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2001-2002 ANNUAL REPORT
IRON GATE HATCHERY STEELHEAD RESIDUALISM STUDY
PROJECT 2b-1

Prepared by

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Steelhead Research and Monitoring Program
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ABSTRACT

Since the early 1990s there has been a concern that the steelhead, *Onchorhyncus mykiss* irideus released from Iron Gate Hatchery may be residualizing in the Klamath River at a greater frequency than wild stocks or other hatchery stocks. Otoliths from 19 known age Iron Gate Hatchery origin steelhead were analyzed with a wavelength dispersive microprobe to determine the strontium/calcium ratio along a transect from the primordia to the outer edge of the of the otolith. Sudden increases in strontium/calcium ratios indicative of ocean entry were observed in eight of the nineteen otoliths. All of these samples were from brood year 1998. Eight of the samples showed no sign of ocean entry and three of the samples were not distinctly anadromous or resident fish. Our sample included examples of anadromous fish with both anadromous and non-anadromous mothers and non-anadromous fish with anadromous and non-anadromous mothers. Data collected from the steelhead returning to the hatchery included fork length, sex, scales, clips observed, clips applied and tissue samples for genetic analysis. From 9/9/01 through 6/29/02, a total of 617 steelhead entered the traps at IGH. The greatest number of fish (n=81) were trapped during the week ending 3/2/02 (Chart 1). A total of 229 scale samples and 225 tissue samples were collected.

[1] Steelhead Research and Monitoring Program report, available from: Department of Fish and Game, 50 Ericson Court, Arcata, California 95521 (707) 825-4850

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IGH Residualism Study 2002

Background

Residualism

Since the early 1990s there has been a concern that the steelhead, *Onchorhyncus mykiss* irideus released from Iron Gate Hatchery (IGH) may be residualizing in the Klamath River at a greater frequency than wild stocks or other hatchery stocks. Residualism occurs when steelhead do not out-migrate to the ocean but instead complete their life cycle in fresh water. The concern that this may be occurring with IGH steelhead is primarily due to the small number of these fish that appear in the Lower Klamath River catch. Limited scale analysis performed in 1993 by the California Department of Fish and Game (CDFG) indicated that of the 12 scale sets examined, only 3 could be interpreted as possessing any ocean growth patterns (Jong, 1994).

Otolith microchemistry has been effectively used to identify anadromous and resident *Onchorhyncus mykiss* (Kalish, 1990; Zimmerman, Reeves 2000). Otoliths are primarily composed of precipitated calcium carbonate and continue to grow throughout the life of a fish. Due to the similarities in ionic radius and valence of the calcium and strontium ions, strontium ions can be incorporated into the calcium carbonate structure of the otolith (Pollard, 1999). Strontium is substituted for calcium in proportion to its concentration in the water (Radtke, 1989), and it is present in much higher concentrations in seawater than in freshwater. An electron microprobe is used to measure the strontium and calcium levels in the otolith. The electron beam from the microprobe causes X-rays of characteristic wavelength for strontium and calcium to be emitted from the points sampled: the greater the rate of X-ray emission, the greater the concentration of the element in the sample. If the strontium/calcium (Sr/Ca) ratios are measured along a transect from the first part of the otolith formed (primordia) to the last area formed at the outer edge of the otolith, it is possible to determine if an individual fish is anadromous and if it is the progeny of an anadromous mother. Rapid increases in Sr/Ca ratios along the transect indicate entry into saltwater. Because the primordia are formed while an anadromous mother is still in the ocean, progeny of an anadromous mother will show a higher Sr/Ca ratio in the primordia than in the freshwater rearing portion of the transect.

The objectives of this study are to describe the characteristics of steelhead returning to IGH including run timing, age composition and fork length and to determine through the analysis of otolith microchemistry, if and to what extent residualism is occurring in IGH origin steelhead.

Methods

Sampling Returning Adults at Iron Gate Hatchery

In the fall of 2001 and the spring of 2002 we sampled the adult steelhead returning to IGH. As in previous years, returning steelhead were sorted by hatchery personnel according to their readiness to be spawned. Fish that were not ready were held in circular tanks at the spawning building and rechecked periodically, after spawning, S-RAMP personnel bio-sampled each fish. Data collected included fork length, sex, scales, clips observed, clips applied and tissue samples for genetic analysis. Otoliths were collected from mortalities.

Otolith Microchemistry Analysis
We collected otoliths in 2000, 2001 and 2002 from steelhead mortalities at Iron Gate Hatchery. Beginning with the steelhead reared in 1997 (brood year 1997) all of the steelhead released by IGH were clipped. Each brood year was assigned a unique clip; adipose, adipose left and adipose right respectively. Nineteen otoliths from known-age IGH steelhead and one wild steelhead from the Shasta River were selected for study.

One sagittal otolith was prepared for each fish in the sample. The otoliths were wet-sanded with 1200 grit paper to within ~ 5 microns of the primordia on the sulcus side. Final polishing was completed with a wet mixture of .05 micron alumina. Microchemistry analysis was performed at the Oregon State University Microprobe Lab using a Cameca SX-50 wavelength dispersive microprobe. A 14KV, 50 nA and a 7 micron beam were used for analysis.

Results
Returning Adults at Iron Gate Hatchery
From 9/9/01 through 6/29/02, a total of 617 steelhead entered the traps at IGH. The greatest number of fish (n=81) were trapped during the week ending 3/2/02 (Chart 1). A total of 229 scale samples and 225 tissue samples were collected. Table 1 shows the number of steelhead of each clip type in the sample and the clip assigned to each brood year. Charts 2a-d show the fork length distribution and mean size for each of the clip types used. The adipose- left maxillary clip was used in both 1998 and 2000.

Table 1. 2002 IGH Steelhead return sorted by clip type

Clip Observed	Number	Percent of Sample	Brood Year
Adipose clip	17	3.8	1997
Adipose clip, left maxillary	114	25.6	1998 and 2000
Adipose clip, right maxillary	253	56.8	1999
No clips	61	13.7	?/wild

Chart 1 IGH Steelhead run timing 2001-2002

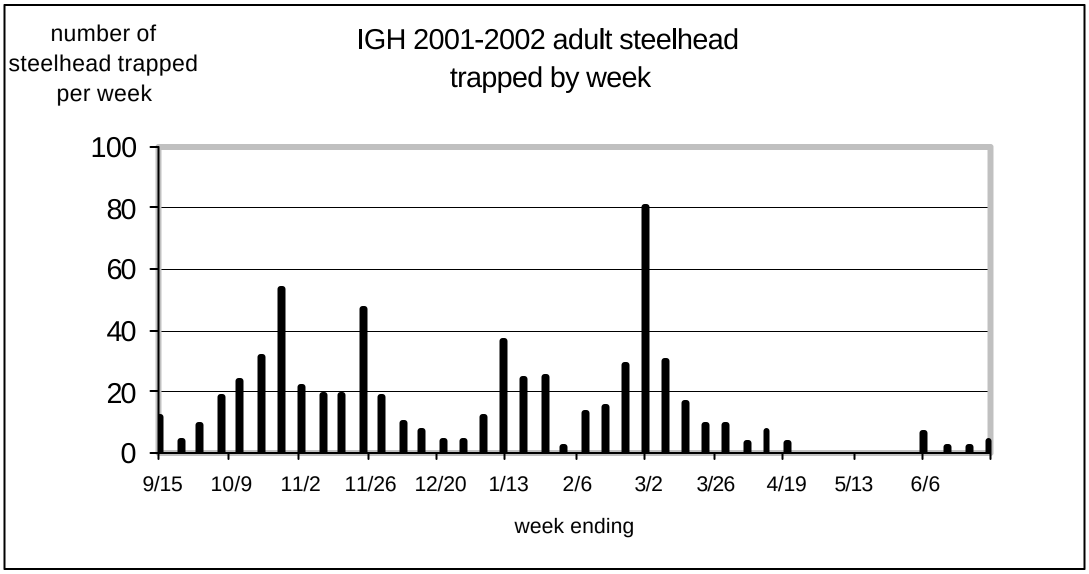
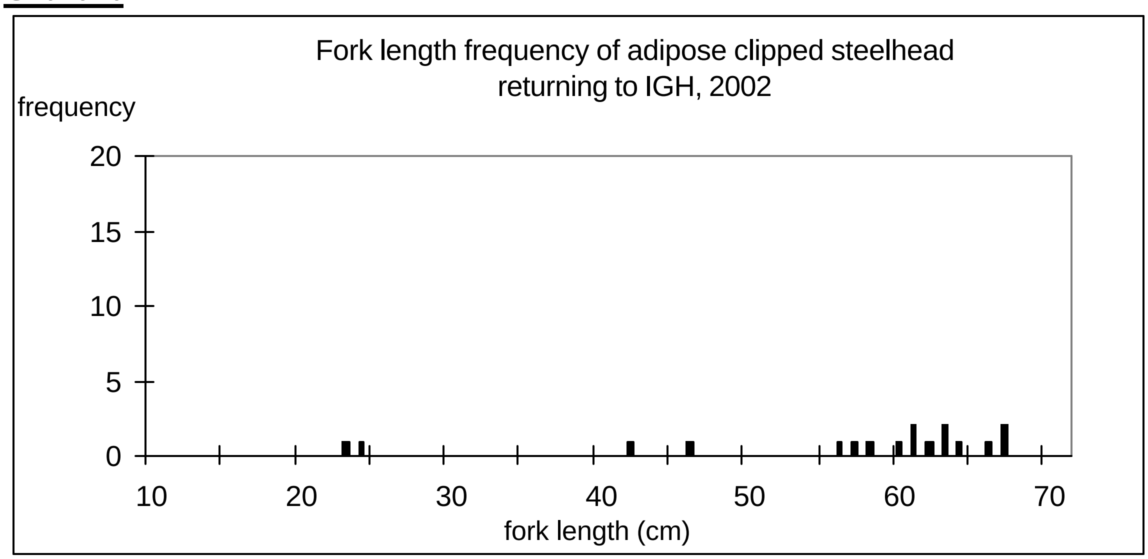


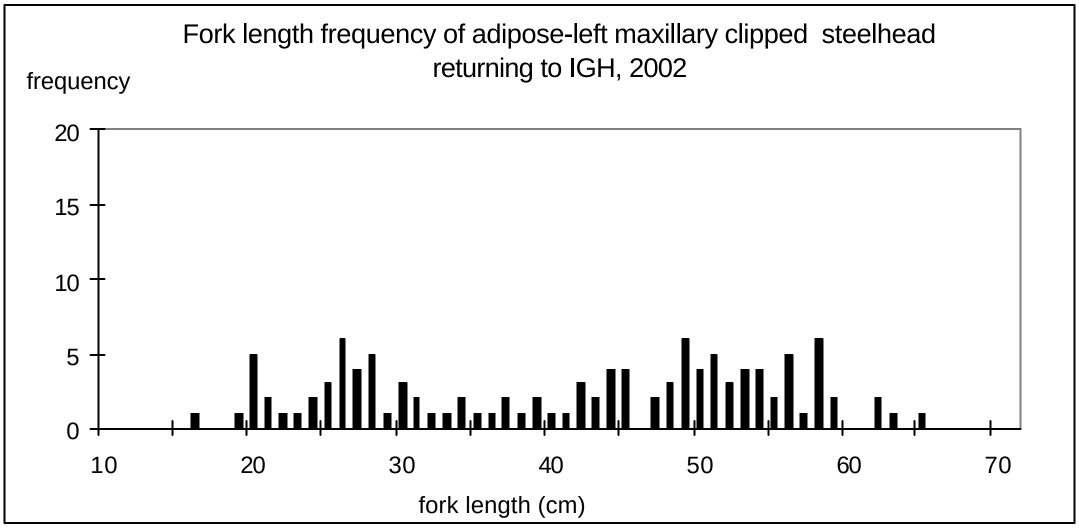
Chart 2a-d Fork length frequency of IGH steelhead by clip

Chart 2a



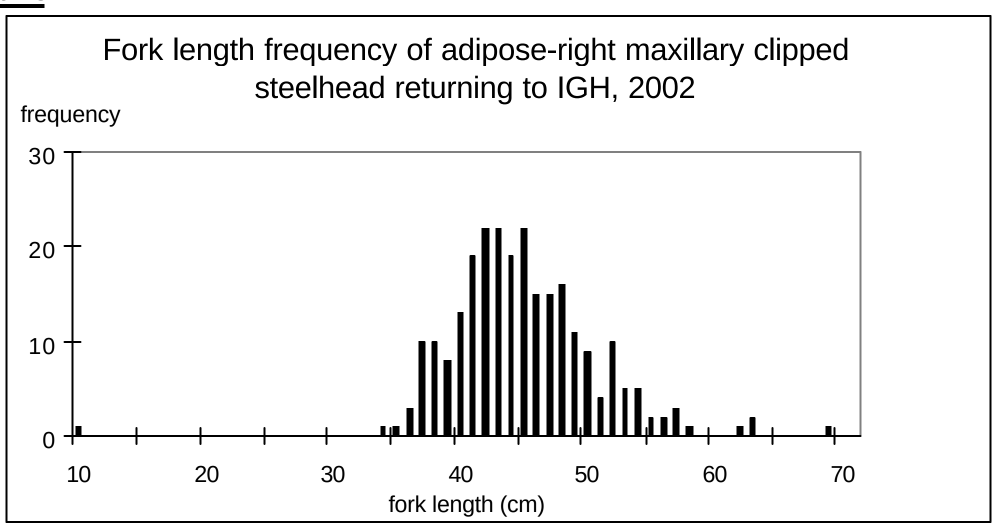
n=17, mean fork length=55.29

Chart 2b



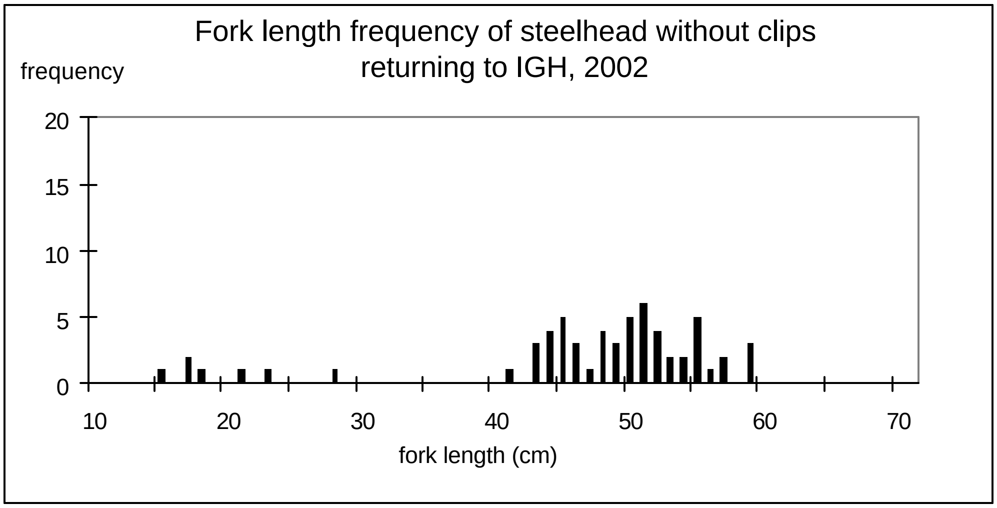
n=114, mean fork length=41.25

Chart 2c



n=253, mean fork length=44.92

Chart 2d



n=61 mean fork length= 46.44

Otolith Microchemistry Analysis

A total of nineteen otoliths from known-age IGH origin steelhead were prepared and processed. Eight of the nineteen otoliths showed rapid increases in the Sr/Ca ratio outside of the point estimated to be the first annulus at 700 microns (charts 3-10). This increase in Sr/Ca ratio occurred for over at least a 100 micron length of the transect and had maximum values greater than 2.0. This pattern is similar with steelhead that were determined to be anadromous from other California hatcheries (Donohoe, 2002). Eight of the otoliths showed little change in the Sr/Ca ratio and were judged to be non-anadromous (Charts 11-18). The remaining three samples had similarities to both the anadromous and the non-anadromous categories and were classified as intermediate (Charts 19-21). On 9/25/02 a wild adult steelhead mortality was found on the Shasta River weir. Chart 22 shows the Sr/Ca ratio for this fish. This fish appears to be anadromous.

Anadromous

Chart 3

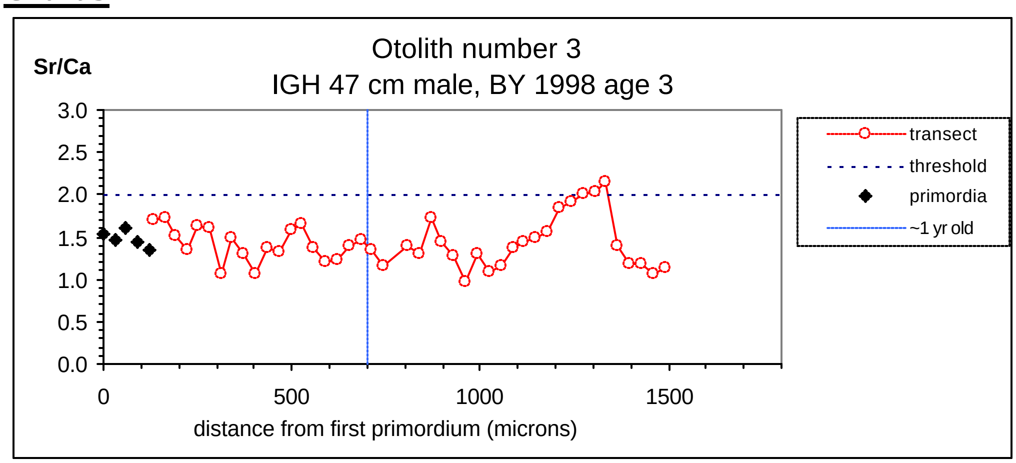


Chart 4

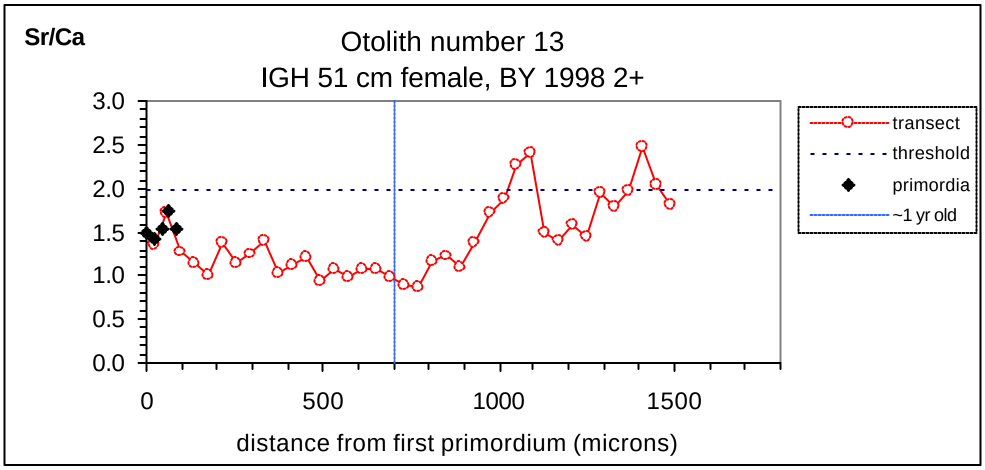


Chart 5

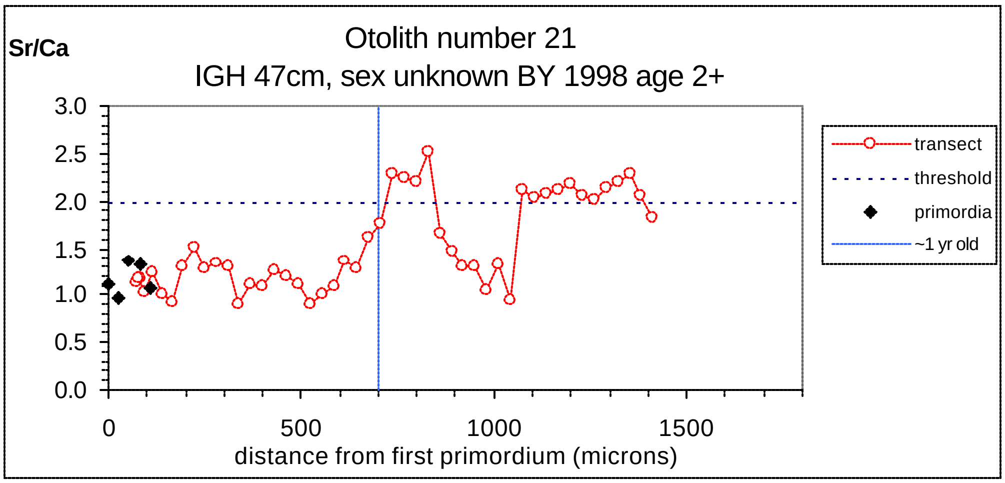


Chart 6

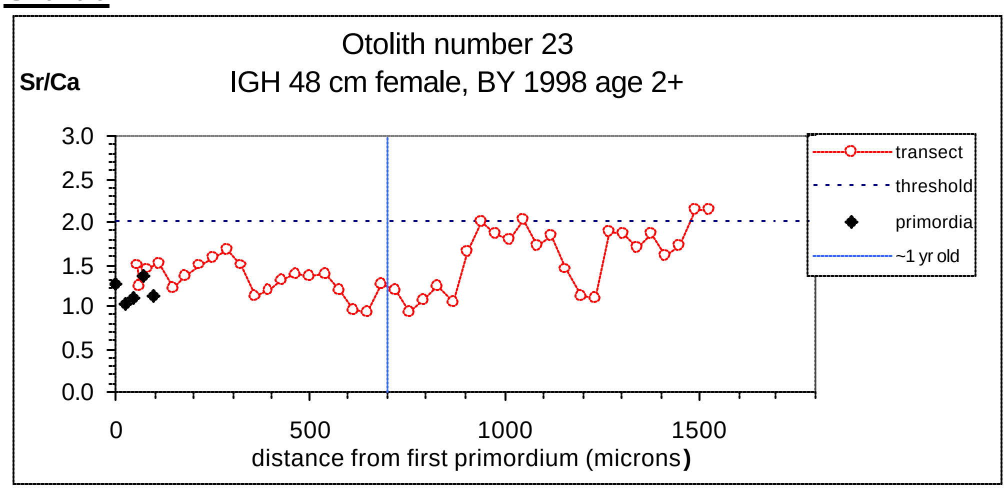


Chart 7

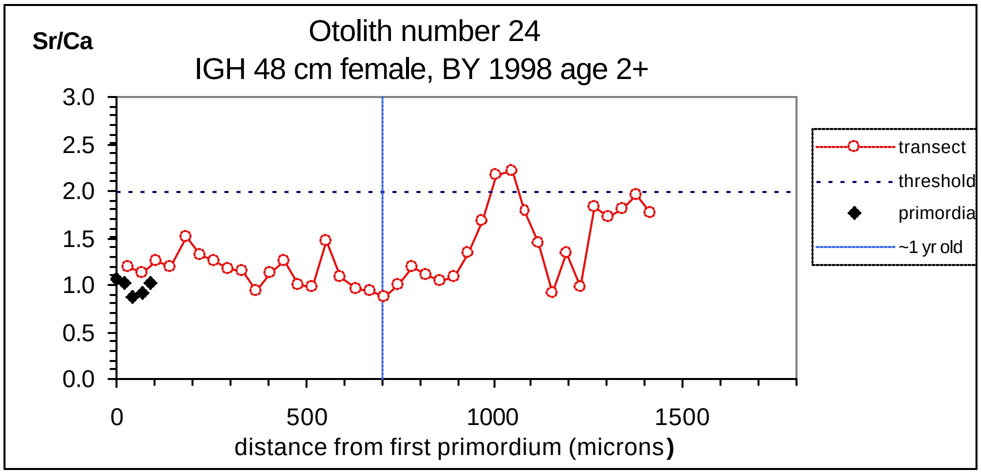


Chart 8

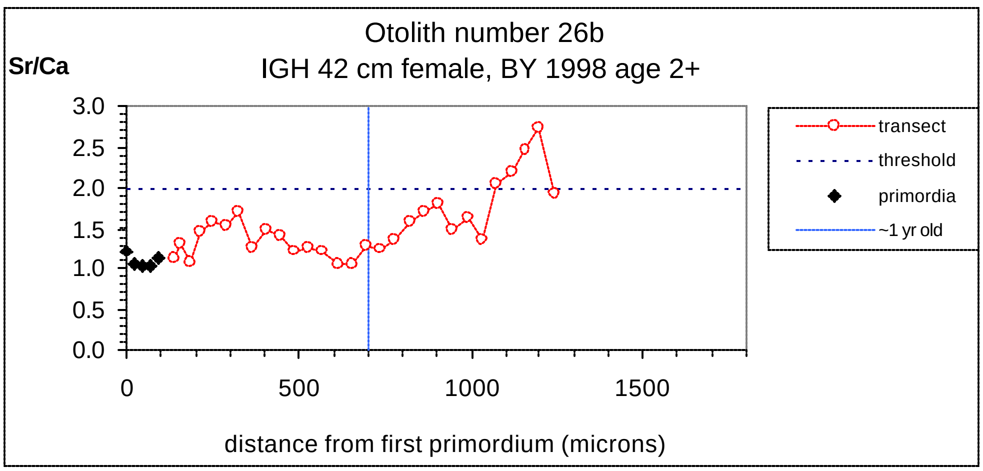


Chart 9

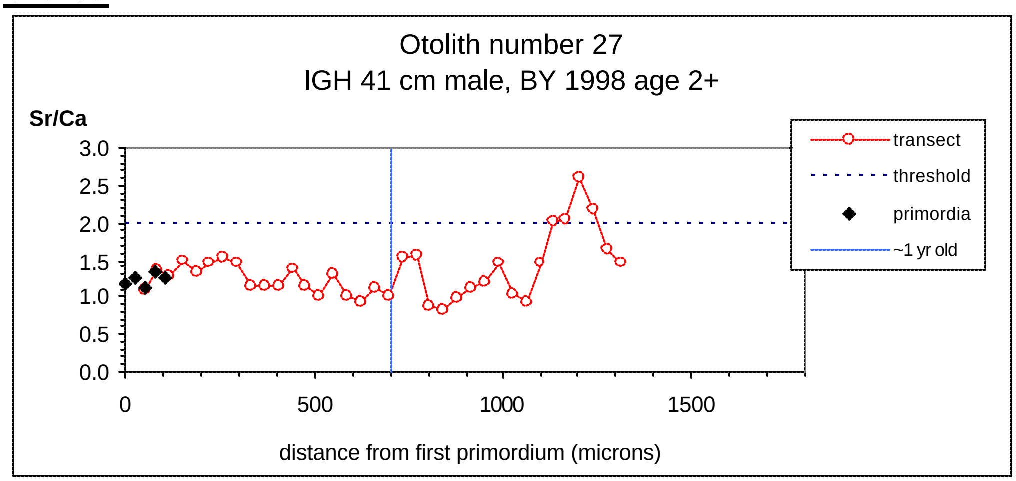
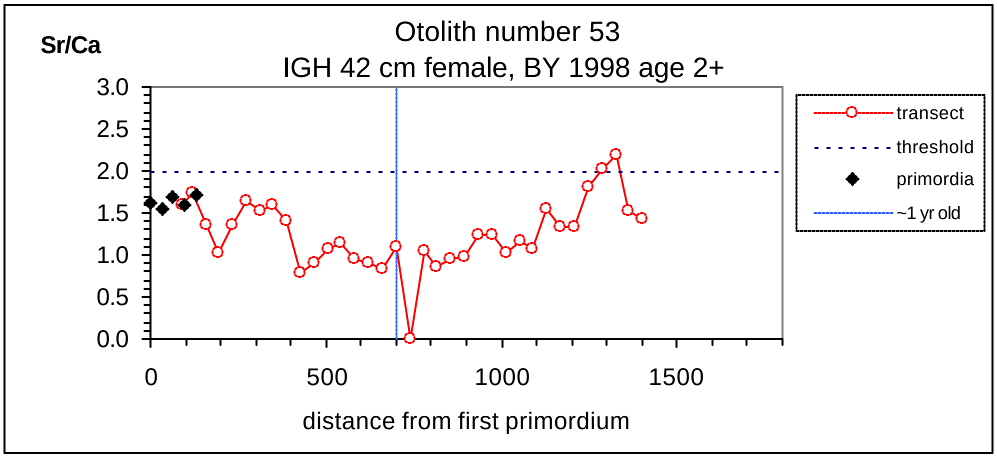


Chart 10



Non- Anadromous

Chart 11

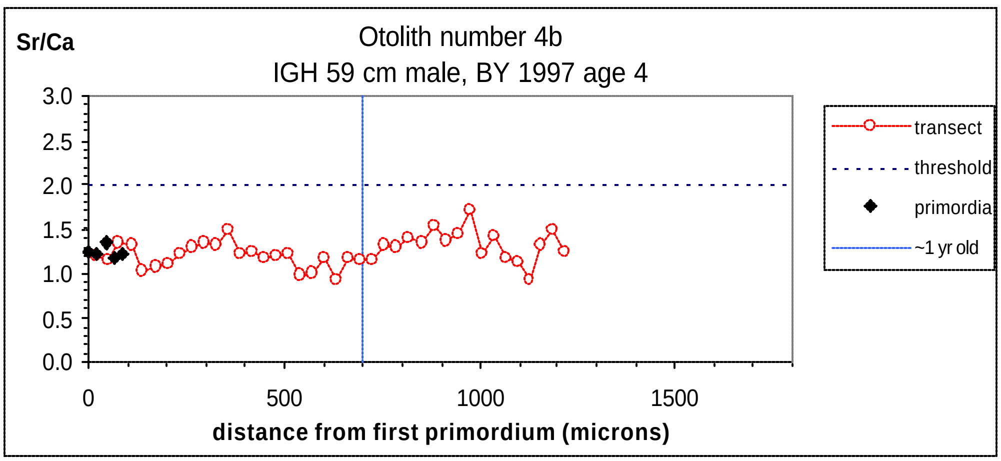


Chart 12

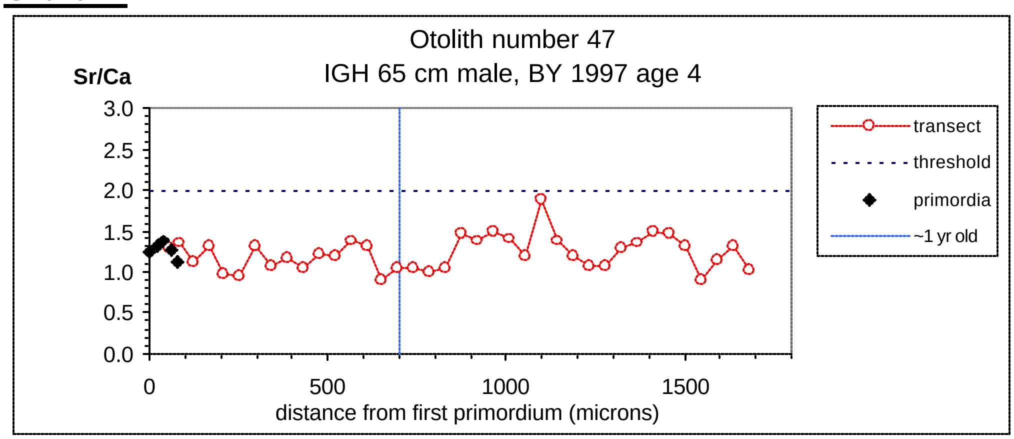


Chart 13

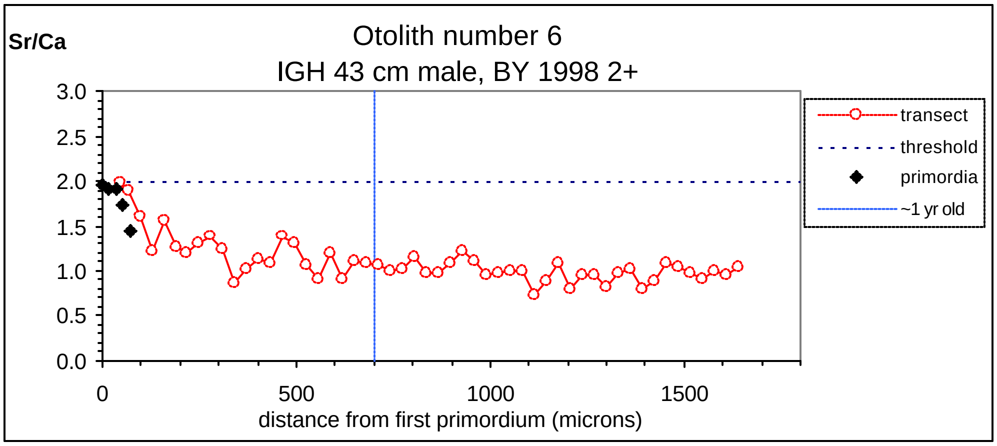


Chart 14

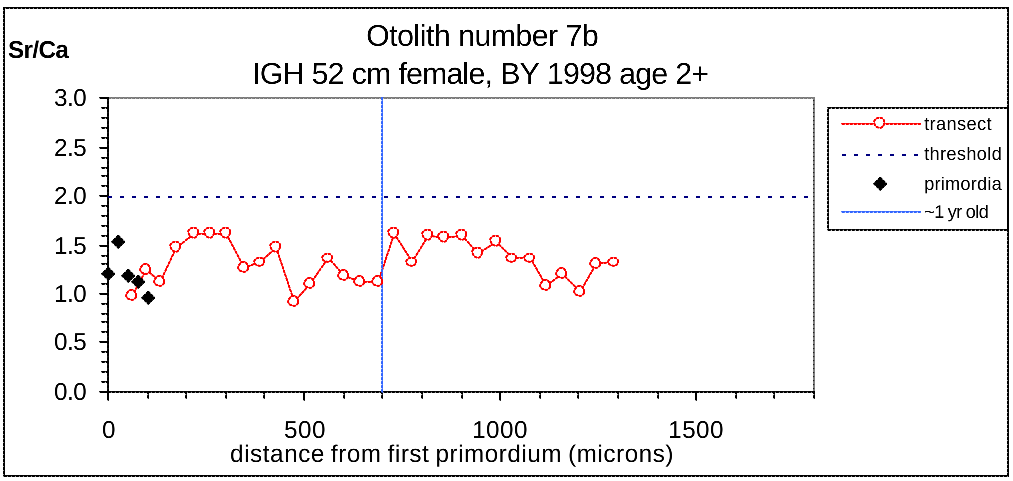


Chart 15

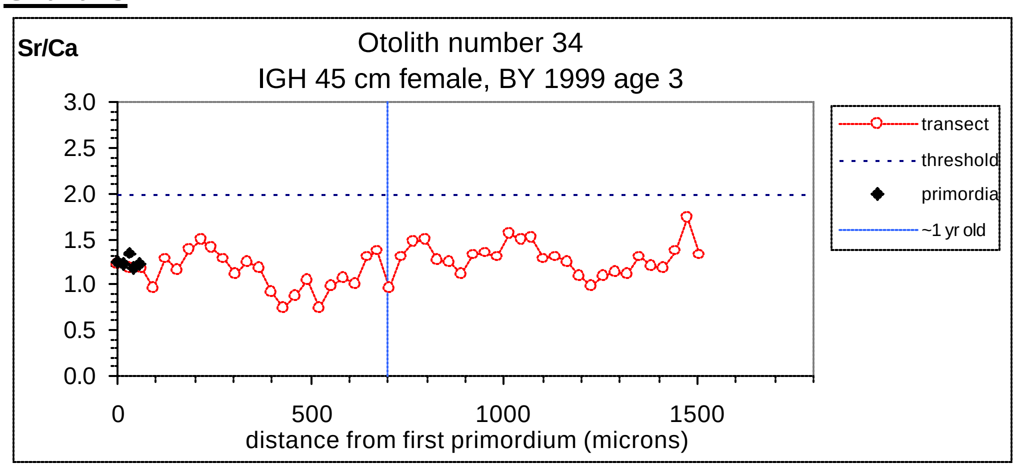


Chart 16

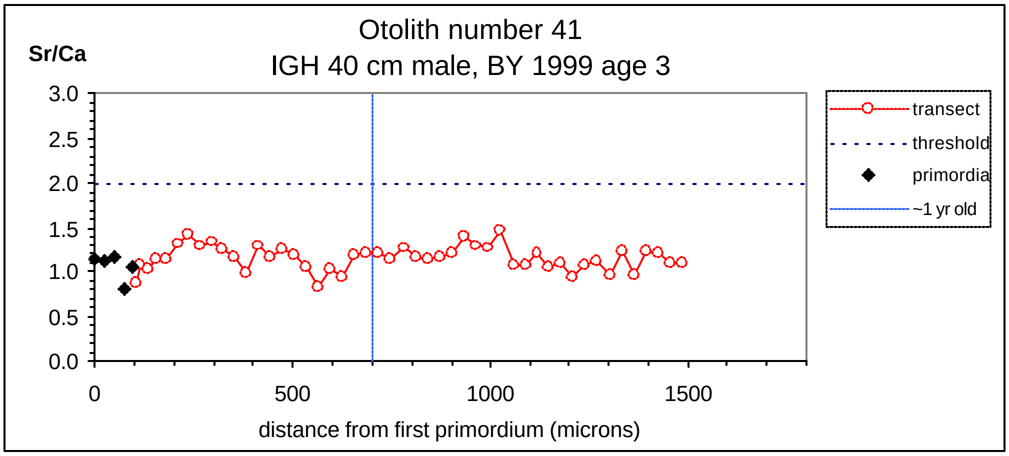


Chart 17

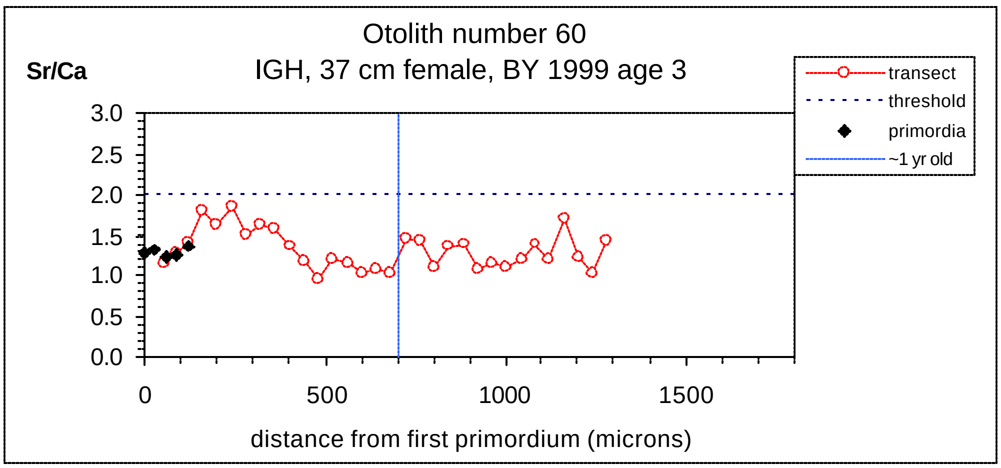
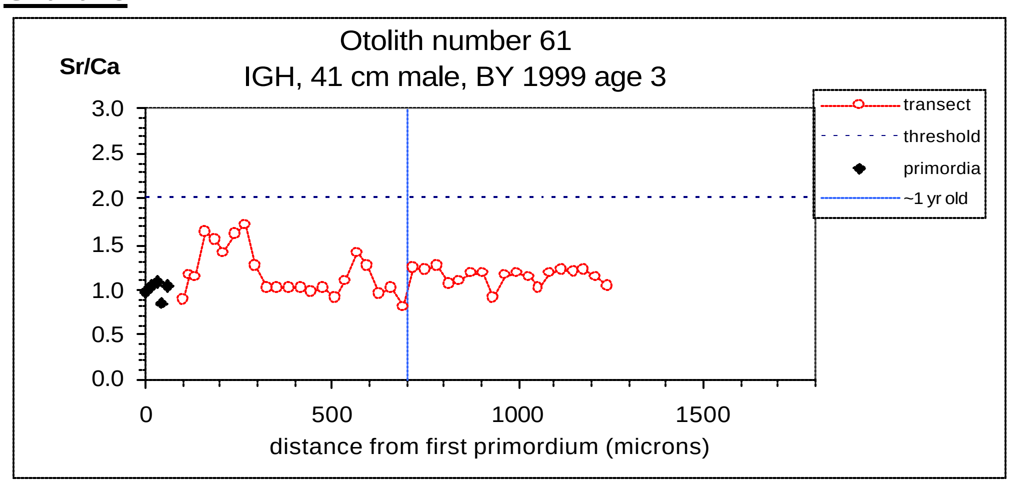


Chart 18



Intermediate

Chart 19

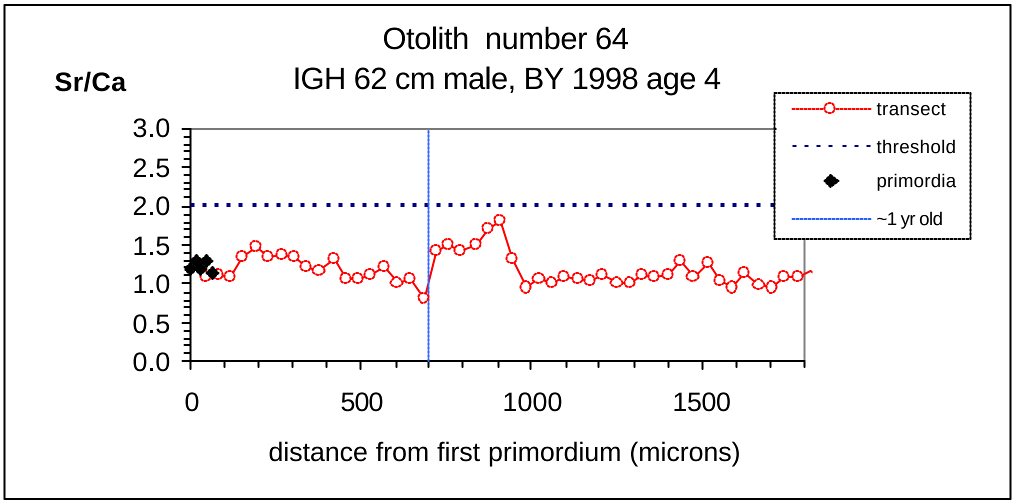


Chart 20

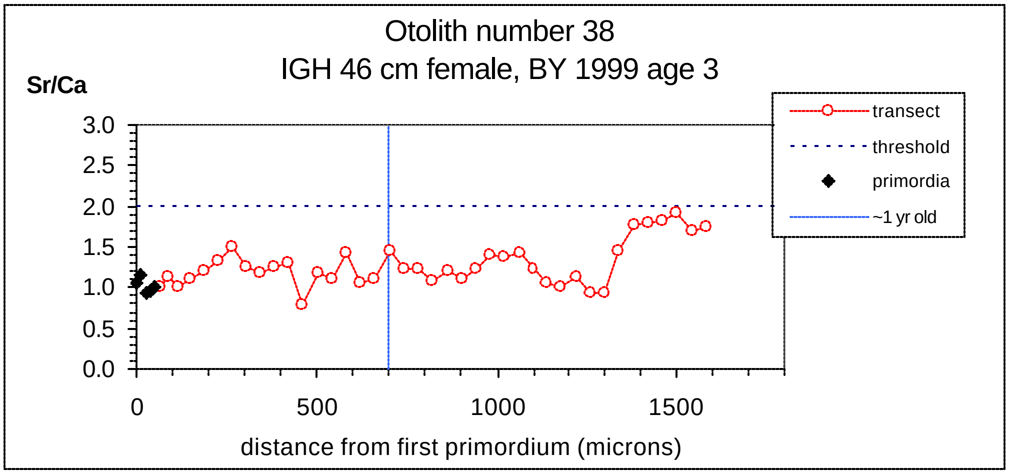
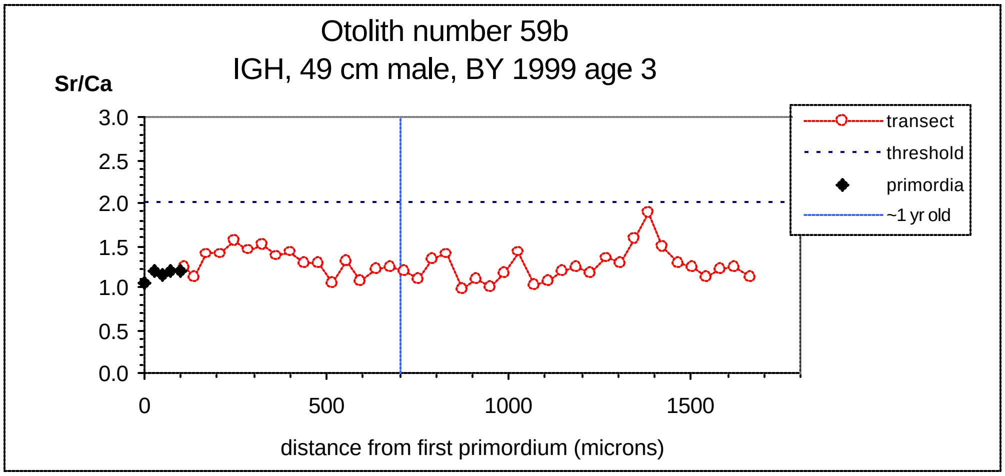


Chart 21



Wild Steelhead

Chart 22

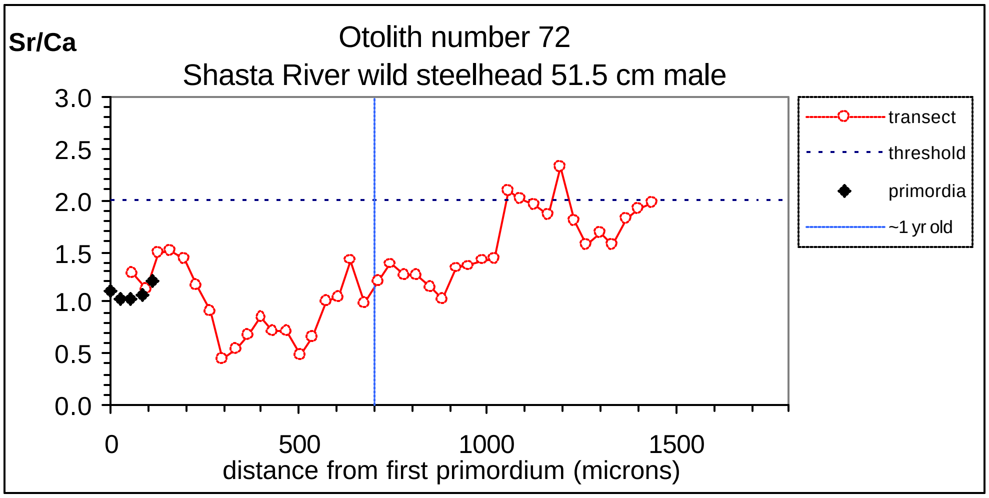


Table 2

Otoliths selected for microchemistry analysis were collected from nineteen IGH origin steelhead sorted by brood year (BY). All eight of the steelhead determined to have anadromous life histories were from (BY) 1998.

Otolith Number	Sex	Fork Length	Fin Clip Observed	Collection Date	Age by Clip	Brood Year	Life History Type
4	m	59	ad	3/22/01	4	1997	non-anadromous
47	m	65	ad	3/22/01	4	1997	non-anadromous
3	m	47	ad -lm	3/29/01	3	1998	anadromous
6	m	43	ad-lm	1/25/2001	2+	1998	non-anadromous
7	f	52	ad-lm	1/11/2001	2+	1998	non-anadromous
13	f	51	ad-lm	11/02/00	2+	1998	anadromous
21	n/a	47	ad-lm	10/09/00	2+	1998	anadromous
23	f	48	ad-lm	10/07/00	2+	1998	anadromous
24	f	48	ad-lm	10/07/00	2+	1998	anadromous
26	f	42	ad-lm	10/07/00	2+	1998	anadromous
27	m	41	ad-lm	10/07/00	2+	1998	anadromous
53	f	42	ad-lm	12/15/00	2+	1998	anadromous
64	m	62	ad-lm	3/22/02	4	1998	intermediate
34	f	45	ad-rm	3/11/02	3	1999	non-anadromous
38	f	46	ad-rm	3/11/02	3	1999	non-anadromous
41	m	40	ad-rm	3/28/02	3	1999	non-anadromous
59	m	49	ad-rm	3/28/02	3	1999	intermediate
60	f	37	ad rm	3/22/02	3	1999	non-anadromous
61	m	41	ad-rm	3/22/02	3	1999	non-anadromous

Chart 23.

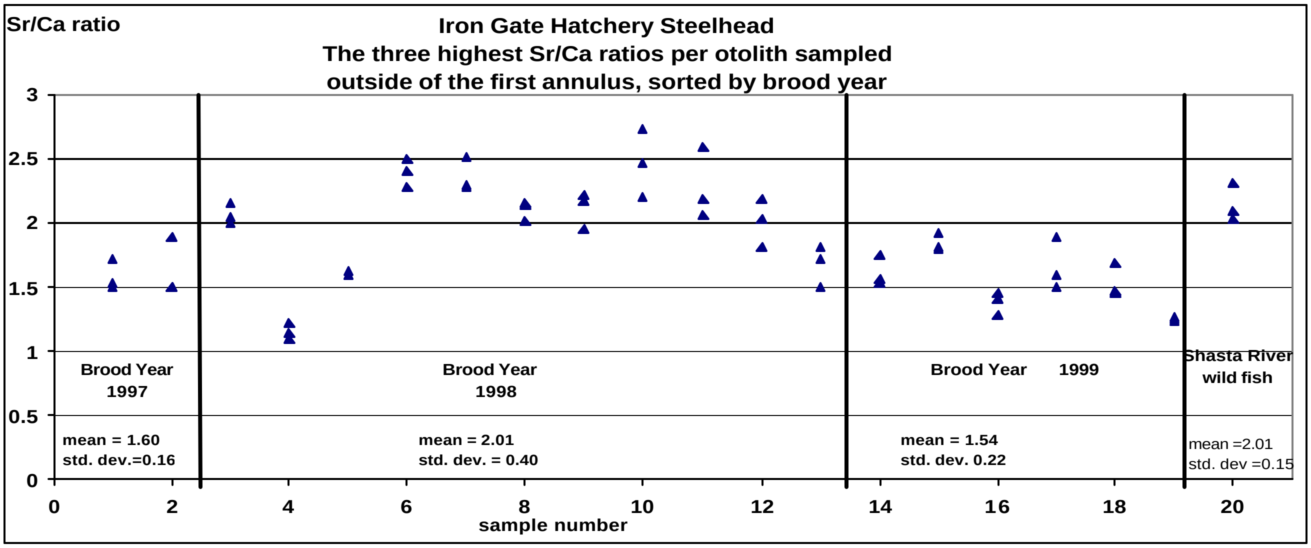


Chart 23 shows the three highest Sr/Ca ratios observed outside of the first annulus for each otolith in the sample. The otoliths are arranged by brood year.

Table 3 Comparison of mean Sr/Ca ratios for each brood year sampled

Brood Year	Mean Sr/Ca ratio	Standard Deviation	n=
97	1.60	0.16	2
98	2.01	0.40	11
99	1.54	0.15	6

Discussion

Scale analysis of Iron Gate Hatchery (IGH) steelhead in 1983 determined that 78.4% of the 119 fish sampled entered the ocean at age 2 (Hopelain, 1998). Since IGH releases steelhead as yearlings, it is expected that many of these fish will remain in fresh water for a year after release. For the purpose of this study we considered a fish to be residualized if it was sexually mature with no evidence of ocean entry.

A variety of life histories were observed in the nineteen IGH steelhead sampled. Based upon a comparison of the mean Sr/Ca values in the primordia and mean values outside of the estimated first annulus, our sample includes anadromous fish with anadromous mothers (example Chart 2), non-anadromous fish with anadromous mothers, (example Chart 5), non-anadromous fish with anadromous mothers (example Chart 11), and non-anadromous fish with non-anadromous mothers (example Chart 14).

Chart 4; anadromous mother, anadromous fish
mean Sr/Ca in the primodia, 1.558 mean Sr/Ca outside of 700 microns, 1.647

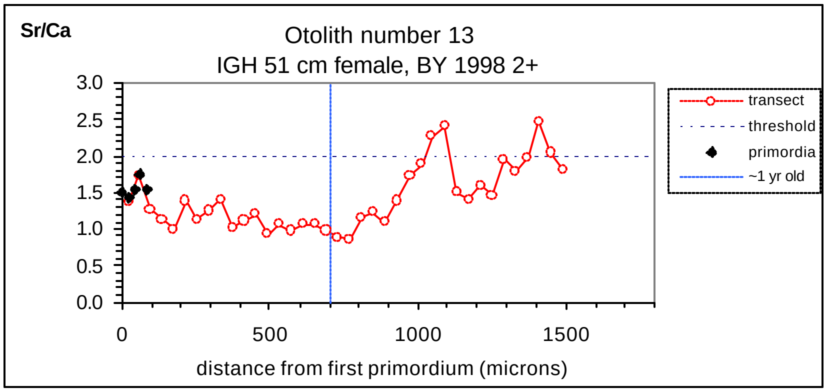


Chart 7; non –anadromous mother, anadromous fish
mean Sr/Ca in the primodia, 0.978 mean Sr/Ca outside of 700 microns, 1.460

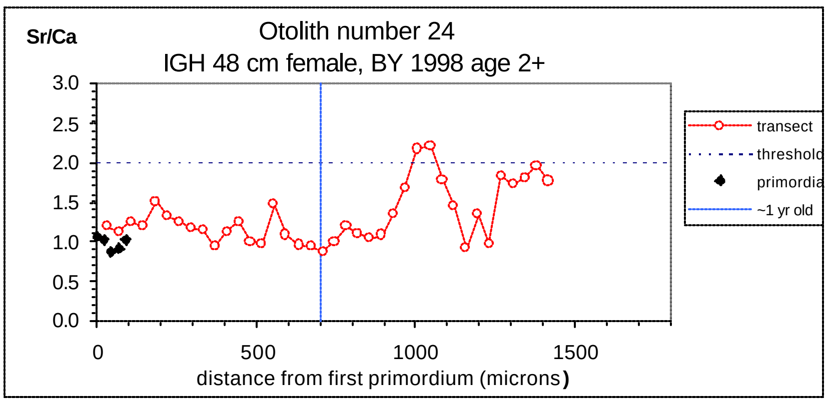
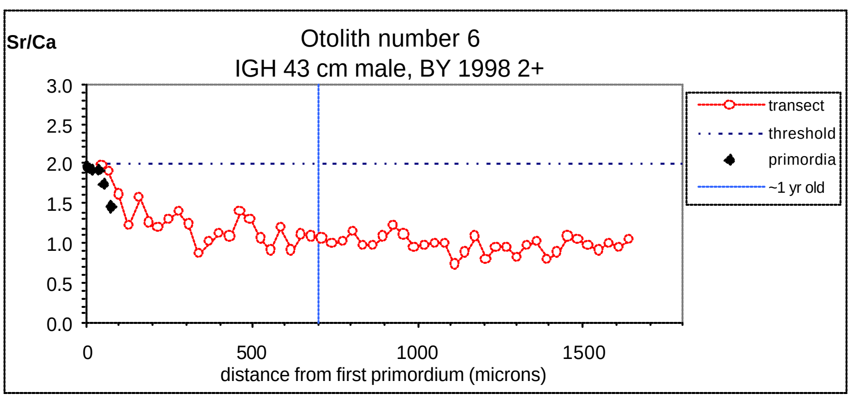
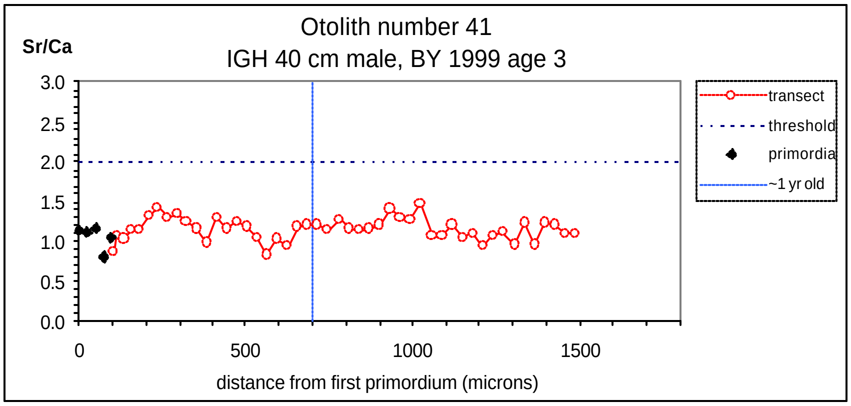


Chart 13; anadromous mother, non-anadromous fish
mean Sr/Ca in the primodia, 1.801 mean Sr/Ca outside of 700 microns, 0.974



<u>Chart16</u>; non-anadromous mother, non-anadromous fish
mean Sr/Ca in the primodia, 1.042 mean Sr/Ca outside of 700 microns, 1.151



Some otoliths in the sample show a substantial increase in the Sr/Ca ratio while the fish are still rearing in the hatchery (example Chart 15, 16). After removal from the incubators, fry are fed Bio Oregon Life Stage Diet for up to three months before switching to Silver Cup. (Philips, pers. comm.2003), This early diet may explain the Sr/Ca increase, since there are varying concentrations of strontium in different types and batches of feed.

The anadromous fish in the sample came from BY 1998. Our sample size for BY 97 and 99 is very small and needs to be increased to determine to what extent anadromous fish are present in other brood years. Numerous factors have been linked to the development of steelhead smolts, including size at time of release, diet, photoperiod, temperature, lunar phase and flow (Meehan and Bjornn 1991). Rearing practices were unchanged during the rearing of brood years 1997 through 1999 (Rushton, pers. comm. 2003) however, the flows in the Klamath River were different during the time each brood year was released (Charts 21, 22, 23). Size at time of release was also different for each brood year (Table 4).

Chart 24

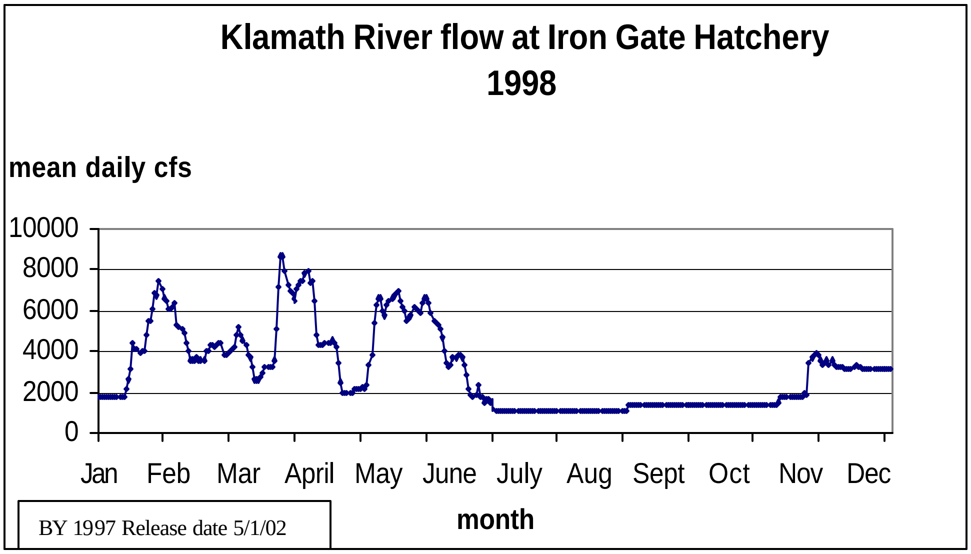


Chart 25

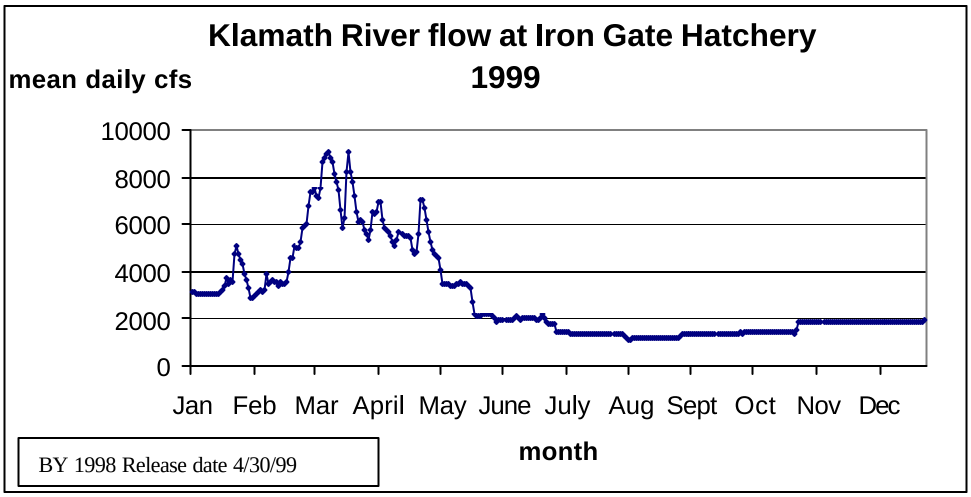


Chart 26

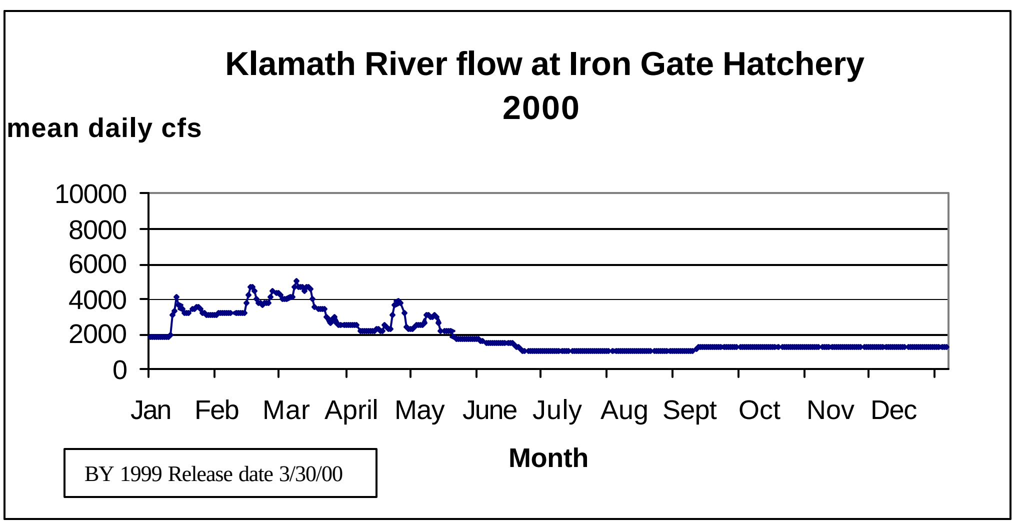


Table 4 Weight per pound, date of release and Klamath flow at release date

Brood Year	Date of release	Mean weight at release	mean flow for month of release	max flow cfs	min flow cfs
1997	5/1/98	10.2/ lb	5559 cfs	6950	2210
1998	4/30/99	12.0/ lb	3103 cfs	4790	2090
1999	3/30/00	14.0/ lb	2567 cfs	3930	2220

Recommendations

Increase the number of otoliths collected and analyzed from each brood year.

Collect otoliths from wild steelhead within tributaries to the Klamath to determine if IGH steehead have similar life histories as wild fish and to establish what are Sr/Ca levels are in the primordia of wild anadromous fish.

Determine the concentration of strontium in different Klamath River tributaries so as to be able to identify the origin and rearing location of steelhead from otolith analysis.

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<u>Personal Communication</u>

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