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Clove oil as an anaesthetic for adult sockeye salmon: field trials

C. A. Woody*, J. Nelson and K. Ramstad

U.S. Geological Survey—Biological Resources Division, 1011 E. Tudor Rd, Anchorage, AK 99503, U.S.A.

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Wild migrating sockeye salmon *Oncorhynchus nerka* exposed to 20, 50 and 80 mg l^{-1} of clove oil could be handled within 3 min, recovered within 10 min, and survived 15 min exposure trials. Fish tested at 110 mg l^{-1} did not recover from 15 min exposure trials. Response curves developed for induction and recovery time considered the following predictors: clove oil concentration, sex, fish length and depth. A significant positive dependence was observed between induction time and fish length for 20, 50 and 80 mg l^{-1} test concentrations; no dependence was observed between induction time and length at 110 and 140 mg l^{-1}. Recovery time differed as a function of clove oil concentration, but not fish size. A concentration of 50 mg l^{-1} is recommended for anaesthetizing sockeye salmon ranging from 400 to 550 mm in length at water temperatures averaging 9–10° C.

Key words: clove oil; sockeye salmon; fish anaesthesia.

INTRODUCTION

Fishery biologists often use anaesthetics to reduce handling stress on fishes. The use of some anaesthetics on food fish, however, is limited. Two anaesthetics are considered safe for use on food fish: MS-222 (3-aminobenzoic acid ethyl ester methanesulphonate) and carbon dioxide (CO_2) (Schnick *et al.*, 1979). The anaesthetic MS-222 is effective (i.e. short induction time and rapid recovery time; Gilderhus & Marking, 1987), but has limited use, because some regulatory agencies such as the U.S. Food and Drug Administration (FDA) require that fishes treated with MS-222 are held for a minimum of 21 days before human consumption. Various derivations of CO_2 used to anaesthetize fishes (Booke *et al.*, 1978; Post, 1979) are ' generally recognized as safe ' for human intake, but are considered only partly effective by many biologists (Marking & Meyer, 1985). Alternative anaesthetics for use on food fishes, which are effective and have a short depuration period would fill a priority need in fisheries science (Gilderhus & Marking, 1987).

One such promising anaesthetic is clove oil, a distillate of herbaceous portions of the clove tree *Eugenia aromatica* containing the active ingredient eugenol (90–95%) (Briozzo *et al.*, 1989). Clove oil has a long history as a local anaesthetic for humans (Soto & Burhanuddin, 1995). It is considered non-mutagenic and a safe substance by the FDA (Nagababu & Lakshmaiah, 1992), with human intake levels established at 2·5 mg kg^{-1} day^{-1} (Expert Committee

*Author to whom correspondence should be addressed. Tel.: +1 907 786 3314; fax: +1 907 786 3636; email: carol_woody@usgs.gov

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on Food Additives, 1982). Soto & Burhanuddin (1995) first evaluated clove oil as an anaesthetic for rabbitfish *Siganus lineatus* Curvier & Valenciennes and found both rapid induction (mean, 108 s) and recovery (mean, 76 s) times at concentrations of 100 mg l^{-1}. Comparative efficacy trials on larval *Pomacentrus amboinensis* Bleeker, a coral reef fish, indicated clove oil was more effective than MS-222, with more rapid induction and less variable response times (Munday & Wilson, 1997). Tests on juvenile and adult rainbow trout *Onchorhynchus mykiss* (Walbaum) indicate short exposures (<5 min) to clove oil concentrations of 40 and 120 mg l^{-1} will not affect subsequent swimming performance of either life stages (Anderson *et al.*, 1997). Taylor & Roberts (1999) examined efficacy of clove oil on juvenile and adult chinook salmon *Oncorhynchus tshawytscha* (Walbaum), juvenile coho salmon *Oncorhynchus kisutch* (Walbaum), juvenile rainbow trout and juvenile and subadult white sturgeon *Acipenser transmontanus* Richardson. All fishes were safely immobilized and held up to 120 min in a concentration of 25 mg l^{-1}, however, median lethal concentrations (10 min exposures) ranged widely between the two genera (e.g. 62 mg l^{-1}, chinook salmon; 526 mg l^{-1}, white sturgeon). Recently, Prince & Powell (2000) demonstrated a clove oil concentration of 30 mg l^{-1} was effective at inducing and maintaining deep anaesthesia for invasive surgery in adult rainbow trout. One potential drawback is that recovery from clove oil anaesthesia can be up to 10 times longer than recovery from MS-222 (Anderson *et al.*, 1997; Keene *et al.*, 1998).

Anadromous sockeye salmon *Oncorhynchus nerka* (Walbaum) support valuable commercial, sport and native-subsistence fisheries in the northern Pacific and therefore are the focus of research that involves anaesthetization (e.g., surgical radio tag implantation). The 21 day withdrawal period for MS-222 and ineffectiveness of CO_2 make use of either anaesthetic problematic for wild salmon studies. Although some research has been conducted on the efficacy of clove oil in salmonids, response curves have not been developed for any species, nor have any studies been conducted on wild homing salmon, which may be more sensitive to clove oil effects than non-homing salmonids. The purposes of this study were: to determine effective concentrations of clove oil for use on mature homing sockeye salmon; to develop induction and recovery response curves that consider interactions between clove oil concentrations, fish sex and size, and finally to examine survival and movements of fish after anaesthetization and radio implantation.

MATERIALS AND METHODS

Field trials using adult sockeye salmon were conducted near the outlet of Lake Clark (61°49′54″N; 154°45′29″W), south-central Alaska. The site was selected because sockeye salmon migrating past the outlet are the focus of regional research requiring anaesthetization and are subsequently subject to human harvest.

Migrating adult sockeye salmon were captured in a small mesh tangle gill net (10 cm mesh, 18 m long, 2·1 m deep). Total catch time (time elapsed from capture to being held in a freshwater tank) varied depending on the number of fish caught and degree of entanglement, but did not exceed 5 min. Sockeye salmon were temporarily held (up to 20 min) in aerated plastic holding containers until an individual was selected and placed in a second plastic tub (660 × 465 × 380 mm) containing 30 l of fresh water with a specific concentration of clove oil solution.

TABLE I. Designated stages of anaesthesia used for Lake Clark sockeye salmon clove oil efficacy tests

Stage	Characteristic behaviour
1	Opercular movement visibly slows or becomes erratic
2	Sporadic loss of equilibrium, difficulty maintaining position while at rest
3	Complete loss of equilibrium; inability to regain upright position
4	No reaction to handling or a sharp prod in the peduncle
Recovery	Ability to remain upright, normal swimming behaviour

Clove oil does not completely dissolve in water below 15° C. Because water temperatures at the study site averaged 9·6° C (S.D. = 0·77), clove oil was mixed with ethanol in a 1 : 9 ratio (Anderson *et al.*, 1997) to facilitate mixing. Ethanol has no known anaesthetic properties on fishes at low doses (Anderson *et al.*, 1997; Munday & Wilson, 1997). Density of clove oil is *c.* 1 g ml^{-1}, thus, 1 ml of the clove oil and ethanol solution contained 100 mg of clove oil (Soto & Burhanuddin, 1995). The stock clove oil and ethanol solution was mixed thoroughly with 30 l of lake water to obtain test concentrations of 20, 50, 80, 110 and 140 mg l^{-1}. Fresh solutions were prepared every other day and were protected from sun and heat to limit photo and thermal degradation (Soto & Burhanuddin, 1995). Water chemistry at the site was reported as: pH 7·2; conductivity, 56 μS cm^{-1}; dissolved oxygen, 13·6 ppm; alkalinity 20 mg l^{-1} as $CaCO_3$, bicarbonate 24 mg l^{-1} as HCO_3 (T. Brabets, pers. comm.).

The efficacy criteria were: ability to handle fish within 3 min, fish recovery within 10 min, and survival of a 15 min exposure trial. Eight different fish were tested at the five concentrations. Fish were placed in the anaesthesia tank and stages of anaesthesia were visually monitored, timed and classified (Table I. Once a fish had reached a state where it did not react to handling (stage 4) it was removed from the anaesthetic bath, placed in a neoprene sling, and body depth (perpendicular to anterior insertion of dorsal fin) and length (mid-eye to hypural plate) were measured (mm). Body dimensions instead of mass were determined since length is commonly collected in field studies and is easier to obtain than masses. Fish that reacted to initial handling attempts were left in anaesthesia and prodded in the caudal peduncle every 20 s until no reaction was elicited whereupon they were removed and measured. After handling, fish were placed in a freshwater recovery tank and monitored until fully recovered. Fish were released after recovery.

Fifteen-minute exposure tests were conducted to estimate the length of time a fish may be safely held in a certain concentration of clove oil. Plastic tanks (660 × 465 × 380 mm) were filled with 30 l of fresh water and a specific concentration of clove oil and ethanol solution. Three fish were individually tested at each concentration. Each fish was left in the bath for 15 min, removed, and body depth and length measured. It was then transferred to an identical aerated fresh water tank and recovery was monitored and timed (Table I).

Mean induction (time from stage 1 to 4) and recovery times were compared among treatment groups using one-way ANOVAs, followed by Tukey's Honestly Significant Difference multiple comparison procedure (Zar, 1984). Fish used in the field trials were a wide range of sizes; therefore, induction and recovery response time for interaction with size were also examined. For each response, the most parsimonious regression model was developed from predictors: clove oil concentration, length, depth, and sex, using partial *F*-tests (Neter *et al.*, 1989). Residual diagnostics were used to assess normality and constant variance assumptions for the final selected models.

RESULTS

Fish in all treatment groups were easily handled in <3 min and recovered equilibrium within 10 min when placed in fresh water (Fig. 1). Induction times

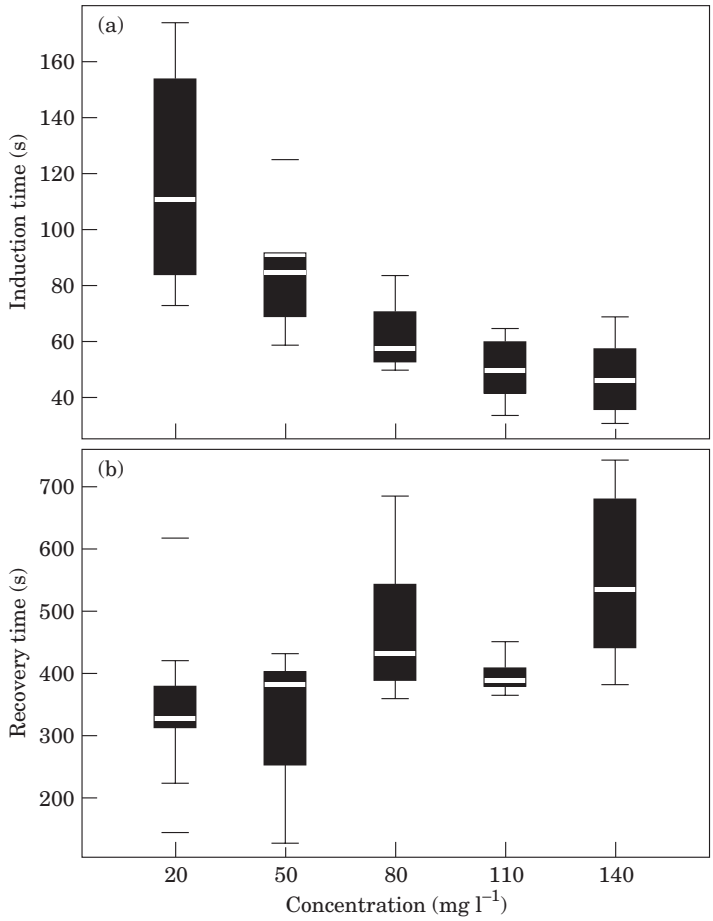

FIG. 1. Box plots of (a) induction and (b) recovery times for adult sockeye salmon anaesthetized at five clove oil concentrations. The upper and lower lines of each plot represent the maximum and minimum times observed, the middle line represents the median, and the grey inner box represents the 25th and 75th percentiles.

were more rapid at high clove oil concentrations (Table II), with fish treated at concentrations >20 mg l^{-1} exhibiting significantly shorter induction times ($F_{4,36}$=16·11, P<0·0001). Recovery time results were somewhat ambiguous, with fish treated at 140 mg l^{-1} taking significantly longer to recover than fish treated at 20 mg l^{-1} (q=5·43, q critical=4·102) and 50 mg l^{-1} (q=5·82, q critical=4·102) concentrations (Fig. 2).

All fish exposed for 15 min to concentrations of 20, 50 or 80 mg l^{-1} recovered from deep anaesthesia; average recovery times were 950, 1673 and 2085 s respectively. Two of the three fish exposed to clove oil at 110 mg l^{-1} suffered mortality while the fish that revived (after 2945 s) never regained equilibrium. It was assumed that fish exposed to higher concentrations would suffer a similar fate; therefore the 140 mg l^{-1} trials were not conducted.

Regression analysis indicated a significant dependence between induction time and fish length for test concentrations of 20, 50 and 80 mg l^{-1}, whereas for concentrations of 110 and 140 mg l^{-1}, no dependence was noted (Table III;

TABLE II. Anaesthetic efficacy trial results for adult sockeye salmon tested at 5 clove oil concentrations. Mean (+S.E.) for time (s) fish spent in each stage of anaesthesia, total time to immobilization, and recovery

Concentration (mg l^{-1})	20 mg l^{-1}	50 mg l^{-1}	80 mg l^{-1}	110 mg l^{-1}	140 mg l^{-1}
Stage 1	27	18	13	10	11
	(3)	(2)	(2)	(1)	(1)
Stage 2	20	16	10	11	10
	(3)	(2)	(2)	(1)	(1)
Stage 3	30	15	23	27	25
	(5)	(2)	(6)	(4)	(5)
Stage 4	43	36	16	3	1
	(9)	(7)	(3)	(1)	(1)
Total time (stage 1–4)	121	84	62	50	48
	(13)	(7)	(4)	(4)	(5)
Recovery time	345	330	472	396	555
	(44)	(38)	(39)	(9)	(48)
n	9	8	8	8	8
Fish length (mm)	483	477	498	449	457
	(12)	(11)	(3)	(13)	(14)
Fish depth (mm)	134	129	140	121	125
	(4)	(5)	(2)	(5)	(6)
Water temperature (° C)	9·9	9·1	9·4	9·6	10
	(0·38)	(0·08)	(0·18)	(0·13)	(0·34)

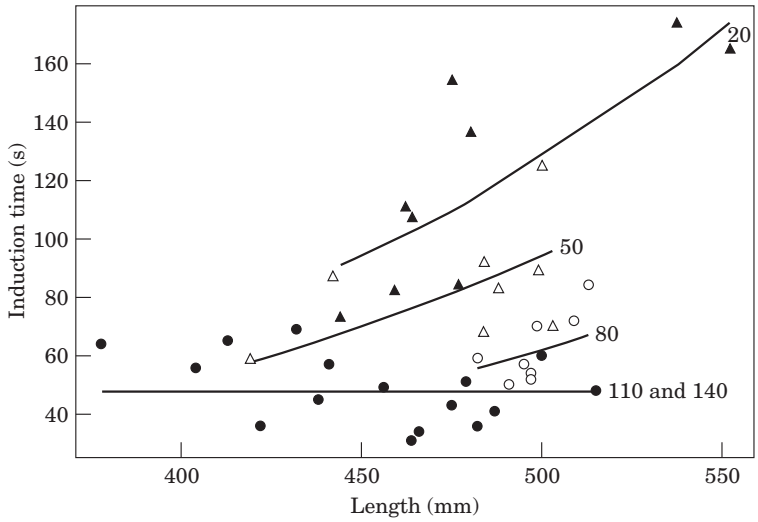

FIG. 2. Induction time response curves as a function of sockeye salmon length for clove oil concentrations of 20 (▲), 50 (△), 80 (○), 110–140 mg l^{-1} (●).

Fig. 2). The slope of the response models did not differ significantly among test concentrations of 20, 50 and 80 mg l^{-1}, indicating a similar relationship of longer total induction times for larger fish (Table III; Fig. 2). Intercepts did

TABLE III. Coefficient estimates for induction response models. *P*-value testing difference of coefficient from that estimated for concentrations 110–140 mg l^{-1} in parentheses. The *P*-values listed under concentrations 110–140 mg l^{-1} are for the test of significant difference from zero. $\log_e$ (total time)$=\beta_0+\beta_1$ (length)

Concentration (mg l^{-1})	β_0	β_1
20	1·836	0·0060
	(0·0035)	(0·0008)
50	1·5333	0·0060
	(0·0015)	(0·0008)
80	1·117	0·0060
	(0·0006)	(0·0008)
110 and 140	3·865	0
	(>0·0001)	(0·864)

differ significantly at these concentrations (Table III), with more rapid induction times observed at higher concentrations. No relationship was observed between induction time and fish lenth at clove oil concentrations of 110 and 140 mg l^{-1} (Fig. 2; Table III). Recovery time differed as a function of clove oil concentration but not fish length, body depth or sex. Fish treated at 20 and 50 mg l^{-1} recovered faster than fish anaesthetized at 140 mg l^{-1}, while results from the 80 and 110 mg l^{-1} groups were ambiguous with the upper and lower extremes (Fig. 1).

DISCUSSION

The field trials in this study indicate clove oil is an effective anaesthetic for handling adult anadromous sockeye salmon. Test concentrations that met the efficacy criteria for handling within 3 min, recovery in 10 min and no mortality after a 15 min exposure were 20, 50 and 80 mg l^{-1}. Fish anaesthetized at 20 mg l^{-1} could be measured and fin clipped easily, though such fish sometimes exhibited reflexive action during handling. Concentrations of 50 mg l^{-1} appear sufficient for measurement; oesophageal implants, and fin clips. Concentrations of *c.* 80 mg l^{-1} may be needed to induce the deeper anaesthesia necessary for surgery and extended handling time although Prince & Powell (2000) preferred low doses (30–40 mg l^{-1}) to anaesthetize adult rainbow trout since recovery times were minimized at lower concentrations.

The response curves developed for this study clearly indicate induction time is related to fish size (Fig. 2) in migrating sockeye salmon, but recovery time is not. The only other study that examined clove oil efficacy on adult salmon was conducted under controlled conditions for 25 and 50 mg l^{-1} concentrations on hatchery chinook salmon (Taylor & Roberts, 1999). Although the chinook salmon anaesthetized at these concentrations generally met the present induction criteria of 3 min (range 2·0–4 min) neither met the recovery criteria of 10 min as the chinook salmon recovery times ranged from 7·5 to 13 min at 25 mg l^{-1} and from 9 to 17·5 min at 50 mg l^{-1} (Taylor & Roberts, 1999). Furthermore, the

relationship between fish length, a common measure used in salmon management, and induction or recovery times at specific clove oil concentrations has not been examined prior to this study. This study clearly demonstrates the effectiveness of clove oil as an anaesthetic for field applications on migrating sockeye salmon. Because this study and that of Taylor & Roberts (1999) indicate variation in effectiveness of clove oil among adults of different salmonid species future studies involving clove oil as an anaesthetic would benefit from efficacy trials prior to concentration selection.

The use of clove oil may not be appropriate for some studies and very little is actually known of its effects on fish physiology (Anderson *et al.*, 1997). The relatively long recovery phase for salmon anaesthetized with clove oil could be problematic for handling large numbers of fishes, particularly where holding facilities are limited. Salmon migration studies involving anaesthetization could be compromised because clove oil may interfere with sensitive olfactory senses. Since adult anadromous salmon rely on their sense of smell to recognize a series of chemical cues imprinted during their early life history to return to natal habitats (Hasler & Schlotz, 1983), any long-term effects on their ability to detect chemical cues would bias data related to migration. Further studies regarding clove oil effects on olfaction in anadromous salmon are needed to alleviate this concern.

Clove oil does appear to have promise as an effective and safe anaesthetic for use on food fishes. However, until further studies are conducted regard ing physiological effects, it should be used with caution and at the lowest concentrations necessary to induce anaesthesia.

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References

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

Booke, H. E., Hollender, B. & Lutterbie, G. (1978). Sodium bicarbonate, an inexpensive fish anaesthetic for field use. *The Progressive Fish Culturist* **40,** 11–13.

Briozzo, J., Nunez, L., Chirife, J., Herszage, L. & D'Aquino, M. (1989). Antimicrobial activity of clove oil dispersed in a concentrated sugar solution. *Journal of Applied Bacteriology* **66,** 69–75.

Expert Committee on Food Additives (1982). Evaluation of certain food additives and contaminants. *World Health Organization Technical Report Series* **20**.

Gilderhus, P. A. & Marking, L. L. (1987). Comparative efficacy of 16 anaesthetic chemicals on rainbow trout. *North American Journal of Fisheries Management* **7,** 288–292.

Hasler, A. D. & Scholz, A. T. (1983). *Olfactory Imprinting and Homing in Salmon*. Berlin: Springer-Verlag.

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

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

Munday, P. L. & Wilson, S. K. (1997). Comparative efficacy of clove oil and other chemicals in anesthetization of *Pomacentrus amoboinensis*, a coral reef fish. *Journal of Fish Biology* **51,** 931–938.

Nagababu, E. & Lakshmaiah, N. (1992). Inhibitory effect of eugenol on non-enzymatic lipid peroxidation in rat liver mitochondria. *Biochemical Pharmacology* **43,** 2393–2400.

Neter, J., Wasserman, W. & Kutner, M. H. (1989). *Applied Linear Regression Models*, 2nd edn. Burr Ridge, Illinois: Richard D. Irwin.

Post, G. (1979). Carbonic acid anesthesia for aquatic organisms. *The Progressive Fish-Culturist* **41,** 142–144.

Prince, A. & Powell, C. (2000). Clove oil as an anaesthetic for invasive field procedures on adult rainbow trout. *North American Journal of Fisheries Management* **20,** 1029–1032.

Schnick, R. A., Meyer, F. P. & Van Meter, H. D. (1979). Announcement of compounds registered for fishery uses. *The Progressive Fish-Culturist* **41,** 36–37.

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

Taylor, P. W. & Roberts, S. D. (1999). Clove oil: an alternative anaesthetic for aquaculture. *The North American Journal of Aauaculture* **61,** 150–155.

Zar, J. H. (1984). *Biostatistical Analysis*. Englewood Cliffs, NJ: Prentice Hall.