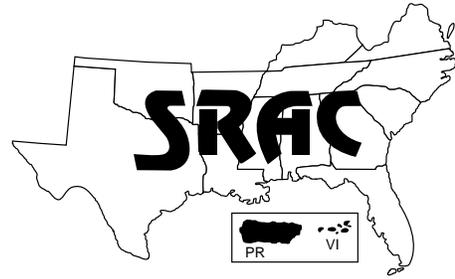


Southern Regional Aquaculture Center



November 2004

Anesthetics in Aquaculture

Shawn D. Coyle, Robert M. Durborow and James H. Tidwell*

Fish are easily stressed by handling and transport and stress can result in immuno-suppression, physical injury, or even death. In aquaculture, anesthetics are used during transportation to prevent physical injury and reduce metabolism (DO consumption and excretion). They are also used to immobilize fish so they can be handled more easily during harvesting, sampling and spawning procedures.

An ideal anesthetic should induce anesthesia rapidly with minimum hyperactivity or stress. It should be easy to administer and should maintain the animal in the chosen state. When the animal is removed from the anesthetic, recovery should be rapid. The anesthetic should be effective at low doses and the toxic dose should greatly exceed the effective dose so that there is a wide margin of safety.

Stages of anesthesia

Induction

Most anesthetics can produce several levels or stages of anesthesia. Stages include sedation, anesthesia, surgical anesthesia and death

(Table 1). The stage achieved usually depends on the dose and the length of exposure. When an anesthetic is first administered (induction) fish may become hyperactive for a few seconds.

Maintenance

Once the desired degree of anesthesia is reached, it may be desirable to maintain fish in that state for some time. Because drug dose and exposure time are often cumulative, it is difficult to maintain a uniform depth of anesthesia. One reason for this is that levels of anesthetic may continue to accumulate in the brain and muscle even after blood levels have attained equilibrium. A desired level of anesthesia can usually be maintained by reducing the dosage.

The condition of the animals must be visually monitored during this maintenance period. A change in breathing rate is the most obvious indicator of over-exposure. If this occurs, animals must be moved or the systems flushed immediately.

Recovery

During the recovery stage the anesthetic is withdrawn and fish return to a normal state. To reduce recovery time, induction should be rapid and handling time should be minimal. Initial recovery may take from a few seconds to several minutes, depending on the anesthetic administered. Typically, the animal will attempt to right itself and will begin to respond to noise and other sensory stimuli. Full recovery can take minutes to hours, depending on the species and drug used.

Table 1. Stages of anesthesia in fish.

Stage	Condition	Behavior/Response
I	Sedation	Motion & breathing reduced
II	Anesthesia	Partial loss of equilibrium Reactive to touch stimuli
III	Surgical anesthesia	Total loss of equilibrium No reaction to touch stimuli
IV	Death	Breathing & heart beat stop Overdose - eventual death

*Kentucky State University Aquaculture Research Center.

Great care should be taken during the recovery stage to minimize stress and prevent mortality. If an animal fails to recover, increasing the flow of anesthetic-free water over the gills will often accelerate and normalize the heart beat. Move the fish backwards and forwards in the recovery bath or gently pass water over the gills with a hose. This increases gill blood flow and eliminates the drug more rapidly.

Legal aspects

Many chemical anesthetics have been used on fish over the years. Most have now been discarded or are not widely used. The U.S. Food and Drug Administration (FDA) regulates which chemicals can be used on food fish. When fish are exposed to an anesthetic, residues or metabolites of the substance remain in the flesh for a period of time until they are excreted or metabolized. Therefore, FDA may require a specific withdrawal time before the animal can be used for food or released into the environment where it might be captured for food.

Anesthetics are licensed for use in food animals only after completing a full drug development program designed to protect the cultured animals, human users, the food chain, and the environment. The program requires a wide range of inputs from the drug company, research scientists, national agencies, and the farming and feed industries. Licensing a new drug is time consuming and costly. Aquaculture is an important industry worldwide, but it is still relatively small compared to other animal production industries and the human medical industry. For this reason, drug companies have not been able to justify the costs of licensing new drugs because the expected financial return is low. The only anesthetic drug currently approved by the FDA for use on food fish is tricaine methanesulfonate (MS-222).

Factors affecting anesthesia

Many factors affect the efficacy of anesthetics in fish. These can be divided into biological and environmental factors.

Often, the rate at which anesthetic drugs become effective is related to the gill area to body weight ratio, which can vary considerably among fish species. Aquatic species also have different metabolic rates that affect the rate at which chemicals are absorbed and anesthesia is induced. For example, cold-water species seem to respond to lower concentrations of anesthetic than warm-water species.

There are also factors that can affect anesthesia within a particular species. Larger individuals generally require a greater concentration of anesthetic than smaller individuals. In contrast, it has also been reported that the larger, more active fish in a group are anesthetized faster than smaller ones. Many drugs such as MS-222 and benzocaine are fat-soluble; therefore, in larger fish or gravid females, anesthesia may last longer and recovery may be slower as the drug is removed from the lipid reserves. Also, diseased or weakened animals are much more susceptible to anesthetic treatment.

Environmental factors can also profoundly affect the efficacy of certain anesthetics. Aquatic invertebrates and fish are ectotherms; their body temperature closely follows that of their environment. As a result, physicochemical passage of the drug into the fish is also temperature related. At lower water temperatures, higher doses or longer exposure times are required with MS-222, benzocaine and 2-phenoxyethanol, presumably because the absorption rate decreases at lower temperatures. The pH of an anesthetic solution also can influence its efficacy, possibly by affecting the ratio of charged to uncharged molecules. This is most pronounced with quinaldine, which loses its efficacy in solutions with low pH.

Anesthesia of fish

Fish are usually anesthetized by immersing them in an anesthetic bath containing a suitable concentration of drug so that the drug is absorbed through the gills and rapidly enters the blood stream. The simplest procedure is to prepare the required drug concentration in an aerated container and quickly but gently transfer the fish to the container. The anesthetic bath and recovery tank should use water (at a similar temperature and chemistry) from which the animals originated. Water quality needs to be carefully controlled, especially where large numbers of animals are being handled and baths are being reused. Main concerns involve maintaining proper temperature, adequate dissolved oxygen, low ammonia and a minimum amount of fecal matter.

Applying an anesthetic solution to the gills with a spray bottle can be useful with large animals or if immersion is impractical. A 100- to 200-mg/L solution of MS-222 is reported to be effective when applied to the gills of salmonid broodstock. This method allows the fish to be handled without immersion, and it has no effect on subsequent egg hatching success.

Anesthesia of aquatic invertebrates

Less is known about anesthetizing invertebrates because it is not done as often. Most operations in crustacean culture can be conducted without anesthesia, although the rapid movement of shrimp can present handling problems and their cannibalistic nature can be a problem during holding and transporting. Consequently, there has been some interest in investigating crustacean anesthetics, particularly for transport. Crustaceans respond differently to anesthesia than finfish, possibly because their synaptic receptor sites are not affected by certain anesthetics. For example, MS-222 is not effective on many crustaceans. It seems that much higher concentrations are required to anesthetize crus-

taceans than fish. Aqui-S™ has been reported to be effective on freshwater prawns (*Macrobrachium rosenbergii*), but only at concentrations 5 to 10 times higher (100 to 200 mg/L) than those used on finfish (20 mg/L). Carbon dioxide is an effective anesthetic for most crustaceans. It is most frequently dispensed as a mixture of baking soda and acetic acid. Cooling is also an effective way to immobilize crustaceans, but one must be careful because cooling can kill the animals.

Anesthetics used in fish

At this time, only MS-222 is registered for use on food fish in the U. S. However, many compounds have been evaluated experimentally and some are being used on nonfood fish and in research. The substances described below have been extensively evaluated in the U. S. or other countries. Effective dosages of these drugs for different fish species are summarized in Table 2.

MS-222

The chemical name for MS-222 is tricaine methanesulfonate. It is sold as Tricaine-S™ and Finquel™. It comes as a white, crystalline powder that can be dissolved in water at up to an 11% solution. It lowers the pH of water, creating an acidic condition that can irritate fish and cause harmful side effects. To prevent problems, the stock solution can be buffered with sodium bicarbonate (baking soda) to achieve a pH of 7. One of the major drawbacks of MS-222 is that even when fish are deeply anesthetized, handling still increases levels of plasma cortisol concentrations, an indicator of stress.

Induction is rapid and can take as little as 15 seconds. Salmonids are quickly anesthetized when immersed in 25 to 50 mg/L. Anesthesia can be maintained at 10 mg/L. Channel catfish (*Ictalurus punctatus*) require 25 to 50 mg/L for sedation and 100 to 250 mg/L for full anesthesia, with a 3-minute induction time. Up to

100 mg/L is required for some species, including tilapia. Generally, concentrations greater than 100 mg/L should not be used for salmonids, and levels higher than 250 mg/L should not be used for warm-water fish.

Recovery is usually rapid and equilibrium can be expected to return after only a few minutes. A recovery time longer than 10 minutes suggests that too much anesthetic is being used or that the exposure time is too long. MS-222 has a good safety margin in fish. In trout, for example, the effective concentration is 40 mg/L and the maximum safe concentration is 63 mg/L. The safety margin narrows as temperature rises and appears to be smaller for smaller fish. The drug is more potent in warm waters with low hardness.

MS-222 is excreted in fish urine within 24 hours and tissue levels decline to near zero in the same amount of time. It is approved for use on food fish in the U. S. and the United Kingdom, but was recently banned in Canada. The withdrawal time for MS-222 required by FDA is 21 days, which makes it impractical as an anesthetic for fish en route to market.

Benzocaine

Benzocaine, or ethyl aminobenzoate, is a white crystal that is chemically similar to MS-222. However, benzocaine is almost totally insoluble in water and must first be dissolved in ethanol or acetone. The standard approach is to prepare a stock solution in ethanol or acetone (usually 100 g/L) that will keep for more than a year when sealed in a dark bottle. In solution, benzocaine is neutral (pH 7) and therefore causes less hyperactivity and initial stress reaction than unbuffered MS-222. Benzocaine is effective at approximately the same doses as tricaine (25 to 100 mg/L). Benzocaine has a fair margin of safety, although this appears to be reduced at higher temperatures. It is not safe for exposures longer than 15 minutes. Its efficacy is not affected by water

hardness or pH. As with MS-222, it is fat-soluble and recovery times can be prolonged in older fish or gravid females. Benzocaine is not approved by FDA for use on food fish in the U. S.

Quinaldine

Quinaldine is a yellowish, oily liquid with limited water solubility that must be dissolved in acetone or alcohol before it is mixed with water. While it is an effective anesthetic, it is an irritant to fish, has an unpleasant odor, and is a carcinogen. The low cost of quinaldine has made it a popular tool for collecting tropical fish for the aquarium trade, as well as in the bait and sport fish industries. Quinaldine sulfonate is a pale yellow, water-soluble powder; it is more costly than quinaldine or MS-222.

Quinaldine solutions are acidic and are usually buffered with sodium bicarbonate. Induction takes 1 to 4 minutes and may cause mild muscle contractions. Recovery is usually rapid. The effective treatment concentration of quinaldine solutions varies with species, but is generally 15 to 60 mg/L. Grass carp (*Ctenopharyngodon idella*) lose equilibrium within 5 minutes of exposure to 15 mg/L. However, quinaldine concentrations of 50 to 1,000 mg/L were required to completely anesthetize tilapia.

Quinaldine may not produce the deep anesthesia needed for surgery because some reflex responsiveness is usually retained. Higher doses (150 mg/L) have been used for surgical procedures, but quinaldine is not usually recommended for these procedures. Fish under full quinaldine anesthesia normally do not stop their gill ventilation so are not as susceptible to asphyxia from respiratory arrest as they are with MS-222. In general, the potency of quinaldine is higher in hard water and warm water. Quinaldine is not approved by the FDA for use on food fish in the U. S.

2-Phenoxyethanol

2-Phenoxyethanol is an opaque, oily liquid. This drug is moderately soluble in water but freely soluble in ethanol. The solution is bactericidal and fungicidal and is, therefore, useful during surgery. It is relatively inexpensive and remains active in the diluted state for at least 3 days. 2-Phenoxyethanol has a relatively large margin of safety and has been reported to produce a range of effects from light sedation to surgical anesthesia at concentrations of 100 to 600 mg/L. Concentrations of 300 to 400 mg/L are useful for short procedures, and lower concentrations of 100 to 200 mg/L are considered safe for prolonged sedation, such as during transport. 2-Phenoxyethanol is not approved by FDA for use on food fish in the U. S.

Metomidate

Metomidate has been used extensively in human medicine. It anesthetizes fish without the usual stress of an elevated heart rate. Induction is rapid—1 to 2 minutes—and recovery is faster than with MS-222. It anesthetizes salmonids at doses of only 2 to 6 mg/L; low doses are also effective in catfish. In salmonids, metomidate is reported to be more potent in larger, sea-water-adapted fish than in freshwater fingerlings or parr. With larval goldfish, *Carassius auratus*, and red drum, *Sciaenops ocellatus*, it has been reported to produce inadequate anesthesia with high mortalities. Metomidate is not approved in the U. S. for use on food fish and is not widely used.

Clove oil

Clove oil has been widely used as an anesthetic in human dentistry and as a food flavoring. The major constituent (70 to 90 percent by weight) is the oil eugenol, but clove oil contains a wide range of other compounds that impart its characteristic odor and flavor. It is an effective anesthesia in carp (*Cyprinus carpio*) at 40 to 120 mg/L. In rainbow trout,

Oncorhynchus mykiss, doses as low as 2 to 5 mg/L produced sedation sufficient to transport the fish, while doses of 40 to 60 mg/L for 3 to 6 minutes gave effective surgical anesthesia. Recovery time increases with higher doses and longer exposure time. Clove oil is also an effective anesthetic for crustaceans at doses of 100 to 200 mg/L. Clove oil has a very high margin of safety; however, it also requires a relatively long recovery time compared to MS-222. The major advantage of clove oil is that it is inexpensive and not unpleasant to work with. Clove oil is not approved for use on food fish in the U. S.

Aqui-S™

Aqui-S™ is a relatively new anesthetic for fish developed by the Seafood Research Laboratory in New Zealand. This compound is approximately 50 percent isoeugenol and 50 percent polysorbate 80. A dosage of 20 mg/L is effective for most fish species and induction is described as “stress free” because the substance suppresses cortisol. A recent study indicated that Aqui-S™ was an effective anesthetic on freshwater prawns, but only at much higher concentrations of 100 to 200 mg/L (S. Coyle and J. Tidwell, unpublished data). Currently, Aqui-S™ is approved for use on food fish in Australia and New Zealand, with no withdrawal period. It is undergoing the New Animal Drug Approval process for use in the U. S., with no withdrawal time. It is used primarily for the “rested harvest” of commercial fish species, where the low stress induction improves the color, texture and appearance of the product. If approved for use in the U. S. with the zero withdrawal time, Aqui-S™ would be a valuable tool to use when transporting live food fish to market.

Carbon dioxide

Carbon dioxide, CO₂, has been used as an anesthetic for many years, especially during transport. It is extremely soluble in water

and can simply be diffused into the water as CO₂ gas. However, it is somewhat difficult to control the final concentration of CO₂. Carbon dioxide anesthesia is effective in rainbow trout at 120 to 150 mg/L for fingerlings and 200 to 250 mg/L for adults. Hyperactivity and subsequent stress can be reduced by buffering the water with sodium bicarbonate. Sodium bicarbonate (NaHCO₃) and acetic acid have also been used to produce CO₂. When dissolved in water, sodium bicarbonate releases carbon dioxide if the pH is acidic. Peak (1998) compared the efficacy of sodium bicarbonate and acetic acid to produce anesthesia in smallmouth bass (*Micropterus dolomieu*), northern pike (*Esox lucius*), and lake sturgeon (*Acipenser fulvescens*). He found that for these cool-water species a 2.6-g/L NaHCO₃ solution (30 L water, 80 g NaHCO₃, and 80 mL acetic acid) performed better than the 1.3-g/L NaHCO₃ solution (30 L water, 40 g NaHCO₃, and 15 mL acetic acid) previously recommended for salmonids. Durborow and Mayer (unpublished data) found that largemouth bass (*Micropterus salmoides*) reached stage 2 anesthesia after 6 minutes when exposed to a 0.67-g/L NaHCO₃ solution (30 L water, 20 g NaHCO₃, and 7.5 mL acetic acid) at 6 °C, and recovered in 10 to 15 minutes after being anesthetized for 1 hour.

The inconvenience of adjusting and maintaining the required pH makes other methods more attractive for most procedures. Carbon dioxide requires a relatively long induction time—generally 5 minutes at concentrations of 120 to 640 mg/L. The main advantage of carbon dioxide is that it is not a controlled substance in the U. S. and is recognized as a “Low Regulatory Priority,” unapproved drug by the FDA. This permits its use in food fish with no withdrawal time. At this time it is the only chemical method available for harvesting or transporting food fish to market.

Table 2. Dose rates of major anesthetic drugs, evaluated experimentally, for a number of commonly cultured fish species.

Anesthetic	Atlantic salmon <i>Salmo salar</i>	Rainbow trout <i>Onchorhynchus mykiss</i>	Common carp <i>Cyprinus carpio</i>	Channel catfish <i>Ictalurus punctatus</i>	Nile tilapia <i>Oreochromis niloticus</i>	Striped bass <i>Morone saxatilis</i>
MS-222	40-50 mg/L	40-60 mg/L	100-250 mg/L	50-250 mg/L	100-200 mg/L	100-150 mg/L
Benzocaine	40 mg/L	25-50 mg/L	ND	ND	25-100 mg/L	50-100 mg/L
Quinaldine	25-40 mg/L	ND	10-40 mg/L	25-60 mg/L	25-50 mg/L	25-40 mg/L
2-Phenoxyethanol	100-200 mg/L	100-200 mg/L	400-600 mg/L	ND	400-600 mg/L	ND
Metomidate	2-10 mg/L	5-6 mg/L	ND	4-8 mg/L	ND	7-10 mg/L
Clove oil	10-50 mg/L	40-120 mg/L	40-100 mg/L	100 mg/L	ND	60 mg/L
Aqui-S™	10-50 mg/L	20 mg/L	ND	20-60 mg/L	ND	ND

ND = not determined. Only MS-222 is approved in the U.S. at the time of this publication.

Non-chemical methods

Hypothermia

Lowering water temperature will tranquilize or immobilize fish. Lower water temperatures also increase the oxygen-carrying capacity of water and reduce the activity and oxygen consumption of fish. Water can be cooled by refrigeration or by adding ice. Gradual cooling is recommended because rapid chilling can produce thermal shock. This technique has been used primarily for transportation. Adult Atlantic salmon (*Salmo salar*) can be hauled long distances when cooled to 0 °C. Carp previously acclimated to 23 °C can be safely maintained in a state of apparent anesthesia for 5 hours at 4 °C, although lower temperatures cause mortality. Hypothermia is commonly used on crustaceans during transportation. Market-size freshwater prawns can be transported at high densities (100 to 200 g/L) if water temperature is cooled to 16 to 18 °C (S. Coyle and J. Tidwell, unpublished data).

When fish are immobilized by lowering water temperature, the safety margin is frequently quite small and deaths occur if the temperature is lowered too far or too quickly. Thus, the rate of cooling should be controlled carefully and

the required temperature should be maintained. As a rule of thumb, the water temperature should not be reduced more than 1 °C every 15 minutes. Hypothermia is often used in combination with chemical anesthesia to reduce the amount of anesthetic required, reduce oxygen consumption, and increase the amount of time the fish are anesthetized.

Electro-anesthesia

Another alternative to chemical anesthesia is the use of electricity. Alternating current (AC) and square waves in the form of chopped direct current (DC) have been used in electro-fishing for many years. Electro-anesthesia is not effective in seawater because seawater is more conductive than fish. In freshwater, the fish are more conductive than the water and the easiest route for electrons is through the fish.

Fish subjected to low-voltage DC become immobile; however, this is effective only while the fish are in the electrical field. If the current stops, the fish will escape almost immediately. This procedure only immobilizes and does not produce true anesthesia.

AC current produces a short-term anesthesia and turning off the supply does not negate the effects.

Larger fish are affected more rapidly than smaller ones; the length of time fish remain in the anesthetized state increases with body length. At 110 to 115 volts, anesthesia was shown to last for less than 1 minute; higher voltages (220 to 240) are preferable and can produce loss of reactivity to touch stimuli for up to 5 minutes.

Electrical stimulation produces violent muscular responses that can disfigure or kill. The safety of the animal and the operator are the greatest concern when using electricity. Electricity is unsafe to use around water; therefore, electro-anesthesia poses special problems. Multiple electrical safety features and strict codes of operation are required to approach a sufficient degree of safety.

Transportation and anesthesia

The stress caused by handling, grading and transporting can be considerable. It may be preferable to anesthetize fish as they are loaded for transport and/or to add ice to the water in transport tanks to reduce metabolic activity. Sedation can be beneficial in the bulk transportation of fish stocks, especially over long distances and when fish density is high. The major concerns in transporting

aquatic animals are the management of handling stress, mechanical shock, heat stress, and water quality.

Fish should not be sedated too deeply or they will sink to the bottom of the hauling tank where very high densities can cause rapid water quality deterioration and suffocation. As previously stated, there are no chemical anesthetics (other than CO₂) approved for use on food fish in the U. S. with zero withdrawal time. Therefore, carbon dioxide and hypothermia are the only legal means of sedation for transporting live food fish to market.

Conclusion

Anesthetics are chemical or physical agents that calm animals and cause them to progressively lose their mobility, equilibrium, consciousness, and finally their reflex action. In fisheries and aquaculture, anesthetics are helpful for reducing the stress caused by handling and transport. Many factors can affect the efficacy of anesthetic

treatments; therefore, experimental dosages should be tested on a small group of non-critical animals before any large-scale anesthetizing is done.

For environmental and human safety, the production, sale and use of chemicals is regulated by government agencies. In the U. S., FDA regulates the use of chemicals on food fish. Currently, the only chemical anesthetic approved by the FDA for use on food fish is MS-222; it requires a 21-day withdrawal period. These regulations are subject to change, and users are encouraged to check with local Extension specialists regularly for new information.

Suggested Reading

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The work reported in this publication was supported in part by the Southern Regional Aquaculture Center through Grant No. 2002-38500-11085 from the United States Department of Agriculture, Cooperative State Research, Education, and Extension Service.