

Comparative efficacy of tricaine methanesulfonate and clove oil for use as anesthetics in red pacu (*Piaractus brachypomus*)

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Objective—To compare the anesthetic efficacy and physiologic changes associated with exposure to tricaine methanesulfonate and clove oil (100% eugenol).

Animals—15 adult cultured red pacu (*Piaractus brachypomus*).

Procedure—Fish were exposed to each of 6 anesthetic concentrations in a within-subjects complete crossover design. Stages of anesthesia and recovery were measured, and physiologic data were collected before and during anesthesia.

Results—Interval to induction was more rapid and recovery more prolonged in fish exposed to eugenol, compared with those exposed to tricaine methanesulfonate. The margin of safety for eugenol was narrow, because at the highest concentration, most fish required resuscitation. Mixed venous-arterial PO_2 consistently decreased with anesthesia, while PCO_2 consistently increased with anesthesia in all fish regardless of anesthetic agent. The increase in PCO_2 was accompanied by a decrease in pH, presumably secondary to respiratory acidosis. Anesthesia was associated with increased blood glucose, potassium, and sodium concentrations as well as Hct and hemoglobin. Fish anesthetized with eugenol were more likely to react to a hypodermic needle puncture than fish anesthetized with tricaine methanesulfonate.

Conclusions and Clinical Relevance—Anesthesia induced with tricaine methanesulfonate or eugenol contributes to hypoxemia, hypercapnia, respiratory acidosis, and hyperglycemia in red pacu. Similar to tricaine methanesulfonate, eugenol appears to be an effective immobilization compound, but eugenol is characterized by more rapid induction, prolonged recovery, and a narrow margin of safety. Care must be taken when using high concentrations of eugenol for induction, because ventilatory failure may occur rapidly. In addition, analgesic properties of eugenol are unknown. (*Am J Vet Res* 2001;62:337–342)

ported, housed in aquaria, and kept by hobbyists. Anesthetic agents also are essential to enable medical evaluation of fish. The use of chemicals introduced into water baths for the purpose of anesthetizing fish has a long history, and practical applications of anesthetic usage involve a number of clinical, management, and husbandry contexts. There have been few controlled systematic investigations of efficacy and physiologic effects for many of these chemicals, and there is a need for more complete and concise ranges of safe and effective concentrations or dosages of anesthetic agents for fish.^{1,2}

Tricaine methanesulfonate is a water-soluble benzocaine-derivative anesthetic used in fish and amphibian species. It is considered the most commonly used anesthetic for fish worldwide.³⁻⁹ It is a water-soluble powdered substance, which is typically buffered with sodium bicarbonate to reduce its acidic properties, and it is commonly delivered in a water bath. It is the only FDA-approved anesthetic for use in fish intended for use as food, with a required 21-day withdrawal period prior to human consumption. There is evidence that chronic exposure in fish, amphibians, and humans can cause reversible retinal deficits.¹⁰

Clove oil recently has become a commonly used anesthetic that can serve as an alternative to tricaine methanesulfonate in commercial (nonfood) fish and fish industries in the United States and Japan.¹¹⁻¹⁴ The active ingredient of clove oil is eugenol (4-allyl-2-methoxyphenol), a phenolic compound. Many aquaculturists and clinicians add it directly to water baths to achieve the desired effect. It is an attractive anesthetic substance for use in fish, because it is commercially available and inexpensive. As a food additive, clove oil is classified by the FDA to be a substance that is generally regarded as safe.¹³ However, the FDA considers the use of clove oil as an anesthetic in fish to be an unapproved use, and it should not be used in fish destined for human consumption. Eugenol also is on the US Environmental Protection Agency's list of exempted least-toxic pesticide active ingredients.¹⁵ The

An efficient, predictable, and safe method for anesthesia of fish is needed, because an increasing number of valuable species are being cultured, trans-

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safety and efficacy of clove oil as an anesthetic agent in fish have not been systematically evaluated. It has been suggested that clove oil may have antipyretic, antibacterial, antifungal, and antioxidant properties as well as anesthetic, myorelaxant, and anticonvulsant activities in laboratory animals.^{16,17} Oral ingestion or IV administration of clove oil can cause CNS depression, urinary abnormalities, and metabolic acidosis in rodents and humans¹⁸; pulmonary edema in humans, dogs, and rodents¹⁹; hepatocellular necrosis in rodents²⁰; permanent localized anesthesia in humans²¹; and death in rodents and rabbits.¹⁸

Objectives of the study reported here were to compare the anesthetic efficacy of eugenol and tricaine methanesulfonate in fish, using 3 concentrations of each agent, by measuring specific behavioral effects and physiologic variables prior to and during anesthesia and to assess the analgesic potential of the 2 agents by observing behavioral reactions to percutaneous puncture with a hypodermic needle.

Materials and Methods

Animals—Fifteen captive-raised red pacu (*Piaractus brachyomus*) of uniform age (approx 4 years old) and similar weight (mean \pm SD, 603.3 \pm 61 g; range, 500 to 727 g) were used in the study. Red pacu were selected, because they are relatively large, hardy, and popular ornamental fish in the United States and are a valuable food source in South and Central America. Prior to the study, fish were maintained in groups in 1,500-L aquariums in an indoor facility; fish had been maintained in this facility for > 3 years. For the purpose of the study, each fish was housed separately in a 60-L aquarium and acclimated to the experimental aquaria for a minimum of 2 weeks. The aquaria shared a common recirculating water system and source of water. Environmental conditions were monitored and maintained within a narrow range of values (Appendix 1). A 12-hour-light:12-hour-dark cycle was maintained. Fish were fed a pelleted diet^a formulated for hybrid striped bass once daily. All fish were healthy prior to and throughout the study.

Study design—A within-subjects complete crossover experimental design was used. Each fish was exposed to each of 6 aqueous anesthetic concentrations (tricaine methanesulfonate^b at 50, 100, and 200 mg/L; pharmaceutical-grade eugenol^c at 50, 100, and 200 mg/L). Each fish was allowed a minimum washout period of 2 weeks between each anesthetic exposure. Because it is incompletely soluble in water, eugenol (1,000 mg/ml) was solubilized in 95% ethanol at a ratio of 1:9 (eugenol:ethanol), providing a final concentration of 100 mg/ml. Tricaine methanesulfonate was solubilized in deionized water and buffered with sodium bicarbonate, using a ratio of 1:1 (sodium bicarbonate:tricaine methanesulfonate powder), providing a final concentration of 10 mg/ml (pH 7.4).

Behavioral measures of anesthesia—Stages of anesthesia and recovery were monitored, and duration of each stage was recorded (Appendix 2).²² For practical purposes, a criterion of 600 seconds was established as a maximum duration from initial anesthetic exposure to induction (stage IV) and for recovery duration after each fish was returned to its home aquarium. Behavioral reactions to insertion of a hypodermic needle during collection of blood samples were documented prior to and during anesthesia to subjectively assess analgesia. All behavioral observations were evaluated and recorded by the same investigator (KKS) throughout the study.

Physiologic measurements—Immediately prior to and

within 60 seconds after induction, mixed venous-arterial blood samples (0.2 to 0.3 ml) were collected from the caudal vein or artery of each fish. This site is commonly used for collection of blood samples in many species of fish, but because of the proximity of the artery and vein, samples often are mixtures of venous and arterial blood. Using the blood samples, the following variables were measured, using a clinical analyzer^d: concentrations of sodium, potassium, glucose, and hemoglobin, Hct, pH, PCO₂, and PO₂. Respiratory rate, assessed by opercular movement, was recorded prior to and during each stage of anesthesia and recovery. Blood gas tensions were corrected for water temperature with the assumption that ambient water temperature and body temperature of each fish were equivalent.

Procedure—Fish were randomly assigned a sequence of anesthetic exposure on each day of data collection, and each fish was randomly assigned to a particular anesthetic concentration. We assumed that at least 1 fish would be exposed to each of the 6 treatment groups on each experimental day. Water used in the plastic containers was obtained from the water system in which the fish normally resided. A baseline respiratory rate was observed for each fish in its home aquarium. A fish was removed from its home aquarium, and a blood sample was collected by percutaneous puncture of the caudal vein or artery within 1 minute after removal from the tank, using a 25-gauge needle attached to a 1-ml syringe containing lyophilized lithium heparin.^e The fish then was placed in a 4-L experimental water bath inside an 8-L plastic bag^f equipped with an air stone, and the fish was allowed to acclimate for 2 to 3 minutes. During this acclimation period, the blood sample was evaluated, using the clinical analyzer.

After the acclimation period, a second baseline respiratory rate was recorded. Five minutes after a fish was placed in the experimental water bath, an anesthetic agent was added, and stages of anesthesia were recorded. When stage IV of anesthesia (complete lack of voluntary movement) or an anesthetic duration of 600 seconds was reached, the fish was removed from the anesthetic bath, a second blood sample was collected immediately from the caudal artery or vein, and the fish was returned to its home aquarium for recovery.

When we did not detect opercular movement in a fish within 5 minutes after reintroduction to anesthetic-free water, resuscitation measures were initiated. Resuscitated fish were manually restrained in a vertical position with a steady stream of water directed through the oral cavity and across the gills. All fish requiring resuscitation resumed spontaneous ventilation during the experiment. Fish that required resuscitation were automatically assigned the maximum duration for recovery (ie, 600 seconds).

Data analysis—Duration for each stage of anesthesia was recorded as the interval from initial exposure to the anesthetic until the end of each stage of anesthesia. Duration for each stage of recovery also was recorded, beginning with reintroduction of the fish to its home aquarium. Respiratory rates were expressed as number of opercular movements per minute. Behavioral reaction to needle insertion was expressed as a binary variable (0 = reaction not detected, 1 = observable reaction). Hemoglobin concentration was automatically calculated from the Hct by the clinical analyzer. Values of physiologic and behavioral variables were compared before and during anesthesia among the 6 treatments. Data were analyzed, using a computer software package.^g All data were normally distributed, as determined by use of the Kolmogorov-Smirnov test for comparing sample data distributions with expected data distributions.²³ None of the sample data sets had distributions that differed from expected normal distributions.

The Friedman test was used to determine differences

among the 6 treatment groups, and the Wilcoxon signed-rank test was used to evaluate pair-wise comparisons.²³ Differences of $P < 0.05$ were considered significant. All data were reported as mean \pm SD.

Results

Changes in behavioral variables were obvious in anesthetized fish. Mean induction times were significantly shorter for fish exposed to the 3 concentrations of eugenol, compared with times for the 3 concentrations of tricaine methanesulfonate (Fig 1). There was a clear linear pattern of decreasing induction time with

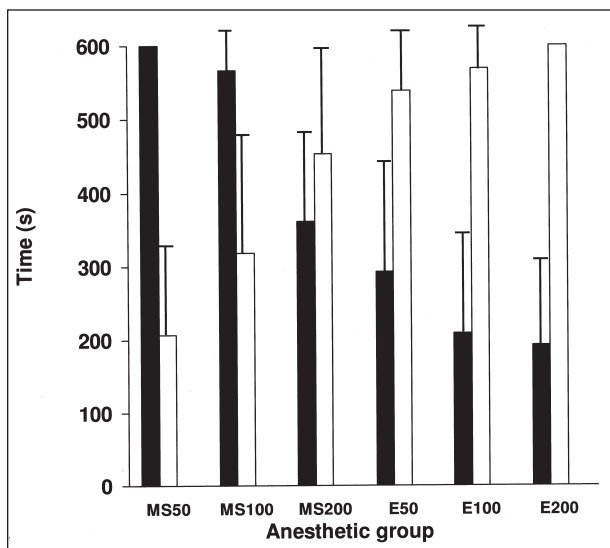


Figure 1—Mean \pm SD interval for induction (■) and recovery (□) of red pacu (*Piaractus brachypomus*) exposed to 6 anesthetic treatments involving tricaine methanesulfonate (50, 100, or 200 mg/L [MS50, MS100, and MS200, respectively] or eugenol (50, 100, or 200 mg/L [E50, E100, and E200, respectively])).

increasing concentration of either anesthetic, with the longest induction times for fish in the group exposed to 50 mg of tricaine methanesulfonate/L (600 ± 117.2 seconds) and the shortest for fish exposed to 200 mg of eugenol/L (186.3 ± 124.6 seconds.). All fish exposed to 50 mg of tricaine methanesulfonate reached the maximum value for induction of 600 seconds, indicating that none of the fish exposed to this concentration of tricaine methanesulfonate was induced. Time to recovery had the opposite pattern, with fish having a more rapid recovery following exposure to tricaine methanesulfonate, compared with recovery times after exposure to eugenol, and a clear linear pattern indicating a more rapid recovery time with lower concentrations of either anesthetic. None of the fish exposed to 200 mg of eugenol/L fully recovered from the anesthetic during the maximum 600 seconds allowed. Each fish had prolonged induction times during exposure to eugenol (100 or 200 mg/L), had a pattern of prolonged recovery times, and required postanesthetic resuscitation. Successful resuscitation efforts were initiated in 11 of 15 fish exposed to 200 mg of eugenol/L; these fish had complete and prolonged ventilatory failure. The remaining 4 fish exposed to 200 mg of eugenol/L recovered without intervention. Similarly, 6 of 15 fish exposed to 100 mg of eugenol/L required resuscitation, whereas the remaining 9 recovered uneventfully.

Reactions to cutaneous needle puncture were more consistent, and often more exaggerated, in fish exposed to eugenol, compared with reactions of fish exposed to tricaine methanesulfonate. Fourteen of 15 fish (100 or 200 mg/L) reacted to needle puncture, and 10 of 15 fish exposed to 50 mg of eugenol/L reacted. This was in sharp contrast to fish exposed to tricaine methanesulfonate. Eight of 15 fish reacted to needle puncture when exposed to 50 mg of tricaine methanesulfonate.

Table 1—Mean \pm SD values for physiologic variables measured before and during anesthesia in pacu (*Piaractus brachypomus*) anesthetized with tricaine methanesulfonate (MS) and eugenol (E) at concentrations of 50, 100, and 200 mg/L for each anesthetic

Group	Glucose (mg/dl)	Sodium (mg/dl)	Potassium (mg/dl)	pH	Po ₂ * (mm Hg)	Pco ₂ * (mm Hg)	Hct (%)	Hemoglobin (g/dl)
MS 50								
Before	56.9 \pm 12.0	140.2 \pm 5.6	2.2 \pm 0.6	7.75 \pm 0.05	26.1 \pm 20.7	4.4 \pm 0.6	18.5 \pm 2.5	6.1 \pm 0.9
During	76.1 \pm 16.4	143.5 \pm 2.6	3.2 \pm 0.4	7.49 \pm 0.13	7.7 \pm 4.5	7.7 \pm 1.9	20.5 \pm 2.6	6.9 \pm 1.0
Change (%)	+24.6 \pm 8.6	+1.9 \pm 3.0	+28.3 \pm 21.8	-3.3 \pm 1.4	-55.4 \pm 33.9	+39.2 \pm 17.8	+9.7 \pm 10.1	+10.0 \pm 12.6
MS 100								
Before	58.7 \pm 7.4	140.9 \pm 4.3	2.6 \pm 0.5	7.74 \pm 0.11	24.1 \pm 26.2	4.3 \pm 0.9	18.9 \pm 3.1	6.3 \pm 1.1
During	74.9 \pm 14.9	142.1 \pm 3.3	3.1 \pm 0.3	7.44 \pm 0.11	3.9 \pm 0.7	9.1 \pm 3.0	20.9 \pm 2.9	7.3 \pm 1.2
Change (%)	+19.4 \pm 15.7	+0.9 \pm 2.4	+15.8 \pm 14.7	-3.9 \pm 1.2	-65.5 \pm 26.8	+48.1 \pm 18.0	+9.1 \pm 11.3	+10.3 \pm 12.9
MS 200								
Before	58.7 \pm 11.9	140.9 \pm 3.5	2.5 \pm 0.5	7.72 \pm 0.06	26.8 \pm 22.5	4.6 \pm 1.1	20.3 \pm 3.8	6.9 \pm 1.4
During	73.4 \pm 15.5	141.8 \pm 4.3	3.1 \pm 0.4	7.45 \pm 0.10	3.93 \pm 0.7	8.6 \pm 1.3	22.7 \pm 3.0	7.7 \pm 1.0
Change (%)	+19.1 \pm 9.8	+0.6 \pm 1.5	+18.2 \pm 11.5	-3.5 \pm 0.8	-76.3 \pm 14.5	+46.1 \pm 7.9	+10.8 \pm 10.1	+10.8 \pm 11.8
E 50								
Before	56.0 \pm 7.7	141.4 \pm 3.0	2.4 \pm 0.4	7.73 \pm 0.06	25.2 \pm 16.6	4.4 \pm 0.8	18.9 \pm 3.1	6.3 \pm 1.1
During	76.8 \pm 15.0	143.1 \pm 4.9	3.3 \pm 0.3	7.41 \pm 0.05	3.5 \pm 0.5	8.9 \pm 1.0	21.4 \pm 2.8	7.2 \pm 1.1
Change (%)	+25.7 \pm 10.0	+1.2 \pm 1.7	+27.2 \pm 12.0	-4.2 \pm 0.6	-77.8 \pm 15.8	+50.4 \pm 9.3	+16.0 \pm 11.7	+15.9 \pm 13.9
E 100								
Before	58.7 \pm 12.7	140.1 \pm 5.4	2.2 \pm 0.4	7.75 \pm 0.06	33.1 \pm 36.8	4.1 \pm 0.4	19.0 \pm 3.2	6.3 \pm 1.1
During	72.3 \pm 20.3	143.3 \pm 3.3	3.5 \pm 0.3	7.44 \pm 0.09	3.93 \pm 1.0	8.4 \pm 1.7	20.6 \pm 3.1	7.1 \pm 1.1
Change (%)	+17.2 \pm 9.9	+2.2 \pm 4.0	+35.2 \pm 13.1	-4.0 \pm 1.1	-73.1 \pm 28.5	+49.4 \pm 10.7	+7.0 \pm 14.8	+9.9 \pm 13.5
E 200								
Before	55.2 \pm 5.7	141.8 \pm 1.9	2.5 \pm 0.5	7.81 \pm 0.30	20.8 \pm 23.97	4.6 \pm 0.9	20.1 \pm 3.2	6.7 \pm 1.0
During	66.0 \pm 10.7	141.5 \pm 3.7	3.5 \pm 0.4	7.50 \pm 0.08	5.2 \pm 2.1	7.4 \pm 1.4	19.3 \pm 2.1	6.3 \pm 0.6
Change (%)	+15.2 \pm 10.3	-0.3 \pm 2.5	+28.4 \pm 13.0	-3.8 \pm 3.2	-59.4 \pm 26.9	+37.7 \pm 13.2	-4.8 \pm 17.8	-7.0 \pm 17.7

Percentage change is indicated as an increase (+) or decrease (-) of values obtained before anesthesia, compared with values obtained during anesthesia. *The Po₂ and Pco₂ values were determined on mixed venous-arterial blood samples.

sulfonate/L, but only 3 of 15 and 1 of 15 reacted when exposed to 100 or 200 mg of tricaine methanesulfonate/L, respectively. Interestingly, reactions to needle puncture were common at the highest concentrations of eugenol, which caused complete ventilatory failure in many of the fish.

Anesthesia induced with tricaine methanesulfonate or eugenol caused physiologic changes in the fish (Table 1). Mean blood glucose concentrations increased significantly when fish were anesthetized. Mean blood glucose concentrations did not differ among anesthetic concentrations for each anesthetic, regardless of type or concentration of anesthetic.

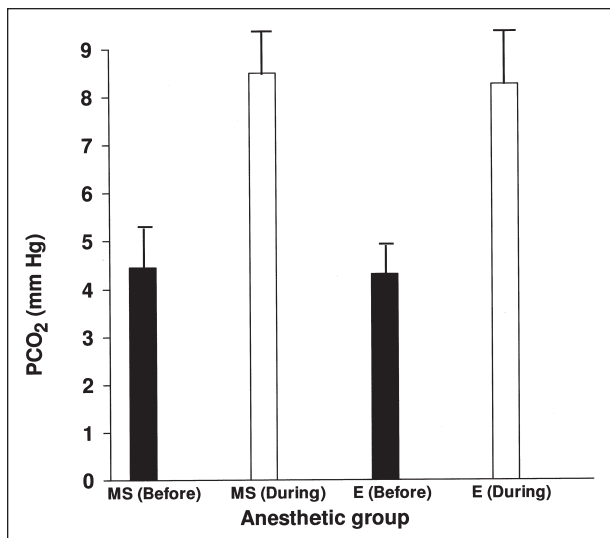


Figure 2—Mean \pm SD PCO_2 for mixed arterial-venous blood samples obtained before and during anesthesia in pacu exposed to tricaine methanesulfonate (MS) and eugenol (E) at each of 3 concentrations/anesthetic. Within each anesthetic, values from before and during anesthesia differ significantly ($P = 0.01$).

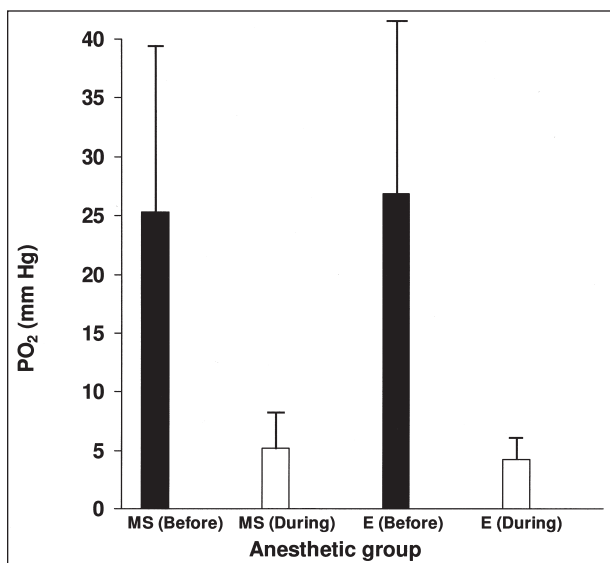


Figure 3—Mean \pm SD PO_2 for mixed arterial-venous blood samples obtained before and during anesthesia in pacu exposed to tricaine methanesulfonate (MS) and eugenol (E) at each of 3 concentrations/anesthetic. Within each anesthetic, values from before and during anesthesia differ significantly ($P = 0.01$).

Similarly, mean blood sodium and potassium concentrations increased, but not significantly, when fish were anesthetized.

Hematocrit and hemoglobin concentration changed in parallel, because hemoglobin was calculated from the Hct value. When treatment groups were combined and values for fish exposed to tricaine methanesulfonate were compared with those for fish exposed to eugenol, hemoglobin concentration and Hct significantly increased when fish were anesthetized. Hematocrit and hemoglobin concentrations did not differ significantly among anesthetic concentrations within each anesthetic.

Blood pH significantly decreased in all anesthetized fish, regardless of anesthetic or concentration used. There was a consistent and significant ($P = 0.01$) increase in mean PCO_2 of mixed venous-arterial blood in anesthetized fish, regardless of anesthetic (Fig 2; Table 1). Mean percentage change in PCO_2 was not significantly different between anesthetics.

Mean PO_2 of mixed venous-arterial blood significantly ($P = 0.01$) decreased when fish were anesthetized, regardless of type or concentration of anesthetic (Fig 3; Table 1). This physiologic variable had more variation than other variables examined, which was expected because of the potential for collection of mixed venous-arterial blood samples. Mean percentage change in PO_2 did not differ significantly between anesthetics.

Discussion

The margin of safety for eugenol exposure was much narrower, compared with that for tricaine methanesulfonate. Most fish exposed to high concentrations of eugenol required resuscitation, and the risk of ventilatory failure increased with increasing dose of eugenol. However, fish that had rapid inductions at high concentrations of eugenol were more likely to recover uneventfully. This outcome may be a function of increased duration of exposure or the physical properties of eugenol oil. Because eugenol is an oil, it has the physical properties whereby it coats anatomic structures, which may be particularly important when it persists on gill epithelia. Consequently, there is prolonged exposure to the chemical and the potential for sustained anesthetic effects. This was supported by our data, which revealed that fish that had the longest inductions at high concentrations of eugenol were exposed to the chemical for a longer time and were more likely to require resuscitation.

An alternative hypothesis to explain the ventilatory failure and medullary collapse in some fish is that eugenol has neurotoxic and hepatotoxic properties in fish similar to those described in mammals.^{18,19,21,24} Toxicity testing has been conducted on juvenile coho salmon and rainbow trout after prolonged exposure to eugenol, and the lethal concentration has been determined.¹⁵ Pathologic changes and fatalities have been described in several mammalian species after exposure to or ingestion of eugenol.¹⁸⁻²¹ Clove oil is a known irritant when applied topically to laboratory rodents, rabbits, and dogs, and it causes inflammation and local cellular necrosis.¹⁶

In the study reported here, fish reacted more con-

sistently to cutaneous needle puncture when anesthetized with eugenol, compared with reactions when anesthetized with tricaine methanesulfonate. Paradoxically, as the concentration of eugenol increased, reactions to needle puncture increased. Because inductions were rapid at high concentrations of eugenol and exposure time to the anesthetic was shorter, it could be speculated that longer exposure times to eugenol may have been required for analgesic properties to be manifested. It also could be speculated that fish exposed to tricaine methanesulfonate perceived this noxious stimulus but were unable to respond.

Although quantitative assessment of the cardiovascular system was not part of our study, cardiovascular function may have been compromised, particularly in fish exposed to high concentrations of eugenol. Collection of 0.3 ml of blood by means of percutaneous venipuncture was subjectively assessed to be more difficult in fish anesthetized with eugenol than in those anesthetized with tricaine methanesulfonate. This observation, along with the higher prevalence of resuscitation in fish anesthetized with eugenol, suggests hemodynamic instability or insufficient oxygen loading or delivery in these fish. It is interesting to speculate whether these possible hemodynamic alterations may have been associated with decreased arterial blood pressure.

Physiologic changes associated with exposure to eugenol such as blood glucose concentration, blood pH, Hct, hemoglobin concentration, and blood gas tensions were generally indistinguishable from physiologic changes associated with exposure to tricaine methanesulfonate. Blood glucose concentrations increased in fish exposed to tricaine methanesulfonate or eugenol. These increases were evident even in fish exposed to the anesthetic bath for relatively short periods (ie, < 3 minutes). These results are consistent with most of the reports on anesthesia of fish^{6,25,26}; in those studies, single or multiple anesthetic events reportedly caused increases in blood glucose concentrations over time. However, transient decreases in blood glucose concentration immediately after induction have led some investigators to argue that commonly used anesthetics such as tricaine methanesulfonate do not alter carbohydrate metabolism in fish.^{6,26} Analysis of our data suggested that blood glucose concentrations increase rapidly after induction, even without prolonged exposure to the anesthetic, and we hypothesize that similar to the situation in many mammalian species, catecholamine-induced gluconeogenesis is the most likely physiologic mechanism for this phenomenon.

Consistent but modest increases in blood sodium and potassium concentrations were observed when fish were exposed to either anesthetic. This could be attributed to retention of sodium or potassium by the gills to compensate for loss of hydrogen ions,²⁷ muscle contractions causing movement of sodium or potassium ions out of myocytes into plasma,²⁷ release of catecholamines into the circulation, which stimulated Na⁺-H⁺ exchange in RBC to maintain constant pH,²⁷ or artifactual increases caused by RBC lysis during the collection of blood samples. The modest electrolyte alterations in the fish also could be explained by normal ion diffusion in the

gills, because a flux of sodium and potassium ions of this small magnitude could happen with many physiologic or environmental manipulations.²⁸

Hematocrit values often are difficult to interpret in fish when multiple blood samples are collected over time because of the potential for a decline in Hct as a result of acute blood loss.^{6,8,29} In the study reported here, Hct and hemoglobin concentration increased with anesthetic exposure despite collection of small blood samples. Because fish were exposed to a maximum anesthetic duration of 600 seconds, the effect on Hct over longer periods and for collection of multiple blood samples was not evaluated. The mechanism of action contributing to an increase in Hct is undetermined, but the rapidity of the response seems to support a hypothesis of splenic contraction, causing an increase in RBC volume. The Hct and hemoglobin concentration of fish exposed to the highest concentration of eugenol did not always increase. This may have been a function of the brief exposure to the anesthetic bath, which may have been of insufficient duration to reliably affect the dynamics that would alter Hct.

Fish had a decrease in PO₂ and a concomitant increase in PCO₂ of mixed venous-arterial blood when anesthetized, regardless of anesthetic or concentration used. Along with hypoxia and hypercapnia, blood pH decreased, which was consistent with respiratory acidosis.

The use of clove oil for immobilizing fish is gaining popularity, because it is considered effective and is readily available as an inexpensive over-the-counter preparation (most commonly as 84% eugenol). However, much of the information regarding its effectiveness has come from anecdotal reports, and there are few reports in which investigators systematically evaluated its physiologic, behavioral, and pathologic effects. In the study reported here, we found 100% eugenol to be an effective agent for immobilization of pacu, characterized by rapid induction and prolonged recovery times, compared with tricaine methanesulfonate. However, pacu exposed to high (100 or 200 mg/L) concentrations of eugenol must be monitored carefully, because ventilatory failure and medullary collapse may occur rapidly. We did not evaluate toxicologic or long-term physiologic effects of eugenol on these fish, but future studies addressing these issues would be of considerable benefit. In addition, it is important to reiterate that clove oil and eugenol are not approved for use in fish intended for human consumption. The search for effective anesthetics suitable for use in fish intended for human consumption without an excessive withdrawal time remains a priority for aquacultural veterinarians. As novel chemicals for immobilization of fish are discovered, evaluation of physiologic effects, toxicosis, and anesthetic efficacy must lend credence to the use of the chemicals. It is imperative that the efficacy and safety of immobilization chemicals for use in fish are systematically, qualitatively, and quantitatively evaluated.

^aHybrid-striped bass pellet, Southern States Cooperative Inc, Richmond, Va.

^bFinquel, Argent Chemical Labs, Redmond, Wash.

[†]Eugenol, Sigma Chemical Co, St Louis, Mo.

[‡]iSTAT Chemical analyzer, Sensor Devices Inc, Waukesha, Wis.

[§]Arterial blood sampler, Chiron Diagnostics Corp, East Wapole, Mass.

[¶]Zip-Loc, DowBrands LP, Indianapolis, Ind.

^{**}Statview software package, SAS Institute, Cary, NC.

Appendix 1

Assessments of water quality for experimental aquaria housing red pacu (*Piaractus brachypomus*)

Variable	Range	Mean \pm SD
Temperature (C)	20–23	21.5
pH	5.5–7.0	5.89 \pm 0.49
Total ammonia (mg/L)	0.7–2.8	1.49 \pm 0.62
Nitrite (mg/L)	0–0.04	0.01 \pm 0.02
Nitrate (mg/L)	5–15	8.18 \pm 3.37
Alkalinity (mEq/L)	35	35 \pm 0
Dissolved O ₂ (%)	> 100%	> 100%

Appendix 2

Behavioral criteria used for evaluating stages of anesthesia and recovery in pacu*

Stages of anesthesia	
I	Onset of erratic opercular movement
II	Partial loss of equilibrium; continued efforts to right itself
III	Total loss of equilibrium; no efforts to right itself
IV	Induction; total loss of voluntary movement and reactivity
V	Medullary collapse; total cessation of opercular movement
Stages of recovery	
I	Reappearance of opercular movement
II	Partial recovery of equilibrium; efforts to right itself
III	Full recovery of equilibrium; successful righting
IV	Response to external stimuli (tapping on glass of aquarium)
V	Behavioral recovery; normal swimming activity

*Modified from Stoskopf MK. Clinical pathology. In: Stoskopf MK, ed. *Fish medicine*. Philadelphia: WB Saunders Co, 1993:81.

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