

Olfactory nerve response of masu salmon (*Oncorhynchus masou* Brevoort) and rainbow trout (*O. mykiss* Walbaum) to clove oil and MS-222

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Abstract

Two anaesthetics, clove oil and methane sulphonate (MS-222), were examined for their effects on the olfactory nerve response of masu salmon (*Oncorhynchus masou* Brevoort) and rainbow trout (*O. mykiss* Walbaum). Exposing both species to clove oil for 3 min at concentrations of 50 and 100 mg L⁻¹, or for 10 min at 50 mg L⁻¹, did not significantly reduce their olfactory response. Directly applying clove oil anaesthesia to the olfactory epithelium significantly reduced olfactory response though after 20 min, olfactory response recovered to 70% and 52% of pre-treatment levels in masu salmon and rainbow trout respectively. Compared with the post-anaesthetic recovery of responses after clove oil (50 mg L⁻¹), buffered MS-222 (100 mg L⁻¹) with NaHCO₃ (100 mg L⁻¹), and unbuffered MS-222 (100 mg L⁻¹) treatment for 3 min, the response after MS-222 treatment declined gradually and significantly, but not after clove oil and MS-222+NaHCO₃ treatments. Clove oil appears to be an effective and relatively safe anaesthetic for salmonids with little long-term impact on their olfactory response, which plays a crucial role in their life history.

Keywords: clove oil, MS-222, anaesthetic, salmon, olfactory nerve response

Introduction

Fisheries research often requires handling research subjects, potentially reducing their immunological

capacity (Ellis 1981; Fries 1986; Schreck, Solazzi, Johnson & Nickelson 1989; Mommsen, Vijayan & Moon 1999). Anaesthetics are routinely used to block the hypothalamus–pituitary interrenal axis and to prevent fish from reacting to additional stressors (Olsen, Einarsdottir & Nilssen 1995). It is generally assumed that anaesthetics do not cause long-term changes in fish behaviour and that normal sensory interpretation, including olfaction, rapidly resumes after recovery. Only one study has shown long-term reduction in olfaction from a widely used fish anaesthetic: tricaine methane sulphonate (MS-222) which reduced olfaction in channel catfish (*Ictalurus punctatus* Rafinesque) for up to 28 days (Lewis, Tarpley, Marks & Sis 1985). Subsequent assays showed no influence of MS-222 on the olfaction-mediated behaviours of homing in chinook salmon (*Oncorhynchus tshawytscha* Walbaum) or avoidance to L-serine in coho salmon (*O. kisutch* Walbaum) (Quinn, Olson & Konecki 1988). MS-222 is currently the only highly effective anaesthetic approved for use on food fish by the US Food and Drug Administration. However, MS-222 is expensive and requires a 21-day depuration period before consumption or release of fish, limiting its applicability and motivating fisheries researchers to examine cheaper alternatives with a zero withdrawal time.

Clove oil is a promising anaesthetic for use on teleosts. Clove oil is an extract of the *Eugenia aromatica* tree whose active ingredient is eugenol (4-allyl-2-methoxyphenol); it is commercially available and

inexpensive. Efficacy studies thus far suggest that clove oil and its derivatives (e.g. AQUI-S™, Ross & Ross 1999; eugenol, and isoeugenol) are effective fish anaesthetics (Soto & Burhanuddin 1995; Taylor & Roberts 1999; Woody, Nelson & Ramstad 2002; Iversen, Finstad, McKinley & Eliassen 2003). Although it appears to be an ideal anaesthetic (see criteria by Marking & Meyer 1985), few studies have examined its effects on fish physiology. The limited studies have indicated both positive (Anderson, McKinley & Colavecchia 1997; Pirhonen & Schreck 2003; Wagner, Singer & McKinley 2003) and negative results (Davidson, Davie, Young & Fowler 2000). Woody *et al.* (2002) raised concern regarding potential negative impacts to salmon olfaction and therefore homing ability. To date, no studies have been conducted on this important question.

The effects of clove oil on olfactory nerve response was examined in masu salmon and rainbow trout, with specific focus on both the short-term time course changes and the time course of olfactory recovery changes after treatment. Olfactory nerve response differences were also compared for masu salmon across treatments of clove oil, unbuffered MS-222 and buffered MS-222 with NaHCO₃.

Materials and methods

Experimental animals

Both 1-year-old masu salmon of the Mori strain and rainbow trout of the Date strain were used in the experiments. All fish were hatched and reared at the Toya Lake Station, Hokkaido University, Hokkaido, Japan. Masu salmon fork lengths averaged 21.42 cm (SE = 2.95 cm) and weights averaged 123.60 g (SE = 23.44 g). Rainbow trout fork lengths averaged 23.8 cm (SE = 4.49 cm) and weights averaged 169.40 g (SE = 5.90 g). All experiments were repeated using three to five fish per experiment; each fish was used for a single trial.

Olfactory stimulants

Fish were tested at water temperatures between 8 and 11 °C. All pond water (PW) used in the study originated from natural spring water. Water chemistry of PW was as follows: 40 mg L⁻¹ total hardness as CaCO₃, pH 6.1, conductivity 0.14 mS cm⁻¹, turbidity 0.0 nephelometric turbidity units, dissolved oxygen 9.1 mg L⁻¹. The compositions of amino acid and

related substances in the PW were as follows (nM): phosphoserine, 8.92; taurine, 5.66; L-aspartic acid, 2.72; L-threonine, 5.15; L-serine, 2.27; L-glutamic acid, 2.07; glycine, 4.97; L-alanine (L-Ala), 3.02; L-valine, 1.05; L-isoleucine, 7.48; L-lysine, 1.15; phosphoethanolamine, 1.73; L-tyrosine, 9.72; L-phenylalanine, 5.36; β-alanine, 6.08; γ-amino butyric acid, 1.45; ethanolamine, 3.4; 1-methyl-L-histidine, 4.2; L-histidine, 9; L-anserine, 1.9; L-glutamine, 1.07.

Mixtures of L-Ala in PW were used as the stimulus to assess olfactory nerve response before (control) and after anaesthetic exposure. L-alanine concentration varied with the experiment. Clove oil, L-Ala and MS-222 were purchased from Wako Pure Chemical Industries (Tokyo, Japan) and Sigma Chemical (St Louis, MO, USA).

Anaesthetics

Clove oil and MS-222 test solutions were prepared with PW and stirred for 30 min to 1 h. New solutions were prepared every other day and stored in a refrigerator between uses. Anaesthetic concentration varied with the experiment.

Experimental protocol

Integrated olfactory nerve response was measured using the electrophysiological techniques of Sveinsson and Hara (1990). Fish were immobilized with an intramuscular injection of gallamine triethiodide (Sigma; 3 mg kg⁻¹ body weight). Gills were bathed through the mouth with an aerated solution of MS-222 (70 mg L⁻¹) which was not allowed to contact olfactory rosettes. A stereotaxic chamber kept exposed portions of the fish moist throughout the experiment.

A portion of the skull was surgically removed to expose the proximal olfactory nerve and bulbs. Twin tungsten electrodes were inserted into the olfactory nerve to record response to olfactory stimulus. A grand electrode filled with 3 M KCl agar (2%) bridged to an Ag–AgCl electrode was placed on the dorsal skin of fish for earthing. Electrodes remained in place for the duration of the experiment. After electrode placement, the olfactory rosettes were rinsed for 30 min with PW.

Recording olfactory nerve response

Olfactory nerve response was determined by monitoring electrical nerve response to different chemical

concentrations and exposure durations. Irrigating and stimulating solutions were applied to the olfactory epithelium via a stainless steel tube. The nerve response signal was amplified using an AC preamplifier (MOD. DAM-5A, W-P Instruments, Sarasota, FL, USA) at 300 Hz–3 kHz and integrated using an electric integrator (time constant = 0.3 s). Integrated olfactory nerve responses were recorded using a pen recorder.

The olfactory response magnitude of a fish was defined as the height of the tallest spontaneous peak in the integrated nerve response. Olfactory response to the stimulus (L-Ala/PW) for 10 s was measured before anaesthetic exposure and taken as the control response magnitude. The olfactory epithelium was washed using PW for 2 min between each treatment. The stimulus was re-applied for 10 s and olfactory response re-measured at various times after exposure to the anaesthetic, as determined by each experiment. The magnitudes of relative response to the stimulus were calculated as ratio to the control response of 10^{-4} M L-Ala.

Pilot study

A pilot study was conducted to determine effective clove oil anaesthetic exposure times and recovery periods in both masu salmon and rainbow trout. Fish were defined as under anaesthesia when they had reached a state where they did not react to handling. When fish had reached a state where they showed ability to remain upright and normal swimming behavior, they were defined as being recovered.

Fish were placed in a plastic tub containing 20 L of fresh water and 50 mg L^{-1} of clove oil, and time to anaesthesia was measured. The fish were then transferred to an aerated fresh water tank, and time to recovery was measured. Average anaesthetization time was about 3 min, so minimum anaesthesia treatment exposure time was set at 3 min. Average recovery time was about 6 min.

Experiments

Four experiments were conducted. Experiment 1 assessed the smallest stimulus concentration in which a change in olfactory nerve response was detected after exposure to anaesthetic. The different concentrations of L-Ala (10^{-3} – 10^{-7} M) exposed to rainbow trout before and after 100 mg L^{-1} clove oil treatment for 3 min and compared the olfactory nerve responses before and after clove oil treatment.

Experiment 2 compared the olfactory nerve response to the stimulus (10^{-4} M L-Ala) before and after exposure to three clove oil treatments: 50 mg L^{-1} exposure for 3 min, 50 mg L^{-1} exposure for 10 min and 100 mg L^{-1} exposure for 3 min. Response was measured at 0, 2, 4, 6 and 8 min using both masu salmon and rainbow trout.

Experiment 3 compared the olfactory nerve response to the stimulus (10^{-4} M L-Ala) before, in the presence of 50 mg L^{-1} clove oil, and 20 min after the anaesthetic treatment. The experiment was conducted using both masu salmon and rainbow trout.

Experiment 4 compared the olfactory nerve response to the stimulus (10^{-4} M L-Ala) before and after exposure to three different anaesthetic treatments: 50 mg L^{-1} clove oil, 100 mg L^{-1} unbuffered MS-222 (MS-222) and 100 mg L^{-1} MS-222 buffered with 100 mg L^{-1} NaHCO_3 (MS-222 + NaHCO_3), each using a 3-min exposure. Response was measured at 0, 10, 20, 30, 40 and 50 min after treatment using masu salmon.

Data analyses

Standardized response magnitudes were averaged across all fish in an experiment and means \pm SE were reported. One-way ANOVA was used to assess changes in mean standardized response magnitudes during each experiment with a significance level of $P = 0.05$. Typical integrated olfactory nerve response signals are displayed for most experiments.

Results

Experiment 1 did not reveal any significant difference in mean olfactory response magnitudes due to clove oil treatment (100 mg L^{-1} for 3 min) at any of the stimulus concentrations (10^{-3} – 10^{-7} M L-Ala) in rainbow trout (Fig. 1a and b). Though non-significant, a slight change in response was observed using the stimulus concentration 10^{-3} M L-Ala between before and after clove oil treatment (Fig. 1b).

Experiment 2 did not reveal any significant changes through time in the relative response magnitudes of either species after exposure to any of the three clove oil treatments (50 mg L^{-1} for 3 min, 50 mg L^{-1} for 10 min and 100 mg L^{-1} for 3 min) in masu salmon (Fig. 2a and b). In each treatment, the relative response magnitudes to compare with the control were reduced to 80–99% at 0 min, and

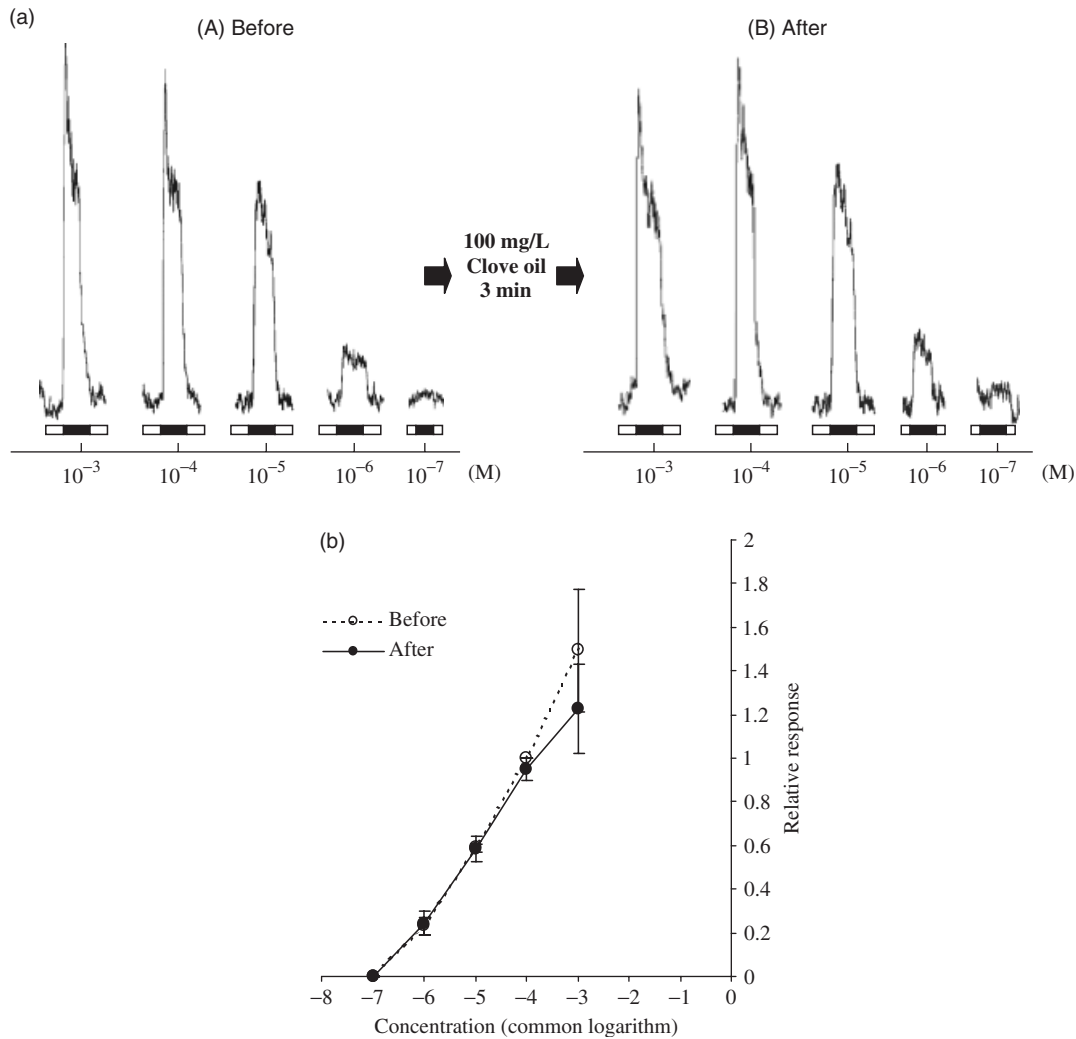


Figure 1 (a) Typical integrated olfactory nerve response of rainbow trout to different concentration of L-alanine before and after clove oil treatment (100 mg L^{-1} , 3-min exposure). (b) Relative magnitude of integrated olfactory nerve response of rainbow trout to different concentrations of L-alanine before and after clove oil treatment. The values are means \pm SE of data obtained from four to five fish.

were continued to 76–90 at 8 min, but these reductions were not significant.

Experiment 3 revealed a significant decline in the relative magnitude of response in both masu salmon ($51 \pm 17\%$) and rainbow trout ($20 \pm 14\%$) in the presence of 50 mg L^{-1} of clove oil (Fig. 3a and b). Twenty minutes after the clove oil treatment, the relative response magnitudes recovered to $70 \pm 31\%$ in masu salmon, but only to $52 \pm 17\%$ in rainbow trout that was significantly lower than before treatment (Fig. 3b).

Experiment 4 showed a significant decline ($70 \pm 3\%$) in the relative magnitude of response after exposure to clove oil at 0 min, but recovered to

$128 \pm 55\%$ at 50 min (Fig. 4a and b). Significant continuous declines through time in the relative response magnitudes were observed after exposure to MS-222 ($85 \pm 16\%$ at 0 min and $24 \pm 13\%$ at 50 min) but only a slight decline after exposure to MS-222+ NaHCO_3 ($99 \pm 6\%$ at 0 min and $86 \pm 4\%$ at 50 min).

Discussion

While many studies have assessed the effect of anaesthetics, including clove oil and MS-222, on swimming performance and handling stress minimization, little is known about their impacts on salmonid olfaction

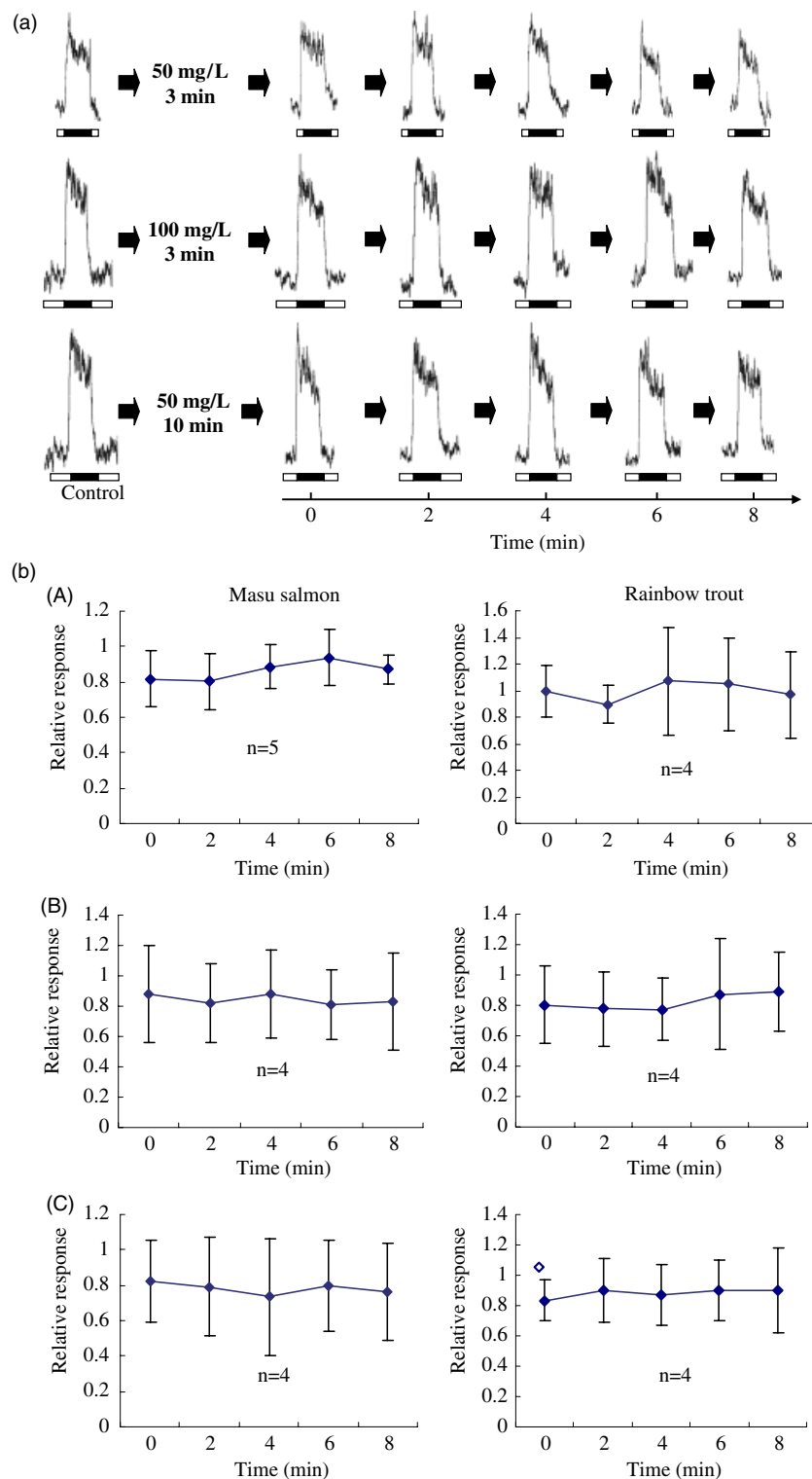


Figure 2 (a) Time course changes in typical integrated olfactory nerve response of masu salmon after exposure to three clove oil treatments. (b) Time course changes in relative magnitude of integrated olfactory nerve response of masu salmon and rainbow trout to different concentrations and anaesthetization times of clove oil. (A) 50 mg L⁻¹, 3 min; (B) 100 mg L⁻¹, 3 min; (C) 50 mg L⁻¹, 10 min. The values are means ± SE of data obtained from four to five fish.

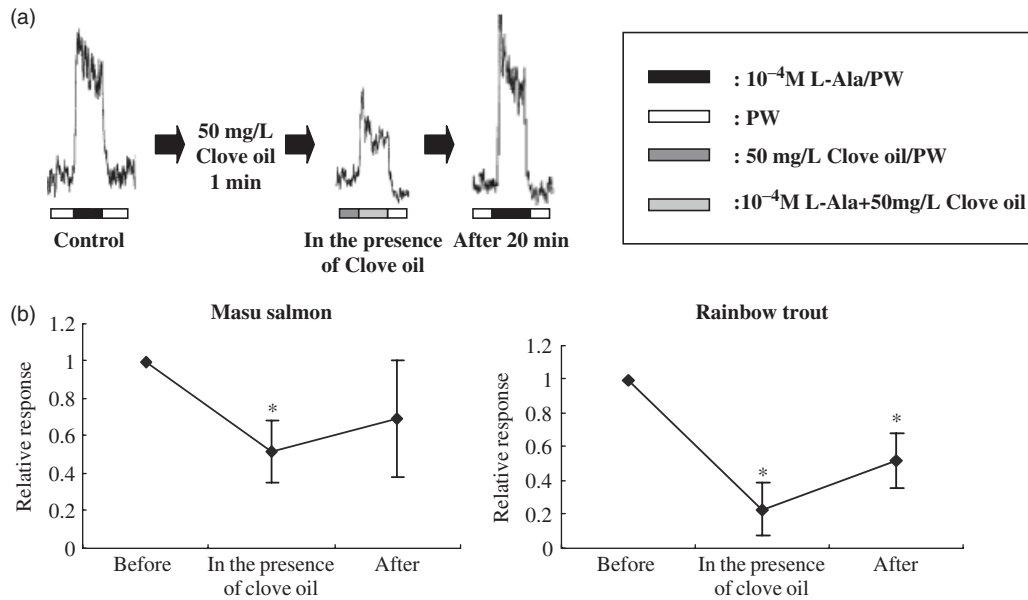


Figure 3 (a) Typical integrated olfactory nerve response of masu salmon before, in the presence of 50 mg L^{-1} clove oil, and after 20 min of the clove oil treatment. (b) Relative magnitude of integrated olfactory nerve response of masu salmon and rainbow trout to 10^{-4} M L-alanine before, in the presence of 50 mg L^{-1} clove oil, and after 20 min of the clove oil treatment. The values are means \pm SE of data obtained from four fish each. Significant decreases are detected in the presence of clove oil in masu salmon and rainbow trout, and after clove oil treatment in rainbow trout to compare with before treatment (* $P < 0.05$ using one-way ANOVA).

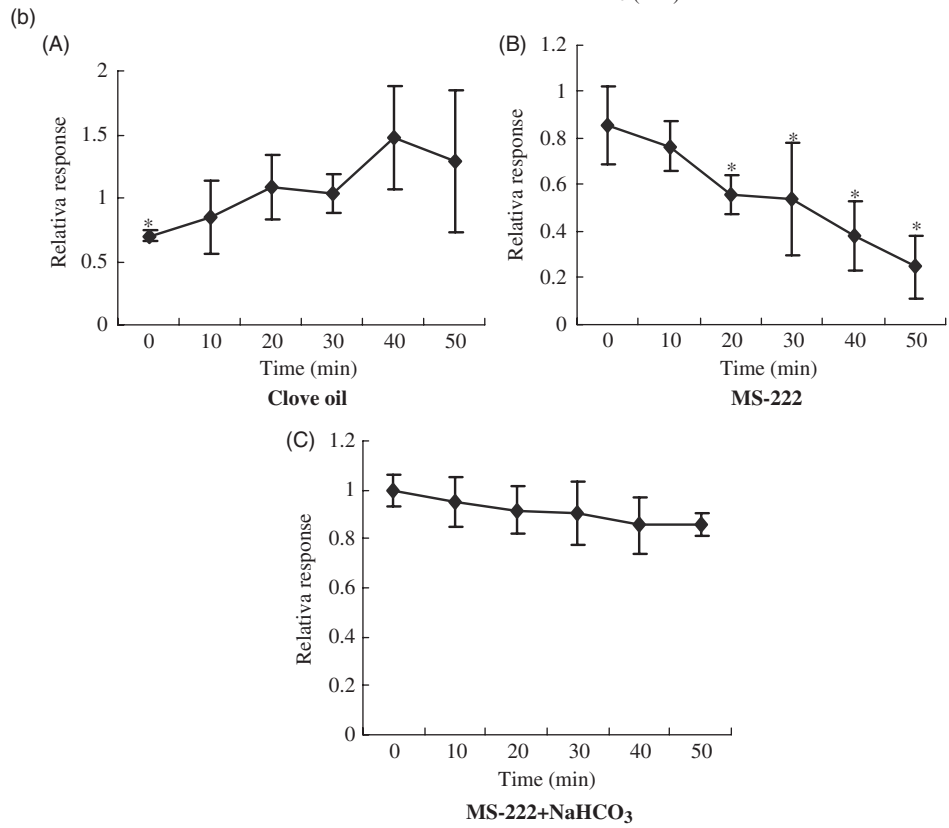
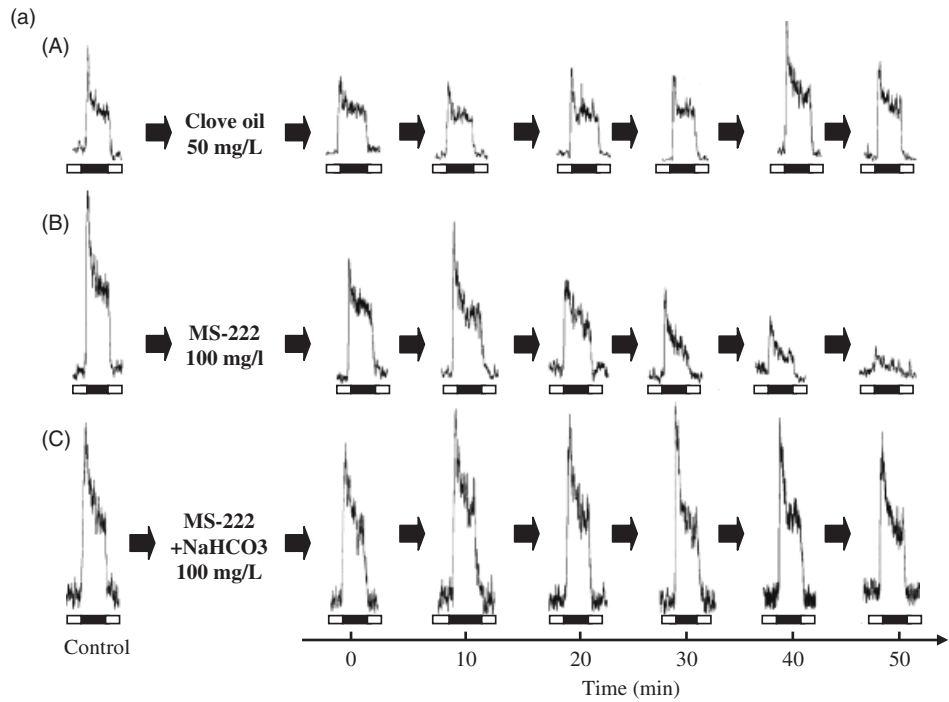
(Ellis 1981; Taylor & Roberts 1999; Woody *et al.* 2002; Wagner *et al.* 2003). The present study shows that clove oil anaesthesia appears an effective anaesthetic for salmonids, with little short-term influence on olfactory nerve response. In masu salmon and rainbow trout, clove oil had relatively little impact on olfactory sensitivity to stimulus (Experiment 1), and a little non-significant reduction on olfactory nerve responses (Experiment 2). Clove oil might have a little inhibitory effect on olfactory response in salmon.

For anadromous salmonids, olfaction is a critical sense for perception in the avoidance of predators (Rehnberg & Schreck 1987; Brown & Smith 1996), the recognition of conspecifics (Quinn & Busack 1985; Griffiths & Armstrong 2000), imprinting and homing to the natal stream (Hasler & Scholz 1983; Dittman & Quinn 1996). Chemicals that elicit the response from the olfactory organs of salmon include

amino acids, steroids, bile acids and prostaglandins (Hara 1992). In general, amino acids are potent odorants for fish. The salmon olfactory organ responds to various species of amino acids, for example, rainbow trout respond to 10^{-8} M L-serine and 10^{-7} M L-Ala (Caprio 1982, 1988; Hara 1982, 1992). And, L-Ala was one of the highly stimulatory amino acids for rainbow trout (Hara 1975). In the present study, we used L-Ala as the odorant stimulus.

When stimulating solutions were applied to the olfactory epithelium during direct anaesthesia using clove oil, olfactory nerve responses declined significantly. But after the clove oil treatment was discontinued, the response recovered to 70% and 52% before treatment in masu salmon and rainbow trout respectively (Experiment 3). While olfactory nerve response recovered within an hour after treatment with clove oil or MS-222 + NaHCO_3 , it gradually and

Figure 4 (a) Time course changes in typical integrated olfactory nerve response of masu salmon in different 3-min anaesthesia treatments: (A) 50 mg L^{-1} clove oil; (B) 100 mg L^{-1} MS-222 and (C) 100 mg L^{-1} MS-222 + 100 mg L^{-1} NaHCO_3 . (b) Time course changes in relative magnitude of integrated olfactory nerve response of masu salmon in different 3-min anaesthesia treatments: (A) 50 mg L^{-1} clove oil; (B) 100 mg L^{-1} MS-222 and (C) 100 mg L^{-1} MS-222 + 100 mg L^{-1} NaHCO_3 . The values are means \pm SE of data obtained from three fish each. Significant decreases are detected in 0 min (A), and 20, 30, 40 and 50 min (B) to compare with the control value (* $P < 0.05$ using one-way ANOVA). MS-222.



significantly declined after MS-222 treatment (Experiment 4). This may be due either to acidic low pH MS-222 solutions that suppress the olfactory response (Hara 1976), or to destruction of cilia on the olfactory sensory epithelia by exposure to MS-222 as occurred in channel catfish in (Lewis *et al.* 1985).

The present study clearly demonstrates the safety of clove oil as an anaesthetic relative to olfaction in masu salmon and rainbow trout and is the first to report on the impacts of clove oil on olfactory nerve response of salmon. Given that clove oil appears more effective than MS-222 at reducing short-term handling stress (Wagner *et al.* 2003), in conjunction with the current results of little significant impact on olfaction suggests that clove oil is a relatively safer anaesthetic in terms of impact on salmon olfactory nerve response. However, the present study was undertaken using only L-Ala as the odorant stimulus. Morphologically, there are three types of odorant receptor neurons (ORNs): ciliated, microvillar and crypt. For salmonids, ciliated ORNs are generalists that respond to all three odorant classes (pheromone, amino acid and bile salt) whereas microvillar ORNs are specialists that respond to amino acids (Sato & Suzuki 2001). It is unknown what odorants correspond to crypt cells (Hansen & Zielinski 2005), although sex pheromones have been hypothesized (Hamdani & Døving 2006). Thus, clove oil might have an effect on the olfactory response to other type of odorants, and therefore further studies should be required to ensure that clove oil does not reduce other type of olfactory stimuli.

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References

- Anderson W.G., McKinley R.S. & 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.
- Brown G.E. & Smith R.J.F. (1996) Foraging trade-offs in fat-head minnows (*Pimephales promelas*, Osteichthyes, Cyprinidae): acquired predator recognition in the absence of an alarm response. *Ethology* **102**, 776–785.
- Caprio J. (1982) High sensitivity and specificity of olfactory and gustatory receptors of catfishes to amino acids. In: *Chemoreception in Fishes* (ed. by T.J. Hara), pp. 109–134. Elsevier, Amsterdam, the Netherlands.
- Caprio J. (1988) Peripheral filters and chemoreceptor cells in fishes. In: *Sensory Biology of Aquatic Animals* (ed. by J. Atema, R.R. Fay, A.N. Popper & W.N. Tavolga), pp. 339–363. Springer, New York, NY, USA.
- Davidson G.W., Davie P.S., Young G. & Fowler R.T. (2000) Physiological responses of rainbow trout *Oncorhynchus mykiss* to crowding and anaesthesia with AQUI-S™. *Journal of the World Aquaculture Society* **31**, 105–114.
- Dittman A.W. & Quinn T.P. (1996) Homing in Pacific salmon: mechanisms and ecological basis. *Journal of Experimental Biology* **199**, 83–91.
- Ellis A.E. (1981) Stress and modulation of defense mechanisms in fish. In: *Stress and Fish* (ed. by A.D. Pickering), pp. 147–169. Academic Press, New York, NY, USA.
- Fries C.R. (1986) Effects of environmental stressors and immuno-suppressants on immunity of *Fundulus heteroclitus*. *American Zoologist* **26**, 271–282.
- Griffiths S.W. & Armstrong J.D. (2000) Differential response of kin and no kin to patterns of water flow: does recirculation influence aggression? *Animal Behavior* **59**, 1019–1023.
- Hamdani E.H. & Døving K.B. (2006) Specific projection of the sensory crypt cells in the olfactory system in crucian carp, *Carassius carassius*. *Chemical Senses* **31**, 63–67.
- Hansen A. & Zielinski B. (2005) Diversity in the olfactory epithelium of bony fishes: development, lamellar arrangement, sensory neuron cell types and transduction components. *Journal of Neurocytology* **34**, 183–208.
- Hara T.J. (1975) Olfaction in fish. In: *Progress in Neurobiology*, Vol. 5 (ed. by G.A. Kerrut & J.W. Phyllis), pp. 271–335. Pergamon Press, Oxford.
- Hara T.J. (1976) Effects of pH on the olfactory responses to amino acids in rainbow trout, *Salmo gairdneri*. *Comparative Biochemistry and Physiology* **54A**, 37–39.
- Hara T.J. (1982) Structure–activity relationships of amino acids as olfactory stimuli. In: *Chemoreception in Fishes* (ed. by T.J. Hara), pp. 135–157. Elsevier, Amsterdam, the Netherlands.
- Hara T.J. (1992) Mechanism of olfaction. In: *Fish Chemoreception* (ed. by T.J. Hara), pp. 150–170. Chapman Hall, London, UK.
- Hasler A.D. & Scholz A.T. (1983) *Olfactory Imprinting and Homing Salmon: Investigations into the Mechanism of Imprinting Process (Zoophysiology)*. Springer, Berlin, Germany.
- Iversen M., Finstad B., McKinley R.S. & Eliassen R.A. (2003) The efficacy of metomidate, clove oil, AQUI-S, and Benzoak® as anaesthetics in Atlantic salmon (*Salmo*

- salar* L.) smolts, and their potential stress-reducing capacity. *Aquaculture* **221**, 549–566.
- Lewis D.H., Tarpley R.J., Marks J.E. & Sis R.F. (1985) Drug induced structural changes in olfactory organ of channel catfish *Ictalurus punctatus* Rafinesque. *Journal of Fish Biology* **26**, 355–358.
- Marking L.L. & Meyer F.B. (1985) Are better anaesthetics need in fisheries? *Fisheries* **10**, 2–5.
- Mommsen T.P., Vijayan M.M. & Moon T.W. (1999) Cortisol in teleost; dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries* **9**, 211–268.
- Olsen Y.A., Einarsdottir I.E. & Nilssen K.J. (1995) Metomidate anaesthesia in Atlantic salmon, *Salmon salar*, prevents plasma cortisol increase during stress. *Aquaculture* **134**, 155–168.
- Pirhonen J. & Schreck C.B. (2003) Effects of anesthesia with MS-222, clove oil and CO₂ on feed intake and plasma cortisol in steelhead trout (*Oncorhynchus mykiss*). *Aquaculture* **220**, 507–514.
- Quinn T.P. & Busack C.A. (1985) Chemosensory recognition of siblings in juvenile coho salmon (*Oncorhynchus kisutch*). *Animal Behavior* **33**, 51–56.
- Quinn T.P., Olson A.E. & Konecki J.T. (1988) Effects of anaesthesia on the chemosensory behaviour of Pacific salmon. *Journal of Fish Biology* **33**, 637–641.
- Rehnberg B.G. & Schreck C.B. (1987) Chemosensory detection of predators by coho salmon (*Oncorhynchus kisutch*): behavioral reaction and physiological stress response. *Canadian Journal of Zoology* **65**, 481–485.
- Ross L.G. & Ross B. (1999) *Synthetic and Sedative Techniques for Aquatic Animals*, 2nd edn. Blackwell, London, UK.
- Sato K. & Suzuki N. (2001) Whole-cell response characteristics of ciliated and microvillous olfactory receptor neurons to amino acids, pheromone candidates and urine in rainbow trout. *Chemical Senses* **26**, 1145–1156.
- Schreck C.B., Solazzi M.F., Johnson S.L. & Nickelson T.E. (1989) Transportation stress affects performance of coho salmon, *Oncorhynchus kisutch*. *Aquaculture* **82**, 15–20.
- Soto C.G. & Burhanuddin C.G. (1995) Clove oil as a fish anaesthetic for measuring length and weight of rabbitfish (*Siganus lineatus*). *Aquaculture* **135**, 149–152.
- Sveinsson T. & Hara T.J. (1990) Analysis of olfactory responses to amino acids in arctic char (*Salvelinus alpinus*) using linear multiple-receptor model. *Comparative Biochemistry and Physiology* **97A**, 279–287.
- Taylor P.W. & Roberts S.D. (1999) Clove oil: an alternative anaesthetic for aquaculture. *North American Journal of Fisheries Management* **61**, 150–155.
- 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.
- Woody C.A., Nelson J. & Ramstad K. (2002) Clove oil as an anaesthetic for adult sockeye salmon: field trials. *Journal of Fish Biology* **60**, 340–347.