# Response of Southern Redbelly Dace to Clove Oil and MS-222: Effects of Anesthetic Concentration and Water Temperature

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*Abstract:* The anesthetic properties of clove oil and tricaine methanesulfonate (MS-222) were tested in a laboratory setting on the southern redbelly dace *(Phoxinus erythrogaster),* a small cyprinid common to upland streams of the Mississippi River basin. We used southern redbelly dace as a surrogate species to indicate the lowest, most effective anesthetic level for our work with the closely related blackside dace *(Phoxinus cumberlandensis),* a federally protected species. Concentrations of 20, 40, and 60 mg $L^{-1}$ clove oil and 20, 40, and 60 mg $L^{-1}$ MS-222 were used to anesthetize southern redbelly dace at water temperatures of 11, 17, and 21 C, representing a natural range of temperatures encountered in research streams from spring through autumn. For clove oil, induction rates were dependent on dose, temperature, and the interaction between these two variables. Recovery rates, on the other hand, were dependent only on temperature (quicker recovery at warmer temperatures). Clove oil concentrations of 40 and 60 mg $L^{-1}$ proved to be effective at all three temperatures, inducing stage 4 anesthesia (total loss of equilibrium) at mean times $<3$ min while allowing stage 4 recovery (reaction in response to external stimuli) in $<5$ min. MS-222 concentrations of 20 and 40 mg $L^{-1}$ were ineffective for producing anesthesia and the 60 mg $L^{-1}$ concentration was only slightly effective. No mortalities were observed with either anesthetic. The 40 mg $L^{-1}$ clove oil concentration was the lowest effective dose at all three temperatures; thus, we recommend this concentration for blackside dace during typical spring, summer, and autumn conditions. Our recent *in situ* use of 40 mg $L^{-1}$ clove oil has confirmed its effectiveness and apparent safety for blackside dace.

*Key Words:* anesthesia, clove oil, dace, MS-222, *Phoxinus*

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Clove oil is receiving increased attention regarding its efficacy as a fish anesthetic in a variety of settings (e.g., Peake 1998, Taylor and Roberts 1999, Griffiths 2000). The oil is derived from clove trees *(Syzygium aromaticum,* also known as *Eugenia aromatica* or *E. caryophyllus)* and contains the phenols eugenol, eugenol acetate, and kariofilen-5 (Soto and Burhanuddin 1995, Taylor and Roberts 1999, Cho

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and Heath 2000). Clove oil has several advantages over other anesthetics, including its safety and low cost, making it favorable in certain fisheries applications (e.g., Anderson et al. 1997, Munday and Wilson 1997, Wagner et al. 2003).

Tricaine methanesulfonate, MS-222, has been perhaps the most commonly used fish anesthetic (Marking and Meyer 1985). MS-222 is only one of two anesthetics approved for aquaculture purposes (Taylor and Roberts 1999, Cho and Heath 2000) and its effects are known for a variety of fish species. A primary disadvantage is that a post-anesthesia withdrawal period is recommended or required; specifically, fish anesthetized with MS-222 must be held for up to 21 d before human consumption or release into the wild (Anderson et al. 1997, Cho and Heath 2000). Other disadvantages are its expense and suspected carcinogenic properties (Cho and Heath 2000).

Many studies comparing clove oil and MS-222 have focused on salmonids (e.g., Anderson et al. 1997, Keene et al. 1998, Cho and Heath 2000) and cyprinids have received relatively little attention, other than the work by Endo et al. (1972) and Hikasa et al. (1986), and no data are available for North American *Phoxinus* species. More information is needed on the effects of clove oil, in particular, on cyprinids and other freshwater nongame species. Such knowledge could be especially useful for the management of endangered fishes.

In the present study, we employ the southern redbelly dace *(Phoxinus erythrogaster)* as a surrogate species to indicate the lowest, most effective anesthetic level for our work with the threatened blackside dace *(P. cumberlandensis).* The blackside dace is a federally-listed species endemic to small upland streams in the upper Cumberland River system of eastern Kentucky and Tennessee (Eisenhour and Strange 1998). The two species are syntopic or sympatric in certain streams. To assist blackside dace recovery efforts, we are concurrently determining its movement patterns, population densities, and habitat affinities, and some of our research activities (marking with tags or fin clips) require anesthesia. Our goal was to determine an anesthetic dose that could be applied in the field during multiple seasons of the year at a range of temperatures.

Thus, our objective here was to determine the effect of anesthetic type, anesthetic concentration, and water temperature on southern redbelly dace anesthesia and recovery in a laboratory setting. We then used the response of the surrogate species to establish a field protocol for the species of concern.

## Methods

We used a 110-volt AC backpack electrofishing unit to collect 143 southern redbelly dace from Mill Creek, Putnam County, Tennessee, on 19 January 2003. Water temperature in Mill Creek was 4 C and surface ice was present along the stream's margins. Dace were transported to the laboratory in stream water that was warmed to 11 C at a rate of 1 C $h^{-1}$. All fish were then transferred to six 38-L glass aquaria positioned in a water-bath raceway supplied with dechlorinated tapwater chilled to 11 C. After 24 h, two-thirds of the fish were acclimated in 19-L plastic buckets at a rate of 1 C $h^{-1}$ to 17 C and then transferred to six additional aquaria bathed in a second race-

way. After another 24 h, half of these fish were acclimated 1 C $h^{-1}$ to 21 C and then transferred to six more aquaria in a third raceway. Aquarium heaters were placed in each aquarium in the third raceway to maintain the 21 C temperature. Dace were acclimated to their respective temperatures and aquaria for 5 d before anesthetic testing. The laboratory ambient air temperature ranged 17–20 C and lights were operated on a 10-h light, 14-h dark photoperiod.

The dace were maintained at densities of 6–9 fish per 38 L and were fed chopped bloodworms to satiation once every 48 h. In each aquarium a cored masonry brick was placed upright on its side for cover, and an air-stone sponge provided aeration and filtration. Values of pH ranged 7.0–7.9 throughout the pre-experimental period. Ammonia, dissolved oxygen, and temperature were checked at least once daily and partial water changes were conducted if ammonia levels exceeded 0.5 ppm $NH_3$. Mean ammonia levels before the experiment were 0.01 (11 C aquaria), 0.24 (17 C aquaria), and 0.15 ppm $NH_3$ (21 C aquaria). Mean dissolved oxygen levels ranged 5.6–7.2 before and 7.0–9.7 mg $L^{-1}$ $O_2$ during the experiment, and mean temperatures were always within 0.6 C of the respective nominal values.

Clove oil (Sigma Aldrich Co.) was first mixed with ethanol to achieve a 1:10 clove oil:ethanol stock solution as described by Anderson et al. (1997) and Keene et al. (1998). Ethanol was used to facilitate clove oil's solubility in water. Clove oil concentrations of 20, 40, and 60 mg $L^{-1}$ were then prepared by mixing 1.6, 3.2, or 4.8 g of the stock solution into 8 L of water. For example, 1.6 g of the stock solution into 8 L of water yielded a 20 mg $L^{-1}$ clove oil concentration. MS-222 (Sigma Aldrich Co.) concentrations of 20, 40, and 60 mg $L^{-1}$ were prepared by adding 160, 320, or 480 mg, respectively, of MS-222 and an equal weight of sodium bicarbonate buffer, $NaHCO_3$, to 8 L of water.

Each anesthetic concentration was mixed in a plastic 19-L aquarium <30 min before testing. A screen (0.5-mm mesh) divided the aquarium into two chambers of roughly equal size, allowing up to two fish to be anesthetized at once. The recovery aquarium also held 8 L of water and was divided by a similar partition. Water in the anesthesia and recovery aquaria was aerated before but not during testing, and changed 100% in between treatments.

The six anesthetic concentrations (20 mg $L^{-1}$ clove oil, 40 mg $L^{-1}$ clove oil, 60 mg $L^{-1}$ clove oil, 20 mg $L^{-1}$ MS-222, 40 mg $L^{-1}$ MS-222, and 60 mg $L^{-1}$ MS-222) were tested at a different temperature (11, 17, or 21 C) each day, yielding a total of 18 treatments. The order of treatments was determined with a random number table on the three test days, as was the order of aquaria within each raceway. Dace in the 11 C raceway were tested on the first day (24 January 2003), 17 C dace were tested on the second day (25 January), and 21 C dace were tested on the third day (26 January), such that all dace were held at their pre-test temperature for a constant length of time, 5 d. Fish were not fed in the 48-h period prior to testing. For each treatment, 6–9 fish in a single 38-L aquarium were transferred to 8 L of continuously aerated water in a plastic bucket where they were captured individually for anesthesia. Only the first five fish caught were anesthetized; the remaining 1–4 individuals were clipped without anesthesia on the lower corner of the caudal fin and returned to their original 38-

L aquarium. These 1–4 individuals were minimally handled and served as controls when monitoring post-anesthesia survival and behavior.

Times to stage 3 (initial loss of equilibrium) and stage 4 anesthesia (total loss of equilibrium; Summerfelt and Smith 1990, Cho and Heath 2000) were timed with a hand-held stopwatch (nearest sec) for each individual fish. After reaching stage 4 anesthesia, the fish was quickly weighed on an electronic balance (nearest 0.01 g), measured (nearest mm total length, TL), and the upper corner of the caudal fin was clipped. The fish was then transferred to the recovery aquarium and times to stage 3 (total recovery of equilibrium) and stage 4 recovery (reaction in response to external stimuli; Summerfelt and Smith 1990, Cho and Heath 2000) were also measured with a hand-held stopwatch (nearest sec). Recovered fish were then returned to their original 38-L aquarium to join the control fish. If two or more fish in a given treatment failed to reach stage 3 anesthesia within 30 min, then no more fish were anesthetized for that treatment. This occurred in most of the 20 and 40 mg $L^{-1}$ MS-222 treatments; in fact, we did not even attempt to anesthetize dace at the 20 mg $L^{-1}$ MS-222 concentration at 11 C. We considered a desirable anesthesia time to be <3 min and recovery time to be <5 min (Marking and Meyer 1985).

Lengths of experimental fish ranged 34–80 mm TL (mean ± SD = 62 ± 12 mm TL) and did not differ significantly among treatments (ANOVA; $F = 0.48$, $P = 0.918$). Weights ranged 0.35–4.46 g (2.09 ± 1.02 g) and also did not differ among treatments (ANOVA; $F = 0.60$, $P = 0.829$). Post-experimental water quality and survival were checked daily for 3–5 d. No mortalities, infections, or unusual behaviors were noted for any control or experimental fish during this observation period.

Clove oil stage 4 anesthesia and recovery times were analyzed separately with two-way ANOVAs, with sources of variation represented by temperature, concentration, temperature x concentration interaction, and error. Treatment mean comparisons were conducted using Tukey's Studentized Range Test. MS-222 data did not require analysis for the 20 and 40 mg $L^{-1}$ treatments, but the 60 mg $L^{-1}$ treatments were analyzed with separate one-way ANOVAs for stage 4 anesthesia and recovery. Statistical significance was evaluated at the 0.05 level in all cases.

## Results

The 40 and 60 mg $L^{-1}$ clove oil concentrations produced stage 4 anesthesia in southern redbelly dace within 3 min at all three test temperatures, but the 20 mg $L^{-1}$ concentration did not (Fig. 1, Table 1). Both water temperature and clove oil concentration had a significant effect on time-to-anesthesia ($F_{temp} = 15.61$, $F_{conc} = 78.64$; $P < 0.0001$), and there was a significant interaction between temperature and concentration ($F_{temp\ X\ conc} = 3.37$, $P = 0.019$). As expected, fish at the two higher clove oil concentrations succumbed to stage 4 anesthesia faster than those at 20 mg $L^{-1}$; however, fish at 17 C reached total loss of equilibrium faster than those at 21 C, followed by those at 11 C. Yet, initial loss of equilibrium was most rapid at 21 C for the 40 and 60 mg $L^{-1}$ concentrations. The time between initial and total loss of equilibrium appeared to be more protracted at 21 C than at 17 C, especially for the 20 mg $L^{-1}$ concentration (Fig. 1).

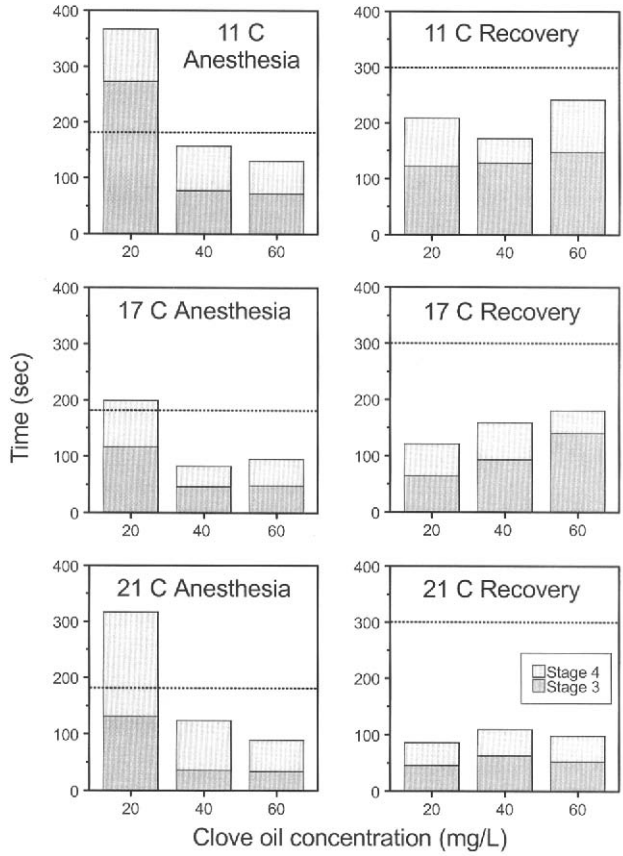


**Figure 1.** Southern redbelly dace time to anesthesia and time to recovery when exposed to 20, 40, or 60 mg $L^{-1}$ clove oil at three different water temperatures. Time to stage 3 anesthesia or recovery is shown by the darker portion of each bar, with time to stage 4 represented by the top of each bar. The dashed lines indicate the 3-min anesthesia and 5-min recovery thresholds recommended by Marking and Meyer (1985).

In contrast to anesthesia, there was no significant difference among the three clove oil concentrations in dace recovery times, but there was a temperature effect as dace reached stage 4 recovery faster as water temperatures increased ($F_{temp} = 13.30$, $P < 0.0001$). Mean recovery times were <5 min in all treatment combinations, with dace achieving stage 4 recovery in 3.5 min at 11 C ($N = 15$), 2.6 min at 17 C ($N = 15$), and 1.6 min at 21 C ($N = 15$; Fig. 1).

At the concentrations tested, MS-222 was a much less effective anesthetic for southern redbelly dace. All three concentrations failed to produce stage 4 anesthesia within 3 min, regardless of water temperature (Table 1). In fact, the 20 and 40 mg $L^{-1}$ concentrations typically required >30 min to induce stage 3 anesthesia and there-

**Table 1.** Times to anesthesia and recovery for southern redbelly dace exposed to clove oil or MS-222 at three different water temperatures. Values are mean ± SD.

| Water temperature (C) | Anesthetic concentration (mg $L^{-1}$) | Time to anesthesia (sec) | | Time to recovery (sec) | |
|---|---|---|---|---|---|
| | | Stage 3 | Stage 4 | Stage 3 | Stage 4 |
| | | Clove oil treatments | | | |
| 11 | 20 | 273 ± 67 | 367 ± 68 | 122 ± 95 | 209 ± 142 |
| | 40 | 77 ± 27 | 157 ± 37 | 128 ± 22 | 172 ± 27 |
| | 60 | 72 ± 16 | 130 ± 42 | 147 ± 23 | 242 ± 58 |
| 17 | 20 | 116 ± 23 | 199 ± 35 | 64 ± 40 | 121 ± 41 |
| | 40 | 46 ± 14 | 81 ± 17 | 93 ± 14 | 159 ± 28 |
| | 60 | 48 ± 9 | 94 ± 19 | 140 ± 48 | 180 ± 39 |
| 21 | 20 | 131 ± 37 | 316 ± 72 | 46 ± 23 | 86 ± 35 |
| | 40 | 36 ± 9 | 123 ± 53 | 63 ± 27 | 109 ± 26 |
| | 60 | 34 ± 10 | 90 ± 35 | 52 ± 22 | 98 ± 20 |
| | | MS-222 treatments | | | |
| 11 | 20 | na[a] | na | na | na |
| | 40 | 403 ± 177 | nm[b] | 33 ± 38 | 76 ± 26 |
| | 60 | 262 ± 165 | 380 ± 160 | 38 ± 48 | 97 ± 47 |
| 17 | 20 | nm[c] | nm | nm | nm |
| | 40 | nm[c] | nm | nm | nm |
| | 60 | 274 ± 123 | 319 ± 97 | 32 ± 4 | 57 ± 20 |
| 21 | 20 | nm[c] | nm | nm | nm |
| | 40 | nm[c] | nm | nm | nm |
| | 60 | 342 ± 180 | 770 ± 168 | 23 ± 10 | 46 ± 25 |

a. This treatment was not attempted ("na") because of the results observed for other treatments during the laboratory trials.

b. At least two fish in this treatment required >30 min to reach this level of anesthesia; "nm" = not measured.

c. At least two fish in this treatment required >30 min to reach this level of anesthesia, and the treatment was abandoned.

fore most treatments at these levels were abandoned. At 60 mg $L^{-1}$, however, stage 3 anesthesia was reached in 4–6 min and stage 4 in 5–13 min. Temperature appeared to have a minimal effect on initial loss of equilibrium, but 21 C dace took longer to reach stage 4 anesthesia than did 11 C or 17 C dace ($F = 14.11$, $P = 0.0007$). Interestingly, as with clove oil, this time between stage 3 and 4 anesthesia was protracted at 21 C. Dace recovery was rapid and more predictable than induction at 60 mg $L^{-1}$ MS-222, averaging 1.6 min at 11 C ($N = 5$), 1.0 min at 17 C ($N = 5$), and 0.8 min at 21 C ($N = 5$); although recovery times tended to be reduced with increasing temperature, this trend was not significant ($F = 3.27$, $P = 0.074$). MS-222 recovery times were generally shorter than those observed for dace exposed to clove oil.

## Discussion

The anesthetic response of southern redbelly dace to clove oil varied with both the anesthetic concentration and the water temperature, yet recovery rates were influenced mostly by temperature. In general, induction time decreased as temperature and dose increased (but see discussion of interaction below), and recovery time de-

creased with increasing temperature. Endo et al. (1972) and Hikasa et al. (1986) also noted a similar temperature pattern for other cyprinids (goldfish, *Carassius auratus;* common carp, *Cyprinus carpio*) and non-cyprinids (rainbow trout, *Oncorhynchus mykiss;* medaka, *Oryzias latipes*) where anesthesia and recovery required longer times at 5 and 10 C than at 20 or 25 C. These trends also generally mimic those observed by Woolsey et al. (2004) for steelhead fry, *O. mykiss,* exposed to 25, 50, and 100 mg $L^{-1}$ clove oil at 11, 15, and 20 C.

Clove oil was much more effective than matched concentrations of MS-222 for inducing southern redbelly dace anesthesia in a short time period, yet recovery times were 2–3 times longer for clove oil than for MS-222. A faster-anesthesia pattern for clove oil vs. MS-222 also has been reported for juvenile rainbow trout at 9 C, at least for matched doses <80 mg $L^{-1}$ (Keene et al. 1998). In addition, the trout took 6–10 times longer to recover with clove oil than with MS-222, which might be explained by the 9 C cold water; clove-oil recovery times were hastened by increasing water temperature in our study. Our average clove-oil recovery time, 1.5–4 min, was similar to that reported for juvenile and adult rainbow trout (3 min at 11 C; Anderson et al. 1997), but it was more rapid than has been reported for common carp (7–12 min at 10 C, 5–11 min at 20 C; Hikasa et al. 1986), or for juvenile rainbow trout in a different study (12–14 min at 9 C; Keene et al. 1998).

An unexpected result of our study was the significant interaction between temperature and clove-oil dose noted during anesthesia. It is not clear why stage 4 anesthesia would be achieved sooner at 17 C rather than at 21 C. However, the time delay between stage 3 and 4 anesthesia across concentrations was more evident at the higher temperature (Fig. 1), thereby contributing to this interaction. Hikasa et al. (1986) also observed a similar stage 3-to-4 time delay with common carp exposed to a 25 mg $L^{-1}$ clove-oil dose at 20 C, but this protraction was absent at 50 and 100 mg $L^{-1}$ doses at 20 C, and also absent for all three doses at 10 C. We also should note that southern redbelly dace at 17 C by coincidence were exposed to slightly higher pre-experimental mean ammonia levels, 0.24 ppm $NH_3$, and slightly lower experimental mean dissolved oxygen levels, 7.0 mg $L^{-1}$ $O_2$, than dace at 21 C for clove oil treatments; the contribution of this water-quality difference to the observed interaction is unknown.

Clove oil emerged from this study as the preferable anesthetic for southern redbelly dace, although concentrations of MS-222 higher than 60 mg $L^{-1}$ were not examined. Clove oil's advantages include consistent anesthesia and recovery times within Marking and Meyer's (1985) guidelines, efficacy at a range of water temperatures, lower expense, and higher user safety. In the field we have used the 40 mg $L^{-1}$ clove oil concentration to anesthetize >1400 blackside dace for elastomer tagging and fin clipping at stream temperatures ranging from 4 to 19 C. This concentration has worked well at these temperatures and blackside dace are responding much like southern redbelly dace did in the laboratory trials. We do not recommend using a clove oil concentration >40 mg $L^{-1}$ with blackside dace, especially in water <10 C, because of the risk of undesirably long recovery times at the colder temperatures. For rapid preparation of 40 mg $L^{-1}$ clove oil solutions in the field, we carry several small

vials each holding 400 mg of the stock solution, a graduated 1-L container for measuring water volume, and a small plastic tub suitable for holding small-bodied fishes. We simply add the contents of one vial to each liter of stream water needed in the tub to achieve a desirable volume in which to anesthetize fish.

In conclusion, experimental analysis of anesthesia using a closely-related species proved quite helpful in our particular situation. But it should be cautioned that a surrogate and a target species might not always respond in a parallel fashion. In general, biologists should proceed conservatively when using untried chemicals with imperiled or otherwise valuable fishes.

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## Literature Cited

Anderson, W. G., R. S. McKinley, and M. Colavecchia. 1997. The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance. North American Journal of Fisheries Management 17:301–307.

Cho, G. K. and D. D. Heath. 2000. Comparison of tricaine methanesulfonate (MS222) and clove oil anaesthesia effects on the physiology of juvenile Chinook salmon *Oncorhynchus tshawytscha* (Walbaum). Aquaculture Research 31:537–546.

Eisenhour, D. J. and R. M. Strange. 1998. Threatened fishes of the world: *Phoxinus cumberlandensis* Starnes and Starnes, 1978 (Cyprinidae). Environmental Biology of Fishes 51:140.

Endo, T., K. Ogishima, H. Tanaka, and S. Ohshima. 1972. Studies on the anesthetic effect of eugenol in some fresh water fishes. Bulletin of the Japanese Society of Scientific Fisheries 38:761–767.

Griffiths, S. P. 2000. The use of clove oil as an anaesthetic and method for sampling intertidal rockpool fishes. Journal of Fish Biology 57:1453–1464.

Keene, J. L., D. L. G. Noakes, R. D. Moccia, and C. G. Soto. 1998. The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture Research 29:89–101.

Hikasa, Y., K. Takase, T. Ogasawara, and S. Ogasawara. 1986. Anesthesia and recovery with tricaine methanesulfonate, eugenol and thiopental sodium in the carp, *Cyprinus carpio.* Japanese Journal of Veterinary Science 48:341–351.

Marking, L. L. and F. P. Meyer. 1985. Are better anesthetics needed in fisheries? Fisheries 10(6):2–5.

Munday, P. L. and S. K. Wilson. 1997. Comparative efficacy of clove oil and other chemicals in anaesthetization of *Pomacentrus amboinensis,* a coral reef fish. Journal of Fish Biology 51:931–938.

Peake, S. 1998. Sodium bicarbonate and clove oil as potential anesthetics for nonsalmonid fishes. North American Journal of Fisheries Management 18:919–924.

Soto, C. G. and Burhanuddin. 1995. Clove oil as a fish anaesthetic for measuring length and weight of rabbitfish *(Siganus lineatus).* Aquaculture 136:149–152.

Summerfelt, R. C. and L. S. Smith. 1990. Anesthesia, surgery, and related techniques. Pages 213–272 *in* C. B. Schreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

Taylor, P. W. and S. D. Roberts. 1999. Clove oil: an alternative anaesthetic for aquaculture. North American Journal of Fisheries Management 61:150–155.

Wagner, G. N., T. D. Singer, and R. S. McKinley. 2003. The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquaculture Research 34:1139–1146.

Woolsey, J., M. Holcomb, and R. L. Ingermann. 2004. Effect of temperature on clove oil anesthesia in steelhead fry. North American Journal of Aquaculture 66:35–41.