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Short Communication

The Efficacy of 2-phenoxyethanol as Anaesthetic for Juvenile Pearl Spot, *Etroplus suratensis* (Bloch)

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Abstract

The use of anaesthetic agents may reduce stress-induced damage to the fish. In the current investigation, anaesthetic efficacy of 2-phenoxyethanol (500 $\mu L \cdot L^{-1}$, 600 $\mu L \cdot L^{-1}$, 700 $\mu L \cdot L^{-1}$ and 800 $\mu L \cdot L^{-1}$) on *Etroplus suratensis* (Bloch 1790) was evaluated. 2-phenoxyethanol appears to be a highly effective anaesthetic in fishes. The time periods necessary for the induction of particular characteristic stages of induction and recovery were determined.The results showed that 600 $\mu L \cdot L^{-1}$ was the effective concentration that induces anaesthesia in *E. suratensis* (induction in 171±13 sec and recovery time 149±21 sec; $p>0.05$). An exponential relationship was observed between concentrations and recovery time, whereas an inverse exponential relationship was observed between concentrations of anaesthetic and induction time.

Introduction

Anaesthesia is usually essential to minimize stress and physical damage during handling of fish for routine husbandry operations (Summerfelt and Smith 1990). When choosing an anaesthetic a number of factors should be considered such as its efficacy, cost, availability, ease of use and side effects on fish, humans and the environment (Marking and Meyer 1985; Mylonas et al. 2005). Overdose of an anaesthetic or retaining the fish in an anaesthetic bath for too long leads to the fading of ventilation, hypoxia, and finally, respiratory and cardiac collapse (Tytler and Hawkins 1981). Commonly used anaesthetics in fishes include MS-222, benzocaine, quinaldine, chlorobutanol, 2- phenoxyethanol, clove oil and metomidate.

Etroplus suratensis (Bloch 1790) known locally as '*Karimeen'* is the largest among Indian cichlids (Perciformes), and a high-valued food fish endemic to Peninsular India and Sri Lanka. Because of their wide salinity tolerance, omnivorous feeding habit and high market price, pearl spot is considered ideal for commercial culture in brackish and freshwaters. 2-phenoxyethanol is used to sedate the fishes during handling and live transport. Recent studies have evaluated the anaesthetic

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efficacy of 2-phenoxyethanol in various fish species (Gilderhus and Marking 1987; Josa et al. 1992; Weyl et al. 1996; Marsic-Lucic et al. 2005; Weber et al. 2009; Pawar et al. 2011). There are no studies available on the use of anaesthetics in handling management of *E. suratensis.*The aim of the present study was to establish the concentration of 2-phenoxyethanol that provides efficient anaesthesia in *E.suratensis*.

Materials and Methods

Pearl spot, *E. suratensis* (average weight=7.12±2.29 g) collected from Vembanad Lake, Alappuzha, Kerala was acclimatised in cement tanks (3000 L) for a period of 2 wk. The fish were fed to satiety twice a day at 09.00 and 17.00 h with commercial formulated feed and were fasted 24 h prior to experiment. Water quality parameters were maintained within a narrow range of values and monitored by using thermometer for temperature; colour comparator solutions (Nice chemicals-India) for pH and ammonia; titrimetric method for alkalinity, hardness, nitrite and Winkler method for dissolved oxygen using standard procedures (APHA 1992). Experiments were carried out under indoor laboratory conditions. All fish were healthy prior to, and throughout the duration of the study. 2-phenoxyethanol (Loba Chemie, Mumbai, India) was used as the anaesthetic agent. A known volume of 2-phenoxyethanol (500 $\mu L \cdot L^{-1}$, 600 $\mu L \cdot L$-1, 700 $\mu L \cdot L^{-1}$ and 800 $\mu L \cdot L^{-1}$) was initially mixed with water (30 mL) in a reagent bottle (50 mL) and then stirred to disperse the chemical to form small droplets before adding to anaesthetic induction tanks (Pawar et al. 2011).

Stages of anaesthetisation include induction, maintenance and recovery (Sajan et al. 2012). For practical purposes, four stages of induction (I_1, I_2, I_3, I_4) and three stages of recovery (R_1, R_2, R_3) were considered in *E. suratensis* (Table. 1). An induction time of 180 sec or less and complete recovery within 300 sec suggested by Marking and Meyer (1985) and Trzebiatowski et al. (1996) were used to record the induction and recovery stages in *E. suratensis*. Dosages of anaesthesia for various teleost (Weber et al. 2009) were used as base information and different concentrations of 2-phenoxyethanol (500 $\mu L \cdot L^{-1}$, 600 $\mu L \cdot L^{-1}$, 700 $\mu L \cdot L^{-1}$ and 800 $\mu L \cdot L^{-1}$) selected to induce anaesthesia in *E. suratensis*. Both treatment and recovery water were taken from the tank, where the fish were maintained and both bath systems were aerated throughout the procedure. When fish reached stage three of anaesthesia (I_3), it was immediately transferred to the recovery tanks for recording recovery stages (R_1, R_2, R_3). The induction and recovery time for each concentration were measured by using an electronic stopwatch. Experiments were repeated four times to verify the findings. The recovered fish were transferred into the observation tanks (2,000 L) for 7 days to assess the post recovery mortality (Pawar et al. 2011).

During the post-recovery period, 50% of the tank water was exchanged daily and the fish were fed twice a day *ad libitum* with commercial formulated feed.

Table 1. Induction and recovery stages of anaesthesia observed in *E. suratensis.*

Induction Stage	Observations
I_1	Initial anaesthetic effect; slow swimming, physiological position
I_2	Partial loss of body balance; body tilting over one side, decreased locomotor activity
I_3	Complete loss of body balance; flank position at the bottom of tank, no reaction to external touch or stimuli; opercular movement is little.
I_4	Cessation of opercular movement (over dose and or longer immersion in anaesthetic solution); Subsequent death
Recovery Stage	**Observations**
R_1	Initial fin movement; Non-moving tilting on flank position.
R_2	Regular breathing; Physiological position, increased locomotor activity, Irregular fin movement
R_3	Ability to swim normally and regular opercular rate

Mean induction time and recovery time of anaesthesia were compared among treatment groups using one-way ANOVA, followed by Tukey's Honestly Significant Difference (HSD) multiple comparison procedure (Zar 1999). All data were reported as mean±S.D. Significant difference was tested at a 5% level of significance, represented as $P<0.05$. The results were processed and analysed with the SPSS (Windows, Version 15.0).

Results and Discussion

Anaesthesia is generally defined as a state caused by an applied external agent resulting in a loss of sensation through depression of the nervous system. 2-phenoxyethanol, a common fish anaesthetic is widely applied in sedation and transportation of fish (Guo et al. 1995). The effective anaesthetic concentration of 2-phenoxyethanol in a number of fish species have been reported to range between 200-600 $\mu L \cdot L^{-1}$ (Gilderhus and Marking 1987; Guo et al. 1995; Weber et al. 2009; Pawar et al. 2011). However, there are no reports on the anaesthetic efficiency of 2-phenoxyethanol in *E. suratensis*.

The responses to the same anaesthetic can vary considerably among different species, so the characterization of the effective dose of the different anaesthetics in a determined species is a rather advisable practice (King et al. 2005). The present study revealed that induction time of anaesthesia in *E. suratensis*is decreased significantly with increasing concentrations of 2-phenoxyethanol ($P<0.05$), which are consistent with previous studies with 2-phenoxyethanol in *Cyprinus carpio* Linnaeus 1758 (Josa et al.1992), *Carassius auratus* (Linnaeus 1758) (Weyl et al. 1996), *Tinca tinca* (Linnaeus 1758) (Hamackova et al. 2004), *Diplodus sargus* (Linnaeus 1758) and *Diplodus puntazzo* (Walbaum 1792) (Tsantilas et al. 2006), *Solea senegalensis* Kaup 1858 (Weber et al. 2009) and *Carasobarbus luteus* (Heckel 1843) (Kaya and Faith 2011)

The different stages of induction (I_1, I_2, I_3) and recovery (R_1, R_2, R_3) observed in *E. suratensis* are presented in Table 2 and Fig.1. The ideal concentration must be the lowest concentration which enables a transition to anaesthesia in 180 sec and a full recovery in 300 sec (Marking and Meyer 1985; Hseu et al. 1998). In this experiment, the lowest induction time (<180 sec) was observed at 600 $\mu L \cdot L^{-1}$ and therefore this dose was considered as the lowest effective concentration for anaesthesia in *E. suratensis*. At 600 $\mu L \cdot L^{-1}$, the time to reach anaesthesia stage (I_3) of induction (171±13 sec) and recovery (R_3) time (149±21 sec) was significantly different ($P<0.05$) from the other dosages 500 $\mu L \cdot L^{-1}$(284±79 sec), 700 $\mu L \cdot L^{-1}$(159±12 sec) and 800 $\mu L \cdot L^{-1}$(128±15 sec) (Table 2). These results are consistent with previous studies in teleost fishes (Gilderhus and Marking 1987; Hseu et al. 1998; Weber et al. 2009; Pawar et al. 2011). A Turkey's HSD test revealed that the induction time 600 $\mu L \cdot L^{-1}$ was significantly different from 500 $\mu L \cdot L^{-1}$ (284±79 sec, $p= 0.004$) and 800 $\mu L \cdot L^{-1}$ (128±15 sec, $p= 0.347$). No significant differences were observed between the concentrations of 600 $\mu L \cdot L^{-1}$and 700 $\mu L \cdot L^{-1}$ (159±12 sec, $p= 0.961$).

Table 2. Induction and recovery times (sec) for *E. suratensis* anaesthetised with different concentrations of 2-phenoxyethanol ($\mu L \cdot L^{-1}$). Data are presented as average±S.D.

Stages of Induction	**2-phenoxyethanol concentration**			
	500	600	700	800
I_1	042±13	029±02	036±03	032±09
I_2	105±19	066±29	113±06	048±06
I_3	284±79	171±13	159±12	128±15
Stages of Recovery	500	600	700	800
R_1	059±21	071±26	093±04	055±03
R_2	101±22	100±35	171±25	129±05
R_3	143±08	149±21	214±15	223±27

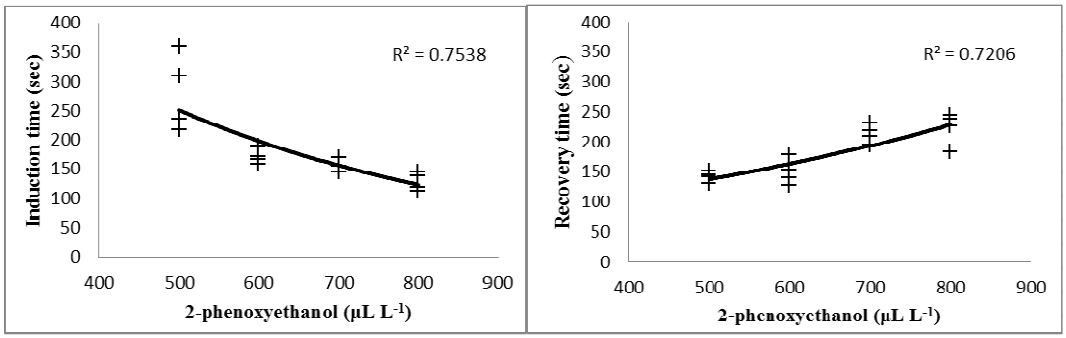

Fig. 1. Induction and recovery times (sec) for *E. suratensis* anaesthetised with different concentrations of 2-phenoxyethanol ($P<0.05$).

The recovery time was directly proportional with increasing doses of 2-phenoxyethanol ($P<0.05$). The longest recovery time was observed at 800 $\mu L \cdot L^{-1}$ and shortest time to reach the total recovery stage was detected at 500 $\mu L \cdot L^{-1}$. Longer recovery time with increased anaesthetic dosage has been reported in *Hippocampus kuda* Bleeker 1852 (Pawar et al. 2011) and *Puntius denisonii* (Day 1865) (Sajan et al. 2012; Mercy et al. 2013). However, experiments by Mylonas et al. (2005) documented recovery times decreasing with increase in anaesthetic concentration in *Dicentrachus labrax* (Linnaeus 1758) and *Sparus aurata* Linnaeus 1758. Such difference in the respective recovery times might be related to species, size, physiological status and environmental conditions as well as temperature, pH, salinity and oxygen and mineral content of the water (Josa et al. 1992; Weyl et al. 1996).

The water quality parameters observed in the experiment were, temperature (27±0.5 ^{0}C), pH (7.3±0.3), dissolved oxygen (6.5±0.5 $mg \cdot L^{-1}$), alkalinity (65±8.0 $mg \cdot L^{-1}$), hardness (70±5.0 $mg \cdot L^{-1}$), nitrite (<0.01 $mg \cdot L^{-1}$) and ammonia (<0.01 $mg \cdot L^{-1}$) that agree with Sajan et al. (2012). In the present study, recovered *E. suratensis* were observed in the post treatment tanks for 7 days and no abnormal behaviour or mortality was observed. Our study indicates that 2-phenoxyethanol is an effective and safe anaesthetic for the handling management of *E. suratensis*. The effective dosage that induces anaesthesia in *E. suratensis* is 600 $\mu L \cdot L^{-1}$ and different dosages did not cause mortality or abnormality to the fish under experiment. Further studies on the effects of anaesthetics on the transportation of juveniles and adult will considerably advance our understanding of anaesthesia in *E. suratensis*.

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