

The Effectiveness of Clove Oil as an Anaesthetic on Adult Common Carp, *Cyprinus carpio* L.

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Abstract: Common carp, *Cyprinus carpio* L. is a commercial aquaculture species and also a pest fish that can destroy local aquatic communities. An effective anaesthetic method has been developed to meet both aquacultural and ecological studies of the fish. Clove oil is a cheap and safe anaesthetic for fish and humans. However, there are few reports on its efficiency on adult carps. In this study, using carp samples (average wet weight = 1.25 kg), effective concentrations of clove oil was determined by to be 50 mg L⁻¹. The induction time taken to reach each of anaesthetic stages appeared to be inversely proportional to clove oil concentration.

Key words: Anaesthesia, clove oil, common carp, effective minimum concentration, induction time, recovery time

INTRODUCTION

Cyprinid fishes are the predominant group in inland fisheries (FAO, 2008) and especially common carp, *Cyprinus carpio* (Linnaeus, 1758) is consumed as an important protein source all over the world. Moreover, carp is intensively raised as ornamental fish in many countries worldwide. However, at the same time, carp is regarded as an invasive species i.e., destroying native aquatic communities (Miller and Crowl, 2006). Therefore, there is an increasing demand for clarifying its physiology and ecology in order to improve aquaculture and manage invasive populations in the wild. For promoting these studies, adequate anaesthetic methods are required for after morphometry taking blood samples, transporting or surgery for tag implantation.

Clove oil is a distillate of flowers, stalks and leaves of the clove tree (Soto and Burhanuddin, 1995; *Syzygium aromaticum* (Linnaeus, 1753) Merr. and Perry, 1939, syn. *Eugenia aromaticum* or *Eugenia caryophyllata*). The active ingredient eugenol is approved as direct food substance Generally Recognized As Safe (GRAS) and its isomer isoeugenol is approved as a food additive in the USA (Roubach *et al.*, 2005). Clove oil has been used as an anaesthetic in a large number of ichthyological studies because it does not have persistent or latent negative effect upon fish physiology or behaviour. Moreover, it costs extremely lower than the most commonly used

anaesthetic MS-222 due to its relatively low dosages required (Keene *et al.*, 1998). Despite its usefulness, clove oil is not approved yet as a anaesthetic for use in aquaculture (FDA, 2007) and accumulation of additional published data on the anaesthetic effects of clove oil is required (Kennedy *et al.*, 2007). To the best of the knowledge, there are few studies on the anaesthetic effect of clove oil on adult carp (Hikasa *et al.*, 1986; Velišek *et al.*, 2005). In the present study, the relationship between clove oil concentration and anaesthetic induction time for adult carp was examined in order to determine the effective minimum concentration.

MATERIALS AND METHODS

Anaesthetic experiments were performed on 14 March 2008 in the Center for Ecological Research, Kyoto University, Otsu, Japan. Experimental fish (n = 10) were purchased on the experimental date from a local fish dealer and transferred to a circular holding tank (1400 L). These fish had been kept in an outdoor pond for a few weeks before purchase. Ten carps in total were used for the whole experiments without identifying their sex. The body wet weight was 1.25±0.16 kg (average weight±SD) and standard length was 34.8±1.6 cm (average standard length±SD). Clove oil was obtained from Nacalai Tesque, Inc., Kyoto, Japan (product code 25604-85).

A variety of clove oil concentrations (20, 30, 40, 50, 60, 80, 100, 120, 150 and 200 mg L⁻¹) were applied to the anaesthetic tank. The highest concentration was determined by reference to Hajek *et al.* (2006). Owing to its low water solubility, the clove oil was strongly shaken with a small amount of water in a glass bottle (<10 mL) to make emulsion of small oil droplets and then poured into the anaesthetic tank (55 L). The clove oil solution in the anaesthetic tank was stirred well using a wooden rod. An electric submersible pump was used to maintain the homogeneity of clove oil in solution throughout the experiment. A recovery tank (100 L) was prepared for observing the recovery process. Tap water, dechlorinated with Tetra Contra Chlorine (Spectrum Brands, Atlanta, USA) was used for the two tanks and as well as for the holding tank and temperature was not controlled. Water temperature was measured every 30 min. During the course of the experiment (6.5 h in total), water temperature was 13.3±0.7, 13.4±0.6 and 13.2±0.5°C (average±SD) in the anaesthetic tank, recovery tank and holding tank, respectively.

The onset of induction stages was examined in conformity with the following classification of Hajek *et al.* (2006):

Stage 1: Sedation-reduced mobility.

Stage 2: Partial loss of equilibrium lateral (side) inclination.

Stage 3: Total loss of equilibrium, almost horizontal position of the fish on the bottom of the tank; weak body movements; removal from the water evoking agitation.

Stage 4: General anaesthesia-no body movement except for regular ventilation, no reaction to being caught and removed from the water.

Stage 5: Medullary collapse-respiratory movement ceases. Though there are five stages in the classification, three stages (from stage 2-4) were adopted for the present study due to their higher simplicity for observation than stage 1. Stage 5 was also excluded because of its danger to make samples die when fish anaesthetised to reach the stage 5. The time required to complete each stage was recorded to an accuracy of one second. Immediately after reaching induction stage 4, the fish were transferred to an aerated recovery tank and the time required for recovery was measured at an accuracy of 1 sec. Two recovery stages 1 and 2 were defined as full recovery of equilibrium

and first burst of tail fin to swim forward, respectively. Carp were introduced individually into the anaesthetic tank using a hand net and time measurement was then started.

To avoid escape and reduce the stress on the carp, the anaesthetic tank had been covered with a blackout sheet until carp stopped strenuous movement. The observation was continued for 30 min in total however, if the fish did not reach any of three stages of induction within 10 min, the concentration was not considered effective for the stage and hence excluded from further data analysis. Induction time to reach general anaesthesia of ideal anaesthetic is <15 min and preferably <3 min (Marking and Meyer, 1985) however, in the present study, some margin was added to the 3 min in reference to Hikasa *et al.* (1986) reporting much longer induction time required for adult carp.

An idealized model that governs anaesthetic induction processes could be given by:

$$D = CvT$$

Where:

- D = The quantity of clove oil absorbed into the carp (mg) to reach each of the three induction stages in the time interval T in seconds
C = The clove oil concentration in the anaesthetic tank (mg L⁻¹)
v = The flow rate of water irrigating the carp gills (L sec⁻¹)

If $k = D/v$, a hyperbolic relationship between T and C i.e., $T = k/C$ is obtained. Using the data obtained in anaesthetic experiments k was determined by the curve-fitting method. All statistical analyses were performed on statistical software package R (ver. 2.7.0, The R Foundation for Statistical Computing).

RESULTS AND DISCUSSION

Carp reached induction stages 2 and 3 at 20 mg L⁻¹ in 735 and 1289 sec (>10 min), respectively. Within 10 min, 30 mg L⁻¹ was the minimum effective concentration for the two stages among the experimental settings. The minimum effective concentration of clove oil at which carp reached induction stage 4 was 50 mg L⁻¹ within 10 min (and also within 30 min).

In all induction stages, there were significant correlations between C and T ($p < 0.05$, \log_{10} Clove oil concentration vs. \log_{10} Induction time; Fig. 1). Values of k were 5.207±0.396, 8.724±0.536 and 25.532±2.202×10³ mg/L/sec (estimated k±SE) in induction stages 2-4, respectively. All carp (at concentrations of 50 mg L⁻¹

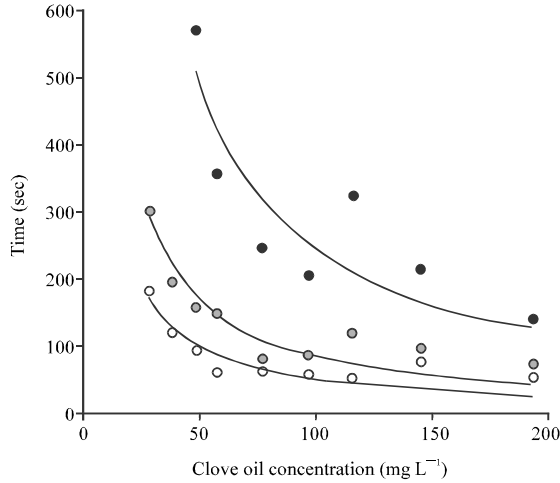


Fig. 1: Relationships between clove oil concentration (mg L^{-1}) and the time required by carp samples to reach the induction stage 2 (○), 3 (●) and 4 (●). Black lines denote the fitted curves (see the main text)

and higher) recovered from stage 4 anaesthesia and reached recovery stages 1 and 2 in 735.9 ± 328.1 and 873.1 ± 370.0 sec (average \pm SD), respectively. No mortality of carp was observed at all concentrations.

The minimum effective concentration of clove oil, 50 mg L^{-1} was consistent with the findings of Hajek *et al.* (2006) for juvenile carp. Keene *et al.* (1998) and Hikasa *et al.* (1986) reported recovery time from clove oil anaesthesia was longer than with MS-222 in case of *Oncorhynchus mykiss* and carp, respectively. The characteristic of clove oil is advantageous when long handling time is required (e.g., surgery). There was no significant correlation between clove oil concentration and recovery time in both recovery stages 1 and 2 ($p > 0.05$, \log_{10} clove oil concentration vs. \log_{10} recovery time) in contrast to that observed by Hikasa *et al.* (1986) who found a positive correlation in adult carp (wet weight, 700-750 g; 10 and 20°C). However, such a correlation was not found for juvenile carp (average wet weight, 32.6 g; 20°C) by Hajek *et al.* (2006). On the other hand, despite the difference in experimental water temperatures and body weight of carp in previous studies (Hikasa *et al.*, 1986; Hajek *et al.*, 2006) and the present study, all three studies suggested a significant correlation between clove oil concentration and induction time. Gill surface area has an allometric relationship with the body weight of carp (Oikawa and Itazawa, 1985) and hence the absorption and releasing rates of clove oil through gills per unit body

weight appears to be constant and independent of body weight. Moreover, physicochemical passage of the anaesthetics into the fish is temperature dependent. Based on the both information not only induction time but also recovery time should depend on anaesthetic concentration but the present results are contradicting to the expectation. Investigation of factors which cause hysteresis effect on metabolic rate during induction and recovery of fish would clarify the inconsistency of the results among previous studies on carp.

The minimum effective concentration of 50 mg L^{-1} was applied for the radio transmitter implantation surgery of carp ($n = 9$; 1.4-5.3 kg wet weight and 38.8-61 cm standard length). All samples were successfully induced to induction stage 4 and recovered even though one fish was kept in this stage for >13 min during the implantation surgery.

The present study provides information on examples of the application of clove oil on large adults of carp and it would be beneficial for future studies, especially for ecological field studies in which carps in a wide variety of body sizes have to be examined.

CONCLUSION

As a result, 50 mg L^{-1} was found to be a practical, effective minimum concentration of clove oil for large adults of carp. Moreover, the effectiveness and safeness of our anaesthetic scheme were confirmed in various sizes of carp (1.4-5.3 kg) as a part of radio-tag implantation study.

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