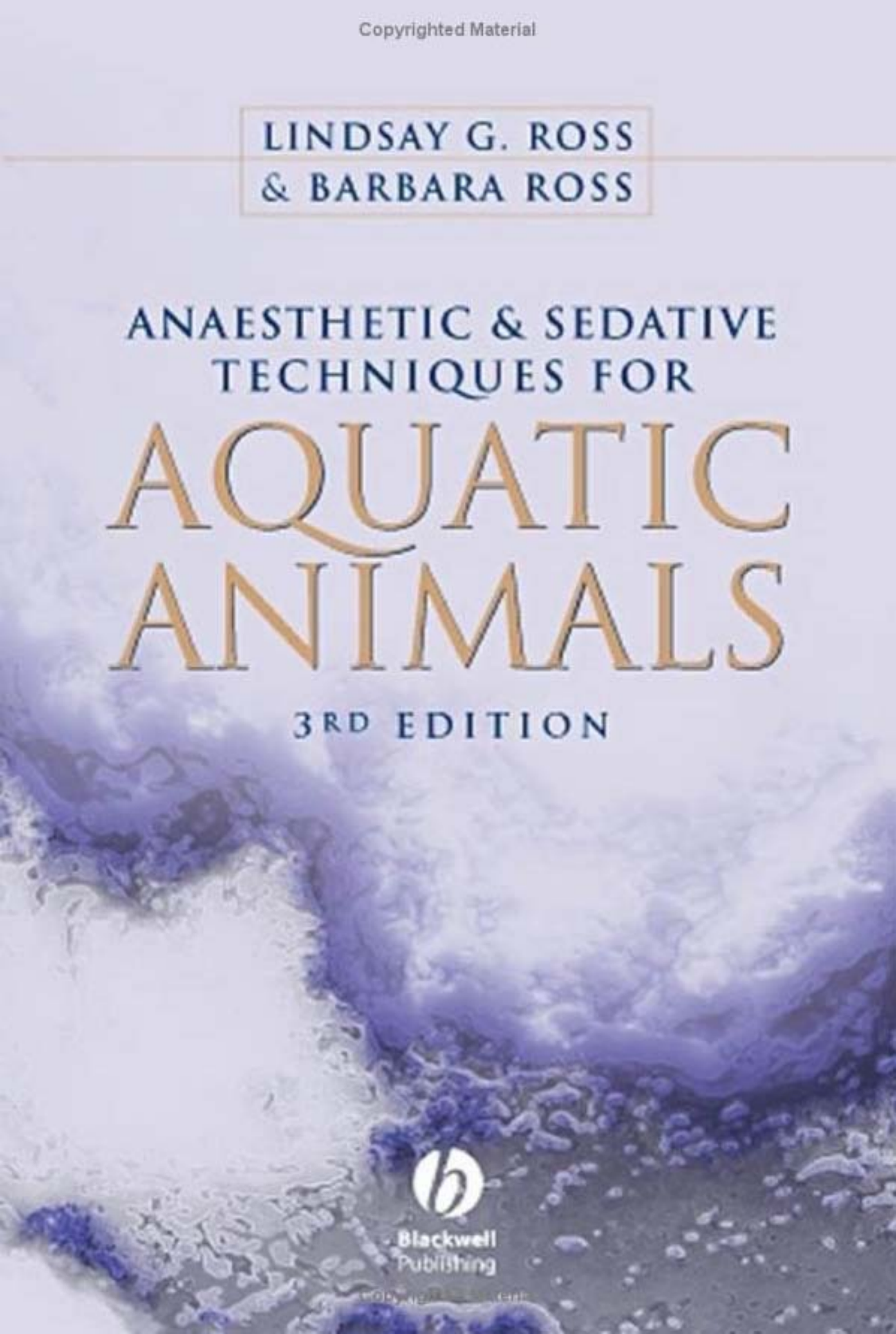
LINDSAY G. ROSS
& BARBARA ROSS

ANAESTHETIC & SEDATIVE TECHNIQUES FOR AQUATIC ANIMALS

3RD EDITION



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Anaesthetic and Sedative Techniques for Aquatic Animals

Anaesthetic and Sedative Techniques for Aquatic Animals Edited by Lindsay G. Ross and Barbara Ross
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Anaesthetic and Sedative Techniques for Aquatic Animals

Third Edition

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Preface to the Third Edition

In 1983 we were invited to a meeting of the UK Veterinary Anaesthetists Association in Edinburgh to talk to an experienced group of researchers about fish anaesthesia, a minor and unusual topic for most veterinary anaesthetists. Both of us had been involved for some time with fish and invertebrate research requiring a range of anaesthetic approaches and had been teaching fish anaesthesia in various courses in the Institute of Aquaculture for some years. From this, we published the first edition of this handbook as one of a series of handbooks from the Institute of Aquaculture. This little handbook was very popular and sold out quickly. Fifteen years later we prepared a much expanded second edition, this time published by Blackwell Science. About 1,000 new articles or monographs were reviewed during its preparation and the content was substantially expanded and updated.

This third edition of the book continues to be the only substantial reference work on anaesthesia of fish. We have again focused on fish, but have also included sections on other cultured aquatic animals. The book is not intended to be an exhaustive review of aquatic animal anaesthesia; rather, we attempt to provide a review of methods and guidelines on the topic aimed at aquaculturists and researchers. Its contents are selective and are aimed at providing an essentially practical reference for the laboratory or the farm. The references provided are also selective, but should be more than sufficient to provide the reader with a starting point in the extensive literature for following up any topic.

In preparing this new edition we have, again, reviewed the content of almost 1,000 new relevant publications. The book has been substantially restructured and a new chapter on pain in fishes is included, written by Bryony Ross. Data on chemical structures, nomenclature and trade names of drugs have been greatly expanded as has the range of informative tables. In the eight years since the publication of the second edition there has been steady progress in the field. Noteworthy advances have been in the use of clove oil and AQUI-S. A small but promising beginning has also been made on use of combined anaesthesia and analgesia.

As noted in the second edition, legal aspects regarding use of anaesthetic drugs with fish have moved on again as the cause of animal welfare has developed and end users can expect to be required to comply with ever more rigorous legislation. We must emphasise that this book is not a guide to current legislation or legal requirements in any given country, nor does it attempt to advise users of their current obligations, which are something of a moving target. We do, however, underline the need to be aware of and to comply with all relevant legislation, wherever in the world the end user is located. This may comprise legislation on animal welfare, safe operator practice for drugs and chemicals and their storage, safety legislation concerning electric fishing and similar electrical apparatus, use of drugs with animals intended for the human food chain and release of drugs into the environment.

The authors and the publisher accept no responsibility for losses of animals, losses of experimental or other data, harm occurring to individuals or any other matter which may arise caused by application of the data contained herein or which may arise as a result of non-compliance with any relevant legislation.

Finally, in attempting to address a wide readership, we have tried to provide sufficient background while maintaining the practical utility of the book. We hope that we have achieved this.

Lindsay and Barbara Ross
Stirling, 2007

Acknowledgements to the Third Edition

While some of the information presented here is based upon our own experiences, much has come from the combined efforts of the aquatic research community who have explored this field over many years. In earlier editions we invited readers to let us know of their problems and experiences and we are grateful to those who have indeed done so as this has kept us in touch with a range of activities world-wide which would not otherwise be reported in the literature.

Some information presented here was generated while the authors were involved with the tropical aquaculture programme supported at Stirling by DFID. We must acknowledge our students, David McCaldon, Remigius Okoye, Elizabeth Robinson, Dr Bernard Ladu, Dr Khaled Al-Abdul Elah, Dr Pepe Aguilarand Judith Sanchez Blanca who worked directly in this field with us over the years. Thanks also to our colleagues, particularly Dr Les Oswald and Dr Malcolm Beveridge, who supplied us with interesting snippets of data gathered from all over the world, and to Richard McKinney.

The second edition was prepared while LGR was on sabbatical leave in Mexico and sincere thanks are due to Dr Carlos Martinez-Palacios, then Director of CIAD, Mazatlan, as well as Dr Innocencio Higuera-Ciapara, then Director-General of CIAD, Hermosillo, Mexico, for their support and generosity.

Lindsay and Barbara Ross
Stirling, 2007

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Chapter 1

Introduction

The handling of aquatic animals both in and out of their natural environment almost always creates great difficulties. Their characteristic struggling during capture and handling usually has strong effects on both physiology and behaviour, and consequently it is almost always necessary to immobilise fish before attempting to perform even the simplest task (Tytler and Hawkins, 1981). Anaesthesia and sedation are valuable tools in wild fish collection and for fisheries management. In the operation of a culture facility it is not always necessary to sedate or anaesthetise stocks. For certain procedures, however, sedation may be essential to minimise stress or physical damage caused by crowding, capture, handling and release and in research work or veterinary practice there may be the additional requirements to render animals unconscious or to alleviate pain.

'Comfort' and animal husbandry

There has been increasing discussion and considerable disagreement on the behavioural and physiological aspects of fish welfare. Huntingford *et al.* (2006) review current thinking on this topic comprehensively and explore whether or not fish and other lower vertebrates 'suffer' in adverse conditions. Arlinghaus *et al.* (2007) challenged their 'feelings-based' approach to fish welfare, considering that many aspects are unquantifiable and hence impossible to understand. They urge for a more objective evaluation of the issues based upon biochemical, physiological and behavioural indicators.

Clearly, this discussion has some way to go and will not be resolved easily until a great deal more is known about the neurophysiology of fish and similar animals. Whatever one's point of view, it should be possible to agree that fish are at least averse to painful stimuli and that they can also experience fear.

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The clear consequence is that any potentially harmful or stressful conditions must be avoided or minimised. This applies not only to fishes but also to other lower vertebrates such as crocodilians and turtles and probably also at least to the higher invertebrates such as the cephalopods. The principal objectives of aquaculture are to grow animals to market size in the minimum of time, with minimum inputs and with minimum cost. This implicitly means that both growth rate and growth efficiency must be maximised and, for this, animals must be unstressed and 'comfortable' within their culture environment. In writing about physiological choices in the natural environment, Balchen (1975) suggested that animals would select, or attempt to select, environments in which they were able to 'maximise their comfort'. This concept can easily be grasped if one considers, for example, the selective movements which a fish would make when presented with a temperature or salinity gradient. A fish would swim into an area of lower or higher temperature which it 'preferred', perhaps in the process optimising feeding or the temperature for some physiological process such as digestion, but inevitably choosing an area of best 'comfort'. This ability to choose applies to a wide range of conditions and situations which may be encountered daily, or seasonally, in the life of an aquatic animal. In the more managed and often confined environments of aquaculture, animals are unable to select their environment in the same way and it is then the task of the aquaculturist to provide an environment in which the cultured animals are as stress-free as possible, and hence 'comfortable'.

Aquatic animals in extensive culture are relatively unstressed, but in more intensive systems they are, almost certainly, in a state of chronic low-level stress caused by the combined effects of some kind of confinement, high culture density, behavioural disruption and possibly compromised environmental factors. These environmental factors can be wide ranging and complex and include poor, or partly degraded, water quality, over-simplistic system design, inadequate provision of biologically meaningful systems and practices and, generally, sub-optimal animal husbandry. Such problems are often the result of trade-offs in design and costs, which lead to convenient and economical systems in terms of their construction, operation and management but which nevertheless are very different from conditions in the natural environment. Little is known of the real extent of this problem in aquaculture, or how it is manifested in different systems and in different species, but many workers now feel that chronic low-level stress is a real and probably constant constraint. Clearly, good animal husbandry can minimise the negative effects of stress although good husbandry may simultaneously tend to suppress or minimise any tell-tale clinical signs which would

normally be apparent. Any additional handling, or similar operation, can cause extra, acute stress which will leave animals in a weakened state, perhaps feeding badly for some time, with poor digestive efficiency and hence growth, greatly predisposed to disease and much less able to deal with environmental fluctuations.

Handling and mechanical damage

There are many instances where some form of restraint or calming is needed to facilitate handling. In struggling aquatic animals there is the additional, and very significant, effect of gross physical damage; a problem which is not usually encountered in handling terrestrial species, at least not to the same degree. The skin of fishes is normally very thin and is external to the scales, making it very easy to abrade. The delicate eyestalks and appendages of crustaceans are also particularly vulnerable and great care must be taken to avoid the damaging abrasion or even possible mutilation which may occur during any handling process. It must be emphasised that, even where animals are not being handled individually, but are involved in bulk operations such as batch weighing, there will be substantial risk of abrasion, asphyxia and stress. This is a factor which is often overlooked in practice, especially where procedures are brief, in the belief that brevity enables evasion of consequences.

Pain

In some areas of aquaculture and in research or veterinary work there may be the additional need to address the problem of pain. The ability of fish and invertebrates to feel pain is frequently contended (Rose, 2002; Chandroo *et al.*, 2004). However, it is clear that all vertebrate animals do react to noxious stimuli and it is well known that most invertebrates also have special receptors for such stimuli. Indeed, because these specific receptors (nociceptors) and their axons can be readily identified in invertebrates, they have been widely used as models in neurophysiological research for many years. Any procedure which involves invasive methods, ranging from fairly simple forms of tagging using needles, to any use of incisions and suturing or to internal surgery, is fairly certain to cause some degree of pain. This is particularly so in vertebrates but probably also to some degree in the invertebrates, and steps must be taken to alleviate any suffering which could be caused. The humane

treatment of experimental or cultured animals must take precedence over other considerations (Green, 1979).

Summary

When aquatic animals are removed from water, individually or in groups, physiological stress is compounded by the risk of serious abrasion and mechanical shock, particularly with a struggling animal. Procedures which can be carried out entirely in water, such as simple tagging, grading using screens or pumped water systems and batch weighing in water, are unlikely to cause serious harm. For example, experienced workers know that careful but firm handling of brood-stock trout can enable procedures such as stripping of eggs and sperm to be carried out without sedation. However, there are many instances in which some form of calming technique, or sedation, will be required; for example, during length–weight studies, sexing, simple injection, withdrawal of body fluids, branding and many types of tagging. In other cases additional time may be needed to handle animals or carry out surgery; for example, during gonadectomy, hypophysectomy, tissue biopsy or prolonged physiological investigations, and then full surgical anaesthesia will be required.

It should be noted that all sedative and anaesthetic procedures themselves induce side-effects, which may not be considered desirable. For example, in 1961, Allison had already shown that sperm motility was greatly reduced in brook trout at 19 mg L^{-1} MS222 and clearly it is inadvisable to expose gametes to anaesthetics. Overall, however, their advantages generally outweigh their disadvantages if the correct technique is used and if sufficient control is maintained.

References

Allison, L.N., The effect of tricaine methanesulphonate (MS222) on the motility of brook trout sperm, *Prog. Fish. Cult*., 23 (1961) 46–48.

Arlinghaus, R., Cooke, S., Schwab, A. and Cowx, I.G., Fish welfare: a challenge to the feelings-based approach, with implications for recreational fishing, *Fish and Fisheries*, 8 (2007) 57–71.

Balchen, J.G., Modelling of the biological state of fishes, SINTEF: The engineering research foundation of the Technical University of Norway, Trondheim, Teknical note 62 for NTNF/NFFR, 1976, 25 p.

Chandroo, K.P., Duncan, I.J.H. and Moccia, R.D., Can fish suffer?: perspectives on sentience, pain, fear and stress, *Applied Animal Behaviour Science*, 86 (2004) 225–250.

Green, C.J., Animal anaesthesia, Laboratory animal handbooks, No 8. Laboratory Animals Ltd., London, 300 p., 1979.
Huntingford, F.A. *et al.*, Current issues in fish welfare, *Journal of Fish Biology*, 68 (2006) 332–372.
Rose, J.D., The neurobehavioral nature of fishes and the question of awareness to pain, *Reviews in Fisheries Science* 10 (2002) 1–38.
Tytler, P. and Hawkins, A.D., Vivisection, anaesthetics and minor surgery, in: Hawkins, A.D. (ed.), Aquarium Systems, New York: Academic Press, 1981.

Chapter 2

Defining Stress in Aquatic Animals

Introduction

Almost any stimulus presented to an animal causes one or more of a number of behavioural and physiological changes. Some of these are normal responses to changes in the environment or circumstances of an animal, while others are beyond this 'normal' range and are collectively referred to as stress. In the strict sense, stress in fishes has been defined as a state resulting from environmental conditions which threaten survival (Brett, 1958). However, it is now widely accepted that conditions which are not life-threatening but which impair feeding, growth, reproduction or other aspects of the normal performance, physiology and activity of an animal are stressful. The stimuli producing these changes are collectively referred to as stressors. Such stress may be counter-productive in the laboratory and in aquaculture it can lead to immediate or delayed mortalities and often causes poor feeding reactions for a day or so, with consequent slower growth and poorer growth efficiency.

It should be borne in mind that, because stress responses are induced in fishes by changes in their environment and by netting and handling, etc., it is almost impossible to investigate the physiology of a truly unstressed fish other than by remote sensing techniques such as biotelemetry.

The adrenergic system and the hypothalamic–pituitary–inter-renal axis

Animals respond to abnormal changes in their environment (stressors) by nerve-mediated motor actions and by releasing one or more hormones into the bloodstream following nervous stimulation of the endocrine organs. Hormonal responses to stress in invertebrates are not well understood, but in fish and other vertebrates the

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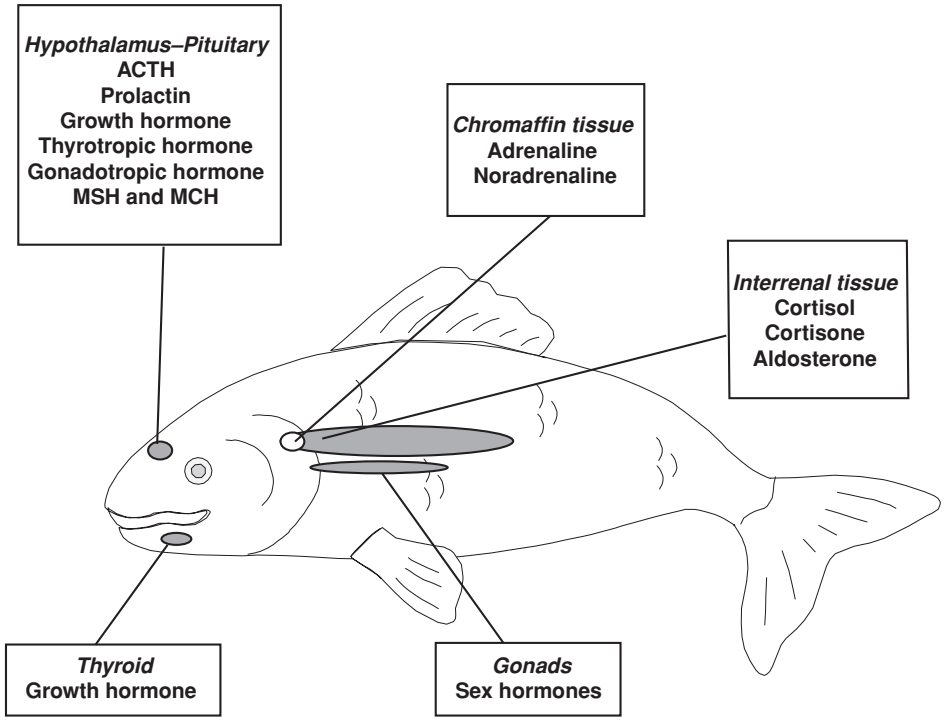


Figure 2.1 The location of the principal endocrine tissues involved in the stress responses in fish.

hormonal actions are mediated by the adrenergic system and the hypothalamic–pituitary–inter-renal axis (HPI). The approximate location of those endocrine tissues involved in the stress response in fish is shown in Fig. 2.1.

The chromaffin tissue is located in the anterior part of the kidney in fish and, following nervous stimulation, it is the source of the catecholamines adrenaline and noradrenaline (epinephrine and norepinephrine) which are released directly into the blood stream. The hypothalamus is located in the base of the brain and the tiny pituitary gland is appended directly to it. Both areas are joined by nerve tracts running in the brain itself. A range of powerful hormones are produced in these tissues and are released into the bloodstream on demand. Many of these hormones have direct metabolic effects but adrenocorticotrophic hormone, ACTH, acts on the inter-renal tissue, which is also located in the kidney, to affect the synthesis and subsequent release of further powerful steroid hormones, principally cortisol. In addition to these major influences, the thyroid hormones and sex hormones also contribute to long-term responses to stress.

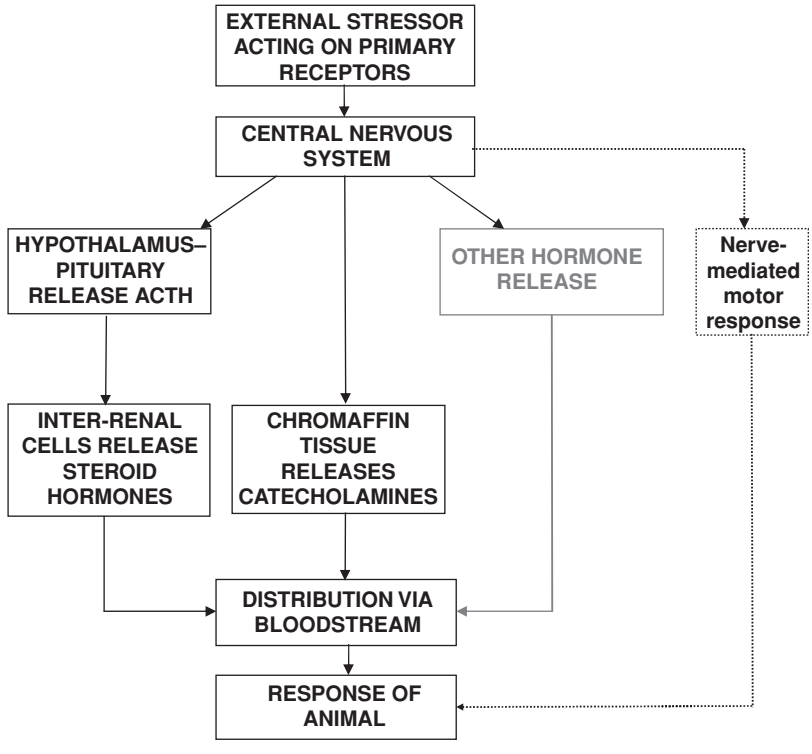


Figure 2.2 Schematic diagram of the stress-response sequence in fish, showing alternative control pathways.

It will be appreciated that the response to a stressor is not a straightforward matter but frequently involves a cascade of events the nature of which can be visualised by reference to Fig. 2.2. The release of hormones from these tissues is influenced by the nature and intensity of the stressor, but may also be influenced by the age and sex of animals and the environmental temperature.

Rapid increase of circulating catecholamines tends to cause immediate reactions (the so-called fight-or-flight reaction), usually within seconds to minutes. By contrast, release of the steroid hormones causes less immediate but usually longer-term effects, lasting from a few hours to several days. The response to these hormones is fairly consistent and, although their release adapts an animal to respond to an environmental change, there is strong evidence that even a minute increase in blood cortisol, when given by injection, ultimately has a deleterious effect in fish. Thus, stress can lead to increased disease susceptibility, growth depression and reproductive failure, all of which appear to be able to be caused by cortisol release, and which in some cases can be directly

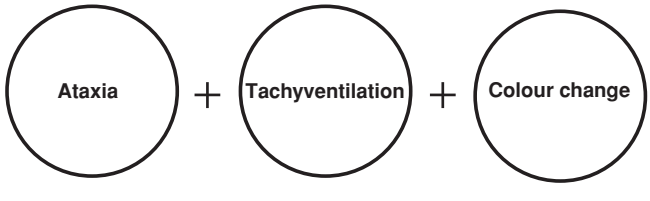
mimicked by direct cortisol injection or implantation (see Pickering, 1993).

The generalised stress response

Over the last 30 years a considerable amount of work has been carried out on stress in fish (e.g. Wedemeyer, 1970; Wardle, 1972; Wardle and Kanwisher, 1974; Casillas and Smith, 1977; Soivio *et al*., 1977; Ross and Ross, 1983; Yaron *et al*., 1983; Pickering, 1993) and although much literature exists on the subject, only limited generalisations can be made. In recent years, however, several authors have attempted to define a general set of responses to stress that would occur in response to all stressors. In general, the generalised stress response (GSR) is characterised by an initial phase of response to the stressor, followed by a period of attempted resistance or adaptation to the stressor. Depending on the magnitude and duration of the stressor, these two phases may be followed by a period of exhaustion, in which the health or even the life of the animal can be strongly compromised. The GSR is the subject of some disagreement among researchers as to its consistency and even its existence in a sufficiently generalised sense. The reason for this is simply that the response to stressors is highly variable. Among aquatic animals, the effects of stressors have been best investigated in fish and may be externally or internally evident.

External signs of stress

There are a number of characteristic external signs of stress in fish including ataxia, obvious tachyventilation and marked colour change which can either be darkening or blanching.



Ataxia is evident as random swimming, often at high speed and in short bursts with frequent changes of direction. This may be accompanied by body-twisting and even swimming upside-down. Brief, very high speed movements, often in response to relatively small stimuli, are especially facilitated in fish by the giant Mauthner axons (Zottoli *et al*., 1995) which run the full length of the spinal chord and whose stimulation may cause widespread and instantaneous stimulation of motor nerves. Good

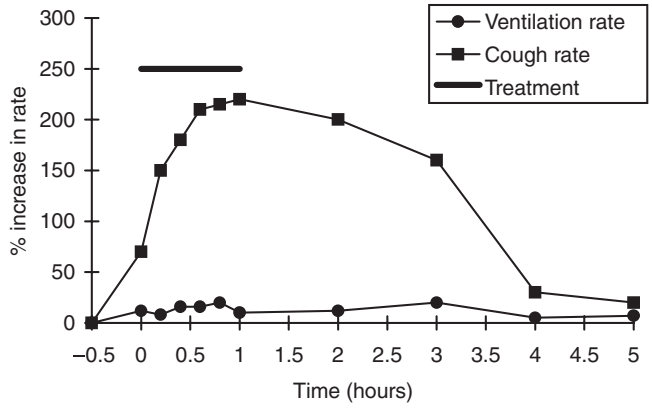


Figure 2.3 The effects of a standard, prophylactic 200 ppm formalin treatment on ventilation rate and cough rate of rainbow trout, *Onchorynchus mykiss*. Based on data from Ross *et al.* (1985).

husbandry and knowledge of normal stock behaviour will enable quick detection and recognition of any changes in motor pattern.

Tachyventilation is evidenced by a rapid movement of the opercula in fish. Although some knowledge of the normal state must be gained before this can be judged by eye, husbandrymen or experienced research workers will have little difficulty in identifying this condition. Ventilatory and other respiratory parameters are good indicators of stress, and although the causes are not fully understood, cough rate has been found to be a better indicator than other general respiratory changes (Sprague, 1971). All fish cough at regular intervals, momentarily reversing the flow of water over the gills; this occurs about once per minute in trout and five times per minute in tilapia. Figure 2.3 demonstrates the effects of a prophylactic formalin treatment on ventilation rate and cough rate of rainbow trout, *Oncorhynchus mykiss*. The massive increase in cough rate can be seen to be many times greater than the relative increase in ventilation rate, making cough rate a more useful measure of stress.

All fish have the ability to change their colour to some degree, within a certain intensity and range of the visible spectrum. They achieve this by controlling the distribution of pigment in the chromatophores of the skin both by nervous and pituitary hormonal means. The chromatophores may contain different pigments and so, in addition to lightening and darkening, some animals may be able to change colour to a varying degree. The virtuosity of flatfishes is well known in this respect and some can even adapt to a chequered background. Some crustaceans have similarly versatile and frequently colourful abilities, principally

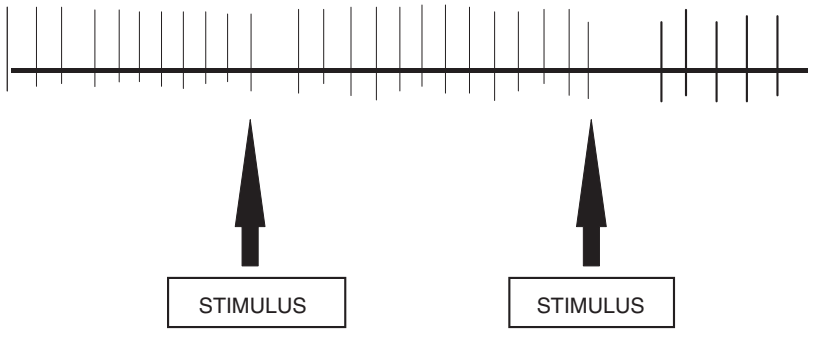


Figure 2.4 The effects of stress on heart rate in saithe (*Pollachius virens*). Momentary bradycardia caused by an observer appearing at the side of the tank.

under hormonal control. However, the prize for the most spectacular and legendary colour change ability goes to the cephalopod molluscs whose nervously controlled colour change is breathtaking both in its speed and colour range. The issue of suitable background colour has been recognised and investigated by aquaculturists and fish keepers with the objective of minimising stress. However, in reality it is still insufficiently understood, no standard approach is taken to the problem and frequently it is simply ignored. Nevertheless, because pigment distributions are controlled by both nervous and hormonal means, it is fair to assume that animals in an extreme state of pigment dispersion or concentration are stressed, at least initially. This stress may be chronic depending on the culture system and some thought should probably be given to matching the background found in the natural environment, as far as possible.

Internal signs of stress

A wide range of internal responses to stress have been described in the literature. These may be effects on the heart rate, effects on blood parameters and metabolic or biochemical effects.

Effects on heart rate

At the simplest physiological level, an external event may inhibit one or more heartbeats in response to a momentary stimulus (Fig. 2.4) although, generally, such a small stimulus would not have a negative effect. More severe stress may, however, produce very prolonged effects, which may or may not revert to normal for more than 24 hours.

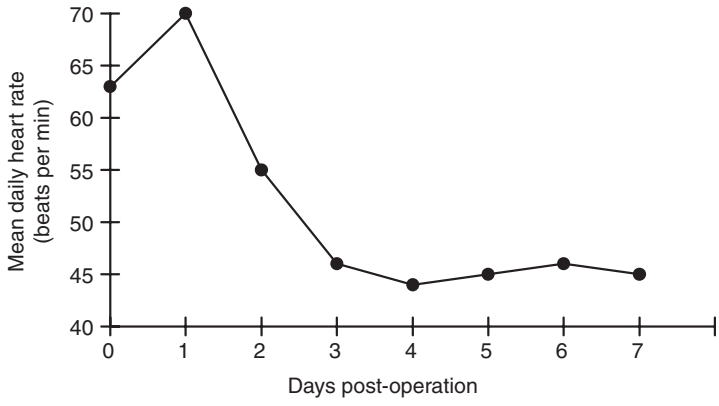


Figure 2.5 The effects of stress on heart rate in saithe (*Pollachius virens*). Chronic tachycardia following anaesthesia and implantation of ECG electrodes.

For example, short-duration bradycardia may be replaced by tachycardia, often of long duration, and Fig. 2.5 shows the effects on heart rate of simple ECG electrode implantation in saithe, *Pollachius virens*. Cardiac rate and ventilatory rate in fish are functionally linked and in many circumstances they are neurophysiologically synchronised, especially when any workload is placed on the animal (Randall, 1970). As will be seen later, this link can be exploited during recovery from certain types of anaesthesia.

Haematological effects

There are a number of well-documented haematological effects induced by stress (Davis, 2006). These may include haemoconcentration or haemodilution, swelling of erythrocytes and increase or decrease in plasma osmolarity, chloride, sodium, potassium and other ion levels. Smith (1982) summarised the work of numerous authors to show the direction of change for a variety of physiological functions under the influence of different stressors. The relative inconsistency of these responses can be seen in Table 2.1. These factors not only affect circulatory physiology, but also clearly will affect osmoregulatory capability.

Hormonal effects

As already discussed, virtually all of the components of any general stress response are mediated by two groups of hormones, the catecholamines and the hormones of the HPI axis, principally cortisol.

Table 2.1 Effects of stress on blood parameters in fish.[a]

Parameter	Effect	Direction of change
Haematocrit (pcv)	Concentration/dilution	↑↓
Haemoglobin	Increase/decrease	↑↓
Individual erythrocyte volume	Swelling of cells	↑↓
Electrolytes	Changes in Na/K/Cl levels Changes in Mg/Ca/PO_4 levels	↑↓
Bicarbonate or CO_2	Variable	↑↓
Glucose	Variable	↑
Lactate	Variable	
Total protein	Decreases	↓
Plasma concentration	Changes in osmolarity	↓
Leucocrit	Changes in cell counts	↑↓

↑indicates an increase and ↓ a decrease in level of a parameter.
[a]Based on data from Smith (1982).

The catecholamines epinephrine and norepinephrine, released by the chromaffin tissue of the head-kidney in response to stimulation of the sympathetic nervous system, produce a series of significant changes which are summarised in Table 2.2. These changes are relatively fast and may begin after less than a second and can last for many minutes up to a few hours.

Release of cortisol from the inter-renal bodies in the middle region of the kidney begins in under an hour but may continue for weeks or even months (Smith, 1982). Figure 2.6 shows the typical rise in serum cortisol induced in response to a very simple stressor. Its effects are wide-ranging, often deleterious if prolonged and are summarised in Table 2.2. Its main effect appears to be an increase in catabolism and an increase in permeability of membranes to ions. Production of cortisol due to stress has been well described by Wedemeyer (1969), Fagerlund and Donaldson (1970), Fryer (1975), Singly and Chavin (1975) and Yaron *et al*. (1983). Note that the increased anabolic effects are accompanied by increased catabolic effects and result in generally inhibited growth. A classical example of this is in migrating Pacific salmon where cortisol levels may be elevated by up to eight times for many weeks. The net result of this is to massively alter metabolism so as to enable the energetically demanding migration and subsequent fighting and spawning activities. The process is irreversible, however, and the extensive steroid-mediated net catabolism that occurs leads to the death of all migrant Pacific salmon; none return to the sea.

Table 2.2 The range of possible effects of catecholamine release and corticosteroid release during stress in fish.[a]

Agent	Effect
The catecholamines (epinephrine and/ or norepinephrine)	Tachycardia and increased cardiac output Tachyventilation Vasodilation or vasoconstriction Increased blood glucose Increased blood lactate Changes in free fatty acids Increase in haematocrit Glucogenesis in liver and muscle Increased peristalsis Increased Protein mobilisation
Steroids cortisol (or cortisone)	Increased protein synthesis Inhibition of growth Reduced utilisation of carbohydrate Increased glucose production from tissue protein Deposition of glycogen in the liver Changes in membrane permeability Increased production of and activity of Na/K-ATPase Increased leucocyte count Immunosuppression

[a]Data drawn from the works of numerous authors.

Acclimation to stressors

Fish, at least, are known to be able to adapt to repeated exposure to acute stressors and usually exhibit a reduced response as the number of exposures increases. The number of mucous cells per unit area of skin

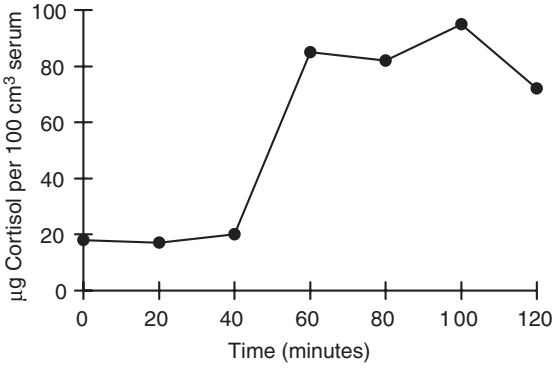


Figure 2.6 Cortisol production in pink salmon, *Oncorhynchus kisutch*, stressed by stirring the aquarium water. After Wedemeyer (1969).

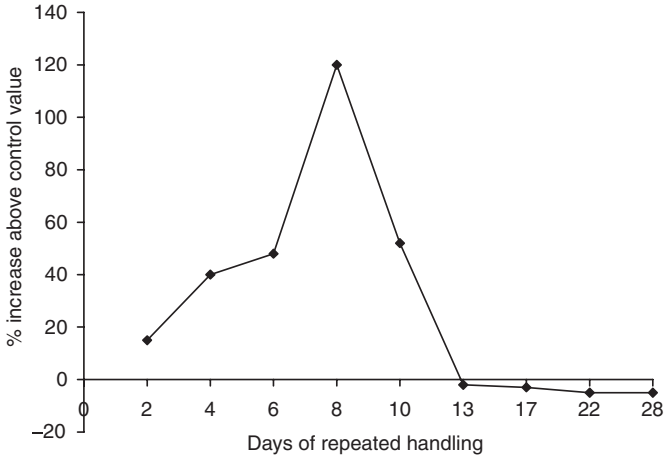


Figure 2.7 Percentage change in superficial mucous cells of the shoulder region following repeated daily handling of rainbow trout, *Onchorhynchus mykiss*. Data from McCaldom (1979).

of rainbow trout increased after repeated handling (Fig. 2.7) but then slowly returned to the original value even though the fish continued to be handled on a daily basis (D. McCaldom and L.G. Ross, unpublished data). By contrast, the magnitude of changes in plasma glucose and lactate did not reduce in the same trial. A further excellent example of this acclimation is shown in Fig. 2.8, in which Pickering (1993) used standardised cleaning actions to stress brown trout and recorded clear acclimation to the stressor as the frequency of exposure increased.

Following prolonged exposure to chronic stressors, levels of plasma cortisol may return to normal even though the stressor is still present. However, Pickering (1993) notes that this is not a standard response and

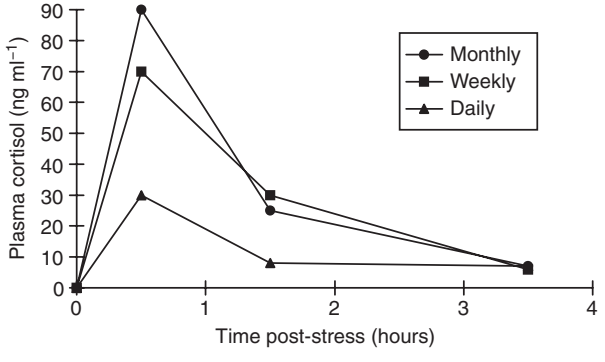


Figure 2.8 Acclimation of brown trout , *Salmo trutta*, to repeated exposure to tank cleaning activities. After Pickering (1993).

Table 2.3 Summary of measures to ameliorate stress during holding, handling and transportation of aquatic animals.[a]

Suggested action	Reason	Application	Qualifications
Reduce duration of exposure to stressor	Stress response is usually proportional to duration of exposure	General	Some effects may result in long recovery times
Work at lower temperatures	Stress-induced mortality increases with water temperature	General	Not always practicable in field conditions
Prevent simultaneous stresses	Stressors may be additive or synergistic	General	Could allow time between exposures to stressors, if in a sequence.
Use dilute salt solutions	Medium becomes more isotonic, reducing osmotic losses and mortality	Transportation	Use with stenohaline freshwater animals may be limited
Withdraw food: 2–3 days in cold water 12–24 hours in tropicals	Reduces oxygen requirements and fouling of medium	General Handling Transportation	Requires additional management input
Reduce stocking density or numbers handled per batch	Reduces interaction and abrasion	General Handling Transportation	May conflict with intensification of aquaculture
Provide adequate, more 'natural', environment	Providing environmental 'comfort' (see preceding paragraphs)	General	Not always possible in some culture systems
Use mild anaesthesia or sedation	Facilitates handling, *temporarily* reduces oxygen consumption, reduces carbon dioxide and ammonia output	Handling Transportation	Anaesthetics can act as stressors

[a] Adapted from recommendations intended for fish by Pickering (1993).

that cortisol levels may stay elevated for considerable periods, ranging from weeks to months.

Stress reduction

Based on this substantial background, it is possible to propose techniques for general stress reduction. Pickering (1992, 1993) summarised current thinking and suggested a number of approaches for use in fish holding, transportation and general aquaculture. Table 2.3 shows a summary of practical measures that can be taken to reduce stress in

any aquatic animal. Not all of these actions may be feasible in practice, although with a little thought and willingness it can be found that many can be used beneficially in a number of situations.

For the future, two routes have been proposed: (a) the breeding of 'low-stress' animals, i.e. animals with a minimal response to stressors; and (b) the use of cortisol-suppressing drugs (see Pickering, 1992). Good examples of the former are found in the poultry industry and there is evidence that the magnitude of stress responses is a heritable trait in fish. Overli *et al.* (2006) showed that rainbow trout selected for their high cortisol response to stress produced more post-stress feed waste and had slower growth than trout with a low cortisol response. Dexamethasone, and other molecules, are cortisol receptor blockers and may have some potential for suppressing the stress response.

Stress reduction during anaesthesia

The use of anaesthesia during normally stressful procedures can alleviate, or delay, many of the normal stress reactions. Ross and Ross (1983) noted that benzocaine anaesthesia gave only a mild reduction in oxygen consumption during handling, but that this deficit had to be made up later, and strongly elevated oxygen consumption levels were recorded during recovery. Hill and Forster (2004) showed that cardiovascular effects consequent upon anaesthesia using MS222, metomidate and AQUI-S were masked by the magnitude of changes brought about by handling and suggested that limiting fish handling and manipulation was more important than the choice of anaesthetic.

Pickering (1993) showed that the normal cortisol response may be suppressed by use of anaesthesia during handling (Fig. 2.9), although, again, this becomes fully manifested once the anaesthetic is withdrawn. This suppression was particularly marked with etomidate, almost certainly because etomidate is now known to inhibit cortisol synthesis.

Harrell (1992) attempted to alleviate stress by using a salt solution during MS222 anaesthesia, assessing stress by measuring plasma corticosteroid and chloride levels. In a comparison of 25 mg L^{-1} MS222, z 25 mg L^{-1} MS222 with 10 g L^{-1} salt and salt alone, he showed that stress was least when only salt was used.

Stress induced by anaesthesia

Anaesthesia itself induces stress reactions although it is difficult to distinguish the direct effects of the anaesthetic method from those of capture or handling. It has been shown by Serfaty *et al*. (1959) and

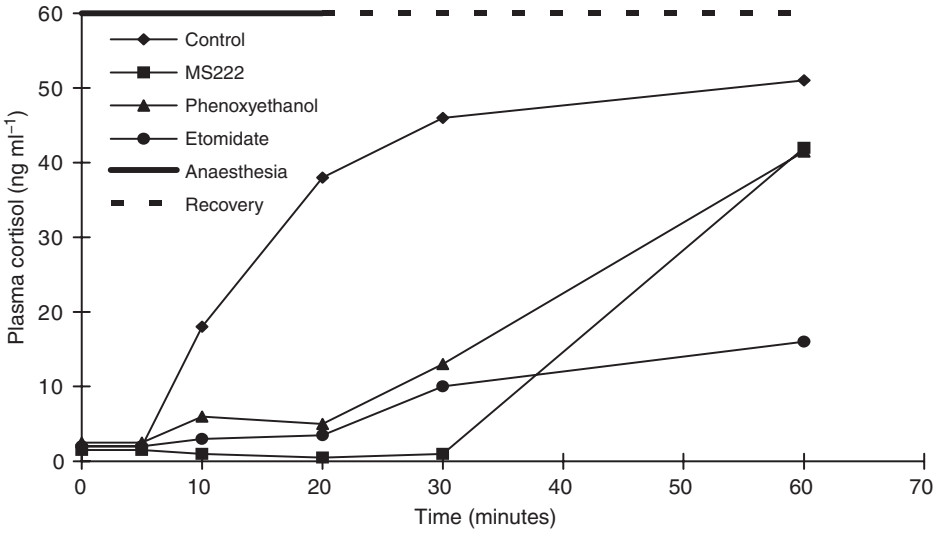


Figure 2.9 The effects of anaesthesia on the cortisol response to handling in rainbow trout. After Pickering (1993).

Houston *et al*. (1971) that MS222 (tricaine methanesulphonate) induction produces both tachycardia and tachyventilation. Randall and Smith (1967) demonstrated the magnitude of the cardio-ventilatory effects of MS222 anaesthesia in rainbow trout (Fig. 2.10) and Houston *et al*. (1971) in a comparable study showed that dorsal aortic blood pressure was similarly affected. Despite this it is clear that anaesthetics are necessary

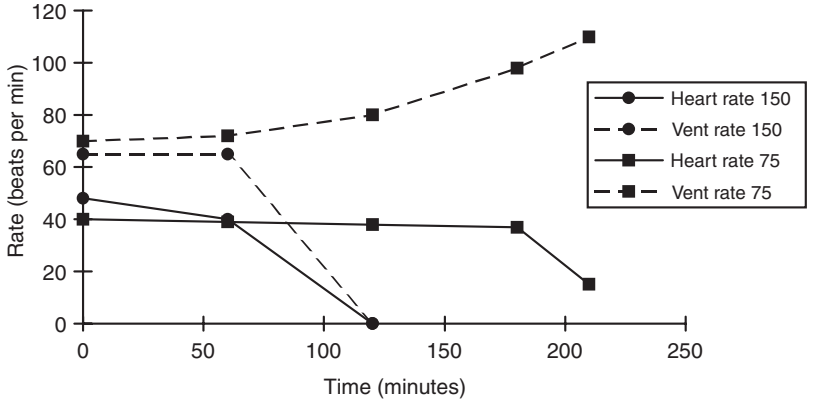


Figure 2.10 Changes in heart rate, ventilation rate and ventilation amplitude at two levels of MS222 anaesthesia, 150 mg L^{-1} and 75 mg L^{-1}. After Randall and Smith (1967).

in many cases to limit the magnitude of the stress response in addition to facilitating handling.

Summary

Clearly, there can be powerful consequences of exposure to a stressor, which can affect physiology, behaviour, feed intake, growth and performance of cultured or experimental animals. Employing the stress-reduction methods that have been proposed will not always be possible and may sometimes appear to be in conflict with other objectives, for example those of aquaculture. Furthermore, obviously, mild anaesthesia will not suffice in all cases. Deeper anaesthesia is required for surgery or invasive work of any kind. A range of techniques are available for the sedation or anaesthesia of aquatic animals in different circumstances and in the remainder of this book we attempt to describe the various approaches to practical anaesthesia.

References

Brett. J.R., Implications and assessments of environmental stress, in: Larkin, P.A. (ed.), Investigations of Fish-Power Problems, H.R. Macmillan Lectures in Fisheries, University of British Columbia, 1958, 69–83.

Casillas, E. and Smith, L.S., The effect of stress on blood coagulation and haematology in rainbow trout, *J. Fish Biol*., 10 (1977) 481–491.

Davis, K.B., Management of Physiological Stress in Finfish Aquaculture, *North Am. J. Aquacult*., 68 (2006) 116–121.

Fagerlund, U.H.M and Donaldson, E.M., Dynamics of cortisone secretion in sockeye salmon (*Oncorhynchus nerka*) during sexual maturation and after gonadectomy, *J. Fish. Res. Board Can*., 27 (1970) 2323–2331.

Fryer, J.N., Stress and adrenocorticosteroid dynamics in the goldfish *Carassius auratus*, *Can. J. Zool*., 53 (1975) 1012–1020.

Harrell, R.M., Stress mitigation by use of salt and anaesthetic fro wild striped bass captured fro broodstock, *Prog. Fish Culturist*, 54(4) (1992) 228–233.

Hill, J.V. and Forster, M.E., Cardiovascular responses of Chinook salmon (*Oncorhynchus tshawytsha*) during rapid anaesthetic induction and recovery, *Comp. Biochem. Physiol. C*., 137 (2004) 167–177.

Houston, A.H., Madden, J.A., Woods, R.J. and Miles, H.M., Some physiological effects of handling and tricaine methanesulphonate anaesthetization upon the Brook Trout. *Salvelinus fontalis*, *J. Fish. Res. Board Can*., 28(5) (1971) 625–633.

McCaldom. D.M., The effect of stress on various blood and skin parameters in rainbow trout, Unpublished B.Sc. thesis, University of Stirling, 1979, 44 p.

Overli, O., Sornesen, C., Kiessling, A., Pottinger, T.G. and Gjoen, H.M., Selection of improved stress tolerance in rainbow trout (*Oncorhynchus mykiss*) leads to reduced feed waste, *Aquaculture*, 261 (2006) 776–781.

Pickering, A.D., Rainbow trout husbandry: management of the stress response, *Aquaculture*, 100 (1992) 125–139.
Pickering, A.D., Husbandry and stress, in: Muir, J.F. and Roberts, R.J. (eds.), Recent Advances in Aquaculture, volume 4, Blackwell Scientific, Oxford, 1993, 340 p.
Randall, D.J., The circulatory system, in: Hoare, W.S. and Randall, D.J. (eds.), Fish Physiology, volume 4, The Nervous System. Circulation and Respiration, Academic Press, New York and London, 1970.
Randall, D.J. and Smith, L.S., The effect of environmental factors on circulation and respiration in teleost fish, *Hydrobiologia*, 29 (1967) 113–124.
Ross, B. and Ross. L.G., The oxygen requirements of *Oreochromis niloticus* under adverse conditions, Proceedings of the First International Symposium on Tilapia in Aquaculture, Nazareth, Israel, 1983, 134–143.
Ross, L.G., Ward, K.M.H. and Ross, B., The effect of formalin, malachite green and suspended solids on some respiratory parameters of rainbow trout, *Salmo gairdneri*, *Aquaculture Fish. Manage*., 16 (1985) 129—138.
Serfaty, A., Labat, R. and Quiller, R., Les reactions cardiaques chez la carpe (*Cyprinus carpio*) au cours d'une anaesthesie prolongee, *Hydrobiologia*, 13 (1959) 134–151.
Singly, J.A. and Chavin, W., The adrenocortical-hypophysial response to saline stress in the goldfish. *Carassius auratus* L, *Comp. Biochem. Physiol*., 51A (1975) 749–756.
Smith, L.S., Introduction to Fish Physiology, T.F.H. Publications, 1982, 352 p.
Soivio, A., Nyholm, K. and Huhti, M., Effects of anaesthesia with MS222, neutralised MS222 and benzocaine on the blood constituents of Rainbow trout, *J. Fish Biol*., 10 (1977) 91–101.
Sprague, J.B., Measurement of pollutant toxicity to fish. III. Sublethal effects and "safe" concentrations, *Water Res*., 5 (1971) 245–266.
Wardle, C.S., The changes in blood glucose in *Pleuronectes platessa* following capture from the wild: a stress reaction, *J. Marine Biol. Assoc. U.K.*, 52 (1972) 635–651.
Wardle, C.S. and Kanwisher, J.W., The significance of heart rate in free-swimming Cod, *Gadus morhua*: some observations with ultrasonic tags, *Marine Behav. Physiol*., 2 (1974) 311–324.
Wedemeyer, G., Stress-induced ascorbic acid depletion and cortisol production in two salmonid fishes, *Comp. Biochem. Physiol*., 29 (1969) 1247–1251.
Wedemeyer, G., The role of stress in the disease resistance of fishes, American Fisheries Society Symposium on Disease of Fish and Shellfish, Special Publication No. 5, 1970.
Yaron, Z., Ilan, Z., Bogomolnaya, J. and Vermaak, P., Steroid hormones in two tilapia species. *O. niloticus*, Proceedings of the First International Symposium on Tilapia in Aquaculture, Nazareth, Israel, 1983.
Zottoli, S.J., Bentley, A.P., Prendergast, B.J. and Rieff, H.I., Comparative studies on the Mauthner cell of teleost fish in relation to sensory input, *Brain Behav. Evol*., 46 (1995) 151–164.

Chapter 3

Pain in Aquatic Animals

Bryony L. Ross

Introduction

In animal research pain is usually described in a operational sense whereby a stimulus is assumed painful if it normally produces pain in humans, can lead to tissue damage and if an escape behaviour is elicited. Most painful stimuli will, or could, produce all three effects. As already noted, pain control may be required in procedures with aquatic animals but despite considerable research, our understanding of the mechanisms of pain detection, transduction and perception in aquatic animals is by no means complete (Lineberry, 1981). It is made all the more difficult as a concept because aquatic animals cover such a wide evolutionary range and so the principal taxa with which the researcher or aquaculturist may engage must each be considered on their own merits. Nevertheless, the possibility of pain is a matter that must be addressed when considering the implications of commercial and experimental handling.

While most animals can detect and react to noxious stimuli, the notion of pain perception is very controversial, and some well-argued but widely differing views are held by those who have engaged with the topic. The recent vigorous discourse on pain perception in fishes gives a useful perspective on the varying viewpoints. Rose (2002) presented a lengthy review proposing that fish are unable to feel pain, principally based on the absence of a neocortex (which is unique to mammals) or any other analogous structure. He considered that pain is a psychological experience and that only animals with consciousness that could experience emotion and fear could be aware of it. He did, however, concede that fish respond to noxious stimuli with a range of aversive and physiological behaviours and that avoidance of stress and good welfare were important issues. Håstein *et al.* (2004) also noted the absence of a neocortex in non-mammalian vertebrates but considered that the higher-level cognitive ability required for sentience, self-awareness and

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hence pain perception could not be simply inferred from neuroanatomy alone. Based on the evidence current at the time, they considered that no point of view was clearly correct or incorrect.

Chandroo *et al*. (2004) critically deconstructed Rose's arguments in the light of contemporary developments in understanding of what constitutes consciousness, self-awareness and hence pain perception in animals. They concluded that many authors fail to consider modern perspectives or data on pain and that consequently, conclusions have frequently been equivocal. They argue that consciousness, and hence one may assume pain perception, in fish is very plausible. Huntingford *et al.* (2006) explored current issues in fish welfare at length and considered that good welfare was manifested by an absence of suffering. They point to the longevity of fishes and the variety and sophistication of their behaviour as indicators of potential complexity of mental processes. Citing numerous examples of extended associative learning, they conclude that there is ample evidence for fish species having sophisticated cognition and behaviours and deduce that pain perception is therefore a strong possibility.

Arlinghaus *et al.* (2007) challenged the 'feelings-based' approach of Huntingford *et al.*, principally from the perspective of recreational fisheries, stating that human interaction with fish was not unnatural and that fish biologists should concentrate their efforts only on understanding the physiological, biochemical and behavioural indicators of fish interactions and not with any aspects of suffering or ethics.

Clearly, there are strongly held opposed views on this topic but, whatever one's viewpoint, experimental or cultured animals must, at the least, be afforded appropriate respect, be provided with good welfare and, until more is known, given the benefit of the doubt as far as protection from pain is concerned.

Defining pain

To understand fully the difficulties in discerning whether aquatic animals are affected by painful stimuli, it is usual to split the process into two parts – pain detection and pain perception. It is commonly accepted that the detection of noxious stimuli (nociception), and pain perception (the unpleasant feeling which occurs following the conscious perception of a noxious stimulus) are two discrete entities. Whilst pain and nociception are often referred to as one and the same, nociception is possible without pain and vice versa (Rose, 2002). For example, nociception can result in a simple, reflex aversive action which restores the *status quo*. Pain, however, is a complex, subjective experience, involving

both the detection and transduction of noxious stimuli, and the cognitive and emotional processing of that stimulus within the brain. Pain is definable in two ways:

- A basic bodily sensation that is induced by a noxious stimulus, is received by naked (free) nerve endings, is characterised by physical discomfort (such as pricking, throbbing, or aching), and typically leads to some evasive action.
- A state of physical, emotional, or mental lack of well-being or physical, emotional, or mental uneasiness that ranges from mild discomfort or dull distress to acute often unbearable agony. This usually produces the reaction of wanting to avoid, escape, or destroy the causative factor and its effects.

Nociception

Nociception is the detection of a potentially painful stimulus usually accompanied by a reflex-style withdrawal from the source of the stimulus (Sneddon, 2004). The painful stimulus may also have the potential to produce some tissue damage, in which case it is described as noxious, although there is no strict adherence to the use of these terms. The stimulus may be produced by physical trauma, mechanical stimulation, thermal, electrical or chemical stimulation, or a combination of some of these. Because of this it is difficult to easily define the stimulus energy required to elicit pain.

Even an *Amoeba* or a *Paramecium* will retract its body from a range of noxious or potentially damaging stimuli. The mechanism for this is clearly not nerve-based but is related to changes in electrical activity in the cell membrane (Naitoh, 1974). More advanced invertebrates have well-developed nervous systems consisting of sets of neurons connected to ganglia with varying levels of complexity. Indeed, because individual nociceptors and their axons tend to be large and can be readily identified in invertebrates, they have been widely used as models in nociception and pain research for many years. However, many authors remain equivocal about the possibility that the very animals on which this research was carried out may be able to perceive pain in one way or another.

The neurological basis of nociception

The biological processes underlying nociception and pain perception are not simple. Even in humans, the exact processes behind the detection

and perception of pain are no longer regarded as merely a 'hard wired system with a pure stimulus response relationship' (Cousins and Power, 2003). In humans, Melzack (2001) has proposed a neuromatrix theory of pain based on a wide, mostly centrally located, neural network modified by sensory inputs, which generates a neural pattern that produces pain. This theory is particularly useful in explaining chronic pain in humans where the pain and its cause cannot be clearly related.

Over a century ago, Sherrington (1906) proposed the existence of 'nociceptors' – sensory nerves activated by noxious stimuli. Nociceptive neurons are distinguishable from other sensory neurons by their high threshold firing values, meaning that they are only activated in response to relatively strong, noxious stimuli, which may have the capacity to cause some tissue damage. These neurons have been characterised into two main types – Aδ and C fibres. Aδ fibres are myelinated medium diameter neurons capable of transmitting signals at a speed of 2–15 $m\,s^{-1}$. It is commonly accepted that these are responsible for the detection of fast or 'sharp' pain. By contrast, C fibres are unmyelinated and polymodal in nature (meaning that they will respond to mechanical, thermal and chemical stimuli), with a small diameter and a conduction velocity of less than 1 $m\,s^{-1}$. They are responsible for the delayed 'dull' pain associated with noxious stimuli (Basbaum and Jessell, 2000).

A lot of research has been carried out into the transduction of noxious stimuli to the brain and higher centres for processing. In higher vertebrates, nociceptors terminate their axons in the dorsal root ganglia of the spinal cord. From there, signals are transmitted up the spinothalamic tract to the brainstem and then on through the thalamus to the limbic system, somatosensory cortex and cingulated cortex (Fig. 3.1). It is within these highly differentiated brain centres that it is thought that the conscious perception of pain occurs. Descending to the lower invertebrates and the invertebrates, analogous structures become increasingly harder to identify and this is one of the sources of much discussion regarding pain perception in these groups.

The central nervous system and pain perception

Pain perception is a complex experience comprising both an unpleasant sensory reaction to a stimulus and an emotional component. Melzack and Wall (1996) considered that a painful stimulus must also be accompanied by an aversive response, usually behavioural, which may be expected to end the unpleasant experience or at least be intended to do so. The problem is much greater to understand in animals than in

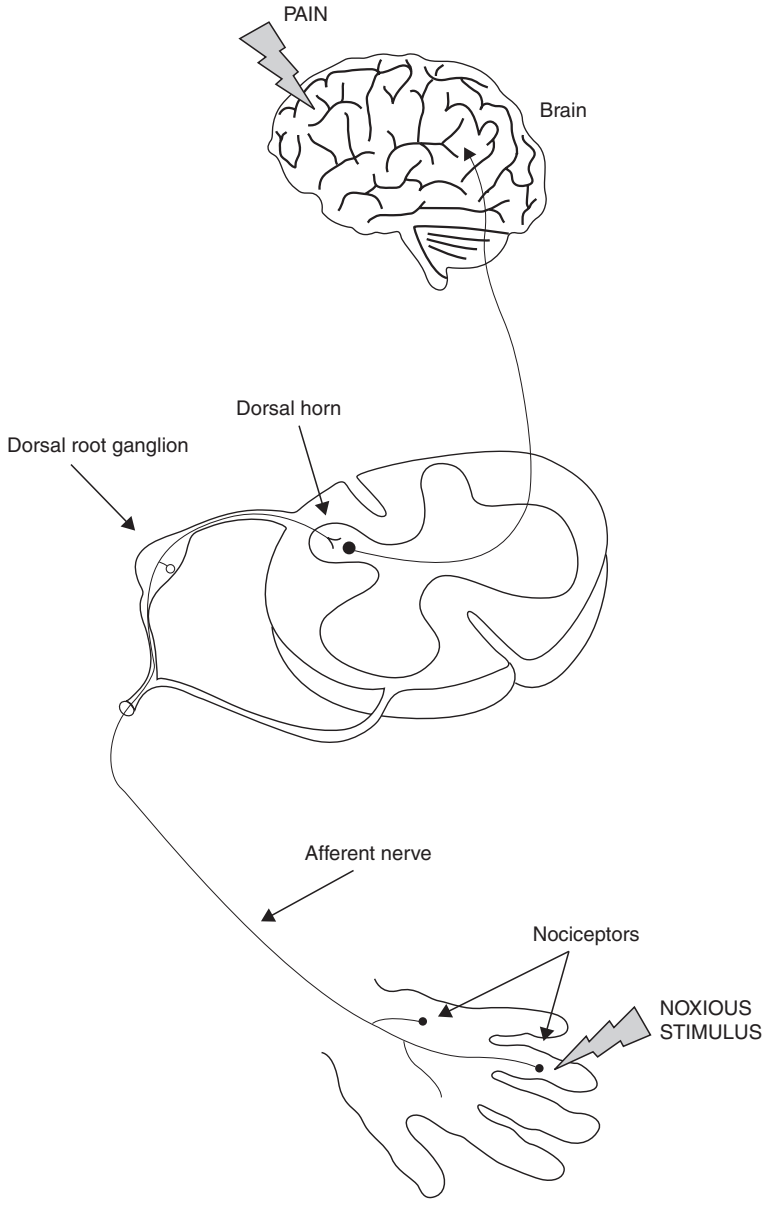

Figure 3.1 An outline of the process of nociception in higher vertebrates.

humans as it is, of course, not possible to converse with animals directly and hence to build up an objective, or even subjective, description of painful events which help the observer to understand the process.

As responses to pain are often more complex than simple nociception, the involvement of more complex pathways and higher centres

Table 3.1 Factors contributing to pain perception in animals.

Smith and Boyd (1991)	Sneddon (2004)
Presence of nociceptors.	Presence of nociceptors
Presence of a central nervous system.	Comparative brain structures
Nociceptors are connected to the CNS	There are pathways to higher brain structures
Endogenous opioids can be produced	Existence of opioid receptors and substances
Responses can be mediated by analgesia	Analgesics reduce nociceptive responses
Response to damaging stimuli is similar to that in humans	Avoidance learning Suspension of normal behaviour

in some animals is implied. This is certainly the case in the lower vertebrates but may also extend to some invertebrates including molluscs and crustaceans. Animals may also learn to avoid particular challenging situations and this has been widely demonstrated in higher crustaceans and molluscs as well as many fish species. This has clear survival value but also suggests that the processing of these events goes beyond simple nociception.

Behavioural responses to noxious stimuli are separate from the experience of pain, which in humans is processed in specific regions of the cerebral cortex. In lower vertebrates the cerebral cortex is reduced or even absent and many have contended that because of this fish, for example, cannot possibly experience pain (Rose, 2002). Rose goes further and contends that it is anthropomorphic to suggest that they can perceive pain based on their observed behaviours, or even on any other basis, and that such responses are merely unconscious physiological stress responses. These views are strongly contested by many authors.

Smith and Boyd (1991) defined six physiological criteria that could be used to assess whether an animal (as distinct from a human) may be capable of perceiving pain and, based on the work of Bateson (1992), Sneddon (2004) also proposed a list of criteria by which to assess the capability of an animal to experience pain (Table 3.1).

While the ultimate point of Smith and Boyd may be thought to be anthropomorphic and hence contentious, the similarity of response to that in humans is widely used in assessing animal pain. There is nonetheless considerable agreement in the two sets of criteria and they form a useful scheme with which to evaluate nociception and pain in aquatic animals. It will be seen that many aquatic animals, even the invertebrates, qualify under most of these criteria and hence are probably capable of perceiving pain.

Nociception and pain perception in aquatic invertebrates

Many invertebrate species are used in toxicity testing and biomedical research, but perhaps the most important aquatic invertebrate groups to consider are members of the Crustacea and the Mollusca.

Nociception

Almost all invertebrates have the ability to detect noxious stimuli such as extremes of temperature, exposure to chemicals, mechanical stimulation or body damage and electric shocks. These conditions are those which would be expected to cause pain in higher vertebrates resulting in avoidance reactions and, at the least, most invertebrates will also avoid such stimuli (Smith, 1991). These avoidance reactions can take a variety of forms but usually there is a clear change in behaviour, which is directly related to the aversive stimulus. Coordinated responses to aversive stimuli have been reported in protozoans, coelenterates, annelids, insects, and gastropod and cephalopod molluscs.

Many invertebrates have nociceptors, specialised nerve cells with a high firing threshold, which respond specifically to noxious stimuli. Some invertebrate nervous systems are so simple that every neuron is known, can be mapped and occurs in every individual. Tobin and Bargmann (2004) described a nematode nervous system comprised of only 320 neurons, but which nevertheless can elicit a range of responses to different stimuli. Higher invertebrates such as *Aplysia* and *Homarus* have been shown to possess nociceptors although there tend to be few synapses between the nociceptor and the effector (e.g. a muscle), and the responses observed upon stimulation are relatively simple withdrawal reflexes.

Brain and central nervous system structure

Invertebrates have no backbone and possess either an exposed nerve net (as in non-segmented invertebrates) or a ventral nerve cord in the segmented invertebrates (as in annelids, molluscs, crustacea) with a very wide range of neural anatomies (Fig. 3.2).

Simpler invertebrates do not have any structures resembling a central nervous system but some have a series of ganglia connected by nerve tracts. More advanced invertebrates have very well developed systems of interlinked ganglia and in crustaceans the supra-oesophageal ganglion forms a brain-like structure in which significant sensory–motor

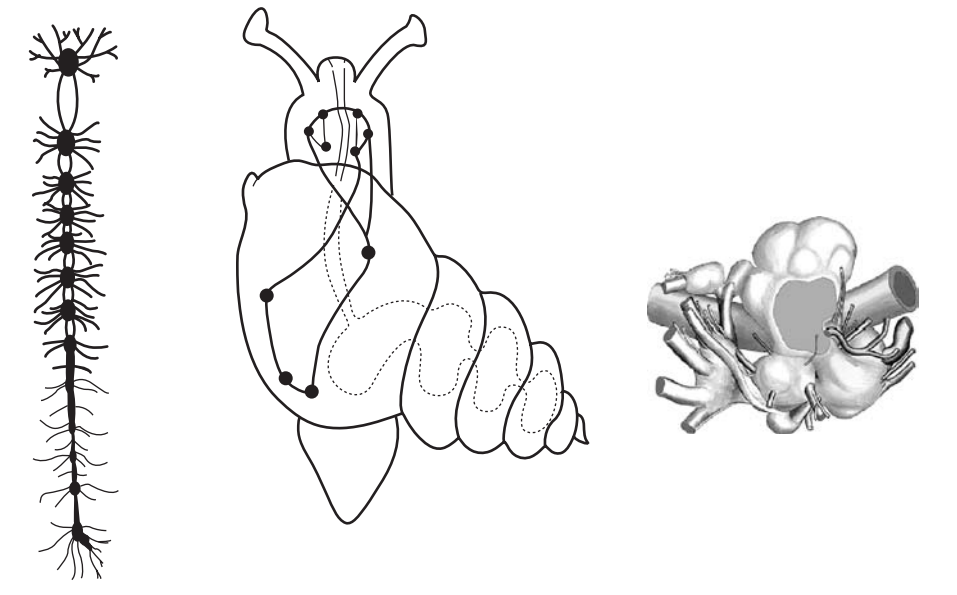

Figure 3.2 Comparative anatomy of crustacean, gastropod and cephalopod nervous systems. (crustacean and gastropod after Håstein *et al*. 2005; cephalopod after Young, 1967).

integration takes place. The cephalopod molluscs have a very well developed brain, formed from the fusion of several circum-oesophageal ganglia and containing about 25 million microneurons (Barnes *et al*., 2001). The brain mass to body mass ratio in cephalopods is well within the vertebrate range, being smaller than that of birds and mammals but larger than most fish and reptiles (Packard, 1972). It is important to recall that these nervous systems have developed separately from the vertebrate plan and consequently it is extremely difficult to compare structure and function among these groups. Cephalopods in particular exhibit a range of advanced behaviours, almost certainly perceive pain and perhaps also fear.

Nociceptor to brain pathways

The simple nerve net of a nematode does not possess any known higher order. However, the circum-oesophageal ganglia or brain masses of the more advanced invertebrates, such as crustacea and molluscs, are relatively complex and it is not unreasonable to assume that some form

of organised afferent connection exists between the nociceptors and the brain.

Opioids

Most vertebrates have been shown to produce opioids (morphine-like compounds), the most common types of which are endorphins and encephalins. These are released in response to noxious stimuli, the adaptive significance of which must be to provide a level of analgesia that helps protect the animal against the adverse effects of tissue damage. The ability to produce these endorphins and encephalins and the presence of specific receptors for them is considered to be evidence for nociception and possibly pain perception. Encephalins and endorphins have been found in a range of invertebrates including earthworms, the bivalve *Mytilus edulis* and some crustaceans. Opioid receptors have been demonstrated in *Mytilus*. The administration of opioid substances has been shown to produce analgesic effects in the terrestrial snail *Cepea* and this demonstrates the presence of specific receptors for these compounds.

Higher centres and learning

In many invertebrate groups responses to noxious stimuli go beyond simple reflex actions and more complex behaviour is seen as well as some degree of learning. In crustaceans learning is simple and can be explained by reflex neural mechanisms. Cephalopods, however, have a wide range of behavioural responses and a remarkable capacity for learning and to be trained. Wells (1978) considered that *Octopus vulgaris* were able to make choices based on past experience and showed strong individuality. While it is difficult, for example, to equate cephalopod behaviour with that of higher vertebrates, the advanced nature of the nervous system is not in doubt and the possibility of pain perception cannot be ruled out.

Overall, invertebrate nervous systems are very different from the vertebrate plan and it is difficult, if not impossible, to compare them directly. While nociception is clearly seen in many groups, it is very difficult, while avoiding anthropomorphism, to be sure whether invertebrates experience pain. Despite this, or perhaps because of it, a precautionary approach should be adopted to handling and experimentation with invertebrates. Smith (1991) concluded that 'the benefit of the doubt' should be given to issues regarding pain and suffering when working with invertebrates and that the standards of husbandry and care

afforded should always be respectful and promote 'general well-being'. It is significant to note that in the UK, *Octopus vulgaris* has now been added to the list of animals (formerly solely vertebrates), which are protected by the Animals (Scientific Procedures) Act 1986 controlling animal experimentation. The position of other advanced invertebrates remains under constant review.

Nociception and pain perception in aquatic vertebrates

In recent years, interest in investigation of nociception in lower vertebrates and invertebrates has grown substantially. Much of this has been aimed at improved animal welfare, in which nociception and pain perception are major factors. The major problem encountered when considering this issue is the wide variety of nervous system anatomies found even within the lower vertebrates.

Nociception

The existence of nociceptive afferent fibres within the lower vertebrates is quite variable.

Studies carried out on lampreys (*Petromyzon marinus*) show that they have no myelinated nerve fibres. They do have free nerve endings, which respond to mechanical and heat stimuli, but the animals do not display any behaviours typically associated with nociception when stimulated (Matthews and Wickelgren, 1978).

Erdmann (1999) considered that the fishes had the anatomical, physiological and biochemical requirements for a nociceptor system. However, work on several elasmobranch species has shown that they have myelinated fibres but appear to have lost the unmyelinated fibres. They have been shown to have free nerve endings with Aδ fibres, but that C fibres are absent. Stingrays do not respond to temperature increases and so there remains some doubt about the capacity for nociception in this group.

By contrast, in the more advanced teleost fishes, the presence of both Aδ and C fibres has been demonstrated (Sneddon, 2002; Sneddon *et al*., 2003). Measurements taken from individual neurons within the head of an anaesthetised fish while they were subjected to mechanical, chemical and heat stimuli also showed that up to 22 neurons were firing at any one time in response to stimulation, some receptors being polymodal (responding to more than one stimulus type) and others being specific mechanoreceptors (Sneddon *et al*., 2003).

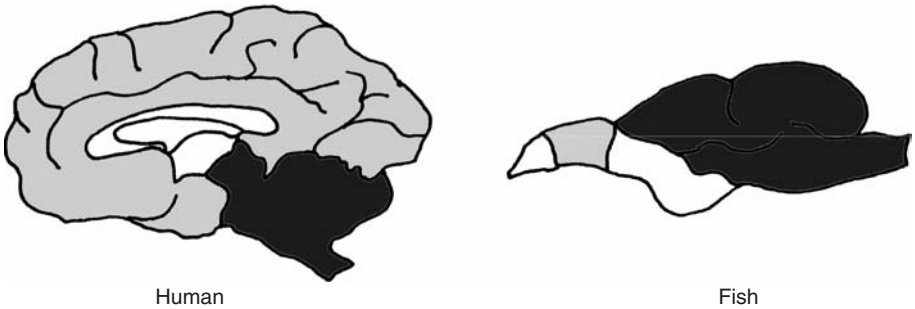

Figure 3.3 Gross structure of mammalian and fish brain, showing the relative differences in size of key regions. Note that a fish brain is considerably smaller than that of a human. Cerebrum = pale grey. (after Rose, 2002)

In amphibia, nociceptor responses are better known and a range of polymodal receptors associated with unmyelinated fibres have been described (Spray, 1976). Action potentials can be triggered in these nerves in response to noxious stimuli including weak acids, strong heating and intense mechanical stimuli. A classic mechanical nociceptor response in frogs is the toe-pinch reflex in which the leg will be retracted towards the body in response to pinching the foot.

Brain and central nervous system structure

Brain structure in the lower vertebrates is variable but is very different from that of mammals. Figure 3.3 shows the relative size of major brain regions in a human and a fish. The most obvious difference is in the size of the cerebrum. The cerebral cortex, and specifically the neocortex, of mammals has been shown to be instrumental in pain perception and its absence in lower vertebrates has become one of the key arguments in suggesting that fish, for example, do not feel pain (Rose, 2002). The cortex of lower vertebrates is certainly much smaller (Fig. 3.4) and less differentiated than birds and mammals but it does exist, particularly in the teleosts (Sneddon, 2004).

Many authors agree that more work needs to be done to improve our understanding of brain structures and their functionality in the lower vertebrates.

Nociceptor to brain pathways

In higher vertebrates, nociceptor processing is handled by the trigeminal nerve and the spinothalamic nerve, respectively serving the head and

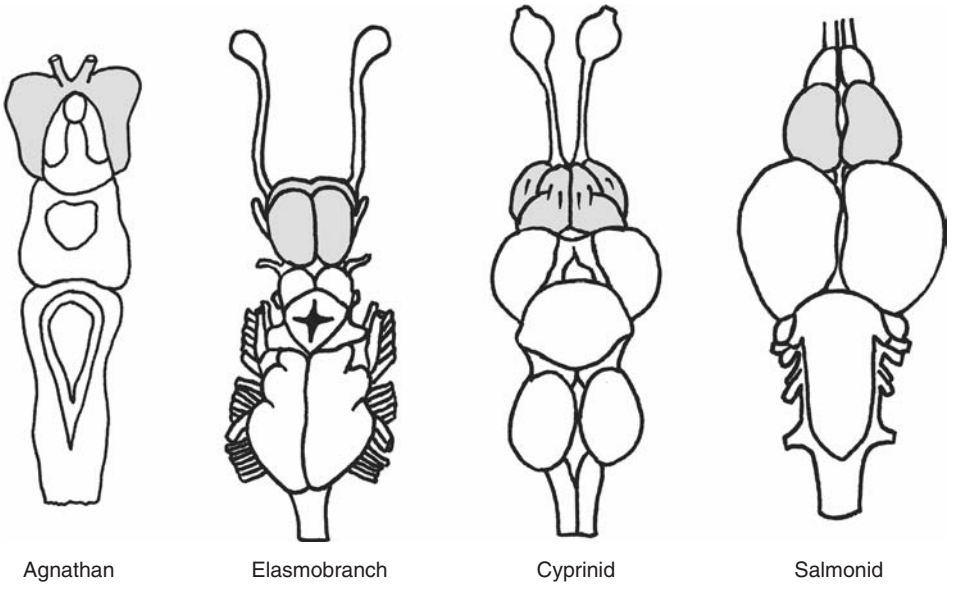

Figure 3.4 Comparative brain structure of agnathans and fishes, showing the relative size of the cerebrum, shaded grey. (Agnathan after Lagler *et al*. 1977; others after Rose, 2002).

body. Hagfish and lampreys have been shown to have these structures and they are similar to those in higher vertebrates. A more advanced arrangement has been shown in the elasmobranch *Scyliorhinus canicula*, the chondrostean *Acipenser oxyrhynchus* and the teleost *Cyprinus carpio*, all of which have two trigeminal nuclei. The evidence suggests that these nuclei evolved later than the agnathans (Sneddon, 2004). The spinal cord of elasmobranchs and teleosts has also been shown to have a complex laminar structure similar to that found in higher vertebrates including mammals. Dunlop and Laming (2005) measured nerve conduction velocities in goldfish and rainbow trout in response to brushing, pin-prodding and heat stimulation. Their data showed that Aδ and C fibres were activated and that there was also activity in all brain areas, supporting evidence for a nociceptive pathway from peripheral nerves to the CNS. It thus seems likely that the nociceptor pathways to the brain in lower vertebrates are very similar to those in higher vertebrates.

Opioids

Most vertebrates have been shown to produce the morphine-like endorphins and encephalins and, as already mentioned, the presence of specific receptors for them is considered to be good evidence for

nociception and possibly pain perception. Receptors for these molecules have been demonstrated in fish (Buatti and Pasternak, 1981) and encephalins have been found in areas of the brain of goldfish, catfish, rainbow trout and the African lungfish, with a similar distribution to that of higher vertebrates. Chernova (1997) showed that cod and salmon exhibited responses to painful stimuli, and that these could be modulated using opiate and monopiate analgesics. Sneddon (2003a, 2003b) showed that the rubbing behaviour exhibited following injection of acetic acid into the lips of trout was reduced when morphine was administered, again confirming the presence of opioid receptors in these fish. Opioid receptors and analgesia have also been clearly demonstrated in amphibians, and Stevens and Rothe (1997) suggested that such mechanisms may be common to all vertebrates. Overall, these substances and their receptors are very similarly manifested in lower and higher vertebrates. Because they provide a degree of pain suppression (analgesia), this strongly suggests that nociception occurs and that pain perception is a consequence.

Higher centres, learning and cognition

Many animals can be conditioned to respond to a stimulus, including a noxious stimulus. This classical conditioning has been demonstrated in many fish species, showing that fish are capable of recognising a noxious stimulus and learning to avoid it. In contrast to the commonly held perception that fish have a short, or even absent, memory it has been shown in several cases that this learning may be retained for many months. Rainbow trout, which had been classically conditioned for investigation of colour vision (Northmore and Muntz, 1974), were able to retrain after some months of rest in a much shorter time than they did originally (Melanie Johnson, personal communication). Dunlop *et al.* (2006) have shown that the response to this type of conditioning can be modified by the presence of other individuals. Trout and goldfish, which had been trained to avoid electric shocks, remained near the noxious stimulus if another individual was also in the zone. This clearly shows that the aversive reaction is not simply a reflex in these species and that a decision process is being used.

Suspension of normal behaviour

This somewhat subjective and potentially anthropomorphic criterion is widely used to assess likelihood of pain in animals. The contention is that if behaviour deviates from the normal following application of

a noxious stimulus, and is more significant than a simple nociceptive reflex, then it is probably painful to the animal. Sneddon *et al.* (2003) demonstrated that rainbow trout ignored a fear-causing novel stimulus when noxiously stimulated by injection of bee venom or acetic acid. They proposed that the noxious stimulus caused pain which overrode other behaviours, and emphasised that this was similar to the observed responses of humans who also performed poorly when in pain.

Summary

Aquatic animals, specifically the lower vertebrates and the higher invertebrates, are clearly capable of nociception with a range of resulting behaviours ranging from simple to complex. They may not perceive or experience pain in precisely the same way as mammals and higher vertebrates do, or by using the same neural structures. Strong views are held on both sides of the debate, and a good deal of anthropomorphism is exercised in interpreting the available data, from all points of view. However, the evidence increasingly suggests that they can experience a 'negative psychological state analogous to human pain in response to noxious stimuli' (Smith *et al.*, 2007). The humane treatment of experimental or cultured animals must take precedence over other considerations (Green, 1979) and from any ethical and moral standpoint good welfare and the freedom from pain must be obligatory.

Any procedure which involves handling or manipulation of aquatic animals or invasive methods, ranging from fairly simple forms of tagging using needles, to any use of incisions and suturing, or to internal surgery, is very likely to cause some degree of pain. This is particularly so in the vertebrates, but probably also to some degree in the invertebrates, and steps must be taken to alleviate any suffering which could be caused. Sedation, anaesthesia and analgesia are tools that contribute to this aspect of good welfare.

In the United States of America, while recognising the current status of the debate, the precautionary principle has been adopted. Guidelines for animal experimentation (US Public Health Services, 1986) state that:

> '*Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals*.'

and further, that:

> '*Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anaesthesia*'

If pain is a possibility following an experimental procedure, then post-procedural analgesics should be given routinely rather than 'as needed'. After most surgical procedures with humans, it is now standard practice to give analgesics during the first 24–48 hours after a potentially painful procedure. An additional focus for potential further research is that of acute pain and clinical pain. It is now accepted that in humans the clinical (post-operative) pain differs markedly from physiological pain (Cousins and Power, 2003). While the mechanisms remain unclear and the extent of pain perception is arguable, it is very probable that as fish and other aquatic animals are able to detect and perceive pain, it may follow that they could also suffer from different 'types' of pain. Were this to be demonstrated, the approach to commercial handling, tagging and marking, surgical procedures, fishing and angling would require substantial revision.

Although pain is a contentious topic, particularly in aquatic animal groups, anyone working in this field or in aquaculture must err on the side of caution until more is known.

References

Arlinghaus, R., Cooke, S. J., Schwab, A. and Cowx, I. G., Fish welfare: a challenge to the feelings-based approach, with implications for recreational fishing, *Fish Fish.*, 8 (2007) 57–71.

Basbaum, A.I. and Jessell, T.M., The perception of pain, in: Kandel, E.R., Schwartz, J H. and Jessell, T M. (eds.), Principles of Neural Science, 4th ed. McGraw-Hill, New York, 2000, pp. 472–491.

Barnes, R.S.K., Calow, P., Olive, P.J.W., Golding, D.W. and Spicer, J.I., The invertebrates: A synthesis, 3rd ed., Blackwell Science, Oxford, 2001, 497 p.

Bateson, P., Assessment of pain in animal, *Animal Behav.*, 42 (1992) 827–839.

Chandroo, K.P., Yue, S. and Moccia, R.D., An evaluation of current perspectives on consciousness and pain in fishes, *Fish Fish.*, 5 (2004) 281–295.

Chernova, L.S., Pain sensitivity and behaviour of fish, *Vosprosy Ikthiologii*, 37 (1) (1997) 106–111.

Cousins, M. and Power, I., Acute and postoperative pain, in: Handbook of Pain Management: A clinical companion to textbook of Pain, Elsevier, Churchill Livingstone, 2003.

Dunlop, R., Milsopp, S. and Laming, P., Avoidance learning in goldfish (*Carassius auratus*) and trout (*Oncorhynchus mykiss*) and implications for pain perception, *Appl. Animal Behav. Sci.*, 98(2–4) (2006) 255–271.

Dunlop, R. and Laming, P., Mechanoreceptive and nociceptive responses in the central nervous system of goldfish (*Carassius auratus*) and trout (*Oncorhynchus mykiss*), *J. Pain*, 6(9) (2005) 561–568.

Hamilton-Paterson, J., Do fish feel pain? in: Granta 83: The magazine of new writing, Granta Publications, 2003.

Håstein, T., Scarfe, A.D. and Lund, V.L., Science-based assessment of welfare: aquatic animals, *Sci. Tech. Rev. Office International des Epizooties*, 24(2) (2004) 529–547.

Huntingford, F. A., Adams, C., Braithwaite, V. A., *et al.*, *Curr. Issues Fish Welfare*, 68 (2006) 332–372.

Lagler, K.F., Bardach, J.E., Miller, R.R. and Passino, D.R.M., Ichthyology, John Wiley, New York, 1972, 506 p.

Lineberry, C.G., Laboratory animals in pain research, in: Gay, William I. (ed.), Methods in Animal Experimentation, volume 6, Academic Press, New York, 1981, 400 p.

Matthews, G. and Wickelgren, W.O., Trigeminal sensory neurons of sea lamprey, *J. Comp. Physiol.* 213 (1978), 329–333.

Melzack, R., Pain and the Neuromatrix in the Brain, *J. Dental Edu*. 65(12) (2001) 1378–1382.

Melzack, R. and Wall, P.D., The challenge of pain, 2nd ed., Penguin books, London, 1996.

Naitoh. Y., Bioelectric basis of behavior in protozoa, *Am. Zool.*, 14 (1974) 883–893.

Northmore, D.P. and Muntz, W. R., Effects of stimulus size on spectral sensitivity in a fish (*Scardinius erythrophthalmus*), measured with a classical conditioning paradigm, *Vision Res.*, 14(7) (1974) 505–514.

Packard, A., Cephalopods and fish – the limits of convergence, *Biol. Rev.*, 47 (1972) 241–307.

Rang, H.P., Dale, M.M., Ritter, J.M. and Moore, P.K., Pharmacology, 5th ed., Churchill Livingstone, 2003, 797 p.

Rose, J.D., The neurobehavioral nature of fishes and the question of awareness and pain, *Rev. Fish. Sci.*, 10(1) (2002) 1–38.

Sherrington, C. S., The Integrative Action of the Nervous System, Scribner, New York, 1906.

Smith, J.A. and Boyd, K.M., Lives in the balance: the ethics of using animals for biomedical research: the report of a Working Party of the Institute of Medical Ethics, Oxford University Press, Oxford, U.K., 1991, 352 p.

Smith, L., Urton, G., Ramnath, K. and Dai, S., 2007. Available from http://www.vet. ed.ac.uk/animalwelfare/fish-pain/Contents.htm. Accessed August 2007.

Sneddon, L.U., anatomical and electrophysiological analysis of the trigeminal nerve in a teleost fish, *Oncorhynchus mykiss, Neurosci. Lett.*, 319 (2002) 167–171.

Sneddon, L.U., Evolution of nociception in vertebrates: comparative analysis of lower vertebrates, *Brain Res. Rev.*, 46(2) (2004) 123–130.

Sneddon, L.U., Braithwaite, V.A. and Gentle M.J., Do fish have nociceptors? Evidence for the evolution of a vertebrate sensory system, *Proc. Royal Soc. London B*, 270 (2003) 1115–1121.

Spray, D.C., Pain and temperature receptors of Anurans, in: Llinas, R. and Precht, W. (eds.), Frog Neurobiology – A Handbook, Chapter 20, Springer-Verlag, Berlin and New York, 1976.

Stevens, C.W. and Rothe, K.S., Supraspinal administration of opioids with selectivity fro mu-, delta- and kappa- opioid receptors produces analgesia in amphibians, *Eur. J. Pharmacol.*, 331(1) (1997) 15–21.

U.S. Public Health Service, Government principles for the utilization and care of vertebrate animals used in testing, research and training, *US Public Health Service Guidance Note*, 1986.

Wells, M.J., Octopus, Chapman and Hall, London, 1978.

Young, J.Z., Homology of retractile filaments of vampire squid, *Science*, 156 (3782) (1967) 1633–1634

Chapter 4

The Nature of Anaesthesia, Sedation and Analgesia

General anaesthesia and sedation

The word anaesthesia ('anesthesia' in USA) has a Greek derivation, meaning loss of sensation or insensibility. It can take a number of forms, which require some definition. In general terms, sedation (defined as a calming effect) is a preliminary state of anaesthesia on a continuum (Fig. 4.1) in which drowsiness is induced, with dulled sensory perception and perhaps with some analgesia (insensitivity to pain), but in which there is no gross loss of sensory perception or of equilibrium. Full general anaesthesia may be defined as 'a reversible, generalised loss of sensory perception accompanied by a sleeplike state induced by drugs or by physical means' (Heavner, 1981). Green (1979) notes that 'general anaesthesia involves a state of general depression of the CNS involving hypnosis, analgesia, suppression of reflex activity and relaxation of voluntary muscle'. The term *narcosis* is also frequently used in discussing anaesthesia and anaesthetic agents. A narcotic is defined as a 'sleep inducing agent whose effect varies according to the strength and amount of the narcotic agent administered'. In animal work, anaesthesia always assumes that recovery will, or could, occur whereas narcosis may be used where recovery is not anticipated. There is clearly a great deal of overlap in the use of these terms, and narcosis and anaesthesia are essentially synonymous.

General anaesthesia and sedation can be produced by a variety of techniques including physical, chemical and psychological methods, which are summarised in Table 4.1. Some of these techniques apply to aquatic animals, although others, notably acupuncture, pressure, rectal drug administration and suggestion, almost certainly do not. Psychological methods operate by drawing the subjects' attention to something other than the noxious or aversive stimulus and it is postulated by some that acupuncture operates in a similar way. There is some support for the notion that acupuncture produces a 'gating' effect in the central

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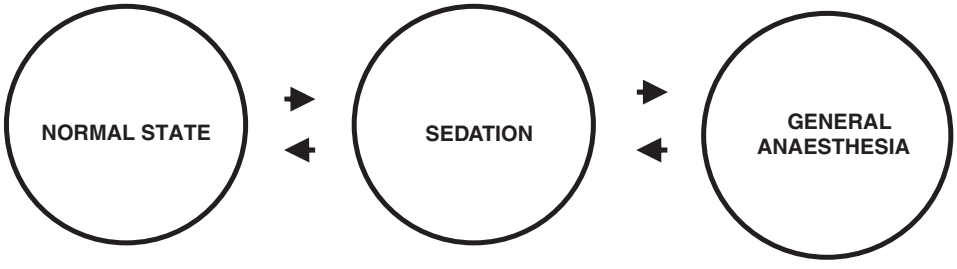

Figure 4.1 The sedation–anaesthesia continuum.

nervous system, either physically or by suggestion or a combination of both. Although some terrestrial animals seem to respond to this form of anaesthesia, there have been no systematic attempts to use it in aquatic animals.

Local anaesthesia

Local anaesthesia can be defined as a technique to produce reversible loss of sensation in a discrete region of the body without affecting consciousness. Normally, a small part of the body is targeted but in regional anaesthesia a larger part of the body may be involved such as an arm or leg. Local anaesthetic drugs are administered directly to the area where they are needed to act and it is not necessary for them to be absorbed into the bloodstream to be effective. They are given either by

Table 4.1 Methods used to produce general or local anaesthesia.

Method	Technique
Physical	Hypothermia Electrical stimulation ***Acupuncture*** ***Pressure***
Chemical (drugs)	Inhalation Parenteral: Intravenous Intraperitoneal Intramuscular Oral (enteral) ***Rectal***
Psychological	***Suggestion/Distraction*** Hypnosis

Adapted from Heavner 1981.
Techniques in bold italics have ***not*** been shown to be effective in aquatic animals.

injection or by applying the drug to the surface, usually the skin – a procedure technically known as topical block. Local anaesthetics produce their effect by blocking propagation of action potentials in receptors and nerves at, and near to, the selected site, effectively preventing transmission of any painful stimulus to the central nervous system and blocking muscle action. In addition to using drugs, local anaesthesia can also be produced by the use of locally-applied hypothermia, electric current, acupuncture, application of pressure and even hypnosis (see Table 4.1) although, clearly, many of these methods would not be effective in aquatic animals.

Analgesia

Analgesia is the relief from pain. It is a block of pain perception with or without the retention of other sensory abilities and with or without continued control of motor function. It typically does not result in loss of equilibrium. In animals it is reflected by a reduced response to noxious stimuli. Again, because of the nature and definition of pain itself, and because animals cannot express the feeling of pain in ways humans can, analgesia in animals may be difficult to judge. It is further complicated by the fact that the response to normally noxious stimuli can change and new responses can be learned. A classical example is that of Pavlov's dogs (Pavlov, 1927) who learned to associate a food reward with an electrical stimulation of the paw. The normal violent reaction to the painful electrical stimulus was soon replaced by tail-wagging and salivation. Research into pain relief in animals has been used as a means of studying pain alleviation in humans. It is, however, difficult on technical grounds and it has become increasingly difficult to justify the use of animal models on ethical grounds.

Overall, pain relief, or analgesia in aquatic animals may be judged to have occurred when the subject no longer responds, or has a markedly reduced response, to normally noxious stimuli following sedation or anaesthesia. Physical signs and motor responses have been used as indicators of analgesia. As physiological clinical equipment has become smaller, more affordable and more reliable, many modern studies assessing analgesia include monitoring of the electroencephalogram.

The mechanism of anaesthesia

General anaesthetic procedures usually act by widespread depression of the central nervous system produced by an action on nerve axons, transmitter release or membrane excitability or a combination of these

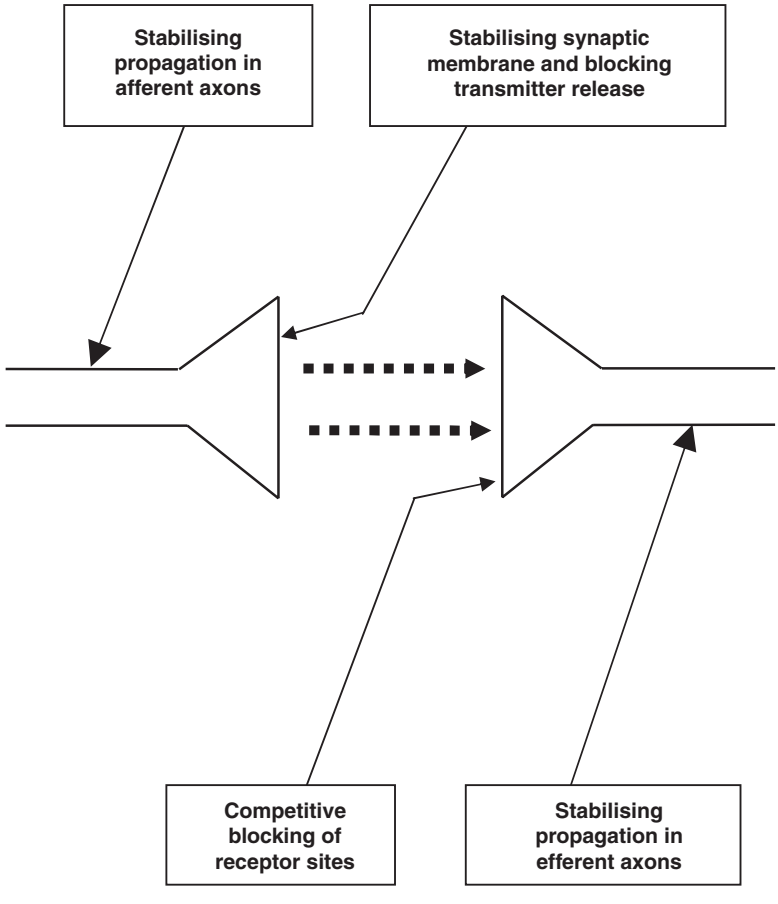

Figure 4.2 Some probable mechanisms of action of anaesthetic agents.

actions (Fig. 4.2). The only unifying principle of general anaesthesia is that anaesthetics interact with membrane components and no single cellular mechanism appears to be able to explain their widespread effects in the central nervous system (Winlow *et al.*, 1992) although they probably all modify the activity of ion channels (Klement and Nilsson, 2003).

It should be noted that little is known about the precise mode of action in invertebrates and fish although with some drugs there appears to be an inverse relationship between the dose level required to induce a given depth of anaesthesia and the animals' evolutionary status. Consequently, a fish may require a larger dose of a drug than a mammal to produce a given effect. It has been suggested that this phenomenon is related to the evolution of molecular mechanisms and may be due to an increasing presence of active sites for any particular molecular form in higher vertebrates.

Table 4.2 Stages of anaesthesia in man.

Stage	Description	Physiological and behavioural signs
I	**Induction:** The period between the initial administration of the induction medications and loss of consciousness	The patient progresses from analgesia without amnesia to analgesia with amnesia. Patients can carry on a conversation
II	**Excitement:** The period following loss of consciousness, marked by excited and delirious activity	Respiration and heart rate may become irregular. In addition, there may be uncontrolled movements, vomiting, breath holding, and pupillary dilation. Since the combination of spastic movements, vomiting, and irregular respirations may lead to airway compromise, rapidly acting drugs are used to minimise time in this stage and reach stage 3 as fast as possible
III	**Surgical:** Follows the excitement stage and is marked by a return of regular respirations	This stage is divided into four planes based on changes to eye reflexes, eye movements, and pupil size. The ideal plane for surgery is plane 3 where the patient has minimal use of the respiratory muscles
IV	**Overdose:** Where too much medication has been given and the patient has severe brain stem or medullary depression	Cessation of respiration and potential cardiovascular collapse. This stage is lethal without cardiovascular and respiratory support

Adapted from several sources.

The stages of anaesthesia

When induction is slow, a series of stages of, first, sedation and then anaesthesia can be observed which occur in most animals. A descriptive scheme for the stages of human anaesthesia is shown in Table 4.2.

The progressive stages of sedation and anaesthesia were first adapted and described for fish by McFarland (1959). The basis of his descriptive scheme is summarised in Table 4.3 and it can be seen that an anaesthetic substance can produce sedation, surgical anaesthesia or death, depending on the combination of the dose level and the length of exposure. In practice, it is often found that a fish species may not conform clearly to every aspect of McFarland's description although there is sufficient general agreement for this to serve as a good preliminary basis.

For practical purposes, anaesthesia reduces to three obvious phases, *induction*, *maintenance* and *recovery*. These principal phases are referred to from time to time in the literature, but unfortunately with no consistency. Each of these varies in duration according to drug or method used, species and conditions.

Table 4.3 Stages of anaesthesia in fish.

Stage	Plane	Description	Physiological and behavioural signs
I	1	Light sedation	Responsive to stimuli but motion reduced, ventilation decreased
	2	Deep sedation	As Stage I Plane 1: some analgesia, only receptive to gross stimulation
II	1	Light anaesthesia	Partial loss of equilibrium; good analgesia
	2	Deeper anaesthesia	Total loss of muscle tone, total loss of equilibrium, ventilation almost absent
III		Surgical anaesthesia	As Stage II, Plane 1: total loss of reaction to even massive stimulation
IV		Medullary collapse	Ventilation ceases, cardiac arrest, eventual death; overdose

After McFarland (1959).

During induction the animal is exposed to the anaesthetic agent in order to achieve the desired stage. Induction is often accompanied by hyperactivity, usually a response of only a few seconds to the sensation or slightly irritant properties of the drug. In general, induction should be rapid and without marked hyperactivity, although there is usually some. The animal will exhibit a succession of the signs indicated in Table 4.3, notably ataxia and loss of the righting reflex, eventually passing into surgical anaesthesia with no reaction to any stimuli.

Maintenance involves extending the achieved stage in a stable manner without detriment to the health of the animal. It should be uneventful and will most probably be effective on a reduced drug dose. In this state the environment of the anaesthetised animal needs to be controlled and physiological needs such as oxygen supply and removal of waste gases and metabolites must be attended to. It is usual to monitor the condition of animals during maintenance. This is achieved either visually or by measurement of ventilation rate or monitoring of ECG or EEG. The latter will clearly not be necessary, or even possible, where batches of animals are being handled. In research, where individual ECG monitoring is undertaken, arrhythmias should be avoided and excessive bradycardia or cardiac arrest requires immediate action to recover the animal. Over-exposure may be accompanied by 'flaring' of the opercula, which may be repeated several times (Klontz and Smith, 1968). Immediate steps should then be taken to recover the animal.

The recovery phase involves withdrawal of the anaesthetic agent and return to a normal state. Initial recovery may take anything from a few seconds to a few minutes, but in general it should be quick and without altered behaviour or other side-effects although there will usually be

some muscle trembling. The animal will attempt to right itself and will begin to respond to noise or other sensory stimuli. In fish, full righting can often be promoted by tapping the holding tank which stimulates the Mauthner axons. The time for a full recovery can be from minutes to days and will depend on the species and drugs used. Sympathetic treatment, handling and general good care are essential during this period to avoid post-operative death.

Dose, exposure time and effect achieved

As already described, the actual anaesthetic effect achieved will depend upon the dose or level of agent used and the duration of exposure to that agent. If the agent is sufficiently strong, the animal will pass rapidly and indistinguishably through all of the possible stages of anaesthesia. At lower concentrations a condition may be achieved where the clearance rate equals the rate of uptake and then an approximately steady state may be maintained. Understanding this relationship between dose, exposure time and anaesthetic stage achieved is very important to ensure good control of a procedure and is particularly important in the bath anaesthesia frequently used with aquatic animals. The degree and nature of analgesia achieved and the ease of recovery is also extremely important. Unfortunately, proper descriptions of all of these features are rarely, if ever, available for most agents.

An example of the stages of anaesthesia achieved or passed through at different drug dose levels is shown in Fig. 4.3 in which Josa *et al*. (1992) recorded the time to achieve certain observable criteria in common carp. The authors considered that there was a good safety margin at the lower doses and also showed a clear potentiating effect of a higher temperature with this species and drug combination. The recovery times are rapid at the two lowest dose levels but become more prolonged as dose level increases (Fig. 4.4). These induction and recovery effects may not be evident in the same way with all agents used but, in general, they show what may be expected.

Assessment of analgesia is very important but is rarely made. In most cases analgesia has been assumed to be effective because the animal is immobilised. This may be a reasonable assumption with some classes of anaesthetic drug, but cannot be relied upon in all cases. Consequently, proper assessment of analgesia should always be made when investigating new agents for aquatic animals. Robinson (1984, unpublished data), immobilised rainbow trout *Oncorhynchus mykiss* using electricity. Based on lack of motor and other responses to stimuli, there was good *apparent* analgesia but this was incomplete in tilapia, *Oreochromis niloticus*,

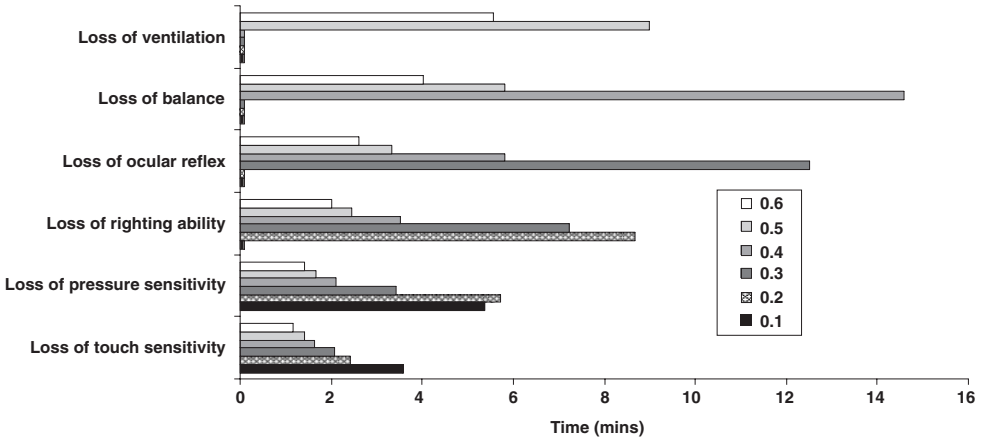

Figure 4.3 Time to induction in carp (*Cyprinus carpio*) using 2-phenoxyethanol at 10°C. Dose levels are in $ml.l^{-1}$. Based on data from Josa *et al*. (1992).

which still responded to pressure and pain stimuli. While useful, such studies do not assess analgesia in neurophysiological terms and there is often doubt as to whether analgesia is really achieved. Animals may be immobilised but not pain free. Some recent studies have used more appropriate physiological indicators such as EEG to assess brain activity (Lambooij *et al.*, 2002; Robb and Roth, 2003). These techniques are more reliable indicators of peripheral and central nervous system responses to an anaesthetic agent.

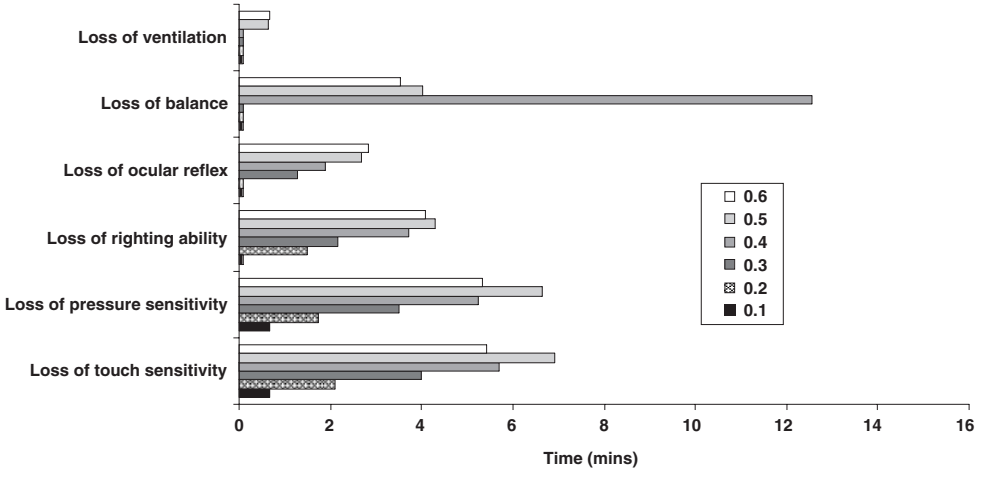

Figure 4.4 Time for recovery of carp (*Cyprinus carpio*) following anaesthesia using 2-phenoxyethanol at 10°C. Dose levels are in $ml.l^{-1}$. Based on data from Josa *et al*. (1992).

Euthanasia

In some cases it will be necessary to kill animals humanely, either to terminate an experimental procedure, harvest tissues, and cull surplus animals or to alleviate distress. This procedure, known as euthanasia, should be carried out with the minimum of physical and mental suffering and so it is best to prepare for this contingency in advance of carrying out any work which may possibly require it. This will involve arranging facilities in advance, as well as ensuring a minimum of disturbance to the subjects, considering safety of personnel and using a method in which you have confidence, i.e. one which is going to work quickly and reliably.

In the UK, legislation determines that only licensed methods can be used and that any other method requires special authority. Similar legislation exists in the USA (AVMA, 2001) and other countries. There are two possible approaches to euthanasia, either physical or chemical. Physical methods involve a sharp blow to the cranium so as to 'cause immediate loss of consciousness and probably death' (HMSO, 1997). This technique is most frequently used with fish and is very effective. Chemical methods involve the use of anaesthetic overdose, administered either by injection or immersion, 'using a route and a dose rate appropriate to the size, stage of development and species of fish'. In the USA recommended agents include barbiturates, benzocaine, 2-phenoxyethanol or MS-222 (AVMA, 2001) although clearly this method cannot be used with animals entering the human food chain.

Decapitation, or pithing in which the brain and anterior spinal cord are destroyed to absolutely ensure death before disposal is effected, should follow all of these methods. Pithing is carried out with a scalpel or sharp blade and a seeker or long needle. In fish this would typically involve cutting through the spinal column just behind the head and then passing a long needle or seeker into the spinal cord to sever connections, followed by passing the needle into the brain cavity and destroying connections by a number of lateral and vertical movements. Clearly, the technique should be adequately practised on cadavers, with guidance and supervision, before use. Once mastered, the procedure is rapid and reliably ensures death.

Holloway *et al*. (2004) compared the effects of euthanasia using MS222, clove oil or cranial concussion (stunning) on levels of plasma cortisol, glucose, growth hormone, and two thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4). Stunning significantly increased plasma cortisol and glucose levels while euthanising fish using either clove oil or MS-222 did not. The levels of growth hormone, T_3 and T_4 were not affected by the method used to kill the fish. These results

showed the importance of investigating the effects of any new anaesthetic or euthanising method or agent on blood and tissue parameters. This is particularly important in research or when comparison is to be attempted with data resulting from use of other techniques.

Summary

A basic understanding of the nature of anaesthesia and how it immobilises and aids in pain suppression is essential to enable users to understand and control the process. This background is also important in allowing workers to judge induction, the depth of anaesthesia and of analgesia, and to be able to adapt the basic scheme to new species and circumstances. It is important to use sedation or anaesthesia which is sufficient for the purpose while avoiding overdosing; an occurrence which is common among practitioners. Many accidents occur as workers implement inadequately tested methods and so we urge judicious pre-experimentation upon all who intend to carry out animal sedation or anaesthesia, of any kind. Anaesthesia and sedation is just part of the aquatic biologist's toolbox, but if mismanaged it can ruin everything.

References

AVMA., 2000 Report of the AVMA panel on euthanasia, *J. Am. Vet. Med. Assoc.*, 218 (2001) 669–696.

Green, C.J., Animal Anaesthesia, Laboratory Animal Handbooks No 8, London, 1979, 300 p.

Heavner, J.E., Animal models and methods in anaesthesia research, in: Gay, William I. (ed.), Methods in Animal Experimentation, volume 6, Academic Press, New York, 1981, 400 p.

HMSO., Code of practice for the humane killing of animals under Schedule 1 to the Animals (Scientific procedures) Act 1986, HMSO, London, 1997.

Josa, A., Espinosa, E., Cruz, J.I., Gil, L., Falceto, M.V. and Lozano, R., Use of 2-phenoxyethanol as an anaesthetic agent in goldfish (*Cyprinus carpio*), *Res. Vet. Sci.*, 53 (1992) 139.

Holloway, A.C., Keene, J.L. Noakes, D.G. Moccia, R.D., Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss* Walbaum, *Aquac. Res.* 35 (2004) 1025–1030.

Klement, A.P. and Nilsson, J., Mechanisms of anesthesia: towards integrating network, cellular and molecular level modelling, *Neuropsychopharmacology*, 28 (Suppl. 1) (2003) 40–47.

Klontz, G.W. and Smith. L.S., Fish as biological research subjects, in: Gay, William I. (ed.), Methods in Animal Experimentation, volume 3, Academic Press, New York, 1968, 469 p.

Lambooij, E., Van De Vis, J.W., Kuhlmann, H., *et al.*, A feasible method for humane slaughter of eel (*Anguilla anguilla* L.): electrical stunning in fresh water prior to gutting, *Aquac. Res.*, 33 (2002) 643–652.

McFarland, W.N., A study of the effects of anaesthetics on the behaviour and physiology of fishes, *Publ. Inst. Mar. Sci.*, 6 (1959) 22–55.
Pavlov, I.P., Conditioned Reflexes, Milford Press, 1927.
Robb, D.H. and Roth, B., Brain activity of Atlantic salmon (*Salmo salar*) following electrical stunning using various field strengths and pulse durations, *Aquaculture*, 216 (2003) 363–369.
Robinson, E., A study of the use of alternating current for electroanaesthesia in *Salmo gairdneri* and *Oreochromis niloticus*, B.Sc. thesis, University of Stirling, 1984, 26 p.
Winlow, W., Yar, T., Spencer, G., Girdlestone, D. and Hancox, J., Differential effects of general anaesthetics on identified molluscan neurons *in situ* and in culture, *Gen. Pharmacol.*, 23(6) (1992) 985–992.

Chapter 5

The Features of Anaesthetic Agents

Introduction

Broadly, there are three main approaches to the sedation and anaesthesia of fish, namely the use of drugs and gases, induction of hypothermia and exposure to electric current. Some of the properties required of an anaesthetic agent are common to all methods in current use, although in some cases special considerations or specific safeguards will be needed. It is unlikely that any agent, either existing or as yet undiscovered, will be able to fulfil all of the ideal pre-requisites.

Desirable features of an anaesthetic agent

The ideal characteristics of anaesthetic agents have been remarked upon by numerous authors, most usually when considering chemical methods (Marking and Meyer, 1985). In general, anaesthesia or sedation should be induced rapidly, preferably in less than 3 minutes, and with minimum accompanying hyperactivity or other stress. The agent or method should be easy to administer, involving no complex procedures. It should be easy to maintain the animals in the chosen state, should provide proper immobilisation and effective analgesia. Recovery should be rapid, substantially complete after about 5 minutes in clean water, and uneventful without prolonged ataxia or other undesirable features.

Where a sedative or anaesthetic drug or gas is used it is preferable that it should be effective at low doses and that the toxic dose should considerably exceed the effective dose providing a wide safety margin. A predictable level of analgesia should be provided. It should also be safe to operators at the dose levels used and should not be irritant or carcinogenic. It is also desirable that the chemicals do not produce hyperactivity during induction of the subject; some materials are much more prone than others to inducing this effect. In addition, the substance

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should be easily soluble in water or in a water-soluble solvent, which can be used as a vehicle. The drug and its solvent should be easy to obtain in bulk and should be relatively inexpensive. In aquaculture and fisheries work large numbers of animals are frequently handled and it is often necessary to dispense large quantities of drugs. The drug should, consequently, remain effective in working solution for more than 24 hours or so and should also be chemically stable over a reasonable period of time in storage. Where a drug is unstable or degraded over a short period cost becomes more important.

When fish are immobilised by lowering of temperature, the safety margin is frequently quite small and deaths occur if temperature is lowered too far, or too quickly. Uncontrolled temperature changes have been shown to be detrimental to osmoregulation in fish. Thus, the main consideration in hypothermia is that the rate of cooling has to be carefully controlled to avoid mortalities and that the required reduced temperature has to be maintained well.

Where immobilisation using electricity is used, it is important that the side effects do not exceed those encountered for other methods and that the electrical stimulation does not induce violent motor responses which can disfigure, or even kill. The safety margin to the animal must be well understood. Overall, operator safety is of paramount importance although it is very difficult to achieve it fully in practice.

The preferred features of anaesthetic agents are summarised in Fig. 5.1.

Toxicity and margin of safety

As already noted, it is preferable that a sedative or anaesthetic drug or gas should be effective at low doses and that the toxic dose should considerably exceed the effective dose, thereby providing a wide safety margin. The effective dose and toxic doses are linked in much of the literature, either qualitatively in the use of the term 'margin of safety' or, quantitatively through the 'therapeutic index'. The therapeutic index is the ratio of the effective safe dose to the dose that first produces undesirable side effects (Loomis, 1968), approximated by 99% survival. Although the therapeutic index will not be available for all drugs and species combinations, it is probably the most meaningful practical index.

An alternative way to express this margin of safety is the LC_{50}, being the concentration of a substance which will kill half of the sample group within a given period of time. The LC_{50} may be available for some drugs, but is arguably of less utility for anaesthetics than the therapeutic index

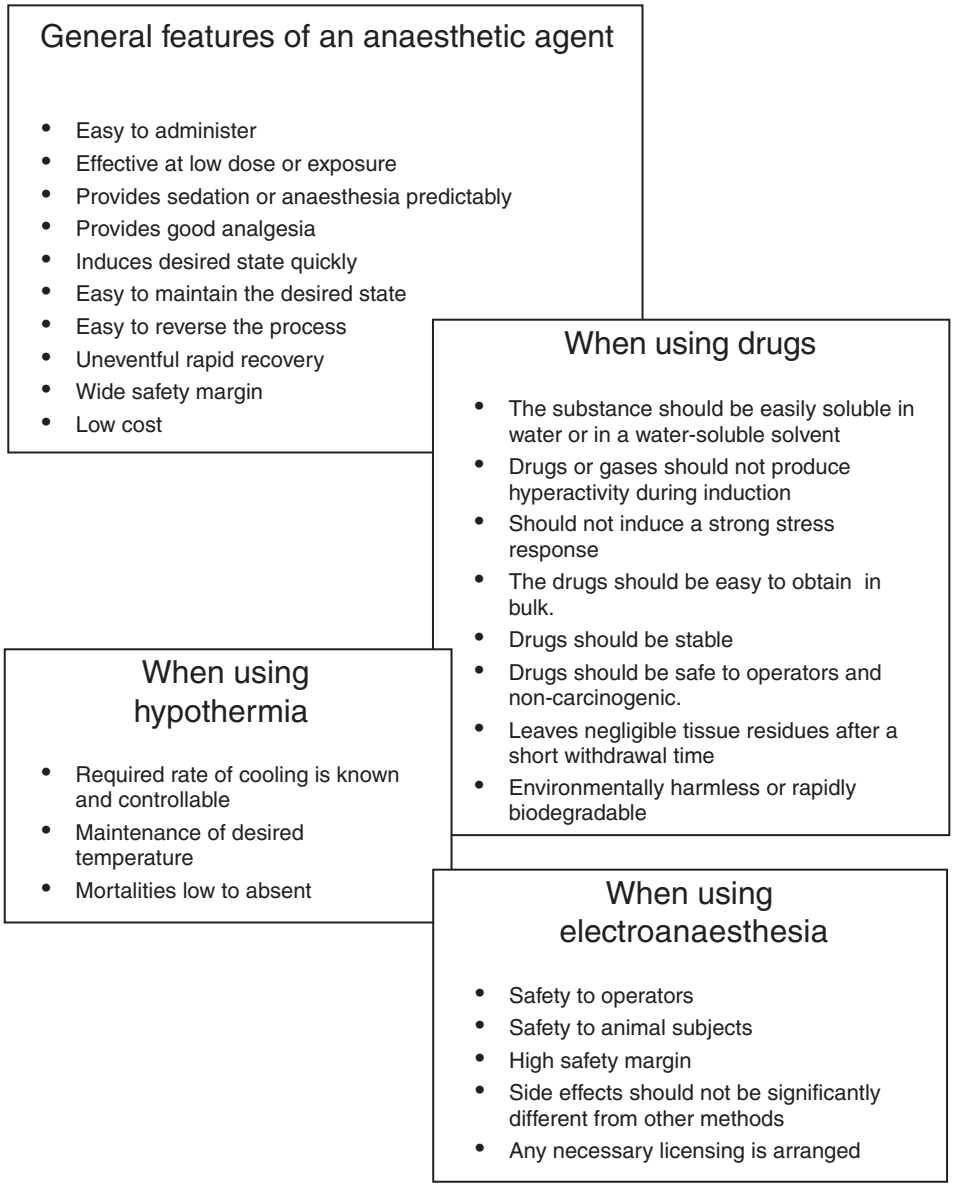

Figure 5.1 The desirable features of anaesthetic agents.

because of the lengths of exposure most frequently used in toxicity testing (24 h, 96 h, 144 h, etc.). Many aquatic animals will be exposed to the working concentration of water soluble anaesthetics for only a relatively brief time, usually considerably less than 30 minutes, making the LC_{50} difficult to interpret.

Additives

A number of proprietary drugs used in anaesthesia and sedation contain additional materials. Antioxidants, such as sodium metabisulphite, are designed to extend shelf-life and methylparaben (methyl-4 hydroxybenzoate) may be used to act as an antifungal agent. Surfactants may be added to improve flow characteristics and injection properties and some drugs may be presented in a carrier vehicle such as 50% glycerine. Some anaesthetics are much more potent at alkaline pH and bicarbonate may be added to proprietary preparations to ensure that substances are in the un-ionised state. Dextrose may be added to liquid preparations to adjust baricity (density relative to human cerebrospinal fluid). All these materials may have undesirable, or unknown, side effects in aquatic animals and where a new preparation is to be evaluated it is essential to have full data on the formulation, if available.

Summary

The desirable features of an anaesthetic agent are a wish list, a perhaps unattainable set of ideal parameters. However, many drugs do realise most of these features and tend to be those in wider use.

References

Alderman, D., Fisheries chemotherapy: A review, in: Muir, J.F. and Roberts, R.J. (eds.), Recent Advances in Aquaculture, volume 3, Croom Helm, London, 1988, 420 p.

Beleau. M.H., 1992, Drug development for aquaculture applications, in: Schaeffer, Dorcas O., Kleinow, Kevin M., and Krulisch, Lee (eds.), The Care and Use of Amphibians, Reptiles and Fish in Research, Proceedings of a SCAW/LSUSVM-sponsored conference, Scientists Center for Animal Welfare, 196 p.

Cowx, I.G., Developments in Electric Fishing, Fishing News Books, 1990, 358 p.

Loomis, T.A., Essentials of Toxicology, Lea and Febiger, Philadelphia, Pennsylvania, 1968.

Marking, L.L. and Meyer, F.P., Are better anaesthetics needed in fisheries? *Fisheries*, 10 (1985) 2–5.

Redshaw, C.J., Ecotoxological risk assessment of chemicals used in aquaculture: a regulatory viewpoint, *Aquacult. Res.* 26 (1995) 629–637.

SOAFD, Marine pollution monitoring management group, Final report of the MPMMG subgroup on marine fish farming, Scottish Fisheries Working Paper No. 3/92, February 1992.

Chapter 6

Anaesthesia and Legislation

Introduction

In recent years, the legislation covering procedures and materials described in this book has been steadily improved and extended, to the general benefit of animal welfare, the user community and the consumer of aquatic products. Although varying widely worldwide, much of the legislation has numerous common features, especially in Europe, North America, Australia and New Zealand. Treves-Brown (2000) provides a thorough summary of the safety and licensing issues applicable to use of fish medicines, including anaesthetics, and explains the currently prevailing situation regarding legal use of drugs with fish. It is important to emphasise, however, that the relevant legislation is subject to constant review and steady revision. This can appear to be glacially slow but changes can occur at any time and so all users of the techniques described here must become aware of the current, relevant legislation for the country in which they operate. Because of this, the disclaimer already included in the preface is repeated here as a reminder:

> *"The authors and the publisher accept no responsibility for losses of animals, losses of experimental or other data, harm occurring to individuals or any other matter which may arise caused by application of the data contained herein or which may arise as a result of non-compliance with any relevant legislation."*

The broad legislative objectives relevant to pharmaceuticals in the United States, and applicable elsewhere, are summarised in Table 6.1 and to this must be added electrical safety.

The overarching legislative issues then fall into the following categories:

- Safe storage of drugs and chemicals.
- Safety of users and practitioners.

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Table 6.1 Legislative targets for licensing of pharmaceuticals in the United States (after Beleau, 1992).

Objective	Protected group
The drug must be shown to be safe to the treated animals	Cultured stock or lab animals
The drug must pose no hazard to the user	Human users (e.g. the fish farmer, laboratory researcher)
No harmful residues must be left which could contaminate the human or animal food supply	Food chain; humans and other food animals
The material must not contaminate the environment either in its use or its manufacture	Environment; all wildlife, all vegetation, humans
The product must be effective for its intended purpose.	Cultured stock or lab animals

- Safety of the consumer from use of drugs with animals intended for the human food chain.
- Safety of the environment from drug discharges etc.
- Safety of the treated animal; animal welfare and experimentation issues.
- Safety legislation concerning electric fishing and similar electrical apparatus.

Safe operator practice for users and safe storage of drugs and chemicals

Use of anaesthetic agents, particularly chemicals, in the aquaculture industry is subject to a further set of constraints relating to safety to the operator. In the UK, use of chemicals in places of work and in research laboratories is regulated by a Health and Safety Executive (HSE), who operates under the overarching legislation on the Control of Substances Hazardous to Health (COSHH). This requires that all users of substances, and their supervisors and co-workers, understand the hazards and risks associated with their use, that drugs and chemicals are stored and handled correctly and that proper procedures are in place for their disposal. A documented risk assessment must be carried out covering all stages from purchase to disposal and users must be formally advised of all potential hazards and be given practical instruction where needed. Any necessary protective clothing, gloves, goggles, etc., must be supplied and must be used. Much of this is also influenced by the Europe-wide directive covering workplace safety and similar processes

that exist in other countries. Steps must be taken to ensure compliance, whether in the laboratory or on the farm.

Most substances used routinely in anaesthesia of aquatic animals pose no excessive dangers to operators in normal use and require relatively simple storage, other than perhaps refrigeration. Clearly, special arrangements will be needed for safe storage of gases and volatile solvents such as the alcohols. Manufacturers provide useful guidelines on safe storage and shelf-life of products and much of this is also freely available from the Internet. Secure storage facilities will be necessary for regulated drugs such as barbiturates. Storage of inflammable, toxic or regulated substances may itself be the subject of regulation.

Food chain safety

The potential dangers of drugs for use in the food chain are in most cases the principal legislative driver and where any risk is identified this will always take precedence over environmental considerations. Anaesthetic agents can be licensed for use in food animals in two ways; either by extending the licensed use of material already approved for use with a terrestrial farmed animal, or by a full drug development programme designed to meet all current legislations. Satisfying this objective requires a wide range of inputs, and cooperation, from the drug companies, research scientists, national agencies and the farming and feed industries. The aquaculture industry is a large one worldwide, indeed it has for some years been the fastest growing animal production sector, but is still relatively small compared to other animal production industries or to human medicine. For this reason, drug companies may have a conflict between supporting the costs of fully licensing a new material for aquaculture and any expected financial returns.

The type of approval varies according to country. A 'fully approved' drug is one for which a substantial amount of data on the quality, efficacy and safety of the material has been amassed, submitted to the national regulatory agency(s) and which has received approval for use, sometimes with conditions applied. There are also a number of drugs that fall into the 'grey areas' of legislation, where their use is generally condoned, although they have not been formally approved. These substances are usually those which, using the definitions of the Food and Drug Administration, USA, may be called *Investigative New Animal Drugs* (INAD) or *Generally Regarded as Safe* (GRAS) and thus are of *Low Regulatory Priority* (LRP). Treves-Brown (2000) outlines the complexity of this process and summarises the law in countries where aquaculture is important.

Table 6.2 Anaesthetic drugs approved for use in aquaculture.

Country	Drug	Withdrawal time	Comment
United Kingdom	MS222	10 days	No temperature specified
	Carbon dioxide	ns	Used in bleeding of salmonids
United States of America	MS222	21 days	
	Carbon dioxide	ns	LRP
	Sodium bicarbonate	ns	LRP; used to generate carbon dioxide for transportation
	Aqui-S	zero	Was GRAS, now INAD.
	Ice (!)	ns	LRP, used in transportation
	Benzocaine	21 days	INAD
European Union	MS222		Variable
Norway	MS222	40 days at <9°C 80 days at >9°C	
	Chlorbutanol	40 days at <9°C 80 days at >9°C	
Philippines	MS222	ns	Food fish
	Quinaldine sulphate	ns	Ornamentals only
New Zealand	MS222	10 days	
	Benzocaine	ns	
	Aqui-S	zero	

ns, not specified.

For some drugs approval may be granted with a specified withdrawal period which specifies the time between the last use of the material and the time at which the animal enters the food chain. This withdrawal period can be set where data is available on the pharmacokinetics and clearance times of the drug from the body, coupled with robust data on the toxicological effects of the drug assessed over a long period of exposure. As fish are ectotherms, this data needs to be specified for relevant temperatures and it should also be specified at relevant salinities in some species. Given the time required and investment costs to amass such a body of data it is not surprising to find that the range of anaesthetics currently approved for food use worldwide is very small indeed (Table 6.2).

It is important to note that for animals entering the food chain no unlicensed drugs can be used legally. While this may not apply to aquaculture products within a given country, the cross-border and exportation implications are clear. As noted by Alderman (1988) and by Treves-Brown (2000), the detection limit for drugs in fish and shrimp

tissue is improving continuously as analytical equipment and technology improve and there is hence a real problem in defining what are the acceptable tissue limits and consequently, of course, appropriate withdrawal times.

Environmental safety

The use of chemicals in aquaculture and fisheries and their release into the environment is of increasing concern. Legislation already exists covering a wide range of on-farm compounds. For example, in the UK, there are clear definitions for a range of materials, including organophosphates, organo-tin compounds, organo-silicon compounds, toxic metals, anti-microbials, biocides, substances affecting taste or odour, ammonia and nitrates, both in terms of discharge limits and groundwater standards. The current concerns and developments in risk assessment procedures for the management of such materials are discussed by Redshaw (1995). Although there is no existing, specific UK legislation regulating discharge of anaesthetic agents, a survey by SOAFD (1992) estimated that 2 tonnes of anaesthetic agents were used in 1992 in Scottish aquaculture alone. In real terms this is a relatively small quantity and, coupled with the wide distribution of the material and the relatively low toxicity of working strengths of anaesthetic, the environmental hazards they pose are probably very low in comparison to most of the other materials mentioned above.

Animal welfare and experimentation

A common approach to regulation of animal experimentation is now taken within the countries of the European Union and a largely similar system exists in many other countries. In the UK, for any procedure to be used, approval must be sought from the Home Office Animals in Scientific Procedures Inspectorate, which controls all such aspects of research with animals under the auspices of the Animals (Scientific Procedures) Act (1987). It is increasingly the case that every procedure to which an experimental animal will be subjected requires prior definition and approval and this now extends to anaesthesia which, as a routine preparatory procedure, was formerly excluded. Consequently, even routine anaesthesia of fish for measurement during a growth trial requires that the operator and the anaesthetic procedure is licensed.

The choice of technique and of drugs for use in research is principally left in the hands of the individual who will use the most appropriate approved methods for the objectives of the work. To gain approval, the

method of handling, technique for exposure to the agent, and expected nature of recovery must be formally documented, along with written contingency actions to be taken in the event of excessive pain or accidental non-recovery. In practice, there are few legal options open to the experimenter, unless anaesthesia itself was to be the subject of the trial and proper approval has been granted. Oversight of these procedures is increasingly stringent and penalties, including disbarment, may be expected for non-compliance. Furthermore, only licensed persons are allowed to use these techniques, although they may be delegated to appropriately trained support staff, under supervision, in certain circumstances.

Safety legislation concerning electric fishing and similar electrical apparatus

Electricity and water do not mix safely and electro-anaesthesia poses special problems. In general, good construction standards, multiple electrical safety features and strict codes of operation are required to approach a sufficient degree of safety, although there can be no absolute protection against deliberate or accidental immersion in the electrical field. The nature of recorded operator accidents during electro-fishing ranges from mild shock to death. There are no official guidelines on this for electroanaesthesia, although a number of useful articles related to use of electric fishing equipment can be found in Cowx (1990). The guidelines for electric fishing equipment and practice, while useful, will be found to require modification for anaesthesia.

Summary

The desirable features of an anaesthetic agent are a wish list, a largely unattainable set of ideal parameters. However, many drugs do realise some of these features and tend to be those in wider use. The additional legislative matters affecting methodology, drug choice and safety are extremely important and are growing. Some indications of the factors that users will need to address have been given here, using UK regulations as examples. Users are urged to adhere to all necessary regulations for the location in which they work.

References

Alderman, D., Fisheries chemotherapy: A review, in: Muir, J.F. and Roberts, R.J. (eds.), Recent Advances in Aquaculture, volume 3, Croom Helm, London, 1988, 420 p.

Beleau, M.H., Drug development for aquaculture applications, in: Schaeffer, D.O., Kleinow, K. M. and Krulisch, L. (eds.), The Care and Use of Amphibians, Reptiles and Fish in Research, Proceedings of a SCAW/LSUSVM-sponsored conference, Scientists Center for Animal Welfare, 1992, 196 p.

Cowx, I.G., Developments in Electric Fishing, Fishing News Books, 1990, 358 p.

Loomis, T.A., Essentials of Toxicology, Lea and Febiger, Philadelphia, Pennsylvania, 1968.

Marking, L.L. and Meyer, F.P., Are better anaesthetics needed in fisheries? *Fisheries*, 10 (1985) 2–5.

Redshaw, C.J., Ecotoxological risk assessment of chemicals used in aquaculture: a regulatory viewpoint, *Aquacult. Res.*, 26 (1995) 629–637.

SOAFD, Marine Pollution Monitoring Management Group, Final report of the MPMMG subgroup on marine fish farming, Scottish Fisheries Working Paper No. 3/92, February 1992.

Treves-Brown, K.M., Applied Fish Pharmacology, Kluwer Academic Publishers, Dordrecht, 2000.

Chapter 7

Factors Affecting the Response of Aquatic Ectotherms to Anaesthesia

Introduction

In common with anaesthesia of other animals, there is a series of factors which can alter or mediate the efficacy of anaesthetic processes in fish. These can broadly be divided into biological and environmental factors; they are summarised in Table 7.1.

Biotic factors

There are about 30,000 species of fish and, unlike other distinct vertebrate taxa, they are very diverse both in body shape and detailed design. A 100-g trout, for example, has a very different body shape and a greater gill area than an eel or a flounder of similar weight. Consequently, they are unlikely to respond similarly to a given anaesthetic treatment. Often the rate at which anaesthetic drugs become effective can be related to gill area to body weight ratios and these can vary considerably between species. Indeed in some species, for example certain air breathers, the gills are reduced to a single arch used only for osmoregulation and excretion. Aquatic species with different habits have intrinsically different metabolic rates. For example, the resting respiratory rate of a very active species, e.g. tuna, may be many times greater than that of a sedentary species, e.g. the monkfish, *Lophius* sp. Based on knowledge of body structure and habit it is usually possible to predict approximately how a given species may respond.

Differences in response to various drugs are well known in higher vertebrates, including different strains of mice, rats, rabbits pigs and dogs (Green, 1979). Such differences have not been described in aquatic animals, but there is every probability that they exist and this should be borne in mind.

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Table 7.1 Factors affecting the efficacy of anaesthetics for fish..

Factor group	Factor	Probable mechanisms
Biological	Species	• Differences in body design and habit
	Strain or genetic variant	• Gill area to body weight ratio
	Size and/or weight	• Physiological variability
	Sex and sexual maturity	• Differences in enzymes
	Lipid content	• Change in metabolic rate
	Body condition	• Lipid content of body, especially of gonads
	Disease status	• Use of lipophilic drugs
	Stress	• Oily fish or older specimens
		• Exhausted animals, post-spawners
		• Weakened or exhausted animals
Environmental	Temperature	• Q_{10} as in all ectotherms (poikilotherms)
	pH	• pK_a effects and ionisation of molecules
	Salinity	• Buffering effects
	Mineral content of environment	• Calcium antagonism

It has been noted by some authors that, within a species, there is a direct relationship between drug dose and body size (Houston and Woods, 1972; Huish, 1972). This empirical general observation would be easy to understand if metabolic rates per unit weight increased with body weight, whereas in fact there is an inverse relationship between metabolic rate and body weight. In practice, large active fish within a group often succumb to chemical anaesthesia before their smaller counterparts and in tilapia it has been noted that fry anaesthetised along with much larger fish recover surprisingly rapidly from the effects of the drug (Ross and Geddes, 1979). The differences noted are probably related to changing liver enzyme activities, fat deposition and surface area : body weight ratios.

Many drugs such as MS222 and benzocaine may be fat soluble. Thus, in larger, older fish with well-developed fat bodies, or in gravid females, duration of anaesthesia may be prolonged and consequent recovery slower as the drug is slowly removed from the lipid reserves for clearance via the gills or kidney or for metabolic degradation.

In common with all vertebrates, diseased or exhausted animals are very susceptible to anaesthetic treatment. Schoettger and Steuke (1970) showed that pike, *Esox lucius,* and walleye, *Stizostedion vitreum,* depleted after spawning, were more susceptible to MS222. Richards (personal communication) found that sea trout infected with *Saprolegnia* did not recover reliably from either MS222 or quinaldine anaesthesia.

Fish culturists are often concerned about the repeated use of anaesthetics and their possible effects on fish growth and performance. McFarland and Klontz (1969) claim that the careful and proper use of tertiary amyl alcohol, methyl pentynol and MS222 is unattended by side effects even with repeated use. Nakatani (1962) induced deep MS222

anaesthesia (100 mg L^{-1}) in trout five times per week for 21 weeks and noted no side effects. Ross and Geddes (1979) described regular use of benzocaine on tilapia with no apparent reduction in growth or spawning ability.

Environmental or abiotic factors affecting anaesthesia

Aquatic invertebrates and fish, with the exception of tunas, are all ectotherms whose body temperatures closely follow that of their environment because of highly efficient heat exchange in the gills and to a lesser extent the skin. Consequently, the effects of temperature on anaesthetic dose can be considerable. Unfortunately, there is no simple underlying relationship and the effect is entirely dependent on the type of drug used. With MS222 and benzocaine, higher doses may be required at higher temperatures to produce the same effect. Sehdev *et al*. (1963) showed a similar effect for 2-phenoxyethanol and they also showed that its therapeutic index was increased at lower temperatures. Obviously, physico-chemical passage of the drug into the fish is also temperature related.

The pH of an anaesthetic solution influences its efficacy, possibly by affecting the ratio of charged to uncharged molecules. In addition, a low-pH medium (e.g. unbuffered MS222; pH 3.8 at 30 mg L^{-1}) induces a stress reaction. Quinaldine, a base with a pK_a of 5.42, forms increasingly ionised solutions as the pH falls and loses its anaesthetic efficacy.

Because of the buffering capacity of sea-water and its ionic constituents the effects of some drugs may be modified in seawater, even in the same species. In general, most anaesthetic drugs are effective in sea-water, but the barbiturates are antagonised by high calcium levels and lose effectiveness (McFarland and Klontz, 1969).

Summary

Although it is useful for operators to be aware of these modifying factors, it is not always possible to take account of all of them in a predictable way. The cumulative effects of these variations are often seen when anaesthetising batches of fish when it frequently becomes obvious that some individuals are responding in a very different manner to the bulk of their fellows.

References

Green, C.J., Animal Anaesthesia, Laboratory Animal Handbooks, No. 8, Laboratory Animals Ltd., London, 1979, 300 p.

Houston, A.H. and Woods, R.J., Blood concentrations of tricaine methanesulphonate in Brook Trout, *Salvelinus fontinalis*, during anaesthetization, branchial irrigation and recovery, *J. Fish. Res. Board Can.*, 29 (1972) 1344–1346.
Huish, M.T., Some responses of the Brown Bullhead (*Ictalurus nebulosus*) to MS222, *Prog. Fish Culturist*, 34 (1972) 27–32.
McFarland, W.N. and Klontz, G.W., Anaesthesia in fishes, *Fed. Proc.*, 28 (1969) 1535–1540.
Nakatani, R.E., A method for force-feeding radio-isotope to yearling trout, *Prog. Fish Culturist*, 24 (1962) 156–160.
Ross, L.G. and Geddes, J.A., Sedation of warm water fish species in aquaculture research, *Aquaculture*, 16 (1979) 183–186.
Schoettger, R.A. and Steucke, E.W., Quinaldine and MS222 as spawning aids for Northern Pike, Muskellunge and Walleyes, *Prog. Fish Culturist*, 82 (1970) 199–205.
Sehdev, H.S., McBride, J.R. and Fagerlund, U.H.M., 2-Phenoxyethanol as a general anaesthetic for Sockeye Salmon, *J. Fish. Res. Board Can.* 20 (1963) 1435–1440.

Chapter 8

Anaesthesia of Fish: I. Inhalation Anaesthesia

Introduction

This most widely used technique depends on the anaesthetic drug being in aqueous solution. The drug solution is ventilated (i.e. inhaled) by the fish and drug molecules diffuse rapidly into the blood spaces in the secondary lamellae, which drain into the efferent arterial blood from where it is a very short route to the central nervous system (Fig. 8.1). This process is analogous to gaseous anaesthesia in terrestrial vertebrates. On return to fresh water the drugs, or their metabolites, are most frequently excreted via the gills and to a much lesser extent the skin. Some materials may also be excreted via the kidney, either intact or as metabolites.

There are certain species of fish, some of which are important in aquaculture, which can breathe air rendering this technique unsatisfactory. The snakehead, *Ophiocephalus* sp. is one example of an obligate air breather. In these species oxygen uptake occurs at alternative respiratory structures which retain their surface area out of water and the gill and accompanying lamellae are much reduced, being used principally for excretion and osmoregulatory purposes. Consequently, although the animal does respond to inhalation anaesthesia, induction is usually lengthy, unpredictable and therefore frustrating. There are many examples of facultative air breathers (e.g. *Umbra*, *Anguilla*, *Clarias*, *Plecostomus*, *Trichogaster*) which use air breathing only when necessary. In the natural environment this occurs mainly when the water becomes hypoxic. Obligate and facultative air breathers have the ability to arrest ventilation for some time when exposed to noxious solutions and this applies to anaesthetic agents in water. When this happens induction can be lengthy, although it is usually still effective. Air breathers may also swim around on the surface of the tank during induction presumably avoiding ventilation of the medium and care should be taken to ensure the fish do not jump out of the container.

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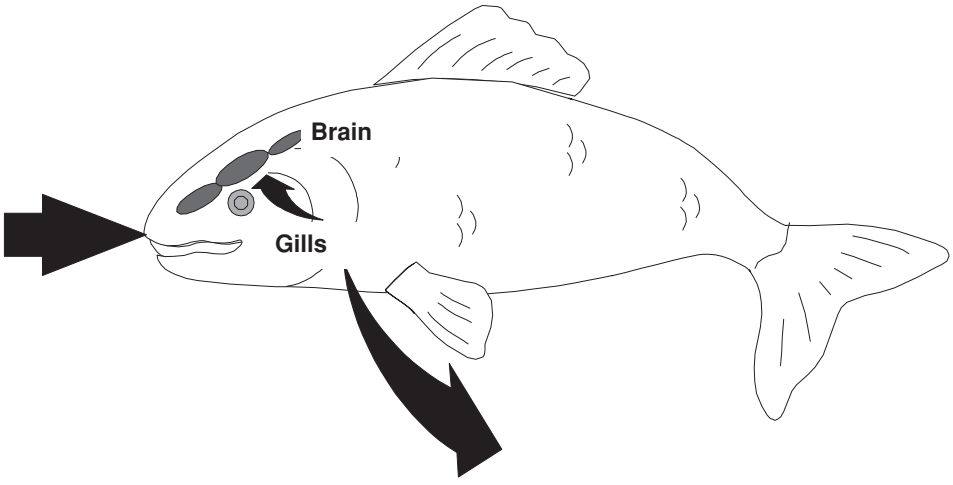


Figure 8.1 Route of inhaled drugs to the central nervous system of fish.

Water quality maintenance during inhalation anaesthesia

It should be obvious that water quality needs to be carefully controlled during inhalation anaesthesia, particularly where large numbers of animals are being handled and where baths of anaesthetic are being reused. This is part of the general management of the anaesthetic procedure and maintenance of the level of anaesthesia where it is continued for more than a brief period. The main problems are those facing all aquatic animals: control of temperature, dissolved oxygen concentration, ammonia levels and build up of faecal matter and other solids in the baths.

The stock baths and recovery vessels should, where possible, all use water from which the animals originate and at the temperature to which the animals are acclimated. Small electrical heaters and coolers can be used if needed although this will rarely be the case for short procedures. Cooling can also be effected with ice although it is best to use bagged ice so as to avoid contact between and mixing of melting ice and the medium. Care should be taken that animals do not overheat or overcool while taken out of the water for handling. In the extreme environmental conditions of the tropics and subtropics or northern winters this can occur very quickly indeed.

The stock containers, anaesthetic bath and recovery vessel should all be equipped with airstones or diffusers. In all cases, aeration is best if it can be effected using a diffuser as the gas exchange efficiency from the finer bubbles produced is much greater than if a simple airstone is used. It is also very important to ensure that high-pressure compressors do

not issue vapourised oil into the water and for this reason it is always best to use low-pressure, high-volume air blowers, if available.

Excreted solids are more difficult to deal with, but water changes can be used and should be made as often as needed by visual inspection. The anaesthetic bath is more costly to replace and if large numbers of animals are to be used some simple filtration system can be used based on pumps or air-lifts. In general, however, working solution replacement is frequently the only practical means.

Ammonia levels can be reduced to some extent by continuously passing the water over zeolites, clinoptilolites or other ion-exchange materials, again using pumps or air-lifts. This extra level of complexity is rarely necessary, however, and the lower metabolic rate induced by the anaesthesia itself will be of some assistance.

The basic procedure

For simple procedures it is usually possible to immerse the fish directly in a suitable concentration of drug so that spontaneous ventilation is maintained, and this approach is widely used in fish farming and for most laboratory studies. The simplest method of achieving this is to make up the required drug concentration in an aerated container and to quickly but gently transfer the fish to the container. Initial capture of fish may require gently crowding fish in their enclosure to facilitate netting. Capture will be easier and more stress-free if the nets used are sufficiently large and are made of soft, knotless mesh to avoid skin abrasion. For most procedures in aquaculture and fisheries management this technique is quite adequate. Induction should be rapid, handling time will be minimal and the fish can then be transferred to well-aerated clean water within a few minutes, having been weighed, measured, marked or whatever is required (Fig. 8.2). Using this procedure it is usually not necessary to exceed stage 1 and plane 2 of McFarland's scheme (Table 4.2) for adequate handling. Consequently, ventilation of the gills will normally be maintained spontaneously and use of well-aerated drug solution and recovery baths will ensure an adequate oxygen supply and good recovery; mortality will be the exception.

Using clean, inexpensive containers, this technique and scheme can easily be adapted for use in the laboratory, on the farm or in the field. Figure 8.3 shows a rainbow trout being fitted with ultrasonic tracking tags in the field, following simple benzocaine anaesthesia.

For successful bulk use of this approach, a scheme of work is useful to ensure that all concerned adhere to a procedure which will safeguard the welfare of the animals. The box below (Fig. 8.4) gives an example

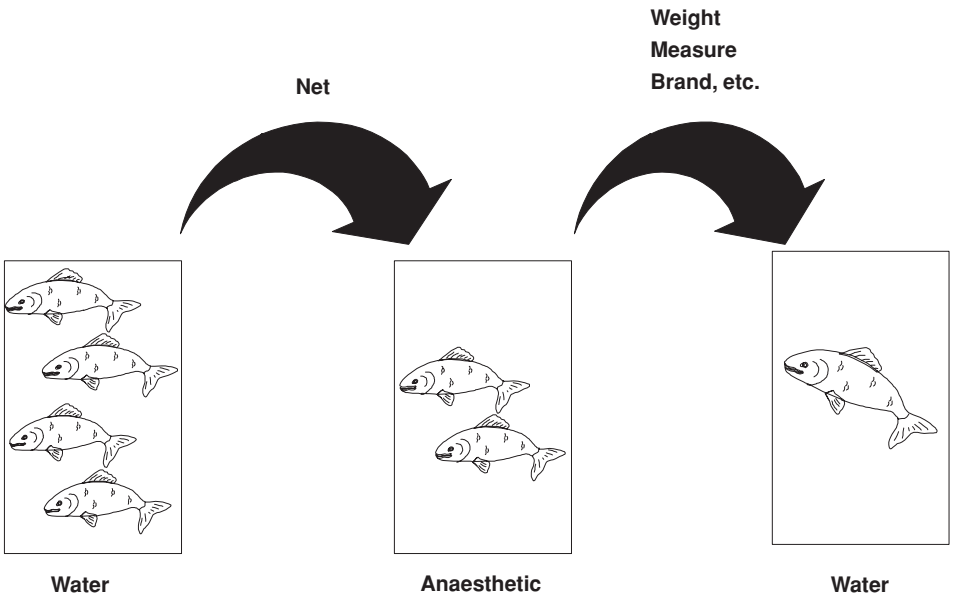


Figure 8.2 Schematic representation of simple inhalation anaesthesia procedure as used in fisheries and aquaculture.

Figure 8.3 Rainbow trout being fitted with ultrasonic tracking tags in the field, following simple benzocaine anaesthesia. The assistant is holding the fish to facilitate tying off of the tag, not for the purpose of restraint.

1. Prepare a stock solution of 100 g of benzocaine dissolved in 1 L of ethanol or acetone. Store in a dark bottle and refrigerate, if possible.

2. Prepare the anaesthetic bath to the appropriate final concentration (see Tables in text) by slowly adding stock solution to water with *thorough stirring* to prevent the drug coming out of solution. Stirring is very importantat this stage. Oxygenate this bath.

3. Prepare a recovery vessel of clean, well-oxygenated water.

4. Ensure that the stock tank, anaesthetic bath and recovery vessel are all at the same temperature. Adjust if necessary.

5. Ensure that all relevant equipment for the intended technique, nets, buckets, syringes, needles, injectable materials, tags, tissue wipes, etc., is prepared and ready to hand before proceeding any further.

6. Quickly, but gently, net out a small batch of fish from stock and transfer them immediately to the anaesthetic bath. The number of fish per batch will depend on the time taken to handle each fish. Even experienced workers should limit themselves to a very small batch in the first netting; say 3 to 4 animals, to allow for set-up errors and to regain handling confidence. On no account should the fish remain in the bath for extended periods. As a rough guide fish should not remain in any anaesthetic bath for longer than 10 minutes.

7. For simple procedures it is usually possible to achieve adequate handling even before the fish have fully lost equilibrium. Operations should commence as soon as a fish can be picked up, gently and without struggling.

8. After handling, the fish should be placed immediately in the recovery tank. Substantial recovery will normally occur within 1 minute although some species may require slightly longer. On no account should the recovery tank be allowed to become over-stocked.

Figure 8.4 Example scheme of work for bulk inhalation anaesthesia using benzocaine.

of a specific scheme for inhalation anaesthesia using benzocaine and outlines the procedure to be followed for simple handling and where no artificial ventilation is required.

Unfortunately it may be difficult to maintain a uniform depth of anaesthesia using this technique. Remember that drug dose and exposure time will act cumulatively to determine the final stage of anaesthesia reached. For example, Houston *et al*. (1971) have shown that levels of MS222 in brain and muscle continue to increase after blood levels

have attained equilibrium. Consequently, a drug dose that is initially satisfactory can produce progressively deeper anaesthesia and eventual ventilatory arrest. The resulting decline of water flow in the buccal cavity contributes to a reflex decline in heart rate and dorsal aortic blood pressure. A progressive hypoxia ensues which is further complicated in some cases by swelling of erythrocytes, causing a decrease in gill capillary blood flows (Soivio *et al.*, 1977). Species with a high body lipid content, or large mature fish, retain fat-soluble anaesthetic agents for a longer period after recovery, further complicating the recovery process.

During simple procedures these complications will not occur, but it will readily be appreciated that lengthy exposure to a dissolved anaesthetic agent should be avoided if steady progress of the animals through the successive stages of anaesthesia is to be prevented. Where an animal fails to recover spontaneous ventilation, the water flow–heart rate reflex can be exploited. As noted earlier, a reduction in buccal water flow reflexly causes a reduction in heart rate. Fortunately, in the anaesthetised animal the reverse is also true, in that, increasing water flow through the buccal cavity will accelerate and regularise heart rate markedly. This increases gill blood flow and eliminates the drug faster, hastening recovery. In emergencies this can be achieved by moving the fish backwards and forwards in the recovery bath, or by *gently* passing clean, well-aerated, drug-free water through the buccal cavity with a narrow hose. Once recovery is advanced sufficiently for the fish to ventilate spontaneously, the process could be left to be completed unattended. During this phase, ventilation can often be very deep with powerful, regular movements of the opercula.

Direct application to the gills

Some workers have used sprays or atomiser bottles to apply drugs directly to the gills in larger fish. This can be useful with large animals where immersion is impractical. The earliest accounts of this were by Gilbert and Wood (1957) who anaesthetised large sharks by holding back the head with a gaff or rope and spraying the gills directly with 1,000 mg L^{-1} MS222. They suggested that a water pistol, syringe pump or spray bottle could be used but good quality, small (say, 1 litre) plastic spray bottles are now inexpensive and widely available. The sharks were maintained in anaesthesia by further spray applications of MS222 or the procedure could be shortened by spraying with drug-free water. Kaneko (1982) describes problems in handling large specimens of air-breathing *Arapaima* and *Lepisosteus* which were incompletely anaesthetised by immersion in 10% quinaldine. He successfully used direct

sprays to apply 200 mg L^{-1} MS222 or 20% fluothane onto the gills. Kidd and Banks (1990) described the use of this method for stripping broodstock cut-throat trout, *Salvelinus namaycush*. They note that the initial handling was possible without drug immersion and that there was no effect on subsequent egg hatching success. Indeed, this approach may be a useful way to isolate eggs from any possible harmful effects of anaesthetic drugs.

Artificially ventilated inhalation anaesthesia

In more complex work, where ventilatory arrest is unavoidable, a system of artificial ventilation should always be used. Numerous descriptions of appropriate systems for ventilated anaesthesia exist in the literature (Bell, 1964; Brown, 1987; Ross, 2001). All that is required is a supply of aerated anaesthetic solution at the correct temperature delivered from a pipe or mouthpiece into the buccal cavity, a collection system for the effluent anaesthetic and preferably a recycling system for used solution. The anaesthetic should be aerated to ensure near-saturation of oxygen and to remove dissolved CO_2. It should also be maintained at the correct temperature using cooling or heating, as required (Figs. 8.5 and 8.6).

The fish is first sedated in a container of appropriate anaesthetic solution, as described in the basic procedure above. The fish is then transferred to a suitable fish holder on an apparatus containing the maintenance solution, which will usually be at a lower concentration than that used for induction. The anaesthetic solution is introduced into the buccal cavity from a soft plastic or rubber tube. If available, silicon rubber is best as it is softer. A useful fish holder can be made by slitting a block of foam plastic half-way through (Fig. 8.7). When this block is opened, it will grip the animal lightly while simultaneously allowing water or anaesthetic solution to flow across the gills. It is most convenient to cover the fish holder with some soft, wettable, disposable material that does not disintegrate readily. Similarly wetted operation cloths may be desirable, for shielding the fish from the heat of lights, soldering equipment, etc. For management of anaesthesia it is also helpful to use a routine ECG monitor; for example, a physiological pre-amplifier and oscilloscope or else a much simpler bleeper triggered by the QRS complex (Ross, and Wiewiorka, 1977), to track progress of the subject. It is easy to detect a simple heart rate signal by using clip electrodes attached to the fins. Dziaman *et al.* (2005) have shown small differences in the electrocardiogram during MS222 anaesthesia of carp

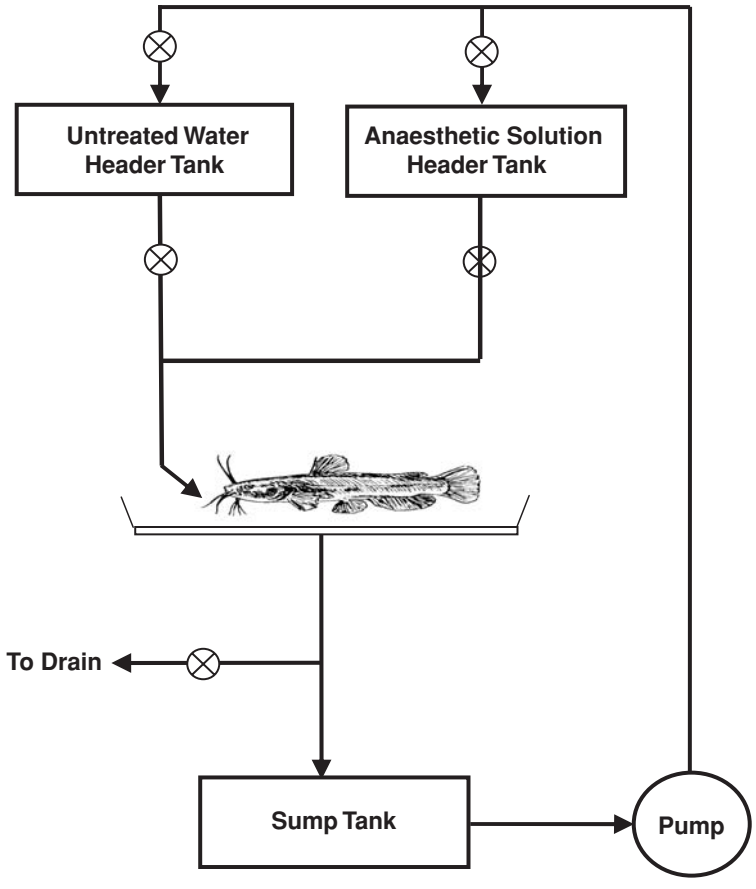


Figure 8.5 Schematic diagram of simple ventilated anaesthesia system.

(*Cyprinus carpio*) depending on the dose rate. They proposed ECG monitoring as an objective method to monitor anaesthesia.

Depending on the drugs used and assuming a good environmental control, fish can be held in this state for several hours. Clearly, in this situation the two prime complicating factors are body temperature maintenance and prevention of excessive drying of the skin. The former is usually alleviated by adequate control of the water temperature using heating and/or cooling equipment, and the latter by spraying the skin surface with water at regular intervals from a wash bottle or, better still, a small portable spray bottle.

To terminate anaesthesia, the drug supply is stopped and clean, drug-free water is passed over the gills until spontaneous ventilation returns. At the first appearance of locomotor activity, the fish may be returned to the stock tank. Those wishing to anaesthetise fish of any value should

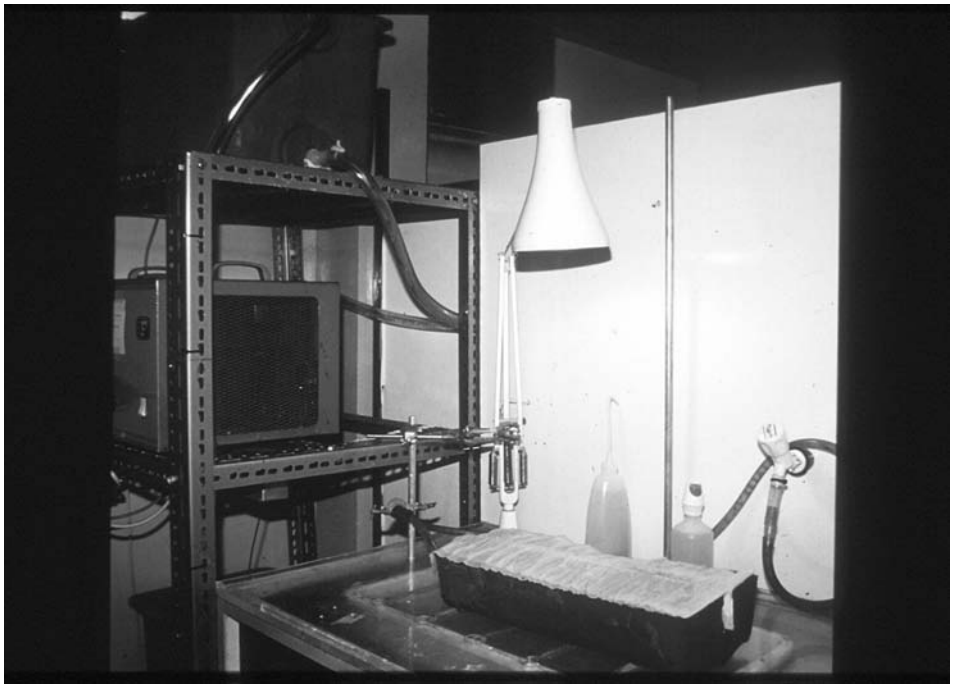


Figure 8.6 Ventilated anaesthesia system used in the author's laboratory. Note the cooler unit and header tank at the left, the anaesthetic delivery tube and the foam block fish holder.

always anticipate ventilatory and/or cardiac arrest and its ensuing complications. Again, it is helpful to remember the ventilatory/cardiac reflex in this context; by simply irrigating the buccal cavity continuously with water, heart rate can be restored and stabilised and excretion of drugs and metabolites back across the gills can be considerably speeded up.

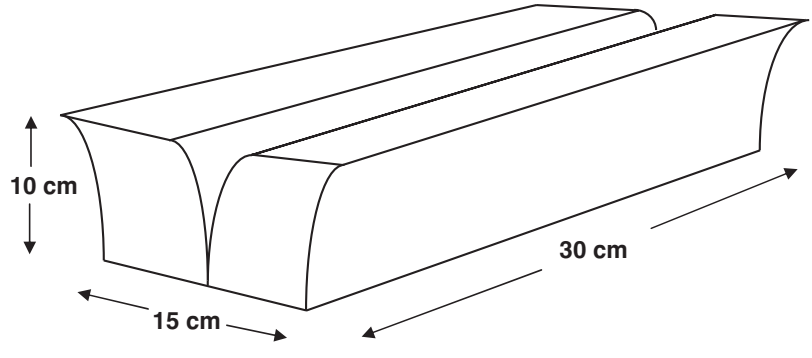


Figure 8.7 A simple fish holder made from a foam plastic block, cut part-way through using a craft knife. Sizes will vary according to fish size. In use the holder is covered with sheets of disposable tissue.

Drugs used for inhalation anaesthesia

An extensive range of drugs has been used in fish anaesthesia. In the sections that follow, a full range of synonyms and trade names has been provided in each case to aid identification. The name(s) in most common use are highlighted in italic. In some cases the drugs are available in more than one form, usually as a sodium salt or hydrochloride either of which is water soluble. These soluble forms sometimes have different names and trade names to the less soluble ones and may be more expensive. Data on these alternatives has been incorporated wherever possible. Full IUPAC chemical nomenclature is provided for each material, as well as the molecular formulae.

Most drugs have potential harmful effects if mishandled and basic safety information has been provided where possible. It should be noted that the harmful effects indicated will only result from mishandling of the bulk compounds or extreme overexposure; harmful effects are very unlikely in normal use.

Widely used drugs for inhalation anaesthesia

There is a relatively small range of drugs that are used most commonly. The following text gives a detailed description of these drugs. Dose rates of these drugs for a range of different fish species are summarised in each section. The examples illustrate the range of doses useable or the variation which may be encountered.

MS222

This compound was first developed by Sandoz in a search for cocaine substitutes. It was initially used as a local anaesthetic, but its effectiveness with ectotherms was recognised and it has since been thoroughly investigated with many species. The base molecule, ethyl 3-aminobenzoate, is a white, crystalline powder which keeps well when dry. The more soluble form, the widely used product MS222, has a sulphonated side-chain which makes it acidic, but as a consequence it is 250 times more soluble in water than tricaine or benzocaine. It can be dissolved in fresh-water and sea-water up to 11% w/v. Although it can be dispensed as a powder, stock solutions may be prepared, usually 10 $g\,L^{-1}$, and these are stable if kept in a dark, stoppered container. Solutions can remain effective for up to 3 months if kept in the dark and cool, but eventually they will develop colour and will slowly lose their potency.

The working solution produced may reduce pH by 0.5–1.0 and has been described as being irritant to fish. In fact, reduction of pH by this amount should have minimal effects on most fish. Palmer and Mensinger (2004) showed that neural activity in the lateral line of toadfish (*Opsanus tau*) was relatively unaffected by lowering the pH alone, while MS222 anaesthesia caused a reduction in spontaneous firing rate. To minimise or prevent this effect, the stock solutions or the working solution can be buffered using sodium bicarbonate or Tris-buffer, usually to pH 7.0–7.5. Smit *et al*. (1977) recommend that distilled or deionised water not be used since neither possesses any buffering capacity.

While MS222 is normally administered by inhalation in a suitable container, Kidd and Banks (1990) reported its successful application directly to the gill of adult lake trout (*Salvelinus namaycush*) using a spray. They note that this technique avoids inadvertent exposure of eggs to the anaesthetic.

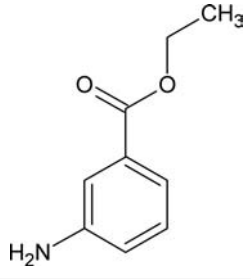
A formidable list of physiological consequences of use of MS222 have been documented, including

Synonyms (non-sulphonate)
Ethyl 3-aminobenzoate Tricaine
m-Ethoxycarbonylaniline
3-(Ethoxycarbonyl)aniline
Ethyl-*m*-aminobenzoate
m-Aminobenzoic acid, ethyl ester
Benzoic acid, 3-amino-, ethyl ester
3-Aminobenzoic acid ethyl ester

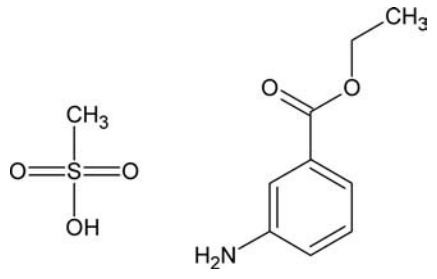
*** Synonyms (methane sulphonate)**
*Finquel
*Metacaine
****MS-222***
*Tricaine
*Tricaine methanesulphonate
*Tricaine methane sulphonate
*2-Methyl 4-sulphonyl aminobenzoate
*Ethyl *m*-aminobenzoate Methanesulphonate

IUPAC name
Ethyl 3-aminobenzoate
*(3-Ethoxycarbonylphenyl) Ammonium; methanesulphonate
Molecular formula
$C_9H_{11}NO_2$
*$C_{10}H_{15}NO_5S$
Structural formula
$H_2NC_6H_4COOC_2H_5$
*$H_2NC_6H_4COOC_2H_5 . CH_3SO_3H$

Structure
Tricaine



Structure
***methane sulphonate derivative**



elevated haematocrit, erythrocyte swelling, hypoxia, hypercapnia, hyperglycaemia, changes in blood electrolytes, hormones, cholesterol, urea, lactate and inter-renal ascorbic acid. It should be borne in mind that all anaesthetics will induce some physiological changes and that handling alone can cause many internal physiological changes.

Induction is rapid and can take as little as 15 seconds. In salmonids it is quickly effective by immersion at about 50 mg L^{-1} although maintenance levels can then be as low as 10 mg L^{-1} (Laird and Oswald, 1975). A dose of up to 100 mg L^{-1} may be required for tilapias and *Clarias* sp. (Ross and Geddes, 1979). Roubach *et al*. (2001) used doses of up to 300 mg L^{-1} to anaesthetise juvenile *Brycon cephalus*, with anaesthesia becoming more rapid as dose increased. They found that at least 150 mg L^{-1} was required for successful anaesthesia of this species. Recovery times are usually rapid and equilibrium and motor activity can be expected to return after only a few minutes.

MS222 has a good safety margin to fish. In trout, Bové (1962) noted that the 30-minute LC_{50} is 82 mg L^{-1} and the maximum concentration tolerated is a little lower at 63 mg L^{-1}. The effective concentration (at which anaesthesia is produced in 99% of fish in 3–4 min) is 40 mg L^{-1} and so the therapeutic index is then $63/40 = 1.57$. This narrows, however, as temperature rises and appears to be less in smaller fish. The drug is more potent in warm waters with low hardness.

Safety & Toxicology Advice:

- Harmful if swallowed, inhaled or absorbed through the skin.
- Irritating to eyes, skin and respiratory tract.

MS222 is not currently known to be toxic to humans at the concentrations used although users should adopt standard precautions including wearing gloves and goggles and washing off any spills onto skin immediately.

MS222 is excreted via the urine within 24 hours and tissue levels decline to almost zero in the same time. The withdrawal time required by the FDA for animals in the food chain is 21 days. A range of effective dose rates is shown in Table 8.1.

Table 8.1 Summary of dose rates for MS222.

Species	Dose	Author
Anguilla rostrata	100–230 mg L^{-1}	Prieto *et al.* (1976)
Brycon cephalus	150 mg L^{-1}	Roubach *et al.* (2001)
Centropristis striata	70 mg L^{-1}	King *et al.* (2005)
Ctenopharyngodon idella	75 mg L^{-1}	Schramm and Black (1984)
Cyprinus carpio	20–85 mg L^{-1}	Takeda *et al.* (1987)
	100 mg L^{-1}	Houston *et al.* (1973)
carp fry	250–350 mg L^{-1}	Jain (1987)
Dicentrarchus labrax	70 mg L^{-1}	Chatain and Corraoa (1992)
Etheostoma fonticola	60 mg L^{-1}	Brandt *et al.* (1993)
Gadus morhua	75 mg L^{-1}	Mattson and Riple (1989)
Hippoglosus hippoglosus	250 mg L^{-1}	Malmstroem *et al.* (1993)
Morone saxatilis	110–123 mg L^{-1}	Henderson-Arzapalo *et al.* (1992)
	150 mg L^{-1}	Lemm (1993)
Mugil cephalus	20–120 mg L^{-1}	Sylvester (1975)
	75–100 mg L^{-1}	Dick (1975)
Onchorhynchus mykiss	100 mg L^{-1}	Soivio *et al.* (1977)
	80 mg L^{-1}	Wedemeyer (1969)
Oncorhynchus sp.	50 mg L^{-1}	Strange and Schreck (1978)
Oncorhynchus tschawytscha	100 mg L^{-1}	Hill and Forster (2004)
Pagrus auratus	60–100 mg L^{-1}	Ryan (1992)
Pagrus major	50–100 mg L^{-1}	Ishioka (1984)
Perca fluviatilis	50–70 mg L^{-1}	Jacquemond (2004)
Polyodon spathula	66 mg L^{-1}	Ottinger *et al.* (1992)
Salmo salar	100 mg L^{-1}	Soivio *et al.* (1974)
Salvelinus alpinus	100 mg L^{-1}	Bystriansky *et al.* (2006)
Salvelinus fontinalis	100 mg L^{-1}	Houston *et al.* (1971)
Sciaenops ocellatus	55 mg L^{-1}	Massee *et al.* (1995)
Sparus aurata	70 mg L^{-1}	Chatain and Corraoa (1992)
Tilapia adults	100–200 mg L^{-1}	Ross and Geddes (1979)
tilapia fry	60–70 mg L^{-1}	
Tinca tinca	25–200 mg L^{-1}	Randall (1962)
Xiphophorus maculatus	30 mg L^{-1}	Guo *et al.* (1995)

Benzocaine

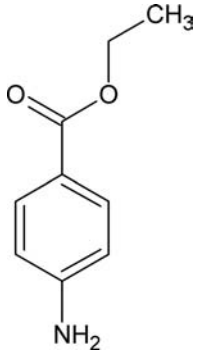
Benzocaine is chemically very similar to MS222. It is a white, odourless, tasteless crystalline ester of *p*-amino benzoic acid and ethanol, and as it does not have the MS222 sulphonyl side-group, it is almost totally insoluble in water (only 0.04% w/v) and must first be dissolved in either acetone or ethanol.

The standard approach is to prepare a stock solution of the solvent, usually 100 g L^{-1}, which, if kept in a dark, stoppered bottle, will stay for long periods (at least a year). Note that this stock solution is 10 times more concentrated than that for MS222 and care should be taken not to confuse the two. It is easier and less wasteful to dispense small volumes

Synonyms:
Americaine
Anaesthesin
Anesthesin
Anesthesine
Benzocaine
Parathesin
Dermoplast
Hurricaine
Identhesin
Parathesine
Ethyl-*p*-aminobenzoate,
4-Aminobenzoic acid ethyl ester

IUPAC name
Ethyl 4-aminobenzoate
Molecular formula
$C_9H_{11}NO_2$
Structural formula
$H_2NC_6H_4COOC_2H_5$

Structure



of this stock than to weigh out very small quantities of solids. Benzocaine hydrochloride is more soluble in water, but is more costly and forms an acidic solution. In solution, benzocaine is neutral, and probably because of this causes less hyperactivity and initial stressful reaction than MS222. However, it should be noted that buffered MS222 is almost identical to benzocaine, both chemically and in terms of physiological reactions. Although many of the side effects of MS222 are still present with benzocaine anaesthesia, the long-term consequences of these side effects do not seem to impair function. Regular anaesthesia of fish in trials at Stirling has not shown any decrement of growth or reproductive capacity.

Benzocaine is effective at approximately the same doses as MS222, generally between 25 and 50 mg L^{-1}, and is useful in freshwater, marine and tropical species, again at higher doses in the latter. A range of effective dose rates is shown in Table 8.2. Gilderhus (1989a, 1989b) induced anaesthesia in *Oncorhynchus tshawytscha*, *O. mykiss* and *Salmo salar* in 3.5 minutes at 25–45 mg L^{-1}, with recovery after 10 minutes following a 15-minute exposure. Salmonids generally require about 40 mg L^{-1} (Laird and Oswald, 1975) and tilapias about 100 mg L^{-1} (Ross and Geddes, 1979). Ortuño *et al*. (2002a) noted that benzocaine depressed complement activity in *Sparus auratus*, resulting in possible immunodepression.

Table 8.2 Summary of dose rates for benzocaine.

Species	Dose	Author
Brycon cephalus	60 mg L^{-1}	Inoue *et al.* (2002)
Clarias batrachus	100 mg L^{-1} (+)	Ross and Geddes (1979)
Colossoma macropomum	100–150 mg L^{-1}	Gomes *et al.* (2001)
Esox lucius	100–200 mg L^{-1}	Webster (1983)
Gadus morhua	40 mg L^{-1}	Ross and Ross (1984)
	40 mg L^{-1}	Mattson and Riple (1989)
Morone saxatilis	55–80 mg L^{-1}	Gilderhus *et al.* (1991)
	55–100 mg L^{-1}	Lemm (1993)
Oncorhynchus mykiss	30–50 mg L^{-1}	Oswald (1978)
Oncorhynchus tshawytscha	25–30 mg L^{-1}	Gilderhus (1990)
Pollachius virens	40 mg L^{-1}	Ross and Ross (1984)
Prochilodus lineatus	100–200 mg L^{-1}	Parma-de-Croux (1990)
Salmo salar (smolts)	40 mg L^{-1}	Ross and Ross (1984)
Salmo trutta	40 mg L^{-1}	Oswald (1978)
Sparus auratus	37 mg L^{-1}	Bressler and Ron (2004)
Sparus sarba	50 mg L^{-1}	Hseu *et al.* (1998)
Tilapia sp.	100 mg L^{-1}	Ross and Geddes (1979)

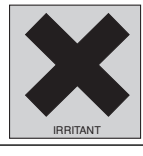
Sorum and Damsgard (2004) considered that benzocaine anaesthesia had only a minor and insignificant effect on feed intake and growth in Atlantic salmon.

Benzocaine has a good margin of safety although this appears to reduce at higher temperatures. Its efficacy is not affected by water hardness or pH. As with MS222, it is fat soluble and recovery times can be prolonged in older or gravid animals, or following long exposure.

Benzocaine is not toxic to humans at the concentrations used and is used widely in many proprietary medical preparations for human use.

Safety and Toxicology advice:

- Harmful if swallowed.
- Irritating to eyes, skin and respiratory tract.
- Use safety glasses



Allen (1988) showed that, following exposure to 50 mg L^{-1} benzocaine for 15 minutes, tissue levels in rainbow trout, *Oncorhynchus mykiss*, and largemouth bass, *Micropterus salmoides*, were 14 and 10 μg g^{-1}, respectively. Levels fell to less than the control values after 8 hours in *M. salmoides* and after only 4 hours in *O. mykiss*, when held in running water. Generally, tissue levels of the drug fall to undetectable levels within approximately 24 hours and fish meal prepared from previously anaesthetised animals contained approximately 45 μg g^{-1} benzocaine on a dry weight basis. However, the withdrawal time required by the FDA is, again, 21 days.

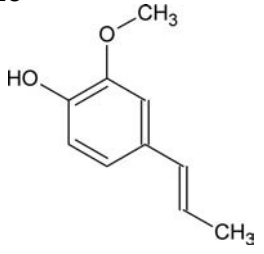
Howe *et al*. (1990) described practical techniques for absorption of benzocaine from farm effluents using activated carbon filtration. This approach has not been widely explored but may be a consideration in obtaining approval for all farm drugs in the future.

Clove oil

Synonyms
(of the principal active ingredient)
Allylguaiacol
Caryophyllic acid
Eugenol
Engenol
p-Allylguaiacol
Eugenic acid
p-Eugenol
4-Allylguaiacol
4-Allyl-2-methoxyphenol

IUPAC name
2-Methoxy-4-prop-2-enyl-phenol
Molecular formula
$C_{10}H_{12}O_2$

Structure



Clove oil is distilled from the flowers, stalks and leaves of *Syzygium aromaticum* (i.e. *Eugenia aromaticum*) or *Eugenia caryophyllata.* It is a dark brown liquid with a rich, aromatic odour and flavour. It has been used as a mild topical anaesthetic since antiquity and has been used to help with toothache, headaches and joint pains. Its effectiveness as a mild anaesthetic in dentistry is well known. The major constituent (70–90% by weight) is the oil eugenol, but raw clove oil also contains acetyl eugenol and a very wide range of turpenoid compounds, which impart their characteristic odour and flavour. While effective, controlled anaesthesia can be achieved with crude clove oil, one of its disadvantages is that it is not recommended for harvesting due to the flavour problems it may cause. However, more purified products may not have this problem. Clove is easily dispersible in water at higher temperatures by vigorous shaking but at lower temperatures it can be prepared as a 10% solution in ethanol. A 10 cm^3 L^{-1} ($\approx$ 10 g L^{-1}) stock solution has been found to be still effective after 3 months' storage at room temperature.

There are many examples of its use as an animal anaesthetic both in invertebrates and vertebrates. There has been a recent upsurge in interest in use of clove oil as a fish anaesthetic and well over

40 articles have been published on this topic since 1995. Endo *et al*. (1972) showed that it is effective in Crucian carp, *Carassius carassius*, and Hisake *et al.* (1986) showed that it gave effective anaesthesia in common carp (*Cyprinus carpio*) at 25–100 ppm. Soto (1995) found doses of 100 mg L^{-1} to be effective in the rabbitfish, *Siganus lineatus.* The fish lost equilibrium after 30–45 seconds and recovery required about 3 minutes. Tamaru *et al.* (1996) found that 25 ppm was sufficient to enable handling in *Siganus argenteus*. In a detailed series of experiments with juvenile (20 g) rainbow trout (*Oncorhynchus mykiss*), Keene *et al*. (1998) showed the 8–96 hours LC_{50} of eugenol to be about 9 ppm. Doses as low as 2–5 ppm produced sedation sufficient for transportation work, while doses of 40–60 ppm for 3–6 minutes gave effective surgical anaesthesia. In all cases recovery was dose- and time-related, increasing exponentially with exposure time. With short exposure times, recovery was uneventful but was always more lengthy than with MS222. Repeated anaesthesia had no detrimental effects and feeding resumed rapidly with no apparent growth inhibition. By contrast, Pirhonen and Schreck (2003) found that although rainbow trout fed well 4 hours after anaesthesia, clove oil reduced feed intake when compared to unanaesthetised controls, but this reduction was similar to that induced by MS222 anaesthesia. Hoskonen and Pirhonen (2006) found that repeated anaesthesia of rainbow trout using clove oil had a significant negative effect on growth. A range of effective dose rates is shown in Table 8.3.

Detar and Matingly (2005) found that induction rates in *Phoxinus erythrogaster* were dependent on the interaction between dose rate and temperature while recovery rate was principally dependent on temperature.

Small (2000) showed that cortisol levels in channel catfish treated with metomidate and clove oil remained at baseline levels during 30 mintues of anaesthesia and were eight times less than those during MS222 anaesthesia. Wagner *et al.* (2003) found that clove oil anaesthesia elevated plasma cortisol in rainbow trout less than MS222 anaesthesia and that, based on blood parameters it is more effective at reducing short-term handling stress. Bressler and Ron (2004) found that clove oil did not exert any immunodepressive effect on the sea bream immune system and suggested that clove oil is the preferred anaesthetic for sea bream aquaculture and research.

Kiessling *et al.* (2004) evaluated the effects of pre-slaughter anaesthesia using iso-eugenol or carbon dioxide on flesh quality in Atlantic salmon. They found no differences in fillet gaping between the two anaesthetic protocols, but anaesthesia using iso-eugenol resulted in firmer, paler flesh with a higher water-holding capacity than salmon exposed to CO_2 anaesthesia.

Table 8.3 Summary of dose rates for clove oil.

Species	Dose	Author
Anguilla reinhardii	2–120 mg L^{-1}	Walsh and Pease (2002)
Carassius carassius	15.5–100 ppm	Endo *et al.* (1972)
Cyprinuis carpio		
Oryzias latipes		
Oncorhynchus mykiss		
Centropristis striata	15–40 mg L^{-1}	King *et al.* (2005)
Colossoma macropomum	65-–100 mg L^{-1}	Roubach *et al.* (2005)
Cyprinus carpio	25–100 ppm	Hisaka *et al.* (1985)
Dicentrarchus labrax	40 mg L^{-1}	Mylonas *et al.* (2005)
Sparus aurata		
Ictalurus punctatus	100 mg L^{-1}	Waterstrat (1999)
Ictalurus punctatus	100 ppm	Small (2003)
Liza parsia	80 ppm	Sonawane and Kulkarni (2001)
Oncorhynchus tschawytscha	20 ppm	Cho and Heath (2000)
Oncorhyncus mykiss	40–60 ppm	Keene *et al.* (1998)
	30 mg L^{-1}	Prince and Powell (2000)
	25–100 mg L^{-1}	Woolsey *et al.* (2004)
Phoxinus erythrogaster	40–60 mg L^{-1}	Detar and Matingly (2004)
Pomacentrus amboiensis	0.013–0.27 mol L^{-1}	Munday and Wilson (1997)
Salmo gairdneri	30 mg L^{-1}	Prince and Powell (2000)
Metynnis schreitmuelleri	80 mg L^{-1}	Vartak *et al.* (2002)
Xiphophorus helleri		
Trichogaster leeri		
Micropterus dolomieui	60 mg L^{-1}	Peake (1998)
Acipenser fulvescens		
Esox lucius		
Stizostedion vitreum		
Seriola dumerii	40 mg L^{-1}	Garcia-Gomez *et al.* (2002)
Dicentrarchus labrax		
Sparus aurata		
Dentex dentex		
Siganus argenteus	25 ppm	Tamaru *et al.* (1996)
Mugil cephalus		
Chanos chanos		
Siganus lineatus	100 mg L^{-1}	Soto (1995)

Safety and Toxicology advice

- Harmful if swallowed, inhaled or absorbed through the skin.
- Irritating to eyes, skin and respiratory tract.
- Minimise direct contact

Clove oil has been proposed as an alternative anaesthetic for wild capture, handling and transportation in the live reef fish industry replacing the use of sodium cyanide (Erdmann, 1999). Ackerman and Bellwood (2002) found that clove oil captured fewer species of reef fishes than rotenone, with fish

recovering too easily, and they considered that rotenone was the preferred method for quantitative sampling. A major advantage of clove oil is that is it relatively inexpensive, not unpleasant to handle and has few harmful effects for humans under normal conditions. Anderson *et al*. (1997) found that clove oil was as effective as MS222 for anaesthetising juvenile and adult rainbow trout and noted that post-anaesthesia critical swimming speed was not affected. Wagner *et al.* (2003) found similar results but also commented that clove oil is more effective at reducing the short-term stress response induced by handling and blood sampling, and they recommended it as a useful alternative to MS222. Cho and Heath (2000) suggested that clove oil is preferable for field-based research because, currently, no withdrawal period is necessary and it poses no known environmental hazard. Munday and Wilson (1997) noted that clove oil has a long history of use in flavouring food and as a topical medicine, which they considered indicative of its potential safety. In fact, clove oil and eugenol are classified by the US FDA as GRAS (Generally Regarded as Safe) for dentistry and as an additive or flavouring agent in human and animal foods, but it is important to note that these materials are not yet approved as animal drugs or as fish anaesthetics and remain under investigation for this application with a withdrawal time of 21 days.

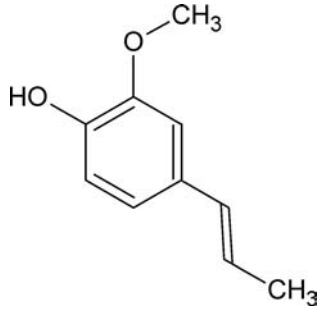
AQUI-S®

The useful features of clove oil prompted the development of a new anaesthetic compound for fish, named AQUI-S®, at the Seafood Research Laboratory in New Zealand. AQUI-S is now commercially available and targeted specifically at 'rested' harvesting for aquaculture as well as transportation for aquaculture and of ornamentals. The active ingredient of the product is isoeugenol, which, although very similar to eugenol, is not present in natural clove oil. It affords a more effective and controlled anaesthesia than the mixture of compounds found in clove oil. In addition to the principal active ingredient, 50% isoeugenol (2-methoxy-4-propenylphenol, the product is also reported to contain polysorbate 80 (sorbitan mono-9-octadecanoate poly (oxy-1,2-ethanediyl) derivatives) which acts as an emulsifier. Polysorbate is widely used in this way in many proprietary pharmaceuticals. Both these materials were in the FDA's GRAS category (i.e. for food use) and required no withdrawal period. However, AQUI-S has recently been placed in the (Investigative New Animal Drug) INAD category.

Synonyms
(of the principal active ingredient)
Isoeugenol
Propenylguaiacol
4-Propenylguaiacol
2-Methoxy-4-propenylphenol phenol
4-Hydroxy-3-methoxypropenylbenzene
1-Hydroxy-2-methoxy-4-propenylbenzene
4-Hydroxy-3-methoxy-1-propenylbenzene

IUPAC name
2-Methoxy-4-prop-1-enyl-phenol
Molecular formula
$C_{10}H_{12}O_2$

Structure



AQUI-S is a clear viscous yellow liquid, which is dispersible in freshwater or seawater to form a stock solution from where it can be dispensed. As a guide, the manufacturers suggest a working concentration of between 15 and 20 mg L^{-1} for most purposes; lighter doses give a sedative effect and higher doses produce a more rapid response. It has a gentle action and fish do not usually show any adverse reaction to its presence. It is particularly useful in harvesting of fish, with slow, stress-free induction in less than 10 minutes. It has been approved for use in New Zealand, Australia and Chile and can be used in those countries for harvesting without any withdrawal period. Systems allowing throughput of 10 tonnes h^{-1} have been developed. The low-stress induction gives improved product quality in terms of colour, texture and external appearance. Although effective, safe to fish and humans and inexpensive, some workers have found that the slow induction achieved at these recommended concentrations is a limitation for some procedures.

Raidal *et al.* (2006) used 10 mg L^{-1} AQUI-S to enable diagnostic imaging in *Cyprinus carpio*, but went on to induce anaesthesia for surgery using MS222. Small and Chatakondi (2005) assessed AQUI-S with channel catfish (*Ictalurus punctatus*) and found that it was equally as effective as MS222, has similar induction times and slightly longer recovery times than MS222 and showed some stress-reducing properties. Davidson *et al.* (2000) examined cortisol levels and other blood parameters in AQUI-S-anaesthetised rainbow trout, with and

Table 8.4 Summary of dose rates for AQUI-S. Note that these dose rates are for the product, AQUI-S; those for the active ingredient will be half of these values.

Species	Dose	Author
Anguilla australis	17–35 ppm	AQUI-S. plc
Cyprinus carpio	10 mg L^{-1}	Raidal *et al.* (2006)
Ictalurus punctatus	20–60 mg L^{-1}	Small and Chatakondi (2005)
Oncorhynchus mykiss	40 mg L^{-1}	Wagner *et al.* (2002)
Oncorhynchus tschawytscha	60 ppm	Hill and Forster (2004)
	120 ppm	Rothwell *et al.* (2005)
Pagrus auratus	120 ppm	Rothwell *et al.* (2005)
Salmonids	17 ppm	AQUI-S Plc
Stizostedeon vitreum vitreum	Up to 20 mg L^{-1}	Stehly and Gingerich (1999)
Perca flavescens	20–35 mg L^{-1}	
Oncorhynchus mykiss	25 mg L^{-1}	
Ictalurus punctatus		
Salvelinus namaycush		
Lepomis macrochirus		

without crowding, and found that AQUI-S did not alleviate stress. Hill and Forster (2005) found that anaesthesia of Chinook salmon using 60-ppm AQUI-S produced a biphasic cardiac response with a 20–50% depression of heart rate, cardiac output and dorsal aortic blood pressure.

Wagner *et al*. (2002) found that recovery time following AQUI-S anaesthesia of rainbow trout was about twice that required following MS222 or CO_2 anaesthesia, and considered that this was an advantage.

AQUI-S has a wide safety margin at low concentrations. Stehly and Gingerich estimated the toxic concentration for several freshwater species to be 2.5 times the effective concentration. A range of effective dose rates is shown in Table 8.4.

Safety and Toxicology advice:

- Harmful if swallowed, inhaled or absorbed through the skin.
- Irritating to eyes, skin and respiratory tract.
- Minimise direct contact

Quinaldine and quinaldine sulphate

Quinaldine is a yellowish-brown oily liquid with limited water solubility and it must first be dissolved in acetone or alcohol before mixing with water. Solutions in water may turn red-brown after air exposure. While it is an effective anaesthetic, it is an unpleasant material with which to work, being irritant to the eyes and skin, insoluble, and corneal damage has been reported following its use with salmonids (R.H. Richards,

personal communication). The low cost of quinaldine has made it a popular choice for collection of fishes from tidal pools and small lagoons.

Quinaldine sulphate is a water-soluble powder, and although it was formerly not easily available commercially (Blasiola, 1976) it is now marketed by a number of companies. Allen and Sills (1973) described the methods for its synthesis in the laboratory. It is a pale yellow to white, crystalline material with little odour and which is easily soluble in water. It is more pleasant to handle than quinaldine but is more costly than both quinaldine and MS222.

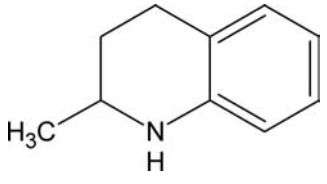
Quinaldine solutions are acidic and are usually buffered with bicarbonate. Stock solutions, usually of 10 g L^{-1}, can be stored in dark, stoppered bottles.

Induction takes 1–4 minutes and recovery is usually rapid and uneventful. Drug susceptibility varies, but quinaldine sulphate induction solutions are in the range 15–60 ppm. Schoettger and Steucke (1970) noted that some reflex responsiveness was retained during surgical anaesthesia in rainbow trout and Schramm and Black (1984) found that quinaldine induction solutions were unsuitable for maintenance during surgery at 29°C in the

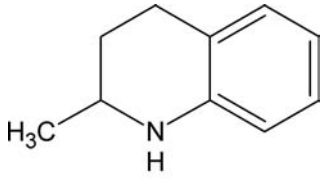
Synonyms
Chinaldine
Khinaldin
Quinaldin
Quinaldine
2-Methylquiniline
Quinoline, 2-methyl-
2-Methylchinolin (Czech)
and
Quinaldine sulphate
2-Methylquinoline sulphate (1:1)
Quinoline, 2-methyl-, sulphate (1:1)

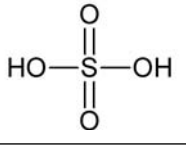
IUPAC name
2-Methylquinoline
Molecular formula
$C_{10}H_9N$
$C_{10}H_9NO_4S^{2-}$
Structural formula
$C_6H_4N{:}C(CH_3)CH{:}CH$

Structure
Quinaldine



Quinaldine sulphate





grass carp, *Ctenopharyngodon idella*. Chellappa *et al.* (1996) found that the relatively high dose of 150 ppm quinaldine was effective in facilitating handling of 7.5–10 kg *Colossoma macropomum* during spawning.

Kumlu and Yanar (1999) anaesthetised juvenile *Sparus aurata* using quinaldine combined with a low dose of diazepam. They found that light anaesthesia was achieved with a 5 ppm quinaldine + 1 ppm diazepam combination, while 10 ppm quinaldine sulphate was required to produce the same effect when used alone. Deep anaesthesia was achieved with a 7.5 ppm quinaldine + 1 ppm diazepam combination, in comparison to 15 ppm quinaldine sulphate when used alone. They found that the addition of diazepam reduced the required quinaldine dose, eliminated any hyperactivity and caused no mortalities.

Cuesta *et al.* (2004) showed that quinaldine sulphate anaesthesia significantly increased immunoglobulin-M levels in *Sparus auratus.* Davis and Griffin (2004) found that exposure to quinaldine increased plasma glucose above control levels.

In general, the potency of quinaldine is less in soft water and more in warm water. Quinaldine is ineffective at pH 5 and below and is more potent at higher pH. A range of effective dose rates is shown in Table 8.5.

Although irritant and having an unpleasant odour, quinaldine is not known to be carcinogenic. Tissue levels of the drug fall to undetectable limits within 24h.

Safety and Toxicology advice

- Harmful if absorbed through the skin or if swallowed.
- May cause burns on skin or eye contact.
- Use safety glasses and adequate ventilation.

2-Phenoxyethanol

This is a clear, colourless or straw-coloured oily liquid with a slight aromatic odour which fairly easily passes into solution if shaken with a small quantity of water. The solution has been used as a topical anaesthetic and is bactericidal and fungicidal and because of this additional feature it is useful during laparotomy or abdominal surgery. It has no great advantages over other drugs, but is relatively inexpensive.

The drug remains effective in working solution for at least 3 days.

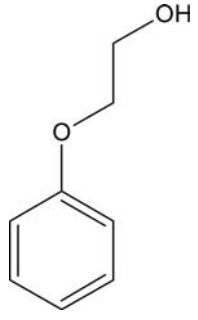
Doses of 0.5 $cm^3\ L^{-1}$ (equal to 385 $mg\ L^{-1}$) produce surgical anaesthesia in rainbow trout, *Oncorhynchus mykiss*, and sedation can be produced at a lower dose although analgesia is sometimes incomplete. Barton

and Helfrich (1981) considered that juvenile salmonids should not be exposed to this dose level, about half the $LD_{50,}$ for more than 13 minutes and that a general dose level of 0.25 $cm^3\,L^{-1}$ was preferable. Takashima *et al*. (1983) showed that yearling rainbow trout of 100–200 g body weight could be held for long periods at 200 ppm. They also showed that serum cortisol levels increased rapidly after immersion of the fish in the drug and concluded that stress was not alleviated in 2-phenoxyethanol anaesthesia. Sehdev *et al*. (1963) found that, at 11°C, the effective dose and lethal dose were 0.09 and 0.29 $cm^3\,L^{-1}$, respectively, in sockeye salmon, *Onchorhynchus nerka*, giving a therapeutic index of more than 3. The potency of the drug increased at 4°C, giving an increased therapeutic index of 5.

Synonyms
Arosol
Dowanol EP
Fenyl-cellosolve
Phenoxyethanol
Phenoxethol
Phenoxetol
Phenoxytol
Phenyl cellosolve
Rose ether
1-Hydroxy-2-phenoxyethane
2-Phenoxyethanol

IUPAC name
2-Phenoxyethanol
Molecular formula
$C_8H_{10}O_2$
Structural formula
$C_6H_5OCH_2CH_2OH$

Structure



McCarter (1992) achieved sedation of broodstock silver carp, *Hypophthalmichthys molitrix*, and grass carp, *Ctenopharyngodon idella*, in a 0.2 $cm^3\,L^{-1}$ solution and this dose did not impair sperm motility. Yamamitsu and Itazawa (1988) induced deep sedation and deep anaesthesia in adult *Cyprinus carpio* at 400 and 800 ppm, respectively, and Josa *et al.* (1992) showed that a range of effects from light sedation to surgical anaesthesia could be induced in carp at doses between 100 and 600 $cm^3\,L^{-1}$. A range of effective dose rates is shown in Table 8.6.

2-Phenoxyethanol caused marked reduction in dorsal aortic blood pressure in rainbow trout (Fredricks *et al.*, 1993). It does not suppress

Table 8.5 Summary of dose rates for quinaldine and quinaldine sulphate.

Species	Dose	Author
Quinaldine		
Anguilla rostrata	8–25 ppm	Hinton and Loyacano (1978)
Blennius pholis	2.5–20 ppm	Dixon and Milton (1978)
Chrysophrys major	20 ppm	Furuichi and Yone (1972)
Clarius gariepinus	6 ppm	Hocutt (1989)
Colossoma macropomum	100–50 ppm	Chellappa *et al.* (1996)
Ctenpharygodon idella	10–50 ppm	Schramm and Black (1984)
Cyprinus carpio	12.5–50 ppm	Osanz-Castan *et al.* (1993)
Tinca tinca		
Epalzeorhynchus bicolor	2–4 ppm	Meenakarn and Laohavisuti (1993)
Ictalurus punctatus	30 ppm	Small (2003)
Large freshwater spp	100–50 ppm	Kaneko (1982)
Lya dussumeri	100 ppm	Sylvester (1975)
Morone saxatilis	25–40 mg L^{-1}	Lemm (1993)
Pomoxis nigromacyulatrus	22 mg L^{-1}	Smeltzer and Flickinger (1991)
Sparus sarba	9 ppm	Hseu *et al.* (1998)
Trichogaster trichopterus	5 mg L^{-1}	Crosby *et al.* (2006)
Tropical marines	200 ppm	Blasiola (1976)
Various spp.	10–30 ppm	Randall and Hoar (1971)
	10–30 ppm	Tytler and Hawkins (1981)
Quinaldine sulphate		
Carassius auratus	60 mg L^{-1}	Massee *et al.* (1995)
Cyprinus carpio	50 ppm	Hettiarachchi and Senadheers (1999)
Sciaenops ocellatus	35 mg L^{-1}	Massee *et al.* (1995)
Sparus aurata	5–7.5 ppm + 1 ppm diazepam	Kumlu and Yanar (1999)
Xiphophorus maculatus	10 mg L^{-1}	Guo *et al* (1995)

involuntary muscle reflexes and recovery is abrupt on occasions (R.L. Oswald, personal communication).

Imamura-Kojima *et al*. (1987) showed that 2-phenoxyethanol was distributed principally in the brain, liver, kidney and gall bladder of rainbow trout and that its biological half-life was approximately 30 minutes.

Safety and Toxicology advice

- Harmful if swallowed, inhaled or absorbed through the skin.
- Severe eye and skin irritant.
- In case of contact, rinse immediately with water.

Metomidate

In the time since the first edition of this handbook was published, a substantial amount of work has been carried out on the two

Table 8.6 Summary of dose rates for 2-phenoxyethanol.

Species	Dose	Author
Acanthopagrus schlegeli	400–600 ppm	Hseu *et al.* (1996)
Carassius auratus	0.3–0.5 $cm^3 L^{-1}$	Weyl *et al.* (1996)
Centropristis striata	200–300 $mg L^{-1}$	King *et al.* (2005)
Ctenopharyngodon idella	0.2 $cm^3 L^{-1}$	McCarter (1992)
Cyprinids	0.1–0.5 $cm^3 L^{-1}$	Osanz-Castan *et al.* (1993)
Cyprinus carpio	400–600 ppm	Yamamitsu and Itazawa (1988)
	0.8–1.2 $cm^3 L^{-1}$	Altun and Danabas (2006)
Dicentrarchus labrax	350 $mg L^{-1}$	Mylonas *et al.* (2005)
Diplodus sargus	0.17–0.2 $cm^3 L^{-1}$	Tsantilas *et al.* (2006)
Diplodus puntazzo		
Epalzeorhynchus bicolor	75–200 ppm	Meenakarn and Laohavisuti (1993)
Hypophthalmichthys molitrix	0.2 $cm^3 L^{-1}$	McCarter (1992)
Oncorhynchus mykiss	200 ppm	Takashima *et al* (1983)
	0.5 $cm^3 L^{-1}$	Hilton and Dixon (1982)
Oncorhynchus nerka	0.1–0.5 $cm^3 L^{-1}$	Klontz and Smith (1968)
Oreochromis niloticus	0.5–1.2 $cm^3 L^{-1}$	Auperin *et al*. (1997).
Pomadasys commersonnii	0.2–0.4 $cm^3 L^{-1}$	Radull *et al*. (2001)
Salmonids	0.25 $cm^3 L^{-1}$	Barton and Helfrich (1981)
Sparus auratus	60–200 ppm	Ortuño *et al.* (2002b)
Sparus sarba	400 ppm	Hseu *et al.* (1998)
Tinca tinca	0.1–0.5 $cm^3 L^{-1}$	Osanz-Castan *et al.* (1993)
Thunnus orientalis	0.2 $cm^3 L^{-1}$	Takii *et al* (2005)
Vimba vimba	0.35–0.48 $cm^3 L^{-1}$	Kaminski *et al*. (2001)
Xiphophorus maculatus	220–440 ppm	Guo *et al.* (1995)

imidazole-based non-barbiturate hypnotic drugs metomidate and etomidate, both of which have been used in human and veterinary medicine. Both drugs produce anaesthesia without the accompanying elevation of blood cortisol. This feature was seized upon in the early 1980s and it was thought that these materials could produce stress-free anaesthesia. Kreiberg (1992) noted the relatively small increase in plasma cortisol in Pacific salmon on handling following metomidate anaesthesia. It has since been shown that this effect is achieved by the suppression of parts of biochemical pathways leading to cortisol synthesis. This is clearly undesirable and the drug etomidate has now been withdrawn from use in human medicine for the same reason.

Metomidate is a water-soluble powder that has been explored for use as an anaesthetic by fish biologists. It has been shown to be very effective, induction normally being achieved in 1–2 minutes with no notable hyperactivity and recovery being faster than after MS222 anaesthesia. Matson and Riple (1989) considered that metomidate is a better choice than benzocaine, MS222, chlorbutanol and 2-phenoxyethanol for cod, *Gadus morhua*, because of this shorter recovery time, although it can be accompanied by muscle twitching. It produces anaesthesia in salmonids

at doses of only 5–6 mg L^{-1} and even lower doses are effective in catfish. Marine tropicals and sharks are anaesthetised at 2.5–5 mg L^{-1}. Using metomidate concentrations of 0.5 and 1.0 mg L^{-1}, Ross *et al.* (1993) working with shad, *Alosa sapidissima,* induced sedation after 9 and 3 minutes with mean recovery times of 6 and 7 minutes, respectively. For fish exposed to 1.0 mg L^{-1} metomidate, normal swimming behaviour was delayed for as long as 4 hours after fish were returned to drug-free water and schooling behaviour was also disturbed for up to 24 hours. Olsen *et al*. (1995) induced anaesthesia in Atlantic salmon, *Salmo salar*, at a range of concentrations from 1 to 10 mg L^{-1} and noted a lack of plasma cortisol response which could not be counteracted by injection of ACTH. This indicated blockage of release from the inter-renal cells. The drug was more potent in larger, sea-water adapted fish than in freshwater parr. Knoph (1995) rapidly induced surgical anaesthesia in 150g Atlantic salmon using a relatively high dose of 40 mg L^{-1} metomidate and was then able to maintain this level using a reduced dose of 10 mg L^{-1}.

By contrast with these positive indications, Massee *et al*. (1995) found doses of 3–4.5 mg L^{-1} to be unreliable with groups of larval goldfish, *Carassius auratus*, and red drum, *Sciaenops ocellatus*, giving a low percentage of anaesthetised animals with a high subsequent mortality.

Small (2003) found that doses of 16 ppm produced significant mortalities in *Ictalurus punctatus* but no mortalities

Synonyms
Hypnodil hydrochloride
Marinil
Metomidate
Methoxymol
Metomidat-hydrochloride
Metomidate hydrochloride
R-7315
1-(1-Phenethyl)-imidazole-5-carboxylic acid, methyl ester, hydrochloride
1 *H*-Imidazole-5-carboxylic acid, 1-(1-phenylethyl)-, methyl ester, monohydrochloride (9CI)
Imidazole-5-carboxylic acid, 1-(α-methylbenzyl)-,methyl ester, hydrochloride
dl-1-(1-phenylethyl)-5-(methoxycarbonyl) imidazole hydrochloride

IUPAC name
Methyl 3-(1-phenylethyl) imidazole-4-carboxylate hydrochloride
Molecular formula
$C_{13}H_{15}ClN_2O_2$

Structure

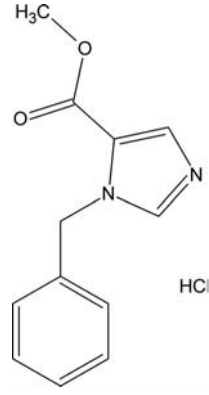


Table 8.7 Summary of dose rates for metomidate.

Species	Dose	Author
Alosa sapidissima	0.5–1 mg L^{-1}	Ross *et al.* (1993)
Centropristis striata	2.0 mg L^{-1}	King *et al.* (2005)
Gadus morhua	5 mg L^{-1}	Mattson and Ripple (1989)
Hippoglosus hippoglosus	10–60 mg L^{-1}	Malmstroem *et al.* (1993)
Ictalurus punctatus	2–8 ppm	Small (2003)
Marine fish	2.5–5 mg L^{-1}	Anon
Morone saxatilis	7.5–10 mg L^{-1}	Lemm (1993)
Oncorhynchus tschawytscha	6–10 ppm	Hill and Forster (2004)
Salmo salar	1–10 mg L^{-1}	Olsen *et al.* (1995)
	10–40 mg L^{-1}	Knoph (1995)
	1–5 mg L^{-1}	Sandodden *et al.* (2001)
Salmonids	5–6 mg L^{-1}	Anon.
Sciaenops ocellatus	7 mg L^{-1}	Thomas and Robertson (1991)
Xiphophorus maculatus	1 mg L^{-1}	Guo *et al.* (1995)

were recorded at 8 ppm. A range of effective dose rates is shown in Table 8.7.

Metomidate has been found to cause drastic reduction in dorsal aortic blood pressure in rainbow trout (Fredricks *et al.*, 1993). Darkening of some species has been reported and this is thought to be due to interference with hormonal control of MSH synthesis. Cortisol synthesis is inhibited during metomidate anaesthesia and Olsen *et al.* (1995) have shown that the cortisol response to handling stress was absent in Atlantic salmon, *Salmo salar,* and that blood lactate and haematocrit also increased.

Safety and Toxicology advice

- Harmful if swallowed or inhaled.
- Use gloves.

Etomidate

This non-barbiturate, imidazole derivative anaesthetic and hypnotic is used intravenously for anaesthesia in humans and laboratory animals. It has little effect on blood gases, ventilation, or the cardiovascular system and is used as an induction anaesthetic. It does not produce analgesia.

Amend *et al.* (1982) noted effective dose rates of 2–4 mg L^{-1} in the aquarium fish *Danio rerio*, *Gymnocorymbus ternetzi*, *Pterophyllum scalare* and *Xiphophorus maculatus* with induction occurring within 90 seconds and recovery within 40 minutes. They suggest that

dose rates up to 20 mg L^{-1} can be tolerated. They found that etomidate was more effective in alkaline water and higher water temperature and that it was not affected by total hardness. Limsuwan *et al*. (1983) induced anaesthesia within 15 minutes using 3 mg L^{-1} etomidate in channel catfish, *Ictalurus punctatus*, and golden shiner, *Notemigonus crysoleucas*, although Plumb *et al.* (1983) note that a working concentration of 0.8–1.2 mg L^{-1} is sufficient. Sedation was obtained at dose rates of 0.2–0.6 mg L^{-1} and continuous anaesthesia could be maintained for 96 hours with 0.6 mg L^{-1} etomidate. They found it to give slower induction and recovery and to be more toxic at lower temperatures. The therapeutic index ranged from 4 to 7 and reduced with exposure time. Falls *et al.* (1988) working with red drum, *Sciaenops ocellatus*, found that at 0.8 mg L^{-1} induction and recovery times were excessively long. They induced anaesthesia at 1.6 mg L^{-1} and were able to maintain anaesthesia at 0.4 mg L^{-1}. A range of effective dose rates is shown in Table 8.8.

Safety and Toxicology advice

- Harmful if swallowed or inhaled.
- Use gloves.

Synonyms:
Amidate
D-Etomidate
(*d*)-Etomidate
(+)-Etomidate
Etomidatum (INN-Latin)
Etomidato (INN-Spanish)
Etomidate
Hypnomidate
Propiscin
Radenarcon
R16659
(+)-Ethyl 1-(α-methylbenzyl)imidazole-5-carboxylate Ethyl 1-(1-phenylethyl)-1 *H*-imidazole-5-carboxylate
R-(+)-Ethyl 1-(1-phenylethyl)-1 *H*-imidazole-5-carboxylate
1-(1-Phenylethyl)-1 *H*-imidazole-5-carboxylic acid ethyl ester
1-(α-Methylbenzyl)-1 *H*-imidazole-5-carboxylic acid ethyl ester
(*R*)-(+)-1-(α-Methylbenzyl)imidazole-5-carboxylic acid ethyl ester

IUPAC name
Ethyl 3-(1-phenylethyl)imidazole-4-carboxylate
Molecular formula
$C_{14}H_{16}N_2O_2$

Structure

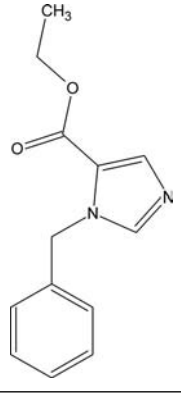


Table 8.8 Summary of dose rates for etomidate.

Species	Dose	Author
Cyprinus carpio	1–2 ml L^{-1}	Hajek and Klyszejko (2004)
Danio rerio	2–20 mg L^{-1}	Amend *et al.* (1982)
Gymnocorymbus ternetzi		
Pterophyllum scalare		
Xiphophorus maculatus		
Ictalurus punctatus	3 mg L^{-1}	Limuswan *et al.* (1983)
	0.8–1.2 mg L^{-1}	Plumb *et al.* (1983)
Morone saxatilis	0.8–1.2 mg L^{-1}	Plumb *et al.* (1983)
Notemigonus crysoleucas	0.8–1.2 mg L^{-1}	Plumb *et al.* (1983)
Oncorhynchus mykiss	2 ml L^{-1}	Kazun and Siwicki (2001)
Sciaenops ocellatus	0.4–1.8 mg l L^{-1}	Falls *et al.* (1988)

Less widely used drugs for inhalation anaesthesia

It is almost certainly the case that the popular drugs already discussed retain their dominance due to the large body of literature in which their use has been described. The following wide range of drugs has been used less frequently. In some cases the drugs are quite effective, but have simply not been widely adopted by fish biologists or veterinarians. In many cases their usefulness is somewhat limited by the lack of knowledge of their precise physiological effects, while others are no longer popular or have unwanted side effects.

4-Styrylpyridine

This is a fine, white powder, easily soluble in water. It is effective in a range of species at 20–50 mg L^{-1} inducing anaesthesia in 1–5 minutes and with a 20–30 minute recovery period (Klontz and Smith, 1969).

Safety and Toxicology advice

- Harmful if swallowed.

Howell and Thomas (1964) tested its efficacy with a range of lampreys and fish species and found that, in general, teleosts and some larval lamprey were more susceptible and larval lamprey were less susceptible to 20 ppm of the drug, while adult sea lamprey (*Petromyzon marinus)* were more resistant. It was effective in the dose range 5–50 ppm and they noted that the upper dose limit of 50 ppm was limited by its solubility in water. Mortality was low. It has also been used for abdominal

Table 8.9 Summary of dose rates for 4-styrylpiridine.

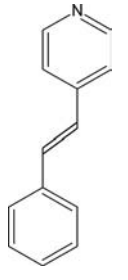
Species	Dose	Author
Ichthyomyzon sp. (larvae) *Lampetra lamottei* (larvae) *Oncorynchus mykiss* *Pimephales promelas* *Lepomis gibbosus* *Perca flavescens* *Catostomus commersoni*	20–50 ppm	Howell and Thomas (1964)
Ichthyomyzon unicupis *Lampetra lamottei* *Petromyzon marinus*	Adults 5–20 ppm	Howell and Thomas (1964)
Salmonids	20–50 mg L^{-1}	Klontz and Smith (1969)

surgery in the lamprey, *Petromyzon marinus,* by Piavis and Piavis (1978). It is considered safe for handling by operators. A range of effective dose rates is shown in Table 8.9.

Synonyms
4-Stilbazole
4-Styrylpyridine
γ-Stilbazole
Pyridine, 4-styryl-
4-(2-Phenylvinyl)pyridine
4-[(E)-2-Phenylethenyl]pyridine
4-(2-Phenylvinyl)pyridine, *trans-*

IUPAC name
4-(2-Phenylethenyl)pyridine
Molecular formula
$C_{13}H_{11}N$

Structure:



Barbiturates

The barbiturates are derivatives of barbituric acid, most of which act as central nervous system depressants probably by potentiating the GABA receptors (inhibitory) and by blocking the glutamate receptor (excitatory). They produce effects ranging from sedation to anaesthesia. Several derivatives are in common human and veterinary clinical use and some of these have proved effective with fish.

Amylobarbitone

Amylobarbitone is used as a veterinary sedative and hypnotic with rapid onset and short duration of action. It is very soluble in water and is effective in salmonids at 7–10 mg L^{-1} (McFarland and Klontz, 1969). Induction requires 30–60 minutes, maintenance is good over long periods but recovery is slow, requiring up to 5 hours. It is antagonised by high calcium levels found in hard fresh-waters and in seawater. It is consequently not of great practical use.

Synonyms

Amobarbital	Isomytal
Amylbarbitone	Mylodorm
Amylobarbitall	Pentymal
Amylobarbitone	Sednotic
Amobarbitone	Amasust
Pentymalum	Amospan
Schiwanox	Sumital
Stadadorm	Amital
Barbamil	Amybal
Barbamyl	Amytal
Binoctal	Robarb
Dorlotyn	Somnal
Dormytal	Talamo
Eunoctal	Barbamyl acid

Isoamylethylbarbituric acid
Ethylisopentylbarbituric acid
Isomytal (TN)
5-Ethyl-5-isoamylmalonyl urea
5-Ethyl-5-isoamylbarbituric acid
5-Isoamyl-5-ethylbarbituric acid
5-Ethyl-5-isopentylbarbituric acid
Barbituric acid, 5-ethyl-5-isopentyl-5-
Ethyl-5-(3-methylbutyl)barbituric acid
Amobarbital sodium for injection
5-Ethyl-5-isopentylbarbitursaeure
Barbituric acid, 5-ethyl-5-isoamyl-
5-ethyl-5-(3-methylbutyl)-2,4,
6(1*H*,3*H*,5*H*)-pyrimidinetrione
2,4,6(1*H*,3*H*,5*H*)-Pyrimidinetrione,
5-ethyl-5-(3-methylbutyl)-5-ethyl-
5-isopentyl-2,4,6(1*H*,3*H*,5*H*)-
pyrimidinetrione
2,4,6 (1*H*,3*H*,5*H*)-Pyrimidinetrione,
5-ethyl-5-(3-methylbutyl)-64–43-7

IUPAC name
5-Ethyl-5-(3-methylbutyl)-1,3-diazinane-2,4,6-trione
Molecular formula
$C_{11}H_{18}N_2O_3$

Quinalbarbitone

Quinalbarbitone is a white powder which is very soluble in water. It is used as a 'short' acting hypnotic and premedicant in human medicine. It is effective as a fish anaesthetic at 35 mg L^{-1}. Induction of anaesthesia is very slow, requiring 30–60 minutes. Maintenance is good over long periods although recovery is very slow. Although an effective fish anaesthetic, McFarland and Klontz (1969) note that it is too slow to be useful in most cases.

Synonyms	**(*as sodium salt):**
Barbosec	*Seconal sodium
Hypotrol	*Dormatylan
Immenoctal	*Immenoctal
Meballymal	*Quinalspan
Quinalbarbital	*Barbosec
Quinalbarbitone	*Evrronal
Secobarbital	*Hypotrol
Secobarbitone	*Imesonal
Seconal	*Sedutain
Thiamylal	*Trisomnin
Trisomnin	

IUPAC names

5-Pentan-2-yl-5-prop-2-enyl-1,3-diazinane-2,4,6-trione

*Sodium 4,6-dioxo-5-pentan-2-yl-5-prop-2-enyl-1 *H*-pyrimidin-2-olate

Chemical formulae

$C_{12}H_{18}N_2O_3$

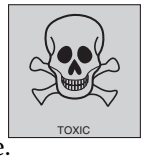
$*C_{12}H_{17}N_2NaO_3$

Pentothal

Pentothal or *Thiopental* is used, sometimes as its sodium salt, intravenously in humans and in veterinary practice for the induction of general anaesthesia or for very short duration anaesthesia. It is also an effective analgesic. In excess, it is used as a lethal injection for executions. Hikasa *et al.* (1986) used 200–300 ppm thiopental sodium in a bath with *Cyprinus carpio.*

Barbiturates are habit-forming drugs and hence are controlled in their distribution and use.

Safety & Toxicology advice



- Toxic if swallowed.
- Irritating to eyes, skin and respiratory tract.
- In case of contact, rinse with wather & seek advice.
- Subject to drug controls in UK, USA and elsewhere.

Synonyms	
thiopental	**(∗as sodium salt)**
Thionembutal	∗Thiopental sodium
Thiomebumal	∗5-Ethyl-5-(1-methylbutyl)-2-thiobarbituric
Intraval	acid sodium salt
Pentothal	∗Barbituric acid,
Pentothiobarbital	5-ethyl-5-(1-methylbutyl)-2-thio-, sodium
Penthiobarbital	salt
Thiopentone	
Thiothal	
Thiopentobarbital	
Thiopentobarbitone	

IUPAC names

5-Ethyl-5-pentan-2-yl-2-sulfanylidene-1,3-diazinane-4,6-dione

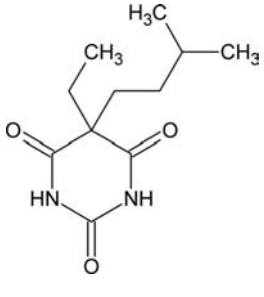
∗Sodium 5-ethyl-6-oxo-5-[(2*S*)-pentan-2-yl]-2-sulfanylidene-pyrimidin-4-olate

Chemical formulae

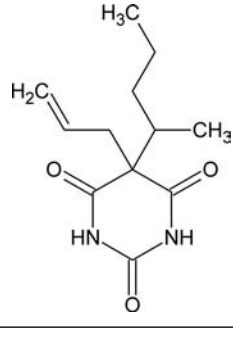
$C_{11}H_{18}N_2O_2S$

$*C_{11}H_{17}N_2NaO_2S$

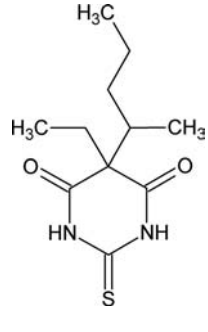
Amylobarbitone structure



Quinalbarbitone structure



Thiopental structure



A compilation of effective dose rates is presented in Table 8.10.

Table 8.10 Summary of dose rates for barbiturates.

Species	Drug	Dose	Author
Fish (salmonids)	Amylobarbitone	7–10 mg L^{-1}	McFarland and Klontz (1969)
Fish (salmonids)	Quinalbarbitone	35 mg L^{-1}	McFarland and Klontz (1969)
Cyprinus carpio	Thiopental	200–300 ppm	Hikasa *et al.* (1986)

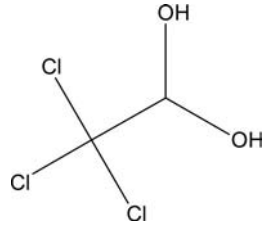
Choral hydrate

This is available as strong-smelling, colourless crystals which are freely soluble in water. It is a good CNS depressant but appears to provide poor analgesia. Surgical anaesthesia is only attained at high doses, at which there may be problems with ventilation or heart action may be arrested. It is effective in fish as a 1% solution, slowly inducing anaesthesia in cyprinids after 25 minutes and in salmonids after 8–10 minutes. Respiratory arrest is likely and it is not recommended to sustain immersion for longer than 10 minutes. Recovery may require from 30 minutes up to 2–3 hours. Its main use has been as a sedative in transportation. A range of effective dose rates is shown in Table 8.11.

Synonyms
Chloraldurat
Chloradorm
Chloral hydrate
Cohidrate
Felsules
Kessodrate
Noctec
Oradrate
Phaldrone
Rectules
Trawotox

IUPAC name
2,2,2-Trichloroethane-1,1-diol
Molecular formula
$C_2H_3Cl_3O_2$

Structure



Safety and Toxicology advice

- Harmful if swallowed.
- Irritating to eyes, skin and respiratory tract.
- Do not inhale.
- Use safety glasses and adequate ventilation

Table 8.11 Summary of dose rates for chloral hydrate.

Species	Dose	Author
Fish	0.8–0.95g L^{-1}	McFarland and Klontz (1969)
Labeo rohita	0.1–0.25g L^{-1}	Sharma and Sharma (1996)
Poecilia reticulata		
Sardina pilchardus sardina	0.2–1.0g L^{-1}	Muzinic (1970)

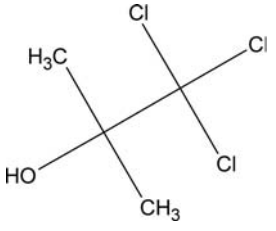
Chlorbutanol

This is available as a colourless to white crystalline compound with a smell and taste similar to camphor. It dissolves easily in hot water but it is slow to dissolve in cold water. 10% aqueous stock solutions remain stable for long periods and it is effective at 8–10 mg L^{-1} (McFarland and Klontz, 1969). Induction is rapid (2–3 min), maintenance is stable and recovery requires 30–60 minutes, although the anaesthetic response has been found to be very variable. It can also be prepared for use as a 30% solution in ethanol which is then added to the water at a rate of 1 cm^3 L^{-1}. It is effective at this dose rate for routine farm operations with Atlantic salmon (Nordmo, 1991). The effective dose rates are shown in Table 8.12.

Synonyms
Chlorobutanol
Chlorbutol
Acetochlorone
Chlorbutanol
Chloretone
Chlortran
Dentalone
Khloreton
Methaform
Clortran

IUPAC name
1,1,1-Trichloro-2-methyl-propan-2-ol
Molecular formula
$C_4H_7Cl_3O$

Structure



Safety and Toxicology advice

- Harmful if swallowed.
- Irritating to eyes, skin and respiratory tract.
- In case of contact, rinse immediately with water.
- Use safety glasses and adequate ventilation.

Table 8.12 Summary of dose rates for chlorbutanol.

Species	Dose	Author
Freshwater fish	1–2 g L^{-1}	Ollenschlkaeger (1975)
Salmo salar	0.33 cm^3 L^{-1}	Nordmo (1991)
Salmonids	8–10 mg L^{-1}	Klontz and Smith (1969)

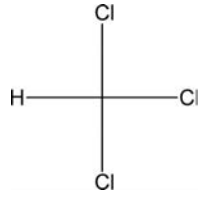
Chloroform

This is a clear colourless fluid with a sweet pungent odour. It is not very soluble in water (0.8% v/v) but it can be an effective agent. Although chloroform has been used with a number of invertebrate species, there are few reports of its use with fish. Mokhayer (1993) used chloroform in combination with acetone and ethanol for surgical anaesthesia of sturgeon during extraction of caviar. However, it is now known to be very hepatotoxic to operators and should not be used in confined spaces, if at all. An effective dose rate is shown in Table 8.13.

Synonyms
Chloroforme
Cloroformio
Chloroform
Formyl trichloride
Methyl trichloride
Methylidine trichloride
Trichloormethaan
Trichloromethane
Trichlormethan
Trichloroform
Tricloro metano

IUPAC name
Chloroform
Molecular formula
$CHCl_3$
Structural formula
$CHCl_3$

Structure



Safety and Toxicology advice

- Probably carcinogenic.
- Harmful if swallowed.
- Irritating to skin.
- Avoid lengthy exposure.
- Use safety glasses and gloves with good ventilation.

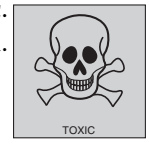


Table 8.13 Summary of dose rates for chloroform.

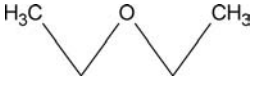
Species	Dose	Author
Gasterosteus aculetaus	0.14 $cm^3\ L^{-1}$	Erichsen Jones (1946)

Diethyl ether

Synonyms
Anaesthetic ether
Anaesthesia ether
Diaethylaether
Diethyl ether
Diethyl oxide
Ether
Ethoxyethane
Ethyl ether
Ethyl oxide
Pronarcol
Solvent ether

IUPAC name
Ethoxyethane

Structure



Safety and Toxicology advice

- Extremely flammable.
- May form explosive mixtures.
- Store and use in well-ventilated space.
- Harmful if swallowed.
- Irritant to skin.
- Use safety glasses and good ventilation.

Ether, or diethyl ether, is slightly soluble in water (8.4% w/w). It is a relatively safe and effective anaesthetic when used at a concentration of 10–50 $cm^3\,L^{-1}$ in water, depending on species, although it should be noted that in terrestrial vertebrates it has a muscle relaxing curare-like action. It has been used to anaesthetise a range of species including eels, goldfish and trout. Anaesthesia is induced in 2–3 minutes and recovery takes 5–30 minutes. It has also been vapourised and bubbled through water for use with *Scyliorhinus canicula* and *Gobius paganellus* (Vivien, 1941). Effective dose rates are shown in Table 8.14.

It should be borne in mind that ether is a mobile, very volatile, highly flammable liquid which forms very explosive mixtures with air and it must be treated with extreme caution. It has been reported to be mildly irritant to skin and membranes and McFarland and Klontz (1969) noted that it was also highly irritant at higher working concentrations and considered that this is a strong deterrent to its use.

Table 8.14 Summary of dose rates for diethyl ether.

Species	Dose	Author
Fish	10–50 $cm^3\,L^{-1}$	McFarland and Klontz (1969)
Fish (salmonids)	10–20 $cm^3\,L^{-1}$	Randall and Hoar (1971)

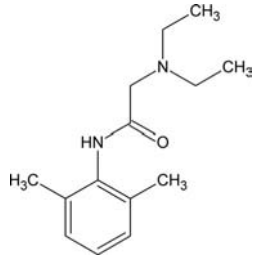
Lidocaine

Lidocaine, also commonly known as Lignocaine or Xylocaine, is insoluble in water but soluble in acetone or alcohol. The hydrochloride salt is freely soluble in water. It is widely used as a relatively short-acting local anaesthetic, cardiac depressant and general analgesic in human medicine. It is relatively cheap, easy to obtain and safe to humans at the concentrations used with fish.

Synonyms
Anestacon
Cappicaine
Esracaine
Gravocain
Leostesin
Lidocaine
Lignavet
Lignocaine
Maricaine
Nopoane
Xylocaine
Xylestesin
Xylotox

IUPAC name
2-Diethylamino-*N*-[2,6-dimethylphenyl acetamide] hydrochloride
Molecular formula
$C_{14}H_{22}N_2O$

Structure



Safety and Toxicology advice

- Harmful if swallowed.

This material has been used at dose levels of 100 $mg\,L^{-1}$ by Houston *et al.* (1973) in brook trout, *Salvelinus fontinalis*, and carp, *Cyprinus carpio*. Rodriguez-Gutierrez and Esquivel-Herrera (1995) showed that repeated xylocaine anaesthesia in carp (*Cyprinus carpio*) had no detrimental effects. Feldman *et al*. (1975) showed that it was effective in goldfish, especially if used in conjunction with sodium bicarbonate, which appears to stabilise the dose response. Rivera Lopez *et al*. (1991) obtained good anaesthesia at 150 $mg\,L^{-1}$ in *Algansea lacustris*, a high-altitude Mexican cyprinid, when the drug was potentiated with sodium bicarbonate at 1 $g\,L^{-1}$. Induction is uneventful and rapid, requiring 1 minute or less, and full recovery occurs after about three to four times the induction period. Similar results using lidocaine and sodium bicarbonate were obtained with *Cyprinus carpio* by Carrasco *et al.* (1984). The safety margin is good. Effective dose rates are shown in Table 8.15.

Table 8.15 Summary of dose rates for lignocaine (lidocaine or xylocaine).

Species	Dose	Author
Algansea lacustris	100 mg L^{-1}	Rivera Lopez *et al*. (1991)
Cyprinus carpio	100 mg L^{-1}	Houston *et al*. (1973)
Labeo rohita	10 mg L^{-1}	Das and Goswami (2003)
Cirrihinis mrigala	(transportation)	
Catla catla		
Salvelinus fontinalis	100 mg L^{-1}	Houston *et al*. (1973)

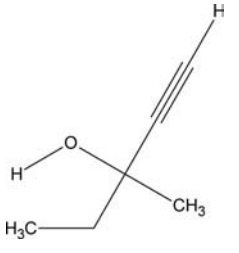
Methyl pentynol

This liquid has an unpleasant, acrid odour. It is soluble in water up to 147 cm^3 L^{-1}. It induces anaesthesia in salmonids in 2–3 minutes at 0.5–0.9 cm^3 L^{-1}, although animals may go into respiratory arrest during maintenance (Klontz and Smith, 1969). An effective dose rate is shown in Table 8.16.

Synonyms
Allotropal
Dormalest
Dormiphen
Dormisan
Melpintol
Meparfynol
Methyl pentynol
Methylparafynol
Metilparafinolo
Metilpentinolo
Noxokratin
Sedapercut

IUPAC name
3-Methylpent-1-yn-3-ol
Molecular formula
$C_6H_{10}O$
Structural formula
$CH_3.CH_2.CCH_3OH.COH$

Structure



Safety and Toxicology advice

- Harmful if swallowed.
- Irritating to eyes, skin and respiratory tract.
- In case of contact, rinse immediately with water.

Table 8.16 Summary of dose rates for methyl pentynol.

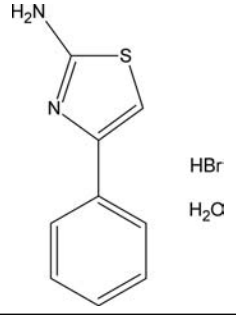
Species	Dose	Author
Fish	0.5–0.9 cm^3 L^{-1}	Klontz and Smith (1969)

Piscaine

Synonyms
APT
Phenthiazamine
Phenthiazamine hydrobromide
2-Amino-4-phenylthiazole,
2-Amino-4-phenylthiazole HBr H_2O
2-Amino-4-phenylthiazole hydrobromide monohydrate,
2-Thiazolamine
4-phenyl-monohydrobromide monohydrate

IUPAC name
4-Phenyl-1,3-thiazol-2-amine hydrate hydrobromide
Molecular formula
$C_9H_{11}BrN_2OS$

Structure



Safety and Toxicology advice

- Harmful if swallowed.
- Irritating to eyes, skin and respiratory tract.
- In case of contact, rinse immediately with water.

This material has been thoroughly investigated for use in fish principally by workers in Japan and a good deal of work has been done on its metabolism in fish tissues. Sekizawa *et al*. (1971) described probable mechanisms for its action and suggested that it acted by depression of the CNS rather than acting peripherally. The carp, *Cyprinus carpio,* could be sedated by immersion in a 12-ppm solution and this could be maintained for up to 72 hours (Kikuchi *et al.*, 1974). Immersion in solutions of 30–40 ppm gave good anaesthesia for 20–40 minutes. Rainbow trout were sedated for up to 24 hours using a 10-ppm solution and were anaesthetised for 40 minutes to 3 hours using a 20–30 ppm solution. The marine yellowtail, *Seriola quinqueradiata*, was sedated at 8 ppm for up to 4.5 hours and anaesthetised at 15–20 ppm for 10–25 minutes. Chiba and Chichibu (1992) successfully induced anaesthesia in the loach, *Cobitis biwae,* in 7 minutes using a 50-ppm solution. A range of effective dose rates is shown in Table 8.17.

Table 8.17 Summary of dose rates for piscaine (APT, phenthiazamine).

Species	Dose	Author
Cobitis biwae	50 ppm	Chiba and Chichibu (1993)
Cyprinus carpio	12–40 ppm	Kikuchi *et al*. (1974)
Oncorhynchus mykiss	10–30 ppm	Kikuchi *et al*. (1974)
Seriola quinqueradiata	8–20 ppm	Kikuchi *et al*. (1974)

Propanidid

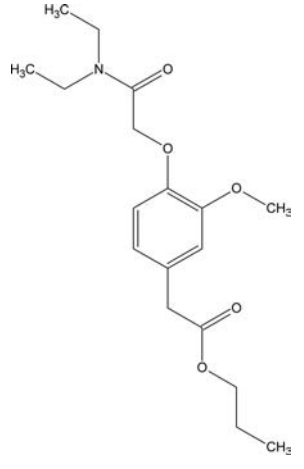
This drug has been used as an intravenous anaesthetic in human medicine for rapid induction of anaesthesia and for maintenance of anaesthesia of short duration. Due to adverse reactions, the drug has been withdrawn for use with humans.

Synonyms
Epontol
Eponthol
Fabantol
Fabontal
Propanidide
Propanidid
Propantan
Sombrevin

IUPAC name
3-Methoxy-4-(*N*,*N*-diethyl-carbamoyl-methoxy)-phenylacetic acid *n*-propyl ester

Molecular formula
$C_{18}H_{27}NO_5$

Structure



Siwicki (1984) noted that as it was used in human medicine it should be relatively harmless for operators and safe for use with fish. A range of effective dose rates is shown in Table 8.18.

Propanidid is also used as an injectable anaesthetic agent presented in the carrier Cremophor EL (polyethoxylated castor oil).

Safety and Toxicology advice

- Harmful if swallowed.
- Irritating to eyes, skin and respiratory tract.
- In case of contact, rinse immediately with water.

Table 8.18 Summary of dose rates for propanidid.

Species	Dose	Author
Cyprinus carpio	4 $cm^3\ L^{-1}$	Jeney *et al*. (1986)
Onchorhyncus mykiss	1–4 $cm^3\ L^{-1}$	Siwicki (1984)
Salmo salar	1–3 $cm^3\ L^{-1}$	Siwicki (1984)

Propoxate

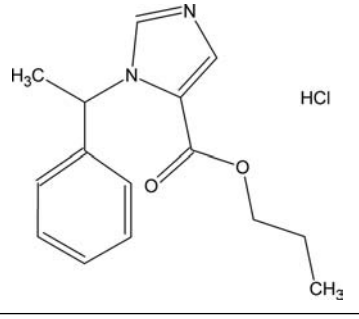
This drug is structurally similar to metomidate and etomidate. It is a crystalline ester of carboxylic acid which is highly soluble in freshwater and sea-water. It is also available as the hydrochloride. It is said to form a stable solution for long periods. It has very impressive anaesthetic properties, being about 100 times more potent than MS222, weight for weight (Thienpont and Niemegeers, 1965). Very rapid induction occurs at high doses (30–60 s at 4 mg L^{-1}), lower doses are effective but induction takes longer (5–9 min at 1 mg L^{-1}). Once anaesthetised, fish can be removed from the drug solution and will remain immobilised, if kept moist, until they are re-immersed in drug-free water. Jirasek *et al*. (1978) noted that propoxate produced anaesthesia at only 0.25–0.5 mg L^{-1} in tench, *Tinca tinca*. Prihoda (1979) found that it is effective in rainbow trout at 1:500000 (2 mg L^{-1}) and that effective sedation for transportation over a 2-hour period was obtained at 1:8000000 (only 0.125 mg L^{-1}). He dispensed it from a 1% stock solution. No mortalities were noted in 5 years of usage. A range of effective dose rates is shown in Table 8.19. Propoxate appears to have a high therapeutic index. Unfortunately, the drug is extremely expensive and probably because of this little is known of its metabolism or physiological effects in fish.

Synonyms
Menocaine
Propoxate hydrochloride
R 7464
DL-1-(1-Phenethyl)imidazole-5-carboxylic acid propyl ester hydrochloride
Propyl (1)-1-(1-phenylethyl)imidazole-5-carboxylate monohydrochloride
1*H*-Imidazole-5-carboxylic acid, 1-(1-phenylethyl)-, propyl ester, hydrochloride, (+–)–
Imidazole-5-carboxylic acid, 1-(α-methylbenzyl)-, propyl ester, hydrochloride, (+–)–
Imidazole-5-carboxylic acid, 1-(α-methylbenzyl)-, propyl ester, monohydrochloride, (+–)–

IUPAC name
Propyl 3-(1-phenylethyl)imidazole-4-carboxylate hydrochloride

Molecular formula
$C_{15}H_{19}ClN_2O_2$

Structure



Safety and Toxicology advice

- Harmful if swallowed.
- Irritating to eyes, skin and respiratory tract.
- In case of contact, rinse immediately with water.

Table 8.19 Summary of dose rates for propoxate.

Species	Dose	Author
Fish (salmonids)	1–10 mg L^{-1}	Randall and Hoar (1971)
Salmo gairdneri	2 mg L^{-1}	Prihoda (1979)
Tinca tinca	0.25–0.5 mg L^{-1}	Jirasek *et al*. (1978)

Sodium cyanide

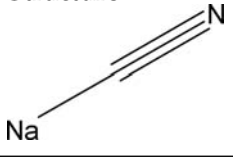
This highly poisonous compound is an inhibitor of many metabolic processes and is a blocking agent for the terminal oxidation enzyme cytochrome oxidase. It is used in many industrial processes and has been widely used to harvest marine tropical fish for supply to the aquarium trade (Hignette, 1984). Hanawa *et al.* (1998) assessed its use with *Dascyllus aruanus* using pulsed doses of 25 and 50 mg L^{-1}. for periods of 10–120 seconds. However, Hignette (1984) notes that it may be responsible for subsequent deaths many weeks later and so its use for any type of anaesthesia is not advised.

The material is very toxic to the user and to aquatic organisms and should not be released into the environment. Overall, it cannot be recommended for use as a fish anaesthetic.

Synonyms
Cymag
Cyanobrik
Cyanogran
Kyanid sodny
Cyanide of sodium
Cianuro di sodio
Cyanure de sodium
Cyanide salts
Cyanasalt H
Cyanasalt S
Sodium cyanide

IUPAC name
Sodium azanylidynemethane
Molecular formula
CNNa
Structural formula
CNNa

Structure



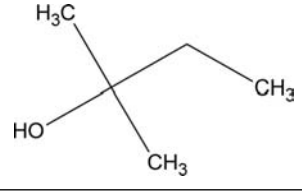
Tertiary amyl alcohol (TAA)

This is a volatile liquid which is fairly soluble in water (125 $cm^3\,L^{-1}$). Randall and Hoar (1971) note that TAA gives slow induction and some hyperactivity during the early stages of recovery. It is effective within 10–20 minutes at 0.5–1.25 $cm^3\,L^{-1}$ in salmonids (McFarland and Klontz, 1969) with recovery requiring 20–90 minutes. Anaesthesia was induced at doses of 400–850 ppm (0.4–0.85 $cm^3\,L^{-1}$) in *Epalzeorhynchos bicolor* (Meenakarn and Laohavisuti, 1993) with an LC_{50} of 1350 ppm. Alvarez-Lajonchere and Garcia-Moreno (1982) produced satisfactory deep sedation in *Mugil cephalus* post-larvae at 0.5 $cm^3\,L^{-1}$ and Hilton and Dixon (1982) induced full anaesthesia in *Oncorhynchus mykiss* using 7 $cm^3\,L^{-1}$. A range of effective dose rates is shown in Table 8.20.

Synonyms
Amylene hydrate
Dimethyl ethyl carbinol
TAA
Tertiary amyl alcohol
tert-Pentanol
t-Amyl alcohol
tert-Amyl alcohol
tert-Pentyl alcohol
tert-Isoamyl alcohol
2-Methyl butanol-2
2-Methylbutan-2-ol
3-Methylbutan-3-ol

IUPAC name
2-Methylbutan-2-ol
Molecular formula
$C_5H_{12}O$

Structure



Safety and Toxicology advice

- Harmful if inhaled.
- Respiratory, skin and eye irritant.
- Use safety glasses and adequate ventilation.

Table 8.20 Summary of dose rates for tertiary amyl alcohol.

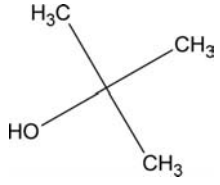
Species	Dose	Author
Epalzeorhynchos bicolor	400–850 ppm	Meenakarn and Laohavisuti, (1993)
Fish	0.5–1.25 $cm^3\,L^{-1}$	McFarland and Klontz (1969)
Fish (salmonids)	5–6 $cm^3\,L^{-1}$	Randall and Hoar (1971)
Mugil cephalus	0.5 $cm^3\,L^{-1}$	Alvarez-Lajonchere and Garcia-Moreno (1982)
Oncorhynchus mykiss	7 $cm^3\,L^{-1}$	Hilton and Dixon (1982)

Tertiary butyl alcohol (TBA)

This is a stable, colourless, non-corrosive liquid which is miscible with water. Alvarez-Lajonchere and Garcia-Moreno (1982) produced satisfactory deep sedation in *Mugil cephalus* post-larvae at 3.5 $cm^3\ L^{-1}$.

Synonyms
Arconol
Butanol tertiaire
TBA
tert-Butanol
t-Butanol
t-Butyl alchohol
t-Butyl hydroxide
tert-Butyl alcohol
Trimethylcarbinol
Trimethylmethanol

IUPAC name
2-Methylpropan-2-ol
Molecular formula
$C_4H_{10}O$
Structure



Safety and Toxicology advice

- Highly flammable.
- Harmful if inhaled.
- Skin and respiratory irritant.
- Severe eye irritant.
- Use safety glasses and adequate ventilation.

Tribromoethanol (TBE)

This is in the form of crystals with a slight aroma which dissolve in water (25 $cm^3\ L^{-1}$). It is effective at 4–6 $mg\ L^{-1}$ and induces anaesthesia in 5–10 minutes. Maintenance is reliable and recovery requires 20–40 minutes (McFarland and Klontz, 1969). The working solution decomposes on exposure to light and forms the irritant products, hydrogen bromide and dibromoacetaldehyde.

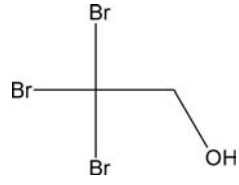
Synonyms
Avertin
Bromethol
Ethobrome
Basibrol
Ethobrom
Narcolan
Narcotyl
Narkolan
Rectanol
Tribromethanol
Tribromoethanol

IUPAC name
2,2,2-Tribromoethanol

Molecular formula
$C_2H_3Br_3O$

Structural formula
CBr_3CH_2OH

Structure



Safety and Toxicology advice

- Harmful if swallowed.
- Irritating to eyes, skin and respiratory tract.
- In case of contact, rinse with water and seek advice.

Urethane

Safety and Toxicology advice

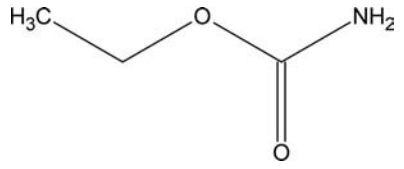
- Harmful if swallowed.
- May be carcinogenic.
- Avoid exposure.
- Use safety glasses and gloves with good ventilation.

Urethane is crystalline and is extremely soluble in water, giving a neutral solution. It has been used in veterinary anaesthesia where it provides a relatively shallow anaesthesia within 2–3 minutes, with few cardiac or respiratory effects. It also has a good therapeutic index. It is effective as a 0.5% solution in *Phoxinus phoxinus*, 2–4% in *Lampetra* and 1–5% in *Salmo trutta* (see Green, 1979) although McFarland and Klontz (1969) suggested dose rates of only 5–50 mg L^{-1}. A range of effective dose rates is shown in Table 8.21.

Table 8.21 Summary of dose rates for urethane.

Species	Dose	Author
Fish (salmonids)	5–40 mg L^{-1}	Randall and Hoar (1971)
Lampetra sp.	2–4%	
Liza aurata	1.5–5.2g L^{-1}	Soldatov (2005)
Mugil saliens		
Trachurus mediterraneus		
Sprattus sprattus		
Scorpaena porcus		
Platichthys flesus		
Phoxinus phoxinus	0.5%	Green (1979)
Salmo trutta	1–5%	

Urethane was formerly widely used but has now been found to be carcinogenic and leucopenic (Wood, 1956). Its use has been generally discontinued and is not advised.

Synonyms, Trade names	**IUPAC name**
Aethylcarbamat	Ethyl aminoformate
Aethylurethan	**Molecular formula**
Ethyl carbamate	$C_3H_7NO_2$
Ethyl urethane	**Structural formula**
Ethyl urethan	$H_2NCOOC_2H_5$
Leucethane	**Structure**
Leucothane	
Pracarbamine	
Pracarbamin	
Urethane	
Urethan	

Inhalation anaesthesia using plant extracts

Many plants contain chemicals which have traditionally been used to harvest fish in almost all parts of the world (for examples see Jenness, 1967). Perhaps the best known are species of *Derris*, which produce rotenone, and species of *Tephrosia*, which contain tephrosin, a substance similar to rotenone. Plant extracts have also been used for predator control in ponds (Baird, 1994). Many of these extracts are extremely toxic to a wide range of animals, not just fish, whereas others are used simply as potentiators of the action of other, more effective plant extracts. The active ingredients are in many cases unknown and, with the exception of derris derivatives and some other materials with a rotenone-like action, little is understood of the mode of action or ecotoxicology of these poisons (Baird, 1994).

Some of these materials, if used in modest concentrations, have biological actions which *resemble* anaesthesia and can be exploited as such. Sinha *et al*. (1992) successfully used extracts of *Cassia fistula* to reversibly anaesthetise guppies, finding the 48-hour LC_{50} of the total alkaloid extract to be 100 mg L^{-1}. Mgebenka and Ejiofor (1998) used an extract of leaves of sassy bark, *Erythrophleum suaveolens*, to anaesthetise the African catfish *Clarias gariepinus* and *Heterobranchus longifilis*. A 2.4 g L^{-1} crude extract gave effective anaesthesia, which was not improved by soaking the leaves for 24 hours. Recovery was good in fresh

water but no fish recovered from doses of 4 mg L^{-1} so the safety margin is small and the authors did not recommend its use.

Little authoritative guidance can be given to the use of plant extracts expect to be cautious, and as their mode of action is poorly understood they should perhaps be employed only as a last resort.

Summary

The tables above show listings of effective dose rates in different species. In some cases a range of values is given which will produce different degrees of anaesthesia in different sized animals. As a guide for those new to fish anaesthesia, or who are intending to anaesthetise a new species, a selection of the preferred drug should first be made for the species closest in structure, body design and habit to the new subject. One or more suggested dose rates should then be tested on a small group of non-critical animals before proceeding with any bulk work or prolonged procedures. It is important to use a number of test animals because of probable individual variation. From this, a dose and time combination can be selected for use with a good degree of confidence.

References

Ackerman, J.L. and Bellwood, D.R., Comparative efficiency of clove oil and rotenone for sampling tropical reef fish assemblages, *J. Fish Biol.*, 60 (2002) 893–901.

Allen, J.L., Residues of benzocaine in rainbow trout, largemouth bass, and fish meal, *Prog. Fish Culturist.*, 50(1) (1988) 59–60.

Allen, J.L. and Sills, J.B., Preparation and properties of quinaldine sulfate, an improved fish anesthetic, *Investigations of the Fisheries Control Bureau of the Sport Fish and Wildlife Service, USFWS, Washington, D.C.*, 47 (1973) 7.

Altun, T. and Danabas, D., Effects of short and long exposure to the anesthetic 2-phenoxyethanol mixed with ethyl alcohol on common carp (*Cyprinus carpio* L., 1758) fingerlings, *Israeli J. Aquac.*, 58 (2006) 178–182.

Amend, D.F., Goven, B.A. and Elliot, D.G., Etomidate: effective dosages for a new fish anesthetic, *Trans. Am. Fish. Soc.*, 111(3) (1982) 337–341.

Anderson, W.G., McKinley, R.S. and Colavecchia, M., The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance, *N. Am. J. Fish. Manage.*, 17 (1997) 301–307.

Alvarez-Lajonchere, L. and Garcia-Moreno, B., Effects of some anaesthetics on postlarvae of *Mugil trichodon* Poey (Pisces, Mugilidae) for their transportation, *Aquaculture*, 28(3–4) (1982) 385–390.

Auperin, B., Baroiller, J.F., Ricordel, M.J., Fostier, A. and Prunet, P., Effect of confinement stress on circulating levels of growth hormone and two prolactins in freshwater-adapted tilapia (*Oreochromis niloticus*), *Gen. Comp. Endocrinol.*, 108 (1997) 35–44.

Baird. D., Pest control in tropical aquaculture: an ecological hazard assessment of natural and synthetic control agents, *Mitt. Int. Ver. Limnologie*., 24 (1994) 285–292.

Barton, B.A. and Helfrich, H., Time-dose responses of juvenile rainbow trout to 2-phenoxyethanol, *Prog. Fish Culturist*., 32(4) (1981) 223.

Bell, G.R., A guide to the properties, characteristics and uses of some general anaesthetics for fish, *Bull. Fish. Res. Board Can.*, No 148 (1964).

Blasiola, G.C., Quinaldine sulphate, a new anaesthetic formulation for tropical marine fish, *J. Fish Biol*., 10(1) ((1976)) 113–120.

Bové, F.J., MS222 Sandoz, The anaesthetic and tranquilliser for fish, frogs and other cold-blooded organisms, *Sandoz News, Basle*, 13 (1962) 24.

Brandt, T.M., Graves, K.G., Berkhouse, C.S., Simon, T.P. and Whiteside, B.G., Laboratory spawning and rearing of the endangered fountain darter, *Prog. Fish Culturist*, 55(3) (1993) 149–156.

Bressler, K. and Ron, B., The effect of anesthetics on stress and the innate immune system of gilthead sea bream, *Sparus aurata, Israeli J. Aquac./Bamidgeh*., 56 (2004) 5–13.

Brown, L., Recirculation anaesthesia for laboratory fish, *Lab. Anim.*, 21(3) (1987) 210–215.

Bystriansky, J.S., LeBlanc, P.J. and Ballantyne, J.S., Anaesthetisation of Arctic charr *Salvelinus alpinus* (L) with tricaine methanesulphonate or 2-phenoxyethanil for immediate blood sampling, *J. Fish Biol*., 69 (2006) 613–621.

Carrasco, S., Sumano, H. and Navarro,-Fierro, R., The use of lidocaine-sodium bicarbonate as anaesthetic in fish, *Aquaculture*, 41 (1984) 395–398.

Chatain, B. and Corraoa, D., A sorting method for eliminating larvae without swimbladders, *Aquaculture*, 107(1) (1992) 81–88.

Chellappa, S., Cacho, M., Huntingford, F., and Beveridge, M., Observations on induced breeding of the Amazonian fish tambaqui, *Colossoma macropomum* using CPE and HCG treatments, *Aquac. Res*., 27 (1996) 91–94.

Chiba, A. and Chichibu, S., High-energy phosphate metabolism in the phenthiazamine hydrobromide anesthetized loach *Cobitis biwae*, *Comp. Biochem. Physiol*., 102C(3) (1992) 433–437.

Cho, G. and Heath, D., Comparison of tricaine methanesulphonate (MS222) and clove oil anaesthesia effects on the physiology of juvenile Chinook salmon *Oncorhynchus tschawytscha* (Walbaum), *Aquac. Res*., 31(6) (2000) 537–546.

Crosby, T.C., Hill, J.E., Watson, C.A., Yanong, R.P.E. and Strange, R., Effects of tricaine methanesulfonate, hypno, metomidate, quinaldine, and salt on plasma cortisol levels following acute stress in Threespot Gourami *Trichogaster trichopterus*, *J. Aquatic Anim. Health*, 18 (2006) 58–63.

Cuesta, A., Meseguer, J. and Esteban, M.A., Total serum immunoglobulin M levels are affected by immunomodulators in seabream (*Sparus aurata* L.), *Vet. Immunol. Immunopathol*., 101 (2004) 203–210.

Davis, K.B. and Griffin, B.R., Physiological responses of hybrid striped bass under sedation by several anesthetics, *Aquaculture*, 233 (2004) 531–548.

Detar, J.E. and Mattingly, H.T., Response of the Southern Redbelly dace to clove oil and MS-222: effects of anaesthetic concentration and water temperature, Proceedings of the annual conference of the South-eastern Association of Fish and Wildlife Agencies, 58 (2005) 219–227.

Dick, G.L., Some observations on the use of MS222 Sandoz with grey mullet (*Mugil chelocuvier*), *J. Fish Biol*., 7 (1975) 263–268.

Dixon, R.N. and Milton, P., Effects of the anaesthetic quinaldine on oxygen consumption in the intertidal teleost *Blennius pholis* (L), *J. Fish Biol.*, 12 (1978) 359, 369.

Dziaman, R., Klyszejko, B. and Hajek, G., The effect of MS-222 on the cardiac and respiratory function and behaviour of common carp (*Cyprinus carpio* L.) during general anaesthesia, *Acta Ichthyologica et Piscatoria*., 35 (2005) 125–131.

Endo, T., Ogishima, K., Tanaka, H. and Ohshima, S., Studies on the anesthetic effect of eugenol in some fresh water fishes, *Bull. Jpn. Soc. Sci. Fish*., 38 (1972) 761–767.

Erdmann, M.V., Clove oil: an 'eco-friendly' alternative to cyanide use in the live reef fish industry? *Live Reef Fish Info. Bull*., 5 (1999) 4–7.

Falls, W.W., Vermeer, G.K. and Dennis, C.W., Evaluation of etomidate as an anesthetic for red drum, *Sciaenops ocellatus*, Red Drum Aquaculture, in: Proceedings of a Symposium on the Culture of Red Drum and Other Warm Water Fishes, *Marine Sci.*, 30 1988, 37–42.

Feldman, S., De Franco. M. and Cascella, P.J., Activity of local anaesthetic agents in the goldfish, *J. Pharm. Sci.*, 64 (1975) 1713–1715.

Fredricks, K.T., Gingerich, W.H. and Fater, D.C., Comparative cardiovascular effects of four fishery anesthetics in spinally transected rainbow trout, *Oncorhynchus mykiss*, *Comp. Biochem. Physiol*., 104C(3) (1993) 477–483.

Furuichi, M. and Yone, Y., The influence of repeated bleeding and anesthetization on change of blood sugar of fish, *Sci. Bull. Faculty Agric., Kyushu Univ.*, 26 (1972) 577–581.

Garcia-Gomez, A., Gandara, F. and Raja, T., Use of clove, *Syzygium aromaticum* L. (Merr. & Perry), oil as a cost-effective anaesthetic for routine tasks in handling cultured marine fish, *Boletin del Instituto Español de Oceanografía*, 18 (2002) 21–23.

Gilbert, P.W. and Wood, F.G., Method of anaesthetising sharks and rays safely and rapidly, *Science*, 126 (1957) 212.

Gilderhus, P.A., Efficacy of benzocaine as an anesthetic for salmonid fishes, *N. Am. J. Fish. Manage.*, 9(2) (1989) 150–153.

Gilderhus, P.A., Benzocaine as a fish anesthetic: Efficacy and safety for spawning-phase salmon, *Prog. Fish Culturist.*, 52(3) (1990) 189–191.

Gilderhus, P.A., Lemm, C.A. and Woods, L.C., Benzocaine as an anesthetic for striped bass, *Prog. Fish Culturist.*, 53(2) (1991) 105–107.

Gomes, L.C., Chippari-Gomes, A.R., Lopes, N.P., Roubach, R. and Araujo-Lima, C.A.R.M., Efficacy of Benzocaine as an Anesthetic in Juvenile Tambaqui *Colossoma macropomum*, *J. World Aquac. Soc.*, 32 (2001) 426–431.

Green.C.J., Animal Anaesthesia, Laboratory Animal Handbooks, No. 8, Laboratory Animals, London, 1979, 300 p.

Guo, F.C., Teo, L.H. and Chen, T.W., Effects of anaesthetics on the oxygen consumption of platyfish *Xiphophorus maculatus* (Günther), *Aquac. Res*., 26(12) (1995) 887–894.

Hajek, G.J. and Kłyszejko, B., The effects of Propiscin (etomidate) on the behaviour, heart rate, and ventilation of common carp, *Cyprinus carpio* L, *Acta Ichthyologica et Piscatoria*., 34(2) (2004) 129–143.

Hanawa, M., Harris, L., Graham, M., Farrell, A.P. and Bendell-Young, L.I., Effects of cyanide exposure on *Dascyllus aruanus*, a tropical marine fish species: lethality, anaesthesia and physiological effects, *Aquarium Sci. Conserv.* 2 (1998) 21–34.

Henderson-Arzapalo, A., Lemm, C., Hawkinson, J. and Keyes, P., Tricaine used to separate phase 1 striped bass with uninflated swimbladders from normal fish, *Prog. Fish Culturist.*, 54(2) (1992) 133–135.

Hettiarachchi, M. and Senadheers, S.P.S.D., Efficacy of quinaldine sulphate as an anaesthetic for the ornamental carp (*Cyprinus carpio*) in simulated packaging for long distance transport, *Sri Lanka J. Aquatic Sci.*, 4 (1999) 13–22.

Hignette, M., The use of cyanide to catch tropical marine fish for aquariums and its diagnosis, *Comptes rendus des journees aquariologiques de l'Institut Oceanographiques*, 10(5) (1984) 585–591.

Hikasa, Y., Takase, K., Ogasawara, T. and Ogasawara, S., Anesthesia and recovery with tricaine methanesulfonate, eugenol and thiopental sodium in the carp, *Cyprinus carpio*, *Jpn. J. Vet. Sci.*, 48 (1986) 341–351.

Hill, J.V. and Forster, M.E., Cardiovascular responses of Chinook salmon (*Oncorhynchus tshawytscha*) during rapid anaesthetic induction and recovery, *Comp. Biochem. Physiol.*, 137 (2004) 167–177.

Hilton, J.W. and Dixon, D.G., Effect of increased liver glycogen and liver weight on liver function in rainbow trout, *Salmo gairdneri* Richardson: recovery from anaesthesia and plasma 35 *S*-sulphobromopthalein clearance, *J. Fish Dis.*, 5(3) (1982) 185–195.

Hinton, M.J. and Loyacano, H.A., Efficacy of quinaldine and MS-222 on American eel, in: Proceedings of the ninth annual meeting, World Mariculture Society, Atlanta, Georgia, January 3–6, 1978, 647–652.

Hisake, Y., Takase, K., Ogasawara, T. and Ogasawara, S., Anaesthesia and recovery with tricaine methanesulfonate, eugenol and thipopental sodium in the carp, *Cyprinus carpio*, *Jpn. J. Vet. Sci.*, 48(2) (1986) 341–351.

Hocutt, C.H., Seasonal and diel behaviour of radio-tagged *Clarias gariepinus* in Lake Ngezi, Zimbabwe (Pisces: Clariidae), *J. Zool.*, 219(2) (1989) 181–199.

Hoskonen, P. and Pirhonen, J., Effects of repeated handling with or without anaesthesia, on feed intake and growth in juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum), *Aquac. Res.*, 37 (2006) 409–415.

Houston, A.H., Madden, J.A., Woods, R.J. and Miles, H.M., Some physiological effects of handling and tricaine methanesulphonate anaesthetization upon the Brook Trout, *Salvelinus fontalis*, *J. Fish. Res. Board Can.*, 28(5) (1971) 625–633.

Houston, A.H., Czerwinski, C.L. and Woods, R.J., Cardiovascular and respiratory activity during recovery from anaesthesia and surgery in brook trout (*Salvelinus fontinalis*) and carp (*Cyprinus carpio*), *J. Fish. Res. Board Can.*, 30 (1973) 1705–1712.

Howe, G.E., Bills, T.D. and Marking, L.L., Removal of benzocaine from water by filtration with activated carbon, *Prog. Fish Culturist.*, 52(1) (1990) 32–35.

Howell, J.H. and Thomas, P.M., Anesthetic effect of 4-styrylpyridine on Lamprey and Fish,Trans. *Am. Fish. Soc.*, 93 (1964) 206–208.

Hseu, J.R., Yeh, S.L., Chu, Y.T. and Ting, Y.Y., Influence of the anaesthetic, 2-phenoxyethanol, on haematological parameters of black porgy *Acanthpagrus schlegeli*, *J. Taiwan Fish. Res.*, 4(2) (1996) 127–132.

Hseu, J.R., Yeh, S.L., Chu, Y.T. and Ting, Y.Y., Comparison of efficacy of five anesthetics in goldlined sea bream, *Sparus sarba*, *Acta Zoolog. Taiwan.*, 9 (1998) 11–18.

Imamura-Kojima, H., Takashima, F. and Yoshida, T., Absorption, distribution and excretion of 2-phenoxyethanol in Rainbow trout, *Bull. Jpn. Soc. Sci. Fish.*, 53(8) (1987) 1339–1342.

Inoue, L., Santos Neto, C. and Moraes, G., Benzocaine as anesthetic for juvenile matrinxa (*Brycon cephalus*), *Boletim tecnico do CEPTA*., 15 (2002) 23–30.

Ishioka, H., Physiological and biochemical studies on the stress responses of the red sea bream, *Pagrus major* (Temminck et Schlegel), *Bull. Nansei Reg. Res. Lab*., 17 (1984) 1–133.

Jacquemond, F., Sorting Eurasian perch fingerlings (*Perca fluviatilis* L.) with and without functional swim bladder using tricaine methane sulfonate, *Aquaculture*, 231 (2004) 249–262.

Jain, S.M., Use of MS-222 as an anaesthetic agent for young common carp *Cyprinus carpio*, *J. Inland Fish. Soc. India*, 19(1) (1987) 67–70.

Jeney, Z., Jeney, G., Olah, J., Siwicki, A. and Danko, I., Propanidid, a new anaesthetic for use in fish propagation, *Aquaculture*, 54 (1986) 149–156.

Jenness, J., The use of plants as fish poison within the Kainji basin, in: Reed, W. (ed.), Fish and Fisheries of Northern Nigeria, Ministry of Agriculture of Northern Nigeria, 1967, 226 p.

Jirasek, J., Adamek, Z. and Giang, P.M., The effect of administration of the anaesthetics MS 222 (Sandoz) and R 7464 (Propoxate) on oxygen consumption in the tench (*Tinca tinca* L.), *Zivocisna-Vyroba*, 23(11) (1978) 835–840.

Josa, A., Espinosa, E., Cruz, J.I., Gil, L., Falceto, M.V. and Lozano, R., Use of 2-phenoxyethanol as an anesthetic agent in goldfish (*Cyprinus carpio*), *Res. Vet. Sci*., 53 (1992) 139.

Kaminski, R., Myszkowski, L. and Wolnicki, J., Response to 2-phenoxyethanol in juvenile *Vimba vimba* (L.), *Arch. Pol. Fish.*, 9(1) (2001) 71–78.

Kaneko, K., On the removal of larger freshwater fishes, *Mar. Parks Aquaria.*, 11 (1982) 39–45.

Kazun, K. and Siwicki, A.K., Propiscin – a safe new anaesthetic for fish, *Arch. Pol. Fish.*, 9 (2001) 183–190.

Keene, J.L., Noakes, D.L.G., Moccia, R.D. and Soto, C.G., The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhyncus mykiss* (Walbaum), *Aquac. Res*., 29 (1998) 89–101.

Kidd, R.B. and Banks, G.D., Anesthetizing lake trout with Tricaine (MS-222) administered from a spray bottle, *Prog. Fish Culturist*., 52(4) (1990) 272–273.

Kiessling, A., Espe, M., Ruohonen, K. and Morkore, T., Texture, gaping and colour of fresh and frozen Atlantic salmon flesh as affected by pre-slaughter iso-eugenol or CO_2 anaesthesia, *Aquaculture*, 236 (2004) 645–657.

Kikuchi, T., Sekizawa, Y. and Ikeda, Y., Behavioural analyses of the central nervous system depressant activity of 2-amino-4-phenythiazole upon fishes, *Bull. Jpn. Soc. Sci. Fish.*, 40(4) (1974) 325–337.

King, W., Hooper, B., Hillsgrove, S., Benton, C. and Berlinsky, D.L., The use of clove oil, metomidate, tricaine methanesulphonate and 2-phenoxyethanol for inducing anaesthesia and their effect on the cortisol stress response in black sea bass (*Centropristis striata* L.), *Aquac. Res*., 36 (2005) 1442–1449.

Klontz, G.W. and Smith, L.S., Methods of using fish as biological research subjects. in: Gay, W.I. (ed.), Methods of Animal Experimentation, Academic Press, New York, 1969.

Kreiberg, H., Metomidate sedation minimises handling stress in Chinook salmon, *Bull. Aquac. Assoc. Can*., 92(3) (1992) 52–54.

Kumlu, M. and Yanar, M., Effects of the anesthetic quinaldine sulphate and muscle relaxant diazepam on sea bream juveniles (*Sparus aurata*), *Israeli J. Aquac./Bamidgeh*., 51 (1999) 143–147.

Laird, L.M. and Oswald, R.L., A note on the use of benzocaine (ethyl *p*-aminobenzoate) as a fish anaesthetic, *J. Inst. Fish. Manage.*, 6(4) (1975) 92–94.

Lemm, C.A., Evaluation of five anesthetics on striped bass, National Fisheries Center Report, No. 193, Kearneysville, WV, 1993.

Limsuwan, C., Limsuwan, T., Grizzle, J.M. and Plumb, J.A., Stress response and blood characteristics of channel catfish (*Ictalurus punctatus*) after anesthesia with etomidate, *Can. J. Fish. Aquatic Sci.*, 40(12) (1983) 2105–2112.

Malmstroem, T., Salte, R., Gjoeen, H.M. and Linseth, A., A practical evaluation of metomidate and MS-222 as anaesthetics for Atlantic halibut (*Hippoglosus hippoglosus*), *Aquaculture*, 113(4) (1993) 331–338.

Massee, K.C., Rust, M.B., Hardy, R.W. and Stickney, R.R., The effectiveness of tricaine, quinaldine sulfate and metomidate as anaesthetics for larval fish, *Aquaculture*, 134(3–4) (1995) 351–360.

Mattson, N.S. and Riple, T.H., Metomidate, a better anesthetic for cod (*Gadus morhua*) in comparison with Benzocaine, MS-222, chlorobutanol, and phenoxyethanol, *Aquaculture*, 83(1–2) (1989) 89–94.

McCarter, N., Sedation of grass carp and silver carp with 2-phenoxyethanol during spawning, *Prog. Fish Culturist.*, 54(4) (1992) 263–265.

McFarland, W.N. and Klontz, G.W., Anesthesia in fishes, *Fed. Proc.*, 28(4) (1969) 1535–1540.

Meenakarn, W. and Laohavisuti, N., Some anaesthetics used in the transport of *Epalzeorhynchus bicolor* (Smith), Proceedings of the Department of Fisheries, Thailand, 1993.

Mgebenka, B.O. and Ejiofor, E.N., Effects of extracts of dried leaves of *Erythrophleum suaveolens* as anaesthetics on Clariid catfish, *J. Appl. Aquac.*, 8(4) (1998) 73–80.

Mokhayer, B., Surgery in Iranian sturgeons, *Iranian Fish. bull., Tehran*, (1993) 2.

Munday, P.L. and Wilson, S.K., Comparative efficacy of clove oil and other chemicals in anaesthetization of *Pomacentrus amboiensisI*, *J. Fish Biol.*, 51 (1997) 931–938.

Muzinic, R., On the use of anaesthetics in the transportation of sardines, *Stud. Rev. Gen. Fish. Counc. Mediterr*, 47 (1970) 1–23.

Mylonas, C.C., Cardinaletti, G., Sigelaki, I. and Polzonetti-Magni, A., Comparative efficacy of clove oil and 2-phenoxyethanol as anesthetics in the aquaculture of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) at different temperatures, *Aquaculture*, 246 (2005) 467–481.

Nordmo, P., Salmon farming in Norway, in: Brown, L. (ed.), Aquaculture for Veterinarians, Pergamon Press, 1991.

Olsen, Y.A., Einarsdottir, I.E. and Nilssen, K.J., Metomidate anaesthesia in Atlantic salmon, *Salmo salar*, prevents plasma cortisol increase during stress, *Aquaculture*, 134 (1995) 155–168.

Ortuño, J., Esteban, M.A. and Meseguer, J., Effects of four anaesthetics on the innate immune response of gilthead seabream (*Sparus aurata* L.), *Fish Shellfish Immunol.*, 12 (2002a) 49–59.

Ortuño, J., Esteban, M.A. and Meseguer, J., Effects of phenoxyethanol on the innate immune system of gilthead seabream (*Sparus auratus* L.) exposed to crowding stress, *Vet. Immunol. Immunopathol.*, 89(1–2) (2002b) 29–36.

Osanz-Castan, E., Esteban-Alonso, J., del Nino Jesus, A., Josa Serrano, A. and Espinoza Velasquez, E., Proceedings of 4th national Congress in Aquaculture, Centre for Marine Research, Spain, 1993, 737–742.

Oswald, R.L., Injection anaesthesia for experimental studies in fish, *Comp. Biochem. Physiol*., 60C (1978) 19–26.

Ottinger, C.A., Holloway, H.L. and Derrig, T.M., Maintenance of juvenile paddlefish as experimental animals, *Prog. Fish Culturist*., 54(2) (1992) 121–124.

Palmer, L.M. and Mensinger, A.F., Effect of the anesthetic Tricaine (MS-222) on nerve activity in the anterior lateral line of the oyster toadfish, *Opsanus tau*, *J. Neurophysiol*., 92 (2004) 1034–1041.

Parma-de-Croux, M.J., Benzocaine (ethyl-*p*-aminobenzoate) as an anaesthetic for *Prochilodus lineatus* Valenciennes (Pisces, Curimatidae), *J. Appl. Ichthyology*., 6(3) (1990) 189–192.

Peake, S., Sodium bicarbonate and clove oil as potential anesthetics for non-salmonid fishes, *N. Am. J. Fish. Manage*., 18 (1998) 919–924.

Piavis, G.W. and Piavis, M.G., Implantation of an abdominal window in the sea lamprey, *Petromyzon marinus* (Pisces: Petromyzontidae), *Copeia*., (2) (1978) 349–352.

Pirhonen, J. and Shreck, C.B., Effects of anaesthesia with MS-222, clove oil and CO_2 on feed intake and plasma cortisol in steelhead trout (*Oncorhynchus mykiss*), *Aquaculture*, 220 (2003) 507–514.

Plumb, J.A., Schwedler, T.E. and Limsuwan, C., Experimental anesthesia of three species of freshwater fish with etomidate, *Prog. Fish Culturist*., 45(1) (1983) 30–33.

Prieto, A., Fajer, E. and Barrera, M., Utilizacion del MS222 en la anguilla americana (*Anguilla rostrata*, Le Suer), Contribuciones del Direccion de Acuicultura, La Habana, Cuba, 1976.

Prihoda, J., Experience with use of propoxate in anaesthesia and transport of salmonids in Slovakian fishery union centres, *Biol. Chem. Vet. Zivocisne. Vyroby*, 15(3) (1979) 283–288.

Prince, A. and Powell, C., Clove oil as an anaesthetic for invasive field procedures on adult Rainbow trout, *N. Am. J. Fish. Manage.*, 20(4) (2000) 1029–1032.

Radull, J., Kaiser, H. and Hecht, T., Effects of 2-phenoxyethanol on stressor-induced changes in metabolic rates of juvenile spotted grunter, *Pomadasys commersonnii*, 2nd Western Indian Ocean Science Association (WIOMSA) Scientific Symposium, Book of Abstracts, 2001, 45–46.

Raidal, S.R., Shearer, P.L., Stephens, F. and Richardson, J., Surgical removal of an ovarian tumour in a koi carp (*Cyprinus carpio*), *Aust. Vet.J*., 84 (2006) 178–181.

Randall, D.J., Effect of anaesthetic on the heart and respiration of a teleost fish, *Nature, London*, 195 (1962) 506.

Randall, D.J. and Hoar, W.S., Special techniques. in: Hoar, W.S. and Randall, D.J. (eds.), Fish Physiology, volume 6, Academic Press, New York, 1971, 559 p.

Rivera-Lopez, H., Orbe-Mendoza, A. and Ross, L.G., Use of xylocaine, potentiated with sodium bicarbonate, as an anaesthetic for fry and juveniles of Acumara, *Algansea lacustris* Steindachner 1895, from Lake Patzcuaro, Michoacan, Mexico, *Aquac. Fish. Manage*., 22 (1991) 15–18.

Rodriguez-Gutierrez, M. and Esquivel-Herrera, A., Evaluation of the repeated use of xylocaine as anaesthtic for the handling of breeding carp (*Cyprinus carpio*). in: The carp. Proceedings of 'Aquaculture' sponsored symposium, Budapest, Hungary, 1993, *Aquaculture*, 129(1–4) (1995) 431–436.

Ross, L.G. and Geddes, J.A., Sedation of warm-water fish species in aquaculture research, *Aquaculture*, 16 (1979) 183–186.

Ross, L.G. and Ross, B., Anaesthetic and sedative techniques for fish, Institute of Aquaculture, Stirling University, Stirling, 1984, 42 p.

Ross, R.M., Backman, T.W.H. and Bennett, R.M., Evaluation of the anesthetic metomidate for the handling and transport of juvenile American shad, *Prog. Fish Culturist.*, 55(4) (1993) 236–243.

Ross, L.G. and Wiewiorka, J., A low-cost audible heart rate monitor for fish surgery, *Lab. Pract.* 26(3) (1977) 188.

Rothwell, S.E., Black, S.E., Jerrett, A.R. and Forster, M.E., Cardiovascular changes and catecholamine release following anaesthesia in Chinook salmon (*Oncorhynchus tshawytscha*) and snapper (*Pagrus auratus*), *Comp. Biochem. Physiol.*, Part A, 140 (2005) 289–298.

Roubach, R., De Carvalho Gomes, L. and Val, A.L., Safest level of tricaine methanesulphonate (MS-222) to induce anesthesia in juveniles of matrinxa, *Brycon cephalus, Acta Amazonica.*, 31(1) (2001) 159–163.

Roubach, R., Carvalho Gomes, L., Leão Fonseca, F.A. and Luiz Val, A., Eugenol as an efficacious anaesthetic for tambaqui, *Colossoma macropomum* (Cuvier), *Aquac. Res.*, 36(11) (2005) 1056–1061.

Ryan, S., The dynamics of MS222 anaesthesia in a marine teleost, *Comp. Biochem. Physiol.*, 101C(3) (1992) 593–600.

Sandodden, R., Finstad, B. and Iversen, M., Transport stress in Atlantic salmon (*Salmo salar* L.): anaesthesia and recovery, *Aquac. Res.*, 32 (2001) 87–90.

Schoettger, R.A. and Steucke, E.W., Synergic mixtures of MS222 and quinaldine as anaesthetics for rainbow trout and northern pike, *Prog. Fish Culturist.*, 32 (1970) 202–205.

Schramm, H.L. and Black, D.J., Anaesthesia and surgical procedures for implanting radio transmitters into grass carp, *Prog. Fish Culturist.*, 46(3) (1984) 185–190.

Sehdev, H.S., McBride, J.R. and Fagerlund, U.H.M., 2-phenoxyethanol as a general anaesthetic for Sockeye Salmon, *J. Fish. Res. Board Can.*, 20 (1963) 1435–1440.

Sekizawa, Y., Kikuchi, T. and Suzuki, A., Electrophysiological surveys on the anaesthetic properties of 2-amino-4-phenylthiazole upon Carp (*Cyprinus carpio*), *Jpn. J. Ichthyology*, 18(3) (1971) 128–138.

Sharma, S.K. and Sharma, L.L., Effect of chloral hydrate on metabolic rate of *Labeo rohita* (Ham.) and *Poecilia reticulata* (Peters), *J. Indian Fish.*, 26 (1996) 121–125.

Sinha, M.J., Kumar, A., Lal, B.K., Sarkhel, B.K. and Munshi, J.D., Toxicity of alkaloid extract of *Cassia fistula* L., on common guppy, *Lebistes reticulatus* (Peters) and on the physico-chemical characteristics of water, *J. Freshwater Biol.*, 4(1) (1992) 7–22.

Siwicki, A., New anaesthetic for fish, *Aquaculture*, 38 (1984) 171–176.

Small, B.C., Anesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinaldine and clove oil anesthetized channel catfish *Ictalurus punctatus*, *Aquaculture* 218 (2003) 177–185.

Smeltzer, J.F. and Flickinger, S.A., Culture, handling, and feeding techniques for black crappie fingerlings, *N. Am. J. Fish. Manage.*, 11 (1991) 485–491.

Smit, G.L., Schoonbee, H.J. and Barham, W.T., Some effects of the anaesthetic MS 222 on fresh water bream, *S. Afr. J. Sci.*, 73(11) (1977) 351–352.

Soivio, A., Malkonen, M. and Tuurala, O., Effects of asphyxia and MS222 anaesthesia on the circulation of the kidney in *S. gairdneri*, Richardson, *Finn. Ann. Zool.*, 11 (1974) 271–275.

Soivio, A., Nyholm, K. and Huhti, M., Effects of anaesthesia with MS222, neutralised MS222 and benzocaine on the blood constituents of Rainbow trout, *J. Fish Biol.*, 10 (1977) 91–101.

Soldatov, A.A., Physiological effects of urethane aesthesia on the organism of marine fishes, *Hydrobiol. J.*, 412 (2005) 113–126.

Sonawane, U.D. and Kulkarni, G.N., Anaesthetic effects of clove oil and sodium bicarbonate on the fry of *Liza parsia*, *Iranian J. Fish. Sci.*, 3 (2001) 49–62.

Sorum, U. and Damsgard, B., Effects of anaesthetisation and vaccination on feed intake and growth in Atlantic salmon (*Salmo salar* L.), *Aquaculture*, 232 (2004) 1–4.

Soto, C., Clove oil: a fish anaesthetic, *West. Indian Ocean Waters*, 6(2) (1995) 2–3.

Stehly, G.R. and Gingerich, W.H., Efficacy and toxicity of Aqui-S as a fish anaesthetic, World Aquaculture Society abstracts, Aquaculture'98, Las Vegas, USA, 1998.

Strange, J.R. and Schreck, C.B., Anaesthetic and handling stress on survival and cortisol concentrations in yearling Chinook salmon (*Oncorhynchus tschawytscha*), *J. Fish. Res. Board Can.*, 35 (1978) 345–349.

Sylvester, J.R., Factors influencing the efficacy of MS222 to striped mullet (*Mugil cephalus*), *Aquaculture*, 6 (1975) 163–169.

Takashima, Y., Wan, Z., Kasai, H. and Asakawa, O., Sustained anesthesia with 2-phenoxyethanol in yearling rainbow trout, *J. Tokyo Univ. Fish.*, 69(2) (1983) 93–96.

Takeda, T., Yamasaki, K. and Itazawa, Y., Effect of MS 222 on respiration and efficacy of forced branchial irrigation with the anesthetic solution in carp, *Bull. Jpn. J. Sci. Fish.*, 53(10) (1987) 1701–1709.

Takii, K., Hosokawa, H., Shimeno, S., Ukawa, M., Kotani, A. and Yamada, Y., Anesthesia, fasting tolerance, and nutrient requirement of juvenile northern bluefin tuna, *Fish. Sci.*, 71 (2005) 499–503.

Tamaru, C.C., Carlstrom-Trick, C. and Fitzgerald, W.J.Jr., Clove oil, minyak cengkeh, a natural fish anaesthetic, *Proc. Pacon Conf. Sustainable Aquac.*, 1996, 365–371.

Thienpont, D. and Niemegeers, C.J.E., Propoxate (R7464): a new potent anaesthetic agent in cold-blooded vertebrates, *Nature, London*, 205 (1965) 1018–1019.

Thomas, P. and Robertson, L., Plasma cortisol and glucose stress responses of red drum (*Sciaenops ocellatus*) to handling and shallow water stressors and anaesthesia with MS-222, quinaldine and metomidate, *Aquaculture*, 96 (1991) 69–86.

Tsantilas, H., Galatos, A.D., Athanassopoulou, F., Prassinos, N.N. and Kousoulaki, K., Efficacy of 2-phenoxyethanol as an anaesthetic for two size classes of white sea bream, *Diplodus sargus* L., and sharp snout sea bream, *Diplodus puntazzo* C, *Aquaculture*, 253 (2006) 64–70.

Tytler, P. and Hawkins, A.D., Vivisection, anaesthetics and minor surgery, in: Hawkins, A.D. (ed.), Aquarium Systems, Academic Press, London, 1981.

Vivien, J.H., Contribution a l'etude de la physiologie hypophysaire dans les relations avec l'appareil genital, la tyroide et les corps supra-arenaux chez les

poissons selaciens et teleosteens, *Bulletin biologique de la France et de la Belgique*., 75 (1941) 257–309.

Wagner, E., Arndt, R. and Hilton, B., Physiological stress responses, egg survival and sperm motility for rainbow trout broodstock anesthetized with clove oil, tricaine methanesulfonate or carbon dioxide, *Aquaculture*, 211 (2002) 353–366.

Walsh, C.T. and Pease, B.C., The use of clove oil as an anaesthetic for the longfinned eel *Anguilla reinhardii* (Steindachner), *Aquac. Res*., 33(8) (2002) 627–635.

Waterstrat, P.R., Induction and recovery from anaesthesia in Channel catfish *Ictalurus punctatus* fingerlings exposed to clove oil, *J. World Aquac. Soc*., 30(2) (1999) 250–255.

Webster, J., The swimbladder as a hydrostatic organ in the Northern Pike. *Esox luscius* (L). PhD thesis, University of Stirling, 1983.

Wedemeyer, G., Stress-induced ascorbic acid depletion and cortisol production in two salmonid fishes, *Comp. Biochem. Physiol.*, 29 (1969) 1247–1251.

Weyl, O., Kaiser, H. and Hecht, T., On the efficacy and mode of action of 2-phenoxyethanol as an anaesthetic for goldfish, *Carassius auratus* (L.), at different temperatures and concentrations, *Aquac. Res*., 27 (1996) 757–764.

Ross, L.G., Restraint, anaesthesia and euthanasia, in: Widgoose, William (ed.), BSAVA Manual of Ornamental Fish, 2nd ed., BSAVA, Gloucester, UK, 2001, 75–84.

Vartak, V.R., Ghughuskar, M.M. and Singh, R.K., Effect of clove oil as an anaesthetic on the fry of fishes, Silver dollar (*Metynnis schreitmuelleri* Ahl), Sword tail (*Xiphophorus helleri* Heckel) and Pearl gourami (*Trichogaster leeri* Bleeker), *Appl. Fisheries Aquac*., 2 (2002) 7–9.

Wagner, G.N., Singer, T.D. and Scott Mckinley, R., The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum), *Aquac. Res*., 34 (2003) 1139–1146.

Wood, E.M., Urethane as a carcinogen, *Prog. Fish Culturist.*, 18 (1956) 135.

Woolsey, J., Holcomb, M. and Ingermann, R.L., Effect of temperature on clove oil anesthesia in steelhead fry, *N. Am. J. Aquac.*, 66 (2004) 35–41.

Yamamitsu, S. and Itazawa, Y., Effects of an anesthetic 2-phenoxyethanol on the heart rate, ECG and respiration in carp, *Bull. Jpn. J. Sci. Fisheries*, 54(10) (1988) 1737–1746.

Chapter 9

Anaesthesia of Fish: II. Inhalation Anaesthesia Using Gases

Introduction

Gaseous anaesthesia as such is virtually impossible in fish. The primary filaments and secondary lamellae of the gills collapse and fold when held out of water, which increases the diffusion distance and decreases the exchange area so much as to render them virtually ineffective (Alexander, 1967). Only in fish which are capable of aerial respiration, such as catfish which have modified structures for gas absorption which do not collapse in air, one-gilled eels, the Symbranchidae, and fish with modified lungs, could gaseous anaesthesia be considered feasible. It should be noted, however, that even in these cases it has not been tried or tested. Consequently, only those gases which are relatively soluble in water can be considered for most fish.

Carbon dioxide

The anaesthetic properties of carbon dioxide, CO_2, are well documented and it has been used as an anaesthetic with almost every animal phylum for many years. It is a colourless, odourless, non-flammable gas which is extremely soluble in water (880 $cm^3\ L^{-1}$) making it a useful alternative for aquatic animals. The anaesthetic technique simply involves bubbling the gas into the medium. It is to some extent inconvenient in that appropriate cylinders and valves may be required and, furthermore, the final concentration of CO_2 in the medium may be difficult to control accurately.

It is an effective anaesthetic in fish (McFarland and Klontz, 1969) but had, until recently, only seen occasional use as a sedative for transportation (Leitritz and Lewis, 1980). Induction and recovery times are relatively long and anaesthesia is shallow in comparison to other drugs (Anderson *et al.*, 1997). In recent years, however, it has been widely

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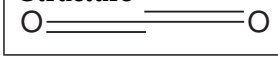
adopted for use in fisheries and aquaculture, particularly in the intensive salmon culture industry where it is used for bulk 'bleeding' of animals to be harvested for smoking. It is popular in these applications because it is gaseous and leaves no tissue residues; consequently, it can be used on animals entering the human food chain without any withdrawal period.

Synonyms
carbon dioxide
carbonic anhydride
Dry ice
carbonic acid gas
dioxidocarbon
Carbonica
Carbon oxide
carboxyl radical
after-damp
methane, dioxo-

IUPAC name
Carbon dioxide

Molecular formula
CO_2

Structure


Interest in carbon dioxide as an anaesthetic, particularly for transportation, increased during the 1980s prompting more work on its mode of action in fish. Mitsuda *et al.* (1988) described its use and potential for anaesthesia of carp, noting that its practical effects compared favourably with those of MS222 and quinaldine. Yoshikawa *et al.* (1991) found that carp, *Cyprinus carpio*, were anaesthetised to stage II or beyond when the pCO_2 of the water was 125 mm Hg or more. Induction took 12 minutes and reduced as the pCO_2 increased to 200 mm Hg. Mean recovery times were from 8 to 22 minutes. Takeda and Itazawa (1983) found that the method was effective but showed that, for fully controlled anaesthesia, the pO_2 (partial pressure of oxygen) needed to be maintained at a high level (400–480 mm Hg) when sedating carp at a pCO_2 of 95–115 mm Hg (1 standard atmosphere = 760 mm Hg). They concluded that precise control of the technique was somewhat impractical because both dissolved oxygen and carbon dioxide levels required to be controlled.

Safety and Toxicology Advice

- Toxic by inhalaton.
- Only use in open spaces with full ventilation.

In general, carbon dioxide anaesthesia is effective in fingerling *Oncorhynchus mykiss* at 120–150 mg L^{-1} and in adults at 200–250 mg L^{-1}. Erikson *et al.* (1997) used CO_2 for preslaughter anaesthesia of *Salmo salar* and noted that rapid anaesthesia was obtained at levels of 284 mm Hg at 7°C.

Exposure of aquatic animals to carbon dioxide in water causes acidosis and a reduction in blood pH. Iwama *et al.* (1991) showed that hyperactivity and subsequent stress experienced when using carbon dioxide anaesthesia in trout could be reduced by buffering the water with sodium bicarbonate. Mitsuda *et al.* (1988) showed that carbon dioxide anaesthesia had a greater disruptive effect on the carp ECG than MS222, producing an extended QRS complex.

There is little doubt that, although it is widely used for harvesting, controlled carbon dioxide anaesthesia is more difficult to achieve than when using other drugs.

Industrial scale anaesthesia using carbon dioxide is known to reduce flesh quality because of the hyperactivity induced in a hypoxic environment (MacKinlay *et al.*, 1994; Roth, 1997). Kiessling *et al.* (2004) used carbon dioxide in combination with iso-eugenol for slaughter of Atlantic salmon and found that the product was firmer, paler and had a higher pH than when stunned with carbon dioxide alone. Erikson *et al.* (2006) outline the successful use of carbon dioxide in combination with chilling as a stunning method for commercial slaughter of Atlantic salmon and considered that this approach gave a superior product. Bretzinger (2001) noted that rainbow trout subjected to carbon dioxide anaesthesia exhibited signs of dyspnoea and aversive reactions until they had been exposed to the anaesthetic for 12 minutes.

It is important to note that the nature of the anaesthesia, and particularly the analgesia, produced with carbon dioxide in these industrial circumstances is the subject of some doubt and continuing debate.

Carbon dioxide from sodium bicarbonate

When dissolved in water, sodium bicarbonate releases carbon dioxide slowly, but will do so much more rapidly if the pH is acidic.

$$NaHCO_3 = NaOH + CO_2\uparrow$$

The use of this carbon dioxide as an anaesthetic is a relatively cheap technique and the use of Alker–Seltzer tablets in water as a source of carbon dioxide is a well-known practice with individual fish. Booke *et al.* (1978) investigated the use of sodium bicarbonate ($NaHCO_3$) as a fish anaesthetic. They found that it was more effective when in water with a slightly low pH. Sodium bicarbonate concentrations of 640 mg L^{-1} at pH 6.5 were most effective, inducing sedation in *Oncorhynchus mykiss*, *Salvelinus fontinalis* and *Cyprinus carpio* within 5 minutes. Locomotion stopped and ventilation rate decreased markedly while equilibrium was maintained. They considered that this sedative

effect resulted from the slow release of CO_2 at a rate controlled by the environmental pH. However, Nagaraj (1990) notes that the inconvenience of adjusting and maintaining the required pH in large water volumes, so as to control the rate of CO_2 release, probably makes other methods more attractive.

Carbon dioxide released from mixtures of sodium bicarbonate and sulphuric acid was advocated for short-duration anaesthesia by Fish (1942).

$$2NaHCO_3 + H_2SO_4 = Na_2SO_4 + 2H_2O + 2CO_2\uparrow$$

He noted that a concentration of 200 ppm for *Oncorhynchus nerka* at 7–10°C produced anaesthesia within 90 seconds of exposure to the gas. They could be safely held in this solution for 5 minutes and recovered 10 minutes after neutralisation of the solution with sodium carbonate to remove the carbon dioxide. Post (1979) also showed that carbon dioxide released from carbonic acid (H_2CO_3) produced by deliberate acidification of sodium bicarbonate solutions was a safe, convenient and easily obtainable anaesthetic for fish. Furthermore, he noted that this is not a controlled substance in the USA and may not require FDA registration for use with food organisms. He described a convenient procedure for producing carbonic acid by adding equal volumes of 6.57% (w/v) sodium bicarbonate and 3.95% (w/v) concentrated sulphuric acid (97–98%) to a known volume of water. He found that baths containing 150–600 mg L^{-1} of carbonic acid were effective and that higher concentrations induce deeper anaesthesia. Hseu *et al.* (1995) used 675 ppm sodium bicarbonate with 395 ppm sulphuric acid–induced anaesthesia of black porgy in 6 minutes with no subsequent mortalities up to 5 days. Prince *et al.* (1995) describe the generation of concentrations of between 195 and 328 mg L^{-1} carbon dioxide from 40 g sodium bicarbonate with 15 cm^3 glacial acetic acid in 30 L of water. Using this technique they achieved surgical anaesthesia of adult Atlantic salmon.

Mishra *et al.* (1983) showed that carbonic acid was an effective sedative for *Labeo rohita* and that fry could be maintained for long periods with very low mortality, significantly better than unanaesthetised controls. Kumer *et al.* (1986) reported successful use of this technique with sub-adult (15 g) Indian major carps, *Catla catla, Labeo rohita* and *Mrigal calabasu* at doses up to 600 mg L^{-1}. However, heavy mortality was noted with fry above 400 mg L^{-1} and the safety margin for fry was very small. Sedation was demonstrated after 5 minutes and anaesthesia after 10 minutes of exposure. At 450 mg L^{-1} the fish could be maintained in the bath for up to 4 hours with 100% survival, although some animals began to revive after this time.

Sodium bicarbonate at 1 g L^{-1} has also been used in combination with lignocaine to give a more stable response (Carrasco *et al.*, 1984; Rivera Lopez *et al.*, 1991).

Overall, these techniques appear useful, and quite inexpensive, providing that some control of pH can be maintained. Because of the sedation it can induce at lower concentrations, sodium bicarbonate is especially useful in transportation. The range of effective dose rates are shown in Table 9.1.

Table 9.1 Summary of dose rates for carbon dioxide and sodium bicarbonate.

Species	Dose	Author
Carbon dioxide		
Cyprinus carpio	125–200 mm Hg 95–115 mm Hg CO_2 + 400–480 mm Hg O_2	Yoshikawa *et al.*(1991) Takeda and Itazawa (1983)
Onchorhynchus mykiss	125 mm Hg 220–275 mg L^{-1}	Bernier and Randall (1998) Wagner *et al.*(2002)
Salmo salar	37–80 mg L^{-1} + 8–10°C cooling	Erikson *et al.*(2006)
Sodium bicarbonate		
Acanthopagrus schlegeli	675 ppm $NaHCO_3$ + 395 ppm H_2SO_4	Hseu *et al.* (1997)
Adult salmon	0.9 g L^{-1}	Booke *et al.*(1978)
Cyprinus carpio	0.64 g L^{-1} $NaHCO_3$	Booke *et al.*(1978)
Oncorhynchus mykiss	@ pH 6.5	
Salvelinus fontinalis *Gadus morhua*		
Labeo rohita	500 ppm	Mishra *et al.*(1983)
Liza parsia	4g L^{-1}	Sonawane and Kulkarni (2001)
Micropterus dolomieui	2.66 g L^{-1}	Peake (1998)
Acipenser fulvescens *Esox lucius* *Stizostedion vitreum* *Oncorhynchus mykiss*		
Fish	150–600 mg L^{-1}	Post 1979

Fluorinated hydrocarbons

These are a range of anaesthetic gases used widely in human and animal medicine. The principal members of this group are

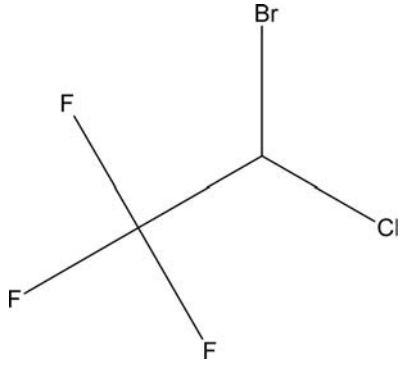
halothane (i.e. fluothane), enflurane (i.e. ethrane) and methoxyflurane (i.e. penthrane). All are potent anaesthetics acting on the CNS and there is some evidence that these compounds work by blocking ion transport, particularly potassium, across cell membranes. These compounds are normally available in pure liquid form and need to be vapourised in a special apparatus at the time of use. Halothane and enflurane have relatively low boiling points of 50°C and 56.5°C, respectively, while that of methoxyflurane is much higher at 105°C.

Synonyms
Halothane
Fluothane
Rhodialothan
Fluorotane
Ftorotan
Ftuorotan
Halotano
Halothan
Narcotan
Narcotane

IUPAC name
2-bromo-2-chloro-1,1,1-trifluoroethane

Molecular formula
$C_2HBrClF_3$

Structure



The best-known anaesthetic of this family, halothane, is a non-flammable, halogenated, hydrocarbon anaesthetic that in higher vertebrates provides relatively rapid induction with little or no excitement. Analgesia may not be adequate and because halothane may not produce sufficient muscle relaxation, supplemental neuromuscular blocking agents may also be required. It has been demonstrated to produce effective anaesthesia in fish (J. Langdon, personal communication) using dose levels of 0.5–2.0 ml L^{-1} added directly to the water. Alternatively, the gas can be vapourised and then dissolved in water to achieve the desired effect. Induction is dose-related and rapid with good surgical anaesthesia being provided and excellent maintenance and rapid recovery (2–5 min). Ghion (1975) successfully anaesthetised *Dicentrarchus labrax* and *Sparus auratus* for up to 8 hours using halothane at 40 ppm. The gas was bubbled into water and narcosis was achieved in 10 minutes.

The gas is, however, relatively insoluble in water and consequently the technique is difficult to control and there is also the risk that fish can receive a fatal dose of pure halothane if the liquid is added directly to the water.

Safety and Toxicology Advice

- Eye irritant.
- Harmful if inhaled, swallowed or in contact with skin.
- May be carcinogenic.
- Use safety glasses with good ventilation.

Although effective, this drug and its technique of application is probably only of minor interest in aquatic animals. Halothane is also relatively expensive. The range of effective dose rates are shown in Table 9.2.

Table 9.2 Summary of dose rates for fluorinated hydrocarbons.

Species	Dose	Author
Onchorhynchus mykiss	0.5–2.0 ml L^{-1} halothane	Langdon (personal communication)
Dicentrarchus labrax *Mugil cephalus* *Sparus auratus*	40 ppm halothane	Ghion (1975)

Summary

Reversible anaesthesia using gases is possible in fish, but of relatively minor importance. There are two important exceptions to this. Carbon dioxide, released from simple chemical reactions, can be a useful, relatively low-cost alternative in isolated locations or when other drugs are unobtainable. The most significant use of gases to sedate fish is during the bleeding process at harvest of cultured salmon for smoking. The fish are temporarily anaesthetised using carbon dioxide, the gills are cut and the animals then bleed to death. The anaesthesia and analgesia provided are unclear and likely to be minimal and so the ethics of this procedure are under scrutiny.

References

Alexander, R. Mc N., Functional Design in Fishes, Hutchinson, London, 1967.

Anderson, W.G., McKinley, R.S. and Colavecchia, M., The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance, *N. Am. J. Fish. Manage.*, 17 (1997) 301–307.

Bernier, N.J. and Randall, D.J., Carbon dioxide anaesthesia in rainbow trout: effects of hypercapnic level and stress on induction and recovery from anaesthetic treatment, *J. Fish Biol*., 52 (1998) 621–637.

Booke, H.E., Hollender, B. and Lutterbie, G., Sodium bicarbonate, an inexpensive fish anesthetic for field use, *Prog. Fish Culturist*, 40 (1978) 11–13.

Bretzinger, Ch., Influence of different pre-slaughter stunning methods on stress reaction and product quality of rainbow trout (*Oncorhynchus mykiss*), *Hieronymus, Muenchen (FRG)*, (2001) 189 p.

Carrasco, S., Sumano, H. and Navarro-Fierro, R., The use of lidocaine-sodium bicarbonate as anaesthetic in fish, *Aquaculture*, 41 (1984) 395–398.

Erikson, U., Hultmann, L. and Steen, J.E., Live chilling of Atlantic salmon (*Salmo salar*) combined with mild carbon dioxide anaesthesia, *Aquaculture*, 252 (2006) 183–198.

Erikson, U., Sigholt, T. and Seland, A., Handling stress and water quality during live transportation and slaughter of Atlantic Salmon (*Salmo salar*), *Aquaculture*, 149 (1997) 243–252.

Fish, F.F., The anaesthesia of fish by high carbon dioxide concentrations, *Trans. Am. Fish. Soc*., 72 (1942) 25–27.

Ghion, F., First promising results in the use of fluothane as general anesthetic for fish, *Riv. Ital. Pisicic. Ittiopathol*, 10 (1975) 111–112.

Hseu, J.R., Yeh, S.L., Chu, Y.T. and Ting, Y.Y., Application of sodium bicarbonate and sulfuric acid for anesthetization of black porgy *Acanthopagrus schlegeli*, *J. Taiwan Fish. Res*., 3 (1995) 151–159.

Iwama, G.K., Yesaki, T.Y. and Ahlborn, D., The refinement of the administration of carbon dioxide gas as a fish anaesthetic.: The effects of varying the water hardness and ionic content in carbon dioxide anaesthesia, ICES Council meeting papers, ICES-CM-1991-F27, 1991, 29 p.

Kiessling, A., Espe, M., Ruohonen, K. and Morkore, T., Texture, gaping and colour of fresh and frozen Atlantic salmon flesh as affected by pre-slaughter iso-eugenol or CO_2 anaesthesia, *Aquaculture*, 236 (2004) 645–657.

Kumer, D., Mishra, B.K., Dey, R.K. and Biswas, B., Observations on the efficacy of carbonic acid as anesthetic of Indian major carps, Network of Aquaculture Cent. in Asia, Bhubaneswar (India), CIFA paper NACA/WP/86/39 (NACAWP8639), 1986, 8 p.

Leitritz, E. and Lewis, R.C., Trout and salmon culture (hatchery methods), Californian Fisheries Bulletin, No. 164, University of California, 1980, 197 p.

MacKinlay, D.D., Johnson, M.V.D. and Celli, D.C., Evaluation of stress of carbon dioxide anaesthesia, Fish Physiology Association, Vancouver, Canada, 1994, 421–424.

McFarland, W.N. and Klontz, G.W., Anesthesia in fishes, *Fed. Proc*., 28 (4) (1969) 1535–1540.

Mishra, B.K., Kumar, D. and Mishra, R., Observations on the use of carbonic acid anaesthesia in fish fry transport, *Aquaculture*, 32 (3–4) (1983) 405–408.

Mitsuda, H., Ishida, Y., Yoshikawa, H. and Ueno, S., Effects of a high concentration of CO_2 on electrocardiograms in the carp, *Cyprinus carpio*, *Comp. Biochem. Physiol*., 91A (4) (1988) 749–757.

Mitsuda, H., Nakajima, K., Mizuno, H., Kawai, F. and Yamamoto, A., Effects of carbon dioxide on carp, *J. Nutr. Sci. Vitaminol*., 26 (1) (1980) 99–102.

Nagaraj, G., Biotechnical considerations in the handling and transport of live fishery products, in: Handling and Processing: The Selling Point in Malaysia Fisheries, Occasional Paper No 3, Malaysian Fisheries Society, Kuala Lumpur, Malaysia, 1990.

Peake, S., Sodium bicarbonate and clove oil as potential anesthetics for non-salmonid fishes, *North Am. J. Fish. Manage*., 18 (1998) 919–924.

Post, G., Carbonic acid anaesthesia for aquatic organisms, *Prog. Fish Culturist*., 41 (3) (1979) 142–144.

Prince, A.M.J., Low, S.E., Lissimore, T.J., Diewert, R.E. and Hinch, S.G., Sosium bicarbonate and acetic acid: an effective anesthetic for field use, *N. Am. J. Fish. Manage*., 15 (1) (1995) 170–172.

Roth, B., The use of electricity as an anesthetic within the slaughtering process of Atlantic salmon (*Salmo salar*), University of Bergen, Department of Fisheries and Marine Biology, Bergen, Norway, 1997, 19.

Sonawane, U.D. and Kulkarni, G.N., Anaesthetic effects of clove oil and sodium bicarbonate on the fry of *Liza parsia*, *Iranian J. Fish. Sci.*, 3 (2001) 49–62.

Takeda, T. and Itazawa, Y., Possibility of applying anesthesia by carbon dioxide in the transportation of live fish, *Bull. Jpn. Soc. Sci. Fish*., 49 (5) (1983) 725–731.

Wagner, E., Arndt, R. and Hilton, B., Physiological stress responses, egg survival and sperm motility for rainbow trout broodstock anesthetized with clove oil, tricaine methanesulfonate or carbon dioxide, *Aquaculture*, 211 (2002) 353–366.

Yoshikawa, H., Yokoyama, Y., Ueno, S. and Mitsuda, H., Electroencephalographic spectral analysis in carp, *Cyprinus carpio,* anesthetized with high concentrations of carbon dioxide, *Comp. Biochem. Physiol*., 98A (3–4) (1991) 437–444.

Chapter 10

Anaesthesia of Fish: III. Parenteral and Oral Anaesthesia

Introduction

In some circumstances, particularly for surgery or lengthy procedures, anaesthesia by injection of drugs may be considered. These techniques require slightly less specialised artificial ventilation apparatus than that required for inhalation anaesthesia as it is not necessary to recirculate an anaesthetic solution. Usually the fish are rapidly sedated by inhalation anaesthesia to minimise handling stress, then the animal is weighed, the dose calculated and then injected. Thus, unless the initial handling of the animal is done without anaesthesia, two drugs would usually be involved. Drugs can also be given orally, although the result is much less satisfactory.

Routes for administration of drugs by injection

Depending on the size of the fish, injection may be intraperitoneal, intravascular or intramuscular. The location of these access points is shown in Fig. 10.1, although not all these routes of access will be easily available in every species and some are clearly size-dependent.

Intraperitoneal injection is the most common and the use of a short, narrow gauge needle ventral to the lateral line will ensure access to the peritoneal cavity. This simple method involves absorption of the drug through visceral blood vessels and induction may consequently be slow.

In larger fish, intravascular injection may be possible and, by using appropriate needles, access may be gained to certain fin sinuses, the orbital sinuses, the caudal artery and vein, the dorsal or ventral aortas or even the heart. The fin sinuses are more effective access points in larger animals. One problem with the caudal artery and vein is that, although the procedure may be successful, some damage may be done to the blood vessels in the process and this may be considered

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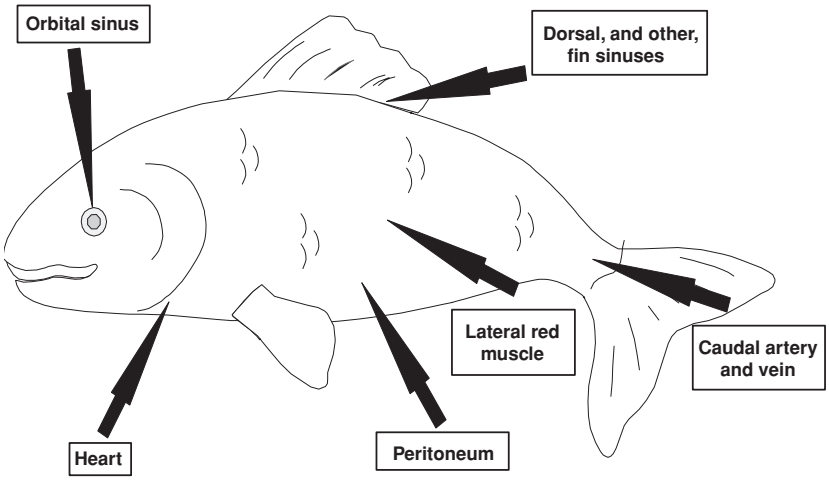


Figure 10.1 Injection sites available for parenteral administration of drugs. Note that not all sites will be available in a given species.

undesirable. However, repeated blood sampling by this route does not appear to cause any problems in fish of 100 g or more. The heart is not a recommended route for inexperienced workers.

Small drug volumes can be very rapidly absorbed if introduced into the well-vascularised red lateral muscle but this technique is only possible in those species that have a substantial block of red muscle (Fig. 10.2). The quantity of red muscle is related to the lifestyle of the fish: more active animals have more red muscle. A narrow gauge needle should be introduced into the muscle at an oblique angle to avoid misplacement of the drug by entering the underlying mosaic, or white, muscle which is poorly vascularised. The approximately 10 times greater blood flow normally found in red muscle ensures rapid movement of drug from the site and hence relatively rapid induction. As in humans and terrestrial animals, precautions must be taken to prevent reflux of the drug from the injection site and this can be simply achieved by holding a finger on the injection point for a few seconds until the drug has been transported away from the site. Injection into the lateral musculature of fish is shown in Fig. 10.3.

Effective injectable anaesthetic drugs

Although numerous drugs are known to be effective, there have been very few systematic investigations of injectable fish anaesthetics, the principal work having been done by Oswald (1978). Most injectable

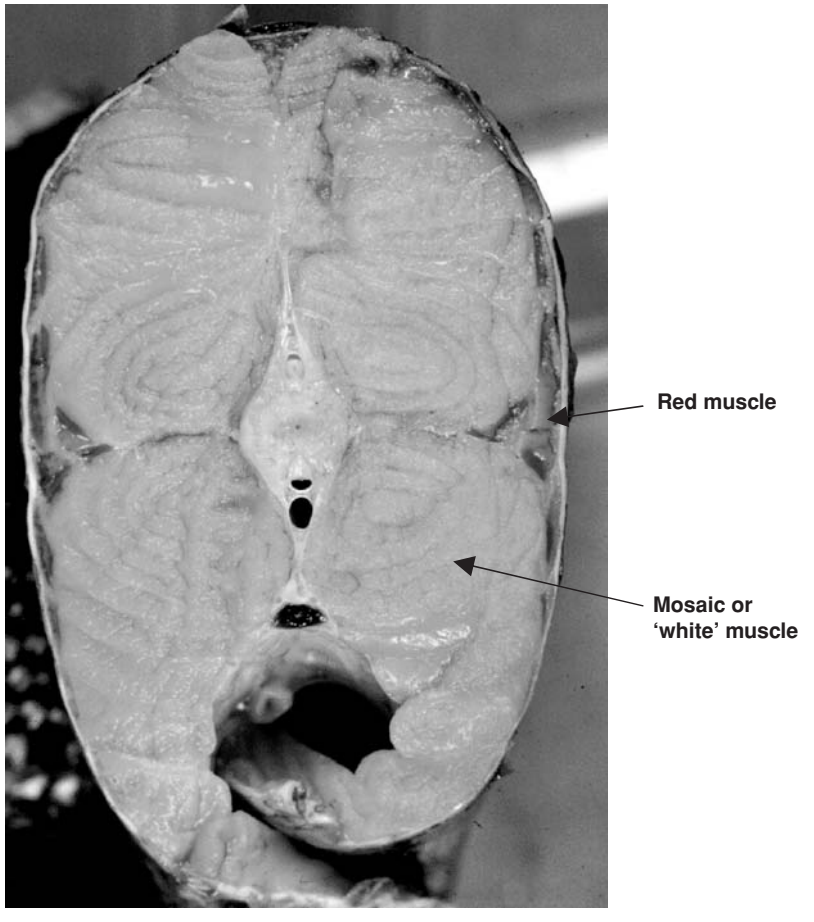


Figure 10.2 The location of red muscle in Atlantic salmon. More active species have more red muscle while sedentary species may have very little.

drugs used with fish originate from veterinary or human medicine and those of major interest are summarised in Table 10.1.

Widely used drugs for injection anaesthesia

From the range of drugs tested, effective practical anaesthesia by injection is probably limited to only a few materials. It is interesting to note that neither benzocaine nor MS222 are effective when given parenterally, possibly because the rate of uptake from the site of injection is effectively lower than the gill clearance rate. The following discussion is limited to those materials known to be used most widely.

Table 10.1 A compilation of drugs effective in inducing anaesthesia in fish when administered parenterally.

Name	Trade name	Characteristic
Alphaxolone–alphadolone	Saffan	Very good; long duration
Propanidid	Epontol	
Ketamine–HCI	Vetalar	
Xylazine–HCI	Rompun	
Etorphine/acetylpromazine	Immobilon	Immobilon and Revivon are used
Diprenorphine–HCI	Revivon	Sequentially
Sodium pentobarbitone	Nembutal	Long duration
Sodium amylobarbitone	Amytal	
Sodium methohexitone	Brevital	Short duration
Sodium thiopentone	Pentothal	
Lignocaine–HCI	Lidocaine	Spinal anaesthesia only
Procaine–HCI		Local analgesic only

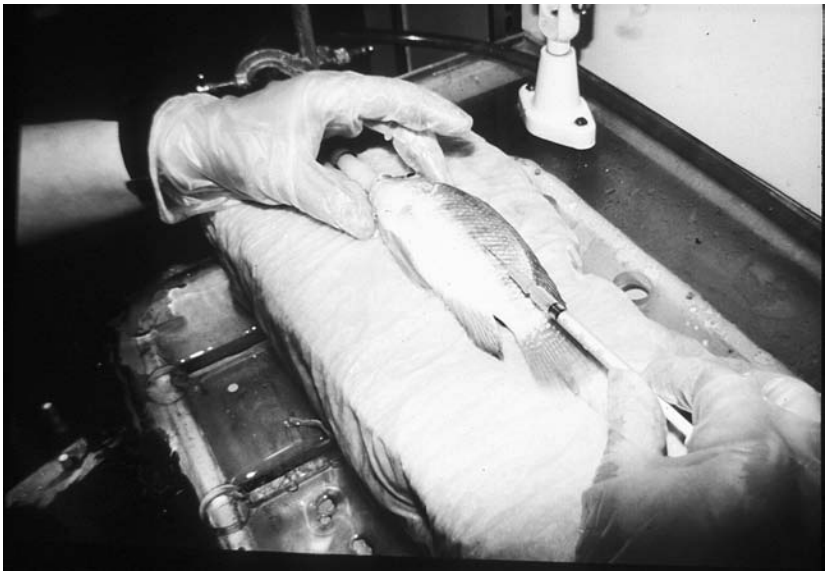


Figure 10.3 Injection of the anaesthetic Saffan into the red muscle of a tilapia, prior to a lengthy procedure. Note that injection is from the rear to allow insertion of the needle under the scales.

Alphaxolone–alphadolone (Saffan)

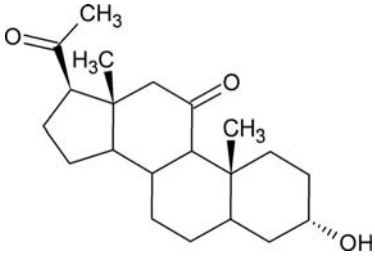
This is a 3:1 mixture of alphaxalone with alphadolone acetate which has been used as a general anaesthetic in veterinary practice. It is an excellent drug for long-term anaesthesia and in recent years has been adopted by some fish neurophysiologists because spontaneous nervous activity is essentially preserved. Its main advantage lies in its

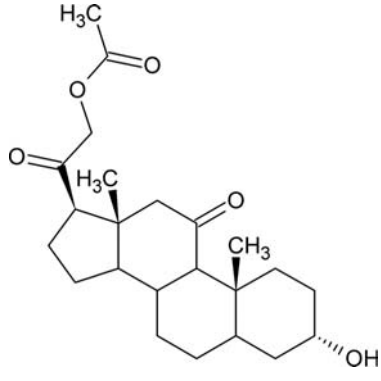
stimulatory effect upon the heart, heart beat becoming very regular and forceful. In addition, it has a general vasodilatory effect both systemically and peripherally, in contrast to the aminobenzoates (Soivio *et al.*, 1977). This effect is very obvious at the gills and this ensures adequate oxygenation of the blood.

Synonyms
Alfathesin
Alphadione
Alfadione
Aurantex
Saffan
Alfatesine (French)
ALTHESIN
CT-1341

IUPAC name
(3*R*,5*S*,8*S*,9*S*,10*S*,13*S*,14*S*,17*S*)-17-acetyl-3-hydroxy-10,13-dimethyl-1,2,3,4,5,6,7,8,9,12,14,15,16,17-tetradecahydrocyclopenta[a]phenanthren-11-one; [2-[(3*R*,5*S*,8*S*,9*S*,10*S*,13*S*,14*S*,17*S*)-3-hydroxy-10,13-dimethyl-11-oxo-1,2,3,4,5,6,7,8,9,12,14,15,16,17-tetradecahydrocyclopenta[a]phenanthren-17-yl]-2-oxo-ethyl] acetate
Molecular formula
$C_{44}H_{66}O_8$

Structure





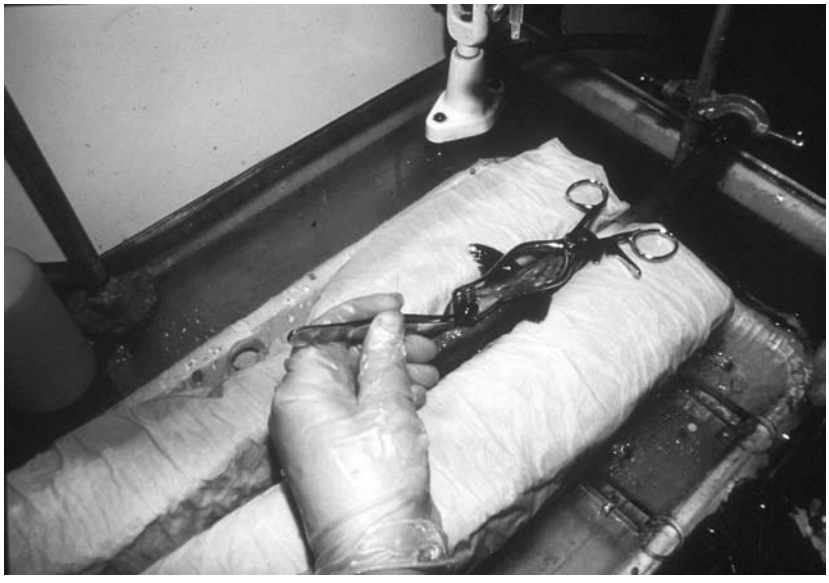


Figure 10.4 Estimation of blood volume in the author's laboratory following alphaxolone–alphadolone (Saffan) anaesthesia.

Safety and Toxicology advice

- Presents little or no hazard to man or the environment in normal conditions of use.
- Potent, so avoid self-injection.
- Use gloves

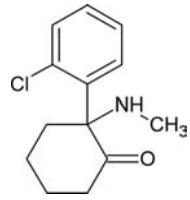
At low doses (12 mg kg^{-1}) it is possible to maintain respiration and circulation at approximately basal level in the cod, *Gadus morhua* (Tytler and Hawkins, 1981). Yacoob *et al*. (2004) anaesthetised Atlantic cod with an intraperitoneal injection of 12 mg kg^{-1} Saffan for studies of olfactory sensitivity. At higher doses, however (over 24 mg kg^{-1} in rainbow trout) ventilation becomes reciprocatory and may be abolished altogether (Oswald, 1978). Consequently, it is always advisable to anticipate ventilatory arrest. Neuromuscular preparations give consistent results over a long period using this anaesthetic. Peters and Denizot (2004) used immersion in 2 mg L^{-1} Saffan to enable studies of electroreceptors in *Gnathonemus petersii*. It can also be extremely valuable in studies of the cardiovascular system and in work with saithe (*Pollachius virens*) it gave stable, long-term anaesthesia with well-maintained cardiac rhythm. This resulted in steady blood flow to the gills and other organs (Ross, unpublished data) (Fig. 10.4). Oswald (1978) noted that there was slight excitement during recovery, but that it was essentially uneventful. Residual neuromuscular blocking effects commonly found in barbiturate anaesthesia are absent.

Ketamine (Vetalar)

Synonyms
ketamine
Ketaject
Ketaset
dl-Ketamine
Ketanest
Ketoject
Ketalar
Ketolar

IUPAC name
2-(2-chlorophenyl)-2-methylamino-cyclohexan-1-one
Molecular formula
$C_{13}H_{16}ClNO$

Structure



Ketamine is available either as a white powder or as a ready-made injectable solution in saline. It has anaesthetic and analgesic properties and has been widely used in human and veterinary medicine. Oswald (1978) found that anaesthesia persisted for only 20 minutes when rainbow trout and brown trout were injected intra-muscularly with ketamine at 130 mg kg^{-1} but sleep times of 50–80 minutes were obtained at 150 mg kg^{-1}. Recovery time was prolonged, taking up to 90 minutes, during which there was notable hyperactivity and ataxia. Bruecker and Graham (1993) induced anaesthesia in the cichlid *Heros citrinellum* after injection of 30 mg kg^{-1} ketamine into the dorsal aorta or caudal vein. Induction was very fast with considerable reduction in ventilation after 1 minute. Anaesthesia lasted for up to 40 minutes and recovery required up to 4 hours. The safety margin was reduced at higher temperatures. Graham and Iwama (1990) successfully used ketamine at 30 mg L^{-1} in *Oncorhynchus mykiss* and *O. kisutch*.

Safety and Toxicology advice

- May be harmful if swallowed.
- Irritating to eyes, skin and respiratory tract.
- In case of contact, rinse with water and seek advice.
- Subject to drug controls in the UK, the USA and elsewhere.

Smith (1992) describes successful use of 12–20 mg kg^{-1} ketamine in combination with xylazine (Rompun) with grey nurse sharks, *Carcharias taurus*. Induction took 10–20 minutes for a sleep time of 10–20 minutes. Williams *et al.* (2004) found that ketamine could be used in combination with the analgesic and sedative medetomidine (4-[1-(2,3-dimethylphenyl)ethyl]-3*H*-imidazole) in bonitos (*Sarda chiliensis*) and Pacific mackerel (*Scomber japonica*). IM administration of ketamine at a

dose of 4 mg kg^{-1} and medetomidine at a dose of 0.4 mg kg^{-1} in bonitos and ketamine at a dose of 53–228 mg kg^{-1} and medetomidine at a dose of 0.6–4.2 mg kg^{-1} in mackerels was safe and effective. For both species, administration of atipamezole (4-(2-ethyl-1,3-dihydroinden-2-yl)-3*H*-imidazole) at five times the dose of medetomidine reversed the anesthetic effects (atipamezole competitively inhibits α2-adrenergic receptors, thereby acting as a reversal agent for α2-adrenergic agonists, e.g. medetomidine).

Nembutal (sodium pentobarbitone)

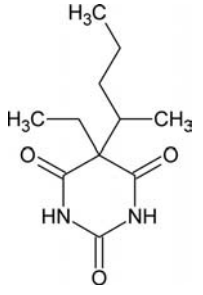
This is a barbiturate, normally used for anaesthesia of short to intermediate duration in mammals. It is effective as a sedative and hypnotic (but not as an anti-anxiety) agent and is usually given orally. It is easily available as a very stable injectable solution sometimes as the sodium salt.

Shelton and Randall (1962) anaesthetised tench, *Tinca tinca,* and roach, *Rutilus rutilus,* using intramuscular injections of 20 mg kg^{-1}. It is effective in rainbow trout and brown trout at higher intraperitoneal doses of 48–72 mg kg^{-1} (Oswald, 1978). Anaesthesia is very lengthy, extending from 6 to 24 hours depending on dose. The main complications are that recovery is very prolonged with persistent ataxia. There is usually intense bradycardia, ventilatory arrest and the drug has some curare-like

Synonyms	**Sodium salt**
pentobarbital	Nembutal
Nembutal	sodium
Mebubarbital	Somnopentyl
Pentobarbitone	Barpental
Dorsital	Biosedan
Ethaminal	Butylone
Nebralin	Carbrital
Rivadorm	Continal
Mebumal	Diabutal
Neodorm	Euthanyl
	Euthatal

IUPAC name
5-Ethyl-5-pentan-2-yl-1,3-diazinane-2,4,6-trione
Sodium 5-ethyl-4,6-dioxo-5-pentan-2-yl-1 *H*-pyrimidin-2-olate
Molecular formula
$C_{11}H_{18}N_2O_3C_{11}H_{17}N_2NaO_3$

Structure



properties. By contrast, in some elasmobranchs Nembutal has been reported to be effective at only 6 mg kg^{-1} but fatal at 60 mg kg^{-1} (Walker, 1972), thus giving a therapeutic index of about 10. Unlike most other anaesthetic agents this drug is not excreted by the gills to any great extent, which may explain its prolonged action. Initial recovery is probably by redistribution within the fish.

Safety and Toxicology advice

- Toxic if swallowed.
- Avoid self-injection.
- Irritating to eyes, skin and respiratory tract.
- In case of contact, rinse with water and seek advice.
- Subject to drug controls in the UK, the USA and elsewhere.

Propanidid (Epontol)

This is a pale yellow liquid which is insoluble in water but soluble in alcohol.

Although doses of 8–9 mg kg^{-1} propanidid are effective in producing very short anaesthesia in mammals (Clarke and Dundee, 1966), Oswald (1978) reported that intraperitoneal doses of 325 mg kg^{-1} were required for effective anaesthesia in rainbow trout and its action lasts for about 2.5 hours – a much longer sleep time than in mammals. By contrast, Siwicki (1984) reported that intraperitoneal injections of 2 mg kg^{-1} induced anaesthesia in salmonids within 2–4 minutes, with recovery in 5–10 minutes.

Synonyms

Epontol	**Propanidide**
Eponthol	Propanidid
Fabantol	Propantan
Fabontal	Sombrevin

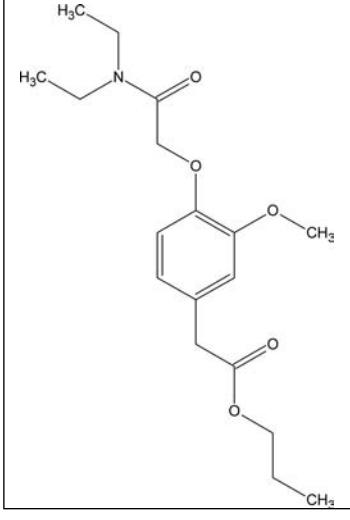
IUPAC name

3-methoxy-4-(*N*,*N*-diethyl-carbamoyl-methoxy)-phenylacetic acid *n*-propyl ester

Molecular formula

$C_{18}H_{27}NO_5$

Structure



Safety and Toxicology advice

- Harmful if swallowed.
- Irritating to eyes, skin and respiratory tract.
- In case of contact, rinse immediately with water.

Its chief advantages are that it does not greatly depress ventilation and has little effect on blood chemistry. Recovery is comparatively uneventful with only a brief ataxic phase and full reaction to stimulation returns after about 1 hour. Oswald (1978) considered that longer sleep times in fish were either due to the intraperitoneal injection route and associated lower clearance rates, or to the lower levels of cholinesterase found in fish plasma.

Less widely used drugs for injection anaesthesia

While the previous section describes the injectable drugs of choice for fish, some other materials have potency and are described briefly here.

Etorphine–acetylpromazine (Immobilon)

Safety and Toxicology advice

- Potent, avoid self-injection or needle scratch.
- MUST be used with the antidote at hand.
- May be addictive, hence controlled drug.

Etorphine is a morphine derivative producing anaesthesia and with an analgesic potency approximately 1,000–3,000 times that of the parent compound. Only 4 mg are required to anaesthetise a 5-tonne elephant. Although binding sites for this drug have been located in the fish brain, as has been found in many other cases, much higher doses of this drug are needed to produce anaesthesia in fish than in mammals. Oswald (1978) found that good anaesthesia was induced in rainbow trout at 8–10 mg kg^{-1}. After up to one hour, the anaesthesia was reversible within 5 minutes by intramuscular injection of equal amounts of the specific antidote diprenorphine (Revivon). Recovery was accompanied by brief ataxia and swimming returned within 6 minutes. A notable point here is that the quantity of Etorphine needed to produce anaesthesia in fish would be fatal to a human. Consequently this drug can only be used with great care, by workers experienced in injection methods and with rapid access to the ready-prepared antidote, diprenorphine (Revivon).

Xylazine (Rompun)

Oswald (1978) tested a range of concentrations of xylazine and found the minimum anaesthetic dose in rainbow trout to be 100 mg kg^{-1}. Ventilatory collapse and convulsions occurred persistently and the ECG was grossly disturbed. Although actually effective, this drug cannot be recommended for use with fish. The only recorded exception to this is the use of xylazine in combination with ketamine with grey nurse sharks (Smith, 1992; above).

Safety and Toxicology advice

- Toxic by inhalation or if swallowed.
- Irritating to eyes, skin and respiratory tract.
- In case of contact, rinse with water and seek advice

Lignocaine (Xylocaine, Lidocaine)

Spinal anaesthesia induced by injection of 0.1 cm^3 lignocaine immobilised rainbow trout in 5 minutes (Oswald, 1978). There was no sensitivity posterior to the injection site and recovery required 45–50 minutes with fish swimming freely after a further 15 minutes.

Safety and Toxicology advice

- Harmful if swallowed.

Procaine (Novocaine)

This is a local anaesthetic agent that has been used to reduce the pain of intramuscular injections in humans and in dentistry. Kisch (1947) produced general anaesthesia lasting for 0.5–1 hour by intracranial injection of procaine. The fish recovered normally.

Safety and Toxicology advice

- Irritating to eyes, skin and respiratory tract.
- In case of contact, rinse with water and seek advice.

Oral anaesthesia: chemicals in food

This is a relatively stress-free method. Murai *et al.* (1979) fed pellets containing the benzodiazepine drug diazepam (Valium) to American shad *Alosa sapidissima* at levels ranging from 0.04 to 0.08 mg kg^{-1} of fish. Diazepam has anxiolytic, sedative and muscle relaxant properties. Although at first sight this method is very attractive there are certain problems, which include the technicalities of incorporating the material uniformly in a specially prepared diet. Furthermore, induction will tend to be slow as the drug is absorbed via the gut and it is not possible to predict accurately the quantity consumed by individuals in the tank.

Safety and Toxicology advice

- Irritating to eyes, skin and respiratory tract.
- May cause allergic reaction.
- In case of contact, rinse with water and seek advice.
- May be addictive.
- Use safety glasses and gloves.

Additives – again

It is important to bear in mind that many commercially available injectable preparations contain additives such as bactericides, detergents, solvents or stabilisers. It is thus necessary to exercise caution so that these components do not damage the gills where recovery is important. For example, both Epontol and Saffan contain the powerful surfactant 'Cremophor EL'. This is known to induce histamine release in dogs but its effect in fish is unknown.

Summary

Effective injection anaesthesia of fish is available, but has the initial disadvantage of requiring immersion or other initial immobilisation to facilitate the injection. Care must then be taken to calculate and administer the dose correctly avoiding loss of material from the injection site. Overall, this method is probably only useful in the physiological laboratory.

References

Bruecker, P. and Graham, M., The effects of the anesthetic ketamine hydrochloride on oxygen consumption rates and behaviour in the fish *Heros (Cichlasoma) citrinellum* (Günther, 1864), *Comp. Biochem. Physiol*., 104C (1993) 57–59.

Clarke, R.S.J. and Dundee, J.W., Survey of experimental and clinical pharmacology of propanidid, *Anaesth. Analg.: Curr. Res*., 45 (1966) 250–276.

Graham, M.S. and Iwama, G., The physiologic effects of of the anaesthetic ketamine hydrochloride on two salmonid species, *Aquaculture*, 90 (1990) 323–331.

Kisch, B., A method to immobilise fish for cardiac and other experiments with Procaine, *Biological Bulletin, Marine Biological Laboratory, Woods Hole, Mass*, 93 (1947) 208.

Murai, T., Andrews, J.W. and Muller, J.W., Fingerling American shad: effect of valium, MS-222, and sodium chloride on handling mortality, *Prog. Fish Culturist*., 41 (1979) 27–29.

Oswald, R.L., Injection anaesthesia for experimental studies in fish, *Comp. Biochem. Physiol*., 60C (1978) 19–26.

Peters, R.C. and Dénizot, J.P., Miscellaneous features of electroreceptors in *Gnathonemus petersii* (Günther, 1862) (Pisces, Teleostei, Mormyri-formes), *Belgian J. Zool*., 134 (2004) 61–66.

Shelton, G. and Randall, D.J., The relationship between heart beat and respiration in teleost fish, *Comp. Biochem. Physiol*., 7 (1962) 237–250.

Siwicki, A., New anesthetic for fish, *Aquaculture*, 38 (1984) 171–176.

Smith, M.F.L., Capture and transportation of elasmobranchs, with emphasis on the grey nurse shark (*Carcharias taurus*), *Aus. J. Marine Freshwater Res.* Special issue: Sharks: Biology and Fisheries, 43 (1992) 325–343.

Soivio, A., Nyholm, K. and Huhti, M., Effects of anaesthesia with MS222, neutralised MS222 and benzocaine on the blood constituents of Rainbow trout, *J. Fish Biol*., 10 (1977) 91–101.

Tytler, P. and Hawkins, A.D., Vivisection, anaesthetics and minor surgery, in: Hawkins, A.D. (ed.), Aquarium Systems, Academic Press, London, 1981.

Walker, M.D., Physiologic and pharmacologic aspects of barbiturates in Elasmobranch, *Comp. Biochem. Physiol*., 42A (1972) 213–221.

Williams, T.D., Rollins, M. and Block, A., Intramuscular anesthesia of bonito and Pacific mackerel with ketamine and medetomidine and reversal of anesthesia with atipamezole, *J. Am. Vet. Med. Assoc*., 225 (2004) 417–421.

Yacoob, S.Y., Browman, H.I. and Jensen, P.A., Electroencephalogram recordings from the olfactory bulb of juvenile (0 year) Atlantic cod in response to amino acids, *J. Fish Biol*., 65 (2004) 1657.

Chapter 11

Anaesthesia of Fish: IV. Non-Chemical Methods

Introduction

It is possible to immobilise fish without the use of chemicals and the two techniques available, namely hypothermia and electro-anaesthesia, may have distinct advantages over chemical methods for certain procedures.

Hypothermia

Lowering the water temperature will tranquilise or immobilise fish (Bell, 1964). Lower temperatures increase the oxygen-carrying capacity of the water and reduce the activity and oxygen consumption of fish. They also reduce general metabolism and hence minimise ammonia and solid waste production. Cooling can be achieved by refrigeration or by the addition of ice or by using dry ice in thermal contact with the water but physically and chemically isolated from it (Solomon and Hawkins, 1981). Although immobilisation is achieved and there is some reduction in sensitivity to stimulation, it must be remembered that true anaesthesia is not likely to be achieved in this way and that it is very unlikely that any true analgesia is achieved.

A given species of fish usually has a fairly wide temperature tolerance. However, the amount of cooling or heating which can be applied instantaneously to a fish depends on its previous temperature history and its acclimation temperature. Thus, although hypothermia can calm and immobilise fish and lower the metabolic rate, the degree of cooling which can be applied in a practical situation may be very limited (Randall and Hoar, 1971). Gradual cooling is preferred as rapid chilling can produce a lethal shock, although for practical purposes rapid chilling will be the preferred method. In addition to immobilising and depressing metabolism, low temperature is also known to cause disruption of osmoregulatory capabilities, which may result in major ion

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imbalances over short periods, resulting in deaths. Randall and Hoar (1971) remarked, "fish can be cooled to about 4°C (NB: this would depend on species and thermal history) to produce a deep narcosis from which recovery is rapid on return to the acclimation temperature". It should be clear that this temperature applies only to temperate animals.

Various authors have used the technique, principally for transportation (Fish and Hanavan, 1948; Anon, 1982). Parker (1939) suggested immersion in crushed ice for 10–15 minutes as a technique for allowing minor procedures and Hawkins (personal communication) has found that adult Atlantic salmon could be cooled to the temperature of iced water, almost 0°C, producing a form of sedation which enabled long-distance transportation of broodstock with no attendant mortalities. Hovda and Linley (2000) exposed adult pink salmon (*Oncorhynchus gorbuscha*) to sub-zero temperatures, from −1.5°C to −6.0°C, achieved using a cooler unit with salt solutions to prevent freezing. Induction time decreased with temperature. They concluded that the technique is effective for short-term handling procedures but that the adverse interaction between hypothermia and osmoregulation may preclude its use in some species.

While simple hypothermia is one of the oldest fish-calming techniques, it is not generally recommended to be used alone. Some attendant mortality has been noted when using hypothermia and it is not clear whether this can be attributed to excessive cooling or the additive action of chemical anaesthetics with which it is often used simultaneously. Yoshikawa *et al.* (1989) found that carp previously acclimated to 23°C could be safely maintained in a state of apparent anaesthesia for 5 hours at 4°C whereas the sedation achieved at 8–14°C could be maintained for 24 hours. The anaesthesia at lower temperatures produced haemorrhaging and deaths in some cases. In practice it was found that an instantaneous temperature decrease of only 6°C can be applied to tilapia fry acclimated to 25°C, greater rates of decrease producing measurable mortalities (Okoye, 1982). When hypothermia was used in conjunction with chemical anaesthesia, the normally effective dose of benzocaine for sedation of tilapia fry (about 30–32 mg L^{-1}) had to be reduced by about 30% to 25 mg L^{-1}. Williamson and Roberts (1981) used cooling after initial MS222 anaesthesia to immobilise dogfish during long surgical procedures. No extra drug was given, electromyographic evidence for blocking of the central nervous system was obtained and eventual recovery was rapid. Ross *et al.* (2007) found that the atherinopsid *Menidia estor* could be cooled from 23°C to 12°C for transportation with no mortalities.

Overall, this technique has good calming properties and allows handling and transportation. When used alone, however, it is not

suitable for any invasive work and almost certainly gives very incomplete analgesia.

Electroanaesthesia

An interesting alternative to chemical anaesthesia is the use of electricity. Direct currents (d.c.) and alternating currents (a.c.) with sinusoidal, triangular and square waveforms are illustrated in Fig. 11.1 and have all been used for induction of anaesthesia at various times. Alternating current and square waves in the form of chopped d.c. have been used in electro-fishing for many years (Vibert, 1967; Cowx and Lamarque, 1990). All four types are effective in freshwater whereas only sine waves or chopped d.c. are fully effective in sea-water. If carefully controlled, all are capable of producing a form of immobilisation in fish (Scheminsky, 1934) but their modes of action differ.

Generally, fish in sea-water are less susceptible to electroanaesthesia. This effect is almost certainly related to body conductivity relative to

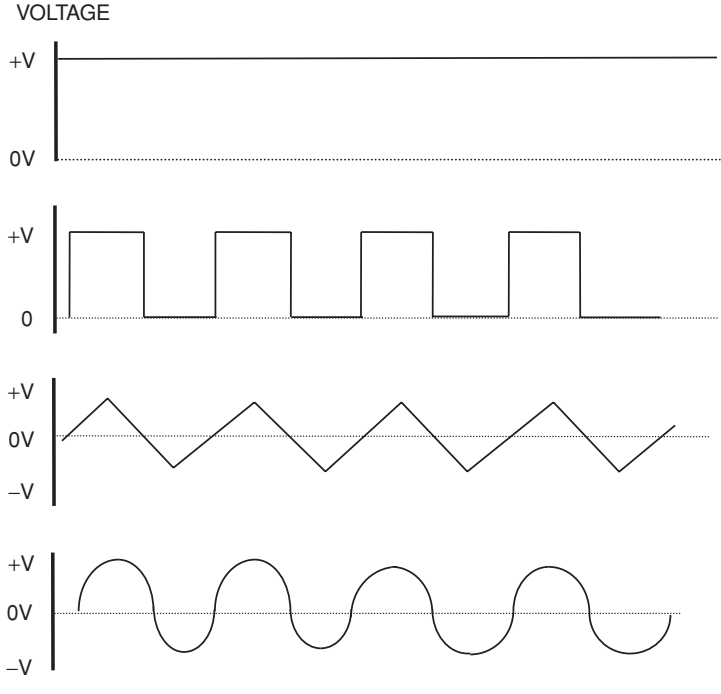


Figure 11.1 Alternating and direct current waveforms. Top to bottom: Direct current (d.c.), square wave (pulsed d.c.), triangular wave a.c., sine wave a.c. Note that square waves are shown here as positive pulses but that pulses can be negative or bidirectional (i.e. alternating).

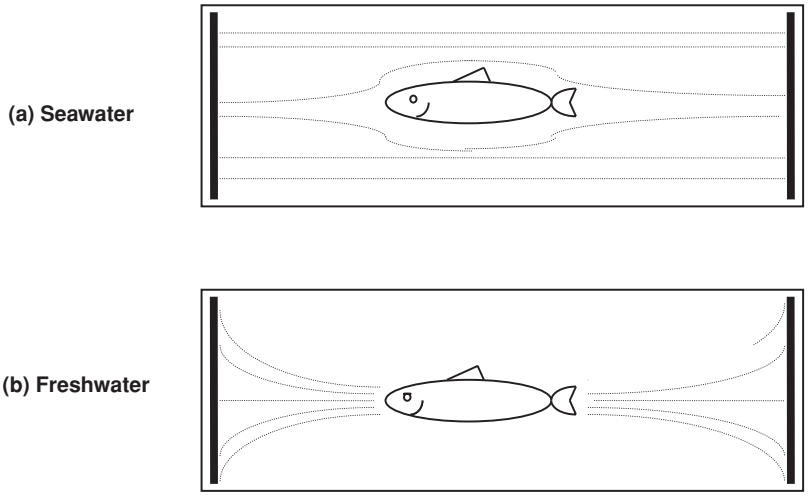


Figure 11.2 Electron flow through and around fish bodies in (a) sea-water (b) fresh-water.

that of the water and is certainly most easily visualised in this way. Because of the high conductivity of the medium, it is more difficult to establish an electric field in seawater which provides an area of effect. Additionally, where the animal is less conductive than the medium (as in saline waters) electrons can flow more easily through the water directly between the electrode plates (Fig. 11.2a). By contrast, in fresh water the fish are more conductive than the medium and the easiest plate-to-plate route for electrons is via the fish (Fig. 11.2b).

The terms *electroanaesthesia*, *electronarcosis*, *galvonarcosis* and *electroimmobilisation* are all used in the literature, sometimes inaccurately, and require some explanation. *Electroanaesthesia* or *electronarcosis* are most often used to describe the effect produced by either a.c.or d.c., whereas, *galvonarcosis* is usually reserved for the transitory immobilising effect produced by d.c. (see below). Both forms produce *electroimmobilisation* but until recently it has not been clear whether either produces real anaesthesia and some therefore consider it prudent to describe both forms as electroimmobilisation. Here, galvonarcosis is used specifically for d.c.-induced effects and electroanaesthesia is used for a.c.-induced effects.

Immobilisation using direct current (d.c.)

This is usually distinguished by the term galvonarcosis (Halsband, 1967). Fish subjected to low-voltage d.c. often undergo a forced

swimming motion towards the anode, but at higher field strengths (>300 mV cm^{-1}) they become immobile and if the field strength is increased further they will undergo tetanic muscle contractions (Lamarque, 1990), a condition which must be avoided if possible. However, this is only effective while the fish are within the electrical field and on turning off the current, or if the fish escapes from the electrical field, it will recover almost immediately.

Kynard and Lonsdale (1975) demonstrated rapid paralysis of yearling rainbow trout subjected to field strengths of 0.6 V cm^{-1} d.c. The fish move initially towards the anode, lose equilibrium and then become immobile in rapid succession (Halsband, 1967). By contrast, tilapias require a d.c. field strength of approximately 3 V cm^{-1} to produce a similar effect (Ross and Ross, 1984). Kolz (1989) immobilised goldfish, *Carassius auratus*, when applied currents exceeded 3 mA and calculated that the minimum electrical power required for d.c. anaesthesia was 20 mW. Recalculation of this data shows that the total intercepted (head to tail) voltage was <1 V cm^{-1} (power = current × voltage) which is quite consistent with Kynard and Lonsdale's results.

Basic galvonarcosis procedure

Power sources for galvonarcosis are usually one or more 12-volt automotive batteries, appropriate cables and a simple immersed electrode pair, or array, often constructed as part of a holding box (Gosset, 1974), net or basket (Sterritt *et al.*, 1994). Electrodes should preferably be of good quality stainless steel for durability. Fish are either guided into the field in water, are netted and placed into it, or are netted in a device which contains the electrodes. The current is continuously applied and immobilisation occurs quickly, often accompanied by minor tail flicks. Having established immobility it is often subsequently possible to reduce the field strength to a point where it is safe for the operator to handle the fish in water with little or no effect. Rubber gloves may be useful, but the holding d.c. field strength will usually be so low as to be undetectable by adult humans. In this condition simple procedures can be carried out so long as the fish remains with its head towards the anode and within the applied electrical field. Gunström and Bethers (1985) describe the use of a landing basket fitted with stainless electrodes powered from a 12-volt car battery. They used this apparatus to immobilise troll-caught salmon and to conduct simple, rapid procedures before turning off the current with immediate recovery. Orsi and Short (1987) describe a similar, portable device fitted with multiple electrodes and note that the smallest *Oncorhynchus tshawytscha* that could

be immobilised was 20 cm in length. Larson (1995) described the use of a padded cradle for larger salmonids and found that this reduced scale loss.

D.C. galvonarcosis has seen most application in fisheries field-work of this type. A major advantage is the instantaneous induction of immobilisation and the immediate recovery. The procedure probably only immobilises and does not induce true anaesthesia; the extent of analgesia is unknown.

Immobilisation using alternating current (a.c.)

A.C. effectively produces a form of anaesthesia and the most usual waveform used is a sine wave. This is most frequently derived from the domestic mains although a portable generator can also be used. Frequencies of 50 or 60 Hz are thus common in such work. Barham *et al.* (1987b) used a signal generator and amplifier in the laboratory to study the effects of waveform and frequency on fish. They showed that frequencies up to 200 Hz have substantially the same effect as lower, mains-derived frequencies. They also showed that square waves are just as effective as sine waves but that triangular waves are less effective. However, both square and triangular waves have the disadvantage that they need to be generated electronically, necessitating more specialist equipment including signal generators and power amplifiers capable of delivering a high current into water.

Following a.c. treatment, the effects are not abolished by switching off the supply and a short-duration sedation or anaesthesia-like state is produced. Ludwig (1930) and Scheminsky (1934) studied the effects of alternating currents on fish and described three successive responses in fish behaviour to increasing electric current. At very low levels, the electrical stimulation results only in *electrotaxis*, but at higher voltages a form of *sedation* occurs which is followed at even higher voltages by *electroanaesthesia*.

The reactions of fish to an electric field can differ markedly and depend on the intensity of the electrical field, the duration of the electrical stimulation and the morphology of the fish's body (Ellis, 1975). It has been demonstrated that, assuming similar orientation, larger fish intercept a greater potential difference than small fish (Holzer, 1932; Halsband, 1967; Vibert, 1967) and consequently larger fish are affected more rapidly by relatively low potentials than small fish. A further consequence, demonstrated in *Oreochromis mossambicus* by Barham *et al.* (1987a) is that, when exposed to a set electrical stimulus, narcosis time increases markedly with body length. It has consistently been shown

that the single most important factor in this is the head-to-tail voltage. It should be clear from this that a longer fish will intercept a greater voltage than a smaller one and that body shape will play an important part in determining the relative effect of a given electric current. Lamarque (1990) relates this effect, in part, to the length of nerve fibres in fish of different body length. Furthermore, the anaesthetic effect will obviously be altered when the fish are *not* orientated roughly parallel to the direction of electron flow. Darroux (1983) showed that a rectangular tank with full-width plate electrodes was the best arrangement to produce a uniform field strength. In circular tanks fish can 'escape' during the electrotaxis phase to an area of low and ineffective field strength.

Barham and Schoonbee (1990) used a fixed stimulus in attempting to induce a short handling period, and found that mature *Oreochromis niloticus* responded better to a 60 V 50 Hz a.c. stimulation than to d.c., but that half-wave rectified a.c. needed a much higher voltage of 200 V. It appears that the sedative or anaesthetic effect is graded according to the duration and magnitude of the applied voltage. Although such data are rarely available for a given species, a full dose–response diagram for tilapia is shown in Fig. 11.3.

Basic method and protocol for electroanaesthesia

The following approach has been used successfully and can easily be modified where needed to suit local circumstances.

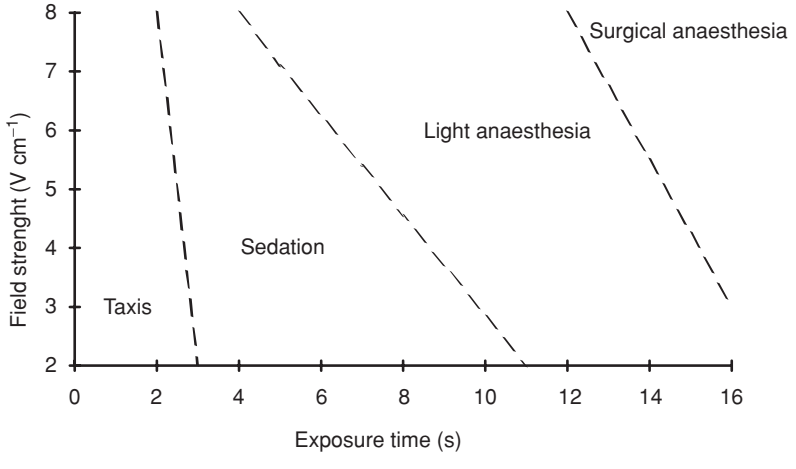


Figure 11.3 Effects of electrical field strength and duration of exposure on *Oreochromis niloticus*. Note that the effects are graded from simple taxis to deep anaesthesia. Data from Robinson 1984.

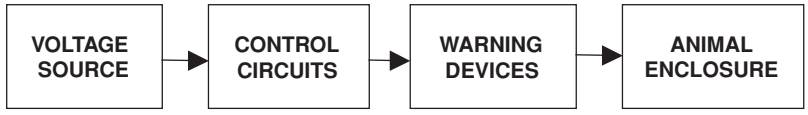
1. The critical factor for successful electroanaesthesia is to achieve a uniform field density and some minimum size-dependent head-to-tail voltage. An approximately uniform field density for anaesthetic purposes can best be achieved in a rectangular tank with maximally sized rectangular stainless steel electrodes placed at each end. Stainless steel electrode plates will not corrode or shed materials into the water. Users will need to experiment with other shapes of tank, bearing in mind that field density could be made more uniform by using multiple electrodes. The maximum length of the tank must not be such as to reduce the head-to-tail voltage for the fish size being used to less than that required for immobilisation.
2. Arrange the tank with the electrodes firmly clamped at each end and in such a way that the fish cannot swim *behind* the electrodes. Aerate and control the temperature of the water, if needed.
3. The most convenient a.c. sources are either the domestic mains or a portable generator providing an equivalent of the domestic mains. The available voltage will thus be in the region of 240–250 V a.c., or as low as 110 V a.c. in some countries. User safety is of paramount importance and, if mains or a generator are used, the voltage should be applied to the electrodes via an isolating transformer of modest current capability thus protecting the operator to some extent. An appropriate isolating transformer will normally have a series of secondary tappings which can be switched thus giving some simple control over the total applied voltage. An alternative a.c. source could be a signal generator and power amplifier, the power stages of which will be current limited depending on the semiconductors used. Pulsed d.c. is generated by electro-fishing gear and the equipment is usually fitted with control circuitry to vary output and pulse frequency.
4. The unit must be operated with appropriate overload circuit breakers on the input (mains or generator) side. However, it is obvious that such units cannot be operated with a traditional earth leakage circuit breaker on the output side as this will cause immediate tripping and defeat the objective. Such protection devices cannot be expected to work correctly in water-filled tanks and so a resistive circuit breaker should be used. The unit should preferably also have overload protection on the output side, which will serve two purposes: first, to protect the output stages from dead-shorting, but second, to interrupt the output should current rise above a certain limit which also partly serves to protect the operator against accidental immersion.
5. In the simplest arrangements, it may be possible to time the application of the current for the few seconds necessary using a stopwatch,

but this is much more reliably achieved using a simple, inexpensive electronic timer controlling the output relay.

6. In addition to these minimum requirements, as much protection as possible should be afforded to those in the vicinity of the operation. This can be achieved by the use of serial lock-out switches on the control box to prevent gratuitous or accidental operation and by the use of audible warning signals and flashing lights. In the authors' laboratory the unit has a four-step operating sequence: (1) selecting voltage and (2) selecting time and then (3) 'arming' the output by pressing a further switch. This 'arming' process illuminates warning lights, sounds a small siren and controls a relay which allows the current to be passed to the final 'firing' relay. The unit can then be (4) 'fired' within 20 seconds of arming. If the unit is not 'fired' within this period, it will time out and need to be fully reset. After firing, the unit has a further protective timer which prevents re-arming within 30–40 seconds. This affords some short-term protection to assistants or handlers who may begin work with the animals immediately after the firing.
7. Users are reminded that no simple electrical or electronic protection can be provided that will fully protect an operator who places hands or body into the water while the current is on and hence a strict working protocol must be adhered to by all involved. Codes of practice for use of electric fishing apparatus exist in most countries and these form an excellent starting point for developing a suitable protocol for electroanaesthesia. These protocols have been thoroughly covered by numerous authors and much useful information can be found in Cowx (1990) and Cowx and Lamarque (1990).

Construction features for an a.c. electroanaesthesia unit

The basic building blocks are as follows:



The diagram in Fig. 11.4 shows the functional units of the electrical system used by the authors for controlling and delivering electroanaesthesia and Fig. 11.5 shows the actual unit. All the components used in such a unit are easily available from major electronic equipment and component distributors.

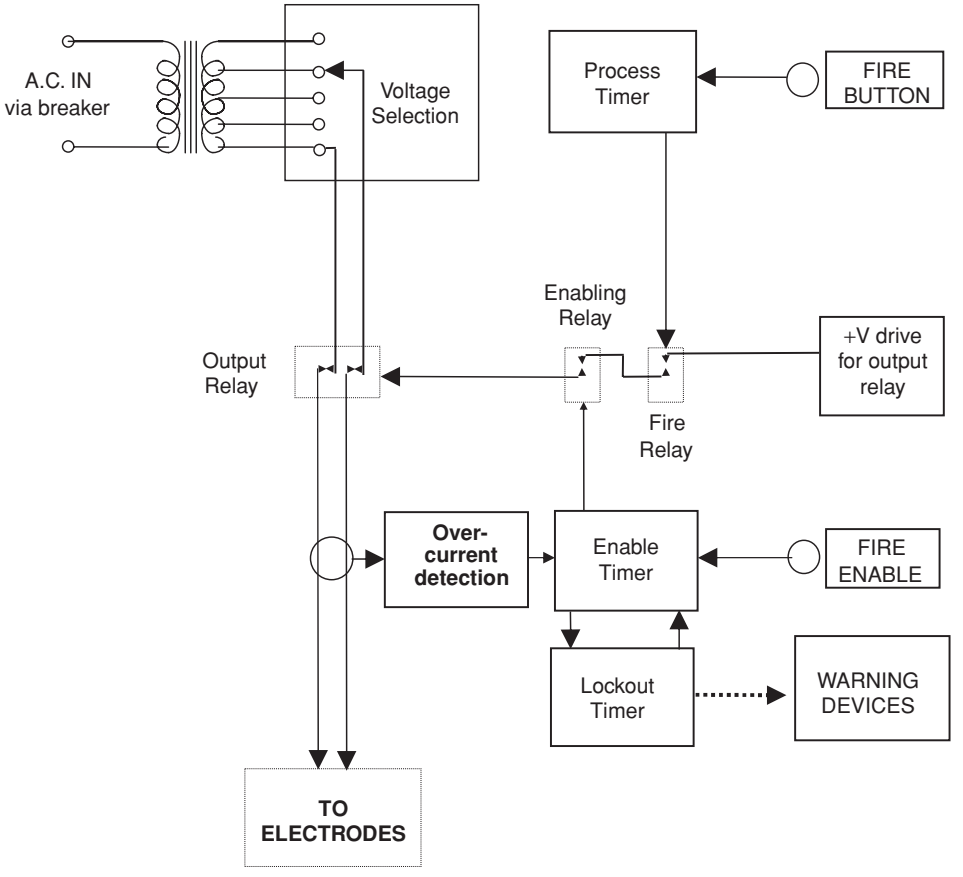


Figure 11.4 Schematic diagram of electroanaesthesia apparatus.

Isolating transformer: this is a transformer of modest current capacity, typically 200 VA, which provides a range of output voltages which can be selected by an appropriate switch. Versions with a 240 V primary will give 240 V output which is sufficient for most electroanaesthesia purposes. In countries with 110–120 V supplies, a step-up isolating transformer capable of giving 240 V output could be used to give greater flexibility of output.

Timers

In this design, use is made of the 555 timer and the dual 556 timer in their CMOS versions. These require fairly low current and minimal additional control components. They are very easily set up to give the range of 'on' times required with good precision and repeatability.

Figure 11.5 The electroanaesthesia control unit and stainless steel plate electrodes used in the author's laboratory. Note the voltage and time selector switches at top right, the indicator panels which light in sequence to guide the user through the firing procedure and the stainless steel plate electrodes used in tanks.

Relays

These are low-voltage types with modestly rated a.c. contacts. Although other timer circuits can be used, the 555 and 556 timers have the advantage of being capable of driving small relays of this type directly.

Switches

The fire button and fire-enable button should be of a prominent push-to-make variety. It is also sensible to have a key-operated master switch, which would prevent unauthorised persons from tampering with or attempting to use the equipment unattended. All switches should be waterproof to IP65 or better.

Warning devices

Simple low-voltage sirens and bright krypton flashers are now available very cheaply and so this simple contribution to safety cannot be ruled out. The sirens can be fitted inside the casing without loss of effect and

a flasher can be mounted on top. The devices should be waterproof to IP65 or better.

Plugs and sockets

All such fittings should be of the shrouded type to avoid accidental contact of fingers with 'live' pins during assembly. They should also be made of high-grade, durable materials, which will last well even if they become damp and should be waterproof to IP65 or better.

Case

This should be waterproof or at least splashproof, i.e. to IP65, 66 or 67, as it is almost impossible to work completely dry.

The physiological effects of electroanaesthesia

Robinson (1984) investigated a.c. electroanaesthesia in tilapia, trout and eels, representing fish with three very different body forms. As may be expected, eels intercept high voltages even in weak fields and tetanise, often grotesquely, with some attendant haemorrhaging. Anaesthesia is not effective at these low field densities and so eels, or indeed any other fish with this body shape are quite unsuited to the technique. Barham *et al.* (1989) noted muscular spasm and haemorrhaging in electrically anaesthetised carp and suggested that this species was also an unsuitable candidate for the procedure.

Robinson (1984) showed that exposure to any voltage produced brief opercular flaring in rainbow trout and tilapia, *Oreochromis niloticus*. At voltages that induced electroanaesthesia, this was followed by decreases in ventilation rate of 50–70%. In some cases ventilation ceased altogether, to be resumed after a brief delay at a much lower rate. Generally, trout become paler and more silvery in appearance, whereas tilapias blanch at first but then darken in the period following the exposure. Black bands frequently appeared on the flank of both species and, although these disappear usually within an hour, in a few cases they stay permanently.

Electroimmobilisation produces haematological changes broadly similar to those from chemical anaesthesia (Madden and Houston, 1976; Schreck *et al*., 1976). Indeed blood electrolytes and body water distribution may undergo large changes (Schreck *et al.*, 1976) and these may last for a day or more. Barham and Schoonbee (1990, 1991a, 1991b) noted that a.c. electroanaesthesia produced changes in blood

parameters which were less than, or broadly similar to those induced by benzocaine, depending on the parameter, but that d.c. galvonarcosis disrupted blood parameters more markedly. Robinson (1984) showed that electroanaesthetised rainbow trout and tilapia, *Oreochromis niloticus*, experienced haemoconcentration and an overall loss of water to the environment. Ladu and Ross (1997) found that chemical and electroanaesthesia had similar effects on haematology and tissue chemistry of rainbow trout. Generally, however, haemoconcentration was minimised by electroanaesthesia and they recommended it as a preferred method for sampling because it is cheap, safe and may be less stressful as it requires no netting.

The great advantage of electroanaesthesia is low netting stress both of the fish and of the operator, as rapid immobilisation of large numbers of animals can be achieved even in relatively large holding facilities. As the head-to-tail voltage is important, it is obvious that tanks over a few metres in length would require prohibitively large voltage sources to achieve anaesthesia and so this approach becomes impractical. The method may also have some advantages in biochemical or nutritional studies where the fish may need to be immobilised instantly. Ottera *et al.* (1999) noted the potential for use of electro-stunning for commercial harvesting of fish, but also commented on the severe muscle tetany, which may result in spinal dislocation and haemorrhaging of the flesh. Although more research is required to refine the techniques of electroanaesthesia, it does have some advantages and would be particularly useful for dealing with airbreathing species.

Analgesia during a.c. electroanaesthesia

In the only study reported to date of the analgesia produced during a.c. electroanaesthesia, Robinson (1984) showed that loss of reactivity to simple touch stimuli and to light topical pain stimuli, judged by contact with a pencil point, lasted for less than 1 minute at lower voltages. However, this could be extended to up to 5 minutes at higher voltage and time combinations.

Pulsed white noise

Numerous authors have noted that electrically anaesthetised fish undergo severe muscle tetany which can result in spinal dislocation and muscle haemorrhage (Mazur *et al*., 1991a). Pulsed white noise, superimposed on a pulsed carrier has been used successfully in humans and other terrestrial animals. White noise can be produced by a pulse

generator and is then amplified for delivery by a wide-band power amplifier. Mazur *et al*. (1991b) showed that this was an effective approach in rainbow trout, *Oncorhynchus mykiss* and Coho salmon, *Oncorhynchus kisutch*. Pulsed rectified white noise at 5–30 kHz produced anaesthesia with reduced tendency to tetanise the fish, although this was not totally abolished.

Summary

Hypothermia is an effective means of calming animals. While it does not provide real anaesthesia, it does effectively sedate and is suitable for transportation. Electroanaesthesia provides good short-term *apparent* sedation and anaesthesia although this is not true anaesthesia. While this enables handling and minor procedures, the data available to date suggest that any apparent analgesia is only effective for a limited period. The technique has some inherent safety problems, although these can be partly addressed electronically and further enhanced by adopting a working protocol. The tetany induced during some forms of electroanaesthesia can be moderated using pulsed white noise. This technique is clearly attractive in many circumstances but its mechanisms and details of management are not understood fully. There is considerable scope for further work in this promising area.

References

Anon., Eel exports, *Fish Farmer*, 5 (4) (1982) 29.

Barham, W.T. and Schoonbee, H.J., Induction behaviour of the tilapia *Oreochromis mossambicus* Peters (Pisces: Cichlidae) subjected to electronarcosis by various alternating or rectified currents, *Water S. Afr*., 16(1) (1990) 75–78.

Barham, W.T. and Schoonbee, H.J., A comparison of the effects of alternating current electronarcosis, rectified current electronarcosis and chemical anaesthesia on the blood physiology of the freshwater bream *Oreochromis mossambicus* (Peters), 2, The effect on haematocrit, haemoglobin concentration, red cell count, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration, *Comp. Biochem. Physiol*., 98A(2) (1991a) 179–183.

Barham, W.T. and Schoonbee, H.J., A comparison of the effect of alternating current electronarcosis, rectified current electronarcosis and chemical anaesthesia on the blood physiology of the freshwater bream (tilapia) *Oreochromis mossambicus* (Peters), 3, The effect on the plasma electrolytes Ca^{2+}, Na^{+} and K^{2+} and on the osmotic pressure of the blood, *Comp. Biochem. Physiol*., 100A(2) (1991b) 357–360.

Barham, W.T., Schoonbee, H.J. and Visser, J.G.J., The use of electronarcosis as anaesthetic in the cichlid *Oreochromis mossambicus* (Peters), I,

General experimental procedures and the role of fish length on the narcotising effects of electric currents, *Onderstepoort J. Vet. Res*., 54 (1987a) 617–622.

Barham, W.T., Schoonbee, H.J. and Visser, J.G.J., The use of electronarcosis as anaesthetic in the cichlid *Oreochromis mossambicus* (Peters), II, The effects of changing physical and electrical parameters on the narcotising ability of alternating current, *Onderstepoort J. Vet. Res*., 55(4) (1987b) 205–215.

Barham, W.T., Schoonbee, H.J. and Visser, J.G.J., Some observations on the narcotising ability of electric currents on the common carp *Cyprinus carpio*, *Onderstepoort J. Vet. Res*., 56(3) (1989) 215–218.

Bell, G.R., A guide to the properties, characteristics and uses of some general anaesthetics for fish, *Bull. Fish. Res. Board Can.*, No. 148 (1964), 11 p.

Cowx, I.G., Developments in Electric Fishing, Fishing News Books, 1990, 358 p.

Cowx, I.G. and Lamarque, P., Fishing with Electricity, Applications in freshwater fishery management, Fishing News Books, 1990, 248 p.

Darroux, F., *Use of Electroanaesthesia in Rainbow Trout*, MSc thesis, University of Stirling, 1983, 52 p.

Ellis, J.E., Electrotaxic and narcotic responses of channel catfish to various electric pulse rates and voltage amplitudes, *Prog. Fish Culturist.*, 37(3) (1975) 155–157.

Fish, F.F. and Hanavan, M.G., A Report on the Grand Coulee Fish Maintenance Project (1939–1947). Special Scientific Report No 55. U.S. Fish and Wildlife Service, 1948, 63 p.

Gosset, C., Galvanonarcotic tank for measuring fish, *Bulletin français de la pêche et de la pisciculture*, 255 (1974) 72–76.

Gunstrom, G.K. and Bethers, M., Electrical anaesthesia for handling large salmonids, *Prog. Fish Culturist.*, 47(1) (1985) 67–69.

Halsband, E., Basic principals of electric fishing, in: Vibert, R. (ed.), Fishing with Electricity – its Applications to Biology and Management, Fishing News (Books) Ltd., London, 1967, 276 p.

Holzer, W., Uber die Stromdichte in Forelleri bei galvanischer Durchrommung in Flussigkecht, *Pflugers Arch*., 232(6) (1932) 835–841.

Hovda, J. and Linley, T.J., The potential application of hypothermia for anesthesia in adult Pacific salmon, *N. Am.J. Aquac*., 62 (2000) 67–72.

Kolz, M.L., Current and power determinations for electrically anaesthetised fish, *Prog. Fish Culturist.*, 51(3) (1989) 168–169.

Kynard, B. and Lonsdale, E., Experimental study of galvonarcosis for rainbow trout (*Salmo gairdneri*) immobilisation, *J. Fish. Res. Board Can.*, 32 (1975) 300–302.

Ladu, B.M.B. and Ross, L.G., The effect of methods of immobilization on the haematology and tissue chemistry of rainbow trout *Oncorhynchus mykiss* Walbaum, *J. Aquatic Sci*., 12 (1997) 31–41.

Lamarque, P., Electrophysiology of fish in electric fields, in: Cowx, I.G. and Lamarque, P. (eds.), Fishing with Electricity, Applications in Freshwater Fishery Management, Fishing News Books, 1990, 248 p.

Larson, L.L., A portable restraint cradle for handling large salmonids, *N. Am. J. Fish. Manage.*, 15(3) (1995) 654–656.

Ludwig, N., Uber electrotaxis und elektronarkose von Fischen, *Pflugers Arch.*, 244(2) (1930) 268–277.

Madden, J. and Houston, A., Use of electroanaesthesia with freshwater teleosts; some physiological consequences in the rainbow trout, *Salmo gairdneri*, Richardson, *J. Fish Biol.*, 9 (6)(1976) 457–462.

Mazur, C.F., Boreham, A., McLean, W. and Iwama, G.K., Improvements on the use of two alternatives to chemical anaesthesia for fish: electroanaesthesia and CO_2 anaesthesia, ICES-Council papers, ICES-CM-1991/F:25, 1991a, 1 p.

Mazur, C.F., Boreham, A., McLean, W. and Iwama, G.K., Rectified wide band white noise as an electroanaesthesia waveform for use with rainbow trout (*Oncorhynchus mykiss*), ICES-Council papers, ICES-CM-1991/F:26, 1991b, 28 p.

Okoye, R.N., *Techniques for Transportation of Juvenile Tilapia*. MSc thesis, University of Stirling, 1982, 39 p.

Orsi, J.A. and Short, J.W., Modifications in electrical anaesthesia for salmonids, *Prog. Fish Culturist*, 49(2) (1987) 144–146.

Ottera H., Roth B., Torrissen O.J., Do killing methods affect the quality of Atlantic salmon? in: Kestin, S.C. and Warriss, P.D. (eds.), Farmed Fish Quality, International Conference on Farmed Fish Quality, 7–9 April, 1999, Blackwell Science, 1999, pp. 398–399.

Parker, G.H., General anaesthesia by cooling, *Proc. Soc. Exp. Biol. Med.*, 42 (1939) 186–197.

Randall, D.J. and Hoar, W.S., Special techniques, in: Hoar, W.S and Randall, D.J. (eds.), Fish Physiology, volume 6, Academic Press, New York, 1971, 559 p.

Robinson, E., *A Study of the use of Alternating Current fro Electroanaesthesia in Salmo gairdneri and Oreochromis niloticus*. BSc thesis, University of Stirling, 1984, 26 p.

Ross, B. and Ross, L.G., Anaesthetic and sedative techniques for fish, Institute of Aquaculture, University of Stirling, 1984, 42 p.

Ross, L.G., Sanchez Blanco, J., Martínez-Palacios, C.A., Racotta, I.S. and Toledo Cuevas, Anaesthesia, sedation and transportation of juvenile *Menidia estor* (Jordan) using benzocaine and hypothermia, *Aquac. Res.*, 38 (2007) 909–917.

Scheminsky, F., Uber die Naturder Wechselstrommnarkose bei Fischen, *Arb der Ungarische Biol. Forschungsinstitut*, 6 (1934) 209–211.

Schreck, C.B., Whaley, R.H., Bass, M.L., Maglon, O.E. and Solazzi, M., Physiological responses of rainbow trout (*Salmo gairdneri*) to electroshock, *J. Fish. Res. Board Can.*, 33(1) (1976) 76–84.

Solomon, D.J. and Hawkins, A.D., Fish capture and transport, in: Hawkins, A.D. (ed.), Aquarium Systems, Academic Press, 1981.

Sterritt, D.A., Elliott, S.T. and Schmidt, A.E., Electrical anesthesia for immobilizing adult coho salmon in freshwater, *N. Am. J. Fish. Manage.*, 14 (1994) 453–456.

Vibert, R., Fishing with Electricity, Fishing News (Books) Ltd., London, 1967, 276 p.

Williamson, R.M. and Roberts, B.L., Body cooling as a supplement to anaesthesia for fishes, *J. Mar. Biol. Assoc. U.K.*, 61(1) (1981) 129–131.

Yoshikawa, H., Ueno, S. and Mitsuda, H., Short- and long-term cold-anaesthesia in carp, *Bull. Jpn. Soc. Sci. Fish.*, 55(3) (1989) 491–498.

Chapter 12

Anaesthesia of Aquatic Invertebrates

Introduction

Invertebrate anaesthesia is not as well developed as fish anaesthesia; one of the principal reasons for this being that there are fewer occasions on which it is used. Despite this, a wide range of substances has been used from time to time with both molluscs and crustaceans. Clearly, in most aspects of mollusc culture there will be little to no requirement to use anaesthesia. The actions of anaesthetic drugs in molluscs have, however, been extensively studied as these animals form excellent model systems for studying the general mechanisms of drug action. Some isolated preparations from crustaceans have also been used as model systems. Most operations in crustacean culture can also be conducted without anaesthesia, although the motor capabilities of shrimp can present problems in handling. There has, consequently, been some interest in investigating crustacean anaesthetics, particularly for transportation.

Anaesthesia of molluscs

A wide range of simple materials have been used to anaesthetise molluscs, principally for museum work and humane dissection, and frequently with no recovery. Smaldon and Lee 1979 note that many of the materials used for such purposes are also effective, reversible anaesthetics, if used at an appropriate dose level. Some guidelines for anaesthetic doses of common materials effective for invertebrates are given by NRC (1989).

General techniques

Ethanol as a 5–10% solution works well with molluscs. Different species respond differently and the agent should be added slowly, with

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mixing, to the chamber containing the animals. Chlorbutanol (chloretone) is effective at 0.05% in cephalopods, but may be used at up to about 0.5% in many other species. A 0.2% solution of chloral hydrate in sea water is also useful. Chloroform, urethane (1%), 2-phenoxyethanol, the barbiturate nembutal (80–350 mg L^{-1}) up to 1% stovaine (amylocaine hydrochloride; 1-dimethylamino-2-methyl-butan-2-yl benzoate) and eucaine (benzamine hydrochloride; 2,2,6-trimethyl-4-piperidinol benzoate) also seem to be effective in molluscs (Smaldon and Lee, 1979).

Clove oil is effective as a muscle relaxant and anaesthetic in a range of molluscs (Araujo *et al*., 1995). Edwards *et al.* (2000) reported successful anaesthesia of abalone, *Haliotis laevigata* and *Haliotis rubra,* using 0.5–1.5 ml L^{-1} clove oil or 50 ppm AQUI-S.

Carbon dioxide, most frequently dispensed in the form of soda water, can be used effectively, adding it to the animal chamber as required.

Magnesium chloride or magnesium sulphate are probably the oldest immobilising agents used with invertebrate animals. Magnesium ions are effective because they block muscle action, competing with the calcium required for synaptic transmission. They can be used as a saturated solution in distilled water for freshwater animals and can be diluted with an equal volume of sea-water for use with marine animals. A more general procedure is to add iso-osmotic 7.5% w/v magnesium chloride, $MgCl_2.6H_2O$, to the medium containing the animal, monitoring the dose level by noting when the animal does not respond to prodding. It is important to note that these agents immobilise and allow handling and simple procedures, but they probably do not provide real anaesthesia and their analgesic properties are unknown.

As may be expected in ectotherms, careful use of cooling produces effective immobilisation, although the true degree of analgesia and anaesthesia is suspect. Many molluscs may be safely cooled to about 2°C, or even to 0°C in some species. Long periods at low temperatures may cause death, especially in warm water species.

Throughout any procedure, some attention must be given to managing the anaesthesia. Water quality should be maintained by oxygenation and filtration. A good oxygen supply with metabolite removal will be particularly important in the case of cephalopods. The temperature should be controlled, or at least managed so that animals do not overheat. Steps may also be needed to maintain a damp skin or integument, usually by spraying with water or sea-water.

Bivalves

Only a limited amount of work has been done specifically on bivalve anaesthesia. 2-Phenoxyethanol at up to a 1% solution is effective in clams

(Owen, 1955). Culloty and Mulcahy 1992 reviewed the effectiveness of Nembutal, magnesium chloride, magnesium sulphate, chloral hydrate, urethane, menthol and benzocaine in the flat oyster, *Ostrea edulis*, at a range of concentrations. They judged anaesthesia to have occurred when the valves open but do not close when the animal is touched; this being the most common bivalve response. Nembutal (0.012–0.12%), menthol (up to 2%) and urethane (0.3–2.0%) gave very poor anaesthesia, and magnesium sulphate required a 30% solution to be effective. Two to five per cent chloral hydrate was effective, as was a 0.1% solution of benzocaine. Overall, they concluded that 3.5% w/v magnesium chloride was the agent of choice for oysters, inducing anaesthesia relatively quickly, lasting for up to 90 minutes and allowing rapid recovery with minimal stress and mortality. Heasman *et al*. (1995) also obtained useful anaesthesia of *Pecten fumatus* using 30 g L^{-1} magnesium chloride, but considered that use of magnesium sulphate resulted in excessive mortalities. The same authors successfully induced anaesthesia in *Pecten fumatus* using chloral hydrate at 4 g L^{-1}. Ehteshami (1993) reports effective anaesthesia of *Pinctada radiata* using MS222 at 1 mg L^{-1}, an extremely low dose.

Gastropods

The freshwater snail *Lymnaea stagnalis* has been used as a model to study general anaesthesia as the snail is reversibly anaesthetised by the fluoranes (Girdlestone *et al*., 1989a). Isolated preparations of its nervous system have also provided good model systems for studying mechanisms of anaesthetic action (Girdlestone *et al.* 1989b). It has large, uniquely identifiable nerve cells some of which produce identified neurotransmitters and simple, monosynaptic connections to other cells, making an ideal study system (Winlow *et al*., 1992). Similar studies have been conducted using neurones from *Aplysia and Helix* (Parmentier *et al*., 1979). *Lymnaea stagnalis* can be reversibly anaesthetised by the gases halothane, enflurane and isoflurane, the effective doses being 0.83%, 1.01%, and 1.09% v/v, respectively (Girdlestone *et al*., 1989a).

In addition to immersion as described earlier, magnesium chloride can be injected as a 10% solution close to the cerebral ganglia, giving quick relaxation for 5–15 minutes (Runham*et al*., 1965). Gastropods can also be reversibly anaesthetised by immersion in solutions of 0.1% Nembutal or 0.3% MS222. Sharma *et al*. (2003) induced anaesthesia in *Haliotis iris* using up to 60 mg L^{-1} sodium pentobarbitone (Nembutal). They judged that anaesthesia was achieved when the animals released their hold on the glass walls of the aquarium. Recovery was slow and

there were no mortalities, although feeding did not resume for up to 2 days post-anaesthesia.

Injection of the acetylcholine analogue, succinyl choline, dissolved in sea-water at 5 g L^{-1} into the space near the cerebral ganglia at 0.5 mg per 10 g live weight has been shown to give good relaxation in many larger gastropods including species of *Aplysia, Aeolidia, Hermissenda, Strombus, Bulla, Pleurobranchia* and *Acmaea* (Beeman, 1969). For research work, injection appears to give more controllable results than immersion.

Cooling in a refrigerator can provide effective immobilisation. However, true anaesthesia is probably not achieved and little or no analgesia may be provided. Molluscs may respond very robustly to cold exposure. *Littorina rudis* chilled to below zero degrees recovered after several days' exposure with a high percentage survival (B. Ross, unpublished data).

White *et al.* (1996) working with *Haliotis midae* of up to 90-mm shell length, obtained useful anaesthesia enabling non-mechanical removal from tanks using magnesium sulphate (40–220 g L^{-1}), 2-phenoxyethanol (0.5–3.0 cm^3 L^{-1}). Procaine and EDTA, which were also tested, were found to be unsuitable. Edwards *et al.* (2000) examined anaesthesia in 40-mm *Haliotis laevigata* and *Haliotis rubra* using a range of drugs. They removed the animals from tanks following anesthesia with ethanol (3%), 2-phenoxyethanol (1 ml L^{-1}), benzocaine (100 ppm), clove oil (0.5–1.5 ml L^{-1}), potassium chloride (10 g L^{-1}) and AQUI-S (50 ppm).

Cephalopods

The cephalopods are not yet cultured although there is considerable commercial interest to do so. They are more widely used as experimental animals, and because of their extremely advanced nervous system and brain, they have in recent years been accorded equal status with vertebrate animals by requiring that such experimental work is humane and properly licensed. They are particularly difficult to handle as they are not only agile and fast, but also have a very delicate integument which is loosely attached to the underlying body.

Gleadall 1991 used a standard procedure to screen the potential of 11 anaesthetic agents for use with *Octopus* sp., having noted that ethanol and urethane are ineffective at temperatures below 12°C. He compared ethanol, urethane, magnesium chloride, MS222, metomidate, propoxate, chloral hydrate, chlorbutanol, menthol, nicotine sulphate and 2-phenoxyethanol, as well as cooling techniques. He showed that some materials were ineffective, toxic or fatal and concluded that properly controlled anaesthesia was not really achieved with any of these agents.

Induction of anaesthesia in *Octopus* sp. using 7.5% magnesium chloride in distilled water, mixed with an equal volume of sea-water for use, requires about 13 minutes and recovery takes about 8 minutes (Best and Wells, 1983). Octopuses cooled to 3–5°C from 24°C are immobilised well, but with less effective muscle relaxation (Andrews and Tansey, 1981).

In squid, 3% urethane is effective in sea-water, providing handling ability after only a few minutes' exposure and a recovery period of 3–15 minutes (O'Dor *et al.*, 1977). However, urethane is now considered an unsuitable material because of its carcinogenic properties. Two per cent ethanol in sea-water is also effective. It should be noted that both of these materials cause initial hyperactivity, which can be traumatic.

O'Dor *et al.* (1990) recommend the use of 7.5% magnesium chloride in distilled water for squid, mixed with an equal volume of sea water for use. Garcia-Franco 1992 found that only 1.5–2% magnesium chloride, 3–4% magnesium sulphate or 1–3% ethanol produced effective anaesthesia in the squid, *Sepioteuthis sepioidea*. Many other agents are ineffective, possibly related to the nature of the nerve transmitters and receptors in these molluscs. O'Dor *et al.* (1990) successfully anaesthetised the squid *Illex illecebrosus* and *Loligo pealei* for brief procedures such as weighing or tattooing using 1.5% ethanol, but cautioned that mortality is high if animals are left in the solution for long periods. Ethanol or magnesium anaesthesia combined with cooling to 5–7°C is better for surgery, lasting for up to 15 minutes. Assisted ventilation is obviously necessary during such longer procedures with cephalopods and cooled, oxygenated anaesthetic should be passed through the mantle cavity continuously.

Bower, *et al*. (1999) transported live squid, *Todarodes pacificus*, by car and aeroplane for up to 10 hours using hypothermia in oxygenated water at 0–1°C.

Anaesthesia of crustaceans

In addition to the standard acetylcholine and noradrenaline transmitters used in nerve cells, crustaceans use 5-hydroxytryptamine, glutamate and possibly polypeptide-like transmitter substances. It is perhaps not surprising then, that crustaceans respond differently to certain anaesthetic agents, possibly because their post-synaptic receptor sites are not affected by some of the commonly used drugs.

General techniques

As in molluscs, a wide range of anaesthetic agents have been used. Ethanol as a 5–10% solution works well. Different species respond

differently and the agent should be added slowly to the chamber containing the animals. Chlorbutanol is effective at as little as 0.05% with some crustaceans, but may be used at up to about 0.5% in other species. Chloroform (1%), urethane (1%), 2-phenoxyethanol (1.5%), ethane disulphonate (2.5 g L^{-1}), isobutyl alcohol (1.5–7 cm^3 L^{-1}), methyl pentynol (5 cm^3 L^{-1}) and MS222 (0.5 g L^{-1}) can also be effective (Smaldon and Lee, 1979).

As in molluscs and many other animals, carbon dioxide is an effective anaesthetic. It is most frequently dispensed in the form of soda water, which may seem a rather imprecise technique, but it is added to the chamber containing the animals in sea-water in approximately equal parts until the desired effect is obtained.

Magnesium chloride or magnesium sulphate are effective as a saturated solution in distilled water for freshwater crustaceans and can be diluted with an equal volume of sea-water for use with marine crustaceans. The more general procedure is to add isosmotic 7.5% w/v magnesium chloride, $MgCl_2.6H_2O$, to the medium containing the animal, monitoring the dose level by noting when the animal does not respond to prodding. It should be noted that, again, these agents merely immobilise and probably do not provide real anaesthesia and their analgesic properties are unknown.

Cooling is also an effective means of immobilisation for crustaceans, although the true degree of analgesia and anaesthesia is unknown. Many crustaceans may be safely cooled to 1–2°C although it should be noted that autotomy of appendages may occur in some species.

Throughout any procedure, some attention must be given to managing the anaesthesia. Water quality should be maintained by oxygenation and filtration. This will be particularly so in the case of the more active species. The temperature should be controlled, or at least managed so that animals do not overheat. Steps may also be needed to maintain a damp exoskeleton, usually by spraying with water or seawater.

Branchiopods

Daphnia magna was shown to be anaesthetised by methoxyflurane, halothane, isoflurane and enflurane when concentrations of 0.095%, 1.005%, 1.170% and 1.42% w/v in 100% oxygen, respectively, were bubbled through the water (McKenzie *et al.*, 1992). The effect was temperature dependent (McKenzie *et al.*, 1992).

Stomatopods

Ferrero and Pressacco 1982 administered isobutanol to the stomatopod *Squilla mantis* by cardiac injection and showed that it was reliable and effective at 200 $\mu L\,kg^{-1}$ at temperatures between 11°C and 17°C. Xylazine, administered by the same route was effective at 100 $mg\,kg^{-1}$, but caused some mortalities. It is thus not considered to be a realistic option for these crustaceans.

Amphipods

The freshwater shrimp, *Gammarus pulex* (Simon *et al*., 1983; Smith *et al*., 1984) has been used to study the ameliorating effect of pressure on anaesthesia, again in an attempt to shed light on the site of action of these agents. Ahmad (1969) used MS222 at 0.5–1.0 $g\,L^{-1}$ to successfully anaesthetise *G. pulex* and noted that induction was slower but that the safety margin was greater at lower temperatures. Species of *Corophium* can also be anaesthetised by MS222 at this dose level (Gamble, 1969).

Decapods

Isolated crayfish and crab axons have been used as models in elucidating the general mechanisms of anaesthetic action, particularly with general anaesthetic agents used in higher animals. Such isolated preparations are effective in identifying whether anaesthetics act by a general action on the lipid bilayers of nerve cells or whether more specific target receptors are involved.

Many decapods have a reflex action of grasping an object; for example, a wet cloth or tissue towel, which is presented to them. They would frequently hold this object tenaciously for some time and, assuming that no anaesthesia or analgesia is necessary, this may be sufficient to carry out many simple procedures. *Carcinus maenas* 'immobilised' in this way were sufficiently distracted to enable the carapace to be drilled to fit near-heart electrodes and to have a small telemetry tag connected and cemented onto their backs (Fig. 12.1). The whole procedure lasted about 10 minutes and no drugs were used at any stage.

In general, decapods may be anaesthetised by exposure to the gaseous anaesthetic agent dispersed in air, by immersion in a solution or by injection.

Obradovic 1986 used halothane at 0.01–0.1.0% by volume in air with the crayfish *Astacus astacus* and found that it was most effective at 0.5%.

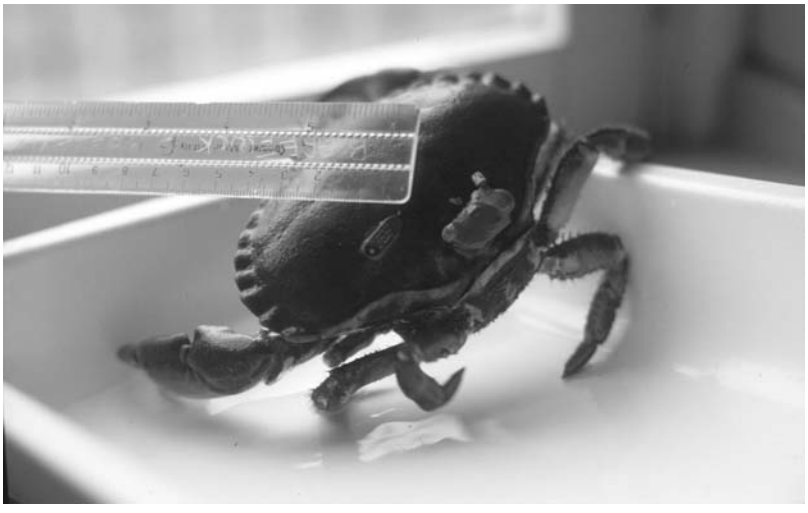


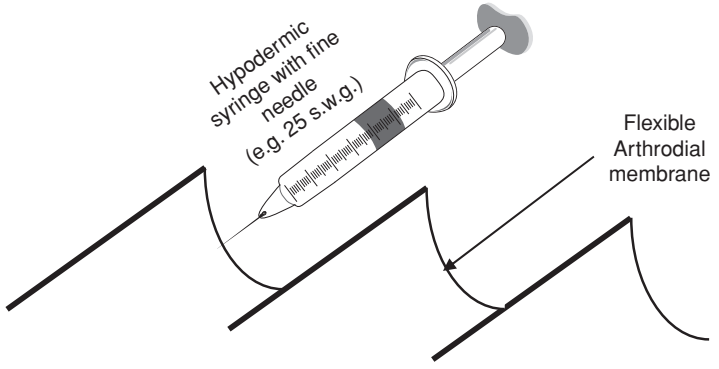
Figure 12.1 Ultrasonic heart-rate telemetry tag fitted to the carapace *of Carcinus maenas* during a procedure using no anaesthetic. The crab lived for many days before leaving the range of the receiving equipment. (L.G. Ross, unpublished data).

This technique would certainly work with other decapods, although it has not been widely used.

Foley *et al.* (1966) found that immersion in isobutyl alcohol at concentrations of 0.5–14.4 $cm^3\ L^{-1}$ anaesthetised the lobster *Homarus americanus.* After brief hyperactivity, ataxia was produced and recovery from this state took 10 minutes in clean, drug-free sea-water. MS222 gives varied results. *Crangon septemspinosa* is anaesthetised by 0.5 $g\ L^{-1}$ MS222. By contrast, Obradovic 1986 showed that MS222 immersion was ineffective in the crayfish *Astacus astacus* at low concentrations (100 $mg\ L^{-1}$) and had only a mild effect at high concentration (1,000 $mg\ L^{-1}$) in these animals. The latter concentration would be rapidly fatal in fish and this perhaps reflects the different neurotransmitters found in crustaceans.

In 1974, Ishioka *et al.* found that juvenile *Penaeus japonicus* could be anaesthetised using 15–200 ppm eugenol, induction time being dose-dependent and there being no effect on growth and survival. Morgan *et al.* (2001) evaluated clove oil as an anaesthetic with three Pacific coast crab species, *Cancer magister*, *Hemigrapsus oregonensis* and *Pugettia producta*. Induction was stress-free and dose-dependent, with effective ranges of 0.5–1.5 $ml\ L^{-1}$, 1–3 $ml\ L^{-1}$ and 0.015–0.25 $ml\ L^{-1}$, respectively, in the three species. They found that recovery was relatively dose independent and suggested that the variation in dose response was related to the different life history strategies of the three species. Vartak and Singh (2006) were able to anaesthetise post-larvae of the freshwater

A. Access through the arthrodial membrane between abdominal or cephalothoracic body segments.



B. Access through the arthrodial membrane at the base of an appendage.

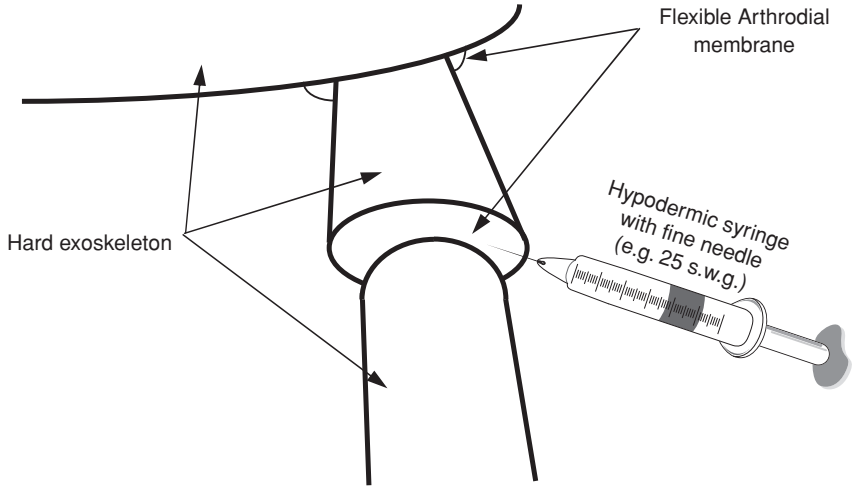


Figure 12.2 Some sites for the injection of drugs into crustaceans through the arthrodial membranes.

prawn *Macrobrachium rosenbergii* using 30–60 mg L^{-1} clove oil and to show that the ethanol used as a vehicle had no anaesthetic effect at the working concentration. Juvenile prawns were anaesthetised using 125–250 mg L^{-1} although in all cases induction was rather slow. Overall, clove oil is a useful decapod anaesthetic as well as having potential as a sedative for live transport of commercially important crustaceans.

Injectable anaesthesia is possible in decapoda and the most comprehensive study in the crabs *Cancer* and *Carcinus* was by Oswald (1977). He tested a range of materials by injection into the haemocoel via the

arthrodial membrane of a posterior leg. There are, of course, many similarly structured, alternative routes to the haemocoel, depending upon species (Fig. 12.2). Oswald showed that MS222, benzocaine, lignocaine, *d*-tubocurarine, gallamine, suxamethonium, decamethonium and guaiacol glyceryl ester were all ineffective and that chlorpromazine induced massive autotomy of limbs. Propanidid (100 mg kg^{-1}), xylazine (70 mg kg^{-1}) and pentobarbitone (250 mg kg^{-1}), were all useful injectable drugs giving sleep times of 60, 45 and 90 minutes, respectively, at these dose rates. Overall, he concluded that alphaxolone-alphadolone (Saffan) at 30 mg kg^{-1} and procaine at 25 mg kg^{-1} were most effective. Procaine produced a brief initial hyperactivity followed by anaesthesia within 30 seconds of injection into crabs and sleep was maintained for 2–3 hours.

Summary

In aquaculture, the need to anaesthetise molluscs or crustaceans will be quite rare, most procedures in husbandry and management being possible without additional aids. However, the ethics of procedures such as the unanaesthetised ablation or cautery of crustacean eyestalks may be questionable. Overall, although sedation and anaesthesia of these groups has not been as extensively investigated as in fish, there are a range of techniques and drugs available which will give good results. More recently, clove oil and AQUI-S have been shown to be effective. Caution should be taken in using any technique with a previously untested species of mollusc or crustacean as these groups are very diverse and the variability in response can be wide and unpredictable.

References

Ahmad, M.F., Anaesthetic effects of tricaine methane sulphonate (MS222 Sandoz) on *Gammarus Pulex* (L.) (amphipoda), *Crustaceana (Leiden)*, 17 (1969) 197–201.

Andrews, P.L.R. and Tansey, E.M., The effects of some anaesthetic agents in *Octopus vulgaris*, *Comp. Biochem. Physiol.*, 70C (1981) 241–247.

Araujo, R., Remon, J.M., Moreno, D. and Ramos, M.A., Relaxing techniques for freshwater molluscs: trials for evaluation of different methods, *Malacologia.*, 36(1–2) (1995) 29–41.

Beeman, R.D., The use of succinylcholine and other drugs for anaesthetising or narcotising gastropod molluscs, *Publications of the Zoological Station, Naples*, 36 (1969) 267–270.

Best, E.M.H. and Wells, M.J., The control of digestion in *Octopus*, I: the anticipatory response and effects of severing the nerves to the gut, *Vie Milieu.*, 33 (1983) 135–142.

Bower, J.R., Sakurai, Y., Yamamoto, J. and Ishii, H., Transport of the ommastrephid squid *Todarodes pacificus* under cold-water anesthesia, *Aquaculture*, 170 (1999) 127–130.

Culloty, S.C. and Mulcahy, M.F., An evaluation of anaesthetics for *Ostrea edulis* (L), *Aquaculture*, 107(2–3) (1992) 249–252.

Edwards, S., Burke, C., Hindrum, S. and Johns, D., Recovery and growth effects of anaesthetic and mechanical removal on greenlip (*Haliotis laevigata*) and blacklip (*Haliotis rubra*) abalone, *J. Shellfish Res.*, 19 (2000) 510 p.

Ehteshami, F., Anaesthetising *Pinctada radiata* with MS 222, *Iranian Fish. Bull.*, 3 (1993) 1.

Ferrero, E.A. and Pressacco, L., Anaesthetic procedures for crustacea, An assessment of isobutanol and xylazine as general anaesthetics for *Squilla mantis* (Stomatopoda), *Memorie di Biologia marina e di Oceanografia*, 12(1) (1982) 47–75.

Gamble, J.C., An anaesthetic for *Corophium volutator* (Pallas) and *Marinogammarus obtustatus* (Dahl), Crustacea, Amphipoda, *Experientia*, 25(1969) 539–540.

Garcia-Franco, M., Anaesthetics for the squid *Sepioteuthis sepiodea* (Mollusca: Cephalopoda), *Comp. Biochem. Physiol.*, 103C(1) (1992) 121–123.

Girdlestone, D., Cruikshank, S.G.H. and Winlow, W., The actions of three volatile general anaesthetics on withdrawal responses of the pond snail *Lymnea stagnalis* (L), *Comp. Biochem. Physiol.*, 92C(1) (1989a) 39–43.

Girdlestone, D., McCrohan, C.R. and Winlow, W., The actions of halothane on spontaneous activity, action potential shape and synaptic connections of the giant serotonin-containing neurone of *Lymnea stagnalis* (L), *Comp. Biochem. Physiol.*, 93C(2) (1989b) 333–339.

Gleadall, I.G., Comparison of anaesthetics for octopuses, *Bull. Mar. Sci.*, 49(1–2) (1991) 663 p.

Heasman, M.P., O'Connor, W.A. and Frazer, A.W.J., Induction of anaesthesia in the commercial scallop *Pecten fumatus*, *Aquaculture*, 131(3–4) (1995) 231–238.

Ishioka, H., Fukuhara, O. and Sakaguchi, S., Studies on the anesthetic effects of eugenol to Kuruma prawn juveniles, *Bull. Nansei Reg. Fish. Res. Lab.*, 7(1974) 31–42.

McKenzie, J.D., Calow, P. and Nimmo, W.S., Effects of general anaesthetics on intact *Daphnia magna* (Cladocera: Crustacea), *Comp. Biochem. Physiol.*, 101C(1) (1992) 9–13.

McKenzie, J.D., Calow, P., Clyde, J., *et al.* Effects of temperature on the potency of halothane, enflurane and ethanol in *Daphnia magna* (Cladocera: Crustacea), *Comp. Biochem. Physiol.*, 101C(1) (1992) 15–19.

Morgan, J., Cargill, C. and Groot, E., The efficacy of clove oil as an anesthetic for decapod crustaceans, Shellfish Health – Risks and Management: Proceedings of a special session held at Aquaculture Canada 2001, Halifax, *Bulletin of the Aquaculture Association of Canada*, 101–103 (2001) 27–31.

National Research Council, Laboratory animal management, Marine Invertebrates, National Academy Press, Washington D.C., 1989, 382 p.

Obradovic, J., Effects of anaesthetics (halothane and MS222) on crayfish, *Astacus astacus, Aquaculture*, 52(3) (1986) 213–217.

O'Dor, R.K., Durward, R.D. and Balch, N., Maintenance and maturation of squid (*Illex illecerebrosus*) in a 15 metre circular pool, Biological Bulletins of Woods Hole. Massachusetts. 153(1977) 322–335.

O'Dor, R., Portner, H.O. and Shadwick, R.E., Squid as elite athletes: locomotory, respiratory and circulatory integration, in: Gilbert, D.L., Adelman, W.J. and

Arnold, J.M. (eds.), Squid as Experimental Animals, Plenum Press, New York, 1990, 516 p.

Oswald, R.L., Immobilisation of decapod crustacea for experimental procedures, *J. Mar. Biol. Assoc. U.K.*, 57(1977) 715–721.

Owen, G., Use of propylene phenoxytol as a relaxing agent, *Nature*, 175(1955) 434.

Parmentier, J.L., Shrivastav, B.B., Bennett, P.B. and Wilson, K.M., Effect of interaction of volatile anaesthetics and high hydrostatic pressure on central neurones, Undersea Biomed. Res., 6(1) (1979) 75–91.

Runham, N.W., Isarankura, K. and Smith, B.J., Methods for narcotizing and anaesthetizing gastropods, *Malacologia*, 2 (1965) 231–238.

Sharma, P.D., Nollens, H.H., Keogh, J.A. and Probert, P.K., Sodium pentobarbitone-induced relaxation in the abalone *Haliotis iris* (Gastropoda): effects of animal size and exposure time, *Aquaculture*, 218 (2003) 589–599.

Simon, S. A., Parmentier, J. L. and Bennett, P. B., Anaesthetic antagonism of the effects of high hydrostatic pressure on locomotory activity of the brine shrimp *Artemia*, *Comp. Biochem. Physiol.*, 75A(2) (1983) 193–199.

Smaldon, G. and Lee, E.W., A synopsis of methods for the narcotisation of marine invertebrates, Royal Scottish Museum Information Series, No. 6, Edinburgh, 1979.

Smith, E.B., Bowser-Riley, F., Daniels, S., Dunbar, I.T., Harrison, C.B. and Paton, W.D.M., Species variation and the mechanism of pressure-anaesthetic interactions, *Nature*, 311(5981) (1984) 56–57.

Vartak, V. and Singh, R.K., Anesthetic effects of clove oil during handling and transportation of the freshwater prawn, *Macrobrachium rosenbergii* (De Man), *Israeli J. Aquac.*, 58(2006) 46–54.

White, H.I., Hecht, T. and Potgeiter, B., The effect of four anaesthetics on *Haliotis midae* and their suitability for application in commercial abalone culture, *Aquaculture*, 140(1–2) (1996) 145–151.

Winlow, W., Yar, T., Spencer, G, Girdlestone, D. and Hancox, J., Differential effects of general anaesthetics on identified molluscan neurones *in situ* and in culture, *Gen. Pharmacol.*, 23(6) (1992) 985–992.

Chapter 13

Anaesthesia of Amphibians and Reptiles

Introduction

Anaesthesia of amphibians and reptiles is used in research and in veterinary practice, but only to a very limited extent in aquaculture. There are, however, circumstances where the aquaculturist may wish to use these techniques for measurement and monitoring. Amphibians and reptiles possess the necessary neuroanatomy and responses to perceive pain; Machin (2001) considered that pain perception in these species is probably analogous to that of mammals. Consequently, although much work remains to be done to understand this field, invasive or painful procedures in lower vertebrates require appropriate anaesthesia and analgesia.

There is little information on anaesthesia of these animals other than for experimental or veterinary purposes and so the following accounts are, necessarily, brief. The data presented is nevertheless based on practical experiences and the techniques have been proven to be effective. Both phyla consist of animals which have lungs and are essentially air breathers, although, with the exception of snakes, lizards and tortoises, much of their lives may be spent in or near water. The life cycles of amphibians are strongly dependent on water; their early life stages (tadpoles) have gills and are entirely aquatic. As both phyla have lungs, the principal approaches to anaesthesia are by gaseous inhalation or by injectable drugs, although drugs can also be absorbed through the skin in amphibians.

Amphibians

General precautions

As amphibians are ectotherms, care must be taken to ensure that they do not overheat or cool down excessively during handling. The skin

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of frogs is very prone to desiccation, therefore drying must be prevented. This can be achieved by using a moistened container, or by direct spraying with water. Maintenance of temperature and humidity not only ensures survival but also maximises drug absorption and excretion. Amphibians can be restrained manually, with wet hands, for short periods although nets may be needed for fully aquatic species and care should be taken to avoid skin damage. During anaesthesia, ventilation of the lungs may cease and gular ventilation (throat movements) may also cease, but the cutaneous circulation is well adapted in these animals and seems to be sufficient to maintain resting metabolism, especially at low temperatures (Crawshaw, 1992).

Anaesthetic gases and agents in solution are rapidly absorbed and transferred through the skin of amphibians, providing a simple route for anaesthetic administration (Green, 1979). Amphibia can be placed in a moist atmosphere containing inhalational drugs, immersed in a drug solution or wrapped in a cloth moistened with a drug solution and these are the preferred methods for adult frogs. Administration of drugs by spraying onto the abdomen is also a relatively low-stress method for frogs (Fig. 13.1).Intraperitoneal injection is also effective and intravascular injection can be easily made into the paired dorsal lymph sacs at the base of the last vertebra. The depth of anaesthesia can be judged from the abolition of the withdrawal reflex when the digits are gently pinched, a diminution of the eye retraction reflex, the level of gular ventilation and a reduction of postural tone in the muscles of the fore-limbs. Frogs should not be allowed to recover while in water as they may drown.

Topical anaesthesia

Topical, local anaesthesia is effectively achieved using 2% lignocaine (lidocaine, xylocaine) (Johnson, 1992). This can be applied directly by swab or by spray (Fig. 13.1) and is useful for minor procedures.

Lignocaine applied to, and absorbed through the skin by metered spray can also be used as a more general anaesthetic and lengthy deep sedation can be achieved in both *Rana catesbiana* and *R. pipiens* (Garcia Aguilar *et al*., 1999). Induction is slow and care must be taken not to overdose because of this.

Smith and Stump (2000) describe application of isofluorane to the skin of *Xenopus laevis* using an absorptive pad saturated with the liquid. The pad is placed on the back of the frog until the desired effect is achieved. Induction was rapid and recovery was safe. Oragel® is a product containing benzocaine and intended for dental use. Cecala *et al.* (2006) assessed its efficacy as an anaesthetic method for the frog

Figure 13.1 Delivering xylocaine as a sedative by metered spray to the abdomen of the leopard frog, *Rana pipiens.*

Acris crepitans, the toad *Bufo fowleri* the axolotl *Ambystoma taklpoideum* and the salamander *Desmognathus fuscus.* They obtained rapid induction and relatively long anaesthesia.

Gaseous inhalation anaesthesia

Rana pipiens can be anaesthetised using ether (diethyl ether) in a bell jar or similar container (Lumb and Jones, 1973). Respiration is depressed and recovery can be prolonged.

Methoxyflurane is a useful material, which can be administered on a ball of cotton wool placed in a suitable glass or clear plastic container. Johnson (1992) notes that 0.5–1.0 cm^3 of 3% methoxyflurane per litre of container induces surgical anaesthesia in about 2 minutes and this is maintained for about 30 minutes. Full recovery is rather prolonged, requiring about 7 hours.

Immersion techniques

This is probably the most effective and easiest technique for use with amphibians, depending on the absorptive characteristics of the well-vascularised skin.

Immersion in a 1% urethane solution induces anaesthesia in frogs in 4–5 minutes and lasts for some hours after removal from the solution (Westhues and Fritsch, 1965). Although not an immersion technique, urethane can also be spread as a powder onto the back of frogs. It is then absorbed through the skin and anaesthesia commences after 8 minutes, at which point the powder must be washed off the animal. The effect can last for up to 3 hours. Users are cautioned concerning the carcinogenic properties of urethane.

Ethanol (10%) applied to the skin induces anaesthesia in about 10 minutes. After removal from the drug, anaesthesia lasts for about 20 minutes and recovery requires about 40 minutes (Kaplan and Kaplan, 1961). Overdoses lead easily to fatalities.

Frogs are anaesthetised after 4–8 minutes in 0.2% chlorbutanol (Lumb and Jones, 1973). Recovery is slow and takes 3–6 hours. Frangioni and Borgioli (1991) also used chlorbutanol successfully with the newt *Triturus cristus carnifex*.

Thienpont and Niemegeers (1965) showed that 16 ppm propoxate anaesthetised *Rana esculenta* after 10 minutes.

MS222 is probably the most widely used immersion anaesthetic for amphibians. A 0.5–1% solution of MS222 is widely used as a bath after buffering with sodium bicarbonate (Crawshaw, 1992) although much lower doses can also be effective. Adult *Rana pipiens* and *R. temporaria* and *Xenopus laevis* can be anaesthetised using solutions of about 0.1%, induction requiring about 5 minutes and recovery 40 minutes, although this is prolonged if the maintenance period is extended. Tadpoles can be safely induced using only 0.03% solutions.

Crawshaw (1992) notes that benzocaine is also effective with amphibians and is considered superior by some workers. Induction requires about 5 minutes and recovery about 15–30 minutes. Xylocaine works well as a sedative agent in both *Rana catesbiana* and *Rana pipiens* (Ross, unpublished observations). Administration as one or two actuations of a 10% xylocaine metered spray system (Fig. 13.1) induces a drowsy state within 1–2 minutes. Sedation sufficient for routine handling lasts for about 10 minutes with a progressive recovery.

Injectable drugs

MS222 is the most widely used amphibian injectable drug. It is used as a 0.1–0.5% solution at a rate of about 13 mg kg^{-1} and gives induction in 5 minutes with recovery after 15–30 minutes. Letcher (1992) reports that use of intracoelomic MS222, at dosages of 100, 250, and 400 mg kg^{-1}, rapidly induced tranquillisation or anaesthesia in *Rana catesbiana* and

Table 13.1 Summary of anaesthetic drugs for use with amphibians.

Mode	Drug	Species or stage	Dose
Inhalation	Ether (diethyl ether)	Frogs	–
	Methylfluorane	Frogs	0.5–1%
Immersion	Benzocaine	Larvae	50 mg L^{-1}
		Frogs and salamanders	200–300 mg L^{-1}
	Chlorbutanol	Frogs	0.2%
	Ethanol	Frogs	10%
	MS222: Bath	Tadpoles, newts	200–500 mg L^{-1}
		Frogs and salamanders	500–2,000 mg L^{-1}
		Toad	1,000–3,000 mg L^{-1}
	Propoxate	Frogs	16 ppm
	Urethane	Frogs	1%
Injection IM or IP	Etorphine (Immobilon)	*R. pipiens*	0.25 mg per frog
	Hexobarbitone	*R. pipiens*	120 mg kg^{-1}
	MS222	*R. pipiens*	100–400 mg kg^{-1}
		R. catesbiana	250–400 mg kg^{-1}
	Pentobarbitone (Nembutal)	*R. pipiens*	60 mg kg^{-1}
	Procaine (5%)	*R. catesbiana*	0.5 cm^3 per frog

Data drawn from numerous authors. IM, intramuscular; IP, intraperitoneal.

R. pipiens; the effects were less pronounced or non-existent at 50 mg kg^{-1}. Depth and duration of anaesthesia were dosage related: the greatest depth of anaesthesia in *Rana pipiens* was attained at the 100, 250, and 400 mg kg^{-1} dosages. This species remained anaesthetised for a significantly longer period than did *R. catesbiana*. Overall, dosages of between 250 and 400 mg kg^{-1} reliably induced deep anaesthesia without mortality in bullfrogs.

Barbiturates give a range of responses. Kaplan *et al*. (1962) reported that hexobarbitone injected into *Rana pipiens* at 120 mg kg^{-1} induced anaesthesia in 20 minutes, lasting for 9 hours, whereas pentobarbitone (Nembutal) at 60 mg kg^{-1} induced in 18 minutes and lasted for 9.5 hours.

Etorphine at 0.25 mg per animal gave anaesthesia with good analgesia in *Rana pipiens*, lasting for 6–12 hours (Wallach and Hoessle, 1970) and 0.5 cm^3 of 5% procaine hydrochloride-induced anaesthesia in *Rana catesbiana* in 3–5 minutes, persisting for 1 hour (Kisch, 1947).

Dose rates for amphibian anaesthesia are summarised in Table 13.1.

Hypothermia

Amphibians are poikilotherms and can be immobilised by lowering their body temperature. This may be useful for handling but it should

be remembered that this is not real anaesthesia and that no analgesia will be provided. No guidelines are available on safe temperature reductions in amphibians and so the technique should be used with care to avoid mortality.

Reptiles

Based on a survey of veterinarians, Read (2004) concluded that provision of controlled anaesthesia in reptiles is more difficult than in other domestic species. The principal management constraint with reptiles is, again, temperature, and steps must be taken to avoid overheating. The problems of skin desiccation do not apply as the integument is heavily keratinised and this presents another problem for the anaesthetist. All reptiles are, strictly speaking, terrestrial animals and those reptiles of major interest to aquatic biologists and aquaculturists are confined to turtles and crocodiles. The snakes and lizards are omitted from this discussion.

Crocodilians

General precautions

Crocodiles and alligators can present considerable danger to operators and any attempt at anaesthesia must be considered carefully in advance to avoid wounding by bites or mechanical abrasion with the scaly skin of crocodilians. It may be necessary to wear strong protective clothing and gloves should be used. Crocodiles and alligators are problematic and the jaws, body and the tail must all be restrained. In smaller animals (approximately up to 50 cm) this can be achieved relatively easily by a skilled assistant holding the snout and tail with gloved hands. Above this size direct handling becomes progressively more difficult and may require several assistants. In these cases jaw restraint must often be managed from a distance using a lasso and body restraint by assistants. With large crocodiles, use of a dart gun with Etorphine will be preferable, avoiding any direct contact with the unanaesthetised animal altogether.

Crocodiles and alligators metabolise drugs quite slowly and recovery is consequently slow.

Local anaesthesia

As in amphibians, topical anaesthesia can be achieved using 2% lignocaine applied directly by swab or spray (Johnson, 1992). This is,

however, more effective in snakes and lizards than turtles and crocodiles due to the nature of the integument.

Oral administration

Pentobarbitone was found to induce deep anaesthesia when fed to small alligators at 45 mg kg^{-1} (Pleuger, 1950).

Injectable drugs

Brisbin (1966) investigated the use of a number of agents with alligators. Pentobarbitone, given intramuscularly to 2–5 kg *Alligator mississippiensis* at 8 mg kg^{-1} recovered after 2–3 hours. MS222 at 80–100 mg kg^{-1} immobilised after 10 minutes, with a 9–10 hour recovery period. Phencyclidine is effective within 60 minutes in alligators at 12–24 mg kg^{-1} with a 6–7 hour recovery period. Wallach *et al.* (1967) showed that intramuscular etorphine sedates crocodiles when used at 0.3 mg kg^{-1}.

Ketamine is the major drug in current use with crocodilians. It is effective at intramuscular weight-related doses between 12 and 80 mg kg^{-1} in a range of species. Induction takes 30 minutes or more, the effect may last for 4–7 hours and full recovery can be lengthy. Heaton-Jones *et al.* (2002) used ketamine in combination with the sedative and analgesic medetomidine injected into either the triceps (upper foreleg) or masseter (jaw) muscles. Juvenile alligators of 1 m total length required 10 mg kg^{-1} ketamine in comparison to 2-m adults which needed only 7.5 mg kg^{-1} for anaesthesia, as assessed by loss of the righting, biting and toe pinch reflexes and absence of corneal and blinking responses. Juvenile animals also required a higher combination of 220 μg kg^{-1} medetomidine with 1,188 μg kg^{-1} atipamezole, while adults of 2 m total length only required 131 μg kg^{-1} medetomidine with 694 μg kg^{-1} atipamezole. This combined system was safe and effective while providing good analgesia.

Johnson (1992) notes that Telazol (tiletamine hydrochloride with zolazepam hydrochloride) may be preferable to ketamine as recovery is shorter. It is also effective at a lower dose (10–30 mg kg^{-1}) but the working solution does not keep well.

Seebacher *et al*. (2005) used Alfaxan-CD RTU, a new alfaxolone-based anaesthetic, with *Crocodylus porosus*. They obtained rapid induction, sleep times from 20 to 100 minutes proportional to dose rates from 1 to 4.5 mg kg^{-1} and rapid and uneventful recovery.

Morgan-Davies (1980) described the use of gallamine triethiodide with *Crocodilus niloticus*. He obtained immobilisation within 15 minutes and considered that it was preferable to etorphine or phencyclidine

because of its availability in the region (West Africa). Suxamethonium chloride (scoline) gave complete muscle relaxation in alligators at 3–9 mg kg^{-1} within 4 minutes, subsequent recovery requiring 7–9 hours (Brisbin, 1966). Similarly, Messel and Stephens (1980) using suxamethonium obtained good immobilisation of *Crocodilus porosus* and *C. johnsoni* within 7–12 minutes. They noted that *C. porosus* required 10 times the weight-related drug dose to achieve the same effect and that the drug was ineffective if the head was held below water. This phenomenon may be related to the vascular shunting, which occurs in the submerged animal. Although both gallamine and scoline are muscle relaxants, which can facilitate handling, they cannot be called anaesthetics.

Turtles

General precautions

Turtles and tortoises have a relatively low metabolic rate and anaesthesia and recovery can be expected to be quite slow. They can be handled for anaesthesia if care is taken to avoid bites and this can be achieved simply by wearing appropriate clothing and gloves. Particular care is needed in the case of the soft-shelled turtles which can move very quickly. In some cases tongs or ropes may be needed for restraint and in aggressive fresh-water species MS222 can be added to the water as a tranquilliser.

Oral or rectal anaesthesia

Drugs can be given by mouth or by rectal instillation, although turtles given urethane by mouth at 2.73 g kg^{-1} were not anaesthetised reliably. Oral administration of pentobarbitone to red-eared turtles, *Pseudemys scripta*, was also considered unpredictable (Vos-Maas and Zwart, 1976). Rectally instilled tribromoethanol, given with amylene hydrate (tertiary amyl alcohol), induced tortoises in 1–2 hours and anaesthesia lasted for over an hour (Hunt, 1964).

Gaseous inhalation anaesthesia

Anaesthesia can be obtained in turtles and tortoises using some fairly simple compounds. Lumb and Jones (1973) administered ether to turtles using a face mask. Induction required 30–40 minutes and recovery up to 10 hours. More recently, Bello and Bello-Klein (1991) administered ether through a plastic cannula placed in the trachea to anaesthetise the

turtle *Chrysemys dorbigni* for up to 90 minutes, with recovery requiring 90 minutes and with no recorded deaths. Methoxyflurane has also been used widely in reptilian work. Induction with a 3% gas:air mixture takes about 8–25 minutes and recovery is variable. A 1.5% mixture can be used for maintenance of anaesthesia. Halothane can also be used for induction and maintenance at the same concentrations and gives induction in 1–3 minutes. Full recovery can be very prolonged, however, and may take up to 24 hours. Johnson (1992) notes that isofluorane is effective at similar doses but that recovery requires only about 3 hours, giving this compound some advantages.

Injection anaesthesia

Turtles have a thick skin, so sharp needles must be used at all times. Intramuscular injections can be given into the muscles of the hind limb and intravascular injection into the heart or the ventral abdominal vein have also been used, both of which are technically difficult and hence not advised. Intraperitoneal routes are via the soft skin at the base of the fore- or hind-limbs.

Pentobarbitone given to turtles by intracardiac injection at 10 mg kg^{-1} induced anaesthesia in 15 minutes, with surgical levels maintained for 3 hours (Young and Kaplan, 1960). Intraperitoneal pentobarbitone at 18 mg kg^{-1} induced sleep in tortoises after 18 minutes and surgical anaesthesia within 80 minutes with prolonged recovery (Hunt, 1964). Ketamine given intramuscularly at 60 mg kg^{-1} gives light anaesthesia after 30 minutes, which lasts for about 60 minutes. Although full recovery may take up to 24 hours, this is probably the method of choice. Wood *et al.* (1982) investigated injectable anaesthetics for the green sea turtle (*Chelonia mydas)* and found effective doses to be 10–25 mg kg^{-1} intravenous Nembutal, 38–71 mg kg^{-1} intraperitoneal ketamine or 19–31 mg kg^{-1} intraperitoneal thiopental. Chittick *et al.* (2002) conducted surgery on loggerhead turtles (*Caretta caretta*) using a more complex combined system of medetomidine (50 μg kg^{-1}, IV) and ketamine (5 mg kg^{-1}, IV), maintained with sevoflurane (0.5–2.5%) in oxygen. Atipamezole (0.25 mg kg^{-1}, IV) was administered at the end of surgery.

In recent years Propofol (2,6-diisopropylphenol) has been used successfully with turtles. Hyland (2002) used 10 mg kg^{-1} Propofol to anaesthetise a manually restrained Macquarrie turtle (*Emydura macquarii macquarii*) prior to maintenance for surgery using 2% isofluorane and oxygen. The turtle was artificially ventilated during surgery.

Dose rates for reptilian anaesthesia are summarised in Table 13.2.

Table 13.2 Summary of anaesthetic drugs for use with 'aquatic' reptiles.

Mode	Drug	Species	Dose ($mg\,kg^{-1}$)	Induction (mins)	Recovery (hours)
Oral	Pentobarbitone	Crocodilia ns ns	45	–	–
	Urethane	Turtles	2,300	–	–
Injection	Etorphine	Crocodilia ns	0.3	–	By antidote
	Gallamine triethiodide: IM	Crocodilia ns		7–12	
	Ketamine: IM	Crocodilia	12–25	30–60	4–7
		ns Turtle/Tortoise	40–80	30	>24
	MS222 IM	Crocodilia ns	80–100	10	9–10
	Pentobarbitone IM	Crocodilia ns Turtles	8–10 20–50	15–18	3
	Pentobarbitone IP	Turtles	18	18	Long
	Phencyclidine IP	Crocodilia ns	12–24	60	6–7
	Propofol	Turtle	10		–
	Succinylcholine chloride: IM	Crocodilia ns Turtle/Tortoise	0.5–2 0.1–1	20–30 20–30	
	Tiletamine-Zolazepam: IM	Crocodilia ns Turtle/Tortoise	15 10–25	>20 5–20	
Inhalation	Ether (diethyl ether)	Turtles	To effect	–	1.5
	Halothane	General	3%	1–30	7–24
	Isofluorane	General	3%	1–15	3
	Methoxyflurane	General	3%	8–25	Variable

Data drawn from numerous authors. IM, intramuscular; IP, intraperitoneal.

Hypothermia

All reptiles are poikilotherms and can be calmed and immobilised by lowering their body temperature. Low temperature also gives some haemostasis. Few guidelines are available on safe temperature reductions in reptiles. However, animals can be placed in a refrigerator or even in crushed ice. Recovery is usually rapid and deaths are rare. This technique is useful for handling but it should be remembered that this is not real anaesthesia and that no analgesia will be provided. Consequently, if minor surgery is contemplated a local anaesthetic should be used at the site.

Summary

The methods of choice for amphibians will depend on circumstances, but the wide range of options means that there will be little difficulty

in selecting a technique. Crocodilians are best anaesthetised by injection and the handling problems inherent in this call for caution. Initial handling for injection calls for some practice in the use of nooses, ropes, etc. Where larger animals are the research subject, there may be little option other than to use a dart gun. Turtles can also be difficult to handle and can inflict severe wounds by biting. The ability to use inhalation as an option to injection may be attractive for this reason but it should be remembered that the low metabolic rates result in longer response times.

References

Bello, A.A. and Bello-Klein, A., A technique to anaesthetise turtles with ether, *Physiol. Behav.*, 50(4) (1991) 847–848.

Brisbin, I.L.Jr., Reactions of the American alligator to several immobilising drugs, *Copeia*, 2 (1966) 129–130.

Cecala, K.K., Price, S.J. and Dorcas, M.E., Measuring and comparing the effectiveness of MS-222 and Oragel® as an amphibian anesthetic, *Herpetol. Rev.*, (2006).

Chittick, E.J., Stamper, A., Beasley, J.F., Lewbart, G.A. and Horne, W.A., Medetomidine, ketamine, and sevoflurane for anesthesia of injured loggerhead sea turtles: 13 cases (1996–2000), *J. Am. Vet. Med. Assoc*. 221 (2002) 1019–1025.

Crawshaw, G.J.,. Medicine and diseases of amphibians, in: Schaeffer, D.O., Kleinow, K.M. and Krulisch, L. (eds.), The Care and Use of Amphibians, Reptiles and Fish in Research, Proceedings of a SCAW conference, Scientists Center for Animal Welfare, Bethesda, MA, 1992, 196 p.

Frangioni, G. and Borgioli, G.,. Effect of spleen congestion and decongestion on newt blood, *J. Zool*., 223(1) (1991) 15–25.

Garcia Aguilar, N., Martinez Palacios C.A. and Ross, L. G.,. Controlled anaesthesia of *Rana catesbiana* and *Rana pipiens* using xylocaine delivered by spray, *Aquac. Res.* 31 (1999) 1–3.

Green, C.J., Animal anaesthesia, Laboratory Animal Handbooks No 8, Laboratory Animal Ltd., London, 1979, 300 p.

Hunt, T.J., Anaesthesia of the tortoise, in: Graham-Jones, O. (ed.), Small Animal Anaesthesia, Pergamon, Oxford, 1964, 71–76.

Johnson, H.J., Anaesthesia, analgesia and euthanasia in reptiles and amphibians, in: Schaeffer, D.O., Kleinow, K.M. and Krulisch, L. (eds.), The Care and Use of Amphibians, Reptiles and Fish in Research, Proceedings of a SCAW conference, Scientists Center for Animal Welfare, Bethesda, MA, 1992, 196 p.

Kaplan, H.M., Brewer, N.R. and Kaplan, M., Comparative value of some barbiturates for anaesthesia in the frog, *Proc. Anim. Care Panel*, 12 (1962) 141–148.

Kaplan, H.M. and Kaplan, M., Anaesthesia in frogs with ethyl alcohol, *Proc. Anim. Care Panel*, 11 (1961) 31–36.

Kisch, B., A method to immobilise fish for cardiac and other experiments with procaine, *Biol. Bull. Marine Biol. Lab., Woods Hole*, 93 (1947) 208.

Letcher, J., Intracelomic use of tricaine methanesulfonate for anesthesia of bullfrogs (*Rana catesbeiana*) and leopard frogs (*Rana pipiens*), *Zool. Biol.*, 11(4) (1992) 243–251.

Lumb, W.V. and Jones, E.W., Veterinary Anaesthesia, Lea and Febiger, Philadelphia, Pennsylvania, 1973.

Machin, K.L., Fish, amphibian, and reptile analgesia. The veterinary clinics of North America, *Exotic Anim. Pract.*, 4 (2001) 19–33.

Messel, H. and Stephens, D.R., Drug immobilization of crocodiles, *J. Wildlife Manage.*, 44(1) (1980) 295–296.

Morgan-Davies, A.M., Immobilization of the Nile crocodile (*Crocodilus niloticus*) with gallamine triethiodide, *J. Zool. Anim. Med.*, 11(3) (1980) 85–87.

Read, M.R., Evaluation of the use of anesthesia and analgesia in reptiles, *J. Am. Vet. Med. Assoc.*, 224 (2004) 547–52.

Smith, J.M. and Stump, K.C., Isoflurane anesthesia in the African clawed frog (*Xenopus laevis*), *Contemp. Topics Lab. Anim. Sci.*, 39 (2000) 39–42.

Heaton-Jones, T.G., Ko, J.C. and Heaton-Jones, D.L., Evaluation of medetomidine-ketamine anesthesia with atipamezole revesal in American alligators (*Alligator mississippiensis*), *J. Zoo and Wildlife Med.*, 33 (2002) 36–44.

Seebacher, F., Smith, J. and Franklin, E., Cardiovascular responses to alfaxolone anaesthesia in the crocodile, *Crocodylus porosus*, *Crocodile Specialist Group Newsletter*, 24 (2005) 14–15.

Thienpont, D. and Niemegeers, C.J.E., Propoxate (R7464): A new potent anaesthetic agent in cold-blooded vertebrates, *Nature, London*, 205 (1965) 1018–1019.

Vos-Maas, M.G. and Zwart, P., A technique for intravenous injection in the red-eared turtle (*Pseudemys scripta elegans*), *Lab. Anim.*, 10 (1976) 399–401.

Wallach, J.D., Frueh, R. and Lentz, M., The use of M99 as an immobilising analgesic agent in captive wild animals, *J. Am. Vet. Med. Assoc.*, 151 (1967) 870–876.

Wallach, J.D. and Hoessle, C., M99 as an immobilising agent in poikilotherms, *Vet. Med. – Small Anim. Clinician*, 65 (1970) 161–162.

Westhues, M. and Fritsch, R., Animal anaesthesia, Oliver and Boyd, Edinburgh, 1965.

Wood, F.F., Critchlow, K. H. and Wood, J.R., Anesthesia in the green sea turtle (*Chelonia mydas*), *Am. J. Vet. Res.*, 43 (1982) 1882–1883.

Young, R. and Kaplan, M., Anaesthesia of turtles with chlopromazine and sodium pentobarbital, *Proc. Anim. Care Panel*, 10 (1960) 57–61.

Chapter 14

Transportation and Anaesthesia

Introduction

Sedation can be beneficial in bulk transportation of stocks, especially where long distances are to be covered, but there may also be advantages in sedating animals for short journeys. There are four main reasons for transporting aquatic animals:

- The movement of fry or juveniles for stocking into farms or restocking into the natural environment.
- The movement of broodstock for management purposes.
- The movement of livestock to markets.
- The movement of animals for research purposes.

For restocking and for aquaculture, the density of transportation may be very high, whereas broodstock and animals transported for research will usually be held at a much lower density. Movement of live fish to market is an increasingly important aspect of aquaculture in many parts of the world and, apart from the technical problems of transportation, any use of drugs raises a number of important questions where food organisms are being considered. As already mentioned, most countries have legislation in place controlling use of drugs with animals entering the food chain and, for the most part, prohibiting their use at harvest.

Some useful reviews of transportation are available (Johnson, 1979; Solomon and Hawkins, 1981; Berka, 1986; Jhingran and Pullin, 1985) which describe in detail many aspects of the technology and approach to live transportation. This chapter discusses the necessary background to the problems of live transportation and focuses on the potential use of anaesthetic and sedative agents in the transportation process.

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The physical and biological problems in transportation

The major issues of concern to those wishing to transport aquatic animals are management of handling stress and mechanical shock, potential heat stress and deterioration of water chemistry. The metabolism of animals will affect water chemistry in a closed environment and the deteriorating water quality will, in turn, affect the physiology of the transported animals. This feedback process can lead to rapid degradation of the transport environment. It will readily be appreciated that control of these factors involves some knowledge of the environmental requirements and physiological responses of the animals, enabling management of the transport environment and maintenance of water quality.

Handling

This is an inevitable precursor to any transportation exercise and may cause mechanical abrasion, induce some degree of stress and result in exhausted animals. In fish, visible damage is frequently seen as scale loss, but it should be remembered that the epidermis will also have been damaged well before scales are shed. Damage to the delicate epidermal layers and mucous covering will allow invasion by pathogens and disrupt osmoregulation. It should be obvious that any handling should be as careful as possible, and when dealing with broodstock or small numbers of animals this will usually be relatively easy to arrange. The practicalities and pressures of dealing with very large numbers of animals tend to militate against careful handling; however, this must be uppermost in the minds of all involved in transportation operations. Alarm pheromones released in crowded conditions may also exacerbate the stress level (Malyukina *et al.*, 1982).

Initial capture can be facilitated by gently crowding fish in their enclosure, from where they can be netted. Good–quality, preferably knotless, nets should be used; low-light environments may also help reduce the capture stress. Good-quality containers and other hardware must be used both at the capture and packing stages and these must be complemented by the extra time and patience required to ensure safe handling and minimise mortalities. A further source of mechanical abrasion, often overlooked, is the animals themselves. This is particularly so where they are either netted in large numbers or too many of them (high density) carried together.

Mechanical stress

During the transportation process itself, there will certainly be some level of vibration, sudden shocks and perhaps a greatly elevated noise level, particularly in vehicles. All these factors will directly cause a stress reaction in most animals and are known to induce a cortisol reaction in fish. Vehicles have to be used and the condition of all roads may not be good, therefore there is often little that can be done to ameliorate these influences, other than be aware of the potential stress they may cause. Interestingly, Laird and Wilson (1979) showed survival benefits from transporting salmonid eggs in a solution of 1% methylcellulose, its viscosity presumably providing some buffering from mechanical shock.

Temperature

Temperature affects all aspects of metabolism and at higher temperatures metabolic rate increases. In general, the transportation water temperature should not be allowed to rise above the starting temperature and should be reduced if possible, within certain limits. This is not easy to achieve in practice, except where specialist equipment is being used; for example, using insulated tanks on purpose-built fish transportation trucks. There are, however, numerous examples of relatively simple cooling and insulation techniques in daily use around the world. The cooling effect of evaporation from earthenware pots covered with wet sacking is a frequently used traditional tool in the Far East for small-scale work. Insulated containers, particularly injection-moulded polystyrene (styrofoam) boxes, are now very widespread and are extremely effective.

There are many ways of using ice, either by direct addition to the medium or by packing fish in plastic bags into crushed ice, the latter being effective for animals requiring saline environments. Cubed, chipped or crushed ice is best, whereas flaked ice probably melts too quickly. Ice blocks can be stored in a deep freeze ready for use. The permanent, recyclable freezable plastic bricks, which are widely available for food use, have found many applications and, particularly in transportation by air, can provide a non-chemical, safe, reduced-temperature environment for many hours. Cooling can also be achieved by the addition of dry ice (solid carbon dioxide at –73°C) in thermal contact with the water but chemically isolated from it (Solomon and Hawkins, 1981).

On the other hand, thermal shock caused by overcooling must be avoided as few species can tolerate an instantaneous temperature

reduction of more than 10°C. As described earlier, tolerable reductions, which guarantee no attendant mortalities, may be as little as 5–6°C.

Dissolved oxygen

Oxygen is the first *limiting* component of the aquatic environment, other than water itself, and ensuring the oxygen supply of transported animals is highly important. Many factors affect the respiratory rate of animals, including stress, temperature, salinity, body weight and feeding level. Oxygen consumption will be high during the earlier part of a journey due to initial stress and this period particularly must be carefully managed. Temperatures should certainly not be allowed to rise and should be reduced if it is practical to do so. Salinity should be maintained or brought towards the iso-osmotic point where it is possible to minimise the osmoregulatory work-load. The minimum tolerated oxygen level varies with species. Tilapias, for example, are relatively hardy and will tolerate dissolved oxygen (D.O.) levels during transportation down to 3 mg L^{-1}, whereas salmonids respond badly below about 5 mg L^{-1}, or higher. Feeding increases metabolism due to the requirements for biochemical processing, an effect known as specific dynamic action or SDA. This can increase metabolic rate and oxygen consumption by more than 50% but fortunately can be minimised during transportation by the simple expedient of starving the animals for a short period prior to travel. The starvation period depends on the gut evacuation rate, which is species and temperature dependent. Typical starvation periods vary from 12 to 24 hours but can be longer in cold-water species with long gut evacuation times.

Salinity

The body fluids of most animals have a concentration between that of fresh-water and sea-water, usually approximately one-third the strength of sea-water. This internal environment is regulated to a greater or lesser degree in different animals, but is very important for proper functioning, health and survival. Salinity is the second most important environmental variable influencing the rate of metabolism. In many cases the salinity of the transportation medium will be identical to the source of the animals. However, it should be borne in mind that salinities of about 15‰ are more iso-osmotic and will reduce osmoregulatory work. Thus, in those animals which can tolerate it, a reduction or increase in salinity towards this iso-osmotic point can notably reduce metabolic rate and hence oxygen consumption and ammonia excretion. Simple dilution of

the environment or addition of sodium chloride or, preferably, sea salts can bring about the required adjustment. Extreme care must be taken in attempting to use this approach with stenohaline animals which do not normally survive such changes well; most carps are good examples of species which cannot benefit as they can tolerate only a few parts per thousand salinity.

Carbon dioxide

Carbon dioxide can accumulate rapidly in the transportation medium, especially at high stocking densities. This may cause some stress in the animals and above about 40 mg L^{-1} it may be fatal. However, it may also have a mild sedative effect at low levels and this can be beneficial. Carbon dioxide levels will be minimised if air bubbling is used for oxygenation, and the container is adequately vented to the outside. At higher densities, however, little can be done to prevent its accumulation.

Ammonia

This constitutes 70%, or more, of the nitrogenous wastes from aquatic organisms. It is excreted directly, and continuously, into the transporting medium, principally via the gills. There is very little urinary excretion of ammonia and hence no periodic elimination as found in terrestrial animals. Ammonia is very soluble in water and in the un-ionised state, as NH_3, it is very toxic, whereas in the ionised state, as NH_4, it is not toxic. The ionisation state of the ammonium ion depends on the pH of the environment and to a lesser extent on the temperature. Higher pH and higher temperatures cause greater ionisation, and hence higher toxicity. Low oxygen levels increase toxicity although this can be ameliorated by high carbon dioxide concentrations, which produce a concomitant reduction in pH. The rate of ammonia excretion is determined by feeding level, dietary protein content and temperature. Consequently, ammonia excretion can be reduced markedly by cooling and withdrawing food from animals to be transported. High pH waters can be buffered down to reduce toxicity.

Suspended solids

Suspended solids irritate the gills, obscure the water and cause stress. Again, the source of suspended solids is faeces and prior withdrawal of

food is the cure. Simple filtration may also be possible in more complex transportation situations.

Basic techniques for transportation

The important issues facing aquaculturists and biologists wishing to transport animals are:

- conditioning
- containers
- control
- sedation

Conditioning

The initial phase is capture or extraction from the holding facility involving netting and handling, which has already been discussed. This is often followed by some form of conditioning which usually consists of a period of starvation in holding facilities with good water exchange to ensure that all faeces are voided and that no solids or metabolites are transferred with the animals. Sampson and Macintosh (1986) note the importance of this conditioning treatment. Jhingran and Pullin (1989) recommended applying additional stress to ensure defecation in carp fry. While there is a certain logic here, this procedure cannot be recommended as a general practice for most aquatic animals and an adequate conditioning period will ensure normal gut evacuation without recourse to such practices. During this period the animals will also substantially overcome the stress of temporarily being held at higher densities. Finally, the animals are placed in the transportation containers.

Containers

There is an enormous range of transportation containers, from simple to complex. As mentioned earlier, in addition to transporting the animals in the minimum amount of water, these containers may be designed to facilitate maintenance of temperature, exchange of gases and perhaps exchange of water.

Many varieties of open, small-scale, simple containers are used in rural settings where small numbers of animals are to be carried, or even large numbers of very young larvae. The containers can be made of plastic, wood or metal; copper must be avoided as it is toxic. As mentioned earlier, earthenware pots or wetted sacking placed over simple

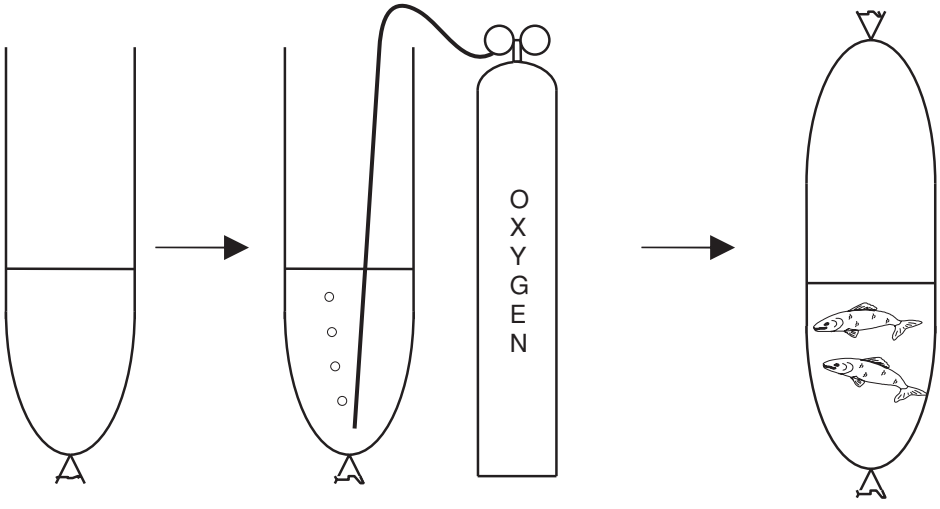

Figure 14.1 The plastic bag technique for transportation of fish and crustaceans. Strong bags may be purchased for use, but plastic tube bought as a roll is frequently the method of choice.

containers can provide significant evaporative cooling. These allow rapid water exchange if needed and the water surface is often continuously agitated by hand to introduce oxygen; this is tedious but effective. Long journeys on foot or bicycle can be made using only this technology. The empirical procedures to ensure maximum survival are well-known to the practitioners; planning the journey carefully is a part of the procedure.

Although sealed containers of plastic, metal or glass have also been used, the most widely used small-scale closed container is now the plastic bag (Fig. 14.1). In this system, animals are placed in a volume of water filling about 20–30% of the bag and the remainder of the bag is filled with pure oxygen, which is bubbled through the water during the filling process. The bag is then tightly sealed for the journey, but often in such a way that more oxygen could be added if required. In this way a large quantity of oxygen is made available to the animals and extended journeys of 24 hours, or sometimes more, can be made. These bags are often used alone or, more frequently, placed in a rigid container of cardboard, wood or plastic to hold them upright. The bag wall provides a smooth, semi-soft surface, which reduces impact abrasion, although care must be taken that small animals do not become trapped in pocketed folds of the bag, especially at the corners. Plastic bags in polystyrene boxes afford excellent thermal protection and intercontinental air transportation of very large numbers of small fish for the aquarium trade takes place in this way daily. Fish fry and young

shrimp for aquaculture also frequently travel in this way. Larger fish or crustaceans can also be carried in plastic bags, either in small numbers or individually.

Large-scale, open containers for transportation of fish fry or adults are often, in fact, semi-closed and will have a vented lid, which effectively isolates the transportation medium from the air. These rigid containers may be up to 4 m^3 in volume and multiple units may be installed on a large road vehicle. The containers themselves are usually well insulated and are equipped with independent compressed air supplies, or oxygen cylinders or even both. As large numbers of high-value animals are involved, monitoring systems are often quite advanced with continuous recording of temperature and dissolved oxygen at least. Although expensive in capital terms, such vehicle-mounted containers are extremely successful, can transport animals over huge distances involving periods of up to two days, or more, and are used in the intensive aquaculture industry in many parts of the world.

Controlling the environment

During transportation, use may be made of any number of a range of additives. The water itself may be exchanged, or partly exchanged, at intervals. The addition of oxygen or compressed air has already been discussed and use has been made of hydrogen peroxide as an additional source of oxygen for small-scale work (Marathe *et al*., 1975; Innes-Taylor and Ross, 1988). Ice or dry ice may be used for cooling. These practices will ensure the basic clean, well-oxygenated, cool environment.

Salts may be added in the form of sodium chloride or calcium chloride to reduce osmotic work. Typical doses range from 1 to 5 g L^{-1} NaCl (Chittenden, 1971; Wedermeyer, 1972) or 50–75 mg L^{-1} $CaCl_2$ for freshwater fish. Weirich *et al.* (1992) showed that elevated calcium levels of 80 mg L^{-1} in the transportation medium boosted survival of *Morone chrysops* × *M. saxatailis* hybrids.

Attempts to minimise the level of dissolved ammonia and ammonium have been made using clinoptilolite, zeolite or other related ion-absorptive resins or activated charcoal, all of which absorb large quantities of ammonia. One of the difficulties in using these materials is ensuring that they are exposed to the water efficiently, but without being dispersed in it. Many varieties of bag and cartridge systems have been used with some success and water can be passed through such containers using pumps or more simply using air-lift mechanisms.

Frequently, antibiotics or disinfectants are used in transportation. These range from methylene blue, malachite green and potassium

permanganate to acriflavin, kanamycin, gentamycin, chloramphenicol, neomycin and furazolidone. Recommended prophylactic doses of these materials vary.

In recent years there has been some interest in using polyvinyl pyrolidone, PVP, for transporting fish. This is a long-chain polymer of varying molecular weight. The belief is that this material coats the skin of fishes and helps protect against abrasion. It is also thought to form a coating over any damaged epithelium, so promoting healing. PVP has been promoted as a commercial product in a formulation, which also contains extracts of *Alöe vera*. *Alöe vera* extract is a well-known material in the traditional or 'herbal' medicine category which has some known anti-microbial properties as well as being a promoter of wound healing. It has also been used alone in fish transportation and, although the scientific evidence for its effectiveness is not yet strongly documented, the preparation is widely in use for transportation of aquarium fish and is being evaluated by aquaculturists, especially for deliveries from hatcheries. Ross *et al*. (2007) used *Alöe vera* extract as a complementary agent in transportation of juvenile *Menidia estor*, a relatively delicate endangered fish species. They showed that when used at 0.02% by volume in combination with benzocaine sedation, there was significantly less scale loss during transportation over an 8.5 hour period. Despite this observation, the utility of *Aloe vera* in transportation is not clear and a more systematic investigation would be worthwhile.

Sedation

Apart from minimising handling time, direct physical injury and stress effects during transportation, sedation is useful in reducing metabolic rate and consequently oxygen consumption, and in reducing excretion of metabolic products into the water. The simplest calming effect is achieved through cooling; more care is needed when using sedative drugs. A combination of some cooling and low-dose drugs can also be a useful approach. Sedation is by no means an essential ingredient of transportation and the decision to use this approach will depend on the individual circumstances. The use of drugs, at however low a dose rate, with animals destined for immediate markets must be avoided and considered along with any necessary customer protection regulations.

Sedation of molluscs

Hovgaard (1984) described a method used in New Zealand for maintaining the keeping quality of the blue mussel, *Perna canalicula*, during

transportation. The mussels are cooled by ice but are not in direct contact with it, and the barrier between the ice and mussels allows the melted water to pass through. This method can maintain the mussels in good condition for about 10–12 days.

Panggabean *et al*. (1989) evaluated storage conditions for *Crassostrea gigas* and noted that larvae stored at 5°C had greater survival than larvae held at room temperature.

Solis and Heslinga (1989) showed that survival of seed of the clam *Tridacna derasa* in pure oxygen was higher than that in the ambient atmosphere over exposure times ranging from 16 to 48 hours. They considered that the low cost of the treatment justifies its routine use for commercial seed shipments.

Bower *et al*. (1999) transported live squid, *Todarodes pacificus*, by car and aeroplane for up to 10 hours using hypothermia in oxygenated water at 0–1°C.

There are no records of the use of sedative drugs in transportation of molluscs. Bivalve molluscs have lengthy survival times in air if kept cool.

Sedation of crustaceans

In many cases, large crustaceans can be transported in air, without the use of anaesthetic drugs, and this is assisted if the animals are kept cool often in damp sea-weed or a similar medium. There are numerous reports of successful transportation using variations of these methods (Meereen, 1991; Meeren and Uglem, 1993) and Whiteley and Taylor (1992) identified the conditioning that affects the success of such operations.

Paterson (1993) describes successful live shipment of *Penaeus monodon* without water following cold 'anaesthesia' at 12°C. Salin and Jayasree-Vadhyar (2001) used chilled sawdust for live transportation of *Penaeus monodon*. They anaesthetised 15-g animals by cooling from 25°C to 14°C before packing in layers of moist, chilled sawdust. The prawns were then held in chilled storage at 14°C for up to 36 hours. 100% survival was obtained after 24, 20 and 16 hours in the slow, medium and fast chilling regimes and they considered that a chill rate of $2.8^{\circ}C\,h^{-1}$ was optimum. Surayanigrum *et al.* (1997) used rapid cooling of 200–250 g lobster (*Panulirus homarus*) to 14°C in 15–20 minutes and found good survival in a dry pack system for up to 20 hours.

Singh *et al.* (1982) showed that chloral hydrate was an effective sedative for the transport of *Penaeus monodon*. A dose of 400 $mg\,L^{-1}$ was found to be the most effective dose for successful transportation of 375

animals per litre for up to 28 hours. Smith and Ribelin (1984) showed that 36-day-old post-larval *P. vannamei* can be packed and shipped at a density of at least 190 animals per litre for 18 hours at 18–20°C with negligible mortality. Adult *M. rosenbergi* packed unconstricted resulted in survival rates substantially higher than those obtained from prawns, which were immobilised by wrapping in mesh (Smith and Wannamaker, 1983).

Morgan *et al.* (2001) evaluated clove oil as an anaesthetic with three Pacific coast crab species, *Cancer magister*, *Hemigrapsus oregonensis* and *Pugettia producta*. They found effective dose ranges of 0.51.5, 1–3 and 0.015–0.25 ml L^{-1}, respectively, in the three species and considered that the lower doses had potential during transportation of these decapods. Vartak and Singh 2006 used 15 mg L^{-1} clove oil to sedate post-larvae of the freshwater prawn, *Macrobrachium rosenbergii*, for up to 3 hours and were also able to show that the ethanol used as a vehicle had no anaesthetic effect at the working concentration. Juvenile prawns were also anaesthetised using clove oil although in all cases induction was rather slow. Overall, clove oil is a useful decapod anaesthetic as well as having potential as a sedative for live transport of commercially important crustaceans.

Sedation of fish

Cooling has been widely used as a calming technique in fish transportation (see Chapter 11). Both the absolute temperature reduction applied and the rate of cooling are important factors. Temperate fishes can often be cooled almost to freezing point while warm-water fishes can also be cooled by a similar amount. Yoshikawa *et al*. (1989) examined techniques for cold anaesthesia of *Cyprinus carpio* during transportation and Nakamura (1992) showed that slow cooling of *Cyprinus carpio* prior to cold-air transportation at 7°C considerably extended tolerance and increased survival. Liu *et al.* (1999) describe maintenance of the flounder *Paralichthys olivaceus* for up to 52 hours at close to freezing point.

The sedative effect of carbon dioxide was described earlier and it is an effective agent during transportation. Itazawa and Takeda (1982) and Takeda and Itazawa (1983) found that carbon dioxide released either from sodium bicarbonate or by bubbling into the medium initially were unsatisfactory methods and that for controlled sedation the gas needs to be bubbled into the medium throughout transportation. However, they also found that for 100% survival, oxygen levels require to be elevated at the same time as carbon dioxide. This tends to make this approach impractical. Yokoyama *et al*. (1989), who used combined techniques,

found that carp could be 'anaesthetised' after about 10 minutes at 4°C and could then be transported safely for up to 9.5 hours at 14°C in 80 mm Hg carbon dioxide. Mishra *et al.* (1983) used the sedative effect of carbonic acid for transportation of *Labeo rohita* fry for up to 215 hours.

A number of workers have reviewed tranquillising drugs for transportation, including McFarland (1960), Bell (1964), Durve (1975) and Rothbard 1988. In most cases only a few agents are recommended and these are summarised in Table 14.1.

Benzocaine hydrochloride at 25 mg L^{-1} has been shown to be effective in transporting fish (Ferreira *et al.*, 1984). Okoye (1984) extended this approach and showed that benzocaine anaesthesia combined with moderate cooling produced substantial calming with 100% survival in *Oreochromis niloticus* juveniles. The highest sustainable dose was 32 mg L^{-1} at 24°C, but combinations of 20–25 mg L^{-1} and 18°C gave the best results. Ross and McKinney (unpublished data) showed that the dose of benzocaine, which could be used in transportation of rainbow trout, was limited by the need to maintain equilibrium and spacing of the animals. If sedated too deeply, fish sink to the bottom of the container where they may be in high densities locally, resulting in massive and rapid local environmental degradation. Benzocaine at 5 mg L^{-1} reduced activity for up to 2 hours while at 7 mg L^{-1} activity was reduced for 3 hours although there was some brief initial hyperactivity at this dose. Doses in excess of 10 mg L^{-1} all eventually resulted in loss of equilibrium and are hence unsuitable for use during long transportation. There was no improvement in water quality over non-sedated fish over 6 hours. Ross *et al.* (2007) used 15 mg L^{-1} benzocaine sedation in combination with hypothermia to transport *Menidia estor* for over 8 h with minimal mortalities.

Teo and Chen (1993) noted the importance of using anaesthetics in transportation of aquarium fishes by air. The same authors showed suppression of oxygen consumption rates of *Poecilia reticulata* using 2-phenoxyethanol in simulated transportation trials. Based on rate of excretion of metabolic wastes over 48 hours and cost, Guo *et al.* (1993) rated 2-phenoxyethanol best for transportation of aquarium fish, followed by quinaldine, metomidate and MS222. Radull *et al.* (2001) considered that 2-phenoxyethanol was safe for the long-distance transportation of juvenile spotted grunter (*Pomadasys commersonnii*) and that a dose of 0.2 cm^3 L^{-1} gave deep anaesthesia without loss of equilibrium.

Cooke *et al.* (2004) used a range of clove oil concentrations for transportation of largemouth bass (*Micropterus salmoides*) and found that 5–9 mg L^{-1} gave rapid induction of Stage 2 anaesthesia with rapid recovery. AQUI-S has been used for transportation of a number of species. Its gentle action and wide margin of safety make it very suitable for

Table 14.1 Summary of dose rates of drugs used in transportation of fish.

Species	Drug	Dose rate	Author
Alosa sapidissima	Metomidate	0.5–1.0 mg L^{-1}	Ross *et al.* (1993)
Aquarium fish	2-phenoxyethanol	0.2 cm^3 L^{-1}	Guo *et al*. (1993)
	Metomidate	1 mg L^{-1}	
	MS222	30 mg L^{-1}	
	Quinaldine	10 ppm	
Carassius auratus	2-phenoxyethanol	0.25–35 cm^3 L^{-1}	Kaiser and Vine (1998)
Cyprinus carpio	Carbon dioxide	95–115 mm Hg + 400 mm Hg pO_2	Takeda and Itazawa (1983)
	MS222	20 mg L^{-1}	Horvath *et al*. (1984)
Fish sp.	Quinaldine	5 mg L^{-1} + 10–30 mg L^{-1} MS222	Solomon and Hawkins (1981)
	Chloral hydrate	250 mg L^{-1}	
	MS222	10–30 mg L^{-1}	
	Tertiary amyl alcohol	0.3–0.8 g L^{-1}	
Hypophthalmichthys molitrix	MS222	10 mg L^{-1}	Horvath *et al*. (1984)
Indian carps	2-phenoxyethanol	0.3 cm^3 L^{-1}	Jhingran and Pullin (1985)
	Amylobarbitone	50–175 mg L^{-1}	
	Barbital sodium	50 mg kg^{-1}	
	Chloral hydrate	0.6 g L^{-1}	
	MS222	10–25 mg L^{-1}	
	Quinaldine	25 ppm	
	Tertiary amyl alcohol	0.45 cm^3 L^{-1}	
	Urethane	100 mg L^{-1}	
	Lignocaine	10 cm^3 L^{-1}	Das and Goswami (2003)
	Thiopentone	15 mg L^{-1}	
	Xylazine	4 cm^3 L^{-1}	
Labeo rohita	Carbonic acid	500 ppm	Mishra *et al.* (1983)
Menidia estor	Benzocaine	15–18 mg L^{-1}	Ross *et al*. (2007)
Mugil trichodon	MS222	20–30 mg L^{-1}	Alvarez Lajonchere and Garcia (1982)
	Tertiary amyl alcohol	0.25–0.5 cm^3 L^{-1}	
	Tertiary butyl alcohol	3.0–3.5 cm^3 L^{-1}	
Oreochromis niloticus	Benzocaine	25 mg L^{-1}	Ferreira *et al*. (1984)
	Benzocaine	20–25 mg L^{-1}	Okoye (1982)
Puntius gonionotus	Etomidate	0.15–0.2 ppm	Gopinath (1990)
Salmonids	2-phenoxyethanol	0.5 ppm	Bell (1964)
		0.25 ppm	Barton and Helfrich (1981)
Sparus aurata	Quinaldine	5 ppm + 1 ppm diazepam	Kumlu and Yanar (1999)
Tinca tinca	MS222	5–50 mg L^{-1}	Jirasek *et al.* (1978)
	Propoxate	0.25–0.5 ppm	Jirasek *et al.* (1978)
Various spp.	Quinaldine	15–30 ppm	Woynarovich and Horvath (1980)

long exposure without attendant mortalities. The manufacturers recommend 1–5 ppm AQUI-S for transportation of live fish under light sedation.

Sado (1985) used quinaldine in transportation of tilapias and the general dose range is 5–30 mg L^{-1}. Kumlu and Yanar (1999) found that 5 ppm quinaldine in combination with 1 ppm diazepam gave light sedation for transportation of juvenile *Sparus aurata*.

Altun and Danabas (2006) used 2-phenoxyethanol dissolved in equal parts of ethanol to sedate *Cyprinus* carpio fingerlings. They found that doses of 0.4–0.6 cm^3 L^{-1} were moist suitable for long duration sedation. Pavlidis *et al.* (2003) successfully used 10–20 ppm 2-phenopxyethanol in 48 h simulated transportation of red porgy fry with good survival at fish transportation densities up to 25 kg m^{-3}. A higher dose of 50 ppm produced significant mortalities.

Prihoda (1979) found that metomidate provided effective sedation for transportation over a 2-hour period at 1:8,000,000, equivalent to only 0.125 mg L^{-1}.

Activity was reduced during short duration transportation of *Labeo rohita*, *Cirrihinus mrigala* and *Catla catla,* using thiopentone (15 mg L^{-1}), lignocaine (10 cm^3 L^{-1}) and xylazine (4 cm^3 L^{-1}) (Das and Goswami, 2003); although longer duration transportation was not tested.

Kacem *et al.* (1988) experimented with a β-blocker, carazolol (1-(9 *H*-carbazol-4-yloxy)-3-(propan-2-ylamino)propan-2-ol), in transportation of *Dicentrarchus labrax*. They found that red cell counts and haemoglobin levels were higher in fish after a 24-hour simulated transportation in 140 mg L^{-1} carazolol than in those treated with 15 mg L^{-1} MS222.

Murai *et al*. (1979) used orally administered valium (diazepam) at 0.04 mg kg^{-1} to enhance survival during transportation of American shad, *Alosa sapidissima*. As noted previously, oral administration has the disadvantage that drugged food may not be consumed equally by all members of the group.

Summary

The principal issues in transportation are minimising the weight of water and equipment carried, minimising the costs of these materials and maximising the survival and health of the animals carried (Taylor and Solomon, 1979). With the exception of a few cases where animals can be transported out of water, maintenance of good water quality is the major factor in transportation and is the prime requirement for success. Some countries have standards for transportation and in Germany the levels of certain water quality factors, the maximum fish density and

duration of the journey are controlled by legislation. While not always necessary, some degree of sedation during transportation can be a useful tool to assist in achieving these objectives.

References

Altun, T. and Danabas, D., Effects of short and long exposure to the anesthetic 2-phenoxyethanol mixed with ethyl alcohol on common carp (*Cyprinus carpio* L., 1758) fingerlings, *Israeli J. Aquac.*, 58 (2006) 178–182.

Alvarez-Lajonchere, L. and Garcia-Moreno, B., Effects of some anaesthetics on postlarvae of *Mugil trichodon* Poey (Pisces, Mugilidae) for their transportation, *Aquaculture*, 28(3–4) (1982) 385–390.

Barton, B.A. and Helfrich, H., Time-dose response of juvenile Rainbow trout to 2-phenoxyethanol, *Prog. Fish Culturist*, 46(4) (1981) 223.

Bell, G.R., A guide to the properties, characteristics and uses of some general anaesthetics for fish, *Bull. Fish. Res. Board Can.*, No. 148, 1964, 11 p.

Berka, R., The transport of live fish. A review, EIFAC Technical paper No. 48, CECPI. FAO, Rome, Italy, 1986, 52 p.

Bower, J.R., Sakurai, Y., Yamamoto, J. and Ishii, H., Transport of the ommastrephid squid *Todarodes pacificus* under cold-water anesthesia. *Aquaculture*, 170 (1999) 127–130.

Chittenden, M.E., Transporting and handling young American shad, *New York Fish Game J.*, 18 (1971) 123–128.

Das, S.K. and Goswami, U. C., Evaluation of a few locally available anaesthetics for potential use in fish seed transportation, *Fish. Technol. Soc. Fish. Technol.*, 40 (2003) 101–104.

Durve, V.S., Anaesthetics in the transport of mullet seed, *Aquaculture*, 5 (1975) 53–63.

Ferreira, J.T., Schoonbee, H.J. and Smit, G.L., The use of benzocaine hydrochloride as an aid in the transport of fish, *Aquaculture*, 42(2) (1984), 69–174.

Gopinath, N., Biotechnical considerations in the handling and transport of live fishery products, in: N. Gopinath and A Abu-Baker, (eds.), Handling and Processing: The Selling Point in Malaysia Fisheries. Occasional publication No. 3, Malaysian Fisheries Society, Kuala Lumpur, Malaysia, 1990.

Guo, F.C., Teo, L.H. and Chen, T.W., Application of anaesthetics in the transport of platyfish by air, in: Carrillo, M., Dahle, L., Morales, J., Sorgeloos, P., Svennevig, N. and Wyban, J. (eds.), From Discovery to Commercialisation, Special publication of the European Aquaculture Society, No. 19, 1993, 228 p.

Hovgaard, P.,. Culture of blue mussel in Ireland, *Norsk Fiskeoppdrettt*, 9(6) (1984) 38.

Horvath, L., Tamas, G. and Tolg, I., Special methods in pond fish husbandry, Seattle, Halver Corporation, 1984 146 p.

Innes-Taylor, N. and Ross, L. G., The use of hydrogen peroxide as a source of oxygen for the transportation of live fish, *Aquaculture*, 70(1–2) (1988) 183–192.

Itazawa, Y. and Takeda, T., Respiration of carp under anesthesia induced by mixed bubbling of carbon dioxide and oxygen, *Bull. Jpn. Soc. Sci. Fish.*, 48(4) (1982) 489–493.

Jhingran, V.G. and Pullin, R.S.V., A hatchery manual for the common, Chinese and Indian major carps, Asian Development Bank/ICLARM, Manila, Philippines, 1985, 191 p.

Jirasek, J., Adamek, Z. and Giang, P.M., The effect of administration of the anaesthetics MS 222 (Sandoz) and R 7464 (Propoxate) on oxygen consumption in the tench (*Tinca tinca.* L.), *Zivocisna-Vyroba*, 23(11) (1978) 835–840.

Johnson, S.K., Transport of live fish, *Aquac. Mag.*, 5(6) (1979) 20–24.

Kacem, N.H., Aldrin, J. F. and Romestand, B., Modifications de certaines reponses secondaires de stress chez *Dicentrarchus labrax* sous l'effect d'un beta -bloquant a base de Carazolol. Application au transport, *Aquaculture*, 68(3), (1979) 277–285.

Kaiser, H. & Vine, N., The effect of 2-phenoxyethanol and transport packing density on the post-transport survival rate and metabolic activity in the goldfish, *Carassius auratus*, *Aquarium Sci. Conserv.*, 2(1) (1998) 1–7.

Kumlu, M. and Yanar, M., Effects of the anesthetic quinaldine sulphate and muscle relaxant diazepam on sea bream juveniles (*Sparus aurata*), *Israeli J. Aquac. /Bamidgeh*, 51 (1999) 143–147.

Laird, L.M. and Wilson, A.R., A method for improving the survival of fish eggs during transportation, *Fish. Manage.*, 10(3) (1979) 129–131.

Liu, Q.Y., Bangzhong; Y.J. and Liang, Mengqing, A waterlessly keep-alive technique for *Paralichthys olivaceus*, *J. Fish. Sci. China*, 6 (1999) 101–104.

Malyukina, G.A., Martem'-yanov, V.I. and Flerova, G. I., The alarm pheromone as a stress factor for fish, *J. Ichthyol.*, 22(2) (1982) 147–150.

McFarland, W.N., The use of anaesthetics for the handling and the transport of fishes, *Calif. Fish Game*, 46 (1960) 407–431.

Marathe, V.B., Huilgol, N.V. and Patil, S. G., Hydrogen peroxide as a source of oxygen supply in the transport of fish fry, *Progr. Fish Culturist*, 37(2) (1975) 117.

Meeren, G.-van-der, Acclimatisation techniques for lobster, Norwegian Society for Aquaculture Research, Bergen, Norway, 1991, 95–97.

Meeren, G. van-der, and Uglem, I., Techniques for transportation and release of juvenile lobsters (*Homarus gammarus*), *Fisken Havet*, 7 (1993) 31 p.

Mishra, B.K., Kumar, D. and Mishra, R., Observations on the use of carbonic acid anaesthesia in fish fry transport, Aquaculture, 32 (1983) 405–408.

Morgan, J., Cargill, C. and Groot, E., The efficacy of clove oil as an anesthetic for decapod crustaceans. Shellfish Health – Risks and Management: Proceedings of a special session held at Aquaculture Canada 2001, Halifax. no., *Bull. Aquac. Assoc. Can.*, 101–3 (2001) 27–31.

Murai, T., Andrews, J.W. and Muller, J.W., Fingerling American shad: effect of valium, MS-222, and sodium chloride on handling mortality, *Progr. Fish Culturist*, 41(1) (1979) 27–29.

Nakamura, K., Effect of precooling on cold air-tolerance of the carp *Cyprinus carpio*, *Bull. Jpn. Soc. Sci. Fish.*, 58(9) (1992) 1615–1620.

Okoye, R.N., Techniques for Transportation of Juvenile Tilapia, M. Sc. thesis, University of Stirling, 1982, 39 p.

Panggabean, L., Waterstrat, P. R., Downing, L. and Beattie, J. L., Storage and transportation of straight-hinge oyster larvae, *J. Shellfish Res.*, 8(1) (1989) 323–324.

Paterson, B.D.,. Respiration rate of the kuruma prawn, *Penaeus japonicus* Bate, is not increased by handling at low temperature (12°C), *Aquaculture*, 114(3–4) (1993) 229–235.

Pavlidis, M., Angellotti, L., Papandroulakis, N. and Divanach, P., Evaluation of transportation procedures on water quality and fry performance in red porgy (*Pagrus pagrus*) fry, *Aquaculture*, 218 (2003) 187–202.

Prihoda, J., Experience with use of propoxate in anaesthesia and transport of salmonids in Slovakian fishery union centres, *Biol.-Chem.-Vet.-Zivocisne.-Vyroby* 15(3) (1979) 283–288.

Radull, J., Kaiser, H. and Hecht, T., Effects of 2-phenoxyethanol on stressor-induced changes in metabolic rates of juvenile spotted grunter, *Pomadasys commersonnii*, 2nd Western Indian Ocean Science Association (WIOMSA) Scientific Symposium, Book of Abstracts, (2001) 45–46.

Ross, L.G., Sanchez Blanco, J., Martínez-Palacios, C.A., Racotta, I.S. and Toledo Cuevas, Anaesthesia, sedation and transportation of juvenile *Menidia estor* (Jordan) using benzocaine and hypothermia, *Aquac. Res*., (2007).

Ross, R.M., Backman, T.W.H. and Bennett, R.M., Evaluation of the anesthetic metomidate for the handling and transport of juvenile American shad, *Progr. Fish Culturist*, 55(4) (1993) 236–243.

Ross, L.G., Sanchez Blanco, J., Martínez-Palacios, C.A., Racotta, I. S. and Toledo Cuevas, M., Anaesthesia, sedation and transportation of juvenile *Chirostoma estor estor* Jordan 1879 using benzocaine and hypothermia, *Aquac. Res*. 38 (2007) 909–917.

Rothbard, S., The use of chemical tranquillisers in fish transport, *Fish and Fish-breeding in Israel*, 21(1) (1988) 28–34.

Sado, E.K., Influence of the anaesthetic quinaldine on some tilapias, *Aquaculture*, 46(1) (1985) 55–62.

Salin, K.R. and Jayasree-Vadhyar, K. Effect of different chilling rates for cold anaesthetization of *Penaeus monodon* (Fabricius) on the survival, duration and sensory quality under live storage in chilled sawdust, *Aquac. Res*. 32 (2001) 145–155.

Sampson, D.R.T. and Macintosh, D. J., Transportation of live carp fry in sealed polythene bags, *Aquaculture* 54 (1–2) (1986) 123–127.

Singh, H., Chowdhury, A.R. and Pakrasi, B.B., Experiments on the transport of postlarvae of tiger prawn *Penaeus monodon* Fabricius, Symposium Series of the Marine Biological Association of India, No 6 (1982) 232–235.

Smith, T.I.J. and Ribelin, B., Stocking density effects on survival of shipped postlarval shrimp, *Progr. Fish Culturist*, 46(1) (1984) 47–50.

Smith, T.I.J. and Wannamaker, A.J., Shipping studies with juvenile and adult Malaysian prawns *Macrobrachium rosenbergii* (de Man), *Aquac. Eng*. 2(4) (1983) 287–300.

Solis, E.P. and Heslinga, G.A., Effect of desiccation on *Tridacna derasa* seed: pure oxygen improves survival during transport, *Aquaculture*, 76(1–2) (1989) 169–172.

Solomon, D.J. and Hawkins, A.D., Fish capture and transport, in: Hawkins, A.D. (ed.), Aquarium Systems, Academic Press, 1981.

Suryaningrum, T.D., Suparno, Wibowo, S. and Erlina, M. D., Anaesthetization of lobster by a direct cooling technique for live transport in a dry pack system. Asia Pacific Fishery Commission. Summary report of and papers presented at the 10th session of the Working Party of Fish Technology and Marketing, Colombo, Sri Lanka, 4–7 June 1996, *FAO Fish. Rep*., (1997) 149–154.

Takeda, T. and Itazawa, Y., Possibility of applying anesthesia by carbon dioxide in the transportation of live fish, *Bull. Jpn. Soc. Sci. Fish.* 49(5) (1983) 725–731.

Taylor, A.L. and Solomon, D. J., Critical factors in the transport of living freshwater fishes. I. General considerations and atmospheric gases, *Fish. Manage*. 10(1979) 27–32.

Teo, L.H. and Chen, T. W., A study of metabolic rates of *Poecilia reticulata* Peters under different conditions, *Aquac. Fish. Manage*. 24(1) (1993) 109–117.

Vartak, V. and Singh, R. K., Anesthetic effects of clove oil during handling and transportation of the freshwater prawn, *Macrobrachium rosenbergii* (De Man), *Israeli J. Aquac*., 58 (2006) 46–54.

Wedermeyer, G., Some physiological consequences of handling stress in the juvenile Coho salmon (*Onchorhynchus kisutch*), *J. Fish. Res. Board Can*. 29 (1972) 1780–1783.

Weirich, C.R., Tomasso, J.R. and Smith, T.I.J., Confinement and transport-induced stress in white bass *Morone chrysops* × striped bass *M. saxatilis* hybrids: effect of calcium and salinity, *J. World Aquac. Soc*., 23(1) (1992) 49–57.

Whiteley, E.M. and Taylor, E.W., Oxygen and acid-base disturbances in the hemolymph of the lobster *Homarus gammarus* during commercial transport and storage, *J. Crustacean Biol*. 12(1) (1992) 19–30.

Woynarovich, E. and Horvath, L., The artificial propagation of warmwater fishes. A manual fro extension, FAO Fisheries Technical Paper, 201 (1980) 138–147.

Yokoyama, Y., Yoshikawa, H., Ueno, S. and Mitsuda, H., Application of CO_2 anesthesia combined with low temperature for long-term anesthesia in carp, *Bull. Jpn. Soc. Sci. Fish*., 55(7) (1989) 1203–1209.

Yoshikawa, H., Ueno, S. and Mitsuda, H., Short- and long-term cold-anesthesia in carp, *Bull. Jpn. Soc. Sci. Fish*. 55(3) (1989) 491–498.

Chapter 15

Concluding Remarks

In any given situation it is always worth reconsidering whether anaesthesia or sedation is strictly necessary. Throughout this book a number of instances have been given where anaesthesia may not be essential or whether there are indeed contraindications to its use. Simple fish holders can be constructed which will constrain small specimens quietly, usually for some tens of seconds, allowing rapid tagging or marking. Handling is greatly facilitated if the head and eyes are covered, light is minimised and if noise is reduced. Many broodstock fish can be stripped without recourse to anaesthetics and without damage so long as the handler is gentle but firm and the fish body is well supported. Transportation and simple handling can be enabled using cooling alone and this is well worth considering. As a general rule it is advisable to avoid the use of chemicals with organisms destined for human consumption for obvious reasons. Legislation in this area is changing, but very slowly and cautiously, and there are still very few drugs that can legally be used with fish intended for the human food chain.

Having decided that some degree of immobilisation is desirable, the selection of a suitable anaesthetic technique will be influenced by the situation in which it will be used and thus no simple guidelines can be given. As this book attempts to show, there are advantages and disadvantages inherent in all of the methods described and these should be weighed against the requirements of the work in hand. In addition to availability of equipment, the availability and cost of drugs may limit the choices. It may be prudent in most cases to have more than one drug available, for example, using benzocaine as a routine but holding small stocks of MS222 for use when no appropriate organic solvent is available. Many workers rely on one drug, presumably adhering to the old axiom 'better the devil you know than the one you don't'. This will serve well in most instances but, again, the pro's and con's of all the available methods should be weighed against the objectives of the work. A single approach will not serve for all ends and it is hoped that

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this book, at the least, may engender some flexibility. Having said this, most legislation only allows use of a very limited range of materials, which have been screened and deemed to be safe under certain conditions of use. However, they may not be the only safe materials and the research literature clearly shows several very promising alternatives. Considerable effort is required to have additional materials approved and licensed for use and so perhaps it is no surprise that change is slow.

There are considerable practical advantages to be gained by using electrical anaesthetic techniques. Following modest initial outlay, running costs can be relatively low. It should be borne in mind that anaesthetic drugs can be expensive, or difficult to obtain in certain parts of the world, whereas electricity is either available or can be made available by the use of inexpensive portable generators. A problem with electrical anaesthesia is that of operator safety and while the use of isolating transformers, circuit breakers and interlocking switches can reduce the dangers, it is virtually impossible to guarantee that electric shocks cannot be sustained. It is therefore most important to adhere to a well-considered code of practice and although the technique for practical implementation has been described here, it should be noted that the authors accept no responsibility for accidents in the use of this equipment, howsoever caused.

It is possible that further research will bring electroanaesthesia into wider use in aquaculture, but at present the major drugs – MS222, benzocaine, quinaldine, 2-phenoxyethanol, clove oil and AQUI-S – are used routinely worldwide for most purposes. For more complex procedures and surgical work chemicals are, and will probably remain, the only choice as much more is known of their physiological effects. It is interesting to note the development of techniques using combined drug treatments in an attempt to provide good anaesthesia with good analgesia and there is much work to be done in this area. The tables provided throughout this book are an attempt to draw together some of the available data on the major drugs in current and past use in order to provide guidelines for inexperienced and experienced operators, alike. As we noted in earlier editions, we would be interested to receive communications regarding problems and experiences from our colleagues, old and new, worldwide.

Glossary of Technical Terms

Acute Having a rapid effect, and not long-lasting (opposite to chronic)

Alternating current, a.c. An electrical current whose voltage regularly varies (alternates) from a positive to negative level. Alternating currents (a.c.) may have a sinusoidal, triangular or square waveform. Domestic mains alternating currents are sinusoidal.

Anaesthesia/Anesthesia (USA) A reversible, generalised loss of sensory perception accompanied by a sleep-like state induced by drugs or by physical means (see Chapter 4)

Analgesia Relief from pain.

Anoxia Exposure to zero environmental oxygen concentration

Ataxia Uncoordinated movements

Biopsy Removal of a sample of live tissue (e.g. liver, spleen, gonad) for biochemical or pathological analysis

Bradycardia Reduced heart rate

Branding A form of medium-term marking of animals using heat or cold damage to the skin

Catecholamines Hormones released by the interrenal bodies (in fish) and having widespread metabolic activity. Also responsible for transmission of nervous signals at synapses

Chopped d.c. An alternating waveform with a rapid rise and fall time, usually varying from 0 V to some positive voltage, created by switching of a d.c. source. Widely used in electric fishing

Chromatophore A cell in the integument (skin) containing dispersible or concentratable pigment in one of a range of colours

Chronic Long-lasting (relatively), opposite to acute

Cough response Momentary reversal of water flow over the gills. Almost all fish species exhibit this function.

Direct current, d.c. An electrical current whose voltage is at a fixed positive or negative level and which does not vary regularly.

Drug A chemical substance which, when consumed by, or applied to, an animal has certain biological effects.

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ECG Electrocardiogram. An electrical record of heart muscle contractions usually recorded from surface electrodes applied to an animal.

Ectotherm An animal whose body temperature is not controlled and closely follows that of the environment, e.g. invertebrates, fishes, amphibia and reptiles.

Electroanaesthesia Anaesthesia brought about by electrical stimulation of an animal, or part of an animal. Evidence suggests that this effect is not 'true' anaesthesia but simply immobilisation.

Electronarcosis As electroanaesthesia.

Galvonarcosis Electroanesthesia brought about by use of a direct current (dc.). Its effects are usually apparent only when current is applied.

Gonadectomy Surgical removal of the gonads

Gular ventilation Ventilation achieved by movement of the base of the throat

Haemoconcentration Increase in concentration of blood by loss of water, gain of ions or addition of cells.

Haemodilution The converse of haemoconcentration

Hypophysectomy Surgical removal of the pituitary body, achieved in fish through the roof of the mouth

Hypoxia Exposure to low environmental oxygen concentration

Intramuscular (IM) Injection given into the muscle

Intraperitoneal (IP) Injection given into the peritoneum, the body cavity.

Local anaesthesia Affecting a localised area, e.g. an area of skin

mg L^{-1} Milligrams of a solute per litre of solvent

Narcosis As for anaesthesia, but does not necessarily presume that recovery will occur

Narcotic A sleep inducing agent

Osmolarity The solute load (e.g. of blood) in kg L^{-1}

ppm Parts per million. Usually refers to concentration of a solution measured either in microlitre of a liquid per litre of solvent (v/v) or milligram of a solid per litre of solvent (w/v).

Pulsed d.c. See chopped d.c.

Sedation A preliminary level of anaesthesia, in which response to stimulation is greatly reduced and some analgesia is achieved, but sensory abilities are generally intact and loss of equilibrium does not occur.

Square wave An alternating waveform with a rapid rise and fall time. Can be generated by chopping of a d.c source or by adding full harmonics to a sine wave.

Steroids A group of powerful hormones having widespread metabolic actions. In fish, released by the inter-renal bodies under control of the hypothalamus/pituitary axis.

Stressor Any environmental variable exceeding normal bounds

Tachycardia Accelerated heart rate

Tachyventilation Accelerated gill ventilation rate (assessed as movement of opercula in fish)

Taxis Motor activity induced by external stimulation

Telemetry Remote sensing of data by radio or ultrasonic means, in this case from an animal

Topical anaesthesia Affecting a localised external area, e.g. an area of skin

Species Index

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Subject Index

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