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Adequacy Study of 2-Phenoxyethanol on *Hypselobarbus kurali* as an Anesthetic

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ABSTRACT

Hypselobarbus kurali, one of the most demanded fresh water hill stream fish belonging to the family cyprinidae. In the case of brood *H. kurali*, injuries inflicted to the sudden death of the fish often pose a major problem. When large brood fish are involved, it can lead to incomplete voiding of eggs and mortality in most cases. Anaesthetizing the fish is a practical option for facilitating ease of handling, improved egg yield and breeding response, besides significantly lowering brood mortality rates. In this context, detailed studies were conducted on the use of a commonly employed anaesthetic 2-phenoxyethanol, in the handling and transportation of the fresh water hill stream fish *Hypselobarbus kurali.* Four levels of anaesthetic were tried viz., 300, 400, 500 and 600 μL L^{-1}. The efficacy of the anaesthetic was assessed by considering four stages of induction (I^1, I^2, I^3 and I^4) and three stages of recovery (R^1, R^2 and R^3). In the present study, the lowest induction time (<180 sec) was observed at 500 μL L^{-1} and therefore, this dose was considered as the lowest effective concentration for anaesthesia in *H. kurali.* At 500 μL L^{-1}, the time to reach anaesthesia stage (I3) of induction (159±13 sec) and recovery (R3) time (133±16 sec) was significantly different ($p<0.05$) from the other dosages 400 μL L^{-1} (193±14 sec), 300 μL L^{-1} (216±78 sec) and 600 μL L^{-1} (190±14 sec).

Key words: Anaesthesia, brooders, fish, *Hypselobarbus kurali*, 2-phenoxyethanol, transportation

INTRODUCTION

Popular fresh water hill stream fish *Hypselobarbus kurali,* has of late, been very much in the news, on account of the severe decimation of this once abundant fish. This omnivorous species, locally known as the kooral is endemic to the Western Ghats Rivers of peninsular India. The earlier abundance of this fish has now declined drastically in the rivers of Kerala. In fact, the fish has been listed as vulnerable as per the IUCN protocols, but if the drastic decimation prevails no sooner this will be under endangered category, warranting the priority implementation of conservation measures and stock replenishment programmes on a priority basis.

This approach in turn, involves induced breeding of the *H. kurali*, as well as transportation of brood as well as seed. Handling of live fish invariably stresses the fish leading to injury, loss of mucus and consequent mortality. In the case of brood *H. kurali*, injuries inflicted to the sudden death of the fish often pose a major problem. When large brood fish are involved, it can lead to incomplete voiding of eggs and mortality in most cases. Anaesthetizing the fish is a practical option for facilitating ease of handling, improved egg yield and breeding response, besides significantly

lowering brood mortality rates. In this context, detailed studies were conducted on the use of a commonly employed anaesthetic 2-phenoxyethanol, in the handling and transportation of the fresh water hill stream fish *Hypselobarbus kurali*. 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 μL L^{-1} (Gilderhus and Marking, 1987; Guo *et al*., 1995; Weber *et al*., 2009; Pawar *et al*., 2011). Anaesthetising the fish is a practical option for facilitating ease of handling, improved egg yield and breeding response, besides significantly lowering brood mortality rates.

MATERIALS AND METHODS

Sample collection: *Hypselobarbus kurali* brood stock collected from downstream area of Kallada river system was used for the study. The average weight of brood stock ranged from 200-250 g. The fish collected were maintained in the wet lab and fed to satiation with commercial formulated feed. The fish were starved for 24 h prior to initiation of the trial.

Methods: Water quality parameters viz., temperature, pH, dissolved oxygen, ammonia, hardness and alkalinity were assessed following standard methods (APHA., 1992). The studies were carried out in laboratory conditions. Healthy fish were selected for the study. The anaesthetic 2-phenoxyethanol (Loba Chemie, Mumbai, India) was used for the study. Four levels of anaesthetic were tried viz., 300, 400, 500 and 600 μL L^{-1}. Each level of anaesthetic was measured out into a 50 mL reagent bottle, mixed with 30 mL of water and stirred to disperse the chemical, before adding to the anaesthesia inducing tanks. The anaesthetization trials were conducted in 10 L plastic buckets. Observations of 20 fish were made at each level assessed. The stages of anaesthetization were differentiated as induction, maintenance and recovery (Sajan *et al*., 2012). The efficacy of the anaesthetic was assessed by considering four stages of induction (I^1, I^2, I^3 and I^4) and three stages of recovery (R^1, R^2 and R^3) in *Hypselobarbus kurali*. An induction time of 180 sec or less and complete recovery within 300 sec suggested by Marking and Meyer (1985) was employed to assess the induction and recovery stages in the *H. kurali*. Dosages of anaesthesia adopted for various teleosts (Weber *et al*., 2009) was adopted as the base information and four concentrations of 2-phenoxyethanol (300, 400, 500 and 600 μL L^{-1}) were selected to assess the inducement of anaesthesia in *H. kurali*. Both treatment and recovery water were taken from the holding tank, where the fish were maintained and both the systems were aerated throughout the study duration. When the test fish reached stage three of anaesthesia (I^3), it was immediately transferred to the recovery tanks for recording the recovery stages (R^1, R^2 and R^3). The induction and recovery time for each concentration was measured by using an electronic stopwatch. Experiments were repeated four times to verify the findings. The recovered fish were transferred into the observation tanks (1000 L) and held for 7 days, to assess the post recovery mortality (Pawar *et al*., 2011).

For transportation, a solution of 1 mL of 2-phenoxyethanol in 5 L of water was prepared and used for the transportation trial, which was conducted in heavy duty polybags. The average weight of the brood fish was 200 g. Five numbers of brood fish were accommodated in a bag for the transportation trial. The duration of the transportation was 3 h. 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. The induction and recovery stages of anaesthesia observed in *H. kurali*.

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). Significant difference was tested at 5% level of significance, represented as $p<0.05$. The results were processed and analyzed employing SPSS (Windows, Version 16.0).

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 μL 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 *H. kurali*.

The responses to the same anaesthetic can vary considerably between different species therefore, the characterization of the effective dose of the different anaesthetics in varied species is a rather advisable practice (King *et al*., 2005). The present study revealed that induction time of anaesthesia in *H. kurali* decreased significantly with increasing concentrations of 2-phenoxyethanol ($p<0.05$), which are consistent with previous studies employing 2-phenoxyethanol in *Cyprinus carpio* (Linnaeus, 1758; Josa *et al*., 1992), *Carassius auratus* (Linnaeus, 1758; Weyl *et al*., 1996), *Diplodus sargus*, *Diplodus puntazzo*, *Solea senegalensis* (Weber *et al*., 2009) and *Carasobarbus luteus* (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 *H. kurali* are presented in Table 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 the present study, the lowest induction time (<180 sec) was observed at 500 μL L^{-1} and therefore, this dose was considered as the lowest effective concentration for anaesthesia in *H. kurali*. At 500 μL L^{-1}, the time to reach anaesthesia stage (I^3) of induction (159±13 sec) and recovery (R^3) time (133±16 sec) was significantly different ($p<0.05$) from the other dosages 400 μL L^{-1} (193±14 sec), 300 μL L^{-1} (216±78 sec) and 600 μL L^{-1} (190±14 sec) (Table 1). 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 Tukey's HSD test revealed that the induction time 500 μL L^{-1} was significantly different from 400 μL L^{-1} (193±14 sec, $p = 0.004$) and 600 μL L^{-1} (190±14 sec, $p = 0.347$).

The recovery time was directly proportional with increasing doses of 2-phenoxyethanol ($p<0.05$). The longest recovery time was observed at 600 μL L^{-1} and shortest time to reach the total recovery

Table 1: Induction and recovery time in sec for *H. kurali* anaesthetized with different concentrations of 2-phenoxyethanol

	2-phenoxyethanol (μL L^{-1})			
Stages	300	400	500	600
Stages of induction				
I^1	28±14	24±03	22±04	20±09
I^2	85±20	71±28	64±07	58±06
I^3	216±78	193±14	159±13	190±14
Stages of recovery				
R^1	47±20	50±27	59±03	69±04
R^2	72±23	79±34	92±24	105±06
R^3	110±07	127±22	133±16	182±26

stage was detected at 300 μL L^{-1}. Longer recovery time with increased anaesthetic dosage has been reported in *Hippocampus kuda* Bleeker (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* 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 (25±0.5°C), pH (7.2±0.3), 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}), which are comparable with the observations of Sajan *et al*. (2012). In the present study, recovered *Hypselobarbus kurali* were observed in the post treatment tanks for 7th days and no abnormal behaviour or mortality was observed. The results obtained in the present study, indicated that 2-phenoxyethanol is an effective and safe anaesthetic for the handling and transport of *H. kurali* brood stock. The effective dosage that induces anaesthesia in *H. kurali* is 500 μL L^{-1} as evidenced by the superior survival at the end of the 7 day, post treatment-observation period as also the absence of any aberrant behaviour. The other levels tested recorded mortality/abnormal behaviour of the test fish. In the trials involving transportation the recovery time in the 0.2 mL L^{-1} treatment after the 3 h transportation period, was 63±20 sec against 40±18 sec in the 0.1 mL L^{-1} treatment. A recovery period of more than 1 sec is desirable in the transportation.

CONCLUSION

Further studies are however, warranted on the effects of this anaesthetic on the transportation of seed as well as adults of *H. kurali* in order to standardize the package of practices. The appropriate use of this anaesthetic will definitely be of great help in facilitating easy handling, propagation and stock revival of this endangered indigenous fish.

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