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Anaesthetic effects of clove oil on seven species of tropical reef teleosts

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The anaesthetic potential of the clove oil was tested on the following species of tropical reef fishes: *Abudefduf saxatilis*, *Stegastes variabilis*, *Pareques acuminatus*, *Acanthurus chirurgus*, *Sparisoma axillare*, *Lutjanus apodus* and *Bathygobius soporator*. Induction and recovery times from anaesthesia were compared using various concentrations (20, 30, 40, 50 and 60 mg l^{-1}). Induction and recovery times were not affected by variations in fish total length. When exposed to any of the five tested concentrations of clove oil, specimens achieved a deep state of anaesthesia, with induction and recovery times of <180 and <300 s, respectively. Nevertheless, to maximize safety and reduce fish mortality and stress, the lowest concentration (20 mg l^{-1}) is recommended during field sampling.

Key words: anaesthesia; Brazil; clove oil; induction; recovery; reef fishes.

INTRODUCTION

Chemical substances have been used to collect fishes for a long time. The ichthyocide rotenone, a non-selective method, is one of the most widely used (Weier & Starr, 1950; Bradbury, 1986; Fajt & Grizzle, 1993). Anaesthetic substances became an alternative method to ichthyocides, and used for collecting, transporting, weighing and measuring fishes, for tagging experiments, and for handling fishes (Moring, 1970; Endo *et al.*, 1972; Bernier & Randall, 1998; Hseu *et al.*, 1998). Marking & Meyer (1985) showed that a number of anaesthetics were used in the U.S. and Canada, the best known and most popular being tricaine methanesulphonate (MS-222) and quinaldine (2-methylquinoline). Clove oil and carbon dioxide are less harmful chemicals to the researcher, the latter being known as a fish anaesthetic for over 50 years (Prince *et al.*, 1995). Carbon dioxide, however, is considered as only partially effective, and is slow in action, and lethal after repeated exposures (Marking & Meyer, 1985).

Clove oil (85–95% eugenol), obtained from the stem, flowers and leaves of clove trees (*Eugenia caryophyllata* Thunberg and *Eugenia aromatica* Baill), has

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been used for centuries as a topic anaesthetic in Indonesia (Soto & Burhanuddin, 1995), and local anaesthetic in dentistry (Curtis, 1990). It is classified as a food additive by the Food and Drug Administration (FDA, U.S.A.) (Anderson *et al.*, 1997). In aquaculture, it has been widely used to anaesthetize freshwater and marine fishes and molluscs (Endo *et al.*, 1972; Soto & Burhanuddin, 1995; Munday & Wilson, 1997; Hseu *et al.*, 1998; Keene *et al.*, 1998; Taylor & Roberts, 1999; Griffiths, 2000; Lellis *et al.*, 2000; Prince & Powell, 2000; Durville & Collet, 2001; Woody *et al.*, 2002). Clove oil has also been recommended for anaesthetizing fishes because it is widely available, relatively inexpensive, is environmentally friendly and is safe for the handler (Iversen *et al.*, 2003). It also imposes short induction and recovery times, but sufficient to identify the fishes and record biological information (Griffiths, 2000). Furthermore, it has been shown that it considerably reduces the risks of pathologies, damage and accidents while handling fishes (Keene *et al.*, 1998). Some studies have compared the efficacy of clove oil to other anaesthetics (Munday & Wilson, 1997; Lellis *et al.*, 2000; Sladky *et al.*, 2001; Ackerman & Bellwood, 2002) and have pointed out that fishes exposed to it exhibited a rapid and much calmer induction to anaesthesia, and longest recovery times; furthermore, clove oil is often effective at much lower concentrations than MS-222, benzocaine and 2-phenoxyethanol, increasing its cost-effectiveness of use.

Most studies testing the efficacy of clove oil on fishes were directed to a single species, and were carried out in subtropical or temperate areas. This study investigated the efficacy of clove oil in a group of tropical reef fishes, and used as a model the studies carried out by Soto & Burhanuddin (1995), Munday & Wilson (1997) and Griffiths (2000). The aims of the study were to compare the induction and recovery times of seven species of tropical fishes subjected to different concentrations of clove oil, in order to better define adequate concentrations for field sampling and handling in tropical areas while minimizing fish mortality and stress.

MATERIALS AND METHODS

The study was carried out at the States of Paraíba (06°58′03″ S; 34°50′04″ W) and Rio Grande do Norte (05°46′03″ S; 35°12′03″ W), north-east Brazil. Seven species belonging to families Pomacentridae [*Abudefduf saxatilis* (L.) and *Stegastes variabilis* (Castelnau)], Sciaenidae [*Pareques acuminatus* (Bloch & Schneider)], Acanthuridae [*Acanthurus chirurgus* (Bloch)], Scaridae [*Sparisoma axillare* (Steindachneri)], Lutjanidae [*Lutjanus apodus* (Walbaum)] and Gobiidae [*Bathygobius soporator* (Valenciennes)] were used.

Species were chosen based on at least one of the following criteria: important to local fisheries (as food fishes or ornamental fishes), abundant in the tidepools found in the study sites or feasible to collect by hand-nets. The first five species are largely collected for the aquarium trade; *L. apodus* is caught as a food fish and is occasionally sold in the aquarium trade. *Bathygobius soporator* is a resident of intertidal regions, and is one of the most abundant fish species in the tidepools at the study sites.

To avoid damage to the specimens, collections were made by using hand-nets designed to capture ornamental fishes. In the laboratory, specimens were acclimatized in 200 l glass aquaria with aeration for 24 h prior to commencement of experiments. Concentrations of clove oil (20, 30, 40, 50 and 60 mg l^{-1}) were chosen based on Soto & Burhanuddin (1995), Keene *et al.* (1998) and Griffiths (2000). Preliminary trials using a 10 mg l^{-1} concentration in the field resulted in predation of some specimens

by more resistant species. This supported the choice of 20 mg l^{-1} as the lowest concentration in the experiments.

Clove oil was dissolved in ethanol (95%), following the procedures outlined by Munday & Wilson (1997) and Griffiths (2000). Ethanol is not a fish anaesthetic when in low doses (Anderson *et al.*, 1997; Munday & Wilson, 1997), and has no anaesthetizing effects on fishes (Griffiths, 2000). This was confirmed by trials conducted in a control tank, where the specimens were submitted to the highest concentration of ethanol used in the experiments.

Efficacy criteria were established based on Soto & Burhanuddin (1995), Munday & Wilson (1997), Keene *et al.* (1998) and Griffiths (2000), except for *B. soporator,* in which efficacy was defined by a cessation of movements by the pectoral and caudal fins. Based on pilot observations, two patterns of recovery were adopted: 1) specimens resumed balance control and a vertical position, and 2) specimens recovered the movements of the pectoral and caudal fins (*B. soporator* only).

Specimens were randomly chosen and individually removed from the acclimatization tank and transferred to 4 l glass aquaria (mean $\pm$ S.D. water temperature 27 $\pm$ 0·6° C) containing the treatments (1 clove oil:5 ethanol), where their induction and recovery times were recorded by using a digital stopwatch. Water temperature was the same in the acclimatization tanks and aquaria where the specimens were tested. Specimens' behaviour was recorded before and during anaesthesia. While anaesthetized, specimens were measured (total length, L_T). Subsequently, they were transferred to an aquarium filled with continuously aerated sea water, where their recovery time was recorded. Water temperature in the tanks was measured both during induction and recovery times. Specimens were monitored for a week, to quantify mortality, record appearance of diseases or changes in behaviour. During that period, fishes were fed twice a day, with *Artemia* sp. or algae.

Experiments were conducted using a completely randomized design. Eight individuals from each species were submitted to each concentration, totalling 40 individuals per species.

ANALYSIS

Data on induction and recovery times were submitted to one-way ANOVA, considering concentrations as fixed factors. Data were $\log_{10}$ transformed to meet the prerequisites of normality and homogeneity (Zar, 1996; Scheiner & Gurevitch, 2001). A Student–Newman–Keuls (SNK) *post hoc* test was used to determine significantly different means (Scheiner & Gurevitch, 2001). The effect of the clove oil concentrations on induction and recovery was tested by linear regression.

The effects of L_T and water temperature on induction and recovery times were evaluated by using Pearson's correlation test.

RESULTS

Induction time decreased inversely to the concentration of clove oil ($y = 2{\cdot}19 - 0{\cdot}009x$; $r = -0{\cdot}48$), the 20 mg l^{-1} concentration producing the highest induction time (mean 127·33 s). Significant differences in the mean induction time ($F_{4,337}$, $P < 0{\cdot}01$) were found only for the 20 mg l^{-1} concentration (SNK test, $P < 0{\cdot}05$); for the other concentrations, two groups were found: 30 and 40 mg l^{-1}; and 50 and 60 mg l^{-1}. The concentrations of 30 and 40 mg l^{-1} anaesthetized the specimens for *c.* 60 and 30 s, whereas the concentrations of 50 and 60 mg l^{-1} kept the specimens anaesthetized for *c.* 60 s.

Recovery times were directly proportional to the concentrations of clove oil ($y = 1{\cdot}98 + 0{\cdot}0056x$; $r = 0{\cdot}34$). The means of the recovery times varied significantly in relation to the concentrations ($F_{4,333}$, $P < 0{\cdot}01$), and revealed the

existence of three overlapping groups (SNK test, $P < 0{\cdot}05$): 20 mg l^{-1}, 30, 40 and 50 mg l^{-1}, and 50 and 60 mg l^{-1}, which resulted in recovery times of *c.* 130, 180 and 220 s in each group, respectively.

Induction and recovery times varied among the species tested ($F_{6,335}$, $P < 0{\cdot}01$ and $F_{6,331}$, $P < 0{\cdot}01$, respectively), suggesting that some of them are more sensitive to the anaesthetic (Fig. 1). Among the species tested, *B. soporator* was the most resistant, requiring 180 s for induction at concentrations of 20 and 30 mg l^{-1} (SNK test, $P < 0{\cdot}05$) and presenting a recovery time >300 s at concentrations of 50 and 60 mg l^{-1} (SNK test, $P < 0{\cdot}05$) (Fig. 1). The most rapidly anaesthetized species were *P. acuminatus* and *S. axillare* (Fig. 1), the former species being the only one to float during anaesthesia.

Acanthurus chirurgus, P. acuminatus and *S. axillare* exhibited patterns of rapid induction to anaesthesia and comparatively long recovery time (Fig. 1), while *L. apodus* and *S. variabilis* presented both rapid induction (*c.* 60 s) and recovery times (<120 s) (Fig. 1). Most species were deeply anaesthetized at all tested concentrations. *Lutjanus apodus* and *S. axillare*, however, did not reach that stage at concentrations of 20 and 30 mg l^{-1}, and kept exhibiting contractions of caudal peduncle and caudal fin during handling.

Mortality was limited to four small-sized ($\leq$4 cm L_T) specimens belonging to two species: *A. saxatilis* and *A. chirurgus* (one specimen). No mortality, behavioural changes or diseases occurred during the monitoring period following completion of the experiments. No correlation was found between induction and recovery times and L_T or water temperature (Table I).

DISCUSSION

The reduction in induction time and the increase in recovery time with increasing concentrations of clove oil observed in this study follows a common pattern obtained in previous studies using clove oil or other anaesthetics (Endo *et al.*, 1972; Soto & Burhanuddin, 1995; Munday & Wilson, 1997; Hseu *et al.*, 1998; Keene *et al.*, 1998; Taylor & Roberts, 1999; Griffiths, 2000; Lellis *et al.*, 2000; Prince & Powell, 2000; Durville & Collet, 2001; Woody *et al.*, 2002). This is also the case for the marked interspecific and the minor intraspecific variation in the induction and recovery times found in this study (Moring, 1970; Endo *et al.*, 1972; Taylor & Roberts, 1999; Griffiths, 2000).

The present results indicated that clove oil was effective for immobilizing the fishes at all concentrations tested (20, 30, 40, 50 and 60 mg l^{-1}), and exhibited a short induction ($\leq$120 s) and recovery time (>120 s). This falls within the efficacy criteria recommended by Marking & Meyer (1985), and the recommendations made by Ross & Ross (1999) that the anaesthetic must be effective at low dose and that the toxic dose may considerably exceed the effective dose.

It has been suggested that the efficacy of clove oil may vary with temperature and also interspecifically (Endo *et al.*, 1972), and that it may be related to branchial area and body mass, both varying considerably in size among species (Ross & Ross, 1999). Griffiths (2000) analysed the effects of clove oil on eight species of intertidal fishes in Australia (water temperature 18·3° C), and indicated that the 40 mg l^{-1} was the most appropriate concentration for anaesthetizing and recovering fish species in a time shorter than 180 and 300 s,

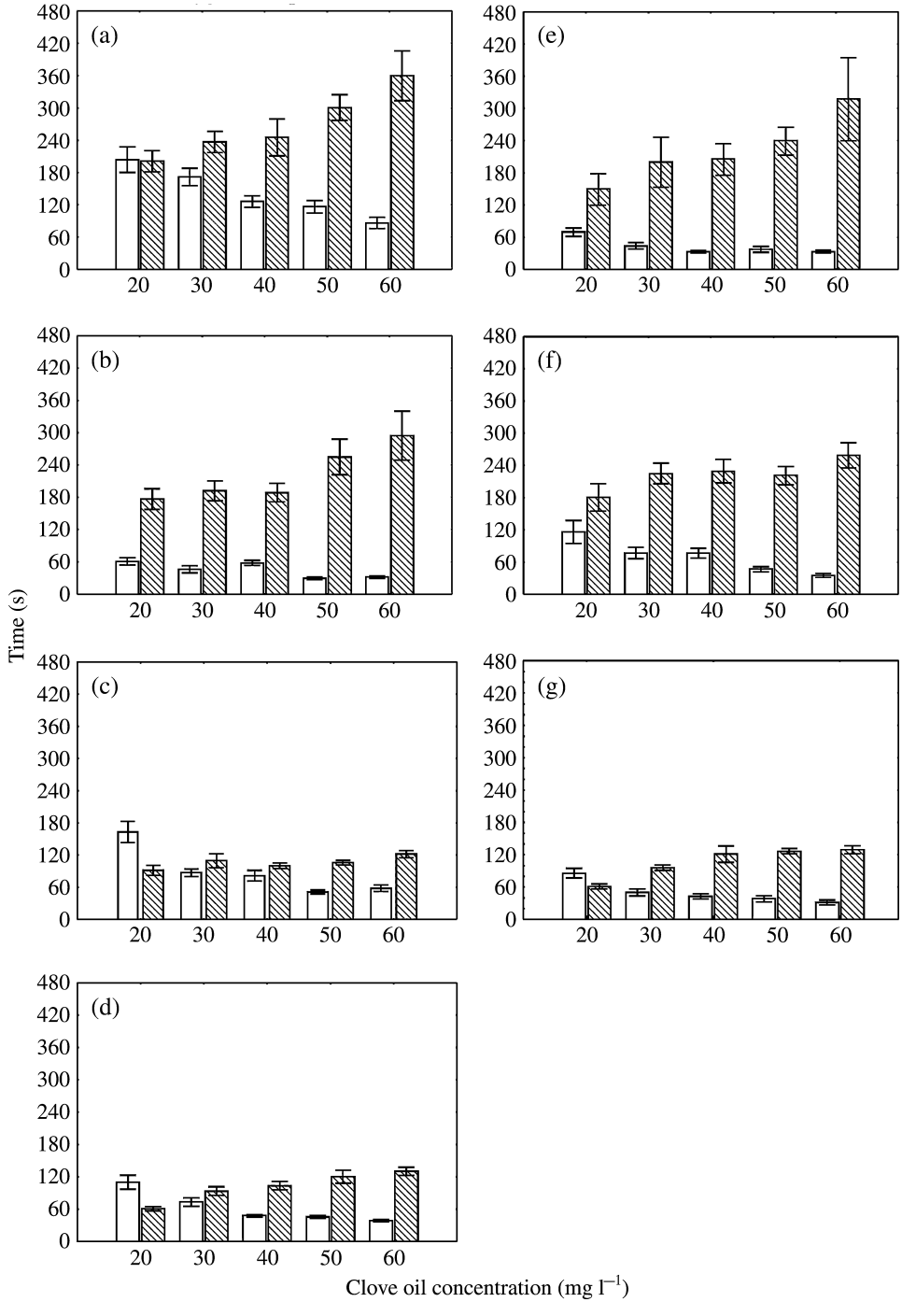

FIG. 1. Mean ± S.E. of induction (□) and recovery (▨) times of (a) *Bathygobius soporator*, (b) *Pareques acuminatus*, (c) *Abudefduf saxatilis*, (d) *Lutjanus apodus*, (e) *Sparisoma axillare*, (f) *Acanthurus chirurgus* and (g) *Stegastes variabilis* submitted to anaesthesia with clove oil at concentrations of 20, 30, 40, 50 and 60 mg l^{-1}.

TABLE I. Coefficients of Pearson correlation (r) between values of induction and recovery times and variation of total length range (means in parentheses) and aquaria water temperature (mean ± S.D.) in each of the seven species submitted to anaesthesia with clove oil

Species	L_T range (mm)	Induction		Recovery		Water temperature (° C), acclimation and tests tanks	Induction		Recovery	
		r	P	r	P		r	P	r	P
Abudefduf saxatilis	17·9–110·0 (66·75)	−0·24	0·13	−0·02	0·90	28·00 ± 0·00	0·04	0·79	0·12	0·38
Acanthurus chirurgus	37·0–180·0 (63·14)	−0·02	0·93	−0·10	0·53	27·73 ± 0·08	0·12	0·46	0·12	0·46
Bathygobius soporator	28·3–98·1 (56·30)	0·02	0·90	−0·28	0·08	25·37 ± 0·06	0·14	0·41	0·20	0·23
Lutjanus apodus	61·0–151·0 (84·22)	0·15	0·36	−0·16	0·34	26·49 ± 0·11	−0·25	0·12	0·19	0·24
Pareques acuminatus	61·1–120·0 (93·57)	0·14	0·38	−0·13	0·43	28·50 ± 0·08	0·10	0·54	−0·17	0·29
Sparisoma axillare	43·0–165·0 (84·69)	0·21	0·19	0·21	0·19	26·70 ± 0·18	0·13	0·42	0·12	0·45
Stegastes variabilis	25·0–84·0 (52·5)	−0·25	0·07	0·14	0·40	26·74 ± 0·08	0·24	0·11	0·14	0·37
All species	17·9–180·0 (71·21)	−0·12	0·05	−0·05	0·35	27·37 ± 0·64	−0·41	0·00	−0·17	0·01

Significance level ($P < 0·05$).

respectively. It is likely that the difference in the lowest effective concentration was related to the distinct group of species used in the present experiments, and the distinct physiological characteristics of tropical fishes. Variations in water temperature (25–29° C), although relevant for the interpretation of the results, did not seem to be directly responsible for changes in induction and recovery times, as experiments using clove oil in the field (27–36° C) to anaesthetize the same species tested in this study produced similar responses to those observed in the experiments, regardless of the water temperature in the pools (pers. obs.) The relationship between temperature and induction and recovery times observed in the present study should be interpreted with caution, due to the low explanatory power of the observed variation and the weak correlations found.

Previous studies have reported various optimum concentrations for anaesthetizing different fish species (Soto & Burhanuddin, 1995; Keene *et al.*, 1998; Cho & Heath, 2000; Prince & Powell, 2000; Woody *et al.*, 2002; Iversen *et al.*, 2003). In the present study, the most anaesthesia-resistant species was *B. soporator*, a typical true resident species of the intertidal zone and quite resistant to adverse environmental conditions (Rosa *et al.*, 1997; Gibson & Yoshiyama, 1999). This agrees with the results obtained by Griffiths (2000) who reported a recovery time >300 s for *Bathygobius cocosensis* (Bleeker) at concentrations of 60 mg l^{-1}.

The present results confirmed the efficacy of clove oil as a fish anaesthetic, and showed that its application at low concentrations leads to a calm induction to anaesthesia; mortality was low and restricted to four small-sized individuals ($\leq$4 cm L_T) of the species *A. saxatilis* ($n = 3$) and *A. chirurgus* ($n = 1$). Some studies have shown that clove oil also reduces stress (Wagner *et al.*, 2002; Iversen *et al.*, 2003), enabling the animals to expel residues accumulated in their tissues in 48 h to an acceptable level for human consumption (Kildea *et al.*, 2004).

The benefits of collecting and analysing fish species in the field, and subsequently returning them promptly to their habitats after measuring the specimens and obtaining species-specific information have clear benefits for researchers and for the species studied. In the present study, clove oil was a useful tool both in the field and in the laboratory. A concentration of 20 mg l^{-1} is suggested as a general guideline when anaesthetizing fishes with clove oil, and the use of higher concentrations on a species-specific basis. It is known that anaesthesia itself induces stress reactions although it is difficult to distinguish the direct effects of the anaesthetic method from those of capture or handling (Ross & Ross, 1999). Thus, it is important to conduct preliminary trials before using anaesthetic substances to sample fishes.

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