Pacific Herring Anaesthetic Trials With Eugenol, Isoeugenol and MS-222 in Association With a Coded Wire Tagging Study

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Field and laboratory trials were conducted in 1999 and 2000 with eugenol, isoeugenol and MS-222 on Pacific herring (*Clupea pallasi*) to simulate treatments for application in a coded wire tag and release study. In the field trials, groups of adult fish were treated in baths starting at 150 ppm for eugenol, 50 ppm for isoeugenol and 100 ppm for MS-222. In the laboratory trials, groups of juvenile fish were treated in baths starting at 30, 45 and 60 ppm. Administration of anaesthetics during tag insertion and release in the field introduced undesirable delays, especially the relatively long recovery periods from eugenol and isoeugenol treatments. The results of the laboratory trials showed that induction periods for the three agents were comparable at similar concentrations. Similar recovery time and behaviour were observed from eugenol and isoeugenol but recovery response from MS-222 was shorter and less variable.

RÉSUMÉ

Flostrand, L., and Schweigert, J.F. 2005. Pacific herring anaesthetic trials with eugenol, isoeugenol and MS-222 in association with a coded wire tagging study. Can. Tech. Rep. Fish. Aquat. Sci. 2578: iv + 16 p.

L’emploi en simulation d’eugénol, d’iso-eugénol et de MS-222 aux fins d’une étude d’étiquetage et de remise à l’eau du hareng du Pacifique (*Clupea pallasi*) a fait l’objet d’essais en laboratoire et sur le terrain en 1999 et 2000. Lors des essais sur le terrain, des groupes d’adultes ont été mis dans des bains contenant respectivement 150 ppm d’eugénol, 50 ppm d’iso-eugénol et 100 ppm de MS-222, et lors des essais en laboratoire, des groupes de juvéniles ont été mis dans des bains contenant respectivement 30, 45 et 60 ppm de ces agents. L’administration d’anesthésique au hareng sur le terrain en vue d’insérer une micromarque magnétisée codée a causé des retards indésirables, en particulier à cause de la période de récupération relativement longue des poissons après l’exposition à l’eugénol et l’iso-eugénol. Les résultats des essais en laboratoire révèlent que la période d’induction des trois agents sont comparables à des teneurs semblables. La période de rétablissement et le comportement des harengs traités à l’eugénol et à l’iso-eugénol étaient semblables, mais la période de rétablissement de ceux traités au MS-222 était plus courte et moins variable.

INTRODUCTON

Anaesthetics are frequently used to try to reduce fish stress and trauma during handling and transport, especially when fish are removed from water (Soto and Burhanuddin 1995; Keene et al. 1998; Peak 1998). An ideal anaesthetic for herring tag and release work would be one which 1) quickly and consistently anaesthetizes fish, 2) has short and predictable recovery periods so that fish can be released to the wild soon after tagging, and 3) does not have a regulated withdrawal period restricting immediate release of fish to the wild after recovery. Group anaesthetic trials can provide useful information for comparing outcomes from different concentrations, different anaesthetics and from re-using induction baths. A common way to measure fish responses to anaesthetic treatments is to time the onset of different induction and recovery behaviours. Several systems have been previously described for classifying stages of anaesthesia in fish (MacFarland 1959; Hisaka et al. 1986; Munday and Wilson 1997; Keene et al. 1998).

Anaesthetic trials with groups of adult or juvenile Pacific herring (*Clupea pallasi*) were conducted to assess different induction methods for their potential application in a coded wire tag (CWT) and release study. The Pacific herring CWT mark and recovery study began in 1999 to investigate inter-annual fish movement and dispersal to spawning grounds in inshore waters of British Columbia (BC) from late February to April (Flostrand and Schweigert 2002, 2003, 2004). Prior to the trials, there was hope that clove oil agents such as eugenol and isoeugenol would be ideal anaesthetics for application in field tagging because they were easy to obtain and had no restrictions or withdrawal periods on time of fish release post treatment. Trials with MS-222 were conducted to provide comparative information on herring response despite the withdrawal period associated with this agent.

METHODS

Induction and recovery stages were timed for groups of herring responding to each of three anaesthetics. Two of the anaesthetics were derivatives of clove oil, these were eugenol (4-allylcatechol-2-methyl ether) and isoeugenol (*cis* and *trans* -1-hydroxy-2 methoxy-4-propenylbenzene) and the third anaesthetic was tricaine methanesulfonate (MS-222). Sources of commercial anaesthetics were the same for field and laboratory trials, using 99% eugenol and 98% isoeugenol from Sigma – Aldrich Canada Ltd (Oakville, Ontario) and MS-222 from Syndel Laboratories (Vancouver, BC). Clove oil derivatives were first dissolved in 95% ethanol in a 1 to 10 ratio before further dilution with sea water to prepare the desired stock concentration. All field and laboratory inductions were done in 25 L baths using 40 L plastic totes. Anaesthesia induction and recovery stages monitored in the herring trials follow those described by Hisaka et al (1986) for carp (*Cyprinus carpio*), refer to Table 1 for abbreviation definitions of response

descriptions. During field trials with adult herring, I4 and R5 were timed whereas during juvenile laboratory trials, I2, I3, R3 and R4 were timed.

FIELD TRIALS

For the field trials, herring aged three years and older were collected by purse seine in the Strait of Georgia, BC, in March of 1999 and 2000. Trials with eugenol occurred on March 18, 1999 with baths at a starting concentration of 150 ppm; trials with isoeugenol occurred on March 15, 2000 with baths at a starting concentration of 50 ppm, and trials with MS-222 occurred on March 19, 1999 with baths at a starting concentration of 100 ppm. For each anaesthetic, there were eight sets of replicate trials each pertaining to three consecutive induction bath treatments. For each induction, we tried to treat each group of fish for the minimum amount of time required for all fish to reach I4. The three consecutive bath trials in each replicate consisted of 1) a freshly prepared bath at a known concentration, 2) a once-used bath and 3) a twice-used bath given an amount of original stock (referred to as a booster dosage). A booster dosage was applied to the third bath to increase the bath's concentration to a similar efficacy as the concentration of the first bath. Induction baths were prepared with fresh stock concentrations of 33% eugenol, 1% isoeugenol or 10% MS-222 and stocks were prepared immediately prior to field trials. Each group of fish added to a bath was one dip-net load weighing 5 to 6 kg and containing 50 to 70 herring. Seawater for the baths was pumped on deck by the vessel's hydraulics and water temperatures of the induction baths were recorded to the nearest degree Celsius. After each group of fish was anesthetised, the fish were distributed on a tagging table where people operating Mark IV CWT injectors (made by Northwest Marine Technology Inc.) inserted a CWT into muscle tissue on the central dorsal region of each fish and tagged fish were immediately placed in a closed, square recovery pen approximately 5.83 m^3 (Flostrand and Schweigert 2002). The recovery pen was partially lifted out of the water so fish at the base of the pen could remain underwater but be better observed. Timing of recovery periods started when the first fish in a group was placed in the recovery pen and ended when all fish in a group displayed R5 recovery behaviour. A representative random sample of 200 herring from each fishing event was frozen and later thawed to determine standard length, weight and condition factor (g/cm).

LABORATORY TRIALS

For the laboratory trials, juvenile herring of approximately twenty months old were collected by purse seine in the Johnstone Strait, BC in mid September, 1999. After capture, fish were brailed into a live holding tank (2 m long x 1 m wide x 2 m deep) and transported for 30 min by boat to a sea pen in Browns Bay, Vancouver Island. Fish were dip-netted out of the holding tank into a sea pen (15 m long x 15 m wide x 8 m deep) and held for approximately four months feeding on wild zooplankton. On January 6, 2000, approximately 700 herring were transferred to an aerated sea water tank (100 cm diameter x 90 cm high) and

transported by truck for three hours to the Pacific Biological Station (PBS) in Nanaimo. At PBS, the fish were dip-netted into a 3 m diameter tank filled 1 m high with a continuous supply of filtered sea water and held without being fed until experimentation days. Trials with eugenol, isoeugenol and MS-222 were conducted on January 14, 15 and 19, 2000, respectively.

Juvenile herring were removed from their anaesthetic baths once all fish in each group reached I3. Fresh induction baths of eugenol, isoeugenol and MS-222 were prepared at 30, 45 and 60 ppm and each group of herring was treated in either a fresh bath (B1) or a once-used bath (B2). One percent stock mixtures of the three agents were prepared daily and kept in opaque bottles at room temperature (17^{o}C). The number of fish treated per bath ranged from 9 to 22 (Table 3), determined by how many fish were collected in one scoop of a 30 x 20 cm rectangular dip net. This was done to try to minimize fish stress during their transfer and it enabled the induction start time to be the same for all fish in a group. After induction, the entire group was removed from the induction bath and placed in a fresh 25 L recovery bath of sea water. To verify that all fish in each group were induced to at least I3, a “touch test” was done whereby a gloved hand gently touched and tried to pick up individual fish to test their equilibrium and reflex activity. Recovery times were recorded from the onset of recovery until the first and last fish in each group reached R3 and R4. Cumulative trial times for each group of fish were also recorded from the onset of induction until the last R4 recovery observation was made. Information on water temperature and oxygen concentration was collected from each induction bath immediately after its use. A representative random sample of 334 herring was frozen on January 19 and later thawed to determine standard length, weight and condition factor (g/cm).

RESULTS

FIELD TRIALS

Fishing, tagging and environmental conditions during all field trials were comparable. Sea water temperatures were 8 to 9 ^{o}C and ambient air temperatures were 8 to 10 ^{o}C. Mean fish lengths, weights and condition factors (and standard deviations) from all representative samples ranged from 17.2 (2.1) to 18.6 (1.8) cm, from 74.8 (27.4) to 88.9 (26.7) g and from 4.24 (1.07) to 4.73 (1.05) g/cm, respectively (Table 2). Tagging occurred at a rate of approximately 800 tags/hour/tag injector with up to four tag injectors in operation. While groups of fish were being tagged, time differences between when the first and last fish in each group was placed in the recovery pen ranged from 2 to 3 min for all trials. No mortality was observed during the trials.

During the 1999 eugenol field trials, group I4 resulted from inductions of 0.8 to 1.5 min when: 1) new baths at 150 ppm were used; 2) once-used baths

were used, or, 3) baths used twice received a booster dosage (5 ml of 33%). Following all eugenol inductions and tag insertion treatments, it took 20 min for all fish to accumulate and display R5 recovery behaviour and there were no notable differences in recovery period from different induction treatments.

During the 2000 isoeugenol field trials, group I4 resulted from inductions of 2.0 to 3.0 min when fresh baths at 50 ppm were used. Subsequent use of baths without booster dosages had notably decreasing efficacies. For example, group I4 resulted from once-used baths after 3.8 to 4.3 min. When booster dosages (25 ml of 1%) were applied to baths that were used twice, inductions took from 3.5 to 4.5 min. Similar to the 1999 eugenol trials, it took 20 min for all treatment groups of fish to accumulate and display R5 recovery behaviour in the recovery pen.

During the 1999 MS-222 field trials, most fish appeared to swim energetically at the surface before losing equilibrium and group I4 resulted from inductions of 0.8 to 1.5 min when: 1) new baths at 100 ppm were used; 2) once-used baths were used, or, 3) baths used twice received a booster dosage (5 ml of 10%). . It took 6 to 7 minutes to accumulate entire groups of fish with R5 responses in the recovery pen and recovery times were not notably different for the different induction treatments.

LABORATORY TRIALS

In the laboratory trials, water temperatures for all baths ranged from 9.0 to 10.5 ^{o}C and oxygen concentrations ranged from 7.4 to 9.4 ppm for B1 trials and 5.5 to 8.9 ppm for B2 trials (Table 3). Mean (and standard deviation) fish length, weight and condition factor of the representative sample were 14.9 (1.1) cm, 23.5 (5.2) g and 1.57 (0.03) g/cm, respectively (Table 2). Observed mortality for the stock of approximately 700 fish was less than 3% from the time of collection from the live bait dealer (January 6) until the last day of the trials (January 19).

Similar induction and recovery time ranges, medians and cumulative times resulted between B1 and B2 trials (Figures 1 and 2). Although I2 and R3 results are presented, reporting focuses on the last fish I3 and R4 because these measure the total induction and recovery times, respectively. There were small decreases in first fish, last fish and median I2 times from increasing concentrations with eugenol and isoeugenol but with MS-222 these trends were not evident (Figures 1, 2 and 3). In MS-222 baths most fish appeared to swim energetically at the surface during I1 and I2 response behaviour. During each of the eugenol and isoeugenol trials, first and last fish I2 and I3 responses were easy to discern because fish showed progressive declines in motor skills and equilibrium. However, this was not the case with MS-222 trials where the transition from I2 to I3 was unclear because it appeared that some fish could temporarily regain partial equilibrium with minimal motor skills just prior to losing all reflex activity. This made it difficult to assess when the first and last fish reached I3; therefore MS-222 first fish I3 observations were not considered

reliable and are not included in the results. To accommodate I3 induction responses to MS-222, last fish I3 times were recorded when no fish in a group averted a touch test and when no fish in a group swam upright for 10 sec. With I3 responses, there were small decreases in induction times with increasing concentration from all anaesthetics except for B2 eugenol trials. Last fish I3 times from B1 trials ranged from 2.5 to 4.3 min for eugenol; from 1.7 to 3.0 min for isoeugenol, and from 1.6 to 4.5 min for MS-222. Last fish I3 times from B2 trials ranged from 2.3 to 4.2 min for eugenol, from 1.5 to 3.5 min for isoeugenol and from 2.5 to 4.5 min for MS-222.

Trends in recovery times to changes in bath concentration were less apparent than for inductions. It was difficult to distinguish MS-222 recovery transitions from R2 to R3 because some fish in each MS-222 recovery bath showed partial recovery of equilibrium (R2) in less than 10 sec. Because of this, we felt we could not accurately distinguish or measure first fish R3 times from R2, therefore we did not present first fish R3 times. Last fish R4 times from B1 trials ranged from 11.5 to 14.1 min for eugenol; from 12.0 to 21.3 min for isoeugenol; and from 2.0 to 4.0 min for MS-222. Last fish R4 times from B2 trials ranged from 11.3 to 12.8 min for eugenol, from 11.5 to 16.5 min for isoeugenol and from 4.0 to 6.3 min for MS-222.

Cumulative trial times from MS-222 treatments were considerably shorter than those from eugenol and isoeugenol (Figure 4). For each anaesthetic, cumulative trial times between different concentrations were comparable with the exception of isoeugenol B1 baths. Cumulative B1 trial times ranged from 15.0 to 17.0 min for eugenol, from 15.0 to 23.0 min for isoeugenol and from 4.3 to 6.5 min for MS-222. Cumulative B2 trial times ranged from 15.0 to 15.2 min for eugenol, from 18.0 to 19.0 min for isoeugenol and from 8.0 to 8.5 min for MS-222.

DISCUSSION

The herring anaesthetic trials were principally undertaken to determine if treatments using one of the clove oil agents could quickly and consistently anaesthetize groups of fish from which they could quickly and predictably recover. Although there was limited evidence to suggest that one anaesthetic was faster at induction than the others, recovery times from MS-222 were consistently shorter and less variable than those from the other two anaesthetics. Results suggest that had we continued to use eugenol or isoeugenol for tagging herring we probably would not have been able to release fully recovered herring in periods less than 20 min.

Differences in behaviour and response time resulting from clove oil and MS-222 treatments were not unexpected based on previously published studies and knowledge that these agents have different chemical properties. Similar to

the herring trials, differences in physical activity resulting from MS-222 and clove oil inductions were also observed by Munday and Wilson (1997), who noted that prior to appearing to lose equilibrium, fish swam at the surface more so from MS-222 inductions than with clove oil. Differences in respiratory and heart rate responses during induction and recovery processes were observed by Hisaka et al. (1986) and Keene et al. (1998). Hisaka et al. (1986) noted that the different chemical properties between anaesthetics may exert different influences on blood-gas and acid-base balances affecting entry and excretion across the gills. Keene et al. (1998) found induction times with rainbow trout (*Oncorhynchus mykiss)* to vary logarithmically with increased dose of eugenol but with MS-222 induction trends were negatively exponential. They also found that onset of induction from eugenol and MS-222 was comparable at concentrations of 80 to 100 ppm but, unlike what was observed in the herring laboratory trials, they found that MS-222 induction was longer than eugenol for concentrations less than 80 ppm.

The relatively long recovery times observed for herring from eugenol and isoeugenol are not atypical for clove oil treatments (Hisaka et al. 1986; Munday and Wilson 1997; Keene et al. 1998; Peak 1998; Prince and Powell 2000). Similar to what the results of the herring trials indicated, in addition to observing faster recoveries from MS-222 than from clove oil, Hisaka et al. (1986) found no prominent differences in carp (*Cyprinus carpio*) recovery times from changing bath concentration of either MS-222 or clove oil (at 50 to 200 ppm and 25 to 100 ppm, respectively). Keene et al. (1998) found rainbow trout recovery to be 6 to 10 times faster from MS-222 than from eugenol at 40 to 100 ppm (9 ^{o}C) whereas Peak (1998) observed marked differences in recovery times between clove oil trials at 40 and 120 ppm with walleyes (*Stizostedion vitreum*) but not between trials at 40, 60 and 80 ppm (10 ^{o}C). Prolonged recovery from clove oil agents following deep stages of anaesthesia may result from an inhibitory effect on the respiratory system (Keene et al. 1998).

Many factors may affect variability in induction and recovery times within and between trial groups. Eugenol and isoeugenol's lower solubility compared with MS-222 may have had confounding effects on results. Relatively cold temperatures would reduce oil solubility and increase adsorption to containers and fish bodies as evidenced by films in the containers and on the water's surface in the recovery pen. Other factors affecting response times include group size, physical activity, physiological states, individual sizes and environmental factors. Variability in induction responses within a group would have confounding effects on recovery times because fish reaching deeper induction stages would require longer recovery times (Peake 1998).

The implementation of the field and laboratory trials may seem somewhat ad hoc but there were reasons why the trials developed as they did. The group trials were not stringently controlled because we wanted to test the versatility of each anaesthetic to fieldwork conditions, including potential sources of error

between trial groups and fishing sessions. For the 1999 field trials, the MS-222 and eugenol bath strengths were chosen after test trials established that relatively high concentrations could induce large groups of adult herring to I4 in approximately 2 min. A 2 min goal was established by the tagging crew in an attempt to have a high turnover of freshly induced fish and to minimize delays during tagging. Shorter induction and recovery times were sought in the laboratory trials compared to the 1999 field trials by using lower concentrations, by having shallower induction stage criteria and by having fewer fish per trial group. Group I3 treatments were thought to be sufficient for tagging juvenile herring because they are easier to pick up by hand and control than adult fish. However, I4 juvenile herring trials would have made the laboratory trial results more comparable to those from field trials and may have helped in avoiding ambiguity in interpreting MS-222 loss of equilibrium responses. The concentrations used in the laboratory trials were lower than those used in the 1999 field trials because studies have indicated that reducing bath concentration reduces recovery times in addition to increasing safety margins from possible over exposure (Keene et al. 1998; Taylor and Roberts 1999). MS-222 was incorporated in the field and laboratory trials because it was conveniently available for providing comparable data; furthermore, in the event that a restriction on its withdrawal period is ever lifted, MS 222 could be considered for future herring tagging because its induction and recovery times were fairly rapid. Isoeugenol was the only anaesthetic used in the 2000 field trials because it was in ample supply and, based on results of the herring laboratory trials and from work of Prince and Powell (2000) on rainbow trout, the assumption was made that herring responses to isoeugenol and eugenol would be relatively equal. Booster dosages were applied in the field trials because they are a quick and inexpensive way to increase bath concentrations for serial inductions. The different volumes and quantities of active ingredients used for booster dosages in the three sets of field trials were chosen after conducting test trials using dosages at concentrations relatively proportional to fresh bath strengths.

In June of 2002, the USA Department of Health and Human Services Food and Drug Administration Centre for Veterinary Medicine prescribed a Guidance for Industry on the Status of Clove Oil and Eugenol for anaesthesia of Fish (150). This states that neither clove oil nor any of its active ingredients (eugenol, isoeugenol, or methyleugenol) is approved for anaesthesia of fish released to the wild unless under an Investigational New Animal Drug Exemption File or after a 21 day withdrawal period. Similar restrictions govern the administration of MS-222 in Canada and the USA as prescribed under the US Code of Federal Regulations (Title 21, Food and Drugs). Therefore, the applicability of the reported herring anaesthetic trials for future herring CWT and release work is no longer valid.

The application of anaesthesia to Pacific herring during early stages of the CWT study was a valid step in the planning and execution of the fieldwork. It was believed a priori that anaesthetizing herring could make them easier to tag in

addition to having animal care benefits. However, production bottlenecks in the tagging and releasing steps in addition to administrative restrictions make it more practical to eliminate anaesthesia from the fieldwork. Further attempts to apply anaesthetics in the Pacific herring CWT study are unlikely unless results suggest that survival, tagging quality or tagging production could be improved with their use and administrative restrictions could be overcome.

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Table 1. Anaesthesia induction and recovery stages as described for carp (*Cyprinus carpio*) in Hisaka et al. (1986).

Stage	Descriptor	Trials**	Description of behaviour
Induction			
0	I0		Normal
1	I1		*Sedation;* Partial or total loss of reaction in response to external stimuli, equilibrium is normal.
2	I2*	Juvenile	Partial loss of equilibrium, erratic swimming.
3	I3*	Juvenile	Total loss of equilibrium.
4	I4*	Adult	*Anaesthesia;* Loss of reflex activity.
5	I5		Medullary collapse: Respiratory movements cease. Fish death.
Recovery			
I	R1		Reappearance of opercular movement.
II	R2		Partial recovery of equilibrium with partial recovery of swimming motion.
III	R3*	Juvenile	Total recovery of equilibrium.
IV	R4*	Juvenile	Reappearance of avoidance swimming motion and reaction in response to external stimuli, but still behavioural response is stolid.
V	R5*	Adult	Total behavioural recovery. Normal swimming.

*Stages monitored in current trials with Pacific herring.
* *Adult trials occurred in the field and juvenile trials occurred in the laboratory.

Table 2. Date and temperature information for the Pacific herring anaesthetic trials and summary fish length (L), weight (W) and condition factor (CF) information from representative samples of size N.

Trials*	Date	Air (°C)	Water (°C)	N	Measure	Min	Max	Mean	SD
Adult eugenol	March 1999	8-10	8 -9	200	L(cm)	12.6	22.6	18.4	1.8
					W(g)	29.0	167.0	88.9	26.7
					CF(g/cm)	2.2	7.49	4.73	1.05
Adult MS-222	March 1999	8-10	8 -9	280	L(cm)	12.6	24.2	18.6	1.8
					W(g)	23.0	139.0	81.2	22.2
					CF(g/cm)	1.8	6.50	4.30	0.85
Adult isoeugenol	March 2000	9-10	9	200	L(cm)	12.1	21.6	17.2	2.1
					W(g)	26.0	164.0	74.8	27.4
					CF(g/cm)	2.1	7.59	4.24	1.07
Juvenile eugenol, isoeugenol and MS-222	January 2000	17	8.9 - 10.5	334	L(cm)	11.1	17.9	14.9	1.1
					W(g)	10.2	49.9	23.5	5.2
					CF(g/cm)	1.0	2.15	1.57	0.03

*Adult trials occurred in the field and juvenile trials occurred in the laboratory.

Table 3. Anaesthetic bath treatments, group sample sizes, anaesthetic bath-water temperatures and oxygen concentrations during juvenile Pacific herring laboratory trials.

Treatment	Bath	N	Temp. (^{O}C)	Oxygen (ppm)
Eugenol				
30 ppm	1	19	9.7	8.9
	2	9	10.5	8.0
45 ppm	1	17	9.0	9.4
	2	19	9.5	8.2
60 ppm	1	14	9.7	8.6
	2	21	10.0	7.5
Isoeugenol				
30 ppm	1	18	9.2	9.1
	2	22	9.5	8.9
45 ppm	1	19	9.4	7.7
	2	19	9.9	7.4
60 ppm	1	19	9.7	7.6
	2	20	10.0	5.5
MS-222				
30 ppm	1	17	8.9	9.2
	2	16	9.3	8.0
45 ppm	1	16	9.5	7.4
	2	16	9.8	7.3
60 ppm	1	19	9.2	8.8
	2	15	9.6	7.8

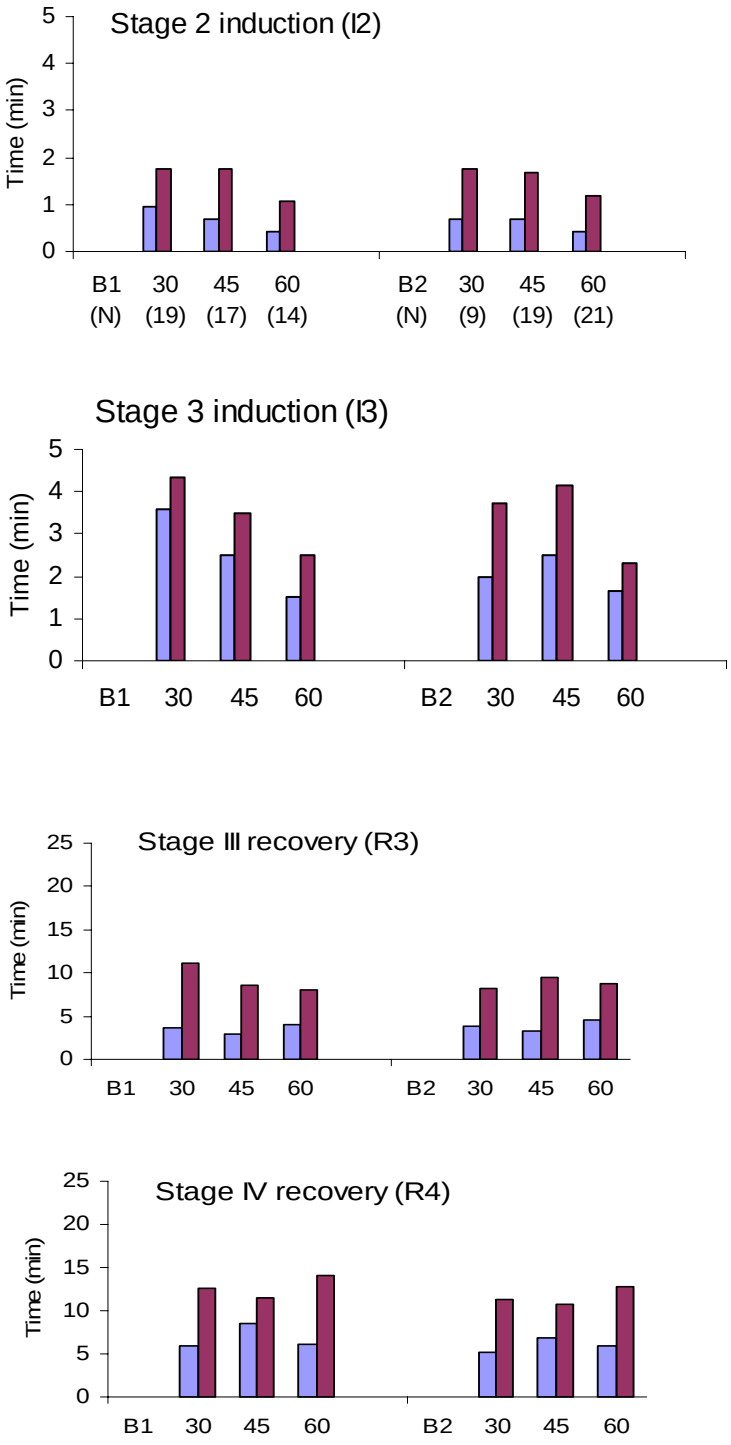

Figure 1. Response times from juvenile Pacific herring group trials with eugenol inductions starting at 30, 45 or 60 ppm. Lighter bars at each bath concentration are first fish response times and darker bars are last fish response times. B1 indicates fresh induction bath; B2 indicates once-used induction bath; numbers in parentheses refer to the sample size (N) in each trial group.

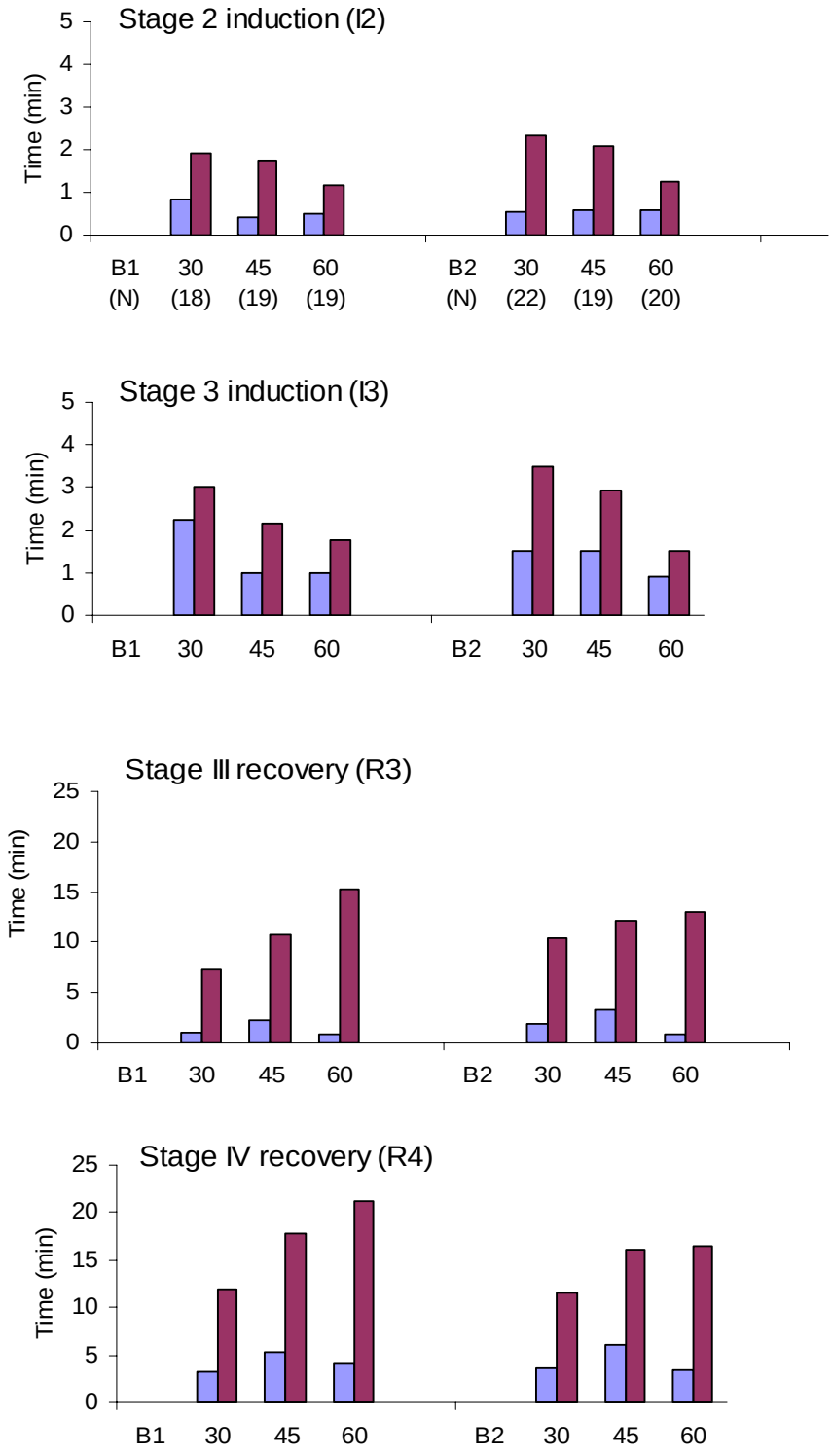

Figure 2. Response times from juvenile Pacific herring group trials with isoeugenol inductions starting at 30, 45 or 60 ppm. Lighter bars at each bath concentration are first fish response times and darker bars are last fish response times. B1 indicates fresh induction bath; B2 indicates once-used induction bath; numbers in parentheses refer to the sample size (N) in each trial group.

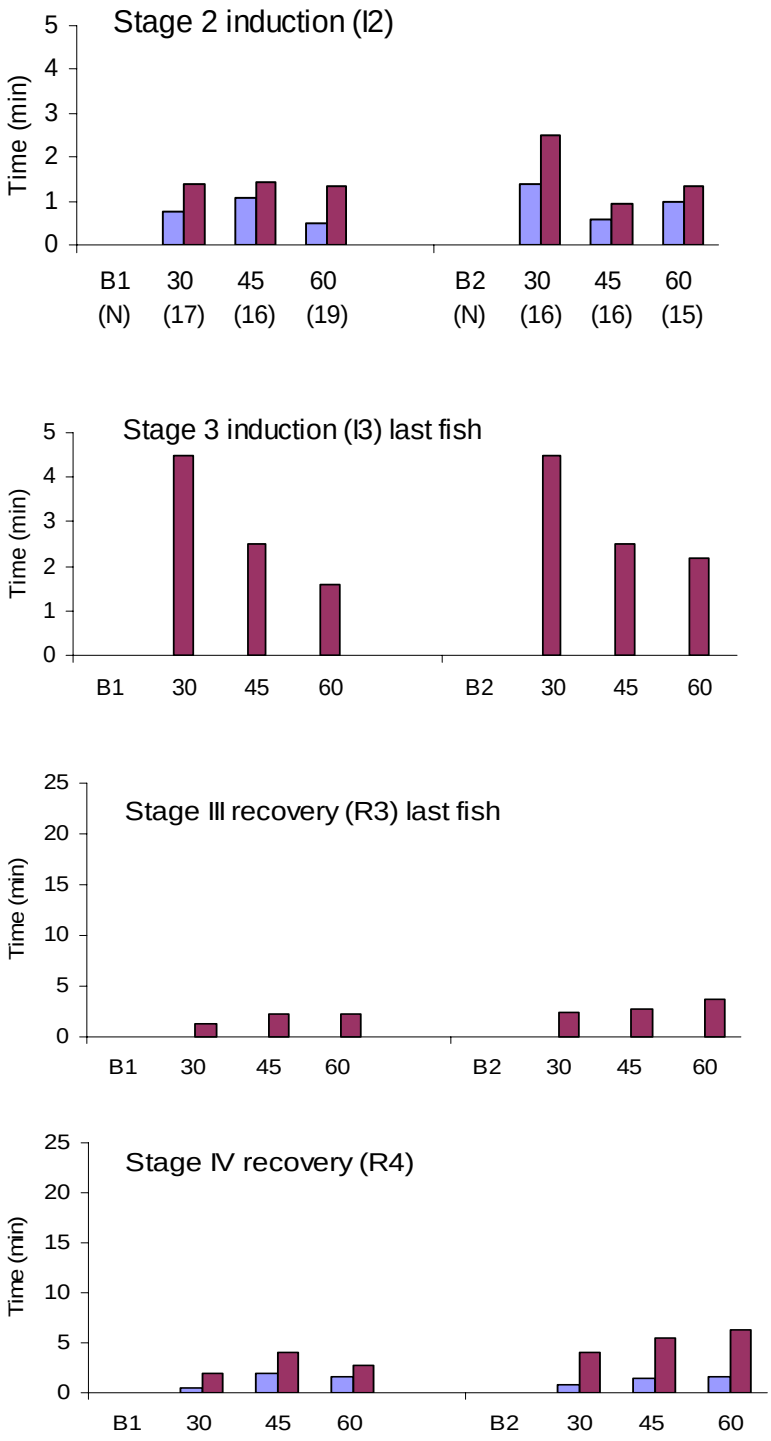

Figure 3. Response times from juvenile Pacific herring group trials with MS-222 inductions starting at 30, 45 or 60 ppm. Lighter bars at each bath concentration are first fish response times and darker bars are last fish response times. B1 indicates fresh induction bath; B2 indicates once-used induction bath; numbers in parentheses refer to the sample size (N) in each trial group.

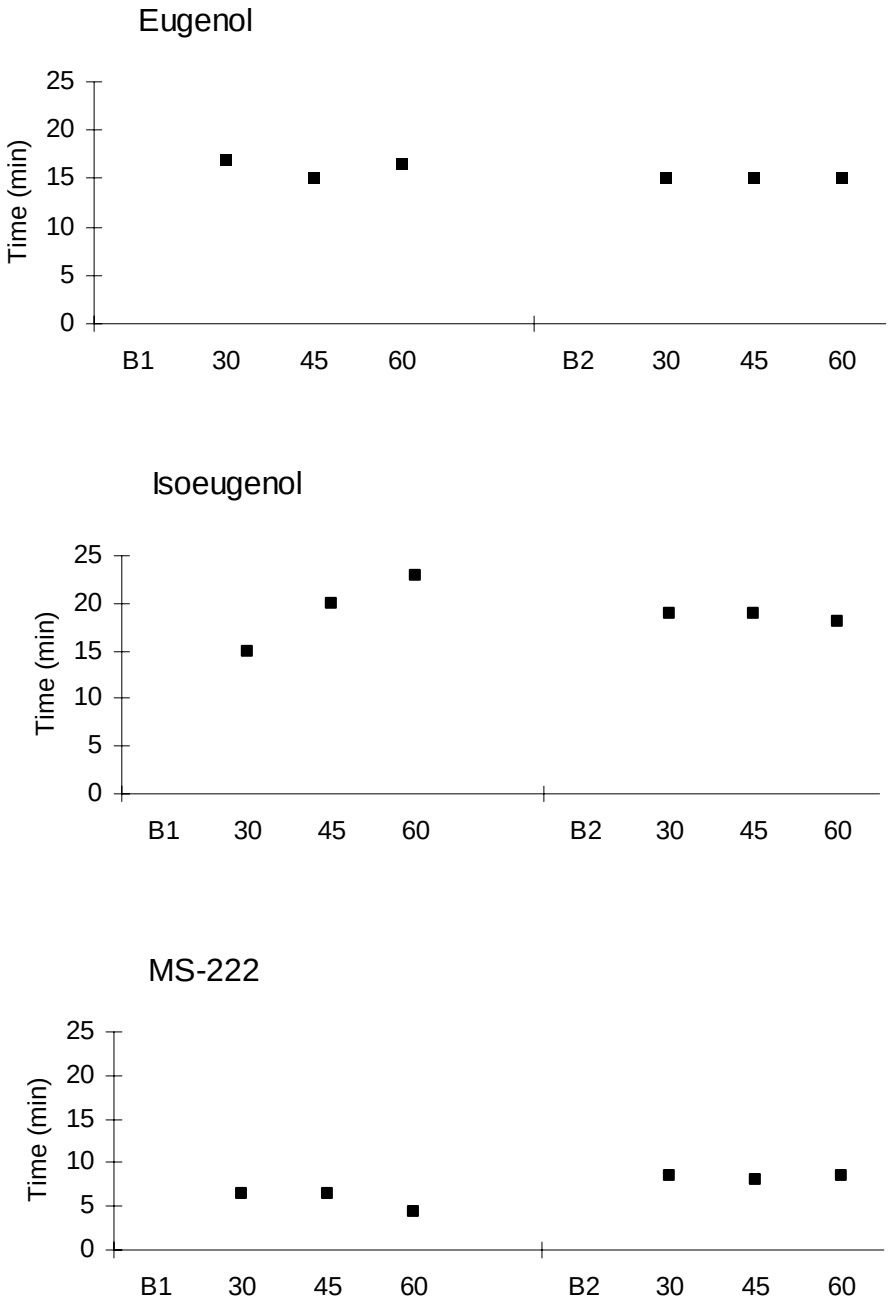

Figure 4. Juvenile Pacific herring cumulative group trial times from induction to recovery with eugenol, isoeugenol or MS-222 inductions starting at 30, 45 and 60 ppm. B1 indicates fresh induction bath; B2 indicates once-used induction bath.