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# ANESTHETIC EFFECTS OF CLOVE OIL DURING HANDLING AND TRANSPORTATION OF THE FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII* (DE MAN)

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**Abstract**

The present work was conducted to determine the effects of clove oil as an anesthetic on postlarvae (mean length 13.42±0.84 mm) and juveniles (43.57±0.94 mm) of the giant freshwater prawn, *Macrobrachium rosenbergii*. In the first of three experiments, exposure to ethanol for 15 min was ineffective in tranquilizing the freshwater prawns at concentrations of 75-625 mg/l for postlarvae and 375-5,000 mg/l for juveniles, indicating that ethanol could be used as a solvent for clove oil without contributing to any anesthetic reaction. In the second experiment, prawns were exposed to one of six clove oil concentrations for 15 min (15-125 mg/l for postlarvae; 75-1,000 mg/l for juveniles). For postlarvae, the fastest induction (0.90±0.05 min) was achieved at the highest concentration (125 mg/l) but all prawns in this treatment died. Induction and recovery times in concentrations of 30, 45, and 60 mg/l were all under 15 min. At 75 mg/l, postlarvae were tranquilized in a relatively short period (3.30±0.10 min) and recovery time was maximum (45.17±0.20 min). For juvenile prawns, concentrations of 750 mg/l and up were fatal while 125 and 250 mg/l induced anesthesia in 78.30±0.20 and 66.83±0.18 min, respectively, with recovery in 4.21±0.12 and 16.91±0.16 min. As far as induction and recovery times are concerned, clove oil was a suitable anesthetic only for postlarvae. The third experiment revealed that a concentration of 15 mg/l could safely be used to transport postlarvae up to three hours.

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## Introduction

Anesthetics are used in aquaculture research for handling fishes and shellfishes during weighing, measuring, marking and tagging, breeding, collection of tissue samples, etc. Anesthetics are also used during transportation of broodstock and seed from hatcheries to grow-out systems. The choice of anesthetic generally depends on availability, cost effectiveness, ease of use, nature of the study, and user safety (Cho and Heath, 2000).

Freshwater prawns (*Macrobrachium rosenbergii*) are commercially important and reared in commercial farms and research centers. Handling of prawns during experiments can involve substantial physical activity such as struggling during capture, fuming, or chafing, and cause death of the prawns. Prawns can be anesthetized to handle them safely during transportation and experimental procedures such as measuring and tagging. Also, as prawns are cannibalistic by nature, a proper and safe anesthetic is essential to prevent cannibalism during transport.

Numerous methods of temporarily paralyzing and even killing crustaceans have been documented. Some methods are slow or inconsistent and appear to cause trauma (Brown et al., 1996). Some anesthetics conventionally used for aquatic vertebrates, such as MS-222 (Sandoz), are ineffective when used by the immersion method for decapods (Oswald, 1977). However, Gardner (1997) found that clove oil is an effective anesthetic for paralyzing crabs by bath treatment.

Clove oil is a low-cost, ready-made, natural, safe, and most important anesthetic oil (Keene et al., 1998). The aim of the present investigation was to evaluate the suitability of clove oil as an anesthetic during handling and transport of postlarvae and juveniles of the freshwater prawn, *M. rosenbergii*.

## Materials and Methods

*Source and acclimation of prawns.* Freshwater prawn, *M. rosenbergii*, seed were procured from a freshwater prawn hatchery in Mumbai. Two age groups were used in the experiment: postlarvae (mean length 13.42±0.84 mm) and juvenile (mean length 43.57±0.94 mm). Both groups were acclimatized to laboratory conditions for five weeks before the experiment in 500-l plastic pools supplied with aerated fresh water at 28±0.9°C and under natural light conditions. Commercial prawn feed was given thrice daily at 10:00, 14:00, and 18:00 at 12% of the body weight, divided into three equal volumes.

*Experiment I. Effect of ethanol as an anesthetic*. Clove oil is not completely soluble in water below 15°C. Five to ten volumes of solvent are required to make it water-soluble. Although the present experiment was conducted in a water temperature of 28°C, when we used the clove oil without any solvent, the oil required vigorous shaking and produced an oily layer on the water surface. When we used ethanol as a solvent, we received a completely dissolved milky mixture without an oily layer that was very easy to use for experimental purposes. Therefore, we used ethanol as a solvent in the present experiment, even though the water temperature was 28°C, and conducted a short experiment to determine whether there were any anesthetic effects of the ethanol on the *M. rosenbergii* postlarvae and juveniles.

Prawns of both size groups were exposed for 15 min to various concentrations of ethanol: 75-625 mg/l for postlarvae and 375-5000 mg/l for juvenile prawns. The desired concentrations were obtained by adding ethanol to test beakers containing water. Then prawns were placed into the beakers. Behavioral changes and induction and recovery times were noted. Twenty prawns of each size were individually tested at each concentration. The purpose of this experiment was to make sure that, in subsequent experiments, the clove oil would be the only ingredient acting as an anesthetic on the prawns.

*Experiment II. Effect of clove oil as an anesthetic*. The experiment was conducted in 2-l glass beakers containing 1.5 l aerated fresh water (28±0.5°C). The desired concentration of clove oil was dissolved in ethanol at a ratio of 1:5 to make it water-soluble. The clove oil and ethanol were added to test beakers containing water and thoroughly mixed. One prawn was randomly selected

from the acclimation pool and transferred by net to the beaker. The prawns were exposed for 15 min to the anesthetic bath. The air supply to the anesthetic bath was disconnected immediately before introduction of the prawn so that behavior could be clearly observed during the induction period. Fresh solutions were prepared for each concentration and beakers were thoroughly cleaned and filled with aerated fresh water between trials.

Twenty prawns of each size group were individually tested in each of six concentrations: 15, 30, 45, 60, 75, and 125 mg/l clove oil. The first four concentrations were ineffective for juvenile prawns and the experiment was repeated using higher concentrations: 75,

Table 1. Induction and recovery stages for anesthetized freshwater prawns (*Macrobrachium rosenbergii*).

| | *Stage* | *Description* | *Behavior* |
|---|---|---|---|
| *Induction* | 1 | Normal | Prawns appeared normal but with a slight loss of reactivity. |
| | 2 | Light sedation | Prawns were stationary, remaining in one place. Increased movement of swimming appendages. |
| | 3 | Deep sedation | Prawns became agitated and swam faster, followed by limpness. Very rapid movement of the swimming appendages. |
| | 4 | Stagnation | Prawns were stationary, remaining in one place. Movement of swimming appendages ceased but movement of chelae, walking legs, and antennae were observed. |
| | 5 | Partial loss of equilibrium | Prawns gradually lost control over walking legs and chelae. Prawns remained motionless at bottom as stage 4 but slight movement of antennae was seen. |
| | 6 | Total loss of equilibrium | Walking legs and chelae became tangled and stiff. Prawns became numb. |
| | 7 | Death | Prawns became rigid, color changed to whitish, prawns died. |
| *Recovery* | 1 | Partial revival of equilibrium | Sporadic movement of walking legs, chelae, and antennae. |
| | 2 | Complete revival of equilibrium | Regular movement of chelae, antennae, swimming and walking legs. Limpness observed. |
| | 3 | Normal | Prawn freely swimming. |

125, 250, 500, 750, and 1000 mg/l. The time required for the prawn to reach a total loss of equilibrium was determined using a stopwatch and behavioral changes were recorded. Total induction times were recorded when the prawns reached stage 6 (Table 1). Once stage 6 was deemed to have been reached, the test prawn was removed by hand and placed in a recovery tank filled with fresh aerated water (28±0.5°C). This was considered the start of the recovery period and total recovery was reached when the prawn turned right side up and started moving in the container. Induction and recovery times were judged visually and measured to the nearest second. Induction and recovery times are expressed as means (min±SE). Recovered prawns were reared separately in tanks for one week to observe post-treatment survival.

*Experiment III. Effect of clove oil as an anesthetic during transport*. This experiment was conducted to evaluate the effect of clove oil as an anesthetic during transport of postlarvae. The experiment was conducted in the laboratory of the Taraporevala Marine Biological Research Station in Mumbai. Water temperature during packing was 28±0.5°C. Double polythene bags of 4-l capacity (194 mm diameter, 165 mm high), one slipped inside the other, were used to prevent water loss from perforations or leakage. The bags were filled with one liter aerated fresh water and the desired concentration of clove oil mixed with solvent. Five concentrations were tested (15, 30, 45, 60, and 75 mg/l) and compared with a control containing no clove oil. The concentrations were chosen based on results of experiment II. Acclimatized postlarvae were transferred to each bag at a rate of 100/l. The bags were squeezed above the water surface to expel air, inflated with industrial oxygen gas, and sealed with rubber bands. The bags were maintained at ambient temperature of the laboratory and not moved. Survival was recorded at 1, 3, 6, and 12 hours.

Water samples were analyzed at the start and end of the experiment for total ammonia using a Spectroquant Nova 30 photometer (Merck KgaA, Frankfurter, Darmstadt, Germany), pH using a pH Scan 1WP1 (range 1.0-15.0, accuracy ±0.2; Eutech Instruments Pvt. Ltd., Singapore), and dissolved oxygen by the titration method (APHA, 1985). Temperature was measured with a digital thermometer (-50-200°C range; Superfit, India). Prawns that survived the 12 hours were held in aquaria supplied with fresh water and aeration for five days to compare survival rates among treatments.

*Statistical analysis.* Significant differences among treatments were analyzed using one-way analysis of variance (Snedecor and Cochran, 1967). Scheffe's multiple comparison tests were used to determine differences between treatment means. Results were considered statistically significant when $p<0.05$.

## Results

*Experiment I*: postlarvae exposed to an ethanol concentration of 625 mg/l and juveniles exposed to 5000 mg/l for 15 min exhibited regular behavior. Therefore, it was concluded that ethanol used as a solvent for clove oil has no sedative effect on prawns.

*Experiment II*: mean induction and recovery periods are given in Table 2.

*Experiment III*: behavior during transport is presented in Table 3, survival in Fig. 1, and water parameters at the start and end of this experiment are given in Table 4 and Fig. 2.

## Discussion

Anesthesia of cultured food animals must allow for immediate release of the anesthetized animal into the food chain and swift induction and recovery from anesthesia, without excessive disturbance of the animal's physiological balance. Since clove oil is organic, a withdrawal period is not required for fish or prawns intended for human consumption and no chemical health hazards endanger the user. The cost of clove oil is low, compared to other anesthetics. Therefore, clove oil was tested as an anesthetic for postlarvae and juveniles of the freshwater prawn, *M. rosenbergii*.

In most cases, rapid induction of anesthesia is desirable. A long recovery period is essential when animals must be handled for

Table 2. Experiment II: Induction and recovery of postlarvae and juvenile freshwater prawns (*Macrobrachium rosenbergii*) treated with clove oil for 15 min.

| | *Concentration (mg/l)* | *Induction period (min)* | *Recovery period (min)* |
|---|---|---|---|
| *Postlarvae* | 15 | Ineffective | Ineffective |
| | 30 | 14.00±0.15[a] | 2.83±0.14[a] |
| | 45 | 12.15±0.13[b] | 4.16±0.14[b] |
| | 60 | 9.50±0.12[c] | 14.56±0.18[c] |
| | 75 | 3.30±0.10[d] | 45.17±0.20[d] |
| | 125 | 0.90±0.05[e] | Death |
| *Juveniles* | 75 | Ineffective | Ineffective |
| | 125 | 78.30±0.20[a] | 4.21±0.12[a] |
| | 250 | 66.83±0.18[b] | 16.91±0.16[b] |
| | 500 | 25.58±0.16[c] | 90.41±0.18[c] |
| | 750 | 10.16±0.12[d] | Death |
| | 1000 | 5.16±0.10[e] | Death |

Means in columns with different superscripts significantly differ ($p$<0.05).

Table 3. Experiment III: Behavioral changes of freshwater prawn postlarvae during transportation using clove oil as an anesthetic.

| *Treatment (mg/l)* | *Stage* after ... hours* | | | |
|---|---|---|---|---|
| | *1* | *3* | *6* | *12* |
| 15 | Stage 1 | Stage 1 | Stage 2 | Death |
| 30 | Stage 6 | Stage 6 | Stage 6 | Death |
| 45 | Stage 6 | Stage 6 | Death | - |
| 60 | Stage 6 | Death | - | - |
| 75 | Stage 6 | Death | - | - |

* see Table 1.

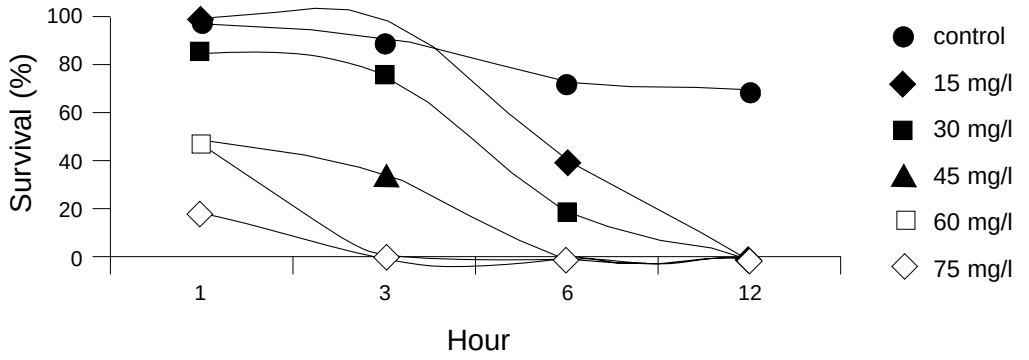


Fig. 1. Experiment III: Survival of postlarvae of the freshwater prawn, *Macrobrachium rosenbergii*, anesthetized by different doses of clove oil.

an extended duration; otherwise, rapid recovery may be preferred (Marking and Meyer, 1985; Stoskopf, 1993). The induction and recovery times, behavioral changes during anesthesia, and survival rate are important factors to consider when anesthetizing animals. Marking and Meyer (1985) outlined the time scale for induction of anesthesia and recovery of fish. According to these authors, three minutes for induction and five minutes for recovery are desirable. The present study did not correspond to this time scale because the minimum induction period for postlarvae was 3.3-14.0 minutes and for juveniles 25.6-78.3 minutes. Gardner (1997) tested various treatments, including freshwater bath, chilling, heating, prolonged exposure to air, hypercapnic seawater bath (carbon dioxide addition), 2-phenoxy-ethanol bath, magnesium sulfate bath, benzocaine bath, MS 222 bath, chloroform bath, clove oil bath, Aqui-s™ bath, Xylazine-HCl by injection, and ketamine-HCl by injection. He found that crabs could be paralyzed within 30 min with clove oil at a dose of 0.125 ml/l, indicating that the time required for induction and recovery with clove oil is relatively useful in crustaceans.

Foley et al. (1966) found that isobutyl alcohol is an effective anesthetic for the lobster, *Homarus americans*, concluding that 7.0 ml/l is required to sedate the lobster for a short period. In the present study, the highest concentrations of ethanol were 625 mg/l for postlarvae and 5000 mg/l for juveniles. These concentrations had no sedative effect on the respective prawns. Oswald (1977) found that 25 mg/kg of procaine injected in hemocoel induced anesthesia in the green crab, *Carcinus maenas*, within 30 s with recovery after 2-3 h at 10°C. In the present study, immersion was chosen as the method of delivery due to the small size of the prawns. The treatment was more effective in postlarvae than in juveniles. Among juveniles, the recovery period was very long (90.4 min) at the most effective dose (500 mg/l), significantly longer than at doses of 125 and 250 mg/l which sedated the prawns for less than 20 min, while the lowest dose (75 mg/l) was ineffective. Jason et al. (2003) also found that clove oil doses ranging 100-300 mg/l were ineffective for sedating juvenile prawns. Among postlarvae, the induction and recovery times were less than 15 min at doses of 30, 45, and 60 mg/l and any of these doses could be used, depending on the needed period of anesthesia. For example, tagging requires that prawns remain numb for a longer duration, such as effected by a concentration of 60 mg/l, while concentrations of 30 or 40 mg/l could be used to anesthetize animals prior to handling for measurement. At 75 mg/l, post-

Table 4. Experiment III: Water parameters (Scheffe's multiple comparison test) during transportation of freshwater prawn postlarvae in various concentrations of clove oil.

| | | *Concentration (mg/l)* | | | | | |
|---|---|---|---|---|---|---|---|
| | *Hour* | *15* | *30* | *45* | *60* | *75* | *Control* |
| *pH* | 0 | 8.00±0.00 | 8.00±0.00 | 8.00±0.00 | 8.00±0.00 | 8.00±0.00 | 8.00±0.00 |
| | 1 | 7.52±0.16 | 7.34±0.05 | 7.12±0.08 | 7.02±0.08 | 6.62±0.21 | 7.88±0.04 |
| | 3 | 7.64±0.05 | 7.22±0.08 | 7.00±0.07 | 6.96±0.11 | 6.28±0.19 | 7.76±0.05 |
| | 6 | 7.28±0.14 | 7.04±0.11 | 6.70±0.15 | 6.68±0.13 | 6.12±0.08 | 7.60±0.07 |
| | 12 | 6.90±0.07 | 6.54±0.20 | 6.16±0.15 | 6.14±0.11 | 5.98±0.08 | 7.38±0.08 |
| *Ammonia (mg/l)* | 0 | 0.02±0.00 | 0.02±0.00 | 0.02±0.00 | 0.02±0.00 | 0.02±0.00 | 0.02±0.00 |
| | 1 | 0.02±0.001 | 0.02±0.001 | 0.03±0.002 | 0.03±0.001 | 0.05±0.007 | 0.02±0.001 |
| | 3 | 0.04±0.001 | 0.05±0.005 | 0.07±0.006 | 0.12±0.02 | 0.22±0.01 | 0.02±0.004 |
| | 6 | 0.08±0.006 | 0.12±0.01 | 0.23±0.02 | 0.35±0.03 | 0.41±0.03 | 0.04±0.005 |
| | 12 | 0.33±0.034 | 0.41±0.01 | 0.50±0.03 | 0.58±0.01 | 0.59±0.01 | 0.09±0.005 |
| *Dissolved oxygen (mg/l)* | 0 | 5.00±0.00 | 5.00±0.00 | 5.00±0.00 | 5.00±0.00 | 5.00±0.00 | 5.00±0.00 |
| | 1 | 4.50±0.07 | 4.22±0.08 | 4.00±0.07 | 3.90±0.15 | 3.54±0.11 | 4.78±0.13 |
| | 3 | 4.06±0.05 | 3.88±0.11 | 3.64±0.11 | 2.00±0.07 | 1.20±0.07 | 4.22±0.08 |
| | 6 | 3.22±0.08 | 2.70±0.15 | 1.76±0.15 | 1.38±0.13 | 1.06±0.08 | 3.98±0.11 |
| | 12 | 1.16±0.11 | 0.92±0.13 | 0.82±0.08 | 0.72±0.08 | 0.28±0.16 | 3.66±0.20 |
| *Temperature (°C)* | 0 | 27.50±0.00 | 27.50±0.00 | 27.50±0.00 | 27.50±0.00 | 27.50±0.00 | 27.50±0.00 |
| | 1 | 27.92±0.08 | 27.92±0.08 | 27.92±0.08 | 27.92±0.08 | 27.92±0.08 | 27.54±0.97 |
| | 3 | 28.20±0.25 | 28.08±0.16 | 28.20±0.25 | 28.14±0.18 | 28.18±0.13 | 28.18±0.08 |
| | 6 | 28.46±0.32 | 28.68±0.21 | 28.78±0.22 | 28.76±0.19 | 28.80±0.15 | 28.40±0.20 |
| | 12 | 28.24±0.15 | 28.24±0.16 | 28.34±0.11 | 28.44±0.16 | 28.58±0.08 | 28.46±0.33 |

| | Hour | Treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | 75 | 60 | 45 | 30 | 15 | Control |
| pH | 1 | ---------- | | | | | |
| | | ---------- | | | | | |
| | | ---------- | | | | | |
| | 3 | ---------- | | | | | |
| | | ---------- | | | | | |
| | | ---------- | | | | | |
| | 6 | ---------- | | | | | |
| | | ---------- | | | | | |
| | 12 | ---------- | | | | | |
| | | Control | 15 | 30 | 45 | 60 | 75 |
| Ammonia (mg/l) | 1 | ---------- | | | | | |
| | | ---------- | | | | | |
| | 3 | ---------- | | | | | |
| | | ---------- | | | | | |
| | | ---------- | | | | | |
| | 6 | ---------- | | | | | |
| | | ---------- | | | | | |
| | 12 | ---------- | | | | | |
| | | 75 | 60 | 45 | 30 | 15 | Control |
| Dissolved oxygen (mg/l) | 1 | ---------- | | | | | |
| | | ---------- | | | | | |
| | 3 | ---------- | | | | | |
| | 6 | ---------- | | | | | |
| | | ---------- | | | | | |
| | 12 | ---------- | | | | | |
| | | ---------- | | | | | |
| | | 15 | 30 | 45 | 60 | 75 | Control |
| Temperature (°C) | 1 | ---------- | | | | | |
| | 3 | ---------- | | | | | |
| | 6 | ---------- | | | | | |
| | 12 | ---------- | | | | | |

Fig. 2. Graphic presentation of Scheffe's multiple comparison test of water parameters in Experiment III. Means of groups underscored by the same line are not significantly different ($p$>0.05).

larvae were tranquilized in a relatively short period (3.30±0.10 min) and recovery time was maximum (45.17±0.20 min).

Cannibalism is the main problem during transport of freshwater prawn seed. There are various methods of preventing cannibalism during transport, for example putting polythene strips into plastic bags or inoculating *Artemia* nauplii. In the present study, clove oil was used to anesthetize the prawns and reduce cannibalism. After 3 h, survival was significantly higher in the 15 mg/l group than in the control as a result of greater cannibalism in the control; there was a greater number of damaged prawns in the control than in the other treatments. However, mortality began in all doses by 6 h and, by 12 h, all prawns were dead in all treatments except the control where survival at 12 h was 69.4%. Thus, the long-term effect of these doses was unsafe. As clove oil is natural and safe up to 6 h, further work should be conducted with lower doses to extend the potential transportation period by using clove oil as a tranquilizer to reduce cannibalism during transport. Until then, a concentration of 15 mg/l may be safely used during transport up to 3 h.

In conclusion, the length of the induction and recovery periods were high for juvenile prawns sedated with clove oil. The required amounts of clove oil are relatively higher for juvenile prawns than postlarvae. Therefore, clove oil was a less effective sedative for juvenile prawns but can be used to tranquilize postlarvae during handling and transport. To increase the tranquilized transportation period, further work is needed using lower doses (below 15 mg/l) of clove oil.

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