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Anaesthetic Effects of Clove Oil and Sodium Bicarbonate on the Fry of *Liza parsia*

U.D. Sonawane and G.N. Kulkarni

Collage of Fisheries, Shigaon Ratnagiri, Pincode: 415 629 India

Email: gnk-fisher@rediffamait.com

Abstract: The gold spot mullet fry, mean length of 1.30±0.048 cm and mean weight of 0.05±0.007 g were collected from coastal creeks of Ratnagiri (Maharashtra, India) and then acclimatized to 20 ppt salinity. They were observed for the response to the two anaesthetics: Clove oil and Sodium bicarbonate. Effective dosage of both the anaesthetics *viz.* clove oil and sodium bicarbonate for the induction and recovery worked out to be 80 and 4000 ppm, respectively. Both the anaesthetics clove oil and sodium bicarbonate were found to be cost effective as compared to MS-222.

KEY WORD: Anaesthetic, clove oil, fry, *Liza parasia*, MS222

Introduction

The grey mullets especially *Liza parsia, L. dussumieri, L. tade,* and *Mugil cephalus* form a cultivable category of fish in brackish waters and thus their fry and fingerlings are stocked in coastal ponds and tanks worldwide. Keeping in view the huge availability of gold-spot mullet (*Liza parsia*) seed along the Ratnagiri coast and increasing demand for the same in coastal and some localities of inland areas for culture, it is being considered as candidate species for monoculture and/or polyculture along with freshwater carp as well as brackish water fin fish and/or crustaceans exploiting the existing salinity phase (Pawar, 2001 and Joshi, 2001).

Among the several anaesthetics tried, tricaine methanesulfonate (trade name: MS-222) and 2-phenoxyethanol were the most commonly used fish anaesthetics, being easy to use and effective (Bergsio, 1974 and Bell, 1987). The effects of MS-222 on mullet seed have been studied by Dick (1975) and Durve (1975). However, the cost and residual effects of these alternatives are being worked out. Peake (1998) tested sodium bicarbonate activated by glacial acetic acid and clove oil as

potential anaesthetics for the nonsalmonid fishes *viz.* walleye, *Stizotedion vitreum*, small mouth bass *Micropterus dolomieu*, Northern pike *Esox lucius* and lake sturgeon *Acipenser fluvescens* and found the technique ideal for use in field, laboratory, fish farms, and hatcheries because it is easy to use and inexpensive, as well as its easy adaptability for other fish species.

Clove oil was being used as fish anaesthetic right from early seventies. Endo *et al.* (1972) studied anaesthetic effects of euganol in some freshwater fishes, such as carp, *Cyprinus carpio*, medaka, *Oryzias latipes* and found that euganol was effective at only one fourth the concentration of MS-222 in *Oryzias latipes*, crucian carp *Carasius auratus* and rainbow trout, *Salmo gaidnerii irideus*. Soto and Burhanuddin (1995) described the successful use of clove oil as a fish anaesthetic for juvenile rabbit fish *Siganus lineatus*. Indonesian clove oil, (brand name: Minyak cengkeh), was found to be an effective anesthetic for use in hatchery practices with rabbit fish (*Siganus argenteus*), milkfish (*Chanos chanos*) and striped mullet (*Mugil cephalus*) at concentration 25 ppm compared to synthetic anesthetics, (Tamaru *et al.*, 1995 and Soto *et al.*, 1995).

Taylor and Roberts (1999) tested clove oil on rainbow trout *O. mykiss,* white sturgeon *Acipense transmontanus*, fall chinook salmon *Oncorhynchus tshawytscha* and coho salmon *O. kisutch* with respect to induction, recovery, median lethal concentration etc. and suggested it as an alternative to chemical anaesthesia in aquaculture. Keene *et al.* (1998) worked out various advantages of clove oil over the other anaestheticsin use. Druville and Collet (2000) conducted experiments using clove oil to find a means of handling fish regularly and efficiently for wild juveniles of *Valamugil cunnesius* and *Monodactylus argenteus*.

Although clove oil has recently been gaining importance as an appropriate anaesthetic for aquaculture and research to be used on food fishes, its use in invasive procedures has not yet been standardized for many important culturable fishes (e.g. salmonids, groupers, mullets, carps, etc.).

In view of the above, the efficiency of two anaesthetics – sodium bicarbonate in combination with glacial acetic acid and natural topical clove oil for the fry of gold-spot mullet (*Liza parsia*) was tested in order to find out a cost effective and efficacious anaesthetic agent, so that handling or transportation of this sensitive fish could become safe and fruitful.

Materials and Methods

Test species and its habitat:

The brackish water fish species *Liza parsia* commonly known as Gold–spot mullet (belonging to Mugilidae family) was selected as the study species, which is widespread and abundant in the creeks, estuaries, back bays, and marshy areas in and around Ratnagiri, West Coast of India (Latitude 16° 59' 10" North and Longitude 73° 16' 25" East). The collected mullet seed (fry, mean length: 1.30±0.048 cm and mean weight: 0.05±0.007 g) were transferred to the large circular plastic pools (6 feet diameter and 4 feet height) and kept ready with water almost having same salinity and other parameters as per the collection site. In these plastic pools, the seed was then acclimatized to the 20 ppt salinity (as per trial and error basis experiments for the survival), slowly by adding fresh water and reared for 8 days. Aeration was provided continuously to the plastic pools by means of air blower. During the acclimation period, the mullet seed was fed with the frozen tubifex worms in the morning and flake feed in the evening.

Because of incomplete solubility in water at temperature below 30°C, the clove oil was first dissolved in the alcohol mix, which contained 98% ethanol and 2% methanol (Munday & Wilson , 1997) at a ratio of 1 part of clove oil to 10 parts of alcohol mix (Keene *et al.*, 1998 and Cho & Heath , 2000). Preliminary trials were taken with the alcohol mix to check out for any sort of visible effect on the experimental fish.

As sodium bicarbonate when used singly, is less effective in immobilizing fish in achieving deep anaesthesia (Bell, 1987), it was tried in combination with glacial acetic acid, which enhances the liberation of CO_2 from a sodium bicarbonate and also stabilizes the pH of the solution (Prince *et al.*, 1995). Peake (1998) suggested addition of 2.66 parts of glacial acetic acid to sodium bicarbonate. This combination was taken as reference dose, and modified dose of 2.33 parts of glacial acetic acid was tried as a treatment dose.

Induction to anaesthesia:

The time taken by the experimental fish right from induction to anaesthesia to total loss of equilibrium were recorded both for clove oil and sodium bicarbonate under the same experimental conditions. The aquaria selected as treatment units

were made of 6 mm-thick glass and size of 9 x 9 x 15 inch, having water capacity of 15 L. The treatment units were made ready by filling them to 7 L with 20ppt salinity water, preferably taken from the acclimation tanks. The treatment tanks were provided with well-aerated water. The experimental fish were selected from the batch prepared.

Prior to the experimentation, 28 fish were randomly selected from 100 fish kept starved out of the total batch of 300 fish, were placed in the treatment tank. Then, the time required for induction, as per the criteria adopted, was recorded to the nearest second using stopwatch. The concentrations tested for induction were 50, 80, 110, 140, 170, 200, 230, 260, 290, 320, 350 and 380 ppm of clove oil.

The solution mixture of sodium bicarbonate and glacial acetic acid was added to the treatment tank, the time required for induction was noted to the nearest second with the stopwatch. The concentrations tested for induction were 50, 100, 200, 400, 600, 800, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500 and 5000 ppm of sodium bicarbonate.

Recovery from Anaesthesia:

The fish treated for induction as per the specific dose was placed in separate recovery tanks containing 7 L of well-aerated water. The time was recorded from the first fish recovered to the last one to the nearest second with the stopwatch. The recovered fish were observed for 96h for any sort of effect of the anaesthetic, such as swimming response, mortality.

Statistical analysis:

Wherever necessary, appropriate statistical tools were used to test the experimental data (Snedecor & Cochran , 1967).

Results

Induction to Anaesthesia:

Of both the anaesthetics tested, clove oil was found to be more effective as compared to sodium bicarbonate. It is clear from Fig. 1 that the clove oil gave fastest induction even at lower dosages, the period being less than 5 min. When

exposed to sodium bicarbonate, the earliest total loss of equilibrium (induction) was achieved at concentration of 2000 ppm.

As seen in Fig. 1, the minimum time of induction for clove oil was 18–25 seconds, with an average of 20.68 seconds at 380 ppm for the two replicates tested. Clove oil gave longest induction at concentration of 20 ppm, ranging from 180–272 with an average of 228.15 seconds.

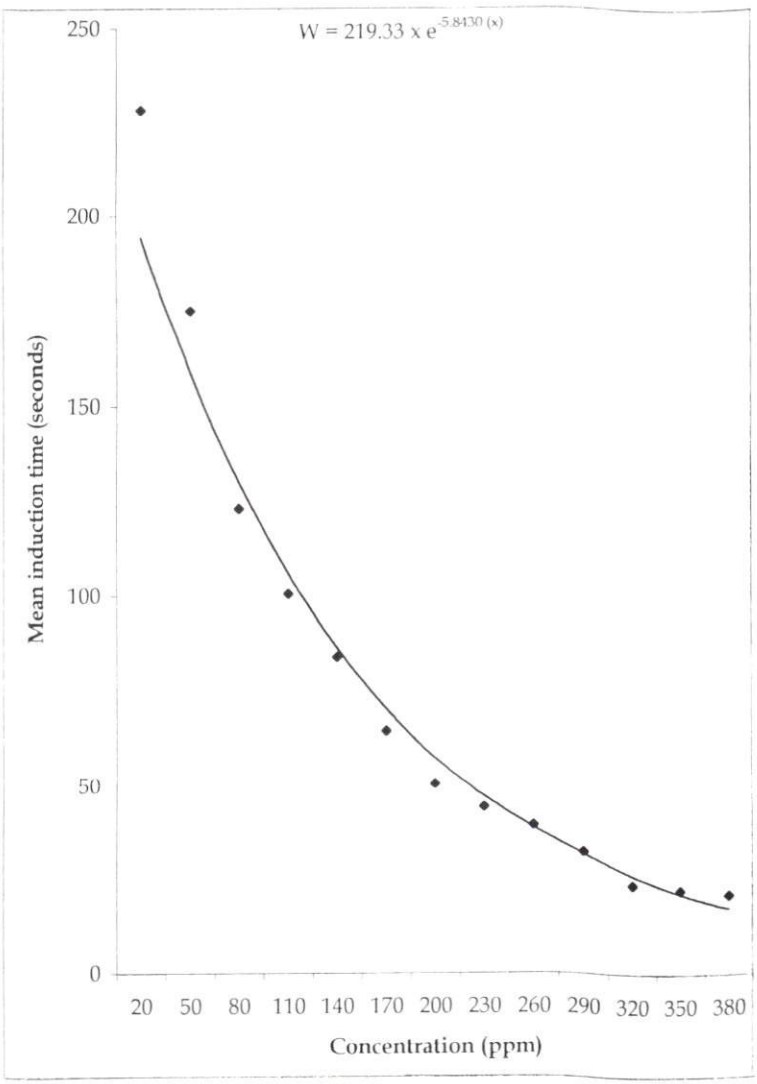

Fig. 1: Induction of fry of *L. parsia* to anaesthesia by clove oil

Negative significant correlation was observed between induction time and concentration of clove oil, the regression equation being $W=219.33 \times e^{-5.8430 (x)}$

At 5000 ppm sodium bicarbonate, the shortest induction was recorded between 62–120 seconds with an average of 87.93 seconds for the two replicates tested. At 2000 ppm of sodium bicarbonate, the longest induction was observed between 350–463 seconds with an average of 402.46 seconds (Fig. 2).

The negative significant correlation was also observed between induction time and concentration of sodium bicarbonate, the regression equation being $W= 1388.577 \times e^{-8.3415 (x)}$. Maximum time of induction was observed for both of the anaesthetics at initial low dosages. In general, the induction time was observed to decrease with increase in concentration.

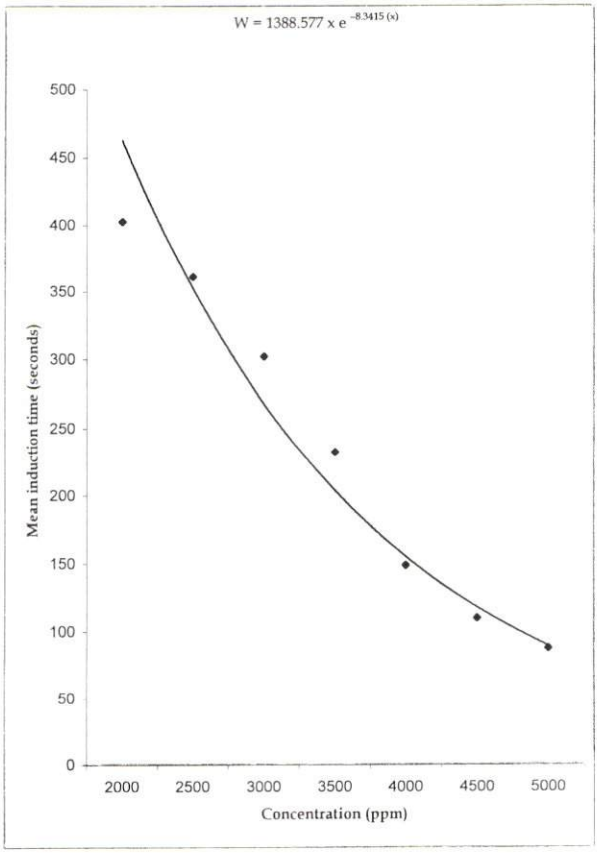

Fig. 2: Induction of fry of *L. parsia* to anaesthesia by sodium bicarbonate

Recovery from Anaesthesia:

Of the 13 concentrations tested, clove oil gave shortest recovery time, i.e. between 125–180 seconds with an average of 153.115 seconds at 80 ppm concentration, beyond which recovery period increased (Fig. 3). At concentration of 380 ppm, the longest recovery time for the clove oil was observed, ranging from 400–1080 seconds with an average of 712.72 seconds for the two treatments tested. For the recovery of clove oil, the parabolic regression was:

$$Y = 265.213 - 1.53330\ X_1 + 0.0071\ X_2$$

In case of sodium bicarbonate, the recovery occurred in less than 5 min at all concentrations tested except at 2000 ppm, where recovery exceeded slightly in one of the trials. The shortest recovery was recorded at 4000 ppm ranging between 136–192 seconds with an average of 156.07 seconds (Fig. 4). The parabolic regression equation for the recovery of fry of *L. parsia* due to sodium bicarbonate use worked out to be:

$$Y = 840.404 - 0.365\ X_1 + 0.00005\ X_2$$

In general, recovery was directly proportional to the dosages.

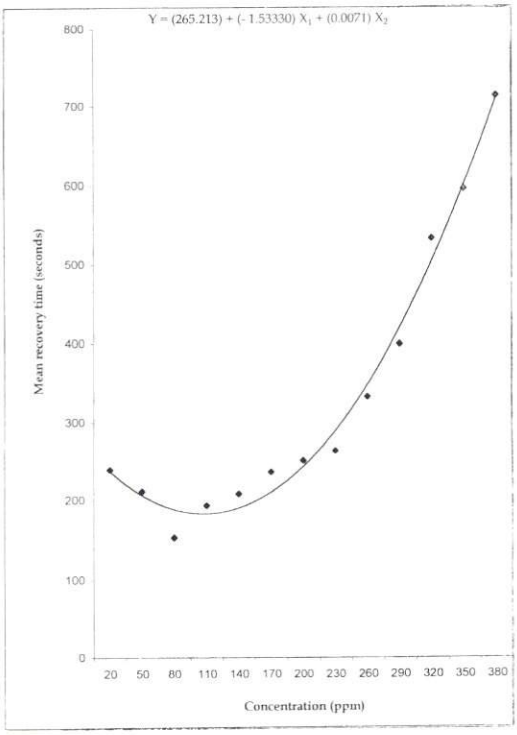

Fig. 3: Recovery of fry of *L. parsia* from anaesthesia due to clove oil

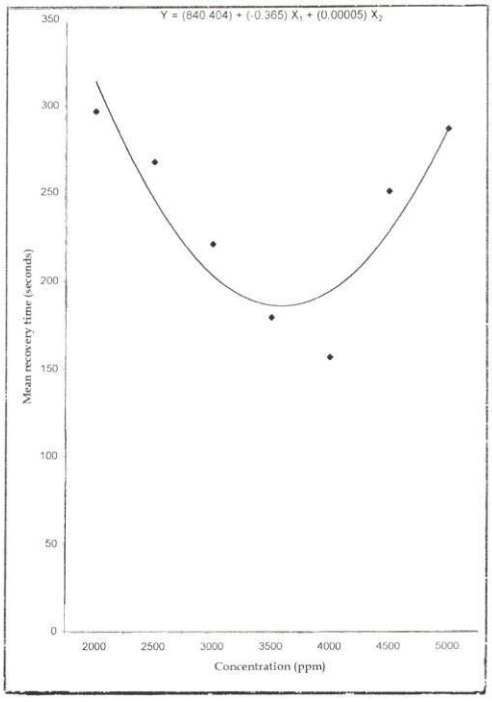

Fig. 4: Recovery of fry of *L. parsia* from anaesthesia due to sodium bicarbonte

Cost analysis:

The relative costs of the two anaesthetics *viz.* clove oil and sodium bicarbonate with necessary ingredients in comparison to MS–222 are given in Tables 1 and 2, pointing out that the clove oil is most economical followed by sodium bicarbonate.

Table 1: Cost of the anaesthetics and ingredients

Anaesthsetics / ingredients	Cost (Rs)
Clove oil/100ml	156
Ethanel / 500 ml	300
Methanol / 500 ml	80
Sodium bicarbonate / 500 gm	125
Glacial acetic acid / 500 ml	87
MS-222 / 10 gm	1835

Table 2: Cost of anaesthetics at their effective concentrations

Anaesthetics (ppm)	Cost (Rs)
Clove oil (80)	2.40
Sodium bicarbonate (4000)	9.70
MS-222 (100)	18.35

Discussion

Induction to anaesthesia:

In the present study, the longest induction time of 180-272 (average 228.15) seconds was recorded at 20 ppm of clove oil while the shortest induction was at 380 ppm, i.e. 18-25 (average 20.68) seconds. Most concentrations of clove oil tested in the present study (i.e., 50-380 ppm) were found to yield induction in less than 3 minutes satisfying the recommendation given by Marking and Meyer (1985) except at 20 ppm concentration.

Soto and Burhanuddin (1995) recorded induction time at various clove oil concentrations *viz.* 33, 50, 67 and 100 ppm respectively as 150-190, 100-125, 60 and 60-75 seconds in *Siganus lineatus*. Taylor and Roberts (1999) exposed juveniles of white sturgeon (*Acipenser transmontanus)* to several concentrations of clove oil from 10-800 ppm and noted the induction ranging between 180-480 seconds up to 250 ppm; beyond 400 ppm, the induction time decreased from 120 seconds to 60 seconds. The induction in coho and chinook salmon exposed to clove oil varied from 240 seconds at 10 ppm to less than 60 seconds at 120 ppm (Taylor & Roberts, 1999).

The present observations on induction by clove oil significantly differ from those noted by other workers. Soto and Burhanuddin (1995) recorded significantly shorter induction time. The observations made by Taylor and Roberts (1999) on juvenile white sturgeon (*Acipenser transmontanus)* revealed significantly longer

induction as compared to the present study, while coho and chinook salmon required shorter time of induction.

Further studies are required in this regard to explain the factors responsible for variations in the induction time for different species. However, the induction time invariably decreases with increase in concentration.

In the present investigation, sodium bicarbonate gave the earliest induction at 2000 ppm, ranging from 350 to 463 (average 402.46) seconds. The shortest induction was recorded at 5000 ppm between 62 to 120 (average 87.93) seconds. The induction time at 4000, 4500 and 5000 ppm sodium bicarbonate coincided with the time limits given by Marking and Meyer (1985). However, the lower concentrations (i.e., 2000-3500 ppm) behaved differently.

Peake (1998) induced walleye (*Stizotedion vitreum*) with sodium bicarbonate at 1330, 2660 and 4000 ppm and recorded induction times as 1040, 420 and 282 seconds, respectively. Using lower doses of sodium bicarbonate, Booke *et al.* (1978) could induce rainbow trout, *Salmo gairdneri* at 442 and 642 ppm after 150-282 seconds and 72-288 seconds, respectively and common carp, *Cyprinus carpio* at 642 ppm in 240-720 seconds.

However, the induction time of fry of *L. parsia* with sodium bicarbonate in the present study does not agree with the above observations. As observed by Peake (1998), the walleye (*Stizotedion vitreum)* took longer induction time than the one recorded in present study. This may be due to the age difference, which is considerable. Water parameters, such as pH and alkalinity, could also play significant roles.

Recovery from anaesthesia:

The recovery duration of the fry of *Liza parsia* in the present study increased with increase in concentration of both the anaesthetics *viz.* clove oil and sodium bicarbonate. In case of clove oil, the shortest recovery was noted at 80 ppm between 125-180 (average 153.115) seconds while the longest was at 380 ppm ranging from 400-1080 (average 712.72) seconds. Considering basis of recovery

time limits given by Marking and Meyer (1985), clove oil follows the trend only up to 230 ppm while the higher dosages result in longer recovery of more than 5 min. This points out that the clove oil is suitable up to 230 ppm only.

In case of sodium bicarbonate, the recovery occurred in less than 5 min. The recovery was the highest at 2000 ppm and ranged from 230-370 (average 296.57) seconds while the shortest was noted at 4000 ppm between 136-192 (average 156.07) seconds.

Based on the criteria for good fish anaesthetics, the induction and recovery times from anaesthesia are reported to be 3 and 5 min, respectively (Marking & Meyer, 1985). The effective dose criteria, i.e. shortest induction and fastest recovery (Durve, 1975 and Keene *et al.*, 1998), worked out to occur respectively at 80 and 4000 ppm for clove oil and sodium bicarbonate during the course of the present investigation. Endo *et al.* (1972) worked out 50 ppm of clove oil as effective concentration for Medaka (*O. latipes*) with induction and recovery times of 180 and 300 seconds, respectively. Prince *et al.* (1995) recommended 1330 ppm of sodium bicarbonate for *O. nerka* and Peake (1998) noted 2660 ppm of sodium bicarbonate as effective concentration for walleye (*S. vitreum*).

The effective concentration of clove oil, i.e. 80 ppm in the present study, was within the range given by Tamaru *et al.* (1998) for *Mugil cephalus, Chanos chanos* and *Siganus lineatus* and by Soto and Burhanuddin (1995) for *Siganus lineatus*. However, the concentrations of sodium bicarbonate adopted by Prince *et al.*, (1995) and Peake (1998) were not strong enough for the fry of *Liza parsia* in the present study, in which a higher dose of 4000 ppm was found out to be the effective concentration.

Cost-effectiveness of anaesthetics:

Keene *et al.* (1998) observed that the clove oil is effective at 1/15th of MS-222's price. In the present study, both anaesthetics *viz.* clove oil and sodium bicarbonate were found cost effective as compared to MS-222. The relative cost of these anaesthetics at their effective dosages was worked out as Rupees (Rs.) 2.40

bicarbonate were found cost effective as compared to MS-222. The relative cost of these anaesthetics at their effective dosages was worked out as Rupees (Rs.) 2.40 for 80 ppm of clove oil, Rs. 9.70 for 4000 ppm of sodium bicarbonate as compared to Rs. 18.35 for 100 ppm of MS-222.

From the present study, it is clear that clove oil can be used as an effective anaesthetic for short-term processes at 80 ppm. Sodium bicarbonate is suggested to be useful only for short-term applications at 4000 ppm. Clove oil has a great financial advantage, giving faster induction even at lower dosages as compared to sodium bicarbonate and MS-222, besides being ecofriendly.

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