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Effectiveness of 2-Phenoxyethanol for Anesthetizing Striped Mullet (*Mugil cephalus*) (Linnaeus, 1758) for Transportation

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Abstract

Anesthetized fish transport is creating new opportunities for aquaculture, as it offers ease of handling and reduction of stress and helps to reduce mortality rates. In this study, 2-phenoxyethanol was administered to Striped Mullet (*Mugil cephalus*) (Linnaeus, 1758) to determine its effects on the ease of handling and transportation. Four dosage levels were tested—100, 200, 300 and 400 mg l^{-1}. Three levels of induction and three of recovery were recorded. The lowest effective concentration was 300 mg l^{-1}. At this dosage, the time required to achieve the final stage of induction was 159±13 s (mean ± SD) and that to achieve the final stage of recovery was 193±16s. These times are significantly different from those for the other dosages.

Keywords: *Mugil cephalus,* species, 2-phenoxyethanol, anesthetizing

Introduction

The Striped Mullet *Mugil cephalus* is a popular marine food fish found in tropical and subtropical waters worldwide. It enjoys high demand owing to the quality of its meat and is currently a candidate for mariculture. Breeding Striped Mullet in captivity is complicated since it does not exhibit clear sexual dimorphism. They need to be examined in the laboratory, which necessitates transporting them with as little stress as possible. The best option is to anesthetize them prior to transport.

The present study focuses on facilitating the transport of adult Striped Mullet from their natural habitat to the laboratory. The major issue arising from the transport of this species is stress, followed by injury, loss of mucus and possible death. Because it is an aggressive species, it responds vigorously to even minor changes in its surroundings. When disturbed it moves rapidly, hitting itself against the sides of the holding tank, causing hemorrhaging that can be fatal. In 2007, the American Veterinary Medical Association reported that breeding shock due to stress results from the transportation of large fishes (AVMA, 2007). To maintain the health and safety of the fish, it is advisable to anesthetize them prior to transportation (AVMA, 2007; Andrews & Jones, 1990). Several agents are available for anesthetizing fish, including clove oil, tricaine methanesulfonate, and benzocaine. In the present study, Striped Mullet were exposed to 2-phenoxyethanol, a known fish anesthetic that results in the loss of sensation owing to the suppression of the action of the nervous system. In studies of anesthesia, induction and recovery times (based on observed fish responses) were compared across the different dosages tested. Typically, induction is divided into three stages (I_1, I_2 and I_3) (Table1), with I_3 being the stage required for fish to be transported. The dosage level of this anesthetic changes from species to species and also with in species depended on the weight and length of the fishes. The present work aims to assess the anesthetic dosage suitable for stripped mullet.

Materials and Methods

The Striped Mullet used in these anesthesia trials (weight, 800–1,500 mg) were collected from cages in Vembanad Lake, Kerala, India. To reduce excretion, they were not fed before the experiment.

Water for the experiment was taken from the site at which the fish were collected. Water quality parameters (dissolved oxygen, pH, temperature, and alkalinity) were estimated following standard

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procedures (APHA 1992). After conducting several trials and a literature review, the effective dose of 2-phenoxyethanol for fish was fixed in the range of 100–600 mg l^{-1} (Gilderhus & Marking, 1987; Guo et al., 1995; Weber et al., 2009; Pawar et al., 2011). Four different concentrations of 2-phenoxyethanol (Loba Chemie, Mumbai, India) were tested: 100, 200, 300, and 400 mg l^{-1}. For each concentration, the anesthetic was measured and placed in a reagent bottle (50 ml), mixed with distilled water (30 ml), and shaken well to distribute the chemical before it was added to the induction tank. Four 500 l tanks were used for the exposures. Altogether, 20 fish were exposed (5 per tank). After being anesthetized, the fish were placed in 15l poly brood storage bags (1 fish per bag) for transport. As suggested above, the water in the bags contained a mild dose of the anesthetic to reduce stress. The bags were oxygen-packed, and the fish were transported in air-conditioned vehicles. At the laboratory, fish were placed in separate recovery tanks according to dosage. The whole experimental sequence was repeated four times so as to confirm the results. After recovery, the fish were shifted to inspection tanks (1,000 l) and observed for 5–7 days for delayed mortality (Pawar et al., 2011). The water in these tanks was replaced at the rate of 50% per day. Fish were fed a commercially formulated feed *ad libitum* during observation.

The efficiency of the anesthetic was assessed in terms of the three stages of induction and three of recovery (Sajan et al., 2012). Comparisons of the mean times to induction and recovery among dosages were performed by one-way ANOVA and Tukey's honestly significantly different (HSD) multiple-comparison procedure (Zar 1999) in SPSS (Windows, version 16.0).

Results and Discussion

The effective dosages of anesthetics vary among fish species, requiring each species to be assessed individually (King et al., 2005). In our study of Striped Mullet, induction times were inversely proportional to the concentration of 2-phenoxyethanol ($p<0.05$), which accords with the results of prior studies with Common Carp (*Cyprinus carpio*), Goldfish (*Carassius auratus*), Ambon Damsel (*Pomacentrus amboinensis*), White Seabream (*Diplodus sargus*), Puntazzo *(Diplodus puntazzo)*, Senegalese Sole (*Solea senegalensis*), European Seabass (*Dicentrarchus labrax*), Gilthead Bream (*Sparus auratus*) and Himri Barbell (*Carasobarbus luteus*) (Josa et al., 1992; Weyl et al., 1996; Munday & Wilson, 1997; Tsantilas et al., 2006; Weber et al., 2009; Mylonas et al., 2005; Kaya & Faith, 2011; Iwama et al., 1989). The various levels of induction and recovery that we observed in Striped Mullet are shown in Table 2.

The minimum effective dose is the smallest concentration of a substance that causes anesthetization within 180s with complete recovery within 300s (Marking & Meyer, 1985; Hseu et al., 1998). In the present study, 300 mg l^{-1} was determined to be the appropriate dosage for Striped Mullet. At that dosage, the time to attain I_3 (159±13s) was significantly different ($p<0.05$) from those for 200 mg l^{-1}

Table 1. Stages of anesthesia induction and recovery as determined by fish responses

Stage	Description
Induction	
I_1	Fish exhibits slow swimming and alteration in physical position
I_2	Fish exhibits partial loss of body balance, followed by tilting over onto one side and decreased locomotive activity
I_3	Fish exhibits a complete loss of body balance and lies on its side at the bottom of the tank, does not react to external stimuli, and has limited opercular movement
Recovery	
R_1	Fish exhibits only slight fin movements, without moving or tilting
R_2	Fish starts regular breathing, assumes its original physical position, and exhibits increased locomotive activity along with irregular fin movements
R_3	Fish resumes its normal position, regains its ability to swim, and exhibits regular opercular movements

Table 2. Induction (measured from the point at which the anesthetic was administered) and recovery (measured from the point at which the fish were again exposed to untreated water) times (s) for Striped Mullet anesthetized with different dosages of 2-phenoxyethanol (mg l^{-1}). Within rows, values with different lowercase letters are significantly different ($p<0.05$).

Stage of induction or recovery	Dosage (mean±SD)			
	100	200	300	400
I_1	68±14 x	74±3 y	62±4 w	80±9 z
I_2	185±20 z	171±28 y	104±7 w	158±6 x
I_3	216±78 z	193±14 y	159±13 w	190±14 x
R_1	47±20 w	50±27 x	59±3 y	69±4 z
R_2	72±23 w	129±34 x	162±24 z	135±6 y
R_3	110±7 w	184±22 y	193±16 z	182±26 x

(193±14s), 100 mg l^{-1} (216±78s), and 400 mg l^{-1} (190 ±14s) (Table 2). These findings are in agreement with those of earlier studies (Gilderhus & Marking, 1987; Hseu et al., 1998; Weber et al., 2009; Pawar et al., 2011).

Recovery time tended to increase with the dosage of 2-phenoxyethanol ($p<0.05$). The lowest time to recovery occurred at 100 mg l^{-1} (110±7s); the lowest time that is consistent with induction occurred at 400 mg l (182 ± 26s). Longer recovery times at lower doses were reported in Yellow Seahorses *Hippocampus kuda* (Pawar et al., 2011) and Denison Barbs *Puntius denisonii* (Sajan et al., 2012; Mercy et al., 2013). By contrast, Mylonas et al. (2005) found decreasing recovery times with increases in sedation in European Seabass and Gilthead Bream. Such variation in the recovery periods may be related to the type, bulk, and physiological grade of the fish as well as to its habitat and water quality parameters such as temperature, pH, salinity, oxygen concentration, and mineral composition (Josa et al., 1992; Weyl et al., 1996). The values of the water parameters in this study were as follows: temperature, 25±0.5°C; pH, 7.9 ± 0.3; salinity, 20 ± 2; dissolved oxygen, 6.7±0.5 mg l^{-1}; alkalinity, 66±8.0 mg l^{-1}; hardness, 70 ± 5.0 mg l^{-1}; nitrite, <0.01 mg l^{-1}; and ammonia,<0.01 mg l^{-1}; these values are comparable to those in Mylonas et al. (2005) and Munday & Wilson (1997).

The conditions under which fish are transported are important, as their health may be seriously affected if the conditions are not suitable. In our study, fish sedated with 2-phenoxyethanol were transported for 90 min, and 7 days dafter recovery they were all healthy and active, indicating that this compound is an effective and safe sedative for the treatment and transport of adult Striped Mullet. The study also suggests that it is possible to safely transport this species for durations longer than 90 min.

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