Research Article

Efficacy of 2-Phenoxyethanol as an Anaesthetic for Adult Redline Torpedo Fish, *Sahyadria denisonii* (Day 1865)

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Received 20 August 2014; Revised 17 October 2014; Accepted 12 November 2014; Published 8 December 2014

Academic Editor: Thomas Iliffe

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Efficacy of 2-phenoxyethanol for redline torpedo fish exposed to five concentrations (200, 300, 400, 500, and 600 μL^{-1}) was evaluated. The time periods necessary for each characteristic stage of induction and recovery were recorded. Results indicated that the induction time of the fish exposed to five anaesthetic concentrations significantly ($P < 0.05$) decreased with increasing concentration but recovery time was independent of concentration. Concentration of 500 μL^{-1} (induction time: 173 ± 7 and recovery time: 129 ± 41 seconds) was determined as the minimum effective concentration that induces anaesthesia in less than 3 minutes.

1. Introduction

Anaesthesia is an effective method to minimize stress or physical damage caused during handling, transport, grading, weighing, and induction of spawning [1]. L. G. Ross and B. Ross [2] defined anaesthesia as a state caused by an applied external agent resulting in a loss of sensation through depression of the nervous system. The use of anaesthetics is primarily for the purpose of holding fish immobile while the animal is being handled for experiments. When choosing an anaesthetic, a number of considerations should be taken into account such as its efficacy, cost, availability, ease of use, and side effects on fish, humans, and the environment [3, 4]. Presently, there are many anaesthetics available for aquatic animals, including MS-222, benzocaine, quinaldine, chlorobutanol, 2-phenoxyethanol, eugenol, and metomidate [3]. 2-Phenoxyethanol is relatively inexpensive and remains viable in long-term exposure [5] and is also easily available compared to other anaesthetics. Deacon et al. [6] recommended 2-phenoxyethanol as a highly suitable anaesthetic for repeatedly exposed fishes. Over the last three decades, studies have evaluated the anaesthetic efficacy of 2-phenoxyethanol in various fish species [7–14]. According to Topic Popovic et al. [15], it is necessary to have a better understanding of safety

margins, induction, and recovery times for many fish species in order to achieve optimal application.

Sahyadria denisonii (or *Puntius denisonii*), an ornamental fish popularly known as redline torpedo fish or Miss Kerala, is endemic to the rivers flowing through the Western Ghats hotspots of India. The species is much sought after in the international ornamental fish trade and is listed as “Endangered” in the IUCN Red List of Threatened Species [16]. Captive breeding is considered to be one of the solutions for ensuring sustainability and conserving wild populations of this endangered species [17]. In fisheries, various approaches have been applied to relieve fishes from regular stressful conditions during captive breeding, and anaesthesia has been used as an applicable way to prevent or reduce stress. Efforts to develop captive breeding technology for *S. denisonii* have shown that the species is very difficult to handle for artificial propagation [17, 18]. Therefore, attempts were made to use anaesthetics to handle the fish during captive breeding. Using clove oil, handling stress was minimized and *S. denisonii* was bred successfully under hatchery conditions [17, 19]. No literature is available regarding the use of 2-phenoxyethanol to anaesthetise *S. denisonii*. In the present study, we determined the effective concentration of 2-phenoxyethanol that

TABLE 1: Stages of anaesthesia used to determine the efficacy of 2-phenoxyethanol for *Sahyadria denisonii* (modified from Marking and Meyer [3], Trzebiatowski et al. [21], and Summerfelt and Smith [22]).

	Observation
Induction stage	
I	Start of anaesthetic effect, slow swimming, and physiological position
II	Partial loss of body balance, body turning to one side, and decreased locomotor activity
III	Complete loss of body balance, flank position at the bottom of tank, and reaction only to very strong tactile pressure
IV	No response to external touch or stimuli and total loss of equilibrium
Recovery stage	
I	Start of fin movement and fish still lays on the bottom of the tank
II	Regular breathing, nonmoving tilting on flank position, and irregular fin movement
III	Physiological position and increased locomotor activity
IV	Ability to swim normally and regular operculum rate

can be used as an anaesthetic for *S. denisonii* during artificial propagation.

2. Materials and Methods

The experiments were conducted using adult individuals of *S. denisonii* with an average weight of 16.5 ± 3.5 gm (mean $\pm$ SD; $n = 40$). Prior to starting the experiment, fishes were acclimatised in cement tanks (2000 litres) for a period of two weeks. Fishes were fed with a formulated diet consisting of crude protein (38%), crude fat (4.0%), crude fibre (3.0%), ash (16%), and moisture (11%) twice a day (at 09:00 and 17:00). Feeding was suspended 24 hours before the start of the experiment. Water quality parameters were maintained within a narrow range of values, temperature ($27.0 \pm 0.5^\circ\text{C}$), 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) and were checked by using a thermometer for temperature; colour comparator solutions (Nice Chemicals, India) for pH and ammonia; titrimetric method for alkalinity and hardness; and Winkler method for dissolved oxygen using standard procedures [20]. 2-Phenoxyethanol (ethylene glycol monophenyl ether, MERCK-Schuchardt) was used as anaesthetic agent. Dosages of anaesthesia for various teleosts [12] were used as base information and concentrations of 2-phenoxyethanol of 200, 300, 400, 500, and 600 μL^{-1} were selected to induce anaesthesia in *S. denisonii*. Both induction and recovery treatment water were aerated throughout the experimental procedure.

The induction time was considered to be the time period from when an experimental fish is placed in the anaesthetic tank until the time it does not respond to external stimuli. The recovery time is the time period from when an anaesthetized fish is placed in a recovery tank until it recovers from anaesthesia with full equilibrium motion. General fish behavior was assessed for each fish throughout induction and recovery. For practical purposes, four stages of induction and recovery were considered in *S. denisonii* (Table 1). An induction time of 180 seconds or less with complete recovery within 300 seconds suggested by Marking and Meyer [3] and Trzebiatowski et al. [21] was used to establish anaesthesia induction and recovery stages presented in Table 1. According

to L. G. Ross and B. Ross [2], deep anaesthesia (stage IV) is normally used for performing biometric procedures.

The experimental induction tank ($1 \times 1 \times 1$ foot) was filled with water from a similar source to that of the experimental fish holding tanks. Each concentration of 2-phenoxyethanol was initially mixed with a little water in a reagent bottle (50 mL) and then stirred to disperse the chemical to form small droplets before addition to the anaesthetic induction tanks [13]. Each fish was netted carefully with minimum stress and placed in the induction tank. When fish reached stage IV of anaesthesia, it was immediately netted and transferred to recovery tanks, to monitor the recovery stages. The induction and recovery time for each concentration were measured by using an electronic stopwatch (Casio, India). Experiments were repeated eight times to verify the findings with each concentration. The recovered fishes were transferred into the observation tanks (1000 L) for seven days to assess the postrecovery mortality [13]. During the posttreatment period, 50% of the tank water was exchanged daily and the fishes were fed twice a day to apparent satiation with pelleted feed. Mean induction time and recovery time of anaesthesia were compared using regression analysis at 5% level of significance ($P < 0.05$), followed by Tukey's honestly significant difference (HSD) multiple comparison procedure [23]. The results were processed and analysed with SPSS (Windows, Version 15.0).

3. Results and Discussion

In the study, induction and recovery times as well as statistical differences for each anaesthetic concentration in redline torpedo fish are shown in Table 2. The results demonstrate that the induction time of anaesthesia in *S. denisonii* was decreased with increasing concentrations of 2-phenoxyethanol ($P < 0.05$). Comparable results were observed in *Carassius auratus* [9], *Tinca tinca* [24], *Diplodus sargus* and *Diplodus puntazzo* [25], *Solea senegalensis* [12], *Carasobarbus luteus* [26], and *Hippocampus kuda* [13]. 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 an advisable practice [14]. The water quality parameters observed in the present experiment were within the range of our previous studies [17, 18].

TABLE 2: Stages of induction and recovery (seconds) exposed to different concentrations of 2-phenoxyethanol (mean $\pm$ SD).

	2-Phenoxyethanol (μL^{-1})				
	200	300	400	500	600
Stages of induction					
I	199 $\pm$ 11 ^c	35 $\pm$ 6 ^b	26 $\pm$ 3 ^a	25 $\pm$ 4 ^a	24 $\pm$ 4 ^a
II	—	92 $\pm$ 12 ^d	59 $\pm$ 16 ^c	49 $\pm$ 13 ^b	39 $\pm$ 5 ^a
III	—	—	168 $\pm$ 65 ^c	120 $\pm$ 51 ^b	85 $\pm$ 23 ^a
IV	—	—	331 $\pm$ 15 ^c	173 $\pm$ 7 ^b	143 $\pm$ 24 ^a
Stages of recovery					
I	—	—	15 $\pm$ 4 ^c	26 $\pm$ 6 ^b	33 $\pm$ 7 ^a
II	—	—	43 $\pm$ 7 ^b	62 $\pm$ 11 ^a	64 $\pm$ 10 ^a
III	—	—	76 $\pm$ 9 ^c	89 $\pm$ 9 ^b	100 $\pm$ 8 ^a
IV	—	—	95 $\pm$ 10 ^b	141 $\pm$ 12 ^a	145 $\pm$ 14 ^a

Numbers in the same row with the same superscript letter are not significantly different ($P > 0.05$).

The ideal concentration must be the lowest concentration which enables a transition to anaesthesia in 180 seconds and a full recovery in 300 seconds [3, 10]. In our experiments, minimum effective concentration was observed at 500 μL^{-1} of 2-phenoxyethanol (induction and recovery occurred at 173 $\pm$ 7 and 129 $\pm$ 41 seconds, resp.) and therefore this dose was considered as the minimum effective concentration for handling of adult *S. denisonii*. These results are consistent with previous studies using 2-phenoxyethanol in teleost fishes [7, 11–14, 27]. The effective anaesthetic concentration of 2-phenoxyethanol in a number of fish species has been reported ranging between 200 and 600 μL^{-1} [7, 10, 11, 13, 27]. At 500 μL^{-1} of 2-phenoxyethanol, the time to reach stage IV of induction (173 $\pm$ 7 seconds) was significantly different from all other concentrations at 331 $\pm$ 15 seconds in 400 μL^{-1} and 143 $\pm$ 24 seconds in 600 μL^{-1} (Table 2). Tukey's HSD test revealed that the induction time (stage IV) at 500 μL^{-1} was significantly different from that at 400 μL^{-1} (331 $\pm$ 15 seconds, $P = 0.000$) and 600 μL^{-1} (143 $\pm$ 24 seconds, $P = 0.128$). 2-Phenoxyethanol at 200 μL^{-1} resulted in sedation (stage I) while at 300 μL^{-1} it resulted in sedation (stage II) only and concentration from 400 μL^{-1} resulted in progressive anaesthesia. Deep anaesthesia (stage IV) is the stage normally used for performing biometric procedures, induced breeding, and handling procedures [2, 28]; therefore, in *S. denisonii*, the minimum effective concentration of 2-phenoxyethanol which meets the requirements for handling during hatchery management was 500 μL^{-1} .

The recovery time was directly proportional to increasing doses of 2-phenoxyethanol ($P < 0.05$). The longest time for recovery was observed at 600 μL^{-1} (145 $\pm$ 24 seconds), while the shortest time occurred at 400 μL^{-1} (95 $\pm$ 3 seconds) (Table 2). Similar results have been reported in *Oncorhynchus nerka* [29], *Rachycentron canadum* [30], *Hippocampus kuda* [13], *Dawkinsia filamentosa* [31], and *Puntius denisonii* [17, 18]. However, experiments by Mylonas et al. [4] documented recovery times decreasing with increase in anaesthetic concentration of clove oil and 2-phenoxyethanol in *Dicentrarchus labrax* and *Sparus aurata*. Such differences in the respective recovery times might be related to species,

size, physiological status, and environmental conditions [2] as well as temperature, pH, salinity, and oxygen and mineral content of the water [9]. The dynamics of recovery times in anaesthetized fish seems to be more complex; for example, in *Solea senegalensis*, recovery times were weight-dependent for metomidate and dose-dependent for clove oil and MS-222 [12].

According to Pawar et al. [13] and Bambang [32], the recovered fishes should be observed for any abnormal behavior or mortality in posttreatment tanks for seven days. In the present study, recovered *S. denisonii* monitored in posttreatment tanks for seven days exhibited normal feeding and physiological behaviour, without any mortality or abnormal behaviour. In conclusion, the results indicated that 2-phenoxyethanol can be used as an efficient anaesthetic for the handling of *S. denisonii* and the lowest effective concentration to induce anaesthesia is 500 μL^{-1} . This lowest effective concentration induced *S. denisonii* through all stages of anaesthesia, without causing mortality, and may be helpful in developing techniques for transportation, captive breeding, and other ex situ conservation plans for this endemic and endangered barb.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors wish to thank the College of Fisheries, Kerala University of Fisheries and Ocean Studies, Panangad, Ernakulam, India, for providing the necessary facilities to carry out this work. The second author gratefully acknowledges the Government of Kerala, India, for a scholarship during his doctoral study (2010–2013). The authors also thank Hatchery manager (RGCA), MPEDA, Tamil Nadu, for his help in providing anaesthetics used for this study. Thanks are due to anonymous reviewers for their criticisms which improved the quality of the paper.

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