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ARTIFICIAL IMPRINTING AND SMOLTIFICATION IN JUVENILE KOKANEE SALMON: IMPLICATIONS FOR OPERATING LAKE ROOSEVELT KOKANEE SALMON HATCHERIES

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EXECUTIVE SUMMARY

In 1987, the Northwest Power Planning Council (NPPC) approved the construction of two kokanee salmon hatcheries on Lake Roosevelt, which would serve as partial mitigation for the loss of anadromous salmon and steelhead caused by construction of Grand Coulee Dam. In 1991, the hatcheries were built by Bonneville Power Administration (BPA) to provide a kokanee salmon (*Oncorhynchus nerka*) and rainbow trout (0. *mykiss*) fishery for Lake Roosevelt. The Sherman Creek Hatchery, located at the north end of the reservoir, provides an egg collection and imprinting site. The second hatchery, the Spokane Tribal Hatchery, located on the Spokane Arm of the reservoir, serves as the production facility. Fish reared there are released primarily as 7-9 month old fry and smaller numbers of 18-20 month old residualized smolts into Sherman Creek and other tributaries in order to imprint them to the water of those streams. However, adult returns have been poor. One reason for these poor returns may be that kokanee imprint to hatchery water at egg or swimup stages before 3 months of age. Therefore, if they are released *as* 7-9 month old fry, they would not be imprinting at the time of stocking. Another reason may be that these fish undergo smolt transformation while they are in the reservoir when they are approximately 1.5 years old. If this happens, they could be emigrating out of the reservoir below Grand Coulee Dam. In support of this idea, past investigations have indicated that some Lake Roosevelt kokanee emigrate downstream as far as Rock Island Dam. Additionally, coded wire tagged (CWT) fish released as residualized smolts have been recovered in Lake Roosevelt in substantially greater numbers than fish released as fry. The ratio of CWT residualized smolts to fry released was approximately 1 to 6 respectively, whereas recoveries were 75 to 1 respectively.

Since it is possible that kokanee imprint before stocking, a study was initiated in 1992 to determine if there was a critical period for thyroxine induced olfactory imprinting in kokanee salmon. Experiments were conducted to determine if kokanee could be imprinted to synthetic chemicals - morpholine and phenethyl alcohol - at different life stages and then home back to their appropriate chemical as mature adults. Fish were exposed to the synthetic chemicals in 1992 and again in 1993. Behavioral tests, to determine which life stages could home to their exposure odor, were conducted in the autumn of 1993 and repeated in the present investigation (1994). The other method for determining the extent of olfactory imprinting was to perform coded wire tagging

investigations involving the release of kokanee into Lake Roosevelt and recapturing them at egg collection sites scented with the chemical during their spawning migration.

Results of previous investigations with kokanee salmon documented that chemical imprinting coincided with elevated thyroxine levels in the 1991 year class kokanee exposed to synthetic chemicals in 1992. The group that had the highest whole body thyroxine content (swimup stage) also had the highest percentage (88%) of fish that were reliably attracted to their exposure odor as sexually mature 2 year old adults in behavioral tests conducted in the autumn of 1993. Recently hatched eggs and alevins also had relatively high thyroxine content and displayed 69% and 81% homing respectively. The other group that displayed accurate homing while being imprinted when thyroxine levels were elevated were 1990 year class fish, exposed to synthetic chemicals in April and May 1992 (smolt stage) and tested as 3 year old adults in the autumn of 1993 (67% homing to exposure odor). Pre-eyed eggs, eyed eggs and four fry stages, ranging from post swimup fry to near fingerling sized fry, all had relatively low thyroxine content and displayed poor homing ability. Homing ranged from approximately 17-32% in these groups.

Imprinting experiments were repeated in the present investigation in order to confirm previous results. Results of the present investigations indicated that imprinting occurred concomitant with elevated thyroxine levels in the 1991 year class kokanee exposed to synthetic chemicals in 1992 and tested in 1994 as age 3 spawners. Imprinting also occurred at the same time as thyroxine peaks in 1992 year class kokanee exposed to synthetic chemicals in 1993 and tested as age 2 spawners. In both groups, fish that had the highest whole body thyroxine content (swimup stage) also had the highest percentage of fish that were attracted reliably to their exposure odor in behavioral tests (92.2% homing for age 3 and 73.2% homing for age 2 spawners). Recently hatched eggs and alevins also had relatively high thyroxine content and displayed accurate homing in behavioral tests (68.2% and 87.5% respectively for age 3 spawners and 62.5% and 70.1% respectively for age 2 spawners). In contrast, pre-hatch eggs and post-swimup fry had relatively low thyroxine content and did not evidence selective attraction to their exposure odor (9.5% and 10.8% respectively for age 3 spawners and 24.3% and 11.4% respectively for age 2 spawners). Therefore, results of the imprinting investigations for both years indicated that kokanee

salmon imprinted to chemical cues during two sensitive (or critical) periods during development, at the alevin/swimup and smolt stages.

In addition to the imprinting experiments described above, a field test was conducted in Lake Roosevelt to determine if fish which were artificially imprinted as juveniles at different life stages in 1992 and 1993 would home to collection sites scented with the appropriate chemical as adults. Results of preliminary investigations with coded wire tagged fish exposed to synthetic chemicals at the smolts stage, then released into Lake Roosevelt as residualized smolts in 1992 and 1993, indicated that approximately 81% of the morpholine exposed fish recaptured were collected in the morpholine scented stream and 82% of the phenethyl alcohol exposed fish recaptured were collected in the phenethyl alcohol scented river. Too few fish exposed to synthetic chemicals at other life history stages and released as fry were recovered to assess imprinting effectiveness. Virtually all recaptures of coded wire tagged (CWT) fish have come from fish released as residualized smolts compared to fish released as fry. From 1992 to 1994 the ratio of fry to residualized smolts released was 2 fry to 1 residualized smolt, whereas the recovery ratio was 1 fry to 107 residualized smolts.

Smoltification investigations were conducted from 1992 to 1994. In 1992, plasma thyroxine levels in yearling (age 1+) kokanee peaked in January and again in May accompanied by pronounced silvering and downstream migration activity. In 1993/1994, smoltification in 12-19 month old fish was determined by measuring thyroxine, silvering, condition factor, salt water tolerance, osmoregulatory capability, gill Na^+-K^+ ATPase activity, intestinal water absorption (J_v), salinity preference and downstream migratory behavior. Results of the smoltification experiments on yearling (age 1) kokanee in 1993 indicated that:

(1) Plasma thyroxine concentration levels peaked in February and April.

(2) Silvering started to increase in February at the time of the thyroxine surge.

(3) Condition factor decreased slightly in April.

(4) Blood plasma osmolarity of fish held in freshwater (25 mOsmol/L), and dilute saltwater (300 mOsmol/L and 600 mOsmol/L) was maintained at approximately 300 mOsmol/L from January through July, but the osmolarity of fish held in full strength sea water (1000 mOsmol/L) was elevated from January through April at about 569 mOsmol/L, then decreased in May and June to approximately 300 mOsmol/L. In salinity tolerance tests, 100% of the fish survived in 25,300, and 600 mOsmol/L salt water every month. In 1000 mOsmol/L salt water, 0% of the fish survived from January through April compared to 90-100% survival from May through July. Gill ATPase peaked in April and intestinal water transport (Jv) decreased steadily throughout the sampling period.

(5) In salinity preference tests, fish showed a slight preference for concentrated seawater in February, but no preference during any other month. Fish displayed an increased tendency to migrate downstream in March (66%) and again in May (65%).

(6) The smolt assessment investigation also ascertained that smolts began to residualize by May or June as indicated by: Thyroxine, which had peaked in February and April, declined by July. Loss of parr marks and silvering began to increase markedly in February and peaked in May when 100% of the individuals exhibited silvery smolt coloration. Parr marks reappeared in June on about 60% of the individuals. Downstream migratory activity peaked in March and May at 65%, but by June only 40% of the fish evidenced downstream migratory activity. Condition factor, which declined during the spring, began to increase in June. Intestinal water uptake peaked in January and was lowest in June. Gill Na+-K+ ATPase activity peaked in mid April. The value then decreased in May and remained low in June. Osmoregulatory activity and salt water tolerance became evident in May. In June, fish still displayed the ability to osmoregulate and survive in full strength sea water.

Smoltification experiments with yearling (age 1) kokanee were repeated in 1994 to confirm the results gathered the previous year. In 1994:

(1) Plasma thyroxine concentration levels peaked in April.

(2) Silvering started to increase in February and continued through April.

(3) Condition factor remained relatively constant throughout the sampling period.

(4) The following physiological transitions were observed during the winter/spring of 1994. Intestinal J_V remained relatively stable from November to June, fluctuating from approximately 8 to 11 $\mu l/h/cm^2$, with no significant peaks. Gill ATPase peaked significantly in March and April. Blood plasma osmolarity of fish held in freshwater (25 mOsmol/L), and dilute saltwater (300 mOsmol/L and 600 mOsmol/L) was maintained at approximately 300 mOsmol/L from October through June, but the osmolarity of fish held in full strength seawater dropped significantly to 378 mOsmol/L and stayed low through April indicating the ability to osmoregulate during these months. In salinity tolerance tests, 100% of the fish survived in 25,300 and 600 mOsmol/L salt water every month. In 1000 mOsmol/L salt water, survival was low during the winter (30-40%) but rose to 95% by April.

(5) The following behavioral transitions were observed during the winter/spring of 1994. In salinity preference tests, fish showed a slight preference for concentrated seawater in November, but no preference during any other month. Fish displayed an increased tendency to migrate downstream in December (56%) and again in April (60%).

(6) Residualization was indicated by: Thyroxine peaked in April and then returned to low levels by June. Silvering peaked in May, but pan marks reappeared in June. Downstream migratory behavior peaked in April at 60% but declined to less than 40% by June. Gill ATPase activity peaked in early spring but by May, it decreased to basal levels. Osmoregulatory activity and salt water tolerance increased in early spring. By May, fish did not continue the ability to osmoregulate or survive in seawater.

Results of the smoltification experiments indicated that kokanee salmon underwent partial smoltification compared to anadromous salmon. They also exhibited residualitation by June. Residualization is a phenomenon where anadromous salmon

remain in freshwater if they do not reach the ocean within about 60 days after the onset of smoltification. If kokanee go through this process, entrainment from Lake Roosevelt could be reduced by releasing fish as residualized smolts instead of fry.

In addition, imprinting experiments confirmed the hypothesis that kokanee could be imprinted to synthetic chemicals. It should be possible to imprint fish to a synthetic chemical at the Spokane Tribal Hatchery, and then decoy the adult fish to the Sherman Creek Hatchery for egg collection.

Based on these results, we recommend the following measures for Lake Roosevelt kokanee hatcheries:

(1) Discontinue fry releases and instead release the fish as residualized smolts. This will require holding over a portion of the kokanee at the Spokane Tribal Hatchery. For this to be accomplished, a new production well capable of delivering 2-4 CFS of additional flow would need to be drilled at the hatchery. This would allow 500,000 fish to be raised to that size instead of the current holdover capacity of 100,000 to 300,000 fish.

(2) Additionally, put 500,000 age 0 fry in net pens from October until the following June or July when the smolts have residualized as was done with the rainbow trout in Lake Roosevelt. Past Lake Roosevelt Monitoring Program floy tag investigations demonstrated that by releasing rainbow trout from net pens in mid May to July, when fish were classified as residualized smolts, recovery rates were higher than with fish classified as smolts and released in March and April. We believe that releasing kokanee after they have residualited could produce benefits similar to those reported for rainbow trout. They would be less likely to emigrate out of the reservoir if they were residualized.

(3) Fish should not be released from the hatchery or net pens until they exhibit signs of residualization, including reappearance of parr marks and reduced downstream migratory activity. In general, the earliest release dates should not occur before mid-June. Late June and July releases are recommended, as this would also coincide with an increased zooplankton abundance within Lake Roosevelt and reservoir refill.

(4) Expose all of the fish to a synthetic chemical from hatch to swimup at the Spokane Tribal Hatchery and re-expose the fish which were being held over at the hatchery or in net pens to the same imprinting chemical a second time at the smolt stage.

(5) Release fish into Sherman Creek Hatchery as residualized smolts. At the time of release, scent the water with the chemical again.

(6) Scent the hatchery ladder with the chemical during the spawning migration.

(7) Intensify efforts to recapture more CWT fish. Sampling frequency should be once per month throughout the year, with continuous monitoring of egg collection sites from September 1 to October 30.

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1 .O INTRODUCTION

1.1 Lake Roosevelt Kokanee Hatcheries

When Grand Coulee Dam was built on the Columbia River in 1939, no fish ladder was constructed to allow adults to return to natal spawning areas upstream from the dam. In 1987, the Northwest Power Planning Council (NPPC) approved the construction of two kokanee hatcheries on Lake Roosevelt, as partial mitigation for the loss of anadromous salmon and steelhead caused by construction of the dam. In 1991, the hatcheries were built by Bonneville Power Administration (BPA) to provide a kokanee salmon (*Oncorhynchus nerka*) and rainbow trout (0. *mykiss*) fishery for Lake Roosevelt.

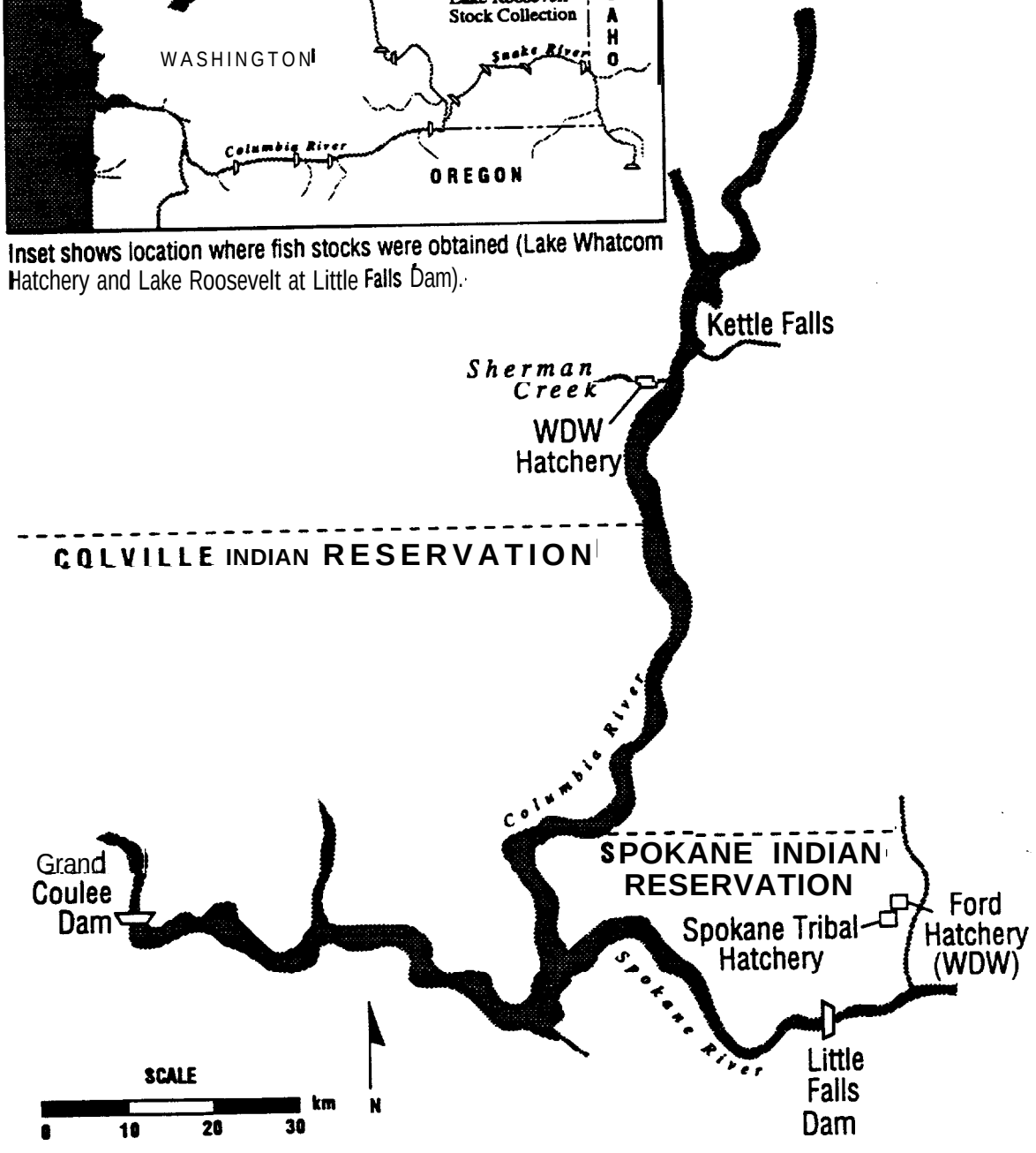
One of the hatcheries was located on the Spokane Indian Reservation (operated by the Spokane Tribe) and the other on Sherman Creek near Kettle Falls, Washington (operated by the Washington Department of Fish & Wildlife--WDFW) (Figure 1). Eggs are collected at the Sherman Creek Hatchery and then transferred to the Spokane Tribal Hatchery for incubation and rearing. Since there are presently not enough adults returning to Sherman Creek for egg collection, the Spokane Tribal Hatchery receives kokanee eggs for rearing from the WDFW Lake Whatcom Hatchery in Bellingham, WA.

Since production commenced in 1991, the Spokane Tribal Hatchery has produced the following fish for outplanting into Lake Roosevelt:

Species/ Location	Number released in 1991	1992	1993	1994
Kokanee released as age 0 fry at:				
Sherman Creek	1,334,845	1,317,220	1,161,822	1,327,522
Spokane River [1]	234,716	201,000	96,002	0
Other tributaries [2]	171,036	463,000	402,186	300,459
Total	1,741,597	1,981,220	1,660,010	1,627,981
Kokanee released as age 1 fingerlings (post-smolt) at:	0			
Sherman Creek	0	34,962	30,907	90,881
Spokane River		104,876	92,721	29,111
Other tributaries	0	0	0	0
Total	0	139,838	123,628	119,992

Figure 1. Location of Lake Roosevelt kokanee hatcheries operated by Spokane Tribe and WDW.

Inset shows location where fish stocks were obtained (Lake Whatcom Hatchery and Lake Roosevelt at Little Falls Dam).



Species/ Location	Number released in 1991	1992	1993	1994
Rainbow trout stocked as age 0 fingerlings into net pens	326,510	424,395	398,226	348,571
stocked as age 0 fingerlings directly into reservoir	0	83,800	185,800	88,874
stocked as age 1 carry over (catchable sized) directly into reservoir	0	24,951	19,772	16,697
Total	326,510	533,146	603,798	454,142

[1] Includes: Below Little Falls Dam and Blue Creek
[2] Includes: Hawk Creek, Barnaby Creek, and Banks Lake

In addition to the production noted for 1994, the hatchery is presently (December 1994) holding over approximately 340,000 kokanee for release into Lake Roosevelt in 1995 as residualized smolts. Thus, current production averages about 1.6 million kokanee fry, 300,000 residualized kokanee smolts and 500,000 rainbow trout for the Lake Roosevelt net pen program.

The rainbow trout net pen program has proven very successful. Past investigations had demonstrated that rainbow trout in Lake Roosevelt experienced a certain degree of smoltification (Peone et *al.* 1990; Griffith and Scholz 1991). In floy tagging studies conducted from 1986 to 1991, rainbow were released from net pens each month from March to July. The fish released in March and April were classified as silvery smolts, while those released from mid May to July were classified as residualized smolts. Griffith and Scholz (1991) found that the recovery rate in Lake Roosevelt was higher when rainbow trout were released as residualized smolts (94%) than those released as smolts (55%). Substantial numbers of fish released in March and April were recovered below Grand Coulee, whereas relatively few fish released after mid-May were recovered below the dam. This indicated that the rainbow trout began to residualize after May and remained in the reservoir better after that. Based on this information, management strategies were changed to release fish primarily in late May and June in an attempt to improve harvest rates in Lake Roosevelt. By releasing

rainbow trout as residualized smolts the harvest rate increased by lOO%, from approximately 70,000 fish per year to 140,000 fish per year.

The hatchery program has improved the kokanee and rainbow trout fishery in Lake Roosevelt. For example, creel surveys conducted before the hatcheries were built estimated the annual harvest of kokanee at 300 - 1000 fish and the annual harvest of rainbow at 1000 - 3000 fish (Beckman et al. 1985). After the hatcheries were built, harvest ranged from 7,500 - 35,000 kokanee and 70,000 to 140,000 rainbow trout (Peone et *al.* 1990; Griffith and Scholz 1991; Thatcher *et* al. 1995). The economic value of the fishery increased from approximately $2.8 million (pre-hatchery) to $5.3 to $12.8 million (post-hatchery).

However, a significant number of sub-adult kokanee released from the hatchery were being lost from the reservoir through Grand Coulee Dam. For example, in 1991, 35,651 kokanee were harvested in Lake Roosevelt (Thatcher et *a/. 1995).* In the same year, loss of kokanee through Grand Coulee Dam was estimated at 25,221 fish based on back-calculated counts of Lake Roosevelt kokanee passing through the fish counting facility at Rock Island Dam. The estimate was made by correcting the number of Lake Roosevelt kokanee observed at Rock Island (721 fish) by the Rock Island counting efficiency (5%) to yield the total number passing Rock Island (14,420). It was then assumed that the fish would suffer an average mortality rate of 15% (NPPC 1987) as they passed over each dam between Grand Coulee and Rock Island, (5 dams total including Grand Coulee and Rock Island), so the number passing over Grand Coulee that would be required to produce the 14,420 kokanee estimated at Rock Island could be back-calculated (Thatcher et *al.* 1995). Unfortunately, the counting facility at Rock Island Dam did not count kokanee passing through in 1993 or 1994.

In addition to the loss of sub-adult kokanee from the reservoir, past releases had produced few adults returning to stocking sites. This led to two hypotheses: (1) kokanee were going through a process of partial smoltification and were emigrating through the dam, therefore being lost from the fishery, and (2) kokanee were not imprinting to the water of the stocking sites and therefore were not homing to release sites. These hypotheses were tested for the first time last year and results were presented in Tilson *et a/.* (1994). The current investigation repeated last year's study in order to determine if results of our initial investigation on kokanee physiology and behavior could be consistently replicated. If so, changes in management protocols for

operating the Lake Roosevelt kokanee hatcheries could be applied with a certain amount of confidence.

1.2 Background

Smoltification is a process in which juvenile salmonids undergo changes in physiology, morphology and behavior which preadapts them to life in the ocean (reviews by Hoar 1976; Folmar and Dickhoff 1980; Hasler and Scholz 1983; Barron 1986). During this time, the juveniles also imprint to the water of their natal stream, and use this olfactory information to home back to their natal tributary during the spawning migration (Scholz et *al.* 1976; Hasler and Scholz 1983).

Changes in body morphology have been used as indicators of smoltification. The stream dwelling parr have characteristic vertical dark bands on the sides of their body called parr marks whereas smolts have a silvery appearance and a streamlined shape (Wedemeyer 1980; Gorbman et *al.* 1982; Winans 1984; Winans and Nishioka 1987). The silver reflecting layers are thought to camouflage the fish in the ocean by reflecting the surface of the water, and therefore protecting against predators below them in the water column. The slimmer, more streamlined shape is reflected by a decrease in the condition factor (K) (Hoar 1939; Fessler and Wagner 1969). These morphological indices were measured in the present investigation to determine whether kokanee exhibit morphological changes typical of anadromous salmon.

Physiological transitions also occur during smoltification so the juvenile anadromous salmon can change from a fresh water fish to one adapted to salt water. When the fish enters seawater, it increases its drinking response, reduces urine output and actively excretes excess salts across the gill. While still in fresh water, it gains water by transporting sodium and chloride ions across the gut wall. This produces a higher concentration in the gut, and allows water to be passively transported from the gut into the body fluids through osmosis (Utida et *al.* 1972). A "preadaptive" increase in intestinal fluid absorption (J_v) before the fish enters salt water has been reported in coho (*Oncorhynchus kisutch*) and Atlantic salmon (*Salmo salar*). This increase in J_v preadapts the fish to seawater (Collie and Bern 1982; Veillette et *al.* 1993). In the present investigation, J_v was measured to determine if kokanee salmon exhibit a similar preadaptive increase in intestinal fluid absorption as has been reported for anadromous salmonids.

Fresh water enters the gut passively by following sodium ions (Na+) which are transported into the gut by an increase in Na+-K+ ATPase enzymes. However, in the gill, these ATPase enzymes also increase in order to pump Na+ out of the fish (Pickford *et al.* 1970; Zaugg et *a/.* 1972; Zaugg and Wagner 1973). Numerous investigators have demonstrated an increase in gill ATPase activity during the parr-smolt transformation (Zaugg and McLain 1970; Johnson et *al.* 1977; Buckman and Ewing 1982; Zaugg 1982b; Hasler and Scholz 1983; Ewing et *a/.* 1979, 1984; Boeuf et *al.* 1985; Rondorf et *al.* 1989; Beeman et *al.* 1990; Birt *et al.* 1991; Franklin et *al.* 1992). This ATPase activity usually peaks during the downstream migration phase, and then after the fish enters seawater it stabilizes at the higher level. However, if the smolt remains in fresh water too long, the gill enzyme activity declines to the fresh water level and the fish becomes less resistant to salt water, assuming some of the characteristics of a parr. When this happens, the fish is termed a "residualized" smolt (Zaugg and McLain 1970; Folmar and Dickhoff 1981). The window of time when ATPase activity peaks has been used as an indicator of smoltification in anadromous salmonids. In the present investigation gill Na+-K+ ATPase activity was measured to determine if kokanee exhibit peaks in activity similar to those reported for anadromous salmonids.

Increased osmoregulatoty capability is another characteristic which smolts use to maintain low ion concentrations in the blood plasma, despite high concentrations in salt water. This ability to hypoosmoregulate develops during the smolt stage in coho salmon (*Oncorhynchus kisutch*) and steelhead trout (0. *mykiss*) (Folmar and Dickhoff 1979, 1980; Wedemeyer et *al.* 1980; Hasler and Scholz 1983) and with this comes the ability to tolerate and survive in full strength sea water. In the present study osmoregulatory capability and salt water tolerance of kokanee were tested to determine if they could adapt to salt water like anadromous salmonids.

Parr-smolt transformation in anadromous salmonids is associated with their downstream migration to the ocean. Monitoring changes in morphology and physiology in kokanee provided information about their metamorphosis into smolts but did not provide direct evidence about their tendency to migrate to the ocean like anadromous salmon. Increased tendency to migrate is the factor that would most likely be associated with increased entrainment at Grand Coulee Dam. Therefore, salt water preference and downstream migratory behavioral tests were conducted in the present investigation to assess entrainment potential through the dam.

Other investigators have reported that anadromous salmon [sockeye (0. *nerka*), coho (0. *kisutch*), chinook (0. *tshawytscha*), chum (0. *keta*) and pink salmon (0. *gorbuscha*)] have a distinctive behavioral preference for seawater at the time of their typical downstream migration, and retain this preference throughout the migration season (Houston 1956; Baggerman 1960; Mclnerney 1964; Conte et *al.* 1966). Hence, salt water preference behavioral tests were conducted with kokanee as part of the present investigation to determine if they showed a preference for salt water at the time of parr-smolt transformation similar to that of anadromous salmon.

The other behavioral test which would indicate entrainment potential was a downstream migratory test. When salmonids are in the parr stage, they are usually territorial, aggressive and orient upstream. When the fish smolt, they begin to migrate downstream toward the ocean (Hoar 1958, 1976; Hasler and Scholz 1983). This migration is nocturnal in Atlantic, sockeye, coho salmon and steelhead trout smolts (Hoar 1958, 1976; Hasler and Scholz 1983). Salmon must remain in visual contact with the bottom in order to orient upstream into a water current (reviewed by Arnold 1974). Hoar suggested that while pan seem to remain near the bottom during both day and night, smolts remain near the bottom during the day but rise to the surface during the night. Smolts positioned higher in the water column lose visual contact with the bottom and are transported downstream (Saunders 1965).

The mechanism of downstream migration has been subject to debate. Some investigators believe that downstream movement is strictly a passive displacement of the fish by the water current (Huntsman 1952; Hoar 1953). However, other investigators believe that the migration is directed and volitional (reviewed by Hartman et *al.* 1967; Chrisp and Bjorn 1978). Stasko et al. (1973) suggested that in many cases smolts display both types of behavior; orienting downstream and swimming faster than the current in low to moderate velocities, and orienting upstream and drifting downstream tail first in conditions of turbulent water. Thus, kokanee were observed in this investigation to determine if they exhibited an increase in downstream migratory behavior similar to other species of salmon. We also determined if their position in the water column (buoyancy and orientation) was similar to that seen by other investigators.

The changes in morphology, physiology and behavior described above are regulated by the neuroendocrine system. Hormone fluctuations are caused by an endogenous rhythm that is resynchronized by environmental cues. Environmental cues such as daylength, lunar cycles, temperature, discharge and water chemistry act to

signal the hypothalamus to release hormones from the anterior pituitary gland. These hormones either act directly on target tissues or activate other endocrine organs such as the thyroid and interrenal glands to secrete hormones, that act directlyon target tissues. Many hormones are thought to be involved in the parr-smolt transformation (Hoar 1976, 1988; Folmar and Dickhoff 1980; Hasler and Scholz 1983; Barron 1986). One of the hormones is thyroxine (T_4), which is a thyroid hormone. Another hormone involved in this process is cortisol.

Cortisol is generally recognized as the seawater-adapting hormone of teleosts. Cortisol appears to influence hypo-osmotic regulatory ability in teleosts by inducing a number of changes, including increased Na^+-K^+ ATPase activity in the gill, and adjustments in water and ion movement in the intestine and urinary bladder (Folmar and Dickhoff 1980; Redding et *a/.* 1984). The net effect is increased Na^+ extrusion (or efflux) of sodium ions. Cortisol may also play a role in other aspects of smoltification such as effects on the immune system, and behavior (reviewed by Specker 1982; Maule et a/. 1987).

Evidence of thyroid involvement stems from investigations which found that thyroid hormones exhibit a stimulatory effect on smoltification. Circulating levels of thyroid hormones including thyroxine (T_4) and triiodothyronine (T_3), have been shown to surge prior to the smolt transformation, which occurs in the spring in many species of salmon and trout. This surge is associated with an increase in silvering, an increase in downstream migratory behavior and with olfactory imprinting (Osborn et *al.* 1978; Folmar and Dickhoff 1979; Dickhoff *et al.* 1982; Hasler and Scholz 1983; Scholz et a/. 1985, 1992; Tilson et *al.* 1994). Thyroxine concentration was determined during this study because a surge could indicate the time of downstream migration. It could also indicate when the critical periods of imprinting occur in kokanee.

Olfactory imprinting occurs during the smolt stage in several species of salmonids. There is evidence that thyroid hormones induce olfactory imprinting (Scholz' 1980; Hasler and Scholz 1983). Coho salmon exposed to a synthetic chemical during the smolt stage were attracted to that chemical as adults (Scholz et *a/.* 1976; Hasler and Scholz 1983). The chemical imprinting appeared to be activated by thyroid hormones. Presmolt coho salmon exposed to synthetic chemicals when plasma thyroid hormones were at basal levels did not imprint to the chemicals; whereas fish exposed during the smolt stage, when thyroid hormones were elevated, did imprint to the chemicals (Scholz 1980; Hasler and Scholz 1983). This imprinting process requires binding of thyroid

hormones to nuclear receptors in neurons (Scholz *et al.* 1985; White et *al.* 1990). Hormone binding was thought to activate transcription of genes that code for nerve growth proteins, which promote neuron differentiation, including arborization of axons and dendrites, and formation of synaptic connections. This neuron differentiation structures the neurons to allow for the permanent storage of the imprinted olfactory memory in the central nervous system (Scholz et *a/.* 1985; 1992). Thyroid hormone receptors have also been identified in the olfactory epithelium and it has been suggested that thyroid hormones may stimulate development of a peripheral olfactory memory by smolts (Nevitt *eta/.* 1994).

However, not all species of salmon stay in their homestream for 1.5 years, and exhibit a distinct smolt stage like coho salmon and steelhead trout. For example, kokanee salmon, a landlocked salmon, emigrate from their natal tributary to a lake soon after they swim up from their gravel redd into the water column. Kokanee remain in the lake, where they grow to adult size before homing to their natal tributary. Thus, if these fish utilize olfactory cues for homing, they must imprint during the egg, alevin, or swimup stages.

Several investigators have reported that whole body thyroxine content fluctuates in developing eggs and larvae of several species of *Oncorhynchus* , peaking at hatch and again at swimup, and then subsequently dropping to very low levels during the fry stage (Tagawa and Hirano 1987, 1990; Leatherland et *al.* 1989; Tagawa et al. 1990; deJesus and Hirano 1992; Scholz et *al.* 1992, 1993; Tilson et *al.* 1994). Tagawa and Hirano (1987) and Kobuke et *al.* (1987) both observed decreases in the thyroxine content of chum and coho salmon larvae after hatching. This disappearance suggested both that thyroid hormone was being cleared from the fish, and that newly developing thyroid follicles had not yet begun thyroid secretion, These results also suggested thyroid hormones are maternally acquired, and used by the developing egg until the embryos begin to produce their own thyroid hormones (Kobuke et *a/.* 1987; Sullivan et *a/.* 1987; Tagawa and Hirano 1987; Greenblatt et *a/.* 1989). In kokanee salmon, a peak in thyroxine concentration was seen at the time of hatch, followed by a decline in alevins, then a second peak at swimup, subsequently followed by a decline to very low levels in post swimup fry (Scholz *et al.* 1992, 1993; Tilson *et al.* 1994). These data indicated that by the swimup stage, kokanee begin to produce their own thyroid hormones. Additionally, there appears to be a peak in thyroid activity at swimup that is similar to the surge reported at smolt transformation for anadromous salmonids. If

olfactory imprinting in kokanee is connected to elevated thyroid hormone levels as it is in other species of salmonids, these thyroid peaks at hatch and swimup may be an indicator of the critical period of olfactory imprinting.

Since it is possible that kokanee imprint as underyearlings, a study was initiated in 1992 to determine if there was a critical period for thyroid induced olfactory imprinting in kokanee salmon (Scholz et *al.* 1993; Tilson et *al.* 1994). Experiments were conducted to determine if kokanee could be imprinted to synthetic chemicals -- morpholine and phenethyl alcohol -- at different life stages and then home back to their appropriate chemical as mature adults. Fish were exposed to the synthetic chemicals in the winter/spring of 1992. The experiment was replicated in 1993. Behavioral tests, to determine which life stages could home to their exposure odor, were conducted in the autumn of 1993 (Tilson *et a/.* 1994) and repeated in the present investigation.

1.3 Summary of Previous Imprinting Investigations with Kokanee

Previous investigations with kokanee salmon documented chemical imprinting concomitant with elevated thyroxine levels. This was indicated by:

1) Whole body thyroxine content in young of the year 1990, 1991 and 1992 year class kokanee peaked at the time of hatch and again at swimup and subsequently declined to low levels in fry of all three year classes (Scholz et *al.* 1992, 1993; Tilson et *al.* 1994). Plasma thyroxine concentration levels measured in yearling presmolt to post-smolt stage kokanee peaked in January and again in May of 1992 in 1990 year class kokanee (Scholz et *a/.* 1993), and in February and April of 1993 in 1991 year class kokanee (Tilson *eta/.* 1994).

2) Chemical imprinting occurred concomitant with elevated thyroxine levels in the 1991 year class kokanee exposed to synthetic chemicals in the winter/spring of 1992. The group that had the highest whole body thyroxine content (swimup stage) also had the highest percentage (88%) of fish that were reliably attracted to their exposure odor as sexually mature 2 year old adults in behavioral tests conducted in the autumn of 1993. Recently hatched eggs and alevins also had relatively high thyroxine content and displayed 69% and 81% homing respectively. The other group that displayed accurate homing while being imprinted when

thyroxine levels were elevated were the smolts (1990 year class fish, exposed to synthetic chemicals in April and May 1992 and tested as 3 year old adults in the autumn of 1993) at 67%. Pre-eyed eggs, eyed eggs and four fry stages, ranging from post swimup fry to near fingerling sized fry, all had relatively low thyroxine content and displayed poor homing ability (homing ranged from approximately 17-32% in these groups).

3) In addition to the imprinting experiments described above, a field test was conducted in Lake Roosevelt to determine if fish which were artificially imprinted as juveniles at different life stages in 1992 and 1993 would home to collection sites scented with the appropriate chemical as adults. During both years, a portion of each group (life stage) released into Lake Roosevelt were tagged with distinctive coded wire tags. Results of preliminary investigations with coded wire tagged fish exposed to synthetic chemicals at the smolt stage, then released into Lake Roosevelt as residualized smolts in 1992 and 1993 indicated that 44 of 54 morpholine exposed fish (81.4%) recaptured were collected in the morpholine scented stream and 103 of 125 phenethyl alcohol exposed fish (82.4%) recaptured were collected in the phenethyl alcohol scented stream. Too few fish exposed to synthetic chemicals at other life history stages and released as fry were recovered to assess imprinting effectiveness. The failure of these fish to home did not appear to be related to an inability to imprint to synthetic chemicals. Instead, the poor returns of these groups were attributed to either low survival in the reservoir or emigration from the reservoir before reaching adult size (Tilson et a/. 1994). The fact that virtually all recaptures of coded wire tagged (CWT) fish have come from fish released as residualized smolts compared to fish released as fry (295 of 299 total recaptures or 98.7% of all tag recoveries, compared to a ratio of 79,149 CWT residualized smolts to 416,705 CWT fry released or 27.9% of all released) pointed to this (Tilson et *al.* 1994). Since the fish released as residualized smolts were recovered at a higher rate relative to the number of fry released, it was speculated that one factor contributing to the poor return of fry was the increased entrainment at Grand Coulee Dam (possibly associated with partial smoltification) at some point after release into the reservoir (Tilson et *a/.* 1994).

1.4 Summary of Previous Smoltification Investigations with Kokanee

Results of the smoltification experiments with yearling kokanee in 1993 indicated that:

1) Plasma thyroxine concentration levels peaked in January and again in May of 1992 (in yearling 1990 year class kokanee) (Scholz et *al.* 1993), and also in February and April of 1993 (in yearling 1991 year class kokanee (Tilson et a/. 1994). In both years, levels declined by July.

2) In 1993, silvering started to increase in February at the time of the thyroxine surge. An increase in silvering at the time of the thyroxine surge was also noted in 1992 (Scholz et *al.* 1993).

3) The following physiological transitions were observed in 1991 year class fish during the winter/spring of 1993. Intestinal water absorption (Jv) peaked in January then steadily declined. Gill ATPase peaked significantly in April. Condition factor decreased slightly in April. Blood plasma osmolarity of fish held in freshwater (25 mOsmol/L), and dilute saltwater (300 mOsmol/L and 600 mOsmol/L) was maintained at approximately 300 mOsmol/L from January through July, but the osmolarity of fish held in full strength sea water (1000 mOsmol/L) was elevated from January through April at about 569 mOsmol/L, then decreased in May and June to approximately 300 mOsmol/L. In salinity tolerance tests, 100% of the fish survived in 25, 300, and 600 mOsmol/L salt water every month. In 1000 mOsmol/L salt water, 0% of the fish survived from January through April compared to 90 - 100% survival from May through July.

4) The following behavioral transitions were observed in 1991 year class fish. during the winter/spring of 1993. In salinity preference tests, fish showed a slight preference for concentrated seawater in February, but no preference during any other month. Fish displayed an increased tendency to migrate downstream in March (66%) and again in May (65%).

5) The smolt assessment investigation also ascertained that smolts began to residualize by May or June (Tilson et *al.* 1994) as indicated by: Thyroxine peaked in February and April, then declined by July. Loss of parr marks

and silvering began to increase markedly in February and peaked in May when 100% of the individuals exhibited silvery smolt coloration. Parr marks reappeared in June on about 60% of the individuals.' Downstream migratory activity peaked in March and May at 65%, but by June, only 40% of the fish evidenced downstream migratory activity. Condition factor, which declined during the spring, began to increase in June. Intestinal water uptake peaked in January and was lowest in June. Gill Na^+-K^+ ATPase activity peaked in mid April. The value then decreased to in May and remained low in June. Osmoregulatoty activity and salt water tolerance became evident in May. In June, fish still displayed the ability to osmoregulate and survive in full strength sea water.

1.5 Study Objectives

Preliminary results of previous imprinting investigations supported the hypothesis that thyroid hormone peaks were correlated with critical periods for imprinting in kokanee and identified critical periods at the alevin/swimup and smolt stages. These results suggested that it should be possible to imprint fish to a synthetic chemical at the Spokane Tribal Hatchery and decoy the adult fish to the Sherman Creek Hatchery for egg collection Tilson *et al.* (1994). In the present investigation we repeated imprinting experiments to confirm the initial results.

Results of previous smoltification investigations indicated that kokanee salmon exhibited smoltification comparable to that of other anadromous salmonids. However, in some aspects, the degree of this transformation was not as pronounced as in other species of salmon. Also, kokanee appeared to go through the process of residualization by June. This phenomenon occurs in many species of anadromous salmonids if they do not reach salt water within about 60 days after the onset of smoltification (Clarke and Nagahama 1977; Folmar et *al.* 1982). These residualized smolts stop their migration, readjust their osmoregulatory systems back to fresh water, and they remain in the river until the following year, or until spawning. If kokanee go through this process, entrainment losses could be reduced if kokanee are released as residualized smolts, instead of presmolt fry or fingerlings. Therefore, it was necessary to confirm the results of the smoltification experiments obtained last year in order to provide reliable outplanting recommendations for the Lake Roosevelt kokanee hatcheries.

2.0 METHODS AND MATERIALS

2.1 Rearlng Conditions

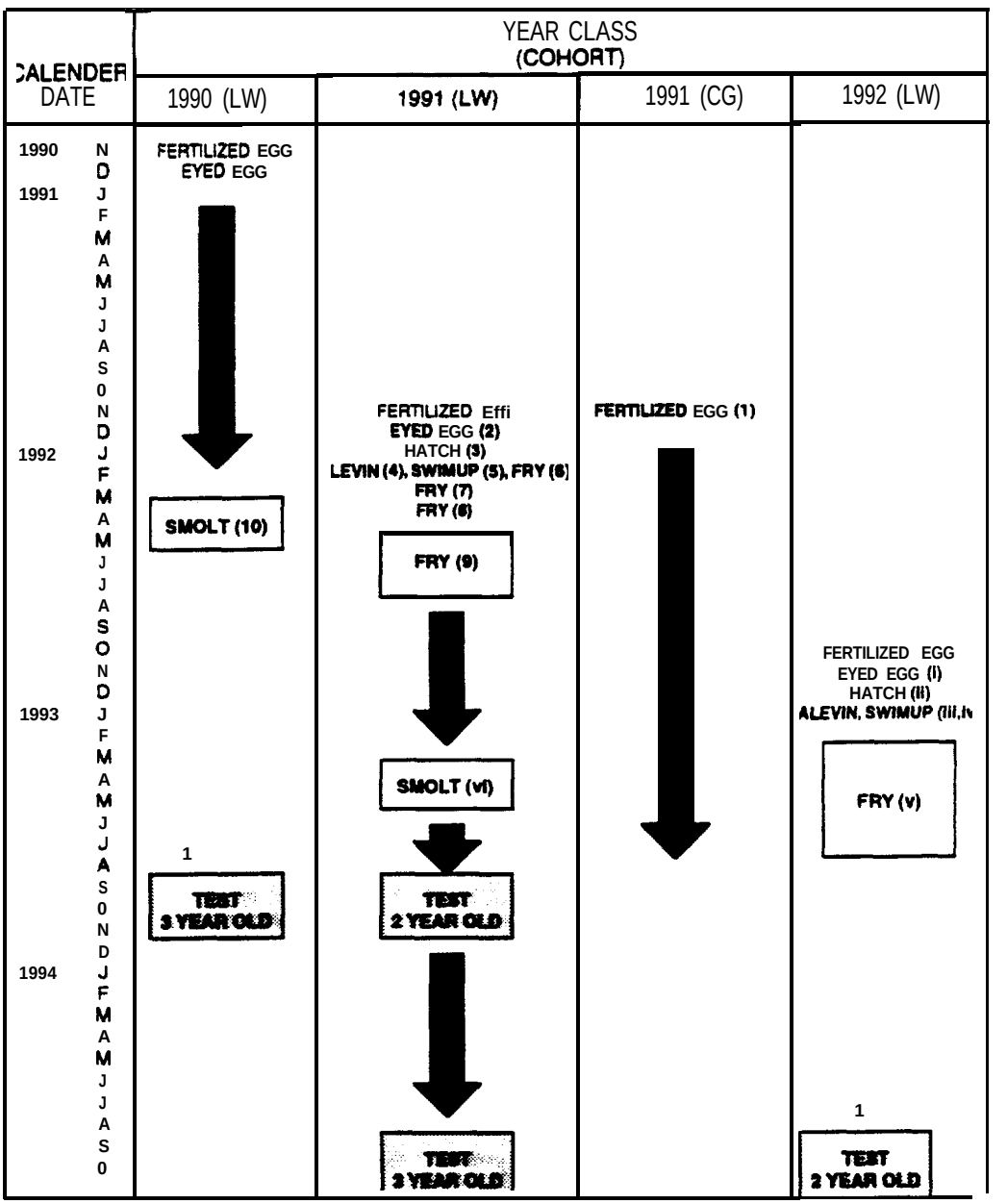
Juvenile kokanee salmon, transferred as eyed eggs from the Lake Whatcom Hatchery in Bellingham, WA, were reared at the Spokane Tribal Hatchery near Wellpinit, WA (Figure 1). Water supply to the raceways was a combination of Metamooteles Springs water and well water at 8-l 1 ° C. After swimup, zero age fry were feed trained on Biodiet semi-moist mash (starter feed). Older fry were fed a combination of Biodiet semi-moist grower feed (1 .0 - 2.5 mm crumbles) and Silvercup size l-4 mm crumbles. Yearling fish were fed Biodry 1000 pellets (3.0 - 4.0 mm) obtained from Bioproducts, Inc. Photoperiod was maintained at natural daylength as each raceway was partially exposed to natural conditions of light and weather.

2.2 Olfactory Imprinting Investigations

To determine when the critical period for olfactory imprinting occurred, whole body thyroxine content was monitored from fertilized egg through fry stage in two year classes of kokanee (1991 and 1992), and plasma thyroxine was monitored from fingerling to post-smolt stage in three year classes of kokanee (1990, 1991 and 1992). Simultaneously, the fish were exposed to either morpholine (C_4H_9NO) or phenethyl alcohol ($C_8H_{10}O$) at various developmental stages (Figure 2). Two imprinting chemicals were employed in this experiment so that one odor could act as a control for the other. These imprinting experiments were conducted twice with 1991 year class egg and fry stage fish and 1990 year class smolt stage fish exposed to the synthetic chemicals in the winter/spring of 1991/1992, and again with 1992 year class eggs and fry and 1991 year class smolts exposed to the synthetic chemicals in the winter/spring of 1992/1993. The fish were then retained at the Spokane Tribal Hatchery without again being exposed to the odors for either 18 or 30 months before they were tested to determine if they could home to their exposure odor when they became sexually mature at either age 2 or age 3.

In the autumn of 1993, the 1990 and 1991 year class fish were tested at age 3 and age 2 respectively. In the autumn of 1994, the 1991 year class fish were tested at age 3 and the 1993 year class fish were tested at age 2. The results of the behavioral testing conducted in 1993 were reported by Tilson *et al.* (1994). Details of the synthetic

Figure 2. **Experimental protocol for synthetic chemical imprinting experiments. Kokanee salmon obtained from Lake Whatcom (LW) or Cabinet Gorge (CG) were transferred to the Spokane Tribal Hatchery as eyed eggs and recently fertilized eggs, respectively. Life stages of each year class in bold type are stages at which synthetic chemical imprinting was attempted. Numbers 1-10 refer to the 10 life stages exposed to synthetic chemicals in 1992. Number i-iv were exposed in 1993. At each life stage one group was exposed to morpholine and a second group to phenethyl alcohol, with the exception of the fertilized eggs from CG (1) which were only exposed to phenethyl alcohol. Stages surrounded by boxes indicate the length of the exposure period.**



chemical imprinting procedure and methods for calculating steady state concentration of imprinting chemicals were described in a previous annual report (Scholz et a/. 1993).

2.2.1 1992 Imprinting Investigations

In 1991/1992, fish were exposed at the following life stages (determined by the number of days post-fertilization):

Life Stage	Days Post-Fertilization
(1) fertilized egg	(O-30)
(2) eyed egg	(30-60)
(3) hatch	(53-63)
(4) alevin	(60-90)
(5) swimup	(88-93)
(6) fry	(93- 125)
(7) fry	(125-155)
(8) fry	(155-185)
(9) fry	(185-227)
(10) smolt	(481-541)

Fish in Groups 1 - 9 were 1991 year class (age 0) when they were exposed to synthetic chemicals. Fish in Group 10 were 1990 year class (age 1) when they were exposed to the chemicals. Details about the first 1.5 years of life of these fish can be found in reports by Scholz et *al.* (1992, 1993) and Tilson et *al.* (1994).

Whole body thyroxine content was determined in twenty 1991 year class fish at approximately weekly intervals from day 0 to 134 days post-fertilization, then at biweekly intervals until 227 days post-fertilization. Blood plasma thyroxine was determined in twenty 1990 year class fish at biweekly intervals from fry to post-smolt stages (235-586 days post fertilization). Procedures were described by Scholz et *al.* (1992, 1993) and Tilson et a/. (1994). Results of these investigations were presented in Scholz et *al.* (1993) and Tilson et *al.* (1994).

At each life stage, one group of fish was exposed to morpholine (15 X 1 0^{-5} mg/L) and a second to phenethyl alcohol (5 X 1 0^{-3} mg/L). The fish were retained at the Spokane Tribal Hatchery until they became sexually mature in the autumn of 1993 without again being exposed to the odors. At that point, some of the fish in groups I-9 became sexually mature age 2 fish, and those in group 10 were sexually mature age 3,

when behavioral tests were conducted. Fish in groups 1-9, not maturing until age 3, were held for another year and were tested in the autumn of 1994 (Figure 2). Behavioral tests were conducted by releasing the fish in a stream below the confluence of two tributaries that formed a natural Y-maze, to determine which fish migrated upstream and selected the arm scented with their appropriate exposure chemical.

2.2.2 1993 Imprinting Investigations

In 1993, fish were exposed at the following life stages (determined by the number of days post-fertilization):

Life Stage	Days Post-Fertilization
(1) eyed egg	(44-64)
(2) hatch	(64-76)
(3) alevin	(70-94)
(4) swimup	(91-98)
(5) fry	(105-252)
(6) smolt	(470-530)

Fish in groups l-5 were 1992 year class (age 0). Fish in group 6 were from the 1991 year class (age 1). In 1993, whole body thyroxine was determined in 20 zero-age fish (1992 year class) at weekly intervals from 44 to 154 days post-fertilization, then at biweekly intervals until 252 days post-fertilization. Also, blood plasma thyroxine concentration was determined in 20 yearling (1991 year class) fish from pre-smolt to smolt stage. A detailed description of the origins of these fish and procedures for thyroxine determination were described in Tilson et *al.* (1994). These fish were divided into twelve groups (two groups from each stage) that were exposed to either morpholine or phenethyl alcohol. They were held at the Spokane Tribal Hatchery until they became sexually mature at age 2 in the autumn of 1994, and behavioral tests were conducted at that time. Behavioral tests were not conducted on 1991 year class fish (age 3) since they were all inadvertently released into Lake Roosevelt.

2.3 Odor Discrimination Behavioral Test Procedure

After synthetic chemical exposure was accomplished in 1992, 400 fish from each of the 19 groups exposed to synthetic chemicals were marked with a specific fin clip that distinctively identified the developmental stage and exposure chemical. These fish were combined in one raceway and held until they became sexually mature and odor

discrimination tests were conducted. After synthetic chemical exposure was accomplished in 1993,200 fish from each of the 10 groups (from eyed egg to fry stage) were marked with a distinctive combination of fin clips and coded wire tags that distinctively identified the developmental stage and exposure chemical. These fish were combined in one raceway and held until they became sexually mature and odor discrimination tests were conducted.

Behavioral experiments were conducted in 1993 and repeated in 1994. Since our test procedure required that the fish be in a migratory state, fish were not tested unless they were sexually mature. In 1993, the following fish became sexually mature and were tested:

Year Class	Age at Sexual Maturity	Life Stage Exposed to Chemical	Exposure Chemical	Number of Fish Tested
1990	3	Smolt	MOR	64
			PEA	65
1991	2	Fertilized egg	PEA	242
		Eyed egg	MOR	33
			PEA	36
		Hatch	MOR	52
			PEA	31
		Alevin	MOR	35
			PEA	35
		Swimup	MOR	28
			PEA	25
		Fry (Feb)	MOR	14
			PEA	17
		Fry (Mar)	MOR	25
			PEA	30
		Fry (Apr)	MOR	22
			PEA	21
		Fry (May-Jul)	MOR	51
			PEA	52
Total				878

In 1994, the following fish became sexually mature and were tested:

Year Class	Age at Sexual Maturity	Life Stage Exposed to Chemical	Exposure Chemical	Number of Fish Tested
1991	3	Eyed egg	MOR	8
			PEA	8
		Hatch	MOR	9
			PEA	8
		Alevin	MOR	10
			PEA	7
		Swimup	MOR	9
			PEA	10
		Fry (Feb)	MOR	5
			PEA	7
		Fry (Mar)	MOR	7
			PEA	5
		Fry (Apr)	MOR	8
			PEA	8
		Fry (May-Jul)	MOR	8
			PEA	8
1992	2	Eyed egg	MOR	125
			PEA	115
		Hatch	MOR	133
			PEA	106
		Alevin	MOR	115
			PEA	82
		Swimup	MOR	88
			PEA	86
		Fry	MOR	12
			PEA	14
Total				1,001

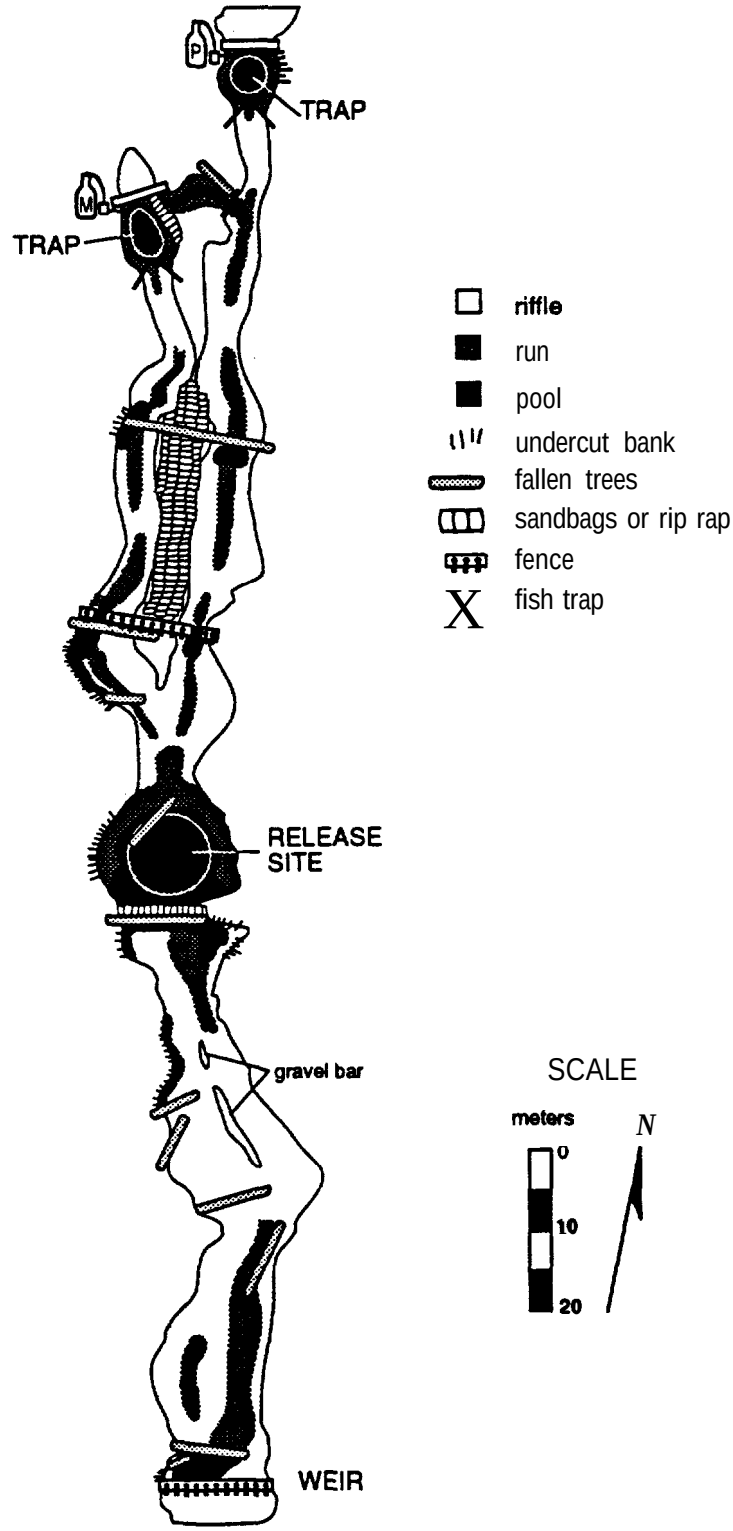
In 1993, mean lengths (±SD) of age 2 (1991 year class) fish tested were 345 ± 33 mm, and age 3 (1990 year class) fish were 423 ± 37 mm. In 1994, mean lengths

(±SD) of age 2 (1992 year class) fish were 306 ± 25 mm and age 3 (1991 year class) fish were 431 ± 38 mm.

In the 1993 experiments, fish were tested daily from September 20 to November 28, 1993. In the 1994 experiments, fish were tested daily, from September 3 to November 13, 1994. Experimental fish were released into a stream 50 meters below the junction of two tributary channels (Figure 3). Fish were randomly selected by dipnetting into the hatchery raceways which contained fish from all exposure groups. Morpholine and phenethyl alcohol were metered randomly into traps installed in each channel to **attract** fish (Figure 3). [Fish trap construction was explained in detail by Tilson et *al.* (1994)]. A response required upstream migration and selection of a channel scented with the correct odor. Hence, this experiment simulated the kind of choice that must be made by maturally migrating adult fish. A weir was set up about 100 m below the release site to prevent fish from escaping downstream. Each trap and downstream weir were checked daily by two observers and the number of each type of fin clipped fish captured at each location was recorded. A blind procedure was used since neither observer knew what chemical treatment or developmental stage was designated by a particular fin clip.

Dye tests were conducted in both years prior to, in the middle and at the end of the experimental period by releasing Rhodamine B dye into the channels. This was done to determine the time it took for odors released at the traps to be cleared from each arm. The mean clearance times (±SD) were 5 min, 50 sec (± 32 sec) from the short arm (n=3) and 8 min, 56 sec (±39 sec) from the long arm (n=3) in 1993. In 1994, mean clearance times were 5 min, 35 sec (f40 sec) in the short arm (n=3) and 15 min, 45 sec (± 1 min, 17 sec) from the long arm (n=3). Odors were switched at 2-5 day intervals and periodically turned off so that no odor was released. In 1993, morpholine was metered into the short arm for a total of 25 days, and the long arm for a total of 20 days. Phenethyl alcohol was metered into each channel in a reciprocal pattern. On nine days, no odor was delivered into either channel. In 1994, morpholine was metered into the short arm for 28 days and the long arm for 32 days. Phenethyl alcohol was metered into each channel in a reciprocal pattern. On 17 days, no odor was delivered into either channel.

Figure 3. **Natural Y-maze used to conduct olfactory discrimination investigations. Bottles labeled M and P indicate where synthetic chemicals were metered into traps on each arm of the Y. The position of the release site and weir used to trap fish migrating downstream are also noted.**



For a fish to be classified as imprinted to their exposure odor, we required that three criteria be met:

(1) Downstream migration if the odor was absent;

(2) Upstream migration if the odor was present; and

(3) Selection of the tributary scented with the fish's exposure odor instead of the tributary scented with the alternate odor.

Fish meeting these criteria were classified as "imprinted" and those not fulfilling them were classified as "not imprinted." Our criteria were derived from the work of Johnsen and Hasler (1980) who observed that sexually mature coho salmon, which had been exposed to morpholine during the smoit stage, migrated upstream if morpholine was present in a stream and downstream if morpholine was absent. These results indicated that the odor acted as a sign stimulus to induce a stereotyped behavior, i.e., swimming against a current (rheotaxis). Thus, Johnsen and Hasler (1980) concluded that the operational mechanism in selection of the home stream by olfactory orientation was positive rheotaxis if the odor was present and negative rheotaxis if the odor was absent.

We tested two hypotheses concerning the capture of fish in traps. The null hypothesis (H_o) stated, "There was no difference in the distribution of fish exposed to different odors at a selected life history stage." This result would have indicated that the fish did not imprint to their exposure odor at that development stage. This hypothesis was supported if:

(1) Fish exposed to the synthetic chemicals were captured principally at the downstream weir in both odor present and odor absent trials. and

(2) Fish migrating upstream with odors present were captured in traps scented with their exposure odor and alternate odor in about equal numbers, which would have indicated that the fish selected tributary channels randomly instead of being attracted to the channel scented with their exposure odor.

The alternative hypothesis (HA) stated, "There was a difference in the distribution of fish exposed to different odors at a selected life history stage." This result implied that fish did imprint to their exposure odor at that developmental stage. This hypothesis was supported if:

(1) Fish exposed to the synthetic chemicals were captured principally in the downstream weir in trials with the odor absent and were captured in upstream traps if the odor was present,

(2) Fish were captured more frequently in the trap scented with their exposure odor compared to the trap scented with their alternative odor, and

(3) Fish switched their channel preference when the odors in the channels were switched.

For each life history stage tested, a chi square statistic using STATVIEW on a Macintosh SE computer was calculated to determine the distributions of morpholine exposed and phenethyl alcohol exposed fish into the morpholine scented trap, phenethyl alcohol scented trap or downstream weir were significantly different ($p \leq 0.05$). This would indicate if morpholine exposed fish were moving into the morpholine scented trap and phenethyl alcohol exposed fish were moving into the phenethyl alcohol trap. Separate statistics were calculated when odors were present and when odors were absent to determine if there was a significant difference in the number of fish moving upstream or downstream under each type of condition.

2.4 Marking Fish for Release into Lake Roosevelt

From 1992 to 1994, most of the fish exposed to the synthetic chemicals were released into Lake Roosevelt at various locations to conduct a field test. A portion of each group released were tagged with distinctive coded wire tags. Tables 1, 2 and 3 provide data regarding the stage exposed, the exposure odor, stocking location, stocking date, and total number of fish with coded wire tags released at each location. During the spawning seasons in 1993 and 1994, morpholine (at a steady state concentration of 5×10^{-5} mg/l) was metered into the hatchery trap at Sherman Creek. Phenethyl alcohol (5×10^{-3} mg/l) was metered into the Spokane River at Little Falls Dam and a met-win trap was installed at that site (in 1993 only). Electrofishing surveys were

Table 1. Summary of kokanee salmon marked with coded wire tags (CWT) in 1992. Eyed egg to fry stages were from the Lake Whatcom 1991 cohort. Smolts were from the Lake Whatcom 1990 cohort.

STAGE EXPOSED	EXPOSURE ODOR	#CWT MARKED	RELEASE LOCATION	DATE RELEASED	STAGE AT RELEASE
Eyed egg	MCR	7,367	Sherman Creek	Jul/Aug	Fry
	PEA	11,393	Sherman Creek	July	Fry
Hatch	MCR	22,222	Sherman Creek	July	Fry
	PEA	23,115	Sherman Creek	July	Fry
Alevin	MCR	11,441	Sherman Creek	July	Fry
	PEA	10,986	Sherman Creek	July	Fry
Swimup	MCR	8,370	Sherman Creek	July	Fry
	PEA	10,71 6	Sherman Creek	July	Fry
Fry (Feb)	MCR	20,194	Sherman Creek	June	Fry
	PEA	6,025	Sherman Creek	June	Fry
Fry (Mar)	MCR	9,798	Sherman Creek	July	Fry
	PEA	10,818	Sherman Creek	August	Fry
Fry (Apr)	MCR	11,445	Sherman Creek	July	Fry
	PEA	11,525	Sherman Creek	July	Fry
Fry (May-Jul)	MCR	6,838	Sherman Creek	July	Fry
	PEA	11,300	Sherman Creek	July	Fry
Smolt	MCR	7,501	Sherman Creek	June	Smolt
	PEA	8,354	Sherman Creek	July	Smolt

Table 2. **Summary of kokanee salmon marked with coded wire tags (CWT) in 1993. Eyed egg to fry stages were from the Lake Whatcom 1992 cohort. Smolts were from the Lake Whatcom 1991 cohort.**

STAGE EXPOSED	EXPOSURE ODOR	#CWT MARKED	RELEASE LOCATION	DATE RELEASED	STAGE AT RELEASE
Eyed egg	MOR	10,961	Sherman Creek	June	Fry
	MOR	10,903	Spokane River	June/July	Fry
	PEA	10,721	Sherman Creek	June	Fry
	PEA	32,953	Spokane River	July	Fry
Hatch	MOR	7,988	Sherman Creek	June	Fry
	MOR	31,416	Spokane River	July	Fry
	MOR	22,026	Barnaby Creek	August	Fry
	PEA	7,988	Sherman Creek	June	Fry
	PEA	21,993	Spokane River	July	Fry
Alevin	MOR	10,938	Sherman Creek	July	Fry
	PEA	11,791	Sherman Creek	July	Fry
Swimup	MOR	10,908	Sherman Creek	July	Fry
	PEA	10,885	Spokane River	July	Fry
Fry (Feb-Jun)	MOR	10,802	Sherman Creek	June	Fry
(Feb-Jul)	PEA	10,896	Sherman Creek	July	Fry
Smolt	MOR	38,345	Sherman Creek	July	Smolt
	PEA	7,753	Sherman Creek	July	Smolt
	PEA	8,196	Blue Creek	May	Smolt

Table 3. Summary of kokanee salmon marked with coded wire tags (CWT) in 1994. Fish released as smolts were from the Lake Whatcom 1992 cohort and fish released as fry were from the Lake Whatcom 1993 cohort. [1,2]

Stage Exposed	Cohort	Exposure Odor	#CWT Marked	Release Location	Date Released	Stage at Release
Hatch	1992	MOR	13,435	Sherman Creek	May	Smolt
		MOR	13,625	Blue Creek	May	Smolt
		PEA	16,158	Blue Creek	May	Smoit
Alevi n	1992	MOR	15,523	Sherman Creek	May	Smolt
Swimup	1992	MOF?	17,917	Sherman Creek	Apr	Smolt
		PEA	38,125	Sherman Creek	Apr	Smolt
Hatch - Swi mup	1993	MOR	51,823	Sherman Creek	Jun/Jul	Fry
		PEA	52,465	Sherman Creek	Jun/Jul	Fry
Alevin - Swimup	1993	MOR	40,112	Sherman Creek	Jun/Aug	Fry
		PEA	20,942	Sherman Creek	Jun	Fry

1 Additionally, 306,466 kokanee from the 1993 cohort were coded wire tagged and held over for outplanting in 1995 as residualized smolts.

2 A total of 22,579 fish from the 1993 cohort were coded wire tagged and released from the Kettle Falls net pen.

also conducted at both locations, and at other sites in the reservoir in both 1993 and 1994. The number of morpholine-exposed and phenethyl alcohol-exposed fish (marked with group specific coded wire tags) returning to each location was counted.

In 1993, groups of fish expected to return in these experiments included 1990 cohort kokanee that had been exposed as smolts in 1992 (returning as 3 year old spawners) and possibly a few 1991 cohort kokanee that had been exposed at egg, alevin, swimup or post-swimup fry stages in 1992 (returning as 2 year old spawners). However, since most kokanee in Lake Roosevelt spawn at age 3 or age 4, the majority of these fish were not expected to show up in either the creel or at egg collection sites until sometime between 1994 and 1996. In 1994, the following groups were expected to return: (1) 1990 cohort kokanee returning as 4 year old fish; (2) 1991 cohort kokanee returning as 3 year old fish; and (3) possibly a few 1992 cohort kokanee returning as 2 year old fish. In 1995, the following groups are expected to return: (1) 1991 cohort kokanee returning as 4 year old fish (2) 1992 cohort kokanee returning as 3 year old fish and (3) 1993 cohort as 2 year old fish. In 1996, 1992 cohort kokanee are anticipated to return as 4 year old spawners and 1993 cohort kokanee are expected to return as 3 year old fish. In 1997, 1993 cohort kokanee are expected to return as 4 year old fish. In this report, we present the results of recoveries of coded wire-tagged fish from 1992 to 1994.

For marking experiments, kokanee were dipnetted out of hatchery raceways and mildly anesthetized with a 50 mg/l concentration of tricaine methanesulfonate (MS 222), then coded wire tags (CWT) were injected into the rostrum using a model MK4 CWT machine (Northwest Marine Technology, Inc.), equipped with two different nose hoods specially fitted for fry and smelt-sized fish. Lengths and weights of fry ranged from 59 to 94 mm and 1.7 to 6.1 g respectively. Lengths and weights of fingerlings ranged from 145 to 183 mm and 25.3 to 48.4 g respectively. Coded wire tagged fish were also given an adipose fin clip as an external identification mark. Marked fish were counted using a tally counter, then released back into hatchery raceways through a quality control device (QCD) (Northwest Marine Technology, Inc.) equipped with a CWT detector. The fish were retained for approximately 30-45 days before release to estimate mortality rates and tag retention. Mortality rates were uniformly low (~0.1%) (T. Peone, Spokane Tribal Hatchery, personal communication).

Tag retention was estimated by two methods. First, the QCD count was compared to the tally counter count to estimate the number of fish that were tagged successfully (>99% in most cases). Second, to account for the possibilitythat tags had been initially injected into the fish but had later been shed, a random sample of fish were rechecked after 30 days using the QCD counter. This was accomplished by randomly collecting 500 fish per each lot of approximately 10,000 fish that were marked and running them back through the QCD CWT detector. Each fish was enumerated using a tally counter before being placed into the CWT detector. Percent tag retention for each lot was determined by dividing the QCD count by the tally counter number. For each lot, the original QCD count obtained on the day the fish were tagged was multiplied by the percentage figure obtained after 30 days to estimate the number of fish in that lot that were released with CWTs. For the 1992 tagging investigations, the mean percent tag retention after 30 days was 91.5%, and ranged from 84 to 97%. The relatively high variation was attributed to the relative inexperience of the fish tagging crew. For the 1993 tagging investigations, the mean percent tag retention after 30 days was 98.5%, and ranged from 97.5 to 99.3%. The relatively low variation was attributed to the expertise of the fish tagging crew, which was composed of the same individuals who accomplished the tagging work in 1992. In 1994, the mean percent tag retention was 96.3%, and ranged from 84.0 to 98.9%. The variation was slightly higher than in 1993 because several groups were tagged with half length CWT's. These groups had a higher rate of shedding CWT's than those tagged with conventional sized CWT's. Tag retention for half length CWT's ranged from 84 to 88% whereas tag retention of fish tagged with conventional CWT's ranged from 91.3 to 98.9%.

In Lake Roosevelt, creel clerks and fisheries technicians checked for adipose clips and used hand held magnetic CWT detectors to check kokanee for CWT's. Heads of kokanee with adipose clips were cut off and sent to Spokane Tribal Hatchery, where CWT's were dissected out and examined with a dissecting microscope to determine the lot code. Percent tag retention of fish after release into Lake Roosevelt was estimated by dividing the number of fish bearing coded wire tags by the total number of fish examined that had adipose clips. From 1992 to 1994, a total of 106 adipose clipped fish marked in 1992 were recovered. Of these, 82 contained CWT's for a 77.4% tag retention rate after approximately 6 to 18 months in Lake Roosevelt. From 1993 to 1994, a total of 203 adipose clipped fish marked in 1993 were recovered. Of these, 192 contained CWT's for a 94.6% tag retention rate after approximately 6-l 8 months in the reservoir. In 1994, a total of 49 adipose clipped fish marked in 1994 were recovered. Of

these, 39 contained CWT's for a 79.6% tag retention rate after 3 to 6 months in Lake Roosevelt.

Fish exposed to morpholine and phenethyl alcohol at different life history stages and released into the reservoir at different locations in 1992 and 1993 were given different CWT lot codes. The number of fish from each lot returning to Sherman Creek near the head of Lake Roosevelt (morpholine scented) or Little Falls Dam/Blue Creek on the Spokane Arm of Lake Roosevelt (phenethyl alcohol scented) was determined.

2.5 Smoltification Studies of Yearling Kokanee

To determine if yearling fish (8 - 18 months old) were going through a parr-smolt transformation, morphological, physiological and behavioral tests were conducted with 1992 year class fish. Condition factor and silvering were used to determine if there were any changes in morphology. Changes in physiology were determined by testing thyroid and cortisol hormone levels, intestinal fluid transport (J_v), gill Na^+-K^+ ATPase activity, osmoregulatory capability and salt water tolerance. Behavioral tests included assessment of salinity preference and downstream migratory tendencies. All statistics were run using STATVIEW I on a Macintosh SE computer. Detailed descriptions of all methods were presented by Tilson et *al.* (1994).

2.5.1 Plasma Thyroxine Sample Collection

For determination of plasma thyroxine concentration from pre- and post-smolts, 20 yearling fish were collected twice per month from October 1993 through June 1994 (1992 year class). Samples were collected at approximately the same time each day (1100 to 1300) to increase the likelihood that any fluctuations observed were related to seasonal rather than diurnal patterns in thyroxine concentration (Grau et *al.* 1981). Fish were anesthetized with 50 mg/L MS-222 and killed by a blow to the head. Blood was collected from the severed caudal peduncle using heparinized capillary tubes. The blood was centrifuged at about 2000 Relative Centrifugal Force (3500 RPM) for 5 minutes, and plasma was stored at -80°C until the time of the T_4 assay.

2.5.1.1 T_4 Radioimmunoassay (RIA)

Circulating T_4 blood plasma levels were measured in ng/ml using a coat-a-count T_4 RIA kit (Diagnostic Products, Inc.). To perform the RIA, 25 µl of each kokanee sample and 1 ml of radiolabeled T_4 (^{125}I-T_4) were added to an antibody (Ab) coated

tube containing T_4 receptors. The actual concentrations of T_4 in unknown kokanee samples was determined by comparison to standards using known amounts of T_4. After an incubation period of 1 hour at 37°C, the excess T_4 and ^{125}I-T_4 was decanted, and the remaining liquid in the tubes was blotted dry. Radioactivity of the bound T_4 was counted for 1 minute using a programmable Cobra QC Model B5002 auto-gamma Counter (Packard Instrument, Co.). This competitive binding technique was described in detail in Scholz *et al.* (1992, 1993) and Tilson eta/. (1994). The mean T_4 (±SD) was determined for fish collected on the same date and plotted vs. date collected. An analysis of variance (ANOVA) was used to determine if there was a significant difference in T_4 concentration over time.

2.5.1.2 Quality Control Procedures

Each T_4 sample was assayed in duplicate to control for procedural errors and the mean percent error between each pair was calculated Unknown quality assurance samples were analyzed at high, medium and low concentrations. The actual concentrations of these standards were obtained from the radioimmunoassay kit manufacturer. Mean concentrations (±SD) were then compared to the company's concentrations to determine assay reliability. Distilled water was added to blank Ab coated tubes and incubated with unknown samples to make sure the gamma counter was subtracting background counts properly. If so, these blanks should contain about O-l 0 cpm. Pipetting accuracy of individuals pipetting the unknown/standard curve samples and ^{125}I-T_4 samples was determined by these individuals pipetting 10 replicate samples of radioactive hormones into uncoated tubes, then counting them in the gamma counter. The mean counts (±SD) and percent error were determined for each person.

Four assays were performed because of the large number of samples involved. To test interassay accuracy, three different interassay pool (IAP) samples of different concentration were inserted into each assay. If these were similar in concentration in all assays, the results could be compared. If not, results could not be compared. Mean values of IAP and standard curve samples were compared by calculating the percent error between assays.

2.5.2 Cortisol Determination

Blood plasma samples were taken from 20 kokanee during each sampling period. Fish were anesthetized with 50 mg/L MS-222 and killed by a blow to the head. Samples were used first for the thyroxine assay, then the remaining blood was pooled into two tubes per period (each tube containing blood from 10 fish). These samples were kept frozen at -80°C until the time of the cortisol assay. Samples thus prepared were shipped on dry ice to Jennifer Specker's laboratory at the University of Rhode Island, Department of Zoology, where they were assayed for cortisol content. Cortisol was assayed using a radioimmunoassay as described by Young (1986) and Bisbal and Specker (1991).

2.5.3 Condition Factor

Condition factor of yearling kokanee was determined in the form of $K_{TL} = (W/L^3) \times 10^5$ where K_{TL} was the condition factor, W was the weight of the fish (g) and L was the total length of the fish (mm). In salmonids, the condition factor typically declines as a fish smolts, because the fish becomes more slender and streamlined (Hoar 1939; Fessler and Wagner 1969).

2.5.4 Silvering

Coloration was visually determined at each sampling period by classifying fish as either parr, transition or smolt. If parr marks were distinct and there was no silvering present, the fish was designated as a parr. If there was a moderate degree of silvering, but parr marks were still evident, the fish was designated as transition. If there were no parr marks present and the silver layer was thick, the fish was designated as a smolt.

2.5.5 Intestinal Fluid Transport

Intestinal fluid transport rate (J_v) was measured in yearling (1992 year class) fish (n=12) at monthly intervals from November 1993 to June 1994. The fish were held in well water at 1 O-l 3°C for 2-5 days prior to sampling. J_v was determined gravimetrically on noneverted gut sacs filled with Ringer solution identical to the solution in which they were bathed. Details of this procedure were described by Veillette *et al.* (1993) and Tilson et al. (1994). In this technique, the gut was dissected out of the fish and its ends tied off, and filled with Ringer's The gut sac was weighed on an analytical balance, and subsequently incubated in a medium identical to the Ringer's solution used to fill the

sac. At 15 min intervals, for 1 h, the sacs were removed from the incubation medium and reweighed. A loss of weight was assumed to be related to water being transported out of the sac (i.e., 1 mg H_2O = 1 µl H_2O). Upon completion of the experiment, the amount of water transported was standardized for the size of the intestinal surface area by making a longitudinal incision of the gut, spreading it open on waterproof paper, then outlining it with a pencil. The image of the sac was scanned using Caere's OMNISCAN software and surface area was computed using the public domain software IMAGE. J_v could then by estimated in µl of solution transported per hour per centimeter intestinal surface area (µl/h/cm^2).

A different Ringer solution was used during the second year of this study than was used the first year. During the first year, the Ringer solution was composed of the following salts: 144 mM NaCl, 5 mM KCl, 2 mM Na_2HPO_4, 20 mM $NaHCO_3$, 1 mM $MgSO_4$, 1 mM $CaSO_4$ and 2.8 mM glucose (Field et *al.* 1978). The incubation medium was aerated with approximately 99.5% O_2 and 0.5% CO_2 which maintained a pH of 8.20 ± 0.05. The Ringer solution used during the first year of this study was used in studying J_v in two earlier studies (Field et *al.* 1978; Kerstetter and White 1994). It was believed that the higher bicarbonate and higher pH were consistent with the solution in the posterior intestine. However, instead of the typical pre-adaptive increase in J_v seen with coho salmon, we observed a decrease in J_v in kokanee (Tilson et *al. 1994).* Therefore, in 1993 we decided to use a Ringer solution developed by Collie and Bern (1982) who had observed a pre-adaptive increase in J_v in coho salmon that were being held in fresh water. In addition, Veillette et *al.* (1993), who used the same buffer with Atlantic salmon, also observed a preadaptive increase in Jv. The Ringer solution they used consisted of the following compounds: 149 mM NaCl, 15 mM $NaHCO_3$, 1.5 mM $CaCl_2$, 1.5 mM KH_2PO_4, 0.8 mM $MgSO_4$, 2.5 mM KCl, 10 mM glucose and 5 mM N-2 hydroxyethylpiperazine - N'-3-propanesulfanic acid (HEPPS), pKa 8.0. Aeration and maintenance of the pH of the incubation medium was similar to the year before except that a pH of 7.8 was used. For both years an incubation temperature of 13°C was used. and the osmolarity of the solution was 300 ± 10 mOsmol/l. An ANOVA was used to document the change in Jv over time. Significant differences were reported by this method when $P \leq 0.05$.

2.5.6 Gill Na^+-K^+ ATPase Activity

Gill Na^+-K^+ ATPase activities of yearling fish were determined using the modified method of Heinonen and Lahti (1981) and Zaugg (1982a). The recipes for chemical

solutions used in this assay are recorded in Table 4. Samples of gill filaments from individual fish (n=20) were collected once a month from October 1993 through June 1994. One ml of SEI (0.03 M sucrose, 0.02 M ethylenediamine tetraacetate, 0.1 M imidazole) was added to each gill sample and kept on dry ice during transportation from the hatchery to the EWU lab. Gill samples were stored at -80°C until the time of the assay.

Enzyme preparations were made by homogenizing thawed samples at 4°C and decanting the homogenate into a centrifuge tube placed in ice water. All homogenates were then centrifuged for 7 minutes at about 2000 Relative Centrifugal Force (3500-3900 rpm). The supernatant solutions were discarded and the pellets were manually resuspended in 0.5-l ml of SEID (SEI plus sodium deoxycholate) using a 10 ml glass tissue grinder. Homogenates were then centrifuged for 6 minutes and an aliquot (0.2 ml) was withdrawn for protein determination.

One 15 µl aliquot of each homogenate was placed into a 16 X 100 mm test tube on ice containing 0.4 ml of Solution A, and another 15 µl aliquot into a tube containing the same volume of Solution B which contained ouabain (See Table 4). Ouabain blocks potassium (K+) binding sites therefore preventing coupled transport of Na^+ and K^+. This inactivates Na^+-K^+ ATPase but not other ATPases. Thus the ouabain sensitive portion of total ATPase activities was designated as Na^+-K^+ ATPase. Added to each of these tubes was 0.05 ml of 0.03 M Na_2ATP. These samples were then placed in a constant temperature water bath (37°C) where they were shaken for one minute, then allowed to remain for a total of 10 minutes. After the reaction time of 10 minutes, the rack of tubes was removed and placed back in the ice-water bath where it was shaken for one minute. Once cold, the reaction was considered essentially stopped.

To determine the amount of phosphate that was released during hydrolysis of ATP, a colorimetric phosphate assay was performed. Samples were acidified with 3 ml of a solution of acetone-acid-molybdate (AAM) to denature the protein. After AAM was added the contents of the tubes were mixed by shaking the rack. The tubes were allowed to set for one minute after which 0.3 ml of 1 M citric acid were added and the samples were shaken vigorously. The amount of phosphate was determined with a Shimadzu UV160 spectrophotometer at 355 nm within one hour. The amount of phosphate hydrolyzed in unknowns was determined by comparing absorbance values to standards using known amounts of phosphate. Activities were presented as µmoles P_i/mg protein/h.

Table 4. Recipe for chemical solutions for Na^+-K^+ ATPase assay taken from W.S. Zaugg (personal communication).

SEI:

0.3 M reagent grade sucrose (102.7 g/L)
0.02 M disodium ethylenediamine tetraacetate (Na_2EDTA, 7.44 g/L); and
0.1 M imidazole (6.8 g/L);
all adjusted to a final pH 7.1 with HCl.

SEID:

SEI + sodium deoxycholate (0.1 g/100 ml)

STOCK A:

4.68 g $MgCl_2 \cdot 6\,H_2O$
9.07 g NaCl
5.6 g KCl
7.83 g imidazole
dissolved in a final volume of 1 L (including final adjustment of pH to 7.0 with HCl).

STOCK B:

0.42 g ouabain
added to 1 L of Solution A.

Na_2ATP (aqueous):

1.84 g/100 ml H_2O
adjusted to pH 7.0 with NaOH.

AAM:

1 volume of 5 N sulfuric acid (100 ml concentrated H_2SO_4 with H_2O for a final volume of 720 ml),
1 volume of 10 mM molybdate (12.36 g $(NH_4)_6Mo_7O_{28} \cdot 4\,H_2O$ in 1 L water),
2 volumes of acetone.

CITRATE:

210.1 g/L citric acid monohydrate.

Protein concentrations were determined by the method of Lowry et *a/.* (1951), modified by Miller (1959), as described in Tilson et *a/.* (1994) except that volumes were reduced. Fifteen µl of the enzyme preparation were placed into a tube containing 0.6 ml water. Then 0.6 ml of the copper tartrate-sodium carbonate solution (see Table 5) were added and allowed to stand for 5 min. Then, 1.8 ml diluted Folin reagent was added and allowed to stand for 75 min in the dark at room temperature. The amount of protein was determined by reading samples at 700 nm. Bovine serum albumin was used as a standard in this assay (Table 5).

Analysis of variance (ANOVA) was used to test for a difference between mean gill Na^+-K^+ ATPase enzyme activities for each group of fish over the sample period of ten months. Significant differences were reported by this method when $p \leq 0.05$.

2.57 Seawater Survival

Freshwater yearling kokanee salmon were tested for salt water tolerance once a month from October 1993 to June 1994. Fish (n=80) were transferred from the Spokane Tribal Hatchery to the EWU lab, and held in Living Streams at a natural photoperiod. After a 1-3 day acclimation period in fresh water, fish were placed in three aerated, flow-through 500 L tanks with either 10 parts per thousand (ppt) (n=20), 20 ppt (n=20) or 30 ppt (n=20) salinity. Osmolarity of tanks was checked with a microosmometer (Precision Systems, Inc.) before fish were transferred, and again after 96 hours. Twenty additional fish were kept in fresh water. Percent survival was determined by calculating the number of individual fish alive after 96 hours divided by the total number of fish in the tank.

2.5.8 Osmoregulatory Capability

The ability of kokanee salmon to osmoregulate was determined using the same fish which were used for salt water tolerance tests. After 96 h or at the time of death, fish were anesthetized with 50 mg/l MS-222. Blood samples were taken via the severed caudal peduncle and plasma was immediately separated by centrifugation for 5 minutes at approximately 2000 RCF and stored at -80°C. Osmolarity was determined using a freezing point microosmometer by Precision Systems, Inc. The instrument was calibrated with 100, 300 and 500 mOsmol/L standards, supplied by Precision Systems, inc., before each assay. Unknown plasma samples were analyzed with known standards interspersed at regular intervals during the assays. Osmolarity was analyzed

Table 5. Recipe for protein determination.

Copper-tartrate-sodium carbonate solution:

1 part Na-K tartrate
(10.0 g/500 ml)

1 part $CuSO_4$
(5.0 g/500 ml)

20 parts alkaline Na_2CO_3
(100 g Na_2CO_3 plus 20 g NaOH in 1 L distilled water)

Made fresh each day.

Diluted Folin

1 part Folin Reagent (Sigma Chemical Co.)

10 parts distilled water

using ANOVA to examine changes over time. Significance was accepted at $p \leq 0.05$. In addition, a t-test was performed to determine differences in osmolarityaf fish held in freshwater compared to the osmolarity of those held in salt water.

2.5.9 Salinity Preference

Salinity preference tests were run once a month from November 1993 through June 1994. Sixteen yearling kokanee were transported from the Spokane Tribal Hatchery to Living Streams at the EWU lab and held for a 1-3 day acclimation period. The salinity preference test followed a modification of the methods of Mclnerney (1964) and permitted salmon to choose between fresh water and 24 ppt salt water, or fresh water and fresh water (control). Two test tanks were arranged side by side in an aerated water bath. An incomplete partition divided the tank into two compartments each containing an airstone and bottom center inlet. To begin the test, the inlets in each compartment were stoppered with rubber plugs, one solid and the other pierced by an 1/4-inch diameter hole leaving the second compartment continuous with the surrounding water bath so that water could be added to the trough (Figure 4B). Next the seawater solution was poured into the sealed compartment to the level of the partition (Figure 4C). The full strength seawater was made by adding instant Ocean salts to dechlorinated tap water to achieve osmotic concentration of 24 ppt.

This year, we omitted the testing of fish in dilute (8 ppt) salt water. Last year, there was a problem with the mixing of salt and fresh water in the smaller (inside dimensions = 26 cm X 25.5 cm X 19 cm) test troughs. Therefore, this year we built bigger troughs in order to prevent the mixing of salt and fresh water (66 cm X 28 cm X 29.5 cm).

After osmotic concentration was achieved, the water bath was filled with fresh water and the unsealed compartment was flooded to within approximately 1/2-1 inch from the top of the partition (Figure 4D). A refrigeration coil was placed in this aerated water bath and was left overnight to allow the temperature to reach 1 O-l 2°C. The following day, eight fish were placed in each of the unsealed freshwater compartments with airstones left on and fish were allowed to acclimate for 1 hour (Figure 4E). After 1 hour, the air supply in the troughs was turned off and the level of the surrounding aerated water bath was again raised so that a freshwater “bridge” was formed across the partition allowing the fish to move freely between the two compartments (Figure 4F).

Figure 4. **Salinity preference tank and test procedure. Refer to text for explanation of figure.**

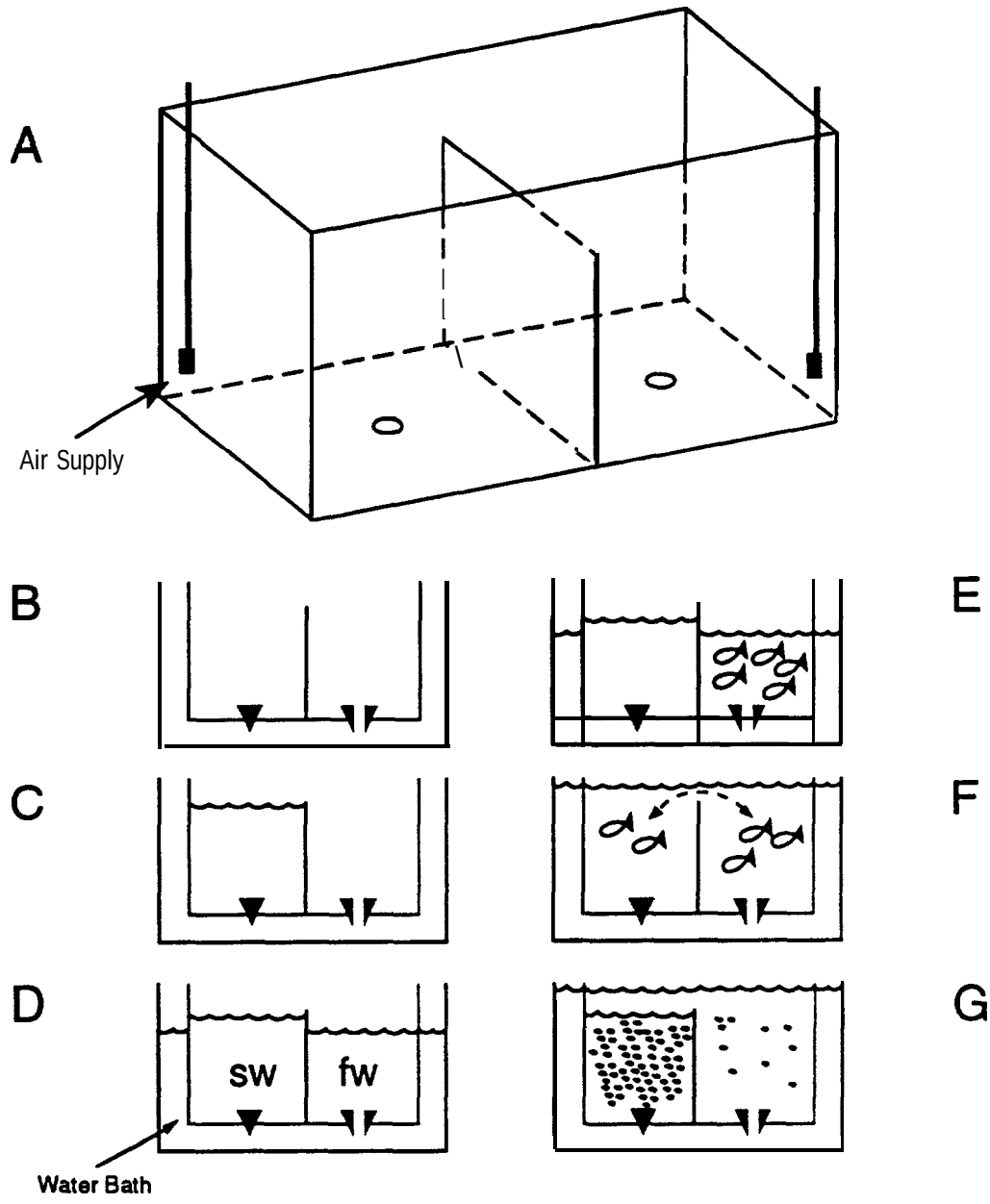


Figure 4G diagrammatically shows the fresh water bridge and stratification layer with the less dense water on top.

Observations of the number of fish in each compartment were made once every 2 minutes for 100 minutes. Observations were taken without knowledge as to which compartment contained the seawater. Evaluations of biological significance were based on an interpretation of the pattern of response to seawater or freshwater. Percent preference was determined by dividing the total number of observations in the salt water compartment by the total number of observations in that trough (fresh and salt water). For example, it there were 178 observations in the salt water compartment after 100 minutes, and 222 observations in the freshwater compartment, percent preference would be 45% (178/(178+222)). A positive preference to salt water was assumed if > 50% of the fish were observed in the salt water compartment. An avoidance to salt water was indicated if < 50% of the fish were observed in the salt water compartment. Neither a preference or avoidance was indicated if 50% of the fish were observed in the salt water compartment. The response to fresh water was used as the baseline or control. Fish in fresh water troughs should have spent approximately 50% of the time in each fresh water compartment.

2.5.10 Downstream Migratory Behavior

Yearling kokanee were transferred from the Spokane Tribal Hatchery to the EWU lab once a month from October 1993 to June 1994. At EWU, the fish were held in Living Streams at a temperature of 9-l 1 °C and kept on natural photoperiod. The Living Streams had an observation window on one side, which ran the length of the tank. To perform behavioral tests, the tank was visually divided into three equal parts by placing tape on the outside window. At night (2000 - 2400 h), the percentage of fish in the upper, middle and downstream thirds of the tank were estimated and recorded every 2-3 min for a total of 15 min. The mean percent of fish in the downstream end was then determined for each sampling period. Orientation (head upstream or downstream) was also recorded, as well as vertical position in the tank (whether fish were on the bottom, middle or top of the water column). The mean percentage (±SD) of fish in the downstream third of the tank was plotted each month to identify trends in behavior.

3.0 RESULTS

3.1 Imprinting Investigations

3.1.1 Thyroxine Content of Age 0 and Age 1 Kokanee

Results of experiments measuring whole body thyroxine content of age 0 kokanee were similar during both years in which they were tested (Figure 5). Thyroxine content of 1991 and 1992 year class kokanee peaked at hatch and again at swimup and subsequently returned to very low levels during the fry stage (Scholz *et a/. 1993).* Similar results were obtained from 1990 year class kokanee (Scholz et *al.* 1992). Plasma thyroxine concentration of age 1 kokanee (1990, 1991 year class) peaked significantly in the winter and in the spring during both years tested (Scholz et *a/.* 1993; Tilson et *a/.* 1994). Thyroxine concentration of 1992 year class kokanee in the present investigation peaked in the spring.

3.1.2 Odor Discrimination Investigations in 1994

Behavioral tests were conducted in 1993 and 1994 with the following groups of fish:

year class	age when tested	life history stage exposed	exposure odor	#tested in autumn 93	# tested in autumn 94	total tested
1991	2,3	Fertilized egg	PEA	242	0	242
		Eyed egg	MOR	33	8	41
			PEA	36	8	44
		Hatch	MOR	52	8	60
			PEA	31	9	40
		Alevin	MOR	35	7	42
			PEA	35	10	45
		Swimup	MOR	28	10	38
			PEA	25	9	34
		Fry (Feb)	MOR	14	5	19
			PEA	17	7	24
		Fry (Mar)	MOR	25	7	32
			PEA	30	5	35

year class	age when tested	life history stage exposed	exposure odor	#tested in autumn 93	#tested in autumn 94	total tested
1991	2,3	Fry (Apr)	MOR	22	8	30
			PEA	21	8	29
		Fry (May-Jul)	MOR	51	8	59
			PEA	52	8	60
					0	
1990	3	Smolt	MOR	64	0	64
			PEA	65		65
1992	2	Eyed egg	MOR		125	125
			PEA		115	115
		Hatch	MOR		133	133
			PEA		106	106
		Alevin	MOR		115	115
			PEA		82	82
		Swimup	MOR		88	88
			PEA		86	86
		Fry	MOR		14	14
			PEA		12	12
Totals				077	1,001	1,070

Results from fish tested in 1993 were reported by Tilson et *al.* (1994) and summarized in the introduction of this report. Results from fish tested in 1994 are reported here.

These tests were conducted in a natural Y-maze, with traps located at each arm and at a position downstream from the release point as described in Section 2.3 and Figure 3. Relatively few fish exposed to synthetic chemicals in 1992 at the fry and smolt stages were tested in 1994 because most of these fry and all the smolts were released into Lake Roosevelt.

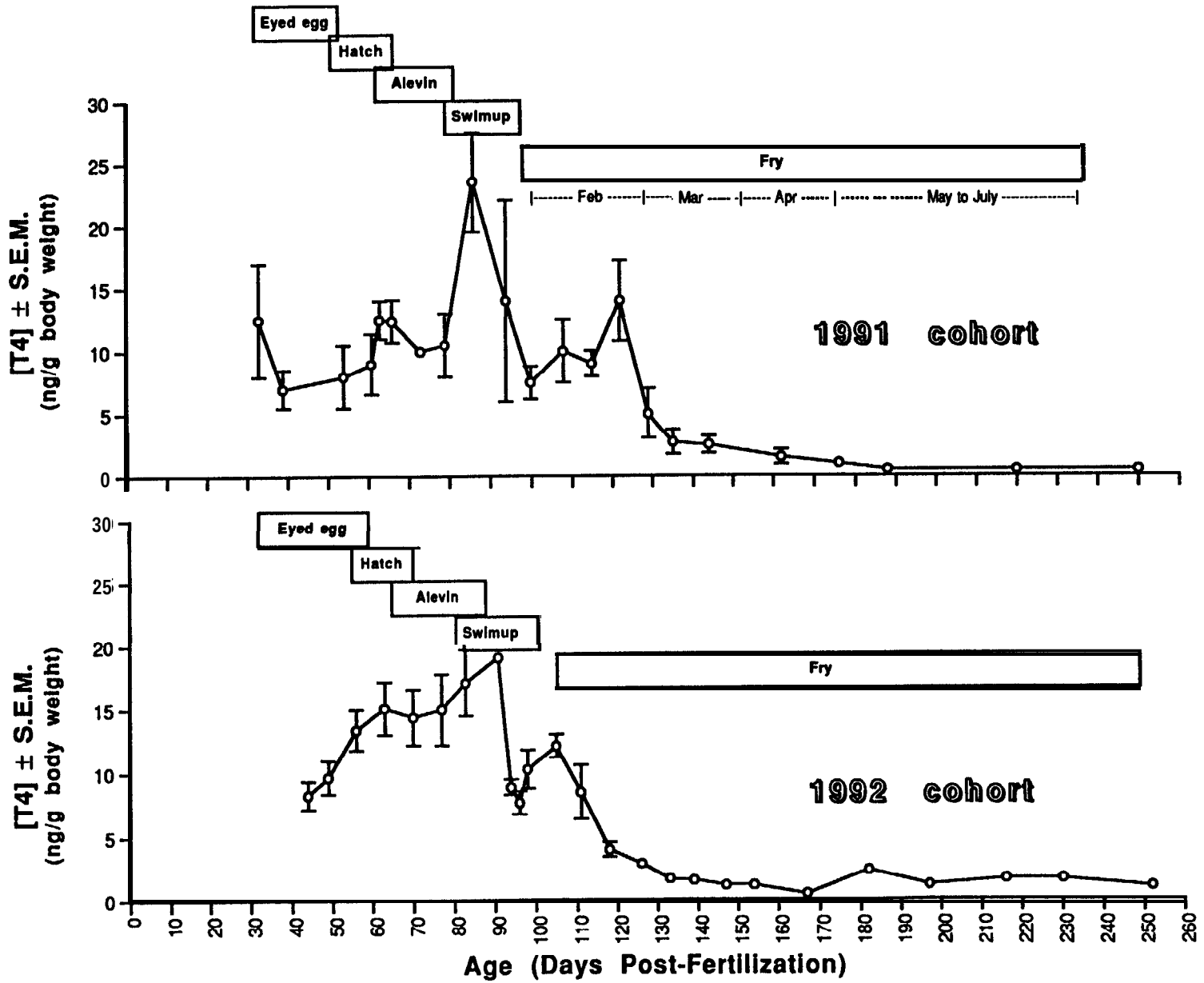


Figure 5. Whole body T_4 concentration of 1991 and 1992 cohort, Lake Whatcom stock kokanee salmon. Each data point represents the mean ± SEM of approximately 10 samples (n=20 fish). Lengths of exposure to synthetic chemicals at different life stages are denoted by boxes.

3.1.2.1 Odor Discrimination in 1991 Year Class Fish (Age 3 Spawners)

For the 1991 year class fish tested at age 3:

1) The group exposed to the synthetic chemicals as eyed eggs (30-60 days post-fertilization) was classified as "not imprinted" because:

   a) The majority (>80%) of the fish were captured at the downstream weir in both odor present and odor absent trials, and

   b) The ability of fish to accurately locate traps scented with their exposure odor was poor, only 3 of 51 total trials or 5.9% (Tables 6, 7, 8, Figure 6)

2) The groups exposed to the synthetic chemicals at hatch, (53-63 days post-fertilization), alevin (60-90 days post-fertilization) and swimup (88-93 days post-fertilization) stages were all classified as imprinted because:

   a) In trials when odors were present, they were captured in traps scented with their exposure chemical more frequently (at rates of 68.2, 87.5, and 92.2% respectively) than either in traps scented with the alternate chemical (rates of 13.6, 6.3 and 5.9% respectively) or at the downstream weir (rates of 18.2, 6.2 and 1.9% respectively) (Tables 6, 7, 8; Figure 6);

   b) In trials when the exposure chemical was absent, fish from all three groups were predominantly captured at the downstream weir, in 93.7%, 82.7% and 85.1% of trials respectively (Tables 6, 7);

   c) In odor present trials, fish from all three groups switched channels at times when the synthetic chemicals were switched. For fish exposed at hatch, seven of eight phenethyl alcohol exposed fish migrated first into the channel scented with phenethyl alcohol, then into the opposite channel after phenethyl alcohol was added to it;

Table 6. **Statistical comparison of the number of 1991 cohort kokanee salmon (tested at age 3 in 1994) exposed to either morpholine (MOR) or phenethyl alcohol (PEA), captured in morpholine or phenethyl alcohol scented traps or at a downstream weir, under odor present and odor absent conditions. Separate tests were made for each condition. The null hypothesis stated that there was no difference in the distribution of the two sets of fish exposed to different odors at a particular life history stage. The null hypothesis was rejected if p ≤ 0.05, denoted by an asterisk. An asterisk signifies these groups of fish imprinted to their exposure odor.**

		Odor Present					Odor Absent				
Exposure Stage	Exposure Odor Chemical	#trials (n)	MOR Trap (#)	PEA Trap (#)	Down Stream (#)	Chi Square	# trials (n)	Trap A (#)	Trap B (#)	Down Stream (#)	Chl Square
Eyed Egg	PEA	29	2	2	25	x^2=0.72	10	0	0	10	x^2=0 [t]
	MOR	22	1	3	18	p=0.7	11		0	11	p=1.o
Hatch	PEA	20	3	15	2	x^2=17.78	9	1 0	8	8	x^2=0 [t]
	MOR	24	15	3	6	p<0.01*	7			7	p=1.o
Alevin	PEA	20	1	18	1	x^2=33.9	8	0	0	8	x^2=2.58
	MOR	28	24	2	2	p<0.01*	15	3	1	11	p-O.27
Swimup	PEA	29	2	28	1	x^2=38.67	16	2 0	1	13	x2=4.7
	MOR	23	21	1	1	p<0.01*	11		1	10	p=0.09
Fry (Feb)	PEA	21	1	2	18	x^2=0.31				11	x^2=0.84
	MOR	20	1	1	18	p=0.86	12	1	0	13	p=0.66
Fry (Mar)	PEA	24	3	1	20	x2=2.22	9	0	1	8	x2=2.22
	MOR	23	3	4	18	p=0.33	11	2	2	7	p=0.33
Fry (Apr)	PEA	21	2	3	18	x2=0.48		0		8	x^2=0 [t]
	MOR	21	3	4	14	p=0.79	9	0	1	8	p=1.0
Fry (May-Jul)	PEA	19	1	4	14	x^2=2.46	9	0	0	9	x^2=0 [t]
	MOR	21	1	1	19	p=0.29	9	1	0	8	p=1.o

[t] Chi square values were calculated for these groups even though the assumption stating: "no more than 20% of expected values may be less than five, and no expected value may be less than 1, was violated.

Table 7. **Thyroxine content and percentage homing to synthetic chemicals of 1991 cohort kokanee salmon exposed to either morphoiine (MOR) or phenethyl alcohol (PEA) at different life stages and tested at age 3 in 1994. Behavioral tests were conducted to determine which fish migrated upstream to the arm with their exposure chemical and which migrated downstream instead. Percentages of fish captured in either MOR or PEA scented traps, or at a downstream weir, under odor present or odor absent conditions were based on the total number trials for each condition.**

					Odor Present				Odor Absent			
Exposure Stage	Days Post-Fertilization	[T4]±S.D. (ng/g body weight)	Exposure Odor Chemical	# fish tested	#trials (n)	MOR Trap (%)	PEA Trap (%)	Down Stream (%)	#trials (n)	Trap A %	Trap B (%)	Down Sream (%)
Eyed Egg	30-60	9.5 ± 2.5	PEA	8	29	6.9	6.9	86.2	10	0	0	100
			MOR	8	22	12.1	6.0	81.9	11	0	0	100
Hatch	53-63	13.1 ± 2.5	PEA	8	20	15.0	75.0	10.0	9	11.1	0	89.0
			MOR	9	24	82.5	12.5	25.0	7	0	0	100
Alevin	60-90	17.5 ± 3.9	PEA	7	20	5.0	90.0	5.0	8	0	0	100
			MOR	10	28	85.8	7.1	7.1	15	20.0	6.7	73.3
Swimup	86-93	22.1 ± 5.2	PEA	10	29	6.9	89.7	3.4	16	6.2	12.5	81.3
			MOR	9	23	91.3	4.3	4.3	11	0	9.1	90.9
Fry (Feb)	94-125	6.5 ± 1.5	PEA	7	21	4.8	9.5	85.7	12	8.4	0	91.6
			MOR	5	20	5.0	5.0	90.0	15	6.7	6.7	86.6
Fry (Mar)	125-155	7.8 ± 1.0	PEA	5	24	12.5	4.2	83.3	9	0	12.2	88.8
			MOR	7	23	13.1	17.3	69.6	11	18.2	18.2	63.6
Fry (Apr)	155165	3.0 ± 0.5	PEA	8	21	9.5	14.3	76.2	9	0	9.1	90.9
			MOR	8	21	14.3	19.0	66.7	9	0	9.1	90.9
Fry (May-Jul)	185-227	1.0 ± 0.1	PEA	8	19	5.3	21.0	73.7	9	0	0	100
			MOR	8	21	4.8	4.8	90.4	9	9.1	0	90.9

Table 8. Classification of 1991 cohort kokanee salmon, exposed to synthetic chemicals (either morpholine or phenethyi alcohol) at different life stages and tested at age 3 in 1994, into imprinted or non imprinted categories. Determinations of upstream and downstream migration when odors were present or absent, and ability to discriminate odors are listed for each life history stage.

Exposure Stage	Days Post-Fertilization	Direction of swimming when odors were absent [1]	Direction of swimming when odors present [2]	Did the fish select the arm scented with the exposure chemical? [3]	Classification [4]
Eyed Egg	30-60	Down	Down	No	Not imprinted
Hatch	53-63	Down	Up	Yes	imprinted
Alevin	60-90	Down	Up	Yes	imprinted
Swimup	88-93	Down	Up	Yes	imprinted
Fry (FEB)	94-125	Down	Down	No	Not imprinted
Fry (MAR)	125-155	Down	Down	No	Not imprinted
Fry (APR)	155-185	Down	Down	No	Not imprinted
Fry (MAY-JUL)	185-227	Down	Down	No	Not imprinted

[1,2] Criteria: When >50% of the individuals from a particular exposure stage displayed upstream migratory behavior, the group was classified as "Up"; when <50% displayed downstream migratory behavior, the group was classified as "Down".

[3] Criteria: Determination of odor discrimination was based on statistical significance (Chi Square test) at p=0.05. If fish selected the arm scented with the exposure chemical, they were considered imprinted. (See Table 6)

[4] Criteria: For a group of fish to be considered imprinted, three criteria had to be met: Downstream movement with odor absent, upstream movement with odor present, and accurate selection of the exposure chemical. All other combinations were classified as not imprinted.

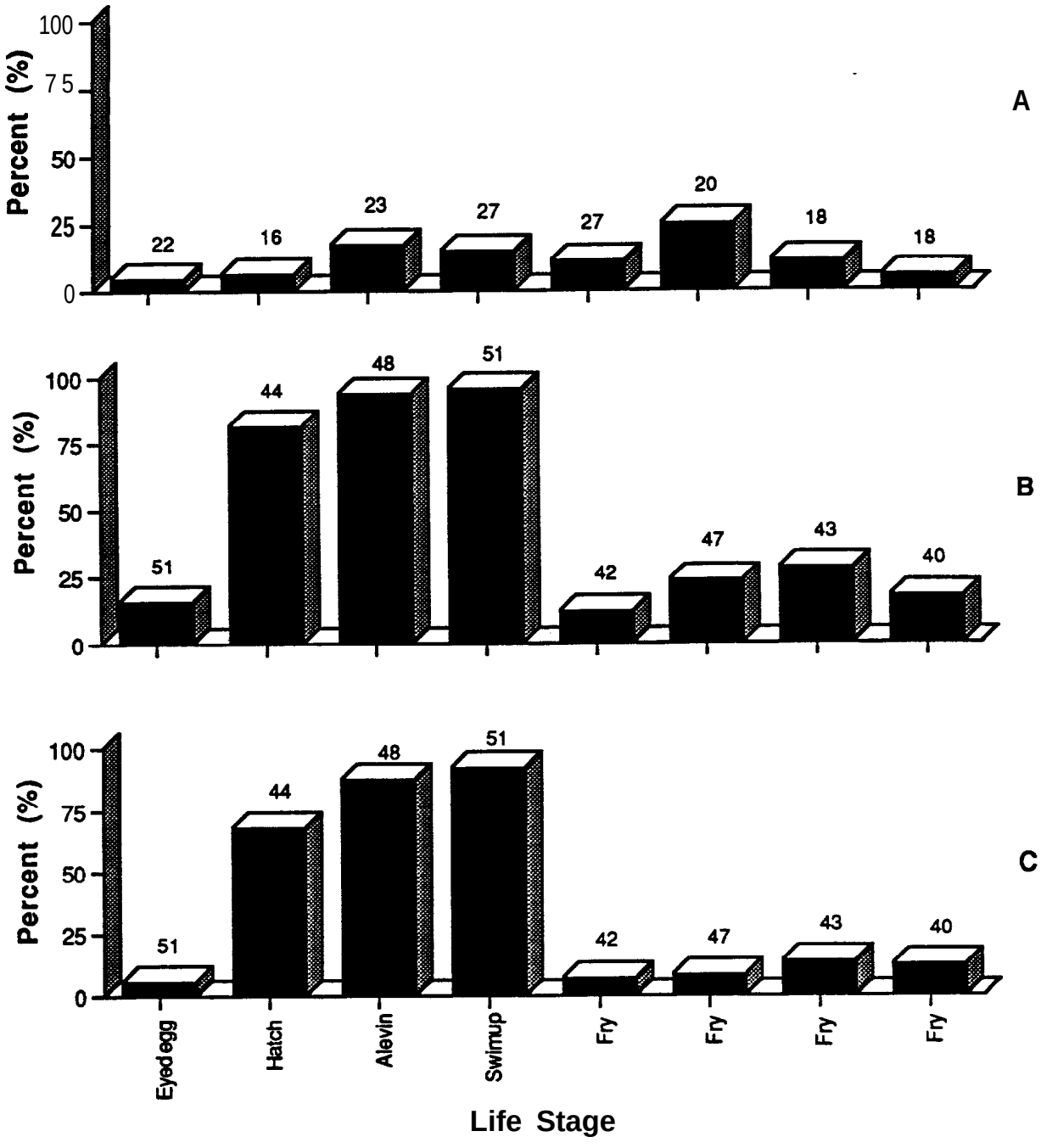


Figure 6. Results of odor discrimination tests with adult 1991 cohort kokanee salmon exposed to synthetic chemicals at different life stages and tested at age 3 in 1994. (A) Percentage that migrated upstream when the synthetic chemicals were absent; (B) Percentage that migrated upstream when the synthetic chemicals were present; (C) Percentage that homed to exposure odor. The values displayed above the bars indicate the total number of trials for that particular life stage.

seven of nine fish exposed to morpholine at hatch migrated first into the channel‘scented with morpholine, then into the opposite channel after morpholine was added to it. Also, six of seven fish exposed to phenethyl alcohol and nine of ten fish exposed to morpholine at the alevin stage, and eight of nine fish exposed to phenethyl alcohol and ten of ten fish exposed to morpholine at the swimup stage, homed first to the channel scented with their treatment odor, then the opposite channel after their treatment odor was added to it; and;

d) Since fish exposed at hatch, alevin and swimup stages all tended to swim upstream to the channel scented with their exposure chemical when odors were present, selected the channel scented with their exposure chemical with precision, switched channels when odors were switched, and swam downstream if the odor was absent, all three groups were classified as “imprinted” (Table 8).

3) The majority of fish exposed to synthetic chemicals as post-swimup fry in February, March, April or May-July (four different groups ranging from 125 to 227 days post-fertilization) were classified as “not imprinted” because:

a) They were captured primarily at the downstream weir in both odor present (88.1%, 76.6%, 72% and 82.5% respectively) and odor absent (88.9%, 75%, 88.9% and 94.4% respectively) trials (Tables 6, 7, 8; Figure 6); and

b) When odors were present, their distribution was random between the two channels, with approximately equivalent numbers of fish from each group captured in the trap scented with their exposure odor and alternate odor (Tables 6, 7). Thus, they appeared to be unable to discriminate their exposure odor (Table 8).

The group of fish that experienced highest whole body thyroxine content (swimup stage) also had the highest percentage of fish homing accurately in the behavioral tests (Figure 6). Recently hatched eggs and alevins also had relatively high ttiyroxine content and displayed accurate homing in behavioral tests (Figure 6). Eyed eggs and post swimup fry had relatively low thyroxine content and exhibited poor homing to exposure odors (Figure 6).

3.1.2.2 Odor Discrimination in 7992 Year Class Fish (Age 2 Spawners)

For the 1992 year class fish tested at age 3:

1) The group exposed to synthetic chemicals as eyed eggs (44-74 days post-fertilization) was classified as non-imprinted because:

   a) the majority of the fish were captured at the downstream weir in both odor present (55.2%) and odor absent (78.2%) trials (Tables 9, 10, 11; Figure 7); and

   b) the ability of fish to accurately locate traps scented with their exposure odor was poor (127 of 545 total trials or 23.3%), with about as many moving to the traps scented with the alternate odor (117 of 545 total trials or 21.5%) (Table 9, 10, 11; Figure 7).

2) The groups exposed to the synthetic chemicals at hatch (64-76 days post-fertilization), alevin (70-94 days post-fertilization) and swimup (91-98 days post-fertilization) stages were all classified as imprinted because:

   a) in trials where odors were present, they were captured in traps scented with their exposure chemical more frequently at rates of 62.5, 70.1 and 73.2% respectively) than either in traps scented with the alternate chemicals (rates of 20.0, 17.6 and 20.6% respectively) or at the downstream weir (rates of 17.5, 12.2 and 6.2% respectively) (Tables 9, 10, 11; Figure 7);

   b) in trials where the exposure odor was absent from the channel, fish from all three groups were predominantly captured at the downstream weir, in 80.0, 82.4 and 86.0% of trials respectively (Table 9, 10, 11; Figure 7);

Table 9. **Statistical comparison of the number of 1992 cohort kokanee salmon (tested at age 2 in 1994) exposed to either morpholine (MOR) or phenethyl alcohol (PEA), captured in morpholine or phenethyl alcohol scented traps or at a downstream weir, under odor present and odor absent conditions. Separate tests were made for each condition. The null hypothesis stated that there was no difference in the distribution of the two sets of fish exposed to different odors at a particular life history stage. The null hypothesis was rejected if $p \leq 0.05$, denoted by an asterisk. An asterisk signifies these groups of fish imprinted to their exposure odor.**

		Odor Present					Odor Absent				
Exposure Stage	Exposure Odor Chemical	#trials (n)	MOR Trap (#)	PEA Trap (#)	Down Stream (#)	Chl Square	#trials (n)	Trap A (#)	Trap B (#)	Down Stream #	Chl Square
Eyed Egg	PEA	225	60	67 57	98	x*=21.53	87	11	9	67	x^2=0.13
	MOR	320	60		203	p<0.01*	115	13	11	91	pro.94
Hatch	PEA	179	33	110	36	x^2=97.27	86	10	9	67	x^2=0 65
	MOR	277	175	58	44	p<0.01*	109	9	11	89	p=0.72
Alevin	PEA	199	29	148	22	x^2=150.8	83	8	6	69	x^2=0.2
	MOR	266	182	53	31	p<0.01*	88	8	8	72	p=0.9
Swimup	PEA				12	x^2=105.8	72	6	5	61	x^2=0.4
	MOR	186	154	139	10	p<0.01*	71	4	5	62	p=0.82
								2			
Fry	PEA				69	x^2=0.29	33	3	4	27	x^2=0.17
	MOR	80	10	10	59	p=0.87	43		4	36	p=0.92

Table 10. **Thyroxine content and percentage homing to synthetic chemicals of 1992 cohort kokanee salmon exposed to either morpholine (MOR) or phenethyl alcohol (PEA) at different life stages and tested at age 2 in 1994. Behavioral tests were conducted to determine which fish migrated upstream to the arm with their exposure chemical and which migrated downstream instead. Percentages of fish captured in either MOR or PEA scented traps, or at a downstream weir, under odor present or odor absent conditions were based on the total number trials for each condition.**

					Odor Present				Odor Absent			
Exposure Stage	Days Post-Fertilization	[T4] (ng/g body weight)	Exposure Odor Chemical	# fish tested	#trials (n)	MOR Trap (%)	PEA Trap (%)	Down Stream (%)	#trials (n)	Trap A (%)	Trap B (%)	Down Sream (%)
Eyed Egg	44-64	8.5	PEA	115	225	27.0	30.0	43.0	87	13.0	10.0	77.0
			MOR	125	320	18.6	18.0	63.4	115	11.3	9.6	79.1
Hatch	64-76	12.5	PEA	106	179	18.4	61.5	20.1	86	11.6	10.5	79.4
			MOR	133	277	63.1	20.9	16.0	109	8.3	10.1	81.6
Alevin	70-94	10.0	PEA	82	199	14.6	74.4	11.0	83	9.6	7.3	83.1
			MOR	115	266	68.4	19.9	11.7	88	9.4	9.4	81.8
Swimup	91-98	22.1	PEA	86				6.5	72	8.3	7.0	84.7
			MOR	88	188	18.6	22.5	5.9	71	5.7		87.3
Fry	105252	2.5	PEA	14	89				32	6.3		
			MOR	12	76	13.2 9.7	9.7 9.2	80.6 77.6	42	7.1	94 7.1	84.3 85.8

Table 11. Classification of 1992 cohort kokanee salmon, exposed to synthetic chemicals (either morpholine or phenethyl alcohol) at different life stages and tested at age 2 in 1994, into imprinted or non imprinted categories. Determinations of upstream and downstream migration when odors were present or absent, and ability to discriminate odors are listed for each life history stage.

Exposure Stage	Days Post-Fertilization	Direction of swimming when odors were absent [1]	Direction of swimming when odors present [2]	Did the fish select the arm scented with the exposure chemical? [3]	Classification [4]
Eyed Egg	44-64	Down	Down	No	Not imprinted
Hatch	64-76	Down	Up	Yes	imprinted
Alevin	70-94	Down	Up	Yes	Imprinted
Swimup	91-98	Down	Up	Yes	imprinted
Fry	105-252	Down	Down	No	Not Imprinted

[1,2] Criteria: When >50% of the individuals from a particular exposure stage displayed upstream migratory behavior, the group was classified as "Up"; when <50% displayed downstream migratory behavior, the group was classified as "Down".

[3] Criteria: Determination of odor discrimination was based on statistical significance (Chi Square test) at p=0.05. If fish selected the arm scented with the exposure chemical, they were considered imprinted. (See Table 9.)

[4] Criteria: For a group of fish to be considered imprinted, three criteria had to be met: Downstream movement with odor absent, upstream movement with odor present, and accurate selection of the exposure chemical. All other combinations were classified as not imprinted.

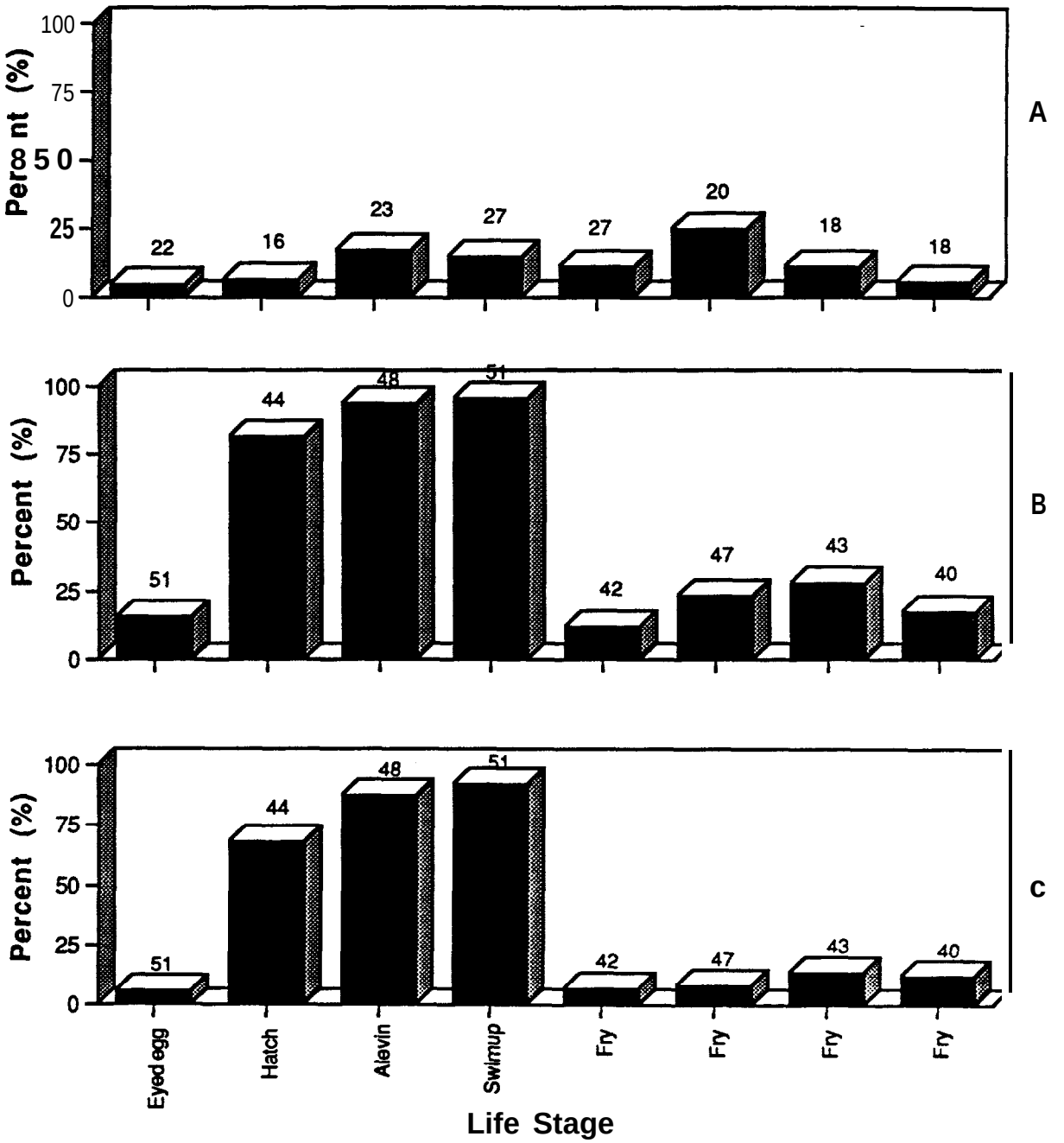


Figure 7. Results of odor discrimination tests with adult 1991 cohort kokanee salmon exposed to synthetic chemicals at different life stages and tested at age 3 in 1994. (A) Percentage that migrated upstream when the synthetic chemicals were absent; (B) Percentage that migrated upstream when the synthetic chemicals were present; (C) Percentage that homed to exposure odor. The values displayed above the bars indicate the total number of trials for that particular life stage.

c) in odor present trials, fish from all three groups switched channels at the time when the synthetic chemicals were switched. For fish exposed at hatch, 93 of 106 phenethyl alcohol exposed fish migrated first into the channel scented with phenethyl alcohol, then into the opposite channel after phenethyl alcohol was added to it. Additionally, 105 of 133 fish exposed to morpholine migrated first to the channel scented with morpholine, then into the opposite channel after morpholine was added to it. Also, 74 of 82 fish exposed to phenethyl alcohol and 105 of 115 fish exposed to morpholine at the alevin stage, and 83 of 86 fish exposed to phenethyl alcohol and 85 of 88 fish exposed to morpholine at the swimup stages, homed first to the channel scented with their treatment odor, then the opposite channel after their treatment odor was added to it; and

d) since fish exposed at hatch, alevin and swimup stages all tended to swim upstream to the channel scented with their exposure chemical when odors were present, selected the channel scented with their exposure chemical with precision, switched channels when odors were switched, and swam downstream if the odor was absent, all three groups were classified as “imprinted” (Table 11).

3) Fish exposed to synthetic chemicals at the fry stage (105-252 days post-fertilization) were classified as “not imprinted” because:

a) they were captured primarily at the downstream weir in both odor present and odor absent trials, at rates of 77.6% and 82.9% respectively (Tables 9, 10, 11; Figure 7); and

b) when synthetic chemicals were present they appeared to be unable to discriminate their exposure odor since the distribution of fish in the traps with the exposure odor and alternate odor was approximately equal (12.1% and 10.3% respectively) (Table 9, 10, 11).

The group of fish that experienced highest whole body thyroxine content (swimup stage) also had the highest percentage of fish homing accurately in the behavioral tests (Figure 7). Recently hatched eggs and alevins also had relatively high thyroxine content and displayed accurate homing in behavioral tests (Figure 7). Eyed eggs and post-swimup fry had relatively low thyroxine content and exhibited poor homing to exposure odors (Figure 7).

These results indicated that fish exposed to morpholine or phenethyl alcohol from hatch to swimup life stages: (1) became imprinted to their treatment odor at that life stage, (2) retained the odor memory during the 18 (1992 cohort) or 30 (1991 cohort) month period between the time of odor exposure and the time that odor discrimination experiments were conducted, and (3) homed to the odor as sexually mature fish.

3.1.3 CWT Returns

Recoveries of kokanee salmon captured in gill net, electrofishing and creel surveys in 1994 are shown in Table 12. Recoveries of ail coded wire tagged fish exposed to synthetic chemicals at different life stages and released from 1992 to 1994 are recorded in Tables 13, 14, and 15. From 1992 to 1994, a total of 4,811,594 kokanee were released into Lake Roosevelt, of which 923,167 were tagged with CWT/finclips. Coded wire tag releases included 618,313 kokanee fry and 304,854. A portion of these, including 582,047 fry and 184,932 smolts, were fish that had been exposed to either morpholine or phenethyl alcohol at different life history stages as part of our imprinting investigations (Tables 13, 14, 15, and 16).

A total of 431 CWT/finclipped fish were recaptured from 1992 to 1994, including 163 observed in randomized creel census or electrofishing/gill net surveys (486 unmarked kokanee were also observed in these surveys), and 263 recaptured at egg collection sites at Sherman Creek (morpholine scented) and Little Falls/Spokane River (phenethyi alcohol scented). Recoveries included 260 fish from the imprinting investigations (Tables 18, 14, 15, and 16). Of the total 431 CWT recoveries, 427 (99.1%) fish had been stocked as smolts and four (0.9%) had been stocked as fry. The relative percentage in each category was in contrast to the relative percentages of fry (67%) and smolts (33%) in the total CWT fish released (923,167).

Preliminary results of coded wire tagging investigations tended to corroborate the results of the olfactory discrimination experiments (see Section 3.1.2) with respect to

Table 12. Kokanee salmon recovered in Lake Roosevelt by electrofishing, gill net and creel surveys in 1994.

	Fish *Surveys*		*Creel* Surveys		Totals	
	Total #	w/Adipose Clips	Total #	#w/Adipose Clips	Total #	w/Adipose Clips
Kettle Falls	17	7	0	0	17	7
Gifford	5	2	1	0	8	2
Hunters	0	0	0	0	0	0
Porcupine Bay	20	16	0	0	20	16
Little Falls	14	9'	50	13[2]	64	22
Seven Bays	113	79	2	0	115	79
Kellers Ferry	0	0	26	0	26	0
San Poil	23	4	0	0	23	4
Spring Canyor	143	13	153	0	167	1
TOTALS	**206**	**118**	**232**	**14**	438	**131**

1 Includes two age 3 fish with A-LP clips and one age 3 fish with A-LV clip.
2 Includes two age 3 fish with A-LP clips and one age 3 fish with A-LV clip.
3 Includes 11 fish captured by TetraTech, Inc., one of which was adipose clipped.

Table 13. Recoveries by location of coded wire tagged kokanee salmon from releases made In 1992. Recoverles are total number recovered from creel and fisheries surveys conducted from 1992 to 1994 [1]..

Cohort	Stage Exposed	Exposure Odor	Release Location	Life Stage At Release	Total # CWT Released	# CWT recovered at		
						Sherman Creek (MOR scented)	Spokane River (PEA scented)	Other
1991	Eyed egg	MOR	Sherman Creek	Fry	7,367	1	0	0
		PEA	Sherman Creek	Fry	11,393	0	0	0
1991	Hatch	MOR	Sherman Creek	Fry	22,222	0	0	0
		PEA	Sherman Creek	Fry	23,115	0	0	0
1991	Alevin	MOR	Sherman Creek	Fry	11,441	0	0	0
		PEA	Sherman Creek	Fry	19,966	0	0	0
1991	Swimup	MOR	Sherman Creek	Fry	8,370	0	0	0
		PEA	Sherman Creek	Fry	10,716	0	0	0
1991	Fry (Feb)	MOR	Sherman Creek	Fry	20,194	2	0	0
		PEA	Sherman Creek	Fry	6,025	0	0	0
1991	Fry (Mar)	MOR	Sherman Creek	Fry	9,798	0	0	0
		PEA	Sherman Creek	Fry	10,818	1	0	0
1991	Fry (Apr)	MOR	Sherman Creek	Fry	11,445	0	0	0
		PEA	Sherman Creek		11,525	0	0	0
1991	Fry (May-Jul)	MOR	Sherman Creek	Fry	6,838	0	0	0
		PEA	Sherman Creek	Fry	11,300	0	0	0
1990	Smolt	MOR	Sherman Creek	Smolt	7,501	10	5	0
		PEA	Sherman Creek	Smolt	8,354	2	3	1

[1] Fish released at fry stages were from Lake Whatcom (1991 cohort) and were recovered as age 2 aduits in autumn 1993 and age 3 adults in autumn 1,994. Fish released as smoits were from Lake Whatcom (1990 cohort) and recovered as age 2 adults in 1992, age 3 adults in 1993 and age 4 adults in 1994.

Table 14. Recoveries by location of coded wire tagged kokanee salmon from releases made in 1993. Recoveries are total number recovered from creel and fisheries surveys conducted in 1993 and 1994'.

Cohort	Stage Exposed	Exposure Odor	Release Location	Life Stage At Release	Total # CWT Released	# recovered at Sherman Creek (MOR scented)	# recovered at Spokane River (PEA scented)	# recovered at Other
1992	Eyed egg	MOR	Sherman Creek	Fry	10,961	0	0	0
		MOR	Spokane River	Fry	10,903	0	0	0
		PEA	Sherman Creek	Fry	10,721	0	0	0
		PEA	Spokane River	Fry	32,953	0	0	0
1992	Hatch	MOR	Sherman Creek	Fry	7,988	0	0	0
		MOR	Spokane River	Fry	31,416	0	0	0
		MOR	Barnaby Creek	Fry	22,026	0	0	0
		PEA	Sherman Creek	Fry	7,988	0	0	0
		PEA	Spokane River	Fry	21,993	0	0	0
1992	Alevin	MOR	Sherman Creek	Fry	10,938	0	0	0
		PEA	Sherman Creek	Fry	11,791	0	0	0
1992	Swimup	MOR	Sherman Creek	Fry	10,908	0	0	0
		PEA	Sherman Creek	Fry	10,885	0	0	0
1992	Fry (Feb)	MOR	Sherman Creek	Fry	10,802	0	0	0
		PEA	Sherman Creek	Fry	10,896	0	0	0
1991	Smolt	MOR	Sherman Creek	Smolt	38,345	35	5	0
		PEA	Sherman Creek	Smolt	7,753	20	25	0
		PEA	Blue Creek[2]	Smolt	8,196	0	92	8[3]

[1] It is anticipated that additional fish from the 1992 cohort tots will be recovered as age 4 adults in 1995.
[2] Blue Creek is a tributary of the Spokane Arm of Lake Roosevelt located about 35 km downstream from Little Falls Dam.
[3] Six fish were collected in Hawk Creek, one at Seven Bays and one in the San Poil River.

Table 15. Recoveries by location of coded wire tagged kokanee salmon from releases made in 1994. Recoveries are total number recovered from creel and fisheries surveys conducted in 1994 [1].

Cohort	stage Exposed	Exposure Odor	Release Location	Life Stage At Release	Total # CWT Released	# recovered at Sherman Creek[1] (MOR scented)	# recovered at Spokane River (PEA scented)	Other
1992	Hatch	MOR	Sherman Creek	Smolt	13,435	4	0	3
		MOR	Blue Creek	Smolt	13,625	0	0	1
		PEA	Blue Creek	Smolt	16,158	0	6	0
1992	Alevin	MOR	Sherman Creek	Smolt	15,523	0	0	5
1992	Swimup	MOR	Sherman Creek	Smolt	17,917	3	0 4	2
		PEA	Sherman Creek	Smolt	38,125	1		0
1993	Hatch	MOR	Sherman Creek	Fry	51,823	0	0	0
	through Swimup	PEA	Sherman Creek	Fry	52,465	0	0	0
1993	Alevin	MOR	Sherman Creek	Fry	40,112	0	0	0
	through Swimup	PEA	Sherman Creek	Fry	20,942	0	0	0

[1] Includes two fish captured at Gifford and thre captured at Colville River.

Table 16. Recoveries by location of kokanee salmon with fin clips. These fish were exposed to synthetic chemicals in 1993, held at the Spokane Tribal Hatchery until July 1993, when they were released into the Spokane Arm of Lake Roosevelt as residualited smolts. Recoveries were made from September to November 1993 and 1994.

Cohort	Stage Exposed	Exposure Odor	Fin Clip	Release Location	Life Stage At Release	Total # Released	# CWT recovered at Sherman Creek (MOR scented)	# CWT recovered at Little Falls (PEA scented)	# CWT recovered at Other	Percent Return
1991	Eyed egg	MOR	RP	Spokane River	Smolt	325	0	1	0	0.3
		PEA	LP	Spokane River	Smolt	325	0	0	0	0
1991	Hatch	MOR	RV	Spokane River	Smolt	325	0	0	0	0
		PEA	LV	Spokane River	Smolt	325	0	5	0	1.5
1991	Alevin	MOR	A-RV	Spokane River	Smolt	325	0	0	0	0
		PEA	A-LV	Spokane River	Smolt	325	0	10	0	3.1
1991	Swimup	MOR	A-RP	Spokane River	Smolt	325	0	1	0	0.3
		PEA	A-LP	Spokane River	Smolt	325	0	14	0	4.3
1991	Fry (Feb)	MOR	LV-RP	Spokane River	Smolt	325	0	0	0	0
		PEA	RV-LP	Spokane River	Smolt	325	0	0	0	0
1991	Fry (Mar)	MOR	D-LV	Spokane River	Smolt	325	0	0	0	0
		PEA	D-LV	Spokane River	Smolt	325	0	0	0	0
1991	Fry (Apr)	MOR	D-RP	Spokane River	Smolt	325	0	0	0	0
		PEA	D-LP	Spokane River	Smolt	325	0	0	0	0
1991	Fry (May-Jul)	MOR	D	Spokane River	Smolt	325	0	0	0	0
		PEA	A	Spokane River	Smok	325	0	0	0	0

homing of fish exposed to synthetic chemicals at the smolt stage and released as residualized smolts. From 1992 to 1994, both morpholine and phenethyl alcohol exposed smolts were released at Sherman Creek or at Blue Creek. Fish-from the group released in 1992 were recovered at age 2, 3 and 4 as sexually mature adult fish in 1992, 1993 and 1994 respectively. Fish from the groups released in 1993 were recaptured as age 2 and age 3 sexually mature adult fish in 1993 and 1994 respectively. Fish from the groups released in 1994 were recovered as age 2 adults in 1994. From 1992 to 1994, 45 morpholine-exposed smolts were recovered as adult spawners at Sherman Creek (morpholine scented) compared to five recovered at Little Falls Dam (phenethyl alcohol scented) and none at other locations. In contrast, 120 phenethyl alcohol-exposed fish were recovered at Little Falls Dam compared to 22 at Sherman Creek and eight at other locations (sum totals from Tables 13 and 14). Thus, 90% of the morpholine exposed smolts were recovered in the morpholine scented river compared to 10% in the phenethyl alcohol scented river and 0% at other locations. In contrast, 14.6% of the phenethyl alcohol exposed smolts were recovered in the morpholine scented stream, compared to 80% in the phenethyl alcohol scented river and 5.4% at other locations.

Too few fish exposed to synthetic chemicals at other life history stages and released as fry were recovered to assess imprinting effectiveness. We do not believe that the failure of these fish to home was necessarily related to their failure to imprint to synthetic chemicals. The reason is that very few fish released as fry have been recovered anywhere in Lake Roosevelt. Instead, we believe that failure of most experimental groups to home and be recovered at locations where synthetic chemicals were metered into the reservoir was related to either their survival in the reservoir or emigration from the reservoir before reaching adult size. Nevertheless, the relatively small amount of data collected from fish exposed to synthetic chemicals at other life stages did tend to support (or at least not conflict with) the results of the olfactory discrimination experiments. Primarily, these results have come from fish that were held at the hatchery for a year after they were exposed to the synthetic chemicals, then released into Lake Roosevelt as post-smolts.

- Eyed egg stage: For fish exposed as eyed (prehatch) eggs, one morpholine exposed fish (50% of the total recovered) was captured in the morpholine scented stream (Sherman Creek) compared to one (50% of total recoveries) in the phenethyl alcohol exposed river

(Spokane River). To date, no fish exposed as eyed eggs to phenethyl alcohol have been recovered (Tables 13 to 16).

- Hatch stage: For fish exposed at hatching, four morpholine exposed fish (50% of total recoveries) have been captured in the morpholine scented stream, compared to none in the phenethyl alcohol scented river and four (50% of total recoveries) at other locations. In contrast, six (100% of total recoveries) fish exposed to phenethyl alcohol at hatch were in the phenethyl alcohol scented river (Tables 13 to 16).

- Alevin stage: For fish exposed at the alevin stage, all five morpholine exposed (100% of total recoveries) fish were recovered at locations other than those scented with the synthetic chemicals, and all ten (100% of total recoveries) of the phenethyl alcohol exposed fish were captured in the phenethyl alcohol scented river (Tables 13 to 16).

- Swimup stage: For fish exposed at the swimup stage, three morpholine exposed fish (50% of total recoveries) were captured in the morpholine scented stream, compared to one (17%) in the phenethyl alcohol scented river and two (33%) at other locations. In contrast, one phenethyl alcohol fish (6.6% of total recoveries) was captured in the morpholine scented stream compared to 14 (93.4%) in the phenethyl alcohol scented river and none at other locations (Tables 13 to 16).

- Fry stage: For fish released as fry, only three have been recovered to date, two morpholine exposed fish and one phenethyl alcohol exposed fish, all recovered in the morpholine scented streams (Tables 13 to 16).

Additional recoveries of 1991 year class CWT fish released in 1992 (as fry) and 1993 (as residualized smolts) are anticipated in 1995 when they will be age 4 spawners. Additional recoveries of 1992 year class CWT fish released in 1993 (as fry) and 1994 (as residualized smolts) are anticipated in 1995 and 1996 at age 3 and 4 respectively. Additional recoveries of 1993 year class CWT fish released in 1994 (as fry) and holdovers already tagged and expected to be released as residualized smolts in 1995 are anticipated in 1995, 1996 and 1997 at age 2, 3 and 4 respectively.

CWT returns also provided data about the growth of kokanee release into Lake Roosevelt. Table 17 shows the results obtained from 1992 to 1994. The weight of kokanee spawners averaged approximately 0.5 lb at age 2, 3.0 lb at age 3 and 3.3 lb at age 4. The largest CWT tagged kokanee recovered in 1994 was an age 4 fish captured on September 13 that was 516 mm (20.3 inches) in total length and 1,954 g (4.3 lbs.) in weight.

3.2 Smoltification Investigations of Yearling Kokanee

Yearling kokanee salmon experienced a peak in thyroxine and an increase in silvering in the spring of 1994. Results also indicated that kokanee experienced increased downstream migratory behavior and an increase in salt water adaptation in the spring.

3.2.1 Thyroxine Content of age 1 (1992 year class) Kokanee

Results of thyroxine experiments conducted in 1993/1994 with yearling kokanee (1992 year class) are shown in Figure 8. On October 20 1993, the mean plasma thyroxine concentration was 10.1 ± 0.13 ng/ml. Thyroxine concentration remained relatively constant until April 1994, when it increased significantly at 11.4 ± 1.7 ng/ml. Levels peaked significantly May 10 at 14.0 ± 1.8 ng/ml (ANOVA, Fisher PLSD; n=10; p=0.0001). The concentration decreased significantly by May 24 at 8.2 ± 2.4 ng/ml (n=10; p=0.0001).

3.2.2 Quality Assurance Results for the Radioimmunoassay (RIA)

Quality assurance results for thyroxine radioimmunoassays conducted in 1993 were previously reported by Tilson et *al.* (1994). Results of blind quality assurance samples for the RIA's in 1994 are recorded in Table 18. The actual concentration of the low T_4 blind sample was 2.3 ng/dl compared to a mean (±SD) measured value of 2.5 ± 0.3 ng/dl (n=4). The actual concentration of the medium T_4 blind sample was 7.3 ng/dl compared to a mean (±SD) measured value of 8.3 ± 1 .O ng/dl (n-4). The actual concentration of the high T_4 blind sample was 11.5 ng/dl compared to a mean (*SD) measured value of 12.0 ± 0.7 ng/dl (n=4).

Mean nonspecific binding for the four assays (n=6) was measured at 639 ± 112 counts per minute (cpm) or about 1.1% of the 60,053 ± 10,337 cpm measured in total

Table 17. Growth of kokanee salmon after release into Lake Roosevelt.

cohort	stage at release	year released	mean length at release (mm)	mean weight at release (g)	year recovered	age at recovery	mean length at recovery (mm)	mean weight at recovery (g)	number recovered with length/weight data (n)
1990	smolt	1992	31	150	1992	2	320	347	2
					1994	4	507	1,502	5
1991	smolt	1993	31	157	1993	2	350	490	66
					1994	3	454	1,181	24
1992	smolt	1994	52	167	1994	2	337	418	37

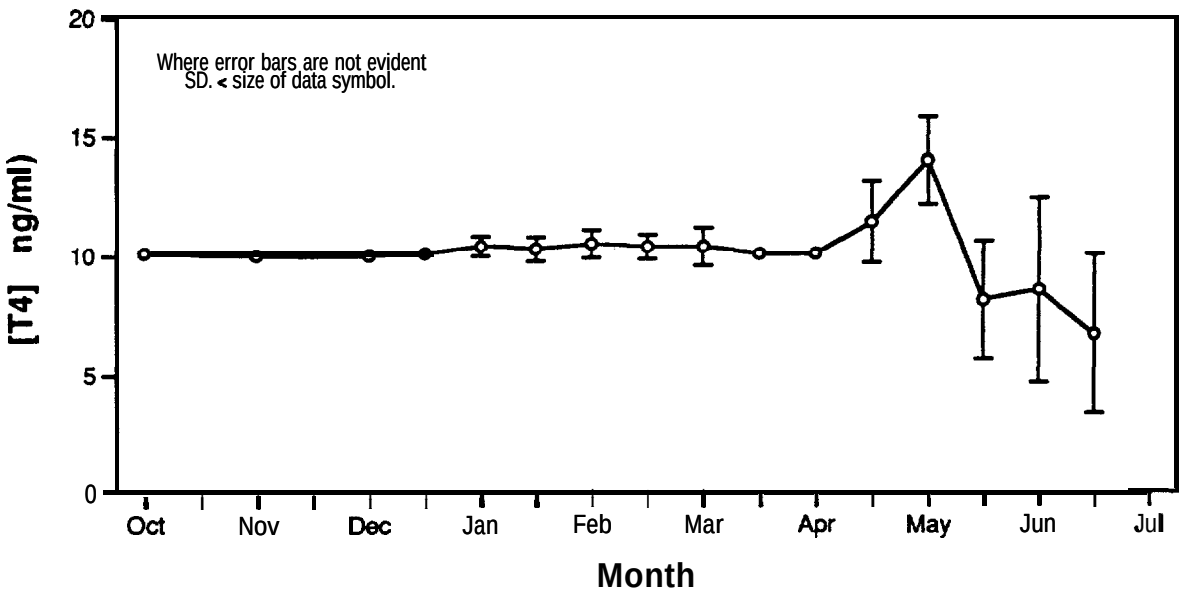


Figure 8. T_4 concentration in blood plasma of 1992 year class kokanee salmon from October 1993 to July 1994. Each point represents the mean ± SD of approximately 20 fish.

Table 18. **Results of blind quality assurance samples. Measured concentrations were the mean ± S.D. of three kokanee assays. 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	2.51 ± 0.31
Medium	7.3	6.2-8.4	8.31 ± 1.04
High	11.5	10.0-13.0	11.96 ± 0.73

Table 19. **Pipetting accuracy of 25 µl and 1.0 ml samples. Counts = counts per minute of radiolabeled T_4. Percent error = standard deviation (S.D.) ÷ mean counts.**

	Assay #	Sample size (n)	Mean # Of counts ± S.D.	Percent error (%)
25 µl	1	10	1823f.98	5
	2			
	3	10 10	1718 ± 238 1269 ± 199	14 16
	4	10	1363 ± 80	6
Mean			1543 ± 154	10
1.0 ml	1	10	67,431 ± 517	1
	2			
	3	10 10	70.8732349 53,271 ± 297	0 1
	4	10	47,801 ± 463	1
Mean			59,844 ± 406	<1

count tubes (n=6) (TCT). The blank tubes had a mean (±SD) of 3.9 ± 3.9 cpm (n=28). Accuracy of pipetting 25 µl and 1 ml samples is recorded in Table 19. The mean cpm (±SD) of tubes that received 25 µl of ^{125}I-T_4 was 1,543 ± 154 for a total error of 10%. The mean cpm of tubes that received 1 ml was 59,844 ± 407 for a total error of 0.8%. A frequency distribution of percent error of duplicate samples measuring blood plasma [T_4] is presented in Figure 9. The mean percent error of 160 samples was 1.4 ± 1.1%.

Results of interassay pool (IAP) samples for each of the four assays are recorded in Table 20. Mean concentration (±SD) for IAP1 was 2.6 ± 0.3 ng/dl, IAP2 was 8.2 ± 0.2 ng/dl and IAP3 had a concentration of 11.4 ± 1.25 ng/dl. The percent error for all IAP samples was <1 0%. The mean concentration (±SD) for standard curve samples (ng/dl) are shown in Table 21. Concentrations between assays were reasonably uniform with a percent error of ≤1 1%.

The actual values of the quality assurance samples were in close agreement to measured values. Blank tubes did not contain any significant contamination. The relatively similar concentration and low standard deviation for IAP samples indicated that all the assays were comparable. These results indicated that the assay was reliable. Therefore, T_4 concentration measurements were accepted as accurate for these kokanee salmon.

3.2.3 Cortisol

Results of mean plasma cortisol (n=2) are shown in Figure 10. Cortisol concentration increased in November to 66.1 ± 17.8 ng/ml. It then decreased to 28.6 ng/ml and remained at that level until April when a peak of 82.9 ± 12.9 ng/ml was seen. Cortisol levels decreased in June to 20.4 ± 5 ng/ml.

Dr. J. Specker (University of Rhode Island, personal communication) informed us that the cortisol assay met her quality control criteria. Mean nonspecific binding (± SD) (n=2) was measured at 281 ± 16 cpm or about 3.9% of the 7,249 ± 93 cpm measured in total count tubes (TCT) (n=2). Percent error between duplicate samples was 2.8 ± 2%. Mean concentration (± SD) for the inter-assay pool sample (IAP) was 24.4 ± 1 ng/dl (n=2), which was uniform to the concentration of the IAP samples that she observed in other cortisol assays she has performed.

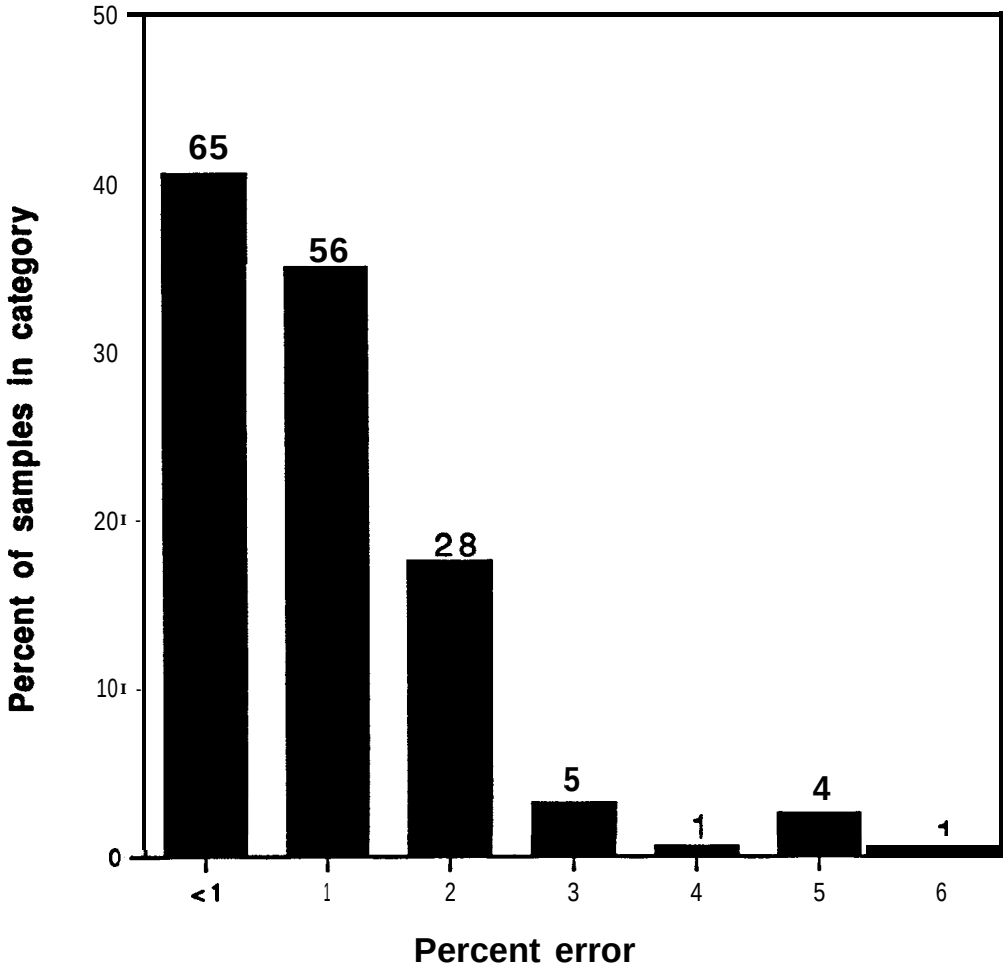


Figure 9. Frequency distribution of percent error in duplicate blood plasma samples from age 1 kokanee salmon. Total sample size was 160 individuals. The number of samples in each category are noted above error bars.

Table 20. **Mean (± S.D.) and percent error of three interassay pool (IAP) samples (ng/dl) run in four assays of kokanee salmon.**

Assay	IAP 1	IAP 2	IAP 3
1	2.4	7.6	11.5
2	2.3	7.9	11.6
3	2.9	9.5	12.8
4	2.8	7.7	9.8
MEAN	2.6	8.2	11.4
S.D.	0.3	0.9	1.25
% ERROR	11%	1 1%	11%

Table 21. **Mean (± S.D.) and percent error of standard curve samples (ng/dl) run in four assays of kokanee salmon.**

Assay	0.5	1.0	4.0	10.0	16.0	24.0	
1	0.6	0.9	3.9	9.5	16.6	25.2	
2	0.6	0.9	3.7	8.7	18.5	24.9	
3	0.5	1.0	4.5	9.3	16.0	23.7	
4	0.5	0.9	4.6	9.4	16.0	25.7	
MEAN	0.5	0.9	4.2	9.3	16.8	24.9	
S.D.	0.05	0.1	0.4	0.4	1.3	0.8	
% ERROR	1 0 %	6%	11%	4%	7%	3%	

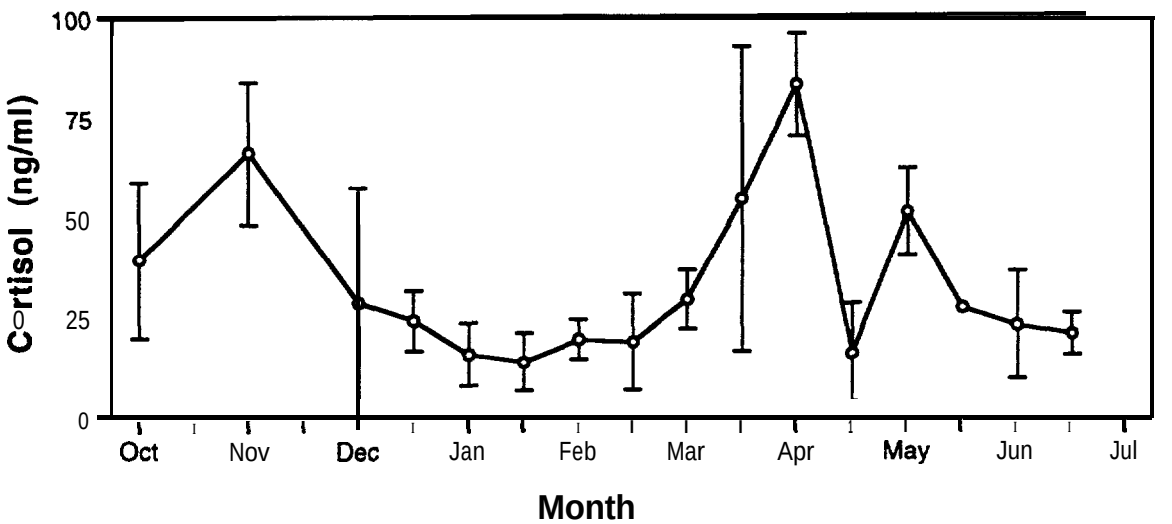


Figure 10. Plasma cortisoi (ng/ml) of 1992 year class age 1 kokanee salmon sampled from October 1993 through June 1994. Each point represents the mean ± SD of approximately 20 fish.

3.2.4 Silvering

Results of monthly visual observations of silvering coloration are shown in Figure 11. In November, most of the fish observed were in transition (80%). In January, most of the fish observed were parr (75%), and then silvering started to increase each month through April when 90% of the fish sampled had developed a silvery coloration. In May, parr marks were evident again signifying a reversion from smolt to parr. The number of fish in the transition phase started to increase at the end of April at the same time the T_4 concentration started to increase.

3.2.5 Downstream Migratory Behavior

Mean downstream migratory activities (% downstream) for yearling kokanee are shown in Figure 11. Fish demonstrated an increase in downstream migratory behavior from November to December 1993 (33 to 56%). This behavior stayed at approximately 50% until April when 60% of the fish exhibited downstream behavior. There was a decrease in downstream behavior from May to June (48 to 32%). Downstream migratory activity increased in April at the same time the thyroxine levels increased (Figure 9 and 12).

The fish did not appear to exhibit increased buoyancy, which is one of the characteristics of smolting anadromous salmonids. They stayed near the bottom or middle of the Living Stream each time they were observed. However, the orientation of the fish changed over time. General observations showed that fish exhibited scattered orientation until March and April when the majority of fish were facing downstream.

3.2.6 Condition Factor

Average coefficients of condition (K_{TL}) for yearling kokanee salmon are shown in Figure 11. The mean condition factor (±S.D.) decreased from 0.88 ± 0.07 in October to 0.77 ± 0.07 in November 1993. It then increased to 0.89 ± 0.05 in December of 1993 and remained consistent until March, 1994 when it increased to 0.96 ± 0.06. Condition factor remained at about 0.90 through June.

3.2.7 intestinal Water Transport

The changes in J_v that occurred for the middle and posterior intestine are shown in Figure 12. The middle intestine J_v decreased from a November J_v of 10.2 $\mu l/hr/cm^2$

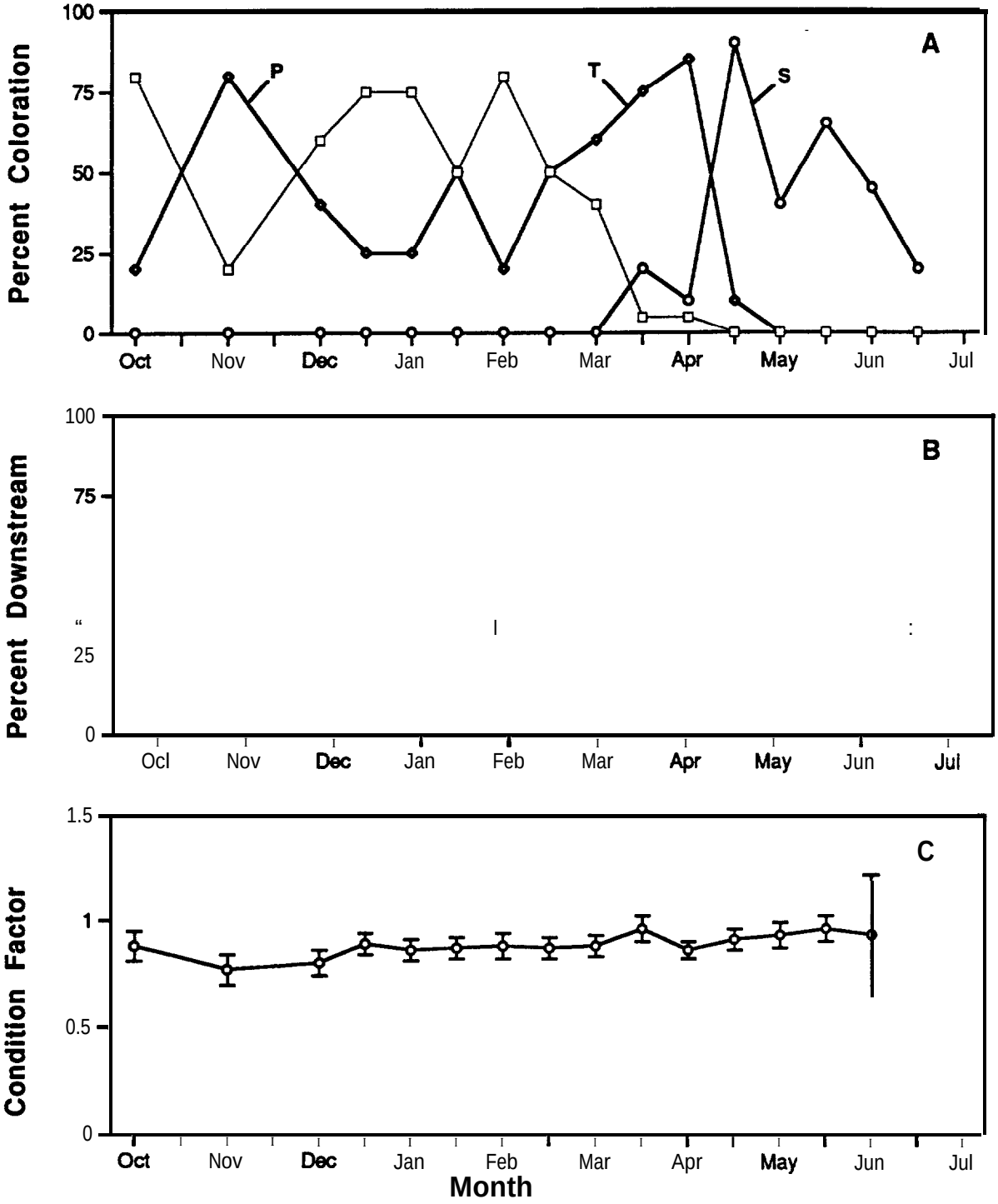


Figure 11. (A) Percentage of fish grouped as parr (P), transition (T), and smoit (S); (B) Percentage of fish in downstream third of tank; and (C) Condition factor (mean ± SD). Data represent age 1 kokanee salmon sampled from October 1993 through June 1994. Sample size (n)=20 fish per data point.

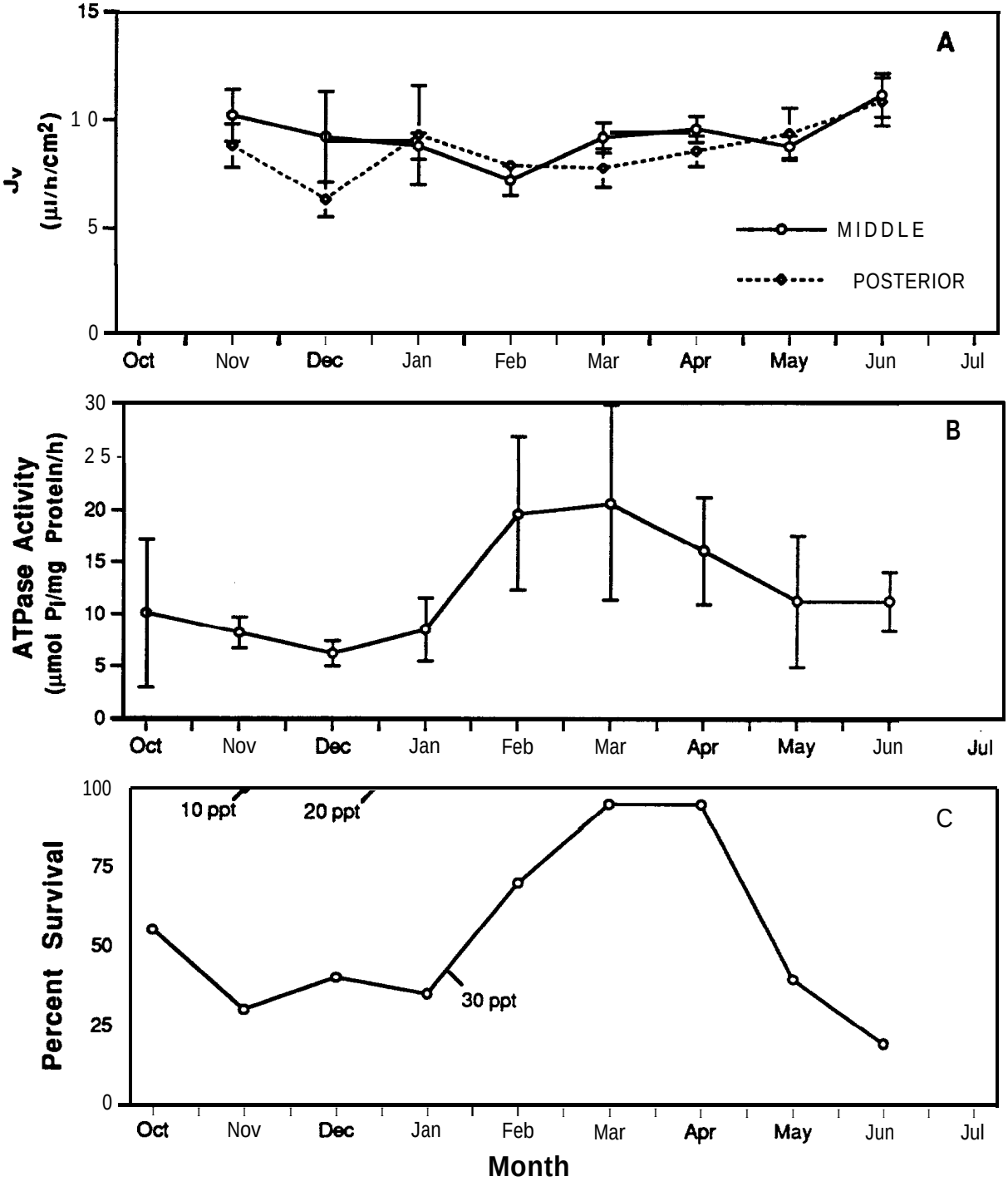


Figure 12. (A) intestinal water transport rate (J_v) (mean ± SEM, n=12); (B) Gill Na^+-K^+ ATPase activity (mean ± SD); and (C) Percent survival after 96 h in salt water for age 1 kokanee salmon sampled from October 1993 through June 1994.

to a February J_v of 7.2 µl/hr/cm^2. The J_v then returned to earlier levels. There was no significant difference in J_v over the 9 month period (ANOVA; Fisher PLSD; $p \geq 0.05$). The posterior intestine J_v showed no significant differences over time, remaining at approximately 8 µl/hr/cm^2 throughout the sample period (ANOVA; Fisher PLSD; $p \geq 0.05$).

3.2.8 Gill Na^+-K^+ ATPase Activity

Gill Na^+-K^+ ATPase levels for yearling kokanee are shown in Figure 12. The average ATPase activity of gill microsomes increased significantly from 8.5 µmoles P_i/mg Protein/h in January 1994 to 20.6 in March (ANOVA; Fisher PLSD, p=0.0001). The value then decreased significantly to 16 µmoles P_i/mg Protein/h in April and dropped to 11 µmoles P_i/mg Protein/h in May and June.

3.2.9 Salt Water Tolerance

Percent survival in salt water for kokanee salmon are shown in Figure 12. There was 100% survival during all months tested for fish held in 330 mOsmol/L (10 ppt) and 660 mOsmol/L (20 ppt) seawater. Fish held in 1000 mOsmol/L (30 ppt) seawater exhibited 55% survival in October 1993. Survival then decreased to 35% in January 1994. In March and April 1994, survival increased to 95% before dropping to 20% survival in June. Table 22 shows the osmolarity of the water taken from each saltwater tank in which fish were held during the experiment. Osmolarity of full strength seawater was consistently between 947-l ,049 mOsmol/L (28 and 31 ppt salinity) during these experiments. Therefore, survival of the different groups of fish tested each month was considered comparable between months.

3.2.10 Osmoregulatory Capability

Blood plasma osmolarity (mOsmol/L) values for yearling kokanee salmon are shown in Table 23. Blood osmolarity levels of fish held in 10 ppt salt water were shown to be statistically different than those of fish held in fresh water during the months of October, November, December, February and March (Table 23). However, we feel that the biological differences of these values were probably not significant since fish in both freshwater and dilute salt water had blood osmotic concentrations near isosmotic levels (330 mOsmol/L). During January, April, May and June, there were no significant differences between the osmolarity of fresh water fish and dilute salt water fish. Blood osmotic concentrations of fish held in 20 ppt were significantly different than those of

Table 22. **Water sample osmoiarity (mOsmoi/L) taken from three fish tanks (low, 10 ppt; medium, 20 ppt; and high, 30 ppt salinity) during salt water tolerance experiments.**

Month	Low Salinity	Medium Salinity	High Salinity
October	315	632	**975**
November	346	685	1015
December	286	639	947
January	320	664	1000
February	349	701	985
March	323	717	1039
April	393	713	1047
May	318	673	1049
June	329	603	1010
MEAN	298	670	1007

Table 23. **Mean blood plasma osmoiarity (mOsmol/L) ± SD of fish held in fresh water (control) compared with osmoiarity of fish in three salt water concentrations (10, 20, 30 ppt) from October 1993 through June 1994. Means represent 10 samples (n=20 fish); t value and probability are given in parentheses below means.**

MONTH	FRESH WATER (0 ppt)	SALT WATER (10 ppt)	(20 ppt)	(30 ppt)
October	289 ± 11	309 ± 12 * (p=0.002)	311 ± 12 * (p=0.0007)	440 ± 27 * (p=0.0001)
November	309 ± 3	320 ± 10 * (p=0.007)	327 ± 16 * (p=0.006)	448 ± 99 * (p=0.002)
December	292 ± 6	300 ± 6 * (p=0.007)	329 ± 42 * (p=0.014)	499 ± 60 * (p=0.0001)
January	315 ± 30	320 ± 32 (p=0.75)	300 ± 16 (p=0.178)	447 ± 75 * (p=0.0001)
February	301 ±7	322 ± 26 * (p=0.02)	318 ± 11 * (p=0.0004)	378 ± 64 * (p=0.001)
March	312 ± 12	329 ± 17 * (p=0.03)	332 ± 13 * (p=0.0055)	364 ± 28 * (p=0.0003)
April	313 ± 27	320 ± 22 (p=0.567)	325 ± 34 (p=0.4.9)	358 ± 28 * (p=0.002)
May	339 ± 37	345 ± 53 (p=0.84)	341 ± 36 (p=0.93)	526 ± 100 * (p=0.005)
June	308 ± 12	338 ± 46 (p=0.07)	322 ± 12 * (p=0.02)	622 ± 26 * (p=0.0001)

* = salt water blood plasma osmolarity was statistically different from fresh water (control) blood plasma osmolarity by unpaired, two-tailed t-test (alpha = 0.05)

fresh water fish during some months (Table 23). However, the osmotic concentrations were again near isosmotic levels and therefore were not considered biologically significant.

Plasma osmolarity of fish held in full strength salt water (30 ppt) were significantly different than those levels of fresh water fish (control) during every month tested. However, mean plasma osmotic concentrations of fish held in salt water declined from January through April approaching the level of the controls. By April, the blood osmotic concentration of salt water fish was close to the control (358 ± 28 mOsmol/L vs. 313 ± 27 mOsmol/l). After April, osmotic concentrations of salt water fish rose again to very high levels.

Figure 13 shows blood plasma osmolarity levels for kokanee over a 9 month period from October 1993 through June 1994. Osmolarity of fish held in fresh water was similar ranging from 289 ± 11 to 339 ± 37 mOsmol/L. Although significant differences were found over time, these levels were all near isosmotic levels and were probably not biologically significant. In 10 ppt seawater, plasma osmolarity levels ranged from 300 ± 6 to 345 ± 53 mOsmol/L. There were no significant differences in mean plasma osmolarity during these months (ANOVA; Fisher PLSD; $p>0.05$). Plasma levels in 20 ppt ranged from 300 ± 16 to 341 ± 36 mOsmol/L. There was a significant decrease from December to January (ANOVA; Fisher PLSD; $p\leq0.05$). However, the osmolarity was near isosmotic levels and therefore was again not considered biologically significant. Plasma osmolarity levels in full strength seawater ranged from 358 ± 28 to 622 ± 26 mOsmol/L. Levels remained elevated from October 1993 to January 1994 (>440 mOsmol/L), then decreased significantly in February and stayed low through April (<380 mOsmol/L) signifying that the fish were osmoregulating (ANOVA; Fisher PLSD; $p=0.0001$). Osmolarity levels then rose significantly in May (526 ± 100 mOsmol/L) and stayed high in June (622 ± 26 mOsmol/L) (ANOVA; Fisher PLSD; $p=0.0001$).

Blood and environmental osmometry samples were run during six days. Standards were run before each assay in order to calibrate the osmometer and to determine if the assays could be compared. Table 24 shows the mean (±SD) of the standard solutions for each assay date. The mean ±SD for the 100 mOsmol/L standard was 106 ± 3 with a percent error of 3%. The 300 mOsmol/L standards showed a mean of 302 ± 2 mOsmol/L and a mean error of <1%. The mean (±SD) of the 500 mOsmol/L

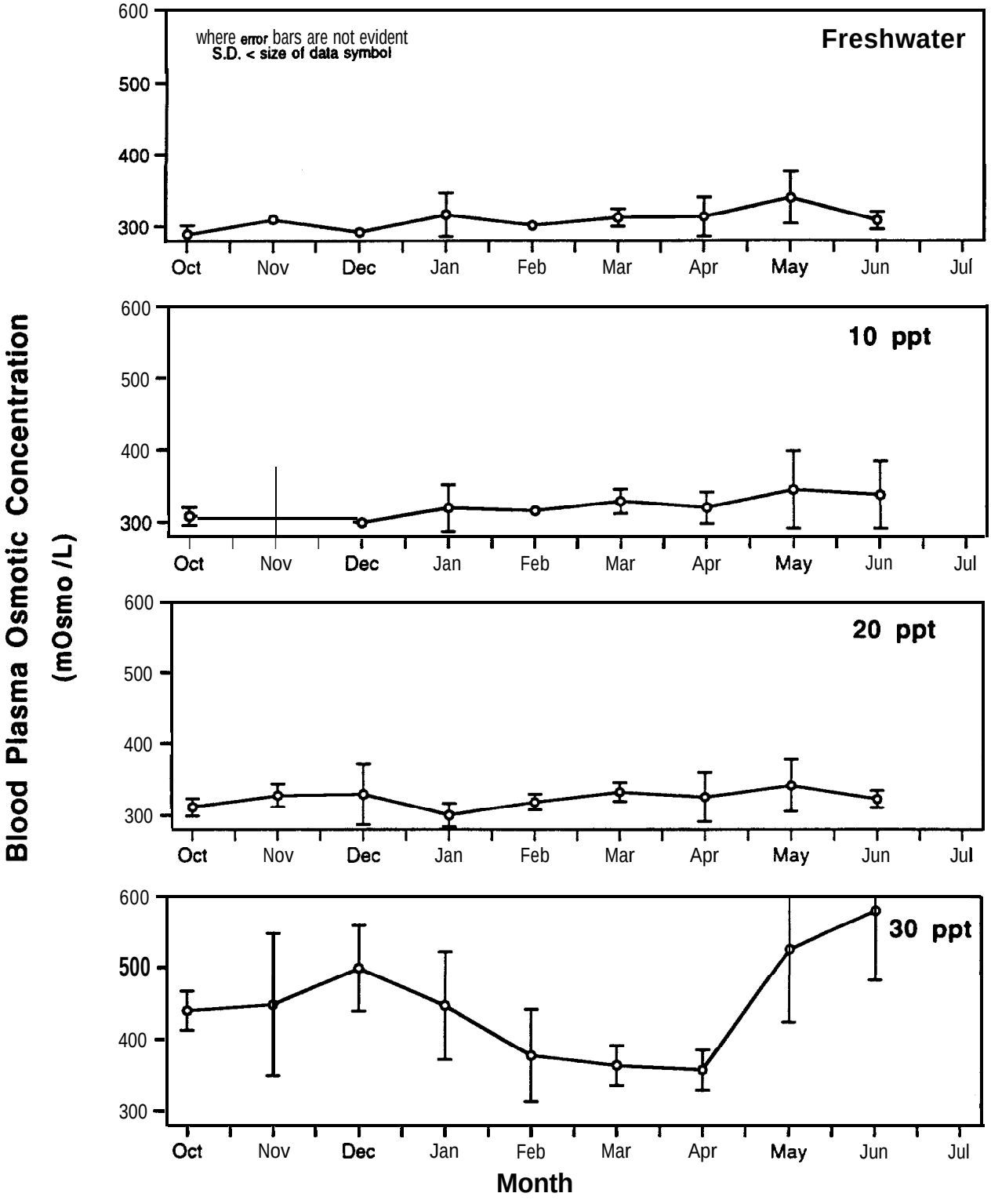


Figure 13. Blood plasma osmoiarity (mOsmol/L) for age 1 kokanee salmon in four different salt water concentrations sampled from October 1993 through June 1994. Each data point represents the mean ± SD of approximately 20 fish.

Table 24. Osmolarity of osmotic concentration standards from six assays. Low (100 mOsmol/L), medium (300 mOsmol/L) and high (500 mOsmol/L) standards were used to calibrate the osmometer before each assay to determine accuracy of the osmometer.

	Assay #	Mean ± SD (mOsmol/L)	Percent Error (%)
100 mOsmol/L	1	108 ± 5	5
	2	109 ± 2	2
	3	109 ± 2	2
	4	111 ± 1	1
	5	97 ± 1	1
	6	102 ± 7	7
Mean ± S.D.		106 ± 3	3
300 mOsmol/L	1		
	2	300 ± 4	1
		303 ± 1	<1
	3	303 ± 3	<1
	4	303 ± 1	<1
	5	299 ± 2	1
	6	302 ± 1	c l
Mean ± S.D.		302 ± 2	<1
500 mOsmol/L	1	499 ± 4	1
	2	500 ± 2	<1
	3	500 ± 4	1
	4	501 ± 2	c l
	5	500 ± 2	<1
	6	499 ± 1	<1
Mean ± S.D.		500 ± 3	<1

standards was 500 ± 3 with an error of ~1%. Since the mean percent error of all assays was less than 5%, the assays were considered comparable.

3.211 Salt Water Preference

During the resting period, fish were not concentrated in any particular area, but were fairly quiet. Upon completion of the fresh water bridge, the fry typically nosed up to the bridge and sometimes schooled and swam rapidly back and forth from fresh water to salt water. This behavior usually lasted about 10 to 20 minutes. Then activity decreased following the break up of schools and usually, individual fish remained in their tank of choice.

Fish showed a preference to concentrated seawater in November (62%) (Figure 14). During December fish exhibited a slight avoidance to salt water (45% preference). During January, February, March, April, May and June, kokanee exhibited an avoidance to salt water with a 23, 2, 9, 0, 15 and 43% preference respectively during these months (Figure 14).

3.3 Evidence of Residuaiization in Smoit Transitions

Many species of anadromous salmonids residualize if they do not reach the ocean within about 60 days after the onset of smoltification. These fish stay in fresh water, readjusting their osmoregulatory systems and either remain in the river for another year or until they spawn. In the present investigation, residualization was indicated by:

(1) Thyroxine peaked in May, then declined by June.

(2) Silvering increased in February and peaked in May when 90% of the individuals exhibited silvery smolt coloration. Parr marks reappeared in June on about 80% of the individuals.

(3) Downstream migratory activity peaked in December and April at 60%. By June only 32% of the fish evidenced downstream activity.

(4) Gill Na^+-K^+ ATPase activity peaked in March at 20.6 µmoles P_i/mg Protein/h. The value then decreased to 11 µmoles P_i/mg Protein/h in May and June.

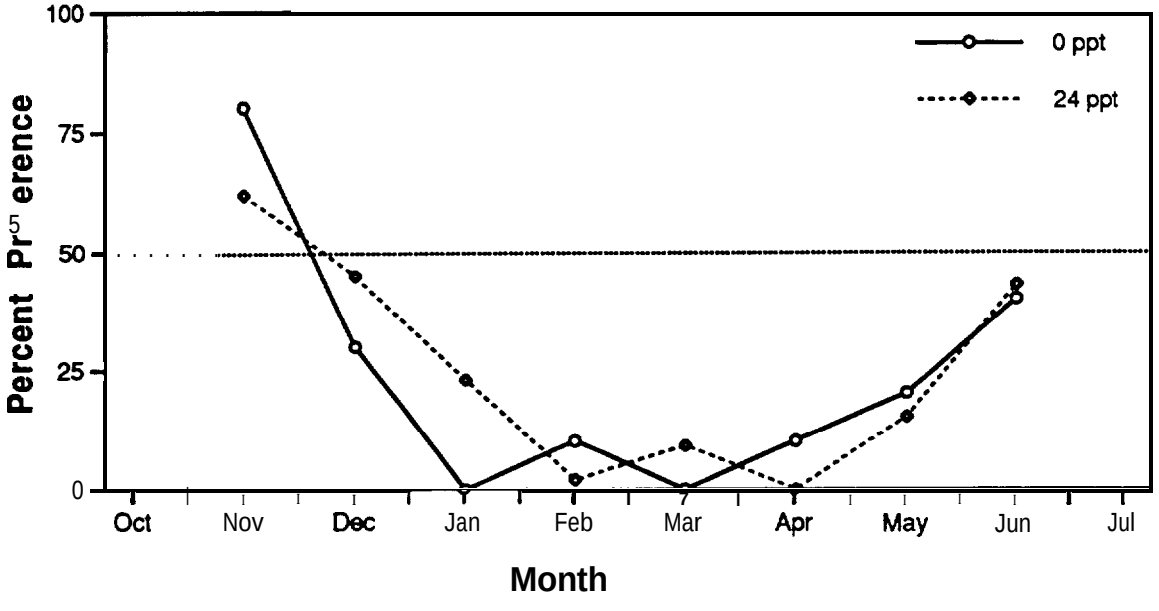


Figure 14. Salt water preference in control tank (0 ppt) and in concentrated seawater (24 ppt) for age 1 kokanee salmon from November 1993 to June 1994.

(5) Osmoregulatory activity and salt water tolerance became evident in February, March and April. By May and June, fish did not display the ability to osmoregulate nor did they show the ability to survive in salt water.

4.0 DISCUSSION

This study has important implications for management of the Lake Roosevelt kokanee fishery. The results of the imprinting investigations replicated the results of our initial experiments conducted in 1993 by again showing that kokanee can be successfully imprinted to artificial odors - morpholine, and phenethyl alcohol - as juveniles from hatch to swimup and again as smolts. In addition, the results of the present study duplicated results collected in 1993 that indicated kokanee underwent partial smoltification and residualization. This information will be helpful in developing management strategies for the Lake Roosevelt kokanee fishery.

4.1 Imprinting Investigations

Olfactory imprinting in coho salmon, Atlantic salmon and steelhead trout is activated by surges in thyroid hormones which occur at the smolt stage (Scholz 1980; Hasler and Scholz 1983; Morin et *al.* 1989a,b). Hasler and Scholz (1983) reported that presmolt coho salmon, which were exposed to morpholine and phenethyl alcohol in February when thyroid hormones were low, did not home to the chemicals as adults; whereas smolts that were exposed to these chemicals in mid-April, during a thyroid surge, did home to them as adults. Also, fish exposed to morpholine and phenethyl alcohol in February while being injected with thyroid stimulating hormone homed with precision as adults in olfactory discrimination experiments, whereas those injected with a placebo did not home to the chemicals.

The imprinting process appeared to require the binding of thyroid hormones to brain cell nuclei (Scholz et *al.* 1985; White et *al.* 1990). This bound thyroid hormone is thought to stimulate the transcription of genes that code for nerve growth factor (NGF) proteins. Activation of NGF causes neuron differentiation and wires a pattern of neuron circuits that makes possible the permanent olfactory memory (imprinting) (Lanier 1987; Scholz et *al.* 1985; 1992).

However, not all species of Pacific salmon stay in their homestream for 1.5 years, then exhibit a distinct smolt stage like coho, steelhead, and Atlantic salmon. For example, anadromous sockeye, and their landlocked kokanee relatives, emigrate from their natal tributary to a nursery lake soon after swimup. Sockeye remain in the lake for about a year, then smolt and migrate to sea. Kokanee undergo incomplete

smoltification and remain in the lake, where they grow to adult size. Both sockeye and kokanee exhibit natal homing to tributaries of lakes experienced only as embryos or larvae (Quinn et a/. 1989), so if these fish utilize olfactory cues for homing, they must necessarily imprint during the egg, alevin, swimup or early fry stages.

Recently, several investigators have reported that whole body thyroxine content fluctuates in developing eggs and larvae of several species of *Oncorhynchus,* including sockeye and kokanee (Kobuke et *al.* 1987; Tagawa and Hirano 1987, 1990; Greenblatt et *a/.* 1989; Leatherland et *al.* 1989; Tagawa et *al.* 1990; deJesus et *al.* 1992; Scholz et *a/.* 1992, 1993; Tilson *et* a/. 1994). Typically, thyroxine content was relatively high in eggs, decreased in post-hatch alevins, then increased in swimup larvae. The disappearance of thyroxine after hatching suggested that it was being taken from the yolk into tissues and then cleared from the fish (Tagawa and Hirano 1990). Thyroid follicles in chum salmon (0. *keta*), another species that emigrates soon after emergence, were found to increase in size and number during yolk absorption, so it was concluded that the increase in thyroxine content at the time of emergence (i.e. swimup stage) was owing to increased production of thyroxine by the embryonic thyroid gland (Tagawa and Hirano 1990). in comparative studies of ail five species of Pacific salmon native to North America, sockeye eggs and larvae had the highest thyroxine content (Leatherland et *al.* 1989). The implication of these studies was that thyroid hormones may be sufficiently high in sockeye/kokanee eggs or larvae to stimulate olfactory imprinting at those developmental stages. Our investigations of thyroid content indicated that early life stages, particularly the period from hatch to swimup, evidenced peaks of thyroid activity that could potentially indicate a critical period for imprinting. Moreover, these patterns appeared to be relatively precise and regular, since we repeatedly observed uniform patterns in two cohorts of Lake Whatcom stock kokanee, one cohort of Cabinet Gorge stock kokanee and one cohort from eggs collected from wild Lake Roosevelt fish (Scholz et *al.* 1992, 1993; Tilson et *a/.* 1994).

4.1.1 Olfactory Discrimination Experiments

Results of the present investigation confirmed our initial findings that chemical imprinting in kokanee salmon occurs concomitant with elevated thyroxine levels. The second year (1994) of olfactory discrimination experiments replicated our first year (1993) to a high degree. Tables 25 to 27 summarize the results of both years of this investigation. Table 25 provides, for each life stage exposed to synthetic chemicals, the numbers captured in morpholine or phenethyl alcohol scented traps or at the

Table 25. Summary of all kokanee salmon tested in olfactory discrimination experiments in 1993 and 1994. Data for each life stage and exposure odor are organized by cohort, year tested and age when tested. Totals for each exposure odor at each life stage tested are denoted In bold letters.

					Odor Present			**Odor** Absent		
Exposure Stage	Exposure Odor Chemical	Cohort	Year Tested	**Age** Tested	MOR Trap (#)	PEA Trap (#)	Down Stream (#)	Trap A **(#)**	Trap B (#)	Down **Sream** (#)
					45					
Pre-eyed Egg	PEA	1991	1993	2	**45**	52	201	5	3	92
		Total	all	all		52	201	5	3	92
					8			**1**		
Eyed Egg	PEA	1991	1993	2	**2**	16	25	**0**	1	10
			1994	3		2	25		0	10
		1992	1994	2	60	67	**98**	**11**	**9**	6
		Total	all	all	70	85	**148**	**12**	**10**	87
	MOR	1991	1993	2	14	11	23	0	1	9
			1994	3	1	3	18	0	0	11
		1992	1994	2	**60**	**57**	203	**13**	11	**91**
		Total	all	all	**75**	**71**	232	**13**	12	**111**
Hatch	PEA	1991	1993	2	9	44	13	2	2	14
			1994	3	3	15	2	1	0	8
		1992	1994	2	33	110	36	**10**	9	67
		Total	all	all	45	189	51	**13**	11	89
	MOR	1991	1993	2	42	12	5	2	2	12
			1994	3	15	3	6	0	0	7
		1992	1994	2	175	56	44	**9**	11	89
		Total	all	all	232	73	53	**11**	13	117
Alevin	PEA	1991	1993	2	3	43	7	0	0	14
			1994	3	1	18	1	0	0	8
		1992	1994	2	29	148	**22**	**8**	**6**	**69**
		Total	all	all	33	209	**30**	**8**	**6**	**91**

Table 25. cont. **Summary of all kokanee salmon tested in olfactory discrimination experiments in 1993 and 1994. Data for each life stage and exposure odor are organized by cohort, year tested and age when tested. Totals for each exposure odor at each life stage tested are denoted in bold type.**

					Odor Present			Odor Absent		
Exposure Stage	Exposure Odor **Chemical**	Cohort	Year Tested	**Age** Tested **2**	MOR Trap **(#)**	PEA Trap **(#)**	Down Stream **(#)**	**Trap** A **(1)**	Trap B (#)	Down Sream **(#)**
	MOR	1991	1993	**3**	39	4	5	**3**	0	16
			1994		24	2	2	**8**	1	11
		1992	1994	2	182	53	**31**	**12**	89	9972
		Total	all	all	245	59	**38**			
				2						
Swimup	PEA	1991	1993	**3**	3	59	6	**1**	**1**	18
			1994		2	26	1	2	**5**	13
		1992	1994	2	35	139	12	**6**		
		Total	all	all	40	224	19	**9**	6	61 92
	MOR	1991	1993	2	52	5	2	**1**	0	15
			1994	3	21	1	1	**0**		10
		1992	1994	2	124	**39**	10		**1**	**62**
		Total	all	all	197	**45**	13	**1**	**5**	**87**
Fry (all stages)	PEA	1991	1993	2	18	28	125	1	4	55
			1994	**3**	7	10	68	**1**		
		1992	1994	**2**	**10**	**10**	**69**	**2**	24	36 27
		Total	all	all	**35**	**48**	**310**	4	IO	118
	MOR	1991	1993	2	18	20	124	**1**	**4**	
			1994	3	8	10	67	**4**	**4**	47 36
		1992	1994	2	10	7	59	3	4	26
		Total	all	all	**34**	37	250	8	12	109
								3		
Smolt	PEA	1990	1993	3	12	37	12	**3**	33	17 17
		Total	all	all	12	37	12			
					39					
	MOR	1990	1993	3	**39**	18	11	**3**	3	16
		Total	all	all		18	11	**3**	3	16

Table 26. **Summary of percentages of kokanee captured In traps with odors present or absent during olfactory discrimination experiments conducted in 1993 and 1994. Data for each life stage and exposure odor are organized by cohort, year tested and age when tested. Totals for each exposure odor at each life stage tested are denoted by bold type.**

					Odor Present			Odor Absent		
Exposure Stage	Exposure Odor **Chemical**	Cohort	Year Tested	**Age** Tested	MOR **Trap** **(%)**	PEA Trap %	Down Stream **(%)**	**Trap** A **(%)**	Trap B **(%)**	Down Sream **(%)**
Pre-eyed Egg	PEA	1991	1993	2	15	17	67	**3**	5	**92**
		Total	all	all	15	17	67	**3**	5	**92**
Eyed Egg	PEA	1991	1993	**2**	16	33	**51**	8	8	83
			1994	**3**	7	7	**86**	0	0	100
		1992	1994	2	27	30	43	**13**	10	**77**
		Total	all	all	23	28	49	**11**	9	**80**
	MOR	1991	1993	2	29	23	48	10	0	90
			1994	3	**12**	**6**	82	**0**	0	100
		1992	1994	2	**19**	**18**	**63**	11	**9**	**79**
		Total	all	all	20	19	**61**	10	**8**	**82**
Hatch	PEA	1991	1993	2	14	**67**	20	**11**	**1**	78
			1994	3	15	**75**	10	**11**	**0**	89
		1992	1994	2	18	**62**	20	12	11	77
		Total	all	all	17	**64**	19	12	9	79
	MOR	1991	1993	**2**	71	20	9	13	12	75
			1994	**3**	63	12	25	0	0	100
		1992	1994	2	**63**	**21**	16	8	10	**81**
		Total	all	all	**65**	**20**	15	8	9	**83**
Alevin	PEA	1991	1993	2	8	81	13	6	0	94
			1994	3	7	90	**3**	6	13	**81**
		1992	1994	2	15	74	**11**	10	7	**83**
		Total	all	all	12	77	11	6	5	87

Table 26 cont. Summary of percentages of kokanee captured in traps with odors present or absent during olfactory discrimination experiments conducted in 1993 and 1994. Data for each life stage and exposure odor are organized by cohort, year tested and age when tested. Totals for each exposure odor at each life stage tested are denoted by bold type.

					Odor Present			Odor Absent		
Exposure Stage	Exposure Odor Chemical	Cohort	Year Tested	**Age** Tested	**MOR** Trap %	PEA Trap **(%)**	Down Stream **(%)**	**Trap** A %	Trap B **(%)**	Down Sream **(%)**
	MOR	1991	1993	**2**	81	8	13	**6**	0	94
			1994	**3**	95	5	0	**0**	9	91
		1992	1994	2	68	20	12	**9**	9	**82**
		Total	all	all	72	17	1 1	**10**	7	**83**
Swimup	PEA	1991	1993	2	4	87	9	5	0	95
			1994	3	7	90	3	6	13	81
		1992	1994	2	19	75	6	**8**		85
		Total	all	all	14	79	7	**8**	3	86
	MOR	1991	1993	**2**	88	9	**3**	**6**	0	94
			1994	**3**	95	5	**0**	**0**	9	91
		1992	1994	2	72	22	**6**	6	**7**	87
		Total	all	all	77	17	**6**	5	**6**	89
Fty (all stages)	PEA	1991	1993	2	11	16	73	2	7	91
			1994	3	**8**	12	80	3	6	91
		1992	1994	2	**11**	11	78	**6**	12	82
		Total	all	all	9	12	79	**3**	8	89
	MOR	1991	1993	2	10	12	78	2	8	90
			1994	3	9	12	79	9	9	**82**
		1992	1994	2	13	9	78	9	12	**79**
		Total	all	all	10	12	78	6	9	8 5
Smolt	PEA	1990	1993	3	27	57	18	**0**	13	87
		Total	all	all	27	57	16	**0**	13	87
	MOR	1990	1993	3	61	20	20	14	14	72
		Total	ail	all	61	20	20	14	14	72

Table 27. Summary of all kokanee tested in olfactory discrimination experiments in 1993 and 1994. Percentage homing correctly at each life stage was calculated by dividing the combined totals of the number homing correctly by the total number of trials. Also presented is the percentage homing correctly for each cohort of fish tested at different spawning ages in 1993 and 1994.

Life Stage	Total # Trials[1]	# Homing Correctly[2]	% Homing Correctly	% Homing Correctly		
				1991 Cohort at age 2 in 1993	1991 Cohort at age 3 in 1994	1992 Cohort at age 2 in 1994
Pre-eyed egg	298	52	17	17	--	--
Eyed egg	681	160	23	30	6	23
Hatch	623	401	64	69	68	63
Alevin	614	454	74	81	87	72
Swimup	538	421	78	88	92	73
Fry (all)	714	82	11	19	11	12
Smolt[3]	129	76	59	59	--	--

1 Combined total trials with odors present of fish exposed to morphoiine and phenethyl alcohol at a particular life stage in 1992 and tested at age 2 and 3 in 1993 and 1994 respectively, plus those exposed in 1993 and tested at age 3 in 1994.

2 Combined totals of morpholine exposed fish captured in the morpholine scented trap(s) and phenethyl alcohol fish homing to phenethyl alcohol scented trap(s).

3 This group was exposed in 1992 and tested at age 3 in 1993.

-- Denotes no fish tested at specified life stage for that cohort.

downstream weir for both odor present and odor absent conditions. The data are organized by cohort, year when olfactory discrimination tests were conducted, and age of the fish at the time it was tested. Combined totals for each life stage were calculated. Table 26 provides similar information but converts numbers captured into percentages. Table 27 provides data about the total percentage of morpholine and phenethyl alcohol fish combined that were captured in traps scented with their corresponding exposure odor.

Combined results from all cohorts tested in both years indicated:

1) Although the percentages of different replicated groups exposed and tested in different years were slightly different, the patterns were remarkably consistent between years. For example, when odors were absent, the majority of fish from each different group from each life stage migrated downstream (range from 77 to 100%) (Table 26). When odors were present, the majority from each different group of fish exposed as prehatch eggs were captured at the downstream weir (range from 43 to 86%) (Table 26), with few fish captured in traps scented with the synthetic chemicals (17% for pre-eyed eggs, 30% for eyed eggs) (Table 26).

2) In contrast, when odors were present, relatively few fish from each different group exposed at hatch, alevin or swimup stages were captured at the downstream weir (range 0 to 25%) (Table 26), and the majority were captured in traps scented with their treatment odor (mean rates of 64, 74, and 78% respectively) (Table 26).

3) In each year, the group of fish that had the highest percent of fish homing correctly were fish exposed at the swimup stage. In 1993, 88% of the fish from the 1991 cohort exposed at the swimup stage in 1992 and tested at age 2, homed to their exposure odor (Table 26) In 1994, 92% of the fish from the 1991 cohort exposed at the swimup stage in 1992 and tested at age 3, as well as 73% of the fish from the 1992 cohort exposed at the swimup stage in 1993 and tested at age 2, homed to their exposure odor (Table 26).

4) Kokanee from all groups exposed at various post swimup fry stages were unable to accurately locate traps scented with their treatment odor (range 9 to 16%) (Table 26), and the majority of these fish migrated downstream (range 73 to 80% when synthetic chemicals were present.

5) Smolts migrated predominantly upstream and were captured more frequently in traps scented with their exposure odor (59%) than in traps scented with the alternate chemical (23%) or at the downstream weir (18%) (Table 26).

6) The groups of fish that had the highest whole body thyroxine content (swimup stage) also had the highest percentage of fish that were attracted reliably to their exposure odor in behavioral tests. Recently hatched eggs and alevins also had relatively high thyroxine content and displayed accurate homing in behavioral tests. Additionally, smolts experienced elevated plasma thyroid levels and tended to be attracted to their exposure odor in the behavioral tests. In contrast, pre-hatch eggs and post-swimup fry had relatively low thyroxine content and did not evidence selective attraction to their exposure odor. These results indicated that kokanee salmon imprint to chemical cues during two sensitive (or critical) periods during development that were correlated with elevated thyroxine levels, at the alevin/swimup and smolt stages.

7) Kokanee (1991 cohort) exposed to synthetic chemicals in 1992 were tested as age 2 spawners in 1993 (approximately 1.5 years after chemical exposure) or as age 3 spawners in 1994 (approximately 2.5 years after chemical exposure). There was no evidence that the homing response became extinguished in older fish despite the extra year between the time they were exposed and the time they were tested. For example, for fish exposed at hatch, alevin and swimup stages in 1992, 69%, 81% and 88% respectively homed correctly as age 2 adults in 1993 to traps scented with their exposure odor (Table 27). in 1993, 68%, 87% and 92% of the fish from each respective group homed correctly as 3 year old spawners in 1994 (Table 27). These data suggested that the

response we observed is related to imprinting rather than a traditional learning process. The distinction is important because imprinting implies a process of rapid learning combined with formation of a permanent (or long-term) olfactory memory, whereas traditional learning requires repeated exposure to the stimulus and is typically coupled with a short-term memory which can be forgotten. Thus, short term memory of a home stream odor is not of much use as a cue for homing salmon, whereas a permanently imprinted memory is.

4.1.2 Coded Wire Tagging (CWT) Investigations

Recoveries of all coded wire tagged kokanee exposed to synthetic chemicals at various life stages and released into Lake Roosevelt from 1992 to 1994 to conduct a field test are summarized in Table 28. Although fish for these experiments were exposed at different life stages and the majority were released as fry, virtually all of the recoveries (256 of 260 fish) have come from fish released into the reservoir as smolts.

Results of coded wire tagging investigations indicated:

1) Relatively few fish exposed at the pre-hatch egg (n=2) or post-swimup fry (n=3) stage have been recovered at any site (Table 28);

2) For fish exposed at hatch, 50% of the total number of morpholine exposed fish recovered (n=8) have been captured in the morpholine scented stream and 50% have been recovered elsewhere; whereas 100% of the total number of phenethyl alcohol exposed fish (n=6) have been recovered in the phenethyl alcohol scented river (Table 28). For fish exposed at alevin stage 100% of the morpholine exposed fish recovered (n=5) have been captured at sites other than the morpholine scented stream and 100% of the phenethyl alcohol fish (n=10) have been recovered in the phenethyl alcohol scented river (Table 28). For fish exposed at the swimup stage, 50% of the morpholine exposed fish recovered (n=6) have been captured in the morpholine scented stream, 17% in the phenethyl alcohol scented river and 33% at other locations; whereas 7% of the total number of phenethyl alcohol exposed fish recovered (n=15) were captured

Table 28. Summary, by location captured -v- life stage/exposure odor, of total recoveries of coded wire tagged kokanee released from 1992 to 1994, indicating life stage when fish were exposed to synthetic chemicals. Data presented were the sum of recoveries made for all fish of that exposure stage and odor released at various locations.

Stage exposed	Exposure odor	Total no. recovered at Sherman Creek (MOR scented)	Spokane River (PEA scented)	Other
Eyed Egg	MOR	1		
	PEA	0	01	00
Hatch	MOR	4	0	
	PEA	0	6	40
Alevin	MOR	0	0	5
	PEA	0	10	0
Swimup	MOR	3	1	2
	PEA	1	14	0
Fry	MOR	2	0	0
	PEA	1	0	0
Smolt	MOR	45		
	PEA	22	120 10	09

in the morpholine scented stream compared to 93% in the phenethyl alcohol scented river and none at other locations;

3) For fish exposed at the smolt stage, 82% of the morpholine exposed fish recovered (n=55) have been captured in the morpholine scented stream, 18% have been captured in the phenethyl alcohol scented river and none at other locations; whereas 15% of the total number of phenethyl alcohol fish recovered (n=151) were captured in the morpholine scented stream compared to 79% in the phenethyl alcohol scented river and 6% at other locations (Table 28).

Thus, results from coded wire tagging investigations paralleled, or at least did not contradict, results observed in olfactory discrimination experiments. The data were reasonably robust for fish exposed and released as smolts but too few fish exposed at other life stages, most of which were released as fry, were recovered to assess effectiveness.

Coded wire tag data also provided the following information about growth rates of kokanee in Lake Roosevelt:

age at recovery	year recovered	total length (mm)	weight (g)	number in sample (n)
2	1992	320	347	2
	1993	350	490	66
	1994	337	418	37
	Mean	336	418	**105**
3	1993	503	1,542	2
	1994	454	1.181	**24**
	Mean	478	**1,362**	**26**
4	1994	447	1 508	**5**
	Mean	447	**1 SO8**	**5**

These observed growth rates of tagged fish generally confirmed kokanee growth rates for Lake Roosevelt reported by Peone *er al.* (1990), Griffith and Scholz (1991) and Thatcher *et al.* (1995) based on age analysis from scales. For example, Peone *et al.* (1990) noted that mean total lengths and weights of kokanee were 367 mm and 493 g at

age 2,463 mm and 1,052 g at age 3, and 525 mm and 1,576 g at age 4, in 1988. Griffith and Scholz (1991) noted that mean total lengths of kokanee were 276 mm and 301 g at age 2,380 mm and 607 g at age 3, and 447 mm and 923 g at age 4, in 1990. Thatcher *et a/.* (1995) noted that mean total lengths and weights of kokanee were 356 mm and 384 g at age 2,422 mm and 718 g at age 3, and 462 mm and 816 g at age 4, in 1992. Relatively large fluctuations in mean length and weight of fish at a particular age of recovery were observed. For example for age 2 fish, mean recovery weight varied from 347 g in 1992 to 490 g in 1993. Since these fish were released at approximately the same weight (31.2 g -v- 32.6 g) at age 1, their difference in weight gain was attributed to either different prey densities within the reservoir or different reservoir operations in each year. These results also paralleled the results based on age analysis by scales (Peone *et al.* 1990; Griffith and Scholz 1991; Thatcher *et a/.* 1995), which indicated wide fluctuations in kokanee growth which appeared to be dependent upon reservoir operating conditions that impacted the zooplankton prey base.

Additional information *was* obtained from CWT investigations that will prove useful for employing adaptive management techniques to enhance Lake Roosevelt kokanee. For example, from 1992 to 1994 a combined total of 4,811,599 kokanee were released from the Spokane Tribal Hatchery into Lake Roosevelt. Of these, 923,167, or 19.1%, were marked with coded wire tags and adipose fin clips. From 1992 to 1994, a total of 604 kokanee were observed in randomly conducted creel census surveys or electrofishing and gill net surveys on Lake Roosevelt. Of these, 158, or 26.1%, bore coded wire tags or adipose fin clips. Since the percentage of marked to unmarked fish recaptured (26.1%) was uniform with the percentage of marked to unmarked fish released (19.1%), these data suggest that the majority of kokanee in Lake Roosevelt were of hatchery origin.

Kokanee releases into Lake Roosevelt from 1992 to 1994 totaled 923,167 CWT/fin clipped fish, including 618,313 (or 67%) planted as fry and 304,854 (or 33%) planted as residualized smolts. Recoveries of tagged fish included those recovered in randomly conducted creel census and electrofishing/gill net surveys noted in the preceding paragraph, plus those recovered in monitoring egg collection sites at Sherman Creek and Little Falls Dam, which were scented with morpholine and phenethyl alcohol respectively. In 1992 and 1993, 299 total CWT fish were captured, including four that had been released as fry and 295 that had been release as

residualized smolts. In 1994, 132 CWT fish were captured. All had been released as residualized smolts. Thus, a total of 431 CWT/fin clipped fish were recaptured from 1992 to 1994, including 4 fish (<1%) that had been released as fry and 427 (>99%) that had been released as smolts. The ratio of fry to smolts was about 2:1 released and 1:108 recovered. These data provided conclusive evidence that fish planted as residualized smolts yield significantly better returns to both the creel and egg collection sites. in fact, fish planted as fry are essentially not contributing to either category. Thus, results from the present investigation confirm that releasing fish as residualized smolts could be an effective measure for enhancing Lake Roosevelt kokanee.

There are three potential explanations for the poor rate of recovery of adult kokanee which were released as fry. The first is mortality caused by walleye predation at the time of stocking. The second is loss of kokanee from the reservoir. The third is related to the amount of effort expended in trying to collect tagged fish.

Walleye predation is known to occur when kokanee fry are released as evidenced by observations of CWT kokanee fry in stomachs of walleye collected at release sites (reviewed by Tilson *et a/.* 1994). Additionally, walleye collected from the reservoir were occasionally reported containing salmonids, presumably kokanee in their stomachs (Peone *et al.* 1990; Griffith and Scholz 1991; Thatcher *et al.* 1995). However, we do not believe that predation is the principle factor accounting for the disappearance of kokanee between the fry and adult stage because stomach contents were analyzed from 1,173 walleye randomly collected in Lake Roosevelt as part of the Lake Roosevelt Monitoring Program from 1988-1992 and only 38 had confirmed salmonids in their gut. Kokanee and walleye clearly occupy different niches within the reservoir, which would tend to reduce predator-prey interactions between these species (Peone *et a/.* 1990, Griffith and Scholz 1991, Thatcher *et al.* 1995). Thus, it appeared that, although walleye may occasionally consume kokanee opportunistically at release sites, they are generally not an important predator on kokanee.

The second reason why we believe walleye predation is not the principle factor accounting for the loss of kokanee is that there is evidence of substantial kokanee entrainment through Grand Coulee Dam. For example, in 1991 a loss of approximately 25,000 subadult kokanee was noted (Thatcher et *a/.* 1995). We believe that such losses are owing partly to fish released as fry undergoing smoltification the following year and emigrating from the reservoir during periods of drawdown and water releases that provide the water budget for anadromous salmonids. The present study confirmed

that Lake Roosevelt kokanee (Lake Whatcom stock) undergo at least partial smoltification as discussed in Section 4.3 below. Releasing fish as residualized smolts should reduce problems with kokanee entrainment. In the event that walleye predation is a greater problem than we presently believe, releasing larger sized post-smolts would also likely reduce predation rates and improve survival.

A related problem is that sampling effort to collect CWT kokanee needs to be intensified. We believe that low effort is partially responsible for the low number of tag returns. At present, funding levels for the Lake Roosevelt Monitoring Program are sufficient to permit only three 10 day electrofishing/gill net sampling periods per year. At a minimum, monthly samples should be collected reservoir wide, and weekly (preferably daily) sampling should be accomplished at egg collection sites. Additionally, although creel surveys are conducted weekly, current funding levels permit only a random survey of anglers. Thus, we are unable to target kokanee at times when substantial numbers are being caught by anglers. Additional creel surveys should be conducted that target kokanee for the purpose of collecting more CWT information.

4.1.3 Conclusions from Imprinting Experiments

The present study confirmed the hypothesis that thyroid hormone peaks are correlated with the critical period for imprinting in kokanee, and identified two critical periods for imprinting at the alevin/swimup and smolt developmental stage. Results of the present investigation suggested that it should be possible to imprint fish to a synthetic chemical at the Spokane Tribal Hatchery at the alevin/swimup stage or smolt stage, and release them at Sherman Creek as residualized smolts and then decoy the adult fish to the Sherman Creek Hatchery for egg collection by scenting the hatchery ladder with the chemical.

4.2 Smoltification Investigations

Because of the significant loss of juvenile kokanee through Grand Coulee Dam in 1991 (Thatcher *et al.* 1995), it was hypothesized that kokanee were undergoing a parr smolt transformation and emigrating downstream out of Lake Roosevelt. Results from the present investigation indicated that kokanee underwent a partial smolt phase. These kokanee experienced thyroid and cortisol peaks, an increase in silvering, increased downstream migratory behavior and an increase in salt water adaptation (including increased Na+-K+ ATPase activity, increased osmoregulatory capability and

increase in salt water tolerance) during the spring of the year, similar to transitions noted for anadromous salmonids. Figure 15 compares the results of the present smoltification investigations (1992 year class yearling kokanee) with last year's investigations (1991 year class yearling kokanee).

4.2.1 T_4 Concentrations

An increase in thyroxine is an important aspect of the smolt transformation. It is not only correlated to the process of olfactory imprinting (Hasler and Scholz 1983), but it is also thought to stimulate certain developmental and behavioral changes in the fish including an increase in silvering and an increase in downstream migratory behavior.

Results of the present investigation (1993-94 season) indicated a significant peak in thyroxine concentration in May 1994 (14.0 ± 1.8 ng/ml) (Figure 15). This spring peak was consistent with the previous year's results. During the 1992-93 season, there was a peak in April of 12.2 ± 2.6 ng/ml (Tilson et *al.* 1994). However, in the present investigation, fish did not experience a T_4 peak in the winter as was seen in both 1992 and 1993 (Scholz et a/. 1993; Tilson et *al.* 1994). We believe that our results were accurate both years and represent true fluctuations in T_4 concentration, because the results of quality assurance procedures indicated that the assay was reliable during both years.

4.2.2 Cortisol

Elevation of plasma cortisol was reported to coincide with smoltification in coho salmon and Atlantic salmon (Specker 1982; Specker and Schreck 1982; Vittanen and Soivio 1985; Young 1986; Langhorne and Simpson 1986). Corticosteroids are thought to prepare the fish for seawater entry by affecting their osmoregulatory capacity (Specker 1982). Hasler and Scholz (1983) found that an increase in cortisol in coho salmon was accompanied by an increase in gill ATPase activity and subsequently an increase in osmoregulatory capability and salt water survival.

In this investigation, plasma cortisol levels were 39.5 ± 19.4 ng/ml in November. The mean concentration then returned to a low level (approximately 20 ng/ml) until March, when the mean concentration increased to 54.4 ng/ml. Plasma cortisol peaked at 82.9 ng/ml in April. This cortisol peak in the spring was similar to peaks observed with coho and Atlantic salmon smolts (Scholz 1980; Specker 1982; Hasler and Scholz 1983; Virtanen and Soivio 1985). Table 29 shows approximate basal and peak cortisol

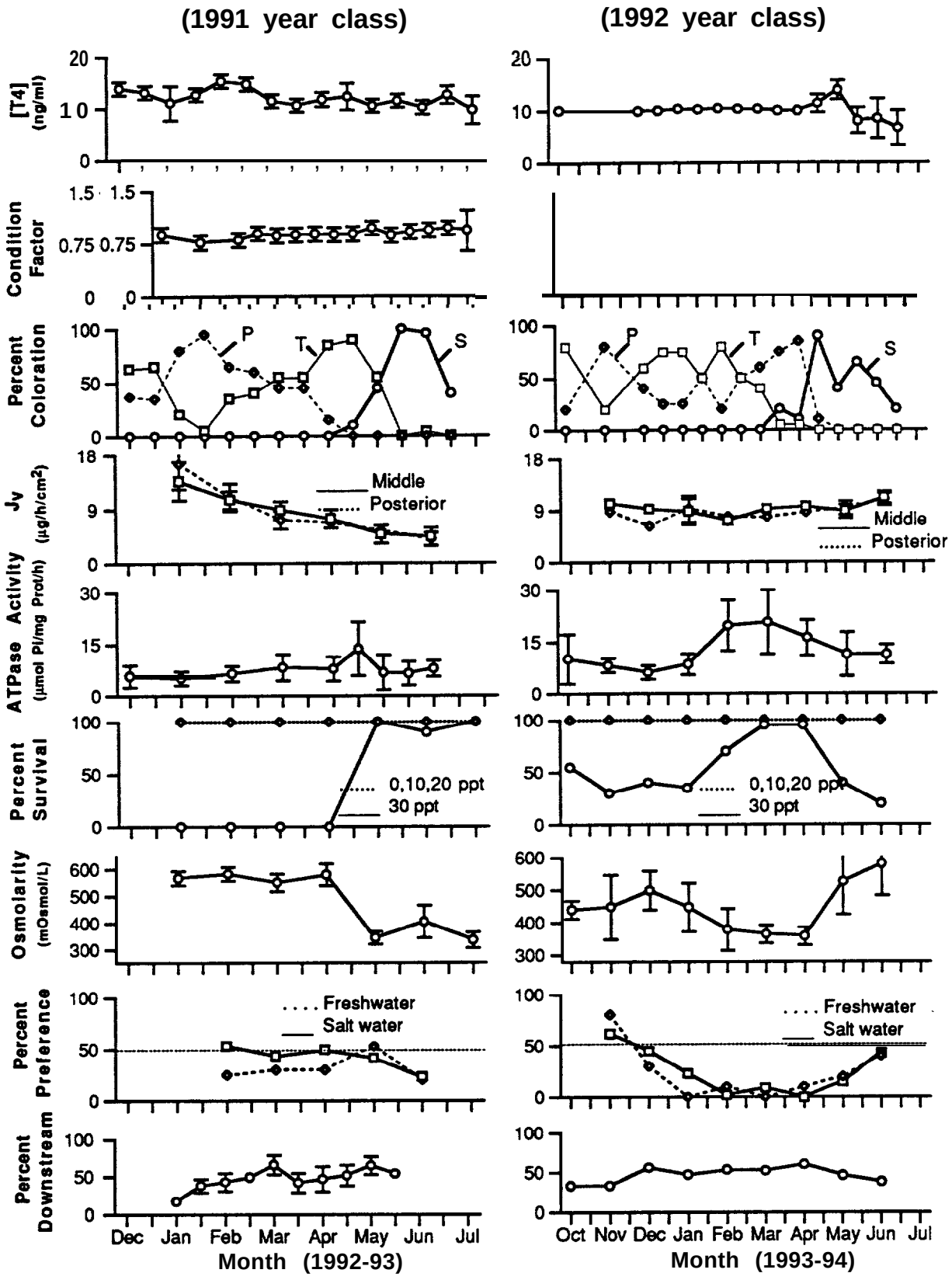


Figure 15. indices of smoltification measured for 1991 and 1992 year class age 1 kokanee salmon.

Flgure 15 cont. Indices of smoltification measured for 1991 year class kokanee sampled from December 1992 through July 1993, and 1992 year class kokanee sampled from October 1993 through June 1994. Measurements included mean plasma T_4 concentration (± SD; n=20); condition factor (± SD); percentage of fish grouped as parr (P), transition (T), and smolts (S); intestinal fluid transport (Jv) (± SEM; n=12); gill Na^+-K^+ ATPase activity (± SD; n=20); percent survival (n=20) after 96 h in seawater; mean plasma osmolarity (± SD; n=20) from kokanee held In 30 ppt saltwater after 96 h; salt water preference (% preference) of fish in control tank (0 ppt) and concentrated salt water (24 ppt); percentage of fish in downstream third of tank.

Table 29. Comparison of cortisol concentrations in yearling salmonids. Values are approximations, taken off graphs of previous investigations.

Species	Basal cortisol (ng/ml)	Peak cortisol (ng/ml)	Ratio	Source
Atlantic	25	75	3:1	Olsen et al. (1993)
Atlantic	21	120	6:1	Cornell *et al.* (1994)
Atlantic	5	85	17:1	Cornell et *al.* (1994)
Atlantic	30	200	7:1	Virtanen and Soivio 1985
Coho	9	24	3:1	Specker and Schreck (1982)
Coho	2	15	7:1	Young (1986)
Coho	6	40	6.5:1	Hasler and Scholz (1983)
Kokanee	**18**	82	**5:1**	**Present investigation**

concentrations from a number of investigations, and the ratio of the peak to basal level. Typical ratios ranged from 3:1 to 17:1. In the present investigation, the ratio was 5:1 which was comparable to that found by other investigators.

4.2.3 Silvering

The silvery coloration characteristic of smolting salmonids is due to the deposition of two purines, guanine and hypoxanthine. In parr, these purines are present in small amounts, but they become quite thick as they are laid down beneath the scales in smolts, resulting in a silvery fish.

In the present investigation, fish started turning silver in February (Figure 15). Silvering continued to increase through April when 90% of the fish sampled were of silvery coloration. In May, fish started reverting back to parr coloration signifying residualization. These results were consistent with the results from last year when silvering began to increase in February and continued to increase through May (Tilson *et al.* 1994).

4.2.4 Condition factor

Condition factor was used as an indicator of whether kokanee undergo a smolt transformation. This factor is a measure of how a fish grows in weight in relation to length. In some cases, the condition factor decreases during the process of smoltification because the fish becomes more slender and streamlined (Hoar 1939; Fesler and Wagner 1969; Saunders and Henderson 1978). In the present investigation, the condition factor remained uniform throughout the sampling period, even when the fish were turning silver in March and April (Figure 15). In the previous year's investigation (Tilson et a/. 1994), condition factor decreased slightly in the spring which was characteristic of smolting anadromous salmon. Condition factor can be affected by variables other than smoltification. For example, fish health and food ration level may affect condition by either decreasing or increasing it. However, T. Peone (Spokane Tribal Hatchery, personal communication) informed us that fish were fed at the same rate each year. Therefore, it could be that because condition factors were generally low, changes were not seen during smoltification.

4.2.5 Downstream Migratory Behavior

Downstream migratory behavior increased from 33% to 56% from November to December 1993, and peaked again in April 1994 (60%) (Figure 15). These results were similar to those obtained during last years investigation. During both years, an increase was seen in the fall and in the spring. However, the migratory disposition was not as high as that seen with coho salmon smolts (Hasler and Scholz 1983). Hasler and Scholz (1983) conducted downstream migratory experiments with coho salmon using test procedures that were similar to those used for this study. In these experiments, they placed fish into Living Streams at night and determined the number of fish which were in the downstream end of the tank. They found that downstream migratory behavior increased from approximately 33% in January through March to 95% in April. It could be that some kokanee experience partial smoltification while others do not experience any. This may explain why only a portion of the fish seem to emigrate through Grand Coulee Dam.

Previous investigators have suggested that increased thyroid activity may be involved in the induction of downstream migratory behavior (Godin *et al.* 1974; Hasler and Scholz 1983), and that this increase in thyroid activity may be due to increased water flow rates or the introduction of novel water chemistry associated with higher flows (Specker and Schreck 1984; Youngson and Simpson 1984; Grau *et al.* 1985; Nishioka *et a/.* 1985; Youngson *et al.* 1985; Hoffnagle and Fivazzani 1990). In our investigation, the highest percentage of kokanee that evidenced downstream behavior was 60% in April. This corresponded to the increase in thyroxine in April and May. This increased thyroxine may have been high enough to stimulate downstream migratory behavior in some of the fish.

4.2.6 Salt Water Preference

In control experiments, when fish had a choice between their original fresh water compartment and the alternate freshwater trough, they remained in the original trough. Only during the months of November, December and June, did the fish seem to spend more equal time in both control troughs. These results were similar to the results obtained in the previous year (Tilson *et a/.* 1994). Ideally, these control fish, which had a choice between fresh water and fresh water, should have spent equal time in both troughs (showing 50% preference). This behavior was a concern because it did not

represent a good control for the experiment and made the results of experiments where fish had a choice between fresh and salt water difficult to interpret.

In the salt water experiments, kokanee showed a slight preference (63%) for salt water (24 ppt salinity) in November, which declined to below 50% from December to May, then rose to near 50% in June. Salinity preference was similar to the results obtained last year (Tilson *et al.* 1994), showing little or no positive response to salt water. This lack of preference was very different from sockeye and other salmonid smolts which have evidenced a strong salt water preference (Baggerman 1960; Houston 1957; Mclnerney 1963, 1964).

4.2.7 Salt Water Adaptation

Four types of tests were performed to assess salt water adaptation in yearling kokanee salmon: intestinal water transport (J_v), gill Na^+-K^+ ATPase activity, salt water tolerance and osmoregulatory capability.

4.2.7.1 Intestinal Water Transport

Preadaptive increases in J_v during smoltification have been observed in Atlantic salmon and coho salmon (Collie and Bern 1982; Veillette *et a/.* 1993). In the present investigation, water transport in the posterior intestine did not evidence a significant increase during the spring, ranging from 8 to 11 $\mu l/h/cm^2$ (Figure 15). During last year's investigation with kokanee, which used a different buffer, the posterior intestine exhibited a decrease from approximately 11 to 4 $\mu l/h/cm^2$ during the spring (Tilson *et a/.* 1994). In contrast, Veillette *et* al. (1993) showed a springtime preadaptive increase in Atlantic salmon of approximately 13 to 25 $\mu l/h/cm^2$. Thus, kokanee did not appear to possess the preadaptive increases in J_v observed in anadromous salmonids.

The middle intestine, in the present investigation, decreased during the winter months, but then increased from approximately 7 to 11 $\mu l/h/cm^2$ from February to June (Figure 15). During last year's investigation, which used a different buffer system, J_v decreased from 10 to 4 $\mu l/h/cm^2$ in the spring. The decrease in Jv shown for the middle intestine during last year's investigation and for the first half of the present study were consistent with the only other studies where middle intestine has been studied in salmon (Veillette *et al.* 1993).

During the first year of our study, measurements of intestinal Jv were done using a 20 mM/liter HCO_3^- Ringer solution taken from Field *et al.* (1978) at pH's of 8.0-8.2 (Kerstetter and White 1994) who worked with salt water fish. However, we did not observe a preadaptive increase in J_v as reported for anadromous species. Dixon and Loretz (1986) noted that HCO_3^- secretion (rather than Na^+ Cl- fluxes) accounted for part of the I_{sc} (short circuit current needed to balance the membrane potential) across the lumen of the goby intestine. They also found that the HCO_3^- secretion involved an active transport in the basolateral membrane with an exchange with Cl^-. These facts suggested that both pH and the amount of HCO_3^- are important factors in establishment of maximum rates of sodium chloride and water transport (Jv). Hence, in the second year of our investigation with kokanee, we decided to switch to a Ringer solution which had a lower bicarbonate and pH, and was identical to that used by Collie and Bern (1982) and Veillette et *al.* (1993) who showed pronounced preadaptive increases in J_v of coho and Atlantic salmon smolts held in freshwater. Again, we found no evidence of a significant increase in J_v.

The absence of a preadaptive increase in posterior intestinal J_v in kokanee indicated a lack of physiological specialization by the gut to function as an osmoregulatory organ in salt water. We are confident that this preadaptive specialization is lacking because we have checked for it with two different Ringer solutions, one of which was the same incubation medium, pH and similar temperature that was used in the coho and Atlantic salmon studies. Further, we are concerned that current efforts underway to crossbreed endangered Snake River anadromous sockeye salmon with Redfish Lake kokanee salmon may be doomed to failure if the Redfish Lake kokanee stock also lack this specialization.

4.2.7.2 Gill ATPase Activity

Numerous investigators have shown that gill Na^+-K^+ ATPase levels increase in salmon smolts (Zaugg and McLain 1972; Ewing *et al.* 1979; Zaugg 1982; Buckman and' Ewing 1982; Hasler and Scholz 1983; Ewing *et a/.* 1984; Boeuff *et a/.* 1985; Birt *et al* 1991; Franklin *et al.* 1992). Typically, in these studies, smolts showed an increase of two to five times the initial level observed in parr. In our investigation, the basal level observed in January was 8.5 ± 3 µmol P_i/mg Protein/h compared to the peak level in March of 20.6 ± 9 µmol P_i/mg Protein/h, exhibiting an increase of 2.4 times the initial level. These results were similar to the previous year's investigation in which an increase 2 times the initial ATPase activity was seen in April (Tilson et *a/.* 1994).

Therefore, it appeared that the "sodium pump" (Na+-K+ ATPase pump) in the gills of kokanee was fully functional and allowed them to survive for 96 h in the salt water challenge tests.

4.2.7.3 Osmoregulatory Capability

In previous osmoregulatory experiments with Atlantic, sockeye, or coho salmon, smolts which were placed in full strength seawater were able to osmoregulate their blood serum concentration while parr were not (Houston 1960, 1964; Parry 1960; Potts 1970; Boeuf et *al.* 1978; Hasler and Scholz 1983). In our investigation, kokanee salmon were able to regulate plasma ion concentrations in early spring. From October 1993 to January 1994, blood plasma osmolarity was significantly elevated in fish held in full strength seawater (about 450-500 mOsmol/L) compared to fish held in fresh water (about 300 mOsmol/L). In February, the osmolarity of fish held in full strength seawater dropped significantly to 378 mOsmol/L and stayed low (about 360 mOsmol/L) through April indicating the ability to osmoregulate during these months. In May and June fish did not continue the ability to osmoregulate signifying reversion back to the parr stage. Plasma osmolarity during these months rose to 526 and 622 mOsmol/L respectively in fish held in full strength seawater. In contrast, blood plasma osmotic concentration of fish held in fresh water (controls) was similar from October 1993 to June 1 994 at about 309 mOsmol/L.

4.2.7.4 Salt Water Tolerance

To determine if kokanee were able to adapt successfully to salt water, we performed salt water tolerance tests. After direct transfer of fish from fresh water to 10, 20, or 30 ppt salt water, percent survival was determined after 96 h. In 10 and 20 ppt seawater, 100% of the fish survived for 96 h. In full strength seawater (30 ppt), 55% of fish transferred in October survived. From November through January, survival dropped to only 30-40%. Survival rose from 70% in February to 95% in April and then dropped to 20% in June, signifying residualization of kokanee smolts. These results were similar to results from last year, when fish held in 10 and 20 ppt seawater survived in all months, and fish held in 30 ppt seawater died from January through April, but exhibited 100% survival from May through July, showing the ability to osmoregulate during those months.

In summary, three of the four tests performed to determine the extent of seawater adaptation (determination of gill ATPase, salt water tolerance and osmoregulatory ability) indicated that kokanee were able to osmoregulate about as well as other anadromous salmonid species. Survival in salt water challenge tests corresponded well to mean monthly gill ATPase activities, with peak survival and ATPase activities occurring concurrently during the months of February, March and April. However, the intestinal water transport experiments (J_v) did not increase like anadromous species of salmon, signifying that kokanee do not experience all of the physiological changes that occur in anadromous salmon. These results were similar to those from experiments performed last year (Tilson *et al.* 1994).

4.2.8 Residualization of Smolt Transitions

If kokanee experience residualization in a manner similar to anadromous salmon, entrainment losses through Grand Coulee Dam could be minimized, especially if kokanee are released as residualized smolts, instead of presmolt fry or fingerlings. The present study confirmed the results of last year's investigation (Tilson et *al.* 1994) indicating that this stock of kokanee began to residualize by June. This was indicated in both studies by:

(1) Thyroxine peaked in the spring during both years (in April 1992-93 and in May 1993-94) and then returned to basal levels by June.

(2) Silvering increased in February and peaked in April 1992-93 and in May 1993-94. Parr marks reappeared in June during both years.

(3) Downstream migratory behavior peaked in the spring at 65% in May 1993 and 60% in April 1994). By June, less than 40% of the fish evidenced downstream migratory activity during both years.

(4) Gill ATPase activity peaked in early spring during both years (in April 1993 at 13.7 µmol P_i/mg Protein/h and 20.6 µmol P_i/mg Prot/h in March 1994). ATPase activity decreased to basal levels by June during both years.

(5) Osmoregulatory activity and salt water tolerance increased in early spring during both years. By May in the present investigation, fish

did not continue the ability to osmoregulate or survive in seawater. Last year, fish still displayed the ability to osmoregulate in June.

Therefore, kokanee appeared to residualize or revert back to parr by June. Behavioral attributes (downstream migratory behavior and salt water preference) were not as pronounced as in anadromous salmon. Although downstream migratory tendencies appeared to rise in the spring, this behavior decreased back to presmolt conditions by June. This study strengthens the theory that if kokanee were released from the hatchery in late June or July as residualized smolts, they would be more likely to remain in the reservoir. Therefore, we recommend that fish be retained at the hatchery or in net pens until they are residualized smolts and released after mid June, and preferably in July.

4.3 Management Recommendations for Outplanting and Imprinting

From information gathered in this report and from the investigations conducted last year (Tilson *et al.* 1994), it was concluded that kokanee salmon exhibited smoltification similar to that of other salmonid species, but that the degree of smoltification was not as pronounced. This was indicated by:

1) Kokanee experienced a thyroxine surge, cortisol surge, increase in silvering, increase in gill Na^+-K^+ ATPase and ability to osmoregulate in full strength seawater similar to anadromous salmonids.

2) Kokanee exhibited increased downstream orientation and migratory activity in spring, however it was not as pronounced as is typically seen in anadromous salmon.

3) Kokanee did not show a springtime preadaptive increase in either salt water preference or intestinal J_v. Although intestinal water transport in sockeye salmon (0. *nerka*), which is the anadromous species closely related to kokanee, has not been tested yet, it is assumed that sockeye would show a preadaptive increase in intestinal water transport similar to that seen with other species of salmon. Typically, when salmonids smolt, they gain water, and become more neutrally buoyant which enables them to raise up in the water column and be more easily displaced downstream with

the current. Kokanee salmon do not appear to gain water and, therefore, are possibly not as subject to downstream displacement as anadromous smolts.

However, kokanee in Upper Columbia lakes and reservoirs are known to emigrate under certain conditions. For example, Thatcher *et al.* (1995) noted that kokanee entrainment losses at Grand Coulee Dam totaled approximately half the fish they could account for in Lake Roosevelt in 1991. They estimated entrainment at 25,221 fish based on a count of 721 subadult Lake Roosevelt kokanee passing through the fish counting facility Rock Island Dam in 1991. The estimate was made by correcting for Rock Island counting efficiency (5%) to yield the total number passing Rock Island (14,420). It was then assumed that the fish would suffer an average mortality rate of 15% (NPPC 1987) as they passed over each dam between Grand Coulee and Rock Island (5 dams total including Grand Coulee and Rock Island), so the number passing over Grand Coulee that would be required to produce the 14,420 kokanee estimated at Rock Island was 25,221. This was a minimum estimate of the loss because it did not include kokanee lost over Grand Coulee that residualized in Rufus Woods Reservoir instead of continuing their migration to Rock Island Dam. Thus, the minimum estimated loss over Grand Coulee of 25,221 kokanee was similar to the total annual 1991 harvest of 31,651 kokanee estimated in 1991 (Thatcher *et a/.* 1995), and actual entrainment could have exceeded the harvest rate.

The pattern of kokanee recoveries at Rock Island in 1991 was also negatively correlated with harvest in Grand Coulee. In the reservoir, harvest rates were relatively high in winter and spring (January to May), but dropped to 0 in June and July. About 700 of 721 total Lake Roosevelt kokanee observed at Rock Island were counted after May 20th. The majority (674 fish) were counted between May 20th and June 30th (Chuck Peven, Chelan County PUD, pers. comm.). Creel records indicated that kokanee were concentrated in the forebay of Grand Coulee Dam between February and May 1991, which is where the majority of the harvest occurred. Thus, the fish were positioned at a location where they could be easily entrained as reservoir elevations dropped and water retention times decreased. In May and June 1991, water retention times ranged from about 20-30 days. From 1988 to 1990, water retention times were generally greater than 30 days during the May to June time period and fewer kokanee were recovered at Rock Island Dam (Thatcher et *al.* 1995). Thus, reservoir operations clearly impacted the magnitude of kokanee entrainment from Lake Roosevelt. The fact

that this stock of kokanee exhibited downstream migration tendencies at a rate of only 60-65%, as determined in the present investigation, could account for why some fish were entrained and others remained in the reservoir.

Other investigators have determined that kokanee emigration, concomitant with periods of high discharge, is a frequent occurrence in Upper Columbia lakes and reservoirs. For example, Ashe and Scholz (1992) noted higher numbers of Pend Oreille Lake kokanee present in the Box Canyon Reach of the Pend Oreille River, which is located below the lake outlet at Albeni Falls Dam, in years of moderate water flow than in years of low water flow. In each year of their investigations, they noted that spring discharge was different. The 38 year mean discharge was about 60,000 CFS in the spring. In their study, mean peak spring discharges were about 25,000 CFS in 1988, 43,000 CFS in 1989 and 65,000 CFS in 1990. This correlated positively with the number of smolt-sized kokanee collected in electrofishing surveys in each year: in 1988 no kokanee, in 1989 twelve kokanee and in 1990 thirty-two kokanee. CPUE was 0 kokanee/minute in 1988, 0.002 kokanee/minute in 1989 and 0.007 kokanee/minute in 1990. Most of these fish were captured in electrofishing transects made perpendicular to the shoreline, across the river, where conditions for electrofishing were poor because water was deep. Thus, even though relatively few fish were captured overall, Ashe and Scholz (1992) believed substantial numbers of kokanee had to be moving through the Box Canyon Reach to catch this many. This indicated that in normal and high water years substantial kokanee entrainment occurs from Pend Oreille Lake.

Skaar (1994) also reported that operations at Libby Dam affected kokanee entrainment in Lake Koocanusa. Peak periods of entrainment were noted from November 1992 to January 1993 and again in June 1993. A similar pattern was observed in the winter of 1994 and May-June 1994. Both peak periods coincided with water releases from Libby Dam, the fall/winter peak for hydropower production, the spring peak associated with water releases to augment flows for endangered Kootenai River white sturgeon and anadromous salmon. Skaar (1994) estimated that a substantial portion of the kokanee population in Lake Koocanusa could be lost at such times. For example, he estimated 297,000 kokanee were entrained on just four nights, the only nights he monitored, in the November to January period in 1992/1993. At that time the estimated kokanee population in Lake Koocanusa was 4.1 million fish, so in just 4 days of operation about 5% of the population was lost. Skaar also noted that Lake Koocanusa experienced wide fluctuations in kokanee abundance from year to year

and speculated that this phenomenon may be related to entrainment losses. We believe that similar losses occur in Lake Roosevelt except that they are more routine and more severe since water retention times are much shorter in Lake Roosevelt than in Lake Koocanusa. This was discussed by Tilson et *al.* (1994) and is summarized below.

Lake Roosevelt has a relatively high flushing rate compared to other systems. The average water retention time is approximately 40 days (range typically 15-66 days) (Griffith and Scholz 1991; Thatcher et *a/.* 1995). Water retention time is the number of days required for a particle of water to travel from the head of the reservoir to Grand Coulee Dam (approximately 140 miles or 232 km). In the spring, when flows are increased for anadromous salmon passage, water retention time is frequently less than 20 days. At the times of these drawdowns, the reservoir behaves more like a run-of-the-river reservoir than a storage reservoir. In contrast, water retention times in other kokanee producing reservoirs and lakes in the Upper Columbia River System (e.g., Libby Reservoir, Kootenay Lake, Pend Oreille Lake, Coeur d'Alene Lake) are generally in excess of 100 days and frequently greater than 360 days. Although some kokanee entrainment has been reported for each of these systems, they do not appear to be as prone to emigrate as Lake Roosevelt kokanee. Thus, we suspect that flow related entrainment may be a more serious problem in Lake Roosevelt than in other lakes and reservoirs of the Upper Columbia Basin. Kokanee from the Spokane Tribal Hatchery are normally released into the reservoir as zero age fry in July of their first year. If kokanee undergo partial smoltification during the following spring, the higher flows (low water retention times) could be sufficient to stimulate downstream displacement causing entrainment through Grand Coulee Dam, especially in that portion (60-65%) of the population that already has a tendency toward downstream migratory activity. It is possible that different genetic stocks of kokanee exhibit different downstream migratory tendencies as smolts. If so, it might be possible to find a stock more compatible with conditions in Lake Roosevelt.

Based upon the results of our investigations, we make the following recommendations for managing Lake Roosevelt kokanee:

(1) Discontinue fry releases and, instead, release the fish as residualized smolts. This will require holding over a portion of the kokanee at the Spokane Tribal Hatchery. For this to be accomplished, a new production well capable of delivering 2-4 CFS of additional flow would need to be drilled at the hatchery (see

Tilson et *a/.* 1994 for further discussion). This would allow approximately 500,000 fish to be raised to residualized smolt stage instead of the current holdover capacity of 100,000 to 300,000 fish. [Note: At present, the hatchery can carryover about 300,000 fish but only until late March or April, which is the height of smoltification. Currently, the hatchery can retain only about 130,000 to 150,000 fish for release as residualized smolts in late June or July.]

(2) Additionally, put 500,000 age 0 fry in net pens from October until the following June or July when the smolts have residualized as was done with the rainbow trout in Lake Roosevelt. Rainbow trout have been known to experience a certain degree of smoltification in March and April (Peone *et al.* 1990; Griffith and Scholz 1991). Past Lake Roosevelt Monitoring Program floy tag investigations demonstrated that by delaying the release of rainbow from net pens until late May to July, when fish were classified as residualized smolts, recovery rates in Lake Roosevelt were higher (>93%) than with fish classified as smolts and released in March and April (<67%). We believe that releasing kokanee after they have residualized could produce benefits similar to those reported for rainbow trout. They would be less likely to emigrate out of the reservoir if they were residualized.

(3) Fish should not be released from the hatchery or net pens until they exhibit signs of residualization, including reappearance of parr marks and reduced downstream migratory activity. In general, the earliest release dates should not occur before mid June. Late June and July releases are recommended as this would also coincide with an increased zooplankton (prey base) abundance within Lake Roosevelt, increasing water retention time, and reservoir refill.

(4) The above recommendations would allow for the release of approximately 1 million residualized kokanee into Lake Roosevelt annually. Assuming that release of residualized kokanee from the hatchery or net pens is comparable to the release of residualized rainbow trout, in terms of both survival until they are recruited into

the fishery, and recovery in Lake Roosevelt, we estimate that approximately 300,000 adult fish would be available for harvest and return to egg collection sites.

(5) Expose all of the fish to a synthetic chemical from hatch to swimup at the Spokane Tribal Hatchery. Re-expose the fish carried over at the hatchery or in net pens to the same imprinting chemical a second time at the smolt stage. Release fish into Sherman Creek Hatchery or other egg collection sites as residualized smolts. At the time of release, scent the water with the chemical again. Scent the hatchery ladder with the chemical during the spawning migration to attract fish. [In 1994, for the first time, we exposed the entire Spokane Tribal Hatchery production of 1,627,381 fry to either morpholine (67%) or phenethyl alcohol (33%) from hatch through swimup life stages. Of these, 310,000 were retained at the Spokane Tribal Hatchery for release as residualized smolts in 1995 and the remainder were stocked into Lake Roosevelt tributaries as fry in 1994. In April/May 1995, the carryover fish will be re-exposed to the same chemical as originally used during the hatch through swimup stage. All 310,000 fish to be released as residualized smolts and 413,745 fish released as fry were coded wire tagged. These fish will return as age 2 to 4 adults from 1995 to 1997.1 Similar imprinting combined with CWT investigations are also planned in 1995.

(6) When stocking fish into tributary streams, stock the fish several kilometers (km), preferably >10 km, upstream from the mouth. Salmonids are known to experience thyroxine surges when exposed to novel waters and may sequentially imprint to cues that help them to identify adopted home streams (reviewed by Scholz et *al.* 1992), so releasing fish further upstream could help to intensify the imprinting experience.

(7) We encourage the fishery managers, as a high priority, to locate alternative stocks of kokanee, with better genetic adaptations than Lake Whatcom fish (e.g., reduced emigration potential) for the Lake Roosevelt Program. This should be one aim of the kokanee stock

assessment in Lake Roosevelt tributaries currently being conducted by the Colville Confederated Tribes. If different stocks are located we further recommend that a smoltification/residualization assessment be conducted to determine which stock(s) may be best suited for Lake Roosevelt. Additional CWT investigations should also be conducted with matched lots of these fish to determine which stocks exhibit lowest entrainment losses, highest harvest rates in Lake Roosevelt, and best return to egg collection sites.

(8) Intensify efforts to recapture more CWT fish during the next four years both by creel survey and fisheries (electrofishing/gill net/trawl) surveys. In particular, sampling frequency for fisheries surveys should be once per month throughout the year, with continuous monitoring of egg collection sites from September 1 to October 30. Additionally, monitor Rufus Woods Reservoir, and coordinate with Rock Island Dam counting facility and McNary Dam counting facility, to determine the number of kokanee entrained through Grand Coulee Dam. This will protect a substantial ratepayer investment in coded wire tags and marking, which has already been or is currently being accomplished, by enhancing the number of CWT fish recovered. This will necessarily require a substantial increase in the Lake Roosevelt Monitoring Program budget.

(9) As part of Lake Roosevelt Monitoring Program, further assess potential impacts of walleye predation at kokanee release sites. This should be accomplished via releasing match paired groups of fry and residualized kokanee and assessing the relative rates of consumption of each life stage by walleye.

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