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RESEARCH ARTICLE

EFFICIENCY OF ANAESTHETICS IN COMBATING STRESS IN FISH TRANSPORTATION OF *ETROPLUS MACULATUS* (BLOCH, 1795)

***,[1]Dalie Dominic, A., [2]Inasu N. D. and [3]Swapna Johny**

[1]Department of Zoology, St. Mary's College, Thrissur-680 103
[2]Former Pro Vice Chancellor, Cochin University of Science & Technology
[3]Department of Zoology, Little Flower College Guruvayoor

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ABSTRACT

Ornamental fishes are show pieces and expressions of aquatic splendor. The ornamental fish industry has now secured an essential section of international trade for which, fish needs to be transported to far away destinations. Ornamental fish packaging systems are characterized by very high fish loading densities and high metabolic wastes in the transport water. Stress created due to this affects the survival of the fish. Therefore new technologies needs to be undertaken for fish transportation. Anaesthetics are nowadays used for fish transportation. These are drugs that cause a reversible loss of consciousness. The fish ventilates these anaesthetics and it enters the blood stream. However knowledge of appropriate concentration to be used is essential for any aquarist. Therefore in the present study efficiency of 2-Phenoxyethanol, Clove oil and Lemongrass oil as anaesthetic for transportation of *Etroplus maculatus*, a popular ornamental fish was done. A concentration of 60mg/l, 12mg/l and 8mg/l were ideal for transportation with 2- Phenoxy ethanol, Clove oil and Lemon grass oil as anaesthetic for the 48 hour transportation.

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INTRODUCTION

Ornamental fish packaging systems are characterized by very high fish loading densities and high metabolic wastes in the transport water (Lim, 2003) creating stress that incur incredible causalities. Stress caused due to handling procedures produce negative impact on fish growth, reproduction, immune function and even the survival on the long run (Ims, 2011). Therefore utmost care implementing the involvement of latest technologies needs to be undertaken for fish transportation. There are several different chemical drugs that can immobilize fish and minimise stress but not all are described as safe and effective for use on fish (Ims, 2011, Iversen *et al.,* 1998; Gomes *et al.,* 2009). Anaesthetics are advantageous for calming excitable fish that injure themselves during transit (Wurts, 1995). They are commonly used in handling and transport of live fish (Berka, 1986) since five decades, as they lower the metabolic activity and mortality (Durve and Dharmaraja, 1966 and Durve, 1975). Anaesthetics need to be applied with extreme cautiousness as even the best drug becomes fatal by wrong administration. Efficacy of a drug varies with species as different taxa of fish have different tolerances to stress (Barton *et al*., 1987). It also depends on body size, loading density and water quality. Hence, it is crucial to select the anaesthetic and the dosage for each species. According to Hseu *et al.* (1998) anesthetic induction time is the period from the time a fish is placed in the anesthetic medium until the time it stops swimming and its tail stops swinging. The recovery time is the period from the time when an anesthetized fish is placed in a recovery tank until it recovers from anesthetization with full equilibrium motion. Summerfelt and Smith (1990) describe six stages of anaesthesia induction that include viz. Light sedation, Deep sedation, Partial loss of equilibrium, Total loss of equilibrium, Loss of reflex reactivity and Medullary collapse. With proper dosing, induction with immersion drugs usually occurs within 5 to 10 minutes (Neiffer and Stamper, 2009) but, stage 2 anaesthesia is regarded as an ideal value for fish transport and general handling (Cooke *et al.,* 2004). Efficiency of anaesthetics in fish transport is a widely studied emerging field that tries to minimise stress in fish. Sladky *et al*. (2001) accounted for the comparative efficacy of tricaine

****Corresponding author: Dalie Dominic, A.***
Department of Zoology, St. Mary's College, Thrissur-680 103

methanesulfonate and clove oil for use as anesthetics, Weyl *et al.* (1996), Tort *et al.* (2002) and Velisek and Svobodova (2004) described the anaesthetic effect of 2-phenoxyethanol. Silva *et al.* (2013) reported the anaesthetic effect of essential oils. Cooke *et al.* (2004), Anderson *et al.* (1997) and Inoue *et al.* (2005) illustrated the effect of clove oil on handling and transportation of Largemouth Bass, *Brycon cephalus* and Siamese Fighting Fish respectively. 2-Phenoxyethanol is a colourless clear oily liquid with a faint aromatic odour. Its anesthetic property causes a short-term immobilization of fish when used as an immersion anaesthetic.

Clove oil is extracted from *Eugenia caryophyllata* of Myrtaceae family. Lemongrass oilis an essentialoil with a musky, lemon scent that is refreshing and relaxing. It is produced from the leaves and stems of Cymbopogon (Lemongrass). *Etroplus maculatus*, commonly called Orange Chromide is a popular fish of the ornamental industry. In contradiction to its popularity the survival study of this fish however indicates that it is highly susceptible to water quality changes and its transportation requires utmost care. Therefore in the present work the determination of the suitable anaesthetic for *Etroplus maculatus* and identification of appropriate dosage of three anaesthetics 2-Phenoxyethanol, Clove oil and Lemongrass oilwere done.

MATERIALS AND METHODS

To estimate the appropriate anesthesia dosage of 2-Phenoxyethanol, Clove oil and Lemon grass oil for short exposure and long term transportation the fishes were observed in different anaesthetic concentrations for a period of ten minutes and 48 hours respectively. Safety concentrations for short exposure of anaesthetics 2- Phenoxyethanol, Clove oil and Lemon grass oil were assessed by determining induction times. Here the behaviour of the fish was observed and the time for inducing anesthesia and recovery was timed with a stopwatch. The second experiment evaluated exposure times to anaesthetic for a period of 48 hours. 2- Phenoxy ethanol is slightly soluble in water so it was first dissolved in equal quantity of ethanol and used for the experiment. Clove oil and Lemongrass oil are immiscible in water therefore it was dissolved in ethanol at a ratio of 1: 9.

The following concentration of each anaesthetic was evaluated for assessing anaesthetic efficiency for short exposure. 2-Phenoxy ethanol (100mg/l, 200mg/l, 300mg/l, 400mg/l and 500mg/l), Clove oil (20mg/l, 40mg/l, 60mg/l, 80mg/l and 100mg/l) and Lemongrass oil (36mg/l, 72mg/l, 108mg/l, 144mg/l and 180mg/l). 3 individuals were exposed to each of the above concentration for ten minutes or until reaching stage 5, the time required for induction was noted down. Immediately after reaching stage five or after ten minutes of anaesthetic exposure the fish were transferred to recovery medium that is water without anaesthetic and the recovery time was noted. Since the fishes need to be transported to long distance, that requires long transportation time, anaesthetization with the above concentration would turn fatal. So in order to determine the anaesthetic dose that maintains fish in first or second stage of anaesthetia for 48 hours another set of experiment was performed with lower concentration 2-Phenoxy ethanol (15mg/l, 30mg/l, 60mg/l, 120mg/l and 240mg/l), Clove oil (4mg/l, 8mg/l, 12mg/l, 16mg/l and 20mg/l) and Lemongrass oil (2mg/l, 4mg/l, 8mg/l, 16mg/l and 24mg/l) and experiment was run for 48 hours at a temperature of 26°C. Experiments were prepared in triplicates and the induction time for all anaesthetics was noted down.

RESULTS

The stages of anaesthesia induced in different concentrations of 2-Phenoxy ethanol for a duration of ten minutes for short exposure (Table 1) indicates that anaesthetizing with 500mg the fish was induced to stage 5 at 10 minutes. A concentration of 300 mg anaesthetized the fish to stage 3 within five minutes in the case of 2-phenoxy ethanol, while for clove oil 80mg and lemon grass oil 144mg anaesthetized the fish to stage 3 within 5 minute (Table 2 & 3). Stages of anaesthesia induced in different concentration of 2-Phenoxy ethanol packed and maintained for a duration of 48 hours of transportation indicate that the effective concentration was 60mg (Table 4). A concentration of 240mg resulted in casuality. For clove oil at a concentration of 12mg the fishes were maintained in stage 1 or 2 anaesthesia for most of the time and they survived (5). A concentration of 20 mg produced mortality at the 18th hour. In the case of lemon grass oil mortality was noticed in 16mg and 24mg concentration while 8 mg concentration maintained the fish in stage 1 or 2 anaesthesia most of the time.

Table 1. Stages of anaesthesia induced in different concentration of 2-Phenoxy ethanol for a duration of ten minutes for short exposure

Concentration of 2-Phenoxy ethanol (mg/l)	Time in minutes										Recovery time (Minutes)
	1	2	3	4	5	6	7	8	9	10	
100	1	1	1	2	2	2	2	2	3	3	4’
200	1	1	1	2	2	3	3	3	3	3	4’
300	1	2	3	3	3	3	3	3	4	4	4’. 10’’
400	2	2	3	3	3	3	3	4	4	4	4’.30’’
500	2	3	3	4	4	4	4	4	4	5	4’. 50’’

Table 2. Stages of anaesthesia induced in different concentration of Clove oil for a duration of ten minutes for short exposure

Concentration of Clove oil (mg/l)	Time in minutes										Recovery time (Minutes)
	1	2	3	4	5	6	7	8	9	10	
20	0	0	1	1	1	1	1	1	1	1	3’
40	0	1	1	2	2	2	2	2	2	2	3’.30’’
60	0	1	2	2	2	2	2	2	2	2	3’.50’’
80	1	2	2	3	3	4	4	4	5	5	7’
100	2	3	4	5	-	-	-	-	-	-	9’

Table 3. Stages of anaesthesia induced in different concentration of Lemon grass oil for a duration of ten minutes for short exposure

Concentration of Lemon grass oil	Time in minutes										Recovery time (Minutes)
	1	2	3	4	5	6	7	8	9	10	
36	0	0	0	0	0	0	1	1	1	1	4'.20''
72	0	0	0	0	0	1	1	1	1	1	5'
108	0	1	1	2	2	2	2	2	2	2	7'.10''
144	1	2	2	2	3	3	3	3	3	3	8'.10''
180	3	4	4	4	4	4	5	-	-	-	10'

Table 4. Stages of anaesthesia induced in different concentration of 2-Phenoxy ethanol packed for a duration of 48 hours for transportation

Concentration of 2-Phenoxy ethanol (mg)	Time in minutes						Time in hours								
	5	10	15	20	25	30	1	6	12	18	24	30	36	42	48
15	0	0	0	0	0	0	0	1	1	1	1	1	1	2	2
30	0	0	0	0	0	0	1	1	1	1	1	2	2	2	2
60	0	0	0	1	1	1	1	1	2	2	2	2	2	2	3
120	0	0	1	1	1	1	2	2	2	2	3	3	3	3	3
240	0	1	1	1	1	2	3	4	5	-	-	-	-	-	-

Table 5. Stages of anaesthesia induced in different concentration of Clove oil packed for a duration of 48 hours for transportation

Concentration of Clove oil (mg)	Time in minutes						Time in hours								
	5	10	15	20	25	30	1	6	12	18	24	30	36	42	48
4	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
8	0	0	0	1	1	1	1	2	2	2	2	2	2	2	2
12	0	0	1	1	2	2	2	2	2	2	2	2	2	2	2
16	0	0	1	2	2	2	2	2	2	2	2	2	3	3	3
20	0	1	2	2	2	2	2	3	4	5	-	-	-	-	-

Table 6. Stages of anaesthesia induced in different concentration of Lemon grass oil packed for a duration of 48 hours for transportation

Concentration of Lemon grass oil (mg)	Time in minutes						Time in hours								
	5	10	15	20	25	30	1	6	12	18	24	30	36	42	48
2	0	0	0	0	0	0	0	0	0	0	1	1	1	2	2
4	0	0	0	0	0	0	1	1	2	2	2	2	2	2	2
8	0	0	1	1	2	2	2	2	2	2	2	2	2	2	2
16	0	1	1	2	2	2	3	3	-	-	-	-	-	-	-
24	0	1	2	2	3	3	-	-	-	-	-	-	-	-	-

DISCUSSION

Ashley (2007) reported that at low density anaesthetic agency reduce the activity of fish and thereby reduce stress. By anaesthetizing the fishes are immobilized enabling capture, handling and packing. Induction time is an important factor in anesthesia treatment. For transportation the ideal stage of anaesthesia is the stage, when fish exhibit reduced reactivity to external stimuli while for handling, the stage which is characterized by partial loss of muscle tone is ideal. According to Cooke *et al.* (2004) stage 2 anaesthesia is regarded as ideal for fish transport. It was identified by Hamackova *et al.* (2004) that 2-phenoxyethanol at 0.6 ml/l and clove oil at 0.033 ml/l produced induction in adult Tench fish. While Simoes *et al.* (2011) identified that the most appropriate clove oil concentration to induce anaesthesia for surgical purpose was 90mg/l and for brief handling 50-60mg/l but high mortality was observed in the same. Cooke *et al.* (2004) reported that a clove oil concentration of 9mg/l produced rapid induction and maintenance in stage 2 anaesthesia for transport of Large Mouth Bass. 0.03-0.05ml/l of Clove oil was recommended for fish according to Hamackova *et al*. (2006). In the present study conducted to evaluate anaesthetic efficiency all three anaesthetics produced anaesthesia in *Etroplus maculatus*. Quick induction is a highly essential factor for an ideal anaesthetic combined with rapid recovery, non-toxicity and cost effectiveness. However, 300mg/l was sufficient to produce anaesthesia by 2-phenoxyethanol and 80 mg/l for Clove oil and 144mg/l for Lemon grass oil. The stages of anaesthesia induced in different concentration of 2-Phenoxy ethanol for duration of ten minutes for short exposure indicates that for handling purposes like for packing for transportation the optimum concentration was 300mg/l. A concentration of 300 mg/l anaesthetized the fish to stage 3 within five minutes and this was selected as the effective concentration for 2-phenoxy ethanol while for Clove oil 80mg/l was identified as the ideal concentration. In the case of Lemon grass oil it was identified that 144mg/l anaesthetized the fish to stage 3 within 5 minute therefore it was selected for anaesthetizing for handling *Etroplus maculatus* for packing procedures.

As the concentration increased the induction was rapid and fish attained stage 5 for all anaesthetics but a lower concentration could induce the fish only up to stage 1 or 2.

Stages of anaesthesia induced in different concentration of 2-Phenoxy ethanol packed and maintained for duration of 48 hours of transportation indicate that the effective concentration was 60mg/l. A concentration of 240mg/l resulted in severe casualty. For Clove oil at a concentration of 12mg/l, the fishes survived and were present in stage1 or 2 anaesthesia for most of the time. This is the ideal concentration for packing, a concentration of 20 mg/l produced mortality at the 18th hour. In the case of Lemon grass oil mortality was noticed in 16mg/l and 24mg/l concentration while 8 mg/l concentration maintained the fish stage 1 or 2 anaesthesia most of the time and this is selected for packing experiments. Various research done on identifying induction time for anaesthesia show that Clove oil concentration of .06 ml/l produced complete immobilization in 4'15'' to 9'.20''minutes according to the study by Zaiko *et al.*(2008) and 0.05ml/l of Clove oil produced induction in juvenile *Valamugil cunnesius* and *Monodactylus argenteus* within 1 minute according to Durville (2001). The present results reveal that 60mg/l, 12mg/l and 8mg/l were the ideal concentrations for transportation for 2- phenoxy ethanol, Clove oil and Lemon grass oil respectively. These concentrations were ideal because they were rapid in action and also safe. The concentrations above this were leading to mortality indicating that these higher concentrations were the upper limits of concentration and the lower doses were ineffective in producing induction.

Although lower concentrations produced anaesthetic induction, the induction time is high in these cases. When induction time is prolonged it may result in prolonged recovery oxygen debt and anoxia (Abdolazizi *et al.,* 2011). Pirhonen and Schreck (2003) identified the recovery time was 3 minutes 30 minutes for clove oil anaesthesia in *Oncorhynchus mykiss*. Pawar *et al*. (2011) reported that recovery time was 271±37 s for 2-phenoxyethanol in *Hippocampus kuda*. In the present study the recovery time varied with variation in concentration. The recovery time was short and ranged from 4 to 4.50 minutes for 2-Phenoxy ethanol but it ranged from 3'.30'' to 9' minutes for Clove oil and 5 to 10 minutes for Lemon grass oil. Sink and Neal (2009) also reported the necessity of long recovery time for Clove oil anaesthesia in transportation of hybrid Striped Bass and Abdolazizi *et al.* (2011) reported that Clove oil is possessing low therapeutic index that is the ratio between therapeutic and toxic concentration. The present results affirm this finding and it is also the case of Lemon grass oil. In the present study the induction time and recovery time had a relation that is, an inverse relationship between concentrations of anaesthetic and induction time, but recovery time increased with the increasing concentration for all anaesthetics evaluated. Therefore the present work proves that transporting *Etroplus maculatus,* in the ideal anaesthetic reduces stress. Anaesthesia lowers the activity of the fish and therefore it lead to a lower O_2 demand and a reduction in CO_2 and waste production maintaining good water quality.

Therefore in the present study the optimum concentration that produces partial loss of muscle tone within 3 minutes was selected as the ideal concentration for handling. The concentration that maintained fish with reduced reactivity to external stimuli was selected as the ideal concentration for transportation.

Conclusion

The lowest effective concentration of anaesthetic for induction for short and long exposure has been identified and therefore these were the ideal concentration for handling and transportation of *Etroplus maculatus.* The finding of the present study has great significance with considerable application in stress management, induced breeding, survival and transport of *Etroplus maculatus.*

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