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COMPARATIVE EFFICACY OF FOUR ANAESTHETICS FOR THE HUSBANDRY OF MELON BARB *HALUDARIA FASCIATA* (JERDON, 1849) (CYPRINIFORMES: CYPRINIDAE)

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Abstract: Anaesthesia is widely used in aquaculture for minimizing stress and preventing mechanical injury. The effective concentration of four anaesthetics (clove oil, benzocaine, 2-phenoxyethanol and quinaldine) for sedation and immobilization of the melon barb, *Haludaria fasciata* (Jerdon, 1849) (Cypriniformes: Cyprinidae), an endemic cyprinid species of peninsular India, was determined. Induction time increased with decreasing concentration of anaesthetics, while increased exposure time resulted in prolonged recovery time (P<0.05). For the husbandry practises of *H. fasciata* the minimum effective concentrations was considered as 58 mg L^{-1} for clove oil (induction 157±2.51 and recovery 210±1.92 sec), 47 mg L^{-1} for benzocaine (induction 158±0.88 and recovery 170±8 sec), 600 µl L^{-1} for 2-phenoxyethanol (induction 156±0.92 and recovery 133±0.80 sec) and 11 mg L^{-1} for quinaldine (induction 162±0.86 and recovery 154±0.15 sec). All four anaesthetics were safe, but 2-phenoxyethanol and clove oil proved to be the most effective for use in husbandry of *H. fasciata*.

Key words: Efficacy, Induction, Barb, Aquarium, Sedation, Fish

INTRODUCTION

In the ornamental fish industry, a number of chemicals have proved effective in anaesthetization of fish for routine husbandry practices such as sorting, grading, vaccination, measuring, sampling for blood or gonadial biopsies, collection of gametes and transportation (Gabriel and Akinrotimi, 2011; Al-yaqout *et al*., 2012 and Ghanawi *et al*., 2013). But, it is necessary to establish safe and effective anaesthetic (Trushenski *et al*., 2013) and the range of concentration as well as induction and recovery time of each anaesthetic because inappropriate concentration of anaesthetic may leads to stress in fish (Hoseini *et al*., 2011 and 2012 and Popovic *et al*., 2012). The reactions of fish to anaesthetics depends on biological factors (Kaminski *et al*., 2000) includes species, the stage of life cycle and age, size and weight, lipid content, body content and disease status and environmental factors also affect the metabolic rate in fish, in addition to changing the uptake across the gills are influences the effectiveness of each anesthetic.

Haludaria fasciata (*Puntius fasciatus*), popularly known as the Melon barb is an endemic freshwater fish occurring in the rivers flowing through the Western Ghats region of India. The species is listed as Least Concern (LC) in the IUCN Red List of Threatened Species™ (Abraham, 2015). Together with other native cyprinids. *H. fasciata* contributes to the thriving aquarium industry of the Western Ghats region (Raghavan *et al*., 2013; Sekharan and Ramachandran, 2005). Although the effects of various anaesthetics have been determined for a number of popular freshwater ornamental fishes of the Western Ghats; for e.g. 40 mg L^{-1} MS-222 and 20 mg L^{-1} benzocaine for the transportation of *Dawkinsia filamentosa,* (Pramod *et al*., 2010); 30 mg L^{-1} clove oil for gen eral handling of *Sahyadria denisonii* (Sajan *et al*., 2012); 4 mg L^{-1} clove oil determined as the optimal anaesthetic concentration for 24 hr sedation of *Barilius bakeri,* (Sindhu and Ramachandran, 2013), there is no information on the use of anaesthetic agents on the husbandry or transportation of *H. fasciata*. In the international market for ornamental fishes, melon barb *H. fasciata* considered as medium desired fish. It is popular due to the similarity with tiger barb. In the present study, we aimed to determine the minimum optimal concentrations of four commonly used anaesthetic agents, clove oil, benzocaine, 2-phenoxyethanol and quinaldine for the husbandry of melon barb, *H. fasciata*.

MATERIALS AND METHODS

Early fingerlings of *H. fasciata* (3.8±0.2 cm and 1.2±0.2 gm) were collected from the Valappatanam River and their tributaries and transported to the indoor holding facility at the Department of Aquatic Biology and Fisheries, University of Kerala, Thiruvananthapuram, India. Fishes were reared in large FRP tanks (500 l) for two weeks with adequate aeration and fed ad libitum twice a day (09:00 and 17:00 h) with commercial pellet. Fishes were fasted for 24 h prior to starting the experiment following Hicks (1989). Temperature (27.0±0.5^{0}C), pH (7.0±0.3), dissolved oxygen (6.50±0.5 mgL^{-1}), alkalinity (65.0±6.0 mgL^{-1}), hardness (70.0±4.0 mgL^{-1}) and ammonia (<0.02 mgL^{-1}) were maintained within narrow ranges.

Four different anaesthetic agents, 2-phenoxyethanol,benzocaine, Quinaldine (Hi-Media Laboratories Pvt. Ltd., Mumbai, India) and clove oil (Micro Fine Chemicals, India) were used. Different concentrations of the anaesthetic were prepared a few minutes before the experiment. Clove oil having active ingredient euginol (1g ml/L), dissolved with 95% ethanol at a ratio of 1: 10 (clove oil: ethanol) to prepare stock solution containing 100 mg ml/L (Sindhu and Ramachandran, 2013; Yildiz *et al*., 2013); 100 g of benzocaine was dissolved in 1 liter of 95% ethanol by following Pramod *et al*. (2010). 2-phenoxyethanol and pure ethyl alcohol were mixed at the rate of 1:1 (2-Phenoxyethanol: ethanol) (Tulay and Durali, 2006) and quinaldine stock solution was prepared by dissolving quinaldine with 95% ethanol (1:10 ratio of quinaldine: ethanol) as described by Harms (2003) and Hseu *et al*. (1998). The minimum and maximum concentration of each anaesthetic for the experiment was selected on the basis of previously published information for cyprinids (Pramod *et al*., 2010; Sajan *et al*., 2012; Mercy *et al*., 2013; Varkey and Sajeevan, 2014). The following concentrations of each agent were finally evaluated, clove oil (46, 58, 62, 73 and 83 mg L^{-1}), benzocaine (35, 47, 58, 70 and 81 mg L^{-1}), 2-phenoxyethanol (400, 600, 800, 1000 and 1200 µl L^{-1}) and quinaldine (1, 11, 21, 32 and 42 mg L^{-1-}). During the experiment, each concentration of the anaesthetic was added to 1 liter of water in the experimental glass tank (2.5 liter) and mixed manually for 1 min with a glass rod. A single fish was collected randomly with hand-net from the rearing tank and placed in the experimental tank. During the induction and recovery period, fish behaviour was monitored individually at each concentration. When an experimental fish reached induction stage III (I^3) they were transferred to a recovery tank and observed until they recovered to the normal condition (recovery stage-III). Separate recovery tanks (5 L) were preferred for each concentration. Eight trials were performed for each concentration using different individual fish following Sajan *et al*., (2012). Behaviour responses of the anaesthetized fishes were assessed according to previous studies with slight modifications based on the species-specific behavioural response (Pawar *et al*., 2011; Varkey and Sajeevan, 2014) (Table 1). Different stages of induction and recovery were illustrated with the behavioural responses of fish.

The water in both the induction and recovery tank was aerated throughout the experiment. The induction time (I^3) was considered to be the time period at which an experimental fish does not respond to external stimuli when it is placed in the anaesthetic tank; and the time period for anaesthetized fish to recover with full equilibrium motion in recovery tank was considered as the recovery time (R^3). An induction time of 180 seconds or less with complete recovery within 300 seconds as suggested by Ghanawi *et al*. (2013), Opiyo *et al*. (2013) and Yildiz *et al*. (2013) were used to determine effective concentration of ana

Table 1. Stages of anaesthetic induction and recovery in *H. fasciata*

Stages	Description	Behaviour criteria
Induction		
0	Normal	Reactivity to external stimuli; opercular rate normal.
I	Partial loss of equilibrium	Reduction of aggressiveness; partial loss of muscle tone, acceleration of opercular rate.
II	Loss of equilibrium	Pressure on caudal peduncle, opercular rate was slow.
III	Total loss of equilibrium	Complete immobilization, opercular rate very slow, fish lie on the bottom and does not react to external stimuli.
Recovery		
0	Total loss of equilibrium	Fish lie on the tank bottom
I	Equilibrium	Start of the operculum and fin movement. Body stay on normal position.
II	Total equilibrium	Slight movements, respond for strong external stimulus.
III	Normal	Swimming and respond to external stimulus

Behavioural responses of *H. fasciata* during induction and recovery time modified from Pawar *et al*. (2011); Varkey and Sajeevan (2014).

esthesia. The induction and recovery time of each treatment was measured with stop watch in terms of seconds (s) and the recovered fishes were separately transferred into10 L glass tanks for seven days observation to assess the post recovery survival rate (Varkey and Sajeevan, 2014).

Statistical analyses were carried out using SPSS 19.0 for Windows. All data were subjected to a one-way analysis of variance (ANOVA) to determine differences in treatments. All data are analysed with Duncan new multiple range test and stated as mean values ± standard error of means (SEM). Significant differences were considered at $P<0.05$ levels among the groups.

RESULTS

The induction and recovery time for *H. fasciata* exposed to the various concentrations of clove oil, benzocaine, 2-phenoxyethanol and quinaldine are given in Table 2. Generally, with increasing concentrations, induction times decreased, whereas recovery time increased significantly for all the anaesthetic agents evaluated in present study.

In the present study, the minimum effective concentrations of anaesthetics were considered as 58 mg L^{-1} (157±2.51 sec) for clove oil, 47 mg L^{-1} (158±0.88 sec) for benzocaine, 600 µl L^{-1} (156±0.92 sec) for 2-phenoxyethyanol and 11 mg L^{-1} (162±0.86 sec) for quinaldine. The induction time of *H. fasciata* significantly decreased with increasing concentration ($P<0.05$) of the four anaesthetics. Time for reach stage III at 58 mg L^{-1} clove oil is (157±2.51 sec) significantly not different ($P>0.05$) with 47 mg L^{-1} benzocaine (158±0.88 sec). *H. fasciata* reared in post-treatment tanks showed no mortality for seven days, and exhibited normal feeding and physiological behaviour.

DISCUSSION

Anaesthetics are an integral component of modern day aquaculture (Pawar *et al*., 2011). The effect of anaesthetics varies between fish species (Zahl *et al*., 2011) and size (Yildiz *et al*., 2013; Opiyo *et al*., 2013) and therefore it is often advisable to identify the lowest effective or appropriate concentrations of various anaesthetics to minimise stress (Feng *et al*., 2011) for different species. Our study revealed that four anaesthetic agents, 2-phenoxyethanol, benzocaine, quinaldine and clove oil are effective for use in the fingerlings of *H. fasciata*.

The lowest effective concentration must enable a transition to anaesthesia in 180 seconds and recovery to normal position within 300 seconds (Ghanawi *et al*., 2013; Opiyo *et al*., 2013; Yildiz *et al*., 2013). In present study, the effective concentration of 2-phenoxyethanol for *H. fasciata* was 600 µl L^{-1} (induction and recovery occurred at 156±0.92 and 133±0.80 seconds, respectively) and therefore this concentration was considered as the

Table 2. Induction and recovery period (stage III) for *H. fasciata* were anaesthetized with five concentrations of four anaesthetic agents. Data are presented as mean ± SEM, n=160.

Anaesthetics		Time (s)	
		Induction (Stage III)	Recovery (Stage III)
Clove oil (mg L^{-1})			
	46	191±5.98	201±1.66
	58	157±2.51	210±1.92
	62	132±1.26*	354±1.13*
	73	137±1.06*	355±0.09*
	83	93±0.56	385±1.08
Benzocaine (mg L^{-1})			
	35	204±0.75	150±1.22
	47	158±0.88	170±1.08
	58	124±0.59	189±0.96
	70	93±0.92	205±0.80
	81	78±0.57	248±1.20
2-phenoxyethanol (µl L^{-1})			
	400	243±1.23	119±1.33
	600	156±0.92	133±0.80
	800	129±1.22	167±0.68*
	1000	86±1.23*	169±0.92*
	1200	85±0.90*	186±0.68
Quinaldine (mg L^{-1})			
	1	191±1.03	148±0.84
	11	162±0.86	154±0.15
	21	106±0.56	159±0.90
	32	57±0.70	168±0.56
	42	44±0.77	245±0.70

Values of each anaesthetic in the same column with * indicates, they are significantly not different (P>0.05).

minimum effective concentration for handling this species. Several studies have proved the effectiveness of 2-phenoxyethanol anaesthesia (Perdikaris *et al*., 2010; Uçar and Atamanalap, 2010; Gholipourkanani *et al*., 2011). Generally, the effective anaesthetic concentration of 2-phenoxyethanol with ethanol ranges from 200 to 600 µl L^{-1} (Pawar *et al*., 2011; Varkey and Sajeevan, 2014).

In our study, the recovery time increased with increasing concentrations s of 2-phenoxyethanol (P<0.05). Similar results have been reported in *D. filamentosa* (Pramod *et al*., 2010), *Siganus rivulatus* (Pramod *et al*., 2010), *Etroplus suratensis* (Sajan *et al*., 2013) *Oncorhynchu smykiss* (Yildiz *et al*., 2013) and *S. denisonii* (Varkey and Sajeevan 2014). Clove oil has been used as potential anaesthetic in *S. denisonii* (Sajan *et al.,* 2012), *Pterophyllum scalare* (Hekimoðlu and Ergun, 2012) *Pampus argenteus* (Al-yaqout *et al*., 2012) and *S. rivulatus* (Ghanawi *et al*., 2013). In the present study, the most effective concentration of clove oil was observed at 58 mg L^{-1} (induction and recovery occurred at 157±2.51 and 210±1.92 seconds, respectively). According to Matin *et al*. (2009) Arzu and Muhammed (2010), Hekimoðlu and Ergun (2012) and Ghanawi *et al*. (2013) clove oil had slightly faster induction and longer recovery time than other anaesthetics. In the present study, it was observed that recovery time was directly related to anaesthetic concentration (P<0.05). The longest time to recovery was observed at 83 mg L^{-1} (385±1.08 sec), while shortest time occurred at 46 mg L^{-1} (201±1.66 sec) (Table. 2). When the dosage of clove oil increased, the induction time reduced and the recovery time notably increased (P<0.05). Similar results have been reported in *O. Nerka* (Woody *et al.*, 2002), *D. filamentosa* (Pramod *et al.*, 2010), *S. denisonii* (Sajan *et al.*, 2012) and *Clarias gariepinus* (Ogretmen and Gokcek, 2013).

In the present study, 47 mg L^{-1} benzocaine (induction and recovery occurred at 158±0.88 and 170±1.08 seconds, respectively) was detected as the safe and effective concentration for handling *H. fasciata.* The same observation was reported in *Salmo salar* (Ross and Ross, 1984) and *Sparus sarba* (Hseu *et al.*, 1998). The result shows that *H. fasciata* has minor resistance to benzocaine anaesthesia with an induction time at 47 mg L^{-1} being 158±0.88 seconds, whereas the induction time obtained in clove oil, 2-phenoxyethanol anaesthesia was 191±5.98 and 243±1.23 seconds respectively at the similar concentration. Our result agreed with Cortes-Garcíaand Rodríguez-Gutiérrez (2015) in *O. mykiss.* This is because of lipid solubility effect of benzocaine, which may lead to effects on the central nervous system (Kiessling *et al.*, 2009; Pramod *et al.*, 2010; Zahl *et al.*, 2011). At the same time benzocaine follows all criteria required for a safe anaesthetic to fish (Hseu *et al.*, 1999; Treves-Brown, 2000; Heo and Shin, 2010) but with a word of caution that safety depends on the species used (Treves-Brown, 2000; Gomes *et al.*, 2001).

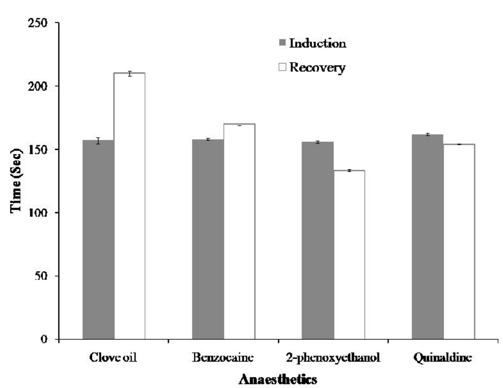

Fig. 1. Induction stage III (I^3) and recovery time of *H. fasciata* exposed in the minimum effective concentration of clove oil (58 mg L^{-1}), benzocaine (47 mg L^{-1}), 2-phenoxyethanol (600 µl L^{-1}) and quinaldine (11 mg L^{-1}) anaesthetics

A concentration of 11 mg L^{-1}quinaldine (induction and recovery occurred at 162±0.86 and 154±0.15 seconds, respectively) was required to anaesthetise *H. fasciata* which was the lowest effective concentration among the four anaesthetics. Schramm and Black (1984) and Osanz-Castan *et al.*, (1993) also recorded similar results. Quinaldine is lipid soluble, and therefore do not accumulate in the brain (Brandenburger Brown *et al.*, 1972); they also depress the sensory centre of the central nervous system (Locke, 1969). It was observed that the induction time was directly proportional to different concentrations of quinaldine (P<0.05). Al-Roumi *et al.*, (2014) reported that the induction time of yellow sea bream (*Acanthopagrus latus*) fingerlings and blue fin sea bream (*Sparidentex hasta*) fingerlings decreased with increasing concentration of quinaldine and increase recovery time.

The recovery time to normal swimming stage was comparatively not longer in quinaldine, benzocaine and 2-phenoxyethanol treatments. Among the minimum effective concentration of four anaesthetics, longer recovery time (210±1.92 sec) was observed with the clove oil treatment (Fig. 1.). According to Keene *et al.* (1998), clove oil has less ability to release excess amount of anaesthetics from the fish body system than other chemicals leading to prolonged recovery. It has been suggested that the recovered fishes should be observed for any abnormal behaviour or mortality in post-treatment tanks for seven days (Pawar *et al.*, 2011). Recovered *H. fasciata* monitored in post treatment tanks for seven days exhibited normal feeding and physiological behaviour, without any mortality or abnormal behaviour.

CONCLUSION

The results of the present study showed that benzocaine and clove oil are effective anaesthetics for the husbandry of *H. fasciata*. Among the effective concentration considered, there was no statistical difference detected between clove oil and benzocaine induction time. Although higher concentrations of 2-phenoxyethanol are required for effective anaesthetization. At the same time quinaldine noticed with lowest concentration for anaesthetization of *H. fasciata.* Need to conduct further studies like cortisol determination for the stress analysis to compare to which anaesthetic is most suitable.

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