UPPER COLUMBIA UNITED TRIBES FISHERIES CENTER
FISHERIES TECHNICAL REPORT NO. 50. JULY 1994.

EVALUATION OF THYROXINE CONTENT AS AN INDICATOR OF IMPRINT TIMING IN JUVENILE BULL TROUT (*Salvelinus confluentus*)

AUTHORS:

HEATHER GALLOWAY
ALLAN SCHOLZ
JENNIFER HENDRICKSON
RONALD WHITE
MARY BETH TILSON
AND
WADE FREDENBERG

DEPARTMENT OF BIOLOGY
EASTERN WASHINGTON UNIVERSITY
CHENEY, WA 99004

ANNUAL REPORT SUBMITTED TO: COEUR D'ALENE TRIBE, PLUMMER, ID, KALISPEL TRIBE, USK, WA AND U.S. FISH AND WILDLIFE SERVICE, CRESTON NATIONAL FISH HATCHERY, KALISPELL, MT

United States Department of the Interior

FISH AND WILDLIFE SERVICE
Creston Fish and Wildlife Center
780 Creston Hatchery Road
Kalispell, Montana 59901

UNITED STATES GOVERNMENT MEMORANDUM

DATE: 12/7/94

TO: Howard Johnson

FROM: Wade F.

SUBJECT: Bull Trout Report

As promised, here is a copy of the report on thyroxine levels and bull trout imprinting. Merry Christmas!

Wade

Wade Fredenberg

Evaluation of Thyroxine Content as an Indicator of Imprint Timing in Juvenile Bull Trout (*Salvelinus confluentus*)

Authors:

Heather Galloway
Allan Scholz
Jennifer Hendrickson
Ronald White
Mary Beth Tilson

Upper Columbia United Tribes Fisheries Center
Department of Biology
Eastern Washington University
Cheney, WA 99004

and

Wade Fredenberg

U.S. Fish and Wildlife Service
Creston National Fish Hatchery
780 Creston Hatchery Road
Kalispell, MT 59901

Upper Columbia United Tribes Fisheries Center
Fisheries Technical Report No. 50
July 1994

ABSTRACT

Whole body thyroxine content was monitored in two stocks of bull trout reared at the Creston National Fish Hatchery in Kalispell, MT to identify potential critical periods for olfactory imprinting. One stock (Lion Creek) was an adfluvial stock which migrated from Swan Lake approximately 80 km up the Swan River into Lion Creek. The other stock (Holland Lake) migrated from Holland Lake into a small inlet tributary, with a migration barrier located about 400 meters upstream from the mouth. Eggs and alevins were incubated in a Heath tray incubator in a chilled water supply (3.0 - 3.4°C). Fry were reared at 4.5°C. Results indicated that thyroxine content of bull trout from Holland Lake was relatively low and stable from fertilized egg to swimup life stages (range 1.5 to 3.4 ng/g body weight). Thyroxine content peaked in post-swimup fry (223 days post fertilization) at 5.1 ng/g body weight and declined thereafter (2.4 ng/g body weight at 237 days post fertilization). Thyroxine content of bull trout from Lion Creek was also relatively low and stable from fertilized egg to swimup life stages (range 1.8 to 3.5 ng/g body weight), except for a brief transitory increase observed in pre-swimup alevins at 183 days post-fertilization when thyroxine content increased to 4.3 ng/g body weight. After declining to 1.8 ng/g body weight at swimup, thyroxine content peaked a second time at 7.6 ng/g body weight in post-swimup fry (224 days post-fertilization) and declined thereafter (3.9 ng/g body weight at 238 days post fertilization). Thus, if thyroxine content is an accurate indicator of the time of imprinting in bull trout, our results indicated that the critical period may be near 223 to 224 days post-fertilization at these incubation and rearing temperatures. However, since thyroxine content was relatively low even during peak periods compared to other species (*e.g.*, kokanee) we have tested, we recommend that monitoring be continued until the smolt stage.

ACKNOWLEDGMENTS

Fish collection for this project was supported by the U.S. Fish and Wildlife Service (USFWS). Laboratory work for this project was supported by a U.S. Congressional Appropriation to the Portland Area Office of the Bureau of Indian Affairs (BIA), to fund the operation of the Upper Columbia United Tribes Fisheries Center at Eastern Washington University. Capital equipment was provided by Eastern Washington University (EWU). The work reported here was conducted as H. Galloway's Senior Research Project in the Biology Department at EWU.

Disclaimer: Use of brand names in this report does not imply endorsement by USFWS, BIA, EWU or the authors.

TABLE OF CONTENTS

1.0 INTRODUCTION

Historically, bull trout (*Salvelinus confluentus)* was a native species, abundant in Coeur d'Alene Lake and the Pend Oreille River. For example, bull trout weighing 20-30 pounds were frequently caught in winter and early spring in Coeur d'Alene Lake (Scott 1968). The first fisheries survey conducted in the Pacific Northwest by the U.S. Fish Commission indicated that bull trout were abundant in the Pend Oreille River (Gilbert and Evermann 1894). However, dam construction and habitat degradation, due to logging, agricultural practices, grazing and mining, have caused bull trout populations to decline significantly at both locations. The bull trout was petitioned for listing as an Endangered Species throughout its historical range in the United States in 1993.

The Coeur d'Alene and Kalispel Tribes are involved in the restoration of this native species in Coeur d'Alene Lake and the Pend Oreille River by restoring habitat and constructing low capital hatcheries to enhance bull trout populations. Since the objective is to utilize hatchery outplantings to restore natural spawning in tributaries formerly occupied but now devoid of bull trout, it will be necessary to develop stocking procedures that ensure the eventual return of fish to stocking sites that are suitable for natural reproduction. Since most species of salmonids home to natal spawning sites, a process that is connected with chemical imprinting at a juvenile life stage (Hasler and Scholz 1983), it will require that hatchery outplanting occur before this critical period so the fish can learn the cues to identify its adopted home stream. Therefore this study was initiated to provide information about the critical period for imprinting in bull trout. Hasler and Scholz (1983) and Tilson *et al.* (in press) have previously shown that the critical period for olfactory imprinting in coho salmon (*Oncorhynchus kisutch*), steelhead trout (*O. mykiss*) and kokanee salmon (*O. nerka*) was induced by thyroxine surges. The critical period for coho and steelhead was at the smolt stage. The primary critical periods for kokanee were from hatch to swimup, with a second period at the smolt stage. The objective of the present study was to determine the thyroxine (T_4) concentrations measured in egg, larvae (alevin), and fry stages of bull trout as an indicator of the critical period for imprinting.

Since there is not presently a bull trout hatchery on either the Coeur d'Alene or Pend Oreille systems, bull trout from Lion Creek stock and Holland Lake stocks in the Swan River portion of the Flathead River drainage, taken from the U.S. Fish and Wildlife Services Creston National Fish Hatchery in Kalispell, MT, were used for this investigation. The USFWS, in collaboration with the Montana Department of Fish, Wildlife, and Parks, and Confederated Salish and Kootenai Tribes of the Flathead Indian Reservation are also attempting recovery of the depleted stocks of bull trout from the Flathead River System (Fraley and Shepard 1989). Part of the recovery effort may involve reintroducing hatchery reared fish. Hence, they were also interested in ascertaining the time of imprinting in bull trout.

1.1 Life cycles of bull trout

Until 1978, bull trout and Dolly Varden (*Salvelinus malma*) were considered to be one species, the Dolly Varden trout. After further investigation they were classified as different species based on morphometric, meristic counts and osteological characteristics (Cavender 1978). Dolly Varden typically inhabit small coastal streams whereas bull trout are generally found further inland in tributary streams of large river systems. However, both species may occur sympatrically within the same river system. Moreover, both species are capable of interbreeding with each other and may spawn multiple times during the course of their life cycle.

Bull trout display three distinct life history patterns. They are:

1. Resident, which inhabit headwater streams, do not migrate, and are normally isolated by a physical barrier. Resident bull trout are smaller, have lower fecundity, and mature at an earlier age than other stocks of bull trout. They may retain juvenile parr marks (Scott and Crossman 1979).

2. Fluvial, which inhabit large streams and main rivers, and migrate from main river into natal tributaries to spawn.

3. Adfluvial, which inhabit large lakes or reservoirs and migrate up main rivers into smaller streams to spawn. They typically rear 1.5

to 2.5 years in the nursery tributary before emigrating to the lake and mature after 2-3 years in the lake before returning to spawn.

In the present study, the Lion Creek stock were considered adfluvial since they move out of Swan Lake, and migrate up the Swan River approximately 80 kilometers into Lion Creek. The Holland Lake trout migrate from Holland Lake into a small inlet tributary which contains a migration barrier 400 meters upstream from the mouth. After hatching, fry are believed to migrate to Holland Lake in the first few months. Therefore the Holland Lake stock are unique and cannot be classified as resident, fluvial or adfluvial since they are basically resident to the lake.

It is well documented that bull trout prefer to spawn at locations where either ground water seepage or smaller tributaries enter their spawning channel (Fraley and Shepard 1989, Martin *et al.* 1992). Thus, these types of spawning locations are ideally suited to provide developing embryo and juvenile offspring with distinctive odor cues that identify the natal spawning area. In bull trout investigations conducted in southeastern Washington streams, we noted that bull trout used the same spawning locations, associated with water seeping in from the stream bank, for three years in a row, but were not able to confirm if the same fish repeatedly used the same spawning sites since the fish were not individually tagged (Martin *et al.* 1992, Underwood *et al.* 1994.). Consequently, we conducted an extensive literature search to determine if bull trout exhibit natal homing. Our hope was that tagging investigations conducted with transplanted fish could help to pinpoint the critical period for imprinting. We were not able to locate any tagging investigations to indicate that bull trout actually home to their natal streams to spawn. However, tagging studies in southeastern Alaska revealed that adult Dolly Varden exhibited both in-season and repeat homing between seasons to the same spawning tributary (Armstrong 1967,1974). In-season displacement experiments conducted in 1967 and 1968 indicated that many large fish (mature) and a few small ones (immature) returned in the same year to the stream of release. Some of the fish tagged in 1967 were recovered that year, as well as during spawning migrations in subsequent years, in the same stream in which they were originally tagged.

Another study of anadromous Dolly Varden migrating from the sea into Hood Bay Creek, Admiralty Island, Alaska, indicated natal homing tendencies (Armstrong 1974). Most Dolly Varden that spawned in the creek had been marked there previously as smolts or as spawners, and none were observed to spawn in a stream other than the stream of origin. Out of the finclipped smolts that were transplanted in May and June 1969 from different (North Arm) streams into Hood Bay Creek, 10.5% returned to Hood Bay Creek to spawn. This was almost identical to the 9.4% of native fish that had been trapped and tagged in Hood Bay Creek as smolts, returning to the creek as adults. Only four spawners out of 1184 transplanted smolts were captured at weirs on the North Arm streams. Thus, Armstrong suggested that imprinting in Dolly Varden occurred sometime during the last day of smolt migration since the transplanted smolts were held in the creek for roughly 24 hours before release. Dolly Varden investigations in Chiniak Bay, Alaska also suggested the return of some fish to their releasing grounds (Marriott 1966). Tagged fish, transplanted into three different recipient streams as smolts, all returned to the stream where they were released as adults spawners (Marriott 1966). We suspect that bull trout are similar to Dolly Varden in their homing tendencies and imprint timing since both species are similar genetically and behaviorally.

2.0 MATERIALS AND METHODS

Bull trout eggs, alevins and fry, were obtained from the Creston National Fish Hatchery (U.S. Fish and Wildlife Service) located in Kalispell, MT. Samples were collected from September 1993 to May 1994 (0-238 days post-fertilization). Eggs from the 6 mated pairs (3 pairs from Holland Lake and 3 pairs from Lion Creek) were incubated in hatchery well water at the Creston Hatchery in a Heath tray incubator with a chilled water supply at 3.2°C (range 3.0 - 3.4°C). Two lots of zero-aged Lion Creek bull trout embryos were fertilized on September 19, 1993 and one lot was fertilized on September 20, 1993. All three lots of Holland Lake stock were fertilized on September 21, 1993. After the late alevin stage, fish were reared in hatchery well water at 4.5°C in two 283 liter, rectangular fiberglass rearing tanks (10 ft. long x 2 ft. wide x 0.5 ft. deep, and painted light blue). One tank was stocked with 1000 swimup bull trout fry from Holland Lake, the other with 1000 bull trout from Lion Creek. Stocking density was approximately 3.5 fry/L. Each tank was partially covered with corrugated fiberglass to simulate filtered natural lighting conditions. Artificial (fluorescent) lights were kept on to increase light intensity during the hours of 7:30 AM to 4:00 PM. A small window allowed natural lighting to maintain the natural photoperiod. The fish were fed Biodiet semimoist biotrainer feed which was administered by automatic belt feeders positioned overhead. The Holland Lake fish were fed during subdued light and darkness (8:00 PM - 4:00 AM) and the Lion Creek fish were fed during intense daylight hours (7:30 AM - 4:00 PM).

Thyroxine (T_4) levels were monitored in Holland Lake stock fish and Lion Creek stock fish at approximately two-week intervals. Mean whole body T_4 content ± SEM (ng/g body weight) was determined in 15 fish (0 - 160 days post-fertilization), 5 fish (174-216 days post-fertilization) and 10 fish (224-238 days post-fertilization) from each mated pair of each stock at approximately bi-weekly intervals. Offspring from each mated pair were collected at each interval. Fish samples at different life stages were collected by U.S. Fish and Wildlife Service personnel. Samples were collected from different egg stages, alevins, swimup fry and post-swimup fry. The time of swimup was difficult to define in both stocks. Thus, the determination of swimup was somewhat arbitrary and not well defined based on the behavior of the fish. Samples were weighed on an

analytical balance (0.1 mg) and flash frozen in an ultracold (-80°C) freezer. They were stored at -80°C until a sufficient number were obtained to ship to Eastern Washington University (EWU) on dry ice via overnight Federal Express mail. Thyroxine was extracted from the samples and thyroxine content determined by investigators at the Upper Columbia United Tribes Fisheries Center, located in the Biology Department at EWU.

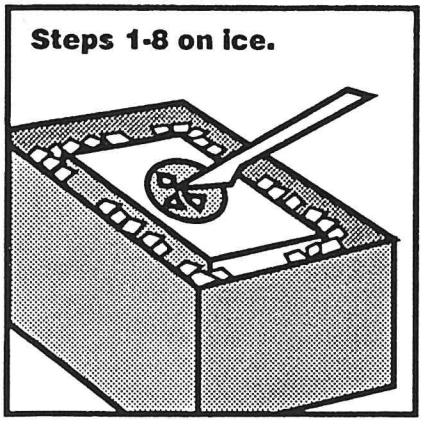
2.1 T_4 Extraction

Thyroxine was extracted from eggs, larvae and fish and reconstituted using a modification of methods from Kobuke *et al.* (1987) and Parker (1988) (Figure 1). Frozen samples were minced and placed into individual 15 x 85 mm boroscillicate glass test tubes with ice cold ethanol (ETOH) containing 1 mM 6-N-propyl-2-thiouracil (PTU; Sigma) at a µl volume equal to twice the mg weight of the fish. Propylthiouracil prevented degradation of T_4 by blocking its enzymatic conversion to triiodothyronine (T_3). Each fish was homogenized for 20 seconds with a Brinkman model Polytron 3000 tissue homogenizer at 20,000 RPM. Following homogenization, each sample was vortexed for five seconds and poured into a centrifuge tube. The test tube was then rinsed at a µl volume equal to the mg weight of the fish and homogenized for an additional 20 seconds at 20,000 RPM. The polytron blade was rinsed with tap water and 95% ETOH between every sample. Samples were vortexed for 5 seconds and added to the centrifuge tube. All the above procedures were performed on ice. Samples were centrifuged for 10 minutes at 3000 RPM at 4°C using a Dynac refrigerated centrifuge (Clay Adams, Inc.). The supernatant was poured into a 10 ml drying tube and the pellet resuspended in 100% ETOH at a µl volume equal to 1.5 times the mg weight of the fish, and vortexed for 10 seconds. This solution was again centrifuged at 3000 RPM at 4°C and the supernatant combined with the supernatant in the drying tube. Samples were dried in a vacuum oven at 60°C at 25 psi for 4-18 hours. The dried samples were stored at 0°C until T_4 content could be assayed.

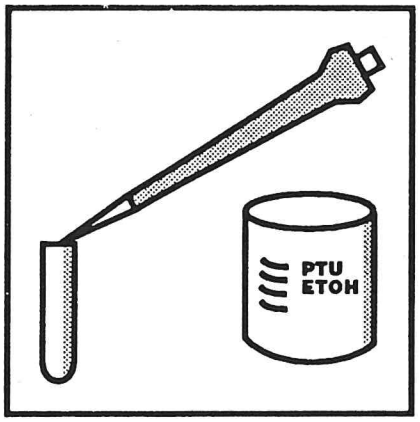
2.2 T_4 Radioimmunoassay

A Diagnostic Products Inc., T_4 radioimmunoassay kit was used for determining T_4 concentrations. Dried samples from fish were reconstituted in

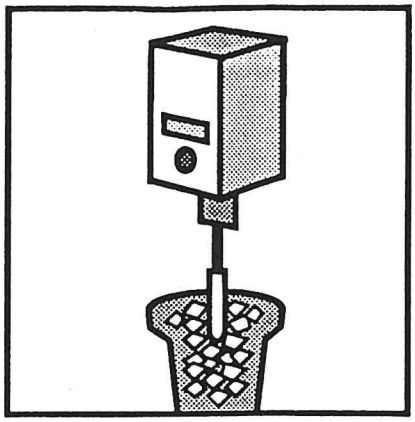
Figure 1. Procedures used for extracting and storing T_4 from bull trout samples.



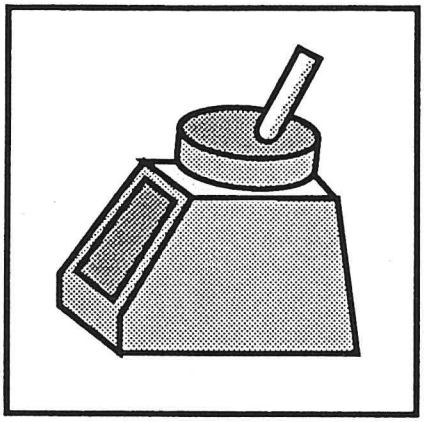
1 Mince sample with scalpel in weighing boat on frozen cutting block. Put into 15 x 85 mm test tube.



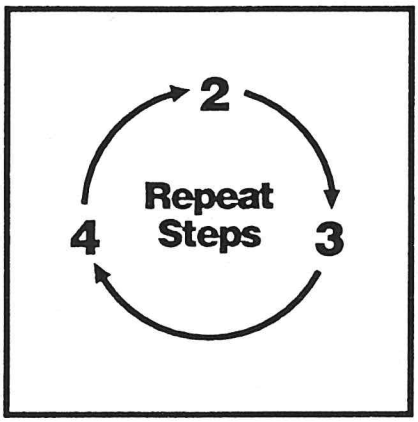
2 Add ice cold 100% ethanol containing 1.0 mM PTU [ETOH-PTU] to tube at a ul volume equal to 2 X the weight of the fish.



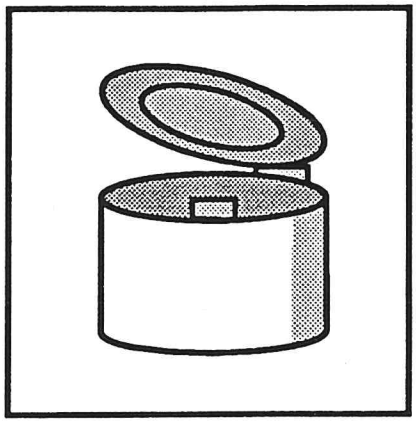
3 Homogenize for 20 sec to 1 min at 20,000 RPM using Brinkman Instrument Polytron 3000 tissue grinder.



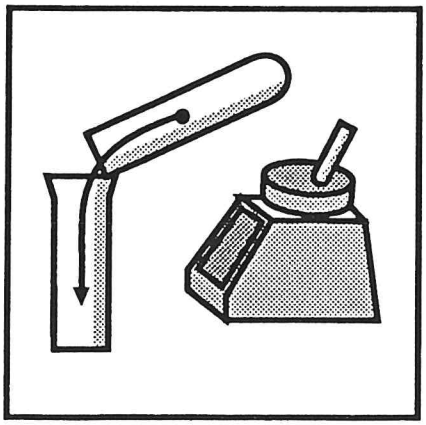
4 Vortex samples for 5 sec using a VWR Vortexer 2 and decant into centrifuge tube.



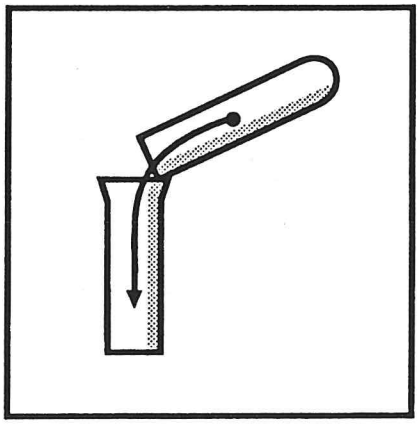
5 Repeat steps 2, 3 and 4 except use a volume of ETOH-PTU equal to 1 X weight of the fish, then vortex for 10 sec.



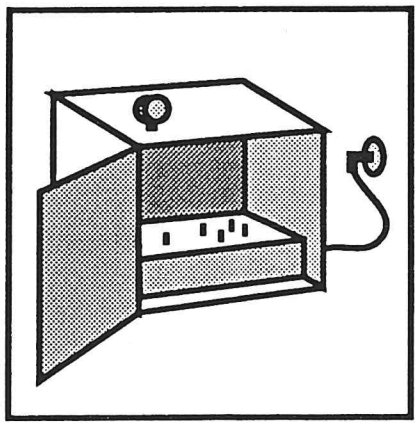
6 Centrifuge for 10 min at 3000 RPM using Clay Adams Dynac refrigerated centrifuge at 4°C.



7 Decant supernatent into a clean drying tube. Resuspend pellet in 100% ETOH (ETOH only, not ETOH-PTU) at a µl volume equal to 1.5 X mg weight of the fish. Vortex for 10 sec and centrifuge at 3000 RPM for 10 min at 4°C.

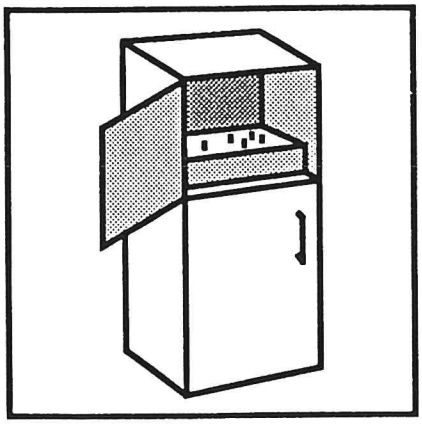


8 Pour supernatent in with first supernatent. [The drying tube contains the extracted T_4].

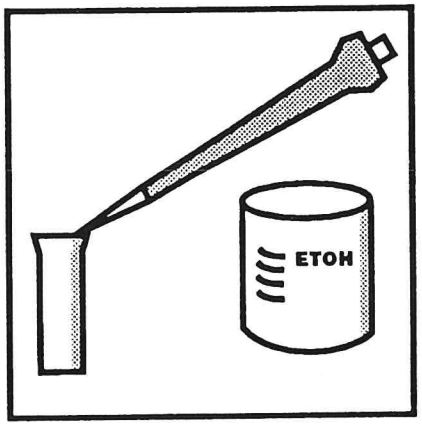


9 Dry in vacuum oven at 60°C and 25 psi until all liquid in the tube is evaporated (usually about 2 h to overnight), then store at 0°C until assayed.

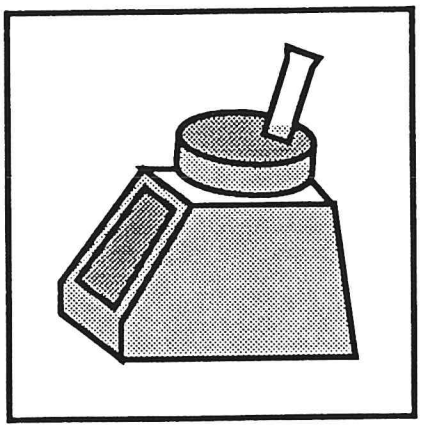
Figure 2. Procedures used to resuspend dried samples for hormone analysis.



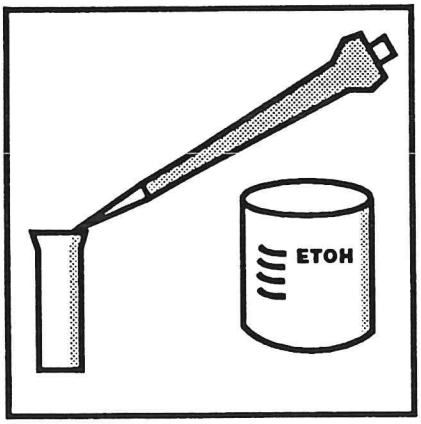
1 Remove dried samples from 0°C freezer.



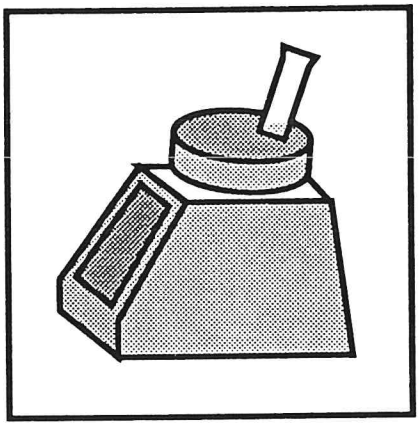
2 Add 250 µl 95% ETOH to each drying tube.



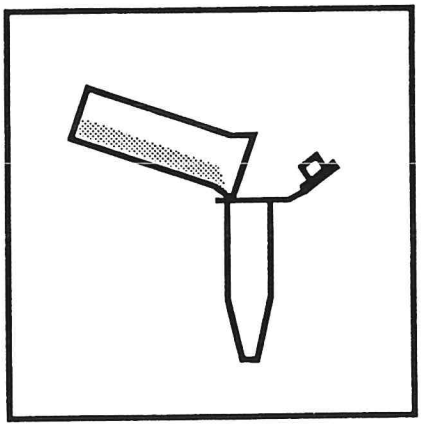
3 Vortex for 15 seconds.



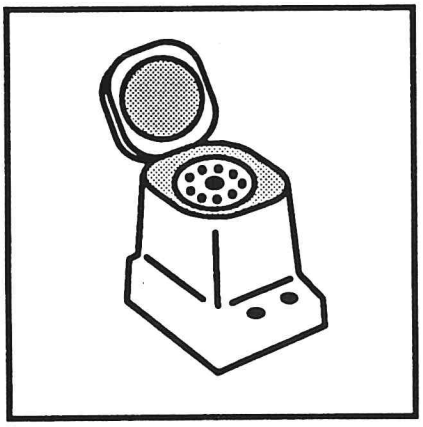
4 Add 250 µl of 0.11 M sodium barbital (pH 8.6) to each drying tube.



5 Vortex for 15 sec.



6 Pour resuspended sample into a 0.5 ml or 1 ml plastic Eppendorf snap cap vial.



7 Centrifuge at 3000 rpm for 10 min using an Eppendorf Model 5415 C refrigerated centrifuge at 4°C.

◄ [This step clears any remaining particulate fraction from the solution by sedimenting it on the bottom of the tube. When pipetting samples into RIA tubes, make sure you don't put the pipette tip into this sedimented material because it interfers with the assay.]

250 µl 95% ethanol (ETOH) and 250 µl 0.11 M sodium barbital buffer (pH 8.6) (Figure 2). Each sample was then vortexed for 15 seconds, transferred into a plastic eppendorf snap cap vial, and centrifuged at 3000 RPM for 10 minutes using an Eppendorf Model 5145C centrifuge refrigerated at 4°C. Reconstituted whole body samples thus prepared were pipetted into duplicate radioimmunoassay (RIA) tubes.

To perform the RIA, 25 µl of each bull trout sample and 1 ml of radiolabeled T_4 (^{125}I-T_4) were added to a tube coated with antibodies (Ab) that contained T_4 receptors. This procedure uses a competitive binding technique in which the ^{125}I-T_4 and unknown T_4 sample compete for Ab binding sites, and bind in proportion to their relative concentrations (Figure 3). Therefore, samples that contain large quantities of T_4 will bind less ^{125}I-T_4 than samples that contain small quantities of T_4. A standard curve was prepared with known concentrations of T_4 ranging from 0 to 24 µg/dl by pipetting 25 µl of 0, 0.5, 1, 4, 10, 16 and 24 µg/dl standard concentrations and 1 ml of ^{125}I-T_4 into Ab coated tubes. The actual concentrations of T_4 in bull trout samples were determined by comparison to the standard curve samples, which were subjected to the same assay procedures.

Unknown bull trout and standard curve tubes were vortexed for five seconds and incubated for 1 hour at 37°C (the equilibrium point) to complete binding of non-radioactive T_4 and ^{125}I-T_4 to Ab. The T_4 and ^{125}I-T_4 that did not bind to the antibody receptors was decanted into a radioactive waste container and the remaining liquid in the tubes was blotted dry on absorbent paper. Radioactivity of remaining bound T_4 was counted for 1 minute using a programmable Cobra QC Model B5002 auto-gamma Counter (Packard Instrument, Co.) (Figure 4).

Maximum binding (MB) of radioactivity was determined from the standard curve tubes containing 0 µg/dl T_4. Percent bound of each of the remaining standard curve and unknown samples was calculated using the equation:

$$\text{Percent Bound} = \frac{\text{counts - NSB counts}}{\text{MB counts - NSB counts}} \text{ X } 100$$

Figure 3. Radioimmunoassay procedure.

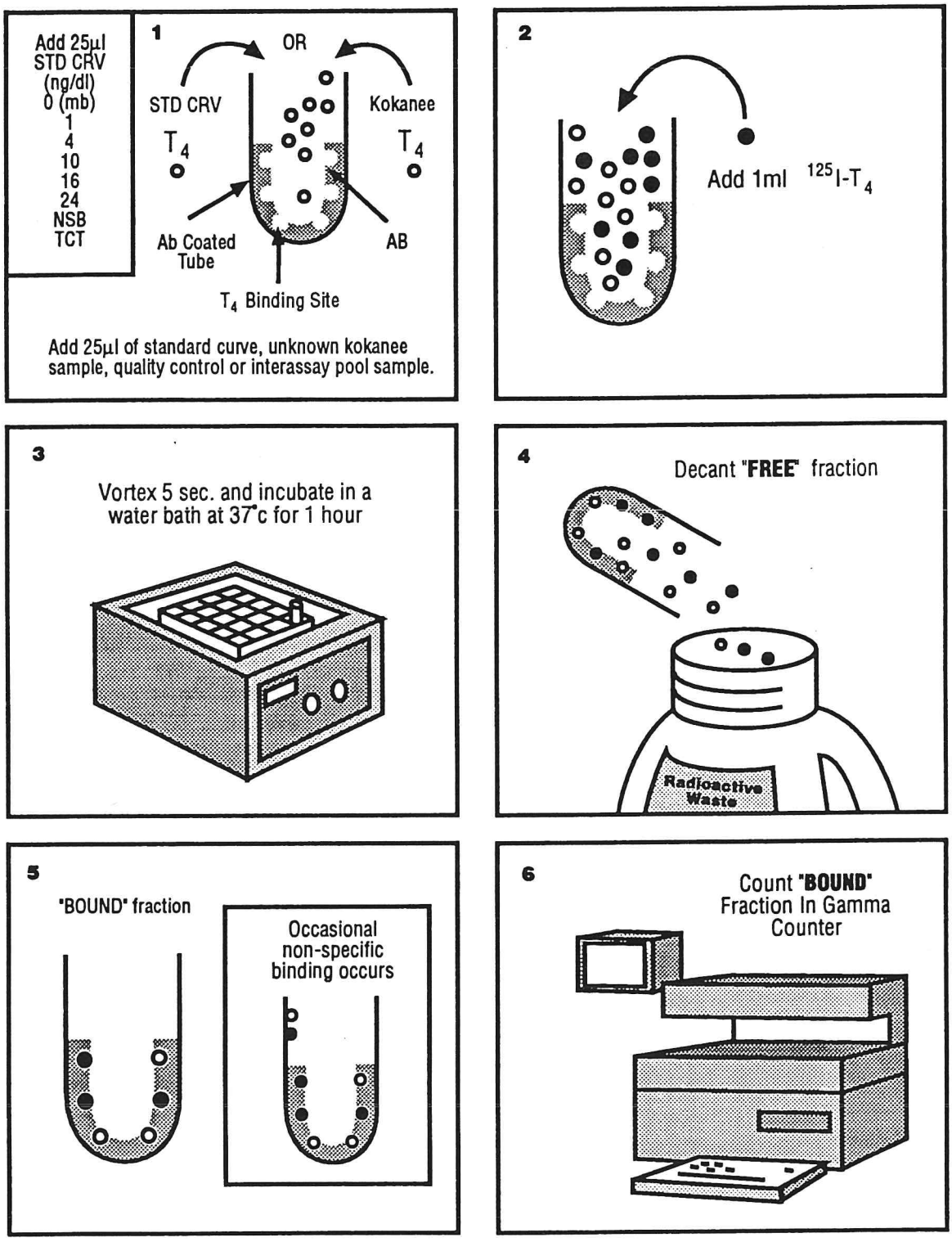


Figure 4. How the RIA technique works.

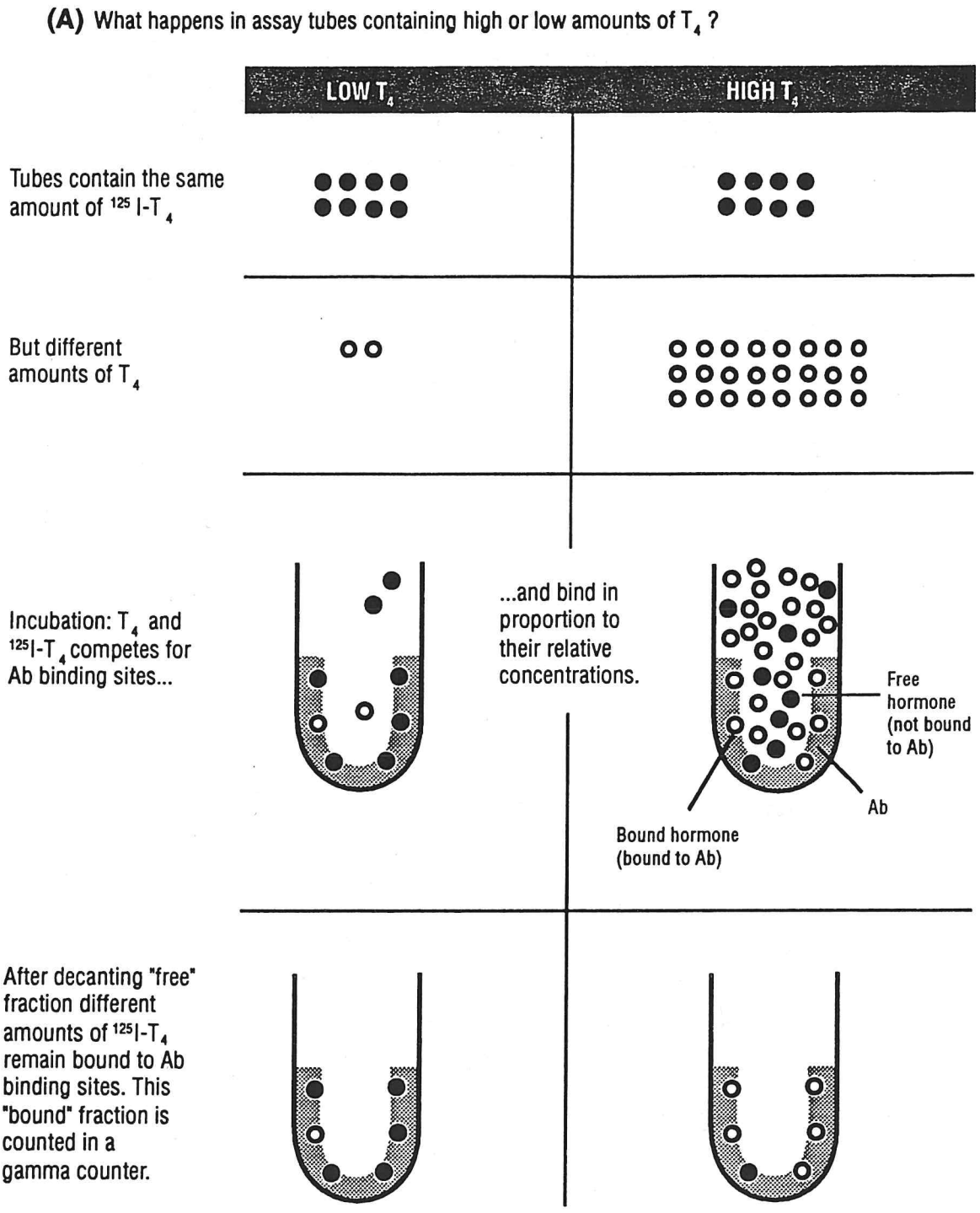
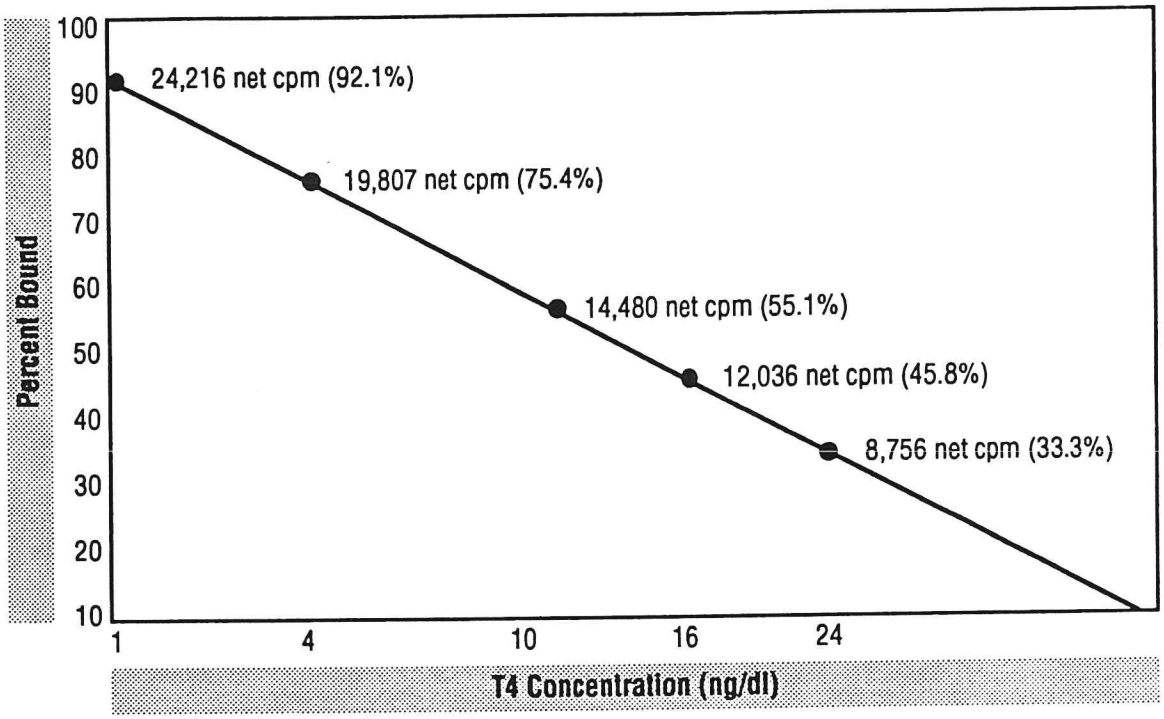


Figure 4. How the RIA technique works.

(B) Sample of log/logit standard curve plot. The plots and calculations were made automatically via computer program contained on a programable gamma counter. To determine the standard curve line the computer program fitted a linear regression line (using least squares) through a log/logit plot of percent bound (y axis) -v- standard curve concentrations (x axis).



(C) Sample calculation to determine unknown concentration. Data were obtained as indicated in Fig 3b.

DATA

Unkown Kokanee Sample (Contains 16,141 cpm)	NSBTube (Contains 219 cpm)

$$\textbf{Percent Bound} = \frac{\textbf{Net Counts x 100}}{\textbf{Net MB Counts (cpm)}}$$

where: Net Counts= Unknown tube count - NSB tube count (16,141 - 219 = 15,922)
Net MB Counts = MB tube count - NSB count (26,507 - 219 = 26,288)

$$\textbf{Percent Bound} = \frac{15{,}922}{26{,}288} \times 100 = 60.8\%$$

Interpolate From Graph
60.8% Bound = 7.8 ng/dl

where: Counts = radioactive counts per minute (cpm) of either a standard curve or unknown tube.

MB counts = radioactive counts per minute (cpm) or maximum binding tube; and

NSB counts = nonspecific binding (cpm) *i.e.*, radioactivity that sticks to the assay tube but does not bind to antibody binding sites. This was determined by adding 1 ml T_4 to uncoated assay tubes that were subjected to the same procedures as antibody coated assay tubes. After decanting the tubes, the remaining radioactivity was considered nonspecific binding.

The gamma counter was programmed to determine the percent bound for each standard concentration and plot a log-logit graph of the standard curve (% bound vs. standard concentration). The program then compared the percent bound of unknown samples to this graph and interpolated their concentrations. The program automatically subtracted NSB from each standard curve and unknown sample before calculating percent bound.

T_4 concentration of whole body samples from zero age fish was calculated by the formula:

$$[T_4]=MC \times \frac{1dl}{100000\mu l} \times \frac{1000ng}{1\mu g} \times \frac{TS_{vol}}{SS_{vol}} \times \frac{1\ sample}{SW} \times \frac{1000mg}{1g} \times \frac{25\mu l}{sample} \times CF$$

where: $[T_4]$ = T4 content (in ng/g body weight);

MC = measured concentration of the sample from RIA analysis (in µg/dl);

TS_{vol} = total volume of reconstituted samples (500 µl);

SS_{vol} = subsample volume of aliquots used for performing the RIA (25µl);

SW = sample weight (mg); and

CF = correction factor for extraction efficiency. This value was determined by spiking one set of samples collected on the same date with three known amounts (low, medium,

and high) of T_4, subjecting them to the same extraction procedures described above, and determining the percentage of each T_4 spike recovered. (See Section 2.4 for details.)

2.3 Quality control procedures

The gamma counter was calibrated before each assay to check efficiency. It remained at a constant 84.5 percent for each of the three assays performed. Background radiation was determined by counting two blank tubes at the beginning of each assay. The counter was programmed to automatically subtract this background level from each tube assayed. Each sample was assayed for T_4 in duplicate to control for procedural errors. Quality assurance samples obtained from the assay kit manufacturer (Diagnostic Products) were assayed in each assay. Mean (± S.D.) and ranges in concentration were then compared to actual concentrations provided by Diagnostic Products, Inc. to determine assay reliability.

During blotting to remove non-bound radiolabeled T_4 in the final step of the assay, the assay tubes were put into a foam rack, turned upside down on absorbent paper, and allowed to drain for two minutes. The rack was then rapped sharply several times to shake out any drops of liquid remaining on the inside of the tube. It is possible that some radiolabeled T_4 dripped onto the outside of the assay tubes during this procedure. To determine the degree of such contamination, six "blank" tubes were inserted randomly into the assay and subjected to all assay procedures except for adding radiolabeled T_4. If no contamination occurred, these blank tubes should read either 0 or no more than a few cpm. These tubes also acted as a check to determine that the counter functioned properly, *i.e.*, automatically subtracted background counts. For example, since background gamma radiation is about 300 cpm in our laboratory, the blank tubes should read about 300 cpm if the counter was not subtracting the background properly. However, the tubes would read about 0 cpm if the counter subtracted background.

Because T_4 concentration was determined for a large number of samples, three assays were performed. To test interassay accuracy, three different

interassay pool (IAP) samples were inserted in duplicate at three different positions into each assay. Also, the same known T_4 samples were used to construct the standard curve for all three assays. If the values for each IAP and standard was uniform for all assays, then the results of each assay could be compared.

2.4 Recovery determination

To determine the efficiency of the extraction process in recovering thyroid hormones, as well as transfer of samples into different types of tubes, a spike recovery experiment was performed. A sample of 40 recently hatched alevins (8 lots x 5 eggs per lot) were collected at one time interval. Unlabeled thyroxine was added to the samples prior to homogenization. Replicate tubes each were spiked with T_4 at concentrations of 0, 2.5, 5.0, and 10 ng/g body weight. The samples were then subjected to the same homogenization, extraction, reconstitution and T_4 assay procedures as the unknown and standard curve samples. Mean ± S.D. were calculated for each set of replicate samples. The percentage of T_4 recovered from each spiked tube was calculated by the formula:

$$T_4(\%R) = ([T_4](sp) - \frac{[T_4](0) \text{ X } Wt(sp)}{Wt(0)}) \div AMT$$

where: $T_4(\%R)$ = The mean percentage of T_4 recovered;

$[T_4](sp)$ = The mean T_4 concentration measured in a particular set of replicate spiked samples;

$[T_4]0$ = The mean T_4 concentration measured in the sample spiked with a concentration of 0 ng/dl T_4;

Wt(sp) = Mean weight (mg) of a particular set of spiked samples;

Wt(o) = Mean weight (mg) of the samples receiving 0 ng/dl T_4; and

AMT = The mean concentration (amount) of T_4 added to a particular spiked sample.

The formula provided a correction for the different weight of each sample. A linear regression was plotted with the amount of T_4 recovered vs. amount of T_4 added to each sample. The r^2 value was examined to determine the consistency of recovery at various spike concentrations.

3.0 RESULTS

3.1 Whole body T_4 concentration in Lion Creek stock bull trout

Lion Creek stock bull trout eggs weighed about 70-75 mg. These fish lost weight at the time of hatch (hatchlings weighed about 65 mg) and gained weight thereafter (Table 1). Mean T_4 concentrations (± S.D.) in eggs ranged from 1.7 ± 0.1 to 3.5 ± 0.4 ng/g body weight. Mean T_4 concentrations peaked before swimup (183 days post-fertilization) at 4.3 ng/g body weight. After declining to 1.8 ng/g body weight on day 194 post-fertilization, T_4 concentration peaked again at 7.6 ± 2.5 ng/g body weight at the fry stage (224 days post-fertilization), then began to decrease (Figure 5).

3.2 Whole body T_4 concentration in Holland Lake stock bull trout

Fertilized eggs of Holland Lake stock bull trout weighed about 90-95 mg (Table 2). The fish lost weight at the time of hatch (76 mg) but then continued to gain weight. Mean T_4 concentration (± S.D.) in eggs ranged from 1.5 ± 0.2 to 3.4 ± 0.7 ng/g body weight (Table 2). T_4 concentration at swimup (182 days post-fertilization) was within the range observed for eggs at 3.2 ng/g body weight. After declining to 1.6 ng/g body weight on day 193 post-fertilization, T_4 concentration rose to 5.1 ± 0.3 ng/g body weight during the fry stage (223 days post-fertilization) and then began to decline (Figure 6).

3.3 Quality assurance results

The accuracy of individuals who pipetted the 25 µl samples and 1.0 ml of radiolabeled T_4 is recorded in Table 3. The mean (± S.D.) cpm of 30 tubes that received 25µl of ^{125}I-T_4 was 1,639 ± 173 for a total error of 10.8%. The mean cpm of 24 tubes that received 1.0 ml of ^{125}I-T_4 was 64,272 ± 395 for a total error of 0.6%.

A frequency distribution of the percent error of duplicate bull trout whole body samples is presented in Figure 7. The mean percent error (± SEM) of 143 samples was 6.8 ± 0.5%.

Table 1. **Mean weights and thyroxine (T_4) concentrations for Lion Creek stock bull trout at various life stages.**

Days post fertilization	Life Stage	Sample Size		Body Weight (mg ± S.D.)	T4 (ng/g weight±SEM)
		# fish	# samples		
0	Egg	15	3	76.5 ± 7.0	1.8 ± 0.2
1	Egg	15	3	75.6 ± 5.9	1.7 ± 0.1
17	Egg	15	3	73.5 ± 5.5	2.7 ± 0.3
30	Egg	15	3	75.7 ± 6.8	2.1 ± 0.5
45	Egg	15	3	74.2 ± 4.6	3.3 ± 0.6
58	Egg	15	3	74.9 ± 7.2	3.1 ± 0.7
72	Egg	15	3	74.5 ± 6.6	1.9 ± 0.3
86	Egg	15	3	73.7 ± 7.4	3.5 ± 0.4
100	Egg	15	3	75.7 ± 6.6	2.9 ± 0.9
111	Egg	15	3	74.9 ± 8.9	3.1 ± 1.3
118	Egg	15	3	74.5 ± 7.8	3.3 ± 0.7
121	Egg	15	3	74.3 ± 8.1	2.5 ± 1.2
125	Pre-hatch	15	3	75.1 ± 7.6	2.7 ± 0.9
128	Hatch	15	3	64.5 ± 3.9	3.2 ± 0.4
135	Post-hatch	15	3	65.7 ± 5.6	2.5 ± 0.7
146	Alevin	15	3	71.2 ± 4.3	2.0 ± 0.3
160	Alevin	15	3	73.6 ± 1.9	2.2 ± 0.4
174	Alevin	5	1	83.8	2.4
183	Alevin	5	1	93.2	4.3
188	Pre-swimup	5	1	82.6	3.7
194	Swimup	5	1	80.4	1.8
202	Post-swimup	5	1	82.0	4.4
216	Fry	5	1	112.4	5.2
224	Fry	10	5	149.9 ± 15.6	7.6 ± 2.5
238	Fry	10	5	230.0 ± 36.2	3.9 ± 0.8

Figure 5. **Whole body thyroxine (T_4) concentration of Lion Creek bull trout. Each point represents the mean ± SEM of 15 fish for days 0-160 post fertilization, 5 fish for days 174-216 post fertilization (SEM not calculated due to small sample size), and 10 fish for days 223-237 post fertilization.**

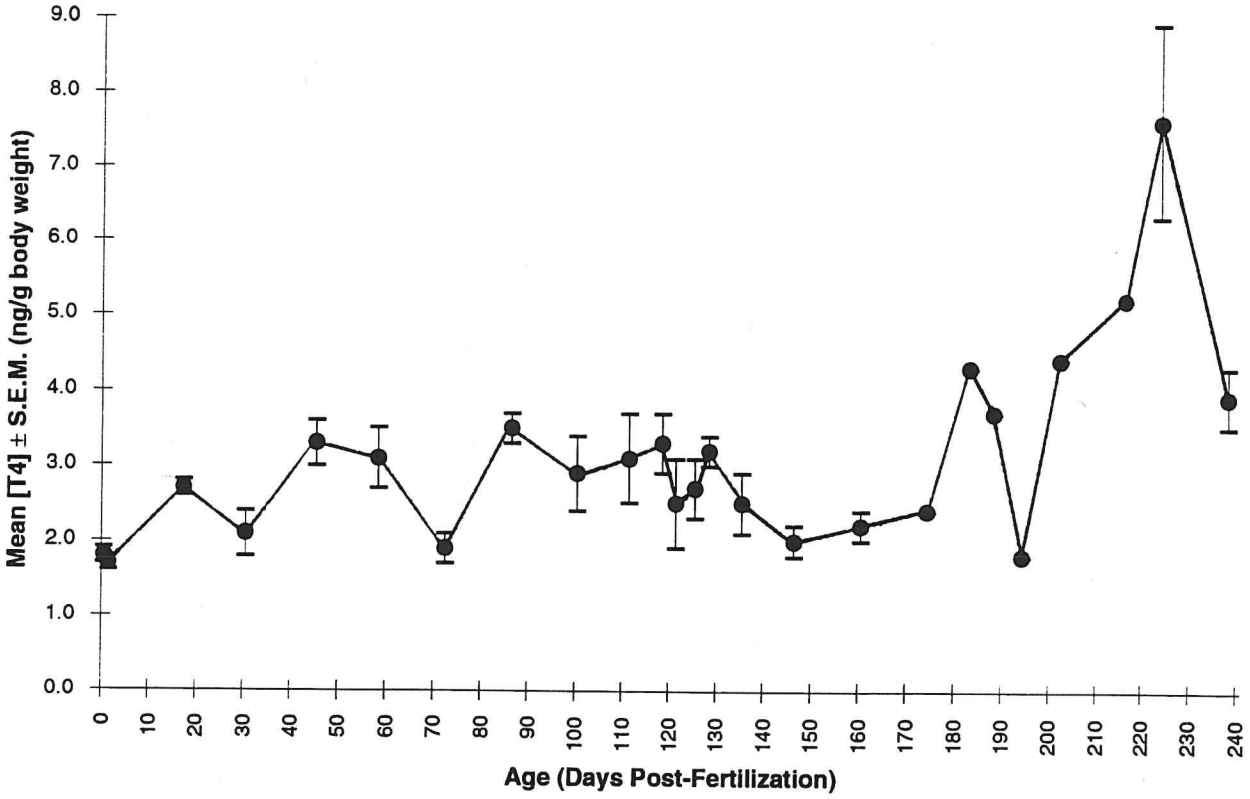


Table 2. **Mean weights and thyroxine (T_4) concentrations for Holland Lake stock bull trout at various life stages.**

Days post fertilization	Life Stage	Sample Size		Body Weight (mg ± S.D.)	T4 (ng/g weight±SEM)
		# fish	# samples		
0	Egg	15	3	92.7 ± 2.1	1.7 ± 0.4
1	Egg	15	3	94.1 ± 2.7	1.6 ± 0.2
16	Egg	15	3	94.6 ± 1.9	1.5 ± 0.2
29	Egg	15	3	92.7 ± 0.6	2.1 ± 0.5
44	Egg	15	3	92.5 ± 1.0	2.5 ± 1.2
57	Egg	15	3	91.3 ± 1.3	1.7 ± 0.1
71	Egg	15	3	91.1 ± 1.5	2.2 ± 0.4
85	Egg	15	3	91.9 ± 1.6	3.4 ± 0.7
99	Egg	15	3	91.0 ± 3.7	2.5 ± 0.5
110	Egg	15	3	92.1 ± 1.1	1.8 ± 0.4
117	Egg	15	3	91.9 ± 1.6	1.6 ± 0.2
120	Pre-hatch	15	3	92.5 ± 1.6	1.6 ± 0.1
124	Hatch	15	3	76.3 ± 4.3	2.1 ± 0.4
127	Post-hatch	15	3	77.3 ± 1.9	2.5 ± 0.3
134	Alevin	15	3	80.9 ± 1.7	1.6 ± 0.1
145	Alevin	15	3	85.5 ± 4.1	1.8 ± 0.2
159	Alevin	15	3	89.0 ± 7.9	2.1 ± 0.4
173	Alevin	5	1	93.6	2.2
182	Pre-swimup	5	1	102	3.2
187	Swimup	5	1	99.6	2.3
193	Post-swimup	5	1	97.4	1.6
201	Fry	5	1	102	3.2
215	Fry	5	1	115.4	2.3
223	Fry	10	5	164.4 ± 17.3	5.1 ± 0.3
237	Fry	10	5	209.0 ± 20.9	2.4 ± 0.4

Figure 6. **Whole body thyroxine (T_4) concentration of Holland Lake bull trout. Each point represents the mean ± SEM of 15 fish for days 0-159 post fertilization, 5 fish for days 173-215 post fertilization (SEM not calculated due to small sample size), and 10 fish for days 223-237 post fertilization.**

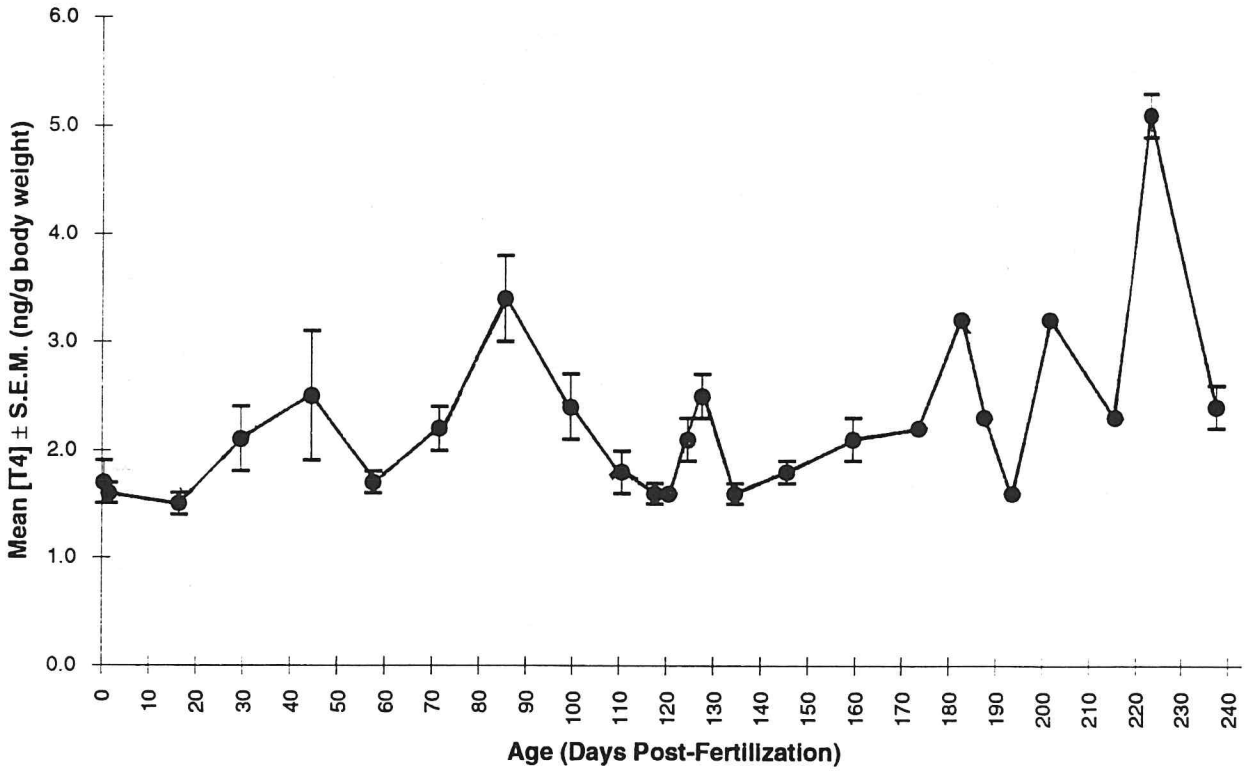


Table 3. **Pipetting accuracy of 25 µL and 1 mL samples. Counts = counts per minute of radiolabeled T_4. Percent error = standard deviation (S.D.) ÷ mean counts.**

Sample	Assay	Sample size (n)	Mean # of counts ± S.D.	Coefficient of Variation (% error)
25 µL	1	10	1,823 ± 98	5.4
	2	10	1,718 ± 238	13.8
	3	10	1,376 ± 182	13.2
Mean			1,639 ± 173	10.8
1.0 mL	1	10	67,430 ± 517	0.8
	2	4*	70,873 ± 349	0.5
	3	10	54,514 ± 319	0.6
Mean			64,272 ± 395	0.6

* Only four tubes were used because the quantity of radiolabeled T_4 was limited.

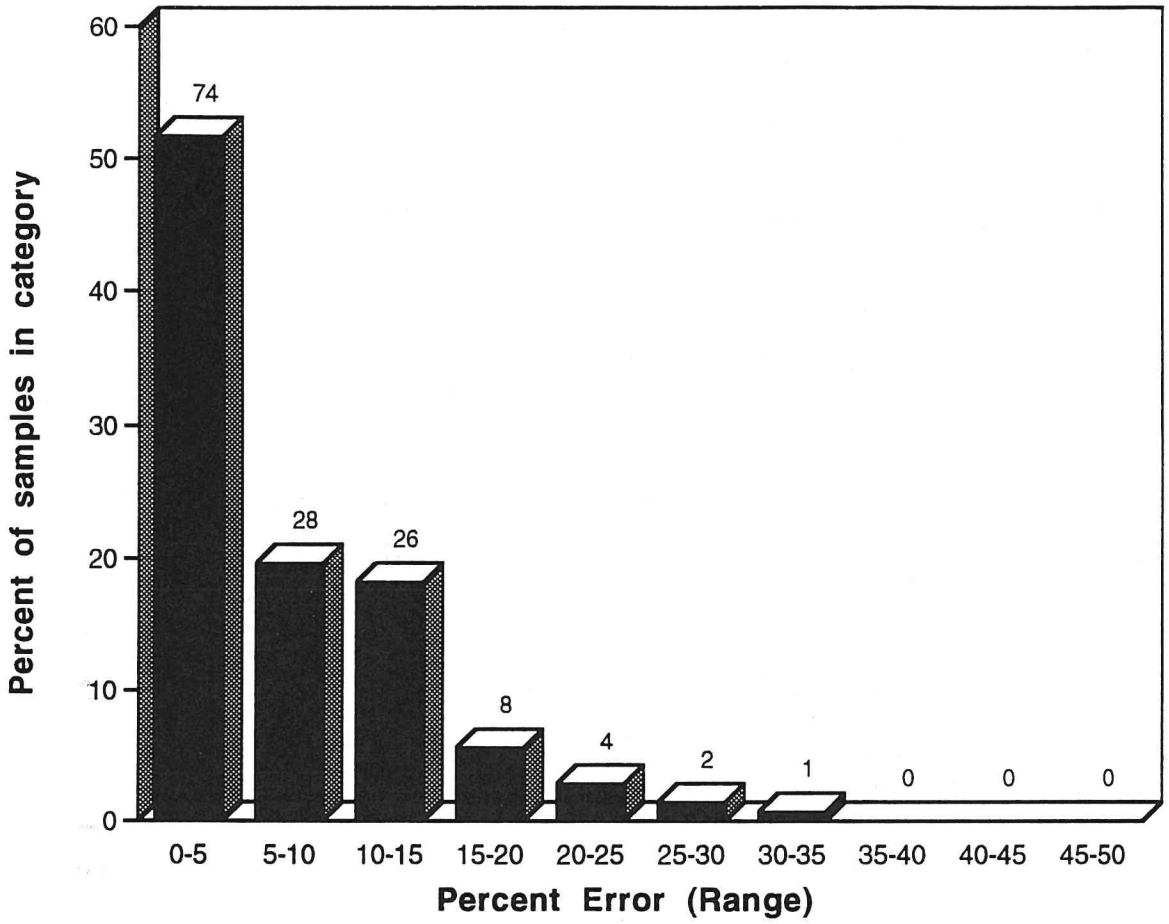


Figure 7. **Frequency distribution of percent error of duplicate Lion Creek and Holland Lake bull trout. The total sample size (n) was 143 duplicate samples. The number of samples in each category are noted above the bar. These numbers were converted to percentages of the total sample for plotting the frequency distribution. Mean percent error (± S.D.) was 6.8 ± 0.5%.**

Results of blind quality assurance samples are recorded in Table 4. The actual concentration of the low T_4 blind sample was 2.3 ng/dl compared to a mean measured value of 2.5 ± 0.3 ng/dl (n=6). The actual concentration of the medium T_4 blind sample was 7.3 ng/dl compared to a mean measured value of 8.1 ± 0.6 ng/dl (n=6). The actual concentration of the high T_4 blind sample was 11.5 ng/dl compared to a mean measured value of 11.4 ± 0.3 ng/dl (n=6).

Mean nonspecific binding (NSB) for eight replicate tubes was measured at 647 counts per minute (cpm) compared to 64,328 cpm measured in total count tubes (TCT) (Table 5), or about 1% NSB. Results and comparisons of interassay pool (IAP) samples and standard curve concentrations for each of the three assays are recorded in Table 6. For IAP samples, mean (± S.D.) for the three assays were 2.5 ± 0.3 ng/dl for the low IAP, 8.1 ± 0.6 ng/dl for the medium IAP and 11.4 ± 0.3 ng/dl for the high IAP. For standard curve samples, the mean (± S.D.) for the three assays were 0.6 ± 0.1 for the 0.5 ng/dl standard, 0.9 ± 0.0 ng/dl for the 1.0 ng/dl standard, 4.0 ± 0.4 ng/dl for the 4.0 ng/dl standard, 9.2 ± 0.5 ng/dl for the 10.0 ng/dl standard, 17.2 ± 0.7 ng;dl for the 16.0 ng/dl standard, and 24.5 ± 1.0 ng/dl for the 24.0 ng/dl standard.

3.4 Recovery determination

Recovery data from bull trout samples spiked with 2.5, 5.0 and 10 ng exogenous T_4 is recorded in Table 7. Results indicated that percent recovery was 86%. The reciprocal of this number was used as a correction factor (multiplier) to calculate T_4 concentrations in the samples. These recovery values were similar to those reported by other investigators, e.g., 83% for coho salmon and 86.8% for kokanee salmon (Kobuke *et al.* 1987; Scholz *et al.* 1992). A regression plot (Figure 8) indicated that the amount of T_4 recovered from each sample was linearly related to the amount of T_4 added to the sample (r^2 = .964).

Table 4. **Results of blind quality assurance samples. Measured concentrations were the mean (±S.D.) of six replicates for each sample. Actual and acceptable ranges were provided by Diagnostic Products Corporation.**

Sample	Actual concentration (ng/dl)	Acceptable range (ng/dl)	Measured concentration (ng/dl)
LOW	2.3	1.9 - 2.7	2.5 ± 0.3
MEDIUM	7.3	6.2 - 8.4	8.1 ± 0.6
HIGH	11.5	10.0 - 13.0	11.4 ± 0.3

Table 5. **Results of non-specific binding compared to total count tubes. Measurements are in count per minute (cpm). Values are mean values of replicate tubes in each assay.**

Assay Number	TCT value	NSB value	% NSB
1	65,990	551	0.83
2	71,023	791	1.11
3	55,972	598	1.06
Mean	**64,328**	**647**	**1.01**

Table 6. Comparisons of mean values between interassay pool (IAP) and standard curve concentration (STDCRV) in three assays. Concentrations were in ng/dL. All values are the mean of duplicate samples for each concentration in each assay.

Interassay Pool

Assay No.	Low	Med	High
IAP 1	2.4	7.6	11.5
IAP 2	2.3	7.9	11.6
IAP 3	2.8	8.7	11.1
Mean (±S.D)	**2.5 ± 0.3**	**8.1 ± 0.6**	**11.4 ± 0.3**

Standard Curves

Assay No.	0.5	1	4	10	16	24
STDCRV 1	0.6	0.9	3.8	9.5	16.6	25.2
STDCRV 2	0.6	0.9	3.7	8.7	18.5	24.9
STDCRV 3	0.5	0.9	4.5	9.5	16.4	23.3
Mean (±S.D)	**0.6 ± 0.1**	**0.9 ± 0**	**4 ± 0.4**	**9.2 ± 0.5**	**17.2 ± 0.7**	**24.5 ± 1.0**

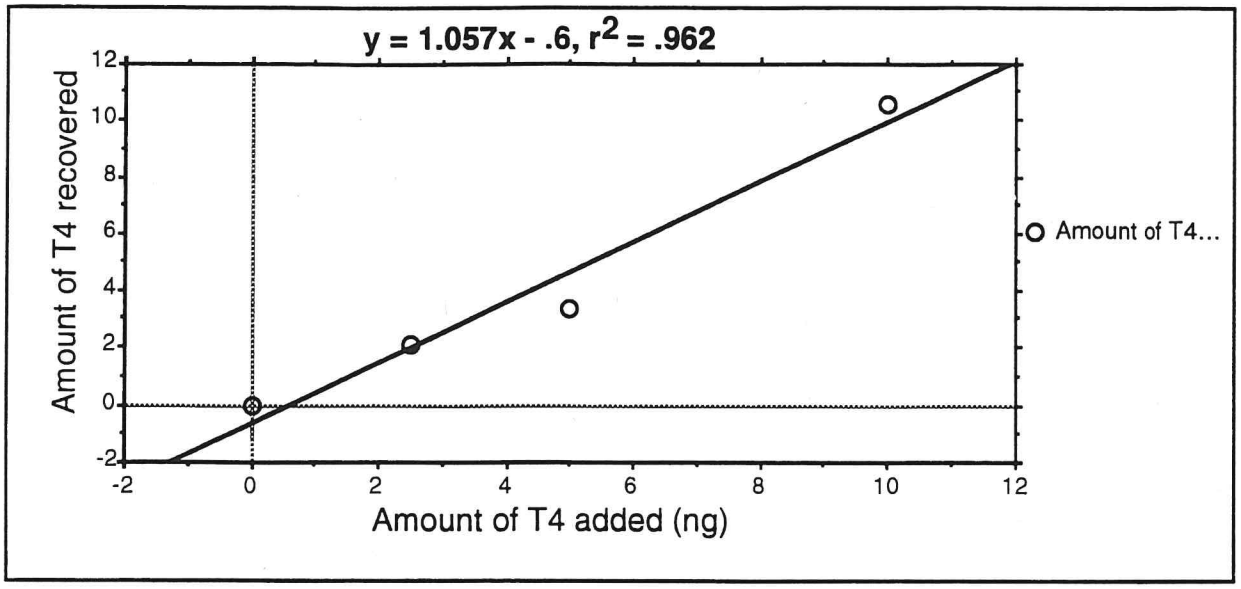
Table 7. **Percent recovery for bull trout samples spiked with nonradioactive thyroxine. Values are means of duplicate samples. NA = not applicable.**

Amount of spike	Amount of T_4 added to sample (ng)	Total T_4 measured in sample (ng)	Amount of added T_4 recovered (ng)[1]	Percent recovered (%)[2]
Control	0	5.5	NA	NA
Low	2.5	7.6	2.1	84%
Medium	5.0	8.9	3.4	68%
High	10.0	16.1	10.6	106%
Mean Percent				**86%**

1 Calculated by subtracting the amount measured in each spike concentration from the amount measured in the control sample.

2 Calculated by dividing the amount recovered by the amount added.

Figure 8. **Linear regression plot of amount of T_4 added versus amount of T_4 recovered from bull trout alevin samples spiked with 0, 2.5, 5.0 and 10,0 ng T_4.**



Simple Regression X_1: Amount of T4 added (ng) Y_1: Amount of T4 recovered

Count:	R:	R-squared:	Adj. R-squared:	RMS Residual:
4	.981	.962	.943	1.098

Analysis of Variance Table

Source	DF:	Sum Squares:	Mean Square:	F-test:
REGRESSION	1	61.116	61.116	50.689
RESIDUAL	2	2.411	1.206	p = .0192
TOTAL	3	63.528		

No Residual Statistics Computed

Simple Regression X_1: Amount of T4 added (ng) Y_1: Amount of T4 recovered

Beta Coefficient Table

Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	-.6				
SLOPE	1.057	.148	.981	7.12	.0192

Confidence Intervals Table

Variable:	95% Lower:	95% Upper:	90% Lower:	90% Upper:
MEAN (X,Y)	1.663	6.387	2.422	5.628
SLOPE	.418	1.696	.624	1.491

4.0 DISCUSSION

4.1 Accuracy of experimental results

Our results indicated that developing Holland Lake bull trout experienced relatively stable thyroxine concentrations from the egg to swimup stages, with some minor fluctuations (1.5 ± 0.2 to 3.4 ± 0.7 ng/g body weight). A distinct peak in post swimup fry was observed at 224 days post fertilization (5.1 ± 0.3 ng/g body weight). T_4 in Lion Creek stock fluctuated between 1.8 ± 0.2 ng/g body weight and 3.5 ± 0.4 ng/g body weight from 0 to 174 days post-fertilization (egg to alevin). At 183 days post-fertilization the T_4 concentration rose above this range to 4.3 ng/g body weight in pre-swimup alevins, then declined during swimup to 1.8 ng/g body weight. A second increase in T_4 concentration at 7.6 ± 2.5 was observed after swimup (224 days post-fertilization).

We believe that these results represent true fluctuations in T_4 concentration because results of quality assurance procedures indicated that the assay was reliable. Measured concentrations of blind quality control samples were within the acceptable range of actual concentrations in six replicates each at the low, medium and high concentration. The interassay pool and standard curve samples from each assay were uniform, so the results from each assay were comparable. The maximum amount of error in the assays was 10.8% associated with pipetting the 25 µl radiolabeled samples to estimate the error of one of the pipettors. The mean percent error of duplicate samples from both stocks of fish was 6.8 ± 0.5%. These errors were well below the 447% difference observed between the lowest and highest T_4 concentrations for Lion Creek stock fish and 340% difference observed for Holland Lake stock fish. Additionally, the percent fluctuation observed during T_4 surges after swimup was 422% (Lion Creek stock) and 319% (Holland Lake stock). Therefore, it is unlikely that the percent error could compromise these observations. We suspect that the error noted in duplicate samples was owing primarily to two factors: (1) Dried samples were resuspended in ethanol solution. Ethanol solutions are less viscous than aqueous solutions and, therefore, more difficult to pipette with uniform accuracy; and (2) a 10.8% error associated with pipetting 25 µl samples. The mean percent error of duplicate samples (6.8 ± 0.5%) could be totally explained by the 10.8% error noted in pipetting the 25 µl samples.

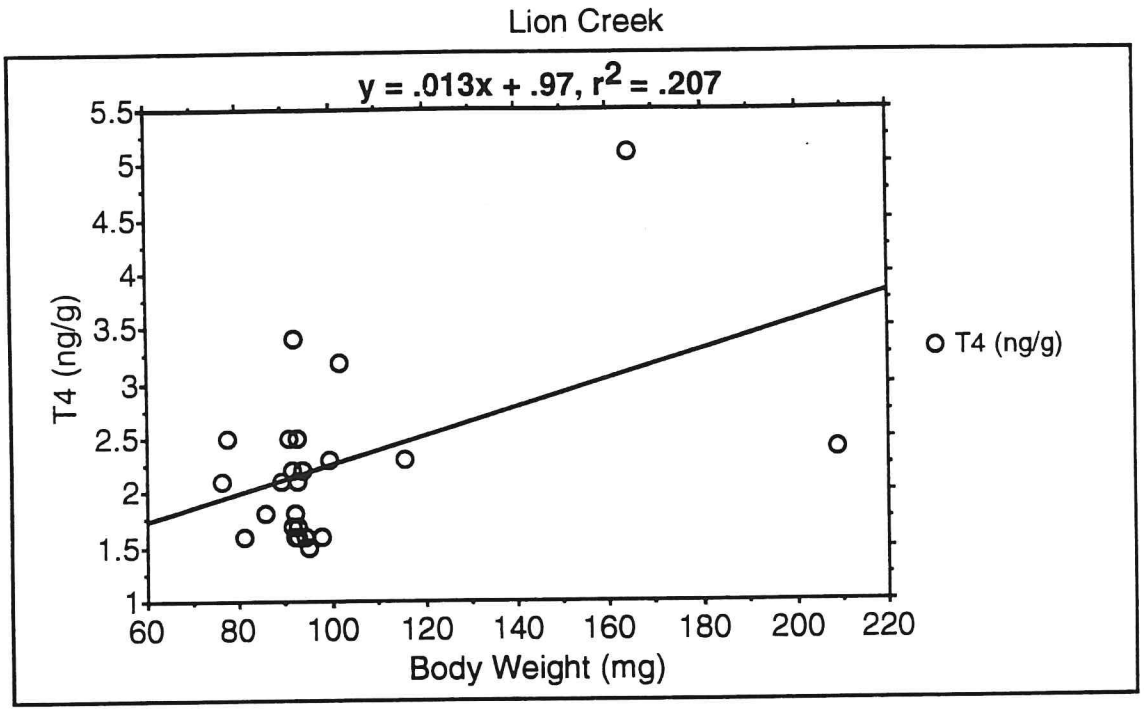
4.2 Comparison of experimental results with kokanee results

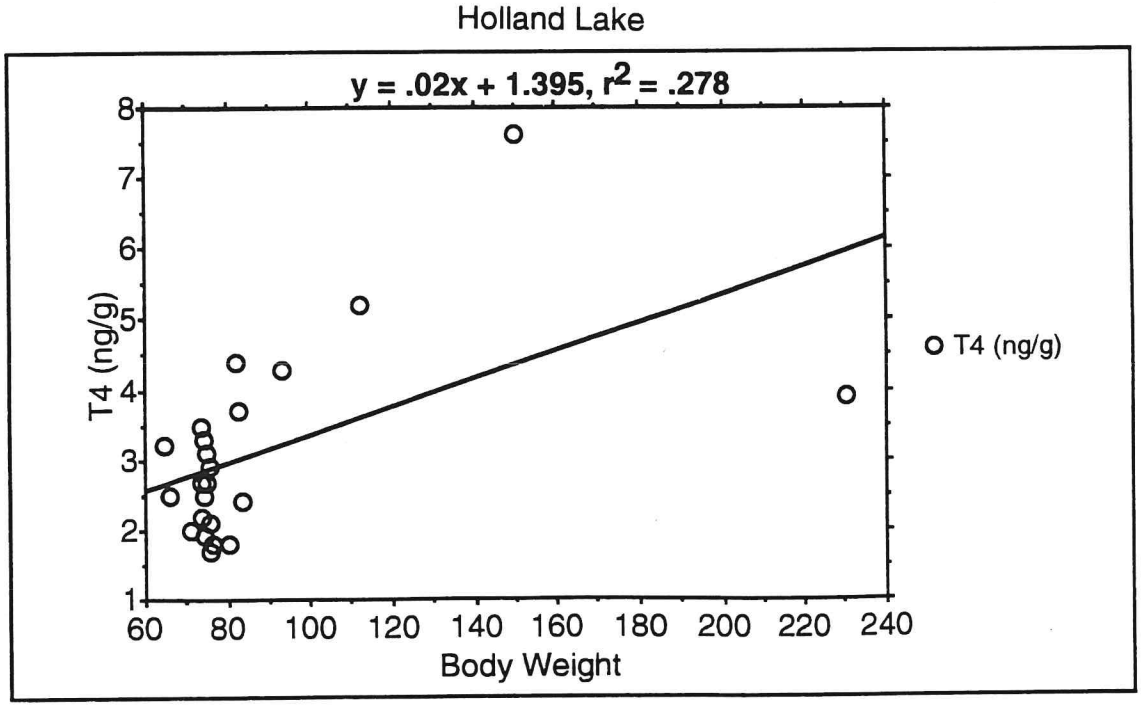
Our results with bull trout reported here were not similar to those reported for kokanee salmon by Scholz *et al.* (1992, 1993). Whole body T_4 concentrations in bull trout eggs and larvae were about 1/5 to 1/2 those reported in kokanee eggs and larvae. Basal T_4 count in kokanee was about 8-12 ng/g body weight compared to 1.5 to 3.0 ng/g body weight in bull trout. At peak concentrations, T_4 content in kokanee was 16-18 ng/g body weight compared to about 5-7 ng/g body weight in bull trout. Also, kokanee salmon experienced distinct thyroid hormone surges at the time of hatch and swimup (Scholz *et al.* 1992, 1993). Subsequently, T_4 rapidly declined to very low levels (<1.0 ng/g body weight) in post-swimup fry. In kokanee fry, T_4 levels were less than 1/20th those observed in eggs and alevins. Also, T_4 content was inversely related to body weight in kokanee. As body weight declined at egg and alevin stages, T_4 levels increased, which indicated that the kokanee retained T_4 at those times.

In contrast, neither stock of bull trout tested in the present study experienced a T_4 surge at the time of hatch and only the Lion Creek stock experienced a T_4 surge at about the time of swimup. Moreover, both stocks experienced T_4 surges in post-swimup fry. Although the T_4 levels subsequently declined, they were still as high as those observed in eggs and alevins. Also, T_4 content did not appear to correlate negatively with body weight in bull trout as it did in kokanee. To illustrate this, we plotted regression of T_4 content versus body weight (Figure 9). In both stocks, there was a weak positive correlation between T_4 content and body weight.

Tilson *et al.* (in press) have documented that the increased level of T_4 at hatch and swimup in kokanee was associated with imprinting. Fish that were exposed to synthetic chemicals from the hatch to swimup stages homed with 86% accuracy to their exposure chemical as sexually mature adults in behavioral experiments, whereas fish that were exposed during the egg and fry stages did not home (14% and 21% accuracy respectively) to their exposure chemical. The highest percent homing by any group was by the group that had the highest T_4

Figure 9. Regression of T_4 content versus body weight in bull trout.





content, which occurred at the swimup stage. If T_4 content is also an accurate indicator of imprinting in bull trout, our results indicated that one potential time could be in the early post-swimup fry stage. Additionally, Lion Creek fish experienced a thyroid surge shortly before swimup, which could also indicate an imprinting period. However, Holland Lake fish did not evidence a T_4 peak at that time. Moreover, because T_4 content at peak times was relatively low when compared to kokanee, we are not certain if it was sufficiently high to stimulate imprinting.

Our ability to discern T_4 peaks at about the time of swimup was seriously compromised in the present study because only one sample (5 fish per sample) from each stock was taken for the days 174 to 216 days post-swimup. This period included the late alevin, pre-swimup, swimup and post-swimup, and earliest fry life stages. Thus, it is conceivable that a peak in T_4 content could have gone undetected during these stages. Therefore, we recommend that if this study is repeated, a larger sample size should be collected for these stages, which were the critical periods in kokanee.

Previous tagging/recapture investigations suggested that imprinting in Dolly Varden occurs sometime during smolt stage (Armstrong 1974). We suspect that bull trout are similar to Dolly Varden in their homing tendencies and imprint timing. Since some stocks of bull trout also smolt as yearlings, it may be that bull trout do not have high thyroid content as eggs. It is thought, therefore, that a thyroxine surge (and associated imprinting) may occur during the smolt stage. Thus, we also recommend that T_4 concentrations of bull trout continue to be monitored until the fish used for the present study have smolted. When the fish become sufficiently large to collect blood from the caudal vein, circulating T_4 concentration instead of whole body T_4 content should be monitored. Blood samples should be collected and centrifuged; plasma should be decanted and stored at -80°C until assayed.

This report is an interim report of the preliminary findings in this investigation. Upon completion of the work described above, a final report will be issued.

LITERATURE CITED

Armstrong, R. H., 1965. Some migratory habits of the anadromous Dolly Varden (*Salvelinus malma*) (Walbaum) in Southeastern alaska. Alaska Department of Fish and Game, Research Report Number 3, 36 pp.

Armstrong, R. H. 1974. Migration of anadromous Dolly Varden (*Salvelinus malma*) in southeastern Alaska. J. Fish. Res. Board Can. 31:435-444.

Cavender, T. M. 1978. Taxonomy and distribution of the bull trout, *Salvelinus confluentus* (Suckley), from the American northwest. California Fish and Game 64:139-174.

Fraley, J. J., and B. B. Shepard, 1989. Life history ecology and population status of migratory bull trout (*Salvelinus confluentus*) in the Flathead Lake and River system, Montana. Northwest Science 63:133-143.

Gilbert, C.H. and B.W. Evermann. 1894. A report upon investigations in the Columbia River Basin, with descriptions of four new species of fishes. U.S. Senate Miscellaneous Document No. 200. pp. 19-54.

Hasler, A.D. and A.T. Scholz. 1983. Olfactory Imprinting and Homing in Salmon: Investigations into the Mechanism of the Imprinting Process. Zoophysiology, Vol. 14. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo. 134 pp.

Kobuke, L, J. L. Specker and H. A. Bern. 1987. Thyroxine content of eggs and larvae of coho salmon, *Oncorhynchus kisutch.* Journal of Experimental Zoology 242:89-94.

Legget, J. W. 1969. The reproductive biology of the Dolly Varden char, *Salvelinus malma* (Walbaum). M.S. thesis, University of Victoria, Victoria, B. C., Canada.

Marriott, R. A. 1966. Inventory and cataloguing of the sport fish and sport fish waters in southwest Alaska *in* Federal aid in fish restoration. Annual report of progress, 1965-1966. 7:43-58.

Martin, S.W., M.A. Schuck, K. Underwood, and A.T. Scholz. 1992. Investigations of bull trout (*Salvelinus confluentus*), steelhead trout (*Oncorhynchus mykiss*), and spring chinook salmon (*O. tshawytscha*) interactions in southeast Washington streams. Annual report to Bonneville Power Administration Division of Fish and Wildlife, July 1992.

McPhail, J. D., and C. B. Murray, 1979. The early life history and ecology of Dolly Varden (*Salvelinus malma)* in the Upper Arrow Lakes. Report to British Columbia Hydro and Power Authority and Kootenay Fish and Wildlife. Victoria, British Columbia, Canada.

Parker, S. J. 1988. Thyroid Hormone Levels in Larval and Juvenile Striped Bass *Morone saxatilis:* Response to Changes in Salinity and Temperature. Ph.D. thesis. University of Rhode Island, Providence, RI. 147 pp.

Scott, O. 1968. Pioneer Days on the Shadowy St. Joe. Caxton Printers. Caldwell, ID. 372 pp.

Scott, W. B., and E. J. Crossman. 1979. Freshwater Fish of Canada. Fisheries Research Board of Canada Bulletin 184: 966 pp.

Underwood, K.D., S.W. Martin, M.A. Schuck, and A.T. Scholz. 1994. Investigations of bull trout (*Salvelinus confluentus*), steelhead trout (*Oncorhynchus mykiss*), and spring chinook salmon (*O. tshawytscha*) interactions in southeast Washington streams. Final report submitted to U.S. Department of Energy, Bonneville Power Administration Contract No. DE-BI79-91BP17758, August 1994.