Mass marking coho salmon, *Oncorhynchus kisutch*, fry with lanthanum and cerium

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Most current salmonid tagging programs identify small proportions of a population. However, under some circumstances it is desirable to mark entire populations. Chemical marking is a technique that can rapidly mark large numbers of fish without individual handling. Marking is accomplished by exposing the fish to biologically rare elements that are subsequently incorporated and retained in certain tissues in which they are not naturally found. Marking of entire hatchery populations could be valuable from a fisheries management perspective for stock identification (hatchery versus wild salmon), assessment of contribution to fisheries, and evaluation of current tagging and sampling techniques.

Ennevor and Beames (1993) have shown that some lanthanide elements (i.e. lanthanum and cerium) are suitable for mass marking juvenile coho salmon, *Oncorhynchus kisutch*. The lanthanide elements are not absorbed from the gastro-intestinal tract (Kyker, 1961; Ellis, 1968; Luckey and Venugopal, 1977), and therefore may be introduced through the fishes' rearing water. Because these are bone-seeking elements (Durbin et al., 1956; Jowsey et al., 1958), administered lanthanides are subsequently incorporated into the bony tissues of coho salmon fry and smolts (Ennevor, 1991; Ennevor and Beames, 1993). Analysis of the vertebral column, otoliths, and scales by inductively coupled plasma-mass spectrometry (ICP-MS) revealed that administered lanthanides are present in these bony tissues 10.5 months post-treatment (Ennevor, 1991; Ennevor and Beames, 1993). ICP-MS is capable of detection and quantification of the lanthanide elements at levels as low as 0.01 $\mu g \cdot g^{-1}$ (Longerich et al., 1987; Houk and Thompson, 1988).

Trials were performed to determine whether immersion into solutions of lanthanide elements would produce recognizable marks on juvenile salmon. These studies were designed 1) to investigate differences in toxicity and uptake between the chloride and acetate forms of lanthanum and cerium, and 2) to assess optimal concentrations and exposure times for marking coho salmon fry in the extremely soft and slightly acidic water at Capilano River Hatchery, British Columbia.

Materials and methods

In the following experiments, lanthanum and cerium were introduced into the rearing water of coho salmon fry at Capilano River Hatchery. The river water at this hatchery is slightly acidic and extremely soft (pH=6.5; hardness as $CaCO_3$=3.8). Concentrated lanthanide stock solutions were metered into the tanks at a rate of 1 $mL \cdot min^{-1}$ and the rearing water was set to flow in at a rate of 1 $L \cdot min^{-1}$. The lanthanide solutions and the rearing water were mixed prior to delivery to the tanks containing the fish.

Two experiments were conducted concurrently with coho salmon fry. One hundred fry, with an average initial weight of 3.2 g, were placed in each 35-L experimental tank. Experiment 1 had 4 treatment groups and a control tank where no lanthanide was administered. The lanthanide treatments and elemental concentrations were: 50 $\mu g \cdot L^{-1}$ of $LaCl_3$, $CeCl_3$, $La(C_2H_3O_2)_3$, or $Ce(C_2H_3O_2)_3$ continuously for 24 days. Experiment 2 involved 7 treatment groups: 50 $\mu g \cdot L^{-1}$ of $La(C_2H_3O_2)_3$ or $Ce(C_2H_3O_2)_3$ continuously for a total of 24 treatment days; 100 $\mu g \cdot L^{-1}$ of $La(C_2H_3O_2)_3$ or $Ce(C_2H_3O_2)_3$ on alternate days for a total of 12 treatment days over a 24-day period; 150 $\mu g \cdot L^{-1}$ of $La(C_2H_3O_2)_3$ or $Ce(C_2H_3O_2)_3$ every third day for a total of 8 treatment days over a 24-day period; and a control tank with no lanthanum or cerium. The treatment days consisted of 24 hours of exposure. Over the treatment period, equal amounts of lanthanum or cerium were administered to each treatment group of fry at the appropriate concentration and duration.

After completion of the lanthanide exposures, the fry were provided with untreated river water for 14 days prior to sampling. Ten fry were randomly sampled from each of the tanks, body weights recorded, and the vertebral columns were removed and prepared for ICP-MS analysis to determine lanthanide accumulation. The majority of flesh was dissected away from the bony tissue and any remaining traces of flesh were digested with a 6% sodium hypochlorite solution. The clean

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backbones were oven-dried at 70°C overnight, ground to a powder, and a 0.01 g subsample from each fish was used for analysis (Ennevor, 1991; Ennevor and Beames, 1993). The prepared samples were submitted to a commercial laboratory in North Vancouver, British Columbia for ICP-MS analyses.

Experiments 1 and 2 were analyzed by analysis of variance with SYSTAT statistical software (Wilkinson, 1989) and differences between means were tested at $P \leq 0.05$ with Tukey's multiple range test. The data were pooled by treatment groups with individual fish as experimental units.

Table 1

Percent mortalities during the 24-day treatment period, mean body weights of coho salmon, *Oncorhynchus kisutch*, fry at time of sampling, mean amounts (ng) ± 1 S.E.M. of lanthanum or cerium in vertebral columns of fry marked in Experiments 1 and 2. Within experiments, mean values sharing a similar superscript letter were not significantly different ($P \leq 0.05$) according to Tukey's Test.

	Treatment group[1]				
Element	Concentration ($\mu g \cdot L^{-1}$)	Duration (d)	Mortalities (%)	Mean fry weight[2] (g)	La or Ce (ng) ± S.E.M.
Experiment 1					
La*	50	24	2	3.7	$7.0^{a} \pm 1.2$
Ce*	50	24	2	4.2	$6.1^{a} \pm 0.9$
La	50	24	0	3.5	$5.6^{a} \pm 0.8$
Ce	50	24	0	4.1	$6.2^{a} \pm 0.8$
Control	0	24	1	3.7	$0.1^{b} \pm 0.0$
Experiment 2					
La	50	24	0	3.5	$5.6^{a} \pm 0.8$
Ce	50	24	0	4.1	$6.2^{a} \pm 0.8$
La	100	12	2	2.7	$4.6^{ab} \pm 0.5$
Ce	100	12	3	3.3	$4.4^{ab} \pm 0.3$
La	150	8	12	4.1	$4.0^{b} \pm 0.5$
Ce	150	8	1	3.7	$4.0^{b} \pm 0.0$
Control	0	24	1	3.7	$0.1^{c} \pm 0.0$

[1] La* or Ce* represents the chloride forms, $LaCl_3$ or $CeCl_3$, respectively; all other treatments used the acetate forms of $La(C_2H_3O_2)_3$ or $Ce(C_2H_3O_2)_3$.
[2] Mean weights of treatment groups after 24-day treatment period and 14-day rinse period completed.

Results

Experiment 1

Lanthanum and cerium administered at 50 $\mu g \cdot L^{-1}$ daily for 24 days had no apparent deleterious effect on the fry. They appeared to be healthy and fry weights between treated and non-treated groups did not differ after the 24-day treatments and 14-day rinse were completed. Few mortalities occurred in all groups (Table 1).

Analysis of the vertebral columns from the marked fry showed each of the lanthanides to be present in approximately equal amounts. Uptake did not differ between the treatment groups. The average concentration of lanthanide in the vertebral columns was 6.1 ng of lanthanum or 6.2 ng of cerium (Table 1).

Experiment 2

Throughout the treatments, mortalities were higher in tanks that contained the 150 $\mu g \cdot L^{-1}$ treatments of lanthanum or cerium (Table 1). Fewer mortalities were observed in the 100 $\mu g \cdot L^{-1}$ treatments; none in the 50 $\mu g \cdot L^{-1}$ treatments. However, after the 24-day treatment period and 14-day rinse period were completed, fry weights did not differ between groups treated with lanthanum or cerium and nontreated groups (Table 1).

Results of the analyses of the vertebral columns from the marked fry showed a trend of significantly ($P \leq 0.05$) decreased uptake of lanthanum and cerium with decreased exposure time regardless of concentration (Table 1). Groups treated with lanthanum or cerium at 50 $\mu g \cdot L^{-1}$ daily had the greatest accumulation, whereas the groups treated with 150 $\mu g \cdot L^{-1}$ every third day had the least. In the groups treated with either element, lanthanum and cerium were accumulated in approximately equal amounts.

Discussion

Coho salmon fry were successfully marked with lanthanum or cerium that was administered through the water supply. The lanthanides were detected in the vertebral columns of marked fry, which is consistent with previous findings (Ennevor, 1991; Ennevor and Beames, 1993) and with the bone-seeking characteristics of the lanthanide elements (Durbin et al., 1956; Jowsey et al., 1958). Ennevor and Beames (1993) have shown that lanthanides that are deposited in the vertebral column, otoliths, and scales remain in these tissues for at least 10.5 months after marking. Michibata (1981) also successfully marked medaka, *Oryzias latipes*, and goldfish, *Carassius auratus*, with samarium, another lanthanide, and these fishes retained detectable amounts of the element in their scales one year after marking.

Coho salmon fry exposed to 50 $\mu g \cdot L^{-1}$ of lanthanum or cerium daily resulted in higher levels of accumulation than fry exposed intermittently to concentrations of 100 $\mu g \cdot L^{-1}$ or 150 $\mu g \cdot L^{-1}$. In tanks with higher concentrations, number of mortalities increased as deposition of the elements in the vertebral column decreased. Therefore, toxicity and accumulation may be related to element concentration during treatments rather than accumulated exposure. A high concentration of lanthanides may impair gill function and prevent further uptake of lanthanides, as well as essential ions and oxygen (Behrens Yamada and Mulligan, 1990). Consequently, marking with a low concentration of lanthanide over an extended period is highly recommended.

A potential concern is the ability to detect the lanthanide mark in the bony tissues of returning adults. Because fish continually accumulate calcium in their bony tissues after marking, the relative amount of lanthanum or cerium will decline gradually as the fish grows (Behrens Yamada et al., 1979; Behrens Yamada and Mulligan, 1982). Marks laid down during freshwater growth stages will be concentrated in the center portion of bony tissues. A possible solution to this dilution problem is to analyze only the center where the element concentration would be about the same as when marked (Behrens Yamada and Mulligan, 1982). Scales of returning adults may be more suitable for sampling and analysis as they retain higher lanthanide concentrations (Ennevor and Beames, 1993). Also, scales are easier for sampling and can be removed for lanthanide determination without sacrificing the fish.

These studies demonstrate the successful marking of experimental groups of fry with lanthanum and cerium applied through the water supply. This technique can be adapted to mark large groups of juvenile salmon at hatchery stages quickly and efficiently without affecting growth or survival. Mass marking with lanthanides can mark large groups of fish for identification without apparent deleterious effects. In addition, the mark remains in the bony tissues for extended periods of time, and samples of bony tissues (i.e. scales and opercular punches) can be taken from marked fish, without sacrificing the fish, for identification by ICP-MS analyses.

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