Notas Científicas

Eugenol as an anesthetic for juvenile common snook

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Abstract – The objective of this work was to evaluate the efficacy of eugenol as an anesthetic for juvenile common snook, and to determine the minimum effective concentration for use in handling procedures. In the first trial, juvenile common snook were subjected to immersion baths at 25, 50, 75, 100, 125, and 150 mg L^{-1} eugenol concentrations, after which induction and recovery times were evaluated. In the second experiment, the lethal exposure time (LT_{50}) at 75 mg L^{-1} was estimated. Minimum effective eugenol concentration was 50 mg L^{-1}, and the stage of deep anesthesia and recovery were, respectively, reached at 126.3 and 208.8 s. At 75 mg L^{-1}, LT_{50} was 1,314 s, and induction time and recovery were also satisfactory; however, fish cannot tolerate over 229 s exposure.

Index terms: *Centropomus undecimalis*, anesthesia, fish farming, fish handling.

Eugenol como anestésico para juvenis de robalo-flecha

Resumo – O objetivo deste trabalho foi avaliar a eficácia do eugenol como anestésico para juvenis de robalo-flecha e determinar a concentração mínima eficaz que pode ser utilizada em procedimentos de manejo. No primeiro experimento, juvenis de robalo-flexa foram submetidos a banhos de imersão com concentrações a 25, 50, 75, 100, 125 e 150 mg L^{-1} de eugenol; em seguida, o tempo de indução e a recuperação foram avaliados. No segundo experimento, o tempo de exposição letal (LT_{50}) a 75 mg L^{-1} foi estimado. A concentração de eugenol mínima e eficaz foi de 50 mg L^{-1}, e o estágio de anestesia profunda e a recuperação foram alcançados, respectivamente, em 126,3 e 208,8 s. A 75 mg L^{-1}, o LT_{50} foi de 1.314 s, e o tempo de indução e recuperação foram satisfatórios; entretanto, os peixes não toleram mais de 229 s de exposição.

Termos para indexação: *Centropomus undecimalis*, anestesia, piscicultura, manejo de peixes.

Anesthesia is a valuable tool for aquaculture management to minimize stress or physical damage caused during handling, transport, grading, weighing, induction of spawning, and tagging and, consequently, to reduce susceptibility to pathogens and infection (Ross & Ross, 2008).

Satisfactory anesthetic effect, rapid induction and recovery times, as well as safety margins are important properties of fish anesthetics (Ross & Ross, 2008). Chemicals such as tricaine methanesulfonate (MS-222), quinaldine, metomidate, 2-phenoxyethanol, menthol, and benzocaine are widely used to anesthetize fish (Pirhonen & Schreck, 2003). However, some of these anesthetics cause unwanted side-effects, such as loss of mucus, gill irritation, and corneal damage (Inoue et al., 2003).

Eugenol has been used as an alternative anesthetic in a large number of ichthyological studies because it does not have persistent or latent negative effects on fish physiology or behavior (Yamanaka et al., 2011), and it is more effective in reducing the short-term stress response induced by handling (Wagner et al., 2003). Eugenol (4-allyl-2-methoxyphenol) is the main active ingredient (70–90%) of clove oil; it is distilled from the stem, leaves, and buds of the clove tree *Syzygium aromaticum* (L.) Merr. & L. M. Perry (Ross & Ross, 2008). Low cost, high efficacy, a large margin of safety for fish, and a lack of toxicity to humans at commonly used concentrations are some of the characteristics that qualify it as a safe anesthetic (Roubach et al., 2005).

Common snook (*Centropomus undecimalis* Bloch, 1792) is a valuable commercial and recreational

resource, widely distributed along the Western Atlantic Coast from North Florida (U.S.A.) to the Southern coast of Brazil (Figueiredo & Menezes, 1980), and is considered as a species with great potential for aquaculture in Brazil. In the nursery, cannibalism is quite common and requires periodic grading. During this procedure, biometrics are also performed to evaluate growth, and anesthesia is important to reduce the effects of handling on juveniles' performance.

The objective of this work was to evaluate the efficacy of eugenol as an anesthetic for common snook juveniles and to determine the minimum effective concentration that can be used for routine grading and biometry.

Two experimental trials were conducted at the Campo Experimental de Piscicultura de Camboriú, of the Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (CEPC-Epagri), in January 2011. Juvenile common snook (7.63 ± 0.15 g, 10.53 ± 0.08 cm), acquired from Danúbio Aquacultura (Balneário Camboriú, SC, Brazil) and already acclimated to freshwater, were stored for two weeks in a circular concrete tank with 3 m³ working volume. Fish were maintained in a continuous flow system with constant aeration (3 L min^{-1} flow; 6.5 ± 0.7 mg L^{-1} dissolved oxygen; 25.2 ± 1.13°C temperature; 7.9 ± 0.06 pH), and fed to apparent satiation twice a day with commercial feed for carnivorous marine fish (50% crude protein and 15% ether extract). Feeding was suspended 24 hours before the start of the experiments.

All trials, both for induction procedures and recovery, were performed in polypropylene circular tanks with 10 L working volume each, at constant aeration. Temperature (25.8 ± 0.40 °C), dissolved oxygen (6.82 ± 0.32 mg L^{-1}), and pH (8.14 ± 0.21) were monitored during the experiments with a multiparameter instrument (YSI 556, Yellow Springs, OH, USA).

Due to its oiliness, eugenol (purity at least 99%, Biodinâmica, Ibiporã, PR, Brazil) was diluted with hydrated ethyl alcohol (92.8°) at 1:10 ratio , resulting in a 100 mg mL^{-1} stock solution (Vidal et al., 2007). Control experiments using a tank containing ethanol alone were not performed because ethanol has no anesthetic properties at a low dosage as those used in the trials (up to 1,400 ppm); also, ethanol produces little or no effect on fish behavior (Grush et al., 2004).

In the first trial, six eugenol treatments were evaluated (25, 50, 75, 100, 125, and 150 mg L^{-1}) after prior testing to assess the influence of eugenol concentration on anesthesia induction and recovery of juvenile common snook. For each treatment, induction and recovery were timed individually, using randomly selected juveniles (n = 10); each fish was considered a replicate, totaling 60 individuals. The induction parameters observed were total loss of equilibrium, lack of movement, and slow but regular opercular rate (deep anesthesia) (Ross & Ross, 2008). When these whole range effects were not detected, an exposure limit of 300 s was adopted. After induction, fish were weighed and measured to simulate biometric procedures. Anesthesia recovery was performed individually in a tank containing eugenol-free water, and total recovery of equilibrium, swimming motion, and escape reflex were used as behavioral parameters indicative of recovery (Vidal et al., 2006). After recovery, fish were transferred to a 3 m³ circular concrete tank with a continuous flow system (3 L min^{-1}) and constant aeration (6.5 ± 0.7 mg L^{-1} dissolved oxygen). Separation of fish from each treatment was maintained for 96 hours for mortality monitoring, in case of fish mortality following the experiment.

In the second trial, to assess the lethal exposure time (LT_{50}; the time expected to cause death to 50% of fish exposed to eugenol), fish were subjected to 75 mg L^{-1} eugenol (defined in the first trial) for varying time periods (300, 600, 900, 1,200, and 1,500 s), using an entirely randomized design in triplicate, with fish (n = 10) randomly sampled per replicate, totaling 150 individuals. At the end of induction, fish from each treatment were transferred to recovery tanks containing eugenol-free water; and, 30 min later, mortality was recorded. Specimens that showed no sign of recovery (opercular movements) in this time interval were considered dead.

Eugenol concentration relationships with induction and recovery times (first trial) was evaluated using nonlinear regression analysis, and the parameters were estimated using the iterative method (Zar, 2010). The Probit method was applied to grouped data from each treatment, in the second trial, to determine the LT50 and 95% confidence intervals (CI95%), following the recommendations of the United States Environmental Protection Agency (2002). The execution of the experiment was in accordance with the Law No.

11.794/08, laying down the procedures for the scientific use of animals. During the induction and recovery procedures and the 96-hour monitoring, no mortality of fish anesthetized with eugenol was observed. Deep anesthesia was induced in all common snook exposed to experimental concentrations of eugenol (Table 1), except for five fish from the 25 mg L^{-1} treatment (data excluded from analysis), which after 300 s reached only narcosis, a condition characterized by Ross & Ross (2008) as partial loss of muscle tone and balance and erratic swimming.

The behavioral response of common snook during the induction procedure was similar to those of other species anesthetized with eugenol, such as rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) (Keene et al., 1998), zebrafish (*Danio rerio* Hamilton, 1822) (Grush et al., 2004), pintado (*Pseudoplatystoma corruscans* Spix & Agassiz, 1829) (Vidal et al., 2006), piavuçu (*Leporinus macrocephalus* Garavello & Britski, 1988) (Vidal et al., 2007), and Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) (Vidal et al., 2008).

When exposed to eugenol, fish of all treatments gradually showed agitated swimming, a regurgitation reflex, loss of equilibrium, and supine positioning. This pattern of behavior intensified with increasing concentrations of anesthetic, and at the highest concentrations (125 and 150 mg L^{-1}) allowed to observe small jets on the water surface from the intense movement of the mouth and operculum.

Time to deep anesthesia induction varied with eugenol concentration (Table 1). There was a significant reduction in induction time above 50 mg L^{-1}, tending to an asymptotic curve at higher concentrations (Figure 1a). The relationship between eugenol concentration and induction time could be described by a power regression model ($R^2 = 0.899$) given by the equation $IT = 4785C^{-0.9190}$, in which IT is the anesthesia induction time (s), and C is the eugenol (mg L^{-1}) concentration.

Table 1. Anaesthesia induction time and recovery of juvenile common snook subjected to different eugenol concentrations.

Concentration	Induction time (s)			CV	Recovery time (s)			CV	n
(mg L^{-1})	Average	Minimum	Maximum	(%)	Average	Minimum	Maximum	(%)	
25	252.6	220	285	10.88	247.6	158	399	41.06	5
50	126.3	76	155	19.91	208.8	112	350	38.79	10
75	94.1	65	116	16.87	231.1	106	333	29.12	10
100	68.3	57	102	21.20	283.7	162	536	35.24	10
125	61.7	35	112	37.95	409.9	207	893	54.18	10
150	45.0	29	58	20.45	344.7	207	681	40.28	10

CV, coefficient of variation (%); N, number of fish.

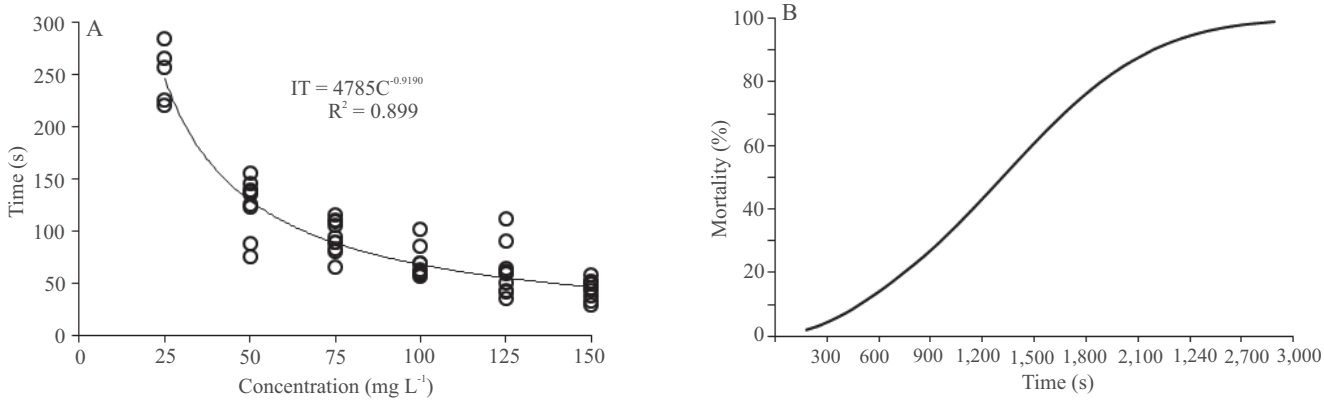

Figure 1. Induction time of juvenile common snook (*Centropomus undecimalis*) subjected to different eugenol concentrations (a), and probability of mortality as a function of exposure time to a eugenol concentration of 75 mg L^{-1}, estimated using Probit (b). IT, anaesthesia induction time; C, eugenol concentration.

Increasing anesthetic concentration did not show a consistent relationship with recovery time (in regression analysis), with averages ranging from 208.8 (50 mg L^{-1}) to 409.9 s (125 mg L^{-1}). The lack of relationship between these variables may be due to a high variability in response time observed in all treatments during recovery, with a coefficient of variation ranging from 29.12 to 54.18% (Table 1).

Independence of recovery time and eugenol concentration have been reported for zebrafish (Grush et al., 2004), common carp (*Cyprinus carpio* Linnaeus, 1758) (Hajek et al., 2006), and silver catfish (*Rhamdia quelen* Quoy & Gaimard, 1824) (Cunha et al., 2010). However, rainbow trout (Keene et al., 1998), piavuçu (Vidal et al., 2007), and cobia (*Rachycentron canadum* Linnaeus, 1766) (Gullian & Villanueva, 2009) showed longer recovery times with increasing concentration, suggesting species-specific differences.

Although the mechanism of action is not fully understood, according to Keene et al. (1998), eugenol exerts an inhibitory effect on the respiratory system, complicating the removal of excess anesthetic through the gills, which may explain the observed high variability in recovery time.

Eugenol and its analogs (iso-eugenol) are known to require more extensive recovery times than other anesthetic agents, such as MS-222, quinaldine, and benzocaine (Keene et al., 1998; Wagner et al., 2003; Roubach et al., 2005). Even with a higher recovery time based on food consumption post-sedation, Pirhonen & Schreck (2003) observed no deleterious effects on fish anesthetized with eugenol compared with those anesthetized with MS-222, which can be advantageous when long periods of management are required (e.g., surgery) (Yamanaka et al., 2011).

Deep anesthesia is the stage normally used for performing biometric procedures and should preferably be induced between 60 and 180 s; time to complete recovery should not exceed 300 s (Ross & Ross, 2008). The average time to reach deep anesthesia at concentrations of 50 (126.3 s), 75 (94.1 s), and 100 mg L^{-1} (68.3 s) was satisfactory; although no relationship was observed between concentration and recovery time, average recovery times were less than 300 s (Table 1). Therefore, the minimum effective concentration, which meets the requirements, was 50 mg L^{-1}.

The effective concentrations achieved in this trial were within the determined range (20–100 mg L^{-1}) for other species as: rainbow trout, 40–60 mg L^{-1} (Keene et al., 1998); matrinxã, 40–50 mg L^{-1} (*Brycon cephalus* Günther, 1869) (Inoue et al., 2003); zebrafish, 60–100 mg L^{-1} (Grush et al., 2004); pintado 50 mg L^{-1} (Vidal et al., 2006); common carp, 30–50 mg L^{-1} (Hajek et al., 2006); piavuçu, 37.5 mg L^{-1} (Vidal et al., 2007); Nile tilapia, 75 mg L^{-1} (Vidal et al., 2008); and silver catfish, 20–50 mg L^{-1} (Cunha et al., 2010).

However, determination of a safe concentration for exposure longer than 180 s should be conducted with caution because of low explanatory power of the observed variation in recovery time. Among the tested eugenol concentrations, 75 mg L^{-1} effectively induced anesthesia. Fish of this treatment showed a lower coefficient of variation during recovery, and 80% of the tested animals had a maximum recovery time of less than 300 s. This concentration was therefore chosen for evaluation of lethal exposure time.

The LT_{50} was estimated at 1,314 s (1,083–1,723 s; CI95%), which is approximately 22 min (Figure 1b). According to modeling results, 1% mortality may occur at 229 s; thus, for prolonged immersion baths exceeding the necessary time to achieve deep anesthesia (94.1 s), the safety time is very short at 135 s. Therefore, eugenol concentration at 75 mg L^{-1} can also be used for grading routines and biometry, but with caution. Because a large number of fish is sometimes handled simultaneously, an appropriate safety margin for concentrations between 50 and 75 mg L^{-1} must be determined for evaluation of lethal exposure times.

Eugenol is an effective anesthetic for juvenile common snook and, based on the requirements for induction and recovery times, the minimum effective concentration for use in handling procedures is 50 mg L^{-1}.

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