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Efficacy of Clove Oil as an Anaesthetic for Two Sizes of *Rutilus frisii kutum*

Susan Javahery, Abdolmajid Haji Moradlu and Rasul Ghorbani

Department of Fishery,
Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Abstract: The object of this study was to evaluate the efficacy of clove oil (*Eugenia caryophyllata*) as an anaesthetic for two size of *Rutilus frisii kutum* with average body of 1.73±0.13 and 15.3±0.5g and establish the minimum concentration producing desirable anaesthetic effects and survival time on those concentrations. Fish were exposed to low concentrations of clove oil varying from 0.044 to 0.4 mg/l with bath immersion under acceptable water quality conditions. Behavioural changes, the time to loss of equilibrium, deep anaesthesia, duration of anaesthesia and also mortality were determined. The behavioural responses to anaesthesia appeared to be the same in both sizes. Induction time decreases with increasing concentrations. The minimum concentration producing desirable anaesthetic effects was 0.044 mg/l for both sizes. In addition, the time to loss of equilibrium, induction time and duration of anaesthesia were increased with increasing body size. No mortality was observed. These findings suggested that clove oil could be use as an effective anaesthetic for longer exposure time in size of 2-13 g for *Rutilus frisii kutum.*

Key words: *Rutilus frisii kutum* · Clove Oil · Anaesthesia

INTRODUCTION

Fish are anesthetized in aquaculture and research to enable handling or surgery. In order to minimize stress and reduce the chance of fish mass mortality [1] from long periods of transportation and handling, an effective high density transporting method is essential to reduce expenses and avoid losses [1, 2].

Several anesthetic agents have been used in fish [3, 4]. An ideal anesthetic for fish should induce anesthesia in less than 3 to 5 min., with total loss of balance and muscle tone, allowing an uneventful and rapid (i.e. less than 10 min) recovery with low tissue residues after recovery, thus being safe to users and consumers. The anesthetic should be inexpensive and easy to use [3-8].

The dosage required to induce general anesthesia varies according to the anesthetic used and other factors such as water temperature, hardness, salinity, oxygen concentration, length of exposure, body weight, the ratio of gill area/body surface area and the species of fish. In general, small fish are more sensitive to anesthesia than larger fish [6].

Clove oil is a natural product that obtained by distillation of the flowers, stems and leaves of the clove tree *Syzygium aromaticum* (i.e. *Eugenia aromaticum* or *Eugenia caryophyllata).* It is a dark brown liquid that has been used as a mild topical anesthetic since antiquity and has been used to help with toothache, headaches and joint pains. Clove oil has a long history as a local anesthetic for humans [9]. Clove oil is actually a mixture of different compounds. The three significant active ingredients are eugenol, isoeugenol and methyleugenol. Clove oil is 85 to 95% eugenol. Isoeugenol and methyleugenol make up 5 to 15% of the remaining ingredients [10].

In fact, clove oil is used for local application to reduce pain and promote healing and exhibits antimicrobial, antioxidant, antifungal and antiviral properties [11]. Caspian Rutilus, *Rutilus frissi kutum* kamenskii 1901, is an endemic fish of Caspian Sea and its populations generally recorded along near the coast, from the Trek River the north to the southern part. It consists more than 70% of fishermen catch in coastal of the Caspian Sea [12].

The objective of this study was to examine the efficacy of low concentrations of clove oil as a calming agent for long transportation and handling using *Rutilus frissi kutum* as a model in two sizes.

Corresponding Author: Susan Javahery, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

MATERIALS AND METHODS

The experiments were undertaken in December 2011, using produced at the Institute of Agriculture Science and Natural Resources, Gorgan, Iran. *Rutilus frissi kutum* (1.73±0.33 and 15.3±0.5g) were used in the study. Prior to the study, fish were maintained in groups in 450-L tanks in an indoor facility; fish had been maintained in this facility for 3 month. Environmental conditions were monitored and maintained within a narrow range of values. A 12-hour-light: 12-hour-dark cycle was maintained. Fish were fed a pelleted diet formulated for once daily. All fish were healthy prior to and throughout the study. Fish fasted and without human contact or disturbance for 24 h before the start of the study. The average water temperature and oxygen level during the experimental period were 23 (±0.2)°C and 12.1 (±0.8) mg /l, respectively. Temperature and dissolved oxygen were monitored daily and water renewal was 25% per a day.

We examined 0.44, 0.1, 0.2, 0.4, mg/l of clove oil for two sizes. Clove oil is poorly soluble in cold water and therefore was initially dissolved in 94% ethanol (ratio of clove oil: ethanol, 1:9). To examine the effect of ethanol exposure, a group of 10 fish of each species were transferred by net into a 20-l bucket with 4 mg/l of ethanol and were observed for 15 min. The end point monitored in the anesthesia experiments were modified from earlier studies [4, 13] (Table 1) and were (a) complete loss of equilibrium (stage A3) and (b) complete loss of responsiveness to tactile stimuli (stage A5).

Behavioural changes, the time to loss of equilibrium (A3), deep anaesthesia (A5) and the duration of anaesthesia in each concentration were recorded to the nearest second using an electronic stop-watch and also mortality were determined for 5 days after anaesthesia.

Statistical analysis of data was done by the main factorial model (General leaner model), by Univariate Analysis of variance using SPSS 16. Statistically significance was set at the level of $p \leq 0.05$ with ± standard deviation (SD).

Table 1: Stages of anesthesia employed as endpoints in the present study [4, 13]

Stage	Description	Notable behavior
Anesthesia		
A3	Loss of equilibrium	Total loss of equilibrium, pectoral fins moving, regular opercular ventilation
A5	Deep anesthesia	No movement, loss of responsiveness to tactile stimuli, slow and irregular opercular ventilation

RESULTS

All fish used in the present study were healthy as was indicated by their activity and exterior appearance. No mortality was observed during the acclimatization period furthermore, no deaths or other adverse. Fishes continued to swim after losing equilibrium and for recovery, they first regained equilibrium and then begun swimming slowly.

Exposure of fries to ethanol (i.e., the anesthetic solvent) did not induce anesthesia, or any apparent modifications in the behavior of the fish. Opercular ventilation rate also was not affected (data not shown), suggesting that the concentration of ethanol used had no, or negligible effect on the fish within the exposure time used in the study.

Stages of anesthesia with different concentrations of clove oil in different sizes of *Rutilus frisii kutum* is presented in Figure 1. The induction period was shorter at higher concentrations and longer at lower concentrations of clove oil in smaller and larger size groups of *Rutilus frisii kutum*. Induction time increased with increasing body size.

DISCUSSION

An ideal anesthetic for fish should induce anesthesia in less than 3 to 5 min, with total loss of equilibrium and muscle tone, should allow an uneventful and rapid (i.e. less than 10 min) recovery, should leave low tissue residues and be safe to users as well as be inexpensive and easy to use [3, 5].

Clove oil are highly lipophilic, therefore they adhere on to and penetrate rapidly the gill epithelium and are absorbed by body tissues, such as the fat and brain [4] once in the blood circulation. The shorter the exposure time to the anesthetic bath the smaller the amount of anesthetic absorbed by the body.

The behavioral response to anesthesia appeared to be the same between the two species. They continued to swim after losing equilibrium, laid quiet on the bottom of the anesthetic tank. With increasing doses a decreasing time to stage A3 and A5 anesthesia is obtained (Fig. 1). Fish from all doses recovered well after the anesthetic experiment and were feeding and behaving normally within 1 day. No further mortality was observed in the following 5 days and no decreases in mean body weight were observed. Therefore, smaller size of *Rutilus frisii kutum* needs lower concentration in comparison with larger fish. Also resistance of anesthesia increases with increasing body size.

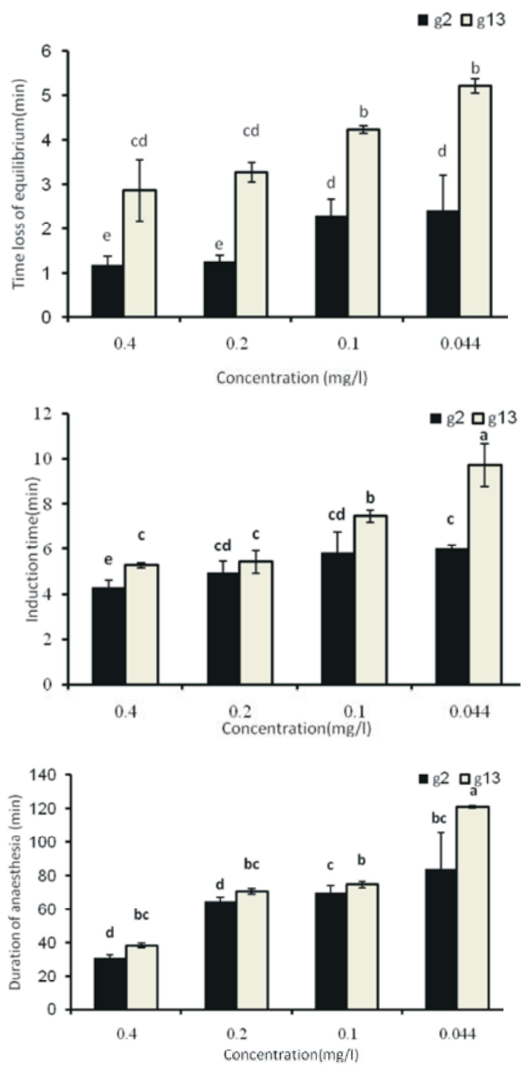

Fig. 1: Mean (±SD) time to loss of equilibrium (stage A3), induction time to anesthesia (stage A5) and duration time of *Rutilus frisii kutum* (n =10) exposed to different doses of clove oil. Data in the different small letter in the error bars are significantly different from each other (P ≤0.05)

Mattson and Riple [14] experienced that more than 90% of the fish lost reflexes and response to external stimulation within 3 min., which were the defined criteria for an effective anesthetic concentration.

The tendency seen here for clove oil was an increasing induction time with increasing weight. This indicates that the rate of absorption of the anesthetic in relation to weight was slower in larger fish and may be a reflection of the smaller gill surface area in relation to body weight in larger fish [15] and thus a smaller area for drug diffusion relative to size. A lower basal metabolism in large fish and thereby a lower oxygen consumption, in relation to body size [16] may also contribute to a slower rate of absorption. It has been shown that *Rutilus frisii kutum* of small size consume oxygen at a greater rate per unit weight than *Rutilus frisii kutum* of large size [17].

The reports regarding the importance of body size for the effect of anesthetics are contradictory. Increased efficacy and sensitivity with increasing body size have been observed in Atlantic salmon anesthetized with metomidate [18] in goldfish (*Carassius auratus*), rainbow trout and brook trout (*Salvelinus fontinalis*) anesthetised with MS-222 [19] and in rainbow trout anesthetized with quinaldine and 2-phenoxyethanol [20]. In these studies larger individuals required more dosages or experienced longer induction and recovery times. The opposite has however also been observed. Furthermore, some studies show that size has no uniform influence on the effect of anesthetics, whereas others reveal variations in relation to size in some, but not all species examined [21, 22, 7].

In red sea bream (*Pagrus major*) there was a positive linear relationship between body size and the dose required to induce a given stage of anesthesia within the same period of time [15]. Similarly, in wild migrating sockeye salmon [9] and whitefish (*Coregonus lavarotus*) [21] there was a clear correlation between induction time to anesthesia and body size, but the opposite was true for rainbow trout [21]. On the other hand, in goldfish (*C. auratus*) [23] long finned eel [24], brown trout (*Salmo trutta*) and Atlantic salmon [21] there was no correlation between size and induction time to anesthesia.

In conclusion, the obtained results from the present study demonstrate that clove oil can be used as an effective and efficient agent for anesthesia in the aquaculture of *Rutilus frisii kutum* in both sizes. In addition, studies should be carried out in order to determine both the short-term effects of anesthetic exposure to the physiology of species [25, 26, 27], as well as the long-term chronic effects of anesthesia.

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