



## Use of Anaesthetics for Transportation of Fish

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### ABSTRACT

The use of anaesthetics facilitates the process of efficient transportation of fish fingerling and broodstock for research purpose which requires invasive studies such as surgical preparations for physiological investigations by keeping immobile for extended duration of time. The sedation with the use of anaesthetics is also used for the manipulation of animals during procedures such as transport, grading or vaccination. Although the use of anaesthetics is primarily for the purpose of holding fish immobile while the animal is being handled for sampling, anaesthetics are also used to lower the level of stress associated with such procedures. The overdose of anaesthetics is also used routinely as an effective and humane means of euthanizing fish.

### INTRODUCTION

The anaesthesia is usually defined as a state or condition caused by an application of external substance which results in a loss of sensation through depression of the nervous system. The anaesthetics may be local or general, depending on their application. The choice of aesthetic depends on many factors.

- For the maintenance of gill ventilation during an experimental procedure, ketamine hydrochloride is suitable which is best administered by injection. It need an initial anaesthetization with another suitable anaesthetic such as buffered TMS (MS-222).
- For the transport stress problem, the low concentrations of an anaesthetic such as TMS buffered with sodium bicarbonate is applied.

### Criteria for selection of sedative/ anaesthetic

- The chemical nature of anaesthetic, mode of application as well as any local regulations and legislation plays key role in the selection of anaesthetic in fish transport and research study.
- The lengthy withdrawal times are mandatory for chemical anaesthesia of food fish prior to harvest such as clove oil.
- The effective and lethal doses for the major chemical anaesthetics used for fisheries activity are well established now.
- The efficacy of most anaesthetics is affected by target species, its body size, density of fish in the bath and water quality (hardness, temperature and salinity).
- It is necessary to perform a preliminary tests with small numbers of the fish to determine the optimal dosage and its exposure time.
- The due care should be taken to control the level of anaesthesia through application of appropriate concentration.

**Criteria for fish transport**

The density depends on the average weight of the fish transported. If the duration of transport is for a maximum period of 48 hours, density may be calculated by the following equation.

$$\text{Fish Density} = 38 \times \sqrt{W_g / l}$$

$$\text{Number of fish} = 38 \div \sqrt{W}$$

$$W_v = 27 \sqrt{W} \text{ ml}$$

Where **W** is the mean weight of individual fish in gram.

**Need of Anaesthetics**

During transportation of brood fishes, sedation is essential because of the following practical benefits:

- Reducing overall stress on the fish;
- Decreasing the rate of oxygen consumption
- Reducing the rate of excretion of CO<sub>2</sub> and NH<sub>3</sub>
- Controlling the excitability of the fish.
- Reducing chances of physical injury.
- Reducing the time required for handling them.

**Properties of Good Anaesthetics**

- It should induce anaesthesia in < 3 minutes and recovery should occur within 5 minutes of placement of the fish in fresh water.
- It should have no toxic side effects for either the fish or the handler.
- It should be biodegradable.
- It should allow the body to clear it from the tissues following exposure.
- It should not persisting physiological, immunological or behavioural effects.
- It should be cost effective.
- It should be easily available.

**Stage of anaesthesia and recovery**

Sl.no	Stage of anaesthesia	Description
1.	I	Loss of equilibrium
2.	II	Loss of gross body movement
3.	II	
<b>Stages of Recovery</b>		
1.	I	Body immobilized but opercular movements slow
2.	II	Regular opercular movements and gross body movements increases
3.	III	Equilibrium regained and pre-anaesthetic appearance develops

**MS 222**

It is the most commonly used tranquilizers in fisheries sector. It is chemically called Tricaine methan sulphonate. It is particularly very effective for rapid induction of deep anaesthesia in fish. It is very mild in nature and fish easily recover from its effects in short time. It is basically a white crystalline powder which is easily soluble in water, with a solubility constant of 1.25 g/mL water, at 20 °C. In general, its doses are usually between 25 to 100 mg/L. It has a haemo-dilution effect on the blood. The initial absorption of it by the fish has an excitatory effect which is reduced by buffering.

**Application method:-**

- The brood fish are kept in 1:20,000 (MS 222 : Water. After 30 minutes, when the fish are fully tranquilized, the solution is diluted by adding water.
- The recommended dilution is 2 times (i.e., 1:40,000) for hardy fish such as common carp and bighead.
- About 2-1/2 times (i.e., 1: 50,000) for less hardy fish like grass carp.
- About 5 times (i.e., 1: 100,000) for least hardy fish like silver carp among cultivated carps.

**Benzocaine**

It is local anesthetic in nature. It is known as p-aminobenzoic acid ethyl ester. It has two forms: i) a crystalline salt with a water solubility of 0.4 g/L ii) a freebase form which dissolves in ethyl alcohol at 0.2 g/ml. The reported doses range from 25-100 mg/L. The induction time is about 5 minutes and when fish are placed in clean water, recovery is usually within 10 minutes. The fish may retain some locomotory functions throughout all stages of this anaesthesia which makes this unsuitable anaesthetic for use in surgery. Benzocaine is also known as <sup>TM1</sup> Anaesthesia, <sup>TM14</sup> Anesthone, <sup>TM2</sup> Americaine, Ethyl aminobenzoate, Orthesin and Parathesin.

**Lidocaine**

It is commonly known as 2-diethyl amino- N-2, 6 di-methyl phenyl acetimide. It is commercially known as <sup>TM3</sup>Xylocaine. It is sparingly in water but highly soluble in acetone /alcohol. It is commonly used in the hydrochloride salt form which is freely soluble in water. It is a cardiac tranquilising which is used do nerve block. It is used in combination with sodium bicarbonate to anaesthetize carp (*Cyprinus carpio*), tilapia (*Oreochromis/Tilapia mossambica*) and catfish (*Ictalurus punctatus*). The addition of sodium bicarbonate, is recommended to enhance its anesthetic effects and variation in dose. E.g, tilapia required in excess of 800% more lidocaine as compare to carp when it was administered in the absence of sodium bicarbonate.

**Clove oil and its derivatives**

It is like an alternative fish anaesthetic. It is pale yellow liquid derivative from leaves, buds and stem of the clove (*Eugenia sp.*). Its prime ingredients is eugenol (4-allyl-2-methoxyphenol) and iso-eugenol (4-propenyl-2- methoxyphenol) which is comprise of 85-95% of clove oil by weight.

It is most effective at concentrations of 50-60 mg/L in salmonids when dissolved in ethanol (e.g., 1:9) before mixing with water. It has a slightly faster induction time and a longer recovery time than similar concentrations of TMS. It is commercially available as <sup>TM25</sup> AQUI-S which is dissolved directly in water at the rate of 20 mg/L for anaesthetizing juvenile Chinook salmon.

### **Non-chemical Anaesthesia**

#### **Electro anaesthesia**

Electrofishing is a common method used to immobilize adult fish for tagging or hatchery brood stock. There are basically 03 types of electric current used to immobilize fish like i) alternating current (AC), ii) direct current (DC), and iii) pulsating forms of AC and DC. The direct current can cause anodotaxis (movement to the anode pole), electro narcosis (stunning) and electrotetany (tetanic muscle contractions).

The alternating current causes only electro-narcosis and tetany. The purpose of electro anaesthesia is to induce electro-narcosis, and avoid severe muscle tetany which can result in spinal injuries. The main advantages of electro anaesthesia over chemical anaesthetics include rapid induction and less recovery times. It also help in immediate release of treated fish without the need for a withdrawal period. Most electro-anaesthesia is now carried out with DC or pulsed DC with a 12-V DC shocking basket. The electroshocking causes abrupt rise in level of plasma corticoid and lactate concentrations. The persistent rise in plasma glucose and corticoids for at least 10 hours following capture, and cardiovascular changes including rhythm changes.

#### **Hypothermia**

Hypothermia is done by lowering the ambient temperature of the fish with dry ice or cold water. The use of dry ice result in hypercapnic (high CO<sub>2</sub>) and acidic conditions in the water, if it is placed in the water. The fish acclimated to higher temperatures may experience stress as a result of cold shock. The hypothermic anaesthesia is more effective for fish acclimated to waters above 10 °C, as the sedative effects does not occur if acclimation temperatures are lower than this. The hypothermia results in a slow, light anaesthesia, which involves absence of motion, reduced power of exertion and diminished nerve sensitivity. It is most useful for transport.

#### **Carbon dioxide**

Carbon dioxide (CO<sub>2</sub>) is a colourless, odourless and non-flammable gas with a water solubility of 1.71 L/L water at 0 °C and 760 mmHg. The CO<sub>2</sub> is safe to use but a level of 10% in the air causes anaesthesia or mortality to the operator; therefore, ample ventilation is necessary. The hydration of CO<sub>2</sub> acidifies the water, and therefore, it is buffered to reduce potential stress to the fish. The exposure of fish to hypercapnia in the water (1 to 3 % ) is a generally done to induce respiratory acidosis in fish, as it produces a consistent and reproducible decrease in blood pH upon exposure to the fish. It is mostly used in fisheries and aquaculture based on its gaseous nature and no residues in the tissues.

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