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Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum

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Abstract

Clove oil has been demonstrated to be an effective, inexpensive anaesthetic and euthanizing agent for a number of fish species, including rainbow trout, used in aquaculture and fisheries research. However, the potential for clove oil to cause perturbations in important plasma hormone concentrations has not been investigated. The effect of anaesthesia and euthanasia in trout with eugenol (the active ingredient in clove oil) on plasma cortisol, glucose, growth hormone (GH) and two thyroid hormones [tri-iodothyronine (T_3) and thyroxine (T_4)] was compared with tricaine methanesulfonate (MS-222) anaesthesia, and stunning by cranial concussion in two experiments. Effects on blood chemistry were different when comparing the particular anaesthetic method being used. Stunning fish significantly increased plasma cortisol and glucose levels (both $P < 0.05$), while euthanizing fish using either clove oil or MS-222 had no effect on these hormone levels. In contrast, the levels of GH, T_3 and T_4 hormones were unaffected regardless of whether fish were euthanized by stunning, MS-222 or clove oil. Variation in effects between hormones were observed using clove oil eugenol. In fish sampled 10 min after anaesthetizing with 150 mg L^{-1} of eugenol, cortisol levels were significantly decreased ($P < 0.03$), while there were no differences in either glucose or GH levels. Tri-iodothyronine and T_4 also showed significantly elevated levels ($P < 0.05$) after 10-min exposure to eugenol. These results highlight the importance of investigating the potential effects of any new anaesthetic or euthanizing compounds on blood plasma parameters, prior to using them in a research setting, or when comparing results to other studies which have utilized alternative anaesthetic compounds.

Keywords: clove oil, eugenol, MS-222, anaesthesia, blood plasma, hormones, trout

Introduction

Anaesthesia, euthanasia and sedation of both wild and captive fish are common requirements in aquaculture and fisheries research around the world. These clinical techniques facilitate a wide variety of activities such as sorting, grading, transportation, tagging, gamete collections, health monitoring, weight/length measurements, blood sampling and invasive surgery to name a few. In all countries with animal care legislation, anaesthetics are routinely required during procedures that are deemed stressful or painful to fish. Several chemical anaesthetics are currently utilized for these purposes, the most common being tricaine methanesulfonate (MS-222), benzocaine, quinaldine, quinaldine sulphate, 2-phenoxyethanol, ethyl aminobenzoate, etomidate and metomidate (Summerfelt & Smith 1990). Most of these compounds meet the basic criteria for efficacy and safety (Marking & Meyer 1985), and produce various stages of controlled anaesthesia from light

sedation to death via full medullary collapse (McFarland 1959; Jolly, Mawdesly-Thomas & Bucke 1972). A controlled overdose of these same compounds is often used for humanely euthanizing fish prior to lethal sampling of tissues or blood in a variety of research studies. Depending on the country, some of these compounds are currently not approved for food-fish use, are extremely expensive, or may present safety hazards to the user (Bernstein, Digre & Creel 1997) or to the fish if handled improperly. Therefore, the search continues for an anaesthetic and euthanizing compound that is singularly safe, inexpensive, readily obtainable and efficacious to a wide variety of fish species. Clove oil, which contains the active ingredient eugenol, has been identified as a possible candidate that may satisfy many of these criteria.

Clove oil is derived from the stem, leaves and buds of the *Eugenia caryophylatta* tree, and it contains the active ingredient eugenol (4-allyl-methoxyphenol) in concentrations of 70–90% by volume (British Pharmacopoeia 1993). Eugenol is commonly used as an analgesic and antiseptic agent in human dentistry (Curtis 1990), and as a food additive for flavouring (Maura, Pino & Ricci 1989), and has been demonstrated to be extremely safe for humans (Miller, Swanson, Phillips, Fletcher, Liem & Miller 1983). Eugenol is rapidly absorbed and metabolized after oral administration and it is almost completely excreted in the urine within 24 h with no apparent ill effects (Fischer, von Unruh & Dengler 1990). Thus, eugenol has long been considered safe for laboratory use (Liu & Gibson 1977). It has also been studied as a potential fish anaesthetic and was shown to be effective in a wide variety of species including, but not limited to, medaka (*Oryzias latipes* Temminck & Schlegel), goldfish (*Carrassius auratus* Linnaeus), carp (*Cyprinus carpio* Linnaeus), rabbitfish (*Siganus lineatus* Valenciennes), channel catfish (*Ictalurus punctatus* Rafinesque), Atlantic (*Salmo salar* Linnaeus) and sockeye (*Oncorhynchus nerka* Walbaum) salmon (Endo, Ogishima, Tanaka & Ohshima 1972; Hikasa, Takase, Ogasawara & Ogasawara 1986; Soto & Burhanuddin 1995; Waterstrat 1999; Chanseau, Bosc, Galiay & Oules 2002; Woody, Nelson & Ramstad 2002). Studies in our laboratory have demonstrated that eugenol is also a highly efficacious and cost-effective anaesthetic for rainbow trout, *Oncorhynchus mykiss* Walbaum (Keene, Noakes, Moccia & Soto 1998). It induces a faster rate of anaesthesia at a lower concentration than does MS-222, and has an effective 96-h LC_{50} of approximately 9 mg L^{-1}. Eugenol meets all of the required criteria for being a suitable anaesthetic. However, as a research tool, it is necessary to ensure that it does not cause perturbations in various biochemical or physiological parameters that may be under evaluation.

The investigation of any impact that eugenol may have on several frequently used blood chemistry indicators is necessary in order to evaluate its potential use for routine aquaculture and fisheries research. Therefore, the purpose of the current study was to evaluate the effect of eugenol anaesthesia and euthanasia on the circulating plasma levels of cortisol, glucose, growth hormone and the two thyroid hormones, thyroxine and tri-iodothyronine (commonly referred to as T_4 and T_3 respectively) in rainbow trout.

Methods and materials

Source and maintenance of fish

Sexually immature rainbow trout, (1+ years old, mean body weight 125 g) were obtained from the Alma Aquaculture Research Station, Alma, ON, Canada. The fish were exposed to natural photoperiod regimens, and were maintained outdoors in a shaded, 10-m circular tank supplied with a continuous flow of high-quality, 8.5 °C-aerated groundwater. Fish were fed from approximately 09:00–21:00 hours, with a commercial trout grower diet appropriate to fish length and weight (Martin's Feed Mills, Elmira, ON, Canada) using 12-h duration belt feeders. These fish had normal background levels of morbidity and mortality, and were in otherwise excellent health.

Preparation of eugenol stock solutions

The eugenol stock solution was prepared by dissolving clove oil (90% eugenol by volume) with ethanol at a ratio of 1 part clove oil to 10 parts ethanol. This solution was then diluted (in fresh water of similar chemistry to that which the fish were held in) to achieve the appropriate final test concentrations.

Blood sampling

Experiment 1

Forty fish were randomly collected and removed from the outdoor tank with a seine net, and then placed into an indoor 200-L holding tank. Fish were then

transferred from the holding tank by dip netting, and randomly split into three groups of 10, 15 and 15 fish each. The fish in these groups were either stunned by a sharp blow to the cranium ($n = 10$), or placed into a solution of either MS-222 (150 mg L^{-1}; $n = 15$) or eugenol (150 mg L^{-1} active ingredient; $n = 15$). Blood samples were then collected into heparinized tubes following caudal severance either after deep, level 5+ anaesthesia (as described in Keene *et al.* 1998) was achieved prior to euthanasia in both the eugenol and MS-222 treatment groups (sampling usually occurred at approximately 2 min in all treatments, including the stunned group). Blood samples were centrifuged for 20 min, the plasma collected, and then stored at -70 °C until analysis.

Experiment 2

Sixty fish were obtained from the same outdoor tank in a manner identical to that described in experiment 1. Animals were randomly transferred from the holding tank and placed into one of six groups, and subjected to one of the following treatment solutions: (A) MS-222 (150 mg L^{-1}; $n = 10$), (B) eugenol (150 mg L^{-1}; $n = 20$), (C) MS-222 (25 mg L^{-1}; $n = 10$), (D) eugenol (25 mg L^{-1}; $n = 10$) or (E) untreated water only (control; $n = 10$). Concentrations of 25 and 150 mg L^{-1} generally represent the lower and higher ends, respectively, of doses used for fish in laboratory studies and were therefore investigated in the present study. After deep anaesthesia (Jolly *et al.* 1972) was achieved (approximately 2 min), blood samples from groups A and B were immediately collected by caudal severance. The 10 remaining fish that were immersed in the 150 mg L^{-1} solution of eugenol (B) were left exposed to the anaesthetic for an additional 10 min, after which blood was collected (10-min exposure group). Blood samples from fish in treatments, C, D and E, were collected after 2 min by venous puncture of the caudal vein with a heparinized syringe. Times of 2 and 10 min after anaesthetic dosing were sampled as they represent times approximating common laboratory procedures that are short and long in duration respectively. Post-anaesthetic sample times of 2 and 10 min also actually represent exact times of >3 and >11 min, respectively, as blood was actually sampled within 1 min of the prescribed treatment times. For simplicity, these times are further referred to as 2 and 10 min respectively. Plasma was collected as described above, and stored at -70 °C prior to analysis.

Hormone and glucose analysis

Plasma growth hormone (GH) concentrations were measured using a non-competitive, enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies developed against salmon GH (Farbridge & Leatherland 1991). Plasma thyroid hormone levels were measured using Amerlex radioimmunoassay (RIA) kits (Johnson and Johnson Clinical Diagnostics, Markham, ON, Canada) modified to use 5 or 25 µL of plasma for the thyroid hormones T_3 and T_4 respectively. Plasma glucose concentrations were measured using a colorimetric assay (Sigma Chemical, St Louis, MO, USA) modified to use 5 µL of plasma. Plasma cortisol concentrations were measured using the Incstar RIA kit (Incstar Corporation, Stillwater, MN, USA) previously validated for use with rainbow trout plasma.

Statistical analysis

The results from experiments 1 and 2 were analysed separately. Mean plasma hormone and glucose levels for each treatment were compared by one-way analysis of variance (ANOVA). Individual means were compared using Tukey's Honestly Significantly Different test when significant differences between treatment means were found using ANOVA. All statistics were assessed using a level of significance of $\alpha = 0.05$. Statistics were performed using the SPSS statistical Package (SPSS, Chicago, IL, USA).

Results

Plasma cortisol concentration

In experiment 1, cortisol levels in the stunned animals were significantly higher than fish anaesthetized with either 150 mg L^{-1} of MS-222 or 150 mg L^{-1} eugenol ($P = 0.004$) while there was no significant difference in mean cortisol level between the MS-222 or eugenol groups. In experiment 2, fish in the 10-min eugenol exposure (euthanized) group had significantly lower plasma cortisol levels than fish in any other treatment group ($P < 0.03$; Table 1). There were no other statistically significant differences in cortisol levels between any other treatment groups.

Plasma glucose concentration

In experiment 1, plasma glucose levels in stunned fish were significantly higher ($P < 0.05$) than those in fish

Table 1 Mean blood plasma levels of cortisol, glucose, growth hormone (GH), tri-iodothyronine (T_3) and thyroxine (T_4) in rainbow trout (*Oncorhynchus mykiss*) anaesthetized with eugenol and tricaine methanesulfonate (MS-222)*

Treatment group (no. animals)	Cortisol (nmol L^{-1})	Glucose (mg dL^{-1})	GH (ng mL^{-1})	T_3 (nmol L^{-1})	T_4 (nmol L^{-1})
Experiment 1					
MS-222	575.22[a]	73.84[a]	4.91	15.39	13.60
150 mg L^{-1} at 2 min (15)	(24.82)	(4.68)	(0.63)	(1.51)	(1.97)
Eugenol	501.99[a]	79.00[a]	3.55	17.03	12.02
150 mg L^{-1} at 2 min (15)	(17.65)	(2.84)	(0.37)	(1.19)	(1.34)
Stunned at 2 min (10)	734.46[b]	105.54[b]	3.94	14.14	14.34
	(49.09)	(9.09)	(0.61)	(1.35)	(1.65)
Experiment 2					
A MS-222	609.52[a]	78.37[ab]	1.72	11.72[a]	7.25[ab]
150 mg L^{-1} at 2 min (10)	(24.43)	(4.69)	(0.29)	(1.94)	(0.57)
B Eugenol	523.04[a]	70.35[ab]	5.17	14.71[ab]	8.67[ab]
150 mg $^{-1}$ at 2 min (10)	(21.34)	(5.13)	(2.92)	(2.08)	(1.09)
B Eugenol	345.87[b]	77.61[ab]	1.63	21.12[b]	10.10[a]
150 mg L^{-1} at 10 min (10)	(36.90)	(5.82)	(0.55)	(3.95)	(1.82)
C MS-222	598.67[a]	70.86[ab]	6.50	11.62[a]	7.03[ab]
25 mg L^{-1} at 2 min (10)	(42.77)	(2.92)	(2.47)	(1.15)	(0.88)
D Eugenol	525.14[a]	88.86[a]	9.93	11.08[a]	9.24[ab]
25 mg L^{-1} at 2 min (10)	(51.41)	(3.27)	(3.10)	(1.15)	(0.86)
E None	513.35[a]	68.59[b]	7.57	12.18[a]	6.40[b]
at 2 min (10)	(41.21)	(4.51)	(1.36)	(1.20)	(0.72)

*Numbers within a column and experiment with the same (or no) superscript, are not significantly different from one another. Experiments 1 and 2 were analysed separately, therefore, the results cannot be compared between the two. Numbers in parentheses represent 1 SEM.

euthanized with either MS-222 or eugenol. In experiment 2, the only significant difference between treatments was that the 25 mg L^{-1} eugenol group showed a significantly higher mean plasma glucose level when compared with the control group ($P = 0.028$).

Plasma GH concentration

There were no statistically significant differences in plasma GH between any treatment groups in either experiment 1 or 2.

Plasma T_3 and T_4 concentrations

There were no significant differences in plasma T_3 between any treatment groups in experiment 1. In experiment 2, the 10-min eugenol exposure group had plasma T_3 levels that were significantly higher than those found in all other treatment groups (all $P < 0.05$), except immediate sampling following immersion in 150 mg L^{-1} eugenol. Plasma T_4 levels were not significantly different between treatment groups in experiment 1, but in experiment 2, plasma T_4 levels in the 10-min eugenol exposure group were significantly higher than the plasma T_4 levels in control fish ($P = 0.028$).

Discussion

Recent work has demonstrated clove oil (eugenol) to be a suitable anaesthetic for aquacultural and fisheries use (Keene *et al.* 1998; Taylor & Roberts 1999), although it is important to note that it has not been approved for such applications in most countries at the present time (FDA 2002). This is due primarily to the lack of animal and human safety testing necessary to support applications for regulatory approval. Clove oil has been found to have differences in effects relative to the more commonly used anaesthetic, MS-222, some of which may make it a more desirable anaesthetic in certain applications. For example, Small (2003) found that plasma cortisol levels remained at baseline levels with clove oil during 30 min of anaesthesia in the channel catfish *Ictalurus punctatus*, while MS-222-anethesized catfish showed an eight-fold increase in cortisol levels over the same period. Wagner, Arndt and Hilton (2002) reported that clove oil was more suitable as an anaesthetic for rainbow trout than either MS-222 or carbon dioxide, due to its longer recovery times and lower cost, when administered in the form of AQUI-S^{TM} (AQUI-S New Zealand, Wellington, New Zealand). It should be noted, however, that AQUI-S^{TM} is not pure clove oil,

it being composed mainly of isoeugenol. Other studies also report that although no anaesthetic completely eliminated a stress response associated with netting fish, those fish anaesthetized using clove oil showed much lower cortisol levels when compared with MS-222, 1 h after the induction of anaesthesia (Wagner, Singer & McKinley 2003).

Although many studies have evaluated the relative anaesthetic effects of clove oil and MS-222 on fish, few, to date, have evaluated the associated physiological impacts of these anaesthetics (Prince & Powell 2000; Wagner *et al.* 2003). Notwithstanding the lack of regulatory approval, due to its proven efficacy and decreased cost for industrial use, clove oil may become more commonly employed in fisheries and aquaculture research. However, caution should be exercised in research applications, since the effects of clove oil on blood plasma hormones (such as those commonly used as health or stress indicators in research) have not been adequately investigated. The effect of any anaesthetic on a given plasma hormone level can range from no effect to a pronounced effect. For example, in the first experiment of the current study, blood glucose and cortisol levels were strongly affected by the method of anaesthesia. In both cases, stunning actually increased plasma levels, while clove oil and MS-222 had no effect. By contrast, levels of GH, T_3 and T_4 were largely unchanged whether a fish was anaesthetized using stunning, MS-222 or clove oil. Also, the direction of the effect of an anaesthetic on hormone and glucose levels (i.e. causes either an increase or decrease in levels) can vary. Consider the 10-min exposure eugenol group in experiment 2 of the current study. In this treatment group, cortisol levels were significantly decreased (relative to controls), yet there were no differences in either glucose or GH levels, while both T_3 and T_4 showed significantly elevated levels. Previous reports have also illustrated that some anaesthetics can significantly alter fish blood plasma chemistry. For example, Harrington, Russell, Singer and Ballantyne (1991) found significant reductions in the amount of absolute and total levels of fatty acids between rainbow trout exposed to MS-222 to the point of respiratory failure, and control groups which were not anaesthetized. The authors noted that depressed levels of non-esterified fatty acid levels were likely in response to reduced lipolysis due to the anaesthetic. Davidson, Davie, Young and Fowler (2000) also reported a significant increase in plasma cortisol, accompanied by an increase in haematocrit, and a decrease in potassium in rainbow trout after administration of clove oil in the form of AQUI-S^{TM}. These changes remained elevated for at least 24–48 h after treatment; however, total protein and sodium levels remained unchanged. Davidson and colleagues (2000) also reported that plasma cortisol concentrations in rainbow trout anaesthetized using AQUI-S^{TM} showed a biphasic response, with an initial increase followed by a decrease in the first few hours after administration. This was then followed by a second increase and subsequent decrease in concentration 24 h post administration. Gingerich and Drottar (1989) reported an increase in blood plasma catecholamine concentrations in trout, most notably epinephrine, during prolonged anaesthesia with MS-222. However, they reported no significant differences, relative to preanaesthesia levels, during early stages of anaesthesia. Such variability in different blood plasma chemistries highlights the importance of knowing the effect of a given anaesthetic, so as to not compromise blood plasma measures that are integral to the experimental design. Researchers should consider potential effects on any blood measures that are important to the results of the experiment, and because of this uncertainty, Houston (1997) questioned the validity of several classical haematological variables which are used to assess fish health. The author suggested that sensitivity of any indicators must be carefully considered before using them to interpret a fish's health status.

The results of the current and previous studies highlight the importance of considering all potential physiological or other effects of a candidate anaesthetic used for aquaculture or fisheries research. It is clear that different anaesthetics can have marked effects upon blood chemistry of fish. Thus, careful consideration should be taken when selecting an anaesthetic, particularly when variations in blood plasma chemistry measures are being used as end point indicators in a study, or when these results are being compared between different laboratories. In such cases, suitable controls should be incorporated into the research projects so as to clarify these effects, and extreme caution exercised when comparing results to other studies, which have employed alternative anaesthetics or euthanizing compounds.

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References

Bernstein P.S., Digre K.B. & Creel D.J. (1997) Retinal toxicity associated with occupational exposure to the fish anesthetic MS-222. *American Journal of Ophthalmology* **124**, 843–844.

British Pharmacopoeia (1993) Department of Health and Social Security, London, UK, 172pp.

Chanseau M.S., Bosc E., Galiay & Oules G. (2002) The use of clove oil as an anesthetic for Atlantic salmon smolts (*Salmo salar* L.) and comparison of its effects with those of 2-phenoxyethanol. *Bulletin Français de la Pêche et de la Pisciculture* **365/366**, 579–589.

Curtis E.K. (1990) In pursuit of palliation: oil of cloves in the art of dentistry. *Bulletin of the History of Dentistry* **38**, 9–14.

Davidson G.W., Davie P.S., Young G. & Fowler R.T. (2000) Physiological responses of rainbow trout *Oncorhynchus mykiss* to crowding and anesthesia with AQUI-S™. *Journal of the World Aquaculture Society* **31**, 105–114.

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

Farbridge K.J. & Leatherland J.F. (1991) The development of a noncompetitive enzyme-linked immunosorbent assay for oncorhynchid growth hormone using monoclonal antibodies. *General and Comparative Endocrinology* **83**, 7–17.

FDA (US Department of Health and Human Services, Food and Drug Administration) (2002) *Guidance for Industry: Status of Clove Oil and Eugenol for Anesthesia of Fish*, Guide 150. Rockville, MD, USA.

Fischer I.U., von Unruh G.E. & Dengler H.J. (1990) The metabolism of eugenol in man. *Xenobiotica* **20**, 209–222.

Gingerich W.H. & Drottar K.R. (1989) Plasma catecholamine concentrations in rainbow trout (*Salmo gairdneri*) at rest and after anesthesia and surgery. *General and Comparative Endocrinology* **73**, 390–397.

Harrington A.J., Russell K.A., Singer T.D. & Ballantyne J.S. (1991) The effects of tricaine methanesulphonate (MS-222) on plasma nonesterified fatty acids in rainbow trout, *Oncorhynchus mykiss*. *Lipids* **26**, 774–775.

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

Houston A.H. (1997) Review: are the classical haematological variables acceptable indicators of fish health? *Transactions of the American Fisheries Society* **126**, 879–894.

Jolly D.W., Mawdesley-Thomas L.E. & Bucke D. (1972) Anesthesia of fish. *Veterinary Record* **91**, 424–426.

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

Liu E.H. & Gibson D.M. (1977) Visualization of peroxidase isozymes with eugenol, a noncarcinogenic substrate. *Analytical Biochemistry* **79**, 597–601.

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

Maura A., Pino A. & Ricci R. (1989) Negative evidence *in vivo* of DNA-damaging, mutagenic and chromosomal effects of eugenol. *Mutation Research* **227**, 125–129.

McFarland W.N. (1959) A study of the effects of anesthetics on the behaviour and physiology of fishes. *Publication of the Institute of Marine Sciences, University of Texas* **6**, 23–55.

Miller E.C., Swanson A.B., Phillips D.H., Fletcher T.L., Liem A. & Miller J.A. (1983) Structure-activity studies of the carcinogenities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. *Cancer Research* **43**, 1124.

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**, 1019–1032.

Small B.C. (2003) Anesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinaldine and clove oil anethesized channel catfish *Ictalurus punctatus*. *Aquaculture* **218**, 177–185.

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

Summerfelt R.C. & Smith L.S. (1990) Anesthesia, surgery and related techniques. In: *Methods for Fish Biology* (ed. by C.B. Schreck & P.B. Moyle), pp. 213–272. American Fisheries Society, Bethesda, MD, USA.

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

Wagner E., Arndt R. & Hilton B. (2002) Physiological stress response, egg survival and sperm motility for rainbow trout broodstock anaesthetized with clove oil, tricaine methanesulfonate or carbon dioxide. *Aquaculture* **211**, 353–366.

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

Waterstrat P.R. (1999) Induction and recovery from anesthesia in channel catfish *Ictalurus punctatus* fingerlings exposed to clove oil. *Journal of the World Aquaculture Society* **30**, 250–255.

Woody C.A., Nelson J. & Ramstad K. (2002) Clove oil as an anesthetic for adult sockeye salmon: field trials. *Journal of Fish Biology* **60**, 340–347.

