Tilapia

Biology, Management Practices and Human Consumption

Renae Wakefield

Editor

NOVA

TILAPIA

BIOLOGY, MANAGEMENT PRACTICES AND HUMAN CONSUMPTION

FISH, FISHING AND FISHERIES

Additional books in this series can be found on Nova's website under the Series tab.

Additional e-books in this series can be found on Nova's website under the e-book tab.

TILAPIA

BIOLOGY, MANAGEMENT PRACTICES AND HUMAN CONSUMPTION

RENAE WAKEFIELD
EDITOR

NOTICE TO THE READER

The Publisher has taken reasonable care in the preparation of this book, but makes no expressed or implied warranty of any kind and assumes no responsibility for any errors or omissions. No liability is assumed for incidental or consequential damages in connection with or arising out of information contained in this book. The Publisher shall not be liable for any special, consequential, or exemplary damages resulting, in whole or in part, from the readers' use of, or reliance upon, this material. Any parts of this book based on government reports are so indicated and copyright is claimed for those parts to the extent applicable to compilations of such works.

Independent verification should be sought for any data, advice or recommendations contained in this book. In addition, no responsibility is assumed by the publisher for any injury and/or damage to persons or property arising from any methods, products, instructions, ideas or otherwise contained in this publication.

This publication is designed to provide accurate and authoritative information with regard to the subject matter covered herein. It is sold with the clear understanding that the Publisher is not engaged in rendering legal or any other professional services. If legal or any other expert assistance is required, the services of a competent person should be sought. FROM A DECLARATION OF PARTICIPANTS JOINTLY ADOPTED BY A COMMITTEE OF THE AMERICAN BAR ASSOCIATION AND A COMMITTEE OF PUBLISHERS.

Additional color graphics may be available in the e-book version of this book.

CONTENTS

PREFACE

This book discusses the biology, management practices and human consumption of tilapia.

Chapter 1 – Aquaculture is one of the fastest growing sectors of agriculture globally. Production in freshwater and marine fisheries has plateaued or is declining, and the increasing demand for seafood and need for affordable protein sources in third world countries will ensure growth of aquaculture in the future. Tilapia are the second most cultured fish worldwide behind the carps, and even though they are easily cultured in a wide variety of environments and are relatively resistant to aquaculture stressors compared to other cultured finfish species, significant losses to disease still occur under intensive culture. Traditionally, antibiotics and other chemicals have been used to treat disease outbreaks in cultured fish species. However, the scope of approved application for most antibiotics is very narrow and concern over development of antibiotic resistant pathogens will further limit use in the future. The focus instead has turned to finding safe and effective means of preventing infectious diseases in cultured finfish, including tilapia. In recent years, there has been considerable interest in the use of probiotic bacteria and prebiotic supplements added to diets to increase immunity as well as improve growth performance in fish. The majority of probiotic application in fish culture has been dietary addition of bacteria isolated from terrestrial species deemed physiologically beneficial to the intended fish host, but recent research has shown probiotics isolated from the targeted species as being more effective for disease resistance and immunity. The diet, and therefore the gut environment, plays a central role in determining successful probiotic colonization. The high cost of feed and changes in ingredient availability can cause fluctuations in dietary formulations and quality of dietary ingredients altering the gut environment and by extension the gut microbiota and health of

the fish. These changes can be minimized by use of dietary prebiotics to provide more consistent gut conditions favorable for growth of targeted probiotic bacteria. Prebiotics are generally non-digestible food ingredients that stimulate growth and/or activity of a limited number of health-promoting bacteria in the intestine while limiting potentially pathogenic bacteria. The logical extension of prebiotic and probiotic use is synbiotics: the simultaneous application of probiotics and prebiotics in diet of tilapia, which should provide more consistent and beneficial results by allowing culturists to control and modify the gut microbiota for a desired outcome. Due to the apparent effectiveness in improving health and growth in tilapia, research and interest in dietary prebiotics and probiotics is likely to continue, which will fill existing research gaps.

Chapter 2 – The demand for attributes beyond quality, such as food safety, respect for the environment and production with social responsibility is increasing in world food trade. In parallel, the fish production chain has been confronted by problems with the lack of quality of their products, many of them related to the quality of the cultivation or capture waters. Nowadays, the water where these organisms are created, presents itself as a critical control point, as well as influencing the quality / safety of the product, the cultivation should be done in an environmentally sustainable manner. In this sense, the fish has been shown to be responsible for public health problems, such as scromboid poisoning, toxicity by mollusks, ciguatera and microbial contamination or through toxic metals such as mercury. Besides the aspects of security and environmental sustainability, the relationship between water quality and the occurrence of compounds capable of negatively alter the taste and smell of fish (off flavour) has been evidenced in many studies. For crop species there is the possibility of controlling the quality of the fish through the proper management, which primarily begins with the quality of the cultivation water. However, questions regarding the best handling practices, processing, storage and marketing also have troubled the consumer, who is more attentive to issues of food safety and quality programs, being, in some cases, willing to pay more for a product of best quality, convenience and posing no danger to their health, the environment or society. It is noted that the tilapia is a species that can guarantee the availability of fish in many regions, since its cultivation has been successful. The advantages of tilapia farming, in relation to other species, are in the easy feeding, hardiness, prolificacy and good adaptation. Based on the chain of production of fish, although the meaning of quality is broad, standing out in this concept features that the consumer believes that the product should have, or should approach its intrinsic composition, nutritional

value, likely to change during the preparation, storage, distribution, sale and presentation to the consumer. Specific actions of environmental and ecological nature should be proposed, with the aim of contributing to the sustainable and rational exploitation of resources, and minimizing the adverse impact that the waste generated by this activity produces on the environment; seeking responsible and sustainable management of fish agribusiness.

Chapter 3 – The semi-arid is not one of the first climates that comes to mind when fish production is imagined. First of all, it is important to describe the semi-arid in Brazil since it enclosures 969.589,4 km^2, about 11% of the Brazilian territory, and more than 1.000 municipalities in 9 different Brazilian States. In this area live over 22 million people, which is about 10% of the Brazilian total population. However the Brazilian semi-arid has some particular characteristics that made it possible.The São Francisco River and large water reservoirs such as Castanhão and Orós are some of the water bodies where tilapia culture has been sucessfull. There are several production systems adopted ranging from tilapia in irrigation reservoirs on small rural properties to intensive cage culture. It does not matter which production system was adopted by the producer, since the area is semi-arid the water quality has to be extremely well managed and cared for in order to preserve it and guarantee the highest possible productivity per cubic meter of water. In order to achieve the best productivity, some management practices must be adopted. One of them is to select the best variety for breending. Several tilapia varieties have been introduced into the Brazilian semi-arid since the 1970's. However Tai tilapia has been the most successful and is cultivated nowadays. Red Koina and Saint Peters have also been introduced, but it did not succeed due to their low growth after 400g. Tilapia GIFT has already been tested in cage culture. The results are promising, but there is a lack of farms to produce this variety in the Brazilian semi-arid.

Tilapia feeding management is an important issue in tilapia culture in this region. Feeding practices may differ from areas within the semi-arid as well as production systems. Several local ingredients have been tested as examples mango meal, cassava chips and leucaena hay. Most of them are used by small producers as tilapia feed complement. Cage culture producers are well instructed to only use balanced extruded feed in order to achieve better growth and maintain the best possible water quality.

Chapter 4 – Physical changes due to degradation or handling to provide improvements in animal husbandry usually modify the aquatic environment and, one of the environmental variables that can be changed is the luminance. Increased light intensity, for instance, can increase the production of reactive

oxygen species (ROS) that cause molecular damage to cellular structures with consequent functional impairment and loss of vital functions. Additionally, increased light intensity affects aggressiveness in territorial fish, leading to different levels of social stress, which can increase the effects of oxidative stress and ROS activity. Thus, the aim of this chapter was to test the influence of light intensity on the agonistic behavior and oxidative stress in female of *Tilapia rendalli*. The authors compared two treatments referred here as low (253.56 ± 62.25 lx) and high light intensity (1435.92 ± 481.40 lx), each one under two conditions: 1. Social Condition, : where animals were isolated for 96 h and paired (resident-intruder paradigm) for 1 h;. and 2. Isolation Condition (baseline) where: fish were isolated for 96 h. The latency to first fighting and for hierarchical settlement was similar in the two light intensities, but the high light intensity decreased the frequency of attacks and displays in winner fish. On the other hand, there was no difference between the frequencies of aggressive events displayed by loser in both treatments. Catalase did not differ between the two intensity or conditions for all animals. Thus, the light intensity reduces the aggressive interactions, and this influence is related to social rank in female of *T. rendalli*. However, the effects of aggressive interactions were not translated into variations of catalase, showing that oxidative stress is not associated to such a behavioral or environmental modifications.

Chapter 5 – This study examined water and sediment characteristics, fish diseases, management and economic data in fish farms (F1, F2 and F3) in the Canoas II reservoir, Paranapanema River Basin, Brazil. We also assessed limnological characteristics at sampling points along the river channel (C1, C2, and C3). In most water evaluations, the high transparency, electrical conductivity and turbidity values indicated the low amount of suspended particulates. No increase in primary production was observed, a fact evidenced by predominance of low chlorophyll *a* values, except for C2 in June 2012. The total nitrogen, total phosphorus, and inorganic forms concentrations remained constantly below the limit of detection for the analytical method used. In the sediment, the association between the highest concentrations of phosphorus and granulometry (fine sediments) in the lacustrine region were was most evident. These results are likely associated with the short residence time of the water in this run-of-river reservoir. The limnological variables are consistent with legal Brazilian standards and with the recommended patterns for cages, except the low temperatures in the winter and autumn. The *Trichodina* sp was the parasite with the highest infestation intensity in all fish farms, followed by monogeneans (Dactylogyridae and Gyrodactilidae) and by the protozoan

Epistylis. The bacteriological analysis demonstrated that the occurrence of each bacterial genus was random. The relationship between limnological characteristics and tilapias diseases was investigated using principal component analysis (PCA), in which 3 groups (G1, G2 and G3) were discriminated. In the PC1 x PC2 biplot, the temperature was positively associated with *Plesiomonas shigelloides*, and negatively associated with *Trichodina* sp, Gyrodactilidae, *Streptococcus* sp, and *Aeromonas* sp predominance, indicating that temperature is a variable with strong influence on the development of these diseases. In the PC1 x PC3 biplot, Dactylogyridae was associated with June and August 2013 in F3, and with April 2013 in F2, coinciding with higher *Trichodina* sp prevalence. The economic analyses demonstrated that the net income is equivalent to USD 365.00 for each 6.0 m³ cage (USD 0.40 for per fish produced) and USD 1,121.00 for each 18.0 m^3 cage (USD 0.54 for per fish produced). The profitability index corresponded to 33% and 41% for the 6.0 and 18.0 m^3 cages, respectively. The time for return on investment after cage installation was 2 production cycles (1.5 years). The minimum quantities of fish that must be produced for economic viability of this activity are 396.00 kg of 6.0 m³ and 882 kg of 18.0 m³.

Chapter 1

POTENTIAL FOR PROBIOTIC, PREBIOTIC, AND SYNBIOTIC USE IN TILAPIA CULTURE

*Thomas L. Welker**

Agricultural Research Service, United States Department of Agriculture,
Hagerman Fish Culture Station, Hagerman, Idaho, US

ABSTRACT

Aquaculture is one of the fastest growing sectors of agriculture globally. Production in freshwater and marine fisheries has plateaued or is declining, and the increasing demand for seafood and need for affordable protein sources in third world countries will ensure growth of aquaculture in the future. Tilapia are the second most cultured fish worldwide behind the carps, and even though they are easily cultured in a wide variety of environments and are relatively resistant to aquaculture stressors compared to other cultured finfish species, significant losses to disease still occur under intensive culture. Traditionally, antibiotics and other chemicals have been used to treat disease outbreaks in cultured fish species. However, the scope of approved application for most antibiotics is very narrow and concern over development of antibiotic resistant pathogens will further limit use in the future. The focus instead has turned to finding safe and effective means of preventing infectious diseases in cultured finfish, including tilapia. In recent years, there has been considerable interest in the use of probiotic bacteria and prebiotic

---

* Corresponding author: E-mail: thomas.welker@ars.usda.gov.

supplements added to diets to increase immunity as well as improve growth performance in fish. The majority of probiotic application in fish culture has been dietary addition of bacteria isolated from terrestrial species deemed physiologically beneficial to the intended fish host, but recent research has shown probiotics isolated from the targeted species as being more effective for disease resistance and immunity. The diet, and therefore the gut environment, plays a central role in determining successful probiotic colonization. The high cost of feed and changes in ingredient availability can cause fluctuations in dietary formulations and quality of dietary ingredients altering the gut environment and by extension the gut microbiota and health of the fish. These changes can be minimized by use of dietary prebiotics to provide more consistent gut conditions favorable for growth of targeted probiotic bacteria. Prebiotics are generally non-digestible food ingredients that stimulate growth and/or activity of a limited number of health-promoting bacteria in the intestine while limiting potentially pathogenic bacteria. The logical extension of prebiotic and probiotic use is synbiotics: the simultaneous application of probiotics and prebiotics in diet of tilapia, which should provide more consistent and beneficial results by allowing culturists to control and modify the gut microbiota for a desired outcome. Due to the apparent effectiveness in improving health and growth in tilapia, research and interest in dietary prebiotics and probiotics is likely to continue, which will fill existing research gaps.

INTRODUCTION

Tilapia, because of their enormous adaptability to a wide range of physical and environmental conditions, ability to reproduce in captivity, relative resistance to handling stress and disease-causing agents compared to other cultured finfish species, good flesh quality, feed on a low trophic level and excellent growth rate on a wide variety of natural and artificial diets, are the most abundantly cultured species worldwide (Welker and Lim 2012). Although they are endemic to tropical freshwater in Africa, Jordan and Israel, their distribution has widened following introductions to other regions of the world in the early part and after the middle of the 20th century. They are presently cultured in virtually all types of production systems, in both fresh and salt water, and in tropical, subtropical and temperate climates (Lim and Webster 2006). Tilapia dominate both small- and large-scale aquaculture in many tropical and subtropical countries, both as low-price commodity mass consumption as a staple protein source and as a high-value, upscale product for export markets. They are the second most cultured freshwater fish in the

world (after carps). However, they are increasingly recognized as the species of choice for intensive aquaculture and are likely to become the most important cultured fish in the world (Fitzsimmons 2006). According to the American Tilapia Association, global farm-raised tilapia production was 4.5 million metric tons in 2012 and is expected to exceed 4.8 million metric tons in 2014. Several species of tilapia are being cultured commercially, but Nile tilapia, *Oreochromis niloticus* and various hybrids are the predominant culture species worldwide.

A major problem associated with intensive fish culture operations is the increased susceptibility of fish to infectious diseases. The total losses from disease outbreaks in aquaculture worldwide have reached billions of dollars annually and have been identified as a major threat to the sustainability of aquaculture industry. Traditionally, antibiotics and chemicals have been used to treat diseases in aquatic animals (Li and Gatlin 2005). However, given the fact that diseased fish eat poorly, a limited number of government-approved and efficacious drugs/chemicals are available, and the increasing problem of emerging drug-resistant pathogens and the resultant food and environmental contamination (FAO 2002), disease prevention, rather than treatment of sick fish, is a better means of controlling infectious diseases. Moreover, the use of antibiotics in animal production, including aquaculture, is increasingly under public scrutiny and criticism in most developed countries. Consequently, there has been considerable interest in recent years to evaluate the feasibility of using non-nutrient dietary additives, particularly prebiotics and probiotics, to enhance growth, stimulate immune system function and/or improve the resistance of fish to infectious diseases. Numerous reviews on these subjects have been published in the past decade (Saka 1999; Gannam and Schrock 2001; Irianto and Austin 2002; Gatesoupe 2005; Kesarcodi-Watson et al. 2008; Wang et al. 2008; Merrifield et al. 2010a; Ringø et al. 2010; Welker and Lim 2012). Although most research has focused on dietary supplementation of prebiotics and probiotics, other means of administration have also been used, e.g., by addition to rearing water (Zhou et al. 2010).

This chapter provides an overview on the use of prebiotics and probiotics in diets and their effects on growth performance, feed utilization efficiency, gut microbiota and morphology, immune responses, and disease resistance of tilapia. Furthermore, the potential for application of prebiotics and probiotics as a synbiotic will also be discussed.

Overview and Definitions

Considerable attention has been given to alteration of the gut microbiota to boost health in humans and other animals in recent years through the use of probiotics and prebiotics (termed biotics). The goal of these dietary supplements is similar, but the manner in which they alter the gut microbial community is varied. Furthermore, some of the probiotic and prebiotic products are similar in composition, containing inactivated microbes or microbial components, which has led to some confusion over what exactly constitutes a "probiotic" or a "prebiotic". The definitions of probiotic and prebiotic have changed to some degree since both terms were first introduced. The most widely used definition of probiotics is given by Fuller (1989) as "a live microbial feed supplement which beneficially affects the host animal by improving intestinal balance". Current probiotic applications and scientific data on mechanisms of action indicate that non-viable microbial components act in a beneficial manner, and this benefit is not limited to the intestinal region (Kesarcodi-Watson et al. 2008), indicating that probiotics may include non-viable microbes and gut colonization is not necessary to produce benefits to the host. There is some debate as to whether the commonly accepted definition should be revised to include these changes. However, for the purposes of this review, living microorganisms, for the most part, will be considered as probionts.

As has been pointed out by numerous researchers (Gatesoupe 2005; Kesarcodi-Watson et al. 2008; Merrifield et al. 2010a; Zhou et al. 2009a; Nayak 2010a; Nayak et al. 2010b), there is an increasing need to find safe alternatives to antibiotics. Intensive fish culture increases the prevalence of stress-related disease outbreaks and associated fish losses. Traditional use of antibiotics to prevent disease in fish has received criticism due to the potential for development of antibiotic resistant bacteria, the presence of antibiotic residues in fish tissue, negative impacts on microbial populations in the aquaculture environment, and suppression of the cultured species' immune system (Ringø et al. 2010; Zhou et al. 2009b). Probiotics have received the most attention as a viable alternative; however, their use poses a number of potential drawbacks: possible negative impact of untested probionts on the environment, regulatory constraints, food safety issues, and maintaining viable probionts through the feed manufacturing process (particularly extrusion) (Ringø et al. 2009). Use of bacterial species known to be safe for humans and the environment may be the most expedient approach for testing of prospective probionts and maintaining viable populations during storage

(Gatesoupe 2005). Although there are a number of drawbacks to implementation of probiotics in fish diets, when used properly, they can be effective in improving growth performance and immunity in tilapia.

Prebiotics, on the other hand, are any non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Ringø et al.2010). There are four primary characteristics a prebiotic must possess: 1) resist gastric acidity, hydrolysis by enzymes, and absorption in the GI tract; 2) fermentation by the intestinal microbiota; 3) selectively stimulate growth and/or activity of intestinal bacteria; 4) improve the health and well-being of the organism (Gibson et al. 2004; Merrifield et al. 2010a). Traditionally, prebiotics had been limited to the oligosaccharides inulin, transg alactooligosaccharide, and lactulose (Gibson et al. 2004) with the addition of fructooligosaccharide by Roberfroid (2007) using the previously listed criteria. However, mannanoligosaccharide, galactooligosaccharides, xylooligo saccharides, arabinoxylooligosaccharides, isomaltooligosaccharides, and the commercially available GroBiotic-A have been considered prebiotics in research with fish (Ringø et al. 2010) and will be considered as such here.

Synbiotics, a relatively recent concept, may prove more effective in controlling the microbiota of the gastrointestinal (GI) tract (Gatesoupe 2005). Synbiotics are supplements that contain both prebiotics and probiotics. An effective synbiotic pairing would allow alteration of the colonic environment by a prebiotic that would select for preferential growth conditions of known beneficial probionts. The benefits of this approach are obvious: fish culturists not only are able to control and provide favorable conditions in the colon but also ensure that a beneficial probiont is present in sufficient numbers. Synbiotics is a relatively new field of study with limited research but large potential for use in promoting health and well-being of aquaculture species.

The study of the effects of probiotics and prebiotics supplemented in the diet of tilapia has not advanced as far as it has in other species, such as salmonids. Merrifield et al. (2010a) and Ringø et al. (2010) provide excellent and detailed reviews of the status of biotics in salmonids and other fish species, respectively. By comparison, the available research with tilapia is severely lacking, underscoring that more research is required. However, research on the supplementation of probiotics and prebiotics in the diets of tilapia is advancing rapidly with the majority of studies taking place since the mid-2000s, and further advances are likely to occur in the coming years (Welker and Lim 2012).

PROBIOTICS

1. Types of Probionts

There are generally two choices when selecting a probiont to supplement in diets of fish: 1) bacteria native to and isolated from the host fish species and 2) commercially available probionts typically of non-fish origin (the majority sold for use in terrestrial animals). The first choice may be the most sensible and potentially successful, since they are capable of colonizing the intestinal epithelial surface and grow within the intestinal mucus, are able to survive the pre-colon digestive tract environment of the host species, are not pathogenic to the host, and are likely environmentally safe. However, for most prospective probionts isolated from fish there are questions about potential safety issues and effectiveness as a probiotic, for example: it is unknown whether they are free of plasma-encoded antibiotic resistance genes, pose a potential health risk to humans and other animals, possess desirable growth characteristics, are easily cultured, and remain viable under normal storage conditions and after the feed making process (Merrifield et al. 2010a). The cost of undertaking the regulatory approval process for these indigenous species may prove to be a major obstacle for commercialization (Gatesoupe et al. 2005). More familiar microorganisms, already tested in other animal species with a body of knowledge supporting their safe use presents a substantial advantage for approval, and many of these probiont species are already available in commercial preparations. Likewise, microorganisms isolated from the gut of fish but belonging to familiar groups (*Bacillus* spp., *Lactobacillus* spp., yeast spp., and others) may also be suitable (Gatesoupe et al. 2005; Merrifield et al. 2010a).

2. Gut Microbiota and Probiotics

Much more is known about the microbiota of the GI tract in mammals and its role in mammalian health compared to fish, but in recent years, substantial research has been conducted to characterize the gut microbiota of fish (Nayak 2010b). The GI microbiota perform a variety of functions that benefit the health of the host species by promoting nutrient supply, enhancing immune function, preventing colonization of pathogenic microbes, energy homeostasis, and maintenance of normal mucosal integrity and function. However, little is known about bacterial communities, their establishment and diversity, and role

in fish nutrition and health. In tilapia, early work focused on describing the gut microbiota in naturally-occurring populations and cultured fish in Japan (Sakata et al. 1980a and 1980b; Sugita et al. 1982a, 1982b, 1983, 1985, and 1987) and only later investigations have centered more on changes in the gut microbial community and health effects through the use of probiotics.

a. Microbial Community of the Tilapia GI Tract

Few studies have characterized the microbial composition of the GI tract of tilapia. The majority of those studies were conducted by Sugita and colleagues in the 1980s using traditional microbiological culture methods. Even more recent examinations of tilapia gut microbiota have continued to rely on traditional culture techniques, rather than more reliable and advanced molecular identification methods. Under normal conditions, the dominant microbial species in the intestine of fish are anaerobic (e.g., *Bacillus*, *Bifidobacterium*, *Lactobacillus*, *Saccharomycetes*, etc.) accounting for 99% of the microbial community; aerobic and facultative bacteria only account for 1% of the population (Zhou et al. 2009a). Facultative anaerobic bacteria from the *Vibrio-Aeromonas* group and obligate anaerobic bacteria, *Bacteroides* type A and type B (Sakata et al. 1980a; Sugita et al. 1982a), *Plesiomonas shigelloides*, *A. hydrophila* (Sugita et al. 1985), and *Cetobacterium somerae* (Tsuchiya et al. 2008) are the primary bacteria comprising the GI tract of cultured Nile tilapia. Sugita et al. (1982b) found the microbiota in freshwater cultured tilapia to be rather stable. However, the rearing environment of tilapia can cause shifts in the gut microbial composition. Obligate anaerobes declined and were replaced by aerobic and facultatively anaerobic Gram negative rods (*Vibrio-Aeromonas* group) when tilapia were adapted from freshwater to seawater (Sugita et al. 1982a). The authors suggest that dominance of the GI microbiota by *Bacteroides* type A and type B is restricted to fish cultured in freshwater. The effect of the salinity of the rearing environment on the intestinal microbiota of tilapia is also illustrated by examining the research of Al-Harbi and Uddin (2003, 2004, and 2005). Gut bacteria in Nile tilapia cultured in brackish water were dominated by *Vibrio* spp. (Al-Harbi and Uddin 2005), while tilapia reared in freshwater, earthen ponds exhibited a greater diversity of gut bacteria, predominately comprised of Gram-negative rods (*Aeromonas hydrophila*, *Shewanella putrefaciens*, *Corynebacterium urealyticum*, *E. coli*, and *Vibrio cholera*) (Al-Harbi and Uddin 2003 and 2004). In these studies, the gut bacterial population correlated well with the predominant species found in the rearing water and pond sediment, showing that the rearing environment plays a large role in the gut microbial composition. Sugita et al. (Sugita et al.

1983) also suggests that the predominant bacterial species in tilapia GI tract, including aerobes and anaerobes, originate from the culture environment. Molinari et al. (Molinari et al. 2003) reported that the GI tract of Nile tilapia is dominated by eight bacterial species, *Aeromonas veronii*, *A. hydrophila*, *Burkholderia cepacia*, *Chromobacterium violaceum*, *Citrobacter freundii*, *E. coli*, *Flavimonas zryzihabitans*, and *Plesiomonas shgelloides*. Although some of these species are also dominant in work by Sugita et al. and Al-Harbi and Uddin, the differences, which are likely affected to some degree by isolation and identification methods (especially the lack of strict anaerobic isolation methods by Al-Harbi and Uddin and Molinari et al. 2003), also suggest the rearing environment should be taken into consideration when selecting probionts for supplementation in diets of tilapia.

Gut microbial composition can also vary seasonally and with changes in diet. Al-Harbi and Uddin (2004) discovered that the bacterial composition of tilapia gut can fluctuate considerably depending on the time of year. Even with the most dominant bacterial species, numbers (total counts and as a percentage of the total population) change dramatically during the course of a year. Modifications of diet can also affect the microbiota composition. In rainbow trout, the gut microbiota make-up is altered when fish were switched from a fish meal to a plant meal based diet (Heikkinen et al. 2006). With continued emphasis on use of alternative feed ingredients in place of fish meal and fish oil, many currently used probiotics may prove ineffective with changes in diet formulation. However, probiotic applications proven successful in improving growth with traditional fish meal-based diets may also improve digestibility and utilization of alternative plant ingredients. Use of established probiotics may need to be re-evaluated with alterations in diet and rearing conditions, and selection of new probionts and/or development of new feeding regimes may be required.

b. Changes in Gut Microbiota

Although a number of studies have been conducted which examine the effect of dietary probiotics on tilapia health, only a few have determined the impacts of supplementation on the microbiota of the GI tract. The aim of probiotic use is to alter the gut microbial composition. The normal microbiota of the tilapia GI tract is established 20 to 60 days after hatching (Sugita et al. 1982b). After the microbiota is established, modifying the microbial composition of the gut is complicated/complex, further complicated by several exogenous and endogenous factors that can also influence establishment or alteration of the GI tract microbiota of fish. Gut structure, rearing

environment, and farming practices can all effect initial colonization and establishment (Nayak et al. 2010b). The goal of probiotic supplementation (immune stimulation, disease resistance, growth performance, etc.) must also be taken into account (Merrifield et al. 2010a). Therefore, proper selection of probionts is critical to success, and as mentioned, use of more than one species or type of probiont in diet may be wise to ensure success with changing conditions and developmental stage, which has proven to be effective in mammals (Timmerman et al. 2004) but remains largely untested in fish.

Establishing the effects of probiotic dietary supplementation on the indigenous microbial population of fish has been difficult because researchers have predominantly enumerated the GI microbial population after probiotic supplementation with little attention paid to the composition of the indigenous microbiota (Merrifield et al. 2010a). This observation also holds true for tilapia, but a few studies have attempted to characterize the GI tract microbial make-up. Nile tilapia fed diets supplemented with viable *Saccharomyces cerevisiae* + *Bacillus subtillus* or non-viable *S. cerevisiae* produced different GI tract microbial compositions (Marzouk et al. 2008). Tilapia fed non-viable *S. cerevisiae* showed no change in the intestinal microbial community, which was dominated by *E. coli*, *Salmonella* spp., *Klebsiella* spp., *Morganella morganii*, *E. tarda*, *Aeromonas sobria*, and *P. fluorescens*. Tilapia fed the diet supplemented with the viable yeast + *B. subtillus* mixture showed no presence of *E. coli*, *Salmonella* spp., *Klebsiella* spp., or *P. fluorescens*. Nile tilapia fed diets supplemented with *Pediococcus acidilactici* exhibited alteration of the indigenous gut bacterial population (Ferguson et al. 2010) (this is the only study which has examined the effects of probiotic supplementation on the gut microbiota in tilapia using a molecular, culture independent method). Although the total counts of aerobes and anaerobes did not change, lactic acid bacteria increased significantly in tilapia fed the probiont. *P. acidilactici* persisted in the gut of Nile tilapia for seventeen days after fish reverted to the control diet. *P. acidilactici* also provided antagonism against an unidentified bacterium. Knowledge on the impact of probiotic supplementation on the indigenous gut microbiota in tilapia and other species is lacking – this information is important to understanding which microbial species are best suited for dietary supplementation and how they may affect fish health.

3. Physiological Changes and Growth Performance

A number of specific modes of action by probiotic microorganisms has been attributed to physiological benefits in fish. Although gut colonization is often identified as the most important characteristic of effective probionts, the reality is that the benefits incurred by the host from probiotic supplementation are likely a synergistic product of multiple biological effects (some of which have nothing to do with gut colonization), including production of inhibitory compounds, competition for chemicals or available energy, competition for adhesion sites, inhibition of virulence gene expression or disruption of quorum sensing, enhancement of the immune response, source of macro and/or micronutrients, enzymatic contribution to digestion, and stimulation of local and systemic immune responses (Merrifield et al. 2010a). Identifying the specific effect(s) produced by probionts that benefit fish can prove difficult. However, such benefits have been documented in many fish species, including tilapia, some of which will be expanded on in this manuscript.

a. Changes in Gastrointestinal Morphology

The endogenous microbiota of the GI tract also affects GI morphology and function of fish. Gnotobiotic studies (animals cultured under axenic conditions) in fish have shown that the GI microbiota community is important in nutrient metabolism and absorption, regulation and energy balance, development and maturation of the mucosal immune system, and epithelial differentiation and maturation (Nayak et al. 2010b). In zebrafish, the absence of GI microbiota results in arrestment of the gut epithelium from a lack of brushborder intestinal alkaline phosphatase activity (Bates et al. 2006). The authors also found that proliferation of goblet cells, enteroendocrine cells, and other secretory cell lines are tied to gut microbiota. Maintenance of a healthy gut microbiota is a likely benefit to the development of the gut epithelial architecture, and because many fish pathogens can disrupt the integrity of the intestinal epithelium, a healthy GI microbial population may reduce mucosal damage, increase absorptive area, and prevent pathogenic disease (Merrifield et al. 2010a). Few studies have examined the effects of probionts on intestinal morphology of tilapia. Ferguson et al. (32) found no effect on intestinal morphology when tilapia were fed diets supplemented with *P. acidilactici*. Pirarat et al. (2011) discovered that dietary supplementation of *L. rhamnosus* promoted the development of the intestinal structure of Nile tilapia. Fish fed the probiotic diet had greater villous height in the proximal and middle sections of the intestine. Similar results were found by Merrifield et al.

(2010b) in rainbow trout, where dietary supplementation with *P. acidilactici* increase microvilli length but not density in the proximal intestine. More research is needed in tilapia and other species to determine the mechanisms by which probiotic microorganisms affect gastrointestinal morphology.

b. Nutrient Utilization, Digestion, and Growth

The gut microbial population is also important to the nutrition of fish by increasing nutrient uptake and utilization, production of enzymes, amino acids, short-chain fatty acids, and vitamins, and improved digestion (Merrifield et al. 2010a; Nayak et al. 2011b). Lovell and Limsuwan (1982) found that the intestinal microbiota of Nile tilapia fed a B_{12} deficient diet were able to produce at least 11.2 ng vitamin B_{12}/g body weight per day, almost ten times that of channel catfish. Sugita et al. (1990 and 1991) reported that obligate anaerobes, primarily *Bacteroides* type A and *Clostridium* spp., were likely responsible for vitamin B_{12} production in the gut of tilapia. Tsuchiya et al. (2008) also found that 17 strains of *Bacteroides* type A isolated from the gut of tilapia were able to synthesize vitamin B_{12} at a rate of approximately 8 ng/mL culture in 48 hr. Tilapia do not require vitamin B_{12} supplementation in diets to prevent deficiency because of the microbial vitamin B_{12} production capability of the gut microbial community. Other B vitamins (nicotinic acid and pantothenic acid) are also produced by intestinal bacteria of fish (Teshima et al. 1967), although it is undocumented whether the same is true for tilapia. Production of other fat or water soluble vitamins by microorganisms in the GI tract of fish has not been reported.

Few studies have examined the impact of probiotics on nutrient uptake and utilization in fish, including tilapia. However, there are reports of improved nutrient utilization through probiotic use in tilapia and other species of fish. Rainbow trout exhibited relief from vertebral column compression syndrome due to improved bone formation/mineral utilization from improved mineral uptake in fish fed diets containing the probiotic *P. acidilactici* (Merrifield et al. 2010b and 2011). The authors hypothesize that improved mineral uptake may have been caused by acidification of the intestinal environment through short-chain fatty acid and lactic acid production by the supplemented probionts. No evidence has been reported supporting improved mineral uptake in tilapia through probiotic use, but probiont-assisted production and utilization of other nutrients has been observed. Premalac[®] or Biogen[®], commercially available probiotic mixtures, supplemented in diets containing varying levels of protein, produced better growth performance in tilapia, suggesting improved protein utilization (Ghazallah et al. 2010). Tilapia

fed the probiotic diet containing lower dietary protein (27.5%) had better growth than fish fed a 30% protein diet without probiotics. No explanation on the mechanism responsible for the improved protein utilization is provided, but bacteria, including Aeromonads commonly found in the gut of tilapia are known to produce proteases (Nayak et al. 2010b). Gut microbes produce amino acids that are utilized by tilapia. Newsome et al. (Newsome et al. 2011) found that tilapia appeared to be able to acquire their essential amino acid requirements directly from the GI microbiota when dietary sources were insufficient. Volatile, short-chain fatty acids can be produced from anaerobic microbes in the gut of tilapia by fermentation of dietary carbohydrates (Kihara and Sakata 1997; Smith et al. 2005). The evidence shows that symbiotic gut microbes in tilapia contribute to nutrient production, and this contribution may be fairly substantial, especially when nutrient requirements are not being fully met by the diet. However, the role of probiotics in the synthesis and/or utilization of most vitamins, minerals, and macronutrients in finfish, including tilapia, are yet unexplored, and future research should emphasize their contribution to physiological maintenance, homeostasis, and growth.

Probiotics may improve digestion by stimulating production of digestive enzymes or through other alterations in the gut environment, which could translate to improved growth performance. Enzymes involved in digestion (carbohydrases, phosphatases, esterases, lipases, peptidases, cellulases, and proteases) are produced by gut microbes in fish, including some species commonly used as probiotics (Nayak et al. 2010b). Amylase, lipase, and protease production was enhanced in tilapia fed diets containing *Bacillus subtilis* and an unidentified "photosynthetic bacteria" (Honsheng 2010). The author attributes improved weight gain and feed efficiency to the increased enzyme production. Essa et al. (2010) also reported improved growth performance of Nile tilapia fed either *B. subtilis*, *Lactobacillus plantarum*, a mixture of *B. subtillis* and *L. plantarum*, or *S. cerevisiae*. Fish fed the bacterial probionts, alone or in mixture, showed improved activity of amylase, protease, and lipase enzymes in the GI tract. Tilapia fed the diet containing *S. cerevisiae* exhibited enhanced amylase but not protease and lipase activities. *Streptococcus faecium* + *Lactobacillus acidophilus* or *S. cerevisiae* supplementation produced significantly higher weight gain and feed utilization efficiency in tilapia fed diets containing 27% or 40% crude protein compared to the control diet (Lara-Flores et al. 2003 and 2010). Disaccharidase but not peptidase activity increased in tilapia fed the probiotic supplemented diets. Fish fed the diet containing *S. cerevisiae* exhibited the greatest increase in growth performance, which was attributed to significantly higher activity of

alkaline phosphatase. The authors suggest that the increase in alkaline phosphatase activity reflects the development of brush border membranes of enterocytes stimulated by the supplemented yeast. Activity of the brush border enzymes (Gawlicka et al. 2000) and alkaline phosphatase (German et al. 2004) can indicate increased nutrient uptake, especially carbohydrate and lipid.

Improved growth performance in tilapia fed probiotic diets has been reported by many researchers (Table 1). Tilapia fed *S. cerevisiae* (Lara-Flores et al. 2003 and 2010), *B. subtilis* + *S. cerevisiae* (Marzouk et al. 2008; Lara-Flores et al. 2003 and 2010), *Micrococcus luteus* (El-Rhman et al. 2009), *Bacillus subtilus*, *Lactobacillus plantarum*, *B. subtilis* + *L. plantarum*, (Essa et al. 2010), *Bacillus pumilus* (Aly et al. 2008a), *Lactobacillus acidophilus*, *Streptococcus faecium* (Lara-Flores et al. 2003 and 2010), the commercial probiotic mixtures Organic Green[®] (Aly et al. 2008b), Biogen[®] (Ghazalah et al. 2010; El-Haroun et al. 2006; Mehrim 2009), and Premalac[®] (Ghazalah et al. 2010) have all shown to increase growth performance in tilapia. However, other researchers report no effect of some dietary probiotics on growth. Non-viable *S. cerevisiae* (Marzouk et al. 2008), *Pseudomonas* spp. (El-Rhman et al. 2009), *Pediococcus acidilactici* (Ferguson et al. 2010), and *Enterococcus faecium* (Biomate SF-20[®]), *B. subtilis* + *B. licheniformis* (Bioplus 2B[®]), and *P. acidilactici* (Bactocell PA10 MD[®]), viable *S. cerevisiae* (Levucell SB 20[®]) (Shelby et al. 2006) have shown to not affect growth of tilapia. Although improved growth has been linked to the production of digestive enzymes stimulated by probionts as reported earlier, metabolite production and improved nutrient utilization may also be responsible for improved feed efficiency and growth performance in tilapia fed probiotics. In most cases, the mechanism for improved growth performance is not known or reported. It is difficult to draw concrete conclusions and provide specific recommendations on the effects of dietary probiotics on growth performance of tilapia given that the studies vary widely with regard to fish age and size, stocking density, diet composition, dietary probiont concentration, feed allowances, feeding duration, and of course, type and source of probiont. However, early studies suggest dietary probiotic supplementation may have beneficial effects when used as growth promoters in tilapia. Furthermore, use of probiotics may allow for the improved utilization of difficult to digest dietary ingredients and nutrients in some fish species. For example, in ruminant and non-ruminant mammals, use of a probiotic that produces high levels of cellulases allows for the increased use of high-cellulose carbohydrate sources (Ushakova et al. 2006). This approach may prove useful in fish to

overcome poor digestibility and anti-nutritional factors in plant-based alternative feed ingredients.

4. Immunity and Probiotics

There is ample evidence to suggest that probiotics supplemented individually or in combination can enhance systemic and local gut immunity and disease resistance in tilapia (Table 2). In most studies, there appear to be some positive effect on either immune function, disease resistance, or both. Variation between studies is likely due to differences in the choice of probiont, dietary concentration, species strain and age/size of fish, feeding management and duration, dosage and virulence of challenge pathogen, and method of challenge. Other factors, such as environmental conditions, handling practices, and stocking densities, may also affect results. All these factors can influence the success or failure of dietary probiotics to affect immunity and/or disease resistance in fish. Furthermore, Merrifield et al. (2010a) suggests the success or potential of probiotics in many studies to prevent disease may be greater than the results show due to the use of intraperitoneal (IP) method of disease challenge. The IP method bypasses one of the most important ways probiotics can prevent infection: competitive exclusion in the GI tract. Intraperitoneal challenges do not reflect the effect of probiotics on resistance to infection – they demonstrate the effect of probiotics on infected fish (Merrifield et al. 2010a). In studies where disease resistance is improved, this suggests that the probiotic may be providing immune stimulation outside the GI tract. This is an important point to highlight in the application of probiotics to boost immunity of tilapia, because the vast majority of challenges performed in tilapia research studies are done by IP injection. Streptococcal disease, caused predominantly by *Streptococcus iniae*, is the biggest disease problem in tilapia culture (Shoemaker et al. 2006); however, it is difficult to reproduce reliably by bath immersion, so researchers have had to rely on IP injection to produce reliable, consistent infection generating the desired mortality rate. A degree of caution should be taken when attempting to apply the results of studies employing IP injection for disease challenge. However, as will be noted, the immune response outside the GI tract is enhanced in many probiotic studies, and probiont colonization of the gut may not be required to prevent infection.

a. Immune Function Enhancement

A large number of probiotic studies have been conducted in fish examining immune function and disease resistance, but their effects on immunity are hard to elucidate (Merrifield 2010a). Certainly, it has been shown that one of the means by which probiotic microorganisms can boost immunity is by antagonistic colonization of the GI tract. However, the effect of the gut microbiota, and therefore probiont colonization, on the immune system is more far-reaching and complex than simple physical displacement of pathogenic microbes in the gut. Along with providing a physical barrier, the epithelium and gut-associated lymphoid tissue (GALT) interact with gut microbes to develop a complex immune response, involving the production of cytokines, chemokines, and different effector and regulatory T-cells (Sanz and De Palma 2009). In mammals, the gut microbiota also regulates immune gene expression of goblet cells, modifies glycosylation patterns (potentially affecting bacterial adhesion) (Caballero-Franco et al. 2007), induces secretion of antimicrobial peptides (Vaishnava et al. 2008), and ensures proper maturation of the GALT (Sanz and De Palma 2009). Although the level of GALT organization in fish is lower than in mammals, the GI microbiota is involved in epithelial differentiation and maturation in gnotobiotic studies in fish (Nayak 2010b) and likely helps in GALT development. Nayak (2010a) also points out that probiotics interact with fish GALT to induce the immune response, increasing Ig+ cells, acidophilic granulocytes, and T-cell counts. Few studies in fish have focused on the effects of probiotic supplementation on gut immunity, and in tilapia, there are far fewer. Pirarat et al. (in press) found an increase in the number of mucous cells in the distal portion of the intestine and a greater abundance of intraepithelial lymphocytes and acidophilic granulocytes in Nile tilapia fed diets containing *Lactobacillus rhamnosus* GG. No disease challenge was performed in the study. Although Ferguson et al. (2010) did not find any changes in the number of leucocytes infiltrating the intestinal epithelium, blood leucocyte numbers and serum lysozyme activity were enhanced in Nile tilapia given the probiotic *Pediococcus acidilactici*.

Probiotic supplementation also improves the systemic immune response in fish. The process and mechanism in which probiotics in the gut stimulate the systemic response are not completely understood in mammals and are clearly undefined in fish. In mammals, the general consensus is that cells in the GALT process and recognize antigens via pattern recognition receptors, such as toll-like receptors (TLRs) (0).

Table 1. Summary of studies examining the effects of dietary probiotic supplementation on growth in tilapia

Probiotic	Origin	Presentation form[1]	Dose	Feeding duration (weeks)	Hematological parameters measured	Effect on growth performance[2]	Notes	References
Bacillus licheniformis + B. subtillus (Biogen)®	Commercial	Mixture	0, 5, 15, 20, 25 g/kg	16	No	BWG (↑), SGR (↑), FCR (↓), FI (↔), PER (↑), ER (↑)	Biogen also contains enzymes, ginseng, and selenium.	El-Haroun et al. 2006
B. licheniformis + B. subtillus (Biogen)®	Commercial	Mixture	3 g/kg	14	Yes	BWG (↑↔), SGR (↑↔), FCR (↑↔), survival (↔)	The effects of Biogen were determined at 6 different stocking densities. See Reference 56 for composition of Biogen.	Mehrim 2009
B. licheniformis + B. subtilis (Bioplus 2B)® , *Enterococcus faecium* (Biomate SF-20)®, *P. acidilactici* (Bactocell PA10 MD)®, *Saccharomyces cerevisiae* (Levucell SB 20)®	Commercial	Individual	3.6×10^9 to 8.5×10^{10} CFU/g	65 or 83 days	No	BWG (↓↔), survival (↑↔)	No effects seen after 39 days feeding, but BWG declined or was unaffected and survival increased or was unaffected after 63 days feeding.	Shelby et al. 2006
B. licheniformis + B. subtillus (Biogen)®, *Bifedobacterium bifedum, Lactobacillus acidophilus, Streptococcus faecium* (Premalac)®	Commercial (Premalac & Biogen)	Mixture	1, 2, & 3 g/kg	28	Yes	BWG (↓↑), SGR (↔), FCR (↔), PER (↔), AD (↓↔)	These probiotic supplements contain other products. Premalac (torula, skim milk, yeast, calcium carbonate, *Aspergillus* extract); Biogen (see Reference 56).	Ali et al. 2010

Probiotic	Origin	Presentation form[1]	Dose	Feeding duration (weeks)	Hematological parameters measured	Effect on growth performance[2]	Notes	References
B. licheniformis +B. subtillus,(Biogen)®, B. bifedum, L. acidophilus, S. faecium (Premalac)®	Commercial (Premalac & Biogen)	Mixture	2 g/kg	16	No	BWG (↑), FCR (↓), PER (↑), AD (↑), FI (↔)	See references 56 & 75 for description of Premalac and Biogen. The probiotics were tested at 25, 27.5, and 30% dietary protein.	Ghazalah et al. 2010
Bacillus pumilus and Organic Green	*O. niloticus* and commercial probiotic mixture	Individual	10^6, 10^{12} CFU/g; 1, 2 g/kg	4, 8, 32	Yes	BWG (↑↔)	Organic Green is a mixture of *L. acidophilus, B. subtilis, S. cerevisae,* and *Aspergillus oryzae*. Probiotic diets fed for 1 mon & then continued on the same diet or switched to the basal diet for 1 mon. Another group was fed the probiotic diets for 2 mon & then basal diet for an additional 6 mon.	Aly et al. 2008a
B. subtillus+ S. cerevisiae (Megalo)®, S. cerevisiae (Diamond V)®	Commercial	Individual & mixture; viable & inactivated cells	1.5, 10 g/kg	6	No	BWG (↑), SGR (↔), FCR (↔), PER (↔), CF (↑)	*S. cerevisiae* was fed as inactivated cells (Diamond V) and as live cells in combination with *B. subtilis* (Megalo). *B. subtilis* was not supplemented alone.	Marzouk et al. 2008

Table 1. (Continued)

Probiotic	Origin	Presentation form[1]	Dose	Feeding duration (weeks)	Hematological parameters measured	Effect on growth performance[2]	Notes	References
B. subtillus, L. acidophilus	Commercial	Individual	0.5×10^7, 1×10^7 CFU/g	8	Yes	BWG ($\uparrow\leftrightarrow$), survival ($\downarrow$)	Growth and survival were measured after 1 and 2 months feeding.	Aly et al. 2008b
B. subtilis, Lactobacillus plantarum, S. cerevisiae	*O. niloticus*	Individual & combination	10^4 and 10^7 CFU/g	2	No	BWG ($\uparrow$), SGR ($\uparrow$), FCR ($\downarrow$), survival ($\leftrightarrow$), PER ($\uparrow$), FI ($\uparrow$), ER ($\uparrow$)	*B. subtilis* and *L. plantarum* were supplemented individually and in a mixture, while *S. cerevisiae* was added to diets alone.	Essa et al. 2010
L. acidophilus, S. cerevisiae, S. faecium	Human	Individual & combination	1 g/kg	9	No	BWG ($\uparrow\leftrightarrow$), SGR ($\uparrow$), FCR ($\downarrow\leftrightarrow$), survival ($\uparrow$), PER ($\uparrow\leftrightarrow$), ANU ($\uparrow\leftrightarrow$), APD ($\downarrow\leftrightarrow$)	*S. faecium* and *L. acidophilus* were supplemented as a mixture, and *S. cerevisiae* was supplemented alone. Fish stocked at 2 densities with 2 dietary protein levels.	Lara-Flores et al. 2003, 2010[3]
Lactococcus lactis	Commercial (isolated from milk)	Water additive	10^7 CFU/ml	6	No	BWG ($\uparrow$), survival ($\leftrightarrow$)	Probiotic added to rearing water.	Zhou et al. 2010
Lactobacillus plantarum	*Anguilla anguilla*	Individual	6×10^5, 6.3×10^8 CFU/g	8 days	No	SGR ($\leftrightarrow$), FCR ($\leftrightarrow$), FI ($\leftrightarrow$)		Galindo et al. 2009
L. plantarum	*O. niloticus*	Individual	10^8 CFU/g	12	Yes	BWG ($\uparrow$), FER ($\uparrow$), survival ($\leftrightarrow$)		Jatoba et al. 2011

Table 1. (Continued)

Probiotic	Origin	Presentation form[1]	Dose	Feeding duration (weeks)	Hematological parameters measured	Effect on growth performance[2]	Notes	References
Lactobacillus rhamnosus	Human	Individual	10^{10} CFU/g	4	Yes	BWG ($\leftrightarrow$), SGR ($\leftrightarrow$), FCR ($\leftrightarrow$)	No effect on growth performance was seen at 14 or 30 days.	Pirarat et al. 2011
Lactobacillus spp.	*O. mossambicus*	Mixture	10^6 CFU/g	25 days	No	Not specified.	Authors state that probiotic groups had significantly better growth & survival compared to the control group.	Vijayabaskar et al. 2008
Micrococcus luteus, Pseudomonas spp.	*Oreochromis niloticus*	Individual & combination	10^7 CFU/g	12	Yes	BWG ($\downarrow\leftrightarrow$), SGR ($\downarrow\leftrightarrow$), FCR ($\downarrow\leftrightarrow$), survival($\downarrow\leftrightarrow$), PER ($\downarrow\leftrightarrow$), FI ($\downarrow\leftrightarrow$)		El-Rhman et al. 2009
Pediococcus acidilactici	Commercial	Individual	10^7 CFU/g	4	Yes	BWG ($\leftrightarrow$), SGR ($\leftrightarrow$), FCR ($\leftrightarrow$), survival ($\uparrow$), PER ($\leftrightarrow$)		Ferguson et al. 2010
S. cerevisiae	Commercial (Super Biobuds)	Individual	1 & 2 g/kg	8	Yes	BWG ($\uparrow$), SGR ($\leftrightarrow$), CF ($\uparrow$), survival ($\uparrow$)		El-Asharam et al. 2008

[1] Probiotic cells were viable unless otherwise stated.

[2] BWG = body weight gain, SGR = specific growth rate, FCR - feed conversion ratio, PER = protein efficiency ratio, FI - feed intake, AD = apparent digestibility, ER = energy retention, CF = condition factor, ANU = apparent nitrogen utilization, APD = apparent protein digestibility.

[3] The same research data was published in two different scientific journals.

Table 2. Summary of studies examining the effects of dietary probiotics on immune function and disease resistance in tilapia

Probiotic	Origin	Presentation form[1]	Dose	Feeding duration (weeks)	Immunological effects[2]	Challenge pathogen (s)[3]	Reduced mortalities	Notes	References
Bacillus firmus, Bacillus pumulis, Citrobacter freundii	*O. niloticus*	Individual & combination	10^7 CFU/g	2	None measured.	*Aeromonas hydrophila*	Yes	All probiotic treatments provided enhanced survival to *A. hydrophila*	Aly et al. 2008c
Bacillus licheniformis + Bacillus subtillus (Biogen)®, Bifedobacterium bifedum, Lactobacillus acidophilus, Streptococcus faecium (Premalac)®	Commercial (Premalac & Biogen)	Mixture	1, 2, & 3 g/kg	28	Differential white blood cell counts ($\uparrow\downarrow$)	No challenge	ND	See references 56 & 75 in Table 1 for description of Premalac and Biogen.	Ali et al. 2010
B. licheniformis + B. subtillus (Biogen)®	Commercial	Mixture	3 g/kg	14	White blood cell counts ($\leftrightarrow$), serum albumin ($\leftrightarrow\uparrow$), serum globulin ($\leftrightarrow\uparrow$)	No challenge	ND	The effects of Biogen were determined at 6 different stocking densities.	Mehrim 2009
B. licheniformis + B. subtilis (Bioplus 2B)®, *Enterococcus faecium* (Biomate SF-20)®, *Pediococcus acidilactici* (Bactocell PA10 MD)®, *Saccharomyces cerevisiae* (Levucell SB 20)®	Commercial	Individual	3.6×10^9 to 8.5×10^{10} CFU/g	65 or 83 days	Lysozyme activity, total immunoglobulin, alternative complement, anti-*S. iniae* antibody levels ($\leftrightarrow$)	*S. iniae*	No	No effects were seen on immune function or survival to *S. iniae*.	Shelby et al. 2006

Probiotic	Origin	Presentation form[1]	Dose	Feeding duration (weeks)	Immunological effects[2]	Challenge pathogen (s)[3]	Reduced mortalities	Notes	References
Bacillus pumilus and Organic Green®	*O. niloticus* and commercial probiotic mixture	Individual	10^6, 10^{12} CFU/g; 1, 2 g/kg	4, 8, 32	White blood cell counts ($\leftrightarrow \downarrow\uparrow$), neutrophil respiratory burst ($\leftrightarrow \downarrow\uparrow$)	*A. hydrophila*	Yes	See reference 54 in Table 1 for Organic Green and feeding regimen.	Aly et al. 2008a
B. subtilis, Clostridium butyricum, L. acidophilus, S. cerevisiae	Commercial	Mixture; viable & inactivated cells; feed & water	10 g/kg	4	Lysozyme activity ($\uparrow\downarrow$), neutrophil migration ($\leftrightarrow$), protease activity($\uparrow$), plasma bactericidal activity ($\uparrow$), neutrophil respiratory burst ($\leftrightarrow$)	*Edwardsiella tarda*	Yes	Reduced mortality & increase in lysozyme and protease only with viable cells.	Taoka et al. 2006
B. subtillus, L. acidophilus	Commercial	Individual	0.5x 10^7, 1x10^7 CFU/g	8	neutrophil respiratory burst ($\uparrow$)			1 and 2 months feeding.	
B. subtillus	Human	Individual; feed & water	0.1 g/L (water); 0.2 g/kg (diet)	8	None measured.	*F. columnare*	Not reported	Fish were treated prior to challenge or after signs of infection. Effects on fish survival were not reported. Clinical signs of disease were alleviated by probiotics.	Mohamed & Refat 2011
Lactobacillus plantarum	*O. niloticus*	Individual	10^8 CFU/g	12	Blood leucocytes and lymphocytes ($\uparrow$), netrophils and monocytes ($\leftrightarrow$)	No challenge	ND		Jatoba et al. 2011

Table 2. (Continued)

Probiotic	Origin	Presentation form[1]	Dose	Feeding duration (weeks)	Immunological effects[2]	Challenge pathogen (s)[3]	Reduced mortalities	Notes	References
Lactobacillus rhamnosus	Human	Individual	10^{10} CFU/g	4	Leucocyte phagocytosis (↔), leucocyte resp. burst (↔), lysozyme activity (↓), serum bactericidal activity (↓), alternative complement (↑), head kidney IL-1 (↑), splene IL-1 (↔), head kidney TNF-alpha (↑), splene TNF-alpha (↔)	No challenge	ND	IL-1 and TNF-alpha measurement is relative gene expression.	Pirarat et al. 2011
L. rhamnosus	Human	Individual	$10^{8}, 10^{10}$ CFU/g	2	Respiratory burst of leucocytes (↔ ↓), lysozyme activity (↔ ↓), alternative complement (↑)	*E. tarda*	Yes	Lowest mortality was in the highest level probiotic group.	Pirarat et al. 2006
Lactobacillus spp.	*O. mossambicus*	Mixture	10^{6} CFU/g	25 days	None measured.	*A. hydrophila*	Yes	Fish were challenged by immersion.	Vijayabaskar et al. 2008
Lactococcus lactis	Commercial (isolated from milk)	Water additive	10^{7} CFU/ml	6	Neutrophil respiratory burst (↑), myeloperoxidase activity (↑), superoxide dismutase (↑), lysozyme acitivy (↑), *in vitro* A. hydrophila inhibition (↑)	No challenge	ND	Probiotic added to rearing water.	Zhou et al. 2011
Micrococcus luteus, Pseudomonas spp.	*Oreochromis niloticus*	Individual & combination	10^{7} CFU/g	12	*In vitro* antimicrobial activity (↑)	*A. hydrophila*	Yes	Reduced mortality with *M. luteus* only.	El-Rhman et al. 2009

Probiotic	Origin	Presentation form[1]	Dose	Feeding duration (weeks)	Immunological effects[2]	Challenge pathogen (s)[3]	Reduced mortalities	Notes	References
P. acidilactici	Commercial	Individual	10^7 CFU/g	4	Gut leucocytes (↔), blood leucocytes (↑), serum lysozyme activity (↑)	No challenge	ND[4]	Improved immunity seen after 14 days feeding.	Ferguson et al. 2010
S. cerevisiae	Commercial (Super Biobuds)	Individual	1 & 2 g/kg	8	Neutrophil respiratory burst (↔)	*A. hydrophila, Pseudomonas fluorescens*	Yes	Mortality from both pathogens was reduced at 1 & 2 g/kg.	El-Ashram et al. 2008

[1] Probiotic added to diets, and cells were viable unless otherwise stated.

[2] Immune parameters were increased (↑), decreased (↓), or unchanged (↔) relative to the control.

[3] Tilapia were challenged by intraperitoneal injection unless otherwise noted.

[4] ND = Not determined.

The TLRs activate immune signaling pathways leading to the production of cytokines and other chemical signals which recruit immune cells (T-cells, natural killer or NK cells, neutrophils, etc.) and affect other immune functions. In tilapia, dietary supplementation of *L. rhamnosus* GG causes an increase in serum complement activity and enhanced phagocytosis and killing ability of head kidney leukocytes (Pirarat et al. 2011). The authors suggest that *L. rhamnosus* GG in the gut affected these peripheral immune responses through an increase in the expression of tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1). A number of other systemic, non-specific immune functions have been shown to be enhanced by dietary probiotic supplementation, including lysozyme activity, peripheral blood immune cell counts, alternative complement activity, phagocytic ability of leucocytes, neutrophil migration and adherence, plasma bactericidal activity, respiratory burst, myeloperoxidase, and superoxide dismutase activities, and others (Table 2). However, not all studies result in enhanced immune function. Shelby et al. (2006) did not find any affect on lysozyme activity, alternative complement, or total serum immunoglobulin in tilapia fed commercial probiotics containing *B. subtilis* + *B. licheniformis P. acidilactici*, and *S. cerevisiae*. There is far less evidence available suggesting that dietary probiotics influence the humoral immune response in tilapia. Shelby et al. (2006) did not find an effect of dietary probiotic supplementation on the antibody response to *S. iniae*. Probiotic use can enhance the immune response of tilapia, and this has been linked to improved disease resistance.

b. Disease Prevention

Although it is important to understand how immune function is affected by probiotic supplementation, tilapia culturists are likely more interested in whether probiotics will prevent disease in fish. The success of probiotic use on disease prevention in fish has been mixed, but overall appears to be fairly effective in tilapia using a variety of probionts against a number of different bacterial pathogens (Table 2). *Streptococcus iniae*, *Aeromonas hydrophila*, and *E. tarda* are the primary bacterial pathogens that have been evaluated in tilapia probiotic studies. All but one study examining the effects of probiotics on disease resistance of tilapia used challenge by IP injection, which as outlined above, bypasses the gut and integument, and may not fully evaluate the potential of probiotics to prevent disease in tilapia. The effectiveness of probiotics in terms of protection against infection is often attributed to enhanced immunity. Pirarat et al. (2006) has suggested that *L. rhamnosus* GG protection against *E. tarda* is accomplished by enhancing the alternative

complement system thereby increasing phagocytic cell aggregation and phagocytic activity. As noted previously, the interaction of probiotics and the immune system is complex and identifying the exact immune mechanism(s) providing protection would be a difficult task. Probiotics can also be ineffective in preventing disease in tilapia. Shelby et al. (2006) found that feeding commercial probiotics for 94 days did not prevent streptococcal disease. Probiotics have proven effective in preventing viral diseases in several fish species. Resistance of grouper (*Epinephelus coiodes*) to iridovirus is enhanced with supplementation of *Lactobacillus plantarum* in diet (Son et al. 2009), and *Pseudomonas* sp., *Vibrio* sp., *Aeromonas* sp., and groups of coryneforms show antiviral activity to infectious hematopoietic necrosis virus (IHNV) (Kamei et al. 1988). Probiotic bacteria have also exhibited antiviral activity against poliovirus (Girones et al. 1989) and *Oncorhynchus masou* virus (OMV) (Direkbusarakom et al. 1998) in fish. However, to our knowledge, the effect of probiotics has not been evaluated on any viral disease in tilapia.

Several factors affect the efficacy of probiotics on disease prevention in fish, especially the type of probiont and dietary dose concentration (dietary concentration + feeding duration). In tilapia, short-term feeding (2 weeks) and long-term (2 months or greater) have all proven to be effective in enhancing disease resistance in tilapia (Table 2).

Few tilapia studies have explored the effects of dose concentration, although several have examined the effect of dietary concentration and feeding duration separately. Published information on immunostimulants suggests that, the larger the dose concentration, the less effective immunostimulants are in protecting fish against infection and can even result in immune suppression (Sakai 1999; Merrifield et al. 2010a). Little data exist to confirm whether this phenomenon also occurs with probiotic supplementation in fish diets, and no studies are known to have investigated the effects of pulsed-feeding (short-term, alternating feeding intervals of basal and probiotic diets) in tilapia, a method commonly used to prevent immune-suppression from excessive dose concentration when feeding immunostimulants to fish (Merrifield et al. 2010a). Aly et al. (2008a) found that supplementing *Bacillus pumilus* at 10^{12}/g diet increased protection of Nile tilapia against *A. hydrophila* after 1 and 2 but not 8 months of feeding. The lower dietary concentration tested (10^6/g) did not provide any protection. In another study, Aly et al. (2008b) also found that dietary supplementation of *L. acidophilus*, *B. subtilis*, or a mixture of the two generally provided greater protection against *A. hydrophila*, *P. fluorescens*, and *S. iniae* after 2 months of feeding compared to 1 month. The form of

probiotic administration can also impact effectiveness in affecting fish health. Improving viability of probionts during the feed making process and during feed storage can be improved by encapsulation in non-nutritive matrices, such as calcium alginate.

Encapsulation of *Shewanella putrefaciens* in calcium alginate improved viability of the bacterium during feed storage, and its presence was found in the gastrointestinal track of Sengalese sole (*Solea senegalensis*) fed encapsulated but not non-encapsulated of *S. putrefaciens* (Rosas-Ledesma et al. 2011). Furthermore, the rout of probiotic administration can also affect the success of probiotic application. Addition of *Lactococcus lactis* RQ516 to rearing water increases the resistance of Nile tilapia to *A. hydrophila* (Zhou et al. 2010). Further research on the effects of dose dependency and form and route of probiont administration on disease resistance are needed for all fish species, including tilapia, in order to provide effective feeding and treatment regimens.

The combination of probiotics and prebiotics, also referred to as synbiotics, has shown promise in treating diseases in humans (Haskey et al. 2006) and other mammals (Zimmerman et al. 2001). The prebiotic is thought to give a probiont a competitive advantage by providing a fermentable energy source enabling it to out-compete endogenous microbial populations (Merrifield et al. 2010a; Gibson and Roberfroid 1995).

The survival rate of probiotics is improved during their passage through the digestive tract, thereby contributing to the stabilization and/or enhancement of the probiotic effects. Few studies have examined the effects of a synbiotic approach on the health of fish. Japanese flounder fed a diet containing *Bacillus clausii* or in combination with prebiotics fructo- or mannan oligosaccharide showed improved non-specific immune function (Ye et al. 2011).

Although the diet containing either prebiotic with *B. clausii* exhibited the highest immune function, activity was not significantly different compared to flounder fed *B. clausii* alone. No disease challenge was conducted in the study. Feeding a synbiotic combination of mannan oligosaccharide and *Enterococcus faecalis* improved survival of rainbow trout challenged with *V. anguillarum* than trout fed the individual prebiotic or probiotic (Rodriguez-Estrada et al. 2009). Given the success observed with synbiotic approaches in mammals, a high priority should be given in future research in tilapia and other fish species.

PREBIOTICS

1. Types of Prebiotics

Prebiotics were first defined as a nondigestible food ingredient that selectively stimulated the growth and/or activity of one or a limited number of bacteria in the colon, thereby improving host health (Gibson and Roberfroid 1995). Prebiotics have traditionally been non-digestible carbohydrates and can be classified by their molecular size and degree of polymerization into monosaccharides, oligosaccharides, and polysaccharides (including dietary fiber) (Ringø et al. 2010). However, not all dietary carbohydrates are prebiotics, and Roberfroid et al. (2007) suggests that prebiotics must meet certain criteria to qualify as prebiotics. They must be: 1) resistant to gastric acidity and enzymatic hydrolysis during digestion, 2) easily fermented by intestinal microflora, and 3) contribute to the health and well-being of an organism by selectively stimulating the growth and activity of beneficial bacteria. This narrow definition for human application has limited the number of substances classified as prebiotics to the oligosaccharides inulin, transgalactooligosaccharide, and lactulose (Gibson et al. 2004) with the addition of fructooligosaccharide by Roberfroid (2007). For practical application in terrestrial and aquatic animals, meeting criterion 2 is not critical, if the prebiotic substance improves the health and well-being of the organism by stimulating growth of beneficial bacteria in the colon. Ringø et al. (2010) includes mannanoligosaccharide, galactooligosaccharides, xylooligo-saccharides, arabinoxylooligosaccharides, isomaltooligosaccharides, and the commercially available GroBiotic-A as prebiotics, which have been examined in fish and will also be considered as such here.

Probiotics are live organisms, and reduced viability in feed during processing and storage and leaching from feed in water can reduce their effectiveness (Merrifield et al. 2010a). As a response, the use of prebiotic substrates to selectively stimulate the growth and activity of probiotic bacteria, such as *Lactobacillus* and *Bifidobacterum*, may improve the probability of establishing these health-promoting bacteria in the GI tract. Thus, the primary benefit of probiotic and prebiotic use is to selectively establish beneficial bacteria at the expense of potentially pathogenic bacteria, such as *Salmonella*, *Listeria*, and *E. coli* (Ringø et al. 2010). Prebiotic stimulated bacteria are considered beneficial to the host by decreasing the presence of intestinal pathogens and/or altering the production of health related bacterial metabolites (Roberfroid 1993; Gibson and Roberfroid 1995; Gibson 1998; Manning and

Gibson 2004; Ringø et al. 2010). Although, alterations in the villi structure and organization in the small intestine can also lead to improvements in growth performance and immunity (Yilmaz et al. 2007). Progress in the research and application of dietary prebiotics has been limited in fish compared to human and terrestrial animals. Comparatively, research on the effects of prebiotics on health of tilapia is even further limited (Table 3) and more investigation is needed to determine if and which prebiotics may prove effective in improving the health of tilapia.

a. Mannanoligosaccharides

Mannanoligosaccharides (MOS) are glucomannoprotein complexes extracted from the cell wall of *Saccharomyces cerevisiae* (Sohn et al. 2000). The mannose receptor is an endocytic receptor of macrophages and endothelial cells whose natural ligands include both 'self' glycoproteins and microbial glycans (Ringø et al. 2010). Immature cultured dendritic cells also express the mannose receptor whereby it mediates efficient uptake of glycosylated antigens (Linehan et al. 2000; Ringø et al. 2010). Mannanoligosaccharides can therefore directly influence the immune system by inducing intracellular signaling and increasing production of inflammatory cytokines, which may benefit fish health, (Ringø et al. 2010). However, dietary addition of MOS can also benefit host fish by improving the gut microenvironment (primarily by improvement in villi structure and organization) (Salze et al. 2008; Yilmaz et al. 2007) and altering the gut microbiota in fish (Ringø et al. 2006; Bakke-McKellep et al. 2007; Dimitroglou et al. 2009). Extensive research on the dietary application of MOS has been conducted in terrestrial animals (Benites et al. 2008; Klebaniuk et al. 2008; Yang et al. 2009). In recent years, the effects of MOS as a prebiotic in diets of fish has received increased attention, especially in salmonids (Merrifield et al. 2010a; Ringø et al. 2010). A number of studies have also been conducted with tilapia (Table 3).

Dietary supplementation of MOS in diets of tilapia has produced mixed results on physiology and health. Genc et al. (2007) supplemented MOS in diet of juvenile hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) at 0, 1.5, 3.0, and 4.5 g/kg for 80 days. The authors found that whole body (WB) dry matter and protein content increased with increasing dietary MOS concentration. The authors suggest improved protein utilization may be linked to enhance amino acid utilization from alterations in the intestinal microflora by MOS.

Table 3. Summary of studies examining the effects[1] of dietary prebiotics on growth performance, immune function, and disease resistance in tilapia

Prebiotic	Tilapia Species	Dose[2]	Feeding duration	Effect on growth performance[3]	Other physiological effects	Immunological effects	Challenge pathogen(s)	Reduced mortality	Notes	References
MOS	Hybrid	0, 1.5, 3, & 4.5 g/kg	80 days	BWG, SGR, FCR, PER (↔)	Villi length (↑), HSI (↔), VSI (↔)	None measured	No challenge	ND[5]	9.8 g initial weight	Genc et al. 2007
	Hybrid	0, 2, & 6 g/kg	58 days	SGR, FCR (↔); SRV (↑)	None	Lysozyme activity, alternative complement (↑)	No challenge	ND	8.1 g initial weight	He et al. 2003
	Nile tilapia	0, 2, 4, 6, 8, & 10 g/kg	45 days	BWG (↔ ↓), FI (↓), SGR, FCR, SRV (↔)	None	White blood cell counts, red blood cell count, HCT, hemoglobin indices (↔)	No challenge	ND	13.7 g initial weight	Sado et al. 2008
	Nile tilapia	0, 2, 4, & 6 g/kg	3 weeks	BWG, SGR (↑), FCR(↓), SRV (↔)	None	None measured	*S. agalactiae*	Yes	All-male, sex reversed fry; 0.013 g initial weight	Samrongpan et al. 2008
	Nile tilapia	0 & 1 g/kg	90 days	BWG, FI, SGR, FCR, PER, SRV (↔), CF (↓), BL (↑)	Villi height (↑), villi epithelium thickness (↓); GI enzyme activity: proteolytic, amylase (↔), lipase (↑)	White blood cell counts (↑↓), red blood cell count, HCT, hemoglobin indices (↔), serum protein (↑)	No challenge	ND	All-male, sex reversed; 24 g initial weight	Liranço et al. 2013

Table 3. (Continued)

Prebiotic	Tilapia Species	Dose[2]	Feeding duration	Effect on growth performance[3]	Other physiological effects	Immunological effects	Challenge pathogen(s)[4]	Reduced mortality	Notes	References
	Nile tilapia	0, 1.5, 3.0, 4.5, 6.0, & 7.5 g/kg	30 days	BWG, SGR, SRV, CF, BL ($\leftrightarrow$), FCR ($\uparrow$)	Intestinal length, villi height, villi density ($\uparrow$)	None measured	No challenge	ND	All-male, sex reversed fry; 0.01 g initial weight	Schwarz et al. 2011
Inulin	Blue tilapia	10 mg/kg BW	IP injection	Not measured	None	None measured	*A. hydrophila, Edwardsilla tarda*	No	21.8 g initial weight; 2 IP injections of inulin given over 2 days	Wang & Wang 1997
	Red tilapia	50 g/kg	60 days	BWG, SGR ($\leftrightarrow$), FCR ($\downarrow$)	None	White blood cell counts ($\uparrow$), red blood cell count, HCT ($\leftrightarrow$)	*S. iniae*	No	All-male, sex reversed; 3.07 g initial weight	Plongbunjong et al. 2011
	Nile tilapia	5 g/kg	2 months	BWG, SGR, SRV ($\uparrow$), FCR ($\downarrow$)	None	HCT ($\leftrightarrow$), NBT, lysozyme activity ($\uparrow$)	*A. hydrophila* by IP injection	No	11 g initial weight; Sampled after 1 & 2 months feeding	Ibrahem et al. 2010
	Nile tilapia	2.5 g/kg inulin + 2.5 g/kg fulvic acid plus lactic acid bacteria (see Notes)	4	SGR ($\uparrow$) with 2.5×10^4 LAB/g diet	None	None	No challenge	ND	All-male, sex reversed; 13.0 g initial weight; diets were supple-mented with *Pediococcus sp. + P. pentosaceus* at 5×10^4, 2.5×10^5, or 5×10^5 CFU/g diet	Cota-Gastélum et al. 2013

Prebiotic	Tilapia Species	Dose[2]	Feeding duration	Effect on growth performance[3]	Other physiological effects	Immunological effects	Challenge pathogen(s)[4]	Reduced mortality	Notes	References
FOS & ScFOS	Hybrid	0, 2, & 6 g FOS/kg	58 days	SGR, FCR (↔); SRV (↑)	None	Lysozyme activity, alternative complement (↑)	No challenge	ND	57 g initial weight	He et al. 2003
	Hybrid	0, 0.8, & 1.2 g ScFOS/kg	8 weeks	BWG, SGR, FCR (↑), SRV, FI, CF (↔)	HSI (↔)	None measured.	No challenge; intestinal counts of *V. parahaemolyticus, A. hydrophila, Lactobacillus sp., S. faecalis* (↑) non-significantly	ND	5.6 g initial weight	Hui-Yuan et al. 2007
	Hybrid	1 g ScFOS/kg	56 days	Not measured	None	Not measured	No challenge; intestinal *Thiothrix eikelboomi, Clostridium spp., Cyanobacterium spp.*, and other uncultured bacterium clones (↑)	ND	1.24 g initial weight	Zhou et al. 2009
GOS	Red tilapia	50 g/kg	60 days	BWG, SGR (↑), FCR (↓)	None	White blood cell counts (↑), red blood cell count, HCT (↔)	*S. iniae*	No	All-male, sex reversed; 3.07 g initial weight	Plongbunjong et al. 2011
IMO	Nile tilapia	0 & 3 g/kg	16 weeks	BWG, SGR, FCR (↑); ADM, APD (↔)	Intestinal perimeter ratios (↔)	None	No challenge	ND	7.6 g initial weight	Ibrahim et al. 2013

Table 3. (Continued)

Prebiotic	Tilapia Species	Dose[2]	Feeding duration	Effect on growth performance[3]	Other physiological effects	Immunological effects	Challenge pathogen(s)[4]	Reduced mortality	Notes	References
DVAQUA	Hybrid	0, 0.125, 0.25, 0.5, 1.0, & 2.0 g/kg	8 weeks	BWG, SGR, FCR, FI ($\leftrightarrow$)	None	Serum lysozyme, alternative complement, head kidney macrophage activity & respiratory burst ($\uparrow$)	No challenge; Intestinal LAB ($\uparrow$) and several potential pathogenic bacteria ($\downarrow$)	ND	50.9 g initial weight	He et al. 2009
Gro Biotic-A	Nile tilapia	0, 10, & 20 g/kg	12 weeks	BWG, FI, SRV, FCR ($\leftrightarrow$)	None	Serum lysozyme, alternative complement, total protein, lysozyme activity, total Ig, Ab titer to *S. iniae* ($\leftrightarrow$)	*S. iniae*	Total mortality ($\leftrightarrow$), cessation of mortality ($\downarrow$)	13.4 g initial weight	Vechklang et al. 2012

[1]Measured parameters were increased ($\uparrow$), decreased ($\downarrow$), or unchanged ($\leftrightarrow$) relative to the control.

[2] Prebiotic added to diets unless otherwise stated.

[3] BWG = body weight gain, SGR = specific growth rate, FCR = feed conversion ratio, PER = protein efficiency ratio, FI = feed intake, CF = condition factor, ADM = apparent dry matter digestibility, APD = apparent protein digestibility; SRV = survival; HSI = hepatosomatic index; VSI = viscerosomatic index; BL = body length.

[4] Tilapia were challenged by intraperitoneal injection unless otherwise noted.

[5] ND = Not determined.

Mean intestinal villi length was significantly longer in fish fed 1.5 g/kg compared to 4.5 g/kg MOS, but villi length in tilapia fed the MOS supplemented diets was not different from the control. MOS did not have any effect on growth performance or hepatosomatic and viscerosomatic indices. Even though intestinal villi structure and organization was not correlated with growth performance in this study, improvements in gut micro structure have been linked to improved growth and nutrient utilization in animals (Bar et al. 2012; Ganguly et al. 2013), and the effect of MOS on this phenomenon should be explored further. He et al. (2003) also found that MOS supplementation in diets of hybrid tilapia did not improve growth performance. However, tilapia fed 0.6% MOS exhibited improved survival and enhanced lysozyme and ACH50 activities. Juvenile Nile tilapia fed MOS at 0.2, 0.4, 0.6, 0.8, or 1.0% of diet for 45 days did not show improvements in growth performance parameters compared to the control, but weight gain and FCR were significantly lower in tilapia fed 0.8% MOS compared to 0.4% MOS and the control diet, respectively (Sado et al. 2008). The reduced growth appeared to be related to a reduction in feed consumption as dietary MOS increased. Hematological parameters were also unaffected by MOS supplementation in this study. Juvenile Nile tilapia reared in cages for 90 days showed significant improvements in weight gain and body length during the final 30 days of the study when fed 0.1% dietary MOS (Liranço et al. 2013). This improvement in growth appears to coincide with an increase in the length and thickness of intestinal villi during the same period. Hematological values were unaffected, but neutrophil and monocyte counts declined while lymphocyte counts increased with dietary MOS supplementation. Dietary supplementation of MOS in diets of tilapia fry during sex reversal by methyl testosterone treatment has also been investigated. Tilapia fry are highly susceptible to disease during this process. Schwarz et al. (2011) found that Nile tilapia larvae fed dietary MOS at 0, 0.15, 0.30, 0.45, 0.60, or 0.75% of diet exhibited a linear increase in intestinal length, intestinal villous height, and villi density as MOS increased in the diet during the 30 day sex reversal process. A quadratic effect by MOS was observed on FCR, and quadratic modeling predicted 0.34% to be the optimal dietary MOS concentration to improve feed conversion and the height and density of intestinal villi of Nile tilapia fry. Other measures of growth performance were unaffected by MOS addition to the diet. Samrongpan et al. (2008) also examined the effects of MOS supplemented at 0, 2, 4, and 6 g/kg diet for 21 days during sex reversal of Nile tilapia fry. Weight gain, length, and average daily growth were significantly higher in tilapia fed 4 and 6 g MOS/kg diet compared to the control diet. Furthermore,

survival of tilapia to *Streptococcus agalactiae* challenge increased as dietary MOS increased in the diet. Although MOS is the most studied prebiotic added to diets of tilapia, more research has been conducted with other species of fish, especially salmonids, and there are many voids in the research record with respect to effects on growth performance, immune function, and disease resistance in tilapia. The effects of MOS on villi structure in the small intestine also needs to be established in tilapia.

b. Inulin

Inulin is a heterogeneous blend of fructose polymers composed of β-D-fructofuranoses attached by β-2-1 linkages and derived from sucrose isolated from vegetable sources (Ringø et al. 2010), such as artichokes, leeks, onion, garlic, asparagus, bananas, and wheat (Roberfroid 1993). In endothermic animals, soluble forms of inulin (α and β) have a beneficial effect on gut microbiota composition (Havenaar et al. 1999; Possemiers et al. 2009). In fish, such as Arctic charr, addition of inulin to the diet can reduce the gut bacterial population and also alter its composition (Ringø et al. 2004 and 2006). Fermentation of soluble inulin is primarily observed in lactic acid bacteria (*Carnobacterium* spp.) (Ringø et al. 2004), but *Streptococcus* spp. and *Staphylococcus* spp. also have this ability (Ringø et al. 2006). Insoluble inulin (γ-inulin), although it does not appear to be fermentable by gut bacteria, can stimulate parts of the immune system of mammals by activating parts of the complement pathway (Silva et al. 2004) and by binding to lectin-like receptors on leucocytes to induce macrophage proliferation (Causey et al. 1998; Sefert and Watzl 2007; Meyer 2008). However, high levels of dietary inulin (15% diet) can cause deformation of hindgut villi, formation of lamellar bodies, and increase vacuolization of the hindgut epithelium of Arctic charr (Olsen et al. 2001). Although elevated levels (7.5% of diet) of dietary inulin also produced an increase in hindgut vacuolization in Atlantic salmon, the level of cellular alteration and damage to intestinal villi was absent or less severe (Bakke-McKellep et al. 2007). The effects of inulin on the cellular structure and organization of intestinal villi has not been established in tilapia, and therefore, caution should be used with high levels of inulin in tilapia diets.

In juvenile blue tilapia (*O. aureus*), Wang and Wang (1997) did not find an effect of giving tilapia 10 mg inulin/kg body weight over two days by intraperitoneal injection (IP) against IP challenge (1×10^7 CFU/fish) with *Aeromonas hydrophila* or *Edwardsiella tarda*. The survival to challenge for both bacteria improved to 20% versus 0% for the control, but the differences were not statistically significant. Juvenile, sex-reversed red tilapia (*O. niloticus*

x *O. mossambicus*) (3.1 g) did not show significant improvements in weight gain, SGR, or survival after challenge with *S. iniae* when fed diets containing 5% inulin (Plongbunjong et al. 2011). Feed conversion ratio was, however, significantly lower in tilapia fed inulin compared to the control diet. Ibrahem et al. (2010) examined the supplementation of inulin at 5 g/kg of diet in juvenile Nile tilapia on growth performance, hematology, non-specific immune function, and resistance to *A. hydrophila* infection. Fish were evaluuated for the effects of inulin on these parameters after 1 and 2 months of feeding the experimental diet. Tilapia showed improved growth performance, nitroblue tetrazolium activity, lysozyme activity, and resistance to challenge with *A. hydrophila* at both sampling times. In the latter, survival was 35.71% better than tilapia fed the control diet. The authors do not speculate on possible mechanisms to explain the improvements in performance.

Dietary inulin may prove useful in aquaculture by stimulating beneficial gut bacteria, suppressing pathogenic bacteria, and enhancing the immune response (Ringø et al. 2010). In tilapia, preliminary data suggest inulin enhances immune function and disease resistance, but more research is needed to confirm this affect and determine proper dosage and feeding duration.

c. Fructooligosaccharides (FOS) and Short-Chain Fructooligosaccharides (ScFOS)

Fructooligosaccharides (FOS) are non-digestible oligosaccharides made from fructose and glucose units (Swanson et al. 2002). Structurally, FOS are composed of short and medium chains of β-D-fructans in which fructosyl units are linked by β-(2-1) glycosidic linkages and attached to a terminal glucose unit (Ringø et al. 2010). β-(2-1) glycosidic bonds and lack of β-fructosidases prevent most animals from digesting FOS (Teitelbaum and Walker 2002). However, FOS can be fermented by many species of *lactobacilli* and *bifidobacteria*, which possess β-fructosidases (Sghir et al. 1998; Manning and Gibson 2004). Many *Lactobacillus* spp. and *Bifidobacterium* spp. have been identified as probiotics, and thus FOS has been used to select for beneficial probiotic bacteria in the GI tract of many species of animals (Burr and Gatlin 2005; Ringø et al. 2010). FOS and ScFOS can enhance immunity in aquatic and terrestrial organisms, and these improvements are most likely due to alterations in the gut microbial composition and not direct activation of immune cells as with MOS, since as Ringø et al. (2010) points out, "no cell receptors specific for FOS have been identified in vertebrate organisms, and it is highly speculative to assign improvements in immunological function or disease resistance by direct action of FOS on host immune cells."

Hui-yuan et al. (2007) first reported that feeding ScFOS in diets of hybrid tilapia (*O. aureus x O. niloticus*) altered the intestinal microbiota. Tilapia were fed diets containing 0 (control), 0.8, or 1.2 g/kg ScFOS for 8 weeks. The authors reported that growth of beneficial probiotic *Lactobacillus* spp. and *Streptococcus faecalis* in the intestine increased. However, there was not a concomitant reduction in pathogenic bacteria, such as *Vibrio parahaemolyticus* and *Aeromonas hydrophila,* compared to the control, which was counter to results found with other monogastric animals (Yao et al. 2000). Growth performance parameters (final body weight, SGR, and feed intake) increased and FCR and HSI decreased significantly as ScFOS increased in the diet in this study. These results are contrary to the findings of He et al. (2003) in which growth performance of hybrid tilapia fed 2 and 6 g/kg ScFOS for 58 days did not improve, although survival and lysozyme and alternative complement activities increased with increasing dietary ScFOS. The levels of ScFOS supplemented in He et al. (2003) were higher than those used in Hui-yuan et al. (2007), which might account for the differential effects on growth performance. Zhou et al. (2009b) also examined the effects of ScFOS on the authochthonous gut microbiota of hybrid tilapia using denaturing gradient gel electrophoresis (DGGE). Tilapia were fed 1 g/kg ScFOS for 56 days. ScFOS produced marked changes in the intestinal microbial composition in which unique bacteria, *Thiothrix eikelboomi*, *Clostridium* spp., uncultured *Cyanobacterium* spp., and other uncultured bacterium clones were identified. However, it is not known whether these bacteria act as beneficial probiotics to the host. The primary benefit of FOS and ScFOS appears to be alteration of the gut microbial composition in organisms. More work is needed to establish these changes and further identify the effects on the health of tilapia.

d. Other Oligosaccharides

Galactooligosaccharides have now been established as a prebiotic ingredient after *in vitro* and animal and human *in vivo* studies (Torres et al. 2010). Galactooligosaccharides are typically created from lactose by hydrolysis with glycoside hydrolases (GH), such as β-Galactosidase, during the process of creating low lactose or lactose-free products. The general mechanism of enzymatic lactose hydrolysis has a transgalactosylic nature, involving a multitude of sequential reactions with disaccharides (other than lactose) and polysaccharides, collectively named galactooligosaccharides (GOS), as intermediate products, which results in mixtures containing GOS of different degrees of polymerization, unreacted lactose, and monomeric sugars (glucose and galactose) (Wallenfels and Malhotra 1960). The GOS

composition is also affected by differences in the position of the glycosidic linkages, which can occur because different hydrolase enzymes are used have different regiochemical selectivity (Mussatto and Mancilha 2007). Depending on oligosaccharide composition, GOS products may vary in terms of prebiotic activity, as well as other physiological effects.

Plongbunjong et al. (2011) examined the effects of GOS added at 1% or 5% in diets of juvenile (approximately 3 g), sex-reversed red tilapia on growth performance and resistance to *S. iniae* challenge. Weight gain, SGR, and FCR improved significantly in tilapia fed GOS for 60 days. Morality (%) and relative percent survival (RPS) to *S. iniae* infection were also improved compared to the control diet, but the changes were not significant. Galactooligosaccharides show promise in improving tilapia growth performance and disease resistance. However, the process to make GOS needs refinement to produce mixtures with greater uniformity in oligosaccharide composition to be useful. More dietary research with GOS is needed in diets of tilapia and other fish species as well.

Isomaltooligosaccharides (IMO) have a specific α1→6 glucosidic linkage (isomaltose, panose, isomaltotriose, and isomaltotetraose) and have been used as a dietary sweetener in Asia for many years (Lee et al. 2008). They are produced using starch (processed from wheat, barley, corn, peas, beans, lentils oats, tapioca, rice, potato, sugar beets, and other sources) as the raw material, which requires a combination of immobilized enzymes in a two-stage reactor (Mussatto and Mancilha 2007). In the first stage, starch is liquefied using α-amylase. The liquefied starch is then processed in a second-stage that involves reactions catalyzed by both β-amylase and α-glucosidase. The β-amylase hydrolyses the liquefied starch to maltose, and the transglucosidase activity of α-glucosidase then produces IMO (Kaneko et al., 1995).

Few studies have examined the effects of IMO on the health of aquatic organisms. In Nile tilapia, Ibrahim et al. (2013) examined the effects of 0.3% IMO in diet for 16 weeks on growth, digestibility, feed utilization, and intestinal perimeter ratios. Differences in growth performance and feed utilization were observed at the end of 14 weeks to the end of study. Protein and dry matter digestibility and intestinal perimeter ratios showed non-significant increases at the end of the study. The researchers did not attempt to determine the effects of IMO on the gut microbial community or immunity. A study conducted by Zhang et al. (2011) shows that IMO may hold promise as a prebiotic in a synbiotic application. The authors investigated the effects of dietary supplementation of probiotics (*Bacillus licheniformis* and *B. subtilis*; 1×10^8 CFU/g feed) in combination with IMO (0.2% diet) on the intestinal

microflora, immunological parameters, resistance of *Penaeus japonicus* against *Vibrio alginolyticus*. Shrimp fed the diet with both *Bacillus* probiotics and IMO significantly improved immune function (phenoloxidase, lysozyme nitric oxide synthase, and superoxide dismutase activities) compared to the control group. Significantly lower cumulative mortality was also observed after challenge with *V. alginolyticus* in shrimp fed the diet containing both *Bacillus* probiotics and IMO than that in the control group. Although these findings are encouraging, similar results need to be established with tilapia to determine if IMO can be used as an effective prebiotic.

e. Other Prebiotics and Prebiotic Mixtures

Commercial dietary supplements comprised of mixtures of yeast (*S. cerevisiae*) fermentation products contain prebiotic components such as MOS. However, these products also contain other products that can positively affect growth and immunity, such as β-glucans, vitamins, proteins, peptides, amino acids, nucleotides, lipids, and organic acids), making identification of the active ingredient(s) which have beneficial affects to the host difficult (Vechklang et al. 2012). Some work has been conducted with these products to determine their prebiotic effects on the gut microbial community.

DVAQUA® is a naturally fermented product consisting of yeast cell walls and cell soluble materials (He et al. 2009; Zhou et al. 2011). He et al. (2009) evaluated the effects of DVAQUA® supplemented at 0 (control), 0.125, 0.25, 0.5, 1.0, and 2.0 g/kg in diets of hybrid tilapia (*O. niloticus x O. aureus*) reared in floating net cages for 8 weeks. Although no effects on growth performance were observed, DVAQUA® stimulated the growth of several potentially beneficial bacterial species, while potentially harmful bacterial species such as *Escherichia coli* serotype O20: H42-like, uncultured *Bacilli* bacterium clone MS030A1_F02-like, and *Pseudomonas fluorescens* strain YC0357-like were depressed. Furthermore, non-speficic immunity (serum lysozyme activity, serum C3 and C4 alternative complement pathways, and head kidney macrophage phagocytic and macrophage respiratory burst activities) were improved by DVAQUA®.

GroBiotic®-A is a commercial mixture of partially autolyzed brewer's yeast, dairy ingredient components, and dried fermentation products (Ringø et al. 2010). Although some prebiotic oligosaccharides are likely present, much of the positive effects of GroBiotic®-A on immunity in fish may be from β-glucans, which are known to have immunostimulatory properties (Sakai 1999), and not from prebiotic properties of the product. Immune cells, such as

leucocytes, have glucan receptors that participate in intracellular signaling and stimulate the immune response in fish (Li and Gatlin 2004).

Juvenile Nile tilapia fed GroBiotic®-A at 0 (control), 1%, or 2% of diet for 12 weeks showed improved serum hemolytic complement activity (Vechklang et al. 2012). Although cumulative mortality from *S. iniae* infection was not affected, tilapia fed 2% GroBiotic®-A showed substantially reduced and earlier cessation of mortality. Growth performance, serum total protein, total immunoglobulin, lysozyme, and agglutinating antibody titer to *S. iniae* were unaffected. Effects on the gut microbiota were not evaluated. Several studies in other fish species have shown that Gro-Biotic® A can improve growth performance (Li and Gatlin 2004 and 2005) and immunity (Li and Gatlin 2004 and 2005; Sink et al. 2007; Sink and Lochman 2008). However, more work is needed to determine if these effects are due to prebiotic activity of the product. As a practical application in aquaculture, establishing the underlying mechanism(s) of action of GroBiotic®-A may not be critical.

2. Future Prebiotic Research

Although commercial production and application of prebiotics to terrestrial livestock has existed for quite some time, it has only garnered interest in aquaculture in recent years. The primary prebiotics examined in diets of tilapia have been MOS, inulin, and FOS, and the primary focus of these studies has been growth performance followed by immune function and disease resistance. Future prebiotic research needs to focus more on immune responses, disease resistance, and beneficial alterations in the gut microbiota (Merrifield et al. 2010a). A better understanding of the underlying mechanisms of how prebiotics improve immunity and alter gut microbial composition may also be helpful in finding new prebiotic substances. Molecular methods, such as DGGE and RNA-seq, rather than traditional culture methods which can only identify aerobic and facultatively anaerobic bacteria should be employed to prevent exclusion of potentially important strict anaerobic probionts in gut microbiota analyses (Merrifield et al. 2010a). Discovery of new prebiotics, refinement of processing methods to produce high quality prebiotics with desirable and uniform oligosaccharide composition are also important goals for application of prebiotics in the future. Use of prebiotics to favorably alter the gut environment for known beneficial probiotics presented together in diet as a synbiotic has shown promise in some aquatic and also terrestrial

organisms. This approach may hold great promise in benefitting tilapia health in the future, and research should be expanded.

3. Synbiotics

The management of gut microbial composition may be possible through the use of synbiotics, where prebiotics and probiotics are supplied together in diet. Prebiotic substrates are added to diet in conjunction with beneficial probiotic bacteria to promote their growth and colonization in the gut. This combination could improve the survival and gut colonization of the probionts added to the diet, because the specific substrate needed for fermentation would be readily available and provide a competitive advantage over other competing endogenous bacteria in the GI tract (Collins and Gibson 1999). As of this writing, only a single synbiotic study has been conducted with tilapia. In this study, three strains of lactic acid bacteria (LAB) (*Pediococcus* spp.) were isolated and cultured from the intestine of Nile tilapia for use as a probiotic (Cota-Gastélum et al. 2013). The LAB strains were combined and added as a single treatment at $5x10^4$, $2.5x10^5$, or $5x10^5$ CFU/g diet in conjunction with 2.5 g/kg inlulin and 2.5 g/kg fulvic acid in diet. Tilapia fed $2.5x10^5$ CFU LAB/g diet with the addition of inulin and fulvic acid showed SGR that was significantly higher compared to fish fed the control diet. Unfortunately, the effects on immune function, disease resistance, or gut microbial composition were not evaluated. Synbiotic application has also shown promise in salmonids. Addition of an *E. faecalis* and MOS synbiotic combination to diets of rainbow trout proffered significant improvements in resistance to *Vibrio anguillarum* challenge and immune function than when the prebiotic or probiotic were given individually. Growth performance was also improved with MOS and MOS + *E. faecalis* supplementation in the diet but not with the probiotic alone. Synbiotic application to tilapia feeds may offer great potential in stimulating immunity and improving growth performance. However, significantly more research is needed to determine which synbiotic combinations are effective in improving the health and well-being of tilapia.

CONCLUSION

Research on the effect of probiotics and prebiotics on the health and growth of tilapia has increased significantly over the past few years, but many

questions remain unanswered and more work needs to be conducted. Little effort has been undertaken to determine the microbial composition of the GI tract of tilapia. This basic information is vital to discovery and selection of potential probionts and also prebiotics. Understanding the underlying mechanisms in which probiotic microbes are able to colonize the gut and alter the gut environment to influence digestion, nutrient absorption, growth performance, and immunity are important for choosing potential probionts. Work in this area needs to continue and be expanded. Furthermore, insight as to how changes in the rearing environment and alterations of diet influence the gut microbial community will help researchers and culturists select microbes with a better chance for success and to develop more effective feeding regimens and diet formulations. Use of probiotic mixtures containing several probionts may be required when this information is not available. Research on the effects of prebiotics on growth, immunity, and the gut microbiota are extremely limited for tilapia, necessitating more research in this area. Development of synbiotic mixtures may prove to be the most effective means of probiotic use, allowing fish culturists to control and provide favorable conditions in the gut and also ensure that a beneficial probiont is present and in sufficient numbers. The success of probiotic and prebiotic dietary supplementation to improve the health and growth performance of tilapia is encouraging given the small amount of research that has occurred. Their effectiveness will surely continue to improve as more questions and uncertainty surrounding probiotic use in tilapia diets are addressed. Future work must focus on applications of probiotics and prebiotics in order to achieve the maximum efficiency, and these treatments must be used in concert with effective farm management and husbandry.

REFERENCES

Al-Harbi, A. H. & Uddin, N. (2003). Quantitative and qualitative studies on bacterial flora of hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) cultured in earthen ponds in Saudi Arabia. *Aquaculture Res*., *34*, 43-48.

Al-Harbi, A. H. & Uddin, M. N. (2004). Seasonal variation in the intestinal bacterial flora of hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*) cultured in earthen ponds in Saudi Arabia. *Aquaculture*., *229*, 37-44.

Al-Harbi, A. H. & Uddin, N. (2005). Bacterial diversity of tilapia (*Oreochromis niloticus*) cultured in brackish water in Saudi Arabia. *Aquaculture*. *250*, 566-572.

Ali, H. M., Ghazalah, A. A., Gehad, E. A., Hammouda, Y. A. & Abo-State, H. A. (2010). Practical aspects and immune response of probiotics preparations supplemented to Nile tilapia (*Oreochromis niloticus*) diets. *Nature and Sci.*, *8*, 39-45.

Aly, S. M., Abdel-Galil Ahmed, Y., Abdel-Aziz Ghareeb, A. & Mohamed, M. F. (2008a). Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of Tilapia nilotica (*Oreochromis niloticus*) to challenge infections. *Fish Shellfish Immunol*. *25*, 128–136.

Aly, S. M., Mohamed, M. F. & John, G. (2008b). Effect of probiotics on the survival, growth and challenge infection in Tilapia nilotica (*Oreochromis niloticus*). *Aquacult. Res.*, *39*, 647–656.

Aly, S. M., Abd-El-Rahman, A. M., John, G. & Mohamed, M. F. (2008c). Characterization of some bacteria isolated from *Oreochromis niloticus* and their potential use as probiotics. *Aquaculture.*, *277*, 1-6.

Bakke-McKellep, A. M., Penn, M. H., Salas, P. M., Refstie, S., Sperstad, S., Landsverk, T., Ringø, E. & Krogdahl, Å. (2007). Effects of dietary soybean meal, inulin and oxytetracycline on gastrointestinal histological characteristics, distal intestine cell proliferation and intestinal microbiota in Atlantic salmon (*Salmo salar* L.). *Br. J. Nutr.*, *97*, 699–713.

Bar, N., Mukhopadhayay, S. K., Ganguly, S., Pradhan, S., Patra, N. C., Pal, S., Goswami, J., Singh, Y. D. & Halder, S. (2012). Study on probiotic effect of xylanase supplementation in broiler feed. *Indian J. Anim. Nutr.*, *29*, 100–103.

Bates, J. M., Mittge, E., Kuhlman, J., Baden, K. N., Cheesman, S. E. & Guillemin, K. (2006). Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. *Dev. Biol.*, *297*, 374-386.

Benites, V., Gilharry, R., Gernat, A. G. & Murillo, J. G. (2008). Effect of dietary mannan oligosaccharides from Bio-MOS of SAF-mannan on live performance of broiler. *J. Appl. Poult. Res.*, *17*, 471–475.

Burr, G. D. Gatlin III. (2005). Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in finfish aquaculture. Journal of the World Aquaculture Society 36: 425-436

Caballero-Franco, C., Keller, K., De Simona, C. & Chadee, K. (2007). The VSL#3 probiotic formula induces mucin gene expression and secretion in

colonic epithelial cells. Am. J. Physiol. Gastrointest. *Liver Physiol*., *292*, G315-G322.

Causey, J. L., Slain, J. L., Tangled, B. C. & Meyer, P. D. (1998). Stimulation of human immune system by inulin *in vitro*. Proc of Danone Conference On Probiotics and Immunity, Bonn, Germany.

Collins, M. D. & Gibson, G. R. (1999). Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. *Am. J. Clin. Nutr*., *69*, 1052S-1057S.

Cota-Gastélum, L. A., Luna-González, A., González-Ocampo, H. A., Flores-Miranda, M. C., Fierro-Coronado, J. A., Escamilla-Montes, R. & Peraza-Gómez, V. (2013). Effect of *Pediococcus sp.*, *Pediococcus pentosaceus*, inulin and fulvic acid added to the diet, on growth of *Oreochromis niloticus*. African *J. Microbiol. Res*., *7*, 5489-5495.

Dimitroglou, A., Davies, S. & Sweetman, J. (2008). The effects of dietary mannan oligosaccharides on the intestinal histology of rainbow trout (*Oncorhynchus mykiss*). Abstracts/Comp. Biochem. *Physiol., A*., S63.

Direkbusarakom, S., Yoshimizu, M., Ezura, Y., Ruangpan, L. & Danayadol, Y., (1998). *Vibrio* spp. the dominant flora in shrimp hatchery against some fish pathogenic viruses. *J. Mar. Biotechnol*., *6*, 266–267.

El-Ashram, A. M. M., Mohammed, M. F. & Aly, S. M. (2008). Effect of Biobuds as a commercial probiotic product in cultured tilapia. 8th *International Symposium on Tilapia in Aquaculture*, 1089-1096.

El-Haroun, E. R., Goda, A. M. A. S. & Kabir Chowdhury, M. A. (2006). Effect of dietary probiotic biogens supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). *Aquacult. Res*., *37*, 1473–1480.

El-Rhman, A. M. A., Khattab, Y. A. E. & Shalaby, A. M. E., (2009). *Micrococcus luteus* and *Pseudomonas* species as probiotics for promoting the growth performance and health of Nile tilapia, *Oreochromis niloticus*. *Fish Shellfish Immuol*., *27*, 175-180.

Essa, M. A., El-Serafy, S. S., El-Ezabi, M. M., Daboor, S. M., Esmael, N. A. & Santosh, P. L. (2010). Effect of different dietary probiotics on growth, feed utilization and digestive enzymes activities of Nile tilapia, *Oreochromis niloticus*. *J Arabian Aquacult*., *Soc*. *5*, 143-161.

FAO (Food and Agriculture Organization of the United Nations), 2002. Antibiotics residue in aquaculture products. Pages 74-82 in The State of World Fisheries and Aquaculture, Rome, Italy.

Ferguson, R. M. W., Merrifield, D. L., Harper, G. M., Rawling, M. D., Mustafa, S., Picchietti, S., Balcázar, J. L. & Davies, S. J. (2010). The

effect of *Pediococcus acidilactici* on the gut microbiota and immune status of on-growing red tilapia (*Oreochromis niloticus*). *J. Applied Microbiol.*, *109*, 851-862.

Fitzsimmons, K. (2006). Prospect and potential for global production. Pages 51-72 in C. Lim and C.D. Webster, Editors. Tilapia: Biology, Culture and Nutrition. The Haworth Press, Inc., Binghamton, New York, NY. Fuller, R., 1989. Probiotics in man and animals. *J. Appl. Bacteriol.*, *66*, 365–378.

Galindo, A. B., Hartemink, R., Schrama, J. W., Verreth, J., Bucio, L. & Zwietering, M. H. (2009). Kinetics of Lactobacillus plantarum 44a in the faeces of tilapia (*Oreochromis niloticus*) after its intake in feed. *Applied Microbiol.*, 1967-1975.

Ganguly, S., Dora, K. C., Sarkar, S. & Chowdhury, S. (2013). Supplementation of prebiotics in fish feed: a review. *Rev. Fish Biol. Fisheries.*, *23*, 195-199.

Gannam, A. L. & Schrock, R. M. (2001). Immunostimulants in fish diets. Pages 235-266 in C. Lim and C.D. Webster, Editors. Nutrition and fish health. Food Product Press, New York, NY.

Gatesoupe, F. J. (2005). Probiotics and prebiotics for fish culture, at the parting of the ways. Aqua Feeds: *Formulation & Beyond*, *2*, 3-5.

Gawlicka, A., Parent, B., Horn, M. H., Ross, N., Opstad, I. & Torrissen, O. J. (2000). Activity of digestive enzymes in yolksac larvae of Atlantic halibut (*Hippoglossus hippoglossus*): Indication of readiness for first feeding. *Aquaculture.*, *184*, 303-314.

Genc, M. A., Yilmaz, E., Genc, E. & Aktas, M. (2007). Effects of dietary mannan oligosaccharides (MOS) on growth, body composition, and intestine and liver histology of the hybrid tilapia (*Oreochromis niloticus* x *O. aureus*). *Isr. J. Aquacult-Bamid.*, *59*, 10–16.

German, D. P., Horn, M. H. & Gawlicka, A. (2004). Digestive enzyme activities in herbivorous and carnivorous prickleback fishes (*Teleostei: Stichaeidae*): Ontogenetic, Dietary, and Phylogenetic Effects. *Physiol. Biochem. Zool.*, *77*, 789-804.

Ghazalah, A. A., Ali, H. M., Gehad, E. A., Hammouda, Y. A. & Abo-State, H. A. (2010). Effect of probiotics on performance and nutrients digestibility of Nile tilapia (*Oreochromis niloticus*) fed low protein diets. *Nature and Sci.*, 8, 46-53.

Gibson, G. R. & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.*, *125*, 1401–1412.

Gibson, G. R. (1998). Dietary modulation of the human gut microflora using prebiotics. *Br. J. Nutr.*, *80*, S209–S212.

Gibson, G. R., Probert, H. M., Loo, J. V., Rastall, R. A. & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr. Res. Rev.*, *17*, 259–275.

Girones, R., Jofre, J. T. & Bosch, A. (1989). Isolation of marine bacteria with antiviral properties. *Can. J. Microbiol.*, *35*, 1015–1021.

Haskey, N. & Dahl, W. J. (2006). Synbiotic therapy: a promising new adjunctive therapy for ulcerative colitis. *Nutr. Rev.*, *64*, 132-138.

Havenaar, R., Bonnin-Marol, S., Van Dokkum, W., Petitet, S. & Schaafsma, G. (1999). Inulin: fermentation and microbial ecology in the intestinal tract. *Food Rev. Int.*, *15*, 109–120.

He, S., Xu, G., Wu, Y., Weng, H. & Xie, H. (2003). Effects of IMO and FOS on the growth performance and non-specific immunity in hybrid tilapia. *Chinese Feed.*, *23*, 14–15.

He, S., Zhou, Z., Liu, Y., Shi, P., Yao, B., Ringø, E. & Yoon, I. (2009). Effects of dietary *Saccharomyces cerevisiae* fermentation product (DVQUA®) on growth performance, intestinal autochthonous bacterial community and non-specific immunity of hybrid tilapia (*Oreochromis niloticus* ♀ x *O. aureus* ♂) cultured in cages. *Aquacult.*, *294*, 99-107.

Heikkinen, J., Vielma, J., Kemilainen, O., Tiirola, M., Eskelinen, P., Kiuru, T., Naviapaldanius, D. & Vonwright, A. (2006). Effects of soybean meal based diet on growth performance, gut histopathology and intestinal microbiota of juvenile rainbow trout (*Oncorhynchus mykiss*). *Aquaculture.*, *261*, 259-268.

Honsheng, M. (2010). Effects of probiotics on activities of digestive enzyme for tilapia. *J. South China Normal Univ.*, *2*, 10-15.

Hui-Yuan, L., Zhigang, Z., Rudeaux, F. & Respondek, F. (2007). Effects of dietary short chain fructo-oligosaccharides on intestinal microflora, mortality and growth performance of *Oreochromis aureus* ♂ x *O. niloticus* ♀. *Chinese J. Anim. Nutr.*, *19*, 1–6.

Ibrahem, M. D., Fathi, M., Mesalhy, S. & El-Aty, A. M. A. (2010). Effect of dietary supplementation of inulin and vitamin C on the growth, hematology, innate immunity, and resistance of Nile tilapia (*Oreochromis niloticus*). *Fish Shellfish Immunol.*, *29*, 241-246.

Ibrahim, M. T., Samy, H. M., Mahmoud, M. M. & Fathalla, G. M. (2013). Evaluation of prebiotic (isomaltooligosaccharide) as a feed additive on growth performance, digestibility and intestinal histology of Nile tilapia (*Oreochromis niloticus*). *Arab. J. Sci.*, *2*, 30.

Irianto, A. & Austin, B. (2002). Probiotics in Aquaculture. *J. Fish Diseases*, *25*, 633-642.

Jatoba, A., Vieira, F. d. N., Buglione-Neto, C. C., Mouriño', J. L. P., Silva, B. C., Seiftter, W. Q. & Andreatta, E. R. (2011). Diet supplemented with probiotic for Nile tilapia in polyculture system with marine shrimp. Fish Physiol. *Biochem.*, *37*, 725-732.

Kamei, Y., Yoshimizu, M., Ezura, Y. & Kimura, T. (1988). Screening of bacteria with antiviral activity from fresh water salmonid hatcheries. *Microbiol. Immunol.*, *32*, 67–73.

Kaneko, T., Yokoyama, A. & Suzuki, M. (1995). Digestibility characteristics of isomaltooligosaccharides in comparison with several saccharides using the rat jejunum loop method. *Biosci. Biotechnol. Biochem.*, *57*, 1190–1194.

Kesarcodi-Watson, A., Kasper, H., Lategan, M. J. & Gibson, L. (2008). Probiotics in Aquaculture: The need, Principles and mechanisms of action and screening processes. *Aquaculture*, *274*, 1-14.

Kihara, M. & Sakata, T. (1997). Fermentation of dietary carbohydrates to short-chain fatty acids by gut microbes and its influence on intestinal morphology of a detritivorous teleost tilapia (*Oreochromis niloticus*). Com. *Biochem. Physiol.*, *118A*, 1201-1207.

Klebaniuk, R., Matras, J., Patkowski, K. & Picta, M. (2008). Effectiveness of Bio-MOS in sheep nutrition. *Ann. Anim. Sci.*, *8*, 369–380.

Lara-Flores, M., Olvera-Novoa, M. A., Guzmán-Méndez, B. E. & López-Madrid, W. (2003). Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). *Aquaculture.*, *216*, 193-201.

Lara-Flores, M., Olivera-Castillo, L. & Olvera-Novoa, M. A. (2010). Effect of the inclusion of a bacterial mix (*Streptococcus faecium* and *Lactobacillus acidophilus)*, and the yeast (*Saccharomyces cerevisiae)* on growth, feed utilization and intestinal enzymatic activity of Nile tilapia (*Oreochromis niloticus*). *Internat. J. Fish. Aquaculture.*, *2*, 93-101.

Lee, C. C., Wang, H. F. & Lin, S. D. (2008). Effect of isomaltooligosaccharide syrup on quality characteristics of sponge cake. *Cereal Chem.*, *85*, 515–521

Li, P. & Gatlin, D. M. III, (2004). Dietary brewers yeast and the prebiotic GroBiotic[®] AE influence growth performance, immune responses and resistance of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) to *Streptococcus iniae* infection. *Aquacult.*, *231*, 445–456.

Li, P. & Gatlin, D. M. (2005). Evaluation of the prebiotic GroBiotic®-A and brewers yeast as dietary supplements for sub-adult hybrid striped bass (*Morone chrysops x M. saxatilis*) challenged in situ with *Mycobacterium marinum*. Aquaculture., *248*, 197-205.

Lim, C. & Webster, C. D. (2006). Tilapia biology, culture, and nutrition. The Haworth Press, Inc., Binghamton, NY.

Linehan, S. A., Martínez-Pomares, L. & Gordon, S. (2000). Macrophage lectins in host defence. *Microbes Infect*., *2*, 279–288.

Liranço, A. D. D. S., Ciarlini, P. C., Moraes, G., Camargo, A. L. S. & Romagosa, E. (2013). Mannanoligosaccharide (mos) and β-glucan (β-glu) in dietary supplementation of Nile tilapia kept in cages. Pan-Am. *J. Aquat*., Sci. 8, 112-125.

Lovell, T. & Limsuwan, T. (1982). Intestinal synthesis and dietary non essentiality of vitamin B12 for *Tilapia nilotica*. *Trans. of Amer. Fish. Soc*., *11*, 485-490.

Manning, T. S. & Gibson, G. R. (2004). Prebiotics. Best Pract. Res. *Clin. Gastroenterol*., *18*, 287–298.

Marzouk, M. S., Moustafa, M. M. & Mohamed, N. M. (2008). The influence of some probiotics on the growth performance and intestinal microbial flora of *Oreochromis niloticus*. Proceedings of 8th International Symposium on Tilapia in Aquaculture, Cairo, Egypt, 1059-1071.

Mehrim, A. I. (2009). Effect of dietary supplementation of Biogen (commercial probiotic) on mono-sex Nile tilapia *Oreochromis niloticus* under different stocking densities. *J Fish. Aquat. Sci*., *4*, 261-273.

Merrifield, D. L., Dimitroglou, A., Foey, A., Davies, S. J., Baker, R. T. M., Bøgwald, J., Castex, M. & Ringø. E. (2010a). The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture*, *301*, 1-18.

Merrifield, D. L., Harper, G., Baker, R. T. M., Ringø, E. & Davies, S. J. (2010b). Possible influence of probiotic adhesion to intestinal mucosa on the activity and morphology of rainbow trout (*Oncorhynchus mykiss*) enterocytes. *Aquacult. Res*., *41*, 1268-1272.

Merrifield, D. L., Bradley, G., Harper, G. M., Baker, R. T. M., Munn, C. B. & Davies, S. J. (2011). Assessment of the effects of vegetative and lyophilised *Pediococcus acidilactici* on growth, feed utilisation, intestinal colonisation and health parameters of rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aqua. Nutr*., *17*, 73-79.

Meyer, D. (2008). Prebiotic dietary fibres and the immune system. Agro. *Food Ind*., *19*, 12–15.

Mohamed, M. H. & Refat, N. A. G. A. (2011). Pathological evaluation of probiotic, *Bacillus subtilis*, against *Flavobacterium columnare* in tilapia nilotica (*Oreochromis niloticus*) fish in Sharkia Governorate, Egypt. *J. Amer. Sci.*, *7*, 244-256.

Molinari, L. M., Scoaris, D., de O., Pedroso, R. B., Bittencourt, N. de L. R., Nakamura, C. V., Ueda-Nakamura, T., Abreu Filho, B. A. de., & Dias Filho, B. P. (2003). Bacterial microflora in the gastrointestinal tract of Nile tilapia, *Oreochromis niloticus*, cultured in a semi-intensive system. *Acta Scientiarum Biological Sciences Maringa.*, *25*, 267-271.

Mussatto, S. I. & Mancilha, I. M. (2007). Non-digestible oligosaccharides: a review. *Carbohydr. Polym.*, *68*, 587–597.

Nayak, S. K. (2010a). Probiotics and immunity: a fish perspective. *Fish and Shellfish Immunol.*, *29*, 2-14.

Nayak, S. K. (2010b). Role of gastrointestinal microbiota in fish. *Aquaculture Res.*, *41*, 1553-1573.

Newsome, S. D., Fogel, M. L., Kelly, L. & Martinez del Rio, C. (2011). Contributions of direct incorporation from diet and microbial amino acids to protein synthesis in Nile tilapia. *Funct. Ecol.*, *25*, 1050-1062.

Olsen, R. E., Myklebust, R., Kryvi, H., Mayhew, T. M. & Ringø, E. (2001). Damaging effect of dietary inulin to intestinal enterocytes in Arctic charr (*Salvelinus alpinus* L.). *Aquacult. Res.*, 32, 931–934.

Pirarat, N., Kobayashi, T., Katagiri, T., Maita, M. & Endo, M. (2006). Protective effects and mechanisms of a probiotic bacterium *Lactobacillus rhamnosus* against experimental *Edwardsiella tarda* infection in tilapia (*Oreochromis niloticus*). *Vet. Immunol. Immunopathol.*, *113*, 339-347.

Pirarat, N., Pinpimai, K., Endo, M., Katagiri, T., Ponpornpisit, A., Chansue, N. & Maita, M. (2011). Modulation of intestinal morphology and immunity in Nile tilapia (*Oreochromis niloticus*) by Lactobacillus rhamnosus GG. *Res. Vet. Sci.*, *91*, e92-97.

Plongbunjong, V., Phromkuntong, W., Suanyuk, N., Viriyapongsutee, B. & Wichienchot, S. (2011). Effects of prebiotics on growth performance and pathogenic inhibition in sex-reversed red tilapia. *Thai J. Agric. Sci.*, 44, 162-167.

Possemiers, S., Grootaert, C., Vermeiren, J., Gross, G., Marzorati, M., Verstraete, W. & Van de Wiele, T. (2009). The intestinal environment in health and disease – recent insights on the potential of intestinal bacteria to influence human health. *Curr. Pharmaceut. Des.*, *15*, 2051–2065.

Ringø, E., Jutfelt, F., Kanapathippillai, P., Bakken, Y., Sundell, K., Glette, J., Mayhew, T. M., Myklebust, R. & Olsen, R. E. (2004). Damaging effect of

the fish pathogen Aeromonas salmonicida ssp. salmonicida on intestinal enterocytes of Atlantic salmon (*Salmo salar* L.). *Cell Tiss. Res*., *318*, 305–311.

Ringø, E., Sperstad, S., Myklebust, R., Mayhew, T. M. & Olsen, R. E. (2006). The effect of dietary inulin on bacteria associated with hindgut of Arctic charr (*Salvelinus alpinus* L.). *Aquacult. Res*., *37*, 891–897.

Ringø, E., Løvmo, L., Kristiansen, M., Bakken, Y., Salinas, I., Myklebust, R., Olsen, R. E. & Mayhew, T. M. (2009). Lactic acid bacteria vs. pathogens in the gastrointestinal tract of fish: a review. *Aquacult. Res*., *41*, 451-467.

Ringø, E., Oldsen, R. E., Gifstad, T. O., Dalmo, R. A., Amlund, H., Hemre, G. I. & Bakke, A. M. (2010). Prebiotics in aquaculture: review. *Aquaculture Nutr*., *16*, 117-136.

Roberfroid, M. (1993). Dietary fibre, inulin and oligofructose: a review comparing their physiological effects. CRC Crit. Rev. *Food Sci. Technol*., *33*, 103–148.

Roberfroid, M. (2007). Prebiotics: the concept revised. *J. Nutr*., *137*, 830S–837S.

Rodriguez-Estrada, U., Satoh, S., Haga, Y., Fushimi, H. & Sweetman, J. (2009). Effects of single and combined supplementation of *Enterococcus faecalis*, mannan oligosaccharide and polyhydrobutyric acid on growth performance and immune response of rainbow trout *Oncorhynchus mykiss*. *Aquacult. Sci*., *57*, 609–617.]

Rosas-Ledesma, R., León-Rubio, J. M., Alarcón, F. J., Moriñgo, M. A. & Balebona, M. C. (2011). Calcium alginate capsules for oral administration of fish probiotic bacteria: assessment of optimal conditions for encapsulation. Aquacult. Res. doi:10.1111/j.1365-2109.2011.02809.x.

Sado, R. Y., Almeida Bicudo, A. J. D. & Cyrino, J. E. P. (2008). Feeding dietary mannan oligosaccharides to juvenile Nile tilapia, Oreochromis niloticus, has no effect on haematological parameters and showed decreased feed consumption. *J. World Aquac. Soc*., *39*, 821–826.

Sakai, M. (1999). Current research status of fish immunostimulants. *Aquaculture*., *172*, 63-92.

Sakata, T., Sugita, H., Mitsuoka, T., Kakimoto, D. & Kadota, H. (1980a). Isolation of obligate anaerobes from the intestinal tracts of freshwater fish. *Bull. Jap.Soc. Sci. Fish*., *46*, 511.

Sakata, T., Okabayashi, J. & Kakimoto D. (1980b). Variations in the intestinal microflora of tilapia reared in fresh and sea water. *Bull. Jap. Soc. Sci. Fish*., *46*, 313-317.

Salze, G., McLean, E., Schwarz, M. H. & Craig, S. R. (2008). Dietary mannan oligosaccharide enhances salinity tolerance and gut development of larval cobia. *Aquacult.*, *274*, 148–152.

Samrongpan, C., Areechon, N., Yoonpundh, R., Sirsapoome, P., 2008. Effects of mannan-oligosaccharides on growth, survival and disease resistance of Nile tilapia (*Oreochromis niloticus* Linnaeus) fry. http://ag.arizona.edu/ azaqua/ista/ISTA8/Abstracts_Papers/Chinnapats%20full%20paper-Thailand.doc.

Sanz, Y. & De Palma, G. (2009). Gut microbiota in modulation of epithelium and gut-associated lymphoid tissue function. *Inter. Rev. Immunol.*, *28*, 397-413.

Schwarz, K. K., Furuya, W. M., Natali, M. R. M., Gaudezi, M. C., Gonçalves de Lima, P. A. (2011). Mannanoligosaccharides in diets for tilapia fry. *R. Bras. Zootec.*, *40*, 2634-2640.

Seifert, S. & Watzl, B. (2007). Inulin and oligofructose: review of experimental data on immune modulation. *J. Nutr.*, *137*, 2563S–2567S.

Sghir, A., Chow, J. M. & Mackie, R. I. (1998). Continuous culture selection of bifidobacteria and lactobacilli from human fecal samples using fructooligosaccharides as selective substrate. *J. Appl. Microbiol.*, *85*, 769–777.

Shelby, R. A., Lim, C. E., Aksoy, M. & Klesius, P. H. (2006). Effect of probiotic diet supplements on disease resistance and immune response of young Nile tilapia, *Oreochromis niloticus*. *J. Appl. Aquac.*, *18*, 23-34.

Shoemaker, C., Lim, C., Yildirim-Aksoy, M.,Welker, T. & Klesius, P. H. (2006). Growth response and acquired resistance of Nile tilapia, *Oreochromis niloticus* (L.) that survived *Streptococcus iniae* infection. *Aquaculture Res.*, *37*, 1238-1245.

Silva, D. G., Cooper, P. D. & Petrovsky, N. (2004). Inulin-derived adjuvant efficiently promote both Th1 and Th2 immune responses. *Immunol. Cell Biol.*, *82*, 611–616.

Sink, T. D. & Lochmann, R. T. (2008). Preliminary observations of mortality reduction in stressed, *Flavobacterium columnare* – challenge golden shiners after treatment with a dairy – yeast prebiotic. N. *Am. J. Aquacult.*, *70*, 192–194.

Sink, T. D., Lochmann, R. T., Goodwin, A. E. & Marecaux, E. (2007). Mortality rates in golden shiners fed high-fat diets with or without a dairy – yeast prebiotic before challenge with *Flavobacterium columnare*. N. *Am. J. Aquacult*, *69*, 305–308.

Smith, T. B., Wahl, D. H. & Mackie, R. I. (2005). Volatile fatty acids and anaerobic fermentation in temperate piscivorous and omnivorous freshwater fish. *J. Fish Biol*., *48*, 829-841.

Sohn, K. S., Kim, M. K., Kim, J. D. & Han, I. K. (2000). The role of immunostimulants in monogastric animals and fish – review. Asian–Australian *J. Anim. Sci*., *13*, 1178–1187.

Son, V. M., Chang, C. C., Wu, M. C., Guu, Y. K., Chiu, C. H. & Cheng, W. (2009). Dietary administration of the probiotic, *Lactobacillus plantarum*, enhanced the growth innate immune responses, and disease resistance of the grouper *Epinephelus coioides*. *Fish Shellfish Immunol*., *26*, 691-698.

Sugita, H., Isida, Y., Deguchi, Y. & Kadota, H. (1982a). Bacterial flora in the gastrointestine of *Tilapia nilotica* adapted in sea water. *Bull. Jap. Soc. Sci. Fish*., *48*, 987-991.

Sugita, H., Enomoto, A. & Deguchi, Y. (1982b). Intestinal microflora in the fry of Tilapia mossambica. *Bull. Jap. Soc. Sci. Fish*., *48*, 875.

Sugita, H., Oshima, K., Tamura, M. & Deguchi, Y. (1983). Bacterial flora in the gastrointestine of freshwater fishes in the river. *Bull. Jap. Soc. Sci. Fish*., *49*, 987-991.

Sugita, H., Tokuyama, K. & Deguchi, Y. (1985). The intestinal microflora of carp *Cyprinus carpio*, grass carp *Ctenopharyngodon idella* and tilapia *Sarotherodon niloticus*. *Bull. Jap. Soc. Sci. Fish*., *50*, 1325-1329.

Sugita, H., Tsunohara, M., Fukumoto, M. & Deguchi, Y. (1987). Comparison of microflora between intestinal contents and fecal pellets of fresh water fishes. *Nippon Suisan Gakkaishi*, *53*, 287-290.

Sugita, H., Miyajima, C. & Deguchi, Y. (1990). The vitamin B12 producing ability of the intestinal bacteria isolated from tilapia and channel catfish. *Nippon Suisan Gakkaishi*, *56*, 701.

Sugita, H., Miyajima, C. & Deguchi, Y. (1991). Vitamin B12 producing ability of the intestinal microflora of freshwater fish. *Aquaculture*, *92*, 267-276.

Swanson, K. S., Grieshop, C. M., Flickinger, E. A., Bauer, I. L., Wolf, B. W., Chow, J., Garleb, K. A., Williams, J. A. & Fahey, J. C. (2002). Fructooligosaccharides and Lactobacillus acidophilus modify bowel function and protein catabolites excreted by healthy humans. *J. Nutr*., *132*, 3042–3050.

Taoka, Y., Maeda, H., Jo, J. Y., Kim, S. M., Park, S. I., Yoshikawa, T. & Sakata, T. (2006). Use of live and dead probiotic cells in tilapia *Oreochromis niloticus*. *Fish. Sci*., *72*, 755-766.

Teitelbaum, J. E. & Walker, W. A. (2002). Nutritional impact of pre and probiotics as protective gastrointestinal organisms. *Ann. Rev. Nutr.*, *22*, 107–138.

Teshima, S. & Kashiwada, K., (1967). *Studies on* the production of B vitamins by intestinal bacteria of fish. 3. Isolation of vitamin B_{12} synthesizing bacteria and their bacteriological properties. *Bull. Jap. Soc. Sci. Fish*. *33*, 979-983.

Timmerman, H. M., Koning, C. J. M., Mulder, L., Rombout, F. M. & Beynen, A. C. (2004). Monostrain, multistrain and multispecies probiotics – a comparison of functionality and efficacy. *Int. J. Food Microbiol.*, *96*, 219-233.

Torres, D. P. M., Gonçalves, M. P. F., Teixeira, J. A. & Rodrigues L. R. (2010). Galacto-oligosaccharides: production, properties, applications, and significance as prebiotics. *Compr. Rev. F. Sci. F. Safe.*, *9*, 438-454.

Tsuchiya, C., Sakata, T. & Sugita, H. (2008). Novel ecological niche of *Cetobacterium somerae*, an anaerobic bacterium in the intestinal tracts of freshwater fish. Letters Appl. *Microbiol.*, *46*, 43-48.

Ushakova, N. A., Kotenkova, E. V., Kozlova, A. A. & Nifatov, A. V. (2006). A study of the mechanisms of probiotic effect of *Bacillus subtilis* Strain 8130. *Appl. Biochem. Microbiol.*, *42*, 252-257.

Vaishnava, S., Behrendt, C. L., Ismail, A. S., Eckmann, L. & Hooper, L. V. (2008). Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. *Proc. Natl. Acad. Sci. USA.*, *105*, 20858-20863.

Vechklang, K., Lim, C., Boonanuntanasarn, S., Welker, T., Ponchunchuwong, S., Klesius, P. H. & Wanapu, C. (2012). Growth performance and resistance to *Streptococcus iniae* of juvenile Nile tilapia (*Oreochromis niloticus*) fed diets supplemented with GroBiotic-A and Brewtech dried brewers yeast. *J. Appl. Aquacult.*, *24*, 183-198.

Vijayabaskar, P. & Somasundaram, S. T. (2008). Isolation of bacteriocin producing lactic acid bacteria from fish gut and probiotic activity against common fresh water fish pathogen *Aeromonas hydrophila*. *Biotechnol.*, *7*, 124-128.

Wallenfels, K. & Malhotra, O. P. (1960). Beta-galactosidase. In: Boyer PD, editor.The enzymes. 2nd ed. New York: Academic Press Inc., 409–30.

Wang, W. & Wang, D. (1997). Enhancement of the resistance of tilapia and grass carp to experimental *Aeromonas hydrophila* and *Edwardsiella tarda* infections by several polysaccharides. Comp. *Immunol. Microbiol. Infect. Dis.*, *20*, 261–270.

Wang, Y. B., Li, J. R. & Lin, J. (2008). Probiotics in aquaculture: Challenges and outlook. *Aquaculture*, *208*, 1-4.

Yao, J., Zhou, Y. & Liu, Q. (2000). Research advances in fructooligosaccharide. Application in monogastric animals. *Anim. Sci. Abroad.*, *27*, 24-27.

Yang, Y., Iji, P. A. & Choct, M. (2009). Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. *World Poult. Sci.*, *65*, 97–114.

Ye, J. D., Wang, K., Li, F. D. & Sun, Y. Z. (2011). Single or combined effects of fructo- and mannan oligosaccharide supplements and *Bacillus clausii* on the growth, feed utilization, body composition, digestive enzyme activity, innate immune response and lipid metabolism of the Japanese flounder *Paralychthys olivaceus*. *Aquacult. Nutr.*, *17*, e902-e911.

Yilmaz, E., Genc, M. A. & Genc, E. (2007). Effects of dietary mannan oligosaccharides on growth, body composition, and intestine and liver histology of rainbow trout, *Oncorhynchus mykiss*. Isr. *J. Aquacult-Bamid.*, *59*, 182–188.

Zhang, Q., Tan, B., Mai, K., Zhang, W., Ma, H., Ai, Q., Wang, X. & Liufu, Z., (2011). Dietary administration of *Bacillus* (*B. licheniformis* and *B. subtilis*) and isomaltooligosaccharide influences the intestinal microflora, immunological parameters and resistance against Vibrio alginolyticus in shrimp, Penaeus japonicus (*Decapoda*: *Penaeidae*). *Aquacult. Res.*, *42*, 943-952.

Zhou, Q., Li., K, Jun, X. & Bo, L. (2009a). Role and functions of beneficial microorganisms in sustainable aquaculture. *Bioresource Tech.*, *100*, 3780-3786.

Zhou, Z., He, S., Liu, Y., Shi, P., Huang, G. & Yao, B. (2009b). The effects of dietary yeast culture or short-chain fructo-oligosaccharides on the intestinal autochthonous bacterial communities in juvenile hybrid tilapia *Oreochromis niloticus* ♀ x *O. aureus* ♂. J. World Aquac. Soc., *40*, 450–459.

Zhou, X., Wang, Y., Jiangtao, Y. & Li, W. (2010). Inhibition ability of probiotic, *Lactococcus lactis*, against *A. hydrophila* and study of its immunostimulatory effect in tilapia (*Oreochromis niloticus*). *Int. J. Engin. Sci. Tech.*, *2*, 73-80.

Zhou, Z., He, S., Liu, Y., Cao, Y., Meng, K., Yao, B., Ringø, E. & Yoon, I., (2011). Gut microbial status induced by antibiotic growth promoter alters the prebiotic effects of dietary DVAQUA1 on *Aeromonas hydrophila*-infected tilapia: production, intestinal bacterial community and non-specific immunity. *Vet. Micro*., *149*, 399-405.

Zimmerman, B., Bauer, E. & Mosenthin, R. (2001). Pro- and prebiotics in pig nutrition − potential modulators of gut health? *J. Animal Feed Sci*., *10*, 47-56.

Chapter 2

TILAPIA PRODUCTION: FROM WATER TO HUMAN CONSUMPTION

Erika da Silva Maciel[1], Luciana Kimie Savay-da Silva[2]†,
Juliana Antunes Galvão[3]‡ and Marília Oetterer[4]§*

[1]The University and Lutheran, Center of Palmas
CEULP-ULBRA, Palmas, TO, Brazil
[2]Department of Food and Nutrition, Nutrition College,
Federal University of Mato Grosso (UFMT), Cuiabá, MT, Brazil
[3]"Luiz de Queiroz" College of Agriculture,
University of São Paulo - USP, SP, Brazil
[4]Department of Agroindustry, Food and Nutrition,
"Luiz de Queiroz" College of Agriculture,
University of São Paulo, SP, Brazil

ABSTRACT

The demand for attributes beyond quality, such as food safety, respect for the environment and production with social responsibility is increasing in world food trade.

---

* E-mail: erikasmaciel@gmail.com.
† E-mail: lukimie@gmail.com.
‡ E-mail: jugalvao@usp.br.
§ E-mail: mariliaoetterer@usp.br.

In parallel, the fish production chain has been confronted by problems with the lack of quality of their products, many of them related to the quality of the cultivation or capture waters. Nowadays, the water where these organisms are created, presents itself as a critical control point, as well as influencing the quality / safety of the product, the cultivation should be done in an environmentally sustainable manner. In this sense, the fish has been shown to be responsible for public health problems, such as scromboid poisoning, toxicity by mollusks, ciguatera and microbial contamination or through toxic metals such as mercury. Besides the aspects of security and environmental sustainability, the relationship between water quality and the occurrence of compounds capable of negatively alter the taste and smell of fish (off flavoUr) has been evidenced in many studies. For crop species there is the possibility of controlling the quality of the fish through the proper management, which primarily begins with the quality of the cultivation water. However, questions regarding the best handling practices, processing, storage and marketing also have troubled the consumer, who is more attentive to issues of food safety and quality programs, being, in some cases, willing to pay more for a product of best quality, convenience and posing no danger to their health, the environment or society. It is noted that the tilapia is a species that can guarantee the availability of fish in many regions, since its cultivation has been successful. The advantages of tilapia farming, in relation to other species, are in the easy feeding, hardiness, prolificacy and good adaptation. Based on the chain of production of fish, although the meaning of quality is broad, standing out in this concept features that the consumer believes that the product should have, or should approach its intrinsic composition, nutritional value, likely to change during the preparation, storage, distribution, sale and presentation to the consumer. Specific actions of environmental and ecological nature should be proposed, with the aim of contributing to the sustainable and rational exploitation of resources, and minimizing the adverse impact that the waste generated by this activity produces on the environment; seeking responsible and sustainable management of fish agribusiness.

Keyword: Tilapia; water quality; fish processing, consumption

1. INTRODUCTION

With further research and development, the production chain of fish is becoming stronger and enabling the delivery of diversified products. Through survey data, it is possible to know the demands of the links on the production

chain and rank important issues facing trends aiming for innovation, sustainability and traceability. Thus, products and coproducts with certificate of origin, identity and innocuousness, when standardized, become competitive to participate in the demanding international market. In this sense, tilapia is highlighted, introduced in Brazil in 1971, the Nile tilapia (*O. niloticus*) has been the kind of freshwater fish cultivated over the country. This interest in tilapia can be explained, in part, for its easy adaptation for possessing the typical requirements of the preferred species by the consumer, such as white meat and easy filleting, in addition to its sensory, nutritional and low calorie characteristics, attributes that have been observed as a market trend in the choice of the species of fish being consumed. Therefore, the development of emerging technologies for tilapia arise as a necessity on the perishability of fish and the fragility of fresh (*in natura*) supplies, dominant in this country. Qualify the raw aquaculture material, standardize products and coproducts, develop modified atmosphere packaging, regulate products from mechanically separated meat (MSM) sources, adjust the processing line focusing on critical points, water management and promoting curbside recycling in the company, are the themes researched and discussed among stakeholders.

This chapter will address issues that range from water quality for tilapia production, processing techniques and benefaction researched in Brazil and a brief account of the case studies in the consumer market in Brazil and Portugal, these items were developed aiming to highlight the importance of communication between the links of the production chain, focusing on the develoment of products with guaranteed quality and meeting the market demands, thereby stimulating the consumption of this protein source associated with a good health.

2. WATER QUALITY IN TILAPIA CULTURE

Water quality is the first attribute to classify the fish as raw material, however, if it is in poor condition, it becomes a PROBLEM to production, it can generate, zootechnical and public health problems, and also interfere with the sensory characteristics of fish, favoring the appearance of undesirable odors or flavoUrs that affect the later acceptance of the fish, and hence the market [1].

The excessive growth of algae and aquatic plants due to eutrophication, is a recurring problem in aquaculture, either by over-fertilization, use of poor

quality rations, and intake of feces and nitrogenous excreta in systems with high stocking density [1,2].

The presence of phosphorus and nitrogen compounds in FRESH water enables the development of actinomycetes and cyanobacteria. Some of these species are potentially producers of toxins, as well as compounds capable of negatively alter the taste and odor of fish, known as *off flavoUr.* Off flavoUr is characterized as odor and taste of "muddy", "earthy", "musty", causing significant economic losses to the fishing industry [1].

Thus, managing to keep the balance between water, fish and microorganisms, in order to maximize productivity in a sustainable manner, has been one of the challenges to be overcome by fish producers [1, 3].

Tilapia are well adapted to the different conditions of water quality, are able to tolerate low dissolved oxygen, can develop in a wide range of acidity and alkalinity, grow and reproduce in brackish and salt waters and tolerate high concentrations of toxic ammonia.

In tilapia culture of freshwater, the water transparency can vary between 38 to 53 cm, range considered ideal in growing high water renewal [4, 5, 6].

In Brazil, for example, national legislation demands that turbidity of freshwater for fish farming (class 2) should be up to 100 nephelometric turbidity units (NTU), regarding the color, the values should not exceed 75 mg Pt / L [7].

Tilapias from fish ponds in Brazil, were found values ranging from 11 to 81.5 NTU for turbidity, and 32-276 mg Pt/L for color. The values for color are 3.68 times higher than those suggested by Brazilian legislation, testifying excess of coloring substances dissolved in water [5].

Tilapia are tolerant to high turbidity conditions of the environment however, high levels of turbidity reduce the incoming light in water bodies and, consequently, the local primary production. Another point to be considered is the behavior of this highly territorialist species, which can be minimized with the turbidity in the water, reducing the field of view of the animals [8].

Regarding Dissolved Oxygen (DO) tilapia tolerate low concentrations of dissolved oxygen in the water and managed to survive the mean values of 0.5 mg/L. When the dissolved oxygen concentration is ranging from 3 to 3.5 mg/liter at 28-30°C, the Nile tilapia begins to reduce its activity and hence oxygen consumption [8].

Tilapia has its thermal comfort in the 27-32 °C range [9], being considered at ideal temperature if within the range of 29 to 31°C [8], temperatures outside the range of comfort reduce appetite, growth, increase stress and disease

incidence [10]. Below $16^{o}C$, the development of tilapia is significantly impaired [8].

Regarding the pH, it has been observed that when below 4, the tilapias presents low survival rate, and is observed increased secretion of mucus, irritation and swelling in the gills in this species. The pH of the cultivation water should be maintained between 6 and 8, 5. Below 4.5 and above 10.5 shows a significant mortality rate [8].

The main chemicals that are influencing the alkalinity bicarbonates (HCO_3^-), carbonates (CO_3^-), and hydroxide (OH^-) [11]. Values lower than 20 mg $CaCO_3$/L correspond to waters with low buffering capacity, that may have significant fluctuations in pH [10], values between 200-300 mg/L, are set as ideal for the cultivation of fish [12,13].

The concentration of free metal ions in water, represented mainly by calcium and magnesium ions, impart hardness at water bodies. It is desirable for the aquaculture water is slightly hard (Table 1), values above 30 mg $CaCO_3$/L. Correction of water with low hardness values, with the addition of calcium chloride is recommended [13], the presence and diversity of phytoplankton is also influenced by the degree of water hardness [14].

Table 1. Classification of water hardness

Water classification	Hardness (mg/L)
Soft	Below 17
Slightly hard	17 to 60
Moderately hard	60 to 120
Hard	120 to 180
Very hard	Above 180

Source: (Galvão et al. 2004).

In regions where the expansion of aquaculture is only possible with the use of brackish or salt water, Tilapia culture has been an alternative. Nile Tilapia reproduce normally in salinities up to 15ppt. Nile Tilapia has low tolerance until 40-45 days of life. The size seems to be more important than age, with regard to tolerance to salinity [15].

The literature differs as to the proper limits of ammonia nitrogen in water cultivation; [16] recommends that you do not exceed the value of 0.05 mg / L for tropical fish. Levels below 0.15 mg of ammonia (NH3) / L are considered safe in the cultivation of tropical fish [15, 11] Specifically for tilapia, the lethal concentration of ammonia (LD 50) is 2.3 to 2.6 mg/L [14]. Measurements of

ammonia should be done weekly on systems with high feeding levels, always in the evening, a time when the values of higher pH potentiate the toxic action of ammonia [11].

For *Oreochromis sp*, the lethal concentration of ammonia to 50% of fish, in 96 hours, is 2.3 to 2.6 mg/L and the critical threshold, above which the performance and health of fish are impaired is 0.20 mg/L [17].

Regarding the electric conductivity, tilapia species is considered tolerant, since it resists conductivity from 0.15 to 0.2 mS/cm [18].

During production in fish ponds, respiration may exceed the photosynthetic activity, increasing the concentration of CO_2 in the system, being able to reach values higher than 25 mg / L, and, as a result, affecting the performance of the fish and leading to mortality [19].

In aquaculture, the main source of phosphorus is feeding, hence the importance of using rations of quality because in the environment, phosphorus is the limiting nutrient to phytoplankton growth [20] and calculations for carrying capacity of the environment are based on the quantification of the concentration and dynamics of phosphorus forms in the ecosystem; therefore, the importance in monitoring water quality [17] (Table 2).

In aquaculture, fertilizer fish ponds is a practice to increase plankton biomass and, consequently, the production of some economically important fish such as tilapia, being produced the natural food of high nutritional value, and tilapia feeding also by filtration, eventually also reduces feed costs. Eutrophication stimulates the development of species of cyanobacteria, which may impair the fertilization programs of nurseries. These organisms are not efficiently utilized by fish, competing for nutrients and can reduce the entry of light into the water, harming the development of phytoplankton truly beneficial to cultivation [1].

The microbiological evaluation of water bodies must be performed periodically so that there is control of the environment [21,22].

There are species of microorganisms that are used as indicators of contamination, these parameters are of great importance for the analysis of water quality with respect to public health.

The colimetric analyzes in areas where aquatic organisms are cultivated is an important tool for quality control of these products, regarding health conditions [23].

Table 2. Suggested frequency for monitoring of some parameters of water´s quality

Frequency	T ºC	Transparency	O.D.	pH	NO_2^-	$NH_3 NH_4^+$	Hardness	Alcalinity	$PO_4^=$	CO_2
Daily	m/a	m	m/a	m/a	-	-	-	-	-	-
Weekly	-	-	-	m/a	m/a	m/a	-	m	m/a	m
Biweekly	-	-	-	-	-	-	m	-	-	-
Monthly	-	-	-	-	-	-	m	m	m/a	m

m = morning; a= afternoon.

Source: Galvão, et al. 2014.

Fecal coliform bacteria are used as indicators because they are eliminated in large quantities in the feces, and are present in sewage. Within the group of fecal coliforms, E. coli is the best indicator of fecal contamination since about 95% of existing in human feces and other warm-blooded animals are constituted of coliforms *E. coli.*, Although this organism can also be come from non-fecal sources [24].

Heterotrophic mesophilic aerobic bacteria are also used as indicators of water quality, being considered the best analysis that estimates the density of bacterial contaminants in not drinkable water [25].

Several Vibrio species are known to be the etiologic agents of outbreaks involving fish products, among species stand out as *Vibrio cholerae, Vibrio parahaemolyticus* and *Vibrio vulnificus*, coming to fish through the water, as these are part of the natural microflora of estuarine and marine waters [26].

Table 3. Characteristics of the major phytoplankton groups

Phytoplankton groups	*Group characteristics*
Cyanobacteria	u, c, f, m, a, ac, h
Bacillariophyceae	u, c, m
Chrysophyceae	u, c, fl
Cryptophyceae	u, fl
Euglenophyceae	u, c, fl
Dinophyceae	u, c, fl
Chlorophyceae	u, c, f, fl, m
Prasinophyceae	u, fl
Conjugatophyceae	u, c, fl

U = unicellular c = colonial; f = filamentous; m = mucilage; a = aerobic; ac = acinetos; h = heterocyst; fl = flagellum.
Source Galvão et al. (2014).

Phytoplankton diversity is an important parameter in assessing the quality of water. Phytoplankton is formed by unicellular or multicellular algae (colonial or filamentous) generally microscopic, that have different characteristics (Table 3) [27].

Cyanobacteria are photosynthetic organisms that make up the phytoplankton of fresh, salt or brackish water and can also be found in soil and rocks materials. Taxonomic class *Cyanophyceae*, preferably occurs in eutrophic aquatic environments with little or no movement of currents. Some species produce toxins that can cause poisoning in humans and animals [1].

The presence of a few dominant species of phytoplankton is influenced by environmental factors, especially the dynamics of phosphorus / nitrogen [28].

Natural predation of phytoplankton also constitutes interference factor in phytoplankton dynamics; tilapia give preference to Cyanobacteria and *Zygnemaphyceae*. Organisms with reduced size and rigid cell walls are more resistant to predation [29].

Several bloom forming species in aquatic environments produce a variety of toxic compounds including hepatotoxins and neurotoxins. Microcystins, characterized as hepatotoxins, have been the most studied for causing problems worldwide [30,31]. Cyanotoxins have been found in muscle tissue of tilapia [32,33]; these data demonstrate the importance of the monitoring of cyanotoxins in water and fish [1].

Always have been attributed to the microalgae the responsibility of the production of the majority of compounds causing taste and odor in water. It is now known that actinomycetes, mainly of the genus Streptomyces are also involved in the production of these compounds [34].

The mechanisms linked to the production of *off flavour* compounds, follow two paths; the first is directly related to the death of microalgae and subsequent release to the liquid phase, of metabolic compounds, among them the geosmin (GEO) and methyl-isoborneol (MIB). The second mechanism is related to degradation of dead cellular material, which can serve as substrate for other microorganisms, in particular, the actinomycetes which synthesize compounds that cause taste and odor [1].

The GEO gives the fish taste "muddy" and / or smell "wet earth", MIB promotes the musty odor even at low concentration[35]. These volatile lipophilic substances, if present in the culture water can be absorbed and bioaccumulate in the fish.

As tilapia absorbs the metabolites produced by algae and other microorganisms, an alternative to ensure the quality of this species is to keep it in clean water for three to five days, when debugging is made in order to minimize and/or eliminate *off flavour* [36].

Fish exposed to the MIB and GEO acquire *off flavour* in a few hours. However, the removal of these compounds can take several days or even weeks. Fish with *off flavour* can be debugged in tanks receiving a continuous flow of clean water [1].

As the tilapia also absorbs off flavour produced by cyanobacteria and other microorganisms, to ensure the quality of tilapia, it must be maintained in clean water for three to five days to depuration the *off flavour* [36]. The number of days that the fish must be submitted for depuration, will depend on

the amount of *off flavour* compounds present in water, as well as its temperature, which influence the process of water filtration by tilapia.

Another problem encountered in the ponds used for tilapia culture is contamination by inorganic molecules, this contamination is probably borne by agriculture, industrial and municipal sewage, water weeds control and disease vectors transactions, or else, on the occasion of accidents [37].

Among the factors that can influence the bioaccumulation of active substances within aquatic systems are water solubility, fat solubility and stability to degradation of the active ingredient in question, whereas the degradation of pesticides, usually expressed by the half-life of the compound in the soil is very variable [1,37].

The water monitoring should be performed periodically, using portable electronic equipment to own field, to ensure a better quality of production. It is a starting point to be deployed traceability in the supply chain in order to obtain quality-processed product.

3. HANDLING AND PROCESSING

Seeking to feed adequately, is among the main concerns of the human being, especially with regard to food quality, nutrient intake for the body's proper functioning and safety of the food being consumed [38].

Fish displays relevant nutritional characteristics, which are an excellent source of proteins, lipids, vitamins and minerals. The protein fraction has all the essential amino acids and lipids, with a number of fatty acids which, apart from its nutritional importance, are also beneficial to health; the low percentage of saturated fatty acids and a high level of poly-unsaturated fatty acids, among which are the series omega-3 long chain. The fisheries products are, overall, a food easily digestible and has low levels of cholesterol [39].

The rich nutritional composition of fish, however, makes it easily degraded by microorganisms and actions of endogenous and/or exogenous enzymes, which begin their activities immediately after the death of fish. Thus, it becomes inevitable the processing of the raw material when it is intended to extend the shelf life of the product.

The tilapia culture has established itself as a successful activity in various regions of the world, therefore, the processing industries should expend efforts for development of products that absorb this production and at the same time, meet the expectations of consumers.

Thus, theprocessing industries of fish play a key role in market research, development, production and distribution of products made of fish that meet the demands of the consumers, who is looking for practical products, convenience, high nutritional value and shelf life, without loss of sensory and healthful characteristics.

In addition, theprocessing of fish also aims to optimize the income of the industrial sector, providing higher profits and also more sustainable production chain, since the industry wastes can be reused for the development of co-products and/or intended proper purposes, without prejudice to the environment.

The development of products and coproducts ends up constituting an essential factor for the survival of food companies that needs to be always launching new products to stay ahead of competition and active in the market. However, for a new product reaches the consumer it is necessary to go a long way, through the identification of the needs or desires of the consumer, the product concept development, adequacy of concepts to business strategy, product development, market testing, and finally launching and monitoring product. These activities require human resources management, time and financial resources [40].

Minimal fishing processingaims at marketing of fish in nature, benefited, from basic procedures of gutting, heading, scaling and filleting or different cuts; even the most elaborate and minced products (mechanically separated meat) of fish, which is the basis for the preparation of processed meats in general, sausage, fishburgers, nuggets, *surimi* and *kamaboko*. For the development of any products and proper completion thereof, modified atmosphere packaging machines (ATM) and vacuum, as well as refrigeration or freezing, are critical. Also being others options for introducing in the processes, smoking, acidification, depuration and irradiation as supporting promoters of longer shelf life.

Therefore, the supply of products derived from fish stemmed lines of diversified processing may contribute to the increased consumption of fish [41].

For the development of fish products, the quality control with the raw material should start in production, with the establishment of Best Management Practices, which should be monitored: the quality of the pond water, the practice of feeding, nutritional and sanitary quality of ration fed to animals, capture processes, stunning and slaughter.

Best Management Practices has as main objective to minimize the stress at which these animals are subjected to, thus preserving their energy reserves of

glycogen in order to delay the onset of rigor mortis and / or prolong the permanency, which will also ensure better quality of physicochemical and sensory characteristics of the final meat.

After fulfilling all of these requirements, the fish will follow in ideal conditions for the processing industry The product marketed refrigerated or frozen still need to undergo handling, that require time and generate inedible waste, as in the case of gutted whole fish. In the industrial processing is possible to obtain sophisticated products, which will be submitted to fully automated processes, have longer shelf life and diversity of presentation to the consumer, for example, minced, *surimi* and its derivatives.

In the literature, the following average values of proximate composition of whole Nile tilapia *in natura* are reported: 70.84% moisture, 19.20% protein, 8.06% fat and 3.41% ash. For fillets of the same species, also *in natura*, these values were found: 77.91% moisture, 25.65% protein, 2.55% fat and 1.04% ash [42]. In another study that evaluated fillets of tilapia *in natura*, both for control treatments and for treatments subjected to 1 or 5 days of depuration, the values were 75.95 to 76.78% moisture, 17.40 to 18.07% protein, 3.15 to 3.67% fat and 0.91 to 0.96 ash [43].

In the flowchart below (Figure 1) there is some possible alternative processing of tilapia in an industry. It appears that the options can come from traditional technologies such as salting and drying, smoking, chilling, freezing and canning; and emerging technologies, such as concentrated protein-minced and *surimi*, irradiation and protein hydrolysates-fermented and silage.

Smoking is is the *process* of flavoring, cooking, or preserving food by exposing it to *smoke* from burning or smoldering material, most often wood to longer shelf life. Nevertheless, the quality of raw materials and handling technique, respecting the rules of good manufacturing practices, are essential to achieving a quality product. The technique of smoking can be combined with different types of presentation such as smoked fish whole gutted, fillets, or put other types of cuts that end up offering greater practicality and convenience to the product [44].

Being a freshwater species, tilapia is more susceptible to absorbing chemicals from the environment, resulting in the development of *off flavor* one of the causes of rejection by the consumer, therefore, smoking is an alternative to reduce these unwanted aromas [45].
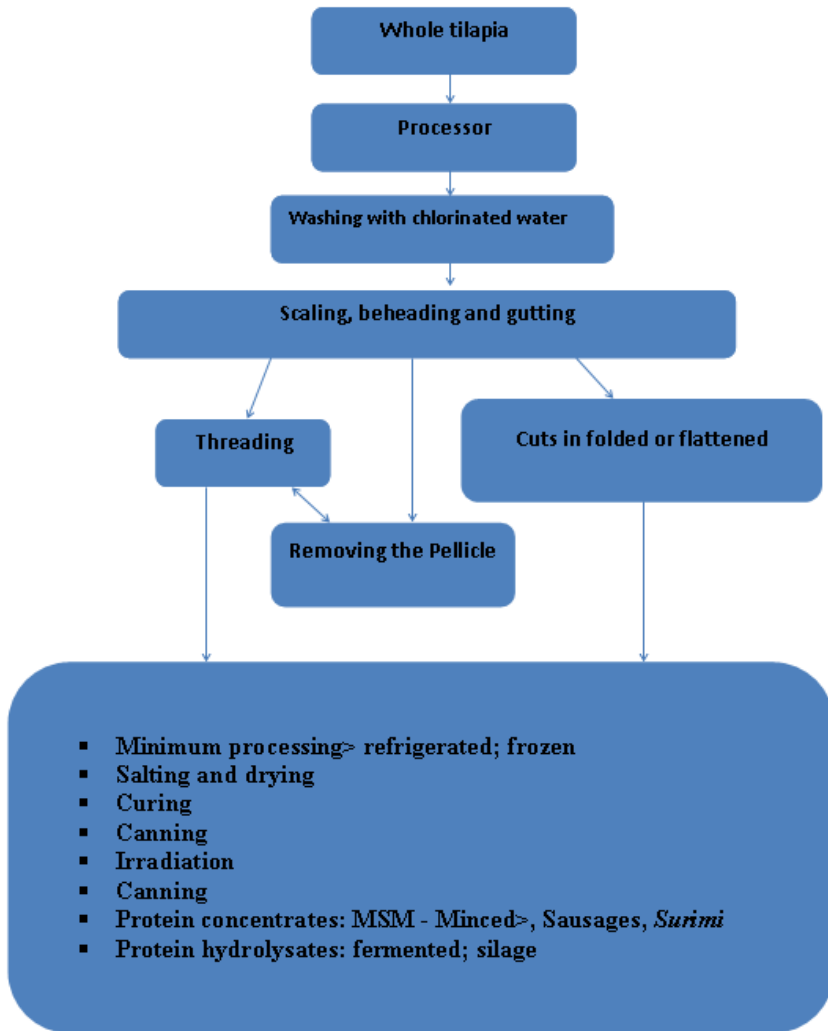
Figure 1. Flowchart of possibilities for tilapia processing.

In an experiment conducted with fillets of Nile tilapia gutted, , *in natura* and smoked, it was observed that the smoking process provided weight loss in some products, which were higher in fillets than in gutted whole fish. In relation to sensory attributes, the smoked fillet had better general acceptance, especially regarding the appearance attribute, although the presentation of smoked whole fish has had greater acceptance for flavor and salt content. With the smoking process, there were changes in the percentages of the components

of the raw material *in natura*, therefore, to reduce moisture from 70.84 to 57.18% and from 77.91 to 63%, respectively, for gutted whole fish and fillet, the protein content went from 6.07 to 7.39%; lipids from 3.25 to 1.92% and ash 3.89 to 4.09% respectively [42].

The canning technology is now widespread throughout the world and is a technique that enables the extension of the shelf life of the fish for many months. The heat generated during cooking and sterilizing cans eliminates all microorganisms capable of reproduction over a long period of storage. Moreover, the final product retains nutrients, aroma and flavor. This processing is widely known and used for marine species, especially tuna and sardines, but currently already exist in academic research and even pilot tests being developed for the canning industry in the tilapia.

In a survey, when assessing sensory, different species of freshwater fish, tilapia (*Oreochromis niloticus*), tetra (*Astyanax spp*) and pacu (*Piaractus mesopotamicus*), submitted to the canning process, there was good acceptance of the products. The majority of the tasters responded that the traditional sardine could be replaced by these species without any rejection of the product. From the species tested, the one that stood out in all the attributes evaluated was the pacu. The tilapia was last in preference of respondents because it had a strong and bitter taste compared to the pacu [46].

The MSM - mechanically separated meat is the first step to obtain *surimi*, which is defined as the flesh of fish suffered several washings with water at temperature 5 to 10 ^{0} C, in order to removal of sarcoplasmic proteins, odoriferous substances and fat; this product washed cryoprotectant substances are added to maintain the elasticity and prevent protein denaturation. From the *surimi*, there can be prepared many different products such as *kamaboko*, which is characterized as a thermostable protein gel with additives that are added to protect the myofibrillar protein structure and improvement of the physical properties of the gel [47, 48].

Apply these types of processing, increase the possibilities of having different forms of presentation as well the availability and regularity of fish products being offered to consumers. There is also an optimization of the company's profits and a reduction of waste generated, since these products can be obtained from the carcass resulting from the filleting process, which also has a large amount of meat attached.

In a study where four different formulations of tilapia fishburguer were prepared (*Oreochromis spp.*) - two of them, the tilapia´s minced was the principal ingredient and the other two was formulated with *surimi*, whereas in one of the formulations each raw material was added flavor of smoked fish -

the samples were subjected to microbiological, physico-chemical and sensory evaluation to characterization of final products. It was found that all samples met the microbiological standards established by current legislation. As for proximate composition, significant difference was observed only between the percentages of lipid samples, probably by the type of feedstock used, since during the washing cycles in the processing of *surimi*, the supernatant fat was removed. The mean values for moisture, protein, lipid and ash were: 75,34; 18,24; 4.7 and 2.12 g/100g, for prepared with minced fish, and 74.24; 17,02; 0.48 and 1.30, for prepared with *surimi*. A significant difference between the samples evaluated by instrumental analysis, in relation to the texture was observed. Both fish-burger made with *surimi* as elaborated with minced fish, were sensory acceptance for flavor, texture and overall, the second of which received top marks in the sensory test, and no statistical difference in acceptance testing between samples that received adding flavor of smoked [49].

In another study, was evaluated the characteristics of *kamaboko* gels made with tilapia *surimi* obtained from the residual ridge of the filleting line. The gels were prepared by adding ingredients in different combinations of salt, starch, carrageenan and dehydrated yolk egg. Then, the samples were submitted to heating in a bain marie with the water temperature maintained at 95°C for 15 and 30 minutes. All samples were in according with the standard limits by law. The *kamaboko* gel samples prepared with *surimi* tilapia showed low lipid content of 0.35% to 0.76% and high protein content of 15.31% to 21.67%, with no overall significant difference between the percentages of protein samples. In sensory analysis, samples with added starch and carrageenan and with added starch and egg white were those that received top scores for attributes firmness, elasticity and chewiness, showed a strong positive correlation between the results of sensory analysis and instrumental analysis [48].

Minced fish is also a raw material for preparation of breaded, processed meats and fish burgers, which are usually well accepted by consumers, especially the characteristics of practicality and convenience of preparation that these products present.

In a study concerning the use of MSM and scrap cut in "V", tilapia fillets, in preparing fish ball usually consumed product in gatherings and parties, it was observed that this process may be feasible. Being verified a higher yield of approximately 11.25% to the fish ball, obtained from cuttings cut in "V" after the process breading and pre-frying, compared to the fish ball made from MSM tilapia, which had a yield of 9.31%. For MSM average values of 79.05%

moisture, 14.63% protein, 0.87% ash, 4.66% lipids and for chips were found, respectively, 81.27, 14.53, 1.13 and 1.93%. For pre-fried fish ball of MSM, were found 57.93% moisture, 15.11% protein, 11.59% ash and 3.36% fat and fried croquettes pre-paring 57.84, 15.34, 9.17 and 3.95%, respectively. These parameters are within recommended by the legislation and also the values reported in the literature. The results of the microbiological analysis of raw materials and pre-fried fish ball were also satisfactory. And the test showed that both fish ball (made with MSM or with chips) were accepted by the tasters moderately, with no significant differences in the characteristic flavor of fish, texture and color of the product. Thus, the preparation of fish ball and breaded in general can be a way of adding value to fish waste [50].

One criticism of breaded products refers to the high sodium content that these products present, mainly by addition of bread flour and other seasonings. However, in a study evaluating the partial replacement of sodium in Tilapia restructured steaks with microbial transglutaminase, it was observed that substitution level of 50% in different formulations with $NaCl + KCl$ and $NaCl + MgCl2$, provided less product acceptance end of the tasters, as these salts affect, besides the taste, the parameters of juiciness and tenderness of the steaks. However, there was no reduction in acceptance rate of finished products with the combination of $NaCl + KCl + MgCl_2$ compared to traditional drawn only with NaCl. For this experiment, polyphosphates, a mixture of spices, onion and garlic powder, water, transglutaminase, and different types of salts were mixed with fillets of Nile tilapia noncommercial sizes. The samples with the combination of the three salts were similar in softness and a higher juiciness and reduced cohesiveness The treatments did not differ when evaluated raw or cooked, as color, taste of tilapia, tilapia flavor, texture and overall. This suggests that although salt replacers have promoted small changes in the results of reviews of instrumental color and texture, the participants of the sensory analysis were not able to differentiate the color attributes and overall texture between the analyzed samples [51].

From the MSM, is also possible to obtain protein concentrate, featured often as a byproduct of the processing of fish. Is usually presented in dried form, and is also known as fish flour for human consumption. According to Souza [52], the product has an average of 75% protein and arose from the attempt to obtain a more concentrated product in terms of protein and that would meet some basic requirements such as chemically stable, low cost, small amounts of moisture and fat, deodorized, easy storage and high digestibility.

On a characterization of fish protein concentrates (FPC) obtained from filleting waste of Nile tilapia, the authors of this paper obtained the first MSM (mechanically separated meat) from the housing resulting from the filleting process. After deodorization process of MSM in solution with 0.002% phosphoric acid, the obtained material was dried and ground to a powder with uniform particle size. The MSM and FPC obtained, respectively, the following percentages of proximate composition: moisture 77.24 and 4.85%; Protein 17.48% and 85.16; lipids of 4.46 and 8.2%; and ash of 1.02 and 2.45%. In the sensory evaluation of the FCP, the general appearance and color attributes obtained the following percentages, respectively, 46.67 and 60%, the acceptable range of the scale. The aroma had great rejection, reaching a frequency of 70%. However, the authors of this study noted that this aroma of fish does not invalidate the use of FPC in the protein enrichment of foods since it will disguise this characteristic in the food with the use of other ingredients [53].

In another similar study, also using Nile tilapia to obtain FPC for human consumption, it was observed that the FPC obtained showed yield of 18.34%, low moisture levels (1.38%) and ashes (2.26%) and high protein content (62.39%) and lipid (32.63%), when compared to the original feedstock (MSM) and low Aw (0.16). In the sensory evaluation of this FPC, the rate of acceptance of the tasters was 60.4% for color, 51.1%, to general appearance and 41.7% to the aroma. The description of aroma in the terms as "characteristic of fish" was reported by 44.2% of the tasters, also suggesting the need for a partial deodorizing material and indicating a balance in relation to the group of the tasters who have not realized the characteristic odor. This research shows adding value to a material disposal, generating possibilities of offering an alternative protein source, of relatively low cost [54].

The protein hydrolyzate is another co-product that can be obtained in the tilapia beneficiation industry. Studies show that the antioxidant activity and sensory properties of hydrolyzed Nile tilapia may differ depending on the enzyme used in the process and the degree of hydrolysis of the product. Prepared hydrolysates elaborated with different commercial proteases (Alcalase (HA), Flavourzyme (HF), Protamex (HPR) and papain (HPA)) showed increases in antioxidant activity as well as increasing the degree of hydrolysis (DH), which reached up to 40% . Among all hydrolysates, whose used the HA protease showed the highest degree of hydrolysis (40%) and high antioxidant activity. When HA was further hydrolysed by papain, the resulting hydrolyzate (APAH) showed the highest antioxidant activity of all test trials .

HAPa showed higher acceptability in sensory tests, than HA, due to low bitterness of the samples [55] .

The use of packaging, active or with modified atmosphere, can also be an alternative to preserve the quality of the product, increase its shelf life and should be taken into consideration when defining how the tilapia should be processed in an industry. Overall, the use of different types of packaging always accompanies other processing technologies in order to maximize conservation actions and control product quality.

The consumer demand for high quality food, with "fresh" or "natural" and require minimal preparation, characteristics led to the development of convenience foods, ready to eat, and which are preserved by milder technologies. The minimally processed products are classic examples of this concept [44].

The main technique of preservation of minimally processed products is chilling, however, due to the difficulty in maintaining the lowlands along the entire chain of production, distribution and storage temperatures as well as the very limited cooling, it is necessary to adopt other obstacles that control the growth of spoilage or pathogenic micro-organisms [56].

To obtain minimally processed tilapia, modified atmosphere packaging may be used, either by inclusion of gases (N_2, CO_2, O_2) or their withdrawal (vacuum packaging) [44].

In an experiment conducted with fillets of tilapia, the authors submitted the samples to different treatments: control (no treatment); immersion in acetic acid; vacuum packing; immersion in acetic acid + vacuum packing; modified atmosphere packaging (MAP), (60% CO2, 40% O2); and immersion in acetic acid + Packing in MAP. By quantifying the Volatile Base Nitrogen Totals (VNBT) present in the samples, it was observed that in all there was an increase in the value displayed throughout the storage period (20 days), however, the samples subjected to the treatment of MAP + acetic acid were those that showed less variation, 12.13 mg N- VNBT/100g on Day 1 storage to 14.93 mg N- VNBT/100g, on the 20th day of storage. Meanwhile, the control samples showed: N- VNBT/100g 14 mg on Day 1 storage and 18.90 mg N-VNBT/100g on the 20th day of storage [60].

In another study, fillets of Nile tilapia were treated by immersing in acetic acid solution and It was also made packing with vacuum, combined with different types of packaging (poly nylon, polyethylene and expanded polystyrene covered with plastic wrap (control). In this experiment it was observed that all samples had excellent freshness condition after 21 days of storage, with regard to the analysis of VNBT, no statistical difference was

observed between the mean values among the different treatments and different days storage evaluated. The highest values were observed for the control samples at 21 ^{0} day of storage, and 14 mg 10.98 N- VNBT /100g [43].

Regarding the sensory tests performed in the last two papers, there are some restrictions, especially for the samples subjected to treatment with acetic acid solution.

In sensory analysis experiment with steaks subjected to pretreatment with acetic acid solution and packed in MAP these were considered unacceptable for consumption, from the 7th day, with notes lower to 5 for the attributes color, aroma and appearance. Since the first day of evaluation, the tasters realized that the fillets were more tender texture, whitish coloring and whitish exudation, when compared to other treatments; some tasters perceived rancidity in treatments with acetic acid, MAP and MAP + acetic acid being associated with the increase in TBARS values. In the vacuum treatment + acetic acid, the presence of rancid was detected only after 20 days evaluation, probably due to lack of O_2 in packaging retarding the oxidative process of polyunsaturated fatty acids [57].

In the second experiment reported, the highest scores in sensory evaluation of minimally processed tilapia fillets, were assigned to the CN (not acidified fillets in packs of poly nylon vacuum) and CP treatments (do not acidified fillets in polyethylene packaging under vacuum) and the lowest, the AN treatments (acidified fillets in packs of poly nylon vacuum) and AP (acidified fillets in polyethylene packaging under vacuum) due to discoloration of fillets, caused by treatment with acetic acid. For tasters, samples AN and AP are among the classification "dislike slightly" to "not liked nor disliked". On the other hand, the CN samples (not acidified fillets in packs of poly nylon vacuum) and CP (do not acidified fillets in polyethylene packaging under vacuum) are among the classifications "enjoyed regular" and "like moderately", indicating the preferably these treatments and packages for consumers [43].

In another study, in which the aim was to evaluate the effects of the processes of irradiation (with a dose of 1.5 kGy) and modified atmosphere packaging (60% N_2 and 40% CO_2), applied alone or in combination, to the extent of the validity commercial fillets of Nile Tilapia cooled and stored for 20 days. The results indicates a progressive reduction in the quality of the product with the retention time, which was significantly faster in the control samples (not subjected to any treatment) than in those subjected to other treatments, particularly the combined treatment (irradiation + modified atmosphere packaging) an extension was observed in the commercial validity

of fillets packaged in modified and / or irradiated 4 days to about two weeks atmosphere. The mean values of Total Volatile Base Nitrogen (TVBN) in fillets control, for example, achieved average 30.87 mg N- TVBN /100g on the sixth day of storage, while the results of samples submitted for other treatments reached values close to those only in 13 (31.5 mg N- TVBN /100g), 15 (30.87 mg N- TVBN /100g) and 18th day (30.87 mg N- TVBN /100g), respectively for treatments packaged in modified atmosphere (40% CO_2 and 60% N_2), irradiation at a dose of 1.5 kGy samples submitted and the combination of these two treatments [58].

4. CONSUMER MARKET FOR FISH - A CASE STUDY OF BRAZIL AND PORTUGAL

The demand for animal protein is increasing significantly in the world, replacing part of the supply stemmed from plant proteins. There is strong correlation between increased income and greater consumption of meat, which, from the reach of a critical point, no more changes can be observed. More mature and developed markets, such as North America and Europe, are close to that point and must not show significant growth. Thus, the largest increase in demand is occurring in emerging markets. In countries like China, India and Brazil, with quantitative high population, the increase in the purchasing power of the poorer sections of the population allowed improvement in diets [59].

Therefore, fish and its derivatives have been gaining prominence in consumer preference that seeks a superior nutritional values compared to other types of meat [60].

However, the striking socioeconomic and cultural differences among consumers within a county, state and country, must be considered. These differences are what determine the different levels of perception each group of consumers have about food, especially the fish. Fish products come from dozens of species and presented in different ways, making the perception of quality even more complex, being constantly shaped by the consumer for each of the products of particular interest [61].

Nonetheless, consumers are more discerning in their choices as to the items of their diet. Food quality issues moved to the forefront of consumer concerns, as part of the strategies of the industry and in some cases also government policy. In Canada, for example, a variety of public policy

initiatives and private sector emerged with the objective to reduce information asymmetry in consumer safety and food quality, in part focused on the attributes of traceability [62].

Such initiatives should be encouraged, given that fish consumption is linked to health issues and these are the main attribute that arouses interest by consumers [63].

The most common misconception in the fish industry is the fact that the focus is on the construction industry and product, development centered on cost center and not the profit center in the case, the consumer or customer. The first task then would be to attract customers, know them, identify their priorities, and then develop loyalty strategies [59].

There is, therefore, the absence of effective policy strategies to organize and promote the development of sector. It is possible to infer that the research on the subject, besides scattered geographically, are characterized by a lack of integration between the sectors that make up the various links of the production chain.

In relation to fish consumption in general, difficulties in quantifying consumption are allocated by the absence of an instrument to collect data that can be used in different languages. Thus, most of the data available are those developed based on the marketing of fish as the data from the Food and Agriculture Organization (FAO) and, in the case of Brazil, the Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatística, IBGE) and the Ministry of Fisheries and Aquaculture (Ministério da Pesca e Aquicultura, MPA).

This difficulty has stimulated the development of proposals for assessment of fish consumption in Brazil and Portugal, reinforcing the premise of the need to develop an instrument to assess fish consumption in context, taking into account the collection of information related to the habit of consumer and preferences of species and preparation methods [64, 65].

Accordingly, we developed an instrument to collect data to assess the perception of fish consumption and its main features, consisting of items that determine the profile of the consumer; consumption habits of fish; desirable characteristics or attributes of the product; factors affecting the purchasing decision; expectation when buying fish tracked; relationship between fish consumption, quality of life and health [64, 66].

Therefore, the construction of the instrument kept in check different stages of development, including technical analysis by a panel of experts in the area, construction of data collection (online), pre-test and analysis system, with the

technique multivariate data analysis, aiming to validate the scale and the constructs of the instrument for final implementation [67] (HAIR Jr, 2009).

Initially the instrument was made available to the University of São Paulo community for validation and review [66].

In this section of the chapter, we present the results regarding the association between nutritional value and importance of marketing-related attributes of the fish.

Subsequently, the instrument was adapted and made available through the Survey Monkey (http://pt.surveymonkey.net/home/) system for consumers in Brazil and Portugal, which could complement our results and allowed us to identify the most preferred species in time of purchase and consumption of fish.

The Survey Monkey system allowed a web interface administrator password for monitoring and sending data. The data are compiled in tables of numerical format. The system is designed to allow the respondent to forfeit from responding at any time, and in the case of responses computed as incomplete questionnaires and, if the case be chosen to respond later, the subject must access the tool again and complete filling.

A home page (http://pt.surveymonkey.com/s/pescado) with a specific link for data collection, which contained information about the research and contact with researchers, and a link for the Research Ethics Committee (CEP) of "Luiz de Queiroz" College of Agriculture was available, the agency that has evaluated and approved the execution of this research.

In this last stage, anyone, age 18, resident in Brazil or Portugal could participate, access links were disclosed on social networks and institutional sites of partner organizations. Thus, the data in all stages were collected through non-probability convenience sampling.

The results were analyzed descriptively and, for this chapter, we used the Spearman correlation analysis between variables: the importance of the nutritional value of fish and the importance of packaging items, brand, origin and availability. Thereby, the hypothesis that the greater the consumer interest by nutritional value of fish was used, consumers would also attach greater importance and concern for packaging items, brand, origin and availability of the product, showing the importance of the investments relation in all parts of the chain to ensure a product that meets the requirements, and especially the consumer expectation.

Statistical tests were performed using SPSS software and included adherence to normal Gaussian distribution, descriptive analysis, frequency analysis and correlation test.

Among the results, it was found that the average age of the volunteers of the research is quite heterogeneous and women are mostly the ones that worked with data collections in both countries (Table 4). Although, there is some similarity between the groups, the number of people who participated in the research in Brazil is substantially higher than those who did so in Portugal.

Table 4. Characteristics of survey participants from Brazil and Portugal

	Brazil 2010.	Brazil 2012.	Portugal 2012
Average age	30,42 (DP 12,41)	16,31 (DP 11,34)	27,16 (DP 9,78)
Income	*	5,80(DP 2,01) SM**	4,96(DP 1,65) SM***
Gender		%	%
Female	61.6	53,7	53.0
Male	38.4,	41.0	41.7
N	1966	944	132

* Not rated ** minimum wage = R $ 622.00 *** minimum wage = 485 Euros.

Regarding the frequency of fish consumption, it is noticed that despite the differences between the number of participating countries, the majority (77%) of the subjects of Portugal has the habit of consuming fish more than twice a week, while in Brazil most participants dispersed between once a week and two to three times a month (Table 5). When performed the correlation test (Table 6) it is noticed that the importance attributed by the consumer to the nutritional value of fish has a very strong positive statistically significant association [68]. With the items importance of packaging, the product brand, the origin of the product and the availability in places of purchase to the research conducted in Brazil in 2010 (n = 1966) in the university community.

As for research in Brazil in 2012, the results indicate a statistically significant correlation, but low to moderate [68] intensity between the importance of the nutritional value and importance of packaging, product branding and product source. Statistically significant correlation was found in the survey with the Portuguese, however, low intensity importance between nutritional value and importance of packaging.

These results suggest that there is an understanding of the necessity of relating to product quality (packaging, brand, origin and availability) are linked to a higher nutritional value attributes in the group of the university community in Brazil, identifying a product with such features as higher quality.

Table 5. Frequency of fish consumption

Researches	Frequency							Missing data
	2 or more x/week	1x/week	2- 3x/month	1x/month	Rarely	No.	Total	
Brazil - 2010	187 [9.51]	533 [27.11]	510 [25.94]	344 [17.49]	291 [14.80]	101 [5.13]	1966 [100]	0
Brazil -2012.	156 [12.0]	372 [28.7]	316 [24.4]	178 [13.7]	170 [13.1]	23 [1.8]	1215 [93.8]	80 [6.2]
Portugal- 2012	134 [77.0]	23 [13.2]	8 [4.6]	3 [1.7]	0 [0]	2 [1.1]	170 [97.7]	4 [2.3]

Values in brackets represent the percentage

For Brazilians who participated in the study in 2012, which forms part of different regions and cultures within the same country, there seems to be a very strong relationship with the nutritional value of fish and the quality of the product. A similar result is found in Portugal, however, for this country, this result can be attributed to the strengthening of the production chain and high quality marketing products, and of course the strong habit of eating fish practiced in this country, which seems to be predominant quality requirements.

Table 6. Spearman's correlation test (r)

Importance:	Correlation Coefficient Sperman r		
Fish nutritional value	Brazil 2010	Brazil 2012	Portugal 2012
packaging	0.939(**)	0.201(**)	0.227(**)
brand	0.937(**)	0.287(**)	0.002
products origin	0.945(**)	0.335(**)	0.138
availability	0.939(**)	0.029	0.080
in	1.966	951	145

** p value =<0,000.

While it is clear that the health effects attributed to fish are the main reason for the recommendations of its consumption [63] it is important to highlight that in certain regions of Brazil, the fish is the main source of animal protein available, as in adjacent regions within the country, this consumption is linked to more economic and social issues than the nutritional recommendations.

A study in Norway among women 30-44 years showed that consumption of fish increases with age. The price was identified as an important attribute imposing barrier to total fish consumption [69].

In research conducted in Belgium, Netherlands, Denmark, Poland and Spain, aiming to identify potential differences in fish consumption, was observed that consumers believed that eating fish is healthy. In the same way, the level of education and age contribute to influence the frequency of consumption, however, when these variables were correlated with consumption the result shows a relationship of low association, indicating that consumption is also influenced by other factors.

Table 7. The five most preferred species by Brazilians and Portuguese consumers

Brazil											
1st option	species	in	%	2nd option	species	in	%	3rd option	species	in	%
1	Salmon	168	21.59	1	Salmon	106	14.83	1	Salmon	107	15.88
2	Tilapia	157	20.18	2	Tilapia	97	13.57	2	Tilapia	82	12.17
3	Hake	101	12.98	3	Hake	92	12.87	3	Hake	66	9.79
4	Whitefish	52	6.68	4	Tuna	56	7.83	4	Whitefish	65	9.64
5	Sardine	40	5.14	5	Whitefish	50	6.99	5	Sardine	56	8.31
Portugal											
1	Whitefish	47	35.34	1	Salmon	25	18.94	1	Salmon	25	19,53
2	Salmon	19	14.29	2	Dourada	22	16.67	2	Bass	21	16.41
3	Bass	17	12.78	3	Whitefish	20	15.15	3	Whitefish	16	12.50
4	Sardine	16	12.03	4	Bass	13	9.85	4	Cod	15	11.72
5	Dourada	10	7.52	5	Sardine	12	9.09	5	Mackerel	10	7.81

The authors suggest that the isolated communication seems to be insufficient to achieve a higher level of compliance with the recommendations for fish consumption, but, recommend that associate the information regarding the perceived benefits related to health may be a more efficient way [70].

Regarding the most preferred species by the participants of the survey in Brazil and in Portugal in 2012 (Table 7) where it was requested that volunteers to list three species in order of preference, the results demonstrates that in both countries the salmon stands as the preferred option.

Nevertheless, it is important to note that the Tilapia occupies a prominent place in Brazil, presenting itself as the second species most preferred by consumers.

These results reinforce the assumptions discussed in this chapter on the importance of this species in the fish trade from Brazil and reinforce the importance of joint actions in every link of the production chain, as well as all the advantages attributed to its cultivation and processing, also adds to consumer preference for this species.

CONCLUSION

Tilapia is a species with favorable adaptation and with great industrial potential. However, we must consider that fish consumption in Brazil is still small.

Thus, it is understood that two main points have to be consider in the sector; the first relates to the need to increase the number of consumers of fish, even more than the per capita quantity consumed; the second refers to the strengthening of the production and distribution chain, identifying interests of consumers and supply of accessible and easy to prepare fish products. Therefore, it is necessary to develop new technologies and products and the transfer thereof to the producers and/or benefactors.

There is still a need for standardization of the aforementioned processes of marketing, the only way to optimize the product and allow for monitoring, implementing the recommended national traceability of fish, and the development of a computerized system which complies with the international market trends to come across market internal and external discerning, focused on quality and safety of the product.

The actions mentioned should be permeated by the concepts of sustainability, in this sense, includes the development of waste disposal, use of by-products arising from the fish, production management, and compliance

with existing environmental standards throughout the production process of fish technology . This initiative, so evident in other sectors, strengthens the sector, corresponds to a collective social desire and allows the desired balance between human actions and the environment.

REFERENCES

[1]   Galvão, J.A.; Oetterer, M.; Matthiensen, A. Sustentabilidade na produção do pescado: qualidade da água. In: Galvão, J.A.; Oetterer, M., editors. Qualidade e processamento do pescado. 1 Edition. Rio de Janeiro: *Elsevier,* 2014: 1- 30

[2]   Pereira, G.P.C; Pequito,M.M.A.; Costa, P.C.R.C. O impacto do incremento das cianobactérias como indicador de toxidade, 1999. 2014 May, 18. Available in: www.fmv.utl.pt/democ/sht/sem9899/G005.htm

[3]   Brunson, M. W., Lutz, C. G., & Durborow, R. M. (1994). Algae blooms in commercial fish production ponds. *Southern Regional Aquaculture Center.*

[4]   Cardoso Filho, R.; Campeche D. F. B.; Paulino R. V. Tilápia em reservatório de água para irrigação e avaliação da qualidade da água. *Revista Brasileira de Ciências Agrárias.* 2010. 5 (1): 117-122

[5]   Galvao, J.A. Rastreabilidade da cadeia produtiva do pescado: avaliação de parâmetros ambientais e sua influência na qualidade da matéria-prima destinada à indústria. *Tese de Doutorado.* Piracicaba: ESALQ /USP, 2011.

[6]   Kubitza, F. Qualidade da água no cultivo de peixes e camarões. *Jundiaí: Degaspari,* 2003.

[7]   Brasil. Conselho Nacional do Meio Ambiente – CONAMA. Resolução nº 357, de 17 de março de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. Available at: http://www.mma.gov.br/port/conama /res05 /res35705.pdf

[8]   Shelton, W.L.; Popma, T.J. Biology. In: Chhorn, L.; Webster, C.D., editors. Tilapia: biology, culture and nutrition. Chicago: *Food Products Press,* 2006: 1-49.

[9]   Justi, K.M.; Padre, R.G.; Hayashi, C.; Soares, C.M.; Visentainer, J.V. Souza, N.E.; Matsushita, M. Efeito da temperatura da água sobre

desempenho e perfil de ácidos graxos de tilápia do Nilo (*Oreochromis niloticus*). *Acta Sci. Anim. Sci.,* 2005; 27 (4): 529-534.

[10] Salvador, R.; Muller, E.E.; Leonhardt, J.H.; Pretto-Giordano, L.G.; Dias, J.A.; Freitas, J.C.; Moreno, A.M. Isolamento de *Streptococcus* spp de tilápias do Nilo (*Oreochromis niloticus*) e qualidade da água de tanques rede na Região Norte do Estado do Paraná, Brasil. *Semina: Ciências Agrárias,* 2003. 24 (1): 35-42.

[11] Boyd, C.E. Manejo do solo e da qualidade da água em viveiros para aquicultura. *Campinas: Mogiana Alimentos*. 1997

[12] Toledo, J.J.; Castro, J.G.D. Parâmetros físico-químicos da água em viveiros da estação de piscicultura de Alta Floresta, Mato Grosso. *Revista de Biologia e Ciências da Terra,* 2001. 1 (3): 2-10.

[13] Buttner, J.K.; Soderberg, R.W.; Terlizzi, D.E. An introduction to water chemistry in freshwater aquaculture. North Darmouth, Massachusetts: Norteastern Regional Aquaculture Center, University of Massachusets, 1993 (NRAC Fact Sheet, 170).

[14] Boyd, C.E. Water Quality Management for pond fish culture. New York: *Elsevier.* 1990.

[15] Kubitza, F. Uma coleção de artigos sobre tilápia. Canada: Southern Ocean Education and Development Project, CIDA/Univ. of Victoria, 2009.

[16] Conceição, C.H.Z. Eficiência da pré-filtração e filtração lenta no controle das características químicas, físicas e biológicas da água para piscicultura. Dissertação (Mestrado), Universidade Estadual de Campinas, 2002.

[17] Daud, S.K.; Hasbollah, D.; Law, A.T. Effects of unionized ammonia on red tilapia (*Oreochromis mossambicus/O. niloticus* hybrid) fry. In: INTERNATIONAL SYMPOSIUM ON TILAPIA IN AQUACULTURE, 2., 1987, Bangkok, Thailand. Proceedings… Mnila, Philippines: *ICLARM,* 1988: 411-413.

[18] Molle, F.; Cadier, E. Manual do pequeno açude. Recife: SUDENE/Orstom/Tapi, 1992.

[19] Ross, B.; Ross, L.G. The oxygen requirements of *Oreochromis niloticus* under adverse conditions. In: INTERNATIONAL SYMPOSIUM ON TILAPIA IN AQUACULTURE, 1983, Tel Aviv, Israel. Proceedings... Tel Aviv, Israel: Tel Aviv University, 1983: 134-143

[20] Barbosa, A.C.A.; Almeida, L.D.L.; Fonseca, R.B. Cultivo de Tilápia Nilótica em gaiolas flutuantes na Barragem de Assu-RN. 27 Edition.

Natal: EMPARN, 2000. 2014, May 18. Available at: file:///C:/Users/Lu %20Kimie/Downloads/cartilha_cultivo_de_tilapias_em_gaiolas.pdf

[21] Cavalcante, C.E.M. De H.; Silva, V.L. Da; Salgueiro, A.A. Avaliação microbiológica da água do riacho Cavouco, Recife – PE. *Higiene Alimentar.* 1998. 12 (57): 45-49.

[22] Guilherme, E.F.M.; Silva, J.A.M.Da; Otto, S.S. *Pseudomona aureginosa*, como indicador de contaminação hídrica. *Higiene Alimentar,* 2000. 14 (76): 43-47.

[23] NATIONAL ADVISORY COMMITTEE ON MICROBIOLOGICAL CRITERIA FOR FOODS. Microbiological criteria for raw molluscan shellfish. *Journal of Food Protection,* 1992. 55 (6): 463-480.

[24] Silva, N.Da; Neto, R.C.; Junqueira, V.C.A., et al. Manual de métodos de análise microbiológica da água. *Campinas: ITAL,* 2000.

[25] AMERICAN PUBLIC HEALTH ASSOCIATION. Standard Methods for the Examination of Water and Watestewater. 18 Edition. Washington: *APHA*, 1992

[26] Cook, D.W. Microbiology of bivalves molluscan shellfish. In: Ward, D.R.; Hackney, C., editors. Microbiology of marine food products. New York: Van *Nostrand Reinhold*, 1991. p.19-34:

[27] Branco, S.M. Hidrologia Aplicada à Engenharia Sanitária. 3 Edition. São Paulo: *CETESB/ ASCETESB*, 1986.

[28] Esteves, F.A. Fundamentos da limnologia. 2 Edition. Rio de Janeiro: *Interciência*, 1998.

[29] Figueredo, C.C.; Giani, A. Ecological interactions between Nile tilapia (*Oreochromis niloticus*, L.) and the phytoplanckton community of the Furnas Reservoir (Brazil). *Freshwater Biology*. 2005. 50: 1391-1403.

[30] Sivonen, K. Cyanobacterial toxins and toxin production. *Phycologia,* 1996. 35 (6 Supplement): 12-24.

[31] Kuiper-Goodman, T.; Falconer, I.; Fitzgerald, J. Human health aspects. In: Chorus, I.; J. Bartram, editors. Toxic Cyanobacteria in Water: a guide line to public healtth significance, monitoring and management. London and New York: *World Health Organization*, 1999: 113-154

[32] Magalhães, V. F.; Soares, R. M.; Azevedo, S. M. F. O. Microcystin contamination in fish from the Jacarepaguá Lagoon (Rio de Janeiro, Brazil): ecological implication and human health risk. *Toxicon,* 2000. 39: 1077-1085.

[33] Galvão, J.A.; Oetterer, M.; Bittencourt-Oliveira, M. C.; Gouvêa-Barros, S.; Hiller, S.; Erler, K.; Luckas, B.; Pinto, E.; Kujbida, P. Saxitoxins

acumulation by freshwater tilapia (*Oreochromis niloticus*) for human consumption. *Toxicon,* 2009. 54: 891-894.

[34] Petersen M. A.; Hyldig G.; Strobel, B. W.; Henriksen N. H.; Jørgensen, N. O. G. Chemical and Sensory Quantification of Geosmin and 2-Methylisoborneol in Rainbow Trout (*Oncorhynchus mykiss*) from Recirculated Aquacultures in Relation to Concentrations in Basin Water. *J. Agric. Food Chem.,* 2011**.** *59* (23): 12561-12568.

[35] Tucker, C.S.; Martin, J.F. Enviromental related off-flavors in fish. Water quality in aquaculture In: Tomaso, J.R.; Brune, D, editors. London: *World aquaculture books,* 1991: 133-179.

[36] Lovshin, L.L. Tilapia farming: a growing worldwide aquaculture industry. In: Cyrino, J.E.P.; Kubtiza, F. SIMPÖSIO SOBRE MANEJO E NUTRIÇÃO DE PEIXES. Piracicaba, 1997. Anais. Campinas: *CBNA,* 1997: 137-164.

[37] Dores, E.F.G.De C.; Freire, E.M.De.L. Contaminação do ambiente aquático por pesticidas: Vias de contaminação e dinâmica dos pesticidas no ambiente aquático. *Pesticidas: Revista de Ecotoxicologia e Meio Ambiente.* 1999. 9: 1-18.

[38] Castro-Gonzalez Mi, Mendez-Armenta M. Heavy metals: Implications associated to fish consumption. *Environmental Toxicology and Pharmacoly*. 2008. 26: 263-271.

[39] Bandarra, N. M.; Batista, I.; Nunes, M. L. O óleo de sardinha e a saúde. 18. Lisboa: *IPIMAR*, 2001.

[40] Wille, G. M. F. C. Desenvolvimento de novos produtos: As Melhores Práticas em Gestão de Projetos em Indústrias de Alimentos do Estado do Paraná. Tese (Doutorado em Tecnologia de Alimentos). Curitiba: Universidade Federal do Paraná. *Curitiba,* 2004.

[41] Ogawa, M; Maia, E.L. Manual da Pesca Ciência e tecnologia do pescado. São Paulo: *Varela*, 1999.

[42] Souza, M. L. R.; Baccarin, A. E.; Viegas, E.M.M; Kronka, S.N. Defumação da tilápia do Nilo *(Oreochromis niloticus*) inteira eviscerada e filé: aspectos referentes às características organolépticas, composição centesimal e perdas ocorridas no processamento. *Revista Brasileira de Zootecnia,* 2004. 33 (1): 27-36.

[43] Savay-Da-Silva, L. K. Desenvolvimento do produto de conveniência: tilápia (*Oreochromis niloticus*) refrigerada minimamente processada embalada a vácuo – padronização para a rastreabilidade. Dissertação (Mestrado). *Piracicaba: ESALQ/USP*, 2009.

[44] Oetterer, M.; Galvão, J.A.; Savay-Da-Silva, L.K. Tilápia: controle de qualidade, beneficiamento e industrialização. Radiações ionizantes e defumação. In: Galvão, J.A.; Oetterer, M. editors. Qualidade e processamento de pescado. 1 Edition. Rio de Janeiro, 2014b: 211-237.

[45] Biato, D.O. Detecção e controle do *off flavor* em Tilápia do Nilo (*Oreochromis niloticus*), por meio de depuração e defumação. Dissertação (Mestrado em Ciência e Tecnologia de Alimentos). *Piracicaba: ESALQ/USP*, 2005.

[46] Dutra, F.M.; Machado, W.J.; Caetano, M.S.; Gobbo, D'A. A. Avaliação sensorial do processamento em conserva, utilizando-se as espécies: tilápia (*Oreochromis niloticus*), lambari (*Astianax spp*) e pacu (*Piaractus mesopotamicus*). *Revista Brasileira de Produtos Agroindustriais,* 2012. 14 (3): 239-244.

[47] Oetterer, M.; Galvão, J.A.; Savay-Da-Silva, L.K. Tilápia: controle de qualidade, beneficiamento e industrialização. Tilápia minimamente processada. In: Galvão, J.A.; Oetterer, M., editors. Qualidade e processamento de pescado. 1 Edition. Rio de Janeiro, 2014a: 183-209.

[48] Mello, S.C.R.P. Caracterização físico-química, bacteriológica e sensorial de "fishburguer", e "*kamaboko*", obtidos da polpa e "*surimi*" de tilápia (*Oreochromis niloticus*). Tese (Doutorado). Rio de Janeiro: Universidade Federal Fluminense, 2009.

[49] Mello, S.C.R.P.; Freitas, M.Q.; São Clemente, S.C.; Franco, R.M.; Nogueira, E.B.; Freitas, D.D.G.C. Development and bacteriological, chemical and sensory characterization of fishburgers made of Tilapia minced meat and *surimi*. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2012. 64 (5): 1389-1397.

[50] Bordignon, A.C.; Souza, B.E.; Bohnenberger, L.; Hilbig, C.C.; Feiden, A.; Boscolo, W.R.. Elaboração de croquete de tilápia do Nilo (*Oreochromis niloticus*) a partir de MSM e aparas do corte em 'V' do filé e sua avaliação físico–química, microbiológica e sensorial. Acta Scientiarum. *Animal Sciences.* 2010. 32 (1): 109-116.

[51] Monteiro, M.L.G.; Mársico, E.T.; Canto, A.C.V.C.S.; Costa-Lima, B.R.C.; Lázaro, C.A.; Cruz, A.G.; Conte-Júnior, C.A. Partial sodium replacement in tilapia steak wirhout loss of acceptability. Food Science and Technology International, 2014. 2014 May, 20. Available in: file:///C:/Users/Lu%20Kimie/Downloads/Monteiro%20et%20al%20201 4%20FSTI-1082013214535229.pdf

[52] Souza, J. F.; Bittencout, N. N.; Gomes, C. S.; Oliveira, J. K. De; Santos, R. M. Dos.; Reis, I. A. O.; Nunes, M. L.; Narain, N. Desenvolvimento e

caracterização físico-química e sensorial de nuggets formulados com concentrado protéico de pescado – MARINE BEEF. *Scientia Plena,* 2010. 6 (3): 1-4.

[53] Rebouças, M.C.; Rodrigues, M.C.P.; Castro, R.J.S.; Vieira, J.M.M. Caracterização do concentrado proteico de peixe obtido a partir dos resíduos da filetagem de tilápia do Nilo. *Semina: Ciências Agrárias,* 2012. 33 (2): 697-704.

[54] Vidal, J.M.A.; Rodrigues, M.C.P.; Zapata, J.F.F.; Vieira, J.M.M. Concentrado proteico de resíduos da filetagem de tilápia-do-nilo (*Oreochromis niloticus)*: caracterização físico-química e aceitação sensorial. *Revista Ciencia Agronômica,* 2011. 42 (1): 92-99.

[55] Yarnpakdee, S; Benjakul, S.; Kristinsson, H.G.; Kishimura, H. Antioxidant and sensory properties of protein hydrolysate derived from Nile tilapia (*Oreochromis niloticus*) by one- and two-step hydrolysis. *Jounal of Food Science and Technololgy*, 2014: 1-14.

[56] Fellows, P.J. Tecnologia do processamento de alimentos: princípio e prática. *Porto Alegre: Artmed*, 2006.

[57] Soccol, M.C.H.; Oetterer, M. Gallo, C.R.; Spoto, M.H.F.; Biato, D.O.; Effects of modified atmosphere and vacuum on the shelflife of tilapia (*Oreochromis niloticus*) fillets. *Brazilian Journal of Food Technology,* 2005. 8 (1): 7-15.

[58] Monteiro, M.L.G. et al., Validade comercial de filés de Tilápia do Nilo (O*reochromis niloticus*) resfriados embalados em atmosfera modificada e irradiados. *Ciência Rural,* 2012. 42 (4): 737-743.

[59] Tomazzelli Junior; Philippi, L.M.N. O brasileiro é um consumidor de pescado? Um olhar sobre o mercado domiciliar no país. *Panorama da Aquicultura,* 2006. 16 (95): 39-45.

[60] Gonçalves, A. A. Tecnologia do pescado: ciência, tecnologia, inovação e legislação. São *Paulo: Editora Atheneu,* 2011

[61] Kubitza, F.; Lopes, T. G. G. Com a palavra os consumidores. *Panorama da Aqüicultura,* 2002. 12 (69): 48-53.

[62] Hobbs, J. E. (2003). Consumer demand for traceability. Retrieved January, *28*, 2006.

[63] Birgisdottir, B. E.; Kiely, M., Martinez; J. A., & Thorsdottir; I. Validity of a food frequency questionnaire to assess intake of seafood in adults in three European countries. *Food control,* 2008. 19 (7): 648-653.

[64] Maciel, E. D. S.; Savay-da-Silva, L. K.; Vasconcelos, J. S.; Galvão, J. A.; Sonati, J. G.; Silva, D. D.; Oetterer, M. Application of exploratory

factor analysis to assess fish consumption in a university community. *Food Science and Technology*, 2013. 33 (1): 99-106.

[65] Maciel, E. Perspectiva do consumidor perante produto proveniente da cadeia produtiva de tilápia do Nilo rastreada (Oreochromis niloticus): consumo de pescado e qualidade de vida. Tese (Doutorado). *Piracicaba:* CENA/USP, 2011.

[66] Maciel, E. S. ; Savay-da-Silva, L ; Vasconcelos, J. S ; Galvão, Juliana Antunes ; Sonati, J. G. ; Silva, D.; Oetterer, M. Designing and validation of the methodology for the Internet assessment on fish consumption at the university. Ciência e Tecnologia de Alimentos (Impresso), 2014. In press.

[67] Hair, J. F., Black, W. C., Babin, B. J., Anderson, R. E., & Tatham, R. L. (2007). *Análise multivariada de dados*. Bookman.

[68] Levin, J.; Levin, J. Por que o pesquisador usa estatística. Estatística aplicada a ciências humanas. 2 Edition. São Paulo: *Harbra,* 1987

[69] Myrland, Ø.; Trondsen, T.; Johnston, R. S.; Lund, E. Determinants of seafood consumption in Norway: lifestyle, revealed preferences, and barriers to consumption. *Food Quality and Preference,* 2000. 11 (3): 169-188.

[70] Pieniak, Z.; Verbeke, W.; Scholderer, J. Health-related beliefs and consumer knowledge as determinants of fish consumption. *Journal of human nutrition and dietetics,* 2010. 23 (5): 480-488.

Chapter 3

TILAPIA PRODUCTION AND FEEDING MANAGEMENT IN THE SEMI-ARID OF BRAZIL: A VIEW OF SOME RECENT DEVELOPED TECHNIQUES

Daniela Bacconi Campeche[1],*, M.Sc.
Ranilson de Souza Bezerra[2], Ph.D.
José Fernando Bibiano de Melo[3], Ph.D.
and Eudes de Souza Correia[4] Ph.D.

[1]Fish Production System Researcher- Embrapa Tropical Semi Arid, Brazil
[2]Professor at the Universidade Federal de Pernambuco, Brazil
[3]Professor at the Universidade Federal do Vale do São Francisco, Brazil
[4]Professor at the Universidade Federal Rural de Pernambuco, Brazil

ABSTRACT

The semi-arid is not one of the first climates that comes to mind when fish production is imagined. First of all, it is important to describe the semi-arid in Brazil since it enclosures 969.589,4 km^2, about 11% of the Brazilian territory, and more than 1.000 municipalities in 9 different Brazilian States. In this area live over 22 million people, which is about 10% of the Brazilian total population. However the Brazilian semi-arid

---

* Corresponding author.

has some particular characteristics that made it possible.The São Francisco River and large water reservoirs such as Castanhão and Orós are some of the water bodies where tilapia culture has been sucessfull. There are several production systems adopted ranging from tilapia in irrigation reservoirs on small rural properties to intensive cage culture. It does not matter which production system was adopted by the producer, since the area is semi-arid the water quality has to be extremely well managed and cared for in order to preserve it and guarantee the highest possible productivity per cubic meter of water. In order to achieve the best productivity, some management practices must be adopted. One of them is to select the best variety for breending. Several tilapia varieties have been introduced into the Brazilian semi-arid since the 1970's. However Tai tilapia has been the most successful and is cultivated nowadays. Red Koina and Saint Peters have also been introduced, but it did not succeed due to their low growth after 400g. Tilapia GIFT has already been tested in cage culture. The results are promising, but there is a lack of farms to produce this variety in the Brazilian semi-arid.

Tilapia feeding management is an important issue in tilapia culture in this region. Feeding practices may differ from areas within the semi-arid as well as production systems. Several local ingredients have been tested as examples mango meal, cassava chips and leucaena hay. Most of them are used by small producers as tilapia feed complement. Cage culture producers are well instructed to only use balanced extruded feed in order to achieve better growth and maintain the best possible water quality.

The semi-arid area is not one of the first climates that comes to mind when thinking about fish production. To understand this, it is important to describe the semi-arid area of Brazil: it covers 969.589,4 km^2, that is about 11% of the Brazilian territory, and includes more than 1.000 municipalities in 9 different Brazilian States. In this area live over 22 million people, i.e., about 10% of the Brazilian total population (ASA BRASIL, 2014). The Brazilian semi-arid area, however, has some specific characteristics that make fish production possible. For instance, it is the semi-arid area with the highest rainfall depth in the world, with average of 200-800 mm of water per year. The rain, however, is irregular when and where it falls. Also, the evaporation index is about three times higher than rainfall. Therefore to make the best use of the water available to raise fish in this region is a challenge, and to produce high quality protein for the local population is a need. As well, increased production helps to offer to the local young generation an opportunity to live and work locally, instead of migrating to other regions.

The Brazilian northeast includes most of the semi-arid area and is responsible for about 25% of the Brazilian total continental fish production (MAPA, 2011). Tilapia is the most cultivated fish species in Brazil and in the northeast. Total fish production in the northeast was 134.293 tons in 2011, but there is no official statistical data about how much tilapia was produced in the area. This semi-arid region has a large river basin the São Francisco River with its lakes and reservoirs, Sobradinho (400 km of extension; 4.214 m^2 of water surface; 34.000.000.000 m^3 of water) and Itaparica (11.000.000.000 m^3 of water capacity) for instance, built for power plant energy. It also has large water reservoirs such as Castanhão (6.700.000.000 m^3 of water capacity), Sítios Novos (126.000.000 m^3) and Orós (2.100.000.000 m^3 of water capacity), all of these these located in the state of Ceará. These are some of the water bodies where tilapia cultivation in cages has been successful. Ceará and Bahia are the Brazilian states with the largest tilapia production. In first place is Itaparica in the São Francisco River Basin, with an annual average production of 24.000 tons/year. Castanhão and Orós Lakes in Ceará State are the number two producers with 18.500 tons/year. This production is in 4 – 240 m^3 cages that can be square or round. A drought in 2013 was responsible for a decrease of about 17% in production. Due to facts like this and to the multiple uses that water reservoirs have in the Brazilian semi-arid area, it is important to be aware of the support capacity for fish production in order to avoid an eutrophication process.

In our research, we have investigated a number of production systems in use, ranging from raising tilapia in irrigation reservoirs on small rural properties and in saline water, to intensive cage culture and tilapia-shrimp integrated production. A case study of tilapia semi-intensive production in irrigation reservoirs (1.170 m^3) and its water parameters have already been evaluated in the Brazilian semi-arid area (Cardoso-Filho et al., 2010). Case studies in rural properties are important in order to evaluate the increase of water productivity. This is especially necessary in the semi-arid area and for small farmers. In the study by Cardoso et al., survival rate (96%) was considered excellent, since the fish were cultivated in earth ponds. Fish final weight (535g) was considered the average after a cycle of 217 days in semi intensive production systems. There was a biomass gain of 18.270 kg.ha^{-1} and an apparent feed conversion rate (FCR) of 0.83. The excellent value of FCR was due to natural food available in the reservoir. Those results can be considered excellent since most of the producers from irrigated areas in the Brazilian semi-arid region do not make use of this resource. Besides the increase of water productivity, extra farm income can be made. During this

trial, water temperature ranged from 23-28°C, which is considered the average for the area. The average value for pH ranged from 7-8.5. Electrical conductivity ranged from 64–173 ìS cm^{-1}. Pond bottom ammonia level ranged from 0.004 – 0.131 mg L^{-1}, and surface ammonia level ranged from 0.007 – 0.143 mg L^{-1}. Nitrite levels in the pond bottom and surface were 0.01 mg L^{-1}. Alkalinity values were of 39.09 mg $CaCO_3$L^{-1} at the bottom and 41.92 mg $CaCO_3$L^{-1} at the surface. Water hardness was 56.77 mg $CaCO_3$ L^{-1} at the bottom and 57.47 mg $CaCO_3$ L^{-1} for surface.

The Brazilian semi-arid region has several saline water reservoirs and water dams. Unfortunately there is no official data about how many there are. These are sometimes located in areas where potable or fresh water is difficult to access. Small farmers who live in these areas need a technology that can make use of saline water, especially for animal production. Since this water is not potable for goats and sheep over a long period, tilapia production is the most indicated type of production. Shrimp is also a possible alternative; but the fact that the larvae must be brought in from far away makes this alternative not viable. It does not matter if the production system adopted by the producer is intensive or not, because in a semi-arid region, the water quality has to be extremely well managed and treated in order to preserve it and guarantee the highest possible productivity per cubic meter of water. Therefore to achieve the best productivity, some management practices must be adopted. One of them is to select the best variety of fish to be raised in saline water. Several tilapia varieties have been introduced into the Brazilian semi-arid since the 1970's. However Thai tilapia has been the most successful and is cultivated nowadays. Red Koina and Saint Peters have also been introduced, but have not succeeded due to their low growth rate after 400g. A study conducted at Embrapa Tropical Semi-Arid Caatinga Experimental Station, comparing growth performance of Red Koina and Thailand tilapia cultivated in brackish water (average salinity of 6.12 g.L^{-1}), showed that fish of the Thailand strain had a significantly better growth (final weight for Thailand and Red Koina: 888.89g ± 20.09[a] and 582.00g ± 78.97[b]; daily weight gain: 7.55g ± 0.180[a]; 4.71g ± 0.89[b]; weight gain: 792.70g ± 18.43[a]; 494.59g ± 93.14[b]; feed consumption: 166.60g ± 2.40[a]; 134.60g ± 3.1[b]) as well as survival (Thailand: 100%; Red Koina: 97.62%) and feed conversion ratio (Thailand: 1.40[a] and Red Koina: 1.56[a]). Performance analysis showed that there was a significant difference ($P < 0.05$) for all the parameters analyzed. Thailand tilapia growth was about 40% higher than for Red Koina tilapia. The sample strains evaluated in this study were introduced in the semi-arid about 10-15 years ago and still show high genetic variability (Campeche et al., 2011b). From the

DNA analysis, all the primers selected produced different patterns of RAPD fragments for both populations. The number of clear reproducible fragments engendered from primers of both stocks showed a variation from 6 to 16 fragments and the size of these amplified products was maintained between 250-2,072 pb. From 70 fragments analyzed randomly, 60 were polymorphic (85.71%) and 10 monomorphic (14.29%). The percentage of polymorphic fragments was higher for Thailand tilapia (84.29%) when compared to Red Koina stock (64.29%). The results from this study proved that genetic variability obtained by the percentage of polymorphic fragments was high showing that the reproduction management for both populations guarantees a conservation of genetic variability. In the Campeche et al. (2001b) study, the Shannon index value found was higher for the Thailand tilapia stock (0.4614) than for Red Koina tilapia stock (0.3526). Therefore, as the percentage of polymorphic locos was high, the genetic variability of the stocks was also high. The need is high for a means of desalination in semi-arid communities isolated from any kind of potable water distribution system. With the aim to increase salt dam water productivity and also, provide an environmentally friendly destination for the water discharged from the desalination process, the Embrapa Tropical Semiarid Station has developed an agriculture-aquaculture production system. The system uses the rejected water from the desalination process to raise tilapia, and the effluent from the tilapia´s pond is used to irrigate a salt bush (*Atriplex nummularia*). This salt bush is used for hay and given to goats and sheep as a feed complement. This shows the importance of testing tilapia performance in brackish and water with high salinity in the Brazilian semi-arid regions.

Tilapia market demand in the northeastern states in Brazil that contain semi-arid areas is different than the market in the south of Brazil. Nowadays in the northeast, the market demands fish that weigh up to 1.3 kg, normally sold as whole fish and not for the fillet industry. Due to feed price, this demand became an important issue for the producers because the feed conversion rate increases after the fish reaches 800g and so the production cost also increases. In order to give to the producers an answer about how to predict fish growth and help to manage fishing enterprise, a recent study was carried out using a mathematical model to predict tilapia growth when raised in 4 m^3 cages in the São Francisco River (Sousa Junior et al, 2014 *posted in* Engenharia Agrícola ISNN 0100-6916). Five non-linear models were tested: Brody (1945) ($Y = A(1 - Be^{-kt}) + \varepsilon;$); Bertalanffy (1957) ($Y = A(1 - Be^{-kt})^3 + \varepsilon;$); Logístico (Nelder, 1961) ($Y = Ae^{-Be^{-kt}} + \varepsilon;$); Gompertz (Laird, 1965) ($= A(1 + Be^{-kt})^{-1} + \varepsilon;$) and Richards (1959) ($Y = A(1 - Be^{-kt})^M + \varepsilon;$). Where Y =

fish weight at certain age (g); A = weight at adult age; B = maturity level of the fish at its birth; k = velocity at which the fish get closer to adult body mass; M is the parameter that gives shape to the curve that corresponds to 1 on the Brody model, which is 3 for Bertalanffy, -1 for the Logístico, a tendency towards ∞ for Gompertz and variable for Richards. The estimated values for fish weight was higher for Brody (13.485 kg), Bertalanffy (4,122 kg), Gompertz (2.383 kg), followed by the models of Logístico (1.521 kg) and Richards (0.972 kg). Based on the average market demand, tilapia raised in cages on the São Francisco River can be harvested when reaching between 600 - 850g at 183; 247; 181; 184 and 183 days for the Bertalanffy, Brody, Gompertz, Logístico and Richard's models. For the demand of 1kg average fish, 244; 546; 244 and 243 days would be necessary according to the models of Bertalanfy, Brody, Gompertz and Logístico. The value (k) that indicates how fast tilapia will reach its market size was also evaluated. The highest k value was obtained by the Logístico (0.0183) model, followed by Richards (0.00856), Gompertz (0.00747), Bertalanffy (0.00369) and Brody (0.00011). In this study only 3 models were converted by the Gauss method. This means that the values estimated are close to what was found in field data. These models were those of Bertalanffy, Logístico and Gompertz. Therefore they were considered the best models to estimate tilapia growth in cage culture in the São Francisco River.

   The semi-arid needs to optimize water productivity. In areas where water is abundant, such as the great water reservoirs built for power plant energy, this need can be overcome in order to generate more employment and food. Therefore, in order to evaluate the best growth for tilapia fingerlings in 4 m^3 cages, a trial was undertaken to test four different densities: 800, 950 and 1100 fish.m^3 (Saraiva, et al., 2009). The trial was carried out in the town of Paulo Afonso, in the State of Bahia, Brazil (9°31'16.2" S e 38°00'32.7" W). Fingerlings (0.85g initial weight) were fed a 55% crude protein diet for the first 20 days and a 45% crude protein diet for the other 34 days. At the end of the trial no statistical difference was observed in the growth performance parameters analyzed. For the three densities evaluated (800, 950 and 1100 fish.m^3), final weight (g) was respectively: 37.50; 37.20 and 32.80; weight gain (g): 36.65; 36.37; 32.00; specific growth rate (%): 7.00; 6.98; 6.76; final biomass (kg. m^{-3}): 28.5; 31.8; 34.1; survival (%): 94.9; 90.1; 94.5 and feed conversion rate: 0.99; 1.09; 1.06. So, the authors concluded that it is possible to stock Thailand tilapia up to 1100 fingerling.m^{-3} in 4 m^3 cages in order to obtain 30g juvenile after 54 days of growth. At this density, water productivity was increased since a greater number of fish were raised per area of water

used. At this trial site, water quality parameters were considered optimal for tilapia growth (temperature: 28°C; pH: 7.5; dissolved oxygen: 6.7 mg.L^{-1}; total hardness: 49.5 mg.L^{-1} CaCO$_3$; alkalinity: 14.8 mg.L^{-1} CaCO$_3$; nitrite: 0.002 mg.L^{-1}; total ammonia: 0.505 mg.L^{-1}).

Thailand tilapia growth performance in cages fed different protein levels was evaluated in another area of the Brazilian semi-arid region. Water quality parameters and transparency constituted the main difference between the water reservoirs where tilapia growth performance cited in this chapter was evaluated. This study was conducted in a water reservoir near the town of Sapé (parallel of 7°05´38´´south latitude and 35°13´58´´ west longitude) in the State of Paraíba (Costa et al., 2009). Cages were also 4m^3 in volume. Growth was evaluated during all phases with different crude protein levels in the feed, using a randomized block experimental design. Commercial diets containing three different crude protein levels (36, 32 and 28%) in three growth phases I – 80 to 300g; II – 301 to 650g and III – 651 to 1000g were tested. In phase I, two protein levels were tested: 32% and 36%. There was no statistical difference as to final weight (311g and 308g); biomass gain per cage (270kg and 267 kg); daily weight gain (5.97g and 6.01g); specific growth rate (3.57% and 3.54%); feed conversion (1.63 and 1.62); feed efficiency (61.21% and 61.23%) and final biomass (99.50 and 98.75 kg. m^3). The only statistical difference (P < 0.05) at this level was observed for the protein efficiency ratio (1.71^a and 1.92^b). Phase II evaluated all fish from the two different groups from phase I (32% and 36% respectively) then were fed 32% of crude protein in the diet. At the end of this phase all the growth performance parameters evaluated were statistically different (P < 0.05), except for the final biomass: final weight (650^a and 723^b g); biomass gain (219^a and 274^b kg); daily weight gain (5.64^a and 6.93^b g.day^{-1}); specific growth rate (1.23^a and 1.42^b %); feed conversion ratio (2.32^a and 1.96^b); feed efficiency (44.05^a and 50.91^b %); protein efficiency (1.71^a and 1.92^b %) and final biomass (99.50^a and 98.75^a kg.m^3). In phase III, the experimental groups were divided into three, where the first two values represent how much CP fish were fed in phases I and II and the last value indicates how much CP fish were fed in phase III: A (fed 32-32-32%); B (fed 36-32-32 %); C (fed 32-32-28%). At the end, all growth performance parameters showed statistical differences. The group that was fed 32-32-32% crude protein showed the best growth performance: final weight (1102^a; 1040^{ab}; 900^b g); biomass gain (159^a; 128^{ab}; 46^b kg); daily weight gain (7.7^a; 5.9^{ab}; 2.5^b g.day^{-1}); specific growth rate (0.82^a; 0.67^{ab}; 0.30^b %); feed conversion ratio (1.63^a; 2.33^{ab}; 2.83^b); feed efficiency (61.15^a; 47.86^{ab}; 37.23^b %); protein efficiency (2.26^a; 1.82^{ab}; 1.57^b) and final biomass (161^a; 152^{ab} ;

143^b kg.m^3). The results observed in this trial were considered optimal for the semi-arid water reservoir. High crude protein diets might indicate that a high amount of nitrogen will be excreted by fish and the as a consequence the water body will become eutrophic, since water exchange in the semi-arid is very slow. At the site of this trial, the water quality parameters were considered optimal: dissolved oxygen at 06:00 am was 5.62 and at 04:00 pm was 9.66 mg.L^{-1}; pH: 7.73; ammonia: 0.05 mg.L^{-1} and nitrite: 0.01 mg.L^{-1}; transparency: 62.33 cm (considered eutrophic); nitrate: 0.03 mg.L^{-1}; inorganic phosphate: 0,12 mg.L^{-1}.

Tilapia feeding management is an important issue in tilapia cultivation in this region. Feeding practices and production systems may differ among areas within the semi-arid regions. A number of local ingredients have been tested as examples of mango meal, cassava chips and leucaena hay. Small producers use most of these as a tilapia feed complement. These fish, however, are not raised for market purposes. Mango is highly produced in one area of the Brazilian semi-arid region and most of the time it is thrown out by the producers for not being accepted by the market. In order to have some use out of it, mango meal was tested on tilapia juvenile (Souza et al., 2013). Four treatments were evaluated: 0%, 33%, 66% and 100% of mango meal replacing corn meal. Results showed that up to 33% of corn meal can be replaced by mango in diets for tilapia juvenile without decreasing growth, weight gain, specific growth rate, carcass yield and survival (Table 1), or increasing feed conversion rate. Final weight and specific growth rate were lower for fish from the groups where 66% and 100% of the corn had been replaced by mango meal as the carbohydrate source. Fish from lower replacement levels had better growth performance results. However feed consumption was significantly lower (p<0.05) only at the 100% replacement level group. This result can be probably due to a palatability issue. The best feed conversion rate was observed with the groups where 0% and 33% of mango meal replaced corn meal. As the level of mango meal increased, energy (kcal/kg) levels in the feed also increased. For this reason it is possible that the source of carbohydrate tested in this trial caused the decrease in tilapia performance due to its fiber level or any anti-nutritional factor. Carcass yield and survival was not affected by the treatments. Fish that were fed diets containing 66% and 100% of mango meal had lower levels of lipid in the carcass. The group that was fed a diet containing 100% of mango meal had the lowest protein in the carcass. Those results proved that mango meal changes the carcass chemical composition. In short, the diets where 100% of corn meal was replaced by mango meal were not nutritionally well balanced.

Table 1. Performance and carcass composition of Nile Tilapia fingerlings fed different levels of inclusion of mango meal replacing corn meal

	Level of inclusion of mango meal replacing corn meal				Regression equation	R^2
	0%	33%	66%	100%		
Final weight (g)	37.51±0.75[a]	35.84±2.11[a]	32.04±1.34[b]	22.58±0.96[c]	Y= -4.376x+42.53	0.081
Weight gain (g)	32.85±0.88[a]	31.17±2.07[a]	27.32±1.34[b]	17.84±0.84[c]	Y= -4.885x+39.51	0.88
Specific growth index	4.63±0.11[a]	4.52±0.12[a]	4.25±0.10[b]	3.46±0.04[c]	Y= -0.378x+5.16	0.85
Feed consumption (g)	33.92±1.42[a]	33.34±0.94[a]	32.46±1.38[a]	26.95±1.33[b]	Y= -2.179x+37.115	0.77
Feed conversion ratio	1.03±0.03[c]	1.07±0.05[c]	1.19±0.01[b]	1.51±0.02[a]	Y= 0.156x+0.81	0.85
Carcass yield (%)	85.29± 1.68	85.36±1.7	86.57±1.74	84.87±1.98		
Survival (%)	97.78± 3.85	97.78±3.8	97.78±3.85	100± 0.00		
Carcass composition (%)						
Crude Protein	59.50±1.24[a]	59.94±0.62[a]	61.69±0.62[a]	54.36±0.16[b]	Y= -1.94x2+8.34x+52.57	0.81
Ether Extract	20.42±0.08[ab]	21.49±0.24[a]	19.71±0.53[b]	19.27±0.08[b]	Y= -0.37x2+1.36x+19.64	0.68
Ash	15.46±0.05	14.62±0.08	15.65±0.08	17.06±1.28		

Average ± SD.

Different letters within the lines indicate significant difference ($P < 0.01$) for the treatment using the Tukey test.

Sousa et al., 2013.

These results are very important, since for small agricultural producers in the Brazilian semi-arid regions, corn is important for the family meal as well as for livestock, such as sheep and goats, feed.

These small agricultural producers are used to adopting any kind of feed available on their property for animal consumption. For this reason, the digestibility of some feed commonly found on small farms was evaluated (Campeche et al., 2011a): cassava (*Manihot utilissima*) chips, sorghum (*Sorghum bicolor* L. Moench) grain, atriplex (*Atriplex nummularia*) hay, gliricidia (*Gliricia sepium*) hay, leucena (*Leucaena leucocephala*) hay and wine residues. Apparent digestibility coefficient (ADC) of dry matter (DM), crude protein (CP) and digestible energy (Kcal kg^{-1}) of the above cited ingredients, were evaluated for Red Koina tilapia. Among all ingredients, the best ADC values for dry matter were for cassava chips (69.96±10.13) and sorghum (74.97±5.4) (Table 2). Although it showed one of the best dry matter ADC values, sorghum is not considered to be one of the best digestible ingredients for tilapia when compared to other energetic conventional ingredients. The best ADC values for crude protein were for cassava chips (88.19±3.98), sorghum (88.56±2.46) and gliricidia hay (88.08±0.91). Atriplex hay was the ingredient with the best digestible energy value (2063.12). Our research concluded that all the ADC values found are considered low when compared to the ingredients conventionally used. However, depending on how much is used, and, in the case of small property fish producers, the ingredients evaluated in this research can be used for tilapia feed.

Table 2. Apparent digestibility of energetic and protein ingredients of vegetable origin ingredients for Red Koina tilapia

Ingredient	Digestibility (%)		
	Dry Matter	Crude Protein	Digestible Energy (kcal kg^{-1})
Cassava chips	69.96±10.13[ab]	88.19±3.98[a]	1609.76[a]
Sorghum seed	74.97±5.4[a]	88.56±2.46[a]	1119.87[c]
Atriplex hay	47.56±0.0[c]	81.37±0.0[bc]	2063.12[a]
Gliricidia hay	59.88±3.09[abc]	88.08±0.91[a]	1059.27[d]
Leucena hay	59.15±3.53[abc]	87.18±1.11[a]	962.98[d]
Wine residue	57.32±0.0[bc]	79.86±0.0[c]	858.18[f]

Values expressed in % of dry matter. Average ± SD.

Different letters within the column indicate significant difference (P < 0.01) for the treatments by Tukey test.

Campeche et al., 2011 a.

Nowadays all the Brazilian shrimp production is destined for the domestic market and Brazil still imports shrimp, principally from Asia (MAPA, 2011). In order to have total production use, to make the aquaculture system in the semi-arid more sustainable and also to deal with the future of fishmeal high prices, the Enzimoly Laboratory of the Universidade Federal de Pernambuco, has conducted a number of trials using shrimp carcass hydrolysate replacing fishmeal for fish feed. The shrimp carcass is a by-product from the shrimp processing industry. A 45-day feeding trial was carried out to evaluate the use of shrimp protein hydrolysate (SPH) in diets for Nile tilapia juvenile. SPH was included in isonitrogenous (average of 35.5% PB) diets replacing fishmeal protein by 0, 5, 10 and 20%. Shrimp protein hydrolysate resulted in a product with 9.7% moisture, 43.63% crude protein, 6.25% ether extract, 7.32% ash, and 3.633 kcal/kg gross energy and a total amino acid content of 46.79 g/100 g (41.2% essential and 58.8% nonessential). The level of SPH incorporated into the diets (0; 1.5; 3 or 6%) did not affect ($P < 0.05$) final fish weight (27.18; 29.46; 26.02 and 25.19 g), weight gain (25.51; 27.73; 24.29 and 23.39 g), average daily gain (0.57; 0.62; 0.54; and 0.52 g day^{-1}) or specific growth rate (7.15; 7.38; 6.85 and 6.73% day^{-1}). Feed conversion ratio (1.15; 1.09; 1.13 and 1.17), protein efficiency ratio (2.26, 2.33, 2.20 and 2.14) and apparent net protein utilization (39.31, 40.39, 38.57, and 34.72) also were not affected by SPH inclusion. The fish fed actively on all diets. An analysis of mathematical models to evaluate length–weight relationships revealed statistical differences ($P < 0.05$) in fish growth. Fish fed SPH5 (1.5% inclusion rate) exhibited the best length–weight relationship. Higher SPH inclusion levels (3 and 6%) did not contribute to fish growth, resulting in similar or worse growth performance than that provided by the SPH0 diet. The inclusion of SPH in Nile tilapia diets significantly affected ($P < 0.05$) final fish body composition. Protein content decreased ($P < 0.05$) when SPH replaced 20% of fishmeal. Fish fed SPH 10 and SPH 20 had greater fat content (58.4 and 59.8 g kg^{-1}, respectively) than fish fed the control diet (51.2 g kg^{-1}) or that with the lowest SPH inclusion level (50.3 g kg^{-1}). Fish fed the diet with no SPH had higher ash content (40.5 g kg^{-1}) than those fed the other diets ($P < 0.05$). This study has demonstrated that SPH is a promising protein feedstuff and could account for as much as 6% of Nile tilapia juvenile diets with no adverse effects on growth and nutrient utilization (Leal et al., 2010).

Cage culture producers are well instructed to use only balanced extruded feed in order to achieve better growth and maintain the best possible water quality. There is only one local fish feed industry that uses local algaroba (*Prosopis juliflora*) meal in the feed formula. This specific feed industry has a

commitment to local social sustainability. Some areas of the Brazilian semi-arid region are intensive producers of fruits and vegetables, since irrigation from the São Francisco River has been widely exploited. Therefore fruit residues are also used by small producers, to complement feed for tilapia in the irrigated area of the Brazilian semi-arid Local universities have also studied the use of fruit meal in fish feed. The results obtained so far are promising.

There is still a lot of field and laboratory research to be done in order to improve tilapia production and management in the Brazilian semi-arid regions. Improvement is certain owing to the number of governmental efforts, private producers, feed industries and others members of the production chain working together towards this cause.

REFERENCES

ASA BRASIL http://www.asabrasil.org.br/Portal/Informacoes.asp?COD_ MENU=105 accessed in May 2014.

Boletim Estatístico Da Pesca E Aquicultura. 2011. Ministério da Pesca e Aquicultura. Governo do Brasil. 60p.

Campeche, D. F. B.; Moraes, S. A.; Lima, V. T.; Sousa, S. M. N.; Oliveira, S. T. L.; Souza, M. G.; Paulino, R. V. 201. Chemical composition and apparent digestibility of feed found in the brazilian semiarid region for tilapia rosa feeding on cultivation. *Ciência Rural*, v.41 (2), p.343-348.

Campeche, D. F. B.; Paulino, R. V.; Lima, V. T.; Ribeiro, R. P.; Gomes, P. C. 2011. Desempenho e variabilidade genética de tilápia Tailandesa e Red Koina cultivadas em água salobra. Boletim de Pesquisa e Desenvolvimento 84. Embrapa Semiárido.

Cardoso-Filho, R.; Campeche, D. F. B.; Paulino, R. V. 2010. Tilápia em reservatório de água para irrigação e avaliação da qualidade da água. Revista Brasileira de Ciência Agrárias, v.5 (1), p. 117-122.

Costa, M. L. S.; Melo, F. P.; Correia, E. S. 2009. Efeitos de diferentes níveis proteicos da ração no crescimento da tilápia do Nilo (*Oreochromis niloticus* Linnaeus, 1757), variedade Chitralada, criadas em tanques-rede. Boletim do Instituto de Pesca, v.35 (2), p.285-294.

Leal, A. L. G.; Castro, P. F.; Lima, J. P. V.; Correia, E. S.; Bezerra, R. S. 2010. Use of shrimp protein hydrolysate in Nile tilapia (*Oreochromis niloticus*, L.) feeds. *Aquaculture International*, 18, p. 635-646.

Saraiva, K. A.; Melo, F. P.; Apolinário, M. O.; Santos, A. J. G.; Correia, E. S. 2009. Nile tilapia *Oreochromis niloticus* (Chitralada strain) fingerlings

reared in cages under stocking densities. Revista Brasileira de Saúde e Produção Animal, v.10 (4), p.963-969.

Souza, R. C.; Melo, J. F. B.; Nogueira-Filho, R. M.; Campeche, D. F. B.; Figueiredo, R. A. C. R. 2013. Influence of mango meal on growth and body composition of Nile tilapia. *Archivos de Zootecnia*, v. 62(238), p. 217-225.

Chapter 4

EFFECT OF LIGHT INTENSITY ON THE AGGRESSIVENESS AND OXIDATIVE STRESS IN FEMALE CICHLID *TILAPIA RENDALLI*

T. B. Carvalho[1], C. C. Mendes[2], E. A. de Almeida[3] and E. Gonçalves-de-Freitas[4]*

[1]Universidade Federal do Amazonas (UFAM),
Departamento de Ciências Fisiológicas, Manaus, AM, Brazil
[2]Programa de Pós-graduação em Ciências da Saúde,
São José do Rio Preto, SP, Brazil
[3]Universidade Estadual Paulista (UNESP),
Departamento de Química e Ciências Ambientais,
São José do Rio Preto, SP, Brazil
[4]Universidade Estadual Paulista (UNESP),
Departamento de Zoologia e Botânica e
Centro de Aquicultura da UNESP,
São José do Rio Preto, SP, Brazil

---

[*] Corresponding author: Thaís Billalba Carvalho, Av. General Rodrigo Octávio Jordão Ramos, 3000, Coroado I, 69077-000, Manaus, AM, Brazil, Instituto de Ciências Biológicas / Departamento de Ciências Fisiológicas, Tel +55 92 3305 4038; Fax +55 92 3305 4231, thaisbillalba@ufam.edu.br / thaisbillalba@yahoo.com.br

ABSTRACT

Physical changes due to degradation or handling to provide improvements in animal husbandry usually modify the aquatic environment and, one of the environmental variables that can be changed is the luminance. Increased light intensity, for instance, can increase the production of reactive oxygen species (ROS) that cause molecular damage to cellular structures with consequent functional impairment and loss of vital functions. Additionally, increased light intensity affects aggressiveness in territorial fish, leading to different levels of social stress, which can increase the effects of oxidative stress and ROS activity. Thus, the aim of this chapter was to test the influence of light intensity on the agonistic behavior and oxidative stress in female of *Tilapia rendalli*. We compared two treatments referred here as low (253.56 ± 62.25 lx) and high light intensity (1435.92 ± 481.40 lx), each one under two conditions: 1. Social Condition, : where animals were isolated for 96 h and paired (resident-intruder paradigm) for 1 h;. and 2. Isolation Condition (baseline) where: fish were isolated for 96 h. The latency to first fighting and for hierarchical settlement was similar in the two light intensities, but the high light intensity decreased the frequency of attacks and displays in winner fish. On the other hand, there was no difference between the frequencies of aggressive events displayed by loser in both treatments. Catalase did not differ between the two intensity or conditions for all animals. Thus, the light intensity reduces the aggressive interactions, and this influence is related to social rank in female of *T. rendalli*. However, the effects of aggressive interactions were not translated into variations of catalase, showing that oxidative stress is not associated to such a behavioral or environmental modifications.

Keywords: Social hierarchy; Luminance; Catalase, Stress, Energetic expenditure

INTRODUCTION

Physical changes due to degradation or handling to provide improvements in animal husbandry usually modify the aquatic environment. These changes are defined by modality, structure and environmental operation and can affect the living organisms in such environments (Wong, 2012). The aquatic environment, for instance, has undergone continuous changes due to human-induced actions. These changes may act as a stressor, affecting physiological and behavioral variables of the aquatic animals, particularly of fishes (Sloman

et al., 2001; Almazán-Rueda et al., 2005). One of the environmental variables that can be changed is the luminance. Considering it regulates many activities and behavioral patterns of aquatic animals (Helfman, 1993), knowing the way this factor affects fish behavior is essential for the fish survival.

An intense and prolonged light can be annoying and harmful to fishes, indirectly affecting physiological parameters regarding to energy expenditure, then acting as a stressor (Boeuf & Le Bail, 1999; Stefánsson et al., 2002; Almazán-Rueda et al., 2005). If increased light can reduce fish's aggressive behavior, it would be a strategy to reduce energy expenditure caused by metabolic changes resulting from potentially stressful environmental changes (Carvalho et al., 2012). In addition, a reduction of aggressiveness in high light conditions acts as a mechanism to reduce the animal conspicuity in the environment (Castro & Caballero, 2004).

Several parameters can be used as stress indicators in fish, such as rate of O_2 consumption, ventilatory rate, plasma cortisol levels, glycogen depletion and glucose levels (Wedemeyer & Mcleay, 1981; Wendelaar-Bonga, 1997; Barreto & Volpato, 2006). In addition, different types of stressors increase the production of reactive oxygen species (ROS) (Yu, 1994; Storey, 1996) that cause molecular damage to cellular structures with consequent functional impairment and loss of vital functions (Wiseman & Halliwell, 1996). Thus, the animal's health is related to the balance between ROS production and antioxidants action (e.g., superoxide dismutase, catalase and glutathione peroxidase); and the discrepancy between these two components is called oxidative stress (Sies, 1993). Therefore, in stressful situation, the radicals' levels can increase and stimulate antioxidant enzymes production. In accordance to this, Mats'opa et al. (2010) observed an increase in catalase and glutathione peroxidase in rats as the duration of the light day increases, protecting cells against lipid and protein oxidation by free radicals. In fish, light incidence can induce the activation of antioxidant enzymes such as catalase to prevent oxidative stress (Shin et al., 2011; Choi et al., 2012). It has been demonstrated that light increases the production of hydrogen peroxide, that acts as the second messenger coupling photoreception to the zebrafish circadian clock leading to a progressive increase in the expression of catalase genes to counteract deleterious effects of hydrogen peroxide (Hirayama et al., 2007).

Increased aggressive interaction also increases social stress in fish (Zayan, 1991). Then, it was expected that high luminance acts as a stressor and interferes on the frequency of aggressive behavior. Considering the aforementioned, the aim of this chapter was to test two different levels of light

intensity and evaluate if they can trigger behavioral and physiological adjustments in fish. For this, the effect of light intensity on the agonistic behavior and oxidative stress were evaluated in female of the cichlid *Tilapia rendalli* (Boulenger, 1897).

Tilapia rendalli was chosen because it is a cichlid species and so, establishes its social organization through aggressive interactions (Baerends & Baerends-Van Roon, 1950), which is an ideal feature for testing the proposed hypothesis. The studied species has biparental substrate-guarding, meaning that male and female take care of the offspring and defend the breeding territory (Ribbink et al., 1981). There are few studies about factors that can affect agonistic behavior and social hierarchy in female cichlids (Weber & Weber, 1976; Gonçalves-de-Freitas & Ferreira, 2004), mostly are performed with male cichlids (e.g., Oliveira & Gonçalves, 2008). In this way, studies that evaluate the modulating factors on aggressive behavior in females are interesting, especially in species such as *T. rendalli*, whose aggressive interaction and social hierarchy have well-defined function. Moreover, it is known in other fish species that males and juveniles are affected by light conditions (e.g., Valdimarsson, & Metcalfe, 2001; Castro & Caballero, 2004; Carvalho et al., 2012; Carvalho et al., 2013), but no information at all about these effects in females. Thus, this study shows for the first time the effect of changing physic aquatic environment on female fish's aggressive response.

MATERIALS AND METHODS

Agonistic profile (Social Condition) and catalase activity were compared in female pairs subjected to two different levels of light intensity, referred here as low and high light intensity. Furthermore, we tested the effect of light intensity as a stressor in isolated animals (Isolation Condition), as a baseline reference.

REARING CONDITIONS

Adult females of *Tilapia rendalli* from Centro de Pesquisa e Treinamento em Aquicultura (CEPTA – IBAMA) de Pirassununga-SP (catalogue number: DZSJRP 11343) were initially kept in a fish pond (a 185 m^3 tank) at UNESP. Before study begun, the fish were transferred to the laboratory and acclimated

in 250 L indoor tanks (ca.1 fish / 5 L) for, at least, 15 days preceding the experiments. During this period, the temperature was close to 27 °C, the light intensity of the room were kept at 664 ± 89.90 lx and the photoperiod in 12 h (0700 h to 1900 h). Biological filters ensured water quality, and food (animal feed for tropical fish with 28% protein) was given *ad libitum* - twice a day (early morning and late afternoon).

SOCIAL CONDITION

Two treatments were tested; in the first one, animals were subjected to a low light intensity (253.56 ± 62.25 lx, n=9) and, in the second one, the high light intensity (1435.92 ± 481.40 lx, n=10). Thirty-eight fish were weighed and measured, then it were isolated by 96 h. Afterwards they were paired for 1 h, an enough period to allow hierarchy establishment (T.B. Carvalho, personal observationscommunication).

Throughout the isolation and pairing period the animals were under luminance, in accordance with the experimental treatment. The animals were grouped using to the resident-intruder paradigm (Figler & Einhorn, 1983) and the individual identification was made by means of small cuts on the tail fin (Hoglund et al., 2005).

Since the size interferes on agonistic profile (Beeching, 1992), the pairs were formed by matched-size individuals (Standard Length: Low intensity: 9.329 ± 28.491 cm; High intensity: 9.386 ± 0.606 cm; independent t-test, t = -0.302, d.f.= 17, $P = 0.764$). The sex identification was made by inspection of the genital papilla, stained with methylene blue, which provides the visualization of the oviduct opening, as performed by Carvalho and Gonçalves-de-Freitas (2008) for Nile tilapia.

Light Treatment

The mean light intensity (in each experimental condition) was obtained from 36 sampling points in the aquarium by using a portable digital light meter (model LD 240). The high light intensity was emitted by two 9W fluorescent bulbs set at 5.5 cm from the surface of the aquarium water. The low light intensity treatment was obtained from the conventional lighting in the laboratory. The light intensity was periodically measured to investigate the

constancy of light emitted by bulbs. The light intensities used correspond to those observed in outdoor ponds and under laboratory conditions.

Aggressive Behavior

The agonistic interactions were video-recorded by 60 min, and initiated immediately after the inclusion of the intruder fish in the aquarium of the resident fish. Quantification of agonistic interactions was done by the frequency of behavioral events of the ethogram described below.

Biting: the aggressor swims towards the opponent and bites its body.
Chase: one fish follows the opponent that swims away from itin opposite direction.
Circling: two fish with erected dorsal fin swim following each other, describing a circle, like a very slow chasing.
Lateral fight: the fish remain alongside each other facing the same or opposite direction and beat their tails sideways.
Lateral threat: one fish with their fins spread and mouth opened approaches laterally to the opponent, which keeps away.
Mouth fight: both fish approach frontally each other with their mouths opened and bite the opponent's mouth. Their mouths are kept tightly together while one fish displaces the opponent backwards.
Tail beating: only one fish beats its tail sideways (undulating the body), without spreading its fins.

Latency to fighting was evaluated in each experimental treatment. The frequency of attacks (biting, chase and mouth fight) and displays (circling, lateral threat, lateral fight and tail beating) were quantified for winner (dominant) and loser (subordinate) to compare possible differences on fight between treatments. The time to settle hierarchy was determined according to Falter (1983), as soon as one animal (subordinate) stops attacking and begins to run away from the another animal (dominant).

Aquaria and Animal Manipulation

The animals were kept in aquaria of 30 x 30 x 40 cm, covered with their side opaque blue plastic on the side and back walls covered by opaque blue

plastic to avoid visualeye contact with fish in neighboring aquariafish. The blue color was used because it reduces the stress in a cichlid species, Nile tilapia (Volpato & Barreto, 2001). The water quality of the aquarium was maintained through constant aeration and use of biological filter. The water temperature was maintained at 27.04 ± 0.84 °C and the photoperiod in 12 h (0700 h to 1900 h). The animals were fed with commercial fooeed, 2% of the biomass. It was given twice daily (early morning and late afternoon).

The fish handling for biometrics, sex identification, and isolation was preceded by anesthesia (benzocaine solution: 12.8 mg/L). The transfer of the intruder animal to the aquarium of the resident was done without anesthesia, but the handling was done carefully to avoid additional stress. At the end of the experiment, the animals were killed with lethal dose anaesthetic (25.6 mg/L) and then dissected to sex confirmation by visualization of the gonads (Weyl & Hecht, 1998). Furthermore, the gills were removed and frozen at -86°C to measure the catalase activity. This tissue was chosen due to its contact with the environment, and thus with the light treatments, that could influences catalase activity.

Catalase

For the measurement of catalase activity, the gills were homogenized (1:4, w/v) in 0.02 M tris buffer containing 0.001 M EDTA, 0.001 M Dithiothreitol, 0.15 M KCl, 0.001 M phenyl-methyl-sulfonyl-fluoride (protease inhibitor_, and 0.5 M sucrose, and centrifuged at 9,168 x g for 20 min at 4°C. The supernatant fraction was then re-centrifuged at 50,000 x g for 60 min at 4°C, and then aliquots of the supernatant were used for catalase measurement.

The technique used to measure the catalase activity was described by Beutler (1975), that quantify the H_2O_2 decomposition speed by the enzyme, using the absorbance decreasing at 240 nm ($\varepsilon = 0.071$ mM^{-1}. cm^{-1}) at 30 °C. The reaction medium contains H_2O_2 10 mM, Tris 1 M and EDTA 5 mM in pH 8.0. The catalase activity values are expressed as U per mg protein. One unit of catalase matches the amount of enzyme which hydrolyses 1 μmol of H_2O_2 per minute at 30 °C and at pH 8.0.

ISOLATION CONDITION

To verify the effect of luminance on oxidative stress in *Tilapia rendalli* were analyzed catalase activity. The isolated animals were subjected to two levels of light intensity (n=8): low (253.56 ± 62.25 lx) and high (1435.92 ± 481.40 lx). The lighting characteristics applied were the same above described for social condition.

The females were sizedmeasured (Low intensity: 9.725 ± 0.859 cm; High intensity: 9.692 ± 0.640 cm), weighed (Low intensity: 32.045 ± 7.773 g; High intensity: 31.102 ± 6.390 g) and isolated for 96 h under light intensity, according to the experimental treatment. After the isolation period, the gills were removed to measure the catalase concentration. The procedures for analyze that parameter, as well as the maintenance, the aquaria, sex determination, handling and death of the animals were identical to those described for social condition.

Data Analyses

The outliers were removed from the raw data and replaced by means. Later, the data were tested for normality, by Shapiro-Wilk test, and for homogeneity of variance, by F max test. Latency to start agonistic interaction and duration of interaction (hierarchical settlement) in low and high light intensities were compared by independent Student's t-test. The frequency of agonistic behavior and was also compared by independent Student's t-test for each social rank (winner and loser). Catalase activity was compared by two-wayrepeated measure ANOVA, rank and light intensity being categorical factors and stressor indicator the repeated measures. It was considered $\alpha \leq 0.05$ for statistical significance; every analysis was based on Zar (1999).

Ethics

This study respects the Ethical Principles in Animal Research adopted by National Council for the Control Brazilian College of Animal Experimentation (CONCEABEA) and was approved by the Ethical Commission of Animal Experimentation of the São Paulo State University (UNESP), Botucatu, SP, Brazil (protocol 052/06).

RESULTS

The latency to fighting (independent t-test, t = -0.04, d.f.= 17, P = 0.97) and hierarchical settlement (independent t-test, t = 0.55, d.f.= 17, P = 0.59) were similar in both light intensity treatment (Figure 1). The winner fish showed a higher frequency of attacks and displays in low light intensity (independent t-test, t > 2.59, d.f.= 17, P < 0.02; Figure 2A).
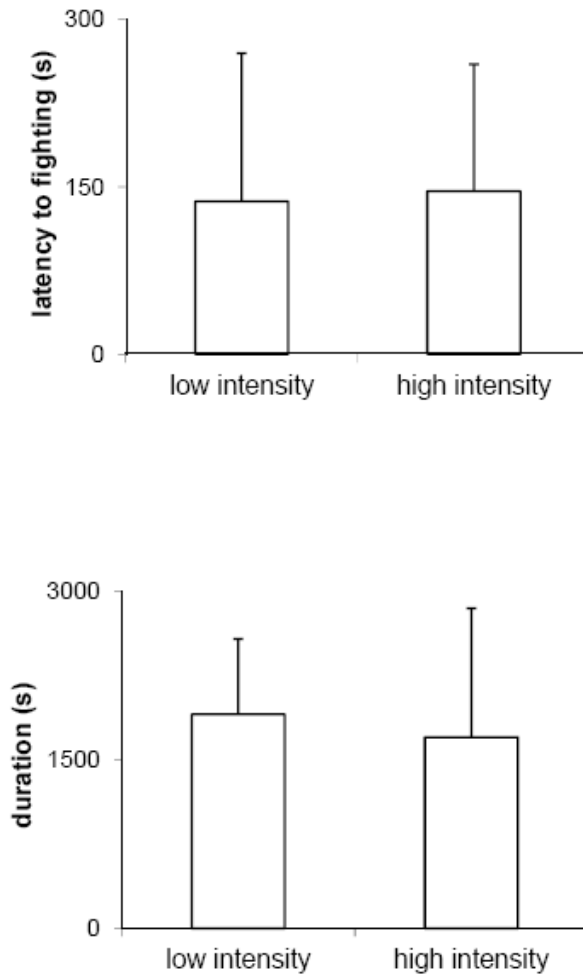
Figure 1. Mean (± SE) latency to fighting (independent t-test, t = 0.04, d.f.= 17, P = 0.97) and duration (independent t-test, t = 0.55, d.f.= 17, P = 0.59) in the low and high light intensity treatments.
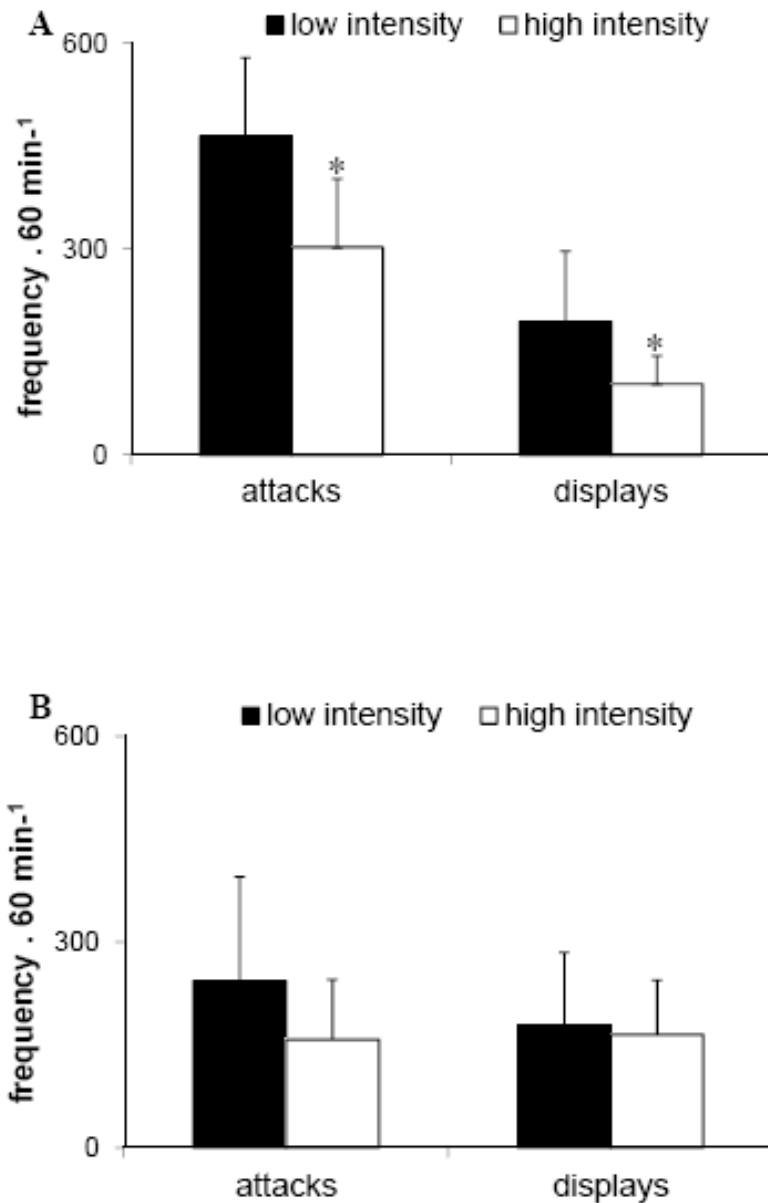
Figure 2. Mean (± SE) frequency of agonistic interactions for winner (A) and loser (B) fish under low and high light intensities. Asterisk indicate significant differences between treatments (independent t-test, t > 2.59, d.f.= 17, *P*< 0.02).
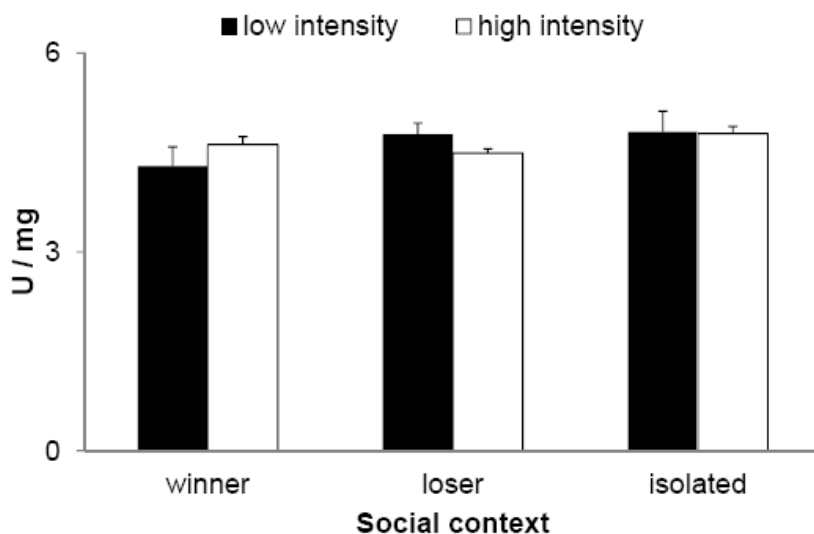
Figure 3. Mean (± SE) catalase activity of the winner, loser and isolated fish under low and high light intensities (repeated measuretwo-way ANOVA, F = 1.56, d.f.= 2, P = 0.23).

On the other hand, there was no difference between the frequency of agonistic interactions showed by the loser fish in both treatments (independent t-test, t < 1.38, d.f.= 17, P > 0.19; Figure 2B). The catalase enzyme concentration was similar to winner, loser, and isolated fish in both light intensities (repeated measuretwo-way ANOVA, F = 1.56, d.f.= 2, P = 0.23; Figure 3).

DISCUSSION

The latency to fighting and the time to settle the dominance hierarchy were similar in the groups subjected to low and high light intensity, indicating that the luminance does not affect the onset and duration of interaction in *Tilapia rendalli*. However, the effect of this environmental condition on agonistic behavior was observed because high light intensity has caused a decreased in the frequency of attacks and displays in the winner fish. On the other hand, the subordinate (loser) fish has showed no difference in the frequency of agonistic units in both experimental treatments. In fact, the

luminance affects the aggressive interaction in fishes, but, the results are contradictory (Valdimarsson & Metcalfe, 2001; Castro & Caballero, 2004) and it is related to species (Carvalho et al., 2012). Moreover, the effect of luminance on aggressive behavior is dependent on social status, so, the dominant fish is the primarily affected in the pair.

Overall, the high light intensity reduces the aggressive behavior in *T. rendalli*, and this response may result from different mechanisms (e.g., Olla et al., 1978; Castro & Caballero, 2004). The greater luminance, for instance, might act as a potentially stressful situation; also the reduction of aggressiveness, in this condition, is an adaptive response, since it would minimize the energy expenditure with the interactions, and consequently would displace energy for physiological adjustments resulting from stress. Indeed, long periods of luminance affect the stress indicative variables in *Clarias gariepinus* (Almazán-Rueda et al., 2005). However, studies relating light intensity and stress had not been performed yet. Thus, this chapter tested if higher light intensity could, in fact, act as a stressor in *T. rendalli*. Catalase is an antioxidant enzyme that can be produced in greater quantities in stressful situations, due to the high formation of radicals in this condition (Sies, 1993; Yu, 1994).

According to Boeuf & Le Bail (1999) and Stefánsson et al. (2002), the prolonged and very intense light can be annoying and harmful to the fishes. Although the light intensity can be considered a stressor for these animals, this response was not observed with the analysis of catalase activity. This may have been due to there was no increase in the formation of radicals in the stressful situation in gills and, consequently, there was no activation of the antioxidant defense system. On the other hand, the catalase may be activated in the longer term; thus, it was nearly impossible to detect its release during the experiment.

We results indicate that the light intensity does not act as a stressor in female of *T. rendalli*. Other possible explanation could be that the two light intensity treatments may be in the range of optimal luminance for this species. Likewise, it shows the lack of studies evaluating the effect of different light levels on variables of stress, also using other parameters such as rate of O_2 consumption, plasma cortisol concentration, glycogen depletion and glucose levels (Wedemeyer & Mcleay, 1981).

Among the hypotheses proposed to explain the aggressive behavior in comparison to the variation of luminance, the chapter concluded that the associated energy expenditure cannot be applied, because, in spite of the luminance does not act as a stressor, an increasing in fish' aggressive behavior

in low light intensity was observed. However, other hypotheses may explain the interaction decreasing in high light treatment. For instance, dominant female cichlids are generally those which reproduce and maintain the offspring care (Weber & Weber, 1976; Brandtmann et al., 1999). Thus, a high luminance would make the animal more conspicuous, and also, would indicate the location of their offspring to potential predators. Considering the subordinate fish, the strategy would not change the aggressive behavior, since submissive animals have already present greater energy expenditure in comparison to the dominants (Volpato & Fernandes, 1994; Alvarenga & Volpato, 1995).

CONCLUSION

The light intensity reduces the aggressive interactions, and this influence is related to social rank in female of *T. rendalli*. However, the effects of aggressive interactions were not translated into variations of catalase, showing that oxidative stress is not associated to such a behavioral or environmental modifications.

ACKNOWLEDGMENTS

REFERENCES

Almazán-Rueda, P.; Van-Helmond, A. T. M.; Verreth, J. A. J. & Schrama, J. W. (2005). Photoperiod affects growth, behaviour and stress variables in *Clarias gariepinus*. *Journal of Fish Biology*, 67, 1029-1039.

Alvarenga, C. M. D. & Volpato, G. L. (1995). Agonistic profile and metabolism in alevines of Nile tilapia. *Physiology and Behavior*, 57, 75-80.

Baerends, G. P. & Baerends-Van Roon, J. (1950). An introduction to the study of the ethology of cichlid fishes. *Behaviour*, 1, 1-242.

Barreto, R. E. & Volpato, G. L. (2006). Stress responses of the fish Nile tilapia subjected to electroshock and social stressors. *Brazilian Journal of Medical and Biological Research*, 39, 1605-1612.

Beeching, S. C. (1992). Visual assessment of relative body size in a cichlid fish, the oscar, *Astronotus ocellatus*. *Ethology,* 90, 177-186.

Beutler, E. (1975). Red Cell Metabolism: A Manual of Biochemical Methods. New York: Grune & Straton.

Boeuf, G. & Le Bail, P. Y. (1999). Does light have an influence on fish growth? *Aquaculture,* 177, 129-152.

Brandtmann, G., Scandura, M. & Trillmich, F. (1999). Female-female conflict in the harem of a snail cichlid (*Lamprologus ocellatus*): behavioural interactions and fitness consequences. *Behaviour*, 136, 1123-1144.

Carvalho, T. B. & Gonçalves-de-Freitas, E. (2008). Sex group composition, social interaction, and metabolism in the fish Nile tilapia. *Brazilian Journal of Biology,* 68, 807-812.

Carvalho, T. B., Ha, J. C. & Gonçalves-de-Freitas, E. (2012). Light intensity can trigger different agonistic responses in juveniles of three cichlid species. *Marine and Freshwater Behaviour and Physiology*, 45, 91–100.

Carvalho, T.B., Mendonça, F.Z., Costa-Ferreira, R.S. & Gonçalves-de-Freitas. E. (2013). The effect of increased light intensity on the aggressive behavior of the Nile tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae). *Zoologia,* 30, 125–129.

Castro, J. J. & Caballero, C. (2004). Effect of the light intensity upon the agonistic behaviour of juvenile of white-seabream (*Diplodus sargus cadenati* de La Paz, Bauchot and Daget, 1974). *Aggressive Behaviour,* 30, 313-318.

Choi, C. Y., Shin, H. S., Choi, Y. J., Kim, N. N., Lee, J. & Kil, G. S. (2012). Effect of LED light spectra on starvation-induced oxidative stress in the cinnamon clownfish *Amphiprion melanopus*. *Comparative Biochemistry and Physiology A,* 163, 357-363.

Falter, U. (1983). Les comportements agonistiques de *Sarotherodon niloticus* (Pisces, Cichlidae) et la signification évolutive de l'incubation buccale. *Buletin de La Classe des Sciences*, 69, 566-593.

Figler, M. H. & Einhorn, D. M. (1983). The territorial prior residence effect in convict cichlids (*Cichlasoma nigrofasciatum* Günther): temporal aspects of establishment and retention, and proximate mechanisms. *Behaviour,* 85, 157-183.

Gonçalves-de-Freitas, E., & Ferreira, A. C. (2004). Female social dominance does not establish mating priority in Nile tilapia. *Revista de Etologia*, 6**,** 33-37.

Helfman, G. S. (1993). Fish behaviour by day, night and twilight. In T. J. Pitcher (Eds.), Behaviour of Teleost Fishes. London: Chapman & Hall.

Hirayama, J., Cho, S. & Sassone-Corsi, P. (2007). Circadian control by the reduction/ oxidation pathway: Catalase represses light-dependent clock gene expression in the zebrafish. *Proceedings of the National Academy of Sciences*, 104, 15747–15752.

Hoglund, E., Bakke, M. J., Øverli, Ø., Winberg, S. & Nilsson, G. E. (2005). Suppression of aggressive behaviour in juvenile Atlantic cod (*Gadus morhua*) by L-tryptophan supplementation. *Aquaculture*, 249, 525-531.

Mats'opa, I. V., Hryhor'ieva, N. P. & Meshchyshen, I. F. (2010). Adaptation of antioxidative system in rat kidneys under varying light conditions during tetrachloromethane intoxication and effect of melatonin. *Ukrainskii Biokhimicheskii Zhurnal*, 82, 75-84.

Oliveira, R. F. & Gonçalves, D. M. (2008). Hormones and social behaviour of teleost fish. In C. Magnhagen, V. A. Braithwaite, E. Forsgren, & B. G. Kapoor (Eds.), Fish Behaviour. New Hampshire: Science Publishers.

Olla, B. L., Studholme, A. L., Bejda, A. J., Samet, C. & Martin, A. D. (1978). Effect of temperature on activity and social behavior of the adult Tautog *Tautoga onitis* under laboratory conditions. *Marine Biology*, 45, 369-378.

Ribbink, A. J., Marsh, A. C. & Marsh, B. A. (1981). Nest-building and communal care of young by *Tilapia rendalli* Dumeril (Pisces, Cichlidae) in Lake Malawi. *Environmental Biology of Fishes*, 6, 219-222.

Shin, H. S., Lee, J. & Choi, C. Y. (2011). Effects of LED light spectra on oxidative stress and the protective role of melatonin in relation to the daily rhythm of the yellowtail clownfish, *Amphiprion clarkia*. *Comparative Biochemistry and Physiology A*, 160, 221-228.

Sies, H. (1993). Strategies of antioxidant defense. *European Journal of Biochemistry,* 215, 213-219.

Sloman, K. A., Taylor, A. C., Metcalfe, N. B. & Gilmour, K. M. (2001). Effects of an environmental perturbation on the social behaviour and physiological function of brown trout. *Animal Behaviour*, 61, 325-333.

Stefánsson, M. O., Fitzgerald, R. D. & Cross, T. F. (2002). Growth, feed utilization and growth heterogeneity in juvenile turbot *Scophthalmus maximus* (Rafinesque) under different photoperiod regimes. *Aquaculture Research,* 33, 177-187.

Storey, K. B. (1996). Oxidative stress: animal adaptations in nature. *Brazilian Journal of Medical and Biological Research*, 29, 1715-1733.

Valdimarsson, S. K. & Metcalfe, N. B. (2001). Is the level of aggression and dispersion in territorial fish dependent on light intensity? *Animal Behaviour,* 61, 1143-1149.

Volpato, G. L. & Fernandes, M. O. (1994). Social control of growth in fish. *Brazilian Journal of Medical and Biological Research*, 27, 797-810.

Volpato, G. L. & Barreto, R. E. (2001). Environmental blue light prevents stress in Nile tilapia. *Brazilian Journal of Medical and Biological Research*, 34, 1041-1045.

Weber, P. G. & Weber, S. P. (1976). The effect of female color, size, dominance and early experience upon mate selection in male convict cichlid, *Cichlasoma nigrofasciatum* Guenther (Pisces, Cichlidae). *Behaviour*, 56, 116-135.

Wedemeyer, G. A. & Mcleay, D. J. (1981). Methods for determining the tolerance of fishes to environmental stressors. In A. D. Pickering (Eds.), Stress in Fish. London: Academic.

Wendelaar-Bonga, S. E. (1997). The stress response in fish. *Physiological Reviews*, 77, 591-625.

Weyl, O. L. F. & Hecht, T. (1998). The biology of *Tilapia rendalli* and *Oreochromis mossambicus* (Pisces: Cichlidae) in a subtropical lake in Mozambique. *South African Journal of Zoology,* 33, 178-195.

Wiseman, H. & Halliwell, B. (1996). Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochemical Journal*, 313, 17-29.

Wong, M. Y. L. (2012). Abiotic stressors and the conservation of social species. *Biological Conservation*, 155, 77-84.

Yu, B. P. (1994). Cellular defenses against damage from reactive oxygen species. *Physiological Reviews*, 74, 139-162.

Zar, J. (1999). Biostatistical Analyses. New Jersey: Printice Hall.

Zayan, R. (1991). The specificity of social stress. *Behavioural Processes*, 25, 81-93.

Chapter 5

Environmental Conditions, Fish Diseases, Management and Economic Evaluation of Tilapia Cages in a Brazilian Hydroelectric Reservoir

Daercy Maria Monteiro Rezende Ayroza[*],
Fabiana Garcia Scaloppi, Luiz Marques da Silva Ayroza,
Fernanda de Paiva Badiz Furlaneto,
Antonio Sergio Ferraudo
and Cacilda Thais Janson Mercante
São Paulo's Agency for Agribusiness Technology
(Agência Paulista de Tecnologia dos Agronegócios) –
APTA/SAA/SP, Brazil

Abstract

This study examined water and sediment characteristics, fish diseases, management and economic data in fish farms (F1, F2 and F3) in the Canoas II reservoir, Paranapanema River Basin, Brazil. We also assessed limnological characteristics at sampling points along the river channel (C1, C2, and C3). In most water evaluations, the high transparency, electrical conductivity and turbidity values indicated the low amount of suspended particulates. No increase in primary production

[*] Corresponding author: Email: dadyroza@apta.sp.gov.br.

was observed, a fact evidenced by predominance of low chlorophyll *a* values, except for C2 in June 2012. The total nitrogen, total phosphorus, and inorganic forms concentrations remained constantly below the limit of detection for the analytical method used. In the sediment, the association between the highest concentrations of phosphorus and granulometry (fine sediments) in the lacustrine region were was most evident. These results are likely associated with the short residence time of the water in this run-of-river reservoir. The limnological variables are consistent with legal Brazilian standards and with the recommended patterns for cages, except the low temperatures in the winter and autumn. The *Trichodina* sp was the parasite with the highest infestation intensity in all fish farms, followed by monogeneans (Dactylogyridae and Gyrodactilidae) and by the protozoan *Epistylis*. The bacteriological analysis demonstrated that the occurrence of each bacterial genus was random. The relationship between limnological characteristics and tilapias diseases was investigated using principal component analysis (PCA), in which 3 groups (G1, G2 and G3) were discriminated. In the PC1 x PC2 biplot, the temperature was positively associated with *Plesiomonas shigelloides*, and negatively associated with *Trichodina* sp, Gyrodactilidae, *Streptococcus* sp, and *Aeromonas* sp predominance, indicating that temperature is a variable with strong influence on the development of these diseases. In the PC1 x PC3 biplot, Dactylogyridae was associated with June and August 2013 in F3, and with April 2013 in F2, coinciding with higher *Trichodina* sp prevalence. The economic analyses demonstrated that the net income is equivalent to USD 365.00 for each 6.0 m³ cage (USD 0.40 for per fish produced) and USD 1,121.00 for each 18.0 m^3 cage (USD 0.54 for per fish produced). The profitability index corresponded to 33% and 41% for the 6.0 and 18.0 m^3 cages, respectively. The time for return on investment after cage installation was 2 production cycles (1.5 years). The minimum quantities of fish that must be produced for economic viability of this activity are 396.00 kg of 6.0 m³ and 882 kg of 18.0 m³.

INTRODUCTION

Brazil is considered the country with the greatest potential for expansion of tilapia cage farming. In 2011, the Brazilian continental aquaculture production increased to 544,490 tons, which is an approximate growth of 38% compared with 2010 [1]. This growth was primarily a result of the expansion of fish farming using the cage system, which was stimulated by incentives from the federal government, by the availability of water dammed in the hydroelectric reservoirs and by the advantages of this breeding system. This

type of farming allows for greater control of production and lower capital investment and production costs compared with other systems, such as raceways (tanks with high water flow) and earthen ponds [2].

In southeastern Brazil, the increase in fish production was due to the expansion of tilapia cage farming in reservoirs of the western and northern regions of the state of São Paulo (Paraná, Tietê and Grande Rivers), the southern state of Minas Gerais (Furnas Reservoir) and the axis of the Paranapanema River (the border between the states of São Paulo and Paraná) [3].

In the Paranapanema Valley, which is the border between the states of Paraná and São Paulo, this activity has generated employment, income and excellent quality food, which has promoted increased interest in the use of reservoirs for fish farming. Because of the increasing importance of this productive segment in the region, the Ministry of Fisheries and Aquaculture (Ministério da Pesca e Aquicultura - MPA) funded a study to demarcate aquaculture parks in 8 reservoirs of the Paranapanema River, and plans are in place for installing 17 aquaculture parks in Paraná and 12 in São Paulo, with 1 in the Canoas II Reservoir. In general, aquaculture parks are spaces that are delimited based on studies conducted by multidisciplinary teams that consider aspects of environmental, social and economic sustainability. Then, the demarcated areas are divided into lots, which will be occupied by fish cage farming after formal proceedings before government agencies. In addition to aquaculture parks, isolated aquaculture projects called aquaculture areas may be authorized in the reservoir.

Despite the existing potential of aquaculture, information and technology for the sustainable development of aquaculture to occur in the Brazilian conditions remain lacking. Thus far, few domestic technologies have been developed; most of these technologies have primarily been imported from Chile, and fish farmers often develop their own farming technology.

In addition, diseases, in general, should be considered a challenge to be overcome in tilapia cage farming because cage farming is an intensive production system with greater health, economic and environmental risks compared with less intensive systems. Determining the carrying capacity of cages and using densities within this limit are the first steps to reducing the occurrence of diseases and, consequently, mortality in cage farming systems. Additionally, water quality and parasites should be monitored to define the critical points of the system (time of year, farming stage, and management, among others) that will determine the recommendations to be made regarding strategic prophylactic products, such as vaccines and special diets containing

probiotics, prebiotics and immune stimulants. These measures should reduce the risks of this farming activity to extend its success, ensuring less environmental impact and greater food safety for tilapia consumers.

A few studies have analyzed the economic viability of fish farms. However, these studies emphasize the importance of periodic evaluations to ensure the economic sustainability of this venture [4].

The present chapter presents the results of a study performed in the Canoas II Reservoir, Paranapanema River, states of São Paulo/ and Paraná (SP/PR), where environmental conditions, fish diseases, and economic and management data of fish cage farms were analyzed. This study was developed to provide support for improvements in farming management and public policies for the management of reservoirs used for fish farming. This study was funded by the Water Resources State Fund (Fundo Estadual de Recursos Hídricos - FEHIDRO).

USE OF RESERVOIRS FOR FISH CAGE FARMING

Hydropower is the primary energy source of Brazil. From the 1950s onward, several dams were built in Brazilian rivers for the purpose of power generation due to the existing water potential, resulting in a water surface area in natural and artificial reservoirs estimated at 5.3 million ha [5]. In 1998, the use of public waters for aquaculture exploitation was regulated through Federal Decree No. 2,869 of December 9, 1998. Despite the advantages and positive effects of fish farming, this intensive system provides increased waste from uneaten food and from metabolic products of farmed fish [6]. In total, 30% of the food supplied to the fish is estimated to become available to the surrounding aquatic ecosystem [7, 8]. The effluent of cages can induce eutrophication processes, such as increases and changes in the abundance of local phytoplankton [9, 10], organic enrichment of the sediment [9, 11-15] and increased abundance of the benthic community [9, 13, 15, 16].

Thus, monitoring the water and sediment quality is an important tool for managing reservoirs, which are used for multiple purposes (water supply, energy production, fish farming, recreation, navigation and sand extraction, among others). Legally, water and effluents must be kept within certain limits, which are currently set in CONAMA Resolution No. 357 of March 17, 2005, as amended by resolutions no. 410/2009 and 430/2011.

Sediment plays an important role in the dynamics of transport, accumulation and availability of nutrients and contaminants in the aquatic

environment [17]. Under certain conditions, the phosphorus accumulated in this compartment can be released into the water column, contributing to eutrophication [18]. The grain-size composition of the sediment may be indicative of its origins, interfere with the storage of chemicals and contribute to the determination of possible anthropogenic changes [19].

Monitoring the aquatic environment is also important for fish farming because water quality is a determining factor for the productive performance of fish. Dissolved oxygen concentrations equal to or greater than 5 mg/L, nitrite concentrations less than or equal to 0.03 mg/L, pH between 6.5 and 8.0, and temperatures between 26 and 30°C are considered suitable conditions for the development of tropical fish [5]. These authors also recommend water transparency values greater than 2.0 m depth at the sites where cages will be installed.

CHARACTERIZATION OF VENTURES IN THE CANOAS II RESERVOIR

The Canoas II Reservoir is part of the complex built in the Paranapanema River for generating electricity, which begins at the Jurumirim hydroelectric power plant (HPP) and ends at the Rosana HPP (Figure 1). The Paranapanema River is classified as a class 2 river, which is water that may be used for aquaculture and for other purposes (Ordinance of the Ministry of Interior No. 13/1976).

This reservoir contains 10 tilapia cage farms, with 6 in São Paulo and 4 in Paraná. The ventures are small to medium size and occupy a volume of approximately 72 to 2,000 m³. The production is performed in small volume (6.0 to 18.0 m³) cages in a multiphase system in which fish are periodically selected and grouped according to their size, until slaughter at a commercial weight between 800 and 900 g. Nine of these ventures are between the dam and the center of the reservoir, as shown in Figure 2, where the fish farms (F1, F2, and F3) and the points along the channel (C1, C2, and C3) evaluated are highlighted. The 10th fish farm occupies a volume of approximately 156.0 m³ and is approximately 5 km upstream.
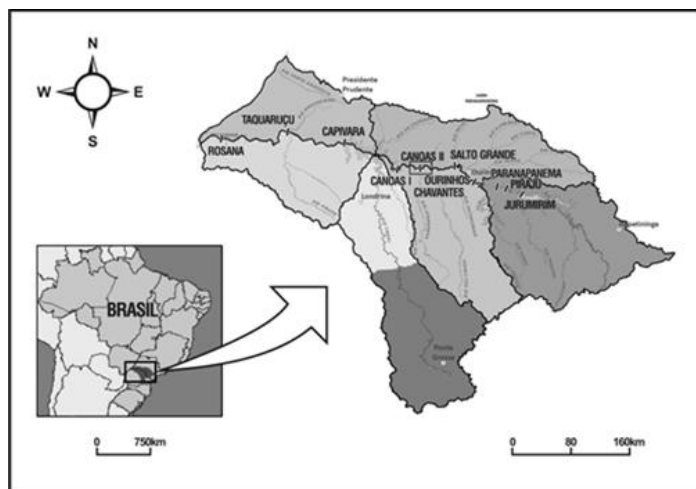
Figure 1. Canoas II Reservoir location in the complex built for power generation in the Paranapanema River, on the border between the states of São Paulo and Paraná.

Figure 2. The fish farms (F1, F2, and F3) and the points along the channel (C1, C2, and C3) evaluated at the Canoas II Reservoir, Paranapanema, SP/PR. Source: © Google; Spot Image, 2013.

Fish farm 1 (F1) was established in 2008 in the state of São Paulo in the transition region of the reservoir. Currently, this farm has 200 and 45 cages with 6.0 m³ and 18.0 m³ of usable volume, respectively (2,010.0 m³). On average, the stocking density is 133 fish/m³, and the production cycle lasts for 7 months. In 2012, this farm's production was 300 tons. Fish farm 2 (F2) was established in 2002 in the state of São Paulo in the dam region. Currently, this farm has 60 and 43 cages with 6.0 m³ and 18.0 m³ of usable volume (1,134.0

m³), with stocking densities of 167 and 128 fish/m^3, respectively, and the mean production cycle duration is 7 months. In 2012, the production in this venture was calculated at 172 tons. Fish farm 3 (F3) was implemented in 2006 in the state of Paraná in the dam region. This farm has 130, 15, and 7 cages with 6.0 m³, 12.0 m³, and 18.0 m³ of usable volume (1,086.0 m³), respectively, with a mean stocking density of 133 fish/m^3. The production cycle duration is 8 months, starting with 30 g juveniles. In 2012, this farm's production was estimated at 118 tons.

EVALUATION OF WATER QUALITY

Bimonthly measures of limnological variables were obtained in the field; samples of surface and bottom water columns were collected using Van Dorn bottles at the centers of the fish farms (F1, F2, and F3) and along the primary channel (C1, C2, and C3). Dissolved oxygen levels and pH, temperature and electrical conductivity values were evaluated using a multiparameter probe (Horiba, model U-10). Water transparency was measured using a Secchi disk, and water depth was measured using a handheld probe (SpeedTech Corp, Taoyuan Hsien, Taiwan). The dissolved oxygen concentration and temperature were also measured at depth intervals of 1.0 m to assess the vertical profiles. The collected samples were sent to the laboratory for determination of total nitrogen, ammonia nitrogen, nitrite (NO_2), nitrate (NO_3), total phosphorus, dissolved phosphorus, chlorophyll *a*, alkalinity, turbidity and biochemical oxygen demand (BOD).

Sample collection and storage followed the recommendations of the National Guide for the Collection and Preservation of Samples [21]. The limnological variables were measured in a laboratory registered with the National Institute of Metrology, Quality and Technology (Instituto Nacional de Metrologia, Qualidade e Tecnologia - INMETRO) following the methodologies of the Standard Methods for the Examination of Water and Wastewater [22].

Weather information obtained from the meteorological station in Palmital, which is part of the Integrated Center for Agrometeorological Information (Centro Integrado de Informações Agrometeorológicas - CIIAGRO), and hydrological information obtained from the concessionaire company website for power generation (http://www.duke-energy.com.br/usinas/Paginas/ Usinas.aspx) were used as support for the interpretation of the limnological variable results.

The average monthly air temperatures ranged between 16.9°C in June and 26°C in December. The average monthly precipitation ranged from 0.0 mm in August 2012 to 242.7 mm in February 2013. In June 2012, a monthly precipitation of 120.4 mm was recorded, with rains during the sampling campaign, although this is considered the dry period according to the Köppen climate classification [23] for the municipality of Palmital (Am).
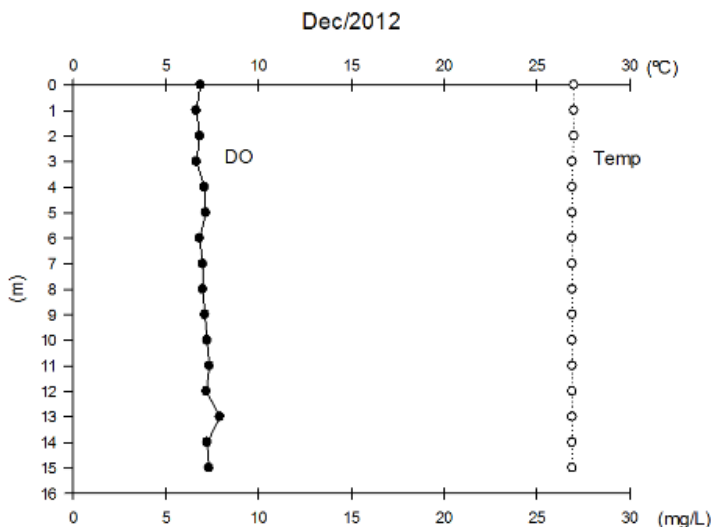
Figure 3. Dissolved oxygen and temperature profiles in a sampling point along the channel in the Canoas II Reservoir in December 2012.

The annual variation in the water level in the Canoas II Reservoir was only 0.22 m. The lowest water level (365.86 m) was recorded in December 2012, and the highest water level (366.08 m) was recorded in February 2013. Although little fluctuation was observed, the lack of agreement between the variation in the water level and the rainfall data demonstrated the influence of operating the hydroelectric plant.

The water depths at fish farms F1, F2, and F3 were 7.0, 11.0 and 10.0 m, respectively. In points C1, C2 and C3 along the channel, the water depths were 10.5, 14.0 and 14.5 m, respectively.

Table 1. Annual means and standard deviations of limnological variables between June 2012 and May 2013. F: fish farms; C: sampling points along the channel

Variáveis	Pontos Amostrais					
	F1	**F2**	**F3**	**C1**	**C2**	**C3**
Transparency (m)	1.63±0.93	1.76±1.15	1.87±1.15	1.66±0.99	1.65±1.00	1.76±1.13
pH	6.97±0.33	6.98±0.41	6.91±0.45	6.96±0.32	6.98±0.34	7.01±0.35
EC (µS/cm)	49.75±3.39	49.33±3.96	50.17±3.54	49.92±3.55	49.50±3.85	49.50±4.10
DO (mg/L)	7.72±1.28	7.28±1.30	6.50±1.16	7.83±1.30	7.77±1.27	7.41±1.26
Temperature (ºC)	23.38±2.70	23.65±3.11	24.13±3.11	23.38±2.80	23.53±2.96	23.69±3.05
Turbidity (NTU)	3.25±2.45	2.25±1.06	5.33±5.00	2.92±1.73	3.42±1.78	2.58±1.24
TN (mg/L)	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0
TAN (mg/L)	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0
NO_2 (mg/L)	0.01±0.01	0±0	0.01±0	0±0	0±0	0±0
NO_3 (mg/L)	0.30±0	0.30±0	0.30±0	0.30±0	0.30±0	0.30±0
TP (mg/L)	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0
DP (mg/L)	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0
Chlo a (µg/L)	1.80±0.79	2.76±2.48	2.17±0.97	1.57±0.33	8.78±15.77	3.02±3.85
Alk (mg/L $CaCO_3$)	23.50±4.27	28.50±3.53	28.08±4.52	26.67±6.37	29.00±4.16	27.42±5.12
BOD (mg/L)	8.38±5.35	11.66±13.21	9.98±8.47	11.62±12.05	10.42±10.65	9.67±11.81

EC: electrical conductivity; DO: dissolved oxygen; TN: total nitrogen; TAN: total ammonia nitrogen; TP: total phosphorus; DP: dissolved phosphorus; Chlo: chlorophyll; Alk: alkalinity, BOD: biochemical oxygen demand.

Table 2. Monthly means and standard deviations of limnological variables from June 2012 to May 2013.
F: fish farms; C: sampling points along the channel

Variáveis	Mês					
	Jun	Aug	Oct	Dec	Feb	Apr
Transparency (m)	1.03±0.05	1.88±0.11	3.83±0.25	1.56±0.37	1.13±0.12	0.88±0.13
pH	6.59±0.17	6.98±0.41	7.19±0.31	6.78±0.32	7.00±0.18	7.29±0.19
EC (µS/cm)	45.17±1.40	49.17±0.72	48.50±0.52	47.33±0.98	52.00±0.85	56.00±0.74
DO (mg/L)	8.54±0.82	8.65±0.79	8.37±0.43	6.89±0.65	5.94±0.53	6.12±0.46
Temperature (ºC)	19.30±0.29	21.57±0.17	24.20±1.01	26.73±0.34	27.23±0.80	22.75±0.49
Turbidiy (NTU)	5.67±4.62	2.33±1.07	1.42±0.51	3.67±2.50	4.25±1.86	2.42±0.90
TN (mg/L)	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0
TAN (mg/L)	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0
NO_2 (mg/L)	0.01±0.01	0±0	0±0	0.01±0.01	0±0	0.01±0.01
NO_3 (mg/L)	0.30±0	0.30±0	0.30±0	0.30±0	0.30±0	0.30±0
TP (mg/L)	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0
DP (mg/L)	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0
Chlo *a* (µg/L)	8.65±15.59	3.82±4.80	1.70±0.91	2.72±1.85	1.60±0.00	1.60±0.00
Alk (mg/L $CaCO_3$)	27.17±2.25	22.08±5.48	28.33±4.16	29.25±4.71	28.25±4.69	28.08±4.85
BOD (mg/L)	4.69±4.94	7.01±4.84	22.81±11.67	3.95±1.42	18.71±10.46	4.55±3.43

EC: electrical conductivity; DO: dissolved oxygen; TN: total nitrogen; TAN: total ammonia nitrogen; TP: total phosphorus; DP: dissolved phosphorus; Chlo: chlorophyll; Alk: alkalinity; BOD: biochemical oxygen demand.

The vertical profiles of temperature and dissolved oxygen obtained in December at sample point C3 displayed destratification of the water column (Figure 3). The residence time in this month was estimated at 3.4 d based on the water level in the Canoas II Reservoir (77.6% of total volume) and on the output flow in the upstream reservoir Salto Grande (393 m³), which was recorded at 6:30 am on d 17. The short water renewal time and the moderate depths contribute to the destratification of the water column, indicating the semilotic nature of the reservoir. The complete circulation of the water mass in the Canoas II Reservoir was previously observed by Pagioro et al. [24]

Table 1 presents the annual mean values and standard deviations of limnological variables, which were obtained bimonthly. Table 2 presents the monthly mean values and standard deviations of the limnological variables. The limits of detection (LOQs) of this method were considered the minimum values, which were 0.002 mg/L for nitrite, 0.30 mg/L for nitrate and 0.01 mg/L for the other nutrients. The surface and bottom data were evaluated together, considering the destratification condition of the reservoir, because the standard deviation values were not primarily associated with the differences in the depth levels at the same sampling point.

In most evaluations, the high water transparency values indicated small amounts of suspended matter, particularly in the lacustrine region (F2, F3, and F3), regardless of the presence of cages, a fact corroborated by the low electrical conductivity and turbidity values. The turbidity data were in accordance with CONAMA Resolution No. 357/2005, which recommends no less than 100 NTU values for class 2 environments.

No increase in primary production was observed, as evidenced by the predominance of low chlorophyll a values, except for in C2 in June. This sampling point is influenced by the Três Ilhas stream (Figure 2). Moretto and Nogueira [25] demonstrated the effect of the entrance of tributaries on the trophic state of the Barra Bonita Dam, Tietê River, São Paulo. Chlorophyll a is an estimate of phytoplankton biomass, indicating that the metabolic processes of the reservoir are more strongly related to a heterotrophic level than to an autotrophic level, i.e., with a predominance of bacterioplankton. CONAMA Resolution No. 357/2005 establishes chlorophyll a concentrations below 30 µg/L for class 2, indicating that the reservoir is in accordance with legal standards.

The joint analysis of the nitrogen and phosphorus data indicated that the reservoir could be classified as oligotrophic. Total nitrogen and phosphorus concentrations remained consistently below the limit of detection of the measurement method, and identical results were observed for inorganic forms.

CONAMA Resolution No. 357/2005 stipulates that total phosphorus concentrations should be less than 50 uμg/L in intermediate environments and in direct tributaries of lentic environments, with residence times ranging between 2 and 40 d. Therefore, for the Canoas II Reservoir, which exhibits a residence time of 3.4 d, the reservoir is considered to be in accordance with the resolution regarding the total phosphorus concentration. Total nitrogen should not exceed 2.18 mg/L for lotic environments, indicating that this variable is also in accordance with this resolution. These results were most likely related to the short water residence time in the reservoir because this factor influences nutrient input, light availability and eutrophication. Shorter water residence times can result in shorter periods of absorption, circulation, sedimentation and reuse of nutrients, thus resulting in increased resistance to eutrophication [26].

Biochemical oxygen demand (BOD) values above the limit of 5 mg/L are not in disagreement with CONAMA Resolution No. 357/2005, considering that the levels of oxygen and nutrients are adequate according to these regulations. BOD allows the approximate determination of the amount of oxygen required for the biological stabilization of organic matter and is commonly used in the dimensioning and establishment of protocols in water treatment plants. However, in describing the aquatic environment metabolism, BOD can receive interference from a series of compounds. For evaluating the organic load resulting from fish farming, the determination of chemical oxygen demand (COD) may often be a better indicator, in addition to the nutrient concentration, conductivity, and chlorophyll *a* measurements.

The values of the limnological variables met the recommended standards for tilapia farming, except for temperature, which was below the desirable levels in the months of June and August 2012 and of April 2013.

EVALUATION OF THE SURFACE SEDIMENT

In fish farms 1 and 2 and in sampling points C1 and C3, composite sediment samples [21] were collected in June 2012 and June 2013 using a modified Van Veen sampler. The samples were sent to a INMETRO accredited laboratory for the determination of total organic carbon (TOC), total phosphorus (TP), and total Kjeldahl nitrogen (TKN), according to the methodologies of the Standard Methods for the Examination of Water and Wastewater [22], and grain size distribution, according to the methodology of the Brazilian Association of Technical Standards [27]. Table 3 presents the results of the laboratory measurements.

Great variability was found among sample data from the first sampling and between samplings; however, little variability was found among the sample data from the second sampling. This variability may have been influenced by the flow rates at the times of sampling, which were 598 and 359 m^3/s in June 2012 and 2013, respectively. Higher flow rates result in greater sediment turbulence, transport and mixing.

Higher percentages of sand were observed in F1 and C2 samples in the transition region of the reservoir, where the water flow is higher, favoring the inflow of eroded material from the surroundings. Higher values of silt, clay and TP were measured in C3 and F2 samples in the reservoir lacustrine region, as expected, because the smaller grain size of the material facilitates its transport. Fine sediments have greater surface contact area, allowing organic matter accumulation, complexation capacity and nutrient and contaminant retention [28, 29]. However, higher TON and TOC values were observed in C2 in June 2012. This increase may have been a result of the input of materials from the tributary, the Três Ilhas stream (Figure 2). In June 2013, this sampling point was moved a few meters upstream, and greater TON concentrations were found in areas with fish farming compared with this point, but lower concentrations than those observed in June 2012.

Table 3. Grain size fractions and chemical variables of the sediment of fish farming areas (F1 and F2) and along the primary channel (C1 and C2) in the Canoas II Reservoir, Paranapanema River (SP/PR), Brazil, in June 2012 (1) and 2013 (2)

Área	Areia Kg/g	Silte Kg/g	Argila Kg/g	TP mg/g	TOC mg/g	TON mg/g
F1_1	675.60	139.80	184.60	0.97	7.41	2.17
F1_2	199.00	787.00	14.00	1.97	21.00	0.97
F2_1	39.50	482.30	478.30	1.78	18.69	2.70
F2_2	42.00	943.00	15.00	2.70	21.00	0.90
C2_1	120.60	638.00	241.50	1.72	22.25	5.09
C2_2	171.00	822.00	7.00	1.55	19.00	1.11
C3_1	29.80	683.00	267.20	0.84	9.30	1.54
C3_2	50.00	918.00	32.00	2.08	21.00	0.67

TP: total phosphorus; TOC: total organic carbon; TON: total organic nitrogen.

Additionally, one must consider that short water residence time does not favor the accumulation and deposition of organic matter and nutrients in the transition region, as recorded by Nogueira et al. [30] in a study in the Salto Grande Reservoir, which is also a run-of-river type reservoir. Therefore, the results are inconclusive for the interference of fish farms because the association between higher concentrations of phosphorus and higher proportions of fine sediments was more evident in the lacustrine region.

The data obtained in the present study were similar to those values recorded by Jorcin and Nogueira [31] in this reservoir. These authors determined TN values ranging between 2.20 and 7.18 mg/g and TP values ranging between 0.71 and 1.60 mg/g. In the sediment in areas with cages in the Itaipu HPP Reservoir in the municipality of Santa Helena/PR, Bueno et al. [32] found a decrease in TP values throughout the farming period and maximum phosphorus (0.01 g/g) values in an area that receives water from an artificial beach. These authors stated that no positive relation was found between phosphorus concentrations and areas with fish farming, most likely due to natural site and farming conditions (management and size of the ventures).

EVALUATION OF THE HEALTH OF CAGE FARMED FISH

Fish health was evaluated every 2 months to identify the causes of fish death and to identify pathogens of fish farms. In the mornings, 12 Nile tilapias were collected from each fish farm. The collected fish could be with or without clinical signs of disease; however, preference was given to moribund fish. These fish were packed individually in plastic bags, stored in an insulated box with ice and transported to the laboratory.

Upon arrival at the laboratory, the fish were weighed, and smears were prepared from the skin and gills for microscopic examination to evaluate the occurrences of parasites. After this procedure, the fish were taken to a laminar flow, and their body surfaces were disinfected with alcohol (70° GL) for 10 min. Microbiological samples consisting of swabs were taken aseptically from the kidneys of the fish. The samples were identified using routine tests, including colony morphology, Gram staining, hemolysis on agar containing 5% v/v sheep blood, catalase, oxidase and phenotypic profiles in API 20 E and API 20 Strep systems (bioMerieux, Marcy l'Etoile, France). The prevalence of pathogens (the number of infected or infested fish/the total number of fish

evaluated) was calculated as described by Bush et al. [33]. The results are shown in Tables 4 and 5.

Table 4. Seasonal prevalence (%) of Nile tilapia ectoparasites from three fish farms in the Canoas II reservoir

Month	Fish farm	Trichodina skin	Gyrodactilidae	Epistylis	Ictio skin	Trichodina gill	Dactylogiridae	Ictio Gill
Jun	F1	100.0	50.0	50.0	8.3	8.3	91.7	8.3
	F2	100.0	75.0	16.7	8.3	8.3	100.0	0.0
	F3	100.0	77.8	66.7	11.1	0.0	100.0	22.2
Aug	F1	100.0	11.1	11.1	0.0	0.0	77.8	0.0
	F2	91.7	16.7	16.7	0.0	0.0	83.3	0.0
	F3	100.0	0.0	55.6	0.0	0.0	100.0	0.0
Oct	F1	77.8	0.0	0.0	0.0	0.0	100.0	0.0
	F2	100.0	22.2	11.1	0.0	11.1	100.0	0.0
	F3	66.7	0.0	22.2	0.0	11.1	100.0	0.0
Dec	F1	44.4	22.2	11.1	0.0	0.0	66.7	0.0
	F2	50.0	0.0	0.0	8.3	0.0	83.3	0.0
	F3	0.0	16.7	50.0	0.0	16.7	33.3	0.0
Feb	F1	58.3	0.0	8.3	0.0	8.3	83.3	0.0
	F2	50.0	41.7	33.3	0.0	0.0	100.0	0.0
	F3	66.7	22.2	0.0	0.0	0.0	66.7	0.0
Apr	F1	88.9	77.8	33.3	0.0	0.0	100.0	0.0
	F2	77.8	22.2	11.1	0.0	0.0	100.0	0.0
	F3	100.0	16.7	16.7	0.0	0.0	100.0	0.0

Table 5. Seasonal prevalence (%) of Nile tilapia bacteria from three fish farms in the Canoas II reservoir

Month	Fish farm	Streptococcus sp	Streptococcus agalactiae	Aeromonas sp	Pseudomonas sp	Plesiomonas shigelloides	Lactococcus lactis	Edwardsiella tarda
Jun	F1	0.0	0.0	8.3	8.3	0.0	0.0	0.0
	F2	0.0	0.0	16.7	16.7	0.0	8.3	0.0
	F3	11.1	0.0	11.1	0.0	0.0	0.0	0.0
Aug	F1	11.1	0.0	22.2	0.0	0.0	0.0	0.0
	F2	8.3	0.0	16.7	0.0	0.0	0.0	0.0
	F3	11.1	0.0	0.0	0.0	0.0	33.3	0.0
Oct	F1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	F2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	F3	0.0	0.0	0.0	0.0	33.3	0.0	0.0
Dec	F1	11.1	0.0	0.0	0.0	0.0	0.0	0.0
	F2	0.0	0.0	0.0	0.0	8.3	0.0	0.0
	F3	0.0	0.0	0.0	16.7	16.7	0.0	16.7
Feb	F1	0.0	0.0	25.0	0.0	8.3	0.0	8.3
	F2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	F3	0.0	0.0	0.0	0.0	11.1	0.0	11.1
Apr	F1	11.1	22.2	11.1	0.0	0.0	0.0	0.0
	F2	0.0	0.0	0.0	0.0	11.1	0.0	0.0
	F3	0.0	0.0	0.0	0.0	16.7	16.7	50.0

All groups of parasites observed in the present study are commonly found in tilapia farms in Brazil [34-39]. The most prevalent parasites during monitoring were the protozoa of the genus *Trichodina* in fish skin and monogenoids of the family Dactylogiridae in fish gills. In June, when the water temperature was lower, multiple parasite infestations occurred, demonstrating the weakness of the host due to unfavorable climatic conditions. Corroborating this result, Jerônimo et al. [40] observed larger infestations by protozoa in months with lower temperatures (autumn and winter) and increased rates of parasitism by monogenoids in months with higher

temperatures (spring and summer) in *O. niloticus* grown in Santa Catarina, Brazil (Table 4).

No relation was found between seasonality and the occurrence of each bacterial genus, which was randomly isolated at each season of the year (Table 5). Importantly, the prevalence of parasites is higher overall compared with that of bacteria, demonstrating that although many fish farmers attribute the cause of mortality of cage farmed tilapia to bacterial diseases, the primary cause is related to parasite infestation, particularly trichodinids protozoa. Trichodinids' adherence to and suction on the epithelium may cause damage and open a port of entry for secondary pathogens [41], such as *Streptococcus agalactiae* and *Aeromonas* sp. In addition, Martins et al. [42] observed that the antibody levels and lymphocyte counts were lower in Nile tilapia parasitized by *Trichodina heterodentata*, *Gyrodactylus cichlidarum* and *Ichthyophthirius multifiliis*.

RELATION BETWEEN LIMNOLOGICAL CHARACTERISTICS AND DISEASES OF NILE TILAPIA

The spatial and temporal variation of superficial limnological characteristics and diseases of Nile tilapia was investigated using principal component analysis (PCA) [43]. The set of variables was distributed in three groups in PCA (PC1×PC2): G1: December 2012 and February 2013; G2: August and October 2012 and April 2013; and G3: June 2012. In PC1, G1 had higher temperature, alkalinity and pH values and *Plesiomonas shigelloides* prevalence compared with G3, which had higher turbidity and DO values and *Trichodina* sp, Gyrodactilidae, *Streptococcus* sp, and *Aeromonas* sp. predominance. In PC2, G2 had higher pH and DO values and *Trichodina* sp. predominance compared with G1 and G3. The temperature was positively associated with *Plesiomonas shigelloides* and was negatively associated with other pathogens, indicating that this variable has strong influence on the development of these diseases. The samples from the month of August to the left of this axis were also associated with *Trichodina* sp., Gyrodactilidae, *Streptococcus* sp., and *Aeromonas* sp. predominance (Table 6 and Figure 4).

Relevant associations were found in the PC1×PC3 biplot graph. Dactylogiridae was associated with June and August 2013 in P3 and with April 2013 in P2, which coincided with larger *Trichodina* sp. prevalence. Electrical conductivity and chlorophyll *a* values were relevant for spatial and

temporal variability; however, these variables were not associated with pathogens (Table 6 and Figure 5).
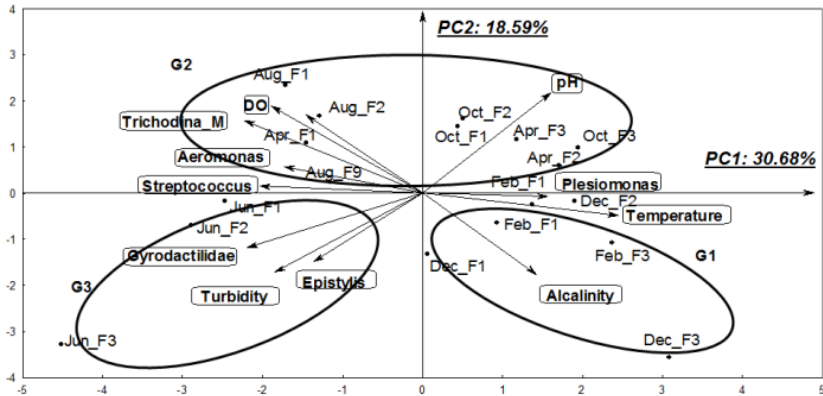
Figure 4. Biplot PC1×PC2 of PCA applied to the limnological variables and fish diseases.

Table 6. Discriminatory power of the variables in principal components 1, 2 and 3

Variable	PC1	PC2	PC3
pH	**0.5141**	**0.7189**	0.1571
EC	0.4186	0.3900	0.6307
DO	**-0.5043**	0.4635	-0.4572
Temperature	**0.8655**	-0.1557	-0.0554
Turbidity	**-0.5796**	**-0.6046**	0.2234
Chlorophyll a	-0.0415	0.1005	**-0.7093**
Alkalinity	0.4105	**-0.6157**	0.1056
Trichodina_M	**-0.6916**	0.5435	0.2771
Gyrodactilidy	**-0.6617**	-0.3322	0.3143
Epistylis	-0.4737	**-0.5067**	0.2617
Dactylogiridy	-0.4115	0.4986	0.5261
Streptococcus	**-0.5908**	0.0264	-0.0492
Aeromonas	**-0.5197**	0.1527	-0.3444
Plesiomonas	**0.6449**	-0.0645	0.0950

EC: electrical conductivity; DO: dissolved oxygen.
The absolute value of 0.5 was adopted as the cutoff point.
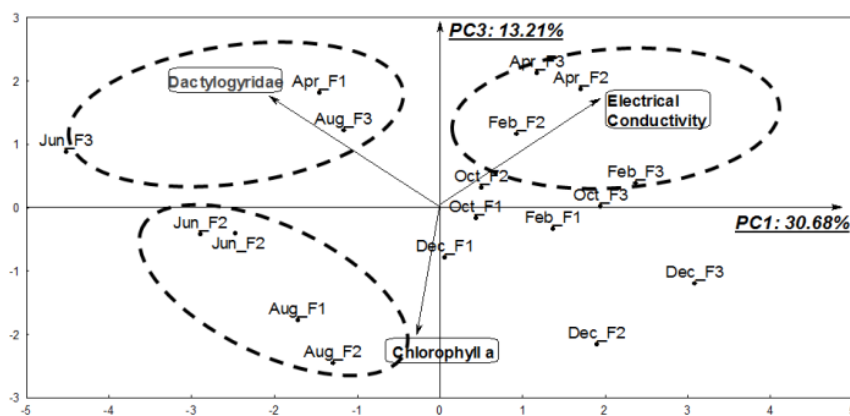
Figure 5. Biplot PC1×PC3 of PCA applied to the limnological variables and fish diseases.

OPERATING COSTS, PROFITABILITY AND TIME FOR RETURN ON INVESTMENT OF TILAPIA CAGE FARMING

Tilapia cage farming in the Canoas II Reservoir was economically evaluated to identify the critical points and to indicate mechanisms for improvement of the production system to minimize costs and to optimize results.

Information related to fish farms was obtained through field survey, in which a questionnaire based on the description of Garcia Filho [44] that consisted of semi-structured questions was applied. Cages with 6.0 m^3 and 18.0 m^3 usable volumes and 2 marketing channels (fishing and processing industries) were analyzed.

Technical data regarding fish farming in 6.0 m^3 and 18.0 m^3 cages corresponded to the following parameters, respectively: a) density: 150 and 115 fish/m³; b) productivity: 600 and 1,500 kg/cycle/cage; c) mortality rate: 10% and 20%; d) feed conversion ratio: 1:1.6 and 1:1.8; and e) harvest: 250 6.0 m^3 cages and 110 18.0 m^3 cages/cycle.

The following variables were considered equal for both cage sizes: a) production cycle: 150 d; b) mean commercial weight: 800 g/unit; c) place of sale of production: 70% for the processing industry and 30% for fisheries; d) mean sale price: USD 1.70/kg for the processing industry and USD 2.06/kg for fisheries; e) mean price of feed: USD 0.72/kg, with USD 0.58/kg for 28 to

32% crude protein (CP) and with USD 0.90/kg for 36 to 42% CP; f) mean price of 1,000 units: USD 100.00 for phase I (up to 20 g) and USD 180.00 for juvenile (45 to 50 g); g) mean monthly price of workforce: USD 672.65 for technical, USD 336.32 for common labor, and USD 15.70 for day laborer; and h) management fees: USD 896.86/mo.

To analyze the viability of this activity, the investment of the cage farming project was standardized for 1 ha of water surface, which corresponds to the installation of 250 cages with 6.0 m^3 of usable volume (2 m long×2 m wide×1.70 m deep, totaling 6.8 m^3) and 110 cages with 18 m^3 of usable volume (3 m long×3 m wide×2.30 m deep, totaling 20.7 m^3). The number of cages per hectare of water surface was obtained using the water dilution ratio of 1:10 such that for each 1 m^2 of cages, a 10 m^2 water surface of the reservoir is used. Note that the usable volume of each cage size is used, i.e., 6.0 m³ and 18.0 m³, for calculating the stocking density.

The methodology for calculating the production cost used was that of the Institute of Agricultural Economics (Instituto de Economia Agrícola - IEA). The following structures were considered in the production system: effective operating cost (EOC), which includes expenditures on labor, machinery/equipment and materials consumed during the production process operations, and total operating cost (TOC), which is the actual operating cost plus direct social-related expenditures (33% of permanent labor), rural social security contribution (*Contribuição Especial da Seguridade Social Rural* – CESSR; 2.2% of gross revenue), financial expenditures of the capital cost (8.75% per year on 50% of the EOC), technical assistance (5% of the EOC), compensation for investment (total operating cost plus the cost of project implementation on productivity) and machinery depreciation.

The profitability analyses used in the study were defined by Martin et al. [45] as follows: a) The gross revenue (GR) is the expected revenue for a given production per hectare for a pre-set or actually received sale price, i.e., GR=Pr×Pu, where Pr: activity production per area unit; Pu: unit price of the product. b) The operating profit (OP) or the net revenue (NR) is the difference between the gross revenue and the operating cost per hectare. The operating profit indicator measures the profitability of the activity in the short term, indicating the financial and operating conditions of the activity; thus, *OP=GR-TOC*. c) The profitability index (PI) shows the relation between the operating profit and the gross revenue as a percentage. The PI is an important measure of profitability of the agricultural activity because this measure shows the activity's available revenue rate after paying all operating costs. Therefore, *PI=(OP/GR)×100*. d) The leveling point (LP) is the cost indicator relative to

the product unit, i.e., this value determines the minimum production required to cover the total operating cost given the unit sale price. Thus, we considered the following equation: *LP=TOC/Pu.*

The cost for tilapia production in 6 m³ cages was higher than the cost of fish farming in 18 m³ cages. This difference was primarily due to expenses associated with feeding, which is the most representative item in the total cost (Table 7).

Table 7. Operating costs of cage farmed tilapias per hectare per 150 d cycle in 2013/2014

Item	6.0 m³ cage		18.0 m³ cage	
	USD	% TOC	USD	% TOC
Labor	2.303.77	1.28	1.946.46	1.11
Fingerlings	9.080.72	5.04	9.189.69	5.24
Feed	147.171.30	81.62	143.359.28	81.77
Fuel	2.414.66	1.34	2.397.15	1.37
Machinery operations	551.61	0.31	535.83	0.31
Effective op. cost (EOC)	**161,522.05**	**89.58**	**157,428.41**	**89.8**
Machinery depreciation	165.48	0.09	160.75	0.09
Direct social expenditures	760.24	0.42	642.33	0.37
CESSR	4.251.23	2.36	3.824.79	2.18
Technical assistance and general expenses	8.076.10	4.48	7.871.42	4.49
Financial expenditures	5.530.52	3.07	5.390.35	3.07
Total op. cost (TOC)	**180,305.62**	**100**	**175,318.05**	**100**
Op. cost per unit	**1.11**	**-**	**1.20**	**-**
Op. cost per cage	**721.22**		**1,593.80**	

USD 1.00=BRL 2.23 in July 2014.

Despite the higher cost in the project with 6.0 m³ cages, the cost per kilogram for the 6.0 m³ cages was lower due to the productivity per m³ obtained at the end of the production cycle. The price per kilogram of fish produced was USD 1.11 and USD 1.20 for 6.0 m³ and 18.0 m³ cages, respectively. Spending per 6 m³ and 18 m³ cage per cycle was equivalent to USD 721.22 and USD 1,593.80, respectively.

Table 8. Profitability of tilapia cage farming per hectare per 150 d cycle in 2013/14

Indicator	Unit	6.0 m^3 cage	18.0 m^3 cage
Productivity	t/ha	150	165
Industry sale price	USD/kg	1.70	1.70
Fishery sale price	USD/kg	2.06	2.06
Weighted average price	USD/kg	1.81	1.81
Sale to industry	kg/ha	105	115,5
Sale to fisheries	kg/ha	45	49,5
Gross revenue	USD/cycle	271,500	298,650
Operating profit	USD/cycle	91,194	123,331
Profitability index	%	33	41
Leveling point	t/ha	99	97
Leveling point	kg/CG	396	882

Obs: sale of production: 70% for processing industry and 30% for fishery.
USD 1.00=BRL 2.23 in July 2014.

The profitability of tilapia production in 6.0 m^3 and 18.0 m^3 cages is feasible considering the sale of 70% of the production for the processing industry and 30% for fisheries. The weighted average sale price was USD 1.81/kg of produced fish, which generated gross revenues of USD 271.500 and USD 298.650/ha for the 6.0 m^3 and 18.0 m^3 cages, respectively. These values represent an operating profit of USD 365 per 6.0 m^3 cages (USD 0.40 of produced fish) and USD 1.121 per 18.0 m^3 cage (USD 0.54 of produced fish). The obtained profitability indices corresponded to 33% (6 m^3 cages) and 41% (18 m^3 cages). The leveling points were equivalent to 99 and 97 t/ha for the 6.0 m^3 and 18.0 m^3 cages, respectively.

The time for return on investment with the implementation of cages was 2 cycles or 1.5 years. The minimum amounts to be produced for the viability of the activity were 396 kg/6.0 m^3 cage and 882 kg/8.0 m^3 cage (Table 8).

REFERENCES

[1]   Ministério da Pesca e Aquicultura, Brazil. Boletim Estatístico da Pesca e Aquicultura [Fisheries and aquaculture statiscal bulletin]. 2011 [Online]. Available from: http://www.mpa.gov.br /index.php/monitoramento-e-controle/informacoes-e-estatisticas.

[2] Halwart, M; Soto, D; Arthur, JR. Cage aquaculture – Regional reviews and global overview. FAO Fisheries Technical Paper. *Rome*, FAO, 2007.

[3] Kubitza, F; Campos, JL; Ono, EA; Istchuk, PI. Panorama da Piscicultura no Brasil: estatística, espécie, pólos de produção e fatores limitantes à expansão da atividade [The Situation of Fish Farming in Brazil: statistics, species, production centers and limiting factors to activity expansion]. *Panorama da Aquicultura*, 2012, 22, 14-25.

[4] Furlaneto, FPB. Análise econômica da produção de tilápia em tanques-rede, ciclo de verão, região do Médio Paranapanema, Estado de São Paulo [Economic analysis of tilapia production in cage farming systems, summer cycle, Middle Paranapanema region, São Paulo State]. *Informações Econômicas*, 2010, 40, 5-11.

[5] Ono, EA; Kubitza, F. Cultivo de peixes em tanques-rede [Fish cage farming]. 3rd edition: Jundiaí: E.A. Ono, 2003.

[6] Stephens, W; Farris, JL. A biomonitoring approach to aquaculture effluent characterization in channel catfish fingerling production. *Aquaculture*, 2004, 241, 319-330.

[7] Beveridge, MCM. Cage aquaculture. 3rd edition. Oxford, UK: Blackwell Publishing Ltd; 2004.

[8] Pillay, TVR. Aquaculture and the environment. 2nd edition. Oxford: Blackwell Publishing; 2004.

[9] Honkanen, T; Helminen, H. Impacts of fish farming on eutrophication: comparisons among different characteristics of ecosystem. *International Review of Hydrobiology*, 2000, 85, 673-686.

[10] Karakassis, I; Pitta, P; Krom, MD. Contribution of fish farming to the nutrient loading of the Mediterranean. *Scientia Marina*, 2005, 69, 313-321.

[11] Carrol, ML; Cochrane, S; Fieler, R; Velvin, R; White, P. Organic enrichment of sediments from salmon farming in Norway: environmental factors, management practices, and monitoring techniques. *Aquaculture*, 2003, 226, 165-180.

[12] Karakassis, I; Tsapakis, M; Smith, CJ; Rumohr, H. Fish farming impacts in the Mediterranean studied through sediment profiling imagery. *Marine Ecology Progress Series*, 2002, 227, 125-133.

[13] Nickell, LA; Black, KD; Hughes, DJ; Overnell, J; Brand, T; Nickell, TD; Breuer, E; Harvey, SM. Bioturbation, sediment fluxes and benthic community structure around a salmon cage farm in Loch Creran, Scotland. *Journal of Experimental Marine Biology and Ecology*, 2003, 285, 221-233.

[14] Pawar, V; Matsuda, O; Fujisaki, N. Relationship between feed input and sediment quality of the fish cage farms. *Fisheries Science*, 2002, 68, 894-903.

[15] Yucel, G; Kucuksezgin, F; Kocak, F. Effects of fish farming on nutrients and benthic community structure in the Eastern Aegean (Turkey). *Aquaculture Research*, 2007, 38, 256-267.

[16] Karakassis, I; Tsapakis, M; Hatziyanmi, E; Papadopoulou, KN; Plaiti, W. Impact of cage farming of fish on the seabed in three Mediterranean coastal areas. *Journal of Marine Science*, 2000, 57, 1462-1471.

[17] Almeida, CA; Rocha, O. Estudo comparativo da qualidade dos sedimentos dos reservatórios do rio Tietê (SP), São Carlos – SP. [Comparative study of sediment quality from the Tietê river reservoirs (SP)] *Journal of the Brazilian Society of Ecotoxicology*, 2006, 1, 141-145.

[18] Esteves, FA. Fundamentos de limnologia [Fundamentals of limnology] Rio de Janeiro: *Interciência*, 1998.

[19] Fonseca, JJ; Gonçalves, JFJ; Callisto, M. C,N,. P e composição granulométrica do sedimento em quatro ecossistemas lóticos amazônicos sob influência de uma mineração de bauxita [C, N, P and granulometric composition of the sediment in four Amazonian lotic ecosystems under the influence of a bauxite mining]. In: Anais do Proceedings of VIII Seminário Regional de Ecologia, 1998, São Carlos, 1998, 8, 1373-1380.

[20] Duke Energy Internacional Geração Paranapanema. Peixes do Rio Paranapanema [Fish of the Paranapanema River]. *São Paulo: Horizonte Geográfico*, 2003.

[21] Companhia Ambiental do Estado de São Paulo (CETESB). Guia nacional de coleta e preservação de amostras: água, sedimento, comunidades aquáticas e efluentes líquidos [National guide to collection and preservation of samples: water, sediment, aquatic communities and wastewater]. São Paulo: CETESB, 2011.

[22] American Public Health Association (APHA). Standard methods for the examination of water and wastewater, 20th edition. APHA: Washington, District of Columbia, 1998.

[23] Köppen, W. Climatologia: con un estudio de los climas de la tierra [Climatology. A study of the Earth's climate]. *Ciudad de México: Fondo de Cultura Econômica*, 1948.

[24] Pagioro, TA; Thomaz, SM; Roberto, MC. Caracterização limnológica abiótica dos reservatórios [Abiotic limnological characterization of reservoirs]. In: Rodrigues, L; Thomaz, SM; Agostinho, AA; Gomes, LC,

editors. Biocenose em reservatórios: Padrões espaciais e temporais [Biocenosis in reservoirs: Spatial and temporal patterns]. *São Carlos: RIMA*, 2005, 17-37.

[25] Moretto, EM; Nogueira, MG. Physical and chemical characteristics of Lavapés and Capivara rivers, tributaries of Barra Bonita reservoir (São Paulo – Brazil). *Acta Limnologica Brasiliensia*, 2003, 15, 27-39.

[26] Ministério da Agricultura, Mar, Ambiente e Ordenamento do Território, Portugal. Ecologia e gestão de albufeiras [Online]. Available from: http://www.afn.min-agricultur.pt/portal/pesca/resource/Ficheiros/gestao-recuros-aquicolas/estudoestrategico/Ecologia-e-Gestao-Albufeiras.pdf /view.

[27] Associação Brasileira de Normas Técnicas (ABNT) [Brazilian Association of Technical Standards]. Norma técnica nº 7181. Solo-análise granulométrica [Technical standard no. 7181. Soil-grain size analysis]. *São Paulo: ABNT*, 1988.

[28] Burton, GA, Jr. Sediment quality criteria in use around of the world. *Liminology*, 2002, 3, 65-75.

[29] Froehner, S; Martins, RF. Avaliação da composição química de sedimentos do Rio Barigüi na região metropolitana de Curitiba [Evaluation of the chemical composition of sediments from the Barigüi River in Curitiba, Brazil]. *Quimica Nova*, 2008, 31, 2020-2026.

[30] Nogueira, MG; Perbiche-Neves, F; Naliato, DAO. Limnology of two contrasting hydroelectric reservoirs (storage and run-of-river). Samadi-Boroujeni, editor. Southeast Brazil, Hydropower – Practice and Application. *Rijeka, Croatia: Intech*, 2012, 167-184.

[31] Jorcin, A; Nogueira, MG. Phosphate distribution in the sediments along a cascade of reservoirs (Paranapanema River, SE, Brazil). *In: Anais do IV International Symposium, Carmona*, 2005.

[32] Bueno, GW; Marengoni, NG; Gonçalves- Júnior, AC; Boscolo, WR: de Almeida Teixeira, R. Estado trófico e bioacumulação do fósforo total no cultivo de peixes em tanques peixes na área aquícola do reservatório de Itaipu [Trophic level and bioaccumulation of total phosphorus in cage fish rearing in the aquaculture area at Itaipu reservoir]. Acta Scientiarum. *Biological Sciences*, 2008, 30.

[33] Bush, AO; Lafferty, KD; Lotz, JM; Shostak, AW. Parasitology meets ecology on its own terms. *Journal of Parasitology*, 1997, 83, 4, 575-583.

[34] Vargas, L; Povh, JA; Ribeiro, RP; Moreira, HLM. Ocorrência de ectoparasitos em tilápias do Nilo (Oreochromis niloticus), de origem tailandesa, em Maringá - Paraná [Occurrence of ectoparasites in tilapias

(Oreochromis niloticus) of Thailand origin, in Maringá - Paraná]. *Arquivos de Ciências Veterinárias e Zoologia da Unipar*, 2000, 3, 31-37.

[35] Tavares-Dias, M; Martins, ML; Moraes, FR. Fauna parasitária de peixes oriundos de "pesque-pague" do município de Franca, São Paulo, Brasil. I. Protozoários [Parasitic fauna of cultivated fishes in feefishing farm of Franca, São Paulo State, Brazil I. *Protozoans] Revista Brasileira de Zoologia*, 2001, 18, 67-79.

[36] Tavares-Dias, M; Martins, ML; Moraes, FR. Fauna parasitária de peixes oriundos de "pesque-pague" do município de Franca, São Paulo, Brasil. II. Metazoários [Parasitic fauna of cultivated fishes in feefishing farm of Franca, São Paulo State, Brazil I. Metazoans]. *Revista Brasileira de Zoologia*, 2001, 18, 81-95.

[37] Martins, ML; Moraes, JRE; Andrade, PM; Schalch, SHC; Moraes, FR. Piscinoodinium pillulare (Schäperclaus, 1954) Lom, 1981 (Dinoflagellida) infection in cultivated freshwater fish from the Northeast region of São Paulo State, Brazil. Parasitological and pathological aspects. *Brazilian Journal of Biology*, 2001, 61, 639-644.

[38] Ranzani-Paiva, MJT; Felizardo, NN; Luque, JL. Parasitological and hematological analysis of Nile tilapia Oreochromis niloticus Linnaeus, 1757 from Guarapiranga reservoir, São Paulo State, Brazil. *Acta Scientiarum Biological Sciences*, 2005, 27, 231-237.

[39] Ghiraldelli, L; Martins ML; Jerônimo, GT, Yamashita MM, Adamante WB. Ectoparasites communities from Oreochromis niloticus cultivated in the State of Santa Catarina, Brazil. *Journal of Fisheries and Aquatic Sciences*, 2006, 2, 181-190.

[40] Jerônimo, GT; Speck, GM; Cechinel, MM; Gonçalves, ELT; Martins, ML. Seasonal variation on the ectoparasitic communities of Nile tilapia cultured in three regions in southern Brazil. *Brazilian Journal of Biology*, 2011, 71, 365-373.

[41] Noga, EJ. Fish disease - diagnosis and treatment. St. Louis: Mosby-Year Book, 1996.

[42] Martins, ML; Shoemaker, CA; Xu, D; Klesius, PH. Effect of parasitism on vaccine efficacy against Streptococcus iniae in Nile tilapia. *Aquaculture*, 2011, 314, 18-23.

[43] Hair, JF; Sant'Anna, AS; Gouvêa, MA. Análise Multivariada de dados [Multivariate data analysis]. 5th edition. *Porto Alegre: Bookman Ed*, 2005.

[44] Garcia Filho, DP. Análise diagnóstico de sistemas agrários: guia metodológico [Diagnostic analysis of agrarian systems: methodological guide]. Brasília: *Projeto de Cooperação Técnica*, INCRA/FAO, 1999.

[45] Martin, NB; Serra, R; Oliveira, MDM; Ângelo, JA; Okawa, H. Sistema integrado de custos agropecuários - CUSTAGRI [Integrated farming costs (Sistema integrado de custos agropecuários – CUSTAGRI)]. *Informações Econômicas*, 1998, 28, 7-28.

INDEX

C

F

G

H

I

Q

R

W

Y