THE FEASIBILITY OF USING LANTHANIDE ELEMENTS TO MASS MARK HATCHERY-PRODUCTION SALMON

By

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ABSTRACT

The development of a marking technique which could be effectively and efficiently used to mark large groups of salmonids, would be a great asset to fisheries management. Six experiments were thus conducted to investigate the feasibility of using the lanthanide elements to mass mark hatchery-production salmon. The lanthanides, introduced through the water supply, appear to be suitable since they demonstrate the characteristics of good elemental markers. Fish marked with these elements can be satisfactorily identified by analysis of bony tissues by inductively coupled plasma-mass spectrometry (ICP-MS).

The lanthanides were shown to be severely toxic to coho and steelhead alevins at concentrations of 100 μg/l in the water supply, but were only slightly toxic to coho fry. Alevin and fry appeared to be more sensitive to the light-weight lanthanides than the heavier lanthanides. Coho smolts showed no adverse effects when exposed to lanthanum or cerium at these concentrations.

Lanthanides, introduced in the form of acetates, were shown to be absorbed from the water supply and subsequently incorporated in the vertebral column, otoliths and scales of coho fry. Coho fry exposed to lanthanum or samarium at concentrations of 100 μg/l in the water for 6 weeks had detectable levels of element in their bony tissues 10½ months

post-treatment. When lanthanum and cerium were added to the water supply at concentrations of 100 µg/l for 4 weeks, higher concentrations of element were accumulated in fry than in smolts, although smolts accumulated greater amounts of element.

Introduction of the lanthanide elements through the water supply, appears to be an effective method for the mass marking of young salmonids.

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INTRODUCTION

Tagging programs allow the collection of important information which can be used to evaluate the overall effects of fisheries management. Juvenile salmon which have been marked before release can be identified when captured in fisheries and as they return to spawn. The results obtained from mark-recovery experiments help to improve and increase salmon production.

In 1988, personnel from the Federal Department of Fisheries and Oceans approached the University of British Columbia to initiate a project to develop a new tagging system. The results of an extensive literature search revealed that the incorporation of the lanthanide elements (rare earth elements) in fish bony tissues by addition to the water supply would be worthwhile to investigate as a possible tagging method.

The tagging method (coded-wire tags) currently being used for identifying hatchery salmon is extremely labour intensive as it requires the individual handling of fry, thereby limiting the number of marked fish to a small percentage of the total production. Marked fish are identified by an adipose fin clip. A method which could greatly increase the numbers marked, and at the same time eliminate handling, would be a great asset to fisheries management and hatchery assessment.

Chemical marking offers a favourable alternative to the labour-intensive mechanical tagging. Chemical marking would

enable hatchery staff to mark large groups of fry for identification before release. However, the marked fish have no external identifier (i.e. no adipose fin clip). The chemicals to be used would have to be:

(i) bone seeking, and persist in the bony tissue for a long time;

(ii) not harmful to fish or humans;

(iii) detectable at very low levels;

(iv) not found in fresh water or salt water in detectable levels; and

(v) relatively inexpensive.

The lanthanides appear to have the characteristics of good elemental markers. These elements, which are incorporated in the bony tissues, can be detected by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) or Neutron Activation Analysis (NAA) at concentrations as low as 0.01 micrograms/litre (Longerich *et al.*, 1987).

Diet incorporation does not seem to be the optimal method to successfully administer the lanthanide elements since they are not readily absorbed by the gastro-intestinal tract (Ellis, 1968; Luckey & Venugopal, 1977; and Kennelly *et al.*, 1980) and, because of this very attribute, have been widely used as indigestible additives for digestibility studies (Huston & Ellis, 1965; Ellis, 1968; Olbrich *et al.*, 1971; Gray & Vogt, 1974; Hutcheson *et al.*, 1974; Luckey *et al.*, 1975, 1977, 1979; Young *et al.*, 1975, 1976; Hartnell & Satter, 1979; Cerasov &

Heller, 1980; Kennelly *et al.*, 1980; Udén *et al.*, 1980; and Arambel *et al.*, 1986). However, it was hypothesized that the addition of a concentrated aqueous solution of the lanthanides to the water supply would be a more effective method of achieving tissue incorporation.

The use of strontium as a chemical marker is currently being investigated by a research group at the Pacific Biological Station, Department of Fisheries and Oceans, Nanaimo. They have demonstrated that strontium, added to the diet, is incorporated into the fish scales. However, since strontium is relatively abundant in sea water and since a multi-element marker would allow for a large number of distinct combinations, the investigation of the lanthanide elements, singly and in combination, appeared to be a more worthwhile project to pursue.

This thesis research was conducted to determine the feasibility of using the lanthanide elements to chemically mark cultured salmon. Coho alevins (newly hatched juvenile salmon with a yolk sac attached) were first investigated because application of the chemical solutions could be more efficient and easier at this stage. Unfortunately, the first set of experiments involving coho and steelhead alevins demonstrated that the lanthanides were toxic, even at low concentrations, to this life stage. The ensuing experiments were carried out to determine if coho fry and smolts were more tolerant stages to work with, and to determine if these chemical marks were

accumulated and retained in their bony tissues. The experiments were conducted as follows:

(i) an investigation using coho alevins exposed to a range of lanthanides;

(ii) a toxicity study using steelhead alevins treated with lanthanum and samarium;

(iii) an experiment using coho fry held in tanks containing static water treated with varying concentrations of lanthanum;

(iv) an experiment using coho fry in a flow through tank with a lanthanum concentration of 100 μg/l;

(v) an experiment treating coho fry with lanthanum and samarium at concentrations of 0, 10, and 100 μg/l for a 3-week period or a 6-week period; fish maintained and analyzed for lanthanide retention throughout a 10½-month period after labelling; and

(vi) combinations of lanthanum and cerium treatments using coho fry and smolts.

LITERATURE REVIEW

Fish Tagging

History

Since the late nineteenth century biologists have been attempting to tag fish stocks, based on the belief that the ability to mark and identify specific groups of fish would be an important fisheries management tool. Without some effective way of quantifying abundance, the overall success of hatchery-production salmon cannot be properly evaluated. Much useful data has been collected from returning marked salmon. Survival rates, growth rates, migration routes, predation effects, success based on size and time of release, effectiveness of different rearing practices, and nutritional requirements are principal variables of interest.

Types of Tagging

Many techniques have been used to mark fish. Some of these include:

(i) physical tags that are attached onto or inserted into the fish;

(ii) mutilation tags where the fins are partially or completely clipped;

(iii) various biological marks (genetic, parasitic, or bacterial tags);

(iv) thermal marks where differentially-spaced growth rings are laid down in the otoliths;

(v) cold-branding or tattooing; and

(vi) genetic markers.

More recently, many types of chemical marks have been used. These involve the application and incorporation of dyes, antibiotics, radioactive isotopes, or stable isotopes of elements. The use of coded-wire tagging and chemical marking with strontium and the lanthanides will be discussed in detail.

Some of the characteristics of an ideal mark include the following:

(i) the mark must remain with the fish throughout its life cycle at detectable levels;

(ii) no harmful effects on fish behaviour, growth, movement or reproduction can be evident as a result of the mark;

(iii) the mark should be relatively inexpensive and easy to apply;

(iv) no natural marks similar to the one applied can be present;

(v) the mark can be effectively and safely used for different ages and species; and

(vi) there are enough possible different combinations of marks so that many groups of fish can be identified separately.

Coded-Wire Tagging

The coded-wire tagging system was developed by Jefferts *et al.*, (1963) and is the method currently being used in Canada and the United States to identify hatchery and wild salmon. This system involves the insertion of a small coded, magnetized wire tag into the nose cartilage and the clipping of the adipose fin for external identification. These tags are 1 mm long x 0.25 mm diameter and are marked with a binary code etched along the side.

The tagged fish are identified by the missing adipose fin and a sensitive magnetic detector is used to detect the metal tag. the tag is dissected out of the cartilage and the code is read using a microscope. Although this system has been in use since the early-1970's and has provided much useful information on the mark-recovery experiments carried out over the last 15 years, it has not proven to be the ideal method to use in all circumstances.

There are some problems associated with the use of coded-wire tags. First, it is extremely labour-intensive to apply and recover the tags. This disadvantage, therefore, limits the numbers of fish that can be marked. Second, there are some problems with tag loss, since the tag may work its way out of the nose cartilage, or it is not always detected in, or recovered from returning adults (Ebel, 1974). Third, upon injection of the tag, it may sever the optic nerve causing impaired vision which will decrease the fish's chances for

survival. Although the incidence of these problems has decreased due to further improvements in the tagging techniques, these problems are still evident.

Chemical Marking

A technique which enables large numbers of fish to be marked at the same time without individual handling would be a more effective marking system than the coded-wire system. Chemical marks which involve the introduction and subsequent incorporation of elements into bony tissues would provide the rapid and reliable method needed to mark large batches of hatchery-production salmonids.

Many of the characteristics of an ideal fish mark are satisfied by chemical marks. These have been described by Trefethen and Novotny (1961) as:

(i) are not harmful to the fish;
(ii) do not cause unusual behaviour patterns;
(iii) have no harmful effects on metabolism;
(iv) can be applied without handling the fish;
(v) are easily and readily applied;
(vi) can be applied to large numbers quickly and easily;
(vii) marked and unmarked population differences are not distinguishable by predators; and
(viii) are inexpensive to apply.

Another important consideration is the safety of the chemical marker in fish destined for human consumption. The disadvantage

of elemental marks is the fact that they are not externally visible and therefore the marked fish are not easily distinguished.

Strontium

The feasibility of using strontium as an elemental mark has been studied extensively. Strontium is a bone seeking element which is chemically similar to calcium (Eisenberg, 1973). Different groups of researchers have induced marks in fish by the addition of strontium to the feed. Ophel & Judd (1968), Behrens Yamada *et al.* (1979), Behrens Yamada & Mulligan (1982), and Guillou & de la Noüe (1987) have used feeds containing strontium for long-term marking of fish. They have successfully demonstrated that strontium is absorbed from the gastro-intestinal tract and subsequently deposited into the bony tissue. These experiments have also shown that there are no adverse effects of strontium on fish survival, metabolism or growth. Strontium appears to replace calcium directly in the bony tissue and there is no evidence of disturbance in calcium metabolism. Furthermore, high strontium content in the fresh water region of the scales (the central area) is detectable up to 18 months after the strontium-enriched diet has been fed (Behrens Yamada *et al.*, 1979; and Behrens Yamada & Mulligan, 1982).

However, strontium is relatively abundant in seawater at approximately 8 mg per litre (Hummel & Smales, 1956). Once the

fish migrate to sea for the adult stage of the life cycle, strontium from the seawater is also incorporated into the scales in the seawater growth region (the outer rings). This additional strontium is of a large enough concentration to mask the mark originally laid down during the freshwater stage. The use of a microanalytical technique, which is capable of analyzing only the central portion of the scale, may allow the differentiation between marked fish and unmarked fish (Behrens Yamada *et al.*, 1979; and Behrens Yamada, 1982).

Another consideration is that multi-element combinations, rather than the use of a single element, enables the identification of a greater number of different groups of hatchery-production salmon. However, a single element could be used to chemically mark fish if the fish are exposed to the element at different time intervals. This type of marking would result in growth rings with an enhanced concentration of the elements spatially arranged. Although this would increase the number of possible marks, the analytical technique (laser ablation by ICP-MS) still needs to be developed for the analysis of small areas in the fish scales.

Lanthanide Elements

Samarium

A very small number of researchers have investigated the possibility of using the lanthanide elements to mass mark fish. Attempts to use samarium were reported by Michibata & Hori

(1981) and Michibata (1981). These researchers state that samarium would be a suitable element to use since it offers the following advantages:

(i) the apparent absence of harmful effects on the fish;

(ii) the small probability of interference from naturally occurring samarium;

(iii) the long biological half-life of the deposited samarium;

(iv) no radioactive contamination of the fish or other organisms; and

(v) safety in handling.

In the first set of experiments, Michibata & Hori (1981), injected doses of samarium into the abdomen of medaka *(Oryzias latipes)* and goldfish (*Carassius auratus*). The elemental mark was detectable for up to two years after the last injection. Samarium was accumulated in the kidneys, intestine, liver, vertebrae, gills, scales, and muscle. Although this method was successful in introducing the element into the fish, it required individual handling and had to be restricted to fish large enough to inject. Since a mass marking method would be more efficient, Michibata (1981) carried out an experiment in which samarium was included in the diet of the two species of fish previously used. They found that the level of samarium decreased within 30 days of completion of feeding, but then remained constant over the next year. The tissues in which the samarium was detected were the fifth branchial arch, the scales,

and the gills. Initially, there was some samarium present in the liver and intestine, but after 90 days the samarium was undetectable in these organs. This indicated that short term storage takes place in the soft tissues and long term storage occurs in the bony tissues. Michibata proposed that the route of entry was though the water, not through the diet. In these experiments, samarium concentration in the water supply could have been elevated as a result of dissolved spilled feed or excrement. In addition, different groups of researchers with various animal species have shown that the lanthanide elements are not absorbed from the gastro-intestinal tract (Ellis, 1968; Luckey & Venugopal, 1977; and Kennelly *et al.*, 1980).

Samarium does appear to be a feasible lanthanide to use as a chemical mark for fish. Samarium administered to fish through the diet or by injection remains in the bony tissues for an extended period of time (Michibata, 1981; and Michibata & Hori, 1981).

Dysprosium

Babb *et al.* (1967) attempted to introduce dysprosium into chinook salmon fingerlings using a variety of methods. These included weekly intramuscular injections, 24 hour dips in dysprosium solution once a week for five weeks, forced feeding of dysprosium-enriched diets, and voluntary feeding of dysprosium-enriched diets. The analytical method used to detect the dysprosium was neutron activation analysis. This method was

not sensitive enough to detect any dysprosium in the fish fed the enriched diets or placed in the solution (< 1 μg/ml), but it did detect the dysprosium in the injected fish. Perhaps a more sensitive analytical method would have been able to detect the smaller amounts of element present in the treatment groups. From these results, the intramuscular injections appeared to be the most effective of the four methods used. However, since injections require individual handling, they are time consuming and result in increased mortalities.

Similar results were found by Miller (1963). In this experiment the treatments included the feeding of dysprosium-enriched diets, submergence of fish in 100 μg/l dysprosium solution, and intramuscular injections; the analytical method used was neutron activation analysis. The chinook fingerlings fed the dysprosium-enriched diets and those submerged in the solution did not deposit detectable amounts of dysprosium into the bony tissue. In contrast, intramuscular injections resulted in the variable absorption and deposition of dysprosium (up to 368 μg/g) in the bones of fish sampled at the end of five weeks. The dysprosium persisted in the bones for up to five months after the injections, indicating that long term storage is in the bony tissues. From these data, it appears that the injection method is the most effective way of inducing a detectable mark. The fish in this experiment were only submerged in the dysprosium solution for 24-hour periods once a week for five weeks with no detectable mark laid down. It is

possible that a longer exposure time would have resulted in detectable amounts of dysprosium in the tissues.

Europium

There are varying reports in the literature on the success of europium as a fish mark. Japanese researchers (Anon., 1974) induced europium marks through the feeding of europium-enriched diets to juvenile chum salmon (*Oncorhynchus keta*). The diets fed to the fish contained 1,000 and 15,000 µg/g of europium. Neutron activation analysis showed detectable amounts of europium in the scales and otoliths for up to 1 year after the labelling. Another researcher, Kato (1985), also succeeded in inducing a europium mark in chum salmon through the diet. He found the element to be present in the fish scales for up to 2 years after feeding of the europium-treated diets was discontinued. His results also indicate long term storage of europium in the bony tissues.

Michibata & Hori (1981) discussed the experiment carried out by Shibuya in 1979 which involved the introduction of europium through the diet. Shibuya discovered that the element was accumulated in the scales, but the mark lasted only 3 months after labelling. The conclusions reached from the results obtained in these experiments vary considerably. Unfortunately, the paper written by Shibuya (1979) was in Japanese and a translation was not available. Consequently, the validity of these results cannot be assessed. However, the other two

researchers have clearly demonstrated that europium was taken up by the fish and was incorporated into the scales.

Terbium

Muncy and D'Silva (1981) conducted an experiment using walleye (*Stizostedion vitreum*) eggs and fry in which varying concentrations of lanthanide solutions were applied for different periods. The solutions added to the water supply were terbium chloride, sodium terbium citrate, terbium dicitrate, europium chloride, and neodymium dicitrate. These researchers determined that terbium dicitrate appeared to be suitable for marking the walleye eggs during the water-hardening process. The other terbium solutions appeared to be ineffective in marking the eggs and fry. In addition, there were no detectable levels of neodymium or europium found in either the eggs or the fry.

Summary of the Lanthanides

The results of all these marking experiments involving the use of the lanthanide elements show that under certain conditions these elements are taken up by the fish and are subsequently incorporated into the bony tissues. Furthermore, long term storage occurs in the bony tissues while the short term storage is in the soft tissues.

Of the various methods used, incorporation of the lanthanides in the diet does not appear to be a suitable method

since these elements are not absorbed through the gastro-intestinal tract at levels which could be detected using the analytical technique available. Injections of samarium and dysprosium did result in considerable concentrations deposited in, and retained by the bone. However, a mass marking method would be preferable to injection. A possible alternative is submergence in a lanthanide solution for extended periods of time. This may result in a sufficient amount of the lanthanide elements being absorbed and stored by the fish for these elements to be used as detectable markers.

Lanthanide Elements

Introduction

The lanthanide elements are also referred to as the lanthanons or the rare earth elements. They are a series of fifteen elements starting with lanthanum (atomic number, 57) and ending with lutetium (atomic number, 71). Their electron configuration places them into subgroup IIIB of the periodic table of the elements. All lanthanides are closely related elements with very similar chemical and physical properties (Kyker, 1961). The three classifications of these elements are as follows:

(i) light lanthanides: lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), and samarium (Sm);

(ii) medium lanthanides: europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), and holmium (Ho); and

(iii) heavy lanthanides: erbium (Er), thulium (Tm), ytterbium (Yb), and lutetium (Lu).

The only radioactive lanthanide is promethium. Yttrium (Y) is a group IIIA element and, although not a true rare earth, it is usually included with the rare earths since it has similar properties to the heavy lanthanides (Vickery, 1961; and Kyker, 1962). The classifications and atomic weights of the lanthanides and yttrium are provided in Table 1.

The title "rare earth" is both misleading and inaccurate. Although concentrated deposits of the lanthanides are rare,

these elements are relatively abundant in the earth's crust. Cerium, for example, occurs in greater amounts than do tin, cobalt, gold, and silver (Taylor, 1964; and Luckey and Venugopal, 1977). Although these elements are metals, they were named earths because they were first identified as a mixture of oxides that closely resembled the alkaline earth oxides (Moeller, 1963). The major mineral source for the lanthanide oxides is monazite sand, where 90% of the lanthanide portion is the light elements. Due to advanced separation and purification methodology, lanthanide compounds are available in highly purified forms from several commercial sources. The odd-numbered and heavier lanthanides are generally the most precious and difficult to purify, therefore these tend to be more expensive.

Table 1. Classification of the lanthanide elements and their elemental characteristics.

Element	Crustal Abundance[1]	Classif-ication	Atomic Number	Atomic Weight	Natural Isotopes
Yttrium (Y)	33.0	Light	39	88.905	1
Lanthanum (La)	30.0	Light	57	138.91	2
Cerium (Ce)	60.0	Light	58	140.12	4
Praseodymium (Pr)	8.2	Light	59	140.907	1
Neodymium (Nd)	28.0	Light	60	144.24	7
Promethium (Pm)[2]	0.0	Light	61	(145)	?
Samarium (Sm)	6.0	Light	62	150.35	7
Europium (Eu)	1.2	Medium	63	151.96	2
Gadolinium (Gd)	5.4	Medium	64	157.25	7
Terbium (Tb)	0.9	Medium	65	158.924	1
Dysprosium (Dy)	3.0	Medium	66	162.50	7
Holmium (Ho)	1.2	Medium	67	164.930	1
Erbium (Er)	2.8	Heavy	68	167.26	6
Thulium (Tm)	0.5	Heavy	69	168.934	1
Ytterbium (Yb)	3.0	Heavy	70	173.04	7
Lutetium (Lu)	0.5	Heavy	71	174.97	2

1 Crustal abundance measured in μg/g (Taylor, 1964).
2 Radioactive lanthanide.

The uses of the lanthanides are diverse and widespread. They include: ceramics, carbon arcs, catalysts, lighter flints, television tubes, mirrors, textile waterproofing compounds, paint driers, electronic equipment, and many others (Kyker, 1961; and Luckey & Venugopal, 1977). As research into these elements continues, it is likely that more applications of the lanthanides will be discovered.

Chemical Properties

The lanthanide elements are inner-transitional elements which share similar chemical and metabolic properties. The atomic weights of the lanthanides increase in very small increments. Various characteristics that are correlated with the increase in atomic number are: (i) increasing acidity, covalent characteristics and stability of complex ions, and (ii) decreasing basicity, ionic characteristics, thermal stability and solubility. Atomic radii decrease with subsequent increases in atomic number from 1.2 angstroms for lanthanum to 0.95 angstroms for lutetium. All of the elements exist in a trivalent (Ln^{3+}) state, however lanthanum, samarium, europium and ytterbium can also exist in a divalent (Ln^{2+}) state, and cerium, praseodymium, terbium in a tetravalent (Ln^{4+}) state (Kyker, 1961).

A wide range of lanthanide compounds can be formed, each with a varying degree of solubility. In general, the nitrates, halides, perchlorates, thiocyanates and acetates are all relatively soluble. The sulphates are moderately soluble, while the oxides, hydroxides, fluorides, oxalates, carbonates, and phosphates are relatively insoluble (Topp, 1965). In aqueous solutions, the halides hydrolyse readily to produce insoluble oxide halides: $LnX_3 + H_2O \rightarrow LnOX + 2HX$, where Ln represents any lanthanide and X represents any halide, for example: $LaCl_3 + H_2O \rightarrow LaOCl + 2HCl$. Lanthanides with organic acids, such as acetates, are relatively stable in aqueous solutions and

do not tend to hydrolyse. The acetate compounds are moderately soluble in water at room temperature (Topp, 1965). From this, it would appear that the acetate form is preferable over the salt form to use in the introduction of the lanthanides to aqueous solutions.

The chemical properties of the lanthanide elements determine their behaviour in living systems. Once lanthanide cations are introduced into the gastro-intestinal tract, they either undergo a hydrolysis reaction or react with the normal biochemical constituents to form complexes of insoluble compounds. At the physiological pH of living organisms, hydrolysis of the lanthanides is highly favoured. This ionic reaction proceeds very quickly and the resulting lanthanide hydroxides and phosphates formed in the gastro-intestinal tract are insoluble and precipitate out. The hydrolysis reaction is as follows: $Ln^{3+} + 3H_2O \rightarrow Ln(OH)_3 + 3H^+$. Since the affinity of lanthanides towards phosphate is high, insoluble phosphate complexes tend to form. Chelating agents such as citrates or lactates may be present in the tissues. The lanthanide cations show a strong tendency to complex with these compounds, thus keeping them in solution. The resulting complexes are stable and are not subject to hydrolysis in biological fluids.

Lanthanides introduced into the gastro-intestinal tract as lanthanide citrate and ethylenediaminetetraacetic acid (EDTA) complexes appear to be absorbed. Since these large complexes resist hydrolysis reactions, insoluble lanthanide compounds do

not form (Luckey & Venugopal, 1977). Since these complex compounds are not readily available, this route was not investigated further.

The lanthanide elements tend to adhere to particulate matter and surfaces with which they come into contact. They exhibit their adsorptive properties at very low concentrations in aqueous solutions (Luckey & Venugopal, 1977). The adsorption of lanthanides to ingesta particles is partially responsible for the passage of the lanthanides through the gastro-intestinal tract. Adherence to surfaces is sometimes referred to as adsorption or "plating-out".

Lanthanides also interact with various tissue components, which can include nucleo proteins, plasma proteins, amino acids, phospholipids, enzymes, and inorganic anions (Kyker, 1961; and Luckey & Venugopal, 1977). The heavy lanthanides tend to complex with complete proteins while the light lanthanides tend to complex with individual amino acids (Graca *et al.*, 1962). Although there are some differences between the light and the heavy lanthanides, the overall chemical reactions are quite similar for the series. Consequently, there is a tendency to group the properties of the lanthanides together.

Metabolism

The biological and chemical characteristics of the lanthanides affect how they are metabolized by living organisms. No lanthanide has been proven to be essential or to have a role

in the metabolism of any plants or animals. These elements are not present in plant or animal tissue (Kyker, 1961). Their absence in plant tissue suggests that plants discriminate against the absorption of lanthanides from soil. This effectively blocks the dietary transfer of lanthanides from soil to animals (Luckey & Venugopal, 1977).

In mammals the gastro-intestinal (GI) absorption of soluble lanthanide salts is negligible - less than 0.05% (Ellis, 1968; Luckey & Venugopal, 1977; and Kennelly *et al.*, 1980). The lanthanide oxides are excellent markers for use in digestibility and rate of passage studies since virtually all the element passes through the tract. Poor absorption of lanthanides has been reported in all species studied: rats and mice (Cochran *et al.*, 1950; Marcus & Lengemann, 1962; Haley, 1965; Hutcheson *et al.*, 1975; and Luckey *et al.*, 1975), cattle (Garner *et al.*, 1960; Huston & Ellis, 1965; Olbrich *et al.*, 1971; Young *et al.*, 1975, 1976; Hartnell & Satter, 1979; Udén *et al.*, 1980; and Arambel *et al.*, 1986), monkeys (Hutcheson *et al.*, 1974), sheep (Ellis, 1968; and Pond *et al.*, 1985), llamas (Cerasov & Heller, 1980), swine (Kennelly *et al.*, 1980), and humans (Hayes *et al.*, 1964; and Luckey *et al.*, 1977, 1979).

Once the simple lanthanide salts enter the gastro-intestinal (GI) tract, they form insoluble hydroxides and phosphates which precipitate out as described in the chemistry section of this review (Kyker, 1961; Ellis, 1968; and Luckey & Venugopal, 1977). The heavier lanthanides tend to separate out

more than the light lanthanides and are even less well absorbed. They can also combine with organic matter to form insoluble phosphates which also pass completely through the GI tract. In addition, the lanthanide hydroxides have very strong adsorptive properties that cause them to adhere to particulate food matter. Once bound to this material, they move through the tract along with the ingesta (Kyker, 1961; and Hutcheson *et al.*, 1975).

The lanthanides are often described as bone-seeking elements. Small doses of lanthanides have regularly been shown to accumulate in the bony tissue (Durbin *et al.*, 1956; Jowsey *et al.*, 1958; Kyker, 1961; Michibata, 1981; and Michibata & Hori, 1981). The elements are distributed rapidly into the liver and kidneys, with gradual uptake and retention by the skeleton (Luckey & Venugopal, 1977). Durbin *et al.* (1956) found that approximately 55% of the dysprosium administered intramuscularly to rats was deposited in the bone. Approximately 50% of the administered lanthanides were deposited in the liver, and the majority of this was excreted within 2 months. Furthermore, young animals absorb and retain the injected lanthanides better than older animals (Luckey & Venugopal, 1977). The highly mineralized non-growing bone surface, not the osteoid tissue, is the site of lanthanide deposition in mammals. Bone mineral is responsible for lanthanide uptake from the plasma and for the subsequent deposition into the bone (Durbin *et al.*, 1956; and Jowsey *et al.*, 1958). The transport of lanthanides from blood to soft tissues and bone depends on the concentration, the

nature of the lanthanide compound, and the mode of administration (Luckey & Venugopal, 1977).

In fish, the gills are the main contact area with the external environment, which implies that the gills also serve as the main uptake site for dissolved compounds, including heavy metals and calcium (Pärt & Svanberg, 1981; and Perry & Wood, 1985). Theoretically, calcium ions dissolved in the water are taken up by the chloride cells in the gills, then transported to other tissues by the circulatory system (Payan *et al.*, 1981).

In a review article, Weiss (1974) states that the La^{3+} ion is a specific antagonist of Ca^{2+} ions, in biological systems. Lanthanum ions have been found to displace or replace calcium in different cell functions, and they prevent the influx of Ca^{2+} ions by competing for available binding sites (Das *et al.*, 1988). La^{3+} ions probably enter the fish by the same route as the Ca^{2+} ions.

Bony Tissue Development

Since the lanthanide elements are bone-seekers, the development of the bony tissues is very important in the success of the chemical labelling. The development and growth of the vertebral column, otoliths and scales may have an effect on the length of time the element remains in the tissue. Little is known about the physiology of calcified material into the fish's bony tissues. However, it has been demonstrated that once the bony tissues are formed, the calcified material appears to

remain in the same location and state as it was when initially deposited.

In general, the bony tissues grow by the continuous, concentrical deposition of calcified tissue. Although there are some differences in calcification between the vertebrae, otoliths and scales, the calcium metabolism is co-ordinated. The growth of these bony tissues is parallel to one another, but the otoliths are the first to develop (Pannella, 1980).

A chemical label which is deposited at sites of calcification provides an elemental mark in the tissue. In this way tetracycline has been used to label various types of hard tissues such as: scales, opercula, vertebrae, spines, and otoliths. This antibiotic remains in the bony tissue, without any harmful effect on the fish, as a result of the concentrical growth and development (Casselman, 1987).

Bone Development

Moss (1961) describes bone as consisting of a collagenous fibre organic matrix with associated mucoproteins which is calcified by hydroxyapatite salts. The bone is made from a calcium phosphate matrix. The deposition of bony tissue is associated with the presence of osteoblasts which are the bone-forming cells.

As with the otoliths and scales, the vertebral column is laid down concentrically. Norris *et al.* (1963) reported that Ca^{45} deposition occurs between the osteoid layers and previously

mineralized bone. This further demonstrates the concentric nature of the bony tissue development.

Otolith Development

There are three pairs of fish otoliths: lapillus, sagitta, and astericulus. The largest is the sagitta (pl. sagittae), and because of its relatively large size it is most often studied. An otolith, unlike regular bone, is composed of calcium carbonate ($CaCO_3$) crystals, in the aragonite form, and a proteinaceous organic material (Gauldie, 1986; and Pannella, 1980).

Pannella (1971) first described the presence of daily growth rings in fish otoliths. Subsequently, a number of researchers have observed these growth rings. The otoliths have been reported to grow concentrically as a result of the continuous calcium carbonate deposition (Mugiya *et al.*, 1979; Wilson & Larkin, 1980; and Neilson & Geen, 1982). The $CaCO_3$ deposited in the otoliths is not recycled and the otoliths remain unchanged throughout the life of the fish. Also, any elements incorporated into the otoliths from the ambient water supply would be reflected in the elemental analysis of the growth rings (Campana & Neilson, 1985).

Scale Development

The formation of scales begins in the area between the dorsal fin and the adipose fin, along the lateral line. They

then extend anteriorly towards the operculum, and posteriorly towards the caudal fin. As a result, the scales along the posterior portion of the lateral line are the largest, decreasing in size away from this region (Jollie, 1984; and Witkowski *et al.*, 1984). The centre of the scale is commonly referred to as the focus and the rings that surround it are called circuli. The scales are composed of calcium phosphate as are the bones. The calcified material is laid down concentrically as in the vertebral column and the otoliths. Any elements incorporated into the central region of the scales should remain there throughout the fish's life history (Lapi & Mulligan, 1981).

Toxicity

When introducing any foreign chemicals into a biological system, possible toxicological effects need to be considered. Unfortunately, no studies on the direct effects of the lanthanides on fish have been reported in the literature. Current knowledge on the toxic effects of the lanthanides is limited to several acute toxicological measurements in mammals. The toxicity studies reviewed here describe the effects of different lanthanide compounds administered by various methods into rodents.

When compared with other elements, the lanthanides are considered to be relatively non-toxic or only slightly toxic. The method of administration of the lanthanide compounds is a

determining factor in the toxicity effects found in mammals. Some of the different modes of introduction used by Venugopal & Luckey (1974) include:

(i) oral administration and absorption from the gastro-intestinal tract (PO);

(ii) intravenous injection with fast distribution to tissues (IV);

(iii) lung inhalation;

(iv) subcutaneous administration (SC);

(v) intraperitoneal injection (IP);

(vi) intramuscular injection (IM); and

(vii) absorption through the skin.

Intravenous injection appeared to lead to greatest toxicity whereas oral administration appeared to have the least effect. Intraperitoneal and subcutaneous administration also resulted in comparatively low toxicity (Laszlo *et al.*, 1952).

Oral toxicity of the lanthanides is very low due to their negligible absorption from the gastro-intestinal tract, which has been shown by many researchers. As described earlier, the lanthanide salts undergo a hydrolysis reaction in living organisms which results in the formation of insoluble hydroxides or phosphates. These compounds are not absorbed from the GI tract (Cochran *et al.*, 1950; Haley, 1965; and Luckey & Venugopal, 1977). Also, the lanthanides tend to adsorb to particulate food ingesta and pass directly through the tract (Venugopal & Luckey, 1974). Mice fed diets containing greater

than 5000 μg/g of lanthanide oxides showed normal general appearance, growth, maturation, and reproduction (Luckey & Venugopal, 1977). Haley *et al.* (1961) found that oral doses of up to 2 g/kg of diet produced no mortalities in rats and there was no evidence of internal damage from the ingestion of samarium and gadolinium.

According to the LD_{50} values of the lanthanides, the medium weight lanthanides are the least toxic, and the light lanthanides are slightly more toxic than the heavy lanthanides. The toxicity of the lanthanides generally decreases with increasing atomic weight (Kyker & Cress, 1957; and Luckey & Venugopal, 1977). Lanthanum is considered to be the most toxic lanthanide because of its electro-chemical characteristics of high electropositivity, high charge density, and high tendency to form complexes through electrostatic bonding (Luckey & Venugopal, 1977). The toxicological data (LD_{50} values) of different lanthanide compounds administered to rats and mice using various methods are summarized in Appendix 1.

According to different groups of researchers, the symptoms of acute lanthanide intoxication in rodents include: writhing, ataxia, sedation, laboured respiration, and immediate defecation (Haley, 1965; Luckey & Venugopal, 1977; and Beliles, 1978). Some of the clinical symptoms include: soft tissue calcification at sites of injection, liver edema and necrosis, pulmonary edema, and hyperaemia (Luckey & Venugopal, 1977). Specific injuries have been reported in experimental animals.

Laszlo *et al.* (1952) observed liver damage in rodents which had received lanthanum chloride by intravenous injection in the toxic dose range. Injections of the lanthanides resulted in the production of fatty livers, which did not occur after oral administration (Haley, 1965). This again demonstrates that the mode of introduction of these elements has a substantial effect on their toxicity.

The above discussion on the toxicity of the lanthanide elements has been limited to the effects observed in small mammals. Since no work has been carried out specifically on fish, any inferences made assume that the effects would be the same in fish as in mammals. This is not a valid comparison since there are different methods that can be used in the introduction of the lanthanides into the fish. For example, lanthanides may be introduced into fish by submerging them in a lanthanide solution or by cannulation of a blood vessel. However, one of the comparisons that can be made between modes of administration between fish and mammals is oral administration. Using this method, the lanthanides were not absorbed by the intestinal tract of either animal, therefore no toxic effects were observed.

Analytical Methods

Very few classic instrumental methods are available for the determination and quantification of the lanthanide elements. Although the spectra of the lanthanides is well known and

documented, spectroscopic methods are generally not sensitive enough for tissue analysis (Kyker, 1961). Methods which lack the necessary sensitivity include Atomic Absorption Spectrophotometry, Emission Spectroscopy and X-ray Fluorescence Spectroscopy (Topp, 1965). Two methods that do offer high sensitivity for lanthanide detection are Neutron Activation Analysis (NAA) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Although each of these methods will be discussed, ICP-MS was the method chosen for this experimental work.

Neutron Activation Analysis (NAA)

The lanthanide elements exhibit certain characteristics which enables neutron activation analysis to detect them at very low levels in tissue samples. These properties include: a large thermal neutron cross-section, a high isotopic abundance, and a suitable decay scheme and half-life (Table 2). Activation analysis consists of exposing a stable isotopic sample to a neutron source and the subsequent measurement of characteristic energies emitted from the radioactive isotopes produced as a result of the nuclear transformations. The nuclear reaction that is important is the neutron, gamma reaction. This involves the absorption of a neutron by the nucleus of a stable isotope, followed by the emission of a gamma photon. The nuclide produced is one mass unit higher than the isotope that absorbed the neutron (Lyon, 1964; Rakovic, 1970; and Kruger, 1971).

Table 2. Characteristics of lanthanide isotopes for determination by neutron activation analysis.[1]

Isotope Produced	Stable Isotopes, % Abundance	Half-Life	Decay Energy (keV)	Neutron Cross-Section (barns)
^{90}Y	^{89}Y = 100	58.8 d	1.545	1.4 ± 0.3 b
^{140}La *	^{139}La = 99.91	40.22 h	3.769	2.8 ± 0.3 b
^{141}Ce *	^{140}Ce = 88.48	33 d	0.581	29 ± 3 b
^{142}Pr	^{141}Pr = 100	19.2 h	2.16	18 ± 3 b
^{147}Nd	^{146}Nd = 17.62	11.1 d	0.91	1.4 ± 0.2 b
^{153}Sm *	^{152}Sm = 26.72	46.8 h	0.801	210 ± 10 b
^{154}Eu *	^{153}Eu = 52.18	16 y	1.97	1500 ± 400 b
^{159}Gd	^{158}Gd = 24.87	18 h	0.95	3.5 ± 1.0 b
^{160}Tb *	^{159}Tb = 100	73 d	1.72	525 ± 100 b
^{165}Dy *	^{164}Dy = 28.18	2.3 h	1.30	2600 ± 200 b
^{166}Ho	^{165}Ho = 100	26.9 h	1.847	61.2 ± 2.0 b
^{169}Er	^{168}Er = 27.07	9.4 d	0.34	1.9 ± 0.2 b
^{170}Tm	^{169}Tm = 100	128.6 d	0.967	92 ± 4 b
^{175}Yb *	^{174}Yb = 31.84	101 h	0.467	46 ± 4 b
^{176}Lu	^{175}Lu = 97.41	3.7 h	1.3	21 ± 3 b

1 CRC Handbook of Chemistry and Physics, 60th Edition (1980).
* Represents the isotopes with the most suitable characteristics for determination by neutron activation analysis.

Neutron activation analysis, based on the activation of stable isotopes of the elements of interest, identifies elements which were analyzed. The use and analysis of stable isotopes, in contrast to the use of radioactive isotopes, eliminates the possibility of radioactive contamination of facilities and

living organisms. Unfortunately, there are a few drawbacks to the feasibility of using NAA for this work. For example, operational nuclear reactors needed for the neutron sources are not widely available, the analytical time may be lengthy depending on the decay scheme of the resulting isotope, the size of sample required is relatively large, and liquid samples cannot be analyzed by NAA. In addition, ICP-MS is more precise and accurate than NAA (Turnlund, 1989).

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

Inductively Coupled Plasma-Mass Spectrometry is a newly available technology for elemental and isotopic analyses. Houk & Thompson (1988) introduced ICP-MS as a method for the determination of isotopes of trace elements. The combination of the characteristics of the two components of this instrument are responsible for the high sensitivity. The ICP is basically used for atomization and ionization of elements carried in an aqueous aerosol from liquid samples, and the MS is for sensitive and selective measurement of all element isotopes (Houk & Thompson, 1988). The first instrument for commercial use was introduced by SCIEX Inc. in 1983. SCIEX and VG Isotopes are the two leading manufacturers of ICP-MS instrumentation. The following figure is a schematic diagram of a typical ICP-MS instrument.

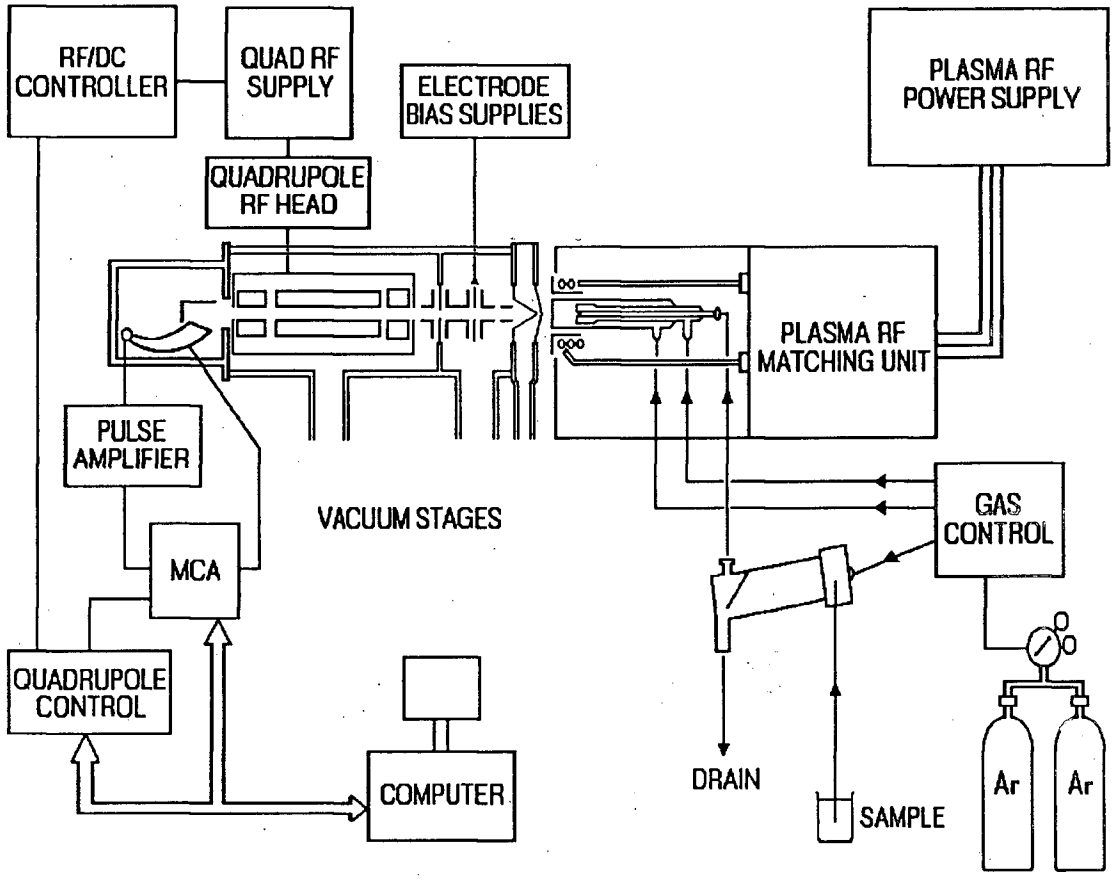

Figure 1. A schematic diagram of a typical ICP-MS showing the arrangement of the various components. Figure redrawn from a diagram supplied by Elemental Research Inc. MCA = Multi-Channel Analyzer; RF = Radio Frequency.

There are some advantages and disadvantages to using ICP-MS instrumentation as described by Douglas & Houk (1985), Gray (1986), Taylor (1986), Selby & Hieftje (1987) and Turnlund (1989). Some of the unique, beneficial features of ICP-MS include:

(i) rapid, multi-element analysis for the simultaneous detection of a wide range of elements;

(ii) high sensitivity resulting in low detection limits in the range of 0.02 to 0.7 μg/l;

(iii) relatively low background levels;

(iv) simple spectra in which there is one peak for each elemental isotope; and

(v) isotope ratio determinations are possible.

Unfortunately, as with all analytical techniques, there are some disadvantages to using ICP-MS:

(i) high initial purchase and maintenance costs; high sample analysis costs;

(ii) a few matrix and spectral interferences;

(iii) variation in instrument performance;

(iv) limited tolerance for analysis of dissolved solids; and

(v) samples are digested and diluted which reduces sensitivity.

Because this is a relatively new technique, research still needs to be carried out to solve some of these problems.

To obtain maximum sensitivity for the detection of a specific element, the plasma and mass spectrometer parameters should be adjusted to the optimum mass range setting for that element. When detection of a range of elements is required, there is some loss in sensitivity for specific elements since a wider mass range is used. However, this decrease is very minor and has not been shown to be a significant problem (Taylor, 1986). The detection limits achieved by ICP-MS are far lower

than those achieved by other techniques; even those of graphite furnace-atomic absorption spectroscopy (Houk & Thompson, 1988). Detection limits are partially determined by the sensitivity of the instrument to the element and by the background noise of other elements (Douglas & Houk, 1985).

The samples are introduced in the form of solutions and are sprayed into the ICP with a nebulizer. The argon ICP is an ionization source which heats the aerosol of the liquid sample to approximately 7,000°K. At that temperature the dissolved solids are vapourized, atomized and ionized. The ICP can effectively and efficiently ionize almost all elements since the average energy of the argon plasma (13.6 eV) is higher than the first ionization potential of most elements (Taylor, 1986). A fraction of the ions are directed into a quadrupole mass spectrometer which separates the ions according to their mass to charge ratio. The ions are then counted by a pulse counter where each count represents one detected ion (Douglas & Houk, 1985; and Houk & Thompson, 1988). The data are then recorded by a data handling system and calculated to a concentration through the appropriate software. Solids can be analyzed by either laser ablation or electrothermal vaporization, without having first to be dissolved (Houk & Thompson, 1988).

Analysis of the Lanthanides by ICP-MS

ICP-MS is an especially sensitive method for detection of the lanthanides. The detection limits for these elements are

very low, ranging from 0.01 to 0.3 μg/g (Table 3). Although the sensitivity and limits are excellent for all the lanthanides, there is some variation between elements (Longerich *et al.*, 1987; and Houk & Thompson, 1988).

Table 3. ICP-MS limits of detection for the lanthanides (3 S.D. of the background). Total dissolved solids are 1 mg/g.[1]

Element	Detection Limit (μg/g solid)	Element	Detection Limit (μg/g solid)
Y	0.05	Tb	0.05
La	0.03	Dy	0.20
Ce	0.03	Ho	0.05
Pr	0.03	Er	0.20
Nd	0.14	Tm	0.03
Sm	0.13	Yb	0.20
Eu	0.05	Lu	0.05
Gd	0.30		

1 Longerich *et al.* (1987).

The factors that affect the sensitivity of ICP-MS are the isotopic abundances and possible interference from mass overlap. The larger the isotopic abundance, the greater the sensitivity to that element - provided that there are no interferences from other elemental isotopes. Table 4 shows the isotopic weights and per cent abundances of the various lanthanides.

Table 4. Isotopic composition of the lanthanide elements and their natural relative abundances.[1]

Ln	# of Isotopes	Isotopic Weight	% Relative Abundance
Y	1	89	100
La	2	138, 139	0.09, 99.91
Ce	4	136, 138, 140, 142	0.19, 0.25, 88.48, 11.07
Pr	1	141	100
Nd	7	142, 143, 144, 145, 146, 148, 150	27.11, 12.14, 23.83, 8.29, 17.26, 5.74, 5.63
Sm	7	144, 147, 148, 149, 150, 152, 154	3.09, 14.97, 11.24, 13.83, 7.44, 26.72, 22.71
Eu	2	151, 153	47.77, 52.23
Gd	7	152, 154, 155, 156, 157, 158, 160	0.20, 2.15, 14.73, 20.47, 15.68, 24.87, 21.90
Tb	1	159	100
Dy	7	156, 158, 160, 161, 162, 163, 164	0.05, 0.09, 2.29, 18.88, 25.53, 24.97, 28.19
Ho	1	165	100
Er	6	162, 164, 166, 167, 168, 170	0.14, 1.56, 33.41, 22.94, 27.07, 14.88
Tm	1	169	100
Yb	7	168, 170, 171, 172, 173, 174, 176	0.14, 3.03, 14.31, 21.82, 16.13, 31.84, 12.73
Lu	2	175, 176	97.40, 2.60

1 Table of Isotopes, 7th Edition (1978).

The monoisotopic lanthanides that have 100% abundance of one isotope are yttrium, praseodymium, terbium, holmium, and thulium. Other lanthanides may have two isotopes, but one of the isotopes will have the greater abundance (^{138}La = 0.09% and ^{139}La = 99.91%). Some elements have as many as seven isotopes;

these include, neodymium, samarium, gadolinium, dysprosium, and ytterbium (Table 4).

In the selection of the isotope to analyze, both the abundance and the possible interferences need to be considered. For the monoisotopic elements there is no problem. For elements with more than one isotope, the most abundant isotope with the least isobaric overlap is the one which is analyzed.

Some examples of preferable isotopes have been presented by Longerich *et al.*, 1987:

(i) ^{89}Y - monoisotopic;

(ii) ^{141}Pr - monoisotopic;

(iii) ^{159}Tb - monoisotopic, with a small interference from $^{143}Nd^{16}O^{+}$ (12.14%);

(iv) ^{165}Ho - monoisotopic, with a small interference from $^{149}Sm^{16}O^{+}$ (13.8%);

(v) ^{169}Tm - monoisotopic, with a small interference from $^{153}Eu^{16}O^{+}$ (52.2%);

(vi) ^{139}La - the most abundant isotope at 99.91%, and the only isotope without interference;

(vii) ^{140}Ce - the most abundant isotope at 88.48%, and the only isotope without interference;

(viii) ^{147}Sm - the most abundant isotope at 15.0% without interference. The second choice would be ^{149}Sm at 13.8% abundance. The third choice would be the most abundant isotope, but it has interference from $^{152}Gd^{16}O^{+}$ (0.20%);

(ix) ^{163}Dy - the four isotopes that have similar abundances of 18.88, 25.53, 24.97, and 28.19%, but the ^{163}Dy (24.97%) isotope has the least interferences; and

(x) ^{173}Yb - this isotopes has only one interference from $^{157}Gd^{16}O^{+}$ (15.7%), which is less than the interference found in ^{171}Yb from $^{155}Gd^{16}O^{+}$ (14.9%).

Any interference caused by these oxides are minimal as the relative abundance of the oxides, on a well-tuned ICP-MS, is approximately 0.1% or less of the element isotope present in the sample.

An example using the samarium spectrum has been given by Longerich *et al.* (1986). The seven isotopes of samarium are found in the mass range of 144 to 154. The spectra of this lanthanide is clearly unique and each peak represents one isotope. The spectra shows the different relative abundances by the varying magnitudes. As can be seen in Figure 2, the ICP-MS spectra are relatively simple.

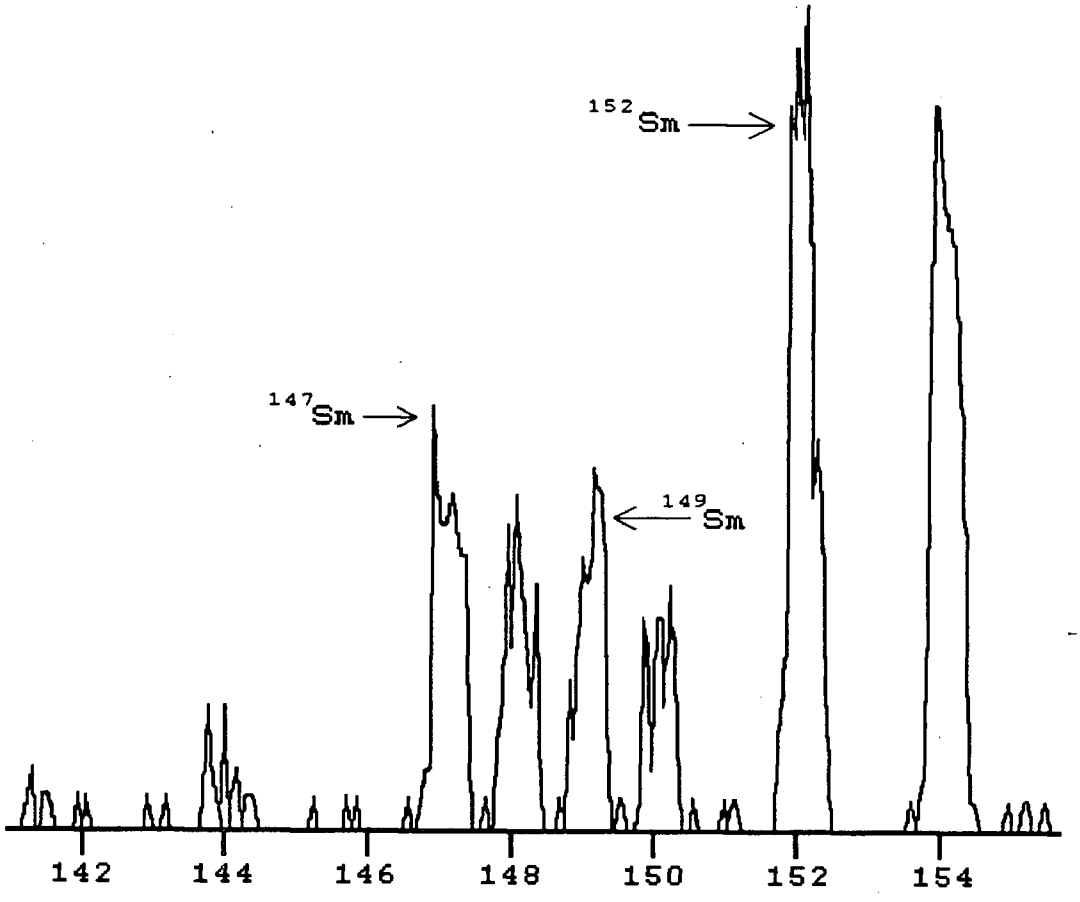

Figure 2. A typical mass spectrum of samarium obtained with ICP-MS instrumentation showing the individual peaks for each of the samarium isotopes. Spectra obtained from experimental data (Experiment 5).

METHODOLOGY

A series of experiments was conducted to investigate the feasibility of using the lanthanide elements to label juvenile coho salmon. Two experiments investigated the toxicity of these elements to coho and steelhead alevins; and four experiments investigated the feasibility of marking coho fry and smolts using the lanthanides. Experiments were conduced at Capilano Hatchery, North Vancouver. All experimental fish were exposed to concentrated aqueous solutions of lanthanide acetates added to the ambient river water. After the completion of the labelling, bony tissues were extracted and analyzed for lanthanide accumulation.

Chemicals Used

For all treatments carried out, the lanthanide elements used were acetates in powder or crystalline form and all were 99.9% reagent quality. The acetate form was chosen because lanthanide salts with organic acids have no tendency to hydrolyse. Lanthanide halides (i.e. chlorides) are very soluble in water at room temperature and hydrolyse readily in solution to produce oxide halides (Topp, 1965). However, when incorporated at low concentrations (100 μg/l) in the water, neither the chlorides nor the acetates should hydrolyse and the salts should dissociate into the Ln^{3+}, Cl^-, and $CH_3CO_2^-$ ions to dissolve and become hydrated ions - $Ln(H_2O)_x^{3+}$. Because of these

properties, both the chloride and acetate forms appear to be suitable for marking fish.

The lanthanides used and the suppliers are as follows: yttrium acetate tetrahydrate, $Y(C_2H_3O_2)_3 \cdot 4H_2O$ (Alfa Catalog Chemicals, Davers); lanthanum (III) acetate hydrate, $La(C_2H_3O_2)_3 \cdot 1\frac{1}{2}H_2O$ (Aldrich Chemical Company Inc., Milwaukee); cerium (III) acetate hydrate, $Ce(C_2H_3O_2)_3 \cdot 1\frac{1}{2}H_2O$ (Alfa Catalog Chemicals, Davers); samarium (III) acetate hydrate, $Sm(C_2H_3O_2)_3 \cdot 3H_2O$ (Aldrich Chemical Company Inc., Milwaukee); dysprosium (III) acetate hydrate, $Dy(C_2H_3O_2)_3 \cdot 4H_2O$ (Aldrich Chemical Company Inc., Milwaukee); and ytterbium (III) acetate tetrahydrate, $Yb(C_2H_3O_2)_3 \cdot 4H_2O$ (Aldrich Chemical Company Inc., Milwaukee). The price of the lanthanide acetates varied greatly from $18/100g for lanthanum (III) acetate hydrate to $170/100g for ytterbium (III) acetate tetrahydrate.

The bleaching solution used to digest the traces of tissue off the vertebral column and otoliths was a 6% sodium hypochlorite solution (BDH Chemicals Inc., Toronto). Analysis on this bleaching solution for lanthanide content are reported in Appendix 2.

Treatment Concentrations

The concentrations in the static tanks were made by adding a measured amount of the concentrated lanthanide acetate stock solution directly into a measured quantity of ambient river water in each tank. Throughout the treatment period the water

in the tanks was not changed and no additional water was added. For the duration of the treatment, water samples were collected for elemental analysis to ensure the concentrations were correct and constant.

Lanthanides added to a flow-through system had to be dripped in at a constant rate while the ambient river water flowed in at a set rate. The concentration of the lanthanide acetate stock solution and the required drip rates were determined using a computer program developed specifically for these types of calculations. The river water was set at a flow rate of one litre per minute. The concentrated lanthanide stock solution was mixed with the river water in a funnel before being delivered to the tank. A modified version of the Merriott bottle was used to deliver the concentrated lanthanide solution into the funnel (Figure 3). Once the bottle had been closed tightly and the drip rate set, air entered the bottle through the rigid tubing and was collected at the top of the bottle. The negative pressure which was created eliminated the effect of gravity, therefore the drip rate remained constant.

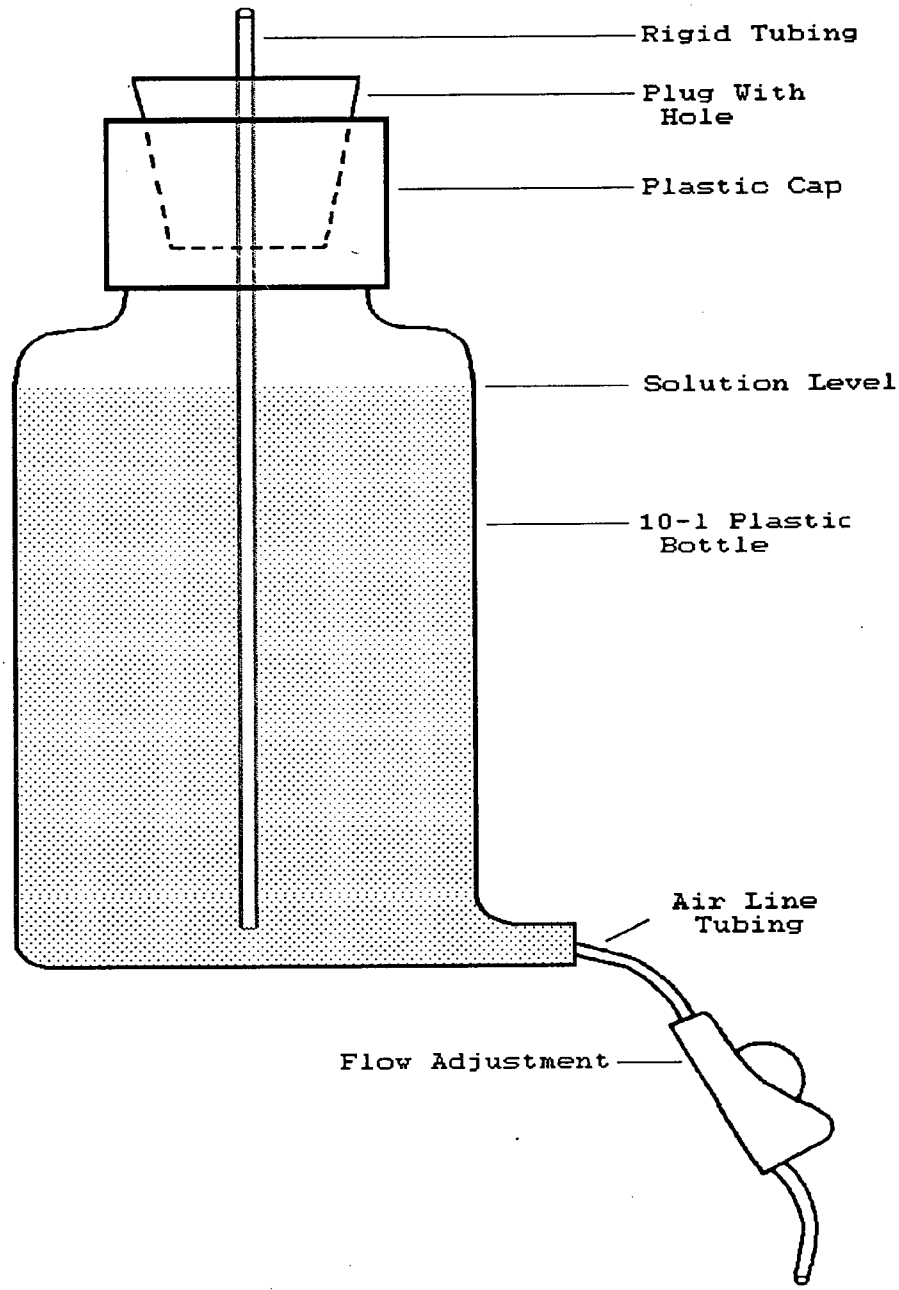

Figure 3. Modified version of the Merriott bottle used to deliver the chemical solutions at a constant drip rate.

Sample Preparation Techniques

Water Samples

For the duration of the treatment period element concentration in the tanks was monitored. Depending on the particular experiment, the water samples were taken every few days or on a weekly basis. For each sample, 10 ml of tank water was collected in a vial and immediately acid-fixed with 3 drops of concentrated nitric acid. The samples were shaken and stored in a cool location until they were submitted for analysis by ICP-MS. To minimize any "plate out" effects, the samples were collected in the morning and delivered to the laboratory (Elemental Research Inc., North Vancouver) for prompt analysis. The water samples were analyzed without modification.

Vertebral Column Extraction

Feed was withheld for the 24-hour period prior to sampling. This helped to reduce stress and decrease the gastro-intestinal tract contents, thus minimizing variation in body weight. After the fish were killed by asphyxiation, the weights of individual fish were recorded and the fish were decapitated. The heads were immediately frozen and stored for otolith removal at a later date. Fins, tail and internal organs were separated and discarded. The majority of the flesh was dissected out from the vertebral column and the remaining traces of flesh were then digested with a 6% sodium hypochlorite solution. Since this bleaching solution would have dissolved the bony tissue as well

as the flesh, the digestion process had to be closely monitored. The length of time required for complete soft tissue digestion increased with the size of fish.

Once the flesh was completely digested, the vertebral column was removed from the digestion solution, rinsed with distilled water and placed in a sample vial. The clean backbones were dried overnight at 70°C and the weights recorded. The samples were then submitted to Elemental Research Inc. (ERI) for ICP-MS analysis. Before the samples were analyzed, the vertebral columns first were digested in concentrated nitric acid and then diluted with distilled water to make a 10 ml sample. Once analyzed, the results were reported as a concentration relative to the sample weight.

Otolith Extraction

For removal of the otoliths, the entire fish head was placed in a petri dish with a 6% sodium hypochlorite solution. As the otoliths are the densest structures in the fish's body and are composed of calcium carbonate, they are able to withstand the digestion procedure (Treacy & Crawford, 1981). After overnight digestion, only the otoliths remained in the dish. The sagittae, the largest of the three pairs of otoliths, were removed and rinsed with distilled water, placed in a vial and dried overnight. Since the sagittae are small, a few were placed in each vial. The sample weight was not recorded.

The prepared samples were then submitted for analysis by ICP-MS. The otoliths were digested in concentrated nitric acid and then diluted prior to analysis. The results were reported as a concentration which was calculated relative to the internal calcium concentration of the otoliths. The calcium concentration of the otoliths was set nominally at 10% by ERI.

Scale Samples

Using a sharp blade, scales were scraped from both sides of the fish's body in the "preferred scale sample area" (Figure 4). This is the area between the lateral line and the dorsal fin. The scales were then placed in a small petri dish filled with distilled water and soaked for up to 24 hours. This served to wash most of the mucus from the surface of the scales, making it easier to remove the individual scales from the dish. Once the scales were relatively clean, they were examined under a dissecting microscope and were then selected to make the sample. The cleanest scales were placed in a sample vial and air-dried overnight. Regenerated scales were avoided. For a fish weighing approximately 10 grams, 80 scales were collected. Since the scales were so small and there was a problem with static electricity, the weight of each sample was not recorded. The samples were then submitted for ICP-MS analysis. As with the other solid samples, the scale samples had to be digested and diluted prior to analysis. The results were then reported

as a concentration calculated relative to the scales' internal calcium concentration, set at 10%.

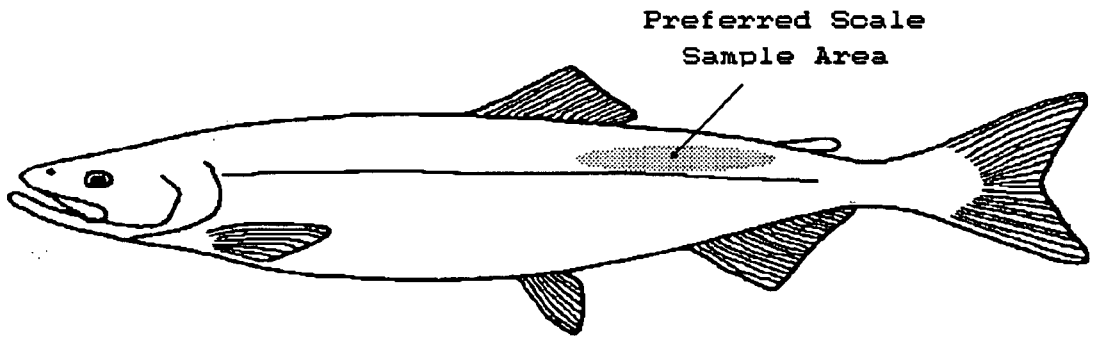

Figure 4. Diagram of a fish showing the "preferred scale sample area".

Sample Analysis

Problems With Analysis

A few problems arose with regard to the analysis of samples collected from the first sets of marking experiments. The severity and nature of the problems were diverse and variable. Some of these will be discussed briefly and generally here, with details included in the results sections of the appropriate experiments.

Values markedly lower than theoretical concentrations were reported for water samples submitted to Elemental Research Inc. (ERI). The company had a problem with the secondary standards

being used to calibrate the ICP-MS instrument. The vials in which the standards were being stored were not airtight. As a result, the solvent gradually evaporated, leaving a more concentrated standard in the vial as time progressed. Because this error was not detected until the third experiment on coho fry (Experiment 5), no corrections were made prior to that time. Consequently, the experimental values reported for the first two experiments using coho fry were lower than the true values. Unfortunately, no records were kept on the age or dates of use of the faulty standards, therefore there was no way of correcting for these errors. The values were estimated to be 10 to 30% too low. When these problems with the secondary standards were discovered the vials used to store the standards were replaced. Since that time the standards were regularly checked for accuracy by the analytical company (ERI).

Another problem was some high anomalous values in some of the submitted samples. ERI suggested that this error had resulted from contamination in the experimental tanks, the dissection lab or the analytical lab. Upon further examination, it was revealed that one of these contaminated samples contained the whole range of lanthanides rather than just the one element being used. However, other contaminated samples contained only the single element which was being investigated. Fortunately, these anomalies were low in frequency and were obvious, and thus easily recognized. No other possible explanations for these anomalies were suggested or identified.

Subsequent to the detection of these problems, blind standards were included with samples submitted for analysis. Either water or vertebrae standards were used, to correspond with the type of sample (liquid or solid). The details of the standards submitted are described below.

Water Standards

Water standards were prepared by the author to check the accuracy of the analysis of regular samples. The concentrations covered the range expected in the samples. The standards were prepared from aliquots of solutions having known concentrations of lanthanides. The final volume of the standards were then made to 10 ml by the addition of distilled water. Three drops of concentrated nitric acid were added to each vial to acid-fix the standards. These standards were then analyzed by ICP-MS together with the experimental water samples.

The possibility of adsorption of lanthanides onto the sides of the sample vials was investigated. Teflon containers and vials have been shown not to be subject to adsorption of these elements. Comparable results were obtained from standards submitted in both teflon and polypropylene vials. Therefore, the adsorption of lanthanides onto the sides of the polypropylene vials must have been negligible.

Vertebrae Standards

Vertebrae standards submitted with selected vertebrae samples were prepared using one of two different methods. The standards for Experiment 5 were made by spiking a vial containing a dried control (lanthanide-free) vertebral column of known weight with an aliquot of the concentrated lanthanide acetate stock solution. The vial was then dried overnight.

The results obtained from these standards were lower than the calculated values. This preparation method was thus discontinued because it was believed that possibly the nitric acid was not able to digest the lanthanide dried onto the side of the vial, with the result that a variable portion of the lanthanide present was not going into solution, and therefore could not be detected.

A new method was then used to prepare the vertebrae standards for Experiment 6. This involved the crushing of several dried vertebral columns into a fine powder using a mortar and pestle. The vertebrae powder was weighed onto a watch glass and a known volume of the lanthanide acetate spike was added. The resulting slurry was then mixed thoroughly and oven dried. The dried spiked powder was then mixed again and divided into vials for analysis.

EXPERIMENT 1 - INVESTIGATION OF COHO (*Oncorhynchus kisutch*) ALEVINS IN RECIRCULATING SYSTEMS CONTAINING A RANGE OF CONCENTRATIONS OF CERIUM, LANTHANUM, DYSPROSIUM, SAMARIUM, AND YTTERBIUM

Introduction

This study was undertaken in an attempt to develop an effective mass marking technique to identify hatchery-production salmon. The feasibility of using the lanthanide elements to label coho alevin was investigated as a first step.

There are four reasons why alevin were investigated first:

(i) mineral uptake from the water supply occurs at the alevin stage;

(ii) alevin require a much smaller volume of water per fish than fry; therefore less water and less lanthanide element solution would be needed per tank;

(iii) recirculating systems could be designed to re-use the lanthanide solutions, so that they would not have to be continually added to the system; and

(iv) an alevin-labelling procedure could be conveniently incorporated into a routine hatchery operation.

The purpose of this experiment was to compare various lanthanides at varying concentrations with respect to uptake and toxicity. Five lanthanides, each at three concentrations, were introduced into the water supply of the alevin. The experimental tanks were set up as upwelling recirculating units.

Materials and Methods

Recirculating Units

Upwelling recirculating systems were set up for these marking experiments to label coho (*Oncorhynchus kisutch*) alevins with the lanthanide elements (Figure 5). The system incorporated a submersible pump which propelled the water up a pipe to the reservoir and to the incubation unit. The flow rate out of the pump was non-adjustable and was controlled by a valve positioned below both the reservoir and the incubation unit. The water was set to flow through the incubation unit at approximately one litre per minute with the surplus being directed to the reservoir. This provided the alevins with a continuous water flow while minimizing the disturbance to the alevins. The reservoir had three important functions: (i) to provide an outlet for the surplus water; (ii) to aerate the water by agitation resulting from spilling over the side of the reservoir; and (iii) to ensure that the incubation unit would never completely drain.

The recirculating system had two safety checks in case of a power failure, where the pump would cease working: (i) a check valve to prevent back flow; and (ii) a reservoir placed higher than the incubation unit. Together, these would limit the back flow of water and ensure that the water would never completely drain out of the incubation unit, thereby leaving the alevins without water.

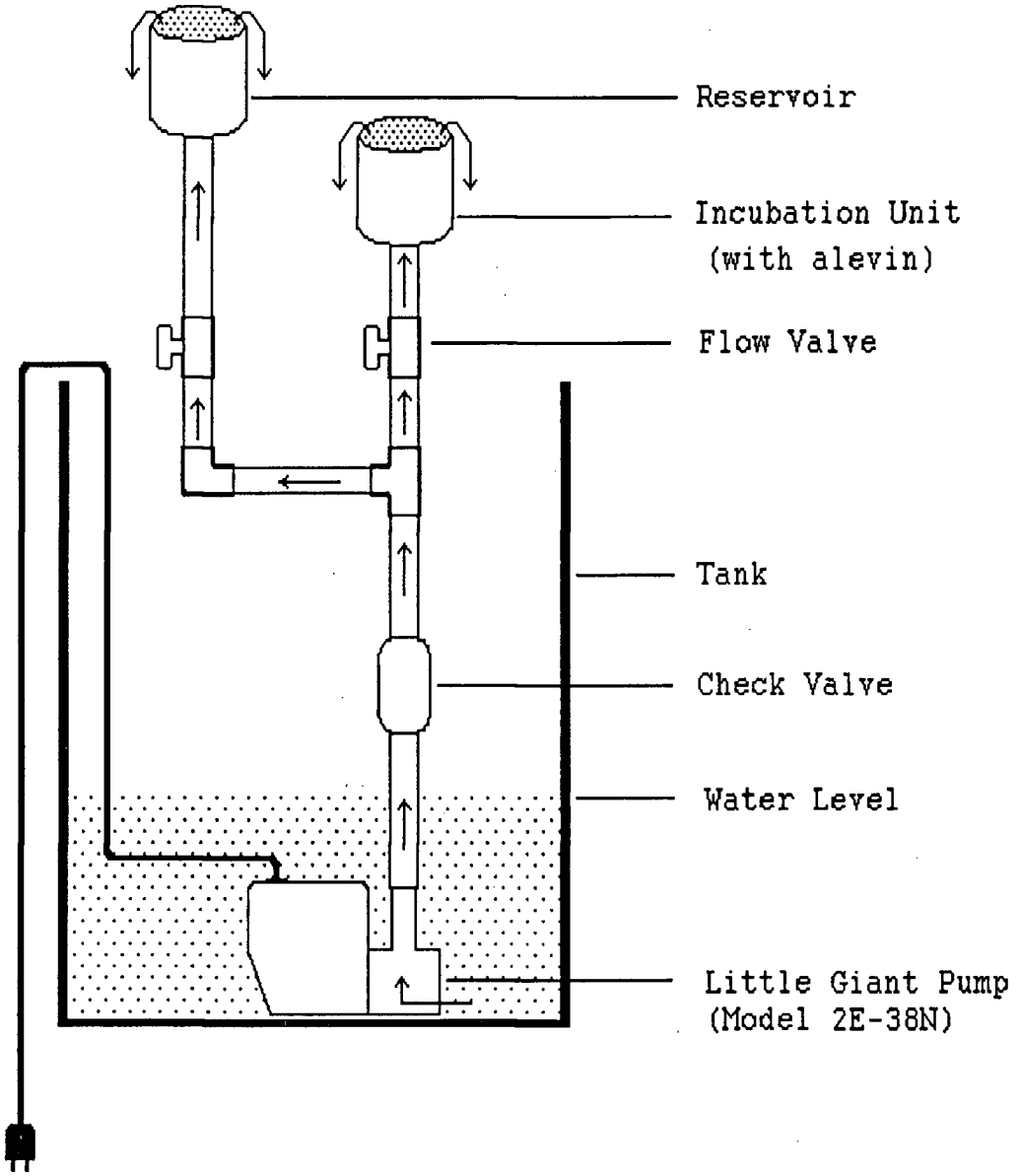

Figure 5. Upwelling recirculating apparatus to recirculate the water in the treatment tanks. Arrows indicate water flow.

Experimental Design

Fifty coho alevins and five coho eggs were placed in the incubation unit of the recirculating apparatus in each of the 14 experimental tanks. A total of sixteen litres of ambient river water (10°C) was added to each tank and a period of two days passed before the chemical treatments were added to the tanks (February, 1989). The treatments consisted of cerium, lanthanum, dysprosium, samarium and ytterbium all at concentrations of 20, 100 and 300 μg/ml, except ytterbium which did not have a 300 μg/ml treatment (Table 6). Because a limited number of recirculating apparati were available, there were no replicates for any of the treatments and there were no negative controls.

The alevins and eggs used in this experiment were coho salmon from Capilano River (B.C.) brood stock. At the start of the experiment, the alevins appeared healthy and normal. They rested on the bottom of the incubation unit, becoming active when disturbed by light or touch, then settling back at the bottom of the funnel soon after the disturbance was discontinued. Although alevin were of primary interest in this experiment, a small number of eyed eggs were also used.

For each treatment group, a measured amount of lanthanide acetate crystals was added to 500 ml of river water and heated until the crystals were completely dissolved. The solution was then cooled in a bucket of ice to 10°C, and poured into the reservoir of the recirculating unit. This method ensured that

the lanthanides slowly mixed with the tank water through the recirculating action of the pump. Approximately 1 ml of concentrated sodium hydroxide was then added to each tank to increase the pH on the assumption that this would help to compensate for any acidity caused by the lanthanide acetates.

Table 5. Lanthanide atomic weights and molecular weights of the corresponding acetates used in the treatment concentration calculations.

Element (Symbol)	Atomic Weight	Salt	Mol. Wt. of Salt	Mol Wt/ At Wt[1]
Cerium (Ce)	140.12	Ce acetate	317.25	2.2623
Lanthanum (La)	138.91	La acetate	316.05	2.2753
Dysprosium (Dy)	162.50	Dy acetate	339.64	2.0901
Samarium (Sm)	150.36	Sm acetate	327.48	2.1780
Ytterbium (Yb)	173.00	Yb acetate	422.24	2.4407

1 Molecular Weight / Atomic Weight.

The atomic weight of each the lanthanide elements and the molecular weights of the corresponding acetates are shown in Table 5. Table 6 shows the amount of lanthanide acetate added to each tank and the theoretical lanthanide treatment concentration.

Sample Treatment Calculation

$$\frac{\text{Grams Salt Used / Litres}}{\text{Molecular Wt. / Atomic Wt.}} \times 1000 = \mu g \text{ lanthanide/ml}$$

Table 6. Lanthanide acetate (grams) added to 16 litres of river water, and the approximate theoretical element concentrations in each tank.

Tank	Compound	Salt Added (g)	Theoretical Concentration
1	Ce acetate	0.675	20 µg/ml
2	Ce acetate	3.393	100 µg/ml
3	Ce acetate	10.181	300 µg/ml
4	La acetate	0.682	20 µg/ml
5	La acetate	3.409	100 µg/ml
6	La acetate	10.235	300 µg/ml
7	Dy acetate	0.623	20 µg/ml
8	Dy acetate	3.132	100 µg/ml
9	Dy acetate	9.422	300 µg/ml
10	Sm acetate	0.655	20 µg/ml
11	Sm acetate	3.264	100 µg/ml
12	Sm acetate	9.806	300 µg/ml
13	Yb acetate	0.733	20 µg/ml
14	Yb acetate	3.653	100 µg/ml

Sampling and Analytical Method

Dead alevins and eggs were sampled from the tanks containing 100 and 300 µg/ml of cerium, lanthanum, dysprosium and samarium. The alevin samples consisted of three whole alevins per vial and one vial with only the contents of yolk sacs from three alevins. The egg samples consisted of three whole eggs per vial and one vial with only three empty egg shells (cases). All samples were oven-dried overnight, dry weight recorded, and analyzed by ICP-MS for lanthanide content.

Results

Water Quality

Within four hours of the addition of the lanthanide solutions and NaOH, a foamy substance developed in the tanks containing the highest concentrations. The amount of foam present varied from none (in the tanks with the lowest concentrations of all elements and the tank with samarium at 300 μg/ml) to a thick layer of bubbles over the surface (in the tanks with the highest concentrations of cerium, lanthanum, and dysprosium). A white precipitate also formed in the 100 and 300 μg/ml tanks for all elements. There was a strong correlation between high lanthanide concentration and presence of foam and precipitate. After 24 hours, the foam in all tanks had dissipated, but the precipitate was still present in the tanks containing 100 and 300 μg/ml of all elements (Table 7).

The water pH in all tanks, after the addition of the lanthanides and NaOH, ranged from 6.20 to 6.67 units. The water in the tanks containing the lowest concentrations of all elements had the highest pH values, while the water in the tanks containing the 100 μg/ml concentrations of all elements had the lowest pH values (Table 7). No other water parameters were tested.

Mortalities

Fish mortality was noted in the tanks containing the highest lanthanide concentrations within four hours of the addition of chemicals. For the 100 μg/ml concentrations of all elements, with the exception of ytterbium, all alevin were dead after four hours. At the 300 μg/ml concentration, mortalities after four hours were present only in the tanks containing cerium and lanthanum (Table 7). All alevins in all tanks were dead after a 24-hour period. Mortality characteristics of the alevins included: arched spine, congealed yolk sac, open mouth, and decomposing flesh with white tips on the fins and tail. After 48 hours, all eggs in all tanks were dead.

Table 7. Foam and precipitate formation, and alevin mortalities observed in the tanks 4 hours and 24 hours after the addition of the lanthanide acetates.

At Time of Treatment		After 4 Hours			After 24 Hours		
Ln µg/ml	pH	Morts	Foam[1]	ppt[2]	Morts	Foam	ppt
Ce - 20	6.30	0 %	0%	-	100 %	0%	-
Ce - 100	6.20	100 %	25-75%	+	100 %	0%	+
Ce - 300	6.29	100 %	25-75%	+	100 %	0%	+
La - 20	6.57	0 %	<25%	-	100 %	0%	-
La - 100	6.33	100 %	>75%	+	100 %	0%	+
La - 300	6.36	100 %	>75%	+	100 %	0%	+
Dy - 20	6.67	0 %	0%	-	100 %	0%	-
Dy - 100	6.24	100 %	<25%	+	100 %	0%	+
Dy - 300	6.34	0 %	25-75%	+	100 %	0%	+
Sm - 20	6.62	0 %	<25%	-	100 %	0%	-
Sm - 100	6.27	100 %	<25%	+	100 %	0%	+
Sm - 300	6.38	0 %	0%	+	100 %	0%	+
Yb - 20	6.37	0 %	0%	-	100 %	0%	-
Yb - 100	6.23	0 %	<25%	+	100 %	0%	+

1 surface area covered by foam expressed as a per cent.
2 precipitate present (+) precipitate absent (-).

Water Analysis

Water samples from the tanks containing the lowest lanthanide concentrations were analyzed for element concentration. In each case the results were lower than the calculated values (Table 8).

Table 8. ICP-MS results of water analysis of the tanks containing the lowest lanthanide concentrations.

Lanthanide	Calculated Concentration	Analysis Results µg/ml
Ce	18.6	15.2
La	18.7	12.5
Dy	18.6	15.4
Sm	18.8	13.5
Yb	18.7	16.6

Residual Element in Tanks

After the alevins had all died, the tanks and recirculating units were rinsed for 24 hours by pumping fresh water through the apparatus at the maximum rate of the pump to remove the lanthanides. Then 16 litres of river water was added to each tank, the recirculating units were re-set up and alevins were placed into the incubation units. No additional lanthanides were added. All alevins in the tanks were dead within 48 hours and water samples were then taken from each tank. It would appear that there was still enough element remaining in each tank after the 24-hour rinse because mortalities still occurred. Concentration of lanthanide in each tank was between 0.424 and 1.250 µg/ml, regardless of the strength of the original treatment concentration (Table 9).

Table 9. Original treatment concentration and residual lanthanide remaining in the tanks after a 24-hour continuous rinse.

Tank	Lanthanide	Original Concentration	Residual Concentration
1	Cerium	20 µg/ml	0.745 µg/ml
2	Cerium	100 µg/ml	0.998 µg/ml
3	Cerium	300 µg/ml	0.941 µg/ml
4	Lanthanum	20 µg/ml	0.707 µg/ml
5	Lanthanum	100 µg/ml	1.030 µg/ml
6	Lanthanum	300 µg/ml	0.731 µg/ml
7	Dysprosium	20 µg/ml	1.070 µg/ml
8	Dysprosium	100 µg/ml	1.250 µg/ml
9	Dysprosium	300 µg/ml	0.586 µg/ml
10	Samarium	20 µg/ml	0.424 µg/ml
11	Samarium	100 µg/ml	0.433 µg/ml
12	Samarium	300 µg/ml	0.708 µg/ml
13	Ytterbium	20 µg/ml	0.498 µg/ml
14	Ytterbium	100 µg/ml	0.499 µg/ml

Acid Rinsing of Tanks

The 24-hour constant flow-through rinse with fresh water was not effective in completely removing the lanthanides from the recirculating apparatus and the tanks. Acid washes with 0.1M and 0.5M hydrochloric acid (HCl) were attempted. After the equipment was treated with the acid, it was again rinsed with fresh water.

The recirculating systems were set-up in each of the tanks after the washing was complete and water samples were taken 24

hours later. The 0.1M and 0.5M HCl appeared to be effective in removing the residual lanthanides from the system (Table 10).

Table 10. Lanthanide concentrations in tanks after a 0.1M or 0.5M HCl wash and a 24-hour flow through rinse.

Residual Lanthanide	Acid Wash	Analysis Results
Ce 0.75 μg/ml	0.1M HCl	<0.01 μg/ml
Ce 0.94 μg/ml	0.5M HCl	<0.01 μg/ml
La 0.73 μg/ml	0.1M HCl	<0.01 μg/ml
Dy 0.59 μg/ml	0.5M HCl	0.01 μg/ml
Sm 0.71 μg/ml	None	0.07 μg/ml
Yb 0.50 μg/ml	None	0.09 μg/ml

Alevin and Egg Analysis

Some of the dead alevins and eggs were sent for analysis by ICP-MS. The results of these analyses are shown in Table 11. The vial containing the three yolks had the lowest concentration of lanthanide present (4.9 μg Dy/g of dry tissue or 0.3 μg Dy/vial). The alevins treated with lanthanum, cerium, and dysprosium at 100 μg/ml had between 34.2 and 76.7 μg lanthanide/g of dry tissue (5.5 to 11.6 μg/vial), while those treated with samarium at 100 μg/ml had only 5.9 μg Sm/g (0.8 μg/vial). Alevin exposed to 300 μg/ml of cerium had the highest concentration of cerium present (242 μg Ce/g). The results obtained for the analysis of the dead eggs and the empty egg shells showed approximately equal amounts of the lanthanides

present in each vial (707 to 1110 μg/vial), but the concentrations present were very different between the two types of samples. The whole eggs treated with dysprosium at 300 μg/ml had a concentration of 3891 μg Dy/g dry weight, while the empty egg shells, treated in the same manner had nearly fifteen times the concentration of dysprosium. The eggs were exposed to all elements for 48 hours and consequently had more element present than the alevins which were exposed for 4 hours (Table 11).

Table 11. ICP-MS analysis of dead alevins and eggs.

Alevin and Eggs Analyzed	Lanthanide, Concentration, and Duration	Sample Weight (g)	Results μg/g	μg Ln Per Vial
3 yolks	Dy 100 μg/ml (4 hrs)	0.061	4.9	0.3
3 alevin	La 100 μg/ml (4 hrs)	0.116	55.2	6.4
3 alevin	Ce 100 μg/ml (4 hrs)	0.161	34.2	5.5
3 alevin	Sm 100 μg/ml (4 hrs)	0.135	5.9	0.8
3 alevin	Dy 100 μg/ml (4 hrs)	0.151	76.7	11.6
3 alevin	Ce 300 μg/ml (4 hrs)	0.162	242.3	39.3
3 eggs	La 300 μg/ml (48 hrs)	0.239	4654.1	1110
3 eggs	Sm 300 μg/ml (48 hrs)	0.236	3791.8	896
3 eggs	Dy 300 μg/ml (48 hrs)	0.192	3891.5	746
3 cases[1]	Dy 300 μg/ml (48 hrs)	0.012	57479.7	707

1 cases = empty egg shells.

Discussion

All concentrations of lanthanides tested were 100% toxic to the alevins. The light lanthanides appeared to be more toxic than the heavier ones. This toxic effect could possibly be attributed to the smaller size and atomic weight of lanthanum and cerium than of samarium, dysprosium and ytterbium. It is possible that the lighter elements may be transported across the gill epithelia and/or the yolk sac membrane at an accelerated rate with a resultant earlier onset of toxicity, although there are no published results to substantiate this hypothesis.

A possible explanation for the toxic effects observed in the alevins could be the lanthanides were accumulated in the alevins at an accelerated rate and were not excreted. The elevated levels of element eventually caused mortality. The absence of mortalities, after 4 hours, in the tanks containing 300 μg/ml of dysprosium and samarium, while there were no survivors in the tanks containing 100 μg/ml of these elements, could have resulted from a reversal of the treatments.

It is not likely that high ammonia levels caused the mortalities since the stocking density was so low and since the alevins were in the tanks for such a short period. Also, aeration of the water would have decreased ammonia levels and increased dissolved oxygen levels.

It was hypothesized that the addition of the lanthanide acetates would increase the acidity of the water and that a base would need to be added to neutralize the water. Because the

extent to which the various concentrations of lanthanides added would affect the pH was unknown, a constant amount of NaOH was added to each tank. Because the resultant variable pH may have confounded results and because markedly lower concentrations of lanthanides were to be used in subsequent experiments, it was decided that NaOH addition would not be practised in subsequent experiments.

The foam and precipitate that developed in the highest concentration tanks may have resulted from the chemical reaction between the lanthanides and the concentrated NaOH or between the lanthanides and organics in the river water. The turbulence caused by the activity of the pumps could have furthered the development of the foam. The foam had completely dissipated within 24 hours. The precipitate that formed could have been lanthanide hydroxide resulting from the following chemical reaction between the lanthanide acetates and the NaOH: $Ln(C_2H_3O_2)_3 + 3NaOH \rightarrow 3NaC_2H_3O_2 + Ln(OH)_3$.

The water samples taken from the 20 μg/ml treatment tanks resulted in lower lanthanide concentrations than those which were calculated. Possible reasons for these low analytical values are not known.

Even with a 24-hour flow-through rinse, there was considerable retention of lanthanides on the sides of the tanks and on the apparatus as evidenced by the lanthanide concentrations in the water in the tanks when they were recharged. A known characteristic of the lanthanide elements is

their tendency to adhere to surfaces at very low concentrations in aqueous solutions (Luckey & Venugopal, 1977). Flushing with water alone was not effective in removing the adhered elements. Regardless of the original treatment concentration in the tank, the residual lanthanide concentrations in the rinsing water were approximately equal, thus indicating an upper limit to the amount of lanthanide that can adhere to the surfaces of the tank and apparatus. However, it was found that this residue could be removed by rinsing the tank and apparatus with 0.1M HCl. During this rinsing, the insoluble hydroxide form was converted to the soluble chloride.

Analysis of dead alevins and eggs showed very high concentrations of lanthanides. This could have occurred as a result of these elements adhering to the mucus coating of the alevins and eggs, rather than being absorbed into the tissues. If this were the case, the resulting concentration would not have been representative of the elements which were actually incorporated into the alevins or eggs. This theory was verified by the comparison of lanthanide content in the whole alevin and in the yolks alone, and by the lanthanide content found in the whole eggs and in the empty egg shells alone (Table 11). As a result of these findings it was decided that, for future experiments, collection of biological samples would be made two weeks post-experiment, during which time untreated water would be provided. It was hypothesized that such a delay would allow

the rinsing away of adhered lanthanides and the deposition of the absorbed lanthanides in the bony tissue.

Because all concentrations tested in this experiment proved to be toxic, it was decided that much lower concentrations would be used for future trials. A 10-day trial was conducted to compare the mortality rates of coho fry and alevins in static tanks containing cerium at concentrations of 0, 0.02, 0.2 and 2 μg/ml. All of the fry and alevins in the tanks with 2 μg/ml died within 24 hours. After 4 days, all of the alevins and only 1 fry were dead in the tank containing 0.2 μg/ml. At the end of the 10-day trial, 4 fry were still alive in the 0.2 μg/ml tank and 5 fry and 2 alevins were still alive in the 0.02 μg/ml tank. Since the alevins were more sensitive than the fry to cerium at these concentrations, fry were chosen for subsequent investigations.

EXPERIMENT 2 - TOXICITY STUDY USING STEELHEAD (*Salmo gairdneri*) ALEVINS TREATED WITH LANTHANUM AND SAMARIUM

Introduction

Experiment 1 showed that coho alevins were sensitive to the lanthanide elements. As a result of the toxic effects observed, the alevin stage was determined to be unsuitable for these marking studies. However, further investigation into the toxic effects of these elements on a different species of salmonid would be useful.

This experiment was designed to investigate the sensitivity of steelhead alevins to lanthanum and samarium at concentrations of 0, 10 and 100 µg/l in the water supply. The concentrations employed in this study were markedly lower than those used in Experiment 1.

Materials and Methods

Experimental Design

This alevin study was carried out concurrently with the third coho fry labelling study (Experiment 5). The design involved a factorial arrangement of two lanthanides each at three concentrations. The treatments used were lower than those of Experiment 1 and consisted of 0, 10 or 100 µg/l of lanthanum or samarium. There were 2 replicates of each treatment. Twenty steelhead (*Salmo gairdneri*) alevins were placed in mesh containers in each of 10 experimental tanks containing 100 coho

fry in July, 1989. The tanks were set up with flow-through systems.

At the start of the experiment, the alevin had absorbed the majority of their yolk sacs and, they had 552 accumulated thermal units (ATUs). Dead alevins were removed and recorded on a daily basis. Because this was a toxicity study, no samples were taken for analysis by ICP-MS.

Results

Mortalities

All steelhead alevins in the tanks containing lanthanum at 100 µg/l died within 8 days, and all those in tanks containing samarium at 100 µg/l died within 19 days (Table 12). No mortalities were observed in any other tanks during this period. Dead alevins were found in an arched position with months open, a congealed yolk sac, and a white colouring.

Table 12. Steelhead alevin mortalities observed, in tanks each initially containing 20 alevins, during 19-day exposure to lanthanum and samarium at 100 µg/l.

Time[1]	La 100 µg/l (Tank 1)	La 100 µg/l (Tank 2)	Sm 100 µg/l (Tank 1)	Sm 100 µg/l (Tank 2)
5	15	18	0	0
6	3	2	0	0
7	1	-	7	7
8	1	-	4	3
9	-	-	6	6
10	-	-	2	2
19	-	-	1	2
Total	20	20	20	20

1 Time = number of days after commencement of treatments.

Discussion

Similar to the results of the first experiment with coho alevins, steelhead alevins were found to be sensitive to the lanthanide elements. The steelhead alevins did not survive at lanthanum or samarium concentrations of 100 μg/l in the water supply. However, no mortalities were observed in the tanks containing 0 and 10 μg/l of lanthanum or samarium.

The alevin in the tanks containing lanthanum at 100 μg/l died first, indicating that they were more sensitive to lanthanum than samarium. Although both lanthanum and samarium are light lanthanides, the toxic effects of lanthanum was more immediate than those of samarium. Lanthanum is a lighter element than samarium and the La^{3+} ions are similar to the Ca^{2+} ions. From this, it would appear that the elements are actively taken up from the water supply as the Ca^{2+} ions are. The increased toxicity of the lighter lanthanides was also observed in the alevins in Experiment 1. The causes of mortality were not investigated.

The sensitivity of both coho and steelhead alevins to the lanthanides observed in these two experiments suggest that a later stage (fry or smolts) might be better alternatives to pursue in these mass marking studies.

EXPERIMENT 3 - INVESTIGATION USING COHO (*Oncorhynchus kisutch*) FRY IN STATIC TANKS CONTAINING LANTHANUM IN A RANGE OF CONCENTRATIONS

Introduction

The first experiment using coho alevins (Experiment 1) showed that the alevin stage was unsuitable, at least at the concentrations employed, for this type of mass marking study. Also, the 10-day cerium trial using coho alevins and fry showed that the fry were less sensitive than the alevins. Consequently, coho fry were chosen for this investigation.

Because the lanthanide elements are not absorbed from the gastro-intestinal tract (Ellis, 1968; Luckey & Venugopal, 1977; and Kennelly *et al.*, 1980), incorporation of the lanthanides into the water supply was tested, to explore the possibility of absorption through the gills or other tissues. For this experiment one element, lanthanum, was introduced into the water supply with different groups of fish exposed to varying concentrations. The levels tested were between 2 and 1000 µg/l. After the labelling was complete, whole body analysis by ICP-MS was carried out on individual fry to determine the amount of element accumulated.

There were two main objectives for this experiment. The first was to determine if the concentrations of lanthanum in the water supply were toxic to the fry. The second objective was to

ascertain whether lanthanum was taken up and subsequently incorporated into the body of the fry in detectable amounts.

Materials and Methods

Experimental Design

Fifteen coho (*Oncorhynchus kisutch*) fry were placed in each of 5 experimental tanks containing lanthanum concentrations of 0, 2, 10, 200, and 1000 µg/l for a 3-week period in March, 1989. Because this was a preliminary experiment, there were no replicates for any of the treatments. For each treatment group, a measured amount of a 20 µg/ml aqueous lanthanum acetate stock solution was added to each of the tanks containing 10 litres of ambient river water to provide the target concentrations. Once the lanthanum was added to the tanks, no water changes were made and no additional element was added. The fry were obtained from Capilano River Hatchery brood stock and were not fed for the duration of the experiment to maintain the ammonia levels in the tank water at a minimum. The experiment was located in a gallery under the hatchery rearing ponds which helped maintain the water temperature at approximately 10°C.

At the conclusion of the 3-week chemical labelling period, the fry were transferred to a flow-through system and provided with untreated river water for the rinse period. Two different durations were used for the rinse period: one week, and two weeks.

Sampling and Analytical Method

The coho fry were sampled at the end of each of the two rinse periods. Seven fry were randomly sampled from each tank for the first set of analyses, while the remaining fry were maintained for the second set of analyses.

The fry were killed by asphyxiation and wet weights were recorded. After overnight oven-drying, they were weighed again and whole individuals were placed in sample vials. They were then analyzed by ICP-MS for whole body lanthanum content.

Results

Mortalities

After two days, all the fish in the 1000 µg/l lanthanum treatment tank were dead. However, there was only one other mortality, this occurring in the 200 µg/l treatment tank. All of the other fry survived the 3-week treatment period and the 2-week rinse period. Although several of the fry were very thin, as a result of the feed being withheld, they survived the treatment and were used as analytical samples.

Water Analysis

Water from each of the tanks was analyzed for lanthanum concentration at the beginning of the experiment and 9 days after the start of the experiment. The results obtained were consistently lower than the theoretical levels (Table 13). The lanthanum concentrations of the water samples taken at the start of the experiment were greater than the concentrations 9 days later.

Table 13. Theoretical lanthanum concentrations, amount of lanthanum added to each tank, and the measured concentrations for the two sample dates.

Tank	Theoretical Concentration	20 µg La/ml S.S.[1] added	Initial [La]	[La] After 9 Days
1	2 µg/l La	1 ml	1.6 µg/l	1.3 µg/l
2	10 µg/l La	5 ml	8.3 µg/l	4.9 µg/l
3	200 µg/l La	100 ml	150.0 µg/l	116.0 µg/l
4	1000 µg/l La	500 ml	816.0 µg/l	n/a[2]
5	0 µg/l La	0 ml	<0.01 µg/l	<0.01 µg/l

1 SS = concentrated lanthanum acetate stock solution.
2 n/a = not analyzed.

Whole Fry Analysis

Analysis of whole fry treated with lanthanum showed a definite accumulation of element, whereas undetectable amounts of element were present in the untreated fry (Figure 6). No fry from the 1000 µg/l treatment group were analyzed as they had all died after only two days of treatment. There was a large increase in the amount of lanthanum found in the fry treated with lanthanum at 200 µg/l compared to those treated with lanthanum at 2 or 10 µg/l after both the 1-week and the 2-week rinse periods (Figure 6). Results for the fry analyzed after the 2-week rinse period were slightly lower than the values obtained for the 1-week rinse period.

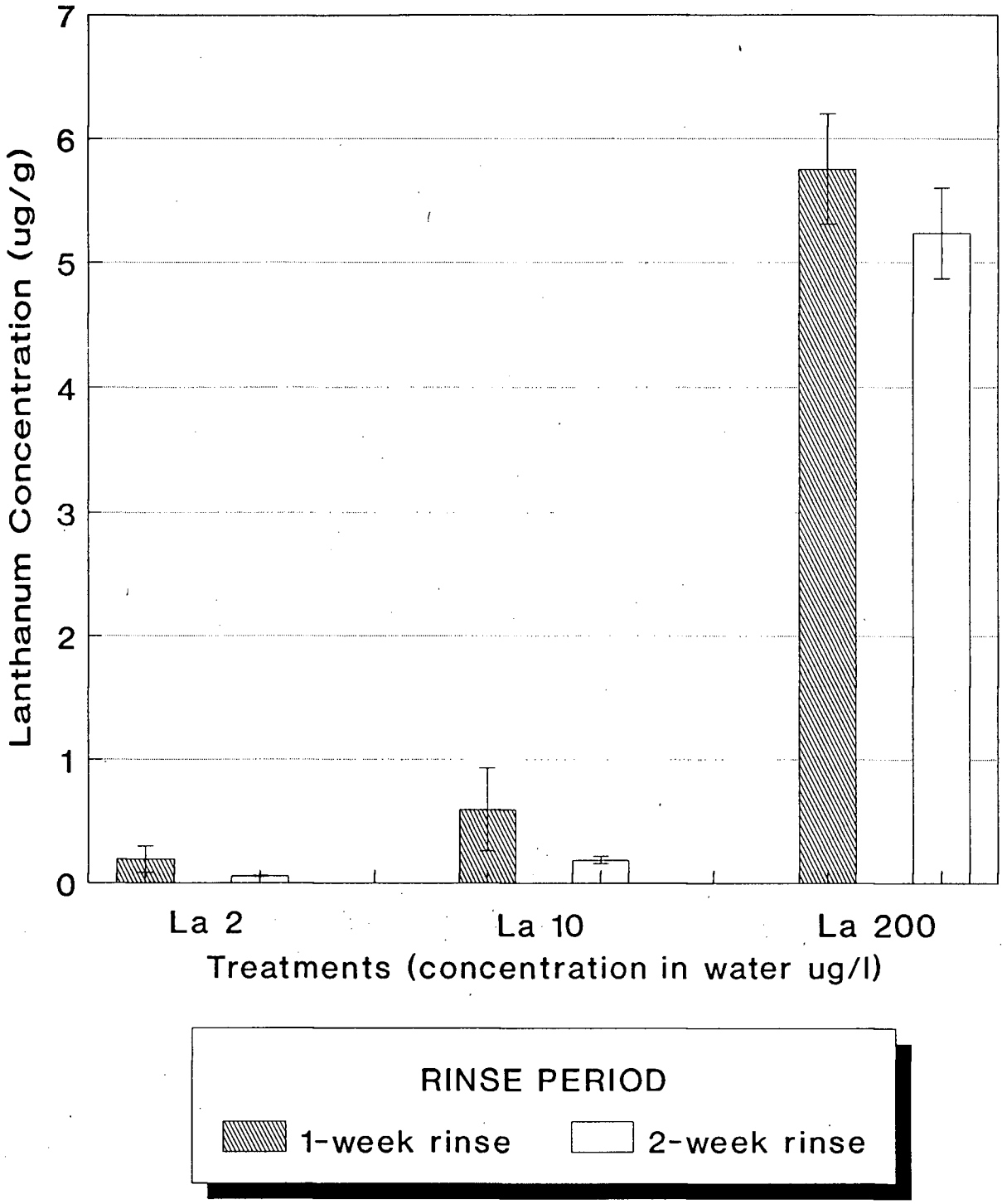

Figure 6. Lanthanum concentration in the whole body of coho fry following a 3-week exposure of lanthanum at 2, 10, and 200 µg/l and a 1-week or 2-week rinse period. Undetectable lanthanum in control tank. Results are reported as mean ± S.E. space in µg of La per g of dry tissue.

Discussion

Introduction of lanthanum through the water supply appears to be a suitable method for marking coho fry. The element is absorbed from the water and incorporated into the body of the fish. Since the rinse periods were of sufficient length for mucus regeneration, the concentrations measured represented amounts of lanthanum accumulated in the fry, not the amounts adsorbed onto the fry.

Some of the anomalies found in the whole body analysis could have resulted from the starved condition of the fry, which could have been associated with a low mucus turnover.

Only the 1000 μg/l concentration was fatally toxic. Since there was only one mortality in the 200 μg/l treatment group, it was considered that this may have been near the upper lethal level. As there were no mortalities observed in the tanks containing 2 and 10 μg/l of lanthanum, hish ammonia levels did not appear to be a factor in the mortalities.

The lowest concentrations, 2 and 10 μg/l, did not result in a sufficient elemental deposition in the whole body. However, the 200 μg/l level did result in a very high element deposition. Considering these results, in combination with toxicity concerns, it was decided that 100 μg/l would be used for future experiments.

The analysis of water samples consistently yielded lower values than those which were expected. The water lanthanum concentrations in the tanks also decreased with time. There are

at least three possible explanations for these low results. First, there may have been an adherance effect whereby the element formed insoluble hydroxides which adhered to the sides of the tanks and to the sides of the sample vials (Luckey & Venugopal, 1977). The second may relate to the static nature of the tank set-ups which would decrease water levels if uptake were occurring. Since the element is not being replaced as the fry absorb the lanthanum from the water supply, a decrease in lanthanum concentration over time would be anticipated. Finally, the company that did the lanthanum analyses had a problem with the secondary standards which were used to calibrate the ICP-MS. The containers that the standards were stored in were not air-tight and the solutions were evaporating over time, thus resulting in low reported values. Since there is no correction factor that can accurately be used for these results, the concentrations reported are only approximate values. The problem would have reduced the reported values for the fry samples as well.

The fry in this experiment were kept in static tanks with no fresh supply of water or lanthanum. This method would have to be altered to a flow-through system for hatchery application. This involves the water inflow to be set at a certain rate with a concentrated aqueous lanthanide stock solution added at a constant rate. This would allow the constant replenishment of element taken up by the fish and would provide suitable water quality to ensure production of high quality juvenile salmon.

Although analyses of whole fry indicated that lanthanum was incorporated into the fry, they did not provide any information on distribution of lanthanum into the individual tissues and organs. Several researchers have described these elements as bone seekers (Durbin *et al.*, 1956; Jowsey *et al.*, 1958; Kyker, 1961; Michibata, 1981; and Michibata & Hori, 1981). Consequently, the next experiment included the analysis of the vertebral column, the otoliths and the scales to determine deposition site.

EXPERIMENT 4 - INVESTIGATION OF COHO (*Oncorhynchus kisutch*) FRY IN A FLOW THROUGH TANK CONTAINING LANTHANUM

Introduction

The previous experiment in which coho fry, maintained in tanks containing static water, were exposed to varying concentrations of lanthanum demonstrated that it is feasible to mark fry using this method. One lanthanide element, lanthanum, was shown to accumulate in the whole body of coho fry. This element was not fatally toxic at concentrations below 200 μg/l during the 3-week period.

The experiment was designed to investigate the uptake of lanthanum by coho fry in a flow-through system with a constant concentration of lanthanum. The distribution of lanthanum into the vertebral column, otoliths and scales was analyzed. Also, the retention of lanthanum in the vertebral columns was measured over a 2-month period.

The following experiment was carried out using a flow-through system to label the fry, with ambient river water flowing into the tank at a set rate while the aqueous lanthanum acetate stock solution dripped in at a constant rate. This method follows standard fish culture procedures more closely. The water quality could be maintained at a high level and the fish could be fed in a normal manner. This procedure maintained the lanthanum concentration at a more constant rate, independent of uptake by fry.

Materials and Methods

Experimental Design

One hundred coho (*Oncorhynchus kisutch*) fry were placed into each of 2 experimental tanks containing lanthanum at concentrations of 0 and 100 µg/l for 3 weeks in April, 1989. Ambient river water flowed into the tanks at a constant rate of one litre per minute. The concentrated lanthanum acetate solution was added directly to the lanthanum treatment tank at a constant rate of 0.7 ml/min. The theoretical lanthanum concentration to which the fry were exposed was calculated to be approximately 100 µg/l. The fry were newly ponded with 993 accumulated thermal units (ATUs) and they were fed Oregon Moist Pellets twice daily for the duration of the experiment.

After the 3-week labelling period, the fry were provided with untreated water for a 2-month rinse period with sampling at 18 days and 2 months. During the rinse period, the flow rate of ambient water into the tanks was increased to approximately two litres per minute.

Sampling and Analytical Method

Forty labelled fry were randomly sampled 18 days after the termination of the lanthanum exposure. Thirty of these were analyzed for whole body lanthanum content. Preparation for whole body analysis for lanthanum content was as described in the previous experiment. The other ten fry were used for bony tissue analysis. The vertebral columns, otoliths, and scales

were removed and analyzed for lanthanum content. The vertebral columns and otoliths were removed and prepared for analysis as described in the Methodology section at the beginning of this thesis. Because the scales from such young fry were too small to handle, the scales had to be collected by taking a scrape from the area around the lateral line, after removal of mucus.

Two months after the termination of the lanthanum treatment, ten more fry were sampled. The vertebral columns were removed and analyzed for lanthanum content.

Results

Mortalities

Throughout the 3-week treatment period, there were only two mortalities; one of these fish was extremely emaciated, the other had a relatively normal appearance. During the two-month rinsing period, there was one other mortality. The fry appeared be healthy and were eating normally throughout the experiment.

Water Analysis

Lanthanum concentration, monitored weekly throughout the treatment period ranged from 46.8 to 53.2 μg/l with an average value of 50.9 μg/l. Although these values were considerably lower than the theoretical concentration of 100 μg/l, they were very consistent throughout the treatment period (Figure 7).

Whole Fry Analysis

The analysis of the thirty whole fry sampled 18 days after removal of treatment further demonstrated that fish do accumulate lanthanum in their body. The results obtained showed low variation between individuals. Eighteen days after the 3-week labelling period the lanthanum concentration in the whole body was 0.86 $\pm$ 0.02 μg/g (mean $\pm$ S.E.).

Bony Tissue Analysis

The analysis of different bony tissues produced some promising and interesting findings. The fry had accumulated concentrations of 0.44 ± 0.02 μg/g in the vertebral columns. The variation between lanthanum concentrations in the individual vertebral columns was low, while the concentrations of element in the scales and otoliths were more variable, with concentrations of 2.08 ± 0.93 μg/g in scales and 0.21 ± 0.07 μg/g in otoliths. Some of the scale scrapings had undetectable amounts of lanthanum present while others had unusually high lanthanum concentrations. The otolith samples showed markedly lower lanthanum concentrations than the vertebral columns; also the results were more variable. Figure 8 shows the concentration of lanthanum accumulated in the various bony tissues.

Analysis of Vertebral Column 2-Months Post Treatment

The vertebral columns from ten treated and five untreated fry were removed and analyzed. The lanthanum-treated fish had large concentrations of lanthanum in their vertebral columns (0.13 ± 0.01 μg/g) compared with untreated fish (<0.01 μg/g). Although there were detectable concentrations of lanthanum in treated fry 2 months after the labelling was completed, the concentration present was markedly lower than the concentration present 18 days after labelling (0.44 ± 0.02 μg/g). This lower value occurred largely as a result of additional calcium being

laid down in the vertebral column. Because no dry weights were taken, the total amount of lanthanum in the vertebral column was not calculated.

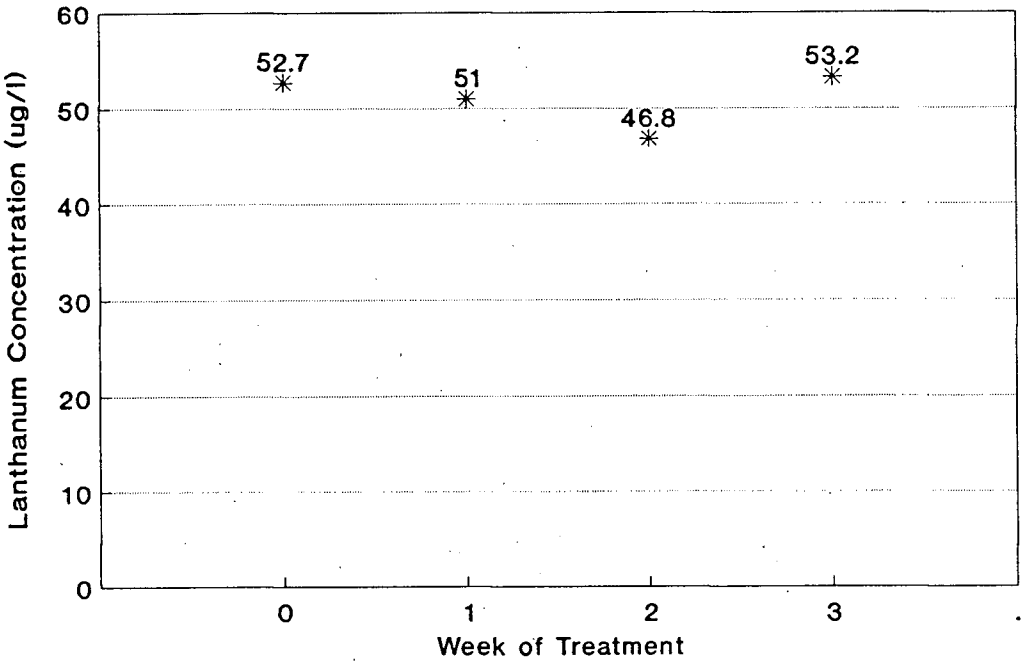

Figure 7. Lanthanum concentrations in the water that coho fry were exposed to over the 3-week treatment period. Lanthanum concentration measured in μg/l.

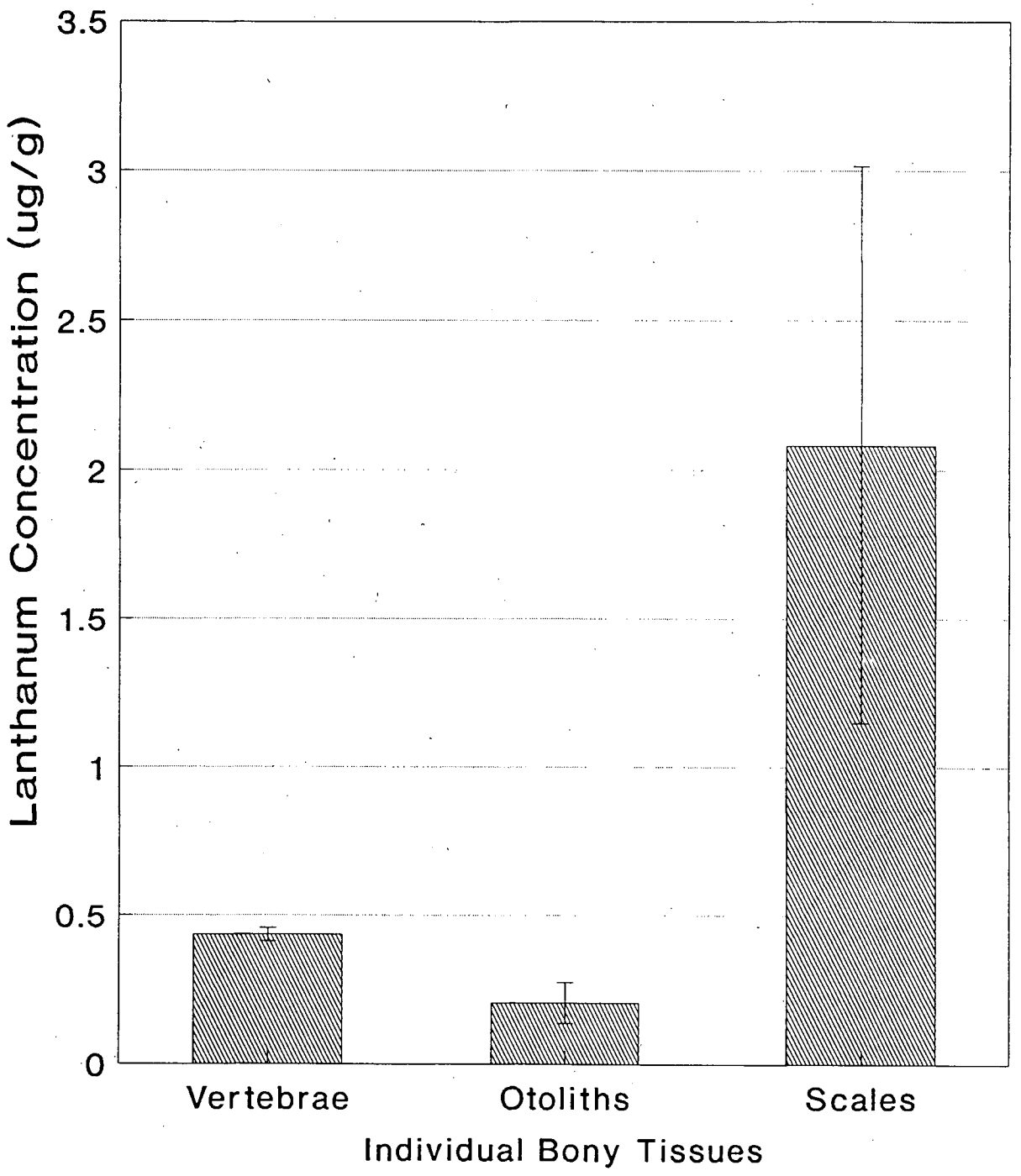

Figure 8. Lanthanum concentrations in the vertebral column, otoliths and scales of coho fry following a 3-week exposure to 100 μg/l La and an 18-day rinse period. Results reported represent the mean ± S.E. in μg of La per g of dry bony tissue.

Discussion

As demonstrated in experiment 3, the fry exposed to the lanthanum at approximately 100 µg/l in the water supply accumulated the element in detectable concentrations. This was shown through a series of different types of samples taken from the labelled fry. There was little variation between the individual fry in lanthanum incorporation into the body tissues.

The accumulation of lanthanum in the various bony tissues supports the findings of Durbin *et al.* (1956), namely, that these elements are accumulated in bony tissues. The vertebral column is a suitable depository of lanthanum, as it incorporated at detectable concentrations with minimal variation between individuals, in the fry stage.

The otoliths also contained detectable concentrations of lanthanum. However, the amounts present were less than those found in the vertebral column and they were more variable. One of the values obtained (38.2 µg/g) was unusually high compared with the other results, which ranged from 0.02 to 0.62 µg/g. This sample may have been contaminated during the sample preparation or analytical procedures. Therefore this value was discarded from the statistical calculations. Another possible problem with the analysis of the otolith samples could have been the relatively small amount of material which was analyzed. In fish of this size, the otoliths weighed approximately 0.2 mg.

The scale scrapings showed relatively high concentrations of lanthanum compared with the vertebral columns and otoliths.

Variation between individual samples was high. Since the fry were wiped clean of mucus prior to the scale scrapings, the high observed variation could not have occurred as a result of lanthanum adhering to the mucus or scales. In addition, the rinse period was sufficiently long to allow for complete regeneration of the mucus. A likely explanation for the high variation between samples is the variation in sample mass. Since the scales were of such a small size, they were just scraped from the lateral line area. No sample weight was taken. Therefore it was impossible to know how many scales were present in each sample. A more reliable method for collecting scales would need to be used for more accurate results, but with fry of this size no satisfactory method was apparent.

From the results of the analysis of various bony tissues, it is apparent that the vertebral column is the most suitable tissue for sampling, because of its larger size, high concentration of lanthanum and low likelihood of contamination.

The lanthanum was still present in detectable concentrations in the vertebral column after 2 months. The total amount of lanthanum in the vertebral column is unknown since the sample weights were not taken. The lanthanum concentration decreased over time due to the dilution caused by additional calcium being laid down. A longer term study would have to be carried out to determine how long the lanthanum would remain in the vertebral column in detectable concentrations.

The water samples taken showed lower concentrations of lanthanum present in the tank water than those which were expected. These results were consistent with the results of Experiment 2. As discussed previously, the plating out theory and the problems with the secondary standards for ICP-MS are both possible explanations for these low values. Because of these analytical problems, concentrations reported are only approximate values.

Problems with the analysis of the bony tissue also existed. Since there was no "expected" concentration, these problems were harder to detect. The problems with the secondary standards cast doubt on the values reported for the tissue samples. However, as a result of this error, the marks laid down in these tissues would have actually been higher than those reported in this thesis.

This experiment has again demonstrated that lanthanum can be used as a chemical marker. Lanthanum is taken up from the rearing water and is subsequently deposited in the fish. The analysis of the vertebral column, otoliths, and scale scrapings indicated that lanthanum was deposited into these bony tissues in varying amounts. Furthermore, the results obtained indicate that storage of lanthanum persists in the bony tissues for at least 2 months.

Of the three bony tissues originally analyzed, the vertebral column appeared to be the one best suited for further

research. However, the scales and otoliths should also be considered further.

EXPERIMENT 5 - THE TREATMENT OF COHO (*Oncorhynchus kisutch*) FRY WITH LANTHANUM AND SAMARIUM AT THREE CONCENTRATIONS FOR 3 AND 6 WEEKS

Introduction

In Experiment 4, in which coho fry were exposed to lanthanum in a flow-through system, lanthanum was incorporated into the vertebral column, otoliths and scales. The element was still present in detectable concentrations in the vertebral column 2 months after the conclusion of the treatment. Also the vertebral column appeared to be the best suited bony tissue for continued research.

This experiment was designed to investigate the effect of varying treatment concentrations and durations of treatment on the uptake and retention of lanthanum and samarium over a 10½-month post-treatment period. The elements were introduced into the water at three concentrations (0, 10, and 100 μg/l) for 3-week and 6-week durations in a flow-through system.

Throughout the 10½-month post-treatment growth period, the fry were sampled and the vertebral columns analyzed for lanthanide content every 2 months. Otolith samples were taken and analyzed for lanthanide content 2 weeks and 10½ months after termination of labelling. Scale samples were taken 10½ months after termination of labelling. Fry weights were also recorded to investigate possible effects of the lanthanides on growth.

Materials and Methods

Experimental Design

The design involved a factorial arrangement of lanthanum and samarium concentrations (3), durations of exposure (2), and time of sampling (6). One hundred coho fry were placed in each of 10 experimental tanks containing 0, 10 and 100 µg/l lanthanum or samarium in June, 1989. The fry were exposed to the lanthanides for 3-week and 6-week durations. There were 2 replications of each of the treatments.

The concentrated lanthanum and samarium acetate stock solutions for the 10 µg/l treatments were prepared with 0.36 g of lanthanum acetate (99.9% purity) or 0.31 g of samarium acetate (99.9% purity) dissolved in 10 l of ambient river water. For the 100 µg/l treatments, 3.56 g of lanthanum acetate or 3.14 g samarium acetate were used (Appendix 3). These concentrated stock solutions were metered into the corresponding treatment tanks at a rate of 0.7 ml/min and were replenished every 10 days. The rearing water flowed in at one litre per minute and the concentrated lanthanide stock solution and river water became mixed in a funnel before being delivered to the tank. Two groups of fry served as negative controls with no lanthanum or samarium introduced to the water supply.

During the first replenishment of stock solution, two of the samarium treatments were inadvertently reversed. This resulted in one group of fry being exposed to samarium at 10 µg/l for 10 days then 100 µg/l for 11 days, with the opposite

reversal occurring with the other group of fry. This also affected the 6-week treatment - samarium at 10 μg/l for 10 days then 100 μg/l for 32 days and visa versa (Table 14).

Table 14. Theoretical lanthanum and samarium treatment concentrations.

3 Week Duration		6 Week Duration	
# Tanks	Treatment	# Tanks	Treatment
2	Control	2	Control
2	La 10 μg/l	2	La 10 μg/l
2	La 100 μg/l	2	La 100 μg/l
1	Sm 10 μg/l	1	Sm 10 μg/l
1	Sm 100 μg/l	1	Sm 100 μg/l
1	Sm 10/100 μg/l*	1	Sm 10/100 μg/l+
1	Sm 100/10 μg/l**	1	Sm 100/10 μg/l++

* 10 μg/l for 10 days, 100 μg/l for 11 days.
** 100 μg/l for 10 days, 10 μg/l for 11 days.
\+ 10 μg/l for 10 days, 100 μg/l for 32 days.
++ 100 μg/l for 10 days, 10 μg/l for 32 days.

The Capilano brood stock (1988) coho fry were ponded approximately 3 months prior to the start of the experiment and, had accumulated 1,365 ATUs at the start of the treatments. The fry weight ranged from 0.51 to 2.89 g with an average of 1.63 ± 0.08 g (mean ± S.E.). During the treatment period, the fry were fed Oregon Moist Pellets twice daily. These diets were analyzed for element content by ICP-MS and found to have undetectable amounts of lanthanides present (Appendix 4). The ambient temperature of the water increased steadily throughout the

labelling period from 7°C to 10°C. Water chemistry data for the ambient river water are given in Appendix 5.

After the 3-week labelling period, 50 fry were removed from each tank and placed in segregated mini-troughs for the grow-out period in the gallery under the hatchery rearing ponds. The remaining fry undergoing the 6-week labelling period in the treatment tanks had continued exposure to the lanthanides for an additional 3 weeks, then were transferred to the mini-troughs. These troughs were divided into four separate 35 litre sections with ambient untreated river water flowing through them at approximately 25 l/min.

The fry were maintained at the Capilano Hatchery for 1 year in fresh untreated water. The fish were fed Oregon Moist Pellets daily and tanks maintained following to normal hatchery procedures. At the end of this 1-year period, 10 of the remaining fish were randomly sampled from 8 of the treatment groups, fin-clipped, and transferred to a fresh water tank at the Department of Fisheries and Oceans West Vancouver Laboratory in June, 1990 (Table 15). After a 3-week acclimation period, each smolt received a 0.01 ml interperitoneal vaccination against vibriosis (*Vibrio anguillarum* and *V. ordalii*), furunculosis (*Aeromonas salmonicida*), and enteric redmouth (*Yersinia ruckerii*). The vaccine used was Ermogen-Furogen-Vibrogen bacterin supplied by Aquahealth Inc. Two weeks later, the fish were gradually transferred to sea water - 33% for 3 days, then 66% for 3 days, then full strength sea water (29-31

g/l). These smolts were to have been maintained for 1 year in sea water, then sampled and analyzed for lanthanide content in July, 1991. Due to an error in aquarium management, these fish were discarded without sampling 10 months after introduction to sea water. Unfortunately, interim samples were not taken because of small numbers of fish.

Table 15. Labelled coho smolts transferred to the Department of Fisheries and Oceans West Vancouver Laboratory sea water tank and the fin-clips used to identify treatment groups.

Treatment	Duration	# Fry	Fin-Clip
Control	6 Weeks	10	Right Ventral
La 100 µg/l	3 Weeks	20	None
La 100 µg/l	6 Weeks	20	Adipose
Sm 100 µg/l	3 Weeks	10	Left Ventral + Adipose
Sm 100 µg/l	6 Weeks	10	Left Ventral
Sm 10/100 µg/l	6 Weeks	10	Right Ventral + Adipose

Sampling and Analytical Method

The initial sample set was taken 2 weeks after termination of the lanthanide exposures and subsequent samples were taken every 2 months for a total of 6 sample times throughout the 10½-month freshwater growth period. For each of the samples sets, 5 fry were randomly sampled from each treatment group for a total of 30 fry per treatment group over the 10½ months.

Wet weights were recorded, and bony tissue samples were taken and prepared for ICP-MS analysis as described in the Methodology section. Vertebral columns were removed from all of the treatment groups at each of the 6 sampling times. Otoliths were taken from all treatment groups at the initial and final sampling times only. Scales were removed from fry treated with lanthanum and samarium at 0 and 100 μg/l for a 6-week period at the final sample time only. All bony tissues were analyzed for lanthanum and samarium content by ICP-MS.

Water samples were taken from each of the treatment tanks at the start of the experiment and then once a week for the duration of the labelling. For each sample, 10 ml were collected, stabilized with 3 drops of concentrated HCl and delivered for ICP-MS analysis on the same day.

Three sets of spiked vertebrae standards and three sets of spiked water standards were prepared and analyzed as described in the Methodology section. Vertebrae standards were analyzed at the same time as the last three treated vertebrae sample sets only. Water standards were prepared fresh as needed using the

same reagents and distilled water and were analyzed concurrently with alternate weekly water samples taken from the treatment tanks.

Statistical Analysis

All results were analyzed using analysis of variance using SYSTAT (Wilkinson, 1989), with differences between means tested at $P \leq 0.05$, using Tukey's multiple range test.

Analysis of variance was carried out on the data to determine if there were any significant tank effects. Since none were shown, subsequent analyses were carried out on the pooled data, using individual fish as the experimental units.

The data from the lanthanum and samarium treatments were analyzed separately. The samarium treatments that were reversed were discarded from the statistical analysis because the concentrations and durations of exposure were not uniform. As a result of these discarded groups, only one replicate of samarium treatments was used. The results for the untreated fish had undetectable levels present and no variance, therefore they were not included in the statistical analyses. The data analyzed were: mortalities; growth (fry weight); lanthanide concentration and amount in the vertebral column; and lanthanide concentration in the otoliths and scales.

Results

Mortalities

The mortalities for each treatment during the labelling period and during the 10½-month growth period are provided in Table 16. There was no significant difference in the number of mortalities between the groups treated for 3 weeks and those treated for 6 weeks. However, there were significantly more mortalities in the treatment tanks containing lanthanum at 100 µg/l during the treatment period than in all other treatment tanks. Throughout the 10½-month growth period, there were only 25 mortalities, with no clear effect of treatment in evidence (Appendix 6).

Table 16. Coho fry mortalities during the treatment period and during the growth period in tanks containing lanthanum and samarium treatments.

Treatment	Treatment Period Mortalities	Grow-out Period Mortalities
Control	2%[a]	1%[a]
La 10µg/l	1%[a]	13%[b]
La 100µg/l	11%[b]	4%[a]
Control	2%[a]	1%[a]
Sm 10µg/l	0%[a]	6%[a]
Sm 100µg/l	0%[a]	0%[a]

Error mean square for lanthanum treatment period = 0.779, total observations (n) = 14; error mean square for lanthanum growth period = 0.526, total observations (n) = 18.

Total observations (n) for samarium treatment period = 2; total observations (n) for samarium growth period = 7.

a, b. Within comparison group, values with unlike superscript letter were significantly ($P \leq 0.05$) different according to Tukey's test.

Growth

Mean weights of fish and significant effects for the 10½ months after discontinuation of the 3-week and 6-week treatments are shown in Tables 17 & 18.

There was no significant effect of treatment or duration on growth (Appendix 7). However, there were significant increases in weights between the initial sample set measured 2 weeks post-treatment and the subsequent sample sets in fish treated with both the lanthanum and with the samarium. In the lanthanum-treated fish, there was a significant increase in weights at the final weighing.

Because the weighing of fish treated for 6 weeks was always done 3 weeks later than the weighing of the fish treated for 3 weeks, comparison of weights between fish treated for each of the two durations was not considered meaningful. The growth rate of the fish treated for 3 weeks was compared with the growth rate of the fish treated for 6 weeks using linear regression analysis. This comparison showed the growth rates between the two groups not to be significantly different.

Table 17. Mean body weights of fry labelled with lanthanum showing the significant effects of sampling time and duration of lanthanum exposures.

		Dur x Time	
	Means	Dur 1	Dur 2
Sample Time			
1	3.62[a]	3.06[a]	4.19[a]
2	7.27[b]	7.17[b]	7.38[bc]
3	7.42[b]	7.94[bcd]	6.90[b]
4	8.05[b]	8.59[bcde]	7.52[bc]
5	8.25[b]	8.92[cde]	7.57[bcd]
6	9.56[c]	9.25[de]	9.87[e]

Overall mean weight = 7.41 g.
Starting weight of fry = 1.63 g.
Error Mean Square = 3.883; total observations (n) = 360.
a, b, c, d, e. Within comparison groups (enclosed by a dashed line), mean values without a common superscript letter were significantly ($P \leq 0.05$) different according to Tukey's test.
Treatment x Duration interaction had no significant differences between means.
Duration 1 = 3 weeks; duration 2 = 6 weeks.
Sample Time 1 = 2 weeks post-treatment; subsequent sample times every 2 months.

Table 18. Mean body weights of fry labelled with samarium showing the significant effects of sampling time and duration of samarium exposures.

		Dur x Time	
	Means	Dur 1	Dur 2
Sample Time			
1	3.45[a]	2.96[a]	3.93[a]
2	7.44[b]	6.72[b]	8.16[b]
3	7.80[b]	8.20[b]	7.41[b]
4	8.16[b]	8.38[b]	7.94[b]
5	7.59[b]	7.53[b]	7.65[b]
6	8.53[b]	8.38[b]	8.69[b]

Overall mean weight = 7.16 g.
Starting weight of fry = 1.63 g.
Error Mean Square = 2.902; total observations (n) = 180.
a, b. Within comparison groups (enclosed by a dashed line), mean values without a common superscript letter were significantly ($P \leq 0.05$) different according to Tukey's test.
Treatment x Duration interaction had no significant differences between means.
Duration 1 = 3 weeks; duration 2 = 6 weeks.
Sample Time 1 = 2 weeks post-treatment; subsequent sample times every 2 months.

Downstream Contamination

Untreated fry placed in tanks receiving the effluent from labelled fry for 2 weeks did not accumulate detectable amounts of element in their vertebral column. The elements do not appear to be "washed off" treated fry and do not contaminate other fry placed directly downstream.

Water samples taken during the 6-week labelling period at 4 different locations along the Capilano River had negligible amounts of lanthanum and samarium present (Table 19). The flow of the river and the flow of the hatchery effluent appeared to dilute the lanthanides in the treatment water to nearly undetectable levels.

Table 19. Concentrations of lanthanum and samarium present in the Capilano River at 4 different locations downstream during the treatment period.

Distance Downstream	La (μg/l)	Sm (μg/l)
10 metres	0.09	<0.03
1 kilometre	<0.04	<0.03
2½ kilometres	0.07	<0.04
3½ kilometres	0.09	<0.02

Water Analysis

The tanks containing lanthanum at the theoretical concentration of 10 μg/l had mean concentrations of 2.6 to 9.5 μg/l, and the tanks containing lanthanum at the theoretical

concentration of 100 μg/l had mean concentrations of 36.9 to 69.7 μg/l (Figure 9). Agreement between duplicates was high.

The tanks containing samarium at the theoretical concentration of 10 μg/l had mean concentrations of 2.7 to 7.2 μg/l, and the tanks containing samarium at the theoretical concentration of 100 μg/l had mean concentrations of 24.7 to 70.1 μg/l. The reversal of the samarium treatments was reflected in the water concentration values (Figure 10).

These measured lanthanide concentrations were consistently lower than the theoretical concentrations, but varied only slightly from week to week. In the tanks containing the highest concentrations of lanthanides the values were lowest for the first two weeks and increased thereafter until the third week when they levelled off. Values for the tanks containing the lowest treatment concentrations remained relatively constant throughout the 6-week period.

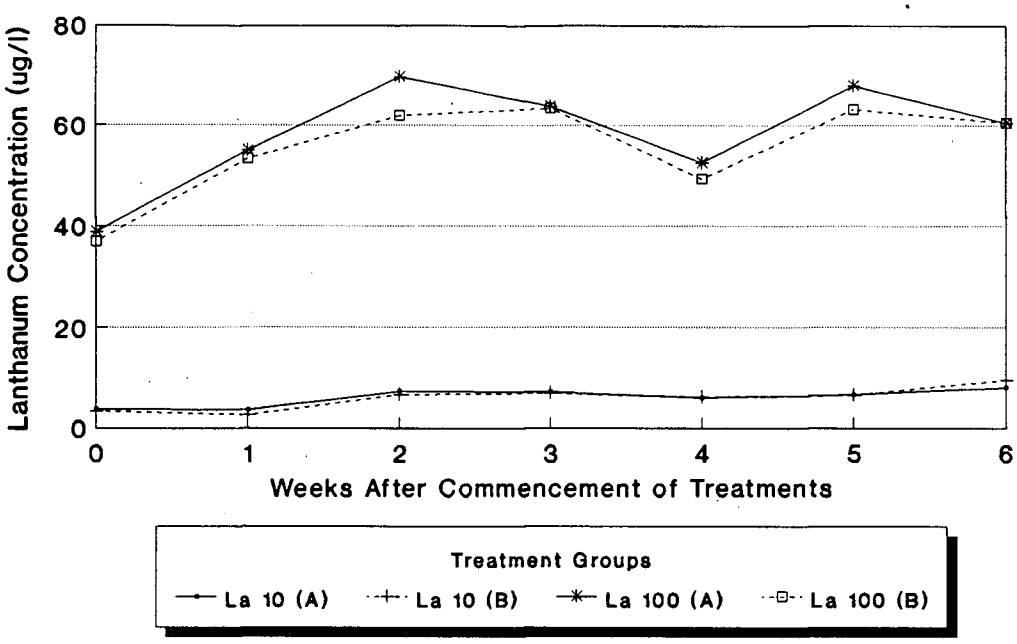

Figure 9. Lanthanum concentrations (μg/l) in the water that coho fry were exposed to over the 6-week treatment period. Undetectable lanthanum in control tanks.

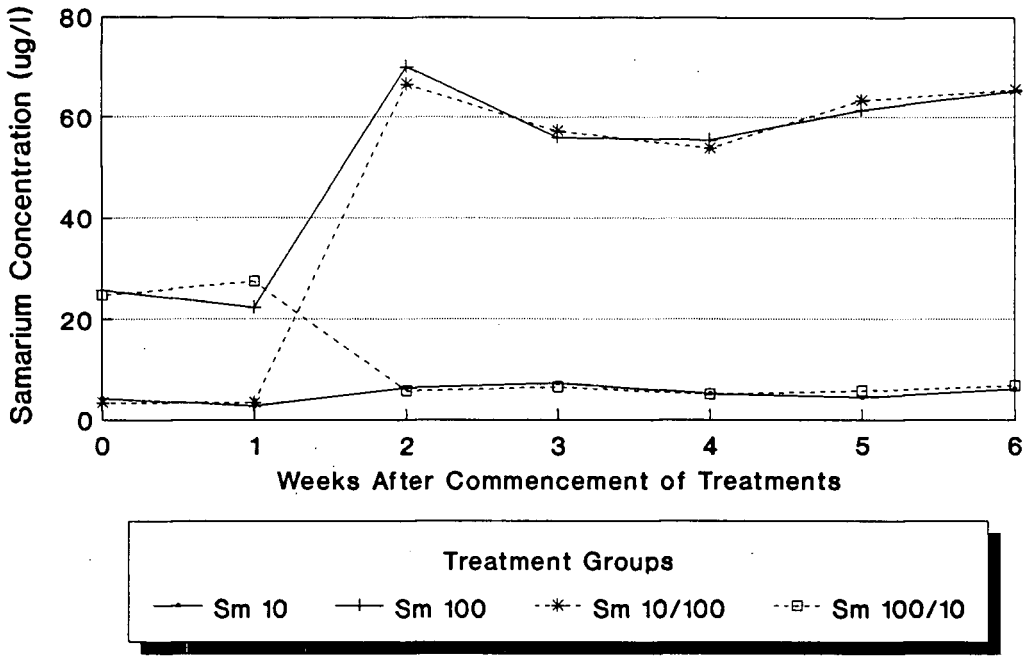

Figure 10. Samarium concentrations (μg/l) in the water that coho fry were exposed to over the 6-week treatment period. Undetectable samarium in control tanks.

Water Standards

The spiked water standards analyzed by ICP-MS had a recovery of approximately 92% and 81% for lanthanum and samarium, respectively (Table 20). These recoveries seemed to be relatively constant between duplicates of the same concentration, but tended to fluctuate between the sets of standards analyzed at different times. The concentration of element present in the standard does not appear to affect the mean recovery rate. However, mean recovery rate of samarium was consistently lower than that of lanthanum.

Table 20. Concentration of lanthanum and samarium in water standards analyzed by ICP-MS.

Time[1]	Calculated Concentration[2]	Measured [La][3]	% La Recovery	Measured [Sm]	% Sm Recovery
2 weeks	10	9.0	90	8.1	81
	50	44	89	40	80
	100	85	85	79	79
Average			88		80
4 weeks	10	9.9	99	8.9	89
	50	49	98	42	84
	100	95	95	84	84
Average			97		86
6 weeks	10	9.7	97	8.2	82
	50	45	90	38	76
	100	88	88	77	77
Average			92		78
Overall Average			92		81

1 Time = number of weeks after commencement of treatments.
2 Concentrations measured in μg/l.
3 All values for duplicates were within 5%.

Vertebrae Standards

Lanthanide recoveries from vertebral columns with known added amounts were low and extremely variable. The lanthanum standards had approximately 77% recovery while the samarium standards had only 49% recovery (Table 21). The lanthanum standards appeared to be more consistent than the samarium standards in terms of percentage recovery. There were fluctuations between the results for the different sample sets, with the recovery varying with the amount of element present. The standards with less lanthanum or samarium had a greater recovery rate than the ones with more element. However, the duplicates within concentrations were reasonably consistent.

Table 21. Concentration of lanthanum and samarium in the vertebrae standards analyzed by ICP-MS.

Time[1]	Calculated Concentration[2]	Measured [La][3]	% La Recovery	Measured [Sm]	% Sm Recovery
6½ months	3	3	100	2	67
	10	6	60	6	60
	20	10	50	11.5	58
Average			70		62
8½ months	3	3	100	2.5	83
	10	7	70	5	50
	20	14	70	9	45
Average			80		59
10½ months	3	4	133	0	0
	10	6	60	7.5	75
	20	9.5	48	7.5	38
Average			80		25
Overall Average			77		49

1 Time = number of months after termination of treatments.
2 Concentration measured in µg/l.
3 All values for duplicates were within 10%.

Vertebral Column Analysis

Analysis of vertebral columns of fry treated with lanthanum or samarium showed the elements to be present, whereas untreated fry had undetectable amounts of element present. The values reported were not corrected for the recovery of the standards, because of the errors associated with the analysis of the standards (previously discussed). A considerably greater number of standards would have had to be prepared and analyzed before enough data would be available to use to correct the values reported. Since the recoveries of the standards were not sufficiently reliable to use to compensate for the results, no

correction was used. The groups treated with lanthanum or samarium at concentrations of 10 μg/l for 3 weeks had undetectable levels present 6½ months after termination of the labelling. The data in Tables 22 and 23 show the significant effects of treatment concentration, durations of exposure, and sampling time on the mean lanthanum and samarium concentrations present in the vertebral columns. ANOVA tables for concentrations and amounts of element in the vertebral column are presented in Appendices 8 and 9.

There were a significantly greater concentrations and amounts of element in fry treated with lanthanum or samarium at concentrations of 100 μg/l for 6 weeks than in all other groups. The concentration of element decreased as time progressed. The group exposed to lanthanum at 100 μg/l for 6 weeks had 0.93 μg of La/g of dry vertebrae tissue 2 weeks post-treatment, but the concentration was only 0.32 μg/g 10½ months post-treatment. All other treatment groups showed similar, steady declines in the concentration of element in their vertebral columns (Tables 22 and 23).

Tables 24 and 25 show the same effects of treatment concentration, durations of exposure, and sampling time on the mean amounts of lanthanum or samarium in the vertebral column of labelled fry. Results similar to those for the concentrations were found in these comparisons. The fish that were exposed to lanthanum at 100 μg/l for 6 weeks had significantly greater amounts of element accumulated in their vertebral columns.

The amount of element in the vertebral columns remained relatively constant throughout the 10½-month growth period. For the fish treated with lanthanum at 100 μg/l for 6 weeks, there was 12.23 ng present 2 weeks post-treatment and 11.83 ng present 10½ months post-treatment. The amount of element present fluctuated only slightly over the period and the average amount in that group over the 10½ months was 14.0 ng. Stable amounts were also observed in all other groups (Tables 24 and 25).

Table 22. Mean lanthanum concentration (μg/g) in vertebral columns of fry labelled with lanthanum showing the significant effects of lanthanum treatment concentration, duration of lanthanum exposures, and sampling time.

Treatment x Duration x Time

	Trt 1		Trt 2	
	Dur 1	Dur 2	Dur 1	Dur 2
Sample Time				
1	0.09^{abc}	0.16^{abcd}	0.47^{efg}	0.93^{h}
2	0.04^{a}	0.12^{abc}	0.22^{bcd}	0.63^{g}
3	0.05^{ab}	0.15^{abc}	0.22^{cd}	0.58^{g}
4	-	0.15^{abc}	0.13^{abc}	0.48^{fg}
5	-	0.10^{abc}	0.15^{abc}	0.41^{ef}
6	-	0.08^{abc}	0.13^{abc}	0.32^{de}
Means	0.06^{a}	0.13^{a}	0.22^{b}	0.56^{b}

	Trt x Time		Dur x Time		
	Trt 1	Trt 2	Dur 1	Dur 2	Means
Sample Time					
1	0.12^{abc}	0.70^{f}	0.28^{ab}	0.54^{c}	0.41^{a}
2	0.08^{a}	0.43^{e}	0.13^{a}	0.38^{ac}	0.25^{ab}
3	0.10^{abc}	0.40^{de}	0.14^{ab}	0.37^{ac}	0.25^{ab}
4	0.15^{abc}	0.31^{cde}	0.13^{ab}	0.32^{abc}	0.25^{ab}
5	0.10^{abc}	0.28^{bcde}	0.15^{ab}	0.25^{ab}	0.22^{b}
6	0.08^{ab}	0.22^{abcd}	0.13^{ab}	0.20^{ab}	0.18^{b}
Means	0.11^{a}	0.39^{b}	0.17^{a}	0.34^{b}	

Overall mean lanthanum concentration = 0.25 μg La/g.
Error Mean Square = 0.009; total observations (n) = 240.
a, b, c, d, e, f, g, h. Within comparison groups (enclosed by a dashed line), mean values without a common superscript letter were significantly ($P \leq 0.05$) different according to Tukey's test.
Duration 1 = 3 weeks; duration 2 = 6 weeks.
Treatment 1 = La at 10 μg/l; treatment 2 = La at 100 μg/l.
Sample Time 1 = 2 weeks post-treatment; subsequent sample times every 2 months.

Table 23. Mean samarium concentration (μg/g) in vertebral columns of fry labelled with samarium showing the significant effects of samarium treatment concentration, duration of samarium exposures, and sampling time.

Treatment x Duration x Time

	Trt 1		Trt 2	
	Dur 1	Dur 2	Dur 1	Dur 2
Sample Time				
1	0.02^{ab}	0.08^{abc}	0.19^{bcd}	0.49^{e}
2	0^{a}	0.05^{ab}	0.10^{abc}	0.31^{d}
3	0.03^{ab}	0.02^{ab}	0.16^{abcd}	0.32^{de}
4	-	0.02^{ab}	0.03^{ab}	0.29^{d}
5	-	0.02^{ab}	0.08^{abc}	0.23^{cd}
6	-	0.02^{ab}	0.08^{abc}	0.22^{cd}
Means	0.02^{a}	0.04^{a}	0.11^{b}	0.31^{b}

	Trt x Time		Dur x Time		
	Trt 1	Trt 2	Dur 1	Dur 2	Means
Sample Time					
1	0.05^{abc}	0.34^{d}	0.11^{ab}	0.29^{b}	0.20^{a}
2	0.02^{a}	0.21^{bcd}	0.05^{ab}	0.18^{ab}	0.12^{a}
3	0.03^{ab}	0.24^{cd}	0.10^{ab}	0.17^{ab}	0.13^{a}
4	0.02^{ab}	0.16^{abc}	0.03^{a}	0.16^{ab}	0.12^{a}
5	0.02^{ab}	0.16^{abc}	0.08^{ab}	0.12^{ab}	0.11^{a}
6	0.02^{ab}	0.15^{abc}	0.08^{ab}	0.12^{ab}	0.11^{a}
Means	0.03^{a}	0.21^{b}	0.08^{a}	0.17^{b}	

Overall mean samarium concentration = 0.12 μg Sm/g.
Error Mean Square = 0.005; total observations (n) = 120.
a, b, c, d, e. Within comparison groups (enclosed by a dashed line), mean values without a common superscript letter were significantly ($P \leq 0.05$) different according to Tukey's test.
Duration 1 = 3 weeks; duration 2 = 6 weeks.
Treatment 1 = Sm at 10 μg/l; treatment 2 = Sm at 100 μg/l.
Sample Time 1 = 2 weeks post-treatment; subsequent sample times every 2 months.

Table 24. Mean amount (ng) of lanthanum in vertebral columns of fry labelled with lanthanum showing the significant effects of lanthanum treatment concentration, duration of lanthanum exposures, and sampling time.

Treatment x Duration x Time

	Trt 1		Trt 2	
	Dur 1	Dur 2	Dur 1	Dur 2
Sample Time				
1	1.35[a]	2.68[ab]	7.81[cd]	12.23[de]
2	0.90[a]	3.84[abc]	4.80[abc]	17.17[f]
3	2.00[a]	4.33[abc]	6.81[bc]	17.24[f]
4	-	4.48[abc]	4.02[abc]	13.40[ef]
5	-	2.68[ab]	4.96[abc]	12.11[de]
6	-	2.88[ab]	4.19[abc]	11.83[de]
Means	1.41[a]	3.48[b]	5.43[c]	14.00[d]

	Trt x Time		Dur x Time		
	Trt 1	Trt 2	Dur 1	Dur 2	Means
Sample Time					
1	2.01[a]	10.02[de]	4.58[ab]	7.45[ab]	6.01[a]
2	2.37[a]	10.99[e]	2.85[a]	10.51[b]	6.68[a]
3	3.17[abc]	12.02[e]	4.40[ab]	10.78[b]	7.59[a]
4	4.48[abcd]	8.71[cde]	4.02[a]	8.94[ab]	7.30[a]
5	2.68[a]	8.54[bcde]	4.96[ab]	7.39[ab]	6.58[a]
6	2.88[ab]	8.01[bcde]	4.19[ab]	7.36[ab]	6.30[a]
Means	2.79[a]	9.71[b]	4.09[a]	8.74[b]	

Overall mean lanthanum amount = 6.25 ng La.
Error Mean Square = 7.369; total observations (n) = 240.
a, b, c, d, e, f. Within comparison groups (enclosed by a dashed line), mean values without a common superscript letter were significantly ($P \leq 0.05$) different according to Tukey's test.
Duration 1 = 3 weeks; duration 2 = 6 weeks.
Treatment 1 = La at 10 µg/l; treatment 2 = La at 100 µg/l.
Sample Time 1 = 2 weeks post-treatment; subsequent sample times every 2 months.

Table 25. Mean amount (ng) of samarium in vertebral columns of fry labelled with samarium showing the significant effects of samarium treatment concentration, duration of samarium exposures, and sampling time.

Treatment x Duration x Time

	Trt 1		Trt 2	
	Dur 1	Dur 2	Dur 1	Dur 2
Sample Time				
1	0.31^{a}	1.43^{ab}	2.60^{ab}	8.73^{cd}
2	0.00^{a}	1.55^{ab}	2.00^{ab}	11.20^{d}
3	1.23^{ab}	0.72^{ab}	4.92^{bc}	11.34^{d}
4	-	0.76^{ab}	1.19^{ab}	10.41^{d}
5	-	0.71^{ab}	2.51^{ab}	7.70^{cd}
6	-	0.86^{ab}	2.64^{ab}	7.26^{cd}
Means	0.52^{a}	1.01^{a}	2.64^{b}	9.44^{b}

	Trt x Time		Dur x Time		
	Trt 1	Trt 2	Dur 1	Dur 2	Means
Sample Time					
1	0.87^{ab}	5.67^{abc}	1.46^{a}	5.08^{a}	3.27^{a}
2	0.78^{ab}	6.60^{bc}	1.00^{a}	6.38^{a}	3.69^{a}
3	0.98^{ab}	8.13^{c}	3.08^{a}	6.03^{a}	4.55^{a}
4	0.76^{ab}	5.80^{bc}	1.19^{a}	5.59^{a}	4.12^{a}
5	0.71^{a}	5.11^{abc}	2.51^{a}	4.21^{a}	3.64^{a}
6	0.86^{ab}	4.95^{abc}	2.64^{a}	4.06^{a}	3.59^{a}
Means	0.84^{a}	6.04^{b}	1.93^{a}	5.22^{b}	

Overall mean samarium amount = 3.44 ng Sm.
Error Mean Square = 2.894; total observations (n) = 120.
a, b, c, d. Within comparison groups (enclosed by a dashed line), mean values without a common superscript letter were significantly ($P \leq 0.05$) different according to Tukey's test.
Duration 1 = 3 weeks; duration 2 = 6 weeks.
Treatment 1 = Sm at 10 µg/l; treatment 2 = Sm at 100 µg/l.
Sample Time 1 = 2 weeks post-treatment; subsequent sample times every 2 months.

Otolith Analysis

The effects of treatment concentration, duration of exposure, and sample time on the mean concentrations of lanthanum and samarium in otoliths (sagittae) of labelled fry are indicated in Tables 26 and 27. Appendix 10 contains the relevant ANOVA tables. The only fry to show any significant accumulation of element were those treated with lanthanum or samarium at 100 μg/l for 6 weeks. In samples taken 10½ months post-treatment, samarium was undetectable in all groups, while lanthanum was detectable, but at a very low level, only in the groups treated with lanthanum at the highest concentration.

Table 26. Mean lanthanum concentration in otoliths of fry labelled with lanthanum showing the significant effects of lanthanum treatment concentration, duration of lanthanum exposures, and sampling time.

Treatment x Duration x Time

	Trt 1		Trt 2	
	Dur 1	Dur 2	Dur 1	Dur 2
Sample Time				
1	0^{a}	0.04^{ab}	0.12^{ab}	0.36^{b}
2	0^{a}	0.01^{a}	0.01^{a}	0.04^{ab}
Means	0^{a}	0.03^{ab}	0.06^{ab}	0.26^{b}

	Trt x Time		Dur x Time		
	Trt 1	Trt 2	Dur 1	Dur 2	Means
Sample Time					
1	0.02^{ab}	0.24^{b}	0.04^{ab}	0.13^{b}	0.09^{a}
2	0.01^{a}	0.02^{ab}	0^{a}	0.02^{ab}	0.01^{b}
Means	0.01^{a}	0.15^{b}	0.02^{a}	0.08^{b}	

Overall mean lanthanum concentration = 0.08 μg La/g dry tissue (relative to calcium).

Error Mean Square = 0.005; total observations (n) = 30 (2 missing values).

a, b. Within comparison groups (enclosed by a dashed line), mean values without a common superscript letter were significantly ($P \leq 0.05$) different according to Tukey's test.

Duration 1 = 3 weeks; duration 2 = 6 weeks.

Treatment 1 = La at 10 μg/l; treatment 2 = La at 100 μg/l.

Sample Time 1 = 2 weeks post-treatment; sample time 2 = 10½ months post-treatment.

Table 27. Mean samarium concentration in otoliths of fry labelled with samarium showing the significant effects of samarium treatment concentration, duration of samarium exposures, and sampling time.

Treatment x Duration x Time

	Trt 1		Trt 2	
	Dur 1	Dur 2	Dur 1	Dur 2
Sample Time				
1	0^a	0^a	0.02^a	0.50^b
2	0.01^a	0^a	0^a	0.01^a
Means	0^a	0^a	0.11^b	0.31^b

	Trt x Time		Dur x Time		
	Trt 1	Trt 2	Dur 1	Dur 2	Means
Sample Time					
1	0^a	0.26^b	0.01^a	0.17^a	0.09^a
2	0^a	0^a	0^a	0^a	0^b
Means	0^a	0.13^b	0^a	0.08^b	

Overall mean samarium concentration = 0.07 μg Sm/g dry tissue (relative to calcium).

Error Mean Square = 0.000; total observations (n) = 16.

a, b. Within comparison groups (enclosed by a dashed line), mean values without a common superscript letter were significantly ($P \leq 0.05$) different according to Tukey's test.

Duration 1 = 3 weeks; duration 2 = 6 weeks.

Treatment 1 = Sm at 10 μg/l; treatment 2 = Sm at 100 μg/l.

Sample Time 1 = 2 weeks post-treatment; sample time 2 = 10½ months post-treatment

Scale Analysis

Significant concentrations of lanthanum and samarium were present 10½ months after the termination of labelling in the scales of fry treated with lanthanum or samarium at 100 µg/l for 6 weeks (Appendix 11). No scale samples were taken from any other treatments. The lanthanum-treated fry had more element accumulated in their scales than the samarium-treated fry or the control fry (Table 28). Because no samples were taken directly after the termination of labelling, the initial concentration of element present is unknown.

Table 28. Concentration of lanthanum and samarium found in scales of coho fry treated with lanthanum and samarium at 0 and 100 µg/l for 6 weeks showing the significant differences.

Treatment	[Lanthanide]
Control	0^a
La at 100 µg/l	1.48^b
Sm at 100 µg/l	0.46^c

Overall mean element concentration = 0.65 µg La or Sm.g dry tissue (relative to calcium).
Error Mean Square = 0.002; total observations (n) = 6.
a, b, c. Within comparison group, mean values with unlike superscript letters were significantly ($P \leq 0.05$) different according to Tukey's test.

Comparing the accumulation of lanthanum in the three bony tissues (vertebral column, otoliths and scales), the scales had the greatest concentration of lanthanum 10½ months post-treatment. The concentrations observed in the vertebral columns

and otoliths decreased as time progressed and the lanthanum in the otoliths was almost undetectable after 10½ months (Figure 11).

As found in the lanthanum-treated fish, the scales of the samarium-treated fish had the greatest concentration of samarium 10½ months post-treatment. The vertebral columns and the otoliths had approximately equal concentrations of samarium 2 weeks post-treatment, with the concentrations decreasing markedly 10½ months post-treatment (Figure 12).

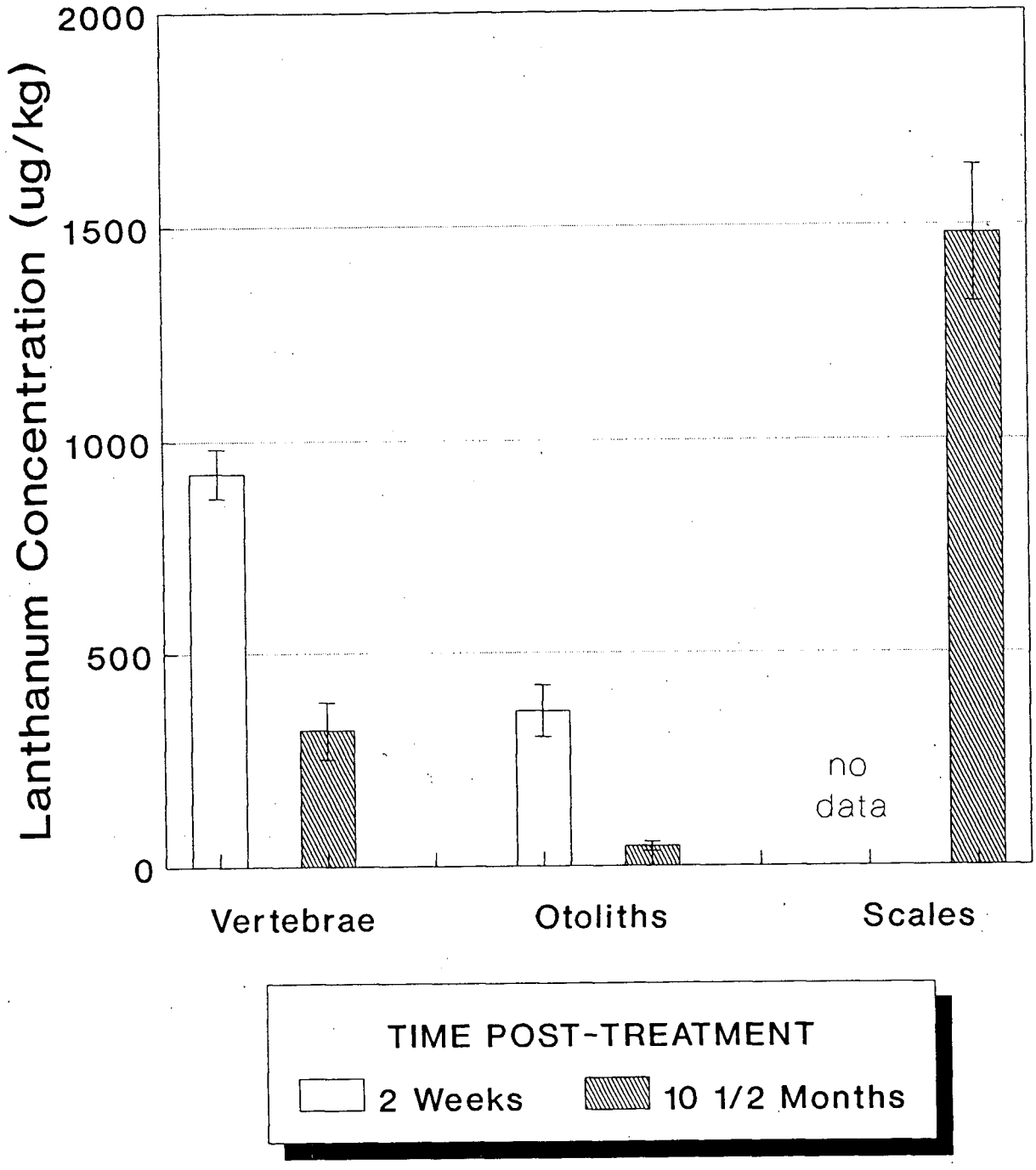

Figure 11. Concentration of lanthanum present in the bony tissues of coho fry 2 weeks and 10½ months after termination of the treatments. Results reported as mean ± S.E. in μg of La/kg of vertebrae, or in μg of La/kg dry tissue (relative to calcium) in otoliths and scales. Undetectable lanthanum in the bony tissues of untreated fry.

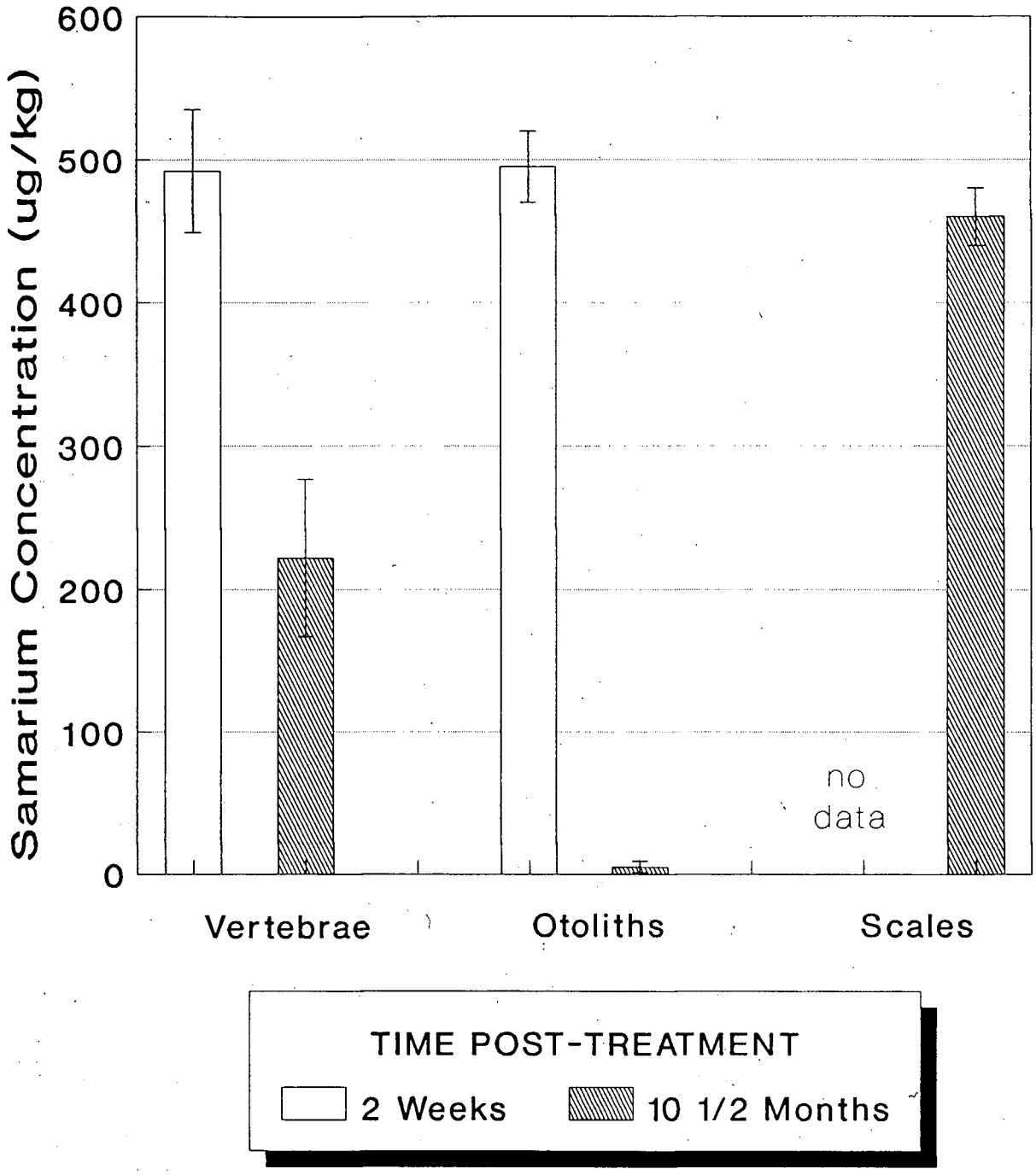

Figure 12. Concentration of samarium present in the bony tissues of coho fry 2 weeks and 10½ months after termination of the treatments. Results reported as mean ± S.E. in μg of Sm/kg of vertebrae, or in μg of Sm/kg dry tissue (relative to calcium) in otoliths and scales. Undetectable samarium in the bony tissues of untreated fry.

Discussion

During the exposure period there were significant mortalities in the groups treated with lanthanum at 100 μg/l in contrast to the negligible mortalities associated with the other lanthanum and all of the samarium treatments. Lanthanum at this concentration may have been slightly high for these young fry, but samarium was better tolerated. A possible explanation for the increased lanthanum toxicity is that light lanthanides may have been accumulated in the fry at an accelerated rate and thereby resulted in increased mortality; however there are no published results to support this hypothesis.

Throughout the 10½-month growth period relatively few mortalities were observed. These mortalities appeared randomly over time and among treatments, indicating that once the exposure to the element had been terminated, the toxic effects were diminished.

There was a significant increase in fry weight two weeks post-treatment, after which the growth levelled off. There are at least three possible explanations for the slow growth rate observed over the 10½ months. First, fish generally tend to consume less food when they are fed in small groups (Brett, 1979). Second, the temperature decreased steadily 4 months post-treatmnet also restricting the growth rates. Third, subdivisions of the mini-troughs into 35 l sections, may not have allowed sufficient space for exercise. Finally, the fry were under stress when they were disturbed during the daily

cleaning, as they had nowhere to hide. Due to the conditions of the experiment, none of these situations could have been avoided.

As found in the previous experiments, the lanthanide concentration in each of the treatment tanks were consistently lower than the theoretical concentration, and the values fluctuated over the 6-week treatment period. The earliest weeks had the lowest relative concentration with the values levelling off after the third week. The low recovery observed in the early weeks could have been a result of the adsorption of the elements onto the sides of the treatment tanks (Luckey & Venugopal, 1977). Once the available adsorption sites had been filled, the lanthanides introduced into the water presumably did not appear to adhere to the sides. Another possibility was variability in ICP-MS analysis.

The ability of ICP-MS to accurately detect the different lanthanides varies, as reported by Douglas & Houk (1985) and Houk & Thompson (1988). The results of the water and vertebrae standards demonstrate this characteristic, where the recovery of lanthanum tended to be greater than the recovery of samarium. Samarium is harder to quantify because there are 7 isotopes in the mass range of 144 to 154, with the most abundant isotope without any interference being ^{147}Sm at 15.0%. This means the instrument is quantifying samarium using a low isotopic abundance and therefore needs greater amounts of samarium in the sample for detection (Table 3). Lanthanum is more reliable to

analyze for because the isotope of interest (^{139}La) has an abundance of 99.91% with no interference. For this reason, lanthanum is preferable to samarium for analysis when using ICP-MS (Longerich *et al.*, 1987).

In addition to variation in detection of elements, both types of standards showed differences between operating days of the instrument. There was a weekly variation in observed recovery for both lanthanum and samarium (Table 20 and 21). This could have occurred as a result of the plasma and mass spectrometer parameters not being adjusted to the optimum mass range, resulting in some loss in sensitivity for specific elements. However, the variation observed was not very large. Published reports indicate that this is not usually a significant problem (Taylor, 1986).

The recovery rate was less for the tissue standards than for the water standards. The recovery of lanthanum and samarium in the water standards was 92% and 81%, respectively, and 77% and 49% in the vertebrae standards, respectively. It is possible that, as a result of the preparation method described in the Methodology section, the nitric acid was not able to digest the entire sample. Future vertebrae standards were prepared using a different method.

Since the results obtained from the standards were variable and since there was an insufficient number of standards analyzed, there was no correction factor that could have been applied with any reliability to the measured concentrations in

the samples. The reported values for the bony tissue samples are probably low also; therefore it would be expected that any marks laid down were actually greater than those reported.

Previous experiments in this thesis and the results of Durbin *et al.* (1956) have demonstrated that the lanthanides are bone seeking elements. The results of the analysis of the vertebral columns, otoliths and scales confirm that lanthanum and samarium are taken up from the water and are deposited in the bony tissues of fry. Greater concentrations of elements and longer exposure periods resulted in increased accumulation of element.

The concentration of lanthanum and samarium in the vertebral column and otoliths decreased over the 10½ months. This was due to the continual deposition of calcium in the bony tissues diluting the original lanthanide deposited. This dilution effect was demonstrated throughout all of the treatment groups. If the initial amount accumulated is not sufficiently large, this dilution effect could reduce concentrations below the limits of detection of the ICP-MS (Longerich *et al.*, 1987).

As had been previously shown with tetracycline, the marks laid down remain in the bony tissues because the bony tissues grow in a concentrical manner (Casselman, 1987). Over the 10½ months, the amount of element present in the vertebral column remained relatively constant. This indicates that once the element has been deposited into the bony tissues it remains there. Although the concentration of element declined steadily,

the actual amount of element present stayed approximately the same.

The results indicate that greater amounts of lanthanum than samarium were accumulated in the bony tissues. However, this finding may be misleading due to the problems of analyzing samarium, as discussed previously. ICP-MS has a greater sensitivity for detection of lanthanum than for samarium (Douglas & Houk, 1985; Longerich *et al.*, 1987; and Houk & Thompson, 1988).

After 10½ months, the scales had markedly higher concentrations of lanthanum than either the vertebral column or the otoliths. However, amount of element accumulated in the otoliths and scales is not known since no weights of these bony tissues were taken.

Once again, this experiment proved that the lanthanide elements are accumulated in the bony tissues and that this storage persists. For the development of an effective marking tool, more than one element would need to be incorporated into the tissue. The following experiment investigated the effect of introducing two light-weight lanthanides (lanthanum and cerium) to the same fry and to the same smolts. Parameters studied included element deposition in bony tissues and toxicity.

EXPERIMENT 6 - THE TREATMENT OF COHO (*Oncorhynchus kisutch*) FRY AND SMOLTS WITH LANTHANUM AND CERIUM IN VARIOUS COMBINATIONS FOR 4 WEEKS

Introduction

Experiment 5 demonstrated that lanthanum and samarium are accumulated in the vertebral columns, otoliths and scales of coho fry, and that the elements remain in these bony tissues for at least 10½ months post-treatment.

To develop an effective mass marking technique, more than one element would need to be incorporated in the bony tissues. The present experiment investigated the effects of introducing two elements to the same groups of fish. The two lanthanides with the lowest atomic weight, lanthanum and cerium, were used because previous studies (Experiments 1, 2, and 5) had shown the light-weight lanthanides to be more toxic than the heavier ones.

Coho fry and smolts were used to investigate the toxic effects of lanthanide administration and the accumulation of the foregoing elements into the bony tissues. Fish were exposed to various combinations of lanthanum and cerium at a concentration of 100 µg/l for 4 weeks.

Materials and Methods

Experimental Design

The design involved a factorial arrangement of stages of development (2) and treatment combinations of lanthanum and cerium (4), with two missing blocks. Thirty coho fry (average weight of 0.52 g) were placed in each of 8 experimental tanks and fifteen coho smolts (average weight of 19.44 g) were placed in each of 4 experimental tanks. Each tank containing various combinations of lanthanum and cerium for a 4 week period starting in April, 1990. The treatment combinations used were: (i) lanthanum at 100 µg/l for 2 weeks then cerium at 100 µg/l for 2 weeks - fry and smolts; (ii) no element for 1 week then lanthanum and cerium each at 100 µg/l for 2 weeks the no element for 1 week - fry only; (iii) lanthanum and cerium each at 50 µg/l for 4 weeks - fry only; and (iv) no element for 4 weeks - fry and smolts. There were 2 replications of each of the lanthanide treatments and of the negative controls (Table 29).

The lanthanides were introduced into the flow-through system as described in Experiment 5. The calculations and concentrations of the treatments are presented in Appendix 12.

Table 29. Theoretical lanthanum and cerium treatment concentrations.

Tanks With Fry		Tanks With Smolts	
Week	Treatment	Week	Treatment
1	0	1	0
2	0	2	0
3	0	3	0
4	0	4	0
1	La 100 µg/l	1	La 100 µg/l
2	La 100 µg/l	2	La 100 µg/l
3	Ce 100 µg/l	3	Ce 100 µg/l
4	Ce 100 µg/l	4	Ce 100 µg/l
1	0		
2	La + Ce 100 µg/l		
3	La + Ce 100 µg/l		
4	0		
1	La + Ce 50 µg/l		
2	La + Ce 50 µg/l		
3	La + Ce 50 µg/l		
4	La + Ce 50 µg/l		

The coho fry used in this experiment were recently ponded with 944 ATUs; and the smolts used were Capilano brood stock (1988) ponded approximately 1 year ago (4,148 ATUs). The water temperature ranged from 4.5 to 6.5 °C throughout the labelling period. The fish were fed Oregon Moist Pellets daily for the duration of the experiment. After the 4-week labelling period was completed, the fish were provided with untreated river water for a 2-week rinse period.

Sampling and Analytical Method

Two weeks after the termination of the treatments (during which time all fish were maintained in untreated water) all fry

and smolts were killed, and 5 fish from each group were randomly selected for analysis. Vertebral columns and otoliths were removed from both fry and smolts. Vertebrae standards with known amounts of lanthanides were prepared as described in the Methodology section. All bony tissue samples and standards were analyzed for lanthanum and cerium content by ICP-MS.

Water samples were taken from each of the treatment tanks at the start of the labelling and then once a week for 4 weeks. Water standards with known amounts of lanthanides were prepared and analyzed at the same time as the weekly water samples taken from the treatment tanks. Preparation of both water standards and samples was the same as for Experiment 5.

Statistical Analysis

All results were analyzed using analysis of variance using SYSTAT (Wilkinson, 1989), with differences between means tested at $P \leq 0.05$, using Tukey's multiple range test. The results for the untreated fish had no variance, therefore they were not included in the statistical analyses. The data analyzed were: mortalities; lanthanide concentration and amount in the vertebral columns; and lanthanide concentration in the otoliths.

Analysis of variance was carried out on the data to determine if there were any significant tank effects. Since none were shown, subsequent analyses were carried out on the pooled data, using individual fish as the experimental units.

Results

Mortalities

Mortalities in the tanks containing lanthanum at 100 µg/l for the first 2 weeks then cerium at 100 µg/l for the last 2 weeks of the treatment were significantly greater than mortalities in all other treatments (Appendix 13). All other tanks had negligible numbers of mortalities (Table 30).

Table 30. Total mortalities during the treatment period in tanks containing lanthanum and cerium treatments.

Treatment	Fry	Smolts
Control	1%[a]	0.5%[a]
La 100µg/l (2 weeks) then Ce 100µg/l (2 weeks)	0%[a]	0.5%[a]
0 (1 week) then La + Ce each at 100µg/l (2 weeks) then 0 (1 week)	12%[b]	-
La + Ce each at 50µg/l (4 weeks)	1%[a]	-

Error mean square = 0.500, total observations (n) = 30.
a, b. Values with unlike superscript letters were significantly ($P \leq 0.05$) different according to Tukey's test.

Water Analysis

The water in the tanks containing lanthanum at the theoretical concentration of 50 µg/l had mean concentrations ranging from 40 to 56 µg/l, and the water in the tanks containing lanthanum at the theoretical concentration of 100 µg/l had mean concentrations ranging from 94 to 107 µg/l. The recovery for lanthanum was relatively constant throughout the treatment period and, had an average value of 98% (Table 31).

The water in the tanks containing cerium at the theoretical concentration of 50 μg/l had mean concentrations ranging from 44 to 59 μg/l, and the tanks containing cerium at the theoretical concentration of 100 μg/l had mean concentrations ranging from 100 to 117 μg/l. The recovery for cerium was relatively constant throughout the treatment period and, had an average value of 107% (Table 31).

Table 31. concentration of lanthanum and cerium in water samples from tanks analyzed by ICP-MS.

Time[1]	Calculated Concentration[2]	Measured [La][3]	% La Recovery	Measured [Ce]	% Ce Recovery
Initial	50	56	111	59	118
	100	94	94	102	102
Average			103		110
Week 1	50	50	100	53	106
	100	98	98	100	110
Average			99		108
Week 2	50	52	104	54	108
	100	107	107	123	123
Average			106		116
Week 3	50	40	80	44	87
	100	94	94	107	107
Average			87		97
Week 4	50	43	86	46	92
	100	100	100	117	117
Average			93		105
Overall Average			98		107

1 Time = number of weeks after commencement of treatments.
2 Concentration measured in μg/l.
3 All values for duplicates were within 5%.

Water Standards

Analysis of water standards produced high and steady recoveries for both lanthanum and cerium. The average values were 105% for lanthanum and 117% for cerium (Table 32). Agreement between duplicates was high (within 5%). The mean recovery rate for cerium was consistently higher than that of lanthanum.

Table 32. Concentration of lanthanum and cerium in water standards analyzed by ICP-MS.

Time[1]	Calculated Concentration[2]	Measured [La][3]	% La Recovery	Measured [Ce]	% Ce Recovery
Initial	50	46	92	55	110
	100	92	92	103	103
Average			92		107
Week 1	50	48	96	55	110
	100	97	97	100	100
Average			97		105
Week 2	50	59	118	64	128
	100	120	120	120	120
Average			113		124
Week 3	50	49	98	60	120
	100	100	100	115	115
Average			99		118
Week 4	50	61	122	66	132
	100	115	115	135	135
Average			119		134
Overall Average			105		117

1 Time = number of weeks after commencement of treatments.
2 Concentration measured in µg/l.
3 All values for duplicates were within 5%.

Vertebrae Standards

Analysis of vertebrae standards spiked with known amounts of lanthanum, cerium and samarium resulted in extremely variable values for each of the elements. The average recovery rates were 88% for lanthanum and 136% for cerium (Table 33). The recovery did not vary with the amount of lanthanide spike present. There was moderately good agreement between duplicates (within 10%).

Table 33. Concentraiton of lanthanum and cerium in vertebrae standards analyzed by ICP-MS.

Conc. (μg/g)	[La][1]	% La	[Ce]	% Ce
0.40	0.34	85	0.57	142
0.80	0.72	90	1.04	130
Average		88		136

1 All values for duplicates were within 10%.

Vertebral Column Analysis

Analysis of vertebral columns of treated fry and smolts showed lanthanum and cerium to be present, whereas untreated fry and smolts had undetectable levels of element present. Values were not corrected for the recovery of the vertebrae standards because of lack of sufficient numbers of standards analyzed and variability of results. The data in Table 34 show the effects of lanthanum and cerium treatments on mean lanthanide concentrations in the vertebral columns of fry and smolts and

those in Table 35 show the effects on mean amounts of lanthanides in the vertebral columns of fry and smolts. ANOVA tables for the concentrations and amounts are presented in Appendix 14.

There were significantly greater concentrations of both lanthanum and cerium in the vertebral columns of fry (0.48 μg/g) than in the vertebral columns of smolts (0.10 μg/g). Comparing the concentrations present in the vertebral columns of fry, there were no differences between treatments or elements (Table 34). Because smolts were exposed to the first treatment only and fry were exposed to all three treatments, comparisons of element present in fry and smolts between the treatments were not considered meaningful.

Amounts of lanthanum and cerium present in the vertebral columns of the fry differed markedly from amounts in smolts with the smolts accumulating markedly greater amounts (8.94 ng) than the fry (1.49 ng). There were no differences observed between lanthanum and cerium or between the three treatments (Table 35). Comparisons of amounts of elements present in fry and smolts between the treatments were not possible.

Table 34. Mean lanthanide concentrations (μg/g) in vertebral columns of fry and smolts labelled with lanthanum and cerium showing the significant effects of lanthanum and cerium treatments.

Treatment x Stage x Lanthanide				
	Fry		Smolts	
	La	Ce	La	Ce
Treatment				
1	0.54^{bc}	0.59^{c}	0.10^{a}	0.09^{a}
2	0.47^{bc}	0.47^{bc}	-	-
3	0.44^{bc}	0.37^{b}	-	-
Means	0.49^{a}	0.48^{a}	0.10^{b}	0.09^{b}

	Trt x Lanthanide		Trt x Stage		
	La	Ce	Fry	Smolts	Means
Treatment					
1	0.32^{a}	0.34^{a}	0.57^{a}	0.10^{a}	0.33^{a}
2	0.47^{a}	0.47^{a}	0.47^{ab}	-	0.47^{b}
3	0.44^{a}	0.37^{a}	0.41^{b}	-	0.41^{ab}
Means	0.39^{a}	0.38^{a}	0.48^{a}	0.10^{b}	

Overall mean lanthanide concentration = 0.40 μg/g.
Error Mean Square = 0.016; total observations (n) = 80.
a, b, c. Within comparison groups (enclosed by a dashed line), mean values without a common superscript letter were significantly ($P \leq 0.05$) different according to Tukey's test.
Treatment 1 = La at 100 μg/l for 2 weeks, then Ce at 100 μg/l for 2 weeks; treatment 2 = La + Ce each at 100 μg/l for 2 weeks, 0 for 2 weeks; treatment 3 = La + Ce each at 50 μg/l for 4 weeks.

Table 35. Mean lanthanide amounts (ng) in vertebral columns of fry and smolts labelled with lanthanum and cerium showing the significant effects of lanthanum and cerium treatments.

	Treatment x Stage x Lanthanide			
	Fry		Smolts	
	La	Ce	La	Ce
Treatment				
1	1.71[a]	1.81[a]	9.19[b]	8.68[b]
2	1.33[a]	1.28[a]	-	-
3	1.52[a]	1.27[a]	-	-
Means	1.52[a]	1.45[a]	9.19[b]	8.68[b]

	Trt x Lanthanide		Trt x Stage		
	La	Ce	Fry	Smolts	Means
Treatment					
1	5.45[a]	5.25[a]	1.76[a]	8.94[b]	5.35[a]
2	1.33[ab]	1.28[ab]	1.30[a]	-	1.30[b]
3	1.52[ab]	1.27[b]	1.39[a]	-	1.39[b]
Means	3.44[a]	3.26[a]	1.49[a]	8.94[b]	

Overall mean lanthanide amount = 2.68 ng.
Error Mean Square = 4.219; total observations (n) = 80.
a, b. Within comparison groups (enclosed by a dashed line), mean values without a common superscript letter were significantly ($P \leq 0.05$) different according to Tukey's test.
Treatment 1 = La at 100 µg/l for 2 weeks, then Ce at 100 µg/l for 2 weeks; treatment 2 = La + Ce each at 100 µg/l for 2 weeks, 0 for 2 weeks; treatment 3 = La + Ce each at 50 µg/l for 4 weeks.

Otolith Analysis

Analysis of otoliths of fry and smolts labelled with lanthanum and cerium showed detectable concentrations to be present, whereas untreated fish had undetectable concentrations of the element. Appendix 15 contains the relevant ANOVA table. There was no significant difference in the concentration of element between the different treatments. The smolts had significantly lower concentrations of element present (0.04 μg/g) than the 0.14 μg/g accumulated in the fry (Table 36).

Table 36. Mean lanthanide concentrations (μg/g) in otoliths of fry and smolts labelled with lanthanum and cerium showing the significant effects of lanthanum and cerium treatments.

Treatment x Stage x Lanthanide				
	Fry		Smolts	
	La	Ce	La	Ce
Treatment				
1	0.14^{b}	0.22^{a}	0.02^{b}	0.05^{b}
2	0.10^{b}	0.12^{b}	-	-
3	0.10^{b}	0.14^{b}	-	-
Means	0.11^{ab}	0.16^{a}	0.02^{b}	0.05^{b}

	Trt x Lanthanide		Trt x Stage		
	La	Ce	Fry	Smolts	Means
Treatment					
1	0.08^{a}	0.14^{a}	0.18^{a}	0.04^{a}	0.11^{a}
2	0.10^{a}	0.12^{a}	0.11^{b}	-	0.11^{a}
3	0.10^{a}	0.14^{a}	0.12^{ab}	-	0.12^{a}
Means	0.09^{a}	0.14^{a}	0.14^{a}	0.04^{b}	

Overall mean lanthanide concentration = 0.11 μg/g.
Error Mean Square = 0.004; total observations (n) = 32.
a, b. Within comparison groups (enclosed by a dashed line), mean values without a common superscript letter were significantly ($P \leq 0.05$) different according to Tukey's test.
Treatment 1 = La at 100 μg/l for 2 weeks, then Ce at 100 μg/l for 2 weeks; treatment 2 = La + Ce each at 100 μg/l for 2 weeks, 0 for 2 weeks; treatment 3 = La + Ce each at 50 μg/l for 4 weeks.

Discussion

The tanks containing fry that were treated with lanthanum at 100 µg/l for 2 weeks followed by cerium at 100 µg/l for 2 weeks had a significantly larger number of mortalities than all other tanks containing fry and smolts. As discussed previously, the light lanthanides as a group may have an increased toxic affect on young fry; however, the elements were better tolerated by smolts.

The spiked water standards and the water samples taken from the treatment tanks showed that the lanthanum concentrations were close to the theoretical values, and that the cerium concentrations were higher than expected. The values were reasonably consistent over the 4-week treatment period. However, these results were inconsistent with those obtained from previous experiments. Two possible explanations for the better recovery rates observed in this experiment are: (i) the ICP-MS instrument may have been better adjusted for the detection of the lanthanides, allowing for more accurate values; and (ii) accuracy of analysis for lanthanum and cerium content was enhanced by the high isotopic abundances of the isotopes used for measurement (^{139}La at 99.91% and ^{140}Ce at 88.48%) and by the lack of interference from other isotopes (Longerich *et al.*, 1987).

As was observed in Experiment 5, analysis of vertebrae standards spiked with known amounts of lanthanides resulted in variable recovery rates. Lanthanum had a low average recovery

(88%), whereas cerium had a high average recovery (136%). This variation was most likely due to the differential ability of ICP-MS in the detection of different elements (Douglas & Houk, 1985; and Houk & Thompson, 1988). Results from the analysis of vertebral column samples showed the cerium levels to be consistently higher than the lanthanum levels. This could have been a result of an analytical problem rather than a difference in the levels of accumulation between the two elements.

Because the analysis of spiked standards produced variable results, no correction factor was applied to the results of the analysis of unknown samples. Reported values for the marks laid down in the bony tissues were therefore only approximate values.

All fry and smolts exposed to lanthanum and cerium received approximately equal doses of each element over the treatment period. The results of the analysis of the vertebral columns showed the fry to contain markedly higher concentrations of lanthanum and cerium than the smolts. However, the smolts had greater amounts of element in their vertebral columns than the fry. The smolts had accumulated more element in their vertebral columns than the fry, but because of the increased size of their bony tissues, the smolts had lower concentrations of element present. The larger fish have a greater daily growth increment, therefore accumulated higher levels of both calcium and the lanthanide elements in their vertebral columns than the fry. Weiss (1974) and Das *et al.* (1988) have suggested that La^{3+} ions and Ca^{2+} ions enter the fish by the same route and that La^{3+}

actively competes with Ca^{2+}. This is a likely explanation for the elevated lanthanum and cerium levels found in the fry and smolts.

There was no significant difference in the lanthanum or cerium concentrations found in the vertebral columns of the fry treated with 50 or 100 μg/l of element. The lanthanides seem to be actively taken up from the water supply at the same rate regardless of the exposure concentration.

The otoliths of both fry and smolts were shown to have lanthanum and cerium deposited in them. The otoliths of the fry had markedly greater concentrations of both elements in contrast to the concentrations in the otoliths of the smolts. The relative amount of element present is not known since the otoliths were not weighed.

This experiment confirms the findings of the previous work reported in this thesis that the lanthanide elements are taken up from the water supply and deposited into bony tissues.

CONCLUSIONS AND RECOMMENDATIONS

The series of experiments carried out for this thesis indicate that the lanthanides are absorbed from the water supply and subsequently incorporated into the bony tissues of coho fry and coho smolts. Longer exposure times and greater treatment concentrations result in increased element accumulation in the bony tissues. Coho fry exposed to lanthanum or samarium at 100 μg/l for 6 weeks had detectable levels of element in their vertebral columns, otoliths, and scales 10½ months post-treatment.

When lanthanum and cerium were introduced into the water supply of coho fry and smolts, both elements were accumulated in the bony tissues in approximately equal concentrations for all methods of application. When the elements were added to the water supply at the same concentration and for the same duration, higher concentrations of lanthanum and cerium were accumulated in the fry than in the smolts; but, the smolts had greater amounts of element present. Since larger fish have a greater daily growth increment, they accumulate more element in their bony tissues. Thus, it may be more effective to expose larger fish to the elements. Not only would this result in increased element deposition, but it would also result in decreased toxic effects.

From analysis of untreated fish it was shown that the lanthanides are not present in the bony tissues in detectable levels and should not interfere in the detection of marked fish.

At concentrations of 100 μg/l in the water supply, the lanthanides were severely toxic to coho and steelhead alevins, but were only slightly toxic to coho fry. The alevins may accumulate the lanthanides at a rate which is faster than their development rate or their excretion processes, thereby resulting in an overdose and death. Coho smolts did not seem to be adversely affected by lanthanum or cerium at 100 μg/l.

The light-weight lanthanides appeared to be more toxic than the heavier ones. The rate of uptake may vary for the individual lanthanides, therefore resulting in differential toxic effects. Because the mechanism for the toxic actions of these elements in fish is not fully understood, a marking strategy to follow may include exposing larger fish to lower concentrations of the elements for longer durations.

Future developments of ICP-MS technology are of great importance to this type of mass marking. Innovations in electrothermal vaporization or laser ablation may lead to greater improvements in sensitivity of detection. Because of the natural dilution of the label by increased calcium deposition as the fish grows, the concentration present in mature fish will be markedly reduced. If sufficient quantities are deposited in bony tissues, and detection limits are

improved, it should be possible to identify marked fish throughout their life cycle.

Although there are 15 lanthanide elements and yttrium (not a lanthanide, but has similar properties), not all of them may be suitable or feasible to use for marking fish. There is a well documented odd/even relationship between the elements in the lanthanide series; the elements with even atomic numbers are more abundant and easier to purify than the elements with odd atomic numbers (Taylor, 1964; Topp, 1965; and Kilbourn, 1988). The implication of this relationship is that the even numbered lanthanides are more commercially available. Also, the light-weight lanthanides tend to be more abundant than the heavier elements; therefore, these would tend to be less expensive. Since these elements are available in large quantities at relatively low cost, it would be very feasible to mark entire hatchery populations.

Along with the natural elemental abundances, the analytical properties need to be considered. The lanthanide elements with high abundance of a single isotope with little or no interference from other isotopes, will be preferable for analysis by ICP-MS. Lanthanides that appear to be feasible to obtain on a commercial basis and to analyze include: yttrium, lanthanum, cerium, praseodymium, neodymium, samarium, gadolinium, terbium, dysprosium, holmium, and ytterbium.

Before this marking technique can be fully utilized on a large-scale, several areas need to be explored. These include:

(i) suitability of all feasible lanthanides for marking, especially the less expensive and more abundant ones;

(ii) species differences in toxicity, uptake and retention of the elements;

(iii) suitability of chlorides as well as acetates because of relatively lower costs, greater availability and ease of handling of chlorides;

(iv) determine the amount of element that would need to be incorporated into the bony tissues of the juvenile salmon to be detectable in the mature adult;

(v) marking of large production groups of both fry and smolts;

(vi) environmental impact study with particular attention to the toxic effects on juvenile salmon and invertebrates exposed to the effluent; also the potential problem of labelling fish downstream;

(vii) effects of lanthanide administration on histopathology and immunocompetence of labelled fish; and

(viii) lanthanide accumulation and retention in the opercula because of the perceived sampling benefits of centre punching the lanthanide-enriched portion.

Introduction of the lanthanide elements into the water supply appears to be a feasible method to use to effectively and efficiently mark hatchery-production salmon. Future experiments and further improvements in sensitivity of detection with electrothermal vaporization and laser ablation will help to

improve the applicability of lanthanides for mass marking programs.

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APPENDICES

Appendix 1. Acute lethal doses of lanthanide elements in rats and mice.

Lanthanide Compound	Animal	Route	Mg metal/kg	Ref
Yttrium Oxide	Rat	IP	395	1
Yttrium Chloride	Rat	IP	132	1
	Mouse	IP	88	2
Yttrium Nitrate	Rat	IP	117	1
Lanthanum Oxide	Rat	PO	>8500	1
Lanthanum Chloride	Rat	PO	2370	1
	Rat	IP	197	1
	Mouse	IP	211	2
	Mouse	SC	>500	2
Lanthanum Sulfate	Rat	PO	>2450	1
	Rat	IP	134	1
Lanthanum Nitrate	Rat	PO	1450	1
	Rat	IP	145	1
	Mouse	IP	131	3
Lanthanum Acetate	Rat	PO	4400	1
	Rat	IP	209	1
Lanthanum Citrate Chloride	Mouse	IP	44	4
Cerium Chloride	Rat	IV	50	2
	Mouse	IP	201	2
Cerium Nitrate	Rat	PO	1355	3
	Rat	IP	93	3
	Rat	IV	2-16	3
	Mouse	IP	151	3
Praseodymium Chloride	Mouse	SC	944	2
Praseodymium Nitrate	Rat	PO	1134	3
	Rat	IP	79	3
	Rat	IV	2-25	3
	Mouse	IP	94	3
Neodymium Chloride	Mouse	IP	200	2
Neodymium Nitrate	Rat	PO	905	3
	Rat	IP	89	3
	Rat	IV	2-22	3
	Mouse	IP	89	3
Samarium Chloride	Mouse	PO	>840	5
	Mouse	IP	241	5

Lanthanide Compound	Animal	Route	Mg metal/kg	Ref
Samarium Nitrate	Rat	PO	901	3
	Rat	IP	96	3
	Rat	IV	3-20	3
	Mouse	IP	106	3
Europium Chloride	Mouse	PO	2075	6
	Mouse	IP	228	6
Europium Nitrate	Rat	PO	>1704	3
	Rat	IP	72	3
	Mouse	IP	109	3
Gadolinium Chloride	Mouse	PO	>850	5
	Mouse	IP	232	5
Gadolinium Nitrate	Rat	PO	>1743	3
	Rat	IP	80	3
	Mouse	IP	105	3
Terbium Chloride	Mouse	PO	2175	7
	Mouse	IP	234	7
Terbium Nitrate	Rat	PO	>1753	3
	Rat	IP	91	3
	Mouse	IP	168	3
Dysprosium Chloride	Mouse	PO	3290	6
	Mouse	IP	251	6
Dysprosium Nitrate	Rat	PO	1103	3
	Rat	IP	105	3
	Mouse	IP	110	3
Holmium Chloride	Mouse	PO	3140	6
	Mouse	IP	243	6
Holmium Nitrate	Rat	PO	1078	3
	Rat	IP	97	3
	Mouse	IP	115	3
Erbium Chloride	Mouse	PO	2700	6
	Mouse	IP	233	6
Erbium Nitrate	Rat	IP	83	3
	Rat	IV	13-19	3
	Mouse	IP	81	3
Thulium Chloride	Mouse	PO	2635	7
	Mouse	IP	204	7
Thulium Nitrate	Rat	IP	104	3
	Mouse	IP	93	3

Lanthanide Compound	Animal	Route	Mg metal/kg	Ref
Ytterbium Chloride	Mouse	PO	2995	7
	Mouse	IP	186	7
Ytterbium Nitrate	Rat	PO	1148	3
	Rat	IP	94	3
	Mouse	PO	93	3
Lutetium Chloride	Mouse	PO	4400	6
	Mouse	IP	195	6
Lutetium Nitrate	Rat	IP	125	3
	Mouse	IP	108-130	3

(1) Cochran *et al*, 1950
(2) Kyker & Cress, 1957
(3) Bruce *et al*, 1963
(4) Graca *et al.*, 1962
(5) Haley *et al.*, 1961
(6) Haley, 1965
(7) Haley *et al.*, 1963

Appendix 2. ICP-MS Analysis for lanthanide content of 6% sodium hypochlorite bleaching solution used to digest traces of flesh off bony tissues.

Lanthanide	Concentration (μg/l)
Yttrium	<0.37
Lanthanum	<0.10
Cerium	0.36
Praseodymium	0.40
Neodymium	2.04
Samarium	<0.98
Europium	<0.27
Gadolinium	0.88
Terbium	0.19
Dysprosium	<0.50
Holmium	0.09
Erbium	0.03
Thulium	<0.10
Ytterbium	0.46
Lutecium	0.07

Appendix 3. Information used and results of computer dripcalc program used for calculation of lanthanide treatments used in Experiment 5.

Lanthanum at 10 µg/l

A. Data Required
Element Name: Lanthanum
Compound Name: Lanthanum acetate
Atomic Weight of Element: 138.91 (La)
Gram Formula Weight of Compound: 343.07 (La acetate)
Increase in Element Required: 10 µg/l = 0.01 mg/l
Solubility of Compound: 168.8 g/l
Stock Solution Container Size: 10 l
Flow Rate: 1 l/min
Number of Units to Treat: 1
Number of Days Stock Solution to Last: 10 days

B. Calculation Results
To Raise La by 0.01 mg/l:
La acetate stock solution: 0.04 g/l
- 10 l requires 0.36 g

Drip rate for stock: 0.7 ml/min
- Unit flow @ 1 l/min
- Stock lasts 10 days for 1 unit

Lanthanum at 100 µg/l

A. Data Required
Element Name: Lanthanum
Compound Name: Lanthanum acetate
Atomic Weight of Element: 138.91 (La)
Gram Formula Weight of Compound: 343.07 (La acetate)
Increase in Element Required: 100 µg/l = 0.1 mg/l
Solubility of Compound: 168.8 g/l
Stock Solution Container Size: 10 l
Flow Rate: 1 l/min
Number of Units to Treat: 1
Number of Days Stock Solution to Last: 10 days

B. Calculation Results
To Raise La by 0.1 mg/l:
La acetate stock solution: 0.36 g/l
- 10 l requires 3.56 g

Drip rate for stock: 0.7 ml/min
- Unit flow @ 1 l/min
- Stock lasts 10 days for 1 unit

Samarium at 10 µg/l

A. Data Required
Element Name: Samarium
Compound Name: Samarium acetate
Atomic Weight of Element: 150.4 (Sm)
Gram Formula Weight of Compound: 381.53 (Sm acetate)
Increase in Element Required: 10 µg/l = 0.01 mg/l
Solubility of Compound: 150 g/l
Stock Solution Container Size: 10 l
Flow Rate: 1 l/min
Number of Units to Treat: 1
Number of Days Stock Solution to Last: 10 days

B. Calculation Results
To Raise Sm by 0.01 mg/l:
Sm acetate stock solution: 0.03 g/l
- 10 l requires 0.31 g

Drip rate for stock: 0.7 ml/min
- Unit flow @ 1 l/min
- Stock lasts 10 days for 1 unit

Samarium at 100 µg/l

A. Data Required
Element Name: Samarium
Compound Name: Samarium acetate
Atomic Weight of Element: 150.4 (Sm)
Gram Formula Weight of Compound: 381.53 (Sm acetate)
Increase in Element Required: 100 µg/l = 0.1 mg/l
Solubility of Compound: 150 g/l
Stock Solution Container Size: 10 l
Flow Rate: 1 l/min
Number of Units to Treat: 1
Number of Days Stock Solution to Last: 10 days

B. Calculation Results
To Raise Sm by 0.1 mg/l:
Sm acetate stock solution: 0.31 g/l
- 10 l requires 3.14 g

Drip rate for stock: 0.7 ml/min
- Unit flow @ 1 l/min
- Stock lasts 10 days for 1 unit

Appendix 4. ICP-MS analysis of Oregon Moist Pellets fed to coho fry during labelling period in Experiment 5.

Total Element Concentrations (μg/g)

Element	Mass	Conc.	Element	Mass	Conc.
Lithium	7	0.103	Beryllium	9	<0.007
Boron	11	<1.43	Sodium	23	773 *
Magnesium	24	9586 *	Aluminium	27	14.2
Silicon	28	178	Phosphorus	31	8740
Potassium	39	3460	Calcium	44	15700
Scandium	45	0.167	Titanium	49	0.93
Vanadium	51	0.547	Chromium	52	0.269
Manganese	55	24.8	Iron	56	58.5 *
Cobalt	59	0.056	Nickel	62	0.083
Copper	63	4.15	Zinc	66	89.3
Gallium	71	<0.007	Germanium	72	<0.004
Arsenic	75	2.16	Selenium	78	1.87
Bromine	79	26.9	Rubidium	85	1.50
Strontium	86	21.7	Yttrium	89	0.008
Zirconium	91	<0.115	Niobium	93	0.006
Molybdenum	100	0.099	Ruthenium	102	<0.002
Rhodium	103	<0.002	Palladium	105	<0.004
Silver	107	<0.002	Cadmium	112	0.118
Indium	115	int.std.	Tin	120	0.133
Antimony	123	<0.006	Tellurium	126	0.012
Iodine	127	8.56	Caesium	133	0.012
Barium	138	2.23	Lanthanum	139	0.010
Cerium	140	0.018	Praseodymium	141	0.004
Neodymium	145	0.014	Samarium	147	0.003
Europium	151	<0.002	Gadolinium	157	<0.003
Terbium	159	<0.002	Dysprosium	161	<0.003
Holmium	165	<0.002	Erbium	166	<0.002
Thulium	169	<0.002	Ytterbium	172	<0.002
Lutetium	175	<0.003	Hafnium	178	<0.002
Tantalum	181	<0.002	Rhenium	185	<0.002
Tungsten	186	0.089	Osmium	190	<0.002
Iridium	193	<0.002	Platinum	194	<0.005
Gold	197	<0.003	Mercury	200	0.020
Thallium	205	0.003	Lead	208	0.237
Bismuth	209	<0.002	Thorium	232	<0.003
Uranium	238	0.035			

* High values for these elements could be a result of digestion problems and matrix interferences in ICP-MS analysis.

Appendix 5. Water chemistry data for the ambient Capilano River water.

I. Parameters and Metals - Cantest Laboratory

A. Parameters

pH (pH units)		6.33
Conductivity (us/cm)		19.0
Hardness	$CaCO_3$	4.0
Alkalinity	HCO_3	4.17
Ammonia	N	<0.01
Chloride	Cl	1.30
Dissolved Oxygen		7.95
Nitrite	N	<0.05
Nitrate	N	0.15
Residual-filterable		12.0
Residual Non-filter		<1.0
Silica	SiO_2	3.12
Sulphate	SO_4	1.08
Turbidity (NTU)		0.55
Fluoride	F	<0.05

B. Metals (mg/l)

Aluminum	Al	<0.15
Arsenic	As	<0.030
Barium	Ba	<0.001
Calcium	Ca	1.23
Cadmium	Cd	<0.02
Cobalt	Co	<0.02
Chromium	Cr	<0.03
Copper	Cu	<0.015
Iron	Fe	0.097
Mercury	Hg	<0.00005
Potassium	K	<1.0
Magnesium	Mg	0.17
Manganese	Mn	0.005
Molybdenum	Mo	<0.04
Sodium	Na	0.53
Nickel	Ni	<0.025
Phosphorus	P	<0.15
Lead	Pb	<0.08
Antimony	Sb	<0.15
Tin	Sn	<0.03
Strontium	Sr	0.006
Titanium	Ti	<0.006
Vanadium	V	<0.01
Zinc	Zn	<0.015

Concentrations reported as mg/l.

II. ICP-MS Analysis for Total Elemental Content - ERI

Element	Mass	Conc.	Element	Mass	Conc.
Lithium	7	<0.03	Beryllium	9	<0.03
Boron	11	<16.3	Sodium	23	140
Magnesium	24	38.6	Aluminium	27	9.83
Calcium	44	181	Scandium	45	<0.17
Titanium	47	<0.32	Vadium	51	0.13
Chromium	52	<0.25	Manganese	55	0.37
Iron	56	9.83	Cobalt	59	0.03
Nickel	60	0.59	Copper	63	<0.05
Zinc	66	<0.04	Gallium	69	<0.14
Germanium	74	<0.05	Arsenic	75	3.04
Selenium	78	0.27	Bromine	79	<0.63
Rubidium	85	0.21	Strontium	88	3.20
Yttrium	89	<0.03	Zirconium	90	<0.05
Niobium	93	<0.02	Molybdenum	100	0.08
Ruthenium	101	<0.03	Rhodium	103	<0.02
Silver	107	<0.02	Palladium	105	<0.03
Cadmium	111	1.64	Indium	115	int.std.
Tin	120	0.04	Antimony	121	<0.03
Iodine	127	0.29	Tellurium	128	<0.08
Caesium	133	<0.02	Barium	138	2.12
Lanthanum	138	0.05	Cerium	140	0.04
Praseodymium	141	<0.02	Neodymium	146	0.03
Samarium	149	<0.03	Europium	153	<0.02
Gadolinium	157	<0.04	Terbium	159	<0.02
Dysprosium	163	0.07	Holmium	165	<0.02
Erbium	166	<0.02	Thulium	169	<0.02
Ytterbium	172	<0.02	Lutetium	175	<0.02
Hafnium	178	<0.02	Tantalum	181	<0.02
Tungsten	184	<0.03	Rhenium	185	<0.02
Osmium	190	<0.02	Iridium	193	<0.02
Platinum	194	<0.03	Gold	197	<0.02
Mercury	202	<0.17	Thallium	205	<0.02
Lead	208	0.55	Bismuth	209	<0.02
Thorium	232	0.02	Uranium	238	0.03

Concentrations reported as μg/l.

Appendix 6. ANOVA tables for mortalities observed in Experiment 5 in lanthanum treatment tanks.

1. ANOVA table of mortalities observed during 6-week treatment period only. $R^2 = 0.779$

SOURCE	SS	DF	MEAN-SQUARE	F-RATIO	P
TRT	16.667	2	8.333	9.091	**0.015**
DUR	2.083	1	2.083	2.273	0.182
TRTxDUR	0.667	2	0.333	0.364	0.709
ERROR	5.500	6	0.917		

2. ANOVA table of mortalities observed during grow-out period only. $R^2 = 0.526$

SOURCE	SS	DF	MEAN-SQUARE	F-RATIO	P
TRT	19.500	2	9.750	2.167	0.196
DUR	0.333	1	0.333	0.074	0.795
TRTxDUR	10.167	2	5.083	1.130	0.383
ERROR	27.000	6	4.500		

Appendix 7. ANOVA tables for growth (fry weight) observed in Experiment 5 in lanthanum and samarium treatment tanks.

1. ANOVA table of fry weights observed during 10½-month grow-out period in lanthanum treatment tanks. $R^2 = 0.536$

SOURCE	SS	DF	MEAN-SQUARE	F-RATIO	P
TRT	7.597	2	3.799	0.978	0.377
DUR	5.649	1	5.649	1.455	0.229
TIME	1205.043	5	241.009	62.072	**0.000**
TRTxDUR	46.308	2	23.154	5.963	**0.003**
TRTxTIME	50.950	10	5.095	1.312	0.222
DURxTIME	80.843	5	16.169	4.164	**0.001**
TRTxDURxTIME	54.670	10	5.467	1.408	0.175
ERROR	1257.999	324	3.883		

2. ANOVA table of fry weights observed during 10½-month grow-out period in samarium treatment tanks. $R^2 = 0.592$

SOURCE	SS	DF	MEAN-SQUARE	F-RATIO	P
TRT	4.286	2	2.143	0.739	0.480
DUR	3.210	1	3.210	1.106	0.295
TIME	520.552	5	104.110	35.881	**0.000**
TRTxDUR	18.938	2	9.469	3.263	**0.041**
TRTxTIME	14.314	10	1.431	0.493	0.892
DURxTIME	26.515	5	5.303	1.828	0.111
TRTxDURxTIME	18.143	10	1.814	0.625	0.790
ERROR	417.824	144	2.902		

Appendix 8. ANOVA tables for lanthanum and samarium concentration in the vertebral columns of fry labelled with lanthanum and samarium in Experiment 5.

1. ANOVA table of lanthanum concentrations in the vertebral columns during 10½-month grow-out period in lanthanum treatment tanks. $R^2 = 0.866$

SOURCE	SS	DF	MEAN-SQUARE	F-RATIO	P
TRT	5.744	1	5.744	631.489	**0.000**
DUR	2.845	1	2.845	312.752	**0.000**
TIME	1.982	5	0.396	43.589	**0.000**
TRTxDUR	0.885	1	0.885	97.239	**0.000**
TRTxTIME	1.013	5	0.203	22.281	**0.000**
DURxTIME	0.129	5	0.026	2.828	**0.017**
TRTxDURxTIME	0.135	5	0.027	2.979	**0.013**
ERROR	1.965	216	0.009		

2. ANOVA table of samarium concentrations in the vertebral columns during 10½-month grow-out period in samarium treatment tanks. $R^2 = 0.809$

SOURCE	SS	DF	MEAN-SQUARE	F-RATIO	P
TRT	1.055	1	1.055	212.979	**0.000**
DUR	0.391	1	0.391	78.961	**0.000**
TIME	0.196	5	0.039	7.898	**0.000**
TRTxDUR	0.224	1	0.224	45.328	**0.000**
TRTxTIME	0.087	5	0.017	3.508	**0.006**
DURxTIME	0.046	5	0.009	1.847	0.111
TRTxDURxTIME	0.017	5	0.003	0.702	0.623
ERROR	0.475	96	0.005		

Appendix 9. ANOVA tables for lanthanum and samarium amounts in the vertebral columns of fry labelled with lanthanum and samarium in Experiment 5.

1. ANOVA table of lanthanum amounts in the vertebral columns during 10½-month grow-out period in lanthanum treatment tanks. $R^2 = 0.801$

SOURCE	SS	DF	MEAN-SQUARE	F-RATIO	P
TRT	3483.696	1	3483.696	472.736	**0.000**
DUR	1928.231	1	1928.231	261.660	**0.000**
TIME	238.865	5	47.773	6.483	**0.000**
TRTxDUR	502.578	1	502.578	68.200	**0.000**
TRTxTIME	52.635	5	10.527	1.429	0.215
DURxTIME	145.757	5	29.151	3.956	**0.002**
TRTxDURxTIME	73.338	5	14.668	1.990	0.081
ERROR	1591.752	216	7.369		

2. ANOVA table of samarium amounts in the vertebral columns during 10½-month grow-out period in samarium treatment tanks. $R^2 = 0.860$

SOURCE	SS	DF	MEAN-SQUARE	F-RATIO	P
TRT	878.038	1	878.038	303.385	**0.000**
DUR	426.851	1	426.851	147.488	**0.000**
TIME	49.081	5	9.816	3.392	**0.007**
TRTxDUR	274.286	1	274.286	94.773	**0.000**
TRTxTIME	24.115	5	4.823	1.666	0.150
DURxTIME	32.593	5	6.519	2.252	0.055
TRTxDURxTIME	22.497	5	4.499	1.555	0.180
ERROR	277.837	96	2.894		

Appendix 10. ANOVA tables for lanthanum and samarium concentrations in the otoliths of fry labelled with lanthanum and samarium in Experiment 5.

1. ANOVA table of lanthanum concentrations in the otoliths 2 weeks and 10½ months post-treatment in lanthanum treatment tanks. $R^2 = 0.794$

SOURCE	SS	DF	MEAN-SQUARE	F-RATIO	P
TRT	0.102	1	0.102	20.486	**0.000**
DUR	0.049	1	0.049	9.842	**0.005**
TIME	0.092	1	0.092	18.566	**0.000**
TRTxDUR	0.023	1	0.023	4.601	**0.043**
TRTxTIME	0.071	1	0.071	14.358	**0.001**
DURxTIME	0.025	1	0.025	5.058	**0.035**
TRTxDURxTIME	0.015	1	0.015	2.990	0.098
ERROR	0.110	22	0.005		

2. ANOVA table of samarium concentrations in the otoliths 2 weeks and 10½ months post-treatment in samarium treatment tanks. $R^2 = 0.995$

SOURCE	SS	DF	MEAN-SQUARE	F-RATIO	P
TRT	0.066	1	0.066	245.649	**0.000**
DUR	0.056	1	0.056	208.904	**0.000**
TIME	0.064	1	0.064	237.122	**0.000**
TRTxDUR	0.059	1	0.059	218.712	**0.000**
TRTxTIME	0.067	1	0.067	247.565	**0.000**
DURxTIME	0.057	1	0.057	210.670	**0.000**
TRTxDURxTIME	0.054	1	0.054	201.046	**0.000**
ERROR	0.002	8	0.000		

Appendix 11. ANOVA tables for lanthanum and samarium concentrations in the scales, 10½ months post-treatment, of fry labelled with lanthanum and samarium in Experiment 5.

$R^2 = 0.997$

SOURCE	SS	DF	MEAN-SQUARE	F-RATIO	P
TRT	2.278	2	1.139	546.792	**0.000**
ERROR	0.006	3	0.002		

Appendix 12. Information used and results of computer dripcalc program used for calculation of lanthanide treatments used in Experiment 6.

Lanthanum at 50 µg/l

A. Data Required
Element Name: Lanthanum
Compound Name: Lanthanum acetate
Atomic Weight of Element: 138.91 (La)
Gram Formula Weight of Compound: 343.07 (La acetate)
Increase in Element Required: 50 µg/l = 0.05 mg/l
Solubility of Compound: 168.8 g/l
Stock Solution Container Size: 10 l
Flow Rate: 1 l/min
Number of Units to Treat: 1
Number of Days Stock Solution to Last: 10 days

B. Calculation Results
To Raise La by 0.05 mg/l:
La acetate stock solution: 0.18 g/l
- 10 l requires 1.77 g

Drip rate for stock: 0.7 ml/min
- Unit flow @ 1 l/min
- Stock lasts 10 days for 1 unit

Lanthanum at 100 µg/l

A. Data Required
Element Name: Lanthanum
Compound Name: Lanthanum acetate
Atomic Weight of Element: 138.91 (La)
Gram Formula Weight of Compound: 343.07 (La acetate)
Increase in Element Required: 100 µg/l = 0.1 mg/l
Solubility of Compound: 168.8 g/l
Stock Solution Container Size: 10 l
Flow Rate: 1 l/min
Number of Units to Treat: 1
Number of Days Stock Solution to Last: 10 days

B. Calculation Results
To Raise La by 0.1 mg/l:
La acetate stock solution: 0.36 g/l
- 10 l requires 3.56 g

Drip rate for stock: 0.7 ml/min
- Unit flow @ 1 l/min
- Stock lasts 10 days for 1 unit

Cerium at 50 µg/l

A. Data Required
Element Name: Cerium
Compound Name: Cerium acetate
Atomic Weight of Element: 140.12 (Ce)
Gram Formula Weight of Compound: 317.26 (Ce acetate)
Increase in Element Required: 50 µg/l = 0.05 mg/l
Solubility of Compound: 200 g/l
Stock Solution Container Size: 10 l
Flow Rate: 1 l/min
Number of Units to Treat: 1
Number of Days Stock Solution to Last: 10 days

B. Calculation Results
To Raise Ce by 0.05 mg/l:
Ce acetate stock solution: 0.16 g/l
- 10 l requires 1.62 g

Drip rate for stock: 0.7 ml/min
- Unit flow @ 1 l/min
- Stock lasts 10 days for 1 unit

Cerium at 100 µg/l

A. Data Required
Element Name: Cerium
Compound Name: Cerium acetate
Atomic Weight of Element: 140.12 (Ce)
Gram Formula Weight of Compound: 317.26 (Ce acetate)
Increase in Element Required: 100 µg/l = 0.1 mg/l
Solubility of Compound: 200 g/l
Stock Solution Container Size: 10 l
Flow Rate: 1 l/min
Number of Units to Treat: 1
Number of Days Stock Solution to Last: 10 days

B. Calculation Results
To Raise Ce by 0.1 mg/l:
Ce acetate stock solution: 0.32 g/l
- 10 l requires 3.24 g

Drip rate for stock: 0.7 ml/min
- Unit flow @ 1 l/min
- Stock lasts 10 days for 1 unit

Appendix 13. ANOVA table for mortalities observed in Experiment 6 during 4-week labelling period.

$R^2 = 0.722$

SOURCE	SS	DF	MEAN-SQUARE	F-RATIO	P
TRT	435.000	3	145.000	290.000	**0.003**
ERROR	1.000	2	0.500		

Appendix 14. ANOVA tables for lanthanum and cerium concentrations and amounts in the vertebral columns of fry and smolts labelled with lanthanum and cerium in Experiment 6.

1. ANOVA table of lanthanum and cerium concentrations in the vertebral columns of fry and smolts. $R^2 = 0.683$

SOURCE	SS	DF	MEAN-SQUARE	F-RATIO	P
TRT	0.258	2	0.129	8.009	**0.001**
STAGE	2.190	1	2.190	136.131	**0.000**
LN	0.005	1	0.005	0.301	0.585
STAGExLN	0.010	1	0.010	0.610	0.437
TRTxSTAGExLN	0.035	2	0.018	1.100	0.338
ERROR	1.158	72	0.016		

2. ANOVA table of lanthanum and cerium amounts in the vertebral columns of fry and smolts. $R^2 = 0.734$

SOURCE	SS	DF	MEAN-SQUARE	F-RATIO	P
TRT	2.350	2	1.175	0.278	0.758
STAGE	514.735	1	514.735	121.993	**0.000**
LN	0.414	1	0.414	0.098	0.755
STAGExLN	0.196	1	0.196	0.047	0.830
TRTxSTAGExLN	0.321	2	0.161	0.038	0.963
ERROR	303.796	72	4.219		

Note: LN = lanthanide

Appendix 15. ANOVA tables for lanthanum and cerium concentration in the otoliths of fry and smolts labelled with lanthanum and cerium in Experiment 6.

$R^2 = 0.533$

SOURCE	SS	DF	MEAN-SQUARE	F-RATIO	P
TRT	0.021	2	0.010	2.768	0.083
STAGE	0.081	1	0.081	21.373	**0.000**
LN	0.014	1	0.014	3.730	0.065
STAGExLN	0.000	1	0.000	0.093	0.763
TRTxSTAGExLN	0.005	2	0.002	0.598	0.558
ERROR	0.091	24	0.004		