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Use of an eco-friendly anaesthetic in the handling of *Puntius denisonii* (Day, 1865) - an endemic ornamental barb of the Western Ghats of India

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ABSTRACT

Anaesthesia is essential to minimise stress and physical damage while handling live fish. Present study evaluated the efficacy of clove oil as an anaesthetic (at four different concentrations *viz*., 20 mg l^{-1}, 30 mg l^{-1}, 40 mg l^{-1} and 50 mg l^{-1}) for handling the endemic ornamental barb *Puntius denisonii*. The onset of individual phases of anaesthesia and recovery rates were studied. Induction time decreased with increasing concentration. Concentration between 30-40 mg l^{-1} of clove oil was found to be the most effective dose for *P. denisonii* and 30 mg l^{-1} was recorded as the lowest concentration that induces anaesthesia in less than 3 min. Clove oil appears to be highly effective as a fish anaesthetic with potentially no side effects, which is safe for both fish as well as human and also less expensive.

Keywords: Anaesthetic, Clove oil, Eugenol, Miss Kerala, *Puntius denisonii*

Introduction

The use of anaesthetics in fisheries and aquaculture research greatly facilitates many procedures including induction of spawning, body weight-length measurement, conducting gonadal biopsies and in transport of live fish. Anaesthesia and sedation is usually essential to minimise stress and physical damage in handling the fish for routine operations (Summerfelt and Smith, 1990; Iwama *et al.*, 1997; Ross *et al.*, 1999). The use of anaesthetics in fish has spanned more than the last five decades and many chemicals (MS-222, benzocaine, quinaldine, chlorobutanol, phenoxyethanol, metomidate *etc*.) have been employed in marine fish. When choosing an anaesthetic, a number of considerations are important such as its efficacy, cost, availability, ease of use and side effects on fish, humans and the environment (Marking and Meyer, 1985). Overdosing 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). The fading of ventilation is an important warning sign suggesting that the exposure should be terminated (Hajek and Klyszejko, 2004; Dziaman *et al.,* 2005).

Clove oil has been used for centuries as a topical anaesthetic for human use as a food additive and a dentistry anaesthetic. Though clove oil has been used as a fish anaesthetic for at least the last quarter-century (Endo *et al.*, 1972; Hikasa *et a.l,* 1986; Hamackova *et al.,* 2001), it has recently been the focus of research aimed at establishing its effectiveness and safety for use by both aquaculturists and fish researchers (Brozova and Sovobodova, 1986; Brown *et al.*, 1988; Soto and Burhanuddin, 1995; Anderson *et al.,* 1997; Keene *et al.,* 1998). The active ingredients are phenol derivatives, essentially the $C_{10}H_{12}O_2$ eugenol compound (Taylor and Roberts, 1999). It has been reported to be effective on several species of fish (Soto and Burhanuddin, 1995; Anderson *et al.*, 1997; Munday and Wilson, 1997; Keene *et al.*, 1998; Peake, 1998; Wagner *et al.*, 2002; Iversen *et al.,* 2003). Its main advantages lie in its low cost and its relative safety to both fish and humans (Keene *et al.*, 1998).

Puntius denisonii (Osteichtheys: Cyprinidae), a much sought after ornamental fish in the global trade is endemic to the Western Ghats of India. It is exported from India through wild collection. The fish is endangered due to its indiscriminate exploitation from the wild. Moreover, for a steady and sustainable supply, it is essential to breed the fish under captive conditions, which is considered as one of the desirable qualities for any ornamental fish. Although this species is well adapted to captivity, it is very sensitive to handling and transportation, frequently experiencing high mortality after transport. So clove oil was tested as an anaesthetic to handle the fish for breeding in captivity. By anaesthetising the fish during handling, stress could be minimised and *P. denisonii* was bred successfully in captive conditions (Anna Mercy *et al.*, 2010). The present study was undertaken aimed at determining the effective concentration of clove oil that can be used for short-term induction of anaesthesia in *P. denisonii* before artificial spawning and whenever the fish is handled out of water.

Materials and methods

Preliminary studies were conducted to evaluate the effect of clove oil on the behavior and performance on *P. denisonii*. Based on these studies, different concentrations were selected for the present experiment. Experiments were carried out using two year old *P. denisonii* with an average weight of 13 ± 2.5 g (Fig. 1). The fishes were collected from the wild and acclimatised to captive conditions for two weeks in cement tanks holding 2000 l of water.

Fig. 1. Two year old *P. denisonii*

Clove oil having the active ingredient euginol (1g ml^{-1}), procured from the Amar products, Pune was used for the experiment. Clove oil does not readily mix with water and hence was diluted with ethanol (95%) in the ratio of 1:9 (clove oil: ethanol) to prepare the stock solution containing 100 mg ml^{-1} (Harms, 2003). Different concentrations of clove oil *viz*., 20 mg l^{-1}, 30 mg l^{-1}, 40 mg l^{-1} and 50 mg l^{-1} were prepared by adding required quantity of the stock solution to the water in the experiment tanks, 5 min. prior to the introduction of fish. Aeration was provided in the tanks throughout the experimental period and water quality parameters recorded are shown in Table 1.

Table 1. Water quality parameters recorded in the experimental tanks

Parameters	Range
pH	6.5 - 7.0
Alkalinity (mg l^{-1})	60 - 80
Hardness (mg l^{-1})	65 - 75
Nitrite (mg l^{-1})	< 0.01
Dissolved O_2 (mg l^{-1})	5 - 6
Temperature (^{0}C)	27
Total ammonia (mg l^{-1})	< 0.01

During the experiment, the guidelines recommended by Hicks (1989) were followed such as: feeding was stopped 24 h before the experiment, proper aeration was provided in anaesthetic baths, same temperature was maintained in bath as well as in holding tanks and recovery bath was thoroughly aerated.

During the experiment, one fish each was netted from the rearing tank and placed in the experimental tank (3 l capacity) in the anaesthetic solution. The induction time for fish to reach anaesthetic stage IV was recorded with a stop watch. When a fish become anaesthetised, it was immediately taken out, weighed and then transferred to a recovery tank (40×30×10 cm) with fresh aerated water. Each fish was used only once and then monitored for any adverse effect for another 24 h. Each trial was repeated eight times with different individuals for each concentration. The time taken for induction and recovery were recorded for each dose of clove oil.

The stages of induction were assessed following the method of Summerfelt and Smith (1990) (Table 2). Analysis of Covariance was used to compare the significant difference between different concentrations.

Table 2. Stages of induction of anaesthesia in fish (Summerfelt and Smith, 1990)

Stages of induction	Description	Behaviour response of fish
0	Normal reaction to external stimuli	Opercular rate and muscle tone normal
I	Light sedation, slight loss of reactivity to external stimuli	Opercular rate slightly decreased: equilibrium normal
II	Deep sedation, total loss of reactivity to all but strong external stimuli	Slight decrease in opercular rate; equilibrium normal
III	Partial loss of equilibrium	Partial loss of muscle tone; erratic swimming; increased opercular rate: reactivity only to strong tactile and vibration stimuli
IV	Total loss of equilibrium	Total loss of muscle tone and equilibrium; slow but regular opercular rate; loss of spinal reflexes
V	Medullary collapse	Respiratory movement ceases

Results and discussion

The clove oil administered at concentrations ranging from 20 to 50 mg l^{-1} resulted in progressive anaesthesia. The induction time is the period from the time when an experimental fish is placed in the anaesthetic tank, to the time it does not respond to external stimuli and the recovery time is the period from the time when an anaesthetised fish is placed in a recovery tank, to the time it recovers from anaesthetisation with full equilibrium motion. Initial recovery took a few seconds to minutes, depending on the concentration of anaesthetic administered.

The lowest effective concentration is the concentration that produces general anaesthesia (Stage IV of anaesthesia) within 3 min and allows the recovery within 10 min (Gilderhus, 1990; Weyl *et al.*, 1996). Stages of anaesthetisation includes induction, maintenance and recovery. The stage achieved usually depends on the dose and the length of exposure. The induction time recorded for *P. denisonii* exposed to various concentrations of clove oil are shown in Fig. 2.

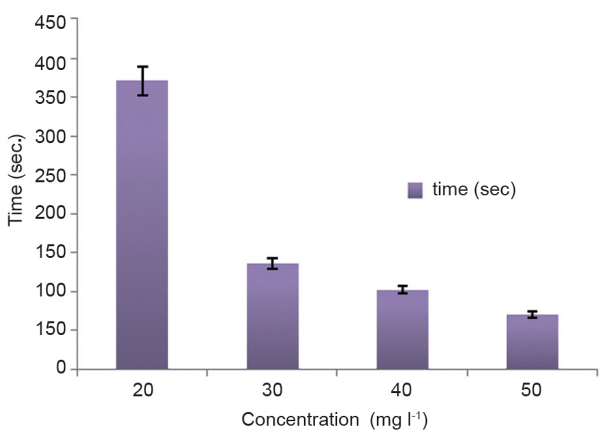

Fig. 2. Average induction time recorded in *P. denisonii* at various concentrations (mg l^{-1}) of clove oil

According to these results, the induction time was less than 3 min at 30 mg l^{-1} and the most effective concentration of clove oil in the induction of anaesthesia for *P. denisonii* appeared to be 30 mg l^{-1}. Time taken to reach different stages of anaesthesia was also recorded. At 30 mg l^{-1}, clove oil induced anaesthesia within 31 sec and the time to reach a complete anaesthesia state (135 secs) was significantly different ($p<0.05$) from the other dosages (20, 40 and 50 mg l^{-1}) (Table 3).

All the fishes used in the experiment recovered within 5 min. Recovery time increased with longer exposure time. At higher concentrations, the time taken to reach stage IV was decreased, but more recovery time was required. At lower concentrations (20 mg l^{-1}) it took more time, 370 sec to reach stage IV and 289 sec for its recovery. The concentration of 10 mg l^{-1} caused equilibrium disturbances in all fish. The increase in the concentration resulted in the shortening of induction time. The significant differences in the recovery times were independent of the anaesthetic solution. No mortalities were recorded within 10 days post-experiment. The study on induction time in terms of fish weight was conducted on 30 fishes weighing 13 ± 2.5 g. A correlation test indicated that there was no significance between induction times and weight of anaesthetised fish.

The definition of efficacy with anaesthetics is more or less subjective (Gilderhus and Marking, 1987). In fact, under different considerations, criteria for an ideal anaesthetic may vary. Generally, an ideal anaesthetic should produce anaesthesia rapidly (*e.g.*, less than 3 or 5 min), allow a speedy recovery, not be toxic to fish and users, leave low tissue residues, and be inexpensive (Marking and Meyer, 1985; Gilderhus and Marking, 1987). In this study, the effective concentration was taken as the minimum concentration that produced anaesthesia within 3 min and allowed recovery within 5 min.

According to Taylor and Roberts (1999) clove oil is an efficient and relatively safe anaesthetic. Munday and Wilson (1997) and Keene *et al.* (1998) found clove oil to be on the whole a superior anaesthetic compared to a number of other chemicals, including MS-222, quinaldine and benzocaine. Clove oil has a slightly faster induction time and a longer recovery time than similar concentrations of TMS (Anderson *et al.*, 1997; Keene *et al.*, 1998). Clove oil is readily available and is less expensive. It is a medicine for direct external use, and is not harmful to human at the concentrations used here.

Table 3. Timing (sec) of anaesthesia and recovery phases in *P. denisonii* exposed to various clove oil concentrations

Stages	Concentrations (mg l^{-1}) (n = 30)			
	20	30	40	50
Light sedation (I)	39.0	31.5	26.21	23.01
Deep sedation (II)	174	58.1	51.24	31.10
Partial loss of equilibrium (III)	264	104	75.2	51.20
Total loss of equilibrium (IV)	370	135	102	70.0
Complete cecovery	289	195	230	150

In the present study, it has been observed that when the exposure time was prolonged, the recovery also became extended. Similar observations were made by Inoue *et al.* (2003) in juveniles of *Matrinxa* and Grzegorz *et al.* (2006) in common carp (*Cyprinus carpio* L.). A solution of 10 mg ml^{-1} of clove oil was found effective in *Valamugil cunnesius* and *Monodactylus argenteus* (Patrick Durville and Adeline Collet, 2001). A concentration of 10 mg l^{-1} caused fish to mainly lose their balance, while 15 mg l^{-1} caused fish to loose consciousness. In carp (*Cyprinus carpio*) at 40 to 120 mg l^{-1}, in rainbow trout, *Oncorhynchus mykiss*, doses as low as 2 to 5 mg l^{-1} produced sedation sufficient to transport the fish, while doses of 40 to 60 mg l^{-1} for 3 to 6 min gave effective surgical anesthesia. The results of the present study has clearly shown that the appropriate safe dose of clove oil as an anesthetic for handling *P. denisonii* is 30 mg l^{-1} .

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