Experimental Bull Trout Hatchery

Progress Report

1993 - 1994

by

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EXECUTIVE SUMMARY

This report is a summary of research findings from experimental bull trout culture work conducted at Creston National Fish Hatchery (CNFH) and the Bozeman Fish Technology Center (BFTC) during 1993 and 1994. Gametes were collected from wild spawning bull trout in the Swan River drainage of northwest Montana during September of 1993 and 1994 and fertilized eggs were produced by paired matings. Eggs were incubated under variable temperature regimes and egg development and survival rates were monitored. Eggs incubated in 1993, in water at 37.6°F, hatched after 126 days with 97.1 percent survival from green egg to hatching. Eggs incubated in 1994, in water at 43.5°F, hatched after 75 days with 95.5 percent survival. Fry rearing experiments at CNFH indicated faster growth rates for fry reared in spring water at a constant temperature of 47°F, than for fry reared at colder temperatures following a simulated natural thermal regime. Bull trout fry growth, particularly early in development, was also negatively impacted by night feeding. Some fish, reared at BFTC, showed symptoms of ascorbic acid (Vitamin C) deficiency from the standard diet when reared under crowded conditions. Results of thyroid imprinting experiments indicated thyroxine content of bull trout eggs and fry was relatively low and stable from fertilized egg through swimup and early development. A mild thyroxine spike in post-swimup fry may indicate some imprinting potential at this time, but further research on older fish is needed. Experiments conducted at BFTC with alternative rearing schemes found that bull trout reared in covered tanks with the presence of structure consistently avoided predation more effectively than fish reared without either structure or cover. Techniques are also being developed at BFTC to use thermal tolerance testing as an indicator of overall physiological fitness. The overall results of this research indicate that hatching and rearing of wild bull trout is relatively routine using standard fish cultural practices. Egg survival was excellent at the range of temperatures investigated and growth was highly temperature-dependent. More investigation at incubation temperatures above 44°F and rearing temperatures in the range of 54°F-to-64°F is warranted. The thyroid hormone experimental work should be continued through the smolt stage to assess hormone peaks and imprinting and smolting behavior in older fish. Initial results of rearing experiments presented in this report indicate bull trout feeding and behavior may be conditioned by the hatchery environment, and survival of stocked fish may be enhanced by incorporation of a natural feeding regime, structure, and cover in the rearing environment.

BACKGROUND

The bull trout (*Salvelinus confluentus*) is quite probably the most widespread native trout or char that has never been extensively cultured in hatcheries. The reason that bull trout culture has not been more widely used is believed to be primarily related to the low esteem accorded this species across its native range. This sentiment was recently summarized by a biologist at the "Friends of the Bull Trout Conference" in Calgary, who noted:

> "The bull trout's failings - its image as a cowardly and lethargic sport fish, its flesh termed "insipid", and its character blighted by a reputation for cannibalism - targeted it, among other species, for eradication by conservationists intent upon creating a perfect underwater world." (Colpitts 1994).

In Montana, bull trout were reared experimentally in hatcheries in the 1940's and 1950's. In 1944 the Montana Fish, Wildlife and Parks (MFWP) collected bull trout eggs from spawners in the Bull River (Clark Fork River watershed). The progeny were planted in the Thompson River and Kootenai River drainages in 1945 (Pratt and Huston 1993).

In 1949 and 1950 MFWP collected over 876,000 bull trout eggs from Bull River, Vermillion River, and Prospect Creek; all in the Clark Fork River drainage; and shipped some of the eggs to Creston National Fish Hatchery (CNFH) in Kalispell. Subsequently, about 10,000 of the resulting fish were planted into Lake Pend Oreille (Pratt and Huston 1993) and about 65,000 into Flathead Lake, tributaries to the North Fork Flathead River, and the Stillwater River during 1950 - 1952 (Creston National Fish Hatchery File Report 1993).

In 1985, bull trout eggs were collected by CNFH and MFWP personnel from Dry Fork Lake Creek (tributary to Saint Mary's Lake on the Flathead Indian Reservation, 10,000 eggs from ten pairs spawned 9/10/85) and Whale Creek (North Fork Flathead River tributary, 20,000 eggs from three pairs spawned 9/11 and 9/16/85). The purpose of the egg takes was for rearing and stocking experiments. The eggs were incubated in an isolation unit at Creston Hatchery in water at 4°C. Adults were sacrificed and passed a health inspection, but subsequently ERM bacteria were detected (causative agent of enteric redmouth disease) and the eggs were destroyed. The program was not repeated.

In 1989-1991 the Idaho Fish and Game conducted a small experimental hatchery program at Cabinet Gorge Hatchery, which was subsequently terminated (Pratt and Huston 1993). Part of the reason for termination was the concern over limited genetic diversity in the parental stock.

In Canada, experimental work with bull trout culture was conducted at Kootenay Trout Hatchery near Wardner, B.C. in the early 1980's. That program was later transferred to the Hill Creek Hatchery near Upper Arrow Lake on the headwaters of the Columbia River drainage. After the construction of Keenleyside and Revelstoke Dams, which cut off major bull trout spawning grounds for fish from Upper Arrow Lake, a mitigation effort was established to compensate for loss of 1,000 adult bull trout (Grant Thorp, B.C. Ministry of Environment, Nakusp,

British Columbia, pers. comm., 1993). A production goal of 100,000 fingerlings was established to meet that loss. Wild fish are captured annually from several tributaries to Upper Arrow Lake, held in a penned-off area of Hill Creek, and spawned. Adults are returned to the lake and juveniles are scatter-planted in the tributaries as 4-inch fingerlings in September. Fish culture success has been good but post-stocking evaluation of the program has not been comprehensive. The program is continuing on a reduced scale, with more emphasis to be placed on evaluation (G. Thorp., pers. comm.).

The bull trout has been increasingly recognized as a species in decline across the Pacific Northwest. In 1980, the bull trout was officially recognized as a separate species from the coastal Dolly Varden by the American Fisheries Society (Pratt 1992). In September 1985, bull trout were officially added to the U. S. Fish and Wildlife Service's Candidate Species List as a Category 2 species (the Service had established there was some evidence of vulnerability but not enough data to support a listing under the Endangered Species Act). On October 30, 1992, the Service received a petition from several Montana conservation organizations to list the bull trout as an Endangered Species throughout its range (USFWS 1994). A 90-day finding, published May 17, 1993, concluded that listing "may be warranted" and the Service initiated a rangewide status review. That review, published in June 1994, found that listing of the species was "warranted, but precluded" by higher priorities. In June 1995, a revised finding was published and warranted but precluded status was continued.

The complexity of threats facing bull trout include:

1. Habitat degradation from forest management practices, road construction, agriculture, irrigation, grazing, hydropower, mining, and other factors.

2. A history of persecution and overharvest by anglers, poachers, and fisheries managers who favored other introduced and/or more "desirable" and less piscivorous species such as rainbow trout and salmon.

3. Isolation, competition, predation, and hybridization with introduced and nonnative species; especially lake trout, brook trout, and brown trout.

The net result has been fragmentation of formerly interconnected populations (metapopulations), isolation and elimination of populations and life history forms (fluvial, adfluvial, resident, and anadromous), and reduced survival and fitness of remaining populations with significantly increased risk of extinction (Rieman and McIntyre 1993).

The Montana Bull Trout Scientific Group, appointed by the Governor of Montana to function as a scientific advisory team for the interdisciplinary Montana Bull Trout Restoration Team, is preparing a scientific evaluation of "The Role of Fish Hatcheries and Fish Transplants in Bull Trout Recovery." That document, currently in draft form, sees a limited but important role for the

use of hatcheries in restoration activities. The report examines a number of potential scenarios and outlines recommended procedures for screening and evaluating hatchery-related research and experimental efforts.

Because of the status of this native species and the lack of sound fish culture experience in propagating bull trout, the Service determined it was prudent to begin experimental work on the culture and rearing of the species. Pending success in that area, research will be conducted on the potential for restoration stocking in programs similar to those used for greenback cutthroat trout (Dwyer and Rosenlund 1988) and other native salmonids.

In 1993, a grant was obtained from the Montana Power Company to retrofit an existing building at the Service's Creston National Fish Hatchery in Kalispell, Montana to allow for experimental work with bull trout to proceed. In August of that year, an isolation facility with a water chiller, Heath incubator, and rearing tanks was constructed. A water line from an existing artesian well, and a holding tank system with chlorination of effluent was developed to reduce the threat of pathogen transfer.

This report discusses the fish culture work conducted at the Bull Trout Hatchery in calendar years 1993 and 1994. In addition, some of the fish from the hatchery were transferred to the Bozeman Fish Technology Center (BFTC) for other types of research and those efforts are summarized here as well. Thyroid hormone analysis was conducted at the Upper Columbia United Tribes (UCUT) Fisheries Research Center in Cheney, Washington, under the direction of Al Scholz.

The material in this report is presented as a summary of study results, and has not been extensively peer-reviewed for publication. Opinions presented are mostly those of the senior author, and do not necessarily represent the position of the U.S. Fish and Wildlife Service.

METHODS

CRESTON NATIONAL FISH HATCHERY

Egg Collection and Incubation

On September 19-21, 1993, a total of 20,800 green eggs were collected from seven wild female bull trout taken off redds in two tributaries to the Swan River drainage of northwest Montana (Table 1). Each female was hand-stripped into a bowl and then sperm from one or two males captured in the vicinity was dripped into the bowl and mixed. After a few minutes, water from the stream was added and the eggs were rinsed and deposited in a bucket containing a solution of Betadyne. Eggs were water hardened in the iodophor solution for one hour, then transported to the hatchery in creek water and placed in the incubator trays; generally within three hours of spawning. Females were captured in varying stages of spawning so the number of eggs collected is not indicative of fecundity.

Most adult spawners were collected by dipnetting the fish off the redd. Initial efforts to capture fish in Lion Creek were made at night, but all nine fish captured were males. The process of crawling through log jams with a lantern held overhead was cumbersome, so subsequently, the females and several more males were captured with the use of a backpack shocker producing pulsed-DC current at 40 Hz. At least one large male and one smaller female suffered electrofishing injury in the process. The use of electrofishing equipment for this purpose is not recommended. In smaller streams, such as Holland Creek, the sneak method of capture with a dipnet proved effective without the use of the electrofisher. In several cases large fish, when disturbed, embedded themselves headfirst into debris jams. We were able to physically grab the head and caudal peduncle and "noodle" the fish out of the stream by hand for spawn-taking purposes.

Eggs from each female captured in 1993 were kept in separate containers in the field and incubated in separate trays at the hatchery in order to assess the range of viability. The eggs from six of the females were bright orange, but the seventh (from Holland Lake) produced eggs that were creamy yellow. One of the females had been dead approximately 1 hour prior to spawning. Egg size ranged from 216 to 304 per ounce, as measured in a Von Bayer trough, and tended to be smaller in the smaller females.

The males captured in Lion Creek by night dipnetting were held in a pen for up to 3 days prior to spawning. Males produced only a few drops of milt in most cases (a few ml at best). We had some concern about whether the sperm volume would be adequate to fertilize the eggs, but results demonstrated these concerns were unfounded.

The egg collections in 1993 occurred in T22N, R17W, Sec. 13 & 14, approximately 5 miles upstream in Lion Creek from the Swan River. Adfluvial adult bull trout, spawning in this portion of Lion Creek, have traveled over 30 miles upstream from Swan Lake. The 1993 egg take was targeted to Lion Creek because annual redd counts of 58 to 100 redds in 1988

Table 1. Vital statistics related to bull trout egg collections for the Creston Experimental Hatchery during 1993.

TRAY	DATE	SOURCE	PARENTS	OUNCES OF EGGS	SIZE OF EGGS	NUMBER OF EGGS
1	9/19	Lion Cr.	21.8" ♀ 29.5" ♂ 25.8" ♂ #2	6.5	253/oz	1,645
2	9/19	Lion Cr.	21.0" ♀ 27.0" ♂ 11.5" ♂ #2	8.0	304/oz	2,432
3	9/20	Lion Cr.	17.8" ♀ 29.0" ♂	5.5	290/oz	1,595
4	9/20	Lion Cr.	22.5" ♀ 22.0" ♂	2.0	238/oz	476
5	9/21	Holland Cr.	29.6" ♀ 30.8" ♂	28.0	238/oz	6,664
6	9/21	Holland Cr.	27.6" ♀ 28.3" ♂	12.0	225/oz	2,700
7	9/21	Holland Cr.	31.8" ♀ 29.3" ♂	24.5	216/oz	5,292
TOTALS				**86.5**		**20,804**

through 1992 indicated a stable or increasing trend in adult spawners. The stream afforded easy access, and is one of three most heavily used bull trout spawning streams in the Swan drainage. Redd counts in 1993 and 1994 were 123 and 141, respectively, consecutive new highs over the 12 year period of record.

Observation indicated that the egg collection effort in Lion Creek during 1993 occurred during the trailing end of the run. Due to the difficulty we experienced in capturing ripe females, we decided to explore other sites. On September 21, 1993 we examined Holland Creek, the inlet stream to Holland Lake, and found 19 completed redds and twelve large spawning adult fish in the creek. We captured three pairs with dipnets, all in the 28-32 inch class, and collected about 14,500 eggs (Table 1). The spawning methods used were the same as Lion Creek.

Due to the steep gradient, Holland Creek is accessible to spawning bull trout only in the lower 3/8 mile where it enters the lake. These fish are presumably residents of Holland Lake, and while still considered an adfluvial population, there is some question whether juveniles rear in this small tributary stream or in the lake. The Holland Lake fish do not exhibit the bright orange flanks and black markings on males found on males in Lion Creek. We kept these two lots of fish separate for purposes of thyroid hormone analysis, in order to observe potential differences in imprinting behavior.

In 1994, a second effort was made to collect bull trout eggs from Lion Creek. The streamflow conditions in 1994 were lower than in 1993 and capture by dipnets or "noodling" were the only techniques used. In 1994, a total of 12,000 eggs were collected from three females captured in Lion Creek (Table 2), at a location a few hundred meters downstream from the 1993 collection site. Due to the heightened concerns about the declining status of bull trout and impending listing decisions, very tight constraints were put on the issuance of the State of Montana Collector's Permit necessary to conduct these spawning operations.

All eggs were incubated in an 8-tray Heath stack. Eggs were examined periodically (at least once every two weeks) during incubation and dead eggs and/or alevins and severely crippled fry were picked off and enumerated.

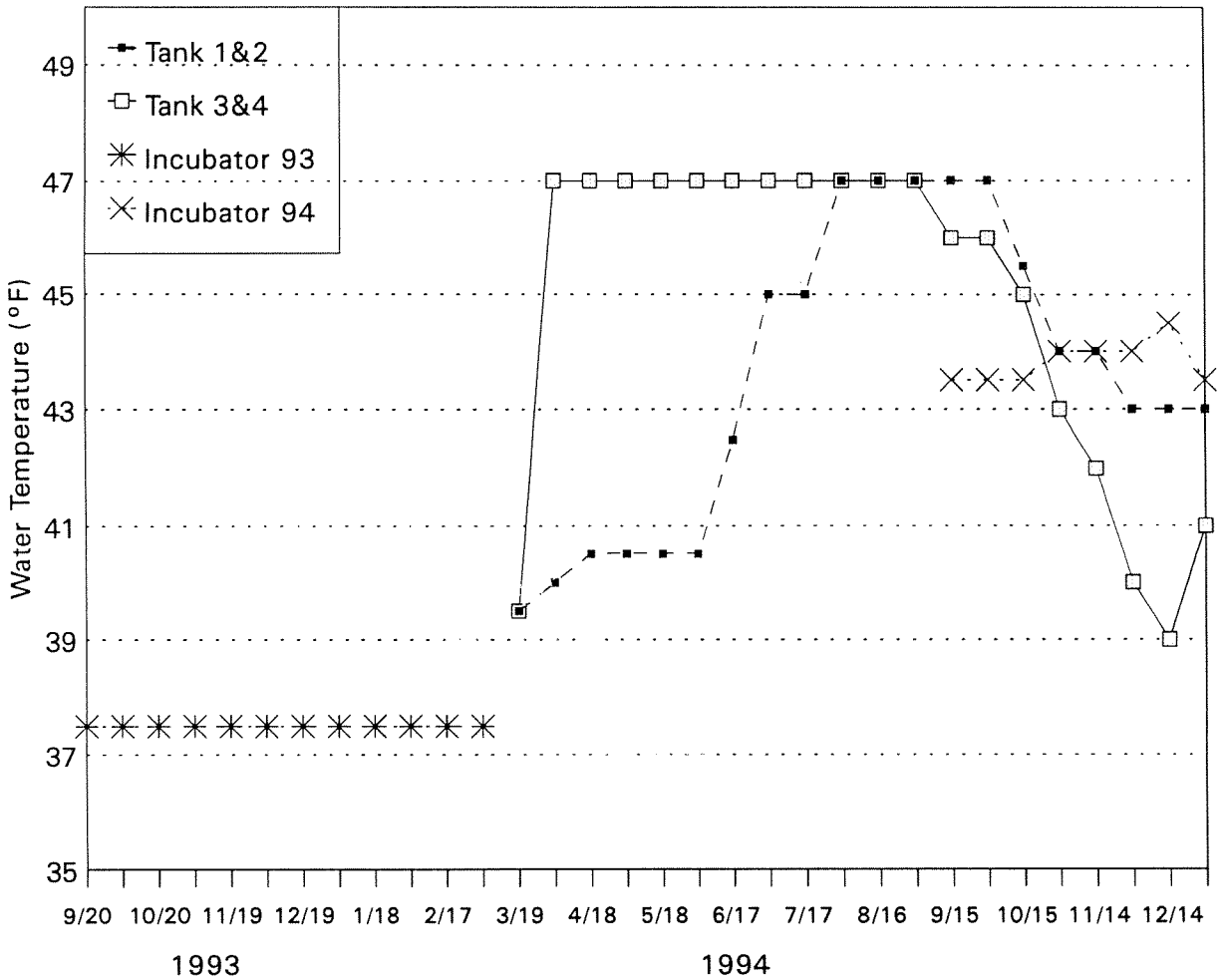
It is well-established that bull trout eggs require very cold incubation temperatures for normal development (McPhail and Murray 1979). Based on the observations of other experimenters, including known water temperatures of streams where eggs have successfully incubated (Tom Weaver, Montana Fish, Wildlife and Parks, Kalispell, pers. comm., 1993) we attempted to incubate the 1993 eggs at a temperature of 37-38°F. This was the minimum temperature we were able to attain with our chiller, cooling the incoming artesian well water which is supplied at a near-constant temperature of 47°F. Incubation temperature in the bottom tray throughout development (September 19, 1993 through March 11, 1994) ranged from 37.4-38.0°F during 1993, averaging 37.6°F, and eggs accumulated 5.6 F° temperature units per day (Figure 1).

Egg Incubation in the bottom trays proceeded slightly faster than in the top trays due to a slight warming (up to 0.5°F) as the water circulated down

Table 2. Vital statistics related to bull trout egg collections for the Creston Experimental Hatchery during 1994.

TRAY	DATE	SOURCE	PARENTS	OUNCES OF EGGS	SIZE OF EGGS	NUMBER OF EGGS
2,3,4	9/16	Lion Cr.	30.5" ♀ 25.0" ♂	22.6	253/oz	5,718
5,6,7	9/16	Lion Cr.	24.0" ♀ 32.0" ♂	18.2	243/oz	4,423
8	9/16	Lion Cr.	22.0" ♀ 24.0" ♂	7.2	268/oz	1,930
TOTALS				**48.0**		**12,071**

Figure 1. Water temperature (°F) used in incubating and rearing bull trout at Creston National Fish Hatchery in 1993 and 1994.



through the uninsulated stack. Eggs were checked periodically and any dead eggs were removed and enumerated. Dead eggs left over a period of several weeks began to fungus, but by picking them every 2 weeks or so, fungicide treatment was avoided.

During 1994, we incubated eggs at a higher temperature to assess the affect on survival, speed egg development, and reduce chiller demand. Eggs were incubated at 43.0-to-43.8°F, with an average temperature of 43.5°F in the bottom tray (September 15, 1994-December 31, 1994), and accumulated 11.5 F° temperature units per day (Figure 1). This was more than double the 1993 rate of thermal unit accrual.

Rearing and Feeding

After the alevins neared swimup in March 1994, they were transferred to rearing troughs. Throughout rearing, feed was delivered with a Ziegler automatic belt feeder placed at the head of the rearing tank. The following experimental design was used for rearing in 1994:

Tank 1 - 10 ft X 2 ft X 0.5 ft (75 gallon) fiberglass trough, painted light blue. Stocked with 1,000 fry (13.3/gallon) from Lion Creek (250 each from four paired matings). Chilled water was used for rearing in a "simulated" natural temperature regime (Figure 1). Some of these fish were also sacrificed to conduct thyroid hormone analysis. Fish in Tank 1 were fed by automated belt feeder during daylight hours (7:30 AM - 4:00 PM).

Tank 2 - 10 ft X 2 ft X 0.5 ft (75 gallon) fiberglass trough, painted light blue. Stocked with 1,000 fry (13.3/gallon) from Holland Creek (333 each from three paired matings). Chilled water was used to simulate natural temperature (Figure 1). Some of these fish were also sacrificed to conduct thyroid hormone analysis. Fish in Tank 2 were fed by automated belt feeder during hours of subdued light and darkness only (8:00 PM - 4:00 AM).

Tank 3 - 12 ft X 2.67 ft X 2 ft (450 gallon) fiberglass half-round tank, painted dark brown. Stocked with 5,870 fry (13.0/gallon), randomly mixed from all egg takes. Constant temperature spring water at 47 °F was used for rearing through September 9 (Figure 1). Fish in Tank 3 were fed by automated belt feeder during daylight hours (7:30 AM - 4:00 PM).

Tank 4 - 12 ft X 2.67 ft X 2 ft (450 gallon) fiberglass half-round tank, painted dark brown. Stocked with 5,870 fry (13.0/gallon), randomly mixed from all egg takes. Constant temperature spring water at 47 °F was used for rearing through September 9 (Figure 1). Fish in Tank 4 were fed by automated belt feeder during darkness hours only (8:00 PM - 4:00 AM).

During the period 3/11/94 through 9/9/94, 50 fish were sampled twice per month from each of the four tanks. Groups of ten fish each were anaesthetized, weighed on an analytical balance to the nearest .001 g, and average weight

calculated. Once per month, a sample of 50 fish was collected from each of tanks 3 and 4. Fish were euthanized and individually measured and weighed to establish length/weight relationships and condition factors.

All fry were fed Biodiet Bio-trainer #1 (Bio-Products Inc., Warrenton Oregon) initially. Rations were carefully weighed out and fish were fed 2.0 - 4.0 percent of body weight daily, with rations determined based on size of fish and water temperature (see chart on page 240-241 *in* Piper et al, 1982). Food size was increased as the fish grew. Tanks # 3 and #4 were switched to Bio-trainer #2 on April 21; Tanks #1 and #2 on May 19. On June 30, all tanks were converted to Sterling Silver Cup Salmon Diet (Nelson and Sons, Inc., Murray Utah). Tanks #1 and #2 were fed Salmon #1 diet June 30-July 27, and Salmon #2 diet July 28-September 9. Tanks #3 and #4 were fed Salmon #2 diet June 30-August 20 and Salmon #3 diet thereafter. A small stick was placed horizontally across the surface of each tank immediately downstream from the feeder, to ensure that the food sank, since bull trout were observed to be reluctant to rise to the surface to feed.

Throughout rearing and incubation, water temperatures were monitored with Hobo-Temp remote sensing devices placed in the water. Data from these devices was periodically downloaded and summarized on a computer file.

The small tanks (Tanks 1 and 2) were partially covered with sections of green corrugated fiberglass to simulate filtered natural light and provide some overhead cover. Sections of this material were placed in the water column in all tanks to provide visual isolation and shelter.

Light was controlled. Since the experimental facility has only one window, and it opens into a darkened feed storage building, we installed overhead fluorescent lighting. Shields were applied to the lights to diffuse direct glare. The lights were put on a timer system so that they mimicked natural day length, with periodic adjustments. Outside these hours the room was either completely dark (if dark outside) or subject to subdued natural lighting (dawn, dusk, or moonlight) filtering through the window.

Fish Health Assessment

Ovarian fluid samples were collected from the adult bull trout spawned in 1993. No specific pathogens were detected. On 8/24/94 the bull trout in the facility were subjected to a complete fish health inspection. Sixty fish were sacrificed and cultures taken to test for causative agents of furunculosis, enteric redmouth, bacterial kidney disease, viral hemorrhagic septicemia (VHS), and infectious pancreatic necrosis (IPN). Following a clean bill of health, the majority of the bull trout were moved to a separate set of rearing tanks in an outside shelter in September, and eventually to a standard 8 X 80 foot raceway in December.

Imprinting Experiments

Whole body thyroxine content was monitored to identify potential critical periods for olfactory imprinting. Samples of whole eggs and fish were collected at least every 2 weeks, more frequently during the period around

hatch and swimup. Samples were weighed to the nearest 0.1 mg on an analytical balance and flash frozen at -80°C in an ultracold freezer. Periodically, these samples were forwarded on dry ice to the UCUT Fisheries Research Center in Cheney, Washington where a procedure involving the use of radioisotopes was used to extract the thyroid hormone (Galloway et al, 1994). Twenty-five sample sets, beginning with the time of fertilization and ending after 237 days of development (post-swimup fry), have been processed to date. Sampling is continuing.

BOZEMAN FISH TECHNOLOGY CENTER (BFTC)

Diet and Growth Evaluation

About 4,800 fry were transferred to the BFTC from CNFH on March 13, 1994, about 6 weeks post-hatch and just prior to swimup. These fish were used to conduct a variety of investigations on diet and growth. We stocked 300 fish per tank in 75 liter (19.8 gal) rectangular aluminum tanks and fed them mechanically with belt feeders. Two diets, Bio-trainer and an experimental Bull Trout Starter (BTS 9401) formulated at BFTC, were fed for the first 8 weeks to 8 tanks each. Fish fed BTS 9401 were switched to Bio-trainer after 8 weeks and results evaluated periodically.

Alternate Rearing Techniques

Preliminary studies of alternate rearing techniques were conducted at BFTC, using fish held over from the diet and growth experiments. The purpose was to evaluate the potential effects of rearing fish with benthic structure to provide visual isolation, and/or overhead cover to reduce light and human disturbance. The fish used in the diet trial (above) were reared in a factorial treatment arrangement (cover only, structure only, cover and structure, control) for a period of 16 weeks in 75 liter (19.8 gal) rectangular aluminum tanks. At the conclusion of the trial period, fish from each of the four treatments were subjected to predation challenges. Ten fish from each of the four treatments were stocked into a 6 x 60 foot raceway, with 12 concrete cinder blocks placed every 5 feet along alternating sides of the raceway with the holes horizontal, to provide structure and cover. After a 24-48 hour acclimation period 4 lake trout that had been trained to prey on small fish were added. After 3-to-5 days the tests were terminated and the survivors counted. The predation challenge was conducted 5 times during October and November 1994.

Ability to Withstand Thermal Stress

A portion of the fish reared for the alternate rearing techniques experiment (described above), were tested after 16 weeks for their ability to withstand high temperatures. The ability of a fish to withstand stress is an indicator of the overall physiological status of a fish. For example, a fish that has been compromised by disease would not be able to withstand stress as well as a healthy fish. Less apparent defects in physiological status can also be important. A method has been developed to provide a temperature stress to fish, that will result in a quantitative evaluation of overall physiological

status as affected by factors such as diet or rearing condition. Ten fish from each treatment (mean weight 7.3 g) were confined in separate containers connected to a recirculating system equipped with temperature control. The temperature was increased incrementally at a rate of approximately 4°C (7.2 °F) per hour. When an individual fish lost equilibrium (was unable to remain upright) it was removed from the system and the temperature recorded. The experiment was analyzed as a 2 by 2 factorial treatment arrangement, with cover and structure being the main effects.

RESULTS

Water Temperature and Egg Incubation - CNFH

Eggs incubated in 1993, at an average and near-constant water temperature of about 37.6°F, were lightly eyed on 11/15 (56 days post-spawn). On 12/14 (85 days) eggs were strongly eyed. They were physically "shocked" at this time and the dead eggs removed.

The first fry hatched January 4, 1993. Early hatching proceeded slowly; by January 17 less than 1 percent of the eggs were hatched. Between Jan. 17 and Jan. 24 about 50 percent of the eggs hatched. Hatching was complete by January 31. Early fry exhibited photonegative behavior. When the tray was opened in a lighted room the fish were very active and agitated for a few seconds, and then settled quietly on the bottom.

In 1994, egg development was accelerated in the warmer (43.5°F) water. Eggs collected 9/16/94 were lightly eyed by November 2, after 47 days. Visible eyeup may have occurred somewhat earlier, but eggs were checked only sporadically. In the warmer water, fungus was more of a problem but treatment was still not necessary as long as eggs were picked weekly. In 1994, eggs were physically shocked at eyeup.

By November 27, 1994 hatching was underway and was complete within the next 10 days. Comparison of the results of the accelerated incubation temperature on the timing and success of hatching and development are presented (Table 3).

Eggs incubated in 1993 at 37.6°F hatched in 126 days, after accruing about 706 °F temperature units. Eggs incubated in 1994 at 43.5°F hatched in 75 days, after accruing about 863°F temperature units. The survival from green eggs to hatch was 97.1 percent in 1993 and 95.5 percent in 1994. Since the eggs from each female were kept separate we were able to calculate the percent viability for each fish. In each year, there was one pair in which percent eyeup was 89 percent; all others exceeded 96 percent.

There was very little mortality beyond the eyed stage. Only a few eggs failed to hatch and in each year a small number of crippled fry occurred. Typically, these deformities were either twins, sharing one yolk sac, or were corkscrew-shaped.

The period of "swimup" was difficult to pinpoint, since behaviorally these fish tend to stay on the bottom of the tank and do not use the upper water column. By April 8, 1994, fry from the 1993 egg collection were being fed and most appeared to be consuming food. This was 200 days from the time of egg collection. Swimup occurred a few days earlier in the fry that had been moved to warmer spring water.

Fry Rearing Experiments - CNFH

In order to evaluate alternative rearing strategies and investigate factors that would affect bull trout fry growth, behavior, and survival, a number of

Table 3. Comparative results of 1993 and 1994 bull trout egg incubation and survival at different water temperatures at Creston National Fish Hatchery.

	1993	1994
Date Spawned	Sept. 20	Sept. 16
Average Incubation Temperature (°F)	37.6	43.5
Calendar Days to First Eyeup	56 (Nov. 15)	47 (Nov. 2)
Degree-Days (°F) to First Eyeup	314	541
Calendar Days to 50% Hatch	126 (Jan. 24)	75 (Nov. 30)
Degree-Days (°F) to 50% Hatch	706	863
Average Temperature - Hatch to Swimup	39.5	43.5
Calendar Days - Hatch to Swimup	74	
Degree-Days (°F) - Hatch to Swimup	555	
% Survival - Green Egg to Eyeup	97.4	96.6
% Survival - Eyeup to Hatch	99.7	98.9
% Survival - Green Egg to Hatch	97.1	95.5

experiments were planned and carried out during 1994. On March 11, 1994 the pre-swimup fry were removed from the incubators, inventoried, and placed in rearing troughs. The following scheme (described in detail in the methods section of this report) was used:

		Temperature	
		Simulated Natural	Constant Spring
Time of Feeding	Day ---	Tank 1 1,000 Fish	Tank 3 5,870 Fish
	Night -	Tank 2 1,000 Fish	Tank 4 5,870 Fish

Fry in tanks 3 and 4 showed advanced development in the warmer water and were started on feed 3/22/94. By March 25 the presence of food in their guts was confirmed. Fry held in the colder water in tanks 1 and 2 were started on feed March 31 and slowly began utilizing it over the next few days. As the fish began to grow a few "pinheads", presumably fish that were physically or developmentally unable to utilize the food source, were apparent. By mid-May, these fish had died and were no longer present in the samples.

Initially, all fry were noted to congregate near the feeder. However, by April 28, 37 days after feeding had commenced, we noticed very different behavior patterns emerging between the day-fed and night-fed fish. In the morning, day-fed fish tended to cluster near the feeder in the head end of the tank. They were not very responsive to overhead movement, were difficult to frighten and were slow to take cover in the artificial substrate. Night-fed fish were evenly dispersed through the tank in the morning, lying mostly on the bottom, and at the first sign of movement overhead showed rapid fright response and sought cover. These differences were particularly apparent in the larger tanks (3 and 4). After several weeks, the differences between the two lots became less noticeable. Observations on June 28 indicated that both groups of fish were more active and became frightened and sought cover rapidly. However, fish fed during the day continued to cluster around the feeder during daylight hours when not frightened.

On June 16, during routine sampling, it was noted that fish in tanks 3 and 4 were beginning to nip on each other's pectoral fins. Incidence of clubbed fins was 6-28 percent in samples collected in June, July, and September. While the incidence appeared to increase over time there was no apparent correlation to day vs night feeding. Curiously, of the 43 fish examined with clubbed pectorals, the problem was consistently worse on the right fin. Fully two-thirds of the time (67 percent) the right pectoral was affected, in 28 percent it was the left pectoral, and in two fish (5 percent) both fins were clubbed. The fins of these fish were otherwise in good shape, with no apparent abnormalities noted in dorsal, pelvic, or anal fins.

Results of the thermal experiment indicated that fish held in the colder water, in a simulated natural temperature regime (Tanks 1 and 2), grew

considerably slower than fish held in constant temperature and warmer spring water (Tanks 3 and 4) (Table 4). After 183 days (September 9) the average weight of day-fed fish held in warm water (4.990 g) was nearly double that of fish fed similarly in colder water (2.868 g). On July 14, 18 weeks into the experiment, the disparity was greatest; the day-fed warmer water fish, averaging 2.67 g. each, were over three and one-half times as heavy as those raised in colder water and also fed during the day (0.748 g. each).

From April 8 through September 9, when all fish were well onto feed, the fish fed during the day in spring water gained an average of 39.3 percent of body weight every 2 weeks (Table 4). Fish in the same warm water, but fed during the night posted an average weight gain of 41.0 percent every 2 weeks. In the colder water, simulating a natural thermal regime, day-fed fish averaged a weight gain of 36.3 percent biweekly, whereas night-fed fish averaged gaining 33.7 percent biweekly. The largest disparity in growth rate gain was primarily during the first few weeks when the water temperature differential was greatest, and in fact the fish held in the natural temperature regime (initially colder) generally outgained the fish held in spring water during the last eight weeks of the experiment when water temperatures were equalized.

Bull trout fry growth, particularly early in development, was negatively impacted by night feeding (Figure 2). Day-fed fish in Tank 3 began to show a growth advantage 6 weeks into the experiment (4+ weeks after starting on feed) and this advantage was continued throughout the experiment. An advantage, equivalent to a 2-week head start, was maintained throughout the experiment.

The deficit in growth between Tanks 3 and 4 was manifested, especially early on, in lower condition factor in fish in Tank 4. Samples of 50 fish from each tank were individually measured, weighed, and condition factor calculated on June 16 (14 weeks), July 27 (20 weeks) and at the conclusion of the test on September 9 (26 weeks). Since both lots of fish were held in the same temperature water, the early disparity in condition factor is attributed to the day vs. night feeding regime. Results (Table 5) show that the condition factor was substantially higher in Tank 3 initially, but had become slightly higher in Tank 4 after 20 weeks, and was about even at the conclusion of the test.

Direct mortality was monitored through recovery of dead fish. Losses were highest in the first few weeks in all tanks, in part because a small percentage of fish did not adapt to feeding (pinheads) and starved to death. After mid-May there were minimal losses in all tanks, never more than five fish per week. There were no problems with disease. On one day in mid-August, 244 fish jumped out of Tank 2 and were found dead on the floor. This was the only incident where fish jumped from the tanks. At the conclusion of the experiment, natural mortalities recovered from each of the four tanks were 41 from Tank 1 (4.1%), 13 from Tank 2 (1.3%), 143 from Tank 3 (2.4%), and 129 from Tank 4 (2.2%). We did not observe any incidence of cannibalism in this period. However, inventory of numbers in each tank near the end of the experiment found as many as 800 fish unaccounted for in Tank 4. It is uncertain how or when these fish were lost. Inventory counts in the other three tanks were close to expected values.

Table 4. Biweekly comparison of mean weight (g) of bull trout reared under two different thermal regimes and fed during the day vs. night at Creston National Fish Hatchery in 1994 (n=50 for all samples).

DATE	TANK 1 DAY FEED COLD WATER	TANK 2 NIGHT FEED COLD WATER	TANK 3 DAY FEED SPRING WATER	TANK 4 NIGHT FEED SPRING WATER
3/11	0.108	0.117	0.114	0.114
3/25	0.096	0.111	0.106	0.110
4/08	0.101	0.112	0.148	0.114
4/22	0.138	0.141	0.249	0.171
5/05	0.184	0.191	0.424	0.340
5/19	0.257	0.249	0.639	0.386
6/03	0.362	0.393	0.901	0.659
6/16	0.502	0.437	1.499	0.863
6/30	0.687	0.639	2.040	1.281
7/14	0.748	0.806	2.674	1.783
8/01	1.181	1.249	3.346	2.417
8/11	1.356	1.389	4.097	3.132
8/25	2.099	2.056	4.283	3.619
9/09	2.868	2.546	4.990	4.293

Figure 2. Average weight (g) of bull trout reared in chilled water (Tank 1 & 2) vs. spring water (Tank 3 & 4) from March 11 through September 9, 1994 at Creston National Fish Hatchery. Fish in Tanks 1 and 3 were fed during daylight hours and Tanks 3 and 4 in darkness.

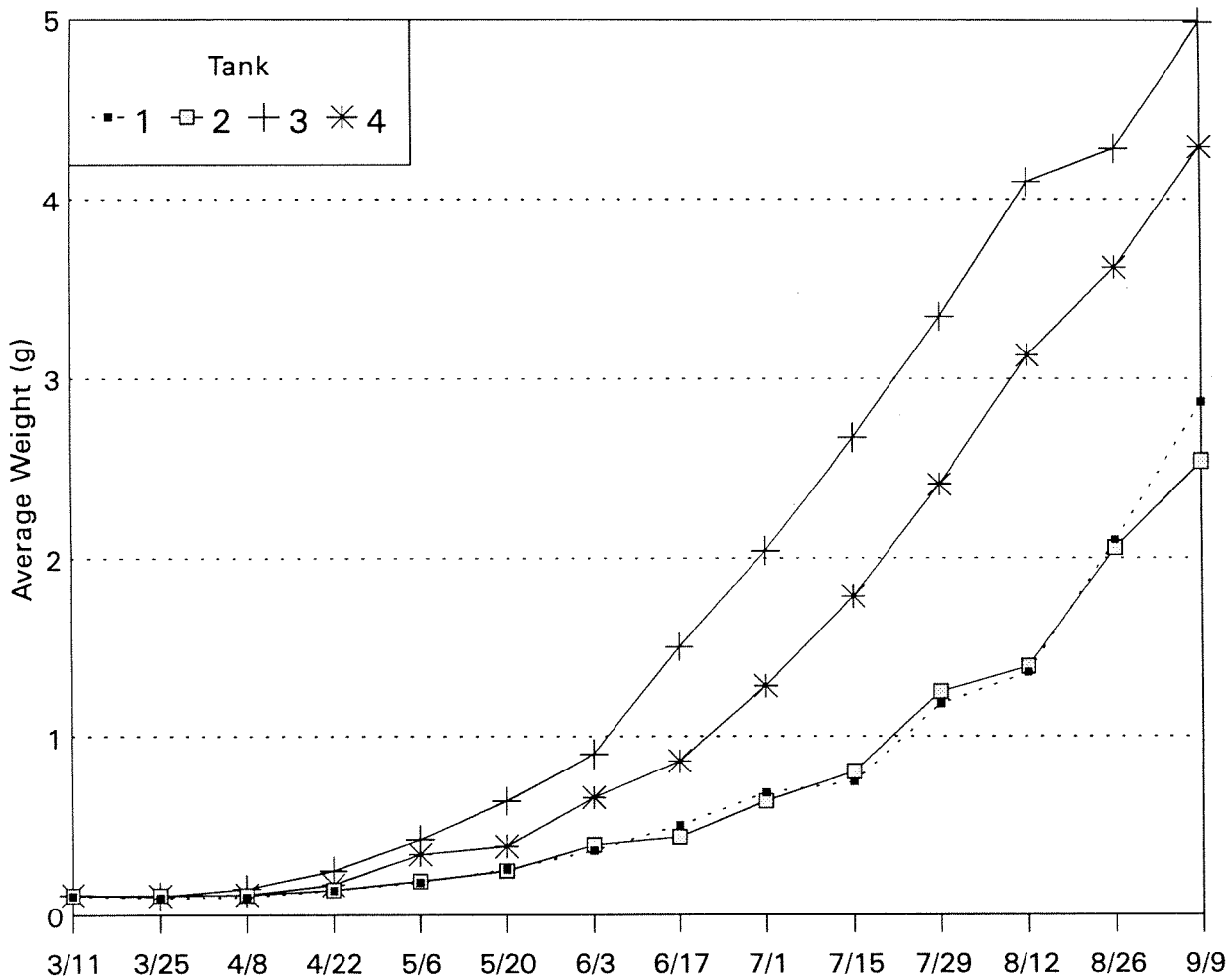


Table 5. Length, weight and condition factor of hatchery-reared bull trout reared in constant temperature spring water at Creston National Fish Hatchery (n=50 for all samples).

	JUNE 16		JULY 27		SEPT. 9	
	TANK 3 DAY-FED	TANK 4 NIGHT-FED	TANK 3 DAY-FED	TANK 4 NIGHT-FED	TANK 3 DAY-FED	TANK 4 NIGHT-FED
MEAN LENGTH (mm)	51.3	43.8	66.0	59.8	79.2	76.0
LENGTH RANGE (mm)	43-57	36-51	59-77	50-72	62-91	65-86
MEAN WEIGHT (g)	1.55	0.90	3.36	2.55	5.18	4.41
WEIGHT RANGE min/max (g)	0.89 2.08	0.50 1.34	2.28 5.33	1.23 3.56	2.43 8.18	2.71 6.28
MEAN CONDITION FACTOR (K)	113.1	105.1	113.5	117.3	101.0	99.3
CONDITION FACTOR RANGE (K)	105-125	93-115	91-135	93-141	86-118	88-109

Growth and Diet - BFTC

At the end of the first 16 weeks of feeding, some of the fish held at the BFTC showed symptoms of an ascorbic acid (vitamin C) deficiency. Quantification of the extent of the deformities revealed an affect of diet during the first 8 weeks, but no affect of the other treatments (structure, cover, etc.). The fish fed the Bio-trainer during the entire 16 week trial had an average of 10.3 percent incidence of scoliosis, whereas the fish started on BTS 9401 for 8 weeks showed an average of only 0.4 percent scoliosis. The increased level of vitamin C in the diet during the first 8 weeks of feeding seemed to prevent the symptoms of scoliosis. Due to the stress involved in holding wild strain fish in confined (75 liter) tanks in the hatchery, the vitamin C requirement for the bull trout may have been increased, thus creating the deficiency. Fish held at Creston Hatchery, fed the same Bio-trainer diet from the same box, but reared in colder water and larger tanks with low levels of light and human disturbance, were not observed to suffer from scoliosis.

Thyroid Imprinting Experiments - CNFH

The first 25 sample sets, comprising the period from fertilization to 237 days post fertilization, have been analyzed (Tables 6 and 7). Results to date indicate that thyroxine content of both stocks (Lion Creek and Holland Lake) were relatively low and stable from fertilized egg to swimup (Galloway et al 1994). In both stocks, thyroxine content peaked in post-swimup fry (Figures 3 and 4) and declined thereafter. Sampling will continue through the "smolt" stage.

Alternate Rearing Experiments and Predation Challenge - BFTC

Experiments conducted at BFTC included development of techniques to evaluate "fish quality" as evidenced by predator avoidance behavior and the variable affects of acclimation to structure and cover. It has been postulated that rearing and stocking techniques may play an important role in the numbers of hatchery fish which may survive in a natural system (Wiley et al 1993). Work with Pacific salmon indicates that rearing density may have an affect on the survival and subsequent rate of return (Ewing and Ewing 1995). Recent work with young grayling has shown that acclimation appears to cause a change in the rate of downstream movement, at least initially (Kaya and Jeanes 1995).

Bull trout reared in the presence of structure and cover consistently avoided predation more effectively than fish reared with only one or neither of these factors (Table 8). In four of the five tests, bull trout reared with structure and cover achieved the highest rate of survival. Overall, bull trout reared with structure and cover were eliminated by predators only 18 percent of the time, compared to 40 percent each for fish reared with only cover or the control group (no structure or cover) and 42 percent for fish reared with structure alone. Results were statistically significant ($P<0.05$).

Ability to Withstand Thermal Stress - BFTC

The presence or absence of covers on the tanks did not affect the ability of the fish to withstand high temperatures. The presence of structure in the

Table 6. Mean weights and thyroxine (T4) concentrations for Lion Creek stock bull trout at various life stages. (Galloway et. al 1994)

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

Table 7. Mean weights and thyroxine (T4) concentrations for Holland Lake stock bull trout at various life stages. (Galloway et al 1994)

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 3. Whole body thyroxine (T4) 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. (Galloway et al 1994)

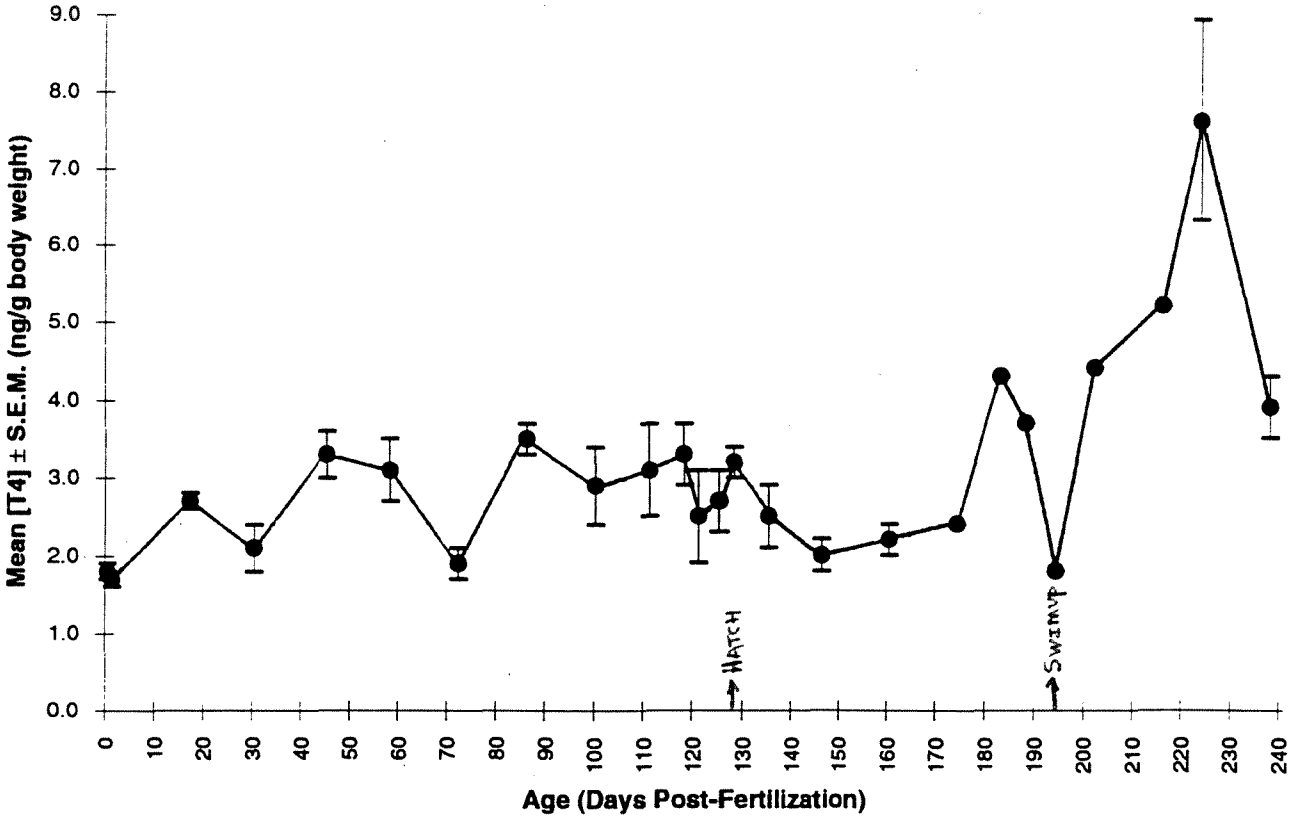


Figure 4. Whole body thyroxine (T4) concentration of Holland Lake bull trout. Each point represents the mean +/- SEM of 15 fish for days 0-159 post fertilization, 5 fish for days 173-215 post fertilization (SEM not calculated due to small sample size), and 10 fish for days 223-237 post fertilization. (Galloway et al 1994)

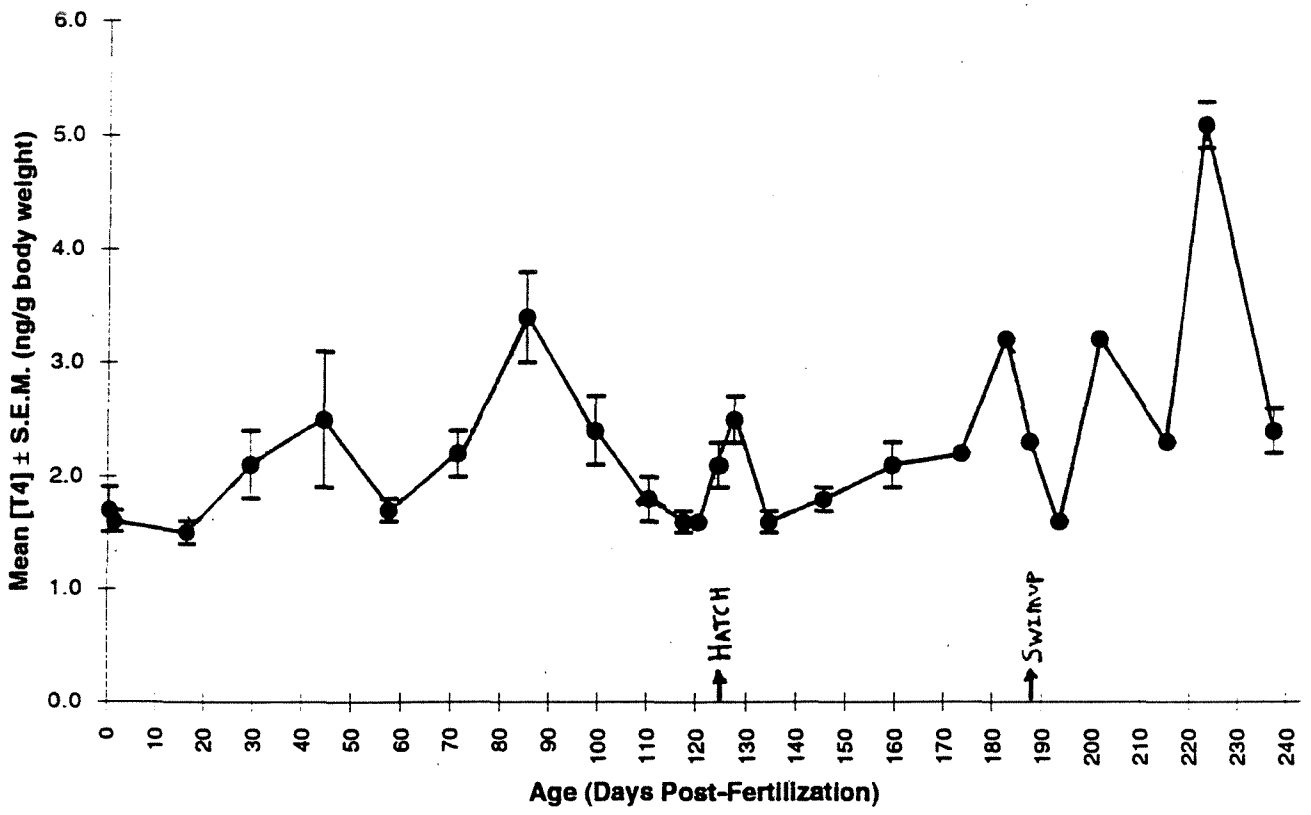


Table 8. Number and percent of bull trout eaten during predation challenge, presented by treatment type and test date (n=10 for each sample).

DATE	TREATMENT TYPE							
	Cover Only		Structure Only		Cover & Structure		Control (No cover) (No structure)	
	#	%	#	%	#	%	#	%
10/09	4	40	3	30	1	10	2	20
10/12	2	20	4	40	1	10	3	30
10/19	5	50	6	60	1	10	4	40
10/30	8	80	5	50	2	20	6	60
11/17	1	10	3	30	4	40	5	50
Total	**20**	**40**	**21**	**42**	**9**	**18**	**20**	**40**

tanks, however, decreased the ability of the fish to withstand high temperatures. The fish tended to pack into the rearing tanks behind the cover that was provided, compared to a more scattered distribution of fish reared without cover. This crowding might have produced stress on the animals, decreasing their overall level of fitness and ability to withstand further stressors (temperature). Structure, per se, may not be a problem but the number of fish relative to the amount of structure provided may be more important. Further study is required with different densities of fish to determine if a beneficial affect of structure, in addition to the predation test, is observed. There was no affect of structure on growth rate, but a negative affect on ability to withstand high temperatures. This data indicates that growth rate, the traditional measurement of quality, should not be the only measurement of physiological quality used when assessing a hatchery-reared product.

DISCUSSION

The initial success achieved in collecting and fertilizing eggs from wild bull trout, and in hatching and rearing the juvenile fish, indicates that culture of this species may be relatively routine. As with the experience of managers in British Columbia (G. Thorp, pers. comm.), it appears that cold incubation temperatures and proper delivery of high quality early diet are key factors in successfully rearing this species.

Egg survival to hatching during both years (96-97 percent) generally exceeded results of previous studies (McPhail and Murray 1979). McPhail and Murray noted a substantial decline in survival of eggs incubated at 6°C versus 4°C, whereas our results show no such decline at 43.5°F (6.4°C) versus 37.6°F (3.1°C). Differences between these studies could be related to the particular strain of bull trout used. McPhail and Murray (1979) also noted that low incubation temperatures (2°-4°C) produced larger fry. We did not evaluate this in our study.

Bull trout in this study accrued 706-863 °F temperature units to 50 percent hatch, with the higher numbers corresponding to warmer incubation temperatures used in 1994. This is equivalent to about 414-511 °C temperature units to complete hatching. Weaver and White (1985) similarly reported embryos required fewer temperature units to hatch at colder temperatures. Hatching was complete after 80 and 133 days, respectively, at 43.5°F and 37.6°F. McPhail and Murray reported slightly quicker development, with hatching completed in 74-78 days at 6°C and 119-126 days at 2°C. Gould (1987) reported hatching nearly complete after 503°C temperature units were accumulated by eggs incubated in 4.5°-8.0°C water.

Experiments conducted at the Creston facility indicate that, as with other salmonids, growth of juvenile bull trout is highly temperature-dependent. Up to 47°F bull trout growth was increased by warmer water temperatures. McPhail and Murray (1979) reported fry did well at temperatures up to 54°F (12°C). Shepard et al (1984) suggested that bull trout typically are not present in streams where water temperatures exceed 64°F (18°C), and that higher juvenile bull trout densities were observed in streams where water temperatures were 54°F (12°C) or less. Future hatchery experiments should incorporate water temperatures in the 54°F-64°F range to assess the effects of warmer water on growth and survival.

If thyroxine content is an accurate indicator of the time of imprinting in bull trout, our test results indicated that one critical period may be just after swimup. However, thyroxine was relatively low in bull trout, even during peak periods, when compared to other species that have been tested. For example, the peak level in bull trout (5.1-7.6 ng/g body weight) was less than half the peak levels observed in kokanee (16-18 ng/g body weight) (Tilson et al 1994). There is little published information on the imprinting period for bull trout, but it is common for imprinting in salmonids to occur at the time of smolting and emigration. We will continue to evaluate the thyroxine levels of these fish in an attempt to identify hormone peaks later in development. The establishment of timing of imprinting is critically

important to proper use of hatchery-reared bull trout in species recovery efforts.

Comparison of day-fed to night-fed fish indicated an early growth deficit resulted from feeding at night, especially during the first month (Figure 2). However, over time the fish seemed to adjust and later growth was equivalent, especially at warmer water temperatures. Goetz (1994) studied the behavior patterns of bull trout fry and juveniles and noted that bull trout fry tended to be most active during the day with peak activity at 1600-1700 hours (4-5 PM). All individuals sought cover approximately two hours before dusk. Juvenile bull trout (age 1 and older) showed the opposite pattern, with peak activity for two to three hours after dusk. It is uncertain when this transition from diurnal to nocturnal activity takes place. Observations of our laboratory fish indicate that the transition probably occurs in the first few months after swimup. Goetz (1994) attributes this partitioning of the resource, between fry (diurnal) and juvenile (nocturnal) bull trout, to developmental differences in the vision of fry and juveniles at different light intensities. In addition, this may be an adaptation for juveniles to avoid predators (which are presumably less active during the day). These behavioral factors could be utilized in developing a protocol for rearing bull trout in a simulated natural environment. Further experiments should be carried out to simulate natural conditions, feeding fry initially during the day, with a gradual transition to feeding juveniles at night. This may maximize survival of stocked fish by stimulating natural behavior tied to predator avoidance.

Experiments conducted at the Bozeman Fish Technology Center demonstrated that there is a conditioning response involved in rearing bull trout to avoid predation. Moring and Hockett (1994) suggested that without the availability of cover and structure, fish in a hatchery environment are unable to complete the fright response. Furthermore, from their tests with Atlantic salmon, they hypothesize that survival of stocked salmon fry and parr in the wild could be improved if fish are first conditioned to develop an antipredator response. Our initial results indicate similar potential. This response may be particularly important to a species like bull trout which are strongly associated with the substrate.

Wiley et al (1993) observes that wild trout in streams seek to maximize survival by minimizing energy expenditure, maximizing feeding when water temperatures permit best growth, avoiding predators, and reproducing in the best locations. To the extent that hatchery trout can be conditioned to maximize natural behavior trends, it is logical to assume their survival will be enhanced. Wiley et al (1993) suggests that hatchery trout should do better in the wild if taught to recognize and forage for natural food and respond to cover. To that end they recommend holding fish in darkened raceways with at least partial cover, automated feeding to reduce stress, and conditioning to wild food by introducing natural food prior to stocking. To the extent possible, we believe a similar protocol should be adopted for hatchery bull trout. The initial results of experiments presented in this report suggest that bull trout may be conditioned and survival of stocked fish enhanced by incorporation of these attributes in a rearing regime.

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