain, total yield, and feed conversion ratio (FCR) for largemouth bass fed prepared diets for one year in ponds. Means within a row followed by the different letters are significantly different ($P < 0.05$).

	Stocking rate (fish/ha)	
	3,750/ha	7,000/ha
Stocking weight (g)	380 ± 24a	366 ± 5a
Final weight (g)	514 ± 38a	559 ± 7a
Weight gain (%)	35 ± 5a	47 ± 5a
Final length (cm)	92.3 ± 4.0a	82.7 ± 16.1a
Survival (%)	341 ± 123b	1,040 ± 66a

	Stocking rate (fish/ha)	
	3,750/ha	7,000/ha
Total yield (kg/ha)	1,758 ± 230b	3,909 ± 19a
FCR	9.6 ± 4.0a	3.8 ± 0.4a

9.8 Effect of reduced fish meal levels on feed consumption and growth

Other factors are known to affect feed acceptance and feeding rate in fish. As a carnivore, large-mouth bass do not always readily accept diets with low levels of fish meal. Cochran et al. (2009) fed diets containing varying levels of fish meal (8, 24, and 45%) to largemouth bass under practical production conditions in ponds. They reported that the average daily feed consumption, average harvest weight, survival, and total production did not differ among fish fed the experimental diets. The feed conversion ratio was very good for fish fed all three diets and was significantly lower (1.34) in fish fed the 24% FM diet than in fish fed the 45% FM (1.43) diets.

The authors in Cochran et al. (2009) noted that the custom manufactured pellets were "slow sink" and were more readily consumed than the commercial floating pellets (used as a commercial control reference diet) especially on bright sunny days. This indicates that physical pellet characteristics might still be a factor to be evaluated in largemouth bass.

The trend toward reducing fish meal in diets for carnivore fishes, such as largemouth bass continues. As discussed, Cochran et al. (2009) demonstrated that practical diets containing 8% fish meal were still consumed well and performed well in ponds. Tidwell et al. (2005) evaluated largemouth bass diets containing 0% fish meal in tanks. Poultry by-product meal (PBM) successfully replaced all fish meal with no significant impacts on growth, survival, or feed conversion ratio. However, PBM is an animal source protein. All plant protein diets were not successfully evaluated.

9.8.1 Feed attractants

One approach to improve the consumption of plant protein-based diets in other species has been the use of chemoattractants or feed enhancers. For the hybrid striped bass, (*Morone saxatilis*) certain amino acids (alanine, serine, glycine, and proline) have been reported to be efficient feed attractants as were fish solubles (Papatryphon and Soars, 2000). Fish solubles (FS) were reported to increase feed intake in red drum (*Sciaenops ocellatus*) fed a soybean based diet (McGoogan and Gatlin, 1997) but were not beneficial for red drum fed diets containing fish meal (Davis et al., 1995).

Kubitza and Lovshin (1997) conducted a series of trials to identify feed enhancers for large-mouth bass. In their Trial 1, fish meal (FM) was decreased (60, 40, 20, and 0%) and replaced by 20, 40, and 60% soybean meal (SBM). As the fish meal was decreased and SBM increased, feed intake declined. In Trial 2, the 0% FM diet was then used as a Control diet to test candidate feed enhancers (including eight amino acids, two nucleotides, and betaine). The betaine and amino acids did not affect feed consumption but nucleotides increased feed intake by 45% compared to the Control diet. A third trial identified the nucleotide inosine-5-monophosphaate (IMP05') as the most effective chemical feed enhancer tested. However, including even 10% fish meal in the formulation was more effective than adding the nucleotide.

A study evaluating attractants in largemouth bass fed 100% plant protein diets compared soluble fish protein (SFP), Fisharon (a commercial product; FA), and fish silage (FS) (Oliveira and Cyrino, 2004). Only the Fisharon produced positive results. Identifying products with feed attractant properties is not always an intentional process. Y.-J. Chen et al. (2012) conducted a study to evaluate possible detrimental effects of oxidized (rancid) dietary lipid on the growth and health of largemouth bass. Unexpectedly, they found that the oxidized oils actually enhanced feed intake, fish growth, and the efficiency of feed conversion. This is unusual as use of rancid oils in fish diets is usually associated with poor palatability of the diet and reduced feed intake (Kestemont and Baras, 2001; Fontagne et al., 2006).

9.9 Pellet characteristics

Early commercial fish diets were manufactured by steam pelleting into densely compressed particles that sink. Later, extrusion processed feeds that float on the water surface became more common. Floating feed is a valuable management tool because it allows the farmer to see how much, and how actively, the fish eat (Mgbenka and Lovell, 1984). Extrusion can also increase the water stability of the pellet (Lovell, 1984). However, the energy costs to produce extruded feeds are higher. In the manufacture of extruded feeds, the ingredient mix is heated to a higher temperature. Also, extruded feeds require heat in drying, while pelleted feeds do not (Mgbenka and Lovell, 1984). However, in some species the lower nutrient density and greater pellet stability of extruded diets prolonged gastric emptying time compared with steam pellets and consequently reduced feed intake (Hilton et al., 1981; Venou et al., 2009) and even growth (Booth et al., 2000, 2002; Honorato et al., 2010).

The size and shape of the feed pellet may also affect the amount of feed consumed (Jobling, 2001). The pellet size of fish feeds should be as large as possible to minimize nutrient leaching (Lovell, 1989). Optimally, pellet size should be 25–50% of the species, mouth width (Jobling, 2001). Kubitza and Lovshin (1997) fed age 1 largemouth bass (initial weight, 62 g) a 7 mm trout pellet and reported that pellet size may have been too small. They indicated that largemouth bass strike at individual pellets and many strikes would be required to satiate a fish. Kubitza and Lovshin (1997) proposed that by feeding a large pellet to largemouth bass, less feeding energy would be expended per meal and faster growth might be attained.

To evaluate the relative performance of largemouth bass fed feeds with different pellet characteristics (sinking or floating and pellet size) under practical pond conditions, Tidwell et al. (2015) produced three experimental diets, all containing 43% protein and 17% lipid. Floating pellets were produced with diameters of 5.5 mm (control) and 13.0 mm (large). Sinking pellets were produced at 5.5 mm. Nine 0.0-ha ponds (three replicates per treatment) were stocked with largemouth bass averaging 185 g at 10,000 bass/ha. Fish were fed once daily (0800) to apparent satiation for 151 days.

9.9.1 Effect of pellet size

Both pellet sizes of the floating diet were well utilized by the fish throughout the experiment. Harvest data indicated that standard size (control, 5.5 mm) versus large (13.0) floating pellets produced no significant impact on production characteristics during second year growth of large-mouth bass in ponds. Although Kubitza and Lovshin (1997) proposed that largemouth bass might benefit from increased pellet sizes, they did not conduct a controlled comparison. While Tidwell et al. (2015) showed no benefit in feeding larger pellets, Nortvedt and Tuene (1995) reported that feeding larger pellets had improved feed conversion efficiencies in Atlantic halibut *Hippoglossus hippoglossus.* Linner and Brannas (1994) reported that in Arctic Char *Salvelinus alpinus* responded more rapidly to larger pellets, but pellet consumption was better at intermediate sizes.

9.9.2 Effect of floating versus sinking pellets

The largemouth bass fed the 5.5 mm sinking pellets were significantly larger at harvest (629 g) than those fed a similar size floating pellet (566 g). Specific growth rate was also significantly higher ($P \leq 0.05$) in fish fed the sinking pellet (206 g/d) than in those fed the floating pellet (2.2 g/d). There was no significant difference ($P > 0.05$) in survival (>90%), total yield, condition factor, FCR, or average daily feed consumption among fish fed the similar size floating or sinking diets. Total yields in our study were higher than for the largemouth bass reported by Cochran et al. (2009), who compared diets containing different fish meal levels but stocked at a lower density (8,650/ha versus 10,000/ha). Fish fed the 5.5 mm sinking pellets, compared with those fed the large (13 mm) floating pellets, had production metric differences similar to the differences between the 5.5 mm sinking versus floating pellets.

The larger average size at harvest and higher SGR of largemouth bass fed sinking pellets are in agreement with findings of Booth et al. (2000, 2002) for silver perch *Bidyanus bidyanus* and with Honorato et al. (2010) for pacu *Piaractus mesopotamicus*. According to Sorensen (2012), the lower bulk density (less weight per unit volume) of floating pellets compared with sinking pel-lets would result in fish fed floating pellets becoming physically satiated at a lower energy intake. This is supported by Mgbenka and Lovell (1984), who reported that sinking feeds had less bulk per unit of weight (that is, were denser) and less digestible energy. Dense pellets with low energy concentrations would allow fish to consume more of the nutrients essential to growth before becoming satiated by energy intake or stomach fullness.

There are also behavioral aspects associated with feeding sinking pellets versus floating. Kubitza and Lovshin (1997) proposed that use of sinking pellets might result in more wasted feed (that is, higher FCR) because largemouth bass were not likely to pick up pellets off the bottom. However, we found FCR was not different among fish fed floating or sinking pellets, indicating the efficient use of sinking pellets. Both studies utilized satiation feeding. Other behavioral traits might also affect the suitability of different pellet characteristics. Kubitza and Lovshin (1997) also observed that, when using floating pellets, largemouth bass were reluctantly forced into surface waters with high light levels and temperatures (≥ 30°C). Cochran et al. (2009) also observed that largemouth bass were hesitant to feed on floating pellets at the surface on bright sunny days, but would readily consume pellets that would "slow-sink" through the water column.

Another potential positive aspect of the use of sinking feeds for largemouth bass is that the manufacture of sinking pellets allows lower dietary carbohydrate levels. To product floating pellets a formulation must contain ≥ 20% carbohydrate for proper expansion (Lovell, 1989). However, as a strict carnivore, largemouth bass have been found to be negatively impacted by carbohydrate levels of 20% (Goodwin et al., 2002; Amoah et al., 2008). Use of sinking pellets would allow more flexibility in feed formulation and reduction of carbohydrate content. Also, it would increase the potential of more localized feed production because steam pelleting facilities are more common and less complex than extruders.

A potential approach to capitalize on the positive results of sinking pellets, without sacrificing the positive feed management characteristics of floating pellets, could be to feed primarily sinking feed but mix in some floating pellets as "indicator pellets" to assist in monitoring feeding activity and consumption, as evaluated by Mgbenka and Lovell (1984) in channel catfish *Ictalurus punctatus*. They proposed the feeding of a 15: 85 ratio of extruded to pelleted diets to reduce costs while providing the same management benefits of feeding observation.

9.9.3 Effect of water temperature on feed consumption

Largemouth bass, as are other fish, are very much influenced by their environment. As poikilothermic animals, water temperature is one of the most powerful environmental variables, largely controlling their metabolic rate and as such, their feed consumption rate. Johnson and Charlton (1960) evaluated the effects of water temperature on the metabolism and activity of fingerling largemouth bass (6–10 g). They reported that food consumption, swimming speed, and scope-for-activity rose steadily from 5℃ to level off over the range of 22–29℃. They reported that the maximum scope-for-activity to be approximately 25℃, the physiological limit of the fish to be approximately 28–30℃ and the upper lethal limit to be around 35℃. According to Johnson and Charlton (1960) largemouth bass are unusual in that their activity rate continues to increase right up to the upper end of their temperature tolerance. In many species, the activity rate drops off well below the upper lethal level.

As part of a series of studies evaluating largemouth bass as a culture species Tidwell et al. (2003) evaluated feeding activity, growth, survival, and body composition of largemouth bass raised at three temperatures (20, 26, and 32°C). Feed-trained juvenile largemouth bass (9 g) were stocked into nine 3,610 l tanks at 140 fish/m^3 (500 fish/tank). The bass were fed a salmonid diet (45% protein, 16% lipid) to apparent satiation twice daily for 12 weeks.

The growth pattern of bass raised at different culture temperatures is shown in Figure 9.7. Consistent differences were established by the first sample date. After 97 d, the average weight of bass cultured at 20°C was significantly lower than fish in the 26 and 32°C treatments, which were not significantly different from each other (Table 9.5). Bass grown at 26 and 32°C had significantly higher weight gain (%), SGR (%), condition factors (K), and production (kg/m^3) than those in the 20°C treatment. There was no significant difference in survival among treatments, which averaged 96.5% overall. However, fish in the 32°C treatment suffered an outbreak of *Aeromonas* sp. This epizootic in the high temperature treatment may indicate that 32°C is high enough to represent stressful conditions for largemouth.

Bass in the 26°C treatment had significantly lower FCR indicating a more efficient feed utilization. Fish raised at 26°C also had a higher percentage protein deposited (PPD) than bass raised at 20 and 32°C, which were not significantly different (Table 9.5). These data indicate that largemouth bass gain weight and convert feed and dietary protein more efficiently at 26°C than at 20 or 32°C. Largemouth bass will feed at 20°C and convert feed efficiently, though appetite is reduced compared to higher temperatures. As culture temperature is increased to 26 and 32°C, appetite and growth both increase. At higher end of that range, feed and protein efficiencies decrease. At 32°C food intake did not increase further, while energy demands for maintenance and activity increase (Weatherly and Gill, 1987) as evidenced by reduced oxygen concentrations in 32°C tanks. Dissolved oxygen concentrations were reduced below levels accounted for by direct effects on the water's ability to dissolve oxygen. These factors could explain the decreased feed conversion efficiency at the high temperature treatment.

Other possible explanations may be shifts of enzyme activities to different isozymes, and stress reactions due to increased occurrence of disease (that is, *Aeromonas* sp.) (Keembiyehetty and Wilson, 1998).

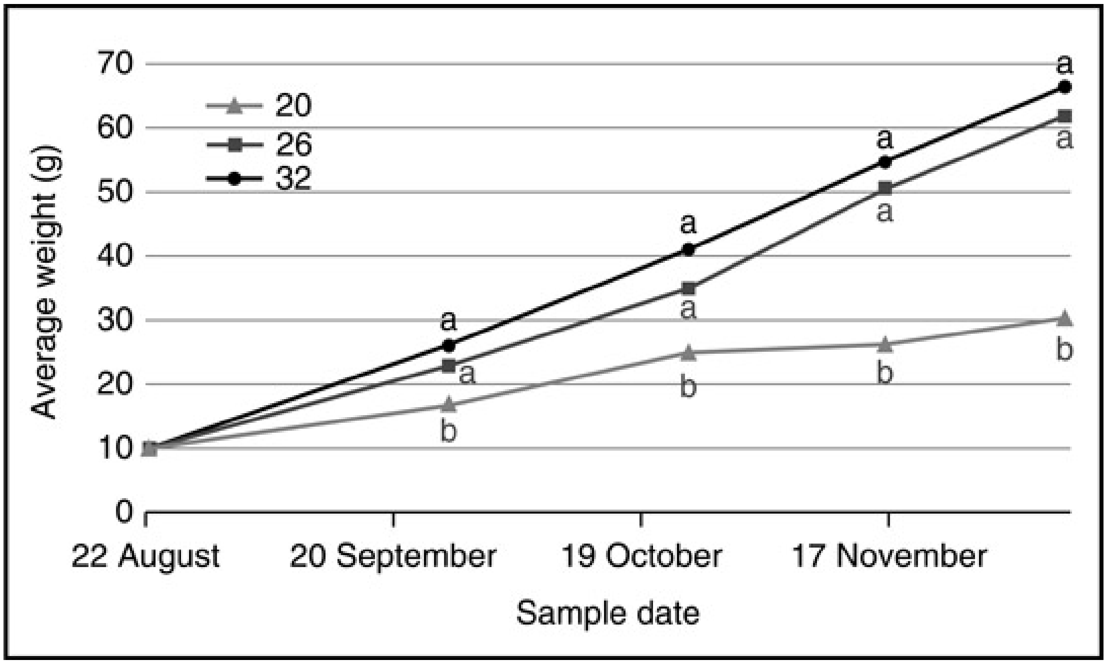

Figure 9.7 Relationship between largemouth bass body weight and sample date when raised at three culture temperatures (22, 26, and 32℃). Each symbol represents the mean of three replicate tanks. Sample means with different letters are significantly different ($P < 0.05$).

Table 9.5 Final average weights, production, feed conversion, survival, weight gains, specific growth rates, and protein efficiency ratio, condition factory (K), and hepatosomatic index (HSI) for largemouth bass raised at three temperatures for 97 d. Values are mean ± SE of three replicates. Means within a row followed by different letters are significantly different ($P < 0.05$) by ANOVA.

	Culture temperature (℃)		
	20	26	32
Average weight (g)	30.5 ± 0.8b	61.9 ± 0.6a	66.1 ± 3.6a
Production (kg/m3)	2.8 ± 0.2b	7.12 ± 0.04a	6.63 ± 0.61a
FCR	1.2 ± 0.0a	1.0 ± 0.0b	1.1 ± 0.1a

	Culture temperature (℃)		
	20	26	32
Survival (%)	96.8 ± 1.9a	97.7 ± 1.3b	86.5 ± 9.8a
Weight gain (%)	325.1 ± 1.8b	666.2 ± 2.9b	627.4 ± 48.9a
Specific growth rate (%/d)	1.3 ± 0.0b	2.0 ± 0.0a	1.9 ± 0.1a
Percent protein deposited	33.4 ± 1.0	39.9 ± 1.6a	32.5 ± 3.5b
K factor	1.3 ± 0.1b	1.4 ± 0.0a	1.4 ± 0.1a
HSI	2.6 ± 0.4a	2.3 ± 0.2a	2.0 ± 0.34a

While it is well known that changes in culture temperature can influence the body composition of fish, reactions among species vary, increasing the need to evaluate impacts on individual species (Cui and Wootton, 1988). In Tidwell et al. (2003) there was no significant difference in the protein, lipid, or ash content of whole body or white muscle of largemouth bass raised at the three treatment temperatures. This differs from channel catfish *Ictalurus punctatus* in which lipid deposition has been shown to increase as culture temperature increases (Andrews and Stickney, 1972). Moisture content of bass tissues was affected by temperature. However, the actual magnitude of changes in moisture levels were quite small and are not likely biologically significant.

Hepatosomatic index (HSI) consistently decreased as culture temperatures increased, though differences were not statistically significant. This agrees with Heidinger and Crawford (1977) who reported increased temperature lowered HSI in largemouth bass. Keembiyehetty and Wilson (1998) reported that in sunshine bass liver metabolism was altered between the two study temperatures (26.7 and 32.2℃) with increased temperatures shifting lipid from the liver to visceral storage. They reported that this may have indicated stress at the higher temperature. In Tidwell et al. (2003) there appeared to be the opposite trend in largemouth bass. Lipid concentrations in the liver were significantly increased at 32℃ (Table 9.5) while whole body lipid concentrations showed a decreasing trend with temperature. Since lipid concentrations in white muscle were low (<0.05%), and did not change with temperature, this indirectly indicates decreased visceral storage. In largemouth bass visceral fat stores are tightly bound to pyloric cecae and accurate direct measurements are extremely difficult.

In Tidwell et al. (2003) at a water temperature near 26°C, feeding, growth, survival, feed conversion, and protein retention appear near optimal. At 20°C, feed conversion and survival remain good but feeding activity and growth rate is reduced approximately 35%. At 32°C, feeding is active and growth is similar to that at 26°C, but feed conversion efficiency and protein retention is decreased. Also, fish may be under chronic stress conditions and more susceptible to disease outbreaks.

Brandt and Flickinger (1987) recommended feeding largemouth bass a semi-moist sinking feed at 1% of BW/day when pond water temperatures are below 10°C every 5–7 d. However, this is seldom practiced in commercial production of largemouth bass.

9.10 Effect of photoperiod on feeding

Photoperiod is also known to influence feed intake and growth of fish. Petit et al. (2003) stated that the largemouth bass is a diurnal species whose activity pattern is based on day-night alterations, and if presented with continuous light, will remain active. They conducted a study to evaluate food intake and growth of largemouth bass held under a 12L: 12D alternating cycle or continuous light. At the end of 12 weeks largemouth bass under continuous light conditions had significantly higher feed consumption, higher average weight, and higher feed conversion efficiency than fish held under day–night alteration.

9.11 Effects of artificial feeding on morphology and fitness

It is accepted that largemouth bass of advanced sizes can be raised much more intensively and cost effectively on formulated feeds than using live forage. However, there have been reports of pellet-raised largemouth bass having morphological differences from those raised on live forage (Wintzer and Motta, 2005). These changes could negatively affect fitness if used in a sport-fish stocking. However, the morphology of the skulls of pellet-reared fish were observed to converge toward wild largemouth bass after being provided with live forage for a period. In a recent study, Keretz et al. (2018) compared survival of pellet-raised largemouth bass with pellet raised which had been “naturalized” by feeding live forage for different periods (1–12 weeks), with all forage fed wild largemouth bass. Fish from these backgrounds were subjected to a series of sublethal stressors. Under the conditions of this study, pellet-reared bass survived as well as naturalized and wild largemouth bass.

9.12 Conclusion

Although the body of research concerning largemouth bass aquaculture continues to grow slowly, there still remains a considerable amount of work to be done. A complete understanding of appropriate feeds and feeding technologies for largemouth bass is still lacking. Fish producers and feed manufacturers still struggle with the notion of the dietary connection to long-term health in largemouth bass. As researchers begin to better understand the relationship between nutrition and health in largemouth bass thus allowing feed manufacturers more flexibility in diet formulations the cost of raising largemouth bass for the food-fish market should decrease over time.

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Chapter 10
Common diseases of largemouth bass

Kathryn M. Mitchell and Robert M. Durborow

Kentucky State University, Aquaculture Research Center, Frankfort, Kentucky, USA

10.1 Introduction

The largemouth bass (*Micropterus salmoides*) is native to North America and is the largest member of the Centrarchidae family. Historically, the primary culture focus has been stock enhancement for the sport-fish industry, but it has also become a popular food-fish sold live in ethnic Asian markets (Brandt, 1991; Cochran et al., 2009). Fish in aquaculture are in much higher densities than in wild populations and suboptimal environments generate a physiological stress response that weakens the immune system. A compromised immune system increases the chance of a disease outbreak; therefore, stress must be minimized throughout production to reduce disease occurrences, but not to the point where the production level becomes unprofitable. Therapeutants applied to the water are used to treat external parasite and bacterial infections. Potassium permanganate ($KMnO_4$) and copper sulfate ($CuSO_4$) are commonly used for this purpose because they are on “deferred status” by FDA and are, therefore, legal for use on food-fish. In the case of a bacterial disease outbreak, necessitating the use of antibiotics (which are formulated in or sprayed on commercial feed), it is important for bass to be feed trained in order to receive medication.

This chapter provides an overview of the maladies and diseases found in largemouth bass, as well as treatment and prevention methods. As of January 2017, in the USA, a Veterinary Feed Directive (VFD) is required to treat fish with antibiotics and a section of this chapter is dedicated to information pertaining to VFDs. This chapter will close with a 28 year record of large-mouth bass disease diagnoses from the archives of the Kentucky State University Fish Disease Diagnostic Laboratory (KSU-FDDL).

10.2 Bacterial diseases

10.2.1 Motile Aeromonas septicemia

One of the most common and problematic bacterial diseases found in farm-raised fish is motile *Aeromonas* septicemia (MAS), also referred to as red sore disease or hemorrhagic septicemia (Huizinga et al., 1979; Hazen et al., 1981). This disease is most often caused by *Aeromonas hydrophila,* however *A. caviae, A. veronii*, and *A. sobria* (all part of the *Aeromonas* Complex bacteria) are also responsible for mortalities. Poor water quality increases the chance of disease, and this is especially true during times of low dissolved oxygen, high ammonia, high water temperatures, or pH instability. Stress caused by overcrowding, spawning, high organic loads, seining, parasite infestation, and transportation also increases disease occurrence (Camus et al., 1998). MAS outbreaks normally occur when water temperatures are between 18℃ and 29℃ (65°F and 85°F) but occurrences have been reported in a wider temperature range. Clinical signs of *Aeromonas* infections are nonspecific, making it challenging to identify and distinguish from other diseases. However, common signs are exophthalmia and hemorrhagic lesions on the skin and at the base of fins (Figure 10.1a). Lesions can develop into hemorrhagic septicemia (Noga, 2010). Fish may also exhibit a distended abdomen due to ascites (Figure 10.1b). Internal organs are usually swollen and appear mottled or hemorrhagic. In systemic infections, fish may swim erratically due to infection within the nervous system and brain. Fish can be asymptomatic carriers of *Aeromonas* bacteria.

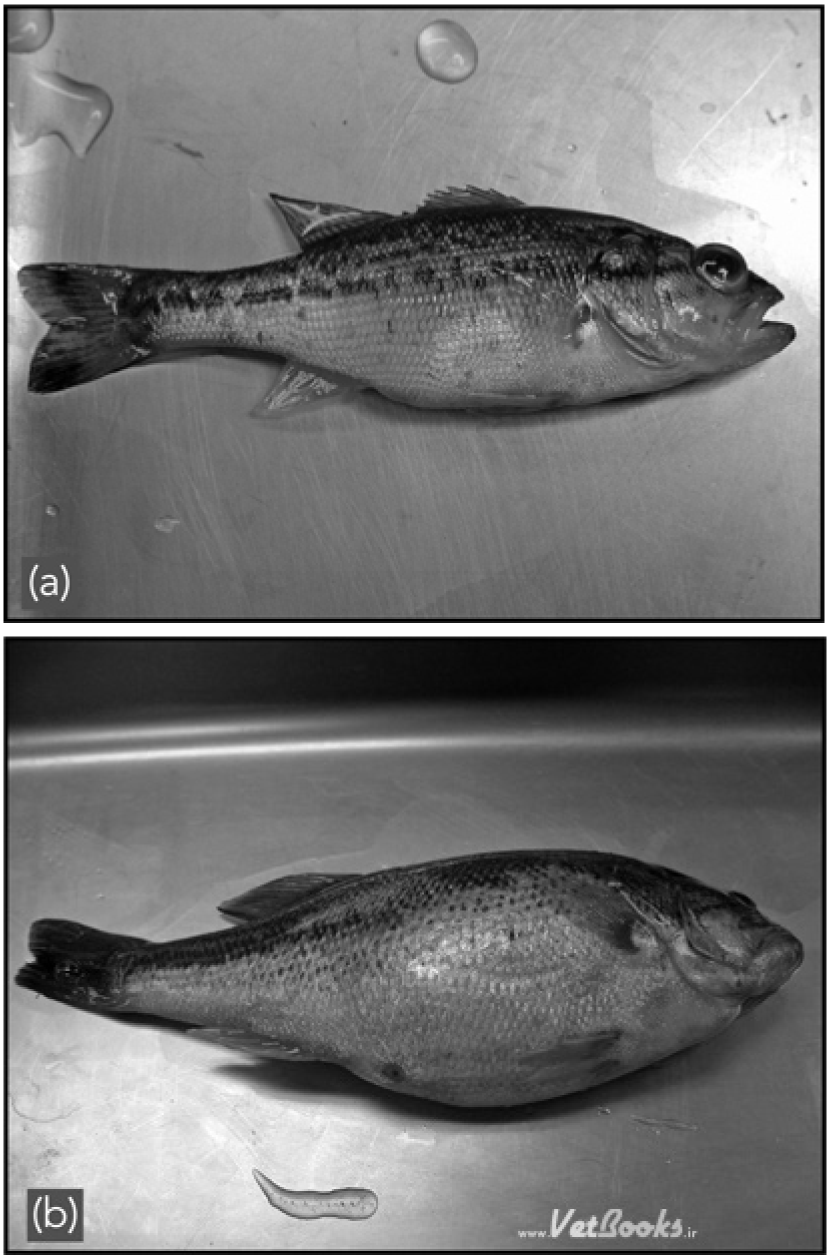

Figure 10.1 (a) Largemouth bass with exophthalmia due to aeromonas infection. (b) Largemouth bass with distended abdomen due to ascites caused by aeromonas.

Aeromonads are gram-negative rods that are facultative aerobes with motility from a single, polar flagellum (Terhune and Beck, 2015). Growth occurs between 5℃ and 37℃ on complex culture media (tryptic soy agar enhanced with 5% sheep blood is commonly used). Individual colonies are visible within 24 h and this rapid growth may explain the fast onset of disease. When infections are limited to the skin, a treatment with 2 to 4 parts per million (milligrams/liter) of potassium permanganate ($KMnO_4$) is effective, if the organic load in the water is not excessive. A demand test should first be used to determine the rate of potassium permanganate that needs to be applied to prevent the organic content of the water from neutralizing the treatment. Camus et al. (1998) reported that *Aeromonas* is frequently resistant to Romet® 30 and oxytetracycline (Terramycin®) but usually sensitive to florfenicol (Aquaflor®); this, however, can be quite variable. An antibiotic susceptibility analysis should be conducted to select the most effective treatment.

Other disease-causing bacteria that have been isolated from largemouth bass with etiologies similar to *Aeromonas* Complex bacteria include *Pseudomonas* spp., *Plesiomonas shigelloides, Vibrio* spp., *A. salmonicida, Citrobacter freundii, Salmonella* spp., *Acinetobacter calcoaceticus*, and *Proteus* spp.

10.2.2 Columnaris

Columnaris is a bacterial disease caused by *Flavobacterium columnare* and most commonly affects the gills, fins, and mucous layer (Figure 10.2a). Primarily an epithelial disease, columnaris causes skin erosion with mold-like growth and gill lesions that can become necrotic (Figure 10.2b). Clinical signs are whitish plaques with a red perimeter that are mainly present on the head, fins, and back. It is frequently referred to as saddleback disease because of the saddle-like appearance when it occurs on the back (Noga, 2010). Columnaris outbreaks have been reported in most freshwater fish and are typically pathogenic in the spring, summer, and fall when temperatures range from 25 to 32°C and become more severe with increasing temperatures (Durborow et al., 1998b; Noga, 2010). Poor water quality and stress increase the chance of an outbreak and this is especially true when largemouth bass are being feed trained. Bass that are not successfully feed trained become more susceptible to columnaris due to starvation (Figure 10.3) (Bebak et al., 2009).

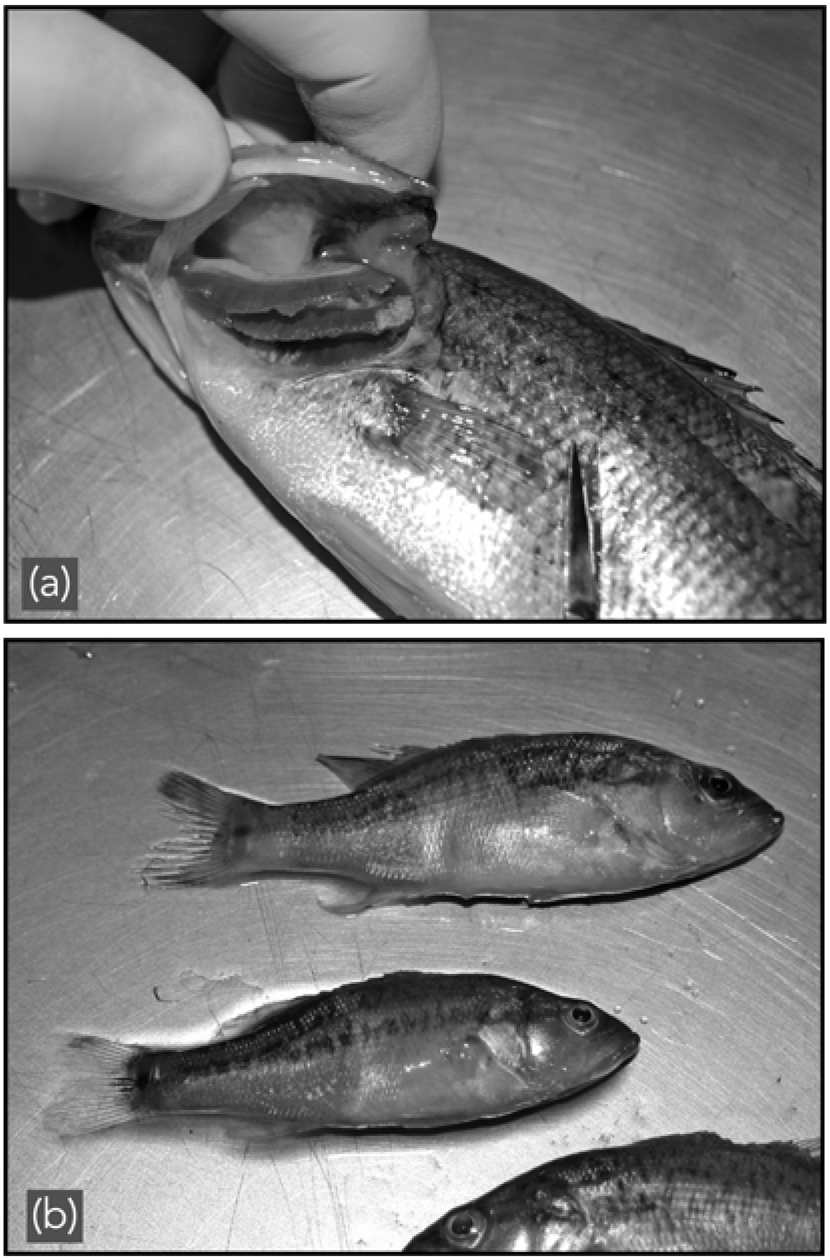

Figure 10.2 (a) Largemouth bass with columnaris infection on the gills. (b) Cotton-like growth on skin of largemouth bass due to columnaris infection.

Flavobacterium columnare is characterized by gram-negative rods ranging from 3 to 10 nm long, visible at 200× magnification and higher. It is aerobic, nonflagellated and uses a flexing or gliding movement for motility. The key diagnostic feature is bacterial cells forming columns or “haystacks” that can be observed microscopically. Bacteria can be grown on low-nutrient media (such as Hsu-Shotts, modified Shieh, cytophaga, or tryptone yeast extract salts [TYES] medias) (LaFrentz et al., 2014) at an optimal temperature of 25°C, but growth occurs between 4 and 30°C. Colonies are dry, yellowish, and rhizoid (Terhune and Beck, 2015).

Figure 10.3 Emaciation in largemouth bass caused by internal columnaris infection.

Columnaris is opportunistic and can be a primary or secondary pathogen. In one case example, largemouth bass from Kentucky State University case

F08–8 were infected with both *Flavobacterium columnare* and *Aeromonas hydrophila*, and florfenicol (Aquaflor®) was successfully used to treat. Other treatment options include oxytetracycline bath, Halamid® Aqua (Chloramine-T), copper sulfate ($CuCO_4$), and $KMnO_4$. Another approach is preventive; Bebak et al. (2009) demonstrated the efficacy of a commercial vaccine for largemouth bass against *Flavobacterium columnare* when challenged naturally (compared to sham-vaccinated bass). Vaccinated largemouth bass had a 43% lower risk of dying from the natural exposure to columnaris bacteria. Lange (2003) also showed effectiveness of a commercially made autogenous vaccine (developed from *Flavobacterium columnare* isolated from the facility where the study was performed) when the largemouth bass were immerse-vaccinated at 6 grams in size but not when the bass were vaccinated at 1 g. An oxytetracycline (OTC) bath at 20 mg/l every third day for the duration of the feed-training period, however, conferred better protection from columnaris infection than the vaccinated or OTC-fed bass for both sizes (1 and 6 g fish; Lange, 2003).

10.2.3 Edwardsiellosis

Edwardsiellosis, caused by *Edwardsiella tarda*, elicits muscle tissue necrosis and is often referred to as fish gangrene due to gas filled pockets that produce a foul odor. *Edwardsiella tarda* is a gram-negative anaerobe that produces hydrogen sulfide and is normally treated with either Terramycin or Romet. The bacterium is an opportunistic and facultative pathogen that has been reported in both saltwater and freshwater warmwater fishes. Disease epizootics involving *E. tarda* normally occur during hot temperatures (above 30℃) and are associated with polluted and stressful environments (Noga, 2010). Infected fish appear listless or lethargic. External hemorrhaging occurs around the abdomen and at the base of the fins, and the anal vent may become swollen and red.

10.2.4 Epitheliocystis

Epitheliocystis has been observed in largemouth bass cases submitted to the KSU-FDDL; it is a tumor-like proliferation of cells occurring on various skin and gill surfaces of fish and is caused by a chlamydia-like bacterium (Draghi et al., 2004). It has been found in otherwise healthy fish but it can cause mortality when hyperinfection occurs. In healthy fish, epitheliocystis is in a benign form but in severe cases, infected cells can cover the gills and produce an inflammatory response. Goodwin et al. (2005) reported successfully treating this disease with a 25 mg/l oxytetracycline tank treatment.

10.3 Viral diseases

10.3.1 Largemouth bass virus

Largemouth bass virus (LMBV) is an iridovirus that was first discovered in Florida and has since been found in other areas of the southeastern USA, Michigan, and Indiana. LMBV is the only known virus that has caused widespread mortalities in wild largemouth bass populations (Grizzle and Brunner, 2003). A study by Maceina and Grizzle (2005) found that LMBV was most prevalent in young to intermediate-age bass ranging from 1.5 to 6 years old (25 to 40 cm in length) and rare in fish smaller than 10 cm and larger than 50 cm. This study indicated that the absence of LMBV in fish greater than 50 cm may be due to larger and older fish dying prior to collection in the Alabama reservoirs that were surveyed. Hanson et al. (2000), however, did not find a correlation between age/size and LMBV occurrence in a Mississippi reservoir, but they were able to detect LMBV in a third of the bass they sampled even 13 months after the outbreak. They also reported a yellow waxy substance (a fibrin clot consisting of erythrocytes and eosinophils) in the swim bladder of infected bass. Southard et al. (2009) also noticed a swim bladder anomaly associated with infected largemouth bass in a Texas lake. Mortalities in adult largemouth bass due to LMBV occur during the summer months when temperatures are greater than 20℃ (68°F) but subclinical infections can occur year-round, and higher temperatures (30℃ [86°F]) cause greater mortalities compared to when temperatures are at 24℃ (75°F) (Grant et al., 2003). The only clinical signs are gross lesions on the swim bladder and/or an overinflated swim bladder. Infected fish appear lethargic, experience a loss of equilibrium, and swim near the surface.

The largemouth bass virus can be identified by using polymerase chain reaction (PCR) (Plumb et al., 1999) and a more recent qPCR (quantitative PCR, or real-time PCR) has been developed by Getchell et al. (2007). The American Fisheries Society (2016) Fish Health Section Blue Book approves BF-2 (bluegill *Lepomis macrochirus* fry) cell line as one of the acceptable cell lines for detecting LMBV (Getchell et al., 2014). In 2014, Getchell et al. reported success in identifying LMBV with a new cell line derived from the largemouth bass ovary. Other cell lines susceptible to the LMBV include fathead minnow (FHM), epithelioma papulosum cyprinid, and channel catfish ovary (CCO) cells (McClenahan et al., 2005). McClenahan et al. (2005) also worked out many of the details for optimizing the detection of LMBV such as how long to allow virus samples to absorb on cell lines and the use of orbital shakers and blind passages to increase the level of detection of the virus. Leis et al. (2018) found that using mucus collected nonlethally from largemouth bass by swabbing and testing it for LMBV in traditional cell culture was just as effective in detecting the virus as when bass tissue samples were inoculated onto the cell cultures (after lethally extracting the tissues from the bass). The mucus samples proved to be more reliable than the conventional tissue samples when conventional and quantitative PCR testing was used to detect the LMBV (Leis et al., 2018).

Beck et al. (2006) showed that LMBV enters largemouth bass quickly (within an hour) and can be found in various organs even when the fish are not moribund or showing clinical signs, but antibody titers were shown to be highest in the swim bladder (Woodland et al., 2002). The virus is capable of infecting bass by an oral route (Woodland et al., 2002). The infection can become undetectable within a month after exposure (Beck et al., 2006). LMBV outbreaks can be caused by holding largemouth bass in fishing boat live wells after they are captured during tournaments. Incidence of LMBV resulting from tournament stress was almost 50% in a study by Schramm et al. (2011), explained in more detail below in the Stress section.

Nath et al. (2010) addressed the potential for LMBV to associate with bacterial biofilms, thus gaining protection from topical disinfectants. Researchers demonstrated that when LMBV is entrapped in *Pseudomonas fluorescens* biofilm it is resistant to sodium hypochlorite (bleach) and povidone iodine (Betadine™) disinfectants but not to ethanol (Nath et al., 2010). They were not able to demonstrate that LMBV is able to exist in natural aquatic environments in association with biofilms.

Boonthai et al. (2018) demonstrated (using experimental infections) that LMBV causes mortality as well as clinical signs in young-of-the-year smallmouth bass (clinical signs consistent to those observed in smallmouth bass die-offs in natural bodies of water, including skin hemorrhaging; a broken, hemorrhaged, and ulcerated mandible; exophthalmia and corneal opacity; ruptured eye with vitreal loss; abdominal distension; enlarged, edematous, mottled, and friable livers; a dark, edematous spleen; and hemorrhaged kidneys and gonads). Higher mortalities were noted at 28 than at 23 or 11°C, and mortalities were compounded when *Flavobacterium columnare* co-infections occurring concurrently with LMBV were "devastating" to the young smallmouth bass even though *A. salmonicida* is a pathogen that prefers cool temperatures (Boonthai et al., 2018).

10.3.2 Viral hemorrhagic septicemia

Viral hemorrhagic septicemia virus (VHSV) has caused mortality and morbidity in multiple species of farmed and wild fish. Kim and Faisal (2010, 2011) demonstrated that muskellunge and largemouth bass were more affected in disease severity and cumulative mortality by VHSV (genotype IVb) compared to other fish species (even at low infection doses). There are VHSV genotypes I through IV with several subtypes. In North America, aside from genotype IVb, all other VHSV genotypes affecting freshwater fish (genotypes Ia, Ic, Id, Ie, and III) affect only trout species. The genotype IVb virus is the genotype that has caused the greatest alarm nationwide in recent years due to large-scale die-offs of many different fish species (28 species) in the Great Lakes region. It has not yet caused a disease outbreak in aquaculture fish and has not been reported south of the states bordering the Great Lakes (Illinois, Indiana, Michigan, Minnesota, New York, Ohio, Pennsylvania, and Wisconsin) and is not usually found in southern parts of these states.

Some states require VHSV-free certification for transportation and sale of live fish. Typically, 60 fish per lot or body of water are required for testing and results must be negative for VHSV in order to legally transport live fish.

10.3.3 Other viruses

In 2008, a ranavirus causing skin and muscle ulcerations and mortality in largemouth bass in China was isolated and sequenced; it was genetically identical to doctor fish virus and closely related to largemouth bass virus (Deng et al., 2011). Gao and Chen (2018) describe a relatively new virus *Micropterus salmoides* rhabdovirus (MSRV) that causes bass to exhibit a crooked body with an irregular, corkscrew swimming pattern. They found that it causes apoptosis in fish skin cells, and they uncovered the transcriptomic profiles using RNA sequencing.

10.4 Parasite infections

10.4.1 Protists

Figure 10.4 Largemouth bass infected with *Ichthyobodo* spp., which can be seen around the dorsal fin and on the head.

The most common parasites found in cultured fish are single-celled organisms or protist ectoparasites, which have a direct life cycle and can occur on the host for the entire or partial life cycle. Common signs of protist ectoparasites are damage to the skin and gills caused by parasitic feeding activity, this irritation can also cause behavioral changes. A photograph from case F08–30 (Figure 10.4) is a largemouth bass with an *Ichthyobodo* spp. infection; irritation and sloughing of mucus can be seen around the dorsal fin. This disease is often called “blue slime” disease. Obligate parasites such as *Ichthyobodo* spp. and *Ichthyopthirius* spp. actively feed on the host epithelium and others such as *Trichodina* spp. and *Ambiphyra* spp. have a commensal relationship with the host but are still capable of causing morbidity and mortality in host fish, especially if their concentrations are heavy enough (MacMillan, 1991). This can lead to irritation of the fish’s skin and/or gills and partial obstruction of oxygen flow to the fish. Although *Trichodina* typically causes problems in an aquaculture setting where fish are much more crowded than in the wild, it can also be a problem in wild fish. A *Trichodina* epizootic in wild largemouth bass from a North Carolina river was reported by Huh et al. (2005). Initial reports from anglers were of a "jelly like slime coat" on the skin. Follow-up electrofishing by these researchers found that 10% of captured bass had trichodinosis clinical signs (a bluish-white mucoid layer characterized by a very thick hyperplastic epidermis with relatively few mucous cells). No other disease organisms besides *Trichodina* were reported (Huh et al., 2005).

10.4.2 Ichthyophthirius multifiliis

Ichthyophthirius multifiliis causes "Ich," which is the most infamous fish parasite. It is highly nonspecific and affects all species of freshwater fish. Ich is recognized by white spots or pustules sprinkled on the skin that are visible to the naked eye. This is the source of the common names of white spot or salt-and-pepper disease. It is found on the skin, gills, and fins and can cause excess mucus production and sloughing of the skin, however, sometimes the only indicator of an Ich outbreak is the presence of dead or dying fish. Fish infected with Ich are often seen rubbing on the pond bottom or hard objects. This is called "flashing" because of the sporadic exposure of the light-colored belly of the fish. In the later stages of the disease, fish may gather around inflowing water, appear lethargic, and stop eating. Microscopically, Ich is sphere shaped and moves around in a rolling motion using cilia and mature Ich have a C-shaped nucleus.

Ich is usually transmitted by a carrier fish, other animals, humans, or from a contaminated water source. Ich has a life cycle that requires 52 or more hours to complete, which slows in cooler weather and cannot reproduce properly above 29℃. Ich leaves the infected fish as a tomont and attaches to the pond bottom or other surface and develops a thin-walled cyst. The tomont divides many times within the cyst, forming as many as 2,000 tomites. Tomites are released from the cyst and elongate into theronts or swarmers. Theronts have a day or two to find a fish host and infiltrate the fish's epithelium using a penetrating gland and ciliary action. When Ich enters the epithelium, it is referred to as a trophont. Trophonts then feed and mature on the fish's epithelium until leaving the fish as a tomont and the cycle continues.

Fish mortality is due to Ich eroding the epithelial tissue, irritation from theront penetration, abrasions from flashing, not eating, anemia, and/or excess mucus production on gills that obstruct oxygen transfer. Ich can be prevented by eliminating entry of wild fish, quarantining new fish, putting filters on water inlets in ponds, and drying or dipping equipment in disinfectant before using between ponds.

Not all stages of the Ich life cycle are susceptible to chemical treatments. Trophonts are protected by the fish's epithelium, therefore chemical treatments are only effective on theronts and tomonts. In order to treat these vulnerable stages, multiple treatments must be administered and depending on temperature, three to seven treatments may be needed to be effective. Chemical drugs approved to treat Ich in food-fish are formalin (Formalin-F^{TM}), copper sulfate, and potassium permanganate. In ponds, formalin should be administered at a rate of 15 to 25 ppm, which is equivalent to 4.5 to 7.5 gallons of formalin per acre-foot (or 13.5–22.5 l of formalin per 1,000m^3 of water). Copper Sulfate ($CuSO_4$) is used as an indefinite treatment and treatment rate is dependent on the total alkalinity of the water. Therefore, the treatment rate of copper sulfate in ppm is equal to the total alkalinity of the water being treated divided by 100 (Durborow et al., 1998a).

Copper sulfate is not typically used when total alkalinity is less than 40 ppm or greater than 300 ppm; copper sulfate is more toxic to fish in low alkalinity waters. In high alkalinity waters, copper may precipitate out of the solution making treatment ineffective. Algae blooms may be killed during copper sulfate treatments so dissolved oxygen concentrations must be monitored. Before stocking a pond, copper sulfate can be applied to kill any theronts or tomonts in the water as a preventive. Potassium permanganate is usually used as an indefinite treatment; it oxidizes organic materials in the water and a demand test should be performed to determine the effective dose for a body of water. The minimum dose recommended is 2 ppm, therefore 2 ppm of potassium permanganate should be added at a time until a wine-red color is attained and to be considered effective, this color should persist for at least 8 h.

10.4.3 *Epistylis* spp.

Epistylis spp. are facultative colonial ciliates with non-contractile stalks. Reproduction occurs by simple binary fission or by formation of teletrochs. Bell-shaped cells form a ring of cilia that flatten to form the teletroch which attaches to hard parts of the fish such as scales, fins, and spines. At the point of attachment, red ulcerative sores develop and the tissue develops a furry white fungus-like growth. The attachment points usually become infected with bacteria that can dissolve spines and scales. Extensive infestations typically indicate polluted waters with poor water quality (Terhune and Beck, 2015). The most common treatment in food-fish is 0.1 to 1.0% salt and/or 15–25 mg/l formalin as an indefinite treatment.

10.4.4 Cestodes (tapeworms)

Cestodes or tapeworms affect all vertebrates, are widely distributed, and normally demonstrate a high degree of host specificity. The bass tapeworm has a complex life cycle that involves two intermediate hosts and a final host. The adult tapeworm lives in the intestinal tract and releases eggs that reach the outside environment through the feces. The eggs then infect cyclopoid copepods and develop into proceroids, the first larval form. When the copepod is consumed by another fish (including bass), the larval tapeworms migrate out of the intestinal tract into the abdominal cavity of the intermediate host and forms the plerocercoid larvae. When the intermediate fish host is eaten by the final host (largemouth bass), the adult tapeworms develop and attach to the host intestinal wall with its head or scolex. Here, it absorbs nutrients and undergoes reproduction (Terhune and Beck, 2015). Adult tapeworms cause minimal damage in light infestations, however, severe infections can lead to nutritional deficiencies or intestinal blockage. Host fish severely affected are more susceptible to other diseases and stressors.

The bass tapeworm, *Proteocephalus ambloplitus*, is of most concern due to the damage and sterilization of reproductive organs in largemouth bass hosts. Bass tapeworm infections can lead to sterilization of largemouth bass broodstock, a result of bass tapeworm plerocercoids (larval stage) migrating through the bass ovaries and causing destructive connective tissue formation. However, bass tapeworms are not a significant problem in largemouth bass fed a pelleted diet. Boonyaratpalin and Rogers (1984) found that mebendazole (methyl-5-benzoyl benziraidazole-2-carhamate) at 200 mg/kg/d (injected or by capsular implant) reduced the tapeworm infection by 95% after six weeks, while 100 mg/kg/d administered orally for 14 consecutive days reduced infection by 90%. These doses did not interfere with reproductive success of the bass brooders but increasing the mebendazole injection dose to 300 mg/kg led to the absence of fry production.

In some molecular-based work, Durborow et al. (1988) demonstrated competitive exclusion interaction between bass tapeworms in largemouth bass and the acanthocephalan *Neoechinorhynchus* sp. High numbers of either parasite significantly reduced the infection rate of the other parasite due to cross-protective immunity/cross-resistance (antibodies against either parasite reacted with antigens of the other). Cross-resistance in largemouth bass's reaction to various species of mussel glochidia has also been observed (Dodd et al., 2005). Durborow (1986) also characterized the molecular weights of largemouth bass antibodies (immunoglobulins) including the heavy and light chains that comprise the bass immunoglobulin.

10.4.5 Crustaceans

Lernaea sp. (Lernaeidae) are parasitic copepods in the order Cyclopoida. These parasites undergo multiple free-swimming life stages before attaching to the host fish and reproducing. Attachment to the host fish normally occurs on the fish abdomen or around the base of the fins and only the females penetrate the skin and mature into adults. Light infestations are normally not of concern unless the fish is small but heavy infestations or infestations around vital organs are problematic. Also, the attachment point can become infected with fungal pathogens or opportunistic bacteria (Noga, 2010). Anchor parasites (*Lernaea cruciata*) were described as a problem in 55% of large (> 100 mm) largemouth bass collected from the Chowan River in North Carolina from May to October (Noga, 1986). The infestation was compounded by secondary bacterial and fungal infections. This parasite was also reported by Timmons and Hemstreet (1980) on bass from a reservoir on the Alabama–Georgia border.

10.4.6 Monogenea

Monogenea or monogenes (phylum Platyhelminthes) are small flatworms that are commonly referred to as skin or gill flukes (although, technically, they are not flukes). Dactylogyroidea and Gyrodactyloidea are the most economically important monogenes in cultured fish; they attach to the epidermal surfaces using a haptor that include 2–4 hamuli (anchors) and 12–16 marginal hooks (Hoffman, 1999). Reproduction varies depending on the family but only one host is required for all monogenes. Gyrodactylids are viviparous and attach to the skin and gills, however, dactylogyrids are oviparous and primarily attach to the gills. Monogenes are problematic when infestations become heavy, which can cause mucus production, respiratory impediment, and inappetence in fish, leading to anorexia. Heavy infestations are more common in waters with poor water quality and high organic content (Noga, 2010).

10.4.7 Digenea

Digenea or digenes (phylum Platyhelminthes) are referred to as grubs and have a complex life cycle that utilizes both asexual and sexual reproduction. Beginning in the definitive host, the adult digene releases a fertilized egg into the water column where it hatches and becomes a free-swimming miracidium. The miracidium is ingested by the second host, a mollusk (snail), and forms a sporocyst. From sporocysts, cercariae develop and leave the snail and have a limited amount of time to find a second intermediate host (fish). The cercariae become metacercariae in the fish. Infected fish are then consumed by a mammal, bird, or another fish, where the digene develops into an adult and the cycle continues. Digenes can be controlled by eliminating snails and/or birds. Snail population can be reduced by removing aquatic vegetation and by targeting snails along the pond margins with copper sulfate or hydrated lime applications. The presence of birds can be discouraged by various scare tactics or by selective killing (with the proper federal permit).

Common digenes found in largemouth bass are yellow grub (*Clinostomum marginatum* or *C. complanatum*), white grub (*Posthodiplostomum minimum*), eye fluke (*Diplostomum spathaceum*), and black spot (*Uvulifer ambloplitis*). Eye fluke cercariae can enter anywhere on the body of the fish and travel to the lens of the eye, causing blindness if infestations are heavy enough (Hoffman, 1999). In most cases, when the cercariae penetrate the fish there is minor damage and minimal stress response. However, heavy infestations may kill the host fish, which is especially true for small fish. In chronic cases, opportunistic bacteria may be able to infect the host through the opening created by the cercariae.

10.4.8 Leeches

Although of limited importance in aquaculture, reports of leech infestations are found in pond-raised and wild bass populations. Leeches feed on a range of aquatic and terrestrial animals and reports of leech infestations of largemouth bass have been documented in the United States. Largemouth bass living in an estuarine environment in coastal North Carolina were observed by Noga et al. (1990) to have bacterially infected mouth ulcerative lesions caused by an infestation of the leech *Myzobdella lugubris* (formerly *Illinobdella moorei*). They attributed the leech infection to the stressful, high-salinity environment, and regarded the concurrent bacterial infection as secondary (Noga et al., 1990).

10.4.9 Fungi

Winter saprolegniasis (winter "fungus" or winter mortality) is the most common fungal pathogen found in largemouth bass. It usually occurs between October and March when water temperatures drop below 15℃ (59°F), however, the disease has been reported as early as September and as late as April and is associated with a sudden drop in temperature that causes immune suppression. *Saprolegnia* spp. are facultative pathogens that obtain nutrients from dead organic matter. Diseased fish have brownish patches of cottony or wooly fungus, skin lesions, endophthalmia, and hemorrhaging around the infection. Lesions start out in small circular, depigmented areas but can become ulcerative and break the skin exposing the muscle tissue. Bacteria can enter through lesions resulting in septicemia or blood poisoning, though death is thought to be associated with the loss of osmoregulation (Durborow et al., 2003). Prevention is the best treatment method, and reducing stress, minimizing handling, and maintaining good water quality, especially during late summer and early fall, can minimize the effect of winter fungus.

Table 10.1 Diseases of importance found in largemouth bass.

Disease	Temperature range (℃)	Reference
Aeromonas	18-20	Camus etal., 1998

Disease	Temperature range (℃)	Reference
Columnaris	25-32	Durborow et al., 1998
Edwardsiellosis	>30	Noga, 2010
LMBV	>20	Grizzle and Brunner, 2003
VHS	~3-18	Kim and Faisal, 2011
Winter Fungus	<15	Durborow et al., 2003
Ich	20-25	Durborow et al., 1998
Protozoan Parasites		Durborow et al., 2003

Table 10.1 provides a list of the most frequently diagnosed diseases, their respective temperature ranges, and references to find additional information.

10.5 Stressors in largemouth bass

Stress in fish leads to impaired reproductive success and decreased survival (Ostrand et al., 2002). Stressors include improper handling, crowding, and poor water quality. Largemouth bass water quality requirements and tolerances are covered in Chapter 4. In general, largemouth bass can tolerate dissolved oxygen (DO) as low as 1.4 mg/l, but DO less than 3–4 mg/l should be avoided. The largemouth bass has a high tolerance to nitrite; the 96 h LC_{50} for nitrite is 460 mg/l in large-mouth bass compared to 25 mg/l in channel catfish (Tidwell et al., 2000). However, largemouth bass are similar to channel catfish in ammonia tolerance, with a 24 h un-ionized ammonia LC_{50} of 1.69 mg/l. Additional stressors to largemouth bass include inadequate diet, rough handling, and high levels of waste solids.

The ideal temperate range for largemouth bass is 20–30℃. Seining and handling should be avoided when temperatures are above 27℃ to prevent stress-related secondary infections. Soft mesh netting should be used to prevent scale loss and infection when fish are seined or netted. A treated net/seine should never be used on largemouth bass because the stiff nature of treated netting causes abrasion. Fish should be purged from feed two days before transport to reduce stress. When transporting largemouth bass, tank water temperature should be between 5 and 15℃, salt concentration should be 3–5 ppt, and stocking density should be 120–240 g of fish per liter of water. Fish can be anesthetized prior to loading; a legal anesthesia for food-fish is a mixture of 1 l of acetic acid (concentrated vinegar) and 1.8–3.6 kg of sodium bicarbonate (baking soda) in 3,785 ml of water. Bass should be adequately sedated for safe handling after 6 min and can safely remain in the solution up to 1 h.

A somewhat related stressor is the holding of largemouth bass in fishing boat live wells after being captured during tournaments. Plumb et al. (1988) demonstrated that survival of largemouth bass caught under tournament conditions and held in live wells was higher (98.9%) when the bass were caught and released (within 30 min) compared to those held for 3 to 9 h (90.8% survival). Schramm et al. (2011) investigated 12 summer tournaments and found that reducing water temperature and increasing salt content in live well holding tank water significantly increased survival of largemouth bass (from 93% to 97%) that were caught and held in these live wells. However, after these captured fish were held for 5 d in net pens after the tournaments, survival averaged 24% regardless of improved live tank holding conditions. Incidence of largemouth bass virus during the five-day post-tournament holding period ranged from 64% to 70%, while LMBV occurrence post-electrofishing during the same holding period was 47%. Survivals were 24% and 41% for the post-tournament and post-electrofishing groups, respectively, showing a correlation between stress and disease/mortality, especially in a lake infected with largemouth bass virus (Schramm and Davis, 2006; Schramm et al., 2011). High mortalities were not observed during bass tournaments in lakes not infected with LMBV.

10.6 Treatments and Veterinary Feed Directive (VFD)

As of 1 January 2017, a VFD must be issued by a licensed veterinarian and a valid veterinarian–client–patient relationship (VCPR) must be established in order to legally treat fish with antibiotics in the USA. A VFD has an expiration date and antibiotics cannot be administered after that date. If there is not an expiration date specified on the product label then it will expire 6 months after the date of issuance, however, the veterinarian can assign a shorter expiration date. Currently no refills are allowed for VFD drugs and if another outbreak occurs, it is up to the treating veterinarian to decide if a new VFD is needed. Medicated drugs approved for use in aquaculture are Aquaflor® (florfenicol), Terramycin® (oxytetracycline), and Romet® TC/Romet-30 (sulfadimethoxine/ormetoprim). Immersion drugs approved for use in aquaculture are Halamid® Aqua (chloramine-T), Formalin-F™/Formacide-B/Parasite-S® (formalin), 35% Perox-Aid® (hydrogen peroxide), and Tricaine-S (tricaine methanesulfonate). Potassium permanganate ($KMnO_4$) and copper sulfate ($CuSO_4$), mentioned earlier in the chapter are often used to treat external bacteria and parasites.

Information on usage and treatment rate for approved aquaculture drugs can be found on the FDA website (https://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/Aquaculture/ucm132954.htm).

The Kirby–Bauer antibiotic testing or agar diffusion test determines the antibiotic susceptibility of bacteria. Bacteria are streaked on a Mueller–Hinton agar plate and antibiotic susceptibility discs are dispensed onto the plate. Bacterial growth up to or close to the disc indicates that the bacteria are resistant to that antibiotic but a zone of growth inhibition around the antibiotic disc indicates that the bacteria are susceptible to that antibiotic.

10.7 Diagnostic case history at Kentucky State University

The KSU-FDDL has diagnosed 355 largemouth bass disease cases over the past 28 years (1990–2017; see Figure 10.5). The most frequent disease diagnosed (75 out of 355 cases) was *Aeromonas* complex, which includes *Aeromonas hydrophila, A. caviae, A. sobria*, and *A. veronii. Columnaris* was the second most common bacterial disease diagnosed at KSU with 25 cases (Table 10.2). Gill monogenes were the most frequently occurring parasite with 16 cases diagnosed, followed by 9 cases of *Trichodina* and 8 cases of Ich (Table 10.3). Of the 355 cases diagnosed, 33 cases were routine checks and 30 cases were diagnosed as unidentified bacteria as cause of death.

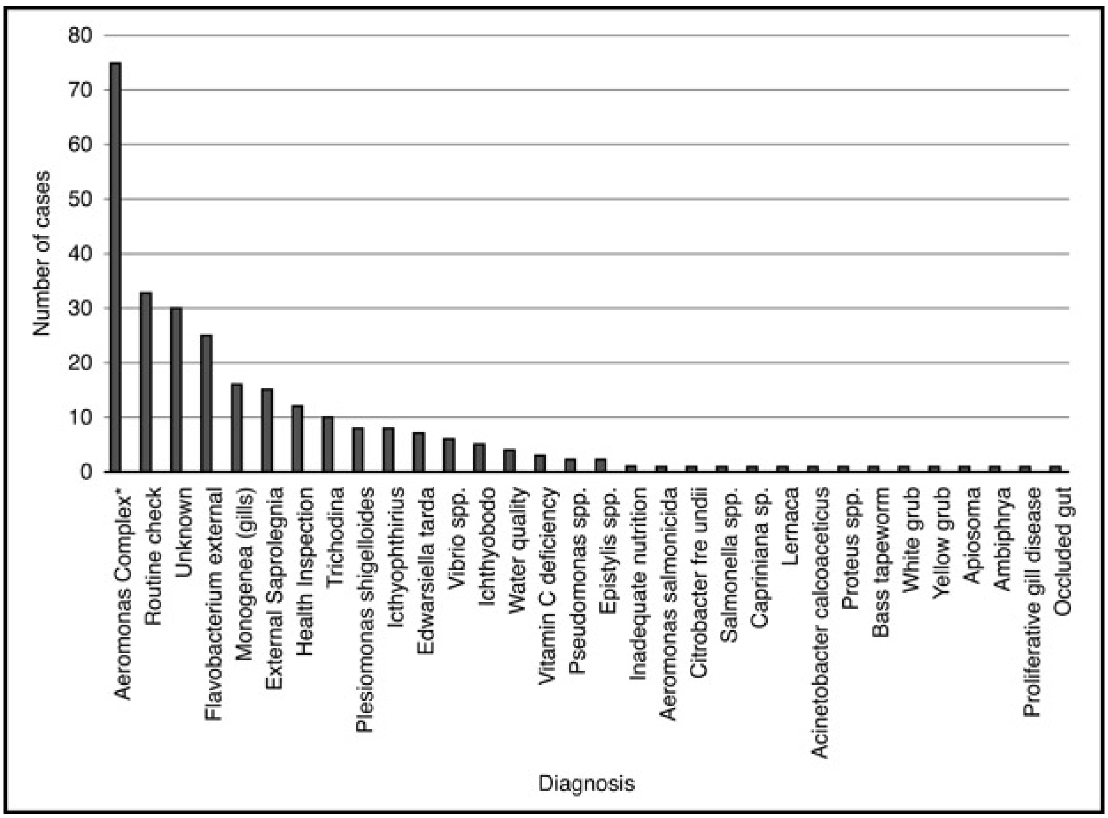

Figure 10.5 Diagnosis of largemouth bass (*Micropterus salmoides*) cases submitted to the Kentucky State University Fish Disease Diagnostic Lab from 1990–2017.

Note: *Aeromonas complex includes *Aeromonas hydrophila, A. caviae, A. veronii,* and *A. sobria.*

Table 10.2 Bacterial diseases diagnosed in largemouth bass cases submitted to the KSU-FDDL

Bacteria	Frequency of Occurrence
Aeromonas hydrophila	60
Flavobacterium columnare	21
Aeromonas sobria	8
Plesiomonas shigelloides	8
Edwarsiella tarda	7
Vibrio spp.	6
Aeromonas veronii	4
Aeromonas spp.	3
Pseudomonas spp.	2
Aeromonas salmonicida	1
Citrobacter freundii	1
Salmonella spp.	1
Unidentified bacteria	30

Table 10.3 Parasites that have been found on largemouth bass submitted to the KSU-FDDL

Parasites	Frequency of Occurrence
Monogenea (gills)	16
External *Saprolegnia*	15
Trichodina	9
Icthyophthirius	8
Ichthyobodo	5
Epistyles sp.	2
Capriniana sp.	1
Lernaea sp.	1
Bass tapeworm	1
White grub	1

Parasites	Frequency of Occurrence
Yellow grub	1
Apiosoma	1
Ambiphrya	1

10.8 Interesting cases

10.8.1 Copper sulfate overdose

In late winter 2006, largemouth bass mortalities (about 30 fish per day) were reported from a 0.4 ha aquaculture pond, averaging 1.2 m deep. Copper sulfate at a rate of about 1.1 mg/l was initially used to prevent aquatic weeds from becoming established in the pond. This treatment rate was double the conventional rate (the pond's total alkalinity was 51 mg/l, and the usual treatment would be 0.51 mg/l for $CuSO_4$ if total alkalinity were divided by 100 to get the recommended rate). Because of this overdose, the bass were exhibiting stressed behavior; this behavior, however, was interpreted by the farmer to be a sign of a disease in the bass that required more $CuSO_4$ treatment. So, another 1.1 mg/l of $CuSO_4$ were added to the pond, adding to the stress on the fish. The cold water also exacerbated the problem; Marple et al. (2001) demonstrated that free copper in the water persisted much longer at cold water temperatures than at warmer temperatures. The compounded overtreatment (4 times the normal rate) led to mortalities and a very peculiar reaction of the bass to loud banging on a plastic five-gallon bucket at the pond's edge. Bass startled by the noise went into convulsions, became stiff with mouth agape and died instantly. The Kentucky Division of Water found dissolved copper concentrations of 57 µg/l in the pond water indicating that high copper levels played a role in the bass mortalities. Gill histopathology revealed an edematous gill epithelium consistent with pathology caused by a water-borne irritant.

10.8.2 Acid sulfate soil in watershed of largemouth bass ponds

In an Ohio fish kill case submitted to the KSU-FDDL in 2006, 12,000 out of 15,000 large-mouth bass died in a series of five ponds receiving water sequentially (water originating from a watershed composed of acid-sulfate soil). The water in these ponds was poorly buffered (total alkalinity values between 0 and 4 mg/l), so the rain runoff from the iron-sulfide-containing soil in the watershed quickly brought water pH values down to 4.1, a level lethal to fish. Soil in the watershed not only contained high levels of iron sulfide, but the soil itself was disturbed during deforestation in this watershed, leaving the iron-sulfide soil exposed to the air, forming sulfuric acid. Rain run-off water acquired an elevated sulfuric acid content as it percolated over this exposed soil, and then ran directly into the top pond. The top pond flowed into the second pond and so on, gradually diluting as it eventually flowed into the last (bottom) pond. The first three ponds in the series had 100% mortality of the largemouth bass; the fourth and fifth ponds had 80% and 60% mortality, respectively; while the last two ponds (sixth and seventh ponds) had no mortalities. The affected water had a reddish orange precipitate characteristic of ferric hydroxide, ferric oxide, and ferric oxyhydroxide that all originate from ferrous iron in the soil. Treating all the ponds with 4,500 to 9,000 kg/ha of pelletized lime and applying 2,250 kg/ha of pulverized lime to the watershed hillside elevated total alkalinity to 20 mg/l and the pH to 7, ending the fish mortalities.

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Chapter 11
Harvest and transport of largemouth bass

Shawn D. Coyle

Kentucky State University, Aquaculture Research Program, Frankfort, Kentucky, USA

Todd S. Harmon

Walt Disney World, Animal Programs, Lake Buena Vista, Florida, USA

11.1 Introduction

Figure 11.1 A largemouth bass caught from a privately owned pond.

Largemouth bass (*Micropterus salmoides*) are produced to supply recreational sport-fish for stocking throughout much of North America and regionally in Europe and Asia, notably Japan. Market demand for largemouth bass as a sportfish encompasses all sizes from fry to trophy size fish (Figure 11.1). Largemouth bass are also produced extensively as food-fish, which are primarily marketed live in major cities with large ethnic Asian populations. Fresh-on-ice largemouth bass can also be found in parts of Mexico. Market demand for the food-fish market is typically for 0.45–0.90 kg fish with a desired minimum market size of 0.6 kg in the USA. The production of food-fish largemouth bass in temperate regions typically involves three production phases: nursery/feed training, first summer growth and second year grow out. Each phase of production involves: harvest, handling, grading and some degree of live transportation. This chapter will primarily focus on the practice of harvesting and live transport of food-size largemouth bass in the USA; although many of the considerations and recommendations are also applicable to smaller sizes of fish and other regions.

11.2 Fish response to the capture and transport process

Handling of fish cannot be avoided in aquaculture and plays a major role in the success of transport. Fish are subject to aerial exposure, crowding, net handling, and environment changes during harvest, sorting, and loading for transport. These handling processes have the potential to cause stress and possible mortality of the fish (Yeager et al., 1990). Fish react to stress with a series of physiological changes. During the primary response, catecholamine and cortisol levels increase. The release of these hormones induces a number of secondary stress responses including: elevated blood glucose (Mazeaud et al., 1977), elevated blood lactic acid (Wedemeyer, 1970), increased glycogen metabolism (Schwalme and MacKay, 1991), and osmoregulatory disturbances (Weirich et al., 1992).

If these biological disturbances are too great or too numerous and the fish is unable to recover, mortality may result. Fish have physiological mechanisms that allow them to cope with stress at a metabolic cost associated with diverting energy from normal metabolic functions. However, these response actions can be forced beyond their normal limits, thus becoming detrimental to the fish and ultimately resulting in mortality (Barton and Iwama, 1991). The severity of the stress response and the length of time before homeostasis is regained are directly related to the duration of the stressor and the characteristics of the recovery water (Reubush and Heath, 1997).

Stressors that affect fish can be categorized into acute (short term) and chronic (long term) stressors (Davis, 2006). Acute stressors include handling, confinement, abrupt changes in water quality and improper acclimation; while chronic stressors include extended periods of poor water quality, improper stocking densities, and improper diets (Harmon, 2009). Severe acute stress might result in immediate mortality, presumably through ion loss (McDonald and Milligan, 1997), whereas chronic stress often results in severely compromised immune system and/or decreased energy stores (Portz et al., 2006). Immunosuppressed fish allow pathogens to initiate a disease that would otherwise normally be resisted by the fish (Wedemeyer, 1997).

"Delayed mortality" can occur days or even weeks after transport depending on the underlying cause and severity (Harmon, 2009). This can make it difficult to verify the exact mechanisms responsible, but generally are considered as mortality due to the transport process. This process starts as the fish are removed from the pond and includes the transport itself and then the acclimation into their new environment. Utilizing good husbandry practices throughout this process is important in the success of harvest and transport.

Relatively little work has been conducted specifically on largemouth bass in documenting the effects of physiological stress. Carmichael et al. (1984a) characterized normal values for blood parameters and evaluated the effects of net confinement and water quality induced stress (oxygen depletion and elevated ammonia levels) in largemouth bass. They reported that plasma glucose and corticosteroid values were good indicators of stress during application of acute stressors, whereas chloride and osmolality were more useful indicators of long-term stress or as stress-recovery indicators.

During the transport and harvesting process reducing the number of stressors is important in getting a good product to the buyer or consumer. Because there is not one large event to focus on, many small practices can prove invaluable in providing healthy fish as an end product.

11.3 Harvest

The food-fish market for largemouth bass is somewhat unique in that the fish are typically marketed live to the consumer. This requires that the physiological condition of the fish following harvest and transport to market is strong enough to endure several days or weeks in retail display tanks. Conversely, channel catfish (*Ictalurus punctatus*) and rainbow trout (*Oncorhynchus mykiss*) harvest and transport procedures are primarily based on live delivery to a nearby processing plant. At the time of writing there is no known commercial processing of largemouth bass in the USA. Since largemouth bass are marketed live, they should be thought of as a perishable product with a shelf life similar to that of produce. As with produce, the clock starts at the time of harvest and even when maintained under optimal conditions the product quality can slowly deteriorate over time. This situation requires that stress be minimized at every step from harvest to retail sale.

Stress from various handling practices associated with harvesting weakens fish that can make them susceptible to disease outbreaks and adverse physiological imbalances (Wedemeyer, 1970). The sensitivity of fish to handling varies among species; where some species are more tolerant while others are delicate and sensitive to what would be generally considered a minor stress (Jensen and Brunson, 1992). The largemouth bass is considered a relatively stress sensitive species with a limited ability to tolerate net confinement, temperature change, or periods of low oxygen or high ammonia concentrations (Williamson and Carmichael, 1986; Suski et al., 2007; Vanlandeghem et al., 2010).

As poikilothermic animals, water temperature and temperature change have a great effect on the physiological stress response in fishes. Largemouth bass do not tolerate rapid changes in water temperature as well as some other commercial aquaculture species such as channel catfish or rainbow trout (Currie et al., 1998). However, the stress response is attenuated at lower water temperatures. Carmichael et al. (1984a) reported a significant reduction in plasma glucose and corticosteroid levels following abrupt temperature change in largemouth bass previously acclimated to lower water temperatures of 10–16°C than for those acclimated to 23°C.

During the summer months, largemouth bass are typically not harvested for food-fish sales due to increased stress and mortality associated with warmer waters in conjunction with harvest, holding and transport. This limits the market window for sale of live largemouth bass. Producers primarily market their fish only when pond water temperatures have stabilized for a couple of weeks below 18°C. Poor survival following harvest at higher water temperatures is thought to be primarily due to an elevated stress response followed by disease outbreaks within days after handling, resulting in financial losses for the buyers. This is of great economic importance to the customer (retail grocer) as the market value of iced fish is less than 20% of the live value in ethnic Asian markets. Some producers cool ponds using ground water or spring water to access live markets during the summer months but at this time accounts for a small percentage of total sales.

Over the past 20 years, the average price paid by live haulers for live largemouth bass picked up at the farm has remained relatively stable at

US$8.80–13.20/kg (personal communication, Robert Mayer, Kentucky, 2017). This suggests that, at this time, supply has not surpassed demand. However, the temperature constrained opportunity for sale and distribution creates a seasonal oversupply as producers attempt to reduce pond holdings before the onset of the coldest months of winter. This generally results in a period of lower prices. This can happen again as the water warms in the spring if producers still have significant holdings of fish for sale. Some producers hold inventory and wait for the price to increase and stabilize, typically around mid-December. They may harvest ponds during ice cover. This is accomplished by breaking the ice around the perimeter of the pond and seining under the ice to an area of the pond where the ice has been melted sufficiently to extract the seine and harvest the fish. Melting of ice in a pond can be accomplished when the air temperatures are > 3°C by use of a surface aerator or tractor powered power take-off (PTO) aerator.

Largemouth bass are usually harvested from ponds by seining the pond with a seine net that is ≥150% of the length and depth of the pond. Different fish species vary greatly in their susceptibility to capture by seine. In purpose built ponds with few obstructions, largemouth bass are relatively easy to capture by seining. Generally, > 90% of the population can be captured in one haul provided the seine is appropriately sized for the pond (personal communication, Robert Mayer, Kentucky, 2017). In contrast, Nile tilapia (*Oreochromis niloticus*) are particularly difficult to seine with reported catch rates of 15–23% in a single haul and with great differences reported between strains (Sifa et al., 1999). Steeby and Lovshin (1993) reported cumulative average harvest efficiencies of 57, 75 and 83% for three seine hauls with channel catfish.

A sufficiently sized collection bag in the seine is necessary as largemouth bass will readily jump over the seine. Seines for largemouth bass should be constructed of the softest knotless mesh available to reduce abrasion to the mucosal layer of the epidermis and underlying scales. Seines designed for channel catfish should not be used as they are coated to prevent their spines from penetrating and can be abrasive. The fish's mucus and skin is the first line of defense against pathogens and disease and any disruption provides an entryway. Largemouth bass do not tolerate skin abrasion associated with rough handling well.

When seining, it is important that the producer captures only the number of fish that they can load into a pond-side transport or holding tank, supplied with pure oxygen, within 30 min. It may be necessary to intentionally only capture a portion of the fish in the pond at one time. Hauling tanks mounted to a tractor or front-end loader that can be lowered into the pond during harvest greatly improves harvest efficiency and reduces aerial exposure time of harvested fish. Largemouth bass should not be overloaded into nets and/or baskets during harvest to prevent fish from bruising and abrading one another. Rubber nets are becoming more popular because they reduce skin injuries to the fish and tend not to rub off the mucous on the skin and limit eye injuries. If handling trophy bass, moving them in water can provide an added layer of protection. Water bags (such as ones used in tournaments) or vinyl fish stretchers can benefit by limiting abrasions and loss of mucous in larger fish, but this process can be time consuming since you are only handling a few fish at a time.

11.4 Holding tanks

Pond harvested largemouth bass food-fish are often moved into holding tanks where they are maintained prior to pick up. Transport from the pond to the holding tank is a crucial first step. Holding densities in pond-side transport tanks should not exceed 240 g/l, and should contain 2–3 g/l salinity and the duration in the tank should be limited to 30 min before they are transferred into larger long-term holding tanks. When water temperatures are > 18℃ and when handling smaller fish, it is beneficial to anesthetize largemouth bass using tricaine methanesulfonate (MS-222) in the pond once the seine net is pulled up to reduce the stress associated with handling.

Most producers of food-fish largemouth bass sell to specialty live haulers that distribute live fish to Asian markets. Live haulers generally prefer the fish to be harvested a couple of days ahead of time and "hardened" in tanks prior to over the road transport. This holding period allows the fish time to purge their stomachs and recover from the stressors of pond harvest. It also allows the producer to inspect the fish for disease and be sure the full number of fish will be available when the live-hauler arrives. When fish are held in tanks prior to transport it is also much easier to grade out undersize fish when loading the truck.

Holding tanks vary in size and shape but are generally supplied with flow through water from either wells, springs or surface water. The quality of the water source and supply dictates many variables associated with successfully holding fish. Temperature change is one of the most important variables to consider, even when water temperatures are within the acceptable range for transport of 3–18℃. It is important to not abruptly change the water temperature more than 4℃/h and no more than 12℃ over a 24 h period. This can be difficult to achieve in situations where the ground water temperature is much higher than the surface water temperature. In the coldest periods of winter, it may be beneficial to use surface water to supply water to holding tanks to more closely align with pond water temperatures from which the fish are harvested.

Water temperature also dictates the allowable biomass densities that can be held with minimal stress. For example, for a farmer using surface water to supply water to holding tanks, as water temperatures decrease from 18 to 3℃ over the course of the winter; the allowable stocking densities in holding tanks may increase from 10 kg/m^3 (10 g/l) to 40 kg/m^3 (40 g/l). Note that the densities in holding tanks are much lower than those often used during live hauling as the duration is often much longer. The holding tank period should be considered a recovery period from the stress of harvest prior to the upcoming stress of live hauling.

The acceptable duration for holding largemouth bass should also be related to water temperature. When water temperatures are at or near the maximum of 18℃ bass should only be held for ≤ 2–3 d before live hauling to market. However, when water temperatures are 3–5℃ bass can be held for up to 2 weeks.

Prophylactic chemical treatments to alleviate stress and prevent disease prior to transport is a common practice. Carmichael et al. (1984b) recommended daily copper sulfate treatments for 10 consecutive days prior to transport of juvenile largemouth bass. Producers routinely use salt (2–10 g/l), copper sulfate (1–3 mg/l), diquat (2–4 mg/l) or formalin (20 mg/l) as daily treatments during holding. Check with your local aquaculture extension agent to ensure compliance with state and federal regulations before administering chemical treatments on fish even if they are not considered food-fish.

11.5 Fish "quality"

The "quality" of fish to be transported is a crucial criterion. The fish must be healthy and in good condition. Weakened individuals and fish with signs of disease or parasites should be eliminated from the consignment, particularly when the temperature during shipment is high or transport times are long. When fish are of poor quality, even reducing biomass densities may not prevent mortality. It is ultimately the responsibility of the live hauler to accept or reject a load of fish before they are loaded for shipment. However, the poor reputation associated with the delivery of undersize or sick fish will ultimately reside with the farm from which they originated. When moving fish from the holding tanks to the truck it is important to recognize unhealthy individuals and not include them in the transport.

The size of the fish is also an important factor considered in the quality of the fish. The live market for food-fish in the USA currently requires largemouth bass to be a minimum size of 560 g. Owing to inherent size variability within largemouth bass cohorts generally the average individual weight of the population has to be > 600 g in order for the smaller fish in the group to achieve the minimum acceptable weight requirement. If a significant number of fish are under-size (> 5%), the producer should remove the submarket size fish from the lot. Graded-off fish can either be restocked for another year of growth or marketed as sportfish for pond stocking. It is important to communicate with the buyer regarding the acceptable percentage of submarket fish. Sometimes the seller or buyer may negotiate a reduced price for the ungraded lot to negate the additional stress associated with grading the fish prior to transport.

Ethnic Chinese customers place a high priority on healthy fish at the point of sale (Jia et al., 2016). Fish quality is generally visually determined by the consumer with main criteria being clear eyes and bright shiny scales. Brandt et al. (1986) evaluated the incidence of corneal cloudiness in transported largemouth bass. After 6 to 28 h of simulated transport, 46 to 83% of fish had cloudy eyes. Generally, opacity increased as the length of the simulated transport increased. Overall mortality in the study was < 1%. The condition was deemed reversible as > 75% of affected fish had recovered within 48 h after the stress was removed and 100% recovered within 7 d. The authors reported that they had not added salt to the water during the simulation which likely increased the occurrence of the abnormality.

11.6 Live transport

11.6.1 Live hauling

Transportation of live fish is a common practice in fish culture operations and the ultimate goal is keeping the fish alive and healthy during and after distribution. The greatest challenge with live fish transport is to minimize stress on the fish. Stress in fish associated with transport is related to disturbances in the normal physiological state as a result of various factors (Francis-Floyd, 2002). Transported fish are often, within a short time period, exposed to multiple stressors such as: high density confinement, physical handling, unfavorable water quality and conditioning to a new environment. Transport associated mortality might be the result of one severe stressor, several mild stressors or infectious disease (Harmon, 2009). Even if the fish are carefully handled and transported, a combination of mild stressors might act together and cause mortality (Carmichael et al., 2001).

Freshwater finfish farmed in China are primarily marketed live to the domestic market where annual mortality of transported fish averages about 7% of the total production (Bureau of Fisheries of Ministry of China, 2013, in Jia et al., 2016). A recent study quantified customer claims of mortality of transported live fish in Beijing, China (Jia et al., 2016). Although physiological and transport conditions were not described, the authors reported an average of 16.1% mortality of largemouth bass. The store managers stated that substandard conditions of transportation were the primary cause. Some mortality during and following fish transport is common and generally accepted as "the nature of the business" as long as mortalities are less than 0.5–1% of the total load. Producers frequently add 1–2% extra fish by weight to compensate for losses due to mortality and "shrinkage" associated with bodily waste elimination.

The primary objectives of the commercial live hauler are to keep the fish alive and healthy but also to deliver them economically. Commercial live haulers desire to haul as many fish as possible as load hauled is directly proportionate to the revenue generated. This creates a situation where fish are often hauled at maximum carrying capacity based on water conditions, distance and experience. The number or weight of fish that can be successfully transported depends on the water quality, the duration of the transport, water temperature, fish size and the species (Harmon, 2009). Piper et al. (1982) suggested that loading rates can be increased 25% for each 5°C decrease in water temperature, but if the duration exceeds 12 h, the loading rate should be decreased by 25%. If environmental conditions are constant, the carrying capacity during transport depends primarily on the species and the size of the fish. Piper et al. (1982) suggested that with trout the maximum permissible weight is directly proportional to their length. Thus, if a tank can hold 10 kg of 5 cm trout, then it can hold 20 kg of 10 cm trout. Similarly, Carmichael (1984) recommended maximum shipping densities of 90 g/l for

12.7 cm largemouth bass and 180 g/l for 20.3 cm fish for long-haul shipping (up to 30 h).

Much of the research conducted on live transport of fish is related to work at government run hatcheries where the transport times are generally < 10 h and the fish are small fingerlings for stocking. Largemouth bass fingerlings are often transported during mid to late summer when water temperatures are relatively warm which decreases acceptable loading densities. Piper et al. (1982) provided recommended carrying capacities for commonly cultured fish and reported that 100 g channel catfish and rainbow trout could be similarly hauled at 400–500 g/l for up to 10 h. The authors reported that transport densities for largemouth bass of similar size and duration should be limited to 240 g/l. Similarly, McCraren and Millard (1978) suggested that advanced fingerling (15–25 cm) largemouth bass could be transported at densities up to 240 g/l for up to 10 h with the use of agitators, bottled oxygen, and circulators (Table 11.1). Wilson (1950) reported that biomass densities during transport of 10 cm largemouth bass should be limited to 120 g/l for up to 16 h at water temperatures ranging from 18 to 24°C (Table 11.1). However, the duration of the transport can exceed 24 h to reach the West Coast of the USA from the South Central region where the majority of largemouth bass production occurs.

Carmichael (1984) and Carmichael et al. (1984a, 1984b) conducted a series of experiments to improve the success of long distance transport of largemouth bass for stock enhancement. Carmichael (1984) showed that lower biomass densities of 80–90 g/l were necessary for long distance transport (> 30 h) of largemouth bass fingerlings (12 cm). Carmichael et al. (1984a, 1984b) evaluated various chemical treatments and methods for alleviation of stress associated with hauling largemouth bass. Stress was reduced significantly and mortality was eliminated when fish were treated for diseases, held 72 h without food prior to transport, anesthetized before they were loaded, hauled at a cool temperature in physiological concentrations of salts with an antibiotic and a mild anesthetic, and allowed to recover in the same medium without the anesthetic.

Table 11.1 Maximum loading densities based on average length for largemouth bass fingerlings transported for 10–16 hours at water temperatures ranging from 18 to 24°C (Wilson, 1950).

Fish length cm (inches)	Number of fish per kg (lb)	Number of fish per l (gal)	Density of fish g/l (pounds/gal)
2.5 (1)	2,200 (1,000)	1265 (333)	40 (0.33)
5.0 (2)	880 (400)	760 (200)	60 (0.50)
7.5 (3)	220 (100)	255 (67)	80 (0.66)
10.0 (4)	55 (25)	95 (25)	120 (1.0)
20.0 (8)[a]	8 (3.6)	16 (7.2)	240 (2.0)

Note: [a]McCraren and Millard (1978).

Carmichael and Tomasso (1988) conducted a survey of public and private hatcheries to assess transportation techniques used, which were highly variable for all species. Largemouth bass were the third most commonly transported species, behind rainbow trout and channel catfish, respectively. Largemouth bass were most commonly transported for durations of < 5 h at water temperatures of 21–26°C and biomass densities of < 60 g/l. All respondents reported using salt at 1–5 g/l, 49% of respondents reported using some type of antibacterial compound, and 8.2% used an anesthetic. The highest reported transport density with largemouth bass was 286 g/l. Generally, densities hauled (g/l) were lower for smaller fish, warmer water, and longer transport times.

Table 11.2 Maximum loading densities based on water temperature and trip duration for food-size (0.45–0.9 kg average individual weight) largemouth bass (Piper et al., 1982).

Water temperature		Trip duration	
°C	g/l @ ≥ 14 h	g/l @ 15-20h	g/l @ 21-25 h
3-8	360[a]	270	180
9-13	270	180	135

Water temperature		Trip duration	
℃	g/l @ ≥ 14 h	g/l @ 15-20h	g/l @ 21-25 h
14-18	180	135	90

Note: [a]Personal communication, Robert Mayer, Kentucky, 2017.

Commercial live haulers of largemouth bass food-fish often haul at lower water temperatures and higher loading densities than those found in the literature. Assuming that all other parameters are suitable in regards to the quality of the fish, water and equipment; a maximum transport density of 360 g/l can be successfully achieved with food-fish size largemouth bass provided that the water temperature is 3–8℃ and the duration of the trip is <14 h (personal communication, Robert Mayer, Kentucky, 2017) (Table 11.2). Water temperature and trip duration should be used to decide allowable transport densities of market-size food-fish within the range of 80–360 g/l for 3–18℃ (Table 11.2). For every 5℃ increase in water temperature the density should be reduced by 25% (Piper et al., 1982). Similarly, for every 6 h increase in transport duration the biomass density should be reduced by 25%. These are estimates based on experience and communications with live haulers and fish farmers; however, there are many unforeseen circumstances that can arise, such as a truck or equipment failure, so it is always advisable to error on the conservative side if possible.

11.6.2 Fry transport

Largemouth bass fry are sensitive to environmental changes. Oxygen levels should be monitored carefully during transport. Colt (2006) recommends avoiding oxygen concentrations above 25 ppm in culture systems. Matthews et al. (2017) showed significant mortality in Guadalupe bass *Micropterus treculii* at 200% saturation and recommend staying below 15 ppm dissolved oxygen when transporting fry of all black bass species. Maintaining dissolved oxygen levels between 10 and 12 ppm is recommended for newly hatch fry. Do not add salt to the water when transporting largemouth bass fry. Transporting fry over distances in hauling boxes is not typically hindered by hauling densities as 1,000,000 largemouth bass fry weigh only 3.64 kg (0.0036 g average weight; 275 fry/g). For on-site moving of fry from the hatchery building out to ponds a density of 1,000 fry/l (4–5 g/l) is acceptable. Oxygen should be checked frequently and turned off once concentrations reach 15 ppm. These densities are intentionally low to enable a slow controlled acclimation. Agitators should not be run due to the physical damage potential to the fry. Largemouth bass fry can be successfully shipped at 10 g/l for 36 h and up to 18 g/l for 24 h in standard plastic fish transport bags containing 10 l of water within Styrofoam shipping boxes. Time in shipping boxes should not exceed 36 h with best results at shipping times of < 24 h. Although fry can tolerate a wide range of water temperatures extra care and time should be taken during acclimation.

11.6.3 Transport water preparation

Several mitigation techniques are commonly used to lessen the severity of the stress response induced by handling, including: a 3 d pre-stress fasting period, anesthetic treatment prior to handling, increasing water hardness, and the addition of salt mixtures (Tomasso et al., 1980). The addition of salts is perhaps the most common technique used with freshwater fish to alleviate the severity of the stress response. Salts passively diffuse from areas of high concentration in the blood to areas of low concentration in fresh water. Therefore, salts are slowly but continuously lost to the environment.

The gills and skin are coated with a thin layer of mucus which helps reduce the loss of salts to the surrounding fresh water. However, netting and handling removes some of the protective mucus coating from fish. Additionally, during excitement and in stressful conditions, epinephrine (adrenaline) is released into the bloodstream increasing the permeability of water across the gill epithelia in fish (Moyle and Cech, 1988). This increases the water gain and blood ion loss in freshwater fish resulting in disturbance of osmoregulatory homeostasis (Portz et al., 2006). Lost salts must be replaced by re-absorbing them from the water or during food digestion; these are active processes requiring significant energy. Ion regulation in fish is physiologically costly accounting for up to 20% of resting metabolism (Febry and Lutz, 1987).

The addition of salt to the transport water limits the loss of salt during transport by reducing or eliminating the concentration gradient between fish blood and the water environment. This reduces energy demands and diffusion leakage while providing a supply of environmental salts for re-absorption and replacement of lost blood salts. Weirich and Tomasso (1991) demonstrated that red drum (*Sciaenops ocellatus*) tolerate confinement and transport stress best in water that is nearly isosmotic with their blood plasma. The authors suggested that fish in the nearly isosmotic solutions were faced with smaller ionic and osmotic gradients and presumably required less energy to maintain proper water and ion balances during confinement.

Sodium chloride is not the only salt important to fish physiology; for example, potassium salts are critical for the normal function of heart, nerve and muscle tissue (Lovell, 1989). The blood of most freshwater fish has a salinity of approximately 9 g/l and the relative percentages of ionic concentrations of plasma fluid are: sodium 47.4, chloride 45.6, potassium 1.1, magnesium 0.44, and calcium 0.76 (Karnaky, 1997). Vanlandeghem et al. (2010) reported that rapid temperature change and exposure to low dissolved oxygen affect potassium balance in largemouth bass suggesting that the addition of potassium chloride to hauling tank water may be advantageous.

Carmichael (1984) described largemouth bass in poor condition following long-distance transport and suggested the cause to be osmoregulatory dysfunction. Carmichael (1984) described the fish as appearing discolored with opaque eyes. The fish were unable to move normally and stiff. Necropsy showed cloudy eyes and turgidity of the body was likely due to osmotic shock. The scales around the caudal peduncle appeared distended and rough to the touch with some hemorrhaging present along the caudal and anal fins. The plasma chloride levels of the fish remained low for 95 h after loading for transport. The turgid condition of affected fish indicates a probable influx of water and loss of the ability to retain homeostasis. Due to their weakened condition a secondary stressor would likely overcome their compensatory ability resulting in death.

Traditionally, 0.5 to 2 g/l sodium chloride solutions have been used to reduce stress during transport of freshwater fish (Tomasso et al., 1980). However, higher salinities may be beneficial when transporting largemouth bass. Carmichael et al. (1984b) reported a reduced stress response and increased survival in largemouth bass exposed to simulated transport conditions when using transport water with a isosmotic salt content (8 g/l) containing salts similar to those in plasma by addition of sodium chloride, potassium chloride, potassium phosphate and magnesium sulfate. Carmichael (1984) used a 5 g/l mixture of salts that closely resembled plasma values for long distance transport of largemouth bass fingerlings and reported better survival rates than in previous trials where the salinity was only 3.8 g/l.

In an unpublished study (Coyle and Tidwell, Kentucky State University), addition of a commercial marine salt premix was found to be effective in reducing mortality and improving osmoregulation of largemouth bass following 24 h of simulated transport. The trial evaluated 0, 8, and 16 g/l salinity and resulted in higher whole blood concentrations of sodium, potassium, chlorine, and lower blood glucose levels in fish at 16 g/l. Results also showed lower oxygen and higher ammonia concentrations in the blood of fish transported at 16 g/l than for fish subjected to 8 g/l, possibly indicating that 16 g/l salinity is too high for largemouth bass. Susanto and Peterson (1996) determined from physiological measurements that largemouth bass adapt well to salinities up to 8 g/l, but reported that higher levels caused measurable osmoregulatory dys-function. The use of marine premixes should more closely match fish blood chemistries than pure salt and may improve osmoregulatory function, but are also much more expensive.

Live haulers typically add evaporated salt or water softener salt to hauling containers at 2–3 g/l (ppt) to aid in osmoregulation and reduce hauling stress during the transport of largemouth bass. The use of higher rates of salinity of 5–8 g/l are generally used only for short periods of up to one hour following harvest and handling procedures such as weighing for sampling and as a prophylactic treatment when bass are held in tanks supplied with flow through water. Increasing salinity also increases foam production in hauling tank water, which may be considered undesirable. However, based on the reported literature higher concentrations of salt (5–8 g/l) could be beneficial during transport of largemouth bass.

11.6.4 Dissolved oxygen

Ideally dissolved oxygen (DO) should be maintained at or near 100% saturation throughout transport. Saturation is the amount of dissolved gas when the water and atmospheric phases are in equilibrium (Piper et al., 1982). The solubility of oxygen is dependent on water temperature, gas composition, salinity and total pressure. The solubility of oxygen decreases as the water temperature, salinity and altitude increase. Gas supersaturation can occur when the dissolved gases are greater than the equilibrium concentration. The gases of importance are nitrogen and oxygen which, in the atmosphere, are at partial pressures of approximately 78% nitrogen and 21% oxygen. However, oxygen is twice as soluble as nitrogen, so in water oxygen (35%) is approximately half as plentiful as nitrogen (65%) (Harvey, 1975).

DO is often the single most limiting factor in any fish-holding and transport system. The initial 30–60 min in the transport container is the most critical for monitoring DO levels because of the increased activity of the fish (Piper et al., 1982). Therefore, it is important to saturate or supersaturate the water with oxygen prior to placing a heavy load of fish into a transport tank. Various methods have been used to achieve and maintain proper DO levels throughout fish transport including compressed oxygen gas, agitators, aerators, circulation pumps equipped with spray bars and liquid oxygen. Commercial fish haulers use pure oxygen injection systems employing either compressed oxygen gas bottles or liquid oxygen Dewar's. Most commercial live haulers use liquid oxygen due to increased volume and reduced refill frequency. However, liquid oxygen Dewar's lose approximately 2% by volume daily so may be economically impractical in situations of long periods of inactivity (Timmons et al., 2002).

Fine pore ceramic diffusers greatly improve the efficiency of using pure oxygen due to the greater air to water surface area of the small bubbles produced. Carmichael et al. (1992) compared efficiencies of oxygen diffusers in fish-hauling tanks and reported oxygen transfer efficiencies of less than 15% for all air diffusers tested at the time. More recently, the development of micro bubble diffusers has greatly increased oxygen transfer efficiency or absorption rates to approximately 50% at 1 m depth and increasing linearly with increased water depth. Most micro bubble diffusers require 25–40 psi (172–275 kilopascal) to operate but are susceptible to cracking at pressures > 50 psi (345 kilopascal). This limits oxygen flow rate potential (l/min) to the surface area of the diffuser so it is important to size the diffuser to the desired flow rate or carrying capacity. Also, since the oxygen flows only from one side of the diffuser it is very important to ensure the diffuser does not flip over during transport thus causing the bubbles to coalesce; this is easily accomplished by attaching a piece of rebar horizontally across the diffuser with a cable tie.

Some live hauling systems use agitation devices, circulation pumps or aeration in combination with pure oxygen injection to strip excess gas and maintain oxygen levels at or near solubility. The primary disadvantage of using agitators and circulation pumps is the heat generated from their operation increases the water temperature of the hauling tanks (0.5°C/h for aerators and 1°C/ha for pumps; Piper et al., 1982). Fries et al. (1993) evaluated aeration systems during high density (521 g/l) transport of channel catfish and reported that after 4 hours water temperatures rose from 21.5 to 23.5°C. Agitators can also cause increased foaming in water containing salt (Carmichael et al., 2001). Carmichael et al. (1992) recommended a combination of agitators and pure oxygen for high density transport of largemouth bass. The advantages of including aeration equipment include carbon dioxide stripping as well as backup in the event of a failure of the primary oxygenation system. However, the associated water temperature increase accompanying the operation of aeration devises is considered a problem for long distance transport of largemouth bass.

While using pure oxygen during transport it is very easy to supersaturate the water. Fish species vary in their ability to tolerate supersaturation of DO which can affect the mechanics and equipment needs during transport. When fish are exposed to supersaturated water the excess gas can form bubbles in various fish tissues which is referred to as gas bubble disease and can result in high mortality. Gas bubble disease can be identified by the appearance of gas bubbles or blisters on the fins and other external surfaces of fish. Bubbles frequently occur behind the cornea and in the connective tissue of the eyes, producing severe exophthalmia or “pop-eye” (Noga, 2000). Piper et al. (1982) suggested maintaining DO levels at just under saturation when transporting salmonids to prevent this condition. Much of the work on gas supersaturation and the resulting gas bubble disease has been conducted on salmonids (Weitkamp and Katz, 1980). Total gas pressure (TGP) and nitrogen (N_2) supersaturation should be maintained below 110% to avoid mortality in salmonids. Largemouth bass are more tolerant to TGP and N_2 supersaturation than salmonids and reportedly tolerate levels up to 120% of saturation (Blahm et al., 1976).

Since oxygen is assimilated metabolically it is less likely than other gases (such as nitrogen) to form persistent bubbles (Noga, 2000). Wiebe and McGavock (1932) reported no mortality in rainbow trout exposed to supersaturation of DO at 300% for 14 d or at 580% for 24 h. Wedemeyer (1996) noted that mortality from gas bubble disease normally does not occur if oxygen (using pure oxygen) supersaturation is <200%. Vanlandeghem et al. (2010) evaluated the physiological response of largemouth bass exposed to oxygen stress. The authors reported that largemouth bass acclimated to 20°C water displayed signs of physiological stress at oxygen levels of 4 mg/l (44% of saturation), however, hyperoxic conditions of 18 mg/l (200% of saturation) did not result in any changes relative to control values. It is likely that largemouth bass are well adapted to conditions of hyperoxia which occurs naturally in eutrophic waters having high amounts of photosynthetic algae often resulting in oxygen supersaturation. As a good practice, live haulers using pure oxygen should maintain 100–150% of oxygen saturation, although 200% of saturation is not likely to cause damage.

11.6.5 Carbon dioxide

Elevated carbon dioxide levels are detrimental to fish and can be a limiting factor in fish transportation. Carbon dioxide is produced as a by-product of respiration. In transport containers carbon dioxide levels slowly increase relative to the density and respiration of the fish. For each 1ml of oxygen a fish consumes approximately 0.9 ml of carbon dioxide is produced (Piper et al., 1982). Increasing levels of carbon dioxide acidifies the tank water thus lowering the pH, which reduces ammonia toxicity (Piper et al., 1982). However, elevated carbon dioxide levels also lowers the oxygen carrying capacity of the blood, further necessitating the importance to maintain DO levels at or above saturation.

Severely elevated levels of carbon dioxide can result in hypercapnia and acidosis leading to narcosis and death (Wedemeyer, 1997). Aeration or agitation are sometimes used to reduce carbon dioxide buildup, however, the friction caused by the operation of electric motors causes an increase in water temperature. Also, if the tank lids are tight fitting without ventilation carbon dioxide gas that has been stripped from the water can build up in the air space between the water and the tank cover reducing the efficiency of using aeration to remove carbon dioxide (Harmon, 2009). Some live haulers, especially long-distance haulers, will have “air stacks” that allow for gases to escape the hauling box, thus not allowing for a build-up of carbon dioxide in the airspace above the water and top of the tank.

As with all water quality parameters, different fish species have different thresholds of tolerance for carbon dioxide. Trout appear to tolerate carbon dioxide at levels less than 15.0 mg/l under the conditions of adequate DO and appropriate temperature but become distressed when carbon dioxide levels reach 25 mg/l (Piper et al., 1982). Wedemeyer (1996) recommended, as a general rule, maintaining carbon dioxide levels below 30–40 mg/l during fish transport but warned that if DO is not saturated this level might be reduced. Carmichael et al. (1984a) evaluated water quality induced stress in largemouth bass by exposing them to elevated carbon dioxide levels for 24 h and measuring their secondary stress response. The authors reported that largemouth bass tolerate moderate levels of carbon dioxide (35 mg/l) with no apparent ill effects and that very high levels of carbon dioxide (135 mg/l) altered plasma corticosteroids and glucose, but had little effect on plasma chloride and osmolality.

11.6.6 Ammonia

When fish are transported, their excretory products accumulate in the tank water. Ammonia is a highly toxic waste product produced by plants and animals and is also generated by the decomposition processes of microorganisms. Ammonia is the primary by-product of metabolic processes in fish and is mainly excreted through the gills. During conditions of live transport largemouth bass may be exposed to higher than normal concentrations of ammonia for prolonged periods. Water temperature and time of last feeding are important factors regulating ammonia excretion. Water temperature during shipping should be as low as can be tolerated by the fish being handled (Piper et al., 1982). The accumulation of ammonia can be minimized by fasting fish prior to transport. Fasting fish for at least 24 h is recommended to reduce the accumulation of feces and ammonia in the tank. Carmichael et al. (1984b) recommended to withhold food from largemouth bass for 72 h before hauling. Wedemeyer (1996) reported that when transporting salmonids a typical protocol is to fast the fish for 48–72 h prior to transport. Piper et al. (1982) noted that trout fasted for 63 h produced one-half the amount of ammonia as recently fed fish.

Suski et al. (2007) evaluated sublethal ammonia toxicity in largemouth bass acclimated to 25°C and reported that total ammonia-nitrogen (TAN) levels as low as 1.7 mg/l cause physiological disturbances that can impair the recovery from exercise. Exposure to 17 mg/l caused significant reductions in ventilation rates and increases in erratic swimming. As a rule of thumb, un-ionized ammonia concentrations should be maintained below 0.2 mg/l during transport to reduce stress.

In water, toxic ammonia (NH^3) exists in equilibrium with non-toxic dissolved ammonium ions (NH^{4+}). TAN is the total amount of nitrogen in the forms of toxic ammonia and non-toxic dissolved ammonium ions. The proportion of toxic ammonia in TAN increases as the pH and temperature of water increases. Lowering of the water temperature not only slows the metabolic activity in the fish but also directly reduces the toxicity of ammonia by decreasing the percentage of un-ionized (toxic) ammonia. Lowering the pH of the water will also reduce the percentage of un-ionized ammonia, however, this is seldom practiced. Maintaining high oxygen levels and low water temperature are the best ways to reduce ammonia toxicity during transport. Chemical agents which stabilize pH and remove ammonia are commercially available and are widely used in the transport of fish (Harmon, 2009). However, before using any chemical agent on food-fish, including small sportfish which may at some point be consumed, it is necessary to check the U.S. FDA regulatory guidelines for food-fish. Your local aquaculture extension agent is usually a good source of information for these regulations.

11.6.7 Water temperature

Maintaining water temperature during transport within a desired range is probably the second most import factor after oxygen. Controlling water temperature fluctuations is generally achieved through the use of insulated hauling tanks and the addition of ice to the transport water; however, temperature controlled box trucks and chillers are also used. Tank materials can have a large impact on maintaining temperature, particularly if the water temperature and the air temperature are very different. Most hauling tanks are made of either aluminum, fiberglass or polyurethane with an insulated layer between the inner and outer surfaces of the tank. Polyurethane has the best insulation properties of the commonly used materials (Harmon, 2009). Because temperature is such an important factor it should be continuously monitored and controlled (Piper et al., 1982).

Since fish are cold blooded, lowering water temperatures reduces metabolic activity, oxygen consumption and waste excretion and also increases the oxygen carrying capacity of water. However, when fish are exposed to lowering of water temperature, the safety margin is quite small and mortality can occur if the temperature is lowered too far or too quickly. Carmichael et al. (1984a) found that abrupt temperature change, above all other factors evaluated, caused the greatest physiological stress response in largemouth bass. Rapid water temperature changes of 12°C resulted in 20% mortality immediately and 100% mortality during subsequent handling. It is well documented that fish may initially survive temporary changes in water quality, temperature or handling but later die of disease (Wedemeyer, 1970; Lewis, 1971). Fish mortalities due to temperature shock have also been attributed to osmoregulatory dysfunction (Maetz and Evans, 1972).

Vanlandeghem et al. (2010) evaluated the physiological effects of immediate temperature shock in largemouth bass acclimated to 20°C. They reported that a large cold shock (12°C) resulted in a six-fold increase in cortisol and a doubling of glucose levels but that a small cold shock (5°C) had no effect on these parameters. Heat shock from 20 to 32°C was not as severe in terms of the cortisol increase, but did produce an increase in plasma glucose similar to that reported for largemouth bass following exhaustive exercise (Suski et al., 2006). Changes in cortisol are considered to be of particular importance because they have lethal and sublethal consequences for fish. Iversen and Eliassen (2009) reported that a 2–3-fold increase in cortisol levels in Atlantic salmon (*Salmo salar*) one hour after transport resulted in a 10-fold increase in 6 d cumulative mortality compared to fish showing no changes in cortisol.

Largemouth bass sold as food-fish are typically only transported live to market when water temperatures are between 4–18°C to reduce stress, disease outbreaks and subsequent mortality (Table 11.2). Largemouth bass marketed as sport-fish are often transported for stocking purposes as soon as the appropriate size is available from production ponds; which is typically during periods of warmer water temperatures (18–28°C) greatly reducing applicable loading densities (Table 11.1). Natural ice is often added to transport tanks to cool the water. Dry ice produced from carbon dioxide or carbonic acid should be avoided. Twenty-five kilograms of ice will cool 1,000 l of water by 2°C. If the ice used is made from chlorinated water add 7.4 mg/l sodium thiosulfate ($Na_2S_2O_3$) for every 1 mg/l chlorine for neutralization (Wedemeyer, 1996). If the water contains fish during the cooling process the temperature drop should not be faster than 4°C/h and not exceed 12°C over a 24 h period for food-size largemouth bass. As a general protocol the water temperature should not be reduced more than 1°C every 15 min.

11.6.8 Use of anesthetics

Anesthetics are chemical agents that calm animals and cause them to progressively lose their mobility, equilibrium, consciousness and finally their reflex action. Anesthetizing fish prior to transport can reduce metabolic rate and associated oxygen demand, reduce general activity, increase the ease of handling and mitigate the stress response (Coyle et al., 2004). Light anesthesia that permits fish to maintain equilibrium and swimming activity can be effective for mitigating stress associated with fish handling and fish transport (Piper et al., 1982). When using anesthetics during transportation it is important that the level of anesthesia is limited to mild sedation so that fish are not physically damaged resulting from collision with the tank walls (Cooke et al., 2004). Chemical anesthetics have been demonstrated to improve transport survival by reducing oxygen consumption and stress in largemouth bass (Carmichael et al., 1984a, 1984b; Cooke et al., 2004).

Tricaine methanesulfonate (MS-222) is the only approved anesthetic registered for use with food-fish in the USA with the condition of a 21 d withdrawal period before the fish can be sold as food. This makes MS-222 impractical for use as an anesthetic for fish en route to market. However, based on increased survival and a reduction in the measured stress response, Carmichael et al. (1984b) suggested that the most effective method for hauling subadult large-mouth bass involved anesthetizing fish before capture (50 mg/l MS-222) and the use of a mild anesthetic (15 mg/l MS-222) during transport. Due to use restrictions, MS-222 can only be used for the rested harvest and transportation of broodfish or small fish that are not immediately destined for the food-fish market.

Other popular anesthetics for sedating fish are clove oil and Quinaldine which have been used as substitutes for MS-222 in the baitfish and tropical fish industries as well as for fisheries management and research procedures. Clove oil and Quinaldine both have a greater margin of safety over MS-222 and are considerably less expensive (Coyle et al., 2004). Quinaldine does not produce deep anesthesia necessary for surgical procedures where clove oil does but has a longer recovery time than either Quinaldine or MS-222. Cooke et al. (2004) reported that 60 mg/l clove oil was sufficient to produce deep anesthesia for surgical procedures in largemouth bass where 5 to 9 mg/l was effective for producing light sedation for handling and transport purposes.

Sodium bicarbonate (baking soda) when mixed with a weak acid will produce carbon dioxide gas; which has been used to produce anesthesia in fish. Durborow and Mayer (unpublished data) found that largemouth bass reached stage 2–3 anesthesia after 6 min when exposed to 0.67 g/l $NaHCO_3$ solution (30 l water, 20 g $NaHCO_3$, and 7.5 ml acetic acid) and recovered 10–15 min after being anesthetized for 1 h. The main advantage of carbon dioxide is that it is not a controlled substance in the USA and is "generally recognized as safe" (GRAS) by the FDA. This permits its use on food-fish with no withdrawal time; which at this time is the only chemical method available for use when harvesting or transporting food-fish to market.

Many factors can affect the efficacy of anesthetic treatments; therefore, experimental doses should be tested on a small group of non-critical animals before any large-scale anesthetizing is done. For environmental and human safety, the production, sale and use of chemicals is regulated by government agencies. In the USA, FDA regulates the use of chemicals on food-fish.

Currently, the only chemical anesthetic approved by the FDA for use on food-fish is MS-222; it requires a 21 d withdrawal period. These regulations are subject to change and users are encouraged to check with local extension specialists regularly for new information.

11.6.9 Other chemical treatments

Chemicals designated as bactericides, bacteriostats, therapeutants, herbicides, and water conditioners are commonly used in aquaculture for routine management practices. It is well known that handling fish predisposes them to attack by various forms of bacteria and protozoans that are seemingly ubiquitous in nature (Maule et al., 1988; Davis, 2006). As a result, disease treatments are at times necessary to treat identified pathogens and sometimes prophylactically used to eliminate disease causing agents on the surface of fish prior to or following a known stressor such as harvest and transport. Prophylactic treatment is a common practice to reduce stress and the subsequent occurrence of disease to mitigate mortality and economic loss.

The use of unapproved drugs or misuse of approved drugs in aquacultured fish poses a potential human health hazard. Chemicals for use in food-fish generally must be approved or conditionally approved by the FDA. Under certain conditions a new drug may be index listed as a legally marketed unapproved new animal drug which may be used in early nonfood life stages of food-producing-species. FDA also restricts the manner in which drugs are used and provides dosage restrictions of their use. These regulations are subject to change at any time, so it is very important to continually review updates prior to using any drug in aquaculture on the FDA website (http://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/Aquaculture/defaul.htm).

The following review of chemicals previously used successfully as treatments for largemouth bass does not imply that they are legal for use on fish produced for either the sport or food markets.

Carmichael et al. (1984b) evaluated protocols to alleviate stress associated with live transport of juvenile largemouth bass for governmental stocking programs. The most effective method involved prophylactic treatment for disease with copper sulfate (10 mg/l) for 1 h/d for 10 consecutive days prior to hauling; anesthetizing before handling with MS-222 (50 mg/l); hauling in water containing: isosmotic salt content (8–10 g/l), a mild anesthetic (15–25 mg/l MS-222) and an antibacterial compound (3 mg/l acriflaven or 10 mg/l oxytetracycline). The post-hauling recovery tanks also contained salts similar to those in plasma by addition of sodium chloride, potassium chloride, potassium phosphate and magnesium sulfate. Post-hauling the prophylactic disease treatment of adding copper sulfate to the recovery tanks at 10 mg/l per day for 10 consecutive days was repeated. The copper sulfate treatments used in this study are greater than normally recommended, however, the alkalinity was also relatively high at 320 mg/l. These treatments reduced hauling-induced mortality to an average of 5% compared to an average mortality of 88% in fish hauled in untreated well water.

Piper et al. (1982) recommended several chemical treatments for external bacteria and parasites of warmwater fish. Diquat (37% active ingredient) has been used effectively at 8.4 to 16.8 mg/l (2–4 mg/l active cation) for columnaris and fungus control. Oxytetracycline is effective as a prolonged bath at 15–20 mg/l active ingredient for columnaris disease. Copper sulfate can be used at whatever concentration is safe in the existing water chemistry. Generally, copper sulfate is safe at 1 mg/l for every 100 mg/l of total alkalinity (that is, if the total alkalinity is 50 mg/l than only 0.5 mg/l of copper sulfate should be used). Potassium permanganate is effective at 2 mg/l in clear water with little organic load; if the color changes in less than one hour it may be necessary to repeat the treatment. Formalin is effective at 125–250 mg/l for 1 h or 15–25 mg/l indefinitely for control of protozoan diseases. More recently Florfenicol, Chloramine-T and hydrogen peroxide have been evaluated to control mortality caused by columnaris (Bowker et al., 2013; Mathews et al., 2013).

Water conditioners are sometimes used during fish transport to aid in osmoregulation, stabilize pH, remove ammonia, as anti-foaming agents or as probiotics to inhibit the growth of pathogenic micorganisms. For example, the hardness and alkalinity of the water is an important consideration during live fish transport. Alkalinity should be >100 mg/l to stabilize pH and can be increased by addition of baking soda. Ideally the calcium concentration should be >100 mg/l for holding and transporting fish to enable the fish to maintain osmotic balance (Grizzle et al., 1985). The use of calcium chloride in low calcium water is an inexpensive means of improving success in fish handling and transportation. Potassium chloride can also be used as an aid in osmoregulation to relieve stress and prevent shock (Carmichael et al., 1984a).

11.6.10 Vitamin C

Vitamin C (ascorbic acid; AA) is essential in the immune responses in fish and is necessary for wound repair (Navarre and Halver, 1989). One of the physiological alterations that occur due to stress is the rapid depletion of interrenal ascorbic acid (Wedemeyer and Yasutake, 1977). Vitamin C deficiency signs are often related to hyperplasia of support cartilage in the gills, spines and fins (Klontz, 1995); which is often observed in largemouth bass after transport when held in retail display tanks for 7–10 d. Since vitamin C is depleted during the initial stress associated with harvest and transport, it is therefore in short supply for normal physiological functions during the live holding period prior to sale.

Halver (Halver et al., 1969, Halver, 2002) suggested that when fish are exposed to stress or are inflicted with muscular wounds, the ascorbic acid requirement would double or triple to aid in physiological stress mechanisms and wound repair. Inadequate dietary vitamin C levels have also been attributed to reduced resistance to bacterial disease (Lovell, 1989). In many cases, physical handling of largemouth bass particularly in warmwater is followed within 2–3 d by a severe episode of a systemic bacterial or viral disease. Given the importance of vitamin C in the stress response of fish, providing a mega dose to largemouth bass just prior to harvest may help alleviate the effects of stress associate with harvest and transport. The protective effect of vitamin C on the innate stress response is an area of research that should be investigated.

11.7 Acclimation

By the time largemouth bass food-fish reach the retail market, they have gone through more than three adjustments in physical and chemical environmental conditions related to pond harvest, holding tanks, transport, and retail display tanks. Fish can become stressed if not acclimated properly to the conditions of the receiving tank. This can be a crucial step in successful delivery as the fish have already been exposed to a series of stressors and stress is known to be cumulative; poor acclimation will likely result in immunosuppressed fish, possibly leading to delayed mortality (Harmon, 2009). Abrupt changes in water quality variables such as temperature, pH, hardness, and salinity should be avoided. If the gradient differential between these parameters is too great the fish should be slowly acclimated to receiving waters to reduce stress. It may be necessary to adjust the receiving water to match as closely as possible the conditions during transport if there is known to be great variation. For example, when holding live bass in retail display tanks for sale it may be preferable to ice the holding tanks down to match the conditions of the hauling tank and let the fish slowly acclimate up to ambient temperature.

Timmons et al. (2002), in a general statement, recommended that temperature change should not exceed 5.5°C in 20 min and if the pH differs by more than one unit to exchange 10% of the tank water every 10–20 min with the receiving water until it is similar. Fish species are known to vary greatly in their tolerance to abrupt changes in water chemistry. Most fish seem to tolerate a rapid decrease in temperature better than an increase in temperature of similar magnitude (Noga, 2000). Salmonids are reported to tolerate a 10°C change in water temperature with only mild stress provided that the fish are healthy and all other parameters are optimal (Wedemeyer, 1996). Carmichael et al. (1984b) reported that following an abrupt 12°C increase in temperature, from 10 to 22°C, 20% of largemouth bass died immediately followed by subsequent 100% mortality of the group following sampling. However, the authors reported that a rapid 6°C change resulted in no mortality and no significant changes in plasma values of corticosteroids, glucose, chloride and osmolality. Because of each unique situation (number of stressors, duration, fish health and so on) even the same species can have various outcomes to acclimation. One has to be practical, but cautious at the same time.

As a general protocol, water temperature change should be limited to 4°C/h and not more than 12°C over 24 h. Even within this recommendation it is preferable to change the water temperature slowly (1°C/15 min) to allow the body of the fish time to acclimate. One thing to consider is that as cold-blooded animals the body temperature of the fish must equilibrate to that of the water environment. Owing to a greater body mass, larger fish may take longer to physically acclimate to changes in water temperature than smaller fish.

Carmichael (1984) described five separate long distance (> 30 h) shipments of largemouth bass fingerlings and made several important observations. The conditions during transport were similar with water temperatures 15–18°C and the addition of 3.8–5 g/l salt. The first two shipments were successful with little mortality; however, fish began dying soon after arrival and mortality peaked 3 to 4 d after shipment with few survivors after 2 weeks of holding in tanks (13 and 2.5% survival). The third lot of fish were significantly larger than the previous shipments (20.3 cm compared to 12.7 cm). This resulted in better survival (55%). Owing to the sequence of unsuccessful stockings, the fourth shipment was tracked by taking blood samples from fish before, during and following transport. Results indicated that plasma corticosteroids and plasma glucose remained high for 24 h and that plasma chloride levels did not return to normal levels until 64 h after transport. Long periods of time (more than 64 h) appear necessary for largemouth bass to fully recover from hauling stress. When the fish are not permitted to recover completely a second, normally nonfatal, stressful occurrence, might now be fatal. Carmichael (1984) recommended following transport that the receiving waters should also contain 3–5 g/l salt to help alleviate transport induced osmoregulatory imbalance. This is not always practical and achievable, however, if it is possible it should be practiced.

11.8 Legal considerations of transport

Restrictive regulations are necessary to protect the interests and safety of humans and the environment. Aquaculture is regulated at various levels of government in the USA. Regulations vary greatly from state-to-state and there is no central source where all such regulations are available. Within the states, local laws may be different in certain municipalities or geographical areas. Additionally, the agencies responsible for aquaculture regulation vary widely between states creating a perplexing combination of regulations with little or no consistency (Rumley, 2012). Different federal agencies are responsible for particular areas of aquaculture regulation: the FDA regulates food safety, the Environmental Protection Agency (EPA) regulates pollution and water discharge and the Fish and Wildlife Service (FWS) regulates injurious wildlife and the Lacey Act. Of particular interest to fish transportation is the Lacey Act.

The Lacey Act is a federal statute regulating the importation and interstate transport of wild-life in the USA with special emphasis on wildlife determined to be injurious and/or endangered species. The original intent of the Lacey Act was to prevent poaching and the illegal commercial trade in wildlife by making interstate commerce of illegally taken wildlife a federal violation, thus removing jurisdictional boundaries for prosecution and levying harsher penalties than imposed by the individual states. Currently, commercially produced aquatic animals legally owned by private fish farmers are considered “wildlife” by FDA and interstate transportation is regulated under the Lacey Act. Since state laws differ greatly regarding species which are considered injurious, this creates a situation where a farmer transporting a legal species in one state into another where they are illegal, can be in violation of federal regulations under the Lacey Act.

Concern over the establishment of nonindigenous species into areas outside of their native range has increased over time leading to increased enforcement and scrutiny over their movement from state to state as well as within specific geographical regions within states. This is further complicated due to the potential for accidental contamination of a load of fish by a species considered indigenous or established in one region and then transported into another region where that species is regulated as nonindigenous and may be listed as injurious.

Regulatory complexity and a lack of clarity in state regulations for interstate transport of fish greatly increases the legal risks associated with transporting largemouth bass to distant markets.

Since largemouth bass are popular as both food-fish and sport-fish, they are commercially shipped live to virtually all states across the continental USA. As a result, farms producing largemouth bass are heavily impacted by regulations related to transporting live fish into the states where their buyers are located as well as through states en route to end markets. Most small and medium sized largemouth bass food-fish producers rely on independent live haulers to transport their product to distant markets. Thus, shifting the responsibility of regulation compliance to the live haulers which act as middleman usually supplying several independent grocery stores in one or more cities. Sport-fish producers are more likely to deliver small quantities of live fish regionally to other farms and to stock private ponds and lakes.

Extreme care when loading fish for transport is necessary to prevent accidental contamination of a load of fish which may result in a Lacey Act violation. The risks associated with the Lacey Act can be minimized by shipping only in-state; however, this may not be feasible for some businesses. Producers involved in interstate transport should know the risks and take steps to mitigate them by having proper documentation for each load and by exercising due care in ensuring compliance with the laws of all states that the shipment passes through.

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Chapter 12
Major markets for largemouth bass

Angela Caporelli

Kentucky Department of Agriculture, Frankfort, Kentucky, USA

12.1 Introduction

The largemouth bass (*Micropterus salmoides*) is a difficult species to secure hard data on in terms of both production statistics, and market scope and characteristics. A number of US states have not allowed the raising or the selling of largemouth bass into the live food markets. It has been considered by many a sport-fish only species, coveted and protected by trophy anglers and sport angler's clubs. It is one of the most popular freshwater fish in North America due to both its wide distribution and reputation as a tough and entertaining fighter (Figure 12.1). As a farmed species, the largemouth bass is one of the highest value freshwater aquaculture species. Competing markets for both sport-fish stocking and as a food-fish help maintain a high selling price.

Figure 12.1 The largemouth bass as a sport-fish.

Largemouth bass are produced by private hatcheries to supply recreational sport-fish stocking throughout much of North America as well as parts of Europe and Asia. In fact, the world record largemouth bass was caught in Japan in 2009 weighing in at 10.12 kg. Market demand for largemouth bass as a sport-fish encompasses all sizes from small fingerlings to trophy-size fish. Millions of largemouth bass fingerlings are also produced by federal and state public hatcheries throughout the USA to meet the demand for recreational use. In the USA, largemouth bass are also produced as food-fish, which are primarily marketed live in major cities with large ethnic Asian populations. In Mexico, largemouth bass are raised and sold fresh-on-ice in certain regions. In China, the species is highly regarded and their production and marketing there are covered in Chapter 3. In the USA, the food-fish market is typically for 0.45–0.90 kg fish with a desired minimum market size of 0.6 kg.

Since the 1970s, there has been market research conducted on both wild caught and farmed fish. These studies have involved both academia and private research entities. The information generated on composition, pricing, and delivery has helped several sectors of the aquaculture industry expand. However, little market related research has been conducted on largemouth bass marketing opportunities to help guide the development of the industry.

Largemouth bass has an optimal growing temperature of 24–28℃. This environmental factor largely defines the geographic regions suitable for production. However, optimal production areas are not always within economical driving distances to the available live food-fish markets or recreational waters for stocking. This marketing consideration is one of several factors affecting the decision to produce them and can have a large impact on farm profitability.

12.2 Sport-fish market

The largemouth bass was scientifically described in 1802 by Lacépède and were harvested commercially and recreationally as a food-fish without oversight until 1871, when the U.S. Fish Commission was created. The Commission sanctioned fish stocking programs by the US government and by 1900, largemouth bass had been stocked far outside of their native range including 26 states and several other countries.

Largemouth bass fingerlings have been produced by state and federal hatcheries since the 1890s (Lydell, 1903; Turner and Kraatz, 1920). Today, state fish hatcheries stock tens of millions of largemouth bass fingerlings into public waters to support the recreational demand. The Texas Parks and Wildlife Department (TPWD) alone reportedly stocks 6 to 8 million largemouth bass in lakes across Texas in a typical year.

Before the 1960s, hatcheries primarily relied solely on live feeds. Unlike channel catfish (*Ictalurus punctatus*) and rainbow trout (*Oncorhynchus mykiss*), newly hatched largemouth bass fry (swim-up stage) cannot be easily trained to accept prepared diets (Brandt et al., 1987; Williamson et al., 1993). Therefore, bass fry is usually raised in fertilized nursery ponds until they reach 25–50 mm TL (1–2 g). These small fingerlings are either sold directly following the nursery phase (untrained) or are habituated to feed on commercially available dry fish-feed (Tidwell et al., 2000). Feed-training increases their value by approximately 30% (Engle and Southworth, 2008).

Almost all larger sizes are produced using "feed-trained" fingerlings as it is much more cost effective than using forage fish to support growth (Engle and Southworth, 2008).

The popularity of largemouth bass as a sport-fish in the USA led to the establishment of the Bass Anglers Sportsman Society (BASS) in 1968. They and other clubs persuaded state fish and wildlife agencies to not allow the farming or sale of largemouth bass as a food-fish in several states. These clubs feared that if allowed, there would be fishing, whether legal or not, in public waters and that "trophy" fish would be sold in the market. This was supported by the estimated value of privately produced fish in 1983 at US$393 million compared to the US$17.3 billion sport-fishing industry which increased to US$28.2 billion by 1985 (personal communication, BASS).

Angler clubs have tripled in number since 1980 and fishing clubs in high schools have increased five times since then. Adding to the public awareness of largemouth bass, professional bass and club tournaments are now widely televised. While this has tended to inhibit the growth of food-fish production, it has likely increased the demand for largemouth bass to stock private and public lakes and ponds. In conversations with several live haulers, it is estimated that the demand for largemouth bass for stocking in sport-fish applications increased by 200% between 2000 and 2017 and suggests that the market continues to grow. They also estimate that demand for large (0.5 to 1.0 kg) largemouth bass for sport-fish stocking will double by 2025 (personal communication, Adam Hatter, Ohio, 2018).

Largemouth bass fingerlings are typically sold into the sport-fish market according to their approximate length. For example, common sizes are 2.5–5 cm (1–2"), 5–10 cm (2–4"), 10–15 cm (4–6"), 15–20 cm (6–8") and 20–25 cm (8–10"). Sizes larger than 25 cm are typically sold by weight. Many sport-fish suppliers purchase adult largemouth bass from food-fish producers. The retail price of largemouth bass sold as sport-fish is typically much higher (~100%) than the wholesale price received for food-fish.

Private hatcheries primarily sell largemouth bass to landowners wishing to enhance recreational opportunities on their lands and increasingly into managed trophy fisheries and pay lake operations. Some state agencies also contract live fish purchases through private producers in addition to or instead of public hatcheries. The actual scale of commercial sales into the sport-fish market is difficult to quantify due to the inherent problems of knowing the ultimate use of a fish, which may be sold multiple times before reaching the end user. However, the number of small largemouth bass fingerlings produced by private hatcheries for recreational purposes is very small compared to the millions produced by state and federal hatcheries for stocking into public and private waters in the USA.

The United States Department of Agriculture (USDA) periodically conducts a Census of Aquaculture in the USA. They have published data sets for 2005 and 2013 (USDA, 2014). Between 2005 and 2013 the total sales of sport-fish increased 32%. In 2013, total sales of sport-fish in the US was US$23,849,000. Of that, US$14,452,000 was generated by largemouth bass, representing 61% of total sport-fish sales. Small fingerlings or fry were raised on 66 farms and 1,964,000 small fingerlings or fry were produced. Average sizes and prices were not reported. Stocker size fingerlings were produced on 52 farms. Sales were 1,122,000 fish with an average weight of 272 g. They generated US$1,868,000 and averaged a selling price of US$10/kg.

12.3 Food-fish market

Largemouth bass has long been prized for not only their recreational but also their culinary qualities. Long et al. (2015) gave a historical perspective of black bass management in the USA. In this article, the authors provide historical reference to the overexploitation of the fishery in the 1800s and the rebuilding efforts that followed. Perhaps the first reference to the food-fish market is from an excerpt from the magazine *Forest and Stream*, which indicated that largemouth bass from Virginia were selling for 18 cents at fish markets in 1875 (Anonymous, 1875). Shortly after, it was reported in the *New York Times* that lakes in Pennsylvania were being “depopulated of its fish” by those wishing to “fill his boat” through the use of dynamite (Anonymous, 1884). The food-fish market for largemouth bass has changed dramatically over the years both in the manner of which they are obtained and the ethnicity of the consumer groups.

State laws differ widely in the USA in their approaches and statutes to protect and regulate fish and wildlife resources. This can have dramatic effects on the market factors for aquacultured species which can also be considered sport-fish. For example, in Mississippi it is illegal to sell sport-fish, including largemouth bass, as a food-fish. Producers are only permitted to sell small fingerlings. However, Mississippi has the largest pond-based aquaculture infrastructure in the USA. The ponds were constructed for channel catfish production, but that industry has contracted approximately 40% in recent years leaving substantial pond acreage unused. Should future state legislation allow Mississippi farmers to produce largemouth bass for sale as food, the scale and pricing structure of largemouth bass production in the USA could change dramatically.

State fish and wildlife agencies' concerns regarding the sale of largemouth bass for food are based on an assumption that anglers would attempt to harvest fish illegally for black market sales. However, as recently as 2013, New York State legislation took effect permitting the sale of large-mouth bass as food-fish. The legislation requires that individual farms obtain a permit from the New York State Department of Environmental Conservation and that the seller must provide a bill of sale to the purchaser and retain a copy of the same. The seller must also provide the purchaser a fish health inspection report certifying the fish to be disease free. The shipping container must also be marked "black bass". Sellers must retain copies of their purchase and sales records for 2 years and sellers (the fish market) are required to kill the fish before transferring possession to the final customer. With this change in regulations, New York City had been the largest market for largemouth bass food-fish in North America. This demonstrates the dramatic effect that state legislation can have on the market potential and growth of the aquaculture industry.

From roughly 2005 to 2016, Toronto, Canada was the largest outlet for US farm-raised large-mouth bass sold as food-fish. In recent years, fewer largemouth bass have been sold into Canada (personal communication, Charlie Conklin, Idaho, 2018). Part of this was due to changes in currency exchange rates. Largemouth bass are the most expensive commercially available cultured freshwater fish. When the exchange rate is above 1.2 (US$1 equals CAN$1.2) Canadian buyers often chose to purchase cheaper alternatives, such as tilapia and carp species. Between 2014 and 2018 the exchange rate increased from 1.0 to 1.33. It is quite fortunate that the New York market was opening up, and distribution channels were developing, just as the Canadian market constricted. This created a situation where regulation change in New York essentially provided a safety net for US largemouth bass producers. The fickle and complex nature of the market for live fish, and for largemouth bass in particular, necessitates that producers develop good relationships and maintain open lines of communication with their buyers and regulators so they can quickly adapt to the changing environment.

The food-fish market for largemouth bass is somewhat unique in that the fish are typically marketed live to the consumer. This requires that the physiological condition of the fish be sufficient to tolerate the steps from harvest to marketing, including holding in retail display tanks over a period of several days to weeks. For other fishes, such as channel catfish (*Ictalurus punctatus*) and rainbow trout (*Oncorhynchus mykiss*), harvest and transport procedures are based only on live delivery to a processing plant, which is usually in relatively close proximity. At the time of the writing of this chapter, there were no known commercial processing of largemouth bass in the USA. Since largemouth bass are marketed live, they should be thought of as a perishable product with a shelf life similar to that of produce. As with produce, the clock starts at the time of harvest and even when maintained under optimal conditions the product quality can slowly deteriorate over time. This situation requires that stress be minimized at every step from harvest to retail sale.

The largemouth bass is a somewhat stress sensitive species with a limited ability to tolerate net confinement, temperature change, periods of low oxygen, or of high ammonia concentrations (Williamson and Carmichael, 1986). Harvesting requires special considerations to limit unhealthy fish being sent to market and subsequent stress associated mortality. As poikilothermic animals, water temperature and temperature change have great effects on the physiological stress response in fishes. Largemouth bass may not tolerate rapid changes in water temperature as well as other aquaculture species such as channel catfish or rainbow trout (Currie et al., 1998). However, the overall stress response of handling is reduced at lower water temperatures.

During the summer months, largemouth bass are typically not harvested for food-fish sales due to increased stress and subsequent mortality. Harvest at high water temperatures likely produces an elevated stress response, followed by disease outbreaks. High mortality is usually seen a few days after delivery to market, resulting in financial losses for the buyers. This is of great economic importance to the customer (retail grocer) as the market value of a dead fish on ice is less than 20% of the live value of the same fish in ethnic Asian markets. Some producers cool ponds using ground water or spring water to access live markets during the summer months but at this time accounts for a small percentage of total sales. Producers primarily market their fish only when pond water temperatures have stabilized for a couple of weeks below 18℃.

The USDA 2013 Aquaculture Census does not list the largemouth bass being produced under its food-fish category. It only lists them under sport-fish production. However, it does have a largemouth bass size category under sport-fish of food-size or market sized. It is safe to assume that most of those fish are not going to be stocked but are actually going to market as a food-fish. The census lists 60 farms raising 1,664,000 food-size largemouth bass, producing a total weight of 975,455 kg. The average individual weight was 590 g. With an average selling price of US$11.70/kg, they generated US$11,424,000. That is six times the income generated for stocker largemouth bass.

Over the past 20 years, the average price paid by live haulers for live largemouth bass picked up at the farm has remained relatively stable at US$8.80–13.20/kg (personal communication, Robert Mayer, Kentucky, 2017).

This suggests that, at this time, supply has not surpassed demand. However, the temperature constrained period for sale and distribution creates a temporary oversupply at the beginning of fall harvest season as producers attempt to reduce pond holdings before the onset of the coldest months of winter. This generally results in a lower price during late fall and early winter. Some producers hold inventory and wait for the price to increase and stabilize, typically around mid-December. This may require that they harvest ponds during ice cover. The vast majority of food-size largemouth bass are sold into the ethnic Asian markets during the months of January and February as they are very popular for use in the traditional Chinese style steamed fish, especially during Chinese New Year celebrations which last for 15 days. Largemouth bass, called "mang cho" in Cantonese, are considered a sign of wealth and prosperity, which promotes continued good fortune into the coming year. If producers still have a significant inventory of fish, a temporary price drop can occur again in spring as water temperatures increase.

Most producers of food-fish largemouth bass sell to specialty live haulers that distribute live fish to Asian markets. Live haulers generally prefer the fish to be harvested a couple of days ahead of time and "to harden" in tanks prior to over the road transport. This holding period allows the fish time to purge and recover from the stressors of pond harvest. It also gives the producer an opportunity to inspect the fish for disease signs and be sure that the number of fish contracted for purchase will be available when the live hauler arrives. When fish are held in tanks prior to transport it is also much easier to grade out undersized fish.

Ethnic Chinese customers place a high priority on healthy fish at the point of sale (Jia et al., 2016). Fish quality is generally visually determined by the consumer with main criteria being clear eyes, bright shiny scales, and vibrant red gills. Weakened individual fish with signs of disease or parasites should be eliminated from the lot. When fish are of poor quality, even reducing biomass densities may not prevent mortality during transport. It is the responsibility of the live hauler to accept or reject a batch of fish before they are loaded for shipment. However, a poor reputation gained from selling undersize or sick fish will ultimately reside with the farm from which they originated.

The size of the fish is an important factor. The live market for food-fish in the USA currently requires largemouth bass to be a minimum size of 0.6 kg. However, because of inherent size variability the average individual weight of the population has to be > 0.6 kg to ensure that the smaller fish in the group also achieve the minimum acceptable weight. If a significant number of fish are undersize (> 5%), the producer should grade and remove the submarket size fish from the lot in order to maintain a high level of quality. Undersized fish can either be restocked for another year of growth or marketed for sport-fish pond stocking. It is important to communicate with the buyer regarding the acceptable percentage of submarket fish. Sometimes the seller or buyer may negotiate a reduced price for the ungraded lot to negate the additional stress associated with grading the fish prior to sale.

There have been some reports of producers selling submarket size largemouth bass to high end restaurants where they are purchased seasonally in small volumes and typically processed by hand in the restaurants (personal communication, Rocky Allen, Kentucky, 2018). Producers often look for another outlet for submarket fish. This situation creates a seasonal opportunity for the sale of pan-sized (400–500 g) largemouth bass at a reduced price, possibly allowing for entry into a fresh-on-ice sales to restaurants at a more competitive price.

12.4 Fillet market

As a carnivorous species, largemouth bass currently have a higher cost of production than some other aquaculture species, such as catfish and tilapia. Largemouth bass require relatively high levels of dietary protein. Engle et al. (2013) estimated the cost of production for largemouth bass to be US$7.27–9.36/kg compared to US$1.21–1.32/kg for channel catfish. Engle et al. (2013) estimated the profit margin typical of catfish food-fish producers to be approximately US$0.11–0.55/kg. Currently the sale prices of largemouth bass food-fish are US$11.02–13.22/kg (personal communication, Robert Mayer, Bardstown, Kentucky, 2018) representing a profit margin of US$1.66–5.95/kg for largemouth bass food-fish producers using production cost data provided by Engle et al. (2013).

Engle et al. (2013) evaluated whole-dress (head and viscera removed) and fillet (shank fillet without belly meat) yield of largemouth bass. Whole-dressed yield from 61–62% of the weight of the whole fish and fillets yield averaged and 34–35% of the original fish weight. Tidwell et al. (1996) reported whole dress and fillet yields (fillet with belly meat) for largemouth bass of 61.2 and 37.5% of the whole weight, respectively. These reported dress-out yields for largemouth bass are basically similar to channel catfish, better than tilapia (with 30% fillet yield) but less than salmon which typically yield fillet weights of > 60%.

Dasgupta and Caporelli (unpublished data) surveyed restaurants in Kentucky to examine chefs' perceptions of farm raised largemouth bass. Two fish, sourced from a commercial farm in Kentucky and a questionnaire were provided to 33 restaurants with 12 restaurants providing responses. The preferred form of largemouth bass was fillets (53%), followed by gutted fish (23%), with 18% preferring whole fish, and 6% desiring live fish. The majority (83%) of respondents indicated that they were unsure whether they would buy the fish. The most preferred product form and price was fillets at US$13.76/kg, the lowest price offered for fillets. Price seemed to be the most important factor. Only 3% of respondents indicated that they would pay US$39.65/kg for largemouth bass fillets, the highest price offered, but also the only price that would be similar to that currently obtained by the live market, considering the cost of processing, a fillet yield of 34–38% and a current sale price of US$11.02–13.22/kg for live largemouth bass sold as food-fish.

12.5 Market research

In the 1970s, increasing demand within the food-fish industry particularly for live fish resulted in increased popularity of commercial production of largemouth bass. Several states, which formerly prohibited the culture of largemouth bass due to their sport-fish status, now allow the sale of largemouth bass raised responsibly under licensed aquaculture methods. By the 1990s, aqua-culture research expenditure in the USA was US$1.04 billion with catfish accounting for 57% of the total. This has led to great returns on investment, as the industry is valued at over US$29 billion. Recently, research expenditures have diversified into several other promising species including largemouth bass (Figure 12.2). Furthermore, the realization that US produced aquaculture products cannot efficiently compete with commodity fish products in the global marketplace has led research and production strategies in the US to focus on supplying live products directly to the consumer and particularly to ethnic Asian markets.

Increases in food-fish demand since the 1980s are partially due to the US population becoming more health conscious as well as more food savvy through more national and international travel and domestic restaurants using more exotic proteins. Ethnic diversity also has become a big factor. There is an increased demand for species that were unheard of in fish markets prior to 1980. Fish farming has expanded the availability of diverse species, but not without struggles. Fish farming has been the target of several environmental groups stating that the feed fed to fish is toxic and those fish raised should not be eaten, and only wild caught fish are healthy. This has been challenged through research (Mozaffarian and Rimm, 2006). They reviewed data from the Environmental Protection Agency (EPA) and calculated that if 100,000 people ate farmed salmon twice a week for 70 years, the extra polychlorinated biphenyl (PCB) intake could potentially cause 24 extra deaths from cancer – but would prevent at least 7,000 deaths from heart disease. They also found that levels of PCBs and dioxins in fish are very low, similar to levels in meats, dairy products, and eggs, and moreover 90% of the PCBs and dioxins in the US food supply come from such non-seafood sources, including meats, dairy, eggs, and vegetables. Therefore, the thought of PCBs and dioxins in fish should not influence any decision about which fish or any other food source to consume. It is all about balance.

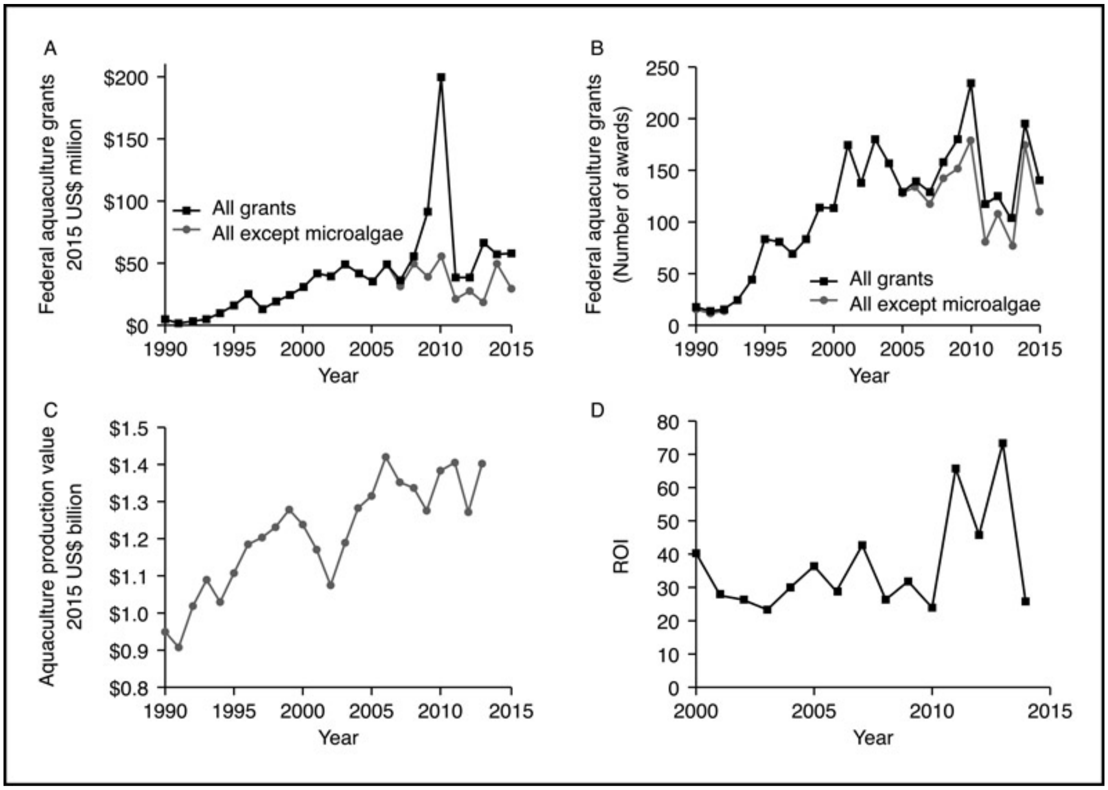

Figure 12.2 Aquaculture research expenditures in the USA. (a) Total grant spending per year, 1990–2015 (cumulative total US$1.04 billion); (b) total number of grant awards, 1990–2015; (c) aquaculture value per year, 1990–2013 (cumulative total US$29.13 billion); (d) return on investment, 2000–2014 (total gain minus total investment divided by the total cost of investment), federal grant funding for aquaculture has had a 37-fold return on investment since 2000.

Source: An Analysis of Nearly One Billion Dollars of Aquaculture Grants Made by the US Federal Government from 1990 to 2015, David C. Love, Irena Gorski, Jillian P. Fry, *Journal of the World Aquaculture Society*, Volume 48, Issue 5, October 2017, pages 689–710.

Cargill (2017) surveyed 1000 US residents in August 2017, led by Grant Murray of Duke University, in their *Feed4Thought Sustainable Seafood Survey* report. The survey found that 72% believed seafood is an important part of a healthy diet and a source of good nutrition. Over 61% agreed that sustainability of source was important when purchasing seafood. They found that 93% of millennials born between 1980–2000 would pay more if certified as sustainably raised and sourced. However, 80% of those surveyed held sensory qualities as most important.

As stated previously, people are becoming much more food savvy and experienced. This in large part due to increased national and international travel. Reynolds (2017) found that in 1974, 2.4 million adults traveled overseas, in 1980 over 3 million and in 2015 over 24 million or 3.5% of the adult population traveled internationally. Most of these travelers are looking for authentic experiences and tasting the real culture. They are bringing these culinary experiences back home and demanding more seafood choices and cultural food experiences.

Fish offerings on menus have changed drastically since the 1970s and 1980s. Gone are the days of breaded fried catfish as the only fish option. Chefs have included squid or calamari to almost every menu. Tilapia, which were not available in the USA until the early 1990s, as of 2018 is the third most popular seafood product in the USA and is available wherever there are more than two fish choices. Inclusion of more exotic species such as squid, whiting, monkfish, and ocean pout (formerly yellow eel) was a direct result of the chef/fisherman collaborative campaign in the 1990s to "Serve By-Catch" in the UK and the "Bite-Back" campaign in the USA. These species were then promoted heavily and served at top restaurants. For the first time ever, commercial fishermen, aquaculturists, regulators, NGOs, and famous chefs came together to help with the overfishing solution that would benefit all by filling the gaps for now regulated wild fish and promoting aquaculture. The marketing campaigns that followed were well thought out and very successful in steering chefs and consumers to think more about their fish and seafood choices. These campaigns also lead to more support for aquaculture and the development of alternative species, such as the largemouth bass.

In order to introduce lesser known species, or seasonally available species, to the public and chefs, a good marketing plan needs to be in place and consistent supply of product needs to be available. As a young industry, aquaculture in the USA is facing the same problems that poultry faced 75 years ago; distribution, processing, quality, consistency and demand. The efforts that have been in place are catching hold through states and grass roots organizations to educate consumers on the benefits of locally raised agriculture products, of which largemouth bass is a valuable one.

The Slow Foods International movement or Buy Local programs in many states have proven to have a positive impact on small farm sales. Both these marketing strategies lend themselves to influencing the consumer that "If it is local, it is fresher and healthier" and has less of an environmental impact than imported products. This is a great advantage for largemouth bass farmers to use these marketing programs to assist them in promoting their products and developing a consistent customer base.

Consumers also want to know the story of where their food comes from and the story of the farmers and the farm history. Several states are employing incentive programs to assist in the marketing and distribution for local producers of all agriculture products including aquaculture. This is especially important for live aquaculture products, as fish sourced from local waters experience less stress, ultimately leading to a better product with a longer shelf life (personal communication, Leo Ray, Idaho, 2018).

Live haulers and grocers offering live food-fish indicate that issues with inconsistent supply and delivery are the main limiting factors to expansion of the market for largemouth bass food-fish (personal communication, Mark Eikenberry, Indiana, 2018). A limited number of farmers can handle and deliver the fish well enough to yield good survival and appearance (personal communication, Adam Hatter, Ohio, 2018). Most live haulers have established relationships with suppliers and are very reluctant to try new suppliers, even when demand requires more product. The primary objectives of the commercial live hauler are keeping the fish alive and healthy and delivering them economically (Figure 12.3). Commercial live haulers of largemouth bass need to haul as many fish as possible as the weight transported is directly proportional to revenue generated. However, this can also potentially contribute to unnecessary stress and mortality. Research is being done on the academic level but farmers and live haulers are often not aware of the best management practices. Any farmer wanting to enter live fish marketing needs to ensure high quality fish, responsible transport and a long shelf life, especially in ethnic markets.

Ethnic diversity is a large reason for the increase in live food-fish demand, especially for largemouth bass. In 2007, the demand for live fish in Ethnic Asian markets was primarily for tilapia and hybrid striped bass (Carlberg et al., 2007). However, was this truly based on demand or primarily availability of these two species as live products? The sale of largemouth bass was not legally permitted in New York State until 2013. Clearly, demand for largemouth bass has increased significantly since 2007.

Figure 12.3 A live haul trailer.

Figure 12.4 Live largemouth bass for sale in a market catering to East Asian customers.

Myers et al. (2009) conducted a survey of ethnic live seafood market operators in the Northeastern US catering specifically to East Asian customers (Figure 12.4). The study evaluated live fish markets in New York, New Jersey, and Pennsylvania. There had previously been little market research on the retail markets selling live seafood. They examined factors that influence live fish sales, expansion opportunities, and characteristics of these businesses and markets. The goal was to develop scenarios that would help local US producers with production and distribution strategies to address these live food-fish markets. A second goal was to identify issues affecting future growth including geographic distribution issues, associated costs and net returns, and risk comparisons. There were 160 markets visited with a 27% response rate of the written survey (35). Thirty-eight percent of the markets used one to three vendors for live-fish supply; half of those were local distributors and 44% were out of state suppliers. Over 90% become associated with their supplier by word of mouth or one-on-one visits from the farmers or live haulers. Tilapia and hybrid striped bass were the top two live finfish species sold (58%). Results indicate that due to demographic changes and trends in seafood demand, growth in the live seafood market is expected. Twenty-four percent of these respondents stated that they plan to expand live-fish sales and tanks in the near future. The unique findings of the study were that: (1) freshness and quality were valued over price; and (2) fish that show good appearance and are energetic will have better quality flesh and longer life in the tanks. Within these ethnic markets, locality or origin was not valued as high or as important as freshness or quality. Sixty-five percent of respondents stated that availability was very important with only 15% stating that regular availability was not important. One-on-one negotiations were the mode of buying from producers or haulers and quality, freshness, and availability were triggers to start price negotiations.

Of the markets responding to Myers et al. (2009), 64% stated that ethnic Asians composed 50–80% of their customers and another 32% of markets stated Asians composed 20–50% customers. Other ethnic groups also purchasing live fish were Hispanic and African Americans. Seasonality was a factor affecting live food-fish sales with June through September having the least sales, and colder months of November through February having the most. Fidler (2000) reported that because of seasonality trends of live seafood, recreational stocking demand and food-fish demand could complement each other. Producers could choose what sector to sell into during different times and profits could increase by restricting supply to one sector over another.

According to the 2010 US Census (U.S. Census Bureau, 2010), the Asian population grew 45.6% between 2000–2010, much more than the 9.7% of the overall US population. Of the total US population, 14.7 million or 4.8%, were Asian and 2.6 million reported Asian combined with another race. This means that 17.3 million or 5.6 % of the US population is Asian or a combination of Asian and another race. Asian populations grew 30% in each state and equate to 50% of the population in Hawaii. Among the 20 metropolitan statistical areas with the largest Asian, alone-or-in-combination, ethnic Chinese was the largest detailed Asian group in 6 of the 20 metro areas (New York City, Los Angeles, San Francisco, San Jose, Boston, and Seattle). The Asian Indian population was also the largest detailed Asian group in six of the 20 metro areas (Chicago, Washington, Dallas-Fort Worth, Philadelphia, Atlanta, and Detroit). The New York /New Jersey area alone had 1.1 million Asian immigrants migrate into the area in the decade 2000–2010.

Research by Puduri and associates (2010) was conducted to characterize the Asian population's preferences in purchasing live seafood products, as well as the availability and the ability of producers to supply these growing markets. Information from producers has been difficult to accrue due to concerns over competition; however, Puduri et al. (2010) found that most producers see the benefit of cooperation to grow the live fish market and increase demand.

Five markets within New York, New Jersey, and Pennsylvania participated in this study and all had over 227 kg (500 lb) of live seafood product sales. A total of 252 consumers were interviewed collecting data on: awareness and perception of live fish sales, importance on availability or seasonality, freshness, quality, health concerns, source of product and characteristics such as size and whether wild or farm raised. In terms of most important attributes, 78% indicated quality and freshness. Another 23% indicated quality and health concerns were important. Hybrid striped bass was preferred by 47% of respondents, 36% wanted wild caught, 26% wanted smaller fish, 53% scaled fish and 45% wanted shellfish. Other findings were on average consumers traveled eight miles, six times per month and spent an average of US$15.19/visit on live food-fish. Ninety-nine percent of the respondents were 20–50 years old with 42% between 21–35 years old and 45% 36–50 years old, 53% had up to college level education, 35% of those had post grad education and 58% of respondents were female. In summation, consumers in the live fish markets prefer year-round availability, if the consumers are more quality and health driven, have college educations, are between age 20–50 and have household incomes of between US$25–75,000 supporting 3.7 people/per household.

The Asian tradition of fresh fish, as the highlight of protein within their daily meals equates to consumption of 21–32 kg/person/year (Degner et al., 1994; Sechena et al., 1999) as compared to non-Asian populations of 7.4 kg/person/year (NMFS, 2008). This along with the fact that in several large cities, Asian communities are growing and their traditional meals and demand for fresh fish will be on the rise. This is a great opportunity for live fish producers to gain market access.

Quagrainie et al. (2011a, 2011b) surveyed shoppers for live seafood products in the Midwestern US, including Chicago, Indianapolis, West Lafayette, Fort Wayne, Evansville, Columbus, Fairfield, Cincinnati, and Cleveland. The study assessed the decisions of shoppers of live seafood and the influence of various factors using binary choice and latent class profit model. The study included 28 ethnic Asian stores. A US$3 coupon was given to participants of which 365 of the 461 approached completed the survey (79%) in person. Results indicated that consumer preferences for live fish species vary by ethnic group in the Midwest and there were two distinct groups of consumers: traditional shoppers and occasional shoppers. Product appearance and weekly frequency of purchase were not important variables to traditional shoppers who were motivated by ethnic traditions and price. Occasional shoppers purchased live seafood less frequently and were more influenced by appearance and distance travelled, as opposed to price.

Thapa et al. (2015) used a binary logit model to study the factors influencing preferences for live and fresh fish products in Asian ethnic markets. This study, conducted in select markets in New York, New Jersey, and Pennsylvania during the summer of 2009, found that market demand varied by ethnic group, but was dominated by the East Asian communities consisting of Chinese, Korean and Japanese. These shoppers visited live fish markets two or three times per week and spent US$16–20 per visit on medium-sized live fish (less than 1 kg live weight). Freshness and appearance of eyes, gills and skin were the most important attributes influencing consumer purchases. Of the ethnic groups within the East Asian communities, Japanese had the highest consumption of fresh fish at 65 kg/person/year, Malaysians at 45 kg/person/year, Thai at 33 kg/person/year, Filipinos 27 kg/person/year, Chinese 25 kg/person/year with an overall average of 27 kg/person/year (Dey et al., 2008). Overall, these are about four times higher than Caucasian consumption of 8 kg/person/year (NMFS, 2008).

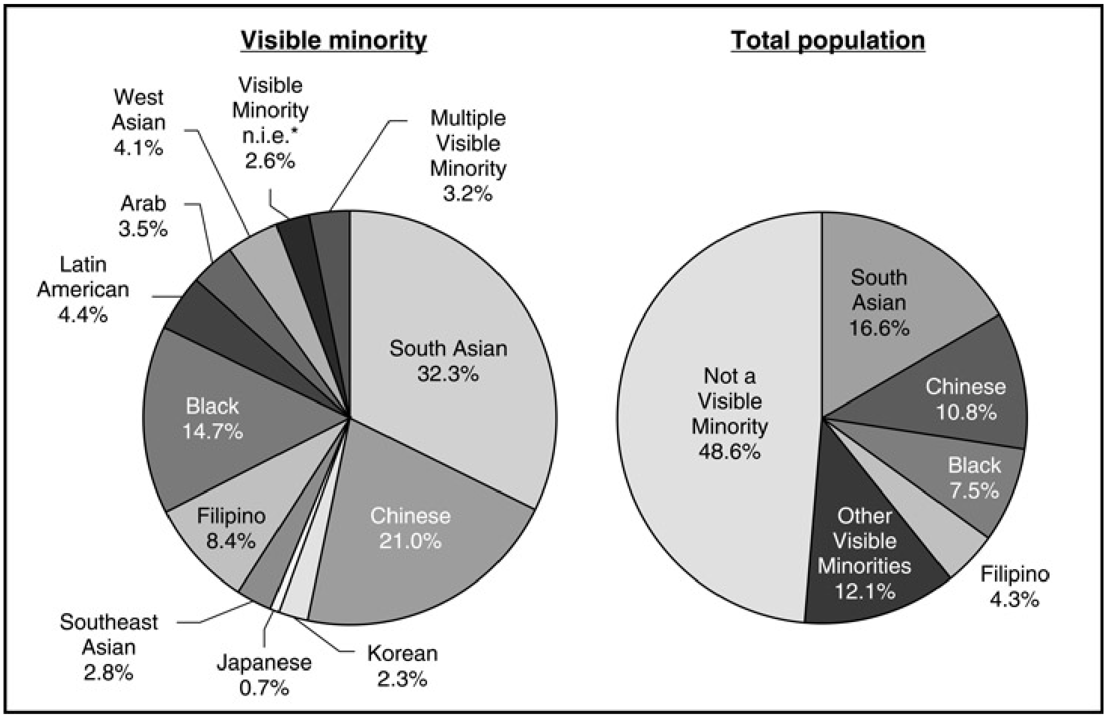

Figure 12.5 Ethnicity of populations in Toronto, Canada as reported by Statistics Canada.

Note: *n.i.e, not identified elsewhere.

Other interesting findings by Thapa et al. (2015) were that the East Asian purchasers of live fish were 83% female, primarily between the ages of 25–55, 34% completed college and 73% were employed full time with 50% making between $40,000–100,000/year. Ninety-one percent preferred to buy live fish at an Asian grocery. However, this last data point may be biased as all participants were surveyed in an Asian grocery. The importance of this research is that in recent decades, a large number of people have come to Canada and large urban areas of the USA from India and other South Asian countries. As of 2011, South Asians alone make up over 15% of the Greater Toronto Area's population and are projected to make up 24 percent of the region's population by 2031 (Gee, 2011).

According to Gee (2011), Toronto, Canada is an ever-changing South-East Asian community. This dramatic growth in Toronto's Indian community and other groups from South Asia are already the biggest visible-minority group in the Greater Toronto Area. There were 684,000 as of the 2006 census, a couple of hundred thousand more than the second biggest group: Chinese. These populations are expected to nearly triple by 2031, reaching around 2.1 million. By that time, a Statistics Canada report said that in 2016 close to one in four people in the Toronto area will be of South Asian heritage (Figure 12.5) and that the Chinese population will expand to 1.1 million, double the current figure.

12.6 Conclusion

Through ethnic migration, future migration and the high amount of live fish demand by this group, we can assume the market will also grow for largemouth bass and other high-quality fish. These markets can be met by existing farmers that want to expand and/or new farmers that may want to diversify or start raising largemouth bass. However, farmers entering into the market need to be well versed in how to raise and handle these fish to ensure only high-quality, healthy fish enter the live market.

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Chapter 13
Composition and product forms

James H. Tidwell

Kentucky State University, Aquaculture Research Center, Frankfort, Kentucky, USA

13.1 Introduction

While today we primarily think of the largemouth bass as a sport-fish, it may actually have a longer history in the USA as a food-fish. Long et al. (2015) cited an 1874 document that reported "large numbers of black bass" were harvested "chiefly with nets" from the Delaware River for food. They also cited a publication stating that in 1875, largemouth bass were selling in fish markets for 18 cents. Later, the Black Bass Act 1926 was promulgated to control market fishing and bootlegging of largemouth bass across state lines. The valuable largemouth bass were known to be smuggled in barrels by sandwiching them between layers of less valuable rough fish.

Even after the Black Bass Act shut down the commercial harvest of largemouth bass, early sport-fishing activities primarily focused on catching the bass for food and their palatability was highly regarded. The concept of "catch and release" had not yet developed. People went fishing for fun, but to also put food on the table. In many areas, the thought of catching fish just to turn it loose would still be regarded as a ridiculous act. In the Great Depression–Dust Bowl years of the later 1930s and early 1940s, when the newly formed US Soil Conservation Service began to promote and support the construction of farm ponds, one of the main purposes of the pond was as a self-supporting source of food. The largemouth bass was one of the species emphasized in the stocking programs.

As we moved into the 1960s and 1970s, sport-fishing of largemouth bass for food was replaced by a greater emphasis on “catch and release” fishing purely for sport. As the most popular sport-fish in North America, there has long been an interest in producing the fish in hatcheries for stock enhancement of sport-fisheries. Robbins and MacCrimmon (1974) reported that > 35 million largemouth bass were stocked annually from 1966 to 1970. Most of these were small fish (5–7.5 cm). Later work showed that larger stocker fish (18–23 cm) survived and performed better. Growing fingerlings to these larger sizes may be well justified as it may take five times as many 5 cm fish to make the same lasting contribution to an age group as it does 10 cm fish (Lochmann, 2013). This demand for large numbers of large largemouth bass stockers led to specialized hatchery and feed-training procedures (see Chapters 6 and 7).

While many of these stocker fish are produced by public hatcheries (state and federal), large numbers are also produced by private aquaculture enterprises. In fact, during the Reagan administration there was a push to shift fingerling production for use in stocking public waters from public to private hatcheries. The 2013 Census of Aquaculture shows, in terms of sport-fish sales, the largemouth bass was produced on more farms (176) and yielded more sales (US$14,452,000) than any of the other sport-fish. Lochmann (2013) indicated that 5 cm fish sold for US$0.46–0.75 each while 10–15 cm fish sold for US$0.81–1.60 each. Heidinger (2000) stated that the wholesale values of small lots of largemouth bass fingerlings were priced individually at US$0.35 for 2.5–5 cm fish, US$0.68 for 5–10 cm fish, US$0.94 for 10–15 cm fish, US$2.07 for 15–20 cm fish, and US$3.74 each for 20–25 cm fish.

Heidinger (1976) referred to largemouth bass being raised as food-fish. However, he said the fish were largely the result of polyculture schemes where they were used to control the populations of large forage organisms. Based on the high prices received for large sizes of large-mouth bass, it was a natural progression for some producers to follow fish beyond the 20–25 cm advanced stockers size and carry fish onto larger sizes. In the 1980s and 1990s a demand for these larger fish was identified among urban ethnic markets in the USA and Canada (Figure 13.1). The largemouth bass was highly regarded in those markets and it was proposed that it resembles the Chinese bass or Mandarin fish (*Siniperca chuatsi*) that the immigrants were familiar with back home (Figure 13.2). Tidwell et al. (2002) estimated that > 500,000 kg of market size largemouth bass (400–700 g) were being sold annually as food-fish. Heidinger (2000) reported that these fish were bringing US$6.60–11/kg live weight for those markets. Most growers do not sell directly to the end user but actually sell to a live-hauler. Some small live-haulers service both markets (food-fish or stocking). However, large-scale haulers tend to focus on food-fish.

Figure 13.1 An ethnic Asian market for live largemouth bass.

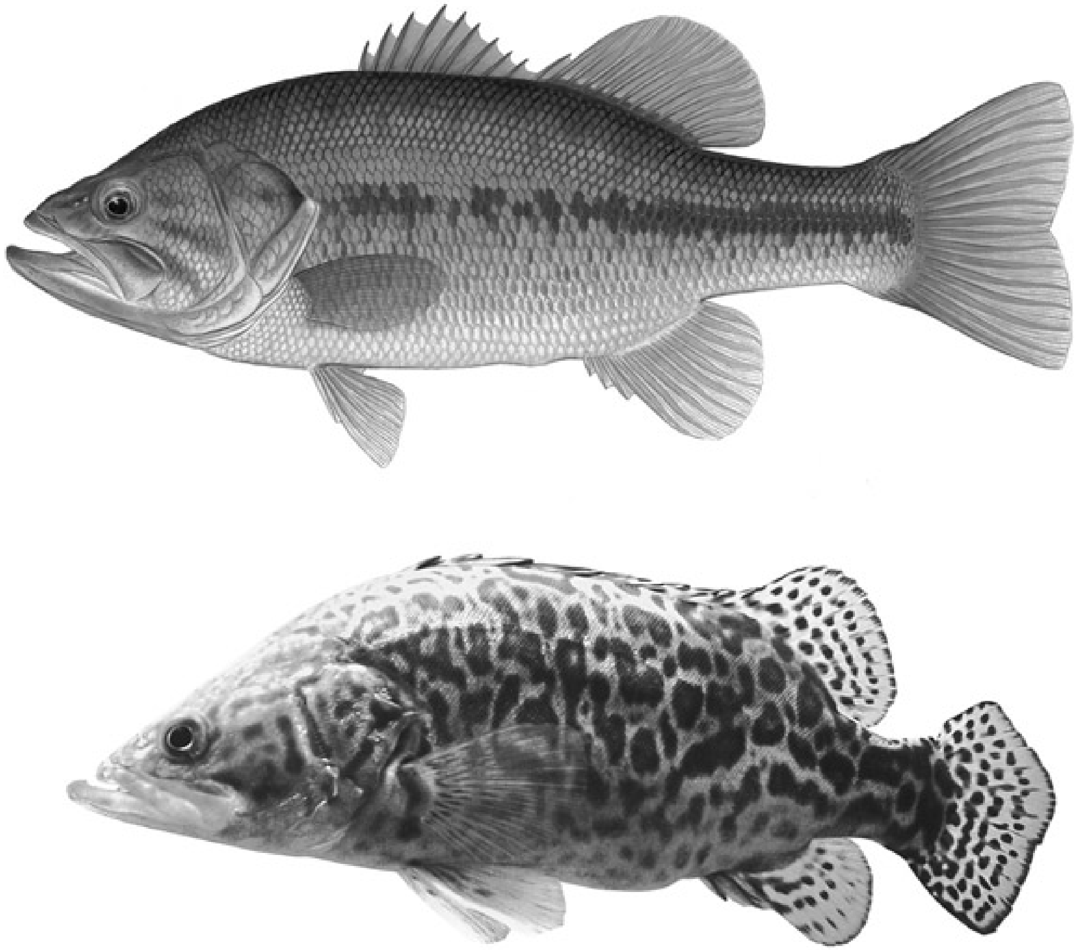

Figure 13.2 The largemouth bass, *Micropterus salmoides* (top) and the Mandarin fish, *Siniperca chuatsi* (bottom).

In the USA, most largemouth bass food-fish are marketed live. As with many things this is both good and bad. The "good" is that it protects domestic production. While well over 90% of the seafood in the USA is imported, primarily from Asia, live is a product form not readily transported such long distances. The "bad" aspect is that it limits the growth of largemouth bass production in the USA. In North America, consumers willing to purchase live fish are largely limited to persons of Asian descent. Outside of this group, North American producers prefer processed product forms, such as fillets. In fact, a shift away from the live whole product forms occurs generationally even among those of Asian descent.

13.2 Proximate composition

In an earlier work, Chatfield and Adams (1940) reported the chemical composition of wild large-mouth bass to be 76.7% moisture, 26.6% protein, 1.8% lipid, and 1.2% ash. Proximate analysis of largemouth bass raised to food-fish sizes on feed were reported in Tidwell et al. (1996). For the whole body, values were 71.2% moisture, 6.0% lipid, 18.9% protein, and 3.8% ash. For the fillet portion only they averaged 76.9% moisture, 1.3% lipid, 20.6% protein, and 1.1% ash. Ash was higher in the whole-body analysis as it includes the frame (skeleton), which is primarily composed of minerals that are retained as ash. The lipid concentration is higher in the whole body than in the fillet as this fish tends to store lipid in the abdomen, in the mesentery and around the pyloric caeca, rather than in the fillet.

Trushenski and DeKoster (2017) compared the dry matter proximate composition of fillets from wild versus pond-raised largemouth bass. Protein, lipid, fiber, and ash average 89.1, 5.1, and 5.8%, respectively, in the wild largemouth bass and 81.8, 10.3, and 5.0% in the farmed largemouth bass. Brecka et al. (1996) evaluated six commercial diets and found that only body lipid and moisture were affected by diet while protein and ash were not. Body lipid decreased and moisture content increased as protein content in the diets increased. Tidwell et al. (1996) reported that the proximate composition of the fish was not significantly affected by dietary protein levels of 42, 44, and 47%.

13.3 Composition by sex

In a study evaluating non-invasive methods to estimate body composition in largemouth bass, Barziza and Gatlin (2000) analyzed 85 fish over a broad range of water bodies, culture systems, body sizes, and various seasons. On a percent wet basis, whole body moisture, lipid, protein, and ash for all fish collected averaged 70.0, 6.8, 17.9, and 4.4%, respectively. When analyzed by sex, males average 69.9, 6.7, 17.9, and 4.6%, respectively, while females averaged 70.1, 6.9, 17.8, and 4.3%, respectively. The authors stated that there were no significant differences between male and female bass in any of the proximate body composition variables when expressed as a percentage of total body weight. It should also be noted that largemouth bass are known to be sexually dimorphic, with females being larger. In the non-selective sampling used by Barziza and Gatlin (2000) to collect the body composition data, males averaged 662 g while females averaged 1,300 g, an almost two-fold increase.

13.4 Lipid characteristics

Lipid characteristics affect many aspects of the final sellable product. For largemouth bass being sold as a sport-fish, "stocker" body lipids can represent stored energy to give the fish time to adapt to the new environment and begin to capture prey. For broodstock, stored lipids again represent stored energy important to enduring the rigors of reproduction. For broodfish, the particular lipids available are also important as they are used to develop the yolk sac which is the sole source of food for the developing larvae during a critical period of growth.

Stored lipids are also important in terms of nutritional attributes of the final product, as well as storage stability. Tidwell et al. (1996) reported that largemouth bass had very high levels of docosahexaenoic acid (DHA) (22:6 n-3) in eggs and muscle tissue. Along with eicosapentaenoic acid (EPA) (20A:5n-3), these are the two primary fatty acids associated with human health benefits (Swanson et al., 2012). The concentration of these important fatty acids as a percentage of total fatty acids is actually higher in largemouth bass than it is in species such as salmon, which are recommended as good dietary sources. However, since salmon have higher total fat levels in the edible portion, salmon can deliver more EPA and DHA per standard portion (85 g). According to USDA analyses (2018), an 85 g portion of Atlantic salmon delivers 1.564 g of EPA and DHA, while largemouth bass delivers 0.206 g for farmed largemouth bass versus 0.197 g for wild largemouth bass (Trushenski and DeKoster, 2017). In terms of storability indicators, there were few if any meaningful differences in farmed versus wild largemouth bass in terms of shelf-life or physical characteristics (Trushenski and DeKoster, 2017).

13.5 Product forms

13.5.1 Frozen whole

Heidinger (2000) stated that to expand largemouth bass production, there was a need to develop frozen and iced food-fish markets. Mraz et al. (1978) reported that largemouth bass, as a frozen product, can be kept for long periods because of its low fat content. However, as stated previously, most food-fish are sold live in ethnic Asian markets in urban areas. In most of these retail outlets, whole frozen largemouth bass (Figure 13.3) are also available but at prices significantly lower than live fish of similar size. These frozen fish likely represent fish stressed by transport or holding conditions and are a "salvage" product. Whole frozen largemouth bass are available for sale on seafood oriented websites (www.cportnet). The country of origin for this product is listed as Taiwan.

13.5.2 Fillet

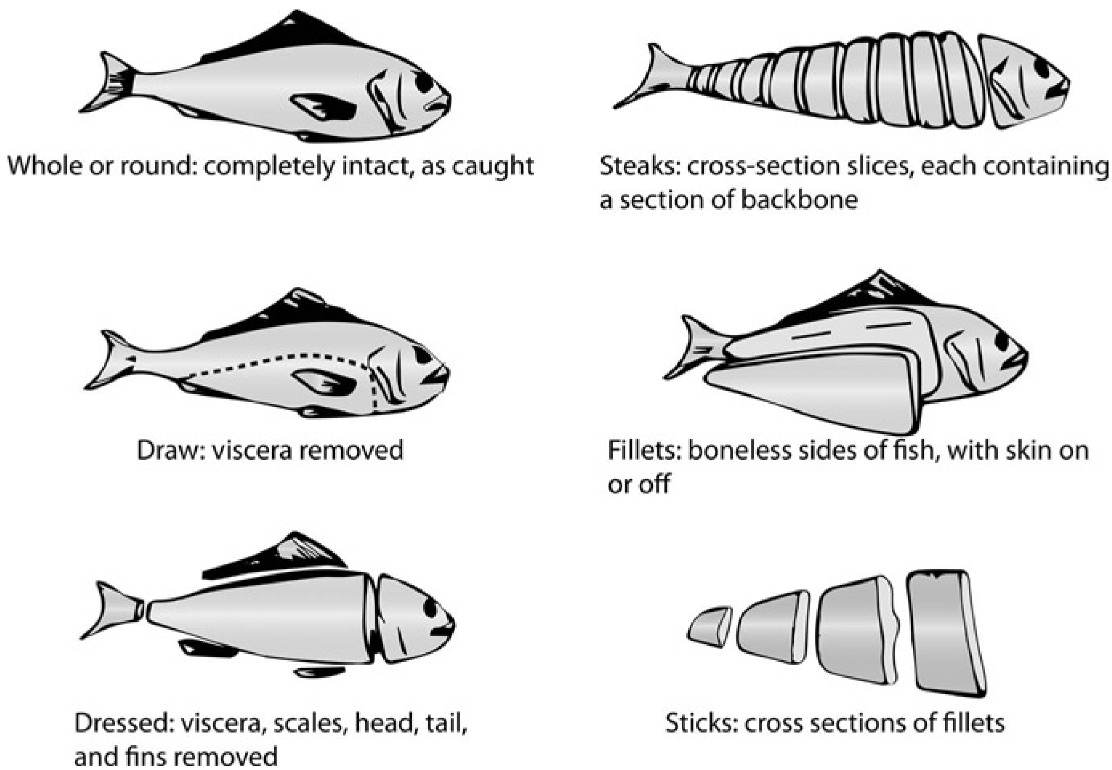

Figure 13.3 Different product forms derived from whole fish.

To expand into more of a mass market in North America, product forms such as fillets (Figure 13.3) or even value-added and/or ready to cook type products would need to be developed. Tidwell et al. (1996) reported that dressout and fillet percentages for largemouth bass (61% and 38%, respectively) were similar to those reported for channel catfish (59% and 40%) (Ammerman, 1985). Engle et al. (2013) investigated the feasibility of producing largemouth bass for a fillet market. They reported that individual fillets of 85–142 g or 142–198 g were the primary sizes utilized in the USA. At 38% fillet yield reported by Tidwell et al. (1996) that would require whole fish weights of 447–474 g for the smaller fillet category and 747–1,042 g for the larger fillets. While total fish harvest weights required for the smaller fillet category are achievable, efficient growth to sizes required for the larger fillets has proven difficult (Tidwell et al., 1998). Steps in filleting a largemouth bass can be found online (such as http://www.wildharvest-table.com/2016/07/14/processing-a-large-mouth-bass/).

Economic analysis by Engle et al. (2013) determined that production costs for largemouth bass fillets would be US$7.26–9.34/kg and that those prices would likely be too high for a feasible fillet market. They indicated that the primary drivers of the high production costs were fingerlings (their budget used US$1.60/fish at a size of 57 g) and feed (US$1,323/MT). It is likely that both of these costs can be significantly reduced with additional research and development efforts.

13.6 Organoleptic traits

With prescience to the future potential of largemouth bass as a food-fish, Snow and Lovell (1974) compared the organoleptic quality of largemouth bass raised on live forage with those raised on an artificial diet. Hatchery reared largemouth bass were raised from an initial size of 5 mm TL to age classes 2, 3, and 4 on either Oregon Moist Pellets (OMP) or live forage (primarily goldfish and tilapia). Fish were whole dressed and frozen. After thawing, fish were filleted and unseasoned fillets were wrapped in foil and baked at 200°C for 20 min. Two evaluations were made. First was a triangle difference test (Larmond, 1977). If a difference was indicated, a, 10-point hedonic rating test was used to evaluate the intensity of differences in flavor, texture, or appearance. The eight evaluators were trained in detecting off-flavors in fish.

The dressing percentage (whole dressed) reported by Snow and Lovell (1974) was 47.6%. This is lower than the 61% reported by Tidwell et al. (1996). One possible difference is that Snow and Lovell (1974) weights were with the skin removed, while Tidwell et al. (1996) included skin. The fish evaluated by Snow and Lovell (1974) also included a very large range of sizes (481–1,492 g). Large fish are known to have lower dress out percentages due to greater weight losses in mature gonads and larger heads.

Organoleptic evaluations readily distinguished between largemouth bass fed the OMP and those raised on live forage (Snow and Lovell, 1974). The largemouth bass raised on forage received higher scores on the 10-point hedonic scale (higher indicating greater preference) than fish fed the OMP. Judges comments indicated that the OMP fed largemouth bass had a "fishoil" flavor. Other flavor comments included "strong" and "pond-like." Comments on the forage fed largemouth bass were usually "mild", "sweet", and "typical good bass flavor."

The authors proposed that the undesirable flavors in the fish fed the artificial diet were due to the very high levels of marine fish oils present in the OMP formula. Texture and appearance were not negatively affected. The researchers proposed that new formulations of artificial diets for largemouth bass be investigated with an emphasis on the organoleptic quality of the largemouth bass being produced.

While aspects of the diet, especially dietary lipids, can affect the taste of the fish being produced, off-flavors can also be absorbed from the culture environment (Tucker and Hargreaves, 2004). Most off-flavors in pond raised fish are caused by naturally occurring organic compounds produced by aquatic algae or bacteria and absorbed into the fish through the skin, gills, or intestines. The most common off-flavor compounds are produced by cyanobacteria (aka blue-green algae) and include geosmin and 2-methylisoborneol (MIB) (Tucker and Hargreaves, 2004). These compounds are known to cause off-flavors in many fish, and even in the water itself.

Although off-flavors are often thought to primarily be associated with pond production environments, fish raised in recirculation systems can also develop off-flavors. These off-flavors are thought to originate in the biofilter (Losordo et al., 2004). Schrader et al. (2005) reported "earthy" and "musty" off-flavors in fillets from largemouth bass cultured in recirculating systems. Instrumental analysis using solid-phase microextraction-gas chromatography-mass spectrometry indicated that these off-flavors were due to geosmin and MIB.

13.7 Conclusion

The primary marketing form for largemouth bass at the present time is live fish in ethnic Asian markets in large metropolitan centers. These markets also offer some whole frozen largemouth bass. The American market is heavily biased toward boneless fillet product forms. Current production costs may make these product forms very expensive. However, developments in fingerling production costs and species-specific feeds could help to address those variable costs in the future.

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Chapter 14
Economics

Carole R. Engle

Engle-Stone Aquatic$, LLC, Adjunct Faculty, Virginia Seafood AREC, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA

14.1 Introduction

The economics of fish production varies a great deal depending on the level of capital investment in different production systems and scales of production, the types of feeds used, and the production performance (for example, yield, growth, survival, feed conversion ratio [FCR]) of different species in different production systems. Largemouth bass are carnivorous fish that require a relatively high protein and high fat diet as compared to many other cultured species. Such a diet is also a higher-cost diet, due to the more expensive ingredients. In addition, largemouth bass fingerlings are more expensive than fingerlings of many other commonly raised warmwater species. The combination of greater feed and fingerling costs results in different cost structures for production of largemouth bass when compared to species such as catfish, baitfish/sport-fish, hybrid striped bass, and trout. The greater feed and fingerling costs also result in relatively greater production costs than for many other commercial warmwater species.

Greater overall costs of production will require careful attention to effective marketing strategies for commercial production of largemouth bass to be profitable. The marketing plan for the business will need to focus on strategies to receive greater market prices that more than cover the relatively greater production costs. Careful thought needs to be given to developing a very comprehensive marketing plan to ensure that adequate numbers of customers are willing and able to purchase a sufficient volume of largemouth bass at prices high enough to result in a profit for farmers raising largemouth bass.

This chapter will focus on the economics of producing largemouth bass. Chapter 12 focuses on a description of major markets for largemouth bass. Those considering investing in and starting a for-profit largemouth bass farm should include both the marketing information from Chapter 12 and the cost information presented in this chapter in developing their overall business plan. Given the general lack of information on the economics of producing largemouth bass, the estimated production costs used in this chapter are those from a number of production studies conducted in ponds in the USA and reflect US prices and costs. Those raising largemouth bass in other countries are encouraged to use what is presented here as guidance only and change prices and line-item costs to reflect economic realities of their production location.

14.2 Food-fish production costs

The majority of food-fish raised in the USA are produced in earthen ponds. According to the 2013 Census of Aquaculture, 48% of all US aquaculture farms (including shellfish and other marine species) use ponds for production (USDA-NASS, 2014). Thus, a great deal is known about farm-level costs associated with raising a variety of food-fish species in the USA (see Engle, 2010, for examples). However, much less is known about the costs of producing largemouth bass, particularly for sale as a food-fish product. Moreover, there are few commercial farms producing largemouth bass as food-fish in the USA, and hence there are no farm-level data from which to draw industry-wide costs.

Several research studies conducted in the USA have measured production parameters for various stocking densities and feeds in research-scale earthen ponds. These studies provide some data on relative production performance characteristics such as yield (kilogram per hectare of fish produced), FCRs, and survival rates. This section will draw upon the data from those studies that have focused on management strategies to produce largemouth bass as food-fish in the USA. Clearly, the information presented here will need to be updated with prices and line-item costs appropriate for their country and location.

It is important to understand that care must be taken in extrapolating research data, such as that used in this chapter, to project expected costs and returns on commercial farms. Reported yields (kilogram per hectare produced) often are greater in research studies, FCRs lower, and survival rates greater than on commercial farms. These differences stem primarily from the necessity for researchers to control and standardize as many variables as possible (other than the treatments being tested) to isolate and identify effects of the specific variable under study. Under commercial farming conditions, many variables that affect fish production cannot be controlled by the farmer and lead to lower yields, higher FCRs, and lower survival rates. Thus, those planning to raise largemouth bass for food-fish should select yields, FCRs, and survival rates that are more conservative than those used in the following analysis. This analysis is intended primarily to show the relative differences among alternative management strategies, although the results perhaps provide some indication of the most optimistic economic outcomes that may be possible.

The five US research studies found in the literature that include pond production performance data were: Tidwell et al. (1996), Tidwell et al. (1998), Cochran et al. (2009), Engle et al. (2013), and Tidwell et al. (2015). Tidwell et al.'s (1996, 1998, 2015) and Cochran et al.'s (2009) studies were conducted in Kentucky while Engle et al.'s (2013) study was conducted in Arkansas, a more southerly state with a longer growing season for warmwater fish. The

Tidwell et al. (1998) study compared effects of two stocking densities (6,175 and 12,350 largemouth bass per hectare) of fingerlings stocked at a mean individual weight of 122 to 124 g (Table 14.1). Engle et al. (2013) followed up on the Tidwell et al. (1998) study and added a higher stocking density of 18,525/ha, but stocked a smaller 57 g fish. Tidwell et al. (1998) compared three protein levels (42%, 44%, and 48% protein) in feed fed to 122 g fish stocked at 12,350 fish/ha. The days to harvest were similar across these three studies with trials lasting approximately one year. However, the Cochran et al. (2009) and Tidwell et al. (2015) studies were stocked in the spring and harvested in the fall of the same year. Cochran et al. (2009) evaluated replacing fishmeal content in the diet by stocking 8,650 largemouth bass per ha at an average size of 210 g, whereas Tidwell et al. (2015) compared various forms of feed pellets to 185 g bass stocked at 10,000/ha.

Table 14.1 Key production performance data from production studies to produce largemouth bass food-fish.

Production parameter	Engle et al. (2013)[a]			Tidwell et al. (1998)[a]		Tidwell et al. (1996)[b]	Cochran et al. (2009)[c]	Tidwell et al. (2015)[d]
Stocking density (head/ha)	6,175	12,350	18,525	6,175	12,350	12,350	8,650	10,000
Mean individual weight fish stocked (g)	57	57	57	124	122	122	210	185
Feed conversion ratio	3.6	2.5	2.5	9.6	3.8	2.3	1.36	1.7
Days to harvest (days)	372	372	372	365	365	365	179	151
Mean final weight harvested (g)	896	827	744	406	406	406	527	566
Survival (%)	71	74	65	92	83	92	95	94
Gross yield (kg/ha)	3,989	7,434	9,096	2,354	4,598	4,613	4,670	5,321

Notes: [a]Stocking density trial. [b]Trial of three protein levels in feed. Used 44% protein feed that had best economic outcome. [c]Trial of three diets. Control did not result in market-sized fish, but diet treatments did. Used mean of dietary treatments. [d]Compared forms of feed. No significant differences due to form. Used values for standard floating pellet.

It is important to note that the minimum market size of largemouth bass sold as food-fish was achieved only in three of these studies: the Arkansas study, in the three treatment diets of the Cochran et al. (2009) study in Kentucky, and in the Tidwell et al. (2015) study of pellet form. The Arkansas production study produced the largest fish (individual mean weights ranged from 744 to 896 g/fish across the three stocking density treatments) of the five studies. Average weights of individual fish in the four Kentucky studies ranged from 406 g to 566 g (Table 14.1). The two most recent Kentucky studies (Cochran et al., 2009; Tidwell et al., 2015) were the only Kentucky studies to achieve average weights of individual fish harvested greater than 500 g, the size considered the minimum market size for largemouth bass food-fish (Watts et al., 2016). The greater growth in the Arkansas study likely reflects a longer growing season with more favorable water temperatures for growth of largemouth bass. All but the Cochran et al. (2009) and Tidwell et al. (2015) studies lasted for approximately 1 year. While the largemouth bass in the Arkansas study were stocked at a smaller size, they still grew to market size in that one-year growing period. While fish in the Cochran et al. (2009) and Tidwell et al. (2015) studies did reach minimum market size in a 6 month period, the starting sizes were larger stockers, 210 g and 185 g, respectively. The greatest gross yield (kg/ha) values were obtained at the two greater densities in the Arkansas study, 7,434 kg/ha and 9,096 kg/ha as compared to gross yields of 2,354 to 4,598 kg/ha in the Kentucky density studies. Survival rates were greater in the Kentucky studies, ranging from 83 to 95% as compared to 65 to 74% in the Arkansas study. While the reasons for such differences are not clear, lower survival rates in Arkansas may have been due to the greater summer temperatures in Arkansas during which there was some degree of chronic mortality observed across all ponds. FCRs were generally better in Arkansas as well, ranging from 2.5 to 3.6 as compared to 3.8 to 9.6 in the Kentucky density studies. However, the best FCRs (1.36 and 1.7) were obtained in the Cochran et al. (2009) and Tidwell et al. (2015) Kentucky studies. Such low FCRs in these two most recent studies may be due to the lack of a winter period in those studies as compared to the three other studies.

To compare cost of production across these four studies, whole-farm enterprise budgets were developed for eight management scenarios for a 32 ha (surface water ha) farm composed of eighty 0.4 ha ponds. Given that the fixed costs associated with land, pond construction, and equipment are similar for pond production of largemouth bass as for production of catfish, the fixed costs were established based on recent fixed cost values from surveys of catfish farms in the south (Kumar, 2017). Fixed costs were held constant across the management strategies to allow for comparison of the effects of the varying yields, survival rates, and FCRs. Representative US costs were used for all line items in the budgets.

Table 14.2 compares annual costs of production associated with results from the four large-mouth bass production studies under standardized assumptions related to the overall farm business. The greatest costs to produce largemouth bass were those of feed and fingerlings. For most of the management strategies evaluated, fingerling cost was greater than that of feed cost. Fingerling costs were proportionately greater in the studies in which larger sizes of largemouth bass were stocked (such as the 210 g fish stocked in Cochran et al. (2009) and the 122 g fish stocked in Tidwell et al., 1996, 1998) as compared to the 57 g fingerlings stocked in Engle et al. (2013).

Total annual ownership costs were held constant across all studies and included farm insurance, legal and accounting costs, and annual depreciation on all depreciable assets such as ponds, vats, buildings, and equipment, and interest on the investment of capital in land as well as the other capital assets (Table 14.2). Total costs are presented with and without the value of the time spent by family labor and management to produce largemouth bass food-fish. While labor and management are necessary inputs to the production of largemouth bass, many operators of small-scale farms, such as a 32 ha farm, do not pay themselves a wage or a salary. However, the value of the time spent by the owner/operator and his/her family is important to consider (Engle, 2017). If those who do the work and make management decisions are not adequately compensated, economics shows that they will not continue in the business. Thus, a cost for family labor and management is included. Total costs were greatest for the strategies with the greatest stocking densities, as would be expected.

Breakeven prices represent the cost per kilogram of largemouth bass produced. Breakeven prices above total costs include all costs necessary to produce largemouth bass food-fish and are calculated on a per kilogram basis. Breakeven prices represent the price per kilogram that must be received for the farm to just break even (above variable costs and fixed costs). It is important to understand that the price received must be greater than the breakeven price for there to be any profit. Another key consideration in interpreting these values is that the quantity of fish used in the calculation must be the weight sold, not the weight produced. Most commercial farms produce more than they are able to sell.

Breakeven prices for the eight research-based scenarios analyzed show that the cost of production ranged from a low of US$7.64/kg to a high of US$14.48/kg (Table 14.2). Given that the often-quoted market price of largemouth bass food-fish is US$10/kg, and that the break-even prices estimated did not include costs associated with grading, transportation, and other

Table 14.2 Key production costs and breakeven prices for largemouth bass food-fish production, based on experimental production results, but without any costs associated with marketing. Costs are total farm costs for a 32 ha farm with eighty 0.4 ha ponds.

Item	Engle et al. (2013)[a]			Tidwell et al. (1998)[a]		Tidwell et al. (1996)[b]	Cochran et al. (2009)[c]	Tidwell et al. (2015)[d]
Variable costs								
Feed	653,744	840,000	985,600	262,027	355,442	357,025	194,040	294,896
Fingerlings	400,000	800,000	1,200,000	540,000	1,080,000	1,080,000	980,000	1,004,152
Seining and hauling	22,338	41,630	50,938	11,766	22,988	23,061	23,341	26,600
All other variable costs[e]	68,400	69,280	69,920	68,400	69,280	69,280	68,400	69,280
Int. on oper. capital	114,488	175,091	230,646	88,219	152,771	152,937	126,578	139,493
Total variable costs	1,258,931	1,926,001	2,537,103	970,412	1,680,481	1,682,302	1,392,359	1,534,421
Total fixed costs[f]	103,421	103,421	103,421	103,421	103,421	103,421	103,421	103,421
Total costs w/out labor and mgmt.	1,362,351	2,029,422	2,640,524	1,073,833	1,783,902	1,785,723	1,452,406	1,637,842
Unpaid labor, mgmt.	30,000	30,000	30,000	30,000	30,000	30,000	30,000	30,000
Total annual costs	1,392,351	2,059,422	2,670,524	1,103,833	1,813,902	1,815,723	1,482,406	1,667,842
Breakeven price above total costs	9.62	7.64	8.09	14.48	12.18	12.15	9.80	9.68

Notes: All figures US$. [a]Stocking density trial. [b]Trial of three protein levels in feed. Used 44% protein feed that had best economic outcome. [c]Trial of three diets. Control did not result in market-sized fish, but diet treatments did. Used mean of dietary treatments. [d]Compared forms of feed. No significant differences due to form. Used values for standard floating pellet. [e]Includes electricity, fuel, pond amendments like salt, repairs and maintenance, predator control, telephone, office supplies. [f]Includes farm insurance, legal and accounting expenses, interest on investment of all capital assets, including land and annual depreciation of all depreciable assets such as ponds, office and storage building, wells, and equipment.

marketing functions, it appears that profit margins for largemouth bass food-fish are fairly thin. The lowest breakeven prices were those estimated from the Engle et al. (2013) study done in Arkansas. This study achieved greater yields than the Kentucky studies, even at similar stocking densities. The greater yields from the Arkansas study likely resulted from the generally warmer weather in Arkansas as compared to Kentucky. However, greater yields do not always result in greater profits. In Engle et al. (2013), the lowest (most profitable) breakeven prices were found in the 12,350/ha density in spite of the greater yields measured in the 18,525/ha treatment. The increased fingerling cost in the highest density was proportionately greater than the increased revenue in that treatment which resulted in a greater breakeven price in the high-density treatment. Breakeven prices were consistently lower at the 12,350/ha density when compared with the 6,175/ha density in both the Arkansas and Kentucky density studies (Tidwell et al., 1998; Engle et al., 2013). Across the Kentucky studies, the lowest breakeven costs were those of the Cochran et al. (2009) and Tidwell et al. (2015) studies. Both these studies resulted in mean weights of fish greater than the minimum market size of 500g (Watts et al., 2016), but importantly, also achieved the lowest FCRs among all eight experimental scenarios.

Table 14.3 presents results related to the relative importance of the various line item inputs used (in terms of costs) in production of largemouth bass from the eight scenarios analyzed. Feed costs ranged from 13% to as much as 47% of total costs. By way of comparison with catfish production costs, feed costs constituted from 43 to 47% across ten different catfish management strategies on a 32 ha catfish farm (Kumar, 2017).

Table 14.3 Percentage of production costs of key inputs for production of largemouth bass food-fish production, based on experimental production results, but without any costs associated with marketing. Costs are for a 32 ha farm with eighty 0.4 ha ponds.

Item	Engle et al. (2013)[a]			Tidwell et al. (1998)[a]		Tidwell et al. (1996)[b]	Cochran et al. (2009)[c]	Tidwell et al. (2015)[d]
Feed	47%	41%	37%	24%	20%	20%	13%	18%
Fingerlings	29%	39%	45%	49%	60%	59%	66%	60%
Seining and hauling	2%	2%	2%	1%	1%	1%	2%	2%
All other variable costs[e]	5%	3%	3%	6%	4%	4%	5%	4%
Interest on operating capital	8%	9%	9%	8%	8%	8%	9%	8%
Total variable costs	90%	94%	95%	88%	93%	93%	94%	92%
Total fixed costs[f]	7%	5%	4%	9%	6%	6%	4%	6%
Unpaid family labor, management	2%	1%	1%	3%	2%	2%	2%	2%

Notes: [a]Stocking density trial. [b]Trial of three protein levels in feed. Used 44% protein feed that had best economic outcome. [c]Trial of three diets. Control did not result in market-sized fish, but diet treatments did. Used mean of dietary treatments. [d]Compared forms of feed. No significant differences due to form. Used values for standard floating pellet. [e]Includes electricity, fuel, pond amendments like salt, repairs and maintenance, predator control, telephone, office supplies. [f]Includes farm insurance, legal and accounting expenses, interest on investment of all capital assets, including land and annual depreciation of all depreciable assets such as ponds, office and storage building, wells, and equipment.

The most striking cost percentage in Table 14.3 is that of fingerlings. The cost of largemouth bass fingerlings constituted from 29 to 66% of total costs. Compared with catfish food-fish production, catfish fingerling costs constituted from 7 to 10% of total costs across five different management strategies for channel catfish and from 12 to 15% for five different hybrid catfish management strategies (Kumar, 2017), including split-pond production (Kumar et al., 2016). Such high costs of largemouth bass fingerlings result, in large part, from the great variability in survival and yields of largemouth bass fingerlings from pond to pond. This results in the necessity for a bass hatchery to charge a sufficiently high price to cover what can be nearly total losses in some ponds on the farm.

Such high feed and fingerling costs for largemouth bass food-fish production resulted in percentages of variable costs that ranged from 88 to 95%. In comparison with catfish production costs, variable costs constituted from 80 to 87% of total costs of production (Kumar, 2017).

Indoor production of largemouth bass in recirculating aquaculture systems (RAS) has been conducted on an experimental basis but studies conducted to date have not resulted in market-sized (> 500 g) largemouth bass (Watts et al., 2016). Thus, no economics work has been done to develop reliable estimates of production costs of largemouth bass produced in RAS. The lack of commercial-scale RAS production of largemouth bass makes it difficult to develop relevant cost estimates. Production costs per kilogram tend to be greater in RAS than in outdoor earthen ponds, given the much greater proportion of capital costs in tanks and equipment that leads to greater annual fixed costs than in ponds. For example, with hybrid striped bass, the cost to produce fingerling hybrid striped bass in indoor tanks was more than three times greater than the production cost in ponds (Eklund et al., 2012). Thus, while it may become possible to produce market-sized largemouth bass food-fish indoors in RAS systems, it is likely that the production cost per kg will be greater than that of largemouth bass produced in ponds.

Clearly, the two drivers of the high production costs of largemouth bass food-fish in the USA are the costs of the fingerlings and the feed. The Cochran et al. (2009) and Tidwell et al. (2015) studies were encouraging in their low FCRs that contributed to lower production costs. Ongoing research efforts to develop lower cost diets for largemouth bass that still result in good growth and production performance are important lines of research that may help to reduce production costs of largemouth bass in the future.

However, there is an even greater need for research efforts to target ways to reduce the cost of producing largemouth bass fingerlings. The next section of this chapter will explore the underlying cost structures for production of largemouth bass fingerlings to shed light on the factors that contribute to this high fingerling cost.

14.3 Fingerling production costs

Largemouth bass fingerlings are produced by a number of public and private hatcheries in the United States, mostly for state-managed stocking programs and for stocking private fishing ponds. However, increased demand for largemouth bass fingerlings for growout to food-fish may increase the proportion of fingerlings sold to food-fish producers over time.

Engle and Southworth (2013) surveyed public and private hatchery producers of large-mouth bass fingerlings in 15 southern states in the USA: Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, Missouri, Oklahoma, North Carolina, South Carolina, Tennessee, Texas, and Virginia. The production data obtained from the survey of these hatcheries was used to develop the cost estimates that will be presented in this chapter. Given the substantial differences found between management strategies used in public and private hatcheries, many of which affect the costs of production, only private hatchery results will be used in this analysis. The northern strain of largemouth bass was the principal strain raised by private hatcheries, and the data used reflect those of northern-strain largemouth bass. Given that most private hatcheries fed pelleted feeds, the following costs will focus on production of feed-trained bass fingerlings for stocking on food-fish growout production. Since these costs were based on US prices, the values presented for fingerling production will need to be adapted to reflect realistic prices in other countries.

Fingerling production begins, of course, with broodstock. Hatcheries need to maintain broodstock in good condition throughout the year to obtain quality eggs and fry. Various sizes of ponds were found in the Engle and Southworth (2013) study to be used to hold broodstock throughout the year. However, this analysis is based on the use of a 0.4 ha pond for holding and maintaining broodstock throughout the year. The cost to hold and maintain broodstock represents an annual cost that needs to then be included in the cost of fry production. The annual holding costs for broodstock on a largemouth bass farm were found to constitute a relatively substantial cost for the hatchery. Table 14.4 shows that the annual costs to hold, feed, and maintain broodstock in good spawning condition was US$11,226/0.40 ha pond, or US$16.86/broodfish. This cost is related primarily to the relatively high cost of feed (45% of total annual costs) required to hold broodfish and the costs of maintaining the entire pond for a relatively low weight of fish.

Largemouth bass can be spawned in ponds, as the more traditional method, or in raceways. Pond spawning costs include the cost of the broodstock stocked into that pond for spawning, fertilizer to promote a phytoplankton bloom, and feed (including the shipping cost) for the broodfish. For the 2 month spawning period, total annual costs were estimated to be US$2,558 per 0.4 ha pond, or US$0.051 per fry (Table 14.5). The cost to hold and maintain broodstock throughout the year constituted 66% of the total costs of fry produced from pond spawning. Some largemouth bass hatcheries spawn largemouth bass in raceways. Raceway spawning costs also include the costs of the broodfish and feed, but include additional costs of the spawning mats, spawning hormones, labor, and other variable costs of electricity, fuel, pumping, and repairs and maintenance. Annual fixed costs of raceway spawning include the annual depreciation and interest on the investment of the raceways that would need to be constructed. Table 14.6 shows that the cost per fry spawned in raceways was much less than in ponds, at US$0.00082 per fry. In raceways, the cost to hold and maintain broodstock throughout the year were only 26% of the total cost to spawn fry due to the greater number of fry produced per broodfish in raceway spawning as compared to pond spawning. Total fixed costs were 28% of the total costs of fry production, as compared to only 6% in pond spawning due largely to the capital investment in concrete raceways. Labor costs were low for pond spawning, constituting only 1% of total costs in pond spawning to obtain fry, but were greater with raceway spawning, constituting 10% of total costs of fry production.

Fry harvested from spawning ponds are typically stocked into fertilized ponds until they reach a size of approximately 5 cm when they can be feed trained. This stage of production was characterized by widely varying survival and yield in the Engle and Southworth (2013) survey, with survival rates that ranged from 0% to 123%, and yields that ranged from 0 to 296,400 fingerlings per hectare and 20 to 481 kg/ha. Respondents attributed these wide ranges to the variability and quality of the phytoplankton bloom produced in ponds following fertilization.

Table 14.4 Holding costs for largemouth bass broodstock, for one 0.4 ha pond stocked at 1,645 broodstock per ha with an average weight of 1.13 kg for 10 months.

Item	Unit	Cost/unit (US$)	Quantity	Total cost 0.4 ha pond (US$)
Variable costs				
Feed	lb/pond/year	0.7	4,996	3,497
Shipping feed	Total	0.3	4,996	1,499
Treatment for filamentous algae	US$/pond	17	1	17
Labor	US$/pond/month	13.75	10	138
Electricity	US$/pond/month	8.00	10	80
Fuel	US$/pond/month	10.83	10	108
Pumping	US$/pond/month	17.58	10	176
Repairs and maintenance	US$/pond/month	8.08	10	81
Bird depredation	US$/pond/month	8.33	10	83
Office supplies	US$/pond/month	0.92	10	9
Interest on operating capital		0.0833[a]	5,687	474
Total variable costs				6,162
Fixed costs				
Telephone	US$/pond/month	1.42	10	14
Farm insurance	US$/pond/month	3.63	**10**	36
Legal/accounting	US$/pond/month	1.57	10	16
Land				
Interest on average investment	US$/pond/month	18.75	10	188
Ponds				
Annual depreciation	US$/pond/month	13.43	10	134

Item	Unit	Cost/unit (US$)	Quantity	Total cost 0.4 ha pond (US$)
Interest on average investment	US$/pond/month	13.43	10	134
Equipment – Ponds				
Annual depreciation	US$/pond/month	15.88	10	159
Interest on average investment	US$/pond/month	5.41	10	54
Broodstock				
Annual depreciation	US$/pond/year	3,330	1	3,330
Interest on average investment	US$/pond/year	9,990	0.1	999
Total fixed costs				5,064
Total annual costs				11,226
Annual cost/kg of broodstock to hold for 10 months (breakeven price/kg) ($/kg)				14.86
Annual cost per broodfish to hold for 10 months (breakeven price per broodfish) ($/fish)				16.86

Note: [a]Monthly rate, based on 10% annual percentage rate.

The cost to produce 5-cm fingerlings in fertilized ponds was estimated to be US$4,356 per 0.4 ha pond with a per-fingerling cost of US$0.118 (Table 14.7). For largemouth bass to accept pelleted feeds requires that the fish be trained to accept a pelleted feed. Feed training is best done in concrete vats where it is possible to crowd the fish and ensure that feed is available to all fish frequently throughout the day. Total costs per vat for feed training were US$1,161 for each 5,700 l vat, and feed training added US$0.0206 per fingerling to the cost (Table 14.8). The greatest costs of feed training were that of krill and krill meal. It should be noted that the costs estimated were based on 100% of the 5 cm fingerlings being trained successfully to feed on artificial diets. Some percentage of these fish will not learn to eat feed; the cost per feed-trained 5 cm fingerling will increase as the percentage of fingerlings that do not learn to eat feed increases.

Table 14.5 Pond spawning annual costs for one 0.4-ha pond; pond used for this purpose for two months.

Item	Unit	Cost/unit (US$)	Quantity	Total cost (US$)
Variable costs				
Broodstock costs	US$/broodfish for holding	16.85	100	1,685
Fertilizer				
Cottonseed meal	US$/pond/application	0.212	200[a]	42.4
Inorganic fertilizer	US$/pond/application	0.54	100[a]	54
Feed	%	1.54	6.12	9.45
Shipping feed	kg	0.66	6.1.2	4.05
All other operating costs				577
Interest on operating capital		0.0083[b]	2,372	39
Total variable costs				2,411
Total fixed costs				147
Total annual costs				2,558

Item	Unit	Cost/unit (US$)	Quantity	Total cost (US$)
Annual cost/kg of Fly (breakeven price/kg) ($/kg)				39.36
Annual cost/Fly (breakeven price/fiy) ($/fry)				0.051

Notes: [a]Four applications per year. [b]Monthly rate, based on 10% annual percentage rate.

Rearing feed-trained fingerlings to 10 cm on feed cost US$4,226 per 0.4 ha pond and fingerlings cost US$0.241 each, with reported yields of 224 kg/ha (data not shown). For production of larger 15 cm fingerlings, respondents reported yields of 2,240 kg/ha (data not shown). Production of 15 cm feed-trained fingerlings cost US$10,826 per 0.4 ha pond, and fingerlings cost US$0.541 per fingerling (Table 14.9). The greatest annual cost of producing 15 cm fingerlings was that of the feed (including its shipping), that constituted 56% of the total costs of production. The second-greatest cost of a 15 cm fingerling was that of the 5 cm fingerlings stocked, that constituted 32% of the cost of producing 15 cm fingerlings.

The fingerling production cost values used in this analysis were based on average yields, survival rates, and FCRs from Engle and Southworth (2013). Largemouth bass hatcheries that sell fingerlings will need to charge a sufficiently high price to recoup losses occurred in various ponds across their farm. The Engle and Southworth (2013) survey documented very high fluctuations in yields and survivals on largemouth bass hatcheries that result in financial risk for hatcheries that must be recovered in prices charged for the fingerlings sold.

14.4 Proportion of farm used for broodstock and fingerling production

For the 32 ha farm modeled for largemouth bass food-fish production, 1.2 ha would be required for broodstock, 8.1 ha for spawning, 5.7 ha for production of 5 cm bass for feed training, and

Table 14.6 Vat/raceway annual costs of spawning; vats used for two months.

Item	Unit	Cost/unit (US$)	Quantity	Total cost (US$)
Variable costs				
Broodfish costs	Broodfish	16.86	240	4,046
Feed – forage fish	Kg	8.82	272.1	2,400
Spawning mats	Each	5	120	600
Spawning hormone	Thousand IU	0.10896	1309	143
Labor	$/hr	9.0	168	1,512
Electricity	US$/vat/month	20	2	400
Fuel	US$/vat/month	27	2	540
Pumping	US$/vat/month	44	2	880
Repairs and maintenance	US$/vat/month	20	2	400
Office supplies	US$/vat/month	0.19	2	3.80
Interest on operating capital	US$	0.0083[a]	10,925	181
Total variable costs				11,106
Fixed costs				
Telephone	US$/month	28	2	*56*
Farm insurance	US$/month	73	2	146

Item	Unit	Cost/unit (US$)	Quantity	Total cost (US$)
Legal/accounting	US$/month	31	2	62
Raceways				
Annual depreciation	US$/month	534	2	1,068
Interest on average investment	US$/month	534	2	1,068
Equipment				
Annual depreciation	US$/month	673	2	1,346
Interest on average investment	US$/month	282	2	564
Total fixed costs				4,310
Total annual costs				15,416
Annual cost/kg of Fry (breakeven price/kg) (US$/kg)				0.62
Annual cost/Fry (breakeven price/fry) (US$/fry)				0.00082

Note: [a]Monthly rate, based on 10% annual percentage rate.

Table 14.7 Fry rearing to 5 cm, annual costs in one 0.4 ha pond; pond used for two months.

Item	Unit	Cost/unit(US$)	Quantity	Total cost(US$)
Fry	each	0.051	74,000	3,774
Fertilizer				
Cottonseed meal		0.212		117
Inorganic fertilizer		0.54	275[a]	149
Interest on operating capital		0.0083[b]	4,177	69

Item	Unit	Cost/unit(US$)	Quantity	Total cost(US$)
Total variable costs				4,246
Total fixed costs				110
Total annual costs				4,356
Annual cost/kg, 5-cm fingerlings (breakeven price, 5-cm fingerlings (US$/kg)				67.65
Annual cost/5-cm fingerling (breakeven price, 5-cm fingerling) (US$/fish)				0.118

Notes: [a]Twice a week for 3 weeks, then once per week. [b]Monthly rate, based on 10% annual percentage rate.

Table 14.8 Feed training annual costs. Costs are for a unit of eight vats used for 0.5 months.

Item	Unit	Cost/unit (US$)	Quantity	Total cost (US$)
Variable costs				
Feed				
Krill	kg	43.00	82.35	3,541.20
Krill meal	kg	20.95	151.86	3,181.55
Crumbles	kg	1.76	146.36	257.60
2.0 mm, 3/32 in	kg	1.76	25.45	44.80
2.5 mm pellet, 1/8 in	kg	1.65	25.45	42
Shipping feed	kg	0.66	432.05	285.15
Labor	US$/h	9.0	96	864
Electricity	US$/month	160	0.5	80

Item	Unit	Cost/unit (US$)	Quantity	Total cost (US$)
Fuel	US$/month	217	0.5	108
Pumping	US$/month	351.7	0.5	175.8
Repairs and maintenance	US$/month	162	0.5	81
Oxygen	US$/month	25	0.5	12.5
Bags to move fry	US$/month	60	0.5	30
Office supplies	US$/month	18	0.5	9
Interest on operating capital	US$	0.0083[a]	8,713	36.16
Total variable costs				8,749
Fixed costs				
Telephone	US$/month	28	0.5	14
Farm insurance	US$/month	73	0.5	36.5
Legal/accounting	US$/month	31	0.5	15.5
Vats				
Annual depreciation				13
Interest on average investment				13
Holding shed				
Annual depreciation				62.50
Interest on average investment				62.50
Equipment				
Annual depreciation				223
Interest on average investment				96
Total fixed costs				536
Total annual costs				9,285
Annual cost per kg (breakeven price per pound) (US$/kg)				15.90

Item	Unit	Cost/unit (US$)	Quantity	Total cost (US$)
Annual cost per feed-trained fish (breakeven price per feed-trained fish) (US$/fish)				$0.0206

Note: [a]Monthly rate, based on 10% annual percentage rate.

8.1 ha for production of 15 cm bass for stocking, leaving only 9.3 ha for final growout. Thus, 71% of the water surface area on the farm is needed to produce enough fingerlings to stock into final growout. The only ponds that generate revenue for the farm would be those used for final growout. With on-farm production of largemouth bass fingerlings, then, only 29% of the water area on the farm would generate direct revenue. It appears that it is not feasible for small-scale producers to raise their own fingerlings. The opportunity cost of on-farm fingerling production is the revenue that would have been received from sale of the food-fish grown in the same ponds used for fingerlings. It is clear that most largemouth bass food-fish producers, unless operating on a very large scale, will need to purchase fingerlings from hatcheries that specialize in production of fingerlings.

Table 14.9 Rearing feed-trained fingerlings to 15 cm, annual costs, in one 0.4 ha pond; pond used for 6 months.

Item	Unit	Cost/unit (US$)	Quantity	Total cost (US$)
Variable costs				
Fingerlings, 5 cm		0.118	25,000	2,950
Feed training		0.0206	25,000	515
Feed (2.4mm)	kg	1.65	909.09	1,500

Item	Unit	Cost/unit (US$)	Quantity	Total cost (US$)
Feed (3.2mm)	kg	1.54	1,818.2	2,800
Shipping feed		0.66	2,727.27	1,800
Fertilizer				
Cottonseed meal	US$/pond	0.212	50	11
Inorganic fertilizer	US$/pond	0.54	25	13
All other operating costs				409
Interest on operating capital		0.0083[a]	9,998	498
Total variable costs				10,496
Total fixed costs				330
Total annual costs				10,826
Annual cost/kg (breakeven price/kg)	(US$/kg)			11.93
Annual cost per 15-cm fish (breakei /*en* price/15-cm fish) (US$/fish)				0.541

Note: [a]Monthly rate, based on 10% annual percentage rate.

14.5 Financial risk of producing largemouth bass

There are a variety of types of risk inherent in any business, especially in a farming business. Production losses due to predators (such as birds and otters), disease, or extreme temperatures clearly result in reduced revenue and income for fish farmers. Similarly, market risks such as fluctuations in price, or changes in demand (that make it difficult to sell volumes sold in the past) will result in reduced revenue for the business. Such risks affect all fish farming businesses.

However, the financial risk in largemouth bass farming appears to be greater than in other pond-based fish farming businesses. The greater risk stems largely from the high cost of fingerlings and feed for bass, market risks associated with live food-fish markets, and uncertainties related to commercial production of a new type of fish crop.

Total production costs estimated in this chapter from research experiments show that per-ha costs ranged from 33,557 to US$82,516/ha. Such per hectare costs are several times greater than those for pond-raised catfish. While the market price for bass is greater than that for catfish, the total amount of capital required per ha represents a greater financial risk. If a pond of largemouth bass is lost due to disease or predators, the amount of that financial loss is greater than that for catfish because more money was spent on the fingerlings and feed. The potential for greater losses is a financial risk that prospective producers must consider carefully.

Cost of feed is a major cost of largemouth bass production. Thus, achieving feed efficiencies is important to the cost of producing largemouth bass food-fish. For example, the FCRs reported in various research studies ranged from a low of 1.36 to a high of 9.6. The higher FCRs reported were from studies in which the fish were carried over the winter. In some of these cases, the hatcheries that supplied fingerlings for the study only sold the appropriate sizes of fingerlings in the fall; thus, fingerlings purchased in the fall would need to be carried over the winter and possibly subjected to the same conditions that likely resulted in higher FCRs. If possible, it clearly would be preferable to avoid stocking fingerlings prior to the winter and wait to stock growout ponds until spring as a strategy to reduce risks associated with higher overwintering FCRs. The studies that did avoid a winter period (Cochran et al., 2009; Tidwell et al., 2015) reported feed conversions of 1.36 and 1.7, respectively. However, given the limited number of hatcheries that produce largemouth bass fingerlings, it may not always be possible to wait until spring to stock ponds. If so, the farmer will need to plan for greater feed costs from poorer FCRs over the winter.

Additional improvements in FCRs during the main production season will also result in reduced production costs and financial risk. For example, the breakeven price in the scenario with a 1.7 FCR was US$9.68/kg. A 25% reduction in the FCR would reduce the breakeven price to US$9.22/kg. Such a reduction in the production cost would increase the profit margin but as importantly would reduce the economic risk by providing a greater buffer against a sudden and unexpected decrease in market price.

The other major cost of producing largemouth bass food-fish is that of fingerlings. While the fingerling production cost also reflects the high cost of feed, the production risk in fingerling production is another contributor to this high cost. Fingerling producers, to be profitable and stay in business, need to cover their risk also. The Engle and Southworth (2013) survey showed widely varying survival rates and yields (kilogram per hectare), particularly in the phase that involves rearing fry to a size of 5 cm. Survivals in this stage of production were reported to range from 0% to 123%. As an example of how this variability can affect production costs, if the yield of 5 cm fingerlings decreases to 24,700/ha from the reported average of 123,500 fingerlings/ha, the cost of 15 cm fingerlings will more than double per fish. The unexplained production losses in the 5 cm production stage were reported by a number of respondents to occur on a frequent basis and are a contributing factor to the high cost of largemouth bass fingerlings. The risk associated with poor production in the 5 cm production phase increases the overall fingerling cost because hatchery operators must earn enough to cover those types of losses. Widely varying yields and survivals that create financial risk were also documented for other stages of largemouth bass fingerling production.

Market risks are also greater when selling into live food-fish markets. In more well-established food-fish industries, there has been increased use of contracts between farmers and processing plants that provide some degree of stability in terms of price and the volume of fish that will be sold. However, live fish markets typically operate as cash markets with few options for contracts, especially those that specify price and volume. Moreover, to maintain market share, a business must maintain adequate supplies to meet the needs of their customers throughout the year. It often is difficult to accurately estimate what demand will be in live fish markets.

Thus, many farms end up producing more fish than they can sell. When the farm cannot sell the more expensive largemouth bass, the financial losses are greater than they would be for less-expensive catfish.

14.6 Research needed to reduce the cost of producing largemouth bass

While much research has been done to develop technologies to successfully rear largemouth bass to a marketable size, there are some key gaps in the research that is currently available. Addressing these research gaps has potential to reduce the costs and risk of producing largemouth bass for food-fish. The primary areas of greatest need include production studies that would lead to reduced farm-level costs of largemouth bass fingerlings and feed, managing strategies to overwinter largemouth bass, and marketing studies that lead to strategies that would reduce market risk.

There is a strong need to identify the causes of variability in yields in the production stage in which fry are stocked into fertilized ponds and raised to a size of 5 cm. This is the size that is appropriate for feed training. On the one hand, there is evidence that it is possible to obtain high yields in this stage of production. Yields of 296,400 fish/ha were reported in some ponds by respondents in the Engle and Southworth (2013) survey. However, total losses in other ponds that were managed in a similar fashion substantially increase production risk associated with this stage of production. Hatchery managers attribute this extreme variability to the variable nature of the types of phytoplankton blooms that develop in different ponds. Work is clearly needed to develop efficient production systems to produce 5-cm fingerlings with much more reliable survival rates and yields.

There also is insufficient research data on production of 15–18 cm largemouth bass fingerlings. If yields in these ponds could be doubled, to 3,570 kg/ha, the cost per fingerling would be cut in half. Hatchery managers reported generally good survival rates and growth in this phase of production (Engle and Southworth, 2013). Given the food-fish production yields above 4,500 kg/ha, it would seem that yields of 3,570 kg/ha in the 15–18 cm production stage should be achievable. However, there is insufficient research data from which to judge what yields are realistic for this stage of production. Additional production trials for this stage of production would also generate additional data related to FCRs. Improvements in feed conversion rates will also reduce costs of 15–18 cm bass fingerlings.

There will be times in temperate regions when largemouth bass need to be in ponds over the winter. There have been no studies to identify optimal management strategies for the winter period. Yet research studies that have included a winter period have shown much poorer FCRs than those conducted within a single growing period.

Largemouth bass currently are sold into live food-fish markets. While there is some evidence of growth in these markets (Thapa et al., 2015), the overall volume of fish sold in live markets is small in the U.S. The growth rates that have been measured in these markets are such that growth will not absorb major increases in production volumes without substantially decreasing prices received by producers. Additional research is needed to understand key factors driving demand for live fish in the USA, including the extent to which this demand is tied to strong preferences for specific species or to a more generic preference for live seafood. If the latter, preferences may be more for a wide variety of live fish, and customers may be more likely to readily substitute among various species. Research is also needed to explore opportunities to develop much larger markets for largemouth bass that may have potential to absorb larger volumes of production without depressing price. In the U.S. and in many countries around the world, the major product form preferred by consumers is that of fillet products. Additional work is needed to explore the potential to market largemouth bass as a fillet product. Such a step would likely require additional decreases in the cost of production, or, alternatively, the creation of market products with strong appeal to specific market segments where a higher price can be achieved. While current price points likely make this difficult, there has been insufficient market research in the USA to understand various market segments well enough to know if there are segments where a fillet product might be introduced successfully. Market prices in other countries will reflect the demand of the relevant consumer groups and will need to be used in economic analyses of largemouth bass production.

14.7 Hidden costs of small-scale production

Small-scale production of an aquaculture crop often entails costs that are not accounted for in the initial plan for the business. As a result, when the need to make these expenditures does arise, the effect on the business may be severe. The hidden costs that are most often omitted from financial planning for aquaculture businesses frequently are those associated with capital assets, such as land, existing farm equipment and buildings. Not accounting for land results in lack of consideration for its opportunity costs. However, it is well understood in economics that if someone can make more from their land from some activity other than raising largemouth bass, they will not continue to raise largemouth bass. Rather, they will switch at some point to the more profitable use of land. If farm equipment such as a truck, tractor, or building already exists on the farm, that investment is considered a "sunk" cost. However, if the farmer does not account for the value of such assets, in proportion to their use in largemouth bass production, then it is not possible to see if the business is generating sufficient revenue to be able to replace those items when they wear out (Engle and Stone, 2014). If they cannot be replaced, then the business will likely fail.

The labor and management skills of the largemouth bass producer and family must also be accounted for in financial planning whether or not the owner pays him/herself or family members a salary or wage. Aquaculture producers must earn enough from their largemouth bass production to be able to save for retirement, to pay for their children's college education, and for an occasional vacation (Engle, 2017). Otherwise, they are unlikely to continue to spend such long hours raising and selling bass.

A common error in budgeting is to assume that all fish produced are, in fact, sold when that often is not the case. In many cases, fish produced are not always in the size range desired by customers. Market demand varies from year to year and producers often overestimate what the demand may be for the coming year. At the same time, it is important for a business to be certain to have an adequate supply on hand so that customers do not begin to search for other suppliers. Thus, there often are fish produced that are not sold. Revenue projections must be based on very conservative projections of volumes of fish that realistically will be sold, with plans for disposing of inventory not sold in that year.

Other common errors when planning a startup aquaculture business are based on use of data from research studies. This most commonly takes the form of applying the yields from research studies as the quantity of fish expected to be sold in financial planning instruments. While the cost estimates presented in this chapter have relied heavily on experimental results from research studies, these should be interpreted by prospective bass farmers with great caution. There is no database of production data from commercial bass farms from which to draw more realistic values. The analysis presented in this chapter did rely on commercial-scale pond-based fish farm data for fixed capital costs and variable costs such as utilities, repairs and maintenance. However, the all-important values for the yield to be sold, FCRs, and survival rates, for food-fish production were based on research data. Yields from research studies and FCRs typically are much better than what commercial farmers can obtain. This has to do with the way that research studies are conducted. Such methods are appropriate for research, but these studies control for many factors that are outside the control of an aquaculture producer. Thus, it is best to use values that are more conservative than what is published in the research literature, especially for yield, FCRs, and survival rates.

14.8 Marketing costs

The estimated costs per kilogram of production of largemouth bass food-fish presented in this chapter do not include costs of selling and marketing live food-fish. The primary markets for live largemouth bass are in urban areas in the northern tier of the USA and in Canada. Thus, for customers to have the opportunity to purchase farm-raised largemouth bass, the fish must be held, size graded, and transported, often to wholesale facilities in cities that have tanks to maintain fish in good condition. From the wholesale facilities, the fish are distributed on smaller transport tanks to the various supermarkets where sold.

Clearly, there are a variety of costs associated with these intermediate market functions of wholesaling, transportation, and distribution. These functions require holding or grading sheds that can be large, open buildings that cover series of concrete vats used to hold, sort, and size grade the fish. These sheds and vats require a supply of oxygen (often oxygen saturators that are installed on each vat), electrical service, and adequate space for farm trucks to offload fish brought in from ponds and to load the larger trucks used to transport live fish to the cities that constitute key markets. Equipment necessary to sell fish thus includes the trucks, oxygen systems on the trucks, nets, and baskets to load and unload fish.

While no studies have been done that estimate these costs for selling live largemouth bass, there are estimates of marketing costs for catfish. Engle and Stone (2014) estimated that annual marketing costs of catfish for direct sales were approximately 0.066 to US$0.2/kg for the equipment and supplies needed to support direct sales. The cost of transporting live fish to markets would be an additional cost. Additional research is needed to estimate the costs of marketing to live food-fish markets in the USA and Canada.

14.9 Conclusion

The research that has been done on production of largemouth bass for sale as a live food-fish has demonstrated that it is possible to raise these fish to an acceptable market size in the USA. There is evidence that it may be possible to do so profitably, although additional work is needed to more fully understand marketing costs, consumer demand, and trends in live fish markets. However, at current production costs, the profit margins appear to be fairly thin. It is also clear that there is considerable financial risk involved in raising largemouth bass, due to the greater costs per ha of feed and fingerlings. Additional research is needed that leads to reduced financial risk in the form of reducing overall farm costs of feed and fingerlings for largemouth bass food-fish producers.

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Chapter 15
Future prospects

James H. Tidwell

Kentucky State University, Aquaculture Research Center, Frankfort, Kentucky, USA

Previous chapters have shown how the largemouth bass was highly regarded as a food-fish in the 1800s and the culture of largemouth bass developed as early as the mid-1800s. Production of large numbers of largemouth bass fingerling expanded during the 1930s to stock new waters being developed during the "New Deal" programs of the Franklin Roosevelt administration in the USA. Most culture activity was based on supporting largemouth bass sport fisheries. Much of the sport-fish management of the 1960s, 1970s, and 1980s relied on supplemental stocking programs using small fingerlings. However, the effectiveness of sport-fish stocking began to be questioned and evaluated. It was shown that the use of larger fingerlings was more effective in terms of long-term impact on the fishery. In the 1970s, Snow and associates developed the Marion Method to produce large numbers of large fingerlings by training the fish to accept artificial diets. During that same decade, largemouth bass began to be sold into ethnic Asian markets as a live food-fish. The production of largemouth bass as a food-fish is now probably 1,000,000 kg in the USA and likely exceeds 150,000,000 kg in China. So where does largemouth bass production go from here?

The current major constraint to the expansion of the largemouth bass as a culture species is summed up as high production costs. This affects almost all considerations as we discuss future directions. In fact, high production costs have a large impact on the current situation. In Chapter 2, a large largemouth bass food-fish producer in the USA described how a shift in currency exchange rates basically caused most Canadian largemouth bass buyers to shift to cheaper alternatives, demonstrating the fragility of the market and constraints of high production costs.

Maybe largemouth bass producers should expand beyond the live-fish markets and into processed markets, such as fillets. Engle et al. (2013) conducted a production and economics study. Their conclusion was that with "production costs of $7.26-$9.34/kg (total costs), that large-mouth bass production for a fillet market is unlikely to be feasible" (Engle et al., 2013: 805).

So how might production costs of largemouth bass be lowered? Engle et al. (2013) reported that at the test density (12,500/ha) similar to commercial densities, the largest single cost item was the cost of feed (41–45% of total costs). Their economic analyses were based on a feed cost of US$1,323/MT. Their sensitivity analysis showed that for every US$100/MT decrease in the price of feed, the breakeven price would decrease by US$0.66/kg. A study by Cochran et al. (2009) demonstrated a reduced fish meal diet (24% FM) that lowered feed costs per unit of gain almost 30%. Lowering the feed cost used in Engle et al. (2013) by even 22% would represent a reduction of US$300/MT and decrease the breakeven price by US$1.98/kg.

Studies have shown that the largemouth bass is able to utilize alternative protein sources, especially animal source proteins (Tidwell et al., 2005). The largemouth bass also does not appear to have significant dietary requirements for the long-chain highly unsaturated fatty acids found in fish oil (AA, EPA, DHA) and perform well on alternative lipids (Tidwell et al., 2007). These traits also make it more likely that feed costs can in fact be substantially reduced.

In the analyses of Engle et al. (2013) fingerling costs were the second largest cost (27–37% of total costs). Their analyses were based on a cost of US$1.60 each for 15 cm fingerlings. Sensitivity analysis showed that for every US$0.10/fingerling decrease in fingerling price, breakeven price would decrease US$0.16–0.22/kg, depending on stocking density.

Part of the relatively high cost of largemouth bass fingerlings is due to the feed training step which is not required for many other culture species such as channel catfish, rainbow trout, and tilapia. However, there is a definite trend in largemouth bass toward this being less of an issue.

Heidinger (2000) reported that after 7–10 d of intensive training (freeze dried krill) ≥ 60% of the fingerlings would have learned to accept a prepared diet. However, if fingerlings are produced from hatchery-reared, feed-trained broodstock, feed-training success increases to > 90%. This is almost certainly the result of domestication. According to information presented in Chapter 6, in the past few years, fingerlings during feed-training are more readily adapting to prepared diets without the stimulant feeds of ground fish, carp eggs, or freeze-dried krill. Fingerlings are also adapting to artificial feeds at smaller sizes. Most largemouth bass feed-training procedures recommend initiating training at 3.8–5.0 cm. However, according to information in Chapter 3, in China fingerlings are being feed trained at < 2 cm. Research also indicates that the nursery pond phase can be skipped entirely (Skudlarek et al., 2007). This leads to the potential of scale-up of intensive fingerling production as discussed in Chapter 6.

Another potential improvement would be reducing the growout period from two years to one. Engle et al. (2013) stated that largemouth bass can reach sizes of 426–515 g in the first year of production. Their sensitivity analysis indicates that this would decrease the breakeven price by US$0.26/kg compared to 2 year production costs.

Not all of these potential improvements would be directly additive. However, it might be useful to consider hypotheticals. As stated earlier, changes in feed costs indicated by Cochran et al. (2009) could lower breakeven costs by US$1.98/kg of live-weight fish. A lowering of fingerling costs from US$1.60 per 15 cm fingerling to US$1.20/fingerling could decrease the break-even cost by US$0.78/kg. Growout in one year rather than 2 years could lower the breakeven cost by US$0.26/kg. If additive, these changes could lower the breakeven cost by US$3.00/kg, approaching a breakeven cost of US$4.25/kg.

It seems that the changes in feed costs could be accomplished in the relative short-term. However, long-term reductions in feed costs, fingerling costs, improved feed conversion ratios, and shortened growout period could all be positively impacted by improved genetics. Documented improvements in feed-training success of fingerlings is largely the result of undirected inadvertent genetic changes through the "Unnatural Selection" of domestication. Just by keeping several generations of a fish in a farm environment, the fish genetically unsuited for that environment do not pass on their genes, while those that are better suited contribute to the subsequent generations.

Through active intervention and selection, strains of largemouth bass could likely be developed which more readily accept artificial diets at first feeding, efficiently utilize lower protein levels and/or higher inclusions of plant source proteins. Strains could also be developed which are disease resistant, grow uniformly, and grow fast enough to achieve market sizes in 1 year. A percentage of fish in the USA already achieve sizes > 500 g in one year, indicating that the genetic potential exists.

The impact of improved genetics is already documented. In China (Chapter 3) the development of an improved largemouth bass strain "Youlu No. 1" was developed by the Pearl River Fisheries Institute. This genetic variety has a good body confirmation, low rate of deformities, good growth with minimal individual size variation, and high production rates. It has already been widely adopted across China and allowed largemouth bass production to expand into new regions as well as into new types of production systems.

Overall, the largemouth bass has already developed from a large public and small private enterprise for producing large numbers of small fish for sport-fish stocking into generating over 150,000 MT of food-fish worldwide. Almost all of this without the research support devoted to other aquaculture food-fish species. With proper support, the fish could be a major contributor to aquaculture growth and diversification in the future.

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