

# Investigating Passage of ESA-listed Juvenile Fall Chinook Salmon at Lower Granite Dam during Winter when the Fish Bypass System is Not Operated

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*Annual Report 2003*

**INVESTIGATING PASSAGE OF ESA-LISTED JUVENILE FALL CHINOOK SALMON  
AT LOWER GRANITE DAM DURING WINTER WHEN THE FISH BYPASS  
SYSTEM IS NOT OPERATED**

April 2004



**Investigating Passage of ESA-listed Juvenile Fall Chinook Salmon at Lower Granite Dam  
during Winter when the Fish Bypass System Is Not Operated**

**ANNUAL REPORT 2003**

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## **Executive Summary**

In August 2002, personnel of the U.S. Fish and Wildlife Service, U.S. Geological Survey, and Washington Department of Fish and Wildlife Service received funding for a new project to study reservoir-overwintering by juvenile fall Chinook salmon and the importance of this life history to adult returns. The first objective of this study was to refine non-lethal methods for identifying the genetic lineage (i.e., fall or spring run) of reservoir-overwintered wild juvenile Chinook salmon smolts for application to existing tissue samples collected from fish at Lower Granite and Little Goose in 1998. This objective was completed in 2003 and the results are reported in Chapters Three and Four of this report. The second objective was to refine non-lethal methods for identifying the age at saltwater entry (i.e., over-wintering history) for unmarked Snake River fall Chinook salmon adults collected at Lower Granite from 1999 to 2005, and then assess the importance of reservoir-overwintering by juveniles to adult returns to the Snake River. The first step in this analysis was completed in 2003 and the results are reported in Chapter Two of this report. The third objective was to determine if reservoir-overwintered fall Chinook salmon smolts pass Lower Granite Dam during winter when the fish bypass systems are not operated. Progress towards this objective is described in Chapter One of this report. The report is divided into sections and self-standing chapters. For detailed summaries, we refer the reader to the abstracts given on the second page of each chapter. The Annual Reporting section includes information collected during the first season of field work. The Journal Manuscripts section includes complete copies of papers submitted or published during 2003. Publication is a high priority of our staff. Publication provides our results to a wide audience, and it insures that our work meets high scientific standards.

## **Acknowledgments**

We thank our colleagues at the Bonneville Power Administration, Fish Passage Center, Idaho Fish and Game, Idaho Power Company, NOAA Fisheries, Nez Perce Tribe, Oregon Department of Fish and Wildlife, Pacific States Marine Fisheries Commission, U. S. Army Corps of Engineers, U. S. Fish and Wildlife Service, U. S. Geological Survey, University of Idaho, and Washington Department of Fish and Wildlife.

# Annual Reporting

## **CHAPTER ONE**

Passage of juvenile fall Chinook salmon at Lower Granite Dam during winter, 2002-2003

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## **Abstract**

Juvenile fall Chinook salmon typically outmigrate from Lower Granite Reservoir as subyearlings in the summer. Preliminary results suggest that many of these fish overwinter in the reservoir and outmigrate during late winter or early spring of the following year when the juvenile bypass system at Lower Granite Dam is not operated. At that time, turbine passage is the only route for fish to pass the dam. During the winter of 2002-2003, we used acoustic telemetry to evaluate the movements and downstream passage of fall Chinook salmon. Fish were captured in the forebay of Lower Granite Dam and implanted with acoustic transmitters (N=40). Fish were primarily of hatchery origin based on scale pattern analysis, and were robust and in good condition. Downstream movement of fish released in the forebay was documented using a stationary array of hydrophones and an acoustic receiver located in the forebay in front of the dam. Of the 12 tagged fish we detected with mobile and stationary hydrophones, 4 fish moved downstream to Lower Granite Dam, 2 fish moved upstream at least 2 km, and 6 fish did not exhibit any significant movement in either direction. We did not document any fish passing Lower Granite Dam during the winter. Quality control tests indicated that acoustic system and tag performance was poor, compromising our ability to effectively monitor fall Chinook salmon movements and passage. We intend to use radio telemetry in 2004 to improve our ability to document fish movement and passage past Lower Granite Dam.

## Introduction

Juvenile Snake River fall Chinook salmon *Oncorhynchus tshawytscha* primarily exhibit an ocean-type life history (Healey 1991). Fry emerge in the spring, grow rapidly, and pass Lower Granite Dam on the Snake River (Figure 1) during their summer outmigration (Connor et al. 2002, 2003; Connor and Burge 2003). However, some of the later emerging and slower growing juvenile fall Chinook salmon do not complete their seaward migration as subyearlings, but over-winter in Lower Granite Reservoir after which they resume seaward migration the following spring as yearlings. This is prevalent for young fall Chinook salmon from the lower Clearwater River, which produces the some of the latest emerging fry of present-day production areas (Connor et al. 2002; in press).

In recent years, we have compiled limited data on juvenile fall Chinook salmon that over-winter in Snake River reservoirs (Sneva and Connor in review; Connor et al. in review; Chapters Three and Four in this report). These data cannot be used to conclusively determine when these fall Chinook salmon juveniles pass Lower Granite Dam (Figure 1), but they likely pass the dam during the winter when fish bypass facilities are not operated. Sneva and Connor (in review) confirmed a reservoir over-wintering history for 38 (34 hatchery, 4 wild) fall Chinook salmon adults collected at Lower Granite Dam that had been tagged with Passive Integrated Transponder tags (PIT tags; Prentice et al. 1990a) as juveniles. Of these, 68% (24 hatchery and 2 wild) were never detected (Prentice et al. 1990b) passing a dam as juveniles. This suggests that juveniles that pass the dams in the winter via the powerhouse, and if so, may experience increased mortality from turbine blade strike.

Although the number of juvenile fall Chinook salmon that survive in Lower Granite Reservoir over the summer is probably small, those that do survive and successfully over-winter likely have high smolt-to-adult return rates because of their large size during outmigration. For example, in 1999 scale samples were collected from 111 wild Snake River fall Chinook salmon adults passing Lower Granite Dam to upstream spawning areas. We determined age at ocean entry and found that a total of 34, or 30.6%, of these adults had over-wintered in freshwater and entered saltwater as yearlings (J. Sneva, Washington Department of Fish and Wildlife, unpublished data).

The major goal of this study is to determine movement patterns and passage rates of juvenile fall Chinook salmon that spend all or part of the winter in Lower Granite Reservoir. Preliminary data suggests that these fish may be located at depths greater than 10 m in the water column. Detecting fish at depths greater than this in front of the powerhouse was an important consideration for this study. Therefore, we believed an acoustic system would be a valuable tool to detect over-wintering fish at Lower Granite Dam. Acoustic systems have already been used at Lower Granite Dam to determine 3-dimensional positions and approach paths of juvenile spring Chinook salmon and steelhead (Cash et al. 2001).

Due to the lack of information on over-wintering juvenile fall Chinook salmon, we sought to acquire baseline data as to the run timing and composition of these fish. These data should prove useful to managers and lead to a better understanding of juvenile salmon life history in the Snake River. In this chapter, we describe our first year efforts to determine if

juvenile fall Chinook salmon pass Lower Granite Dam during the winter when the fish bypass system is not operated. Our objectives for work in 2002-2003 were to: 1) determine the suitability of an acoustic system to detect fish at Lower Granite Dam; 2) use a fixed hydrophone array at Lower Granite Dam to determine if and when passage occurs at Lower Granite Dam during the winter; and 3) describe movements of holdover juvenile fall Chinook salmon in Lower Granite Reservoir.

## Methods

### *Study area*

Lower Granite Dam is located 173 km upstream of the Snake and Columbia river confluence. It is the fourth upstream dam on the lower Snake River and the first dam encountered by outmigrating juvenile salmonids originating from the Snake and Clearwater rivers. Lower Granite Dam impounds the Snake River to form Lower Granite Reservoir and is located 51 km below the Snake and Clearwater river confluence (Figure 1).

### *Acoustic Telemetry System*

Unlike more traditional radio telemetry, acoustic telemetry uses sound waves to convey information about research subjects. Acoustic applications are particularly useful to aquatic researchers working in deep (>10 m) or highly conductive (turbid, saline) waters. Additionally, acoustic receivers can listen for multiple frequencies simultaneously, and acoustic tags do not have an antenna extending from the body cavity of test fish. For our work in 2002-2003, we used a Lotek Map\_600 (Lotek Wireless Inc., Newmarket, Ontario) acoustic system in Lower Granite Reservoir. The two types of acoustic receivers used in our study were a Map\_600 RT and a Map\_600 Multiport. All hydrophones used in our study were model TC 4014 (Reson Inc.). The Map\_600 Multiport had eight hydrophones and cables and was used in a stationary configuration to detect presence and passage of fish at Lower Granite Dam. The Map\_600 RT was used as a boat-mounted mobile detection system to determine fish presence and movement patterns of tagged fish before they reached the Lower Granite Dam forebay. Preliminary testing indicated that the detection range of tags would be at least 400 m.

The Map\_600 Multiport system was configured with 8 hydrophones, each connected to 305 m of hydrophone cable. This system was located on the powerhouse at Lower Granite Dam. We deployed four hydrophones in the forebay and four hydrophones in the tailrace of the dam to document presence in the forebay and passage of tagged fish into the tailrace. Because the juvenile fish bypass system was shut down during our study and there was no spill, the primary route of passage through the dam was via the powerhouse turbines. All cables were connected to a centrally located Map\_600 receiver (Figure 2). In the forebay, a hydrophone was placed at the southern end of turbine unit 1, at the southeast corner of the Surface Bypass Collector (SBC) in front of turbine unit 3, at the north end of the Removable Spillway Weir (RSW) in front of spillbay 1, and on a pier nose between spillbays 6 and 7. Tailrace hydrophones were placed at the downstream end of the training wall, in the middle of the training wall on its south side,

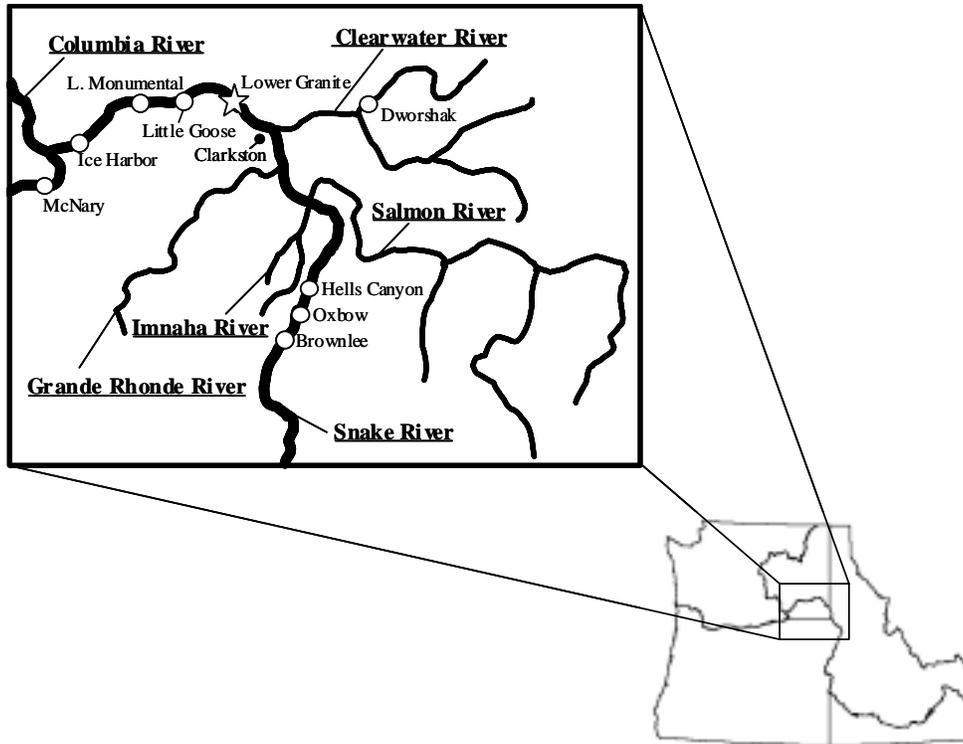


Figure 1. Map of major tributaries and hydroelectric projects in the Lower Snake River Basin.

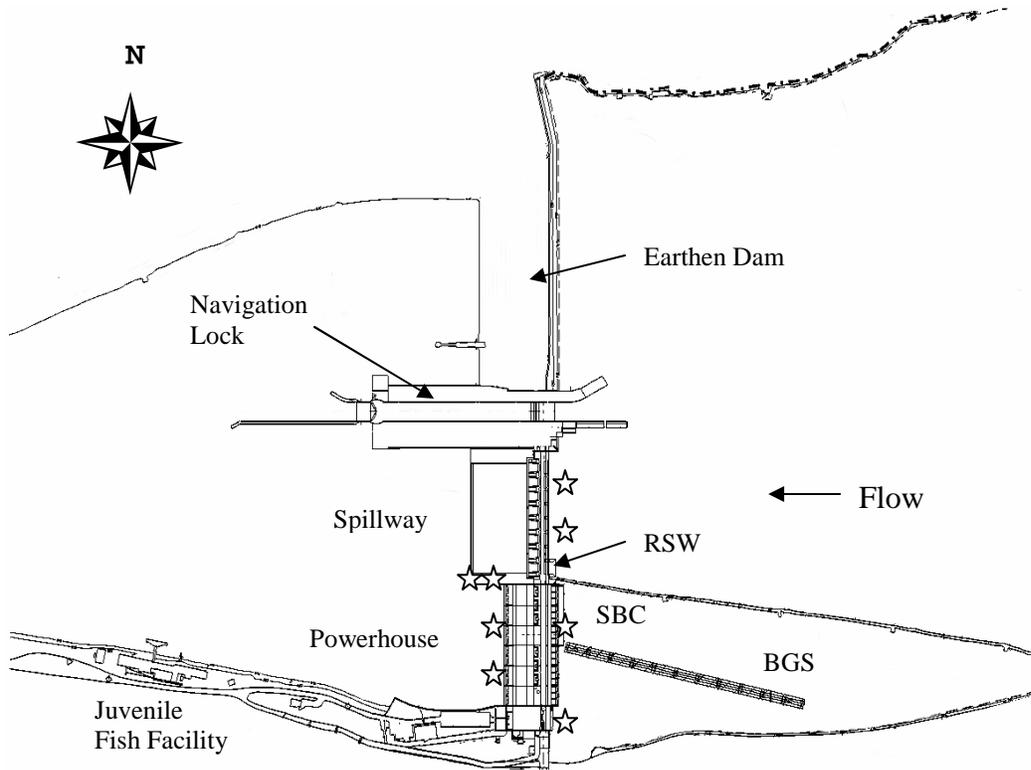


Figure 2. Plan view of Lower Granite Dam with hydrophone locations (stars) during 2002-2003.

between the outflows of turbine units 4 and 5, and between the outflows of turbine units 2 and 3. Hydrophone mounts were anchored to the dam with steel 'I-beam' and rock bolts.

The Map\_600 RT system was configured with two hydrophones and 10 m cables and used as a boat-mounted mobile telemetry system. Single hydrophones were mounted on the port and starboard sides of a 7-m inboard jet boat. Hydrophones used in mobile tracking were equipped with plastic baffles covering half of the hydrophone tip. This was done so that directionality could be determined from a received acoustic signal in relation to the unbaffled side of the hydrophone. This setup allowed us to determine the location of tagged fish by moving in the direction of the stronger of two acoustic signals received from either the port or starboard side of the boat. All mobile telemetry was conducted downstream of Wawawai boat ramp (rkm 178.6, 5.6 km upstream of Lower Granite Dam). The search pattern for locating test fish was to traverse the north shore from Wawawai boat ramp down to the Lower Granite Dam forebay and then return up the south shore while trying to maintain a distance of 100-150 m from shore. After locating an individual fish, unique tag symbol, GPS position, water depth, and estimated distance to shore were recorded. Fish locations were also plotted on a map of the lower reservoir. Repeat locations of test fish were recorded 3 h after the initial detection for each day.

### *Fish Capture and Tagging*

Staff used hook and line to collect juvenile Chinook salmon in Lower Granite Reservoir in 2002-2003. Three rods were each equipped with leaded line and silver "cowbell" flashers. The flasher was weighted with 71 g of lead and tipped with a size 8 "wedding ring" spinner baited with either maggots or corn. Terminal gear was let out approximately 27 m and then trolled at slow to moderate speeds while searching for fish using a fish finder. The ability to capture holdover juvenile fall Chinook salmon was tested in January (hereafter, pilot sampling). Staff fished on four separate occasions from January through mid March, 2003 (hereafter, sampling periods 1, 2, 3, and 4). Fishing was conducted from approximately 0930 to 1430 hours. All fish were captured 2.4 km upriver from Lower Granite Dam. Fish less than 170 mm were deemed too small to be over-wintering fall Chinook salmon and were released. A GPS was used to record each fish capture location.

Captured fish were anesthetized in water containing approximately 66 mg/L MS-222, measured to the nearest millimeter fork length, weighed (g), and scanned for the presence of a PIT tag. Up to 15 scales were collected from below the posterior edge of the dorsal fin and above the lateral line, and field mounted on gummed cards. The left ventral fin was removed and placed in a 1.5-ml centrifuge tube containing 180  $\mu$ L of lysis buffer (Rasmussen et al. 2003, Chapter Two in this report). Twenty  $\mu$ L of protease K stock solution was dispensed into the centrifuge tube. The tube was capped and the contents were mixed. The solution was stored at ambient temperatures for approximately 72 h.

We processed scales and fin tissue to obtain age and genetic information. We made acetate impressions of the scales with a heated press (Clutter and Whitesel 1956) and viewed the impressions on a microfiche reader at 48X magnification. We examined each scale for a hatchery release check (always indicative of hatchery origin), and then measured radial distance

from the focus to the scale's edge to the nearest 0.05 mm (Sneva and Connor in review). Juvenile Chinook salmon that over-wintered in a stream or a reservoir are 100% distinguishable based on this measurement. Fish that over-winter in streams have scales with a radial distance from the focus to the scales edge (hereafter, total radial distance) less than 0.65 mm, whereas this distance is 0.75 mm or more on scales fish that over-winter in reservoirs (Sneva and Connor in review). After approximately 72 h, the fin tissues were incubated 16 to 24 h at 56°C. The genetic lineage (i.e., spring-summer run or fall run) of fish will be identified from the fin tissue using the dual-primer product of a nuclear DNA marker (93% accurate; Rasmussen et al. 2003, Chapter Two in this report).

After capture and processing, fish were transferred to a net pen (1.8 m long, 1 m deep, and 0.6 m wide) stationed offshore in the forebay of Lower Granite Dam for a holding period of 3-5 d. No mortality or injury was observed. All acoustic tags used in our study were surgically implanted into fish using procedures described by Adams et al. (1998) and held for a 24-h in-river recovery period prior to release. We used Lotek acoustic transmitters that were 20 mm in length, 8 mm in diameter, weighed 1.8 g in air (1.1 g in water), and had a tag life of 15 d. All tags were individually assigned a symbol by the manufacturer and operated on a frequency of 200 kHz. All fish were released in the forebay of Lower Granite Dam with half released near the north shore and half released near the south shore upstream of Offfield Landing (rkm 175.4, 2.4 km upstream of Lower Granite Dam).

### *Quality control*

Because this was our first use of a Lotek 200 kHz acoustic system for data collection, we implemented extensive quality control tests to verify the accuracy and validity of the data we collected. We tested the functionality of tags by activating individual tags and testing them in air and water to insure that tags were switching on and remaining on when activated, and also to insure proper coding of tag symbols. The receiver interprets symbols of individual tags when a particular sequence of three numbers (code) is detected. Each tag has a unique sequence of codes.

We also conducted range tests to insure that the manufacturer's detection range of the system (400 m) was being achieved in our study area. This was important because we wanted to insure detection of all test fish entering the Lower Granite Dam forebay and passing the dam into the tailrace. We tested the detection range of Map\_600 hydrophones located in the forebay by measuring the detection signal strength of test tags deployed from a boat anchored at increasing distances (10, 25, 50, and 100 m) from individual hydrophones. We tested the range of tailrace hydrophones by releasing a floating buoy with test tags attached and measuring the distance from hydrophone to floating buoy and measuring tag signal strength. A floating buoy system was used in the tailrace because boat access was restricted due to turbulent conditions. Finally, we tested the mobile Map\_600 RT system by attaching test tags to a floating buoy and incrementally increasing the distance (10, 25, 50, and 100 m) between the buoy and the boat and recording the corresponding signal strength. We tested the Map\_600 RT system both at Lower Granite Dam and at the confluence of the Snake and Clearwater rivers area to eliminate the possibility of ultrasonic interference from the dam environment on the acoustic system. All distances from hydrophone to test tags were determined using a laser range finder. Test tags were taped to the

end of the nylon cable ties, which were attached to heavy monofilament fishing line. A 9 kg lead weight was affixed 6.1 m (20 ft) below the deepest test tag and was used to keep the test tags at the desired depth for at least 5 min. Tag depth, tag orientation, and signal strength of detection were recorded. After five minutes at a particular depth, the test tag was lowered to a greater depth (1, 2, 3, 4, 5, 10, and 20 m) and the process was repeated. We used vertical and horizontal tag orientations during testing.

### *Data analysis*

We used our catch data from fish collection to assess population trends and relative condition of juvenile fall Chinook salmon in Lower Granite Reservoir during winter. We calculated catch per unit effort (CPUE) using the hours fished per rod during collection of test fish. We also calculated condition factors (K) for each sample period. The condition factor (K) was defined as  $K = WX/L^3$ , where W=weight, X is a scaling unit, and L= length. These numbers were used to assess the relative change in abundance and relative condition of fish captured in our study. Detections from our fixed array were used to calculate travel time, travel rate, and passage through Lower Granite Dam. Multiple mobile telemetry detections were used to evaluate upstream and downstream fish movement in our study area.

## **Results**

### *Fish Capture and Tagging*

Catch per unit effort of holdover fall Chinook salmon was highest in January and February, but declined in March. During pilot sampling, fish were observed at a depth of 9-10 m where total water depths were approximately 30 m, and they were located offshore in an area approximately 5-10 km upstream of Lower Granite Dam. A total of 37 fish were captured at a rate 0.8 per rod hour (Table 1). During sampling periods 1-4, 4-20 fish were captured at rates ranging from 0.4 to 1.0 fish per rod hour. The fish were observed at depths of 9-10 m and they were pelagically oriented. All fish were large, robust, and had condition factors exceeding 1.0 throughout our study period.

Table 1. Information on juvenile Chinook salmon captured by hook and line sampling in Lower Granite Reservoir during winter, 2002-2003. The minimum and maximum water temperature recorded at the catch location, number of fish captured (N), number of fish captured per rod hour (CPUE), mean fork length (FL $\pm$  SD), mean weight (Wt  $\pm$  SD), and condition factor (K  $\pm$  SD).

| Sample Period | Date             | Temperature |     | N  | CPUE | FL (mm)      | Wt (g)           | K                 |
|---------------|------------------|-------------|-----|----|------|--------------|------------------|-------------------|
|               |                  | Min         | Max |    |      |              |                  |                   |
| Pilot         | 13-Jan to 15-Jan | 5.0         | 5.7 | 37 | 0.8  | 196 $\pm$ 10 | 76.9 $\pm$ 11.5  | 1.022 $\pm$ 0.049 |
| 1             | 22-Jan to 23-Jan | 4.1         | 4.4 | 20 | 0.7  | 196 $\pm$ 10 | 82.1 $\pm$ 12.3  | 1.083 $\pm$ 0.043 |
| 2             | 19-Feb           | 5.1         | 5.1 | 15 | 1.0  | 203 $\pm$ 11 | 91.3 $\pm$ 13.8  | 1.108 $\pm$ 0.226 |
| 3             | 04-Mar           | 5.7         | 5.7 | 4  | 0.4  | 192 $\pm$ 13 | 69.2 $\pm$ 16.3  | 1.014 $\pm$ 0.402 |
| 4             | 12-Mar           | 6.2         | 6.2 | 8  | 0.5  | 212 $\pm$ 8  | 107.0 $\pm$ 32.3 | 1.098 $\pm$ 0.059 |

Of the 47 fish captured during sampling periods 1-4, a total of 46 had scales that could be read. Two of the 47 were hatchery fish that had scale patterns indicating that they had over-wintered in a stream (total radial distances of 0.60 and 0.70 mm). One of these fish was 188 mm long when captured on 19 February 2003. The second was 181 mm when captured on 4 March 2003. Of the remaining 44 fish with scale patterns indicating that they had spent the winter in the reservoir, 7 (15.9%) were determined to be wild and 37 (84.1%) were determined to be of hatchery origin (Table 2). Total radial distance was similar on scales of the wild and hatchery fish (Table 2). One of the hatchery fish was PIT tagged prior to being transported from Lyons Ferry Hatchery for release at rkm 265 in the Snake River on 11 June 2002. This fish was 77 mm long when released and it had grown to 213 mm by the time of its capture in Lower Granite Reservoir on 12 March 2003.

Table 2. Results of scale pattern analysis on 44 juvenile Chinook salmon that over-wintered in the reservoir including total radial distance (mm  $\pm$  SD) and the minimum total radial distance (mm).

| Origin   | N  | Percentage of sample | Total radial distance (mm) | Minimum total radial distance (mm) |
|----------|----|----------------------|----------------------------|------------------------------------|
| Wild     | 7  | 15.9                 | 0.93 $\pm$ 0.11            | 0.8                                |
| Hatchery | 37 | 84.1                 | 0.91 $\pm$ 0.09            | 0.8                                |

We released a total of 40 tagged fish over four release periods. Release periods were January 23-24, February 20, March 5, and March 13, 2003 (Table 3). Of the 50 acoustic tags received from Lotek, 10 (20%) failed. Six of these tags were initially working but then switched off after being implanted in fish. From the first batch of 24 tags, 7 (29%) did not work and were returned to Lotek. From the second batch of 36 tags, 3 (8.3%) did not work.

### *Range Testing*

Data collection using the Map\_600 Multi Port System began at Lower Granite Dam on 23 January 2003. The acoustic system was calibrated following the procedures provided by the manufacturer, first with no tag in the water and then with a tag in the water at close range (1 m). Initial testing in the forebay area near the dam indicated that test tags were being detected by all hydrophones but that ranges generally did not exceed 50 m. The maximum range to any of the hydrophones was 105 m, which is short of the 400 m anticipated. Most tags were not detected beyond a range of 50 m as the tags were moved progressively farther away from the array. The vertical and horizontal tag orientations we tried did not consistently influence detection range. We tried various gain settings in our testing to improve detectability, but we generally did not use gains greater than 70, as recommended by the manufacturer.

Table 3. Release date, release time, release location, fork length (mm), and weight (g) for acoustic tagged juvenile fall Chinook salmon released in Lower Granite Reservoir, 2003.

| Release Date | Release Time | Release Location | FL (mm) | Weight (g) |
|--------------|--------------|------------------|---------|------------|
| 1/23/03      | 10:12        | North            | 193     | 81.7       |
| 1/23/03      | 10:12        | North            | 197     | 83.1       |
| 1/23/03      | 10:12        | North            | 204     | 97.2       |
| 1/23/03      | 10:12        | North            | 186     | 69.8       |
| 1/23/03      | 10:32        | North            | 199     | 84.8       |
| 1/23/03      | 10:37        | North            | 193     | 78.1       |
| 1/23/03      | 08:09        | North            | 208     | 92.7       |
| 1/23/03      | -            | South            | 192     | 74.5       |
| 1/23/03      | -            | South            | 195     | 79.3       |
| 1/23/03      | 11:11        | South            | 187     | 70.0       |
| 1/23/03      | 11:11        | South            | 197     | 81.3       |
| 1/23/03      | 11:11        | South            | 170     | 51.4       |
| 1/24/03      | 10:31        | -                | 216     | 105.2      |
| 1/24/03      | 10:31        | -                | 189     | 75.3       |
| 1/24/03      | 10:31        | -                | 195     | 79.7       |
| 1/24/03      | 10:31        | -                | 206     | 90.7       |
| 2/20/03      | 09:27        | North            | 212     | 103.0      |
| 2/20/03      | 09:27        | North            | 198     | 89.9       |
| 2/20/03      | 09:27        | North            | 204     | 85.3       |
| 2/20/03      | 09:27        | North            | 195     | 82.7       |
| 2/20/03      | 09:27        | North            | 202     | 84.3       |
| 2/20/03      | 10:08        | South            | 201     | 94.7       |
| 2/20/03      | 09:27        | North            | 216     | 108.6      |
| 2/20/03      | 10:08        | South            | 213     | 107.2      |
| 2/20/03      | 10:08        | South            | 188     | 74.7       |
| 2/20/03      | 10:25        | South            | 188     | 73.9       |
| 2/20/03      | 10:25        | South            | 195     | 79.8       |
| 2/20/03      | 10:25        | South            | 217     | 110.1      |
| 3/5/03       | 08:27        | North            | 204     | 89.5       |
| 3/5/03       | 08:27        | North            | 181     | 61.5       |
| 3/5/03       | 08:27        | North            | 203     | 93.6       |
| 3/5/03       | 08:27        | North            | 181     | 60.9       |
| 3/13/03      | 08:54        | North            | 215     | 114.6      |
| 3/13/03      | 08:54        | North            | 216     | 116.8      |
| 3/13/03      | 08:54        | North            | 203     | 83.5       |
| 3/13/03      | 08:54        | North            | 188     | 68.7       |
| 3/13/03      | 09:04        | South            | 213     | 105.6      |
| 3/13/03      | 09:04        | South            | 196     | 81.5       |
| 3/13/03      | 09:04        | South            | 216     | 111.7      |
| 3/13/03      | 09:04        | South            | 247     | 173.8      |
| Mean         |              |                  | 200.5   | 88.8       |

We conducted range testing using the Map\_600 RT mobile system on 13 February, 19 February, and 13 March 2003 just below the confluence of the Snake and Clearwater rivers. The maximum detection ranges achieved on these test dates were 30 m, 75 m, and 135 m, respectively. These detection ranges were achieved with high channel gain settings (75-85), and detection ranges were smaller at lower gain settings.

We tested the detection range of tailrace hydrophones on 13 March 2003. Detection of tags in the Lower Granite Dam tailrace was poor in areas of high turbulence with entrained air bubbles from powerhouse discharge. Tags were detected by all hydrophones in calm water especially as they drifted into the spillway area, however, tags were not detected in the turbulent flow upwelling from the turbines. In calm water, the maximum detection range was about 80 m.

#### *Fixed site detections*

Our acoustic array at Lower Granite Dam did not document the passage of any tagged fish into the Lower Granite Dam tailrace. We detected only four (10%) tagged fish with our stationary array. Three of the four tagged fish were from the first release period and one fish was from the second release period. The fish we detected in the forebay of Lower Granite Dam were not detected in the tailrace and spent an average of 14 h 28 min (range 5 min to 36 h 34 min) in our forebay hydrophone array before disappearing from the detection record. Mean residence time of fish from release to first detection in the forebay was 97 h, 8 min (range 30 h 18 min to 196 h 1 min). With a distance of 0.75 miles from release site to Lower Granite Dam, we documented travel rates of 0.15, 0.44, and 0.96 km/d for the three fish from which we could calculate travel rates.

#### *Mobile tracking detections*

We conducted mobile tracking of tagged fish in Lower Granite Reservoir on 14 days from 21 February 2003 through 18 March 2003. Mobile tracking indicated that tagged fish either did not move appreciably from their release location or moved upstream. Two fish exhibited upstream movement in excess of 2 km, but we did not document any downstream movement during mobile tracking. During this tracking effort, we recorded 20 locations of 8 tagged fish. We detected 4 fish from the second release period, 2 fish from the third release period and 2 fish from the fourth release period. We conducted no mobile tracking during the first release period. One fish was repeatedly detected throughout our mobile tracking period from 21 February 2003 through 18 March 2003.

### **Discussion**

The success of this study was partially contingent on capturing juvenile Chinook salmon to evaluate movement and dam passage. This was a critical uncertainty because hook and line sampling was the only viable means of collecting fish since the fish bypass system was not operated during the winter. In spite of the relatively small number of fish that likely over-winter in Lower Granite Reservoir, we successfully captured fish by hook and line sampling during the winter of 2002-2003. No mortality was observed. The large size and high condition factor of

the juveniles is consistent with previous observations of over-wintered juvenile Chinook salmon (Connor et al. in review). This shows that fish that manage to survive harsh summer conditions over-winter in good condition. Capture locations and depth suggest that the fish were pelagically oriented. All fish were captured in the forebay of Lower Granite Dam, but we did not sample other locations in the reservoir.

Scale pattern analysis showed that collected fish were predominantly of hatchery origin. These fish were likely Lyons Ferry Hatchery subyearlings released from acclimation facilities along the Snake and Clearwater rivers to supplement wild production or as part of ongoing research conducted with hatchery fish. Total radial distance measured on the scales of both wild and hatchery fish were similar to those reported by Sneva and Connor (in review). Relatively small total radial distances indicated that two of the fish had over-wintered in streams. Given the large size of these two fish, they were likely Lyons Ferry Hatchery fall Chinook salmon that spent the summer, fall, and early winter in the lower Clearwater River. The results of genetic analyses will be reported when available to confirm this hypothesis.

A key component of this study was to identify if and when over-wintering juvenile fall Chinook salmon pass Lower Granite Dam. We detected only a small number tagged fish (4) in our forebay acoustic array and did not document fish passage past the dam. Given our small detection ranges, it is probable that fish do pass Lower Granite Dam during the winter via the turbines. At the time of this writing, we have documented winter passage of juvenile fall Chinook salmon at Lower Granite Dam using radio telemetry. Our 2003 data indicate that juvenile Chinook salmon that delay their summer migration and survive into the fall move downstream at least to the Lower Granite Dam forebay during the winter months. This movement may be related to large runoff events that occur periodically during the winter as was observed in February 2003 when flows increased from 20 kcfs to 72 kcfs.

Our mobile acoustic telemetry results indicated that most of the fish detected in our study moved upstream or did not move appreciably from their release location. Two fish traveled upstream at least 2 km, and 6 fish did not exhibit any substantial movement. Because flows during the winter are at annual lows, fish may lack the velocity cues to continue downstream movement. This may be particularly true in the forebay where water velocities are relatively low compared to the entire reservoir. Others have noted altered migratory behavior in forebay environments as seen in migratory delay and the propensity to move back upstream in subyearling Chinook salmon (Venditti et al. 2000; Tiffan et al. 2003) and juvenile steelhead (Plumb et al. 2003). In addition, fish may have been less inclined to move at the 3.9-5.5°C water temperatures that prevailed during our study. Fish are typically lethargic during the winter as metabolic processes are slow at cold temperatures.

The Lotek Map\_600 acoustic systems performed poorly during field quality control testing and deployments to document fish passage at Lower Granite Dam. The small detection ranges we measured limited our ability to collect sufficient data to document passage and fish movements. We achieved a maximum range of about 105 m with the Map\_600 acoustic systems and 200 kHz tags, but more typical ranges were 30-50 m. At times, ranges inexplicably declined to less than 30 m. In a few cases, we could not detect tags at a distance of 10 m. There were no range differences between the RT and Multiport acoustic receivers. Tag orientation seemed to

affect detectability, however, no consistent patterns were observed. Although no problems were found in subsequent tests of both systems by the manufacturer, it was discovered that the hydrophones contained the wrong signal amplifiers and amplifier power consumption likely affected hydrophone performance. Tests by the manufacturer are ongoing to resolve these issues.

In addition to poor detection ranges, we experienced an overall tag failure rate of 20%. Many of these tags failed after surgical implantation. The latter scenario was troublesome because initial tests indicated the tags were working properly. Due to observed tag failure following surgical implantation into test fish, it is likely that additional tag failures occurred following release of fish in the reservoir. This may have further reduced our ability to monitor fish movement and detect fish that approached our acoustic array in the forebay of Lower Granite Dam.

The concept of using the Map\_600 acoustic system as a detection tool at Lower Granite Dam originated as an effort to detect fish traveling deeper in the water column. However, the Lotek Map\_600 acoustic system did not perform as expected in 2002-2003 due to high tag failure rates and low detection ranges. The likelihood of detecting tagged fish in the tailrace was further hampered by air bubbles and turbulence from powerhouse discharge. For field work during the winter of 2003-2004, we intend to use radio telemetry to evaluate passage and movement of reservoir over-wintered Chinook salmon through Lower Granite Reservoir and Dam. Given the large size of juvenile fall Chinook salmon over-wintering in the reservoir, we will be able to use tags with a 70-d life span, which will increase the probability of detection and passage. The USGS has successfully used radio telemetry at Lower Granite Dam for many years to monitor fish movement and passage and detection efficiencies in the tailrace often exceed 90%. The USGS also maintains a radio telemetry infrastructure at the dam that will allow a greater analysis of fish movement behavior in the forebay. At the time of this writing, we have used radio telemetry to successfully document passage of over-wintering juvenile fall Chinook salmon past Lower Granite Dam during the winter of 2003-2004.

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## **CHAPTER TWO**

Identifying First Year Over-wintering History of Fall Chinook Salmon  
by Use of Scale Pattern Analysis

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*Abstract.*—Juvenile Snake River fall Chinook salmon typically spend their first winter in saltwater. Dams in the Snake River basin have, however, caused some juvenile fall Chinook to overwinter in reservoirs. In this paper, we identify reservoir-overwintered fall Chinook salmon juveniles and adults by use of scale pattern analysis. We hypothesize that differences in scale patterns reflect time spent in specific rearing environments during the first year of life. We found that the radial distance from the focus to the scale edge was significantly ( $P < 0.0001$ ) larger on scales from reservoir-overwintered fall Chinook salmon juveniles ( $N = 45$ ; mean,  $1.02 \pm 0.11$  mm SD) than on scales from stream-overwintered spring-summer Chinook salmon juveniles ( $N = 20$ ; mean,  $0.53 \pm 0.07$  mm SD). This difference was the result of reservoir-overwintered fish spending more time in habitat with high growth opportunity. Of the fall Chinook salmon adults that spent their first winter in a reservoir ( $N = 45$ ), 59% had scales with an annulus formed at the time of ocean entry. A total of 41% had scales with a freshwater annulus that resulted in a narrow spring growth zone typical of a stream-type life history. These visual characteristics contrasted with the wide first year ocean zones on scales of fall Chinook salmon adults that spent their first winter in saltwater ( $N = 98$ ). To quantitatively identify the first year over-wintering history of adults, we fit a logistic regression model from measurements of radial distance to ocean entry. The probability of the scale being from an adult that spent its first winter in a reservoir increased as radial distance to ocean entry increased. Classification accuracy was 100% when predicting first year over-wintering history for adults using an out-of-sample validation data set ( $N = 22$ ). The large radial distances to ocean entry for adults that spent their first winter in a reservoir was caused by growth that occurred in freshwater prior to ocean entry. We conclude that differences in scale patterns reflect time spent in specific rearing environments and growth during the first year of life.

## Introduction

Scale pattern analysis can be used to identify salmonid stocks (Cook and Lord 1978; Patton et al. 1998), life history type (i.e., stream-type or ocean-type [Healey 1991]; Koo and Isarankura 1967; Unwin and Lucas 1993), and origin (i.e., hatchery or wild; Schwartzberg and Fryer 1993; Stokesbury et al. 2001). The high utility of scale pattern analysis results from a dependable response of scale growth to water temperature and food availability. Fine ridges called circuli form a circular pattern on the anterior portion of the scale and intercirculus spacing increases as growth rate increases within optimum ranges of food availability and water temperature (Bilton and Robbins 1971a, b; Bigelow and White 1996). A disruption in growth at any time can cause decreases in intercirculus spacing resulting in an accessory mark or check (Jearld 1983; Bigelow and White 1996). Seasonally decreasing water temperatures and the concurrent decrease in growth results in a group of closely spaced circuli called an annulus (Jearld 1983). Increases in water temperature during the spring or exposure to productive ocean conditions causes a marked increase in intercirculus spacing (Koo and Isarankura 1967; Clutter and Whitesel 1955).

Our study was initiated to use scale pattern analysis to describe the first year overwintering history of the Snake River stock of fall Chinook salmon *Oncorhynchus tshawytscha* listed for protection under the Endangered Species Act (NMFS 1992). Snake River fall Chinook typically have an ocean-type life history. Adult fall Chinook salmon spawn in the mainstem Snake River and lower reaches of its tributaries including the lower Clearwater River primarily in November (Groves and Chandler 1999). Fry emerge in spring (Connor et al. 2002, in press), grow rapidly (Connor and Burge 2003), emigrate from the spawning areas before the end of summer, and then enter Lower Granite Reservoir (Figure 1) during early seaward migration as subyearling juveniles (hereafter, subyearlings; Connor et al. 2003a). Subyearlings pass Lower Granite Dam (Figure 1) primarily during the summer (Connor et al. 2002). Roughly 98% of the unmarked subyearlings from the Snake River never reach the Columbia River. Mortality is high and of those fish that survive to pass Lower Granite, Little Goose, and Lower Monumental dams, 50% are collected at each dam and trucked for release downstream of Bonneville Dam (e.g., Ward et al. 1997; Smith et al. in press; Figure 1). Some of the remaining 2% of subyearlings that enter the Columbia River are collected and trucked at McNary and John Day dams (Figure 1), thus few subyearlings actually emigrate in-river to the Columbia River estuary and Pacific Ocean.

Dams in the upper and lower Snake River (Figure 1) changed the spawning, rearing, and migrational habitats of Snake River fall Chinook salmon thereby reducing growth opportunity (Thorpe 1989; Metcalfe and Thorpe 1989) and delaying movement of subyearlings to the sea (Connor et al. 2002). Water is released from upstream reservoirs into Lower Granite Reservoir to increase seaward movement and provide thermal refuge (Connor et al. 2003b). According to mark-recapture data, however, seaward movement is still delayed to the extent that a small number of subyearlings that survive harsh summer conditions adopt a modified stream-type juvenile life history. These fish spend their first winter in the lower Snake River reservoirs and resume seaward movement the following spring (Connor et al. 2002).

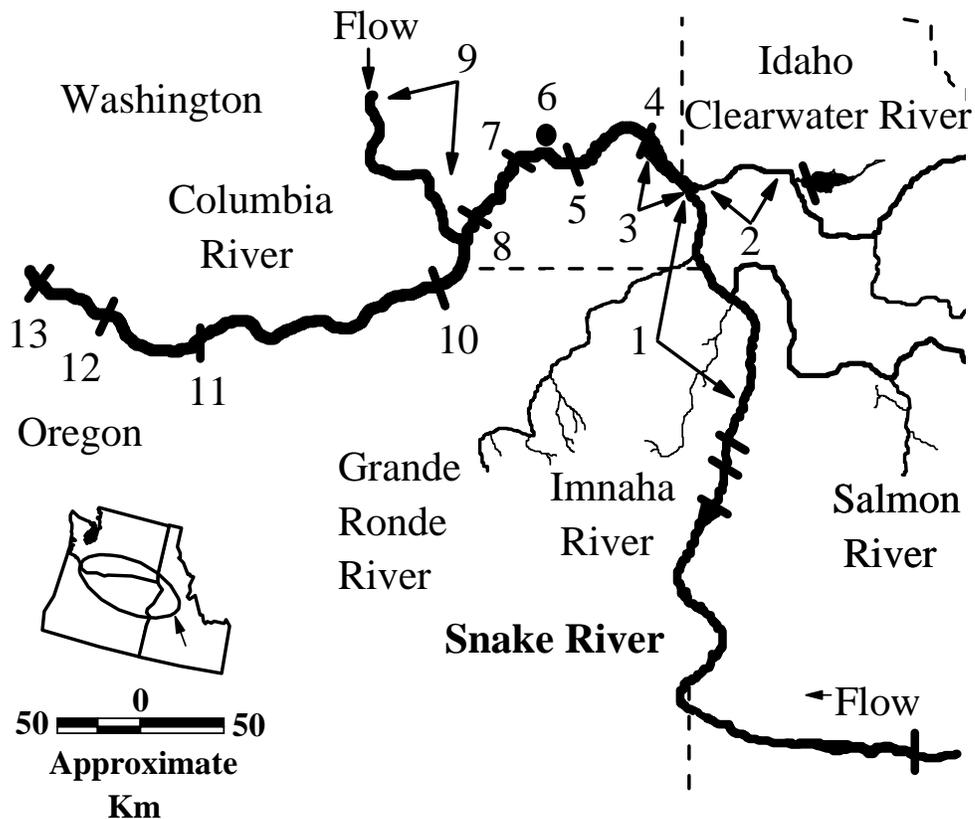


Figure 1.—The free-flowing Snake and lower Clearwater rivers where adult fall Chinook salmon spawn and their offspring were captured, tagged, and released and where subyearling hatchery fall Chinook salmon were released. The Hanford Reach of the Columbia River where wild adult fall Chinook salmon carcasses were recovered and other landmarks mentioned in the text are also given. The locations are as follows: 1 = free-flowing Snake River; 2 = free-flowing lower Clearwater River; 3 = Lower Granite Reservoir; 4 = Lower Granite Dam (PIT-tag monitoring); 5 = Little Goose Dam (PIT-tag monitoring); 6 = Lyons Ferry Hatchery; 7 = Lower Monumental Dam (PIT-tag monitoring); 8 = Ice Harbor Dam; 9 = free-flowing Hanford Reach; 10 = McNary Dam (PIT-tag monitoring); 11 = John Day Dam (PIT-tag monitoring), 12 = The Dalles Dam; and 13 = Bonneville Dam (PIT-tag monitoring).

Recovery of Snake River fall Chinook salmon is partly dependent on increased smolt survival and a commensurate increase in adult returns. There is no published information on survival of subyearlings that overwinter in reservoirs, or on the importance of this life history to adult returns. This information was necessary to assess management actions implemented to recover Snake River fall Chinook salmon. In contrast to the subyearlings studied by Connor et al. (2002), the vast majority of Snake River fall Chinook salmon are not marked. Thus, a method to identify first year overwintering history of unmarked juveniles and adults was needed to fully understand the importance of reservoir-overwintering to production of Snake River fall Chinook salmon.

In this paper, we identify reservoir-overwintered fall Chinook salmon juveniles and adults by use of scale pattern analyses. We hypothesize that differences in scale patterns reflect time spent in specific rearing environments during the first year of life. Our objectives are to use scale patterns to discriminate between: 1) juvenile fall Chinook salmon that overwintered in a reservoir and juvenile spring-summer Chinook salmon that overwintered in a stream; and 2) adult fall Chinook salmon that overwintered as juveniles in a reservoir, entering saltwater the following spring, and adult fall Chinook salmon that entered saltwater as subyearlings.

## Methods

*Analyses on juveniles.*—We evaluated scale patterns of hatchery and wild juvenile Chinook salmon that had been tagged with Passive Integrated Transponders (PIT tags; Prentice et al. 1990a) during life history and survival studies conducted in the free-flowing Snake and lower Clearwater rivers during 1993-2002 (e.g., Connor et al. 2002; Smith et al. in press). The hatchery fish were transported from Lyons Ferry Hatchery (Figure 1) for release as subyearlings into the Snake River. Lyons Ferry Hatchery fall Chinook salmon are genetically and phenotypically similar to wild Snake River fall Chinook salmon (Bugert et al. 1995; Marshall et al. 2000). Wild fall Chinook salmon were of the Snake River genetic lineage (Marshall et al. 2000) and wild spring-summer Chinook salmon were stream-type fish from the Imnaha, Salmon, Grande Ronde, and Clearwater river sub-basins (e.g., Achord et al. 1996; Connor et al. 2001; Figure 1).

We collected a sub-sample of the PIT-tagged juveniles by use of PIT-tag diversion devices (Marsh et al. 1999; Downing et al. 2001) as the juveniles moved downstream in the fish bypass systems of Lower Granite, Little Goose, Lower Monumental, and McNary dams (Figure 1) during the spring and summer. Fish that were not collected were routed by electronic gates back to the river to resume seaward movement. See Prentice et al. (1990b) and Muir et al. (2001a,b) for a description of fish bypass and PIT-tag monitoring systems. Operation of the fish bypass systems at the four dams typically begins in March and ends in November or December. The only route of dam passage during the winter months when the fish bypass systems were not operated was via the powerhouse.

Overwintering history (i.e., reservoir or stream), origin (i.e., hatchery or wild), and genetic lineage (i.e., spring-summer or fall) were confirmed by use of PIT-tag records and by use of genetic analyses (Marshall et al. 2000; Rasmussen et al. 2003). Reservoir overwintering was confirmed when a subyearling was initially tagged and released in a free-flowing river in the

spring or summer of year  $t$  and subsequently collected in the spring of year  $t+1$  at one of the dams. Stream overwintering was confirmed when a wild yearling spring-summer Chinook salmon emigrating from natal tributaries was tagged in the free-flowing Snake River or a natal tributary and collected at a dam during year  $t$ . Stream overwintering was also confirmed when a subyearling spring-summer Chinook salmon was initially tagged in a natal stream during the fall of year  $t$  and subsequently collected at a dam in spring of the year  $t + 1$ ,

For each PIT-tagged juvenile collected at a dam, we scrape sampled up to 15 scales below the posterior edge of the dorsal fin and above the lateral line (INPFC 1963). We field mounted the scale samples on gummed cards. We made acetate impressions with a heated press (Clutter and Whitesel 1956) and viewed the impressions on a microfiche reader at 48 power. We examined the largest non-regenerated scale for an accessory check and an annulus as generally described by Jearld (1983). We measured radial distance from the focus to each of these two scale features when present. We measured radial distance from the focus to the scale edge (hereafter, total radial distance). All measurements were made to the nearest 0.05 mm with a calibrated ruler. We took digital photographs of juvenile scales to illustrate these scale features.

We pooled the data collected on hatchery and wild fall Chinook salmon and then tested ( $\alpha = 0.05$ ) the following hypothesis by use of a  $t$  test. There is no difference in total radial distance on scales from juvenile Chinook salmon that overwintered in a reservoir or in a stream. When the variances were unequal between the subyearling and yearling total radial distances, we adjusted the degrees of freedom for the  $t$  test (Satterthwaite 1946).

*Analyses on adults.*—Lyons Ferry Hatchery and wild Snake River fall Chinook adults were collected during August-October as they passed upstream to spawn during 1998-2002 by use of a PIT-tag diversion device (Downing et al. 2001) at Lower Granite Dam. We used juvenile PIT-tag detection histories to confirm that these adults spent their first winter in a reservoir. A detection in year  $t + 1$  for a juvenile tagged in year  $t$  provided conclusive evidence for reservoir overwintering. A PIT-tagged adult that was never detected as a juvenile provided a second method for confirming first year reservoir overwintering because the probability of a PIT-tagged juvenile passing the six dams equipped with PIT-tag monitoring equipment (Figure 1) during March-December without being detected is low. For example, the probability of a PIT-tagged subyearling surviving to pass Lower Granite, Little Goose, or Lower Monumental dams during 1995 to 2001 was approximately 0.50 per dam and the probability of this fish passing without detection at each dam was approximately 0.50 (Smith et al. in press). Therefore, the probability of a fish surviving to pass all three of these dams without being detected before the juvenile bypass systems were shut down in late fall was only  $0.50^6$  or 0.016 (i.e., 1.6%). This means that the fish either passed all the dams in January and February (an unlikely event), overwintered in a reservoir and then resumed seaward migration before juvenile fish bypass operation resumed in early spring, or overwintered in a reservoir and passed every dam in the spring via the spillways.

We were unable to use PIT-tag detection histories to identify Snake River fall Chinook salmon adults that conclusively spent their first winter in saltwater because a detection at Bonneville Dam (Figure 1) was necessary for confirmation. Few PIT-tagged juvenile fall Chinook salmon released in the Snake or lower Clearwater rivers survive to pass Bonneville

Dam as subyearlings because of the previously mentioned practice of routing every PIT-tagged fish back to the river at each dam. Thus the odds of collecting a PIT-tagged adult at Lower Granite Dam that entered saltwater as a subyearling were low.

To provide surrogates for Snake River adults, we analyzed scale patterns on a sample of wild (natural origin) adult fall Chinook salmon that spawned in the Hanford Reach of the Columbia River (Figure 1) during 1998-2002. These adults had been tagged with coded wire (Jefferts et al. 1963) as juveniles during 1994-2001. Hanford Reach fall Chinook salmon are genetically and phenotypically similar to the Snake River fall Chinook salmon (Marshall et al. 2000). The subyearlings pass Bonneville Dam in July and August (Ken Tiffan, U.S. Geological Survey, unpublished data) and they enter saltwater on a late summer-early fall time schedule that is similar to unmarked Snake River fall Chinook salmon subyearlings.

Scale samples from adults were enveloped and up to six scales per fish were later cleaned and mounted on gummed cards. We made acetate impressions, viewed the impressions, and measured scale features as described for juveniles. We measured the radial distance to any accessory check when present, radial distance from the focus to the first annulus (hereafter, radial distance to the first annulus), and the radial distance from the focus to a check coincident with the point of ocean entry (hereafter, radial distance to ocean entrance).

The point of annulus formation and ocean entrance were easily discerned on adult Snake River scales because freshwater zone circuli are more narrowly spaced and finer than circuli in ocean zones (Rich and Holmes 1929). We also had juvenile and adult Snake River scales with known first year reservoir-overwintering histories and known total age (i.e., we knew how many annuli should be present on a scale). First year scale features were readily apparent on adult Hanford Reach scales because the aforementioned difference in freshwater and ocean circuli spacing were evident and we knew the total age of each fish based on coded-wire tag codes.

We examined each acetate impression to determine if an annulus was formed at the time of ocean entrance. We measured the distance between the first annulus and the point of ocean entry. For adults that spent their first winter in a reservoir, we refer to this measurement as the spring growth zone (Clutter and Whitesel 1956). For adults that spent their first winter in saltwater, we refer to this measurement as the first year ocean zone. The presence of an annulus at ocean entrance, or a spring growth zone, provided a visual method for identifying adults that spent their first winter in a reservoir. We made digital photographs of adult scale impressions to illustrate these scale features.

We also developed a quantitative method for identifying first year overwintering history of adults. We began by running a two-way analysis of variance (overwintering history and year;  $\alpha = 0.05$ ). We tested two null hypotheses. There is no difference in the radial distance to the first annulus on scales from fall Chinook salmon adults that spent their first winter in a reservoir or in saltwater. There is no difference in the radial distance to ocean entrance on scales from fall Chinook salmon adults that spent their first winter in a reservoir or in saltwater. We used Fisher's test for least significance difference (Ott 1993) to make pair-wise comparisons ( $\alpha = 0.05$ ).

We set aside the 1999 data for validation purposes because relatively large sample sizes were available for adults of both first year overwintering histories. We then fit a logistic regression model (Johnson 1998) to determine if radial distance to ocean entry could be used to discriminate between adults that spent their first winter in a reservoir or in saltwater. Logistic regression produces a logit function. In our analysis, the form of the logit function was  $g(x) = B_0 + B_1 O_e$  where  $B_0$  was the intercept,  $B_1$  was the slope coefficient, and  $O_e$  was the radial distance to ocean entry. We used the logit function to calculate  $P_i$  for fish as:

$$P_i = \frac{e^{g(x)}}{1 + e^{g(x)}}$$

We interpreted  $P_i$  as the probability that an adult Chinook salmon spent its first year in a reservoir. An estimate of  $P_i$  above 0.5 indicated that the adult spent its first winter in a reservoir. An estimate of  $P_i$  less than or equal to 0.5 indicated that the adult spent its first winter in saltwater. We used the likelihood ratio test ( $\alpha = 0.05$ ; SAS 1990) to determine if a model with a coefficient for radial distance to ocean entry was different from the constant-only model. We calculated classification accuracy as the total number of correct predictions of first year overwintering history divided by the total number of predictions multiplied by 100. Finally, we ran the model on the 1999 data and calculated classification accuracy to validate the model for use on out-of-sample data.

## Results

### *Analyses on Juveniles*

During 1993-2002, PIT-tagging records confirmed the overwintering history of 65 juvenile Chinook salmon collected at all dams combined (Table 1). Reservoir overwintering was confirmed for 43 hatchery and 2 wild fall Chinook salmon juveniles (Table 1). Stream overwintering was confirmed for 20 wild spring-summer Chinook (Table 1).

The presence of accessory checks on scales from juveniles (Figure 2) was dependent on origin. All 43 of the scales from reservoir-overwintered hatchery fall Chinook salmon had an accessory check within 0.50 mm of the focus (mean,  $0.30 \pm 0.07$  mm SD). No accessory checks were observed on scales from reservoir-overwintered wild fall Chinook salmon or stream-overwintered wild spring-summer Chinook salmon.

The presence of an annulus (Figure 2) was not dependable for distinguishing between reservoir-overwintered and stream-overwintered juveniles. An annulus was present within 1.0 mm (mean,  $0.85 \pm 0.10$  mm SD) of the focus on 29% (13 of 45) of the scales sampled from reservoir-overwintered hatchery and wild fall Chinook salmon combined. An annulus was present within 0.40 mm (mean,  $0.32 \pm 0.06$  mm SD) of the focus on all 20 scales from stream-overwintered wild spring-summer Chinook salmon.

A visual difference in total radial distance on scales from reservoir-overwintered and stream-overwintered juveniles was apparent (Figure 2). Total radial distance was significantly ( $P < 0.0001$ ) larger on scales from reservoir-overwintered fall Chinook salmon (mean,  $1.02 \pm 0.11$  mm SD) than on scales from stream-overwintered spring-summer Chinook salmon (mean,  $0.53 \pm 0.07$  mm SD; Figure 3). There was no overlap between the minimum total radial distance

for reservoir-overwintered fall Chinook salmon (0.75 mm) and the maximum total radial distance for stream-overwintered spring-summer Chinook salmon (0.65 mm; Figure 3).

Table 1.—Sampling information for PIT-tagged juvenile Chinook salmon that overwintered in reservoirs or streams including the number collected at the dams (*N*), 1993—2002. Spring-summer Chinook salmon of undetermined stock were tagged in the Snake River after moving downstream from tributaries of the Imnaha, Salmon, Grande Ronde, or Clearwater rivers.

| Overwintering history | Run           | Origin           | Stock        | Year tagged | Year collected | <i>N</i> |
|-----------------------|---------------|------------------|--------------|-------------|----------------|----------|
| Reservoir             | Fall          | Hatchery         | Snake River  | 1995        | 1996           | 6        |
|                       |               |                  |              | 1997        | 1998           | 31       |
|                       |               |                  |              | 2001        | 2002           | 6        |
|                       |               | Wild             | Snake River  | 2001        | 2002           | 2        |
|                       |               | Total            |              |             |                | 45       |
| Stream                | Spring-summer | Wild             | Undetermined | 1993        | 1993           | 4        |
|                       |               |                  |              | 1995        | 1995           | 4        |
|                       |               |                  |              | 1996        | 1996           | 9        |
|                       |               |                  |              | 1998        | 1998           | 1        |
|                       |               | Imnaha River     | 1993         | 1994        | 1              |          |
|                       |               | Clearwater River | 1994         | 1994        | 1              |          |
|                       |               | Total            |              |             | 20             |          |

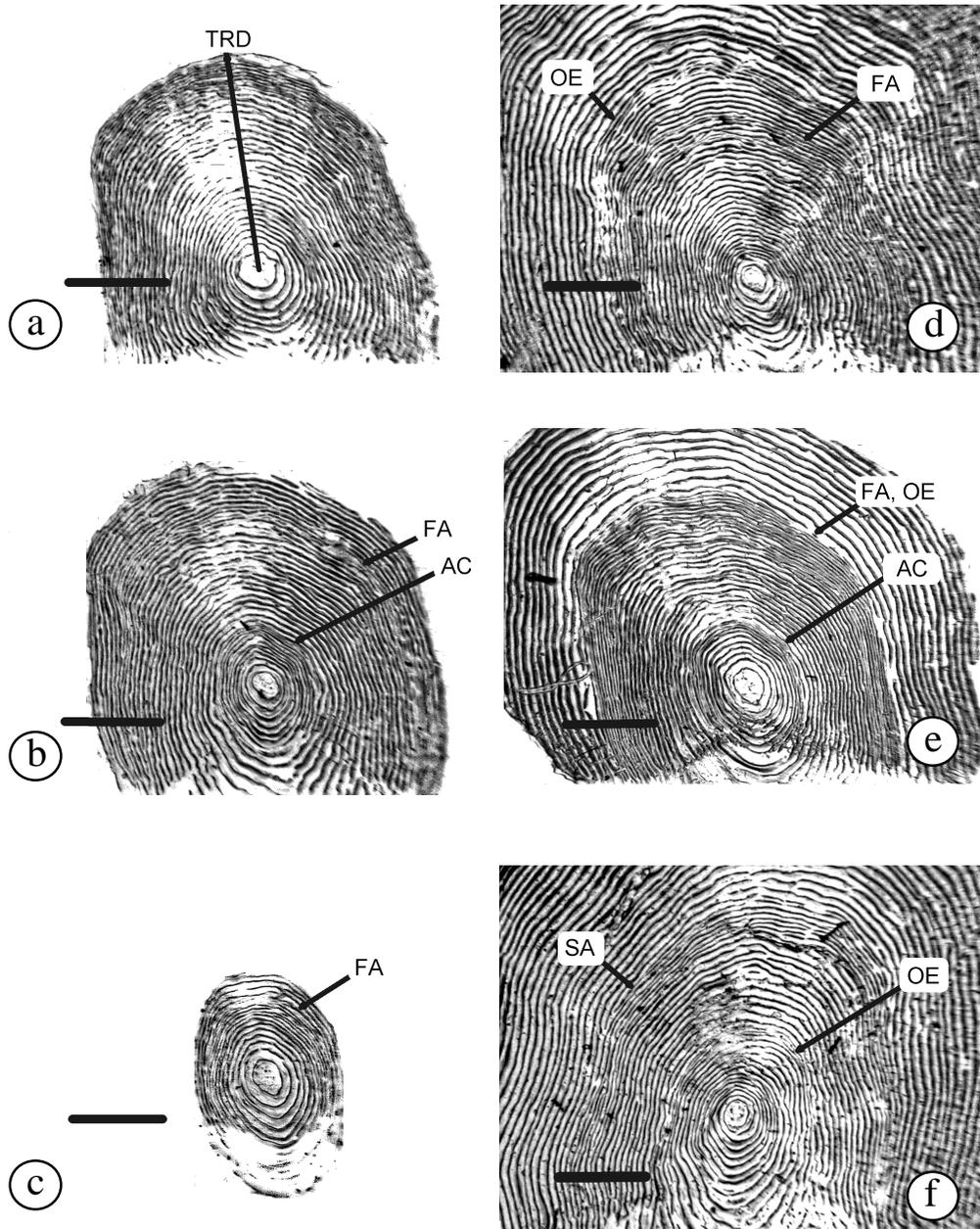


Figure 2.—Examples of scales from juvenile (left side) and adult (right side) Chinook salmon including scales collected from: (a) a reservoir-overwintered wild fall Chinook salmon juvenile lacking an accessory check and a freshwater annulus; (b) a reservoir-overwintered hatchery fall Chinook salmon juvenile with an accessory check and a freshwater annulus; (c) a wild spring-summer Chinook salmon juvenile lacking an accessory check, with a freshwater annulus; (d) a wild fall Chinook salmon adult that spent its first winter in a reservoir, lacking an accessory check, with a freshwater annulus prior to ocean entry that formed a spring growth zone; (e) a hatchery fall Chinook salmon adult (jack) that spent its first winter in a reservoir, with an accessory check and a freshwater annulus coincident with ocean entrance; and (f) a wild fall Chinook salmon adult that entered saltwater as a subyearling, with a check coincident with ocean entrance and a saltwater annulus forming a first year ocean zone. The images were enhanced with a best fit local equalization filter and the horizontal lines on the left side of each scale are scale bars equal to 0.5 mm. Abbreviations: TRD = total radial distance; AC = accessory check; FA = freshwater annulus; OE = ocean entrance; and SA = first saltwater annulus.

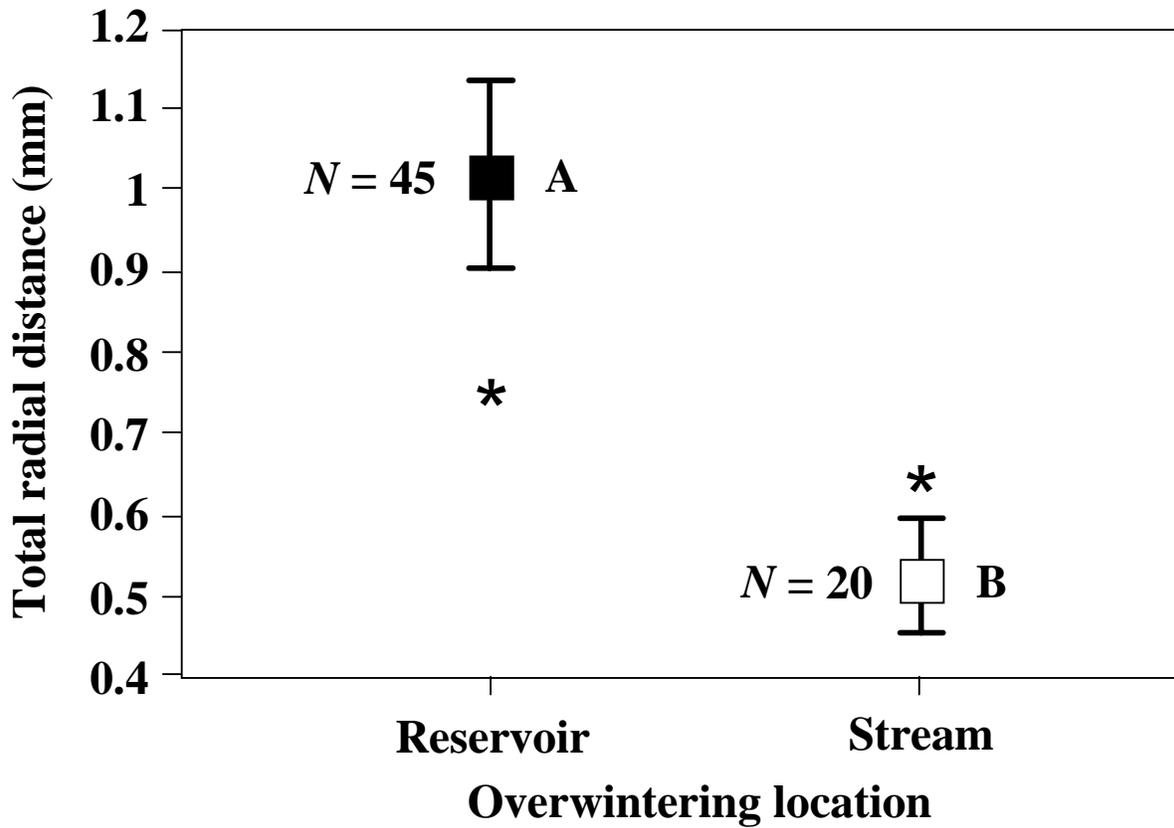


Figure 3.— Mean total radial distance (mm  $\pm$  SD) measured from the scale focus to the scale edge on scales from reservoir-overwintered juvenile fall Chinook salmon and stream-overwintered juvenile spring-summer Chinook salmon. The asterisks are the maximum and minimum values and the letters beside each plot show that mean total radial distance varied significantly ( $P < 0.0001$ ) between scales from fish of these first year overwintering histories.

*Analyses on Adults*

During 1998-2002, 38 (34 hatchery, 4 wild) PIT-tagged fall Chinook salmon adults were collected at Lower Granite Dam and 98 coded-wire tagged wild fall Chinook salmon adults were collected along the Hanford Reach (Table 2). First year reservoir overwintering by 10 hatchery and 2 wild Snake River fall Chinook salmon adults was confirmed because the adults were tagged as subyearlings in year  $t$  and detected passing downstream at one of the dams during year  $t + 1$ . First year reservoir overwintering was confirmed for 24 hatchery and 2 wild PIT-tagged Snake River fall Chinook salmon adults (68% of total sample) because they were never detected passing a dam as juveniles.

Table 2.—Sampling information for adult Snake River fall Chinook salmon that were PIT tagged as juveniles, spent their first winter in a reservoir, and were collected ( $N$ ) at Lower Granite Dam en route to spawning areas, and for adult Hanford Reach fall Chinook salmon that were coded wire tagged as juveniles, presumably spent their first winter in saltwater, and were collected after spawning along the Hanford Reach of the Columbia River, 1998-2002.

| Overwintering history | Origin   | Stock       | Return year | $N$  |
|-----------------------|----------|-------------|-------------|------|
| Reservoir             | Hatchery | Snake River | 1998        | 3    |
|                       |          |             | 1999        | 7    |
|                       |          |             | 2000        | 20   |
|                       |          |             | 2001        | 2    |
|                       |          |             | 2002        | 2    |
|                       | Wild     | Snake River | 1998        | 2    |
|                       |          |             | 1999        | 1    |
|                       |          |             | 2000        | 1    |
|                       |          |             | Total       | 38   |
|                       |          |             | Saltwater   | Wild |
| 1999                  | 14       |             |             |      |
| 2000                  | 17       |             |             |      |
| 2001                  | 15       |             |             |      |
| 2002                  | 36       |             |             |      |
| Total                 | 98       |             |             |      |

The presence of accessory checks (Figure 2) on scales from adults was dependent on origin. All 34 of the hatchery fall Chinook salmon adults had an accessory check within 0.50 mm of the scale focus (mean,  $0.34 \pm 0.08$  mm SD). No accessory checks, other than an ocean entry check, were observed on scales from the 98 wild fall Chinook salmon adults.

First year overwintering history was usually discernable on scales from adults based on time of annulus formation and the presence of a spring growth zone or a first year ocean zone (Figure 2). Of the 38 of hatchery and wild Snake River fall Chinook salmon adults that spent their first winter in a reservoir, 21 (55%) had scales with an annulus formed at the time of ocean entry. The remaining 17 (45%) had scales with a freshwater annulus that resulted in a narrow spring growth zone less than or equal to 0.35 mm wide (mean,  $0.18 \pm 0.07$  mm SD). Of the 98 scales from wild Hanford Reach fall Chinook salmon adults that presumably spent their first winter in saltwater, 95 (97%) had a check coincident with ocean entrance and a saltwater annulus that resulted in wide first year ocean zones (mean,  $0.54 \pm 0.17$  mm SD). Only 13 (13%) of 98 fish had first year ocean zone widths less than or equal to 0.35 mm, and only 3 (3%) had an annulus formed at ocean entry resulting in the absence of a first year ocean zone.

First year overwintering history was not visually discernable based on radial distance to the first annulus (Figure 2). Results from two-way analysis of variance showed that the main effects of first year overwintering history ( $P = 0.5187$ ), year ( $P = 0.1218$ ), and the interaction between overwintering history and year ( $P = 0.0579$ ) had no significant effect on radial distance to the first annulus. Mean radial distance to the first annulus was  $1.05 \pm 0.15$  mm SD on scales from adult fall Chinook salmon that spent their first winter in a reservoir ( $N = 38$ ) and  $1.07 \pm 0.17$  mm SE on scales from adult fall Chinook salmon that spent their first winter in saltwater ( $N = 98$ ).

Radial distance to ocean entry appeared larger on scales from adult fall Chinook salmon that spent their first winter in a reservoir than on scales from adult fall Chinook salmon that spent their first winter at sea (Figure 2). Two-way analysis of variance on radial distance to ocean entry measured on scales from adult fall Chinook salmon showed that the interaction between first year overwintering history and year was non significant ( $P = 0.2073$ ). The main effects of overwintering history ( $P < 0.0001$ ) and year ( $P < 0.0001$ ) were significant. Mean radial distance to ocean entry was greater on scales from adults that overwintered in freshwater ( $N = 38$ ; mean,  $1.12 \pm 0.15$  mm SD) than for adults that spent their first winter in saltwater ( $N = 98$ ; mean,  $0.53 \pm 0.11$  mm SD; Figure 4). There was a slight overlap between the minimum radial distance to ocean entry for adults that overwintered in a reservoir (0.80 mm) and the maximum radial distance to ocean entry for adults that presumably spent their first winter in saltwater (1.00 mm; Figure 4).

After setting aside the 1999 data for validation, the logit function for the logistic regression model fit to discriminate first year overwintering history of adult fall Chinook salmon from radial distance to ocean entry was  $g(x) = -18.0818 + 21.1747 Oe$ . The model with a coefficient for radial distance to ocean entry was significantly ( $P < 0.0001$ ) different from the constant-only model. The positive slope coefficient for radial distance to ocean entrance shows that the probability of the scale being from a fall Chinook salmon adult that spent its first winter in a reservoir increased as radial distance to ocean entry increased. Classification accuracy for this

model when predicting first year overwintering history from the data used to fit the model was 98.3%. The model miss-classified 1 hatchery adult that actually spent its first winter in a reservoir, and 1 wild adult that from that Hanford Reach that presumably spent its first winter in saltwater. Classification accuracy for this model when predicting first year overwintering history for adults in the 1999 validation data set was 100% ( $N = 22$ ).

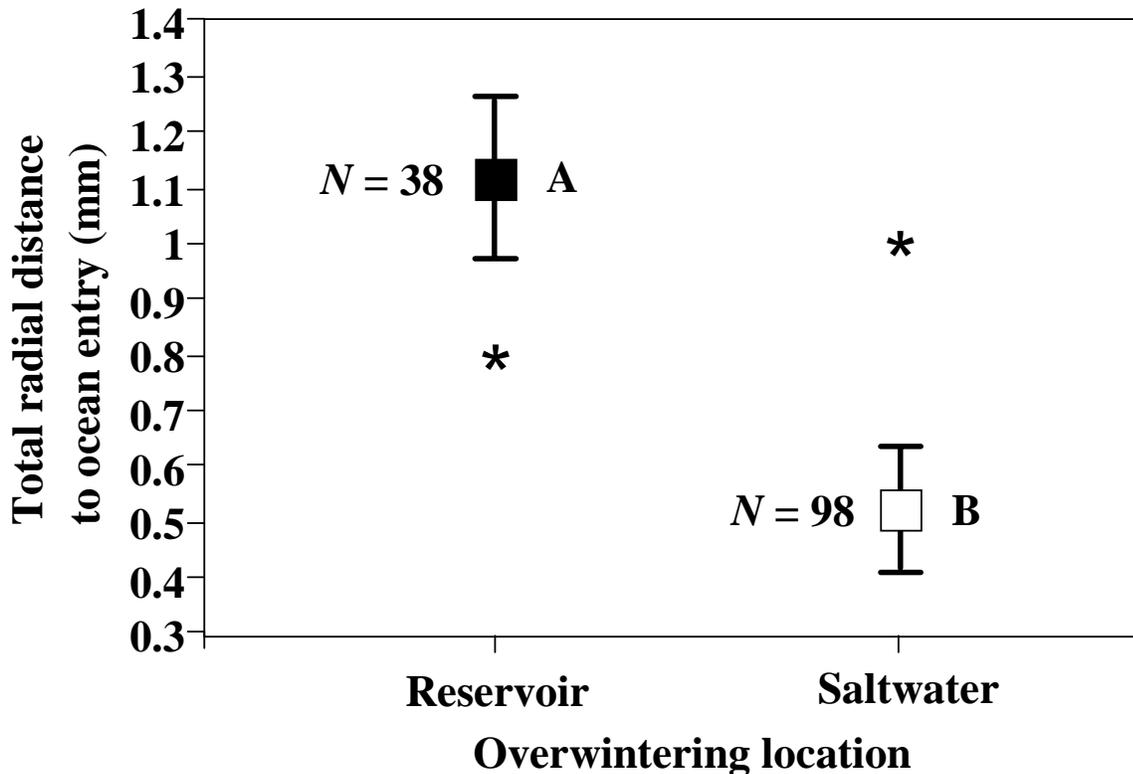


Figure 4.— Mean radial distance to ocean entry (mm  $\pm$  SD) measured from the scale focus to the point of ocean entry on scales from adult fall Chinook salmon that spent their first winter in a reservoir and adult fall Chinook salmon that spent their first winter in saltwater. The asterisks are the maximum and minimum values and the letters beside each plot show that mean radial distance to ocean entry varied significantly ( $P < 0.0001$ ) between scales from fish of these first year overwintering histories.

### Discussion

To assess the findings reported, an understanding of the limitations and assumptions of our analyses is necessary. Samples of Snake River fall Chinook salmon adults that entered saltwater as subyearlings were unavailable. The first year scale patterns of adult Hanford Reach fall Chinook salmon should be similar to Snake River adults, as they were similar to the ocean-type patterns described for adult Chinook salmon throughout the Columbia River basin by Gilbert (1913) and Rich and Holmes (1929). One exception was that we did not observe a

separate estuary growth zone on scales of Hanford Reach adults, just a single check prior to the first annulus that we interpreted as the ocean entrance check. We assumed that none of the Hanford Reach adult fall Chinook salmon we used as ocean-type baselines overwintered in a reservoir. This assumption probably was not met because 3 (3%) of the Hanford Reach adults did have a first year ocean zone suggesting that they had spent their first winter in a reservoir or in the 234 km stretch of the Columbia River downstream of Bonneville Dam. Notably, the primary author has observed scales collected from other Columbia River ocean-type stocks that had patterns reflective of reservoir overwintering (Washington Department of Fish and Wildlife, unpublished data). Thus, it would not be surprising for a small percentage of Hanford Reach adults to have a first year reservoir-overwintered life history. Finally, we did not explore other scale characteristics such as inter-circulus spacing because it was our intent to identify first year overwintering history by procedures that could easily be repeated by biologists with less experience analyzing scale patterns than the primary author.

In spite of the above limitations and assumptions, distinct differences in scale patterns of juvenile and adult fall Chinook salmon clearly reflected first year overwintering history. Juvenile fall Chinook salmon that overwintered in a reservoir had scales with total radial distances of 0.75 mm and above, whereas juvenile spring-summer Chinook salmon that overwintered in a stream had scales with total radial distances less than 0.65 mm. An annulus formed at ocean entrance, or a narrow spring growth zone, provided a visual indicator of first year reservoir overwintering by fall Chinook salmon adults. The absence of an annulus formed at ocean entrance and a wide first year ocean zone visually indicated that adult fall Chinook salmon spent their first winter in saltwater. The logistic regression function fit from radial distance to ocean entry quantitatively identified first year overwintering history of adults with a high level of accuracy.

Scale patterns of juvenile Chinook salmon reflected differences in time spent in specific rearing environments and differences in growth rates, during the first year of life. The majority of reservoir-overwintered fall Chinook salmon juveniles did not have scales with an annulus by comparison to 100% of the stream-overwintered spring-summer Chinook salmon that had scales with an annulus. Part of this may be due to sampling the reservoir-overwintered juveniles in April and early May, while most of the stream-overwintered juveniles were sampled in late May to early July. The lower rearing temperatures during fall through spring for stream-overwintered juveniles probably promotes earlier annulus formation. Spring growth probably begins as these fish move downstream into habitats with greater growth opportunity during late winter and early spring. Higher reservoir temperatures during the fall through spring time period probably extends growth opportunity, leading to later annulus formation for reservoir-overwintered juveniles. Also, since smolt length correlates to survival, additional spring growth is probably more advantageous to stream-overwintered juveniles than to reservoir-overwintered juveniles. Total radial distance on scales from reservoir-overwintered fall Chinook salmon juveniles was larger than observed on scales of stream-overwintered spring-summer Chinook juveniles because reservoir-overwintered juveniles spent more time in habitat with high growth opportunity. Temperatures in high-order stream reaches such as the lower Snake River and its impounded lower stretch are typically warm and stable throughout the year by comparison to low-order streams inhabited by juvenile spring-summer Chinook salmon (e.g., Vannote et al. 1980). Others have credited the productive rearing environment in Lower Granite Reservoir for rapid growth

observed in wild juvenile Chinook salmon (Connor et al. 2001; Connor and Burge 2003).

Similarities and differences in scale patterns of adult fall Chinook salmon reflected time spent in specific rearing environments during the first year of life. Growth opportunity for subyearlings in the Snake River and downstream reservoirs was apparently similar to growth opportunity in saltwater because there was no difference in radial distance to the first annulus on scales from adults of the first year overwintering histories studied. Others have reported similar results. Connor et al. (2001) and Connor and Burge (2003) compared growth rates of 0.8-1.4 mm/d for subyearlings in the Snake River and Lower Granite Reservoir to growth rates of 0.4-1.3 mm/d for subyearlings in saltwater habitat reported by Healey (1980), Kjelson et al. (1982), and Buckman and Ewing (1982). The narrow spring growth zone of adults that overwintered in a reservoir reflected a relatively short period of growth in fresh water by fish that became yearlings prior to ocean entry. The wide first year ocean zone of adults that spent their first winter in saltwater reflected a relatively long period of growth in saltwater for subyearlings prior to becoming yearlings.

Radial distance to ocean entrance was larger on scales of adults that spent their first winter in a reservoir than on scales of adults that spent their first winter at sea because of time spent in specific rearing environments during the first year of life. Adults that overwintered in a reservoir had large radial distances to ocean entry because they did not enter saltwater until they were yearlings, so more time elapsed between scale formation and ocean entrance than in the case of ocean-overwintered fish. Adults with a reservoir-overwintering life history also experienced conditions favorable for growth during their extended period of residence as subyearlings in fresh water, so they were much larger than typical ocean-type subyearlings at ocean entrance. For example, fork lengths of reservoir-overwintered wild Snake River fall Chinook juveniles averaged 226 mm in April (Connor, unpublished data) by comparison to an average of 141 for wild subyearling Snake River fall Chinook salmon measured prior to ocean entrance during the summer (Connor and Burge 2003).

The findings in this paper have three management implications. First, scales from juveniles and adults can be used to estimate the prevalence of reservoir-overwintered fish in larger samples of unmarked juveniles and adults. This atypical life history would be important to fall Chinook salmon recovery if reservoir-overwintered juveniles make up only a small percentage of the smolts but a large percentage of adults that return to spawn. The second management implication is that 68% of the PIT-tagged Snake River fall Chinook salmon that overwintered in a reservoir were never detected as juveniles. It is possible that some young fall Chinook salmon listed under the Endangered Species Act are passing the dams during the winter via the powerhouse and the turbine blades. Mortality of yearling spring Chinook salmon due to turbine blade strike ranges from 7.3-13.5% (Muir et al. 2001a). Information is needed to determine when and how reservoir-overwintered fall Chinook salmon juveniles pass dams in the Snake and Columbia rivers. Finally, in the late 1990s the practice of marking 100% of the hatchery subyearling fall Chinook salmon released into the Snake River to supplement production in the wild was discontinued. In our study, hatchery Chinook salmon juveniles and adults were 100% identifiable by the presence of an accessory check within 0.50 mm of the scale focus. This accessory check was most likely a "hatchery release check" caused by a disruption in growth associated with pre-release transportation stress and by the period of time required for

hatchery subyearlings to learn how to feed in the wild. The presence of a hatchery release check on the scales of returning unmarked adults at Lower Granite Dam will help managers determine the proportion of hatchery and wild adults in the run.

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### CHAPTER THREE

#### Fork Length, Condition Factor, and Abundance of Reservoir-overwintered Fall Chinook Salmon Juveniles in the Snake River

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Running head: Reservoir Overwintering of Juvenile Salmon

Key words: Chinook Salmon, Overwintering, Snake River Reservoirs

*Abstract.*—Juvenile Pacific salmon *Oncorhynchus* sp. deviate from typical juvenile life histories in response to environmental conditions. We studied wild and hatchery juvenile fall Chinook salmon *O. tshawytscha* that departed from the typical ocean-type life history by overwintering in the first two reservoirs passed en route to the sea. In early spring 1998, we systematically collected 90 juvenile Chinook salmon at Little Goose Dam (forms the downstream most of the two reservoirs studied). Scale pattern showed that 39 of these fish were reservoir overwintered and 51 were stream overwintered. Scale pattern and genetic analysis showed that 62% of the reservoir-overwintered fish were wild fall Chinook salmon, 36% were hatchery fall Chinook salmon, and 2% were hatchery spring-summer Chinook. Stream-overwintered fish were 94% wild spring-summer Chinook salmon and 6% wild fall Chinook salmon. Reservoir-overwintered juveniles were significantly longer ( $P < 0.0001$ ;  $226 \pm 12$  mm SD) and had significantly higher condition factors ( $P = 0.0012$ ;  $1.04 \pm 0.09$  SD) than stream-overwintered juveniles (mean fork length,  $137 \pm 17$  mm SD; mean condition factor,  $0.98 \pm 0.08$  SD). A logistic regression model fit from fork length classified overwintering history of the 90 fish with 100% accuracy. The model was also 100% accurate at classifying overwintering history of 52 juveniles collected at Lower Granite Dam (forms the upstream most reservoir studied). We used the model to help calculate abundance indices at Lower Granite Dam for brood year 1996 fall Chinook salmon. Typical ocean-type subyearling migrants in 1997 made up and estimated 91% of the total brood year 1996 outmigration, whereas reservoir-overwintered juveniles in 1998 made up 9%. We conclude that relatively few fall Chinook salmon survive to overwinter in Snake River reservoirs, but the survivors might reach the sea at high rates because of their early migrational timing and large size.

Overwintering location of young Pacific salmon *Oncorhynchus sp.* varies within the genus. Young sockeye salmon *O. nerka* typically overwinter in freshwater lakes (Burgner 1991). Coho salmon *O. kisutch* and stream-type Chinook salmon *O. tshawytscha* usually spend one winter in natal freshwater tributaries before completing seaward migration during the spring as yearlings (Sandercock 1991; Healey 1991). Pink salmon *O. gorbuscha* and ocean-type Chinook salmon typically spend their first winter at sea (Heard 1991; Healey 1991). Deviation from these typical juvenile life histories can reflect adaptation to environmental conditions (e.g., Randall et al. 1987; Quinn and Unwin 1993; Quinn 1999).

Wild spring-summer Chinook salmon produced in tributaries of the Imnaha, Salmon, Grande Ronde, and Clearwater rivers (Figure 1) usually have a stream-type life history. They overwinter in natal or larger-order tributaries as subyearlings and then pass Lower Granite and Little Goose dams (Figure 1) as yearlings in April and May (e.g., Chapman and Bjornn 1969; Bjornn 1971; Achord et al. 1996). Some spring-summer Chinook salmon, however, diverge from the typical stream-type life history by dispersing into Lower Granite Reservoir in the summer as subyearlings (Connor et al. 2001a,b). Wild fall Chinook salmon produced in the main-stem Snake river and the lower reaches of its tributaries (Figure 1) typically have an ocean-type life history (Connor et al. 2002). Young fall Chinook salmon move downstream from the free-flowing river in late spring, spend prolonged periods in Lower Granite Reservoir (Figure 1) during seaward emigration (Connor et al. 2003a), and then pass Lower Granite and Little Goose dams as subyearlings primarily in summer (Connor et al. 2002). Some fall Chinook salmon depart from an ocean-type juvenile life history by overwintering as subyearlings in reservoirs before resuming seaward emigration the following spring (Figure 2; Connor et al. 2002).

Reservoir-overwintering by wild fall Chinook salmon and hatchery fall Chinook salmon subyearlings released into the Snake River to supplement natural production might be important to recovery of the Snake River stock that is listed under the Endangered Species Act (NMFS 1992). Information was needed to help determine if reservoir-overwintered juveniles make up only a small percentage of fall Chinook salmon smolts, but return at high rates as adults two to four years later. Sneva and Connor (in review) found that the radial distance from the focus to the scales edge was wider on scales from reservoir-overwintered fall Chinook salmon juveniles than on scales from stream-overwintered spring-summer Chinook salmon juveniles suggesting higher growth of reservoir-overwintered fish. We hypothesized that this difference in growth caused by rearing location would be evident in fork length or condition factor of juvenile Chinook salmon when they pass Lower Granite and Little Goose dams. A distinct difference in either growth measure could be used to identify overwintering history and to help calculate an abundance index for reservoir-overwintered juveniles passing Lower Granite Dam.

In this note, we compare fork length and condition factor between reservoir-overwintered and stream-overwintered juvenile Chinook salmon collected at Little Goose dam in 1998. We also calculate abundance indices for brood year 1996 fall Chinook salmon juveniles that entered Lower Granite Reservoir and survived to pass the dam as subyearlings in 1997 or in 1998 after overwintering in the reservoir.

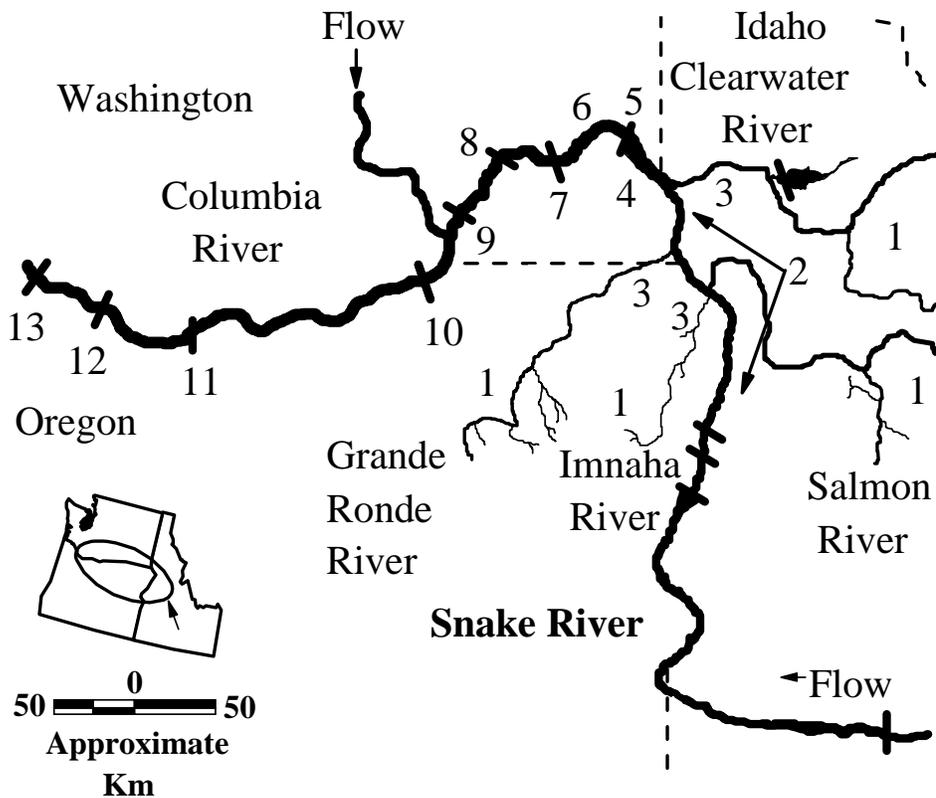


Figure 1.-Tributary streams where spring-summer Chinook salmon spawn and their offspring typically overwinter, the main-stem Snake River where fall Chinook salmon spawn and their offspring rear prior to migrating seaward primarily as subyearlings, and reservoirs and dams subyearling and yearling smolts encounter en route to the sea. The locations are as follows: 1 = tributary subbasins that sustain spring-summer Chinook production; 2 = the primary fall Chinook salmon spawning area in the Snake River; 3 = larger tributaries where fall Chinook salmon spawn; 4 = Lower Granite Reservoir; 5 = Lower Granite Dam (juvenile bypass); 6 = Little Goose Reservoir; 7 = Little Goose Dam (juvenile bypass); 8 = Lower Monumental Dam (juvenile bypass); 9 = Ice Harbor Dam (juvenile bypass); 10 = McNary Dam (juvenile bypass); 11 = John Day Dam (juvenile bypass); 12 = the Dalles Dam; and 13 = Bonneville Dam (juvenile bypass).

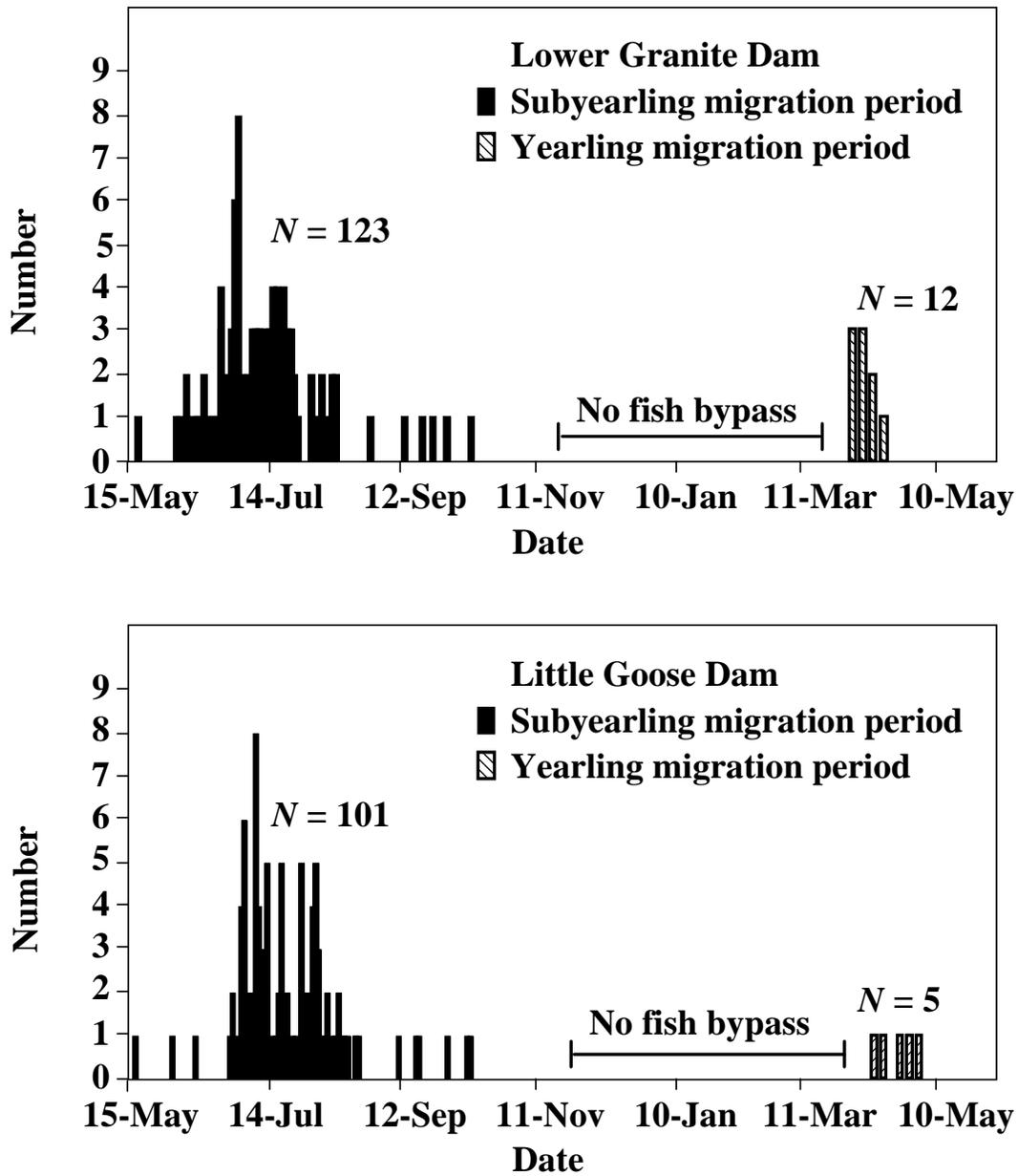


Figure 2.—Passage dates at Lower Granite (top panel) and Little Goose (bottom panel) dams for wild subyearling Chinook salmon (mostly fall run) from brood year 1996 that were captured, tagged with passive integrated transponders (Prentice et al. 1990a), released in the free-flowing Snake River in 1997, and then detected (e.g., Prentice et al. 1990b) passing the dams during the 1997 subyearling migration period or the 1998 yearling migration period (data from Connor et al. 2002). Notably, PIT-tagged fish are routed back to the river unlike untagged fish so they are not collected and trucked for release downstream of Bonneville Dam (e.g., Ward et al. 1997; Figure 1) thus some of the PIT-tagged fish in this figure were detected at both Lower Granite and Little Goose Dams. Timing of juvenile bypass operations is also shown.

## Methods

*Fork length and condition factor.*—Juvenile bypass systems (e.g., Muir et al 2001a,b) are operated annually at several dams in the lower Snake and Columbia rivers (Figure 1). Juvenile bypass operations cease in late fall at the end of the subyearling migration period, and then resume in early spring at the onset of the yearling migration period (Figure 2).

From 30 March to 27 April during the 1998 yearling migration period, personnel of the Oregon Department of Fish and Wildlife diverted samples of juvenile Chinook salmon from the juvenile bypass system Little Goose Dam to a sampling room (e.g., Muir et al. 2001b) for routine smolt monitoring. We collected a length, weight (g), fin clip, and a scale sample from every fish in these samples that was longer than 170-mm fork length and had an adipose fin. Each time a fish longer than 170-mm fork length was collected, we randomly collected a fish less than 170-mm long that had an adipose fin for a length, weight, fin clip and scale sample. Chinook salmon without adipose fins were usually hatchery spring-summer Chinook salmon and were not important to our study.

We made acetate impressions of the scales with a heated press (Clutter and Whitesel 1956) and viewed the impressions on a microfiche reader at 48 power. We examined each scale for a hatchery release check (always indicative of hatchery origin), and then measured radial distance from the focus to the scales edge to the nearest 0.05 mm (Sneva and Connor in review). Juvenile Chinook salmon that overwintered in a stream or a reservoir are 100% distinguishable based on this measurement. Stream-overwintered fish have scales with a radial distances from the focus to the scales edge less than 0.65 mm, whereas this distance is 0.75 mm or more on scales of reservoir-overwintered fish (Sneva and Connor in review). The genetic lineage (i.e., spring-summer run or fall run) of fish was identified from the fin clips by use of the dual-primer product of a nuclear DNA marker (93% accurate; Rasmussen et al. 2003).

We calculated condition factor as weight divided by fork length<sup>3</sup> multiplied by 10<sup>5</sup>. We tested ( $\alpha = 0.05$ ) the following hypotheses by use of *t* tests: 1) there is no difference in the mean fork length of Chinook salmon that overwintered in a reservoir or a stream; and 2) there is no difference in mean condition factor of Chinook salmon that overwintered in a reservoir or a stream.

We fit a logistic regression model (Hosmer and Lemeshow 1989) to determine if fork length could be used to discriminate between Chinook salmon that overwintered in a reservoir or a stream. Logistic regression produces a logit function. In our analysis, the form of the logit function was  $g(x) = B_0 + B_1 FL$  where  $B_0$  was the intercept,  $B_1$  was the slope coefficient, and FL was the fork length measured on fish collected at Little Goose Dam. We used the logit function to calculate  $P_i$  for fish as:

$$P_i = \frac{e^{g(x)}}{1 + e^{g(x)}} .$$

We interpreted  $P_i$  as the probability that Chinook salmon overwintered in a reservoir. An estimate of  $P_i$  above 0.5 indicated that the juvenile overwintered in a reservoir, whereas an

estimate of  $P_i$  less than or equal to 0.5 indicated that the juvenile overwintered in a stream. We used the likelihood ratio test ( $\alpha = 0.05$ ; SAS 1990) to determine if a model with a coefficient for fork length was different from the constant-only model. We calculated classification accuracy as the total number of correct predictions of overwintering history divided by the total number of predictions multiplied by 100.

*Abundance indices.*—We sampled juvenile Chinook salmon at Lower Granite Dam during the 1997 subyearling migration period (6 April–1 November) and the 1998 yearling migration period (27 March–30 April). During these two periods, we randomly diverted fish on a daily basis for 0.5–24 h a day (daily sample rates of 1–100%) from the juvenile bypass system. We counted every Chinook salmon that had an adipose fin. We divided each daily count by the daily sample rate. These expanded daily counts were summed for each migration period to provide expanded seasonal counts.

We used a statistical model of release-recapture data for a single release of fish (Cormack 1964; Skalski et al. 1998) to calculate the percentage ( $\pm$  SE) of juveniles that survived to the Lower Granite Dam that were routed into the juvenile bypass (hereafter, collection efficiency). Data for calculating collection efficiency were providing by tagging groups of fish with passive integrated transponders (PIT tags)(Prentice et al. 1990a). We PIT tagged and released 640 wild subyearling fall Chinook salmon into the free-flowing Snake River from April to July 1997, some of which were detected (e.g., Prentice et al. 1990b) passing Lower Granite Dam during the subyearling migration period. We also PIT tagged and released 9,942 yearling hatchery fall Chinook salmon into the free-flowing Snake River in April 1998, some of which were detected passing Lower Granite Dam during the yearling migration period. We divided the expanded seasonal counts by the estimate of capture efficiency for each respective migration period to provide an index of abundance for each migration period.

We divided the index of abundance for the 1998 yearling migration period at Lower Granite Dam into reservoir-overwintered and stream-overwintered components as follows. We measured fork length on a random sample of the fish that we diverted and counted at Lower Granite Dam. To validate the logistic regression model, we also collected scales on some of these measured fish to confirm overwintering history. We ran the logistic regression model on these validation data, and then ran the model on all the fish measured. We calculated the proportions of the measured fish that were predicted to be reservoir and stream overwintered. These proportions were then multiplied by the index of abundance for the yearling migration period to provide an index of abundance for reservoir-overwintered fish that passed Lower Granite Dam when the juvenile bypass was operated.

When calculating the final abundance index for reservoir overwinter juveniles, we had to account for fish that passed the dam during the winter when the juvenile bypass operations were not operated. Sneva and Connor (in review) found that 66% of the 38 adults collected at Lower Granite Dam during 1998–2001 that were PIT tagged as juveniles, and had a reservoir-overwintered juvenile life history, likely passed downstream during late winter when the juvenile bypass systems were not operated. This indicates that the probability of a fish passing the dam when the juvenile bypass was operated was 0.32 (1.0 - 0.68). We calculated the final estimate

for abundance of reservoir-overwintered juveniles by dividing the index of abundance for reservoir-overwintered fish that passed Lower Granite Dam when the juvenile bypass was operated by 0.32.

## Results

### *Fork Length and Condition Factor*

Wild and hatchery fall Chinook salmon made up the majority of the reservoir-overwintered juveniles collected at Little Goose Dam in 1998, whereas wild spring-summer Chinook salmon made up the majority of stream-overwintered juveniles (Table 1). Juvenile Chinook salmon that overwintered in a reservoir were significantly ( $P < 0.0001$ ) larger than fish that overwintered in a stream (Table 1). Juvenile Chinook salmon that overwintered in a reservoir had significantly ( $P = 0.0012$ ) higher condition factors than juveniles that overwintered in a stream (Table 1).

We selected fork length as the variable for logistic regression modeling because there was less overlap in fork length between reservoir-overwintered fish and stream-overwintered fish than there was in condition factor (Table 1). The logit function fit from fork length data collected at Little Goose Dam was  $g(x) = -60.0711 + 0.3219 \text{ FL}$ . The model with a coefficient for fork length was significantly ( $P < 0.0001$ ) different from the constant-only model. The positive slope coefficient for fork length shows that the probability of a juvenile Chinook salmon having overwintered in a reservoir increased as fork length increased. Classification accuracy was 100%. The model predicted that fish with fork lengths above 187 mm were reservoir overwintered.

Table 1.–Information on juvenile Chinook salmon collected at Little Goose Dam during the 1998 yearling migration period including overwintering history, the number of fish with each overwintering history collected ( $N$ ), origin and run composition (%), mean fork length (mm  $\pm$  SD), and mean condition factor ( $K \pm$  SD) at time of collection. The letters above mean fork length and mean condition factor indicate a significant difference.

| Overwintering history | $N$ | Origin and run composition |               |                    |                        | Fork length    | $K$               |
|-----------------------|-----|----------------------------|---------------|--------------------|------------------------|----------------|-------------------|
|                       |     | Wild fall                  | Hatchery fall | Wild Spring-summer | Hatchery Spring-summer |                |                   |
| Reservoir             | 39  | 62                         | 36            | 0                  | 2                      | $226 \pm 12^A$ | $1.04 \pm 0.09^A$ |
| Stream                | 51  | 6                          | 0             | 94                 | 0                      | $137 \pm 17^B$ | $0.98 \pm 0.08^B$ |

## Abundance Indices

We diverted and counted 26,446 juveniles at Lower Granite Dam in daily samples made during the 1997 subyearling migration period (Table 2). The expanded seasonal count for the 1997 subyearling migration period was 90,770 (Table 2). The estimate of capture efficiency during the 1997 subyearling migration period was  $0.51 \pm 0.04$  SE (Table 2), thus the abundance index for subyearlings in 1997 was 177,981 (Table 2).

We diverted and counted 3,781 juveniles at Lower Granite Dam during the 1998 yearling migration period (Table 2). The expanded seasonal count for the 1998 yearling migration period was 137,641 (Table 2). Estimated capture efficiency was  $0.50 \pm 0.01$  SE, thus the abundance index for the 1997 yearling migration period was 275,282 (Table 2).

We measured fork length on 1,970 juveniles during the yearling migration period (Figure 3), of which scales were taken from 52 fish. The logistic regression model classified overwintering history correctly for all 52 of these fish (reservoir-overwintered,  $N = 22$ ; stream-overwintered,  $N = 30$ ). The logistic regression model predicted that 2.0% or 40 of the 1,970 juveniles measured were reservoir overwintered (Figure 3). We multiplied 275,282 (Table 2) by 0.02 to estimate that 5,507 reservoir-overwintered juveniles passed Lower Granite Dam during the 1998 yearling migration period when the juvenile bypass was operated. Dividing 5,506 by 0.32 (i.e., the probability of a fish passing the dam when the juvenile bypass was operated) gave the final abundance estimate of 17,209.

Adding the abundance index for the 1997 subyearling migration period of 177,981 (Table 2) to the final abundance estimate of 17,209 reservoir-overwintered fish indicated that a total of 195,190 fall Chinook salmon juveniles from brood year 1996 that entered Lower Granite Reservoir during 1997 survived to pass the dam. Of these survivors, 91% (177,981 / 195,190) passed the dam during the 1997 subyearling migration period and 9% (17,209 / 195,190) overwintered in Lower Granite Reservoir and resumed seaward migration in 1998.

Table 2.—Information used to estimate abundance of juvenile Chinook salmon that passed Lower Granite Dam during the subyearling (6 April–1 November 1997) and yearling (27 March–30 April 1998) migration periods including the number of fish diverted and collected during daily sampling, the expanded seasonal count calculated by summing daily counts adjusted for sampling rate, estimated capture efficiency ( $\pm$  SE), and the abundance index calculated by dividing the expanded seasonal count by capture efficiency.

| Migration period | Number of fish diverted and counted | Expanded seasonal count | Capture efficiency | Abundance index |
|------------------|-------------------------------------|-------------------------|--------------------|-----------------|
| Subyearling      | 26,446                              | 90,770                  | $0.51 \pm 0.04$    | 177,981         |
| Yearling         | 3,781                               | 137,641                 | $0.50 \pm 0.01$    | 275,282         |

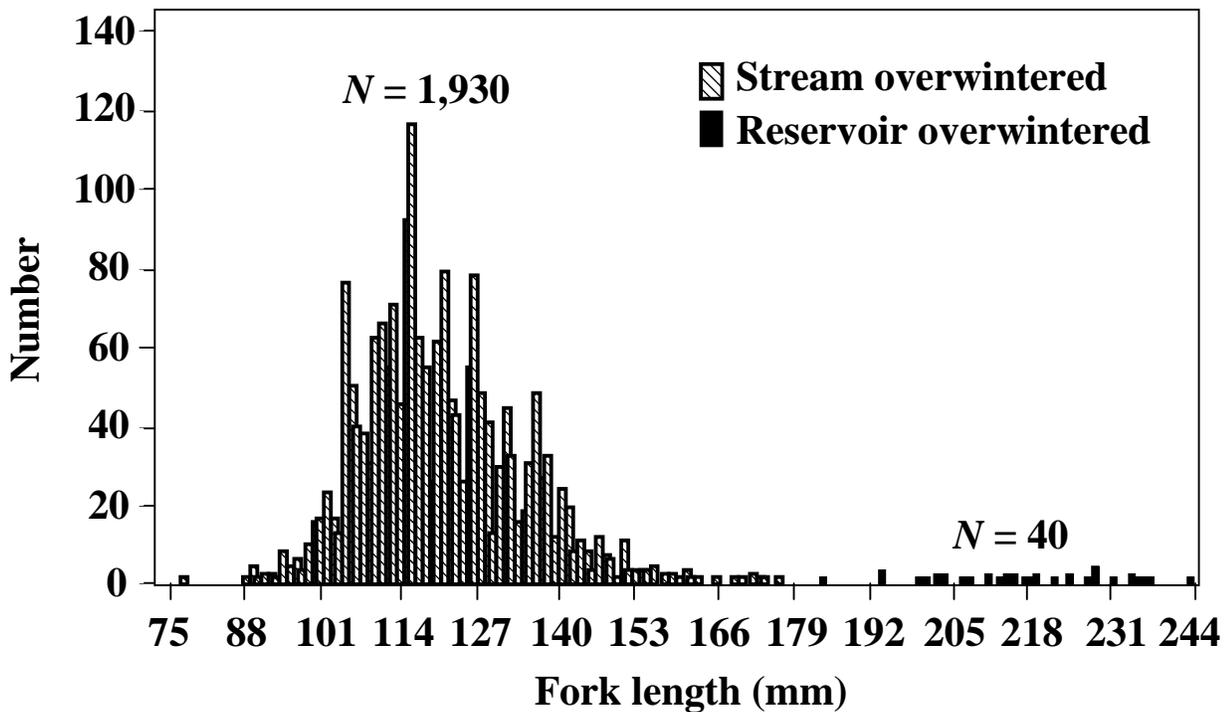


Figure 3.-A fork length distribution for juvenile Chinook salmon diverted and measured at Lower Granite Dam during the 1998 yearling migration period. The fish that were classified as reservoir-overwintered and stream-overwintered by use of logistic regression are indicated.

### Discussion

We were limited to studying the upper two Snake River reservoirs that fall Chinook salmon smolts pass en route to the sea. However, the number of reservoir-overwintered juveniles that pass Snake River dams decreases as fish pass downstream (Figure 2) and few juvenile fall Chinook salmon enter the Columbia River. For example, the probability of a subyearling surviving to pass Lower Granite, Little Goose, and Lower Monumental dams during 1995 to 2001 was approximately 0.50 per dam (Smith et al. in press). The probability of the survivors passing each of these dams via the juvenile bypass system was also near 0.50 (reported in this note; Smith et al. in press). Thus the probability of a fish surviving to pass all three of these dams without entering the juvenile bypass systems was  $0.50^6$  or 0.016 (i.e., 1.6%). All of the subyearlings that are not PIT tagged that enter the juvenile bypass systems are collected and trucked for release downstream of Bonneville Dam (e.g., Ward et al. 1997; Figure 1). Therefore, only 2 out of every 100 run at large subyearlings (i.e., not PIT tagged) that passed the lower Snake River dams during the subyearling migration period had the opportunity to overwinter in reservoirs in the Columbia River provided that they survived to pass Ice Harbor Dam (Figure 1).

In spite of the above limitation on our study, a distinct difference in fork length allowed the accurate identification of overwintering history of juvenile Chinook salmon that passed Lower

Granite and Little Goose dams in the spring of 1998. Reservoir-overwintered fish (predominantly wild and hatchery fall Chinook salmon) were larger in fork length and had higher condition factors than stream-overwintered fish (predominantly spring-summer Chinook salmon). These findings supported our hypothesis that differences in growth caused by rearing history would be evident in the fork length or condition factor of juvenile Chinook salmon when they pass Lower Granite and Little Goose dams.

The differences reported in fork length and condition factor between reservoir-overwintered and stream-overwintered Chinook salmon juveniles were likely caused by the length of the growing season and growth opportunity (e.g., Thorpe 1989; Metcalfe and Thorpe 1990). Temperatures in high-order stream reaches such as the lower Snake River stay relatively warm and stable throughout the year by comparison to low-order streams in the Imnaha, Salmon, and Grande Ronde, and Clearwater river basins (e.g., Vannote et al. 1980; Connor et al. in press) where spring-summer Chinook salmon usually spend their first fall and winter of life. This explanation is consistent with empirical estimates of growth. Subyearling spring-summer and fall Chinook salmon in the lower Snake River grow rapidly by comparison to subyearling and yearling spring-summer Chinook in the aforementioned tributary subbasins (Achord et al. 1996; Connor et al. 2001a, in press b; Connor and Burge 2003).

Of the estimated 195,190 brood year 1996 juveniles that entered Lower Granite Reservoir during 1997 and survived to pass the Lower Granite Dam, only 9% were estimated to have overwintered in the reservoir. Apparently, few juvenile Chinook salmon survive harsh summer conditions (e.g., Connor et al. 1998, 2003b) to overwinter in Lower Granite Reservoir. The survivors, however, might reach the sea at high rates because they pass downstream earlier in the year than subyearlings migrants and are larger than subyearling migrants that average only 122–142 mm fork length (Connor et al. 2001a; Connor and Burge 2003). Others have found that yearling releases of juvenile Pacific salmon have higher adult return rates than subyearling releases (Reisenbichler 1981; Martin and Wertheimer 1989; Bugert et al. 1997). Yearlings probably return at higher rates than subyearlings for two reasons. They pass downstream in Snake River reservoirs during periods of high spill and flow associated with survival levels of 75–98% by comparison to subyearlings that pass during periods of no spill and low flow and survive at levels of 10–90% (Muir et al. 2001a,b; Connor et al. 2003b, in press b; Smith et al. in press). The second reason for higher smolt survival of yearlings is that they are less susceptible to size-selective predation (Poe et al. 1991; Tabor et al. 1993).

We suggest that the indices of abundance reported for brood year 1996 juveniles could be used to generally assess the importance of reservoir-overwintering to adult fall Chinook salmon production. Brood year and juvenile overwintering history of a sample of fall Chinook salmon adults passing Lower Granite Dam during 1999–2002 could be determined based on scale pattern (Sneva and Connor in review). A disproportionate percentage of adults with a reservoir-overwintered juvenile life history (e.g., 9% of the juveniles versus 30% of the adults) would support reservoir-overwintering as an important juvenile life history. The indices of abundance we reported, however, cannot be used to calculate absolute values of smolt-to-adult return rates for fall Chinook salmon because of previously mentioned limitations on our study. Also as

shown in this note and by others (Marshall et al. 2000; Connor et al. 2001b; Tiffan et al. 2001), fall Chinook salmon do not make up 100% of the fish during the subyearling migration or 100% of the reservoir-overwintered juveniles.

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## **CHAPTER FOUR**

## Identification of a Genetic Marker That Discriminates Ocean-Type and Stream-Type Chinook Salmon in the Columbia River Basin

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**Abstract.**—A marker based on randomly amplified polymorphic DNA (RAPD), OT-38, was discovered that nonlethally discriminates between stream-type and ocean-type populations of chinook salmon *Oncorhynchus tshawytscha* in the Columbia River basin, including the threatened fall-run (ocean-type) and spring-run (stream-type) Snake River populations. This marker was developed by amplifying chinook salmon genomic DNA with a single RAPD primer, sequencing the termini of the polymorphic products, and designing primer pairs for allele-specific amplification. It was used to assay 18–80 individuals from several wild and hatchery populations differing in year-class, freshwater life history, and location along the Columbia River. OT-38 unambiguously distinguished ocean-type from stream-type populations in 93.1% of the chinook salmon sampled.

The Columbia River basin has historically supported more chinook salmon *Oncorhynchus tshawytscha* than any other river system in the world (Netboy 1980; Raymond 1988; Utter et al. 1995). Today, chinook salmon populations in this basin are in a state of decline due to habitat loss, overfishing, and obstruction of water flows and fish passage (Raymond 1988; Williams 1988; Schaller et al. 1999). Fish populations are further stressed when water is diverted from rivers for hydropower, irrigation, and human consumption, which are often in conflict with the habitat needs of the fish.

Management of chinook salmon is complicated by the genetic and ecological diversity within and between populations. Chinook salmon have a number of life history traits, such as natal homing (Kristiansson and McIntyre 1976; McIsaac and Quinn 1988; Adams et al. 1994; Danzmann et al.

1994) and the timing of juvenile and adult spawning migrations, that have led to genetic isolation and local adaptation among populations. A number of studies suggest that North American chinook salmon, including those in the Columbia River, can be segregated into two major evolutionary lineages defined by the timing of juvenile migration (Utter et al. 1989; Waples et al. 1991; Utter et al. 1995; Myers et al. 1998; Teel et al. 2000). The ocean-type lineage is comprised of juvenile fish that migrate to estuaries within the first year of life and spend an extended period of time in the estuary before entering the ocean, while stream-type juvenile chinook salmon spend one or more years in their natal rivers before migrating seaward (Healey 1991). Ocean-type chinook salmon tend to be found in smaller coastal rivers and the lower reaches of major rivers, while stream-type fish are found closer to the headwaters of major rivers and predominate in northern waters (Healey 1983). These two lineages are thought to have arisen as a result of geographic isolation during the Pleistocene gla-

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ciation and subsequent recolonization following contraction of the ice sheets (Teel et al. 2000). Temporal differences in adult spawning migrations have also been observed, and these differences have been used to designate salmon runs as spring, summer, fall, or winter.

Early work in the Columbia River drainage using allozymes found highly significant differences between spring- and fall-run chinook salmon (Kristiansson and McIntyre 1976). More recently, allozyme studies have indicated that the fall-run populations found in the Columbia River basin belong to the ocean-type lineage, which also includes spring-run fish from the lower Columbia region (Myers et al. 1998). Spring-run fish from the middle and upper Columbia and Snake rivers belong in the stream-type lineage (Utter et al. 1995). Currently, maximum likelihood estimation (MLE) mixture analysis of 40 allozyme loci is used to distinguish between fall-run (ocean-type) and spring- and summer-run (stream-type) chinook salmon in these rivers (Utter et al. 1995; Marshall et al. 2000).

Management of chinook salmon is further complicated by the fact that threatened and endangered populations coexist with other stable populations within a river system. Thus, the development of nonlethal diagnostic systems that discriminate populations, life histories, gender, and genetic diversity will aid in the management of all chinook salmon populations. Recently, DNA markers have complemented allozymes as attractive tools in salmon management, particularly with threatened and endangered species, because of their less-stringent storage requirements and potentially higher resolution. The goal of this work was to develop DNA markers that can be used nonlethally to assess genetic diversity as well as to discriminate between populations of chinook salmon. In this paper, we describe the use of randomly amplified polymorphic DNA (RAPD) products to identify a genetic marker (OT-38) that discriminates between the major chinook salmon lineages in the Columbia and Snake River basins.

### Methods

*Samples and sample locations.*—Tissue samples from chinook salmon were obtained from the Quinault River on the Olympic Peninsula in Washington State, the Sacramento River in northern California, and nine locations within the Columbia River basin. Fifty-nine fall- and spring-run chinook salmon samples were obtained from the Sacramento River and 24 fall-run samples from the

Quinault River. In all, 591 individuals from wild and hatchery populations differing in year-class, run timing (fall or spring), and location were sampled from the Columbia River basin, including 184 from the threatened fall- and spring-run Snake River populations. Samples were obtained over a 4-year period from 1993 to 1997 from both wild and hatchery locations spanning 522 river km of the Columbia River and 173 river km of the Snake River. These locations are shown in Figure 1A, and a detailed map with the locations in the Columbia River basin from which samples were obtained is given in Figure 1B. As indicated in Table 1, the tissue samples used in this analysis were obtained from individuals that differed in the timing of adult spawning migration, collection year, maturity, number, and stock type (hatchery or wild populations). Tissue was obtained from the fin, muscle, or liver or a portion of whole fry of cultured chinook salmon from the following locations in the Columbia River basin: Abernathy Salmon Culture Technology Center, Carson National Fish Hatchery (NFH), Little White Salmon NFH, Warm Springs NFH, and Lyons Ferry State Fish Hatchery. Cultured chinook salmon samples were also obtained from Quinault NFH and Coleman NFH (Sacramento River, California). Wild chinook salmon samples were obtained below Lower Granite Dam and Little Goose Dam on the Snake River, Washington, from the Tucannon River (a tributary of the Snake River, Washington), and from Warm Springs NFH. Approximately 350 mg of tissue was stored in 500  $\mu$ L lysis buffer (50 mM tris-HCl (pH 8.0), 150 mM EDTA, and 2% Sarkosyl) at 8°C until the DNA was extracted as previously described (Clifton and Rodriguez 1997; Rodriguez 1993).

*Polymerase chain reaction amplification.*—Both single-primer and dual-primer polymerase chain reactions (PCRs) were performed using approximately 20 ng of DNA isolated from individual chinook salmon. A typical 25- $\mu$ L reaction contained 10 mM tris-HCl (pH 9.0), 0.2% Triton x-100, 50 mM KCl, 2.5 mM MgCl<sub>2</sub>, 200  $\mu$ M deoxynucleotide triphosphates, 20 pmol of each primer, and 1 unit of *Taq* polymerase. DNA amplification using RAPD primer 102, 5'-GGTGGGGACT-3' (University of British Columbia Nucleic Acid-Protein Service Unit), was achieved with 35 cycles of denaturation at 93°C for 15 s followed by primer annealing at 44°C for 1.5 min and extension at 72°C for 1.5 min. Ramp or slope steps of 0.4°C/s were included between the denaturation and annealing steps and between the annealing and extension steps. Amplifi-

cation was preceded by denaturation at 93°C for 2 min. Amplification with the dual OT-38 primers, 5'-CCAGTGTGTGGAAGTGTACA-3' and 5'-GC CACTCTCTCACTATCTCC-3', was performed in a similar manner except that the primer annealing temperature was 68°C and no slope steps were included. PCR amplification was performed using either a Thermolyne Amplitron II or Temp-tronic Thermocycler (Barnstead Thermolyne Corp.). Amplification products were resolved by electrophoresis at 100 V on 2% SeaKem LE Agarose (FMC Bioproducts) in 0.5 X TAE (20 mM tris-acetate, 0.5 mM EDTA) for 1.5 h.

*Cloning and sequencing of amplified products.*—PCR-amplified bands were excised from 2.0% agarose gels and the DNA purified using either the GeneClean Kit (Bio 101, Inc.) or the Wizard PCR DNA Purification Kit (Promega, Inc.) according to the manufacturer's instructions. The amplified product was then either sequenced directly or cloned into pT7Blue using the T-Vector cloning kit (Novagen, Inc.) or the pCR II/Topo cloning kit (Invitrogen, Inc.). Plasmid DNA was isolated using the Plasmid Mini kit (Qiagen, Inc.). Plasmids and PCR products were sequenced by the Center for Gene Research (Oregon State University, Corvallis) or using the ABI Prism Dye Terminator Cycle Sequencing Reaction Kit and analyzed on an ABI Prism 310 Genetic Analyzer (Perkin-Elmer, Inc.). Sequences were analyzed using Sequencher (GeneCodes, Inc.), MacVector 6.0 (Oxford Molecular Group), and BLAST (Altschul et al. 1990).

## Results

### *Development of OT-38*

Total genomic DNA was extracted from each individual fish, and a subsample of the populations listed in Table 1 were subjected to PCR amplification with approximately 100 different RAPD primers. The RAPD primer 102 (p102) amplified DNA products that were polymorphic within chinook salmon populations. As shown in Figure 2A, amplification with p102 produced bands in the size range of 0.9–1.2 kilobases (kb). Several of these polymorphic bands were excised from agarose gels, cloned, and sequenced. Using the sequence data (see below), we developed a dual-primer set, referred to as pOT-38, for marker-specific amplification. PCR amplification with pOT-38 produced several bands in the 0.9–1.2 kb size range similar to the polymorphic bands produced using the RAPD primer p102 (Figure 2B). Preliminary se-

quence data led us to believe that the pOT-38 amplification products were alleles of the same locus. However, PCR amplification of several individuals produced three bands instead of the expected one or two bands (see Figure 2B, lanes 2, 5, 6, and 9). We suspect that these products were artifacts generated by the annealing of the different-length variants to each other after PCR amplification.

This hypothesis was tested by mixing PCR products from two individuals with two different single-length variants (genotypes Fa1 and Fa2; see Table 2) and performing a PCR amplification in the absence of *Taq* polymerase. The results indicated that allelic-length variants were indeed responsible for the larger additional band (data not shown). The production of electromorphs or non-parental RAPD bands due to length variation in alleles has been reported (Nagamine et al. 1989; Ayliffe et al. 1994). Several protocols were used in an attempt to eliminate these bands, including (1) heating the PCR reaction at 95°C for 10 min at the end of the amplification and then very slowly cooling the reaction to 15°C in the thermal cycler, (2) boiling the PCR reaction in a water bath for 5–10 min and then allowing it to cool to room temperature very slowly in the water bath, and (3) incubating the PCR reactions at room temperature overnight. None of these methods resolved the electromorphs. Cloning of the PCR reaction products from individuals with electromorphs resulted in only two distinct clones, again suggesting that the third band was due to the annealing of length variants of the OT-38 alleles.

### *Discrimination of Chinook Salmon Lineages by OT-38*

A 2% agarose gel loaded with pOT-38 PCR amplification products from 10 fall (ocean-type) chinook salmon from the Abernathy Salmon Culture Technology Center and 10 spring (stream-type) fish from Carson NFH is shown in Figure 3. The size difference between the spring and fall OT-38 alleles is evident. Analysis of all the chinook salmon samples listed in Table 1 revealed that although the OT-38 locus had a number of alleles, the size differences in the amplification products appeared to discriminate between the ocean- and stream-type lineages. We detected four stream-type genotypes, four ocean-type genotypes, and eight rare genotypes that we were unable to confidently classify. The four ocean-type genotypes ranged in size from 920 to 1,074 base pairs (bp) and the four stream-type genotypes from 1,113 to 1,152 bp. These genotypes were distributed between wild

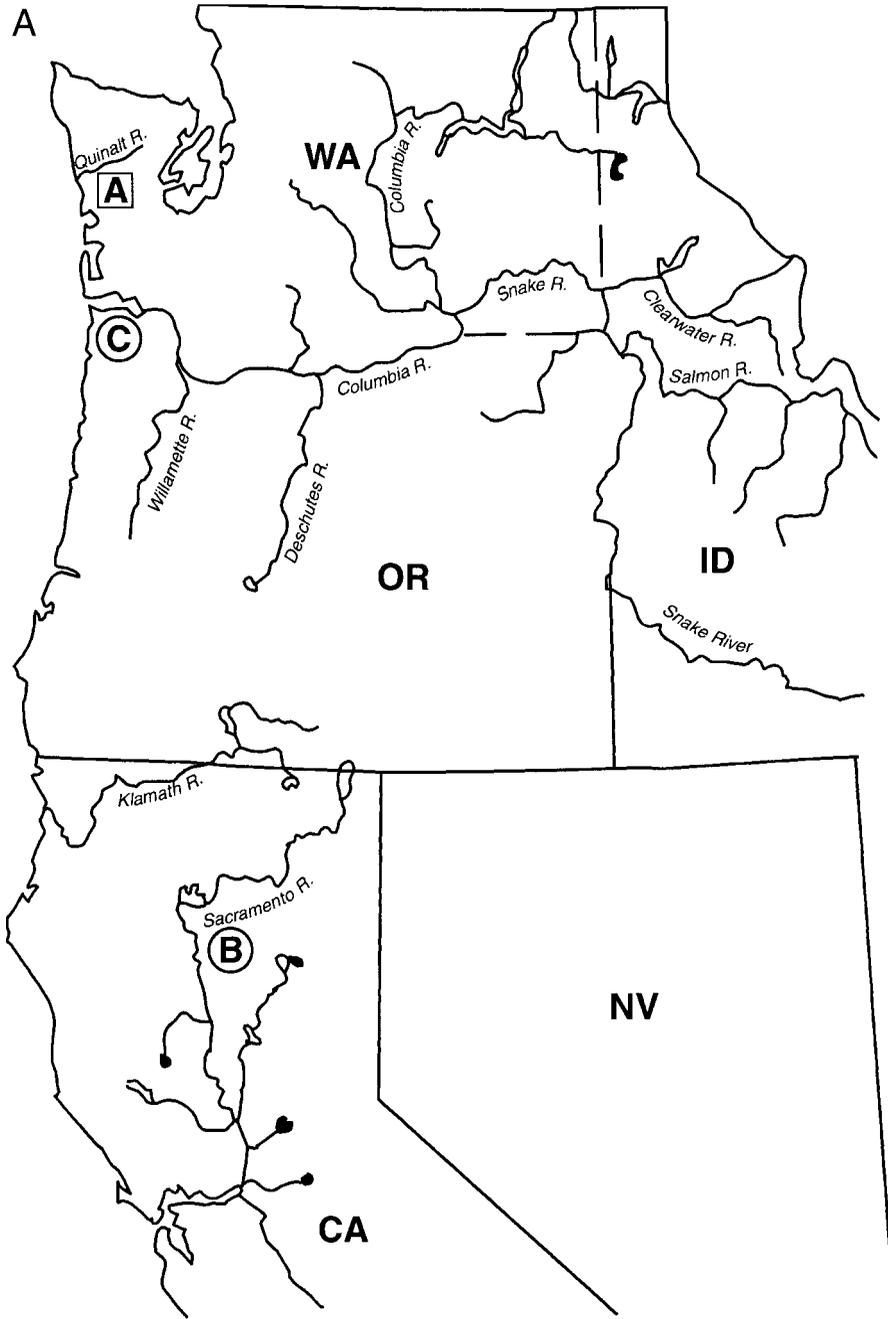


FIGURE 1.—Geographic locations from which chinook salmon samples were taken in western river basins (see Table 1 for detailed information on these populations). In panel (A), the boxed A indicates fall-run chinook salmon samples obtained from the Quinalt National Fish Hatchery (NFH), the circled B indicates fall- and spring-run samples obtained from Coleman NFH, and the circled C indicates spring and fall samples obtained from a number of locations within the Columbia River basin. The enclosed numbers in panel (B) correspond to sample locations in Table 1. Numbers within boxes indicate fall samples, numbers within triangles spring samples, and numbers within circles both fall and spring samples from these locations.

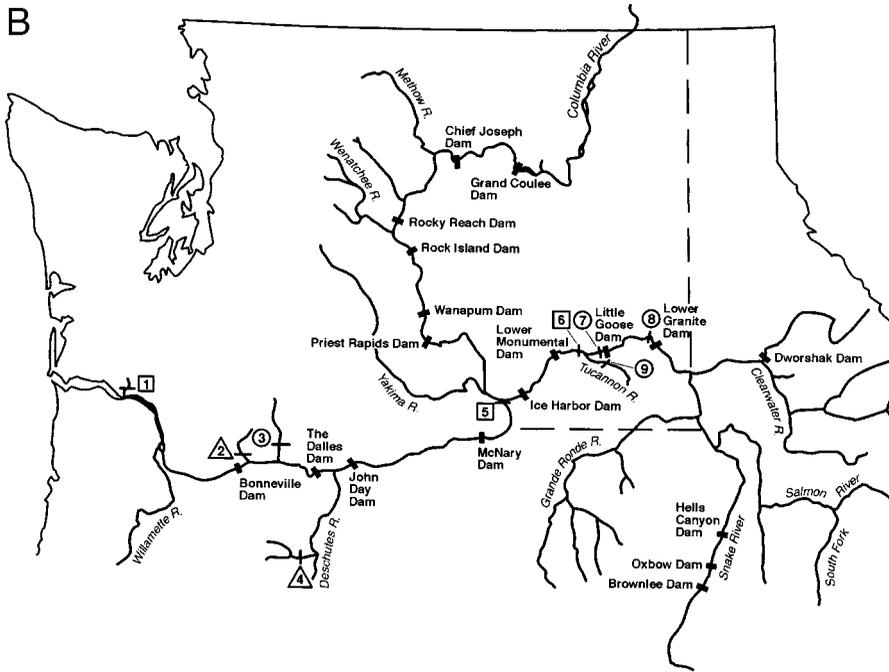


FIGURE 1.—Continued.

TABLE 1.—Descriptions of the chinook salmon populations that were sampled. The letters A, B, and C correspond to geographic locations shown in Figure 1A. Samples obtained from the Columbia River basin (indicated by the letter C) were given numbers corresponding to geographic locations shown in Figure 1B. The abbreviation NFH stands for National Fish Hatchery. The column headed by *N* shows the total number of individuals assayed with the OT-38 primer set. The asterisk indicates that the Snake and Tucannon River samples comprised a mixed population containing both fall and spring chinook salmon; for simplicity, the Tucannon River samples include the two spring and three fall hatchery individuals added by the Washington Department of Fish and Wildlife. The OT-38 column shows the number of individuals that produced OT-38 genotypes.

| Sampling location                              | Year | Run    | Stock type | Maturity  | <i>N</i> | OT-38 |
|--|------|--------|------------|-----------|----------|-------|
| C1. Abernathy Salmon Culture Technology Center | 1995 | Fall   | Cultured   | Adults    | 31       | 28    |
| C2. Carson NFH                                 | 1994 | Spring | Cultured   | Adults    | 82       | 79    |
| C3. Little White Salmon NFH                    | 1996 | Fall   | Cultured   | Juveniles | 20       | 19    |
| C3. Little White Salmon NFH                    | 1996 | Spring | Cultured   | Juveniles | 20       | 20    |
| C4. Warm Springs NFH                           | 1996 | Spring | Cultured   | Adults    | 76       | 70    |
| C4. Warm Springs NFH                           | 1996 | Spring | Wild       | Adults    | 18       | 17    |
| C5. Hanford Reach, Columbia River              | 1996 | Fall   | Wild       | Juveniles | 81       | 79    |
| C6. Lyons Ferry State Fish Hatchery            | 1996 | Fall   | Cultured   | Juveniles | 79       | 77    |
| C7. Little Goose Dam                           | 1997 | Fall   | Wild       | Juveniles | 41       | 41    |
| C7. Little Goose Dam                           | 1997 | Spring | Wild       | Juveniles | 82*      | 28    |
| C8. Lower Granite Dam                          | 1993 | Fall   | Wild       | Juveniles | 16       | 16    |
| C8. Lower Granite Dam                          | 1993 | Spring | Wild       | Juveniles | 26       | 26    |
| C8. Lower Granite Dam                          | 1994 | Fall   | Wild       | Juveniles | 17       | 17    |
| C8. Lower Granite Dam                          | 1994 | Spring | Wild       | Juveniles | 33*      | 13    |
| C9. Tucannon River                             | 1997 | Fall   | Wild       | Juveniles | 7        | 7     |
| C9. Tucannon River                             | 1997 | Spring | Wild       | Juveniles | 20*      | 13    |
| A. Quinault NFH                                | 1994 | Fall   | Cultured   | Adults    | 24       | 24    |
| B. Coleman NFH                                 | 1994 | Spring | Wild       | Juveniles | 29       | 29    |
| B. Coleman NFH                                 | 1993 | Fall   | Cultured   | Adults    | 30       | 28    |



TABLE 3.—PCR amplification products of stream-type chinook salmon from the Columbia River basin. Genotypes Sp1 through Sp4 were considered scorable; the remaining genotypes are likely stream type. See Table 2 for additional details.

| Sampling location       | Year | Run    | N  | Genotype and PCR product lengths |                 |                           |                           |                         |                         |                                   |
|-------------------------|------|--------|----|----------------------------------|-----------------|---------------------------|---------------------------|-------------------------|-------------------------|-----------------------------------|
|                         |      |        |    | Sp1<br>(1,113*)                  | Sp2<br>(1,152*) | Sp3<br>(1,113/<br>1,151*) | Sp4<br>(1,050/<br>1,100@) | Sp5<br>(900/<br>1,100/) | Sp6<br>(950/<br>1,100/) | Sp7<br>(950/<br>1,050/<br>1,300@) |
| Carson NFH              | 1994 | Spring | 82 | 42                               | 6               | 31                        |                           | 3                       |                         |                                   |
| Little White Salmon NFH | 1996 | Spring | 20 | 14                               |                 | 6                         |                           |                         |                         |                                   |
| Warm Springs NFH        | 1996 | Spring | 76 | 46                               |                 | 24                        |                           |                         | 4                       | 2                                 |
| Warm Springs NFH        | 1996 | Spring | 18 | 11                               | 1               | 5                         |                           |                         | 1                       |                                   |
| Little Goose Dam        | 1997 | Mixed  | 82 | 16                               | 3               | 8                         | 1                         | 4                       |                         |                                   |
| Lower Granite Dam       | 1993 | Mixed  | 49 | 16                               | 3               | 7                         |                           |                         | 1                       |                                   |
| Lower Granite Dam       | 1994 | Mixed  | 33 | 5                                | 1               | 7                         |                           |                         | 1                       |                                   |
| Tucannon River          | 1997 | Mixed  | 20 | 8                                | 3               | 2                         |                           |                         |                         |                                   |

lack of representation in hatchery populations of fall-run fish. Therefore, a total of six fish from fall-run hatchery populations could not be unequivocally classified as ocean-type chinook salmon.

The most common stream-type genotypes (Sp1, Sp2, and Sp3) comprised more than 99% of the classifiable stream-type individuals assayed, while a fourth rare genotype (Sp4) accounted for the remaining pattern. The additional patterns listed in Table 3 are likely stream type but due to size ambiguity and the rarity of these genotypes in spring-run hatchery populations they could not be confidently labeled as such. This resulted in nine fish from spring-run hatchery populations that could not be unambiguously assigned to a lineage. In addition, 26 wild fish from mixed spring and fall runs had ambiguous genotypes. More samples of these rare genotypes from hatchery and wild populations of known run timing need to be obtained to allow them to be categorized and to determine whether there is overlap between the stream- and ocean-type pOT-38 amplification products. Once they have been categorized, sequencing of these rare genotypes may also be helpful in establishing patterns for lineage discrimination.

Although pOT-38 amplification products clearly distinguished ocean- and stream-type populations in the Columbia River basin, we could not determine whether OT-38 distinguished between ocean- and stream-type chinook salmon from other river systems. Limited sampling of chinook salmon from the Sacramento River, where all populations exhibit an ocean-type lineage (Myers et al. 1998), indicated that 29 spring-run fish had two ocean-type alleles while 28 of 30 fall-run individuals exhibited the same two ocean-type alleles (data not shown). The pOT-38 amplification products were highly polymorphic in the Quinault River

fall-run population, where nine different alleles were observed. However, there was no clear pattern or frequency distribution segregating the pOT-38 amplification products into ocean- or stream-type patterns since the alleles were a mixture of those determined to be ocean- and stream-type in the Columbia basin. More extensive sampling will be needed to determine whether OT-38 can distinguish ocean- and stream-type lineages throughout the North American range of chinook salmon.

#### Segregation Analysis Using OT-38

To determine whether the OT-38 alleles segregated in a Mendelian fashion, progeny were obtained from hatchery matings between fall (ocean-type) parents and between spring (stream-type) parents. Segregation analysis was performed on 22–24 progeny from three fall crosses and three spring crosses. The parental OT-38 genotypes and an example of each progeny type are shown in Figure 4A and B. Goodness-of-fit (chi-square) tests indicated that OT-38 alleles segregated in a Mendelian fashion in all crosses (data not shown). No significant ( $P < 0.05$ ) departures from expected values were observed.

#### Comparison of Allozyme, Fork Length Measurement, and OT-38 Run Discrimination Methods

The Snake and Tucannon River samples were collected from chinook salmon populations containing both stream- and ocean-type fish. Classification of 15 wild chinook salmon samples from the Tucannon River was based on fork length measurements taken by the Washington Department of Fish and Wildlife (WDFW) hatcheries program (Joe Bumgarner, personal communication). Three of the 15 fish had fork lengths that could not be

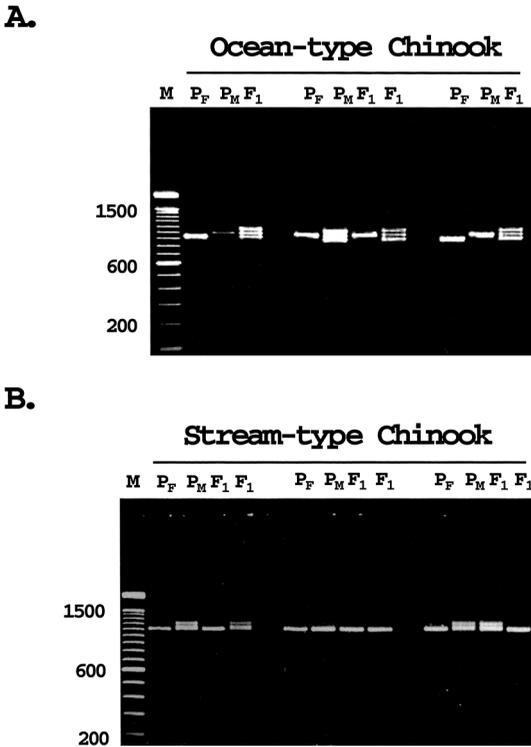


FIGURE 4.—Mendelian inheritance of pOT-38 amplification products. Panel (A) shows the amplification products of fall (ocean-type) chinook salmon parents and their progeny, panel (B) the amplification products of spring (stream-type) parents and their progeny. In each of the three crosses shown, the parental genotypes are indicated in the lanes marked with a P ( $P_F$  = female,  $P_M$  = male). Examples of the genotypes of the progeny are shown in the lanes marked  $F_1$ . The numbers on the left indicate the sizes of selected bands from the Gibco-BRL 100-base-pair ladder shown in lane M.

accurately used for run determination. We compared the results of the fork length analysis with those using pOT-38 amplification. Our results were in complete agreement on a fish-by-fish basis (Table 4). Moreover, the OT-38 marker showed that the three samples that could not be classified by fork length analysis were spring-run (stream-type) chinook salmon. Furthermore, in a blind test of OT-38, WDFW placed five additional fish, two spring hatchery fish and three fall hatchery fish, in the 1997 Tucannon River samples to test our method of run discrimination. Using OT-38, the three fall hatchery fish were found to have an ocean-type pattern and the two spring hatchery fish amplified stream-type alleles.

Juvenile chinook salmon samples from the Snake River were assayed by WDFW using allozymes. Snake River fish can be putatively assigned to fall or spring lineages based on their paired genotypes for two allozyme loci, *MEP1* and *PGK2* (Marshall et al. 2000). Although this initial discrimination works reasonably well, it is not completely accurate and therefore is followed by maximum likelihood estimation mixture analysis using baseline data from 40 allozyme loci obtained from Upper Columbia and Snake River populations collected over a number of years (Marshall et al. 2000; Utter et al. 1995). We compared the results from using OT-38 with those of the allozyme analysis performed by WDFW on the same 161 individuals. The total number of individuals assayed and the number of fish determined to be spring or fall by both methods are shown in Table 4. Again, our results are very much in agreement on a fish-by-fish basis. Of the 161 juvenile chinook salmon that were compared, only 6 individuals (3.7%)

TABLE 4.—Comparison between OT-38 and Washington Department of Fish and Wildlife (WDFW) determinations of spring and fall chinook salmon. The WDFW performed MLE mixture analysis on the same individuals from the Snake River and fork length analysis on the same individuals from the Tucannon River. All samples contained both spring and fall fish;  $N$  indicates the total number of individuals assayed. Unscorable fish were not included in tallying the disagreement between the run discrimination methods.

| Location                 | $N$             | OT-38 |        | WDFW analysis |        | Disagreement between methods |
|--------------------------|-----------------|-------|--------|---------------|--------|------------------------------|
|                          |                 | Fall  | Spring | Fall          | Spring |                              |
| Snake River              |                 |       |        |               |        |                              |
| Lower Granite Dam (1993) | 49 <sup>a</sup> | 16    | 26     | 23            | 26     | 0                            |
| Lower Granite Dam (1994) | 33 <sup>b</sup> | 17    | 13     | 18            | 15     | 0                            |
| Little Goose Dam (1997)  | 82 <sup>c</sup> | 41    | 28     | 49            | 33     | 6                            |
| Tucannon River (1997)    | 20 <sup>d</sup> | 7     | 13     | 7             | 10     | 0                            |

<sup>a</sup> Seven fish were unscorable by OT-38.

<sup>b</sup> Three fish were unscorable by OT-38.

<sup>c</sup> Thirteen fish were unscorable by OT-38.

<sup>d</sup> Three fish were unscorable with fork length analysis.

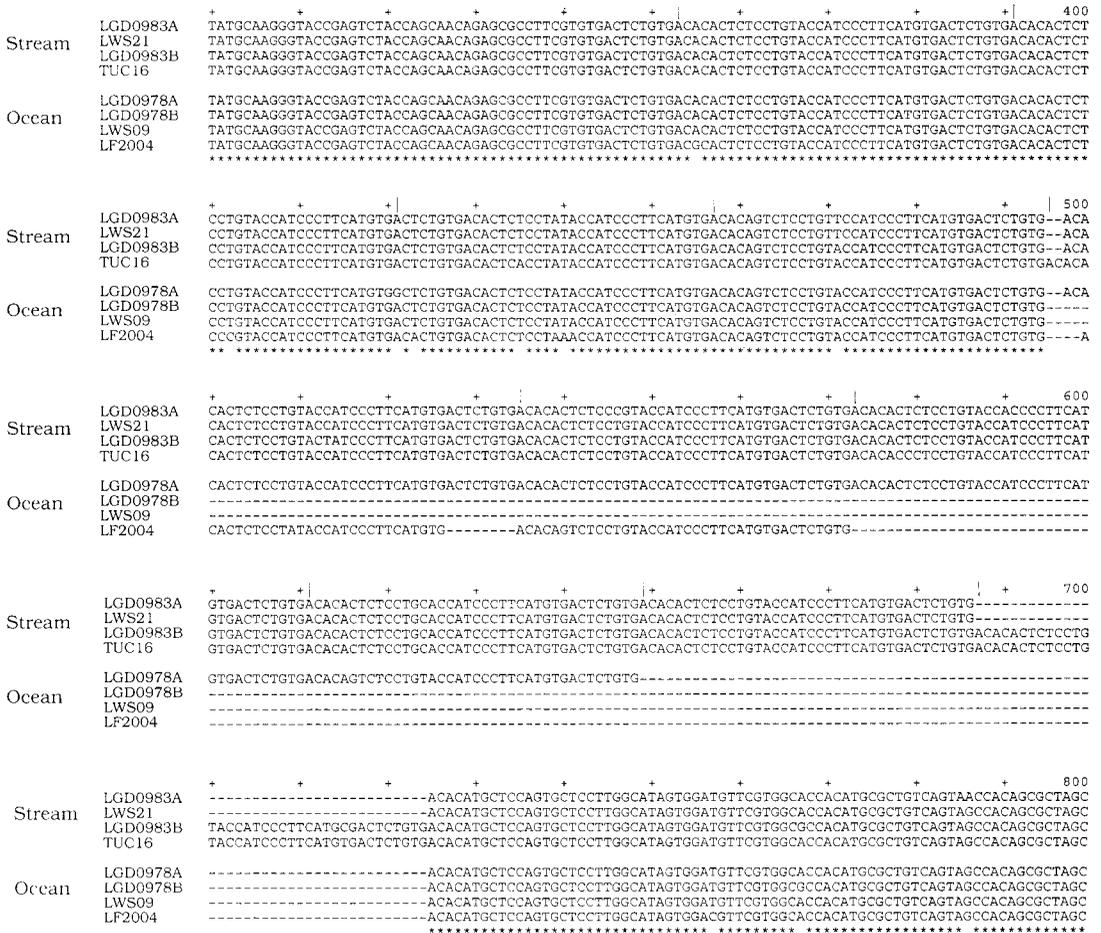


FIGURE 5.—Alignment of the common OT-38 stream- and ocean-type chinook salmon alleles. Clustal W alignment (Higgins et al. 1992) of the common spring and fall OT-38 alleles using default settings and gap-opening penalties of 10. This alignment shows the internal and flanking nucleotide regions where the stream- and ocean-type sequences differ substantially from each other. Stream-type sequences for Sp1, Sp2, and Sp3 are from individuals sampled from Little White Salmon NFH (LWS21; GenBank accession no. AF387898), the Tucannon River (Tuc16; AF387897), and Little Goose Dam (LGD0983 A and B alleles; AF388305 and AF388306), respectively. Ocean-type sequences for Fa1, Fa2, and Fa3 are from individuals sampled from Little White Salmon NFH (LWS09; AF387899), Lyons Ferry (LF2004; AF388302), and Little Goose Dam (LGD0978 A and B alleles; AF388303 and AF388304), respectively. The asterisks underneath the aligned sequences indicate identity. The vertical lines above the sequence indicate the positions of the 38-base-pair repeats. The plus symbol is positioned every 10 nucleotides starting at position 300 in the sequence alignment. The complete alignment is available at <http://biology.usgs.gov/wfrc/pubs2003.htm>.

were scored differently. If rare alleles were observed upon amplification with pOT-38 or a fish had an allozyme genotype that was ambiguous for race according to MLE analysis, we did not include it in our analysis.

*Sequence Analysis of OT-38 Ocean- and Stream-Type Alleles*

The pOT-38 amplification products from the three most common ocean- and stream-type chi-

nook salmon genotypes were sequenced in their entirety. In addition, we sequenced the 5' and 3' ends of the less-common alleles from both wild and hatchery individuals when available. Aligned sequences (data not shown) indicated that OT-38 primers were amplifying the same locus in the different individuals. Figure 5 shows the alignment of the internal and flanking region of the three most common ocean-type alleles and the three most common stream-type alleles where the sequences differ

substantially from each other. (For the complete alignment of the major alleles see the U.S. Geological Survey Web site <http://biology.usgs.gov/wfrc/pub2003.htm>.) The complete alignment shows that both sequences are nearly identical (98.2–99.7%) with the size polymorphisms and that this is largely due to differences in the number of 38-bp repeat units present in the internal region of the pOT-38 amplification products. The ocean-type alleles contain 3–7 of these repeat units and the stream-type alleles 8–9 copies. In addition, each sequence contains a 28-bp sequence that is similar to the 38-bp repeat but with a 10-bp deletion. The sequence of all the pOT-38 amplification products showed numerous stop codons in all reading frames, suggesting that the OT-38 primers do not amplify a coding region. Blast searches were performed using these sequences, and no significant similar sequences were found in the GenBank database.

### Discussion

The OT-38 marker was developed by PCR amplification of chinook salmon genomic DNA with a single RAPD primer and sequencing of the termini of the polymorphic products to obtain primer pairs for allele-specific amplification. This technique of converting single-primer to dual-primer PCR has been used to develop other markers, including a quantitative DNA marker that discriminates sex in chinook salmon (Clifton and Rodriguez 1997) and species-specific markers for rainbow trout *O. mykiss* and cutthroat trout *O. clarki* (Ostberg and Rodriguez 2002), which suggests that this is a valuable technique for developing functional markers.

OT-38 is a single locus that nonlethally discriminates between ocean- and stream-type chinook salmon in the Columbia River basin, including the threatened fall- and spring-run Snake River populations. However, due to the rarity or lack of representation of certain alleles in hatchery populations, 41 individuals (6.9% of the total sample) had genotypes that could not be clearly identified. More sampling from hatchery and wild populations of known juvenile and adult migration timing may result in samples that contain more individuals with these rare alleles and enable us to definitively label these rare genotypes as stream or ocean type.

OT-38 was used to distinguish between ocean- and stream-type wild chinook salmon from the Snake and Tucannon rivers that were also assayed by WDFW using MLE mixture analysis of 40 al-

lozyme loci (Utter et al. 1995; Marshall et al. 2000). Our results are very much in agreement with those obtained independently by WDFW; of the 161 juvenile chinook salmon assayed, only 6 (3.7%) were scored differently. The few differences that we found may be due to the ambiguity of some of the genotypes observed in the samples obtained for allozyme analysis (A. Marshall, WDFW, personal communication). For instance, in the 1997 Little Goose Dam samples, an individual determined to be spring run by DNA analysis was scored as fall run by allozyme analysis. Allozyme analysis also showed that this individual had a rare allele at a particular locus that had not been seen in previous Snake River juvenile samples, suggesting that it was a stray from another river system. Chinook salmon straying from other river systems into the Columbia River basin could confound the allozyme or OT-38 DNA analysis. The straying of fish from other river systems is thought to be uncommon, but one fish that reared in the Trinity River in California and another in the Cole River in Oregon have strayed up the Columbia and Snake rivers to the Dworshak hatchery in Idaho (R. Roseberg, Dworshak Fisheries Complex, personnel communication). Similar ambiguities in DNA analysis could also be responsible for the differences produced by our two methods.

The OT-38 DNA analysis and the MLE mixture analysis of 40 allozyme loci are in agreement for 96.3% of the chinook salmon samples scored, suggesting that the methods have equal discriminating power. However, PCR amplification of the OT-38 locus is a rapid method that requires only small amounts of tissue that can be obtained nonlethally. Although recent work has shown that a number of allozymes can be sampled nonlethally from fin tissue (Van Doornik et al. 1999), some allozymes (including *MEPI*) require greater amounts of tissue, making it difficult to sample juvenile fish in a nonlethal manner (A. Marshall, WDFW, and D. Van Doornik, National Marine Fisheries Service, personnel communications). There was no difference in the PCR amplification quality of pOT-38 when DNA samples were obtained by lethal sampling of whole fry or muscle or liver tissue as opposed to our method of using fin clips.

Sequence analysis suggested that the pOT-38 amplification products are alleles of a single locus that do not have significant similarities to sequences in the GenBank database or contain an obvious coding region. Evidence that the phenotypic differentiation between ocean- and stream-type chinook salmon populations has a genetic basis was

provided by progeny obtained from crosses between ocean- and stream-type fish (Clarke et al. 1992; Clarke et al. 1994). The results of these experiments were consistent with Mendelian inheritance of a single locus with two alleles: a dominant allele that is responsible for the photoperiod-independent parr-smolt transformation of fall chinook salmon and a recessive allele that appears to control the photoperiod-dependent parr-smolt transformation of spring chinook salmon.

Size differences in OT-38 appeared to distinguish between the ocean- and stream-type lineages in the Columbia River basin. However, our sample size was limited and did not include spring-run chinook salmon populations from the lower Columbia River. (Although some spring-run samples obtained for this analysis were from lower Columbia hatcheries [Carson NFH and Little White Salmon NFH], the broodstocks of these hatcheries originated from middle and upper Columbia stocks [Wahle and Chaney 1981; Howell et al. 1984].) Spring-run populations from the lower Columbia, including the Cowlitz, Kalama, Lewis, and Willamette rivers, have been shown to belong to the ocean-type lineage (Myers et al. 1998). More sampling in the Columbia basin (including that of spring-run populations in the lower Columbia River) and in other river systems throughout the North American range of chinook salmon will determine how accurately OT-38 discriminates between the ocean- and stream-type lineages.

Nevertheless, the OT-38 marker appears to be a valuable tool for the management of the Columbia and Snake River chinook salmon stocks. The viability and recovery of salmon populations depend on life history characteristics such as body size, age structure, run timing, fecundity, and some behaviors. For the recovery of fish stocks to succeed, fisheries managers need to know where and when particular populations reside in the tributaries, main stems, and estuaries of particular rivers. The noninvasive OT-38 marker can be used to determine the life history and habitat use of juvenile and adult stream- and ocean-type chinook salmon within the Columbia River basin. Once we have determined the stream and main-stem utilization by different lineages of chinook salmon, we can implement water flow strategies that are suited to the populations residing in these habitats at particular times of the year and thus assist the recovery of populations in danger of extinction. As chinook salmon populations decrease, the need for nonlethal methods of assaying important population parameters for management is increasingly

necessary. OT-38 can be used to nonlethally assess run timing in ocean- and stream-type chinook salmon from the Columbia River basin and thereby provide fisheries managers with a tool to more effectively preserve and manage these fish.

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