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Sodium Bicarbonate and Clove Oil as Potential Anesthetics for Nonsalmonid Fishes

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Abstract.—The use of chemical anesthetics, such as tricaine (MS-222), on fish that might subsequently be consumed by humans or animals is currently banned in Canada and restricted in the United States. Although "nonchemical" substitutes such as sodium bicarbonate or clove oil preparations have been used, appropriate concentrations have not yet been determined for nonsalmonid species native to North America. In this study, we found that solutions of 2.66 g sodium bicarbonate/L or 60 mg clove oil/L were optimal as anesthetics for use on walleyes *Stizostedion vitreum* at 10°C. At these concentrations, complete immobilization required 7.0 ± 0.5 (SE) and 4.3 ± 0.4 min, respectively, and recovery occurred in 4.9 ± 0.5 and 10.9 ± 1.2 min, respectively, for sodium bicarbonate and clove oil. These concentrations were also found to be suitable for use on smallmouth bass *Micropterus dolomieu*, northern pike *Esox lucius*, and lake sturgeon *Acipenser fulvescens*. Clove oil is recommended over sodium bicarbonate for surgical procedures; however, recovery took substantially longer when time under clove oil anesthesia exceeded 5 min.

Traditionally, anesthetics such as quinaldine, ethyl oxide, urethane, benzocaine, 2-phenoxy-ethanol, metomidate, tricaine (MS-222), and carbon dioxide (CO_2) have been used to reduce movement and handling stress in fish (Iwama et al. 1989). Five distinct stages of anesthesia have been described: (1) partial loss of equilibrium with normal swimming motion, (2) total loss of equilibrium with normal swimming motion, (3) partial loss of swimming motion, (4) total loss of swimming motion with weak opercular motion, and (5) no opercular motion (Yoshikawa et al. 1988). Stage-4 anesthesia is generally required for immobilization and surgical procedures such as cannulation or biotelemetry transmitter attachment (Prince et al. 1995).

Although MS-222 is commonly used in laboratory studies, Canadian regulations currently prohibit the use of all chemical anesthetics on potential food fish or on wild fish that will be subsequently released. In the United States, MS-222 can be used; however, treated fish must not be consumed for 21 d (Prince et al. 1995). Traditionally, these regulations have limited aquaculturists and field researchers to CO_2 anesthesia. Carbon dioxide is introduced into the water either directly through an air stone or indirectly by addition of sodium bicarbonate ($NaHCO_3$). While this is somewhat effective in immobilizing fish, deep anesthesia is difficult to achieve (Prince et al. 1995), and the CO_2 acidifies the water, which can stress the fish (Wedemeyer 1970). Recently, Prince et al. (1995) found that acetic acid enhanced liberation of CO_2 from a sodium bicarbonate–water preparation (1.33 g/L) and stabilized the pH of the solution. The authors reported that stage 4 could be achieved in adult sockeye salmon *Oncorhynchus nerka* within about 6 min; however, it was noted that the procedure should be tested on nonsalmonids to determine appropriate concentrations for these species.

Another compound that seems to be an effective fish anesthetic is clove oil. Soto and Burhanuddin (1995) found that clove oil (100 mg/L) caused rabbitfish (=goldenspot spinefoot) *Siganus lineatus* to lose consciousness in slightly more than 2 min, which allowed the fish to be measured and weighed. Anderson et al. (1997) demonstrated that clove oil (40 and 120 mg/L) could be used to anesthetize rainbow trout *Oncorhynchus mykiss* and that exposure had no effect on swimming performance shortly after recovery. Finally, Munday and Wilson (1997) reported that clove oil was more effective than benzocaine, MS-222, and 2-phenoxyethanol for anesthetizing a damselfish *Pomacentrus amboinensis*. As with the sodium bicarbonate–acetic acid treatment, however, appropriate concentrations for use on freshwater nonsalmonid species are not known.

The objectives of this study were to (1) determine concentrations of clove oil and sodium bicarbonate–acetic acid that would minimize time to stage-4 anesthesia and time to recovery for a representative nonsalmonid (walleye *Stizostedion vitreum*), (2) assess the suitability of these anes-

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TABLE 1.—Constituents of each clove oil and sodium bicarbonate concentration tested. Ranges for lengths of walleyes used for each test are also provided.

Ingredient or measurement	Amount per clove oil concentration (mg/L) of:				$NaHCO_3$ concentration (g/L) of:		
	40	60	80	120	1.33	2.66	4.00
Clove oil (mL)	1.2	1.8	2.4	3.6			
Ethanol (mL)	10.8	16.2	21.6	32.4			
Water (L)	30	30	30	30	30	30	30
$NaHCO_3$ (g)					40	80	120
Acetic acid (mL)					15	30	45
Fork length (cm)	36–69	38–61	41–74	40–75	38–64	38–64	38–67

thetics for use in surgical procedures, and (3) confirm that the concentrations determined for walleyes are also appropriate for three other nonsalmonid species (smallmouth bass *Micropterus dolomieu,* northern pike *Esox lucius,* and lake sturgeon *Acipenser fulvescens*).

Methods

Experimental fish.—Walleyes were caught from Falcon Lake in southeastern Manitoba, Canada, in May 1996 with a large trap net. Northern pike and smallmouth bass were angled from nearby West Hawk and South Cross lakes. All fish were transported (<20 km) to the Whiteshell Fish Hatchery where experiments were conducted. Fish were held at a low density in large (2,000 L) outdoor fiberglass tanks. Water from a nearby lake continuously flowed through the holding tanks at a rate of about 1 L/s. Water temperature was maintained at a constant 10°C. After experiments, fish were held for 1 week and any deaths were noted.

Experiment 1: sodium bicarbonate on walleyes.—Three groups of 12 walleyes were treated with sodium bicarbonate (Anachemia Canada) concentrations of 1.33, 2.66, or 4.00 g/L. Amounts of sodium bicarbonate, acetic acid, and water used for each concentration, and fork lengths (FLs) of walleyes within each treatment group, are given in Table 1. Fish were transferred from a holding tank into a tub containing 30 L of anesthetic solution, and the time required to reach each stage was recorded. After stage 4 was achieved, FL was measured and the walleyes were returned to anesthetic-free water. Time from this point to recovery (when the fish regained its equilibrium and was able to swim on its own) was recorded.

Experiment 2: clove oil on walleyes.—Four groups of 12 walleyes were anesthetized with clove oil (Hilltech Canada) concentrations of 40, 60, 80 or 120 mg/L. Due to its poor solubility in water, clove oil was first dissolved in ethanol at a ratio of 1 : 10 (clove oil: ethanol; volume: volume). Amounts of clove oil, ethanol, and water used for each concentration, and FLs of walleyes within each treatment group, are given in Table 1. Time to each stage of anesthesia and for recovery were measured as described for experiment 1.

Experiment 3: prolonged anesthetic exposure on walleyes.—Twenty-four walleyes (mean FL: 43.6 cm, range: 42.1–45.4 cm) in three groups of eight fish were used to examine exposure to a solution of 2.66 g $NaHCO_3$/L (found to be optimal in experiment 1). Each group was each kept under stage-4 anesthesia for either 0.5, 5.0, or 15.0 min. Each fish anesthetized to stage 4 was removed from the solution and placed dorsal side up on a foam surgical tray. Throughout the assigned exposure period, aerated anesthetic solution (10°C) was pumped from a reservoir, over the gills of the fish, and back into the reservoir. The entire body of the fish was covered in damp paper towels to prevent desiccation. When the exposure period was complete, the anesthetic solution in the reservoir was replaced with anesthetic-free water and the gills were irrigated until the fish began to move. The walleye was then removed from the surgical tray and placed in a holding tank containing freshwater to recover. Exposure time began when the fish was first placed on the surgical tray. Recovery time began when freshwater replaced the anesthetic solution and ended when the fish regained equilibrium and began to swim.

Prolonged exposure to 60 mg clove oil/L (found to be optimal in experiment 2) was also examined by using the exact protocol described above for sodium bicarbonate.

Experiment 4: effects on other nonsalmonid species.—Time to reach each stage of anesthesia and time to recovery were also measured for eight smallmouth bass (mean FL: 26 cm, range: 21–33 cm), eight lake sturgeon (mean FL: 27 cm, range: 26–29 cm), and eight northern pike (mean FL: 30 cm, range: 27–34 cm) exposed to solutions of either 2.66 g sodium bicarbonate/L or 60 mg clove

TABLE 2.—Mean (SE) induction and recovery times for walleyes anesthetized with sodium bicarbonate, acetic acid, and water or clove oil, ethanol, and water in varying concentrations. Within each column and for each anesthetic, mean times without lowercase letter in common are significantly different ($P < 0.05$).

Anesthetic concentration	Time (min) to anesthesia stage: 1	2	3	4	Recovery time (min)
			Sodium bicarbonate		
1.33 (g/L)	4.8 (0.5) z	5.8 (0.5) z	6.5 (0.5) z	14.0 (1.1) z	6.4 (0.5) z
2.66 (g/L)	3.6 (0.3) y	4.0 (0.3) y	4.4 (0.3) y	7.0 (0.5) y	4.9 (0.4) zy
4.00 (g/L)	2.6 (0.2) y	3.0 (0.2) y	3.9 (0.3) y	4.7 (0.3) y	4.4 (0.3) y
			Clove oil		
40 (mg/L)	1.8 (0.1) x	2.5 (0.2) x	4.0 (0.5) x	6.2 (0.6) x	7.1 (0.9) x
60 (mg/L)	1.5 (0.2) x	2.0 (0.3) x	3.7 (0.5) xw	4.3 (0.4) w	10.9 (1.2) xw
80 (mg/L)	1.4 (0.1) x	1.9 (0.1) x	3.4 (0.3) xw	4.3 (0.3) w	10.3 (1.7) xw
120 (mg/L)	0.9 (0.1) w	1.1 (0.1) w	2.2 (0.3) w	2.8 (0.3) w	12.5 (1.3) w

oil/L. Experiments were conducted exactly as previously described for walleyes in experiments 1 and 2.

Statistical analysis.—We used analysis of variance (ANOVA) and the Bonferroni multiple-comparison test to determine differences among treatment groups because all data were found to approximate the normal distribution. We used linear regression analysis to determine correlations; *P*-values of 0.05 or lower indicated statistical significance.

Results

Experiment 1

Walleyes placed in the sodium bicarbonate solutions initially thrashed violently for 20–30 s before relaxing and losing their equilibrium (i.e., entering stage 1). Fish also tended to struggle during posttest FL measurements even though stage-4 anesthesia had apparently been reached. Regression analysis indicated that time to stage-4 anesthesia decreased as sodium bicarbonate concentration increased ($r^2 = 0.67$) but was independent of fish length over the range used. Recovery time increased with sodium bicarbonate concentration ($r^2 = 0.21$) but was independent of fish length.

Walleyes exposed to 1.33 g $NaHCO_3$/L required significantly more time (36–196%) to reach each stage of anesthesia than those treated with the concentrations of 2.66 or 4.00 g/L (Table 2). No significant differences among mean induction times were found between the 2.66- and 4.00-g/L solutions. Mean recovery times for walleyes anesthetized with the 1.33- and 2.66-g/L preparations were not significantly different; however, walleyes treated with 1.33 g $NaHCO_3$/L required significantly more time (45%) to recover than those treated with anesthetic at 4.00 g/L (Table 2). Time to recovery was significantly lower than time to stage 4 for the 1.33-g /L solution; however, this relationship was not found for the 2.66- and 4.00-g/L solutions. No deaths were recorded during or after the study period.

Experiment 2

Walleyes placed in clove oil solutions did not thrash or struggle during posttest measurements. In addition, stages 4 and 5 (cessation of ventilation) occurred virtually simultaneously. Regression analysis indicated that time to stage 4 decreased as clove oil concentration increased ($r^2 = 0.31$) but was independent of fish length over the range used. Recovery time increased with clove oil concentration ($r^2 = 0.11$) but was also independent of fish length.

Mean time to stages 1 and 2 were not significantly different among groups exposed to the 40-, 60-, and 80-mg/L solutions; however, fish exposed to these concentrations required significantly more time (60–115%) to achieve stages 1 and 2 than those treated with the 120-mg/L anesthetic (Table 2). Mean time to stage 3 for the 40-mg/L group was significantly greater (79%) than that of the 120-mg/L group. Mean time to stage 4 for the 40-mg/L group was significantly greater (44–123%) than that for the other concentrations. Finally, recovery times were not significantly different among fish treated with the solutions of 40, 60, and 80 mg clove oil/L; however, walleyes treated with the 120-mg/L anesthetic took significantly more time (75%) to recover than the 40-mg/L group (Table 2).

Recovery time was not significantly greater than time to stage 4 for the clove oil solution at 40 mg/L; however, walleyes took significantly more time

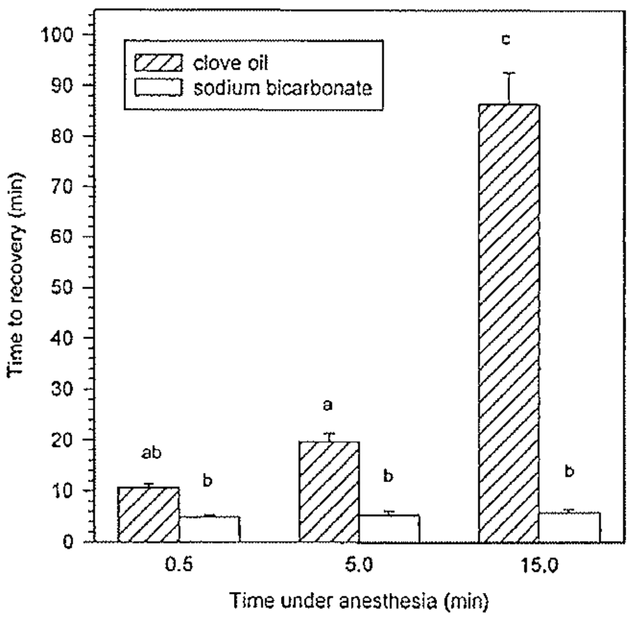

FIGURE 1.—Mean (±SE) time to recover for walleyes immobilized with a solution of sodium bicarbonate (2.66 g/L) or clove oil (60 mg/L) at 10°C and held under anesthesia for varying periods of time. Means without a lowercase letter in common are significantly different ($P <$ 0.05).

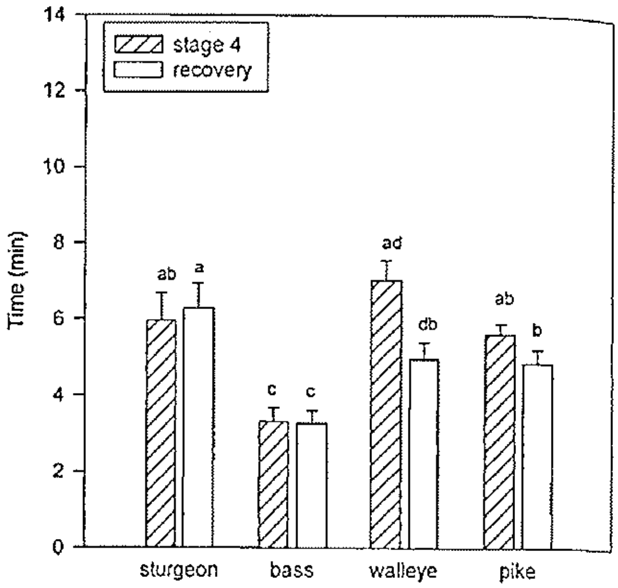

FIGURE 2.—Mean (±SE) times to induction to stage-4 anesthesia and times to recovery for lake sturgeon, smallmouth bass, walleyes, and northern pike treated with 2.66 g $NaHCO_3$/L at 10°C. Means without a lowercase letter in common are significantly different ($P < 0.05$).

to recover than to reach stage 4 when exposed to 60, 80, or 120 mg/L. No fish deaths were recorded.

Experiment 3

Walleyes anesthetized with sodium bicarbonate (2.66 g/L) routinely flinched during the first 30–60 s on the surgical tray. This movement often caused the tube irrigating the gills to be displaced. However, fish that remained on the tray for more than 1–2 min remained motionless for the rest of the experiment. When the flow of anesthetic solution over the gills was replaced with freshwater, walleyes typically began to move within 5 min, and full recovery occurred shortly thereafter (Figure 1). No significant differences in mean recovery times were found among treatment groups.

Walleyes treated with clove oil were completely immobilized during the entire test period. Fish that spent 15 min on the surgical tray required significantly more time (330–760%) to recover than those left for 5 and 0.5 min (Figure 1). Mean recovery times among the 5- and 0.5-min treatment groups were not significantly different. For fish left under anesthesia for 0.5 min, times to recovery from sodium bicarbonate and clove oil anesthesia were not significantly different; however, fish exposed to clove oil for 5 and 15 min required significantly longer times (300 and 1,330%, respectively) to recover than those exposed to sodium bicarbonate solutions for the same periods (Figure 1). All fish survived the experiment.

Experiment 4

Lake sturgeon exposed to 2.66 g $NaHCO_3$/L also displayed the thrashing observed in walleyes; however, immersion also caused prolonged ejection of the mouth parts (this normally occurs only during feeding). Nevertheless, the solution was effective in causing stage-4 anesthesia within 1.9–8.4 min (Figure 2). Lake sturgeon required 4.0–8.7 min to recover. Smallmouth bass treated with 2.66 g $NaHCO_3$/L reached stage 4 in 2.7–5.8 min and recovered in 1.9–4.9 min. Northern pike achieved stage 4 within 4.5–7.0 min and recovered in 3.6–6.2 min. For all species, time to recovery was not significantly different from time to reach stage 4. No mortalities were observed.

Lake sturgeon exposed to 60 mg clove oil/L required 4.2–8.3 min to reach stage 4 and recovery took 6.0–12.0 min (Figure 3). Smallmouth bass reached stage 4 in 2.0–4.0 min and recovered within 4.7–7.5 min. Northern pike required 3.2–10.9 min to enter stage 4 and 5.4–10.8 min to recover. As with sodium bicarbonate, times to reach stage 4 and times to recover from clove oil were not significantly different among these three nonsalmonids. No fish deaths were recorded.

Discussion

Because induction and recovery times tend to fluctuate with anesthetic strength, optimal concen-

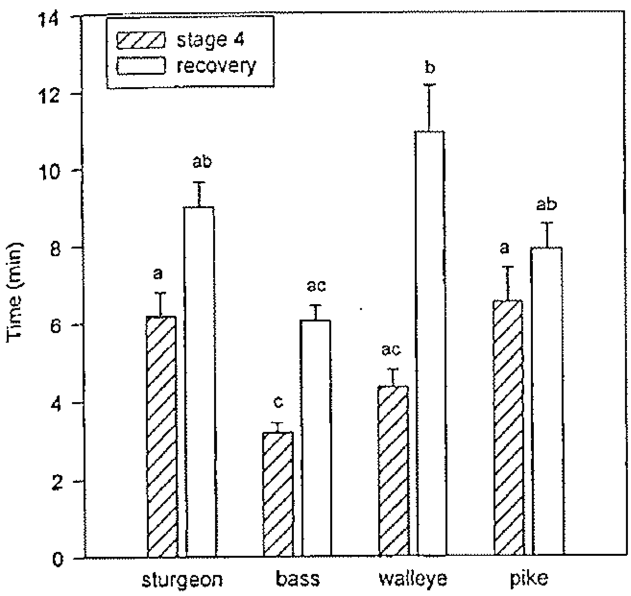

FIGURE 3.—Mean (±SE) times to induction to stage-4 anesthesia and times to recovery for lake sturgeon, smallmouth bass, walleyes, and northern pike treated with 60 mg clove oil/L at 10°C. Means without a lowercase letter in common are significantly different ($P < 0.05$).

tration will vary with study objectives. As such, results for each concentration tested are provided in Table 2 to allow researchers to choose the preparation with properties best suited to their needs. For the purposes of this study, however, the most efficient concentration is defined as the one that minimizes time to immobilization, time to recovery, and materials used to prepare the solution. We found that 1.33 g $NaHCO_3$/L, recommended by Prince et al. (1995) for adult sockeye salmon, was not strong enough to anesthetize walleyes in a timely fashion. Whereas 4.00 g $NaHCO_3$/L yielded mean induction and recovery times less than 5 min, the 2.66-g/L treatment gave statistically identical results but with less material. As such, this concentration is recommended for use on walleyes.

This study also found that 40 mg clove oil/L resulted in induction and recovery times of about 6 and 7 min, respectively. Anderson et al. (1997) found similar results for juvenile rainbow trout anesthetized in 40 mg clove oil/L. Despite this, 60 mg/L is recommended for walleyes because time to stage 4 was lower than that for 40 mg/L and recovery times were not significantly different between the two concentrations of clove oil. Soto and Burhanuddin (1995) recommended 100 mg clove oil/L for rabbitfish; however, they also reported satisfactory induction and recovery times for fish tested in a 67-mg/L preparation.

The clove oil and sodium bicarbonate concentrations recommended for walleyes also appear to be suitable for other nonsalmonid fishes; however, there were species-specific differences in mean induction and recovery times. Smallmouth bass exposed to sodium bicarbonate anesthetic required less time to reach stage 4 than all other species (Figure 2), and they recovered more quickly than all other species. Smallmouth bass anesthetized in clove oil also required less time to achieve stage 4 than lake sturgeon or northern pike and recovered faster than walleyes. Although the reasons for these differences are unclear, they demonstrate that different fish species can react differently to the same anesthetic.

Clove oil and sodium bicarbonate also proved to be effective anesthetics for use in surgical procedures, although fish treated with clove oil did not struggle as did those anesthetized with sodium bicarbonate. Munday and Wilson (1997) also noted that damselfish treated with clove oil exhibited a much calmer induction than those treated with quinaldine. Despite this benefit, prolonged exposure to clove oil resulted in very long recovery periods (Figure 1) and we recommend that circulating clove oil solutions be replaced with anesthetic-free water after about 5 min. This should ensure that the fish will remain immobilized for the entire procedure but will also recover shortly after the work is completed. Long recovery times appear to be typical for clove oil anesthetic (Anderson et al. 1997; Munday and Wilson 1997); however, Soto and Burhanuddin (1995) reported that rabbitfish recovered very quickly (20–160 s) after exposure to a bath of 100 mg clove oil/L, again demonstrating that results can be species specific.

Although the primary benefit of clove oil and sodium bicarbonate anesthetics is that they are safe to use on potential food fish, laboratory researchers may also want to use these substances in place of MS-222, which can be more expensive. Anderson et al. (1997) compared induction and recovery times and postanesthesia swimming performances between MS-222 and clove oil; they found that induction time for clove oil tended to be faster than for MS-222, whereas recovery times and postanesthesia swimming performances were similar between the two anesthetics. Munday and Wilson (1997) also noted that clove oil was more effective than MS-222 at low concentrations. Despite this, the physiological effects of clove oil have not been investigated whereas those of MS-222 (and most other chemical anesthetics) have been thoroughly studied (see Strange and Schreck 1978; Barton and Peter 1982; Iwama et al. 1989).

In summary, we recommended that preparations of 2.66 g $NaHCO_3$/L or 60 mg clove oil/L be used to anesthetize the species tested in this study. Results also indicated that the anesthetic concentration need not be adjusted for fish size; however, this is in contrast to the findings of Prince et al. (1995) for sodium bicarbonate used on sockeye salmon. Because there was some variation in induction and recovery times among species, it is also recommended that preliminary trials be conducted if species other than those tested in this study are used.

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References

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.

Barton, B. A., and R. E. Peter. 1982. Plasma cortisol stress response in fingerling rainbow trout *Salmo gairdneri* Richardson to various transport conditions, anaesthesia, and cold shock. Journal of Fish Biology 20:39–51.

Iwama, G. K., J. C. McGreer, and M. P. Pawluk. 1989. The effects of five fish anesthetics on acid-base balance, haematocrit, blood gasses, cortisol, and adrenaline in rainbow trout. Canadian Journal of Zoology 67:2065–2073.

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.

Prince, A. M. J., S. E. Low, T. J. Lissimore, R. E. Diewert, and S. G. Hinch. 1995. Sodium bicarbonate and acetic acid: an effective anesthetic for field use. North American Journal of Fisheries Management 15:170–172.

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

Strange, R. J., and C. B. Schreck. 1978. Anesthetic and handling stress on survival and cortisol concentration in yearling chinook salmon *Oncorhynchus tshawytscha*. Journal of the Fisheries Research Board of Canada 35:345–349.

Wedemeyer, G. 1970. Stress of anesthesia with MS-222 and benzocaine in rainbow trout *(Salmo gairdneri)*. Journal of the Fisheries Research Board of Canada 27:909–914.

Yoshikawa, H., Y. Ishida, S. Ueno, and H. Mitsuda. 1988. Changes in depth of anesthesia of the carp anesthetized with a constant level of carbon dioxide. Bulletin of the Japanese Society of Scientific Fisheries 54:457–462.

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