COMPARATIVE STUDY OF 2–PHENOXY ETHANOL AND CLOVE OIL ON ITS EFFICIENCY AS ANESTHETICS IN ANESTHETIZING *Hypselobarbus Kurali*

**DOI**: 10.7904/2068–4738–VI(12)–15

Vishnu Nair M.S., Sherly Williams E.

The Environmental sciences, Aquaculture and Fish biotechnology lab, PG and Research Department of Zoology, Fatima Mata National College, KOLLAM; msvishnunair@gmail.com

**Abstract.** A study was conducted to assess the efficiency of 2–phenoxy ethanol and clove oil as anesthetic to anesthetize *Hypselobarbus kurali* while transportation. It was observed that for induction and recovery experiments 2–phenoxy ethanol showed constant range of peaks at 5 μL L⁻¹, but for clove oil induction had hike at 500 μL L⁻¹ and for recovery it was for 600 μL L⁻¹, which is not a sign of a good anesthetic. Along with this the clove oil purchased at different time had different concentration ranges as it is a natural product and thus the dosage rate will not be stable from one trial to the next.

**Keyword:** 2–phenoxy ethanol, clove oil, anesthesia, *H. kurali*, 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 Ghat 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 least concern 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.

Anaesthetising the fish is a practical option for facilitating ease of handling, improved egg yield and breeding response, besides significantly lowering brood mortality rates [FERENCZ et al., 2012].

In this context, detailed studies were conducted on the use of two commonly employed anaesthetics 2–phenoxyethanol and clove oil, in the handling and transportation of the fresh water hill stream fish *Hypselobarbus kurali* and there by comparing its efficiency for this species.

**Material and methods**

*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 to 250 g. The fish collected were maintained in the wet lab and fed to satiation with commercial formulated feed. The fishes were starved for 24 h prior to initiation of the trial. Water quality parameters viz., temperature, pH, dissolved oxygen, ammonia, hardness, alkalinity were assessed following standard methods [APHA, 1992, BUTU et al., 2014a].

The studies were carried out in laboratory conditions. Healthy fish were selected for the study. The anaesthetic 2–phenoxyethanol (Loba Chemie, Mumbai, India) and clove oil (Arya Vaidhya shala, Kottakkal) was used for the study. Four
level of anaesthetic were tried viz., (300 μL.L⁻¹, 400 μL.L⁻¹, 500 μL.L⁻¹ 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 anaesthetisation trials were conducted in 10 L plastic buckets. Observations of 20 fishes were made at each level assessed.

The stages of anaesthetisation were differentiated as induction, maintenance and recovery [SAJAN et al., 2012].

The efficacy of the anaesthetic was assessed by considering four stages of induction (I1, I2, I3, I4) three stages of recovery (R1, R2, R3) in Hypselobarbus kurali. An induction time of 180 sec or less and complete recovery within 300 sec suggested by [MARKING and MEYER 1965; TRZEBIATOWSKI et al., 1996] 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 μL.L⁻¹, 400 μL.L⁻¹, 500 μL.L⁻¹ 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 fishes reached stage three of anaesthesia (I3), it was immediately transferred to the recovery tanks for recording the recovery stages (R1, R2, and R3). 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 post recovery mortality [PAWAR et al., 2011, RASHED and BUTNARIU, 2014].

For transportation a solution of 1mL of 2–phenoxyethanol and clove oil in 5L of water was prepared and used for the transportation trial, which was conducted in heavy duty poly bags. The average weight of the brood fish was 200 g.

Five numbers of brood fishes 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 are detailed below.

**Induction stage–observations**
- I1–Initial anaesthetic effect; slow swimming, physiological position.
- I2–Partial loss of body balance; body tilting over one side, decreased locomotor activity.
- I3–Complete loss of body balance; flank position at the bottom of tank, no reaction to external touch or stimuli; opercular movement very limited.
- I4–Cessation of opercular movement (over dose and or longer immersion in anaesthetic solution); Subsequent death.

**Recovery stage–observations**
- R1–Initial fin movement; Non–moving tilting on flank position.
- R2–Regular breathing; Physiological position, increased locomotor activity, Irregular fins movement.
- R3–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, BUTU et al., 2014b]. Significant difference was tested at 5% level of significance, represented as $P < 0.05$. The results were processed and analysed employing SPSS (Windows, Version 16.0).

**Results and discussion**

Studies were conducted on the efficiency of 2–phenoxy ethanol and clove oil for four dosages on both induction and recovery separately and had compared both anesthetics so as to conform which one is better anesthetic. Water quality assessments were also done so as to evaluate the most suitable concentration...
and condition for the fishes to thrive in relation to its control. The observations so obtained are explained below: Duncan’s test was done to assess the most suitable concentration for induction and recovery (Figure 1).

Figure 1. Means for groups in homogeneous subsets for induction (2–phenoxy ethanol)

An efficient concentration of anesthetic agent must have lowest subset value of alpha compared to all other concentrations for the induction time and must have a high subset value of alpha compared to all concentrations for the recovery time. As per the Duncan’s test for homogeneous subset it was observed that 500 μL.L⁻¹ of 2–phenoxy ethanol had the lowest value of 160.75 followed by 600 μL.L⁻¹ with a value of 190.00 followed by 400 μL.L⁻¹ and 300 μL.L⁻¹ having 192.00 and 215.25 respectively (Figure 2).

Figure 2. Means for groups in homogeneous subsets for recovery (2–phenoxy ethanol)

For the recovery means for groups in homogeneous subset was observed to be higher for 500 μL.L⁻¹ which was 181.25 followed by 600 μL.L⁻¹ having 133.50 followed by 400 μL.L⁻¹ and 300 μL.L⁻¹ having 126.5 and 110.00 respectively (Figure 3). Thus as per the results obtained the dosage with lowest mean value for groups in homogeneous subsets for induction and highest mean value for groups in homogeneous subset for recovery was 500 μL.L⁻¹ of 2–phenoxy ethanol and this dosage could be considered as a standard dose.
The results obtained by working out Duncan’s test to identify the homogeneous subset for induction of clove oil revealed that the lowest means for groups in homogeneous subsets was that for 500 µL.L⁻¹ which was 191.75 followed by 600 µL.L⁻¹ with 195.50, 400 µL.L⁻¹ with a mean value of 213.00 and 300 µL. L⁻¹ 218.50 (Figure 4).

The recovery means for groups in homogeneous subsets were highest for 600 µL. L⁻¹ 137.50, followed by 126.25 for 400 µL.L⁻¹, 120.25 for 500 µL. L⁻¹ and the least were for 300 µL. L⁻¹ with a value of 109.75. As per the result for induction and recovery clove oil had no correlation with induction and recovery values. Both showed a significance range beyond 0.05.

The reason for this result may be due to the variation in the natural composition and concentration as it’s a natural product. 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 the fish species have been reported to the range between 200–600 µL.L⁻¹ [WEBER et al., 2009; PAWARI et al., 2011; GUO et al., 1995; GILDERHUS and MARKING, 1987, PUTNOKY et al., 2013].

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 [KING et al., 2008], therefore the characterization of the effective dose of the different anaesthetics in varied species is a rather advisable practice.
Conclusions
The results in the variation of dosage for induction and recovery for clove oil compared to 2-phenoxethanol may be due to its change in concentration of natural contents like eugenol. The concentration varies once it is exposed to air so the same bottle from which we conducted the first trial will also give a different result for the next exposure. But it is to be specified that both 2-phenoxethanol and clove oil are potent anaesthetics.

References

Received: May 20, 2015
Article in Press: October 26, 2015
Accepted: Last modified on: November 20, 2015