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Fish Ecology Division

Northwest Fisheries Science Center

National Marine Fisheries Service

Seattle, Washington

by
Eric E. Hockersmith, Richard S, Brown, and Theresa L. Liedtke

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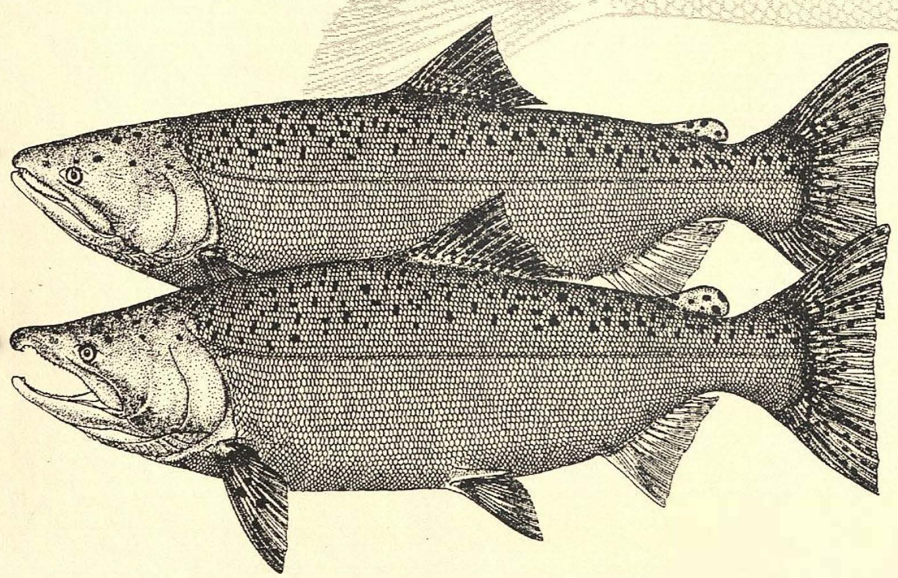

Comparative Performance of Acoustic-Tagged and Passive Integrated Transponder-Tagged Juvenile Salmonids

Eric E. Hockersmith,[1] Richard S. Brown,[2] and Theresa L. Liedtke[3]

Report of research by

[1]Fish Ecology Division
Northwest Fisheries Science Center
National Marine Fisheries Service
National Oceanic and Atmospheric Administration
2725 Montlake Boulevard East
Seattle, Washington 98112

[2]Ecology Group
Pacific Northwest National Laboratory
P.O. Box 999, MS K6-85
Richland, Washington 99352

[3]Western Fisheries Research Center
Columbia River Research Laboratory
U.S. Geological Survey
5501A Cook-Underwood Road
Cook, Washington 98605-9717

for

Environmental Resources Branch
Planning and Engineering Division
Portland District
U.S. Army Corps of Engineers
Robert Duncan Plaza
333 S.W. 1st Avenue
Portland, Oregon 97208-2946
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PREFACE

Numerous research tools and technologies are currently being used to evaluate fish passage and survival to determine the impacts of the Federal Columbia River Power System (FCRPS) on endangered and threatened juvenile salmonids. Among these are the PIT tag, balloon tag, hydroacoustic evaluation, radio telemetry, and acoustic telemetry. Each has advantages and disadvantages, but options are restricted in some situations because of limited capabilities of a specific technology, lack of detection capability downstream, or availability of adequate numbers of fish. In these situations, alternative telemetry technologies have been used to evaluate passage behavior and estimate survival. However, there remains concern about the effects of different tags or tagging procedures on fish performance.

The recently developed Juvenile Salmonid Acoustic Telemetry System (JSATS) transmitter is approximately 40% smaller than transmitters previously available to researchers throughout the Columbia River Basin. The JSATS acoustic transmitter measures 13-17 mm long × 5-6 mm wide and tapers from 4 to 2 mm high. The tag weighs 0.60-0.66 g, and its coding method provides over 65,000 individual tag codes.

In addition to its small size, the acoustic tag does not require the trailing antenna associated with radio transmitters, which may affect swimming performance and survival. Determining whether fish tagged with a JSATS acoustic tag can provide unbiased estimates of passage behavior and survival within the performance life of the tag is important to regional managers.

Studies conducted in 2002 and 2003 evaluated the effects of the JSATS acoustic tag on predator avoidance, growth, mortality, and tag expulsion in a laboratory setting for up to a 30-d period (McComas et al. 2007). These studies found that growth and survival were similar between JSATS acoustic-tagged juvenile Chinook salmon and controls. However, effects of the JSATS tag on fish performance have not been evaluated in the field, and tag effects have not been evaluated for periods longer than 30 d.

To provide additional insight on potential JSATS tag effects, a multi-agency collaborative study involving both field and laboratory evaluations was undertaken during 2006. Field work was headed by the National Marine Fisheries Service (NMFS), and laboratory evaluations by Pacific Northwest National Laboratories (PNNL) and the U.S. Geological Survey (USGS). The NMFS and PNNL compared survival and behavior of acoustic-tagged yearling Chinook salmon to those tagged with PIT tags as they migrated through the FCRPS. Separate laboratory studies by PNNL and USGS were conducted concurrently with the field work to evaluate the effects of acoustic tags on tissue response and tag loss for periods of up to 90 d. In addition, the USGS conducted

laboratory evaluations of predator avoidance, and PNNL evaluated tag effects on growth and mortality and the minimal fish sizes appropriate for implantation of the JSATS tag.

This document contains four individual reports detailing each of these studies. Each report begins with a summary of the major findings, and a collective summary of major findings from all studies is presented in a single section, conclusions and recommendations. Results of this work will aid in determining the suitability of the JSATS acoustic tag to estimate short- and longer-term (30 to 90 d) survival of juvenile salmonids through Columbia and Snake River reservoirs and dams and through the Columbia River below Bonneville Dam.

McComas, R. L., L. G. Gilbreath, S. G. Smith, G. M. Matthews, and J. W. Ferguson. 2007. A study to estimate salmonid survival through the Columbia River estuary using acoustic tags, 2005. Report of the National Marine Fisheries Service to the U.S. Army Corps of Engineers.

CONTENTS

FIELD EVALUATION OF ACOUSTIC TELEMETRY TAGS IN JUVENILE SALMONIDS

Eric E. Hockersmith,[1] Richard S. Brown,[2] R. Lynn McComas,[1] Brian J. Bellgraph,[2] Benjamin P. Sandford,[1] Jessica A.Vucelick,[2] Brad Ryan,[1] and Geoffrey A. McMichael[2]

Report of research by

[1]Fish Ecology Division
Northwest Fisheries Science Center
National Marine Fisheries Service
National Oceanic and Atmospheric Administration
2725 Montlake Boulevard East
Seattle, Washington 98112

and

[2]Ecology Group
Pacific Northwest National Laboratory
P.O. Box 999, MS K6-85
Richland, Washington 99352

Executive Summary

The goal of this study was to determine whether fish tagged with the Juvenile Salmonid Acoustic Telemetry System (JSATS) tag could provide unbiased estimates of passage behavior and survival within the performance life of the tag. We conducted field studies to assess tag effects using hatchery reared Snake River spring/summer Chinook salmon *Oncorhynchus tshawytscha*. Tag effects were also evaluated in cooperative laboratory studies by the Pacific Northwest National Laboratory and the U.S. Geological Survey.

For the field evaluation, we released a total of 996 acoustic-tagged fish in conjunction with 21,026 PIT-tagged fish into the tailrace of Lower Granite Dam on 6 and 13 May 2006. The acoustic tags were 16.9 mm in length, 5.5 mm in diameter, and weighed 0.66 g in air (an average of 2.7% of the fish weight). A PIT tag was inserted along with the acoustic tag at the time of tagging. Travel times, detection probabilities, and survival were estimated from PIT-tag detections of individual fish at Little Goose, Lower Monumental, McNary, John Day, and Bonneville Dams. Migration rates, detection probabilities, survival, and avian predation rates were compared between fish tagged with both a JSATS and PIT tag and those tagged with only a PIT tag.

Travel times between release and downstream dams were not significantly different between acoustic-tagged and PIT-tagged fish for the majority of reaches evaluated. For fish released on 6 May, we observed some significant differences in travel times; however, these differences were generally 1 d or less and may have been related to sample sizes of acoustic-tagged fish. PIT-tag detection probabilities for acoustic- and PIT-tagged fish were similar, and differences were 2% or less. Estimated survival was not statistically different among tag types between the tailrace of Lower Granite Dam and downstream sites except in the first reach (Lower Granite Dam tailrace to Little Goose Dam tailrace) where acoustic-tagged fish had higher survival than PIT-tagged fish. Avian predation rates were similar between acoustic-tagged and PIT-tagged fish.

Introduction

In recent years, radio and acoustic transmitters have been miniaturized sufficiently for use in smaller fish such as juvenile salmonids. Telemetry has been used extensively in the Snake and Columbia Rivers to evaluate surface bypass collectors (Adams et al. 1996, 1997; Hensleigh et al. 1997), turbine survival (Absolon et al. 2003), and dam passage behavior and survival (Eppard et al. 1998, 2002, 2005a,b; Anglea et al. 2001; Ploskey et al. 2001; Axel et al. 2003, 2004a,b; Hockersmith et al. 2005).

Adams et al. (1998b) found reduced swimming performance in both gastrically and surgically radio tagged Chinook salmon less than 120 mm FL. For fish greater than 120 mm FL, swimming performance in surgically implanted fish was reduced after 1 d, but not after 21 d. For gastrically implanted fish, the opposite was observed: swimming performance was not effected after 1 d, but was significantly lower after 21 d. Fish with either gastric or surgical implants had significantly reduced predator avoidance capabilities. Adams et al. (1998a,b) and Martinelli et al. (1998) concluded that surgical implantation was the preferred method for most studies, although gastric implantation might be preferred for studies of short duration.

Hockersmith et al. (1999) compared the performance of surgically radio-tagged fish to PIT-tagged fish from release at Lookingglass Hatchery on the Grande Ronde River to Lower Granite Dam on the Snake River, a distance of 238 km. Their results indicated the presence of a radio tag significantly affected growth, travel time, and survival. Radio-tagged fish passed Lower Granite Dam sooner, at a smaller size, and with reduced survival compared to PIT-tagged fish. These results are not surprising since conditions smolts encounter in the wild, such as feeding and predator avoidance, would be expected to be less forgiving than in a laboratory setting. The negative effects of the radio tag on fish performance in this study may have been exaggerated by the great distance over which performance was measured or by the size of the radio tag relative to fish size.

Hockersmith et al. (2003) compared relative performance of yearling Chinook salmon that were either PIT-tagged, radio-tagged gastrically, or radio-tagged surgically. They found that fish surgically and gastrically tagged with a 1.4-g sham radio transmitter had survival and migration rates similar to PIT-tagged fish over a period of 6 d or less and a migration distance of 106 km. However, they found that regardless of tagging method, radio-tagged fish had lower survival than PIT-tagged fish when the migration distance increased to 225 km and travel time was greater than 10 d.

Steig et al. (2004) compared migrational behavior and survival between acoustic- and PIT-tagged yearling Chinook (145-200 mm FL), juvenile steelhead (150-220 mm), sockeye salmon (116-150 mm), and subyearling Chinook (120-152 mm) passing Rocky Reach Dam on the Columbia River. Similar to the findings by Hockersmith et al. (2003), they did not identify any differences in travel times, passage behavior, or survival between acoustic- and PIT-tagged juvenile salmonids over relatively short distances.

In 2006, we compared survival and behavior of acoustic-tagged yearling Chinook salmon to those tagged with PIT tags as they migrated through the Federal Columbia River Power System (FCRPS). Results of this study will aid in determining the suitability of acoustic telemetry to estimate short-term (30 to 60 d) survival of juvenile salmonids through Columbia and Snake River reservoirs and dams, and through the Columbia River below Bonneville Dam (Figure 1).

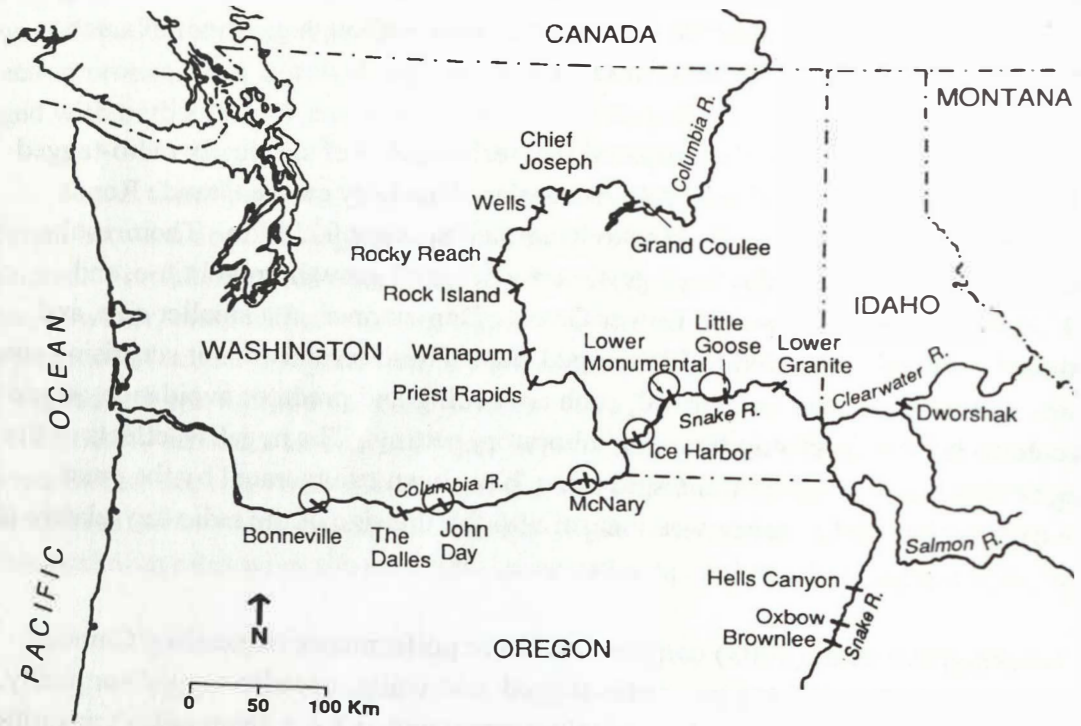

Figure 1. Detail of the field study area showing release location at Lower Granite Dam and PIT tag detection facilities. Circles (O) show dams where detections of fish were used to estimate and compare travel times, detection probabilities, and survival between PIT-tagged and acoustic-tagged yearling Chinook salmon in 2006.

Methods

Study Area

The study area included a 695-km river reach from Lower Granite Dam on the lower Snake River to the mouth of the Columbia River (Figure 1). Lower Granite Dam is the fourth dam upstream from the mouth of the Snake River and is located in Washington State, 173 km above the confluence of the Snake and Columbia Rivers.

Fish Collection, Tagging, and Release

Acoustic transmitters (model E101) were purchased from Sonic Concepts.[†] Each acoustic tag measured 5.5 mm wide, 16.9 mm long, 4.0 mm high (thick), and weighed 0.66 g in air. Each tag transmitted a uniquely coded 31-bit binary phase-shift keyed signal at a frequency of 416.7 kHz and a source level of 150 dB (relative to 1 μPascal at 1 m). The pulse rate interval was 10 sec, and minimum tag life was 55 d. Tags were activated 1-2 d prior to tagging by a small solder connection, which was then sealed by UV-activated epoxy.

River-run, hatchery yearling Chinook salmon were collected from the smolt collection facility at Lower Granite Dam from 5 to 13 May. We used only hatchery yearling Chinook salmon that were not previously PIT tagged, that had no visual signs of disease or injury, and that weighed 10 g or more. Fish were anesthetized with tricaine methane sulfonate (MS-222) and sorted in a recirculating anesthetic system. Treatment fish for acoustic tagging were randomly selected from the sample for a study of latent mortality (Bonneville Power Administration Project 2003-041-00) and transferred to a 75-L holding tank. Following collection and sorting, fish were maintained via flow-through river water and held a minimum of 18 h prior to acoustic tagging.

Treatment fish were surgically tagged with an acoustic transmitter using techniques described by Anglea et al. (2004). Fish were placed in an anesthetic tank prior to surgery. Anesthetic tanks were prepared using MS-222 in quantities of 80-100 mg/L and PolyAqua (0.15 mL/L). After a fish lost equilibrium in the anesthetic tank, it was immediately weighed and measured. The fish was then placed on a surgery table and given anesthesia through rubber tubing from a gravity-fed bucket. Anesthesia consisted of MS-222 in quantities of 40 mg/L during the surgical procedure.

[†] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA, Pacific Northwest National Laboratory, or the U.S. Geological Survey.

With the fish facing ventral side up, a 5-7 mm incision was made 2-5 mm from and parallel to the mid-ventral line anterior of the pelvic girdle. The acoustic tag was implanted, and a PIT tag was also inserted with the acoustic transmitter so that test fish could be separated by code in the fish collection system and returned to the river at downstream dams (Marsh et al. 1999). Combined tag mass was 0.77 g, and combined tag volume was approximately 400 mm^3. The incision was closed with 2, 5-0 Vicryl sutures. Surgical tagging was conducted simultaneously at three tagging stations with approximately 60 fish tagged per h. Surgical instruments were sanitized in 70% ethyl alcohol between surgeries and rinsed in distilled water prior to reuse. All surgical tools were autoclaved before surgeries started each day.

Immediately following tagging, treatment fish were placed into 75-L, aerated recovery containers and held a minimum of 2 h for recovery and determination of post-tagging mortality. After recovery, acoustically tagged fish were transferred water-to-water to an 18,500-L holding tank supplied with flow-through river water.

Control groups consisted of hatchery Snake River spring/summer Chinook salmon collected and PIT-tagged by hand, with tags coded for no transport (Prentice et al. 1990a,b). These fish were released into the tailrace of Lower Granite Dam and were used for the latent mortality study. To reduce the likelihood of disease transmission, all needles were soaked in 70% ethyl alcohol for at least 10 min before being reloaded with a PIT tag. Collection and handling techniques, including use of the recirculating anesthetic water system, followed the methods described in Marsh et al. (1996, 2001). After PIT tagging, control fish were held in the same 18,900-L holding tank as treatment fish.

After a post-tagging recovery period of 24 h, both PIT- and acoustic-tagged fish were released by connecting the holding tank to the juvenile bypass pipe with a 10.2-cm diameter flexible hose. A valve was opened that allowed the tank and its contents to go out the juvenile bypass pipe. To ensure temporal mixing of PIT- and acoustic-tagged fish, all fish were released simultaneously from the same holding tank into the juvenile bypass river-return pipe.

The PIT tags of released fish were passively interrogated by automatic PIT-tag detectors (Prentice et al. 1990a,b,c) within the bypass/detection systems at Little Goose (rkm 635), Lower Monumental (rkm 589), Ice Harbor (rkm 538), McNary (rkm 470), John Day (rkm 347), and Bonneville Dams (rkm 235; Figure 1). The majority of detected PIT-tagged fish were diverted back to the river by slide gates (rather than being barged or trucked downstream), which provided the potential for detection of individual fish at multiple sites downstream from release (Marsh et al. 1999). Downstream from Bonneville Dam, a large surface pair-trawl fitted with a PIT tag detection antenna detected PIT-tagged fish (Ledgerwood et al. 2005). In addition, acoustic-tagged fish were detected on two arrays of autonomous hydrophones near the mouth of the Columbia River.

Data Processing and Analysis

Travel times were calculated through the following reaches: release (Lower Granite Dam tailrace) to Little Goose Dam (60 km), release to Lower Monumental Dam (106 km), release to McNary Dam (225 km), release to John Day Dam (348 km), and release to Bonneville Dam (460 km). Travel time through a reach included both delays associated with residence time in forebays before passing dams, and those within the bypass systems.

The true travel time for a release group would include travel times of both detected and non-detected fish. However, travel time could not be determined for fish that traversed a river section but were not detected at one or both ends of the reach. Thus, travel-time statistics were estimated from travel time rates for detected fish only, with computations representing a subsample of the complete release group.

Median travel times between release and each downstream dam were compared using a bootstrap technique to determine *P*-values for a test of the null hypothesis, that there was no real difference in travel time between acoustic- and PIT-tagged groups. A bootstrap technique was also used to construct 95% confidence intervals for the true difference (Efron and Tibshirani 1993). Bootstrap resamples for both groups were taken 1,000 times, and the differences between group medians were calculated. The 25^{th} and 95^{th} values of the ordered differences were used as 95% confidence intervals, and the doubled proportion of the values below zero was used as the *P*-value. We estimated medians for each release date separately due to differences in travel times associated with temporal differences in river flow.

PIT tag detection data for all release groups were retrieved from the PTAGIS database (PSMFC 1996) and checked for errors. Estimates of survival and detection probabilities were based on detection histories using the Cormack-Jolly-Seber (CJS) or single-release model (Cormack 1964; Jolly 1965; Seber 1965) as implemented in the statistical computer program *Survival with Proportional Hazards* (Smith et al. 1994). Detection history was a record of detections for each fish at each downstream location (and whether the tagged fish was incidentally removed from the system due to the transportation program). Estimates of survival probabilities under the single-release model are random variables, subject to sampling variability. When true survival probabilities are close to 1.0 and/or when sampling variability is high, it is possible for estimates of survival probabilities to exceed 1.0. Standard errors for these estimates were also obtained from the model.

Survival from release to each downstream dam, and detection probability at each downstream dam for acoustic-tagged and PIT-tagged groups were compared using *t*-tests and 95% confidence intervals on the ratios of the estimates. Since ratios of proportions can be assumed to be log-normally distributed (Snedecor and Cochran 1980), we transformed them using the natural log. Therefore, to test the null hypothesis that survival was not different between tag groups (i.e., that the ratio was different than one or the log of the ratio was different than zero), we calculated the test statistic:

$$t = \frac{LN\left(\frac{\hat{S}_{Acoustic}}{\hat{S}_{PIT}}\right)}{\sqrt{\frac{\hat{SE}_{S_{Acoustic}}^{2}}{\hat{S}_{Acoustic}^{2}} + \frac{\hat{SE}_{S_{PIT}}^{2}}{\hat{S}_{PIT}^{2}}}}$$

We then compared it to the normal variant corresponding to $\alpha = 0.05$ (i.e. 1.96). We used the same approach for comparing relative detection proportion. The confidence intervals were of the form:

$$\left[e^{LN\left(\frac{\hat{S}_{Acoustic}}{\hat{S}_{PIT}}\right) - 1.96 \times SE}, e^{LN\left(\frac{\hat{S}_{Acoustic}}{\hat{S}_{PIT}}\right) + 1.96 \times SE} \right]$$

where *SE* is the denominator in the calculation for *t* above (i.e., the standard error for the LN ratio).

Predation rates from Caspian terns *Sterna caspia,* double-crested cormorants *Phalacrocorax aurtius,* and gulls *Larus* spp. were compared between tag treatments. Avian predation rates were determined by PIT-tag detections or acoustic tag recoveries on piscivorous bird colonies in the Columbia River Basin. Tag detection and recovery on piscivorous bird colonies was conducted during fall 2006, after the birds had abandoned their nesting colonies. Detection and recovery data were provided by the National Marine Fisheries Service (NOAA Fisheries) and Real Time Research, Inc. (A. Evans, Real Time Research, Inc., personal communication). There is an ongoing monitoring effort to detect PIT tags from active Caspian tern colonies in the region conducted by NOAA Fisheries and by the Columbia Bird Research group.

PIT tag recovery data from piscivorous bird colonies were assumed to be binomially distributed. We calculated *P*-values for the null hypothesis, that there was no difference between acoustic- and PIT-tagged groups in vulnerability to avian predation, using the test statistic:

$$t = \frac{\hat{P}_{Acoustic} - \hat{P}_{PIT}}{\sqrt{\frac{\hat{P}_{Acoustic}(1-\hat{P}_{Acoustic})}{N_{Acoustic}} + \frac{\hat{P}_{PIT}(1-\hat{P}_{PIT})}{N_{PIT}}}}$$

where P_i was the observed detection rate for a particular group i. We then compared t to the normal variant corresponding to $\alpha = 0.05$ (i.e., 1.96). We constructed 95% confidence intervals for the differences between acoustic-tagged and PIT-tagged groups as:

$$\left[\left(\hat{P}_{Acoustic} - \hat{P}_{PIT}\right) - 1.96 \times SE,\ \left(\hat{P}_{Acoustic} - \hat{P}_{PIT}\right) + 1.96 \times SE\right]$$

where SE is the denominator in the equation for t.

Results

Fish Collection, Tagging, and Release

Our experimental design included total releases of 3,500 acoustic-tagged fish and 16,800 PIT-tagged fish into the tailrace of Lower Granite Dam. Releases were scheduled for 14 days partitioned over a 30-d period, from 14 April to 15 May. However, a much lower number of acoustic transmitters were available for the study due to manufacturing and delivery problems. Therefore, the experimental design schedule could not be met. Only 1,000 acoustic transmitters were available during the entire study period (14 April-15 May), and these were not available until the last week of the study. Thus, on 6 and 13 May, we released a total of 996 surgically acoustic-tagged and 21,026 PIT-tagged hatchery yearling Chinook salmon into the tailrace of Lower Granite Dam (Table 1).

Table 1. Number of hatchery yearling Chinook salmon tagged and released at Lower Granite Dam to evaluate tag effects of the JSATS acoustic transmitter on behavior and survival, 2006.

		Number released	
Tag date	Release date	Acoustic-tagged	PIT-tagged
5 May	6 May	237	9,582
11 May	13 May	297	
12 May	13 May	293	11,444
13 May	13 May	169	
Total		996	21,026

The release on 6 May corresponded with the 58th percentile of the cumulative smolt index for yearling Chinook salmon passing Lower Granite Dam in 2006, and the release on 13 May corresponded with the 85th percentile (Figure 2). Overall handling and tagging mortality averaged 1.3% for PIT-tagged fish and 1.8% for acoustic-tagged fish.

Average fish sizes were similar among tagging methods (Table 2). The combined tag (acoustic transmitters and PIT tag) weighed 0.77 g, resulting in an average tag burden (tag weight/fish weight) of 3.22% (range 1.54-7.33%). Less than 2% of the acoustic tagged fish had a tag burden greater than 5% (Table 3). River discharge during the study in both the Snake and Columbia Rivers was above the 10-year average for the entire study period in 2006 (Figures 3 and 4).

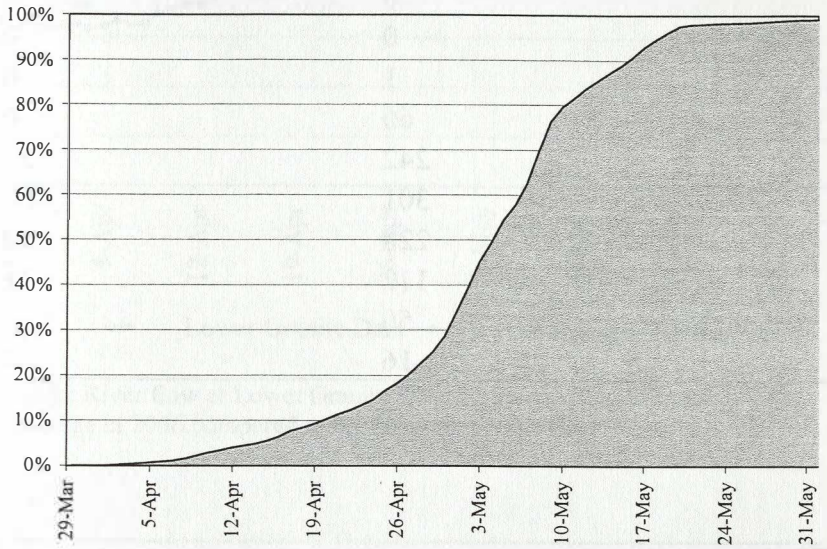

Figure 2. Cumulative passage distribution of yearling Chinook salmon at Lower Granite Dam during 2006.

Table 2. Fish size (fork length and weight) for acoustic-tagged yearling Chinook salmon released at Lower Granite Dam for evaluation of tag effects of the JSATS acoustic transmitter on behavior and survival, 2006. Only length is given for PIT-tagged study fish, since weights were not collected.

		Length (mm)			Weight (g)		
Release date	N	Mean	SD	Range	Mean	SD	Range
				Acoustic-tagged			
6-May	237	137.4	11.3	113-240	24.4	4.9	12.6-37.7
13-May	759	137.1	8.8	105-160	23.7	4.6	10.5-50.1
Overall	996	137.2	9.4	105-240	23.9	4.7	10.5-50.1
				PIT-tagged			
6-May	9,582	136.0	10.5	86-258			
13-May	11,444	136.2	8.9	89-284			
Overall	21,026	136.1	9.7	86-284			

Table 3. Number and percent of fish that were acoustic tagged for various levels of tag burden. The tag burden was the weight of the combined tags (acoustic transmitter and PIT tag) (0.77 g) relative to the weight of the fish.

Tag burden (%)	Number of fish tagged	Percent of fish tagged (%)
0.0-0.4	0	0.0
0.5-0.9	0	0.0
1.0 -1.4	0	0.0
1.5-1.9	1	0.1
2.0-2.4	66	6.5
2.5-2.9	242	23.8
3.0-3.4	301	29.6
3.5-3.9	228	22.4
4.0-4.4	112	11.0
4.5-4.9	50	4.9
> 4.9	16	1.6

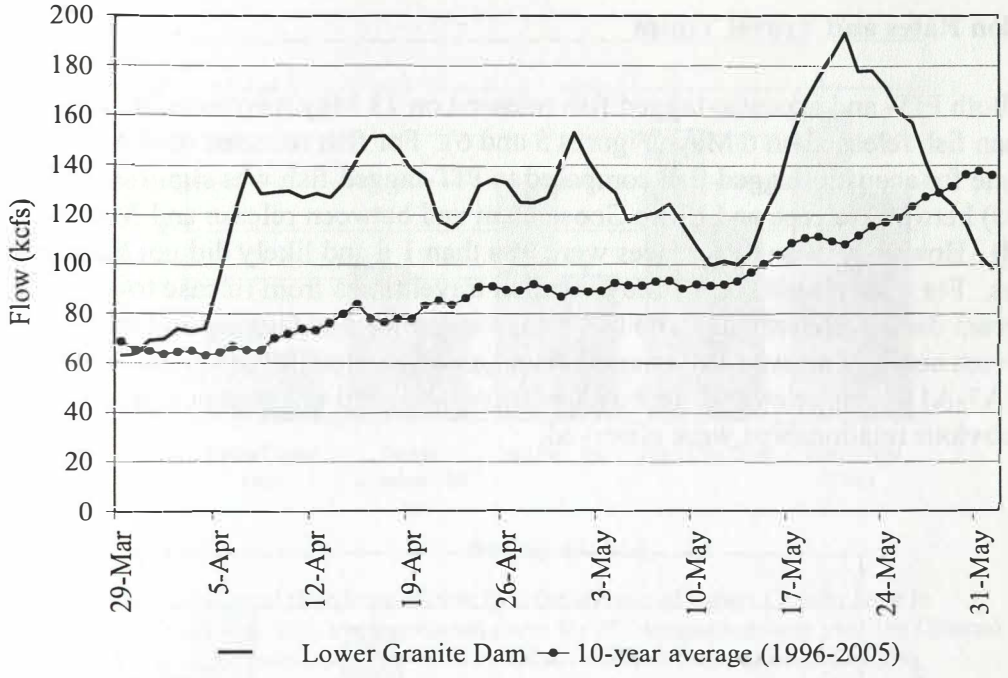

Figure 3. Snake River flow at Lower Granite Dam during yearling Chinook salmon passage in 2006 compared to the 10-year average flow.

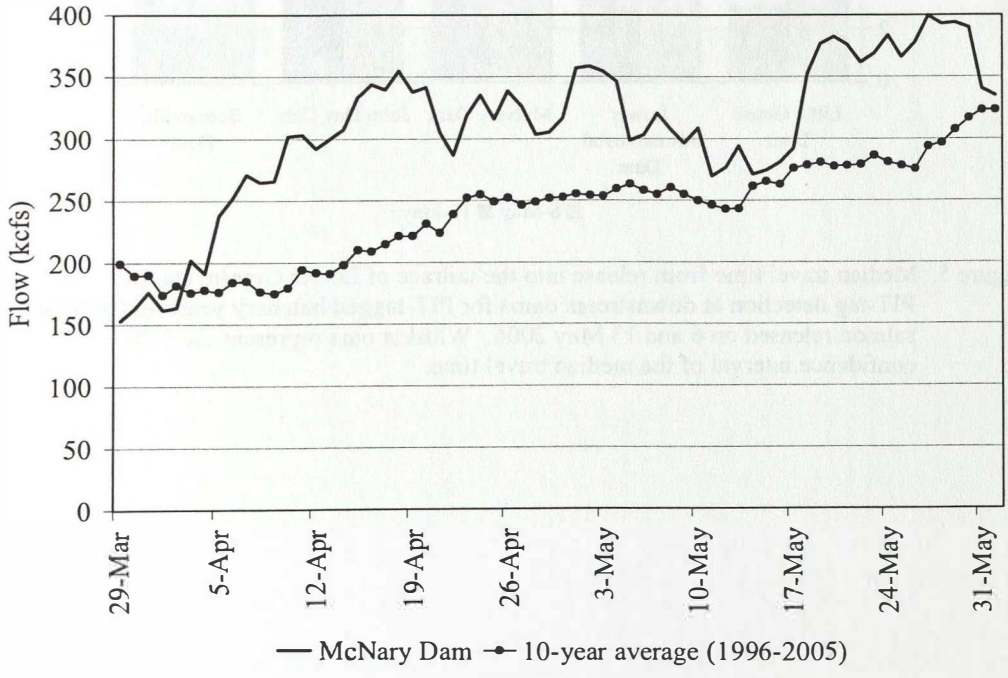

Figure 4. Columbia River flow at McNary Dam during yearling Chinook salmon passage in 2006 compared to the 10-year average flow.

Migration Rates and Travel Times

Both PIT- and acoustic-tagged fish released on 13 May traveled significantly faster than fish released on 6 May (Figures 5 and 6). For fish released on 6 May, median travel time for acoustic-tagged fish compared to PIT-tagged fish was significantly slower ($\alpha = 0.05$) between release and Little Goose Dam and between release and McNary Dam (Table 4). However, these differences were less than 1 d and likely did not bias survival estimates. For fish released on 13 May, median travel times from release to all downstream dams were similar. The tag burden at the time of tagging and travel time to downstream acoustic arrays were examined and are presented in Appendix Figures A7-A11. The relationships between travel time and tag burden appeared random, and no obvious relationships were observed.

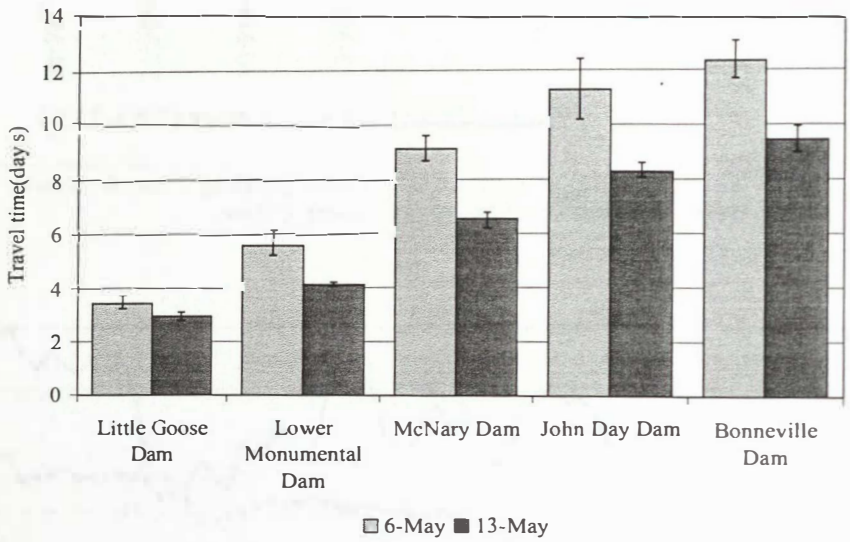

Figure 5. Median travel time from release into the tailrace of Lower Granite Dam to PIT-tag detection at downstream dams for PIT-tagged hatchery yearling Chinook salmon released on 6 and 13 May 2006. Whisker bars represent the 95% confidence interval of the median travel time.

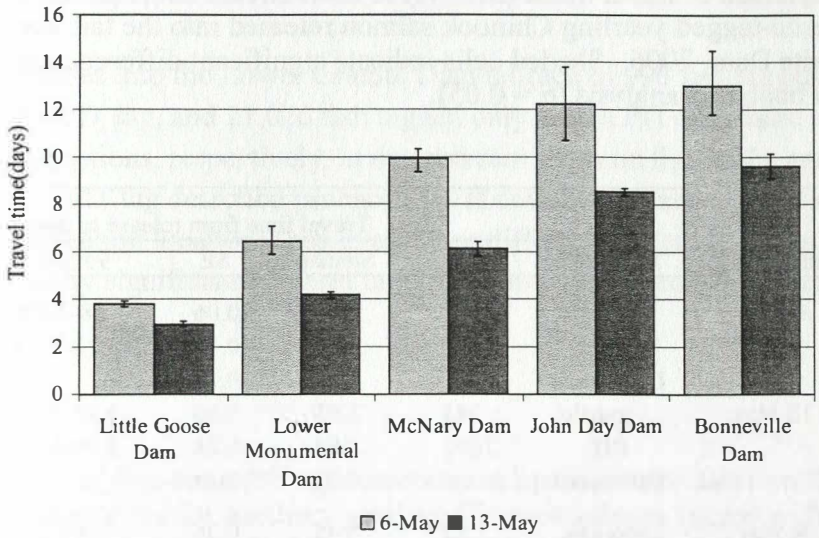

Figure 5. Median travel time from release into the tailrace of Lower Granite Dam to PIT-tag detection at downstream dams for PIT-tagged hatchery yearling Chinook salmon released on 6 and 13 May 2006. Whisker bars represent the 95% confidence interval of the median travel time.

Table 4. Comparison of travel times (in days) to downstream dams for PIT- and acoustic-tagged yearling Chinook salmon released into the tailrace of Lower Granite Dam, 2006. Shaded cells indicate significant difference in travel times from bootstrap analysis ($\alpha = 0.05$).

			Travel time from release to dam (d)				
Location	Release date	Treatment	N	Median	SE	95% CI	*P*-value
Little Goose Dam	6-May	Acoustic	71	3.80	0.06	3.68-3.929	
		PIT	2590	3.43	0.13	3.23-3.74	
		Difference		0.38	0.14		0.030
	13-May	Acoustic	243	2.95	0.06	2.87-3.08	
		PIT	3252	2.94	0.08	2.79-3.09	
		Difference		0.01	0.11		0.958
Lower Monumental Dam	6-May	Acoustic	52	6.45	0.36	5.9-7.07	
		PIT	1933	5.56	0.29	5.20-6.14	
		Difference		0.88	0.47		0.084
	13-May	Acoustic	172	4.19	0.06	4.05-4.31	
		PIT	2338	4.13	0.03	4.07-4.21	
		Difference		0.07	0.06		0.220
McNary Dam	6-May	Acoustic	53	9.93	0.25	9.37-10.34	
		PIT	2027	9.13	0.25	8.69-9.62	
		Difference		0.81	0.36		0.040
	13-May	Acoustic	52	6.15	0.14	5.81-6.42	
		PIT	733	6.56	0.15	6.22-6.8	
		Difference		-0.41	0.20		0.076
John Day Dam	6-May	Acoustic	13	12.23	1.18	10.7-13.79	
		PIT	918	11.29	0.54	10.24-12.42	
		Difference		0.94	1.30		0.528
	13-May	Acoustic	62	8.56	0.08	8.39-8.69	
		PIT	976	8.28	0.14	8.08-8.62	
		Difference		0.27	0.16		0.106
Bonneville Dam	6-May	Acoustic	17	13.01	0.68	11.87-14.49	
		PIT	702	12.33	0.39	11.76-13.06	
		Difference		0.69	0.79		0.434
	13-May	Acoustic	30	9.61	0.21	9.1-10.13	
		PIT	556	9.46	0.29	9.00-9.95	
		Difference		0.16	0.36		0.722

Detection Probability

From releases into the Lower Granite Dam tailrace of 996 fish tagged with both an acoustic and a PIT tag, and 21,026 fish tagged only with a PIT-tag, there were 839 and 17,424 unique detections, respectively, at downstream dams on the Snake and Columbia Rivers (Table 5). PIT tag detection probabilities at downstream sites varied among treatments and detection locations; however, these differences were less than 8% and were not statistically significant between tagging treatments at each detection site (Table 6).

Table 5. Numbers of first-time PIT tag detections at hydroelectric dams on the Snake and Columbia Rivers for hatchery yearling Chinook salmon tagged with either an acoustic transmitter and a PIT tag or a PIT tag only and released into the tailrace of Lower Granite Dam, 2006.

Release date	Little Goose Dam	Lower Monumental Dam	Ice Harbor Dam	McNary Dam	John Day Dam	Bonneville Dam	Estuary pair trawl (TWX)
				Acoustic-tagged			
6 May	71	52	11	53	13	17	2
13 May	243	172	57	52	62	30	4
Overall	314	224	68	105	75	47	6
				PIT-tagged			
6 May	2,590	1,933	499	2,027	918	702	89
13 May	3,252	2,338	642	733	976	556	169
Overall	5,842	4,271	1,141	2,760	1,894	1,258	258

Table 6. Estimated PIT-tag detection probabilities (p) and standard error of each estimate for hatchery yearling Chinook salmon tagged with either JSATS acoustic transmitters or PIT tags and released into the tailrace of Lower Granite Dam, 2006. Detection probabilities (estimated using the single-release model) were compared using a t-test ($\alpha = 0.05$). See Table 1 for release numbers.

	Little Goose Dam		Lower Monumental Dam		McNary Dam		John Day Dam		Bonneville Dam	
Release date	p	SE	p	SE	p	SE	p	SE	p	SE
					Acoustic tagged					
6-May	0.28	0.04	0.24	0.04	0.33	0.05	0.08	0.03	0.16	0.04
13-May	0.32	0.02	0.27	0.02	0.10	0.02	0.15	0.02	0.08	0.02
Overall	0.31	0.02	0.26	0.02	0.16	0.02	0.13	0.02	0.10	0.02
					PIT tagged					
6-May	0.30	0.01	0.24	0.01	0.25	0.01	0.16	0.01	0.16	0.04
13-May	0.32	0.01	0.27	0.01	0.10	0.01	0.13	0.01	0.09	0.02
Overall	0.31	0.00	0.25	0.01	0.17	0.01	0.15	0.01	0.12	0.02
					Ratio					
6-May	0.92	0.12	1.02	0.16	1.33	0.19	0.49	0.17	0.97	0.33
13-May	1.03	0.07	0.99	0.09	1.00	0.18	1.12	0.19	0.91	0.28
Overall	1.01	0.06	1.04	0.08	0.91	0.11	0.88	0.13	0.88	0.20
t	0.15		0.51		0.82		0.86		0.57	
P-value	0.877		0.611		0.413		0.387		0.567	

Survival Estimates

Estimated survival between release and downstream dams was not statistically different among tagging treatments, except from release to the tailrace of Little Goose Dam (Table 7). Estimated survival for fish tagged with JSATS tags relative to those tagged with only PIT tags was slightly higher within the Snake River, whereas within the Columbia River it was slightly lower than for fish tagged with only a PIT-tag. Tag life testing for all studies using JSATS transmitters in 2006 were conducted by PNNL and are presented in Ploskey et al. 2008.

Table 7. Comparison of estimated survival probabilities (S) for hatchery yearling Chinook salmon tagged with either JSATS acoustic transmitters or PIT tags and released into the tailrace of Lower Granite Dam, 2006. Survival probabilities were estimated using the single-release model and compared using a t-test ($\alpha = 0.05$); the standard error for each estimate is shown. Shaded cells indicate a significant difference in survival was observed.

	Lower Granite to Little Goose		Lower Granite to Lower Monumental		Lower Granite to McNary		Lower Granite to John Day		Lower Granite to Bonneville	
Release date	S	SE	S	SE	S	SE	S	SE	S	SE
	Acoustic tagged									
6-May	1.07	0.09	0.92	0.09	0.70	0.06	0.72	0.15	0.46	0.04
13-May	0.98	0.04	0.87	0.05	0.71	0.07	0.59	0.05	0.50	0.04
Overall	1.00	0.04	0.88	0.04	0.70	0.05	0.61	0.05	0.48	0.03
	PIT tagged									
6-May	0.89	0.01	0.85	0.02	0.90	0.04	0.63	0.05	0.47	0.11
13-May	0.89	0.02	0.78	0.02	0.66	0.04	0.69	0.06	0.55	0.13
Overall	0.89	0.01	0.83	0.02	0.79	0.03	0.65	0.04	0.54	0.09
	Ratio (Acoustic/PIT only)									
6-May	1.20	0.10	1.08	0.11	0.78	0.08	1.14	0.26	0.99	0.26
13-May	1.10	0.05	1.12	0.07	1.07	0.13	0.85	0.11	0.90	0.23
Overall	1.12	0.04	1.07	0.06	0.88	0.07	0.94	0.09	0.90	0.16
t	2.88		1.22		1.63		0.62		0.60	
P-value	0.004		0.222		0.102		0.538		0.547	

Avian Predation

Predation by avian predators (Caspian tern, double-crested cormorant, and gulls) from PIT-tag detection or tag recovery on piscivorous bird colonies in the Columbia River Basin were compared between PIT-tagged and acoustic-tagged fish. Overall, avian predation rates were not significantly different between tagging treatments ($P = 0.584$; Table 8). Significantly more PIT tags were recovered on cormorant colonies from PIT-tagged only fish than from acoustic/PIT-tagged fish. Significantly more PIT tags were also recovered from Island 18, the Potholes, and East Sand Island from PIT-tagged only fish than from acoustic/PIT-tagged fish (Table 9). These differences were probably due to the relatively small acoustic/PIT-tagged fish sample sizes relative to the PIT-tagged only fish sample sizes in conjunction with relatively low overall avian predation rates.

Table 8. Comparison of PIT tags recovered from piscivorous bird colonies in the Columbia River Basin by avian colony for hatchery yearling Chinook salmon. Fish were tagged with both an acoustic transmitter and a PIT tag or with only a PIT tag and released into the tailrace of Lower Granite Dam, 2006. Colony detections as a percent of fish released are shown in parenthesis. Proportions recovered were compared using a *t*-test ($\alpha = 0.05$). Shaded cells indicate a significant difference in recovery rates.

Release date	Cormorant colony	Gull colony	Caspian tern colony	Overall
		Acoustic tag		
6 May	4 (1.7%)	0 (0.0%)	2 (0.8%)	6 (2.5%)
13 May	5 (0.7%)	4 (0.5%)	9 (1.2%)	18 (2.4%)
Overall	9 (0.9%)	4 (0.4%)	11 (1.1%)	24 (2.4%)
		PIT tag		
6 May	123 (1.3%)	18 (0.2%)	91 (0.9%)	232 (2.4%)
13 May	204 (1.8%)	17 (0.1%)	111 (1.0%)	332 (2.9%)
Overall	327 (1.6%)	35 (0.2%)	202 (1.0%)	564 (2.7%)
		Ratio		
Difference (overall)	-0.7%	0.2%	0.1%	-0.3%
SE	0.3%	0.2%	0.3%	0.5%
t	2.09	1.16	0.43	0.55
P-value	0.037	0.245	0.671	0.584

Table 9. Comparison of PIT tags recovered from piscivorous bird colonies in the Columbia River Basin by location for hatchery yearling Chinook salmon. Fish were tagged with either an acoustic transmitter and a PIT tag or with only a PIT tag and released into the tailrace of Lower Granite Dam, 2006. Percent of the fish released in parenthesis. Proportions recovered were compared using a *t*-test ($\alpha = 0.05$). Shaded cells indicate significant difference in recovery rates.

Release date	Crescent Island	Foundation Island	Island 18	Rock Island	Potholes	East Sand Island	Overall
				Acoustic tag			
6 May	1 (0.4%)	3 (1.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.8%)	6 (2.5%)
13 May	7 (0.9%)	2 (0.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	9 (1.2%)	18 (2.4%)
Overall	8 (0.8%)	5 (0.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	11 (1.1%)	24 (2.4%)
				PIT tag			
6 May	39 (0.4%)	48 (0.5%)	2 (0.0%)	1 (0.0%)	2 (0.0%)	140 (1.5%)	232 (2.4%)
13 May	41 (0.4%)	34 (0.3%)	2 (0.0%)	1 (0.0%)	3 (0.0%)	251 (2.2%)	332 (2.9%)
Overall	80 (0.4%)	82 (0.4%)	4 (0.0%)	2 (0.0%)	5 (0.0%)	391 (1.9%)	564 (2.7%)
				Ratio			
Difference (overall)	0.4%	0.1%	0.0%	0.0%	0.0%	-0.8%	-0.3%
SE	0.3%	0.2%	0.0%	0.0%	0.0%	0.3%	0.5%
t	1.48	0.49	2.00	1.41	2.24	2.20	0.55
P-value	0.139	0.623	0.045	0.157	0.025	0.028	0.584

Discussion

Field studies like ours, whose objective was to provide inference to general populations, are most appropriately conducted using as much experimental replication as possible. The field portion of this study was originally designed to have much more temporal replication (i.e., blocking through time) than two replicate pairs. This would have provided larger sample sizes of acoustic-tagged fish and would have allowed for comparison between acoustic-tagged and PIT-tagged groups that accounted for empirical (replicate) variability.

However, since we were able to release only two replicate pairs, we were constrained to the use of theoretical sampling variability (e.g. parametrically with binomial for survival and detection probabilities, and non-parametrically with bootstrapping for travel time). Therefore, the 2006 field portion of this study should be considered a pilot study and viewed as a "snapshot in time" of tag effects on hatchery yearling Chinook salmon. An expanded study with more temporal replication would provide more accurate and unbiased inference to the entire migrating population, and provide more realistic, and possibly narrower statistical bounds.

The basic premise in telemetry research is that tagged individuals behave and survive like non-tagged individuals. In 1956, the first telemetry application to study fish used acoustic telemetry to investigate the passage of adult Chinook salmon at Bonneville Dam (Trefethen 1956). For the next 14 years, acoustic telemetry was used extensively to examine adult fish passage issues in the Columbia River Basin. In 1970, acoustic telemetry was replaced by radio telemetry because acoustic telemetry worked poorly in turbulent areas such as those downstream from dams, especially during periods of spill. Over the following 30 years, telemetry studies in the Columbia River Basin primarily used radio telemetry.

Beginning in 2001, NOAA Fisheries Service, Pacific Northwest National Laboratory, and the U.S. Army Corps of Engineers began development of an acoustic telemetry system to study behavior and estimate survival for juvenile salmonids through the FCRPS and to the mouth of the Columbia River (McComas et al. 2005). The use of acoustic telemetry to evaluate survival within and through the FCRPS is appealing because of the relatively small sample sizes required compared to other methods (PIT tags, coded-wire tags, or nitrogen freeze brands), the potential for use in areas without sufficient detection or recapture capabilities downstream, and the ability to detect acoustic-tagged fish in seawater.

Sample sizes for telemetry studies are smaller than for other methods because detection probabilities for active tags (radio and acoustic transmitters) are usually very high (Skalski et al. 1998). However, as with PIT tag studies, certain assumptions of survival models must be met for valid survival estimation using telemetry. Two stated assumptions from Skalski et al. (1998) are:

A1) *Individuals marked for the study are a representative sample from the population of interest*, and

A2) *Survival and capture probabilities are not affected by tagging or sampling.*

That is, tagged animals have the same survival probabilities as untagged animals.

Juvenile fish are likely to be more sensitive to the presence of a transmitter and attachment methods than adult fish, since the weight and size of the transmitter are a greater percentage of juvenile fish body weight and volume. The weight or volume of the transmitter may reduce swimming performance, foraging ability, predator avoidance, and ultimately survival.

Validating the first assumption may be difficult in acoustic telemetry studies with juvenile Chinook salmon because a portion of the population is smaller than the minimum size appropriate for tagging. However, if the mean size of fish in the tagged sample is similar to that of the population, then the tagged sample should be representative of the majority of the population. The minimum size fish that can be tagged with the JSATS acoustic transmitter has yet to be determined.

In this field study, we tagged fish as small as 105 mm in length and as little as 10.5 g in weight. The tags (acoustic tag and PIT tag) used had a combined tag mass of 0.77 g and the combined tag volume was approximately 400 mm^3. Tag burden ranged from 1.54 to 7.33% and averaged 3.22% of body weight. Less than 2% of the acoustic tagged fish in our study had a tag burden greater than 5%. Adams et al. (1998a) demonstrated that the growth, feeding behavior, and survival of juvenile Chinook salmon were unaffected by surgically implanted radio transmitters that weighed from 2.3 to 5.5% of the fish's weight 54 d after tagging. Although our transmitters are very small, battery technology, tag-life requirements, and transmission capability may limit the manufacture of even smaller transmitters.

The second assumption requires that the presence of the tag and the tagging procedure do not significantly affect the fish's performance. If the behavior of a smolt is altered by the tag, then application of survival estimates or passage timing using tagged smolts to the general (untagged) population would be invalid. For example, a tagged fish might swim at a different depth than non-tagged fish, and therefore could be differentially

susceptible to juvenile fish bypass systems, spillway passage, or surface bypasses. Marked fish may also be more susceptible to injury, infection, or predators (Maynard et al. 1996).

In our field study, detection probabilities and survival were not significantly different among tag types between the release site and Bonneville Dam (a distance of 460 km), except within the first reach (release to Little Goose Dam). Muir et al. (2001) reported that temporal survival estimates varied considerably (83 to 99%) for PIT-tagged yearling Chinook salmon released into the tailrace of Lower Granite Dam and detected downstream in this reach. It is likely that the survival estimates we observed, which were higher-than-expected for acoustic-tagged fish (100%), and lower-than-expected for PIT-tagged fish (89%), were affected by small sample sizes. More replicates and larger sample sizes are needed to accurately assess tag effects.

Travel times on 6 May were significantly longer from release to Little Goose and McNary Dams for acoustic-tagged yearling Chinook salmon. There were no differences among travel times through all other reaches for fish released on this date or for fish released on 13 May. For acoustic-tagged fish, sample sizes released on 6 May were relatively small compared to those released on 13 May, and the significantly longer travel times may have been an artifact of the sample sizes used. Additional replicates are needed to determine if there is an effect on travel time from the JSATS acoustic tag.

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EVALUATION OF GROWTH, SURVIVAL, TAG EXPULSION, AND TISSUE REACTION IN ACOUSTIC-TAGGED JUVENILE SALMONIDS

Richard S. Brown,[1] Kathleen M. Carter,[1] Katherine A. Deters,[1] David R. Geist,[1] Geoffrey A. McMichael,[1] Craig A. McKinstry,[1] and Ralph Elston[2]

[1]Ecology Group
Pacific Northwest National Laboratory
P.O. Box 999, MS K6-85
Richland, Washington 99352

and

[2]AquaTechnics Inc.
P.O. Box 687
Carlsborg, Washington 98324

Executive Summary

Growth, mortality and tag expulsion were examined over a 90-d period in yearling and subyearling Chinook salmon. Fish (840 yearling and 947 subyearling) were randomly assigned to one of four treatments: a) non-tagged control, b) tagged only with a passive integrated transponder (PIT) tag, c) tagged with an integrated transmitter (i.e. acoustic transmitter with a PIT tag adhered); and d) tagged with a non-integrated transmitter (separate acoustic transmitter and PIT tag, as used in the field).

After implantation, treatments were divided and held for 21, 30, 60, or 90 d. During this time, temperatures were adjusted to simulate river temperatures experienced by migrating run-of-river fish. Fish were monitored daily for mortality and tag expulsion. At the end of each holding period, fish were euthanized, a necropsy was performed, individual growth recorded, and a histological analysis conducted.

Among both yearling and subyearling fish, there was no significant difference in mortality among treatments and no trend of differences in growth among treatments. Only yearling fish with integrated and non-integrated transmitters experienced mortalities, and these were low (< 4.5%). Mortality among subyearling control and PIT-tag treatments ranged up to 7.7%, while integrated and non-integrated treatments had slightly higher rates (up to 8.3 and 7.9%, respectively). No yearling fish shed acoustic transmitters during the 90-d study, while up to 7.8% of subyearling fish expelled transmitters, with tags expelled from 5 to 63 d post-surgery. Average time to expulsion was 27 d; few fish expelled transmitters within 14 d or less of implantation.

Histological results suggest that inflammation associated with implantation of an acoustic transmitter can produce fibrous tissue, which can invade and possibly damage internal organs soon after implantation. Reactions severe enough to damage organs, however, were limited to only ~20% of subyearling Chinook salmon, all of which were under 101 mm and 12 g at tagging. Infiltration of fibrous tissue into organs was observed most often in fish held for 21 d and appeared to decrease for subsequent holding times.

Several indices were examined to determine if implantation of an integrated acoustic transmitter and PIT tag would have less negative influence than the current method of inserting the acoustic transmitter and the PIT tag separately into the surgical incision. There was no difference in growth or survival found between these two treatments. However, since expulsion of PIT tags is generally very low, but that of acoustic transmitters was up to 7.7% of subyearling fish studied, use of integrated transmitters is not recommended. The increased loss of PIT tags could hinder the collection of data for other research objectives. In addition, integrated transmitters were more often found in the anterior part of the body cavity than the non-integrated, which may lead to expulsion through the surgical incision.

Introduction

Numerous laboratory studies have been conducted on the effects of externally attached and gastrically or surgically implanted radio and acoustic tags on swimming performance, growth, feeding behavior, predator avoidance, and survival (Adams et al. 1998a,b; Anglea et al. 2004; Brown et al. 1999, 2006; Greenstreet and Morgan 1989; Lucas 1989; Martinelli et al. 1998; Mellas and Haynes 1985; Moore et al. 1990; Moser et al. 1990). However, these evaluations were conducted in laboratory tanks, or if conducted in the field, did not compare performance between electronically tagged and non-tagged fish.

Studies examining the effects of acoustic transmitters on growth, survival, and swimming performance of juvenile salmonids have been conducted by Anglea et al. (2004) and Brown et al. (2006). Anglea et al. (2004) found no significant difference in the critical swimming speed of Chinook salmon tagged with an acoustic transmitter weighing 1.5 g in air and representing 1.6-6.7% of fish body weight. Similar results were found by Brown et al. (2006) for Chinook salmon (94-125 mm FL) implanted with a 0.75-g acoustic transmitter which represented 3.2-10.0% of fish body weight. However, Brown et al. (2006) found that growth rate decreased for acoustic-tagged Chinook salmon compared to control fish.

We conducted laboratory studies of the effects of acoustic tags on the growth, mortality, and tag loss of yearling and subyearling Chinook salmon concurrently with field research to provide additional insight on potential tag effects. These laboratory studies and those in the following report on minimal fish size for acoustic tagging, were the only assessment of tag effects on subyearling Chinook salmon conducted during 2006. In addition, the laboratory studies examined potential tag effects over a period longer than the field study (90 vs. 60 d).

Research was conducted using both hatchery reared and run-of-the-river Chinook salmon. Hatchery stocks of Chinook salmon are commonly used successfully in laboratory experiments such as this, however, some researchers have had difficulty maintaining run-of-the-river Chinook salmon in the laboratory. Use of run-of-the-river fish would be preferred since it may provide a more realistic representation of fish studied in field research. Thus, a pilot scale effort was made to use run-of-the-river Chinook salmon for these laboratory studies.

Work reported here was conducted in the Aquatic Laboratory at Pacific Northwest National Laboratory (PNNL) in Richland, Washington. All fish maintenance, handling, and testing procedures were reviewed and approved by the PNNL Animal Care Committee. Results of this study will aid in determining the suitability of acoustic telemetry to estimate short- and longer-term (30 to 90 d) juvenile salmonid survival at Columbia and Snake River dams and through the lower Columbia River.

Methods

Fish Acquisition, Holding, and Surgical Protocols

Experimental animals—Yearling and subyearling hatchery-reared Chinook salmon were subjected to several treatments to determine the influence of implantation of acoustic transmitters and passive integrated transponder (PIT tags) on their growth, survival, transmitter retention, and tissue reaction. Yearling Chinook salmon were obtained from Dworshak National Fish Hatchery, Ahsahka, Idaho. Yearling fish ranged in fork length from 98 to 152 mm and weighed from 9.2 to 46.1 g (Table 1). Subyearling hatchery Chinook salmon were obtained from Little White Salmon National Fish Hatchery, Cook, Washington. Subyearling fish ranged in fork length from 93 to 126 mm and in weight from 7.7 to 23.7 g (Table 1).

A smaller number of run-of-the-river (ROR) fish were obtained to determine their suitability as laboratory experimental specimens. Yearling ROR Chinook salmon were obtained from the Lower Granite Dam juvenile fish facility on 5 May 2006, while subyearling ROR fish were obtained on 12 June 2006. Yearling ROR Chinook salmon fork length ranged from 106 to 158 mm and their weight from 11.6 to 36.4 g. Subyearling ROR Chinook salmon ranged in fork length from 95 to 127 mm and in weight from 7.3 to 20.7 g.

Table 1. Sample size, mean and range of length, weight and tag burden (% tag weight to body weight in air) for each treatment of juvenile Chinook salmon, 2006.

				Fork Length (mm)		Weight (g)		Tag Burden (%)	
Source	Year Class	Treatment	N	Mean	Range	Mean	Range	Mean	Range
Hatchery	Subyearling	Control	236	103.1	93 - 119	12.1	7.7 - 18.4	NA	NA
Hatchery	Subyearling	PIT	237	102.5	95 - 119	11.9	7.7 - 19.7	0.8	0.5 - 1.2
Hatchery	Subyearling	Integrated	236	103.2	95 - 115	12.1	8.5 - 17.7	6.1	4.0 - 8.6
Hatchery	Subyearling	Non-Integrated	238	103.4	95 - 126	12.2	8.6 - 23.7	5.8	2.9 - 8.8
Hatchery	Yearling	Control	210	129.2	110 - 151	23.8	13.1 - 40.4	NA	NA
Hatchery	Yearling	PIT	210	126.9	100 - 150	22.9	12.2 - 46.1	0.4	0.2 - 0.8
Hatchery	Yearling	Integrated	211	130.2	99 - 152	24.5	14.3 - 43.0	3.1	1.5 - 5.4
Hatchery	Yearling	Non-Integrated	209	128.9	98 - 150	23.6	9.2 - 37.4	3.1	1.7 - 7.3
ROR	Subyearling	Control	58	106.8	97 - 118	12.1	8.5 - 18.0	NA	NA
ROR	Subyearling	PIT	58	106.1	95 - 119	11.7	7.3 - 18.5	0.8	0.5 - 1.3
ROR	Subyearling	Integrated	58	106.9	95 - 127	11.9	8.2 - 20.7	6.3	0.5 - 9.2
ROR	Subyearling	Non-Integrated	58	107.3	96 - 122	12.3	7.8 - 19.1	6.1	3.8 - 9.1
ROR	Yearling	Control	43	136.7	120 - 157	23.0	13.2 - 35.5	NA	NA
ROR	Yearling	PIT	49	132.6	106 - 158	22.3	11.6 - 36.4	0.4	0.3 - 0.8
ROR	Yearling	Integrated	38	136.8	121 - 156	23.2	17.0 - 31.4	3.2	2.3 - 4.4
ROR	Yearling	Non-Integrated	51	134.6	114 - 156	22.9	13.5 - 34	3.2	1.9 - 5.3

Experimental treatments—Fish were given one of four treatments: non-implanted (control), implanted with a PIT tag only, implanted with a PIT tag and acoustic transmitter integrated into a single unit (integrated), or implanted with a PIT tag and acoustic transmitter that had not been integrated (non-integrated). In the Columbia River Basin, all fish implanted with JSATS acoustic transmitters currently also are implanted with a separate PIT tag through the surgical incision; these are represented by the non-integrated treatment in this study.

Integrated transmitters consisted of a PIT tag held to a dummy acoustic transmitter with biocompatible epoxy (FDA2,‡ Tra-Con, Bedford, Massachusetts). Integrated transmitters had a mean mass of 0.73 g in air and 0.45 g in water. Non-integrated transmitters consisted of either a dummy transmitter (mean mass in air 0.65 g and 0.38 g in water) or a Juvenile Salmon Acoustic Telemetry System (JSATS) transmitter with an expired battery (mean mass 0.62 g in air, 0.37 g in water) and a separate PIT tag (mass 0.085 g in air, 0.056 g in water).

Animals assigned to the control group were anesthetized in a manner similar to that for treatment fish. However, once the fish had lost equilibrium, a combination of colored visible implant elastomer (Northwest Marine Technology, Inc., Shaw Island, Washington) was injected into the right and left adipose eyelids and a clip of one of the other pelvic fins was made to identify control fish.

After implantation, fish were held for one of four durations: 21, 30, 60, or 90 d, after which they were euthanized with an overdose of tricaine methanesulfonate (MS-222, 250 ppm). At this time, each fish was again measured and weighed and underwent a detailed necropsy. In addition, subsamples of fish were sent for histological examination.

Surgical technique—Surgical implantation of integrated and non-integrated transmitters and PIT tags was conducted using methods similar to those described in Brown et al. (2006). Each fish was anesthetized with a 100-mg/L solution of MS-222. The fork length (nearest millimeter) and mass (grams) for all treatment groups, including controls, were measured after fish were anesthetized. While still anesthetized, each fish to be implanted was placed ventral side up in a groove within a piece of wet foam saturated with a solution of PolyAqua (Kordon Aquarium Products, Hayward, California). A small tube inserted in the fish's mouth during surgery provided a continuous solution of 40-mg/L MS-222. An incision 4-7 mm long was made 3-5 mm

‡ Reference to trade names does not imply endorsement by the National Marine Fisheries Service, the Pacific Northwest National Laboratory, or the U.S. Geological Survey.

from and parallel to the mid-ventral line, anterior to the pelvic girdle. A PIT tag and transmitter were inserted into the peritoneal cavity. Incisions were closed with two interrupted sutures for all fish that underwent surgery using absorbable violet-colored coated 5-0 braided polyglactin 910 sutures (Vicryl, Ethicon, USA). After surgery, fish recovered in buckets or tanks with fresh, oxygenated water and were monitored until they achieved equilibrium.

PIT tag implantation—Single PIT tags were implanted according to the *PIT Tag Marking Procedures Manual* (CBFWA 1999). Briefly, fish were anesthetized with a 40-mg/L solution of MS-222. Once fork length and mass were recorded, a single PIT tag was injected into the fish through the ventral surface between the posterior tip of the pectoral fin and the anterior point of the pelvic girdle. A 12-gauge veterinary-grade needle was used for the tag injections.

Housing—During the study period, test populations were held in three types of holding tanks—circular tanks (1.8 m in diameter and 0.76 m deep, holding 1,996 L of water); oblong tanks (1.3 m long, 0.8 m wide, and 0.6 m deep, and holding 378 L of water); or troughs. Troughs were sectioned to hold two groups of fish at once so that one group was closer to the water inflow (upper) and one group was closer to the water outsource (lower). Each section of trough was 0.2 m long, 0.8 m wide, and 0.6 m deep, and held 80 L of water. All hatchery-reared yearling fish were held in circular tanks. Hatchery-reared subyearling fish were held in circular or oblong tanks. All ROR yearling fish were held in troughs, and all ROR subyearling fish were held in either troughs or oblong tanks.

Fish were fed Biodiet moist pellets at 2.5 to 3% of body weight per day (Bio-Oregon, Warrenton, Oregon). All fish were fed by hand. Fish were acclimated to human presence by leaving tank lids open for extended periods. Run-of-the-river Chinook salmon did not eat well after arriving and after handling. These groups were fed two to three times daily in an attempt to increase their daily intake of food. Despite this, ROR fish usually ate only 1.5 to 2.0% of their body weight per day, although their consumption increased with time and acclimation. Hatchery fish were fed only once per day and ate all food presented to them. Food was withheld from fish selected for a given test for 24 h before and 48 h after surgery.

Fish were held at water temperatures that reflected water temperatures in the Snake and Columbia Rivers during the migration of juvenile Chinook salmon. Therefore, water temperature was maintained between 11 and 17°C for yearling fish and between 15 and 21°C for subyearling fish (Figure 1).

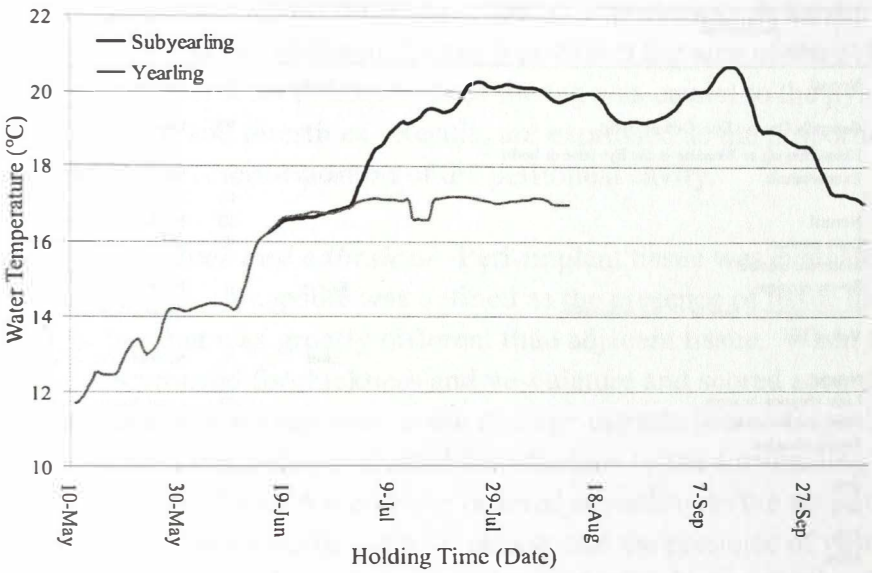

Figure 1. Holding water temperatures for yearling and subyearling juvenile Chinook salmon, 2006.

Necropsy—At the end of each holding period, necropsies were performed to evaluate the basic health of each fish. As part of the necropsy process, five examinations were conducted to determine the influence of implantation on healing and tissue reaction.

General health index—The general health index score consisted of 10 components (Table 2). Scores for each condition were adapted from Adams et al. (1993) and ranged from 0 to 30 with 0 being the best or normal. At necropsy, each component was evaluated and scored. Scores were then summed for each fish. Results are reported as the mean.

Table 2. General health index. Each fish was examined at necropsy and scored. The score values progress from best to worst with 0 being best and 30 being worst.

External			Internal		
Component	Score	Condition	Component	Score	Condition
Eyes	0	Normal	Spleen	0	Normal appearing spleen
	30	Generally Opaque Eye (One or Both)		30	Enlarged or otherwise not normal
	30	Hemorrhaging or Bleeding in the Eye (one or both)			
	30	Exophthalmia	Hindgut	0	Normal with no redness or swelling
				10	Light redness or swelling
Fins	0	Normal		20	Moderate redness or swelling
	10	Light erosion		30	Severe redness or swelling
	20	Moderate erosion			
	30	Severe erosion	Kidney	0	Normal
	30	Hemorrhaging at base of fin but no erosion		30	Other/not normal
	30	Yellow fins			
			Liver	0	Normal solid or light red color
Skin	0	Normal		30	"Fatty" liver/coffee with cream color
	10	Light abrasion or scars		30	Focal discoloration
	20	Moderate abrasion		30	General discoloration covering entire liver
	30	Severe abrasion			
Descaling	0	25%			
	10	50%			
	20	75%			
	30	100%			
Gills	0	Normal Gill Appearance			
	30	Fraying or erosion of lamella tips leaving a ragged appearance			
	30	Swelling to the tips of the lamella giving a clubbed appearance			
	30	Pale color along the tips of the lamella			
	30	Pale color of entire gill complex			
Parasites	0	No parasite			
	30	Parasite present			

Incision healing—The incision of fish surgically implanted with acoustic transmitters was assessed for healing progression and graded according to conditions described in Table 3. Only fish implanted with integrated or non-integrated acoustic transmitter treatments were evaluated.

Table 3. Rubric for incision healing evaluation of fish implanted with integrated and non-integrated transmitters.

Score	Definition
0	Completely closed
1	Healing - tissue growth at incision site
2	Sides of incision in apposition but no tissue growth across incision site
3	Open and gaping

Suture retention—The number of sutures retained (out of two) by each surgically implanted fish (integrated and non-integrated treatments only) was recorded. Results are shown as the percentage of sutures remaining.

Transmitter location—At necropsy, the location of acoustic transmitters and PIT tags within the peritoneal cavity was determined. Acoustic transmitter location analysis was performed only on fish implanted with integrated and non-integrated transmitters. Likewise, PIT tag location was determined only for treatments with independent PIT tags (PIT and non-integrated acoustic treatments). The location of the transmitter was quantified as either anterior (within the pyloric caeca) or posterior. A transmitter was considered anterior if more than 50% of the tag was within the area of the pyloric caeca. It was considered posterior if more than 50% of the tag was caudal to the pyloric caeca, thus nearer to the spleen and intestines. Results are expressed as the proportion of transmitters found in the anterior portion of the peritoneal cavity.

Capsule appearance and adhesion—Peri-implant tissue was evaluated for appearance and thickness. A capsule was defined as the presence of tissue directly around the transmitter that was grossly different than adjacent tissue. When a capsule was present, it was evaluated for thickness and vasculature and scored according to the criteria in Table 4. Results are reported as the average capsule score. Capsules surrounding transmitters were also evaluated for adhesion to the surrounding tissues according to definitions in Table 5 which are ordered according to the severity of the reaction to the implant. Increases in tissue thickness and the presence of vasculature implies increased difficulty in the body's attempt to return to homeostatsis after implantation.

Table 4. Rubric for evaluation of capsule appearance around implanted acoustic and PIT tags.

Score	Definition
0	No capsule
1	Clear
2	Clear with vasculature
3	Thick with vasculature

Table 5. Rubric for adhesions associated with implanted acoustic and PIT tags.

Score	Definition
0	Capsule is adhered to a portion of the body wall but does not include the incision.
1	Capsule is adhered to the incision.
2	Capsule is adhered to organs only. Adhesion does not involve the body wall.

Histology—A total of 12 yearling and 25 subyearling fish were collected randomly across all holding times for histological examination (conducted by AquaTechnics Inc., Sequim, Washington). Yearlings were examined for condition of the internal organs, the tissue encapsulating the transmitter, tissue reaction to the transmitter, and any conditions or effects related to the transmitter or the implantation process. Subyearlings were examined similarly. In addition, the nature of the tissue reaction forming the transmitter capsule was quantified by systematically enumerating cell types from cross sections of the transmitter capsule.

Statistical Analysis

Logistic regression was used to assess differences in mortality rates and tag expulsion rates among the three treatment groups and one control group of juvenile Chinook salmon. Four separate studies followed these four treatment groups for holding times of 21, 30, 60, and 90 d; each holding time group was analyzed separately. The two binary endpoints (mortality and tag expulsion) also were analyzed separately; the control group was omitted from the tag expulsion analysis.

The same basic statistical analysis methodology was applied to all studies. The methodology used a logistic regression model with a binary response variable (e.g., mortality or tag expulsion) and was fitted to an independent factor variable that classified fish into their respective groups. The coefficient estimates and their standard errors from the fitted logistic regression model were used to compute pairwise statistical tests for differences in rates.

Each tagged treatment group was compared directly with the control group, and the integrated tag group was compared directly to the non-integrated tagged fish in the analysis of mortality rates. The integrated and non-integrated tagged groups each were compared with the PIT-tagged group and with each other on the incidence of expelled tags. All pairwise comparisons were constructed from the regression coefficient estimates and standard errors used to form Wald chi-square statistics to test for significant differences.

In each analysis, the data were aggregated on holding time group, with the number of mortalities or dropped tags counted along with the total count of fish (N) for that group. In many cases, there were no incidences of mortality or dropped tags for one or more treatment groups, which created very poor estimates of the regression coefficients with largely inflated standard errors. In these cases, a small bias value (e.g., 0.1) was substituted for the 0 before the model was fit. By relaxing the requirement for unbiased estimators, a better estimate of the variance was achieved, which facilitated a

more reliable statistical test for differences. This technique falls in the category of *ridge regression* and is detailed in Montgomery and Peck (1992).

Days to mortality and days to expelled tag events define left-censored data best modeled using survival analysis methods. Model fitting was attempted using a proportional hazards model but was unsuccessful in generating legitimate estimates of differences because of too few events. In addition, 18% (six fish) of the hatchery-reared subyearlings were found at necropsy to have lost their transmitter. However, the date to expulsion was not known for these fish. Because of these complications, these analyses are not shown in the Results section.

The change in weight of tagged juvenile Chinook salmon was assessed for yearling and subyearling Chinook salmon 21, 30, 60, and 90 d after tagging. Comparisons of growth were made among the four treatment groups. One-way ANOVA models and F-tests were used to assess the overall significance of differences among tag treatments by age class and holding times separately. Least-squares means and standard errors also were estimated from the fitted models, and chi-square tests for significant differences between paired treatments within age class and holding time were performed.

Fish were numerically scored for health index (HI) on 10 health indices (described previously) following completion of their holding period (21, 30, 60, or 90 d) The total HI score is the sum of these 10 indices. Higher HI scores indicate lower health status of fish. Only fish with non-missing values on all 10 indices were used in this analysis.

Health scores had skewed distributions; most HI scores were at lower values, including many HI scores of 0. The skew and presence of multiple HI scores of 0 in these distributions precluded the use of log transformation and normal-based parametric statistical tests, while the presence of multiple ties in HI scores between holding time and treatment groups precluded the use of rank-based nonparametric methods.

The alternative approach was to compare count distributions across HI scores among treatment groups. Count distributions were statistically compared using a log-linear model, with counts as the response variable taken as Poisson distributed. Because tagging and holding time studies were conducted as separate experiments, treatments were compared within each holding time. Specific comparisons were made between each tag treatment and the untagged control group and between the integrated and non-integrated PIT and acoustic tags.

The classification results for suture retention, capsule appearance, and capsule adhesion defined in the preceding section were analyzed using multinomial response models. Transmitter locations for acoustic and PIT tags were defined as binary outcomes and were analyzed using logistic regression. Statistical results reported in the Results section for these classifications are from the fitted multinomial regression models based on the likelihood ratio chi-square tests on the null hypothesis of all groups being equal versus the alternative of at least one group being not equal to the others.

Results

Mortality

Mortality was low for all yearling hatchery-reared Chinook salmon. No mortality was observed among any control or PIT-tagged test groups, and mortality among groups implanted with acoustic and PIT tags ranged from only 0 to 4.5% (Table 7). There were no significant ($P > 0.05$) differences in mortality among yearling test groups that were held 21, 30, 60, or 90 d after surgical implantation (see Appendix B, detailed P values). No mortality was observed among any of the hatchery-reared groups held 21 d.

Mortality in subyearling hatchery-reared Chinook salmon was higher than yearlings, ranging from 0 to 8.3% (Table 7). Similar to hatchery-reared yearling fish, there were no significant ($P > 0.05$) differences among treatment groups in mortality during any holding time. Additionally, there was no difference in mortality between fish implanted with integrated and non-integrated transmitters ($P > 0.05$).

Mortality was higher for yearling ROR fish than for hatchery-reared fish. While mortality ranged from only 0 to 4.5% for yearling hatchery-reared fish, it ranged from 0 to 27.3% for yearling ROR fish (Table 7). Mortality of ROR subyearling fish (ranging from 23.1 to 87.5%) was higher than both yearling ROR fish and higher than all hatchery-reared groups (Table 7). This was much higher than the 0-8.3% mortality observed in hatchery-reared subyearling Chinook salmon. Due to the low numbers of test animals, no statistical analyses were performed on ROR fish.

Table 7. Mortality (%) of hatchery-reared and run-of-the-river (ROR) juvenile Chinook salmon implanted with PIT tag, integrated or non-integrated acoustic transmitter and PIT tag, or not implanted (control) and held for 21, 30, 60, or 90 d after implantation, 2006.

Source	Year class	Days held	% Mortality			
			Control	PIT	Integrated	Non-integrated
Hatchery	Yearling	21	0	0	0	0
Hatchery	Yearling	30	0	0	2.3	4.5
Hatchery	Yearling	60	0	0	0	1.7
Hatchery	Yearling	90	0	0	1.7	1.6
Hatchery	Subyearling	21	3.8	7.7	5.8	7.8
Hatchery	Subyearling	30	0	0	5.7	3.7
Hatchery	Subyearling	60	0	0	8.2	4.6
Hatchery	Subyearling	90	1.5	0	8.3	7.9
ROR	Yearling	21	0	0	0	27.3
ROR	Yearling	30	9.1	10.0	20.0	0
ROR	Yearling	60	0	7.1	14.3	6.7
ROR	Yearling	90	20.0	6.7	20.0	13.3
ROR	Subyearling	21	46.2	53.8	46.2	23.1
ROR	Subyearling	30	69.2	53.8	46.2	69.2
ROR	Subyearling	60	37.5	37.5	43.8	37.5
ROR	Subyearling	90	87.5	84.6	86.7	83.3

Days to Mortality

Mortality of yearling hatchery-reared Chinook salmon occurred 14-67 d after implantation (Table 8). The majority of fish died between 14 and 23 d after implantation. Only one yearling fish died more than 23 d after implantation, and none of the yearling fish died less than 14 d after implantation.

Table 8. Mean days to mortality (± SD [range]) for hatchery-reared and run-of-the-river (ROR) juvenile Chinook salmon implanted with PIT tag, integrated or non-integrated acoustic transmitter and PIT tag, or non-implanted (control) and held 21, 30, 60, or 90 d after implantation, 2006.

Source	Year class	Days held	Days to Mortality			
			Control	PIT	Integrated	Non-integrated
Hatchery	Yearling	21				
Hatchery	Yearling	30			23 ± 0	14.5 ± 0.7 [14-15]
Hatchery	Yearling	60				23 ± 0
Hatchery	Yearling	90			67 ± 0	17 ± 0
Hatchery	Subyearling	21	13.5 ± 9.2 [7-20]	17.5 ± 3.3 [13-21]	13 ± 7.5 [6-21]	21 ± 0
Hatchery	Subyearling	30			5.7 ± 3.5 [2-9]	5.5 ± 0.7 [5-6]
Hatchery	Subyearling	60			6.9 ± 3.6 [4-13]	36 ± 14 [20-46]
Hatchery	Subyearling	90	77 ± 0		7.7 ± 6.9 [4-20]	19.4 ± 12.7 [6-35]
ROR	Yearling	21				10 ± 1.7 [9-12]
ROR	Yearling	30	10 ± 0	14 ± 0	10 ± 0	
ROR	Yearling	60		5 ± 0	22 ± 0	55 ± 0
ROR	Yearling	90	76.3 ± 10.7 [64-83]	61 ± 0	56.6 ± 42.1 [9-89]	33.3 ± 49.1 [5-90]
ROR	Subyearling	21	13 ± 2.5 [11-17]	14.7 ± 3.6 [10-20]	17.3 ± 3.3 [11-20]	15 ± 3.5 [13-19]
ROR	Subyearling	30	20 ± 7.6 [10-30]	19.1 ± 6.2 [10-30]	15 ± 4.3 [11-21]	19.8 ± 7.2 [10-30]
ROR	Subyearling	60	32.3 ± 17.1 [21-52]	43.0 ± 15.5 [15-59]	46.2 ± 5.7 [38-54]	46.6 ± 6.0 [39-55]
ROR	Subyearling	90	25.7 ± 9.6 [14-43]	34.3 ± 8.8 [19-46]	30.2 ± 20 [1-81]	35.4 ± 14.6 [18-63]

Hatchery-reared subyearling fish died 2-46 d (mean 14 d) after implantation. Even in fish held for 60 and 90 d, 50% of mortalities occurred before day 14, and 77% occurred before day 21. Because of low numbers of occurrences, statistical analysis could not be performed to determine differences between days to mortality of treatment fish.

Mortality among yearling ROR Chinook salmon was more variable than in hatchery fish. Run-of-the-river yearlings incurred 63% (12 fish) of mortalities before day 21. Mortality was observed again between days 55 and 89. These mortalities occurred shortly after water temperatures were raised to 17°C. Subyearling ROR Chinook incurred mortalities throughout the study. No clear pattern of mortality could be seen in these subyearling ROR fish.

Growth

Implantation of hatchery-reared Chinook salmon with either PIT tags or acoustic transmitters and PIT tags affected growth for only one of the four holding periods (Table 9). For hatchery-reared yearling Chinook salmon, significant ($P < 0.05$) differences in growth among tag treatments were seen only 60 d after tagging; no differences were seen 21, 30, or 90 d after tagging. At 60 d after tagging, control fish had significantly ($P < 0.05$) greater growth than both PIT-tagged fish and fish implanted with integrated transmitters, but not fish implanted with non-integrated transmitters (Table 9). Fish implanted with non-integrated transmitters had significantly ($P < 0.05$) higher growth than those implanted with integrated transmitters.

Table 9. Growth, measured as a change in weight (grams ± SE) for hatchery-reared and run-of-the-river (ROR) juvenile Chinook salmon held for 21, 30, 60, or 90 d after implantation. Treatments include non-implanted control fish and fish implanted with PIT tag, integrated PIT tag and acoustic transmitter, or non-integrated PIT tag and acoustic transmitter, 2006. Values along a row with a letter in common are not significantly different ($\alpha = 0.05$).

Year class	Origin	Days held	Growth			
			Control	PIT	Integrated	Un-integrated
Yearling	Hatchery	21	8.2 ± 0.3z	8.7 ± 0.3z	8.5 ± 0.3z	8.8 ± 0.3z
Yearling	Hatchery	30	13.5 ± 0.4z	13.2 ± 0.4z	13.8 ± 0.4z	13.3 ± 0.4z
Yearling	Hatchery	60	33.6 ± 0.6z	31.5 ± 0.6y	31.1 ± 0.6y	33.1 ± 0.6yz
Yearling	Hatchery	90	48.4 ± 1.3z	48.4 ± 1.2z	47.1 ± 1.2z	48.4 ± 1.2z
Subyearling	Hatchery	21	2.3 ± 0.2z	2.7 ± 0.2z	2.6 ± 0.2z	2.8 ± 0.2z
Subyearling	Hatchery	30	8.2 ± 0.2yz	8.3 ± 0.2z	7.7 ± 0.2xy	7.5 ± 0.2x
Subyearling	Hatchery	60	17.8 ± 0.5z	17.8 ± 0.5z	18.7 ± 0.5z	18.1 ± 0.5z
Subyearling	Hatchery	90	27.5 ± 1.0z	28.3 ± 0.9z	28.0 ± 1.0z	30.0 ± 1.0z
Yearling	ROR	21	5.3 ± 0.5	4.9 ± 0.6	5.3 ± 0.7	4.8 ± 0.5
Yearling	ROR	30	9.4 ± 0.8	9.1 ± 0.7	8.9 ± 0.4	8.4 ± 1.0
Yearling	ROR	60	25.6 ± 1.7	25.0 ± 2.2	22.5 ± 0.7	24.5 ± 1.3
Yearling	ROR	90	35.9 ± 1.8	36.0 ± 2.4	34.4 ± 2.4	33.8 ± 2.3
Subyearling	ROR	21	1.2 ± 0.7	2.1 ± 0.6	0.0 ± 0.7	-0.1 ± 0.8
Subyearling	ROR	30	3.5 ± 0.8	4.4 ± 1.0	3.3 ± 0.7	0.0 ± 1.4
Subyearling	ROR	60	6.2 ± 1.3	5.5 ± 1.0	5.6 ± 1.2	4.9 ± 0.9
Subyearling	ROR	90	8.2 ± (0)*	15.4 ± (0*)	7.1 ± 2.1	7.2 ± 2.2

* Only 1 fish survived to necropsy

For hatchery-reared subyearling Chinook salmon, differences in growth among tag treatments were seen 30 d after tagging but not 21, 60, or 90 d after tagging. At 30 d after tagging, control fish had significantly ($P < 0.05$) greater growth than fish implanted with non-integrated transmitters (Table 9). PIT-tagged fish also had significantly ($P < 0.05$) higher growth than fish implanted with either integrated or non-integrated transmitters.

Growth for ROR juvenile Chinook salmon was lower than for hatchery-reared fish (Table 9). Fish implanted with integrated and non-integrated transmitters showed less growth over each holding time compared to control fish. Within the 21-d holding time group, fish implanted with acoustic transmitters showed no growth. Due to small sample sizes, no statistical analyses were performed for differences in growth between treatments in ROR fish.

Tag Expulsion

No acoustic transmitters were expelled by hatchery-reared yearling Chinook salmon (Table 10). There was only a very small occurrence of PIT tag expulsion (1.6%; one tag) among yearling PIT-tagged fish held 90 d. This fish lost its PIT tag 7 d after implantation (Table 10). This lack of dropped tags precluded any meaningful statistical analyses.

Expulsion of transmitters was more common among subyearling than yearling Chinook salmon (Table 10), with rates ranging from 1.5 to 7.8%. Neither fish implanted with integrated transmitters nor fish implanted with non-integrated transmitters had significant ($P > 0.05$) differences in the expulsion rate compared to PIT-tagged fish. There also was no significant ($P > 0.05$) difference in tag expulsion between integrated and non-integrated transmitters. No fish longer than 108 mm (tag burden of 4.8%) at time of implantation expelled transmitters, although hatchery-reared subyearling fish ranged in size from 93 to 126 mm.

The length of time that it took for subyearling hatchery-reared fish to expel their acoustic transmitters varied from 5 to 63 d (Table 11). Only three subyearling fish expelled their transmitters in 14 d or less, and only six fish expelled transmitters before day 21. Mean time to expulsion for hatchery-reared subyearling Chinook salmon was 27 d. Although statistical analysis could not be performed to determine differences in days to expulsion between treatments, we did not observe differences in trends between integrated and non-integrated treatment groups.

Table 10. Percentage of tags expelled for hatchery-reared and run-of-the-river (ROR) Chinook salmon implanted with PIT tag, integrated PIT tag and acoustic transmitter, or non-integrated PIT tag and acoustic transmitter and held 21, 30, 60, or 90 d after implantation, 2006.

Source	Year class	Days held	Expulsion (%)		
			PIT	Integrated	Non-integrated
Hatchery	Yearling	21	0	0	0
Hatchery	Yearling	30	0	0	0
Hatchery	Yearling	60	0	0	0
Hatchery	Yearling	90	1.7	0	0
Hatchery	Subyearling	21	0	7.7	2.0
Hatchery	Subyearling	30	0	1.9	5.6
Hatchery	Subyearling	60	0	7.7	1.5
Hatchery	Subyearling	90	0	4.3	7.8
ROR	Yearling	21	0	0	0
ROR	Yearling	30	0	0	0
ROR	Yearling	60	0	0	0
ROR	Yearling	90	6.7	0	0
ROR	Subyearling	21	0	0	0
ROR	Subyearling	30	0	0	7.7
ROR	Subyearling	60	6.3	12.5	0
ROR	Subyearling	90	0	0	0

The rate of transmitter expulsion among ROR fish was similar to that of hatchery-reared fish. None of the yearling ROR fish expelled acoustic transmitters. However, similar to the hatchery fish, some (6.7%) PIT-tagged fish expelled their tags. Run-of-river subyearling fish had a slightly higher range of transmitter expulsion (0-12.5%) than hatchery-reared subyearling fish (0-7.8%). There was a negative relationship between tag burden and days to expulsion among hatchery-reared subyearling fish implanted with integrated and non-integrated transmitters (Figure 2). Fish with relatively high tag burdens expelled their transmitters sooner than those with low tag burdens.

Table 11. Mean days to tag expulsion (±SD [range]) for hatchery-reared and run-of-the-river (ROR) Chinook salmon implanted with PIT tag, integrated PIT tag and acoustic transmitter, or non-integrated PIT tag and acoustic transmitter and held 21, 30, 60, or 90 d after implantation, 2006.

Source	Year class	Days held	Days to Expulsion		
			PIT	Integrated	Non-integrated
Hatchery	Yearling	21			
Hatchery	Yearling	30			
Hatchery	Yearling	60			
Hatchery	Yearling	90	7 ± 0		
Hatchery	Subyearling	21		18.3 ± 3.1 [15-21]*	unknown*
Hatchery	Subyearling	30		10 ± 0	7.5 ± 3.5 [5-10]*
Hatchery	Subyearling	60		31.3 ± 11.6 [22-47]	15 ± 0
Hatchery	Subyearling	90		50 ± 0*	39.4 ± 20.5 [26-63]*
ROR	Yearling	21			
ROR	Yearling	30			
ROR	Yearling	60			
ROR	Yearling	90	61 ± 0		
ROR	Subyearling	21			
ROR	Subyearling	30			
ROR	Subyearling	60		20 ± 0	
ROR	Subyearling	90			

* Not all dates of tag expulsion are known.

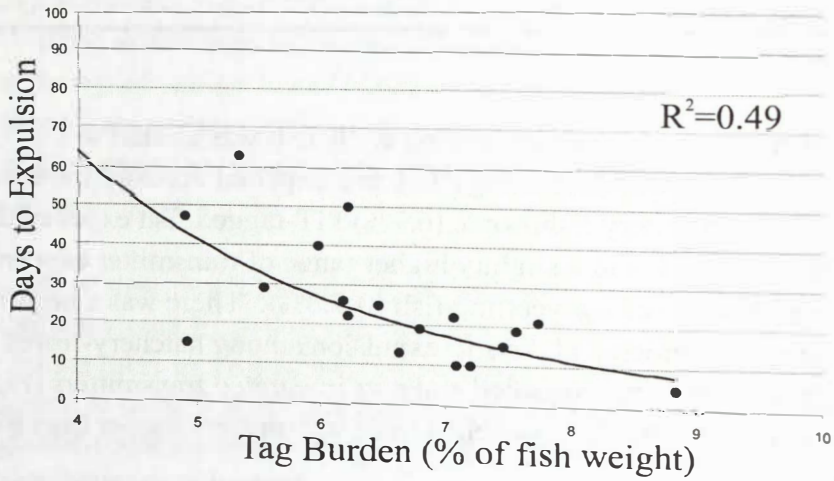

Figure 2. Relationship between days to expulsion and tag burden in subyearling hatchery-reared Chinook salmon, 2006.

Necropsy

General health—Fish health index scores did not vary significantly ($P > 0.05$; see Appendix B for detailed statistics) among treatment groups for either hatchery-reared yearling or subyearling Chinook salmon. Fish health, however, did appear to change over time (Figure 3) for all groups of fish. Hatchery subyearling health improved over time, while hatchery yearling health declined over time. Yearlings held for 21 and 30 d had better health scores than subyearlings held for the same period. However, by days 60 and 90, health scores for yearling and subyearling fish were similar.

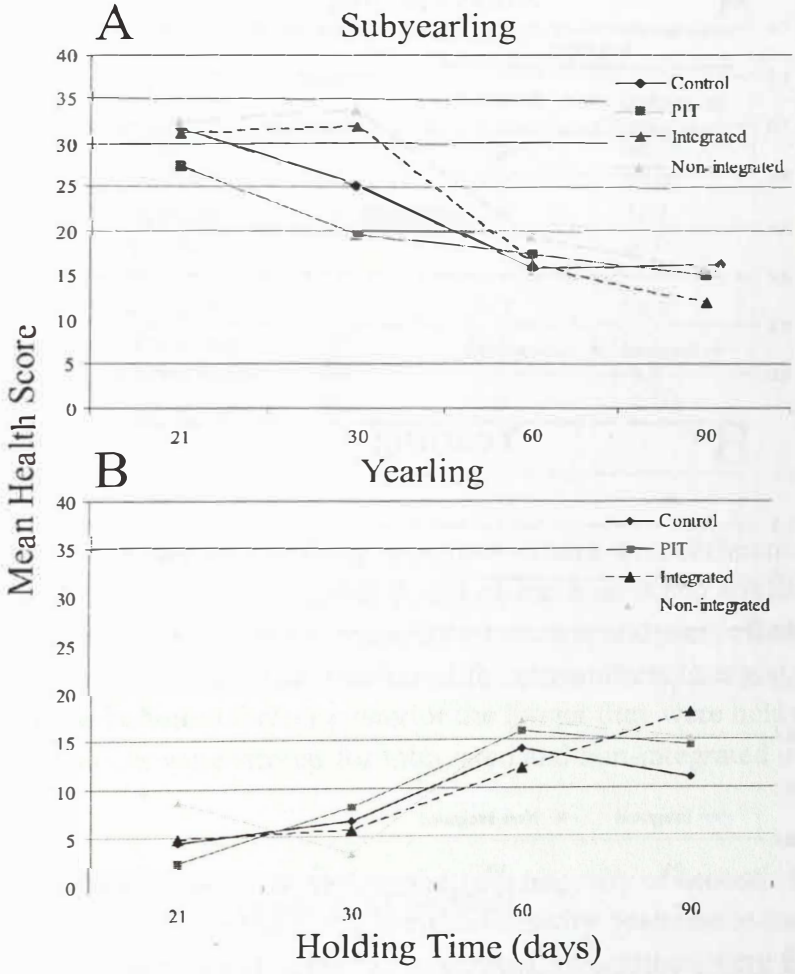

Figure 3. Mean health scores for hatchery-reared subyearling (A) and yearling (B) Chinook salmon implanted with PIT tag only (grey square), integrated (black triangle) or non-integrated (grey triangle) acoustic transmitters, or control (black diamond), 2006.

Incision healing—The incisions of hatchery-reared yearling fish healed more slowly than those of subyearling fish (Figure 4). By day 60, most subyearling fish had completely healed. In comparison, incisions in yearling fish generally showed tissue growth across the incision, but the incision was still distinct and clearly visible. Fish implanted with integrated and non-integrated transmitters showed differences in healing, although there was no consistent trend in differences.

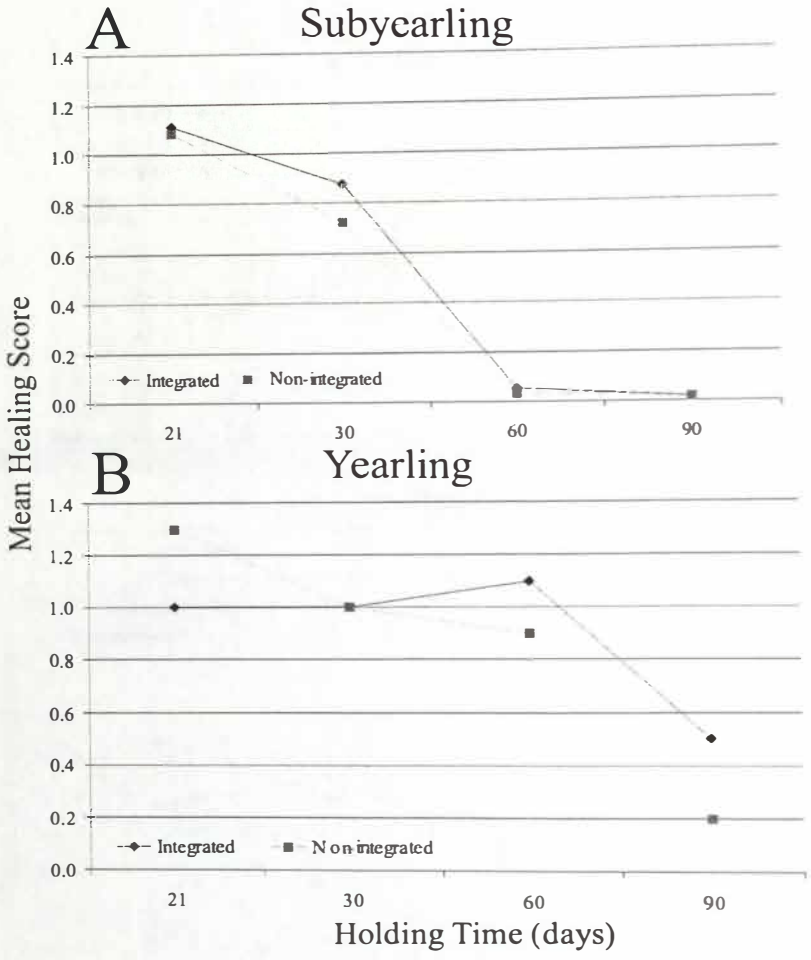

Figure 4. Healing scores for subyearling (A) and yearling (B) aged hatchery-reared Chinook salmon implanted with integrated PIT and acoustic transmitter (black square) or non-integrated PIT and acoustic transmitter (grey diamond) and held 21, 30, 60 or 90 d after implantation, 2006.

Suture retention—Suture retention varied based on fish age and transmitter configuration. Yearling fish implanted with non-integrated transmitters retained sutures significantly longer than those implanted with integrated transmitters ($P < 0.001$). However, no significant difference was seen in subyearling fish between these two groups ($P = 0.079$). In addition, hatchery-reared yearling fish shed sutures more slowly than subyearlings at every holding period (Table 12).

Table 12. Percentage of sutures remaining for yearling and subyearling hatchery-reared Chinook salmon implanted with integrated or non-integrated acoustic transmitter and PIT tag and held for 21, 30, 60, or 90 d, 2006.

Age class	Holding time	Treatment	
		Integrated	Non-Integrated
yearling	21	97.1	100.0
yearling	30	100.0	97.6
yearling	60	57.4	41.1
yearling	90	37.3	20.0
subyearling	21	26.7	29.2
subyearling	30	40.8	23.5
subyearling	60	7.3	4.8
subyearling	90	3.8	0.0

Acoustic transmitter and PIT-tag location—There were differences in locations of transmitters within the peritoneal cavity based on treatments and holding times. In hatchery-reared yearling fish, acoustic transmitters were found most often in the anterior portion of the peritoneal cavity. The location of the transmitters changed significantly over time, tending to be found farther posterior the longer they were held ($P < 0.001$) . Locations of transmitters were similar for integrated and non-integrated treatment groups ($P = 0.73$).

Among hatchery-reared subyearling fish, the majority of acoustic transmitters were found in the posterior portion of the peritoneal cavity posterior to the pyloric caeca 30 d or more after implantation (Figure 5). Integrated transmitters were found in the anterior portion of the peritoneal cavity significantly more often than non-integrated transmitters ($P = 0.005$).

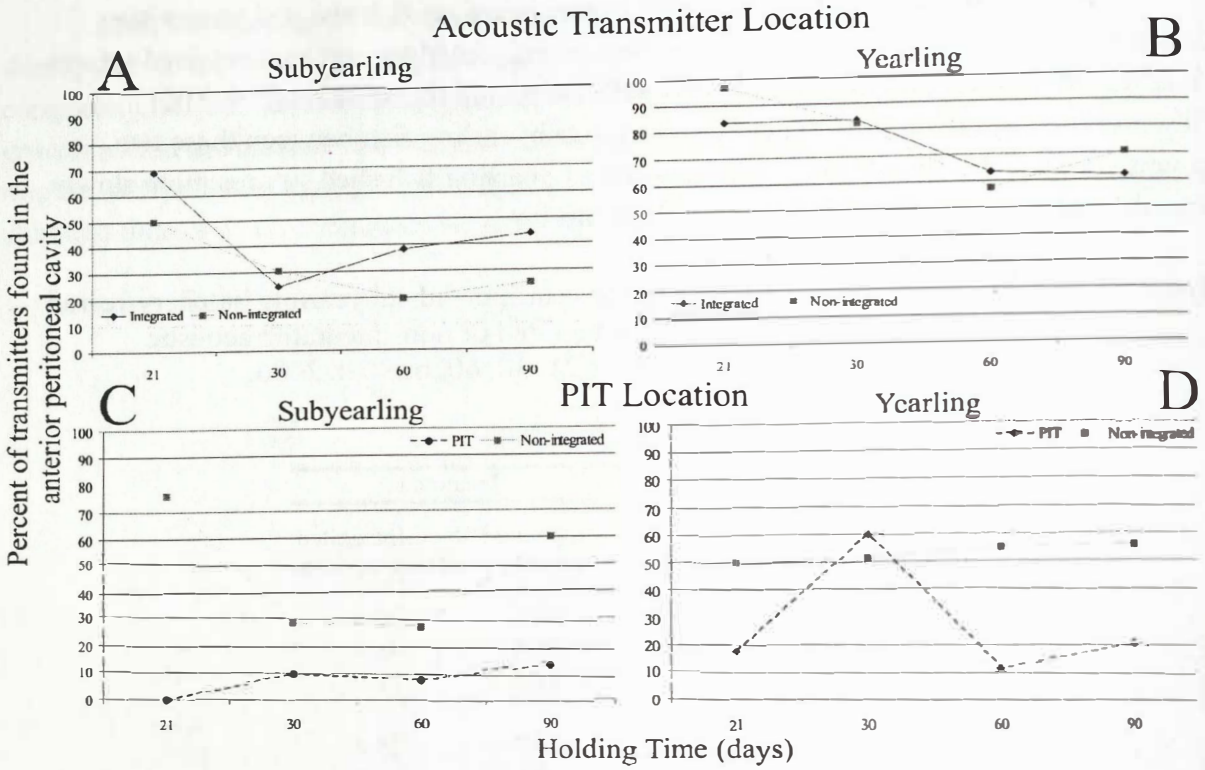

Figure 5. Percentage of integrated and non-integrated acoustic transmitters (A) and PIT tags (B) found in the anterior portion of the peritoneal cavity for yearling and subyearling fish, 2006.

We also observed differences in PIT tag location between treatment groups. In general, both yearling and subyearling hatchery-reared fish implanted with only PIT tags carried these tags in the posterior portion of the peritoneal cavity (Figure 5). The location of PIT tags was significantly more anterior in fish implanted with non-integrated transmitters and PIT tags than in those implanted with PIT tags only ($P < 0.001$ for both yearling and subyearling fish).

Transmitter capsule appearance—The majority of hatchery-reared fish had grossly visible tissue encapsulating the transmitter (Figure 6). Only 1.2% of subyearlings and 2.3% of yearlings had no apparent capsule. When present, the capsule around the transmitter ranged from clear with no vasculature (a score of 1; see Figure 7) to thick with vasculature (a score of 3).

Capsule appearance at times varied with transmitter configuration. The capsule was significantly ($P < 0.034$) thicker among subyearling fish implanted with

non-integrated transmitters than for subyearling fish implanted with integrated transmitters. These differences are obvious only 60 and 90 d after implantation. There was no significant ($P = 0.819$) difference between these two groups for yearling fish.

Among yearling hatchery-reared Chinook salmon, the capsule was often clear with no vasculature, as designated by a score of 1 (Figure 7). Subyearling hatchery-reared fish had thicker and more vascularized capsules compared to yearling fish at every holding time. In addition, the transmitter capsule appearance was more variable over time in the subyearling fish.

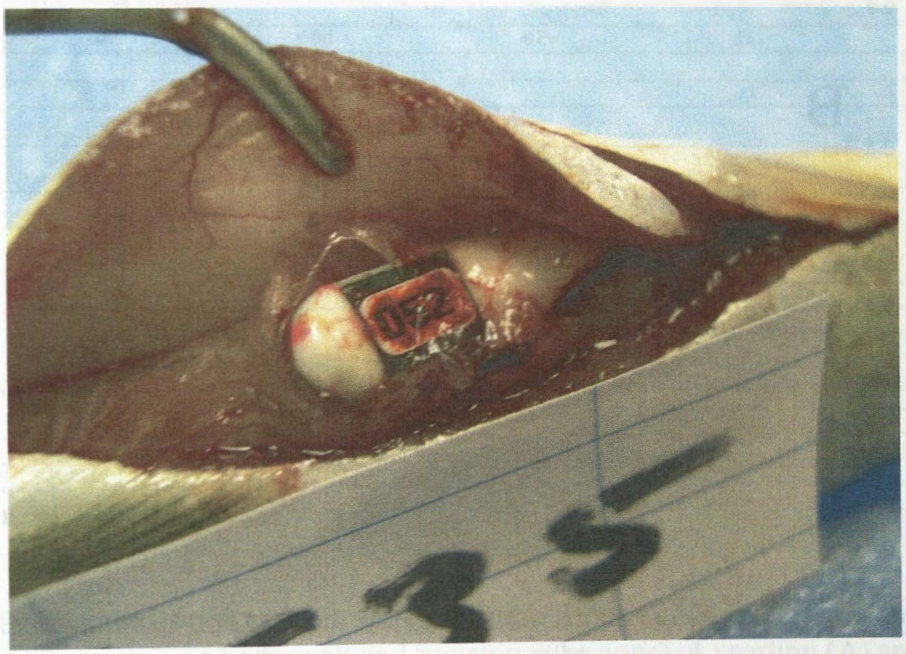

Figure 6. JSATS acoustic transmitter implanted in a yearling Chinook salmon, 2006. The transmitter is covered with a thin capsule of clear fibrous tissue, seen tearing as the body cavity is opened. This fish was held for 21 d before necropsy.

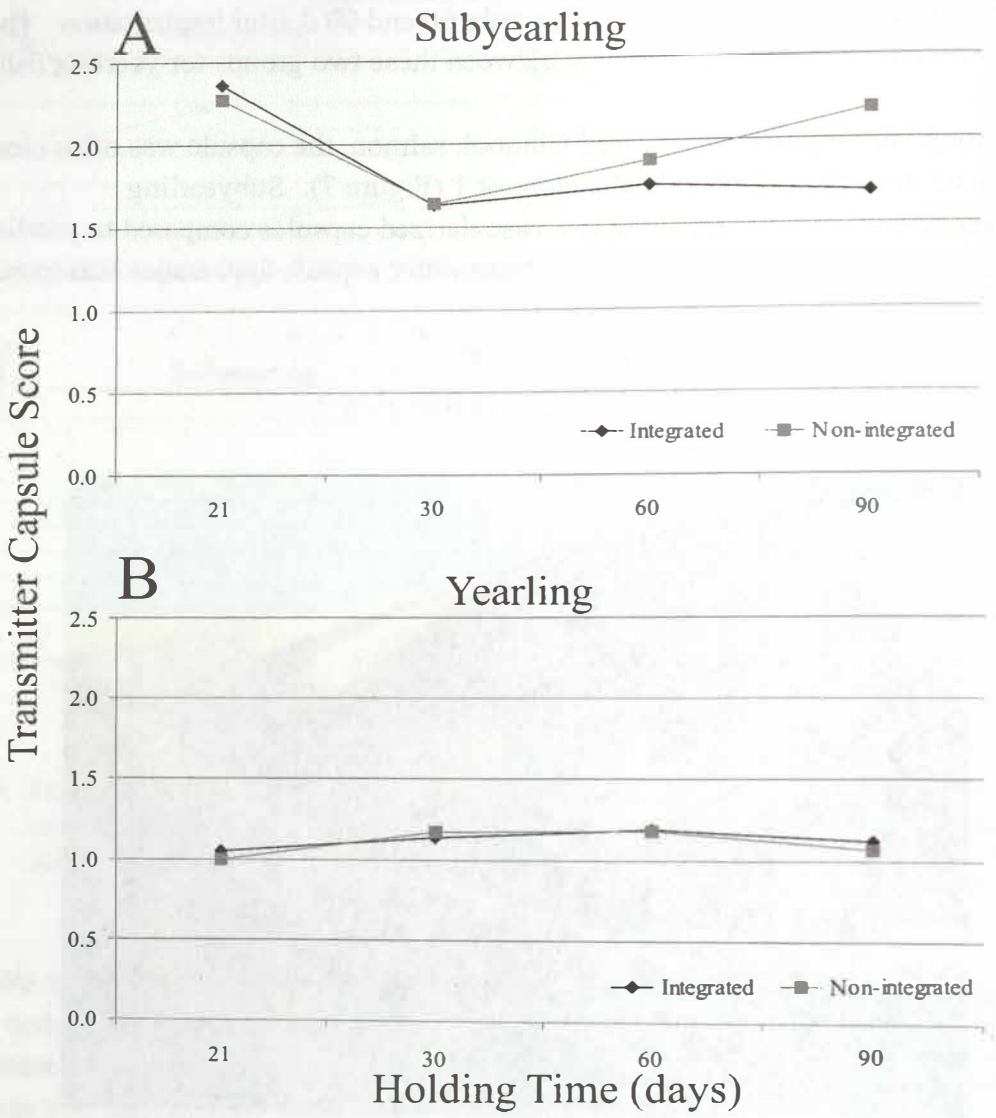

Figure 7. Capsule appearance score for subyearlings (A) and yearlings (B) implanted with integrated and non-integrated acoustic transmitters and PIT tags, 2006.

Transmitter adhesions—Both hatchery-reared yearling and subyearling Chinook salmon commonly had fibrous adhesions between the capsule around the transmitter and the surface of the peritoneal cavity. Adhesions to the peritoneal cavity surface or body wall appeared to decrease over time in yearling fish, while this trend was not observed in the subyearling fish.

The location of fibrotic adhesions involving the incision varied with year class and with time. After day 21, transmitters were more likely to be adhered to the incisions of subyearling fish than to those of yearling fish (Figure 8). This may be related to the amount of tag expulsion observed in subyearling fish. Although adhesions to the incision were common among yearling fish 21 d after implantation, such adhesions markedly declined the longer after implantation the fish were held. In contrast, adhesions to the incision of subyearling fish persisted over the 90-d study period.

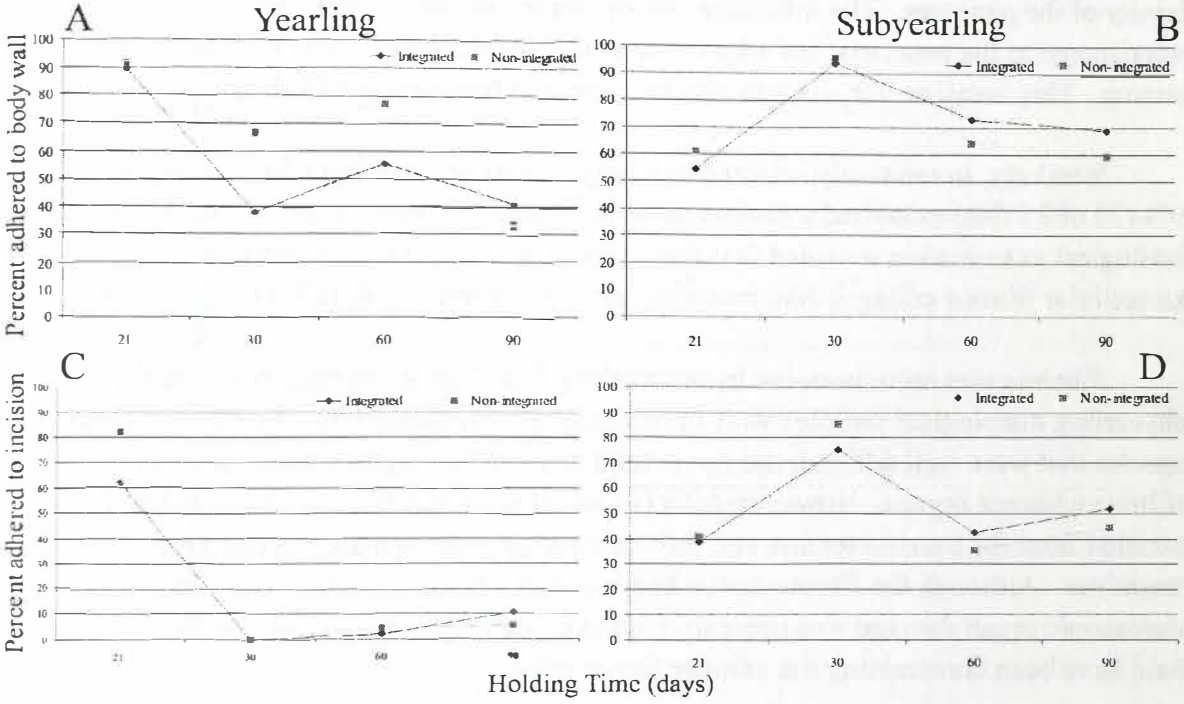

Figure 8. Percentage of transmitters adhered to the body wall (surface of the peritoneal cavity; a sum of body wall and incision adhesions) of yearling (A) and subyearling (B) hatchery-reared Chinook salmon implanted with integrated and non-integrated transmitters. The percentage of adhesions to the incision alone also is shown for yearlings (C) and subyearlings (D), 2006.

Fish were examined also to determine if there was a difference in adhesions due to transmitter configuration. There was no significant difference in the location of adhesions between fish implanted with non-integrated acoustic transmitters and those implanted with integrated transmitters ($P = 0.42$ for subyearlings; $P = 0.067$ for yearlings).

Histology

Among randomly selected hatchery-reared yearling Chinook salmon, all transmitters were surrounded by fibrotic capsules consistent with an inflammatory response by the immune system to a foreign body. The majority of submitted specimens (75%; 9 fish) exhibited tissue reactions that were well contained to the transmitter area and did not infiltrate adjacent organs. In the remaining 25% of fish (3 fish), the fibrous tissue, like that comprising the transmitter capsule, had infiltrated organs immediately around the transmitter. Typically, the invasive fibrosis was found in adipose tissue in the vicinity of the pancreas. The inflammation of this tissue did not appear to be associated with damage to the pancreas, but adipose cells may have been constrained by the tissue reaction. This invasive fibrosis was observed only in fish held for 21 d.

Similarly, in randomly selected hatchery-reared subyearling Chinook salmon, 80% (20 of 25 fish) exhibited a fibrous capsule around the transmitter. A more detailed histological examination revealed that this capsule was composed of fibrocytes, or extracellular fibrous collagen-like material, and interspersed with inflammatory cells.

Fibrosis was more invasive in subyearling fish than in yearling fish. Of the subyearling histological samples with fibrous capsules present, 25% (5 of 20 fish) had capsules that were well defined, did not extend beyond the capsule itself, and did not infiltrate adjacent organs. However, 55% (11 of 20 fish) had fibrous tissue that had extended from the transmitter site and infiltrated organs immediately adjacent to the transmitter. Although the fibrous tissue had invaded adjacent organs, no clear or obvious microscopic organ damage was present. However, as noted above, the fibrotic reaction could have been constraining the adipose tissue cells.

An additional 20% (4 of 20 fish with capsules; 16% of all 25 fish examined;) had fibrotic tissue of capsular origin that was more extensively invasive in the peritoneal cavity and clearly caused organ damage. This damage consisted of compression of the pancreas and cellular changes associated by such compression. The results suggest that fish resolved such invasive fibrosis and organ damage because the observation was more common in fish held 21 d than those held 30, 60, or 90 d. All fish that had fibrotic infiltration of organs were less than 101 mm fork length and 12 g at tagging (range 95-101 mm and 9.7-12 g at tagging), while all fish submitted to histology ranged from 95 to 126 mm and from 9.7 to 23.7 g.

Fibrocytes, which secrete collagen that makes up the fibrous capsule, were the most common cell found in the capsule tissue and peaked at 21 d after implantation (Figure 9). Macrophages, which remove and digest foreign material, and lymphocytes, another inflammatory cell, also were present. All types of inflammatory effector cells (macrophages, lymphocytes, and eosinophilic granular cells) were relatively rare compared to fibrocytes.

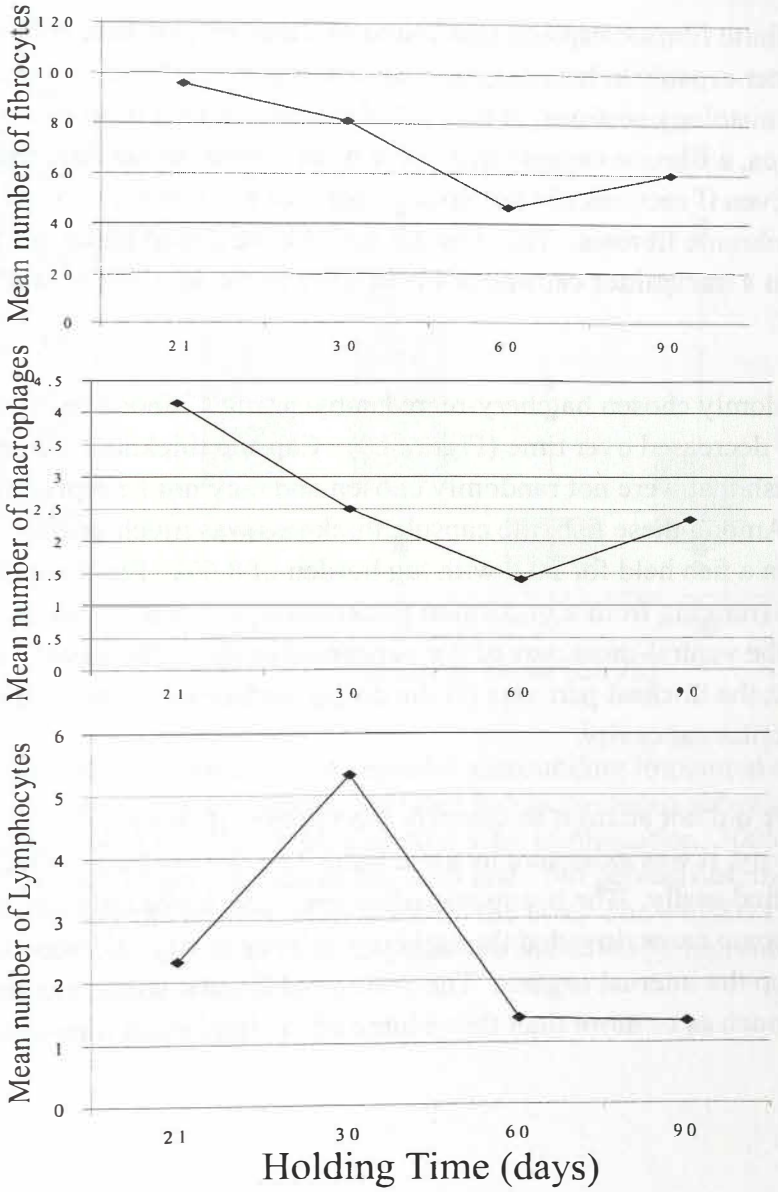

Figure 9. Mean number of three different cell types found during histological analysis, 2006.

Numbers of macrophages and eosinophilic granular cells also peaked at 21 d and became less common over time (30, 60, and 90 d after implantation). Lymphocyte numbers were highest 30 d after implantation, which could indicate the attempt to resolve infection from contaminants or pathogens implanted with the transmitter. Necrotic cells found in the transmitter capsules were most abundant 21 d after implantation and decreased with time. The decrease in the cellular components of the transmitter capsule over time may signify a resolution of the noxious stimulus caused by implantation.

While a definite fibrotic capsule was found in a majority of fish, 20% of fish did not have a transmitter capsule in histological sections. This may be an underestimate, however, since the histology sections, at times, did not have tissue from directly around the transmitter. Thus, a fibrotic capsule may have been present in the fish, but absent from the sample. Even if sections did not contain parts of the fibrotic capsule, many contained areas of chronic fibrosis. This fibrosis could have arisen either from the incision site or from a transmitter capsule not contained in the sections submitted for histology.

Among randomly chosen hatchery-reared subyearling Chinook salmon, capsule thickness generally decreased over time (Figure 10). Capsule thickness was examined also among other fish that were not randomly chosen and may not be representative of all fish in this study. Among these fish, the capsule thickness was much greater, up to 2,064 μm for one radius in a fish held for 30 d with tag burden of 8.6%. For three fish with very thick capsules (ranging from a maximum thickness of 1,236 to 2,064 μm), the transmitter was in the ventral-most part of the peritoneal cavity. The fibrotic capsule was not uniformly thick; the thickest part was on the dorsal surface of the transmitter, below the organs of the peritoneal cavity.

Although we did not attempt to quantify the volume of fibrous tissue growth within the body cavity, it was extensive in some fish. The total volume of the fibrous tissue is not quantified easily. The tissue was often invasive; it not only covered the transmitter but, in some cases, invaded throughout the body cavity and was interspersed with cells making up the internal organs. The volume of fibrous tissue in some cases appeared to be as much as or more than the volume of the implanted transmitter.

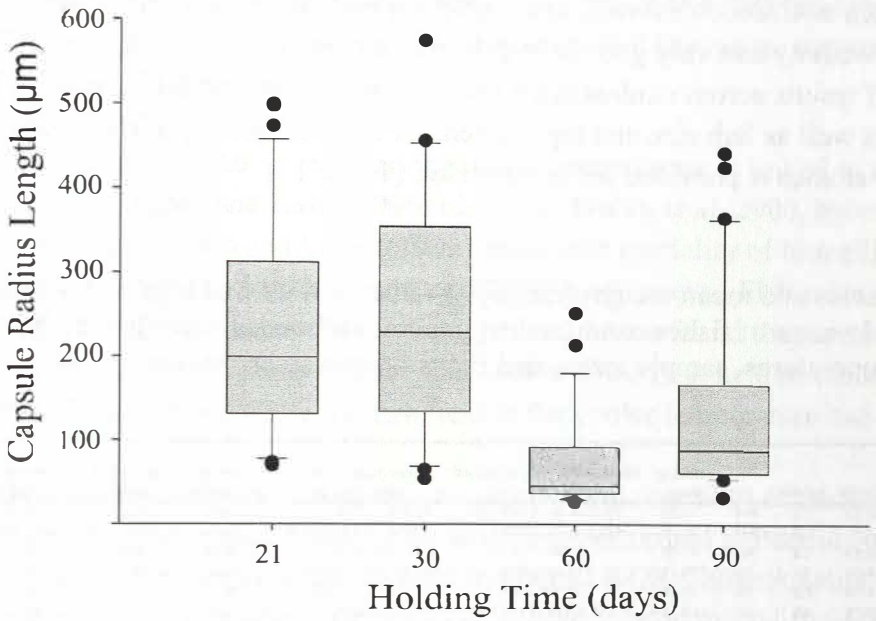

Figure 10. Thickness of fibrous tissue capsules surrounding implanted acoustic transmitters from randomly selected hatchery-reared subyearling Chinook salmon held for 21, 30, 60 and 90 d after implantation, 2006. The boundary of the box in grey indicates the 25th and 75th percentiles; the median is marked by the horizontal line within the box. The whiskers indicate the 10th and 90th percentiles. Outlying data are indicated by individual points above and below the whiskers.

Discussion

Mortality

No differences were observed among test groups (control, PIT-tagged, implanted with an integrated or non-integrated PIT tag and acoustic transmitter) in mortalities due to implantation of acoustic transmitters or PIT tags for hatchery-reared yearling or subyearling Chinook salmon. Seemingly conflicting results exist in the literature concerning mortality rates among implanted salmonid species. However, poikilothermic organisms, such as Chinook salmon, are highly dependent on their environment, and physiological activity can vary greatly in different environmental conditions. Thus, comparison of results across studies must take into consideration differences in holding temperature as well as fish size and tag burden. A detailed description of these aspects of this and other studies is provided for comparison (Table 14).

Table 14. Species and mean weight (range) of fishes and size of tags and percentage of body mass of fishes examined by several authors and for this study. Holding temperatures, sample sizes, and fish source also are shown.

Authors	Source	Species	N	Holding Temp.	Mean (range) FL of tagged fish (mm)	Mean (range) mass of tagged fish (g)	Antenna length (cm)	tag mass in air (g)	tag excess mass (g)	% tag mass in air
This study main part	H SY	CS	947	15-20	103.2 & 103.4 (95-126)	12.1 & 12.2 (8.5-23.7)	None	0.62-0.73	0.37-0.45	2.9 to 8.8
This study main part	H Y	CS	841	11-17	128.9 & 130.2 (98-152)	23.6 & 24.5 (9.2-43.0)	None	0.62-0.73	0.37-0.45	1.5 to 7.3
This study size limit	H SY	CS	908	17-20	(80-109)	(4.7-16.3) †	None	0.62	0.37	4.3 to 15.1
Brown et al. 2006	ROR	SS	50	10-13	113 (105-123)	12.2 (10.2-16.0)	None	0·75	0·41	4.6-7.2
Brown et al. 2006	ROR	CS	50	13-16	105 (93-116)	12.4 (7.5-16.8)	None	0·75	0·41	4.3-9.7
Robertson et al. 2003	ROR	AS	19	13.8-18.4	150 & 152 (134-191)	37.8 & 45.5	28	0·75	0·5	means 1.6-2.0*
Adams et al 1998a	H	CS	43	12	135	28	31	1	0.7	3.6
Martinelli et al. 1998	H	CS	60	14	119	20.5	30	1.3	0.9	7
Moore et al. 1990	H	AS	11	11.2-13.5	172.7	59.4	None	1.3	*	2.2*

*Not reported by Author

CS=Chinook salmon; AS = Atlantic salmon, SS= sockeye salmon

H = Hatchery; ROR = Run-of-the-river; SY= subyearling; Y=yearling

Studies examining mortality in intraperitoneally implanted salmonids of similar size to yearlings in the current study (98-152-mm fork length) have found little to no mortality despite differences in temperature and tag burden. Martinelli et al. (1998) found no mortality in hatchery-reared juvenile Chinook salmon implanted with a radio transmitter and held 5 or 21 d (see Table 14 for fish sizes, tag burdens, and holding temperatures). Robertson et al. (2003) did not observe any mortality from implantation of juvenile ROR Atlantic salmon *Salmo salar* with radio transmitters. Moore et al. (1990) also found no mortality in Atlantic salmon surgically implanted with acoustic transmitters and held 150 d. Adams et al. (1998a) reported 2% mortality (1 fish out of 43) 36 d after implantation of hatchery-reared juvenile Chinook salmon.

Fewer studies have examined the effects of implantation on smaller salmonids. The implanted hatchery subyearling Chinook salmon examined during this study experienced higher mortality (3.7-8.3%) than implanted hatchery yearling Chinook salmon (0-4.5%), although there was no difference in mortality between implanted and control animals. Brown et al. (2006) saw 24% mortality over 21 d in similarly sized implanted ROR Chinook salmon (held in 13-16°C water; see Table 14 for fish sizes and tag burdens), but no mortality in subyearling-size ROR sockeye salmon *Oncorhynchus nerka* held at 10-13°C. Adams et al. (1998b) saw no mortality in implanted hatchery-reared subyearling Chinook held at 12°C, much colder than temperatures used during this study. These differing results suggest that fish of this size have the potential to be negatively influenced by transmitter implantation, and that water temperature plays an important part in the fish's reaction.

Other researchers also have found that water temperature is linked to the survival of implanted fish (Knights and Lasee 1996, bluegills; Walsh et al. 2000, hybrid striped bass). For example, Knights and Lasee (1996) examined mortality of bluegill *Lepomis macrochirus* (mean weight 133g) held at 6 and 20°C for 8 weeks. Fish were surgically implanted with 2.81-g radio transmitters. Knights and Lasee found the mortality rate was 10% for fish held at 20°C but only 0% for fish held at 6°C. Fish held in the warmer water also had 15% tag loss, while the fish held at the cooler temperature had no tag loss.

Temperature was also a probable factor in the high mortality of the ROR Chinook salmon during this experiment. Previous experiments performed at Pacific Northwest National Laboratory (Brown et al. 2006) held implanted ROR Chinook salmon at lower temperatures with lower mortality. Brown et al. (2006) saw no mortality for implanted ROR sockeye salmon and only 24% mortality in implanted subyearling ROR Chinook salmon—mortality rates much lower than those seen in this study (0-27.3% for implanted yearlings and 23.1-86.7% for implanted subyearlings).

Growth

This portion of the study found no trend of differences in growth among PIT tag implanted, control, or integrated and non-integrated transmitter implanted hatchery-reared Chinook salmon that ranged in size from 95 to 158 mm. For yearling hatchery reared fish, there were differences among tag groups only 60 d after tagging. At 60 d after tagging, there was no difference between control fish and those implanted with non-integrated transmitters. Because fish are implanted with non-integrated transmitters during field studies, no relevant growth effect on yearling fish was seen. For subyearling hatchery-reared fish, there were differences among tag groups only 30 d after implantation. Both control and PIT-tagged fish had higher growth at this time than fish

implanted with non-integrated transmitters. However, because there were no differences among tag groups 21, 60, or 90 d after implantation and no differences between control fish and those implanted with integrated transmitters on day 30, there is likely no biological difference among tag groups.

A similar lack of difference in growth was found by Brown et al. (2006) for subyearling-size ROR juvenile sockeye and Chinook salmon implanted with transmitters and held 21 d (see Table 14 for fish sizes, holding temperatures, and tag burdens). Martinelli et al. (1998) also found no difference in growth between control and surgically implanted juvenile Chinook salmon after being held for 5 or 21 d. Similarly, Moore et al. (1990) examined the growth of juvenile Atlantic salmon 14 and 28 d after surgical implantation and did not find any difference in growth among control, tagged, or sham tagged groups.

In contrast, Robertson et al. (2003) found negative effects over 45 d from implantation of a radio transmitter in juvenile Atlantic salmon with a mean tag burden of only 1.6-2.0%. Similarly, Adams et al. (1998a) found that surgically implanted juvenile Chinook salmon had lower growth 21 d after implantation than control fish, but that there was no difference between the two groups 54 d after implantation.

Implanted ROR subyearlings in the current study showed less growth over all holding periods than controls, and this effect was most pronounced at 21 and 30 d post-implantation. However, statistical evaluation of these results was not possible due to low sample sizes. Robertson et al. (2003) also found diminished growth of ROR juvenile Atlantic salmon up to 36 d after radio transmitter implantation comprising up to 3.6% of the fish's body weight. However, no difference in food consumption between control and implanted fish was noted.

In the current study, we were not able to measure differences in food consumption between implanted and control animals within a holding period because all treatment groups were housed together. However, we found ROR fish in a captive setting did not eat as readily as hatchery fish.

Tag Expulsion

Acoustic transmitter expulsion was observed in only subyearling fish under 108 mm (tag burden > 4.9%). Other researchers have seen a lack of transmitter expulsion in juvenile salmon. Martinelli et al. (1998) saw no transmitter expulsion over the 21 d in surgically implanted Chinook salmon (see Table 14 for fish and transmitter details). Adams et al. (1998a) also did not find any radio transmitters expelled during their 54-d study of juvenile Chinook salmon. Robertson et al. (2003), however, did find transmitters expelled from juvenile Atlantic salmon 20-29 d after implantation.

Our research suggests that there may be a negative relationship between the burden of an implanted transmitter and the time to transmitter expulsion. In subyearling Chinook salmon, we observed transmitter expulsion as early as 5 d after implantation in a 95-mm fish with a transmitter burden of 8.8%. The exact cause of expulsion in these smaller fish is most likely a combination of several factors that relate to the size of the fish at implantation and result in differences in tissue reaction, as seen in our necropsy results. Tag expulsion may be a problem for smaller subyearling fish which may compromise in-river survival studies. Further research on the cause of this expulsion and the relationship between tag burden and expulsion are needed.

Necropsy

General health index—General health index scores indicated that hatchery-reared yearling fish were in good condition when implanted and gradually declined in health, while the opposite was true for subyearling fish. Because this was the case for all treatment groups, it is unlikely this was related to either PIT tagging or implantation of transmitters. The health of yearling fish may have declined as water temperatures increased over the 90-d holding period. The reason the health index of subyearling fish improved from day 21 to day 90 is unclear.

Incision healing—The surgical incisions of hatchery-reared yearling fish healed more slowly than those of subyearling fish. While there were few differences between the age classes 21 d after implantation, subyearling incisions were more healed by day 30; most were completely healed by day 60, while yearling fish incisions healed more slowly.

Other authors, however, have stated that juvenile Chinook salmon incisions were well healed by 21 d after surgical implantation of radio transmitters (Martinelli et al. 1998). Similarly, Adams et al. (1998a) found that most juvenile Chinook salmon they studied (surgery incisions from sham surgeries or transmitter implantation) were well

healed by day 21, but incisions on 29% of the fish were slightly inflamed, reddened, or incompletely healed 21 d after surgery. They noted that by 54 d after surgery, only 21% of fish had similar incisions. When provided a shorter period of time to heal (5 d), Martinelli et al. (1998) found that 50% ($N = 6$) of the Chinook salmon had slightly red or swollen incisions. This inflammation was gone 21 d after implantation.

Healing of incisions was likely related to water temperature. Yearling fish were held under lower water temperatures than subyearling fish. There is evidence that healing and resolution of inflamed lesions is correlated with temperature in salmonids (Anderson and Roberts 1975). Thus, lower water temperature likely slowed the healing process in the yearling fish.

Suture retention—Similar to the rate of incision healing, yearling Chinook salmon retained their sutures for a much longer period than did subyearling fish. This is likely due to the slower metabolic rate of yearlings because of colder holding water temperature. Because the water temperature in the laboratory reflected water temperatures in the Snake and Columbia Rivers, this rate of suture retention would likely be observed during field studies.

Yearling Chinook salmon implanted with integrated transmitters retained sutures longer than those implanted with non-integrated transmitters. The reason for this is unclear. However, because the retention of sutures during the first 30 d was near 100% for both groups, it is unlikely fish would have lost transmitters due to a lack of suture retention.

Acoustic transmitter and PIT tag location—When implanted alone, PIT tags were located most often in the posterior portion of the body cavity. However, this was not the case for acoustic transmitters. The location of acoustic transmitters (either integrated or non-integrated) generally began near the anterior part of the body cavity but was farther posterior in the body cavity as the fish were held longer following implantation.

There was a trend of smaller implanted objects being positioned farther back within the body cavity than larger implanted objects. PIT tags were inserted next to the acoustic transmitter during surgery (placed slightly anterior to the pelvic girdle) but later were found mostly posterior in the body cavity. Among subyearling fish, non-integrated transmitters (those without a PIT tag adhered) were found farther back in the body cavity than the large integrated transmitters (transmitters with a PIT tag adhered).

It follows that because of their size, the larger transmitters may be unable to migrate farther back into the body cavity of small fish. It may be preferable for implants to reside farther back in the body cavity of smaller fish so they do not inhibit the filling of the stomach. Alternatively, the expansion of the stomach from feeding may shift implants farther back within the body cavity when there is enough physical space. Because acoustic transmitters were found more often anterior in yearling fish but posterior in subyearling fish, there may be enough space within the body cavity of the larger fish so the expansion of the stomach does not push the implant farther back within the body cavity.

When surgically implanted with a transmitter, PIT tags were farther anterior within the body cavity than when injected alone. When included during surgery, the PIT tag may become adhered to the fibrous tissue associated with the implantation of the transmitter and remain farther anterior.

Tissue reaction—Cutting the skin or introducing foreign material into the body causes a disruption of normal function in tissues surrounding the foreign object and, therefore, a loss in homeostasis. Inflammation is the process by which the body attempts to return itself to a homeostatic state. Both the breaching of the body wall and the presence of an implant results in inflammation. In addition, if foreign material (such as bacteria or a biotic microscopic debris) is inadvertently implanted, the inflammatory response will be exacerbated.

As part of the return to homeostasis, the inflammatory response consists of a progression of cell types that clean the site, prepare the site for reconstruction of tissue, and then aid in reconstruction. The following describes a simplistic explanation of this process; for a more in-depth review, see Lorenz and Longaker (2003) and Coleman et al. (1974).

The first (acute) step in the return to homeostasis happens during the first few days. This stage serves to minimize damage and prepare the site for repair. Then, after several days, tissue repair begins and, if present, the sequestration of an implant begins. This marks the beginning of chronic inflammation.

During the chronic inflammation stage, macrophages (which can include multinucleated giant cells) continue to clean the site by engulfing necrotic cells, bacteria, and small foreign bodies. In addition, they prepare the site for the deposition of the fibrous matrix needed to repair the damaged tissue. Fibrocytes then are able to deposit collagen, which makes up the fibrous tissue.

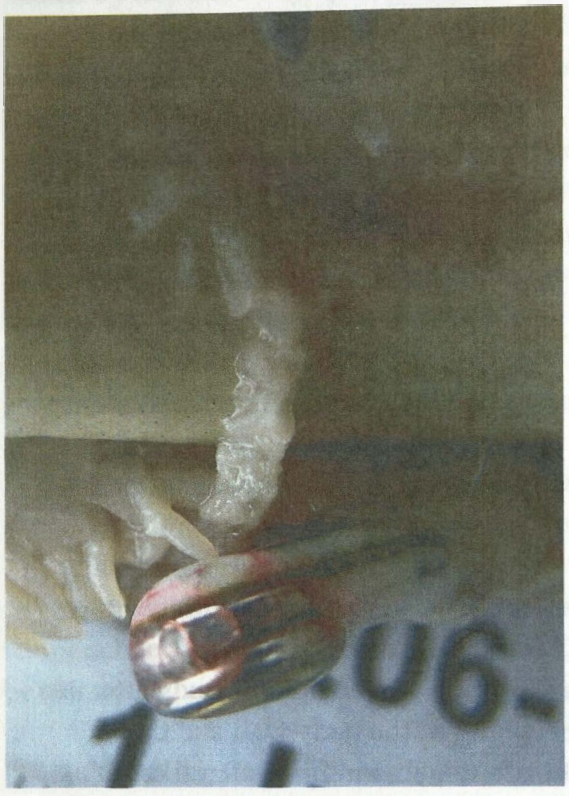

Figure 15. Bundle of fibrotic strands, consisting primarily of collagen and fibrocytes extending from the surgical incision to the capsule covering the transmitter and adhering to the pyloric caeca.

This fibrous tissue allows the two sides of the incision to fuse. In addition, this tissue surrounds the implanted transmitter and can adhere to internal organs, the body wall, or other tissue. Fibrosis is the common name for the formation of this fibrous tissue composed of collagen. Long strands (as seen in Figure 15) of this fibrous tissue can emanate from either the surgical incision or the capsule covering the transmitter and adhere to internal organs or other tissue.

Transmitter capsule appearance— Almost all of the surgically implanted fish had fibrous tissue encapsulating the acoustic transmitter or, in the case of integrated transmitters, both the acoustic transmitter and its adhered PIT tag. Similarly, Martinelli et al. (1998) found that 21 d following implantation in juvenile Chinook salmon, radio transmitters were encapsulated in a fibrous tissue. Other researchers observed that transmitters implanted in juvenile Atlantic salmon were encapsulated in tissue 45 d after implantation (Robertson et al. 2003) and 21 d or more after implantation (Moore et al. 1990).

This research revealed that the appearance of the fibrous capsule surrounding the transmitter can vary between yearling and subyearling fish. Subyearling hatchery-reared fish had thicker and more vascularized capsules compared to yearling fish. The thicker capsules may be due to a more intense reaction to the implantation and the high tag burden. Capsules may also have been thicker in subyearling fish than yearling fish because the subyearling were held in higher water temperatures.

There was no general trend in the appearance of capsules over time, especially among yearling fish. The gross appearance of the capsules in subyearling fish was thinner 30 d after implantation than 21 d after implantation but then tended to be thicker after 60 and 90 d. However, histological analysis indicated that the thickness of capsules

over time decreased. The lack of change or decrease in capsule thickness over time seen in this research on Chinook salmon differs from the findings of Moore et al. (1990). They state that 21 d after Atlantic salmon were implanted with transmitters, capsules were clear, but 90 d after implantation, the capsules had thickened.

Transmitter adhesions—The growth of fibrous tissue was not isolated to the capsule surrounding the implanted transmitter or PIT tag (less commonly seen). Fibrous tissue also connected the capsule to the body wall, to adipose tissue (fat), and to internal organs. In some fish, these adhesions were quite extensive and, although not quantified, appeared to occupy as much volume as the transmitter itself.

Ideally, the process of implanting a telemetry device would involve the production of a minimal fibrotic tissue that would surround the implant only. However, the inadvertent introduction of other microscopic material, such as viruses, fungi, scales, or other foreign materials into the body cavity, can lead to an acute and chronic inflammatory response.

From the research we conducted, it is not possible to determine the exact cause or causes of the fibrosis (at times extensive) that extended from the transmitter capsules and into other organs or tissue. Further research should be conducted to determine the cause and identify solutions to minimize the development of fibrosis and adhesions because of the large volume this reactive tissue can occupy. We suggest experiments in which sham surgeries are conducted to determine the extent of fibrosis present without the implantation of a transmitter. In addition, research into various tag coatings and volumes and implantation methods should be conducted to determine if the foreign body response could be minimized.

The capsule surrounding the implanted acoustic transmitters was often, but not always, attached to the body wall of juvenile Chinook salmon and, in many cases, was adhered to the incision. Among juvenile Atlantic salmon, Robertson et al. (2003) noted that 45 d after implantation, all radio transmitters were encapsulated in a fibrous capsule on the body wall. However, we have not found research that details the locations where capsule adhesions reside within the body cavity.

Adhesions to the incision were commonly seen and varied over time. Twenty-one days after implantation in yearling fish, most of the capsules were adhered to the incision. However, as yearling fish were held for longer durations, very few of the capsules remained adhered to the incision. There appears to be a correlation between the location of the transmitter within the body cavity and the likelihood of the capsule being adhered to the incision. The longer yearling fish were held, the more likely transmitters were

located farther posterior within the body cavity, away from the incision, and adhesion to the incision was less likely. No relationship between adhesions to the incision and length of recovery time was apparent among subyearling fish.

Histology—Histological examination of subyearling and yearling fish revealed that in some cases, fibrous tissue had invaded surrounding tissue and organs of the peritoneal cavity. This occurred more often among subyearling fish than yearling fish. The fibrosis of surrounding organs was observed only 21 d after implantation in yearling fish. In subyearling fish, it was observed at 21, 30, 60, and 90 d after implantation.

Adhesion of the transmitter capsule to surrounding organs has been reported by other researchers (Lucas 1989; Moore et al. 1990). Infiltration of the reactive fibrous tissue into surrounding organs has been reported by others as well, although the potential impact of such infiltration is not known (Bauer and Loupal 2007). Additionally, because there was no obvious cellular damage in fish examined as part of this study, minimal loss of function of the affected organs is suggested, although the effect of compression or constraint of the tissues by the invasive fibrotic tissue is not known. The fact that the reactions tend to resolve over time suggests limited effect.

As the length of time after implantation increased, the type of cells present in the capsule changed in subyearling fish. At day 21, the number of fibrocytes was the largest, and they became less common at days 30, 60, and 90. This may be a relative effect in which the mass of the capsule tends to increase in content of extracellular collagen over time and thus the mass of fibrocytes is relatively decreased. In addition, the fibrotic reaction may have stabilized over time and become less significant due to normal growth of the fish. The secretion of collagen may also have decreased more than 21 d after implantation. Although the rate of fibrosis and collagen deposition may decrease, the rate of resorption or resolution of previously formed material is not known.

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DETERMINATION OF A MINIMUM FISH SIZE FOR IMPLANTATION WITH A JUVENILE SALMONID ACOUSTIC TELEMETRY SYSTEM (JSATS) TAG

Richard S. Brown, Kathleen M. Carter, Katherine A. Deters, and Craig A. McKinstry

Ecology Group
Pacific Northwest National Laboratory
P.O. Box 999, MS K6-85
Richland, Washington 99352

Executive Summary

We evaluated the minimum fish length at which a Juvenile Salmonid Acoustic Telemetry System (JSATS) transmitter could be surgically implanted without negative effects on growth and survival. Tests were conducted with 908 yearling Chinook salmon from Priest Rapids Hatchery (458 implanted and 450 non-tagged). Test fish were held in indoor circular tanks at the Pacific Northwest National Laboratory Aquatic Laboratory in Richland, Washington.

For juvenile Chinook salmon, the minimum fish length at which surgical implantation of a JSATS transmitter and a PIT tag did not negatively influence growth (change in weight) was 88.3 mm (95% CI, 80-97 mm). The minimum fish length at which surgical implantation of a JSATS transmitter and a PIT tag did not negatively influence survival was 95 mm fork length.

For fish used during this study, this was equivalent to a tag burden (combined burden of acoustic transmitter and PIT tag) of approximately 7.6% of body weight for a 95-mm fish, which would weigh approximately 9.2 g. Acoustic transmitter expulsion was observed only in subyearling fish under 108 mm (tag burden > 4.8%).

Introduction

The goal of this evaluation was to determine the minimum fish length at which surgical implantation of a Juvenile Salmonid Acoustic Telemetry System (JSATS) transmitter and a PIT tag would not negatively influence the growth and survival of juvenile Chinook salmon between 80 and 110 mm fork length. Currently, field studies using the JSATS transmitter do not implant fish under 95 mm fork length. However, to use telemetry as a tool to study the entire run of subyearling Chinook salmon, fish smaller than this will need to be implanted.

Studies reported here were conducted in the Aquatic Laboratory at Pacific Northwest National Laboratory (PNNL) in Richland, Washington. All fish maintenance, handling, and testing procedures were reviewed and approved by the PNNL Animal Care Committee. Results of this study will aid in determining the suitability of acoustic telemetry to estimate short- and longer-term (30 to 90 d) juvenile salmonid survival at Columbia and Snake River dams and through the lower Columbia River.

Methods

Fish Acquisition, Holding, and Surgical Protocols

To determine the minimum size at which surgical implantation of an acoustic transmitter and PIT tag would not have a negative influence on growth and survival, tests were conducted with 908 hatchery-reared juvenile Chinook salmon (Table 6; 458 implanted fish, 450 control fish). Fish were obtained from Priest Rapids Hatchery. During the study period, the test populations were held in indoor circular tanks (1.29 m diameter × 0.59 m deep; 770 L). Fish were fed Biodiet[§] moist pellets ad lib. Fish were subjected to a 12:12 photoperiod, and water temperature was maintained between 17 and 20°C. Fish selected for a given test were not fed 24 h before and 48 h after surgery.

The 908 fish were distributed evenly across a length range extending from 80 to 109 mm by placing fish into 2-mm size bins (Table 6). Therefore, each 2-mm bin had $N = \sim 30$ control and treatment fish. Tag burden ranged from 4.3to 15.1% of the fish's mass in air.

§ Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA, Pacific Northwest National Laboratory, or the U.S. Geological Survey.

Juvenile Chinook salmon were either surgically implanted with a PIT tag and JSATS transmitter (using methods previously mentioned) or left as controls with no implant. The acoustic transmitter and PIT tag were left separated, similar to the non-integrated treatment previously described and representing the current technique used in field implantation. Each treatment fish was implanted with a JSATS transmitter having an expired battery.

Table 6. Number of fish in each of three size categories either implanted with an acoustic transmitter and a PIT tag or served as a control.

			Fork Length (mm)		Weight (g)		Tag Burden (%)	
Size class	Treatment	N	Mean	Range	Mean	Range	Mean	Range
80-89 mm	Control	150	84.5	80 - 90	6.6	5.1 - 8.9	NA	NA
	Implanted	150	84.5	80 - 90	6.7	4.7 - 8.4	11.2	8.6 - 15.1
90-99 mm	Control	150	94.4	90 - 99	9.1	6.0 - 11.9	NA	NA
	Implanted	158	94.3	90 - 99	9.1	6.8 - 12.4	7.8	5.4 - 10.6
100-109 mm	Control	150	104.6	100 - 109	13.0	7.5 - 16.8	NA	NA
	Implanted	150	104.6	100 - 109	13.1	8.6 - 16.3	5.5	4.3 - 8.3

Animals assigned to the control group were anesthetized similar to treatment fish. After losing equilibrium, each fish received an injection of colored visible implant elastomer (Northwest Marine Technology, Inc., Shaw Island, Washington) in the right and left adipose eyelids and a clip to one of the pelvic fins for identification as a control fish.

All treatment and control fish were housed for 30 d, then euthanized with an overdose of MS-222 (250 ppm), weighed, and measured.

Statistical Analysis

Growth—Because fish had already been sorted into size groups for holding, comparisons within size groups of 80-89, 90-99, and 100-109 mm were examined first. Linear regression models were used to assess trends in growth patterns between surgically tagged (treatment) and untagged (control) juvenile Chinook salmon, and to find evidence of adverse growth effects among surgically implanted fish. Each model took the change in fish weight (g) 30 d following surgery as the dependent variable, with independent variables of fork length at time of tagging and a dichotomous indicator variable (tx) categorizing fish as either in the treatment or control groups. An interaction term between tx and fork length also was included in the model.

Significance of both coefficient estimates for fork length and the interaction term would suggest a significant difference in slopes between two groups and thus a real difference in growth trend lines (Neter et al. 1990). Groups showing a significant difference in growth trends are shown in a figure with fitted regression lines overlaid for the treatment and control groups. The fork length at the intersection point of the two regression lines was calculated from the fitted regression equation, and an approximate 95% confidence interval around this fork length was calculated using the inverse regression estimation procedure (Neter et al. 1990).

Mortality—Estimation of differential mortality rates between surgically tagged and untagged fish was carried out in three separate tasks using fish in 10-mm size groups of 80-89, 90-99, and 100-109 mm. For each group, Fisher's exact test was applied on a 2×2 contingency table formed by cross-tabulating dichotomous variables for mortality and surgically tagged or untagged fish.

The influence of fork length at time of tagging on mortality was examined to approximate the minimum length at which surgical implantation of an acoustic transmitter and PIT tag would have minimal adverse effects on mortality in juvenile Chinook salmon. This was done by comparing the observed mortality rates of surgically tagged and untagged fish. The mortality rates were computed across an interval of fork lengths for surgical (treatment) and control fish. Upper 95% confidence bounds were estimated using the binomial variance and assuming the normal approximation to the binomial distribution.

Results

Growth

The first phase of analysis was to examine growth within 10-mm size groups to determine the size at which surgical implantation of an acoustic transmitter and PIT tag negatively influenced growth of juvenile Chinook salmon. There was no significant difference in growth trends between control and treatment fish within either the 90-99 mm ($P = 0.22$) or the 100-109-mm groups ($P = 0.26$).

However, this analysis did indicate that within the 80- to 89-mm size group, growth trends were significantly different between treatment and control fish ($P = 0.007$). The significance of the interaction term in this analysis indicates that the slopes of the growth were different between treatment and control fish ($P = 0.01$; see Neter et al. 1990). This difference in slopes led us to conduct further analysis to determine where the critical difference in growth existed within the 80- to 89-mm size range.

The second step in the analysis was to examine more closely the regression plots for the control and treatment fish within the 80-89 mm size range (Figure 1). Within this group, diminished growth rates in the surgically implanted group were most evident for the smallest fish (toward the left side of Figure 1).

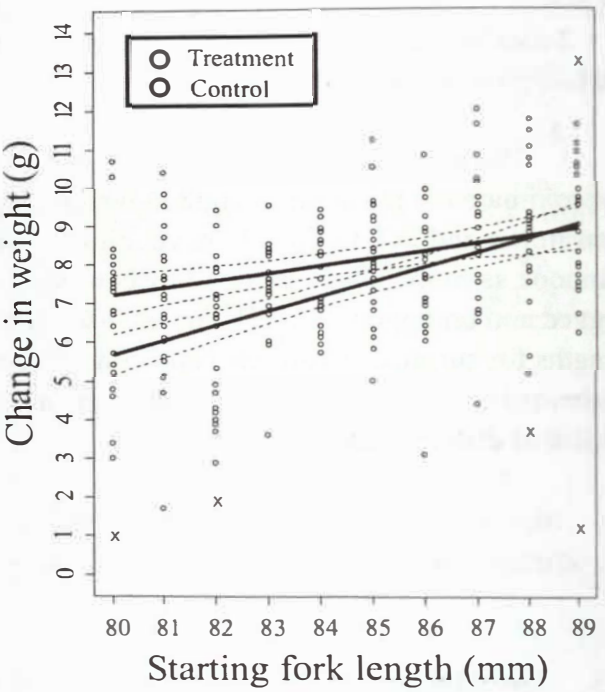

Figure 1. Weight increase by initial fork length for juvenile Chinook salmon between 80 and 89 mm, 2006. Regression lines are overlaid for implanted fish (solid red) and control fish (solid black); 95% confidence intervals are indicated by dashed lines. Plotted X marks show observations removed due to regression diagnostics criterion.

However, as fish size increased, the influence of tagging on growth decreased to coincide with the growth trends of the control group (toward the right side of Figure 1). The fitted regression equation was used to estimate a fork length at which the treatment and control regression lines intersect—88.3 mm—and a 95% confidence interval around this point of 80 to 97 mm, using the inverse prediction method (Neter et al. 1990).

Survival

The first phase of the survival analysis was to examine mortality within the 10-mm size groups to determine the size at which surgical implantation of an acoustic transmitter and PIT tag negatively influenced survival of juvenile Chinook salmon. A Fisher's exact test showed that there was no significant ($P = 0.68$) difference in mortality rates between control and treatment fish within the 100- to 109-mm group (Table 13). There was, however, a significant ($P = 0.045$) difference in mortality between treatment and control groups for fish in the 90- to 99-mm group. The Fisher's exact test could not be performed for the 80- to 89-mm group due to zero (0) mortality among control fish. However, the difference in mortality rates between surgically implanted (9.3%) and control fish (0%) was clear (Table 1; Figure 2).

Table 1. Mortality rates of juvenile Chinook salmon surgically implanted with an acoustic transmitter and a PIT tag, or non-implanted (control), 2006.

Fish size group	Treatment	Mortality (%)	*P*-value
80-89 mm	Control	0	
	Implanted	9.3	*
90-99 mm	Control	9.3	
	Implanted	16.5	0.045
100-109 mm	Control	2.9	
	Implanted	2.7	0.684

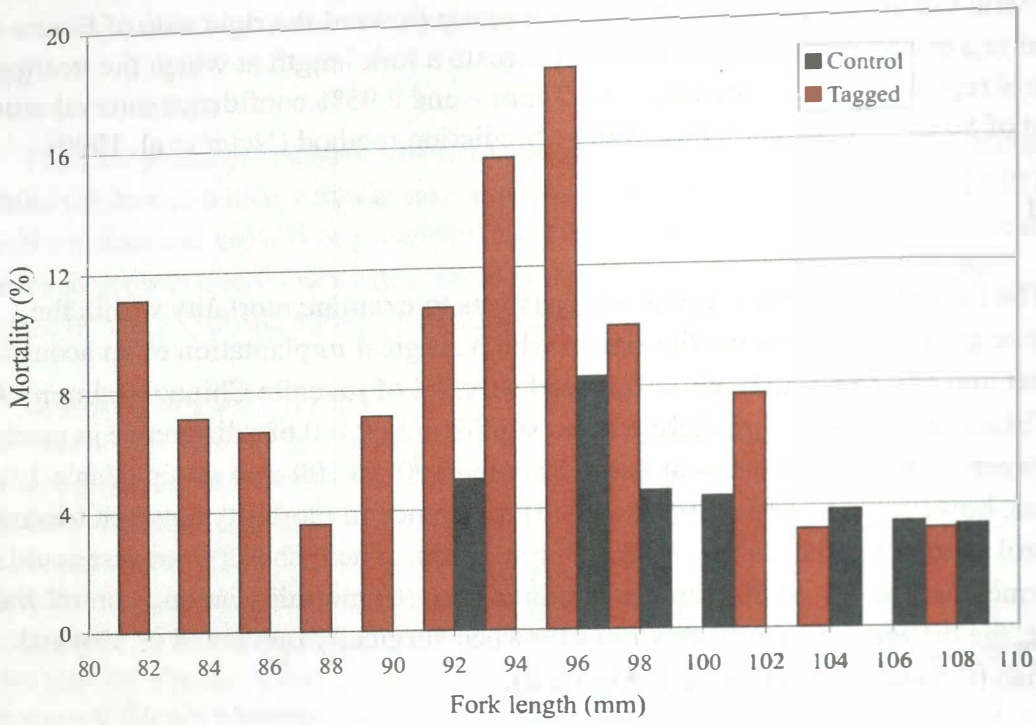

Figure 2. Percentage mortality of juvenile Chinook salmon either implanted with an acoustic transmitter and a PIT tag or not implanted (control), 2006.

The next step was to examine the probability of mortality for both implanted and control fish within the 90- to 99-mm length range. Probabilities of mortality for both control and implanted fish were very similar among fish ranging from 95 to 100 mm long (Figure 3). The lines intersect at a fork length slightly less than 95 mm. Because these lines were not derived from a fitted regression equation, no confidence intervals were computed for the 95-mm point estimate.

A conservative policy would establish a minimum threshold at 95-mm fork length for surgical implantation with the expectation of minimally affecting the mortality of tagged fish as compared to untagged fish. For fish used during this study, this is the equivalent to a burden (combined burden of acoustic transmitter and PIT tag) of approximately 7.6% for a 95-mm fish that would weigh approximately 9.2 g (Figure 4).

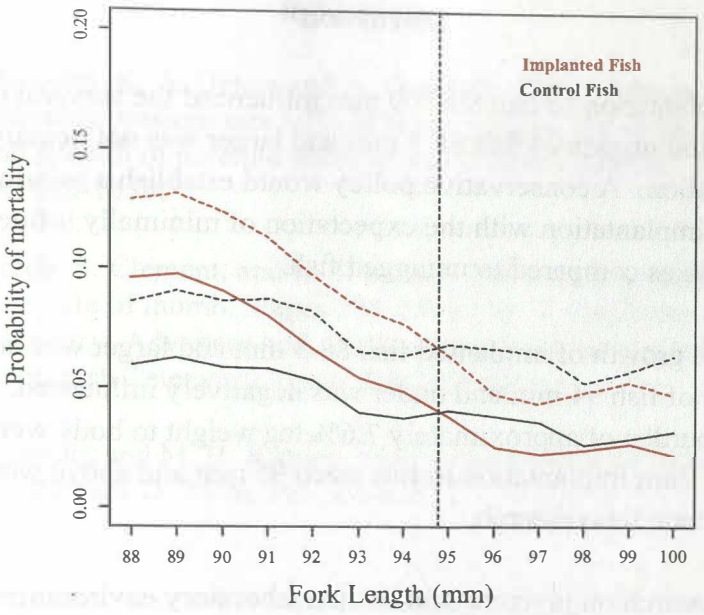

Figure 3. Probability of mortality for both control (black solid line) and implanted (red solid line) juvenile Chinook salmon, 2006. The two trend lines intersect at ~94.8 mm. The 95% confidence intervals are indicated by dashed lines.

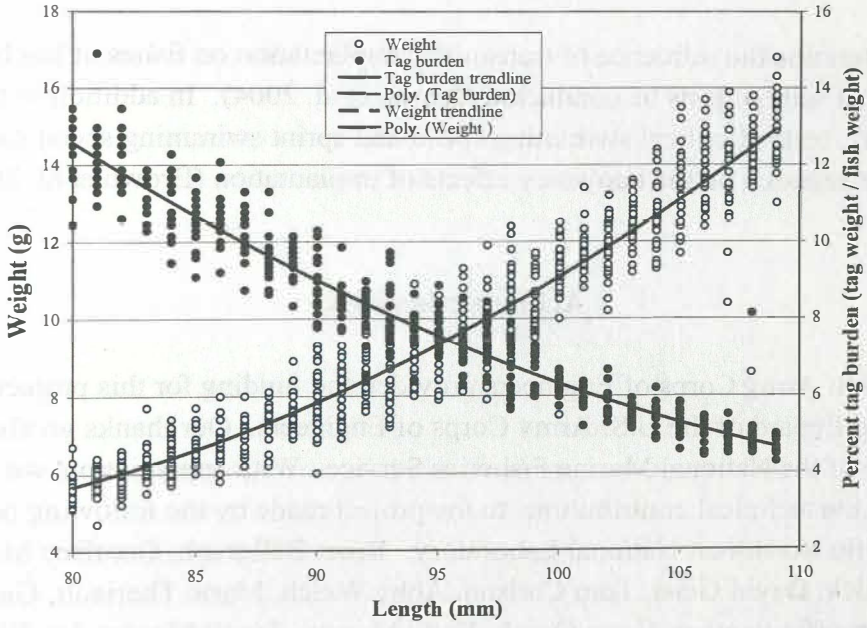

Figure 4. Regression plots of the weight of juvenile Chinook salmon and their tag burden (a sum of acoustic transmitter and PIT tag burden) at different fork lengths, 2006.

Discussion

Surgical implantation of fish 80-109 mm influenced the survival of fish more than the growth of fish, and growth of fish 88.3 mm and larger was not negatively influenced by surgical implantation. A conservative policy would establish a minimum threshold at 89 mm for surgical implantation with the expectation of minimally influencing the growth of tagged fish as compared to untagged fish.

Although the growth of implanted fish 88.3 mm and larger was not negatively influenced, survival of fish 94 mm and under was negatively influenced. This is equivalent to a tag burden of approximately 7.6% tag weight to body weight. Thus, the current guideline to limit implantation to fish sized 95 mm and above would appear to be appropriate, based upon this research.

Although research on juvenile salmon in a laboratory environment can definitely provide insight into the survival and behavior of implanted fish within the Snake and Columbia Rivers, differences exist between holding, feeding, and other conditions between the two environments. Thus, we suggest field research be conducted which would examine the survival and migration rates of implanted juvenile Chinook salmon less than 95 mm to those 95 mm or greater.

To determine the influence of transmitter implantation on fishes, it has been suggested that a suite of tests be conducted (Jepsen et al. 2004). In addition to the studies conducted here, tests of critical swimming speed and sprint swimming speed can provide insight, as can research on the buoyancy effects of implantation (Brown et al. 2006).

Acknowledgments

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EVALUATION OF PREDATOR AVOIDANCE ABILITY, TAG LOSS, AND TISSUE RESPONSE OF ACOUSTIC-TAGGED JUVENILE SALMONIDS

Theresa L. Liedtke, Lisa P. Gee, Matthew G. Mesa, John W. Beeman, Diane G. Elliott, and Carla M. Conway

U.S. Geological Survey
Western Fisheries Research Center
Columbia River Research Laboratory
5501A Cook-Underwood Road
Cook, Washington 98605-9717

Executive Summary

This study was one of two laboratory-based research efforts intended to complement and support the field evaluation of the Juvenile Salmonid Acoustic Telemetry System (JSATS). We evaluated the effects of JSATS acoustic tags on predator avoidance ability, tag loss, and tissue response of yearling and subyearling Chinook salmon *Oncorhynchus tshawytscha* relative to those implanted with PIT tags, over a 90-d period. We found little evidence that acoustic-tagged fish had reduced performance relative to PIT-tagged fish in either predator avoidance ability, tag loss, or tissue response tests.

All individual and pooled trials of predator avoidance tests showed the same trend, with random predation for both yearling and subyearling Chinook salmon. Tag loss and tissue response experiments revealed no grossly observable differences between PIT-tagged and acoustic-tagged fish, but some tissue-level differences in response were noted upon microscopic examination. For example, local fibrous tissue and inflammation were greater in acoustic-tagged fish. There were no indications of processes to initiate transmitter loss in either group, and no transmitters were shed during our 90-d holding period.

Our pilot effort to hold run-of-the-river fish for extended periods showed that elevated background mortality will be a complication if active migrants are used for laboratory evaluations. Although river-run fish would theoretically have been ideal study animals for this study, we observed 28-92% mortality in control groups over 34 d. Such high mortality levels in untagged fish will reduce or eliminate the ability to determine any effect of tagging.

We also had concerns that the "dummy" tags produced for this study did not have the same size and shape specifications as the active transmitters used for field studies. As mentioned, close alignment on transmitter specifications is needed to extrapolate from laboratory to field settings. A final concern related to the JSATS transmitter is based on microscopic examination of acoustic-tagged subyearling Chinook salmon, which showed a foreign body response that may be related to the adhesive used on the tags. Some loose glue particles were noted in the body cavities of fish, and some transmitters, removed from fish after 21 to 90 d, showed cracking and peeling glue on the surface of the tag. Further histological evaluation of this adhesive may be warranted if the ultimate goal for this transmitter is to evaluate fish over long time periods.

For future work we recommend continued and increased coordination regarding specifications of the transmitter and techniques used in separate parts of the study, and to continue using hatchery-reared fish for laboratory trials.

Introduction

This study was part of a multi-agency, collaborative effort to evaluate the effects of implanting juvenile salmon with the Juvenile Salmonid Acoustic Telemetry System (JSATS) acoustic tag, relative to fish implanted with PIT tags. The study was one of two laboratory-based research efforts intended to complement and support a field evaluation. We evaluated the effects of acoustic tags on predator avoidance ability, tag loss, and tissue response of yearling and subyearling Chinook salmon *Oncorhynchus tshawytscha* relative to those implanted with PIT tags over a 90-d period.

This study design allowed evaluation of transmitter effects over a period similar to those of field studies monitoring fish through the hydropower system and into the Pacific Ocean. Transmitters and implantation procedures used in this study were matched with those used in the field evaluation. The acoustic-tagged group was surgically implanted with both an acoustic tag and a PIT tag, and the PIT-tagged group was injected with a PIT tag. Results of this study will aid in determining the suitability of acoustic telemetry to estimate short- and longer-term (30 to 90 d) survival of juvenile migrant salmonids at Snake and Columbia River dams and through the Federal Columbia River Power System (FCRPS).

The best possible match between field and laboratory evaluations for this study would involve the use of river-run fish in the laboratory elements. This approach would provide the greatest interpretive power from laboratory studies to the field. Traditionally, fish for laboratory studies have been of hatchery origin due to the challenges of holding river-run fish for long periods and their undefined health and migratory history. We initiated a pilot study to evaluate whether river-run fish, collected at Lower Granite Dam, could be held for extended periods in a laboratory setting. The goal of this pilot effort was to assess the feasibility of using river-run fish in future laboratory evaluations.

We compared the performance of acoustic-tagged subyearling and yearling Chinook salmon relative to PIT-tagged fish for extended periods (30-90 d). Specifc objectives were to evaluate:

1) predator avoidance ability 30 d after transmitter implantation,
2) tag loss and tissue response over a 90-d period, and
3) whether river-run fish could be held for extended periods of time in a laboratory setting.

Methods

Fish Collection and Rearing

Yearling Chinook salmon were collected in April 2006 from Dworshak National Fish Hatchery, and subyearling Chinook salmon were collected in May 2006 from Little White Salmon National Fish Hatchery. Fish were transported to the Columbia River Research Laboratory in Cook, Washington. Study fish were reared in 1.5- × 1.0-m diameter tanks at low density (4-5 g/L) using Little White Salmon River water heated to 14 ± 1°C for yearling salmon and 17 ± 1°C for subyearling Chinook salmon. All fish were held indoors under a light regime that simulated natural photoperiod. Fish were fed a growth or maintenance ration of 2.5-mm diameter commercial fish food. These stocks of fish were used for both the predator avoidance tests and to assess tag loss and tissue response.

Subyearling Chinook salmon underwent smoltification during rearing in July and August 2006. Based on elevated background mortality and reduced feeding activity, we submitted fish for an evaluation by the U.S. Fish and Wildlife Service Lower Columbia River Fish Health Center in Willard, Washington. Fish health specialists found no signs of disease in any stocked fish, but did note evidence of smoltification. After several weeks, fish condition improved, and we continued our planned experiments, although sample sizes were reduced in some cases.

Smallmouth bass *Micropterus dolomieu* were collected from the Columbia River (Bonneville Reservoir) by electrofishing and angling in March and April 2006. We used a minimum predator size of 300 mm FL to increase the likelihood they would seek prey in appropriate size classes (i.e., yearling and subyearling Chinook salmon large enough to accept transmitters).

Following collection, predators were held for 20-30 d to acclimate in two 3.75-m diameter × 1-m deep tanks lined with small and large cobble substrate. Netting (1.3-cm mesh) was placed over each tank, and the tanks were separated by floor-to-ceiling curtains to minimize disturbance. Lighting over the tanks was from six 75-W incandescent bulbs spaced evenly around the tank and controlled by timers to simulate natural photoperiod. During acclimation, bass were fed a maintenance diet of juvenile salmon, and predation trials were not initiated until the predators were feeding each day. Trials used 10 bass in each of the two experimental tanks, and additional predators were kept in reserve to replace experimental animals as needed.

Transmitter Implantation

To meet our study objectives, we used three groups of fish that were either 1) surgically implanted with both PIT and acoustic transmitters (hereafter called acoustic-tagged), 2) PIT-tagged by traditional injection techniques, or 3) minimally handled and left untagged (control). Based on a decision by the U.S. Army Corps of Engineers to consistently deploy the Juvenile Salmonid Acoustic Telemetry System (JSATS) transmitter with a PIT tag, none of the study groups were used to evaluate implantation of an acoustic tag by itself.

For our evaluations, we used non-functional (dummy) JSATS transmitters manufactured to replicate the transmitters used by the field study. The range of tag sizes we received was large (0.46-0.78 g), although the mean size of tags used for yearling and subyearling Chinook salmon experiments was the same (0.6 g). The difference in tag weight from smallest to largest was 58% for yearling Chinook salmon, and 63% for subyearling Chinook salmon. Even without knowledge of tag weight, tags could be visually categorized into smaller and larger tags. Transmitter coating material varied; at least three different coatings were observed. Since transmitter production was proprietary, we were unable to determine the chemical nature of the different coatings. Due to these differences, we recorded tag weight and coating material for each tagged fish.

Fish were anesthetized using 50-70 mg/L of sodium bicarbonate-buffered tricaine methane sulfonate (MS-222) for no more than 5 min. Acoustic-tagged fish were tagged using the surgical implantation technique described by Martinelli et al. (1998), with two exceptions. First, no antenna exit site was needed since the acoustic tag did not have an antenna, and second, we omitted our traditional use of oxytetracycline for all but one study element (i.e., a small group of subyearling Chinook salmon used to evaluate tissue response) to match the procedure used in the field. The PIT-tagged group had PIT tags implanted by injection according to the technique described by Prentice et al. (1990). Immediately following tag implantation or handling, fish were allowed to regain equilibrium in a 19-L bucket containing river water and constant oxygen flow. After recovering, fish were transferred into circular tanks (61 cm diameter × 45 cm deep, 150 L) at 14°C for yearling Chinook salmon and 17°C for subyearling Chinook salmon.

Study Design

Predator Avoidance Ability—Predator avoidance ability of acoustic-tagged and PIT-tagged fish were compared 30 d after tag implantation. We chose the 30-d period because other studies have addressed the question for shorter time periods, and we wanted to look at the longer-term effects of the transmitter on fish performance.

Predation experiments used a series of replicate trials. Each trial consisted of adding up to 20 prey (6-10 from each of the two groups being compared) into a tank with predators. To best simulate natural river conditions, we used smallmouth bass as the test predator. The null hypothesis for these experiments was that no difference exists in predator avoidance ability between the two test groups. If this was the case, predation would be random, with consumption being 50% acoustic-tagged fish and 50% PIT-tagged fish.

Tagging and holding of fish began 30 d ahead of our target time periods for predation trials, occurring in May for yearling Chinook salmon and in July for subyearling Chinook salmon. For each predation trial, we placed equal numbers of acoustic-tagged fish and PIT-tagged fish into a tank with smallmouth bass.

Predation trials were conducted under natural photoperiod conditions in Little White Salmon River water heated to 14 ± 1°C for yearling Chinook salmon or 17 ± 1°C for subyearling Chinook salmon. To start a trial, prey were netted from their holding tanks, placed behind a moveable mesh partition (61 × 61 × 91 cm) positioned in the predator tank, and allowed to adjust for 5 min (Anglea et al. 2004). Following the adjustment period, the partition was removed and the trial began. Predation was allowed to continue until 50% of the prey were consumed. At the end of each trial, all surviving prey were netted out of the predator tank and identified to group based on their PIT tag. A concealed platform overhead was used to observe predator/prey interactions in the tanks and to count prey. We made observations at 30-min intervals for the first 2 h of the trial, and as often as needed after that to ensure that the trial ended when 50% of the prey were consumed.

To facilitate active feeding during a predation trial, smallmouth bass were not fed for 24 h before the start of a trial, and trials were separated by 2-3 d, depending on feeding activity (i.e., the number of prey eaten per feeding). We started trials between 0730 and 1000 PDT, and trials were complete by 2000 PDT. None of the yearling or subyearling Chinook salmon predation trials included a crepuscular period.

Analysis of predation trial data was similar to Mesa (1994). A heterogeneity *G*-test was used to determine if the individual trials were homogeneous (Sokal and Rohlf 1995). A *G* goodness-of-fit test was then used on pooled data to test whether predation was random (i.e., 50% PIT-tagged, 50% acoustic-tagged). Trials where less than 25% or more than 75% of the prey were consumed were omitted from analysis to account for changes in prey availability during a trial (i.e., the decreasing abundance of the preferred group; Coutant 1973; Mesa and Warren 1997).

Tag Loss and Tissue Response—We evaluated tag loss and tissue response in three groups of fish: 1) acoustic-tagged, 2) PIT-tagged and 3) non-tagged (control). Following handling or tagging, fish were held in tanks (61-cm diameter × 45-cm deep, 150 L) for 90 d. At 21, 30, 60, and 90 d post-tagging, 5-10 fish from each of the three groups were removed from the tanks, examined for any signs of tag loss, and prepared for histological evaluations.

Sampling intervals were set to facilitate comparison with other studies and the anticipated life of transmitters. The 21-d period represented the typical life of active transmitters currently used in the region and was comparable to examination times by Martinelli et al. (1998) and Adams et al. (1998). The 30-d period represented the anticipated tag life of the JSATS and other acoustic transmitters suitable for use in small juvenile salmonids in studies to estimate survival through the Federal Columbia River Power System. The 60- and 90-d periods represented the tag life of existing and future acoustic tags that could be used to study migration and survival through the hydropower system and in the estuary and ocean.

To minimize disturbance to fish and accommodate repeated sampling from tanks, we held each test group separately. Tanks held 40 yearling Chinook salmon at 14 ± 1°C or 80 subyearling Chinook salmon at 17 ± 1°C. To avoid possible confounding effects with different transmitter coatings, all fish used for these experiments had a single coating type. At each sampling period, fish were netted from each of the three treatment tanks into separate 19-L buckets containing a lethal dose of MS-222 (300 mg/L) buffered with an equal concentration of sodium bicarbonate. Study fish were necropsied, examined for tag location and encapsulation, observed for gross tissue response, and prepared for histological evaluation.

At each sampling, fish were preserved for histological evaluation. The spinal cord was severed by an incision immediately posterior to the opercula, and visceral organs were exposed to fixative by a cut through the skeletal muscle along the lateral line from the vent to the operculum. Fish were placed into formalin fixative and stored at

4°C. Prior to dissection, the location and appearance of tissue at the injection or incision sites were noted. Gross observations of any tissue response to the tags were recorded and digital photos taken.

Tissues collected included skin and skeletal muscle at the injection or incision sites, tissue surrounding the tags, gill, thymus, heart, liver, spleen, pyloric caeca, pancreas, intestine, and kidney. Intact skin and skeletal muscle were sampled as controls. Tissues were subjected to routine processing, embedded in paraffin, and sectioned at 5 μm. For histopathological examination, tissue sections were deparaffinized, rehydrated through graded alcohols, and stained with Gill's hematoxylin and eosin.

To compare the histological response of subyearling Chinook salmon to surgical implantation procedures with and without the use of the antibiotic oxytetracycline, we conducted a small pilot study. Small numbers of hatchery-reared subyearling Chinook salmon (2-4 fish per group) were surgically implanted with both acoustic transmitters and PIT tags either with or without the use of 50 mg/kg oxytetracycline. Following holding periods of 21, 30, 60, or 90 d at 17 ± 1°C, fish were necropsied, and sent for detailed histological examination. The histological examination was blind to the antibiotic treatment and focused on the healing of the incision. All holding and handling procedures for this pilot study matched those described for the main tag loss and tissue response experiments.

Responses noted during histological review included: epidermal erosion, epidermal inflammation, dermal inflammation and fibrosis, muscle inflammation and fibrosis, body wall inflammation and fibrosis, and body wall adhesion (abnormal union of adjacent tissues). Each of the five tissue response variables was subjectively rated (by an experienced histologist) on distribution (focal = 1 through diffuse = 3), size (small = 1 through large = 3), and severity (minimal = 1 through severe = 4). The metric used to compare between the antibiotic treatment group and the controls was the severity score, which is the sum of the scores for each of the five tissue-response variables and levels. No statistical comparisons were made because sample sizes were too low to draw meaningful conclusions.

Extended Holding of River-Run Fish—River-run yearling and subyearling Chinook salmon were collected on 6 May and 12 June 2006, respectively, from Lower Granite Dam and transported to The Dalles Dam for holding, tagging, and observation. Active migrants collected at Lower Granite Dam were a mix of hatchery and wild stocks. Although the goal for this study objective was to assess long-term holding of river-run fish in our laboratory at Cook, WA, we held fish at The Dalles Dam due to fish health concerns. The fish showed no overt signs of illness or disease, but hatchery stocks above

Lower Granite Dam could not be certified to be disease-free, and therefore we were not authorized to hold them at our facility.

At The Dalles Dam, study fish were held in a large, rectangular tank (5.6- × 2.1- × 1-m deep, 8,705 L) with circular water flow (75.7 L/min), shade cover, and river-rock substrate. The interior corners of the tank were rounded, and pumps were installed behind perforated metal in the corners, directing water along the length of the tank to create the circular flow. The tank was supplied with Columbia River water maintained within 0.5°C of ambient river temperatures. Fish were offered daily rations of a commercial fish food.

Following transport to The Dalles Dam, fish were held for at least 48 h prior to handling and assignment to one of three groups: 1) acoustic-tagged, 2) PIT-tagged, and 3) untagged (control) fish. We tagged yearling Chinook salmon on 11 May 2006, using 17-18 fish per group. Subyearling Chinook salmon were tagged on 15 June 2006, using 60 fish per group. Samples sizes for yearling Chinook salmon were reduced due to a pump failure during holding, which resulted in reduced fish condition and mortality. Following tagging, we noted general behavior and condition of fish daily, and tallied mortalities as they occurred.

Results

Predator Avoidance Ability

Smallmouth bass adjusted well to the laboratory environment and soon began feeding on a maintenance diet of live juvenile salmon. Mean predator size was similar for yearling and subyearling trials (Table 1). After the trials were complete, we necropsied six predators from each of the two experimental tanks and found no signs of intestinal blockage. We retrieved PIT and acoustic tags from the predator tanks daily, suggesting that gut evacuation was occurring regularly.

Table 1. Summary of smallmouth bass mean fork length (FL) and gender for yearling (CH1) and subyearling (CH0) Chinook salmon predator avoidance trials, 2006.

Run	*N*	Mean FL (mm)	Female *N*	Male *N*
CH1	20	389.0	13	7
CH0	20	388.5	16	4

We conducted 18 predation trials with yearling Chinook salmon from 21 May through 13 July 2006 (Table 2). Mean water temperature was 14.2°C, and mean trial duration was 5.2 h. Smallmouth bass consumed, on average, 47.5% of the yearling Chinook salmon per trial (Table 2), although the percent prey consumed by treatment group varied (Figure 1). The mean weight of yearling Chinook salmon was larger for fish in the acoustic-tagged group (17.6 g) than for those in the PIT-tagged group (16.6 g; Table 3; $P = 0.002$). Weights of acoustic-tagged and PIT-tagged fish ranged from 8.2 to 30.2 g (Figure 2). Mean tag-weight to body-weight ratio of acoustic-tagged fish was 3.6% and ranged from 1.9 to 6.3%.

There was no significant difference in predator avoidance ability of acoustic-tagged and PIT-tagged yearling Chinook salmon, despite appropriate statistical power ($1 - \beta = 0.94$; Figure 3). The 18 trials were homogeneous ($P = 0.68$), and none differed significantly from random (Table 2).

Table 2. Predation of yearling Chinook salmon PIT-tagged by injection or surgically implanted with both acoustic and PIT tags (acoustic-tagged) and exposed to predation by smallmouth bass, 2006.

		Number consumed (exposed)			Statistics		
Trial date	Trial duration (h)	PIT-tagged	Acoustic-tagged	Percent consumed	df	G-test	P
05/21/06	0.2	6 (10)	4 (10)	50	1	0.40	0.53
05/25/06	0.5	4 (10)	7 (10)	55	1	0.83	0.36
05/25/06	0.2	8 (10)	4 (10)	60	1	1.36	0.24
05/31/06	0.5	4 (10)	7 (10)	55	1	0.83	0.36
05/31/06	7.4	4 (10)	5 (10)	45	1	0.11	0.74
06/04/06	0.9	4 (10)	6 (10)	50	1	0.40	0.53
06/04/06	7.4	3 (10)	5 (10)	40	1	0.51	0.48
06/16/06	11.0	7 (10)	4 (10)	55	1	0.83	0.36
06/16/06	11.0	4 (10)	5 (10)	45	1	0.11	0.74
06/22/06	11.7	3 (10)	4 (10)	35	1	0.14	0.71
06/22/06	8.9	3 (10)	6 (10)	45	1	1.02	0.31
06/30/06	12.2	5 (10)	2 (10)	35	1	1.33	0.25
06/30/06	12.2	5 (10)	1 (10)	30	1	2.91	0.09
07/06/06	4.7	4 (10)	6 (10)	50	1	0.40	0.53
07/06/06	0.6	5 (10)	5 (10)	50	1	0.00	1.00
07/09/06	0.7	6 (10)	4 (10)	50	1	0.40	0.53
07/09/06	2.6	5 (10)	5 (10)	50	1	0.00	1.00
07/13/06	0.4	8 (10)	3 (10)	55	1	2.36	0.12
Total / Mean	5.2	88 (180)	83 (180)	47.5	18	13.94	0.73
Pooled					1	0.15	0.70
Heterogeneity					17	13.80	0.68

Subyearling Chinook salmon predator avoidance ability was tested in 21 trials completed from 11 August through 12 October 2006 (Table 4). Mean water temperature was 16.6°C and mean trial duration was 2.6 h. An average of 50% of subyearling Chinook salmon were consumed during each trial (Table 4), although the percent prey consumed by treatment group varied (Figure 4).

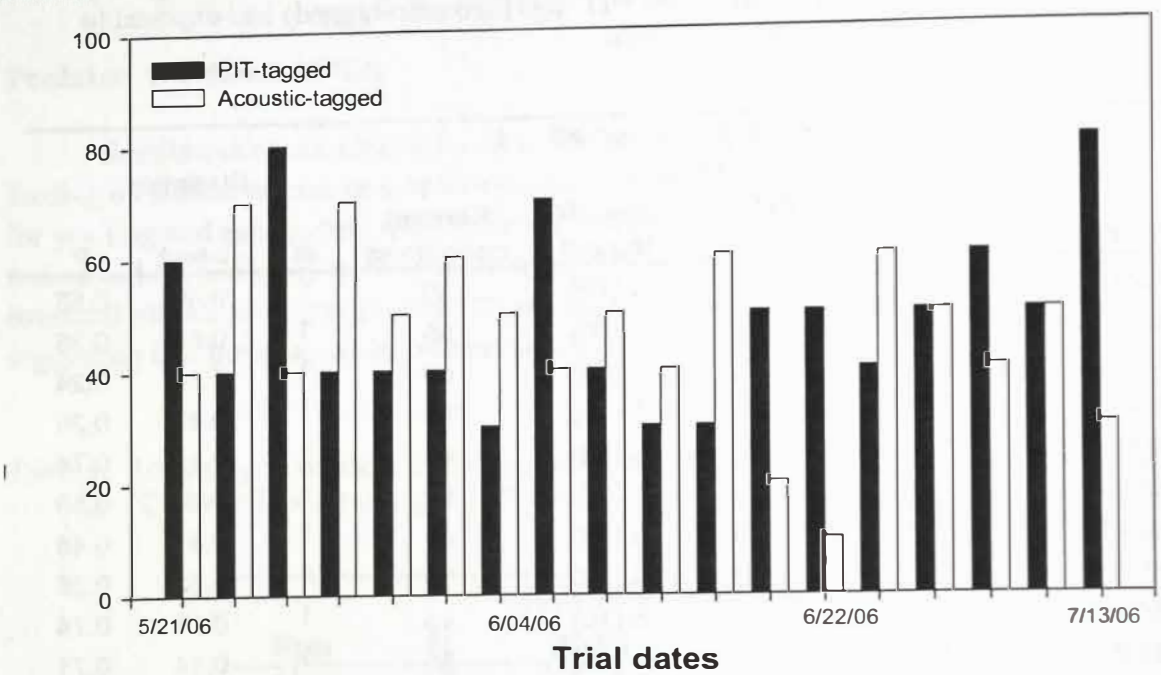


Figure 1. Percent of yearling Chinook salmon consumed during 18 predator avoidance trials where two treatment groups were exposed to smallmouth bass, 2006. The PIT-tagged treatment was injected with a PIT tag and acoustic-tagged fish were surgically implanted with both PIT and acoustic tags.

Table 3. Sample size (N) and weight (g) summary for yearling (CH1) and subyearling (CH0) Chinook salmon in two treatment groups used in predator avoidance trials with smallmouth bass, 2006. The PIT-tagged treatment was injected with a PIT tag, and the acoustic-tagged treatment was surgically implanted with both PIT and acoustic tags.

			Weight (g)			
Run	Treatment	*N*	Mean	SD	Min	Max
CH1	PIT-tagged	180	16.6	4.1	8.2	30.2
	Acoustic-tagged	180	17.6	3.6	10.2	30.1
CH0	PIT-tagged	179	12.1	1.9	9.5	21.1
	Acoustic-tagged	179	12.5	2.4	9.6	26.2

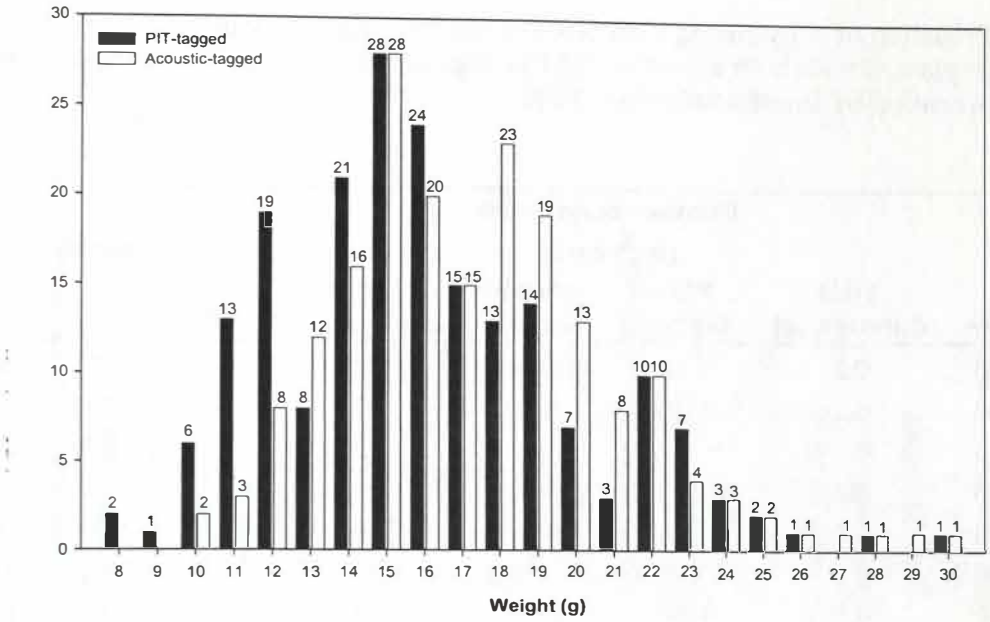


Figure 2. Distribution of individual fish weights (g) for yearling Chinook salmon consumed during 18 predator avoidance trials where two treatment groups were exposed to smallmouth bass, 2006. The PIT-tagged treatment was injected with a PIT tag and acoustic-tagged fish were surgically implanted with both PIT and acoustic tags.

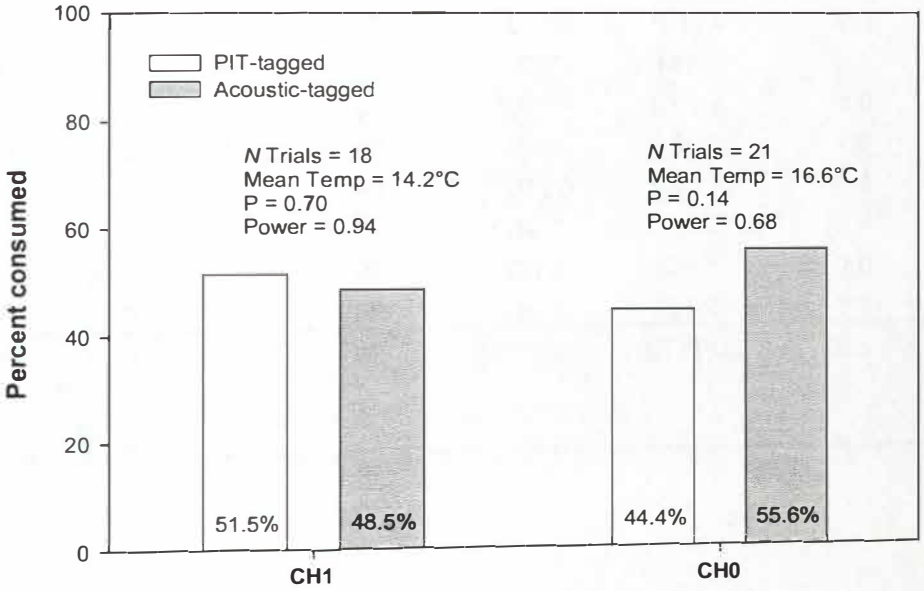


Figure 3. Percent of yearling (CH1) and subyearling (CH0) Chinook salmon consumed during predator avoidance trials where two treatment groups were exposed to smallmouth bass, 2006. Fish in the PIT-tagged group were injected with a PIT tag, and fish in the acoustic-tagged group were surgically implanted with both PIT and acoustic tags.

Table 4. Predation of subyearling Chinook salmon PIT-tagged by injection or surgically implanted with both acoustic and PIT tags (acoustic-tagged) and exposed to predation by smallmouth bass, 2006.

		Number consumed (exposed)			Statistics		
Trial date	Trial duration (h)	PIT-tagged	Acoustic-tagged	Percent consumed	df	G-test	P
08/11/06	0.2	8 (9)	5 (9)	72	1	0.70	0.40
08/17/06	0.2	2 (6)	4 (6)	50	1	0.68	0.41
08/20/06	0.3	5 (10)	7 (10)	60	1	0.33	0.56
08/28/06	0.4	6 (10)	5 (10)	55	1	0.09	0.76
08/31/06	8.5	2 (9)	4 (9)	33	1	0.68	0.41
09/03/06	9.1	3 (10)	3 (10)	30	1	0.00	1.00
09/07/06	4.9	4 (9)	5 (9)	50	1	0.11	0.74
09/07/06	0.5	6 (10)	6 (10)	60	1	0.00	1.00
09/11/06	0.5	5 (9)	5 (9)	56	1	0.00	1.00
09/15/06	1.3	3 (9)	6 (9)	50	1	0.14	0.70
09/15/06	3.0	3 (7)	4 (7)	50	1	1.02	0.31
09/18/06	0.5	3 (6)	3 (6)	50	1	0.00	1.00
09/21/06	1.1	6 (8)	4 (8)	63	1	0.40	0.53
09/21/06	6.9	4 (10)	6 (10)	50	1	0.40	0.53
09/25/06	1.5	2 (9)	7 (9)	50	1	2.94	0.09
09/25/06	9.5	5 (10)	4 (10)	45	1	0.11	0.74
09/26/06	0.7	3 (8)	5 (8)	50	1	0.51	0.48
09/29/06	0.8	4 (10)	6 (10)	50	1	0.40	0.53
10/06/06	1.8	3 (8)	2 (8)	31	1	2.91	0.09
10/06/06	0.9	1 (6)	5 (6)	50	1	0.20	0.65
10/12/06	1.1	2 (6)	4 (6)	50	1	0.68	0.41
Total / Mean	2.6	80 (179)	100 (179)	50	21	12.32	0.93
Pooled					1	2.23	0.14
Heterogeneity					20	10.09	0.97

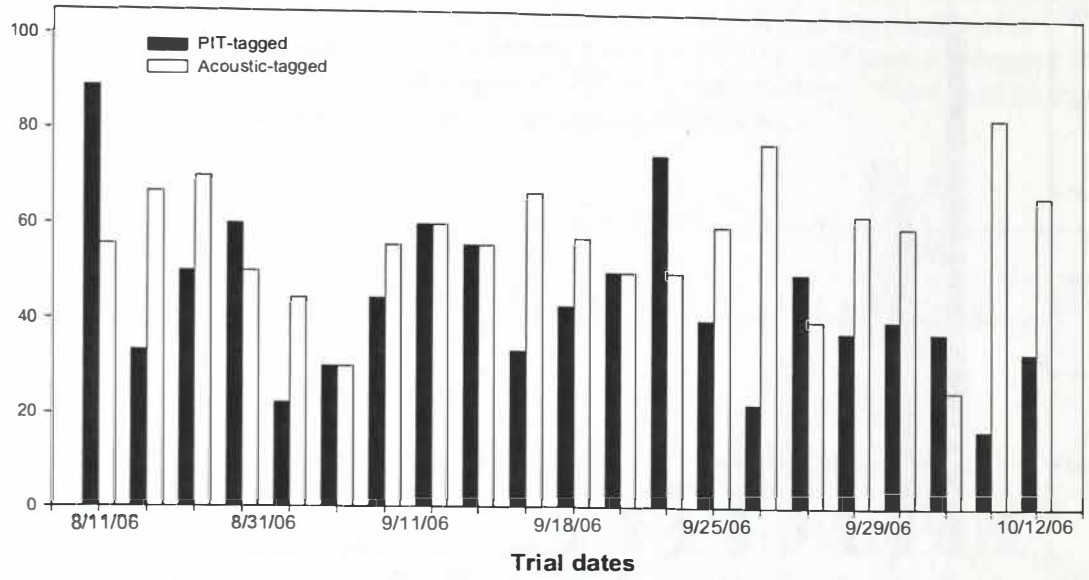


Figure 4. Percent of fish consumed during twenty-one predator avoidance trials using subyearling Chinook salmon. Control fish were injected with a PIT tag and test fish were surgically implanted with both an acoustic and a PIT tag.

The mean weight of subyearling Chinook salmon in the acoustic-tagged group (12.5 g) was not significantly different from that of the PIT-tagged group (12.1 g; $P = 0.12$; Table 3). Weights of acoustic-tagged and PIT-tagged fish ranged from 9.5 to 26.2 g (Figure 5). Mean tag-weight to body-weight ratio of acoustic-tagged fish was 5.1%, and ranged from 2.3 to 7.3%.

The predator avoidance ability of subyearling Chinook salmon implanted with acoustic tags was not significantly different from that of fish implanted with PIT tags ($P = 0.14$; Figure 3). Statistical power for this comparison was 0.68. The 21 trials were homogeneous ($P = 0.97$), and none were found to differ significantly from random (Table 4).

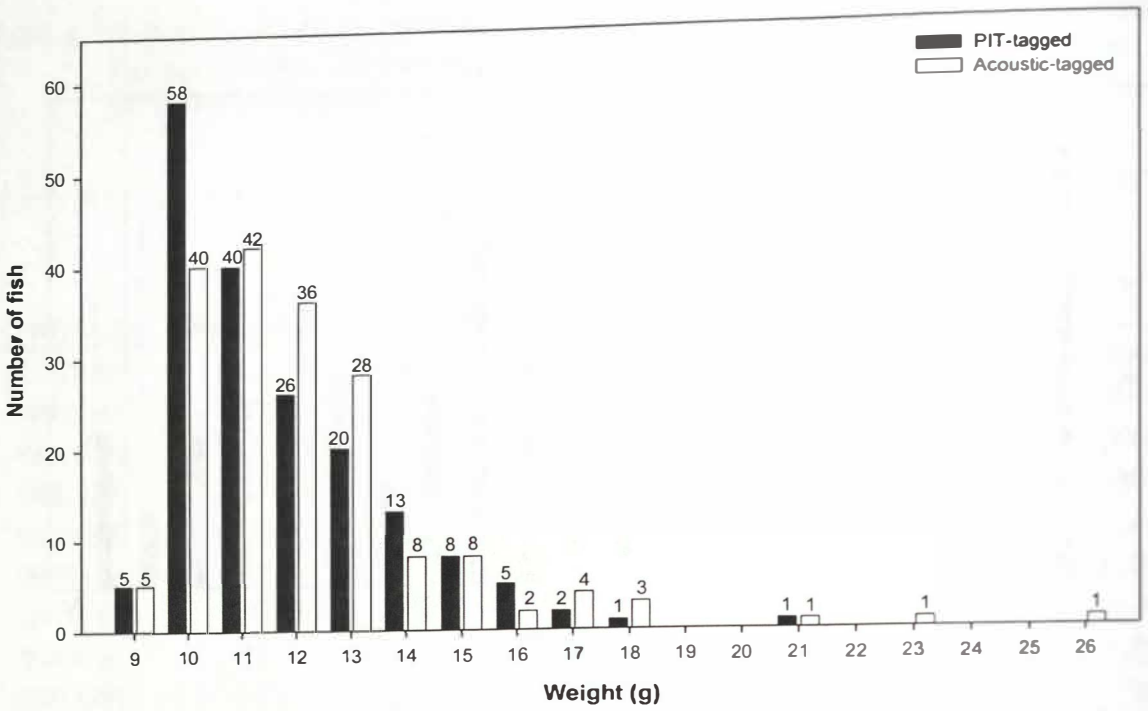


Figure 5. Distribution of individual fish weights (g) for subyearling Chinook salmon consumed during 21 predator avoidance trials where two treatment groups were exposed to smallmouth bass, 2006. The PIT-tagged treatment was injected with a PIT tag and acoustic-tagged fish were surgically implanted with both PIT and acoustic tags.

Tag Loss and Tissue Response

Over the 90-d experiment, no tags were lost from the 110 yearling Chinook or the 59 subyearling Chinook salmon in our test groups. Gross observation of fish during sampling showed that surgical incisions and PIT-tag injection sites were healing or healed, and there were no obvious signs of impending tag loss (e.g., no bulging masses, protruded tags, separated incision or injection points).

Yearling Chinook salmon trials were conducted between 15 May and 15 August 2006, and the mean initial weight of fish was 16.0 g (Table 5). The initial mean weight of fish was not significantly different by group ($P = 0.14$). There were no significant differences in final fish length among the three treatment groups, except at 30 d after tagging, when control fish were smaller than the other groups (Table 6). The mean tag-weight to body-weight ratio for fish in the acoustic-tagged group was 3.6% and ranged from 2.0 to 5.4%.

Table 5. Sample size (N) and mean weight (g) of yearling (CH1) and subyearling (CH0) Chinook salmon in three treatment groups at the start of a 90 d experiment to evaluate tag loss and tissue response, 2006. Control fish were minimally handled, PIT-tagged fish were injected with a PIT tag, and acoustic-tagged fish were surgically implanted with both PIT and acoustic tags. Treatment groups were compared with Analysis of Variance (ANOVA).

	CH1			CH0		
Treatment	***N***	**Mean (g)**	**SD**	***N***	**Mean (g)**	**SD**
Control	40	15.1	3.85	79	11.1	2.64
Pit-tagged	40	16.1	3.78	80	12.1	1.68
Acoustic-tagged	40	16.7	3.41	80	12.9	1.75
Total	120	16.0	3.72	239	12.0	2.18
ANOVA finding			0.14			0.001

Table 6. Mean fork length and sample size (N) for yearling Chinook salmon in three treatment groups, measured 21, 30, 60, and 90 d after tagging or handling in an experiment to evaluate tag loss and tissue response, 2006. Control fish were minimally handled, PIT-tagged fish were injected with a PIT tag, and acoustic-tagged fish were surgically implanted with both PIT and acoustic tags. Treatment groups were compared with Analysis of Variance (ANOVA).

Treatment	**21 d (*N*)**	**30 d (*N*)**	**60 d (*N*)**	**90 d (*N*)**
Control	116.7 (10)	112.2 (10)	123.9 (10)	134.0 (6)
Pit-tagged	116.9 (10)	119.6 (10)	128.5 (10)	137.3 (6)
Acoustic-tagged	119.5 (10)	120.6 (10)	126.3 (10)	129.5 (8)
ANOVA finding	0.66	0.03	0.59	0.33

Subyearling Chinook salmon trials were conducted between 9 August and 14 December 2006, and the mean weight of fish at the start of the trials was 12.0 g (Table 5). The initial mean weight of fish was significantly different by group ($P = 0.001$), as control fish were smaller than PIT-tagged or acoustic-tagged fish (Table 5). There were no significant differences in final fish size among the three treatment groups at any sample period (Table 7). The mean tag-weight to body-weight ratio for fish in the acoustic-tagged group was 4.9 % and ranged from 3.6 to 6.8%.

Table 7. Mean fork length and sample size (N) for subyearling Chinook salmon in three treatment groups, measured 21, 30, 60, and 90 d after tagging or handling in an experiment to evaluate tag loss and tissue response, 2006. Control fish were minimally handled, PIT-tagged fish were injected with a PIT tag, and acoustic-tagged fish were surgically implanted with both PIT and acoustic tags. Treatment groups were compared with Analysis of Variance (ANOVA).

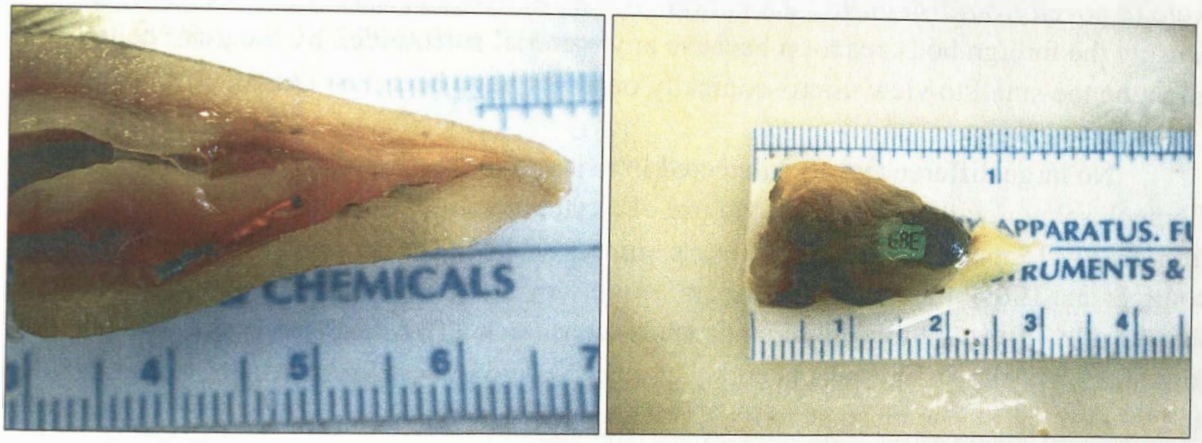
Treatment	21 d (*N*)	30 d (*N*)	60 d (*N*)	90 d (*N*)
Control	111.0 (5)	116.4 (5)	120.4 (5)	113.0 (3)
Pit-tagged	103.0 (5)	115.8 (5)	117.2 (5)	117.5 (4)
Acoustic-tagged	102.2 (5)	115.4 (5)	124.2 (5)	123.0 (7)
ANOVA finding	0.11	0.98	0.68	0.70

Review of gross tissue response by yearling and subyearling Chinook salmon showed that tags became encapsulated in membranes, and that fish developed these membranes around acoustic tags more quickly than PIT tags. The type of transmitter influenced the magnitude and timing of the encapsulation response, but both runs of fish had similar tissue response. In PIT-tagged fish, a thin transparent membrane formed around the tag early in the experiment (Figure 6). Although the membrane got thicker as the experiment progressed, not all PIT-tagged fish developed membranes, and none of the fish developed semi-opaque membranes. In acoustic-tagged fish, the encapsulating membrane was initially similar to that of PIT-tagged fish, but a greater proportion of the fish developed them, and by 90 d after tagging all fish had thick, semi-opaque membranes tightly adhered to the acoustic tag.

In all fish, both PIT and acoustic tags were generally located near the ventral midline, between the body wall and the intestines (Figure 6). Some tags were located in the pyloric caecae (Figure 7), which appeared to slow development of the encapsulating membrane. Some acoustic tags were near the spleen, and in some cases the membrane around the tag was also adhered to the spleen. For acoustic-tagged fish, which received both PIT and acoustic tags, we noted that tags were not usually in close proximity to each other. Only one fish was observed to have a single membrane surrounding both the PIT tag and the acoustic tag.

(A) (B)

Figure 6. Photographs of transmitter encapsulation and position in yearling Chinook salmon 60 d after transmitter implantation, 2006. Image (A) shows a PIT-tagged fish and image (B) shows an acoustic-tagged fish. Note the position of the tags in the body, and the encapsulating membranes surrounding the tags.



(A) (B)

Figure 7. Photographs of transmitter encapsulation and position in subyearling Chinook salmon after transmitter implantation, 2006. Image (A) shows a PIT-tagged fish, 30 d post-tagging, and image (B) shows an acoustic tag near the pyloric caecae, 90 d post-tagging. Note the position of the tags in the body, and the encapsulating membranes surrounding the tags.

Microscopic examination of acoustic-tagged subyearling Chinook salmon sampled 60 d after tagging showed more histopathological reactions to the tags and the tagging process than did PIT-tagged fish. In PIT-tagged fish, the injection site was completely covered by normal epidermis, and regenerating scales were present. In acoustic-tagged fish, regenerating epidermis had completely covered the incision site, and scale regeneration was visible only near the edges of the incision sites, suggesting that scale pockets in the center of the incision had been destroyed.

An encapsulation reaction occurred in the adipose tissue adjacent to tags in both tagged groups. In this tissue, areas of fibrosis (fibrocytes) surrounded the tag and inflammatory cells (primarily macrophages) were present. Acoustic-tagged fish had large areas of fibrous tissue that surrounded one or both tags. Some of the macrophages present in these areas had fused to form multinucleate giant cells, which were not observed in PIT-tagged fish. Multinucleate giant cells are common in certain chronic inflammatory conditions such as responses to the presence of nonlysable foreign bodies. It is possible that these cells had surrounded small pieces of material sloughed from the surfaces of the acoustic tags.

Loose material that appeared to be glue was noted when some of the acoustic tags were removed from fish during dissection. It may be difficult to identify the material causing the foreign body reaction because any material surrounded by the giant cells might be too small to view microscopically or might have been lost during processing.

No large differences were detected in tissue response between subyearling Chinook salmon with and without the use of oxytetracycline. Fish sizes (initially) ranged from 97 to 115 mm FL. Mean sizes were similar between the groups: 105.8 mm FL for controls and 106.4 mm FL for treatment (oxytetracycline) fish. Sample sizes were low for this pilot effort (22 total fish), and trends were weak. The mean severity score was lowest (best healing) for controls at day 21 and 30, and lowest for treatment fish at day 60 and 90 (Table 8). The mean severity score gradually decreased over time for treatment fish, but was more variable for controls, with higher scores initially and at 60 d. The largest difference in mean severity score between test groups was at 60 d, where the treatment group showed the best healing (Table 8).

Although the relatively large difference between the treatment and control groups at 60 d suggests a positive influence of oxytetracycline, the mean score for control fish at this sample period was higher than would have been expected based on the anticipation that the incision was healing through time. Specifically, the mean score for control fish was near 30 for the sample periods before and after this comparison, but the comparison was based on a mean score of 56 (Table 8). The overall mean severity score was lower

for treatment fish, but the difference was nominal. The largest difference between treatment and controls in individual tissue response measures was for epidermal erosion, with more erosion observed in controls. The only response variable that showed a better outcome for controls was muscle inflammation and fibrosis, with a small differential between groups.

Table 8. Mean values for five tissue response variables and mean severity score for subyearling Chinook salmon implanted with an acoustic tag and a PIT tag with (treatment) or without (controls) the use of the antibiotic oxytetracycline. Tissue response was noted 21, 30, 60 and 90 d after tagging. Each of the five tissue response variables was subjectively rated for distribution (focal = 1 through diffuse = 3), size (small = 1 through large = 3), and severity (minimal = 1 through severe = 4). The tabled values for each tissue response variable are the mean value across the distribution, size, and severity ratings. The severity score is a sum of the scores for each of the five tissue response variables and levels.

Sample period	Group	*N*	Epidermal erosion mean	Epidermal inflammation mean	Dermal inflammation mean	Muscle inflammation and fibrosis mean	Body wall inflammation and fibrosis mean	Mean severity score
21 d	Control	3	1.7	1	1.5	1.3	2	41.7
21 d	Treatment	2	0	0.7	2.2	2.9	3.3	53.2
30 d	Control	2	0	1.1	1.6	1.6	0.9	34.3
30 d	Treatment	3	1	1.1	1.4	1.6	2.3	38.9
60 d	Control	2	2	2.2	2.4	2.1	2.4	56.2
60 d	Treatment	3	0	1	1.7	1	2.2	31
90 d	Control	4	0	0.8	1.3	0.7	1.9	34.9
90 d	Treatment	3	0	0.4	1.5	0.9	1.5	27
Overall	Control	11	0.9	1.3	1.7	1.4	1.8	41.8
	Treatment	11	0.3	0.8	1.7	1.6	2.3	37.5

Extended Holding of River-Run Fish

Both yearling and subyearling Chinook salmon were in good condition following transport from Lower Granite Dam, handling, and tagging. There were no mortalities during transport and no overt signs of disease when fish were handled individually. Fish showed schooling behavior in the large holding tank and fed on commercial fish food.

Yearling Chinook salmon were tagged on 11 May 2006 and held for 32 d before the experiment ended on 12 June 2006. Mean water temperature during the trial period was 14.8°C, and daily mean water temperatures are shown in Figure 8. Mean weight of fish was 23.0 g, and weights of control fish, PIT-tagged fish, and acoustic-tagged fish were not significantly different ($P = 0.99$; Table 9). Mean tag-weight in air to body-weight ratio of acoustic-tagged fish was 2.7% and ranged from 1.7 to 2.8%. Overall mortality was highest for the acoustic-tagged fish (64.7%), but the controls (27.7%) and PIT-tagged group (35.3%) also had elevated overall mortality (Table 10).

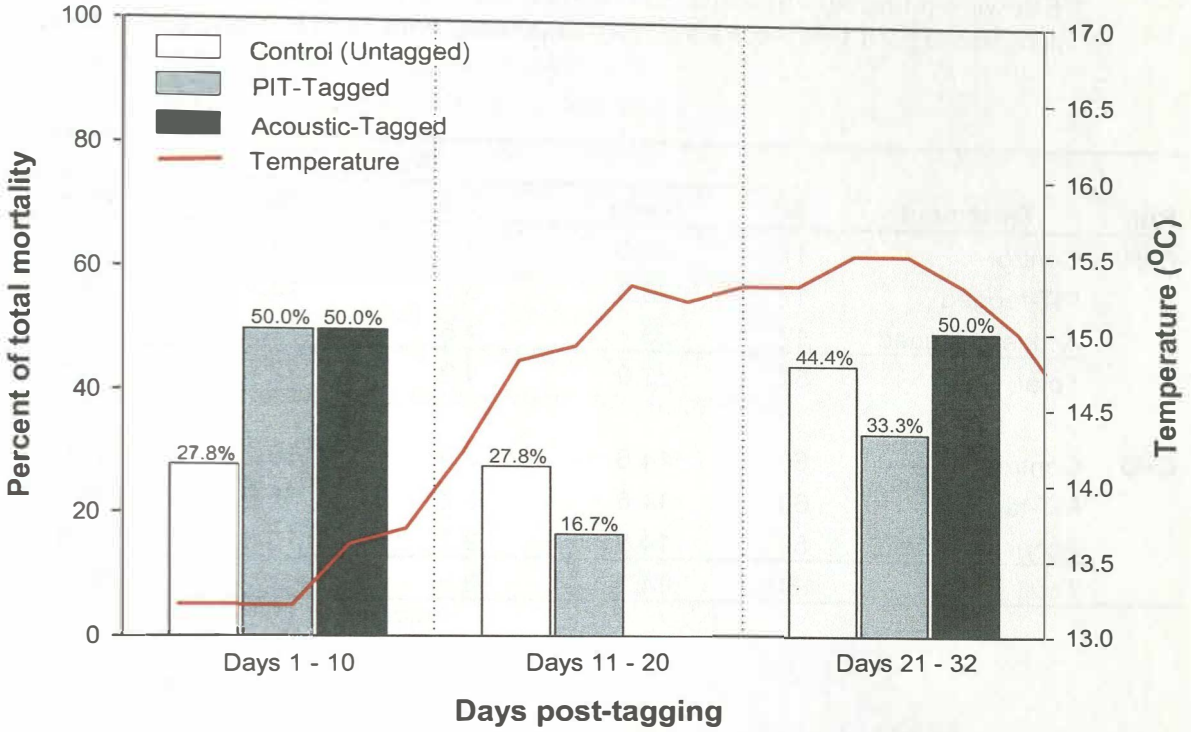


Figure 8. Percent of total mortality and mean daily tank temperatures for river-run yearling Chinook salmon in three treatment groups, 2006. Mortality is reported for three time periods, based on days post-tagging, through the 32-d experiment. Control fish were minimally handled, PIT-tagged fish were injected with a PIT tag, and acoustic-tagged fish were surgically implanted with both PIT and acoustic tags.

Mortality was highest during the first 10 d and last 11 d of holding (Figure 8.). Temperatures were lowest during the first 10 d of the experiment, when mortality was between 28 and 50%, and temperatures were rising during days 11-20 when mortality was lowest (Figure 8).

Table 9. Sample size (N) and mean weight (g) of river-run yearling (CH1) and subyearling (CH0) Chinook salmon in three treatment groups, 2006. Control fish were minimally handled, PIT-tagged fish were injected with a PIT tag, and acoustic-tagged fish were surgically implanted with both PIT and acoustic tags.

			Weight (g)			
Run	Treatment	N	Mean	SD	Min	Max
CH1	Control	17	23.0	4.6	14.9	30.3
	PIT-tagged	17	23.0	5.7	13.5	33.0
	Acoustic-tagged	17	23.1	4.5	12.5	28.8
	Total	51	23.0	4.9	12.5	33.0
CH0	Control	60	14.6	2.9	10.2	21.6
	PIT-tagged	60	14.6	3.2	9.7	25.8
	Acoustic-tagged	60	14.8	2.7	10.3	22.9
	Total	180	14.7	2.9	9.7	25.8

Table 10. Sample size (N) and total mortality for river-run yearling (CH1) and subyearling (CH0) Chinook salmon in three treatment groups, 2006. Yearling Chinook were held for 32 d and subyearling Chinook were held for 34 d to evaluate mortality through time. Control fish were minimally handled, PIT-tagged fish were injected with a PIT tag, and acoustic-tagged fish were surgically implanted with both PIT and acoustic tags.

Run	Treatment	*N*	Number of mortalities	% Mortality
CH1	Control	18	5	27.7
	PIT-tagged	17	6	35.3
	Acoustic-tagged	17	11	64.7
CH0	Control	60	55	91.7
	PIT-tagged	60	49	81.7
	Acoustic-tagged	60	50	83.3

The subyearling Chinook salmon holding experiment started on 15 June 2006, and ended 34 d later on 19 July. Mean water temperature during the experiment was 18.4°C, and daily mean water temperatures are shown in Figure 9. The mean weight of fish in the experiment was 14.7 g, and weights of control fish, PIT-tagged fish, and acoustic-tagged fish were not significantly different ($P = 0.92$; Table 8). The mean tag-weight to body-weight ratio for acoustic-tagged fish was 4.1% and ranged from 2.8 to 6.1%. Overall mortality for subyearling Chinook salmon was 91.7% for controls, 81.7% for PIT-tagged fish, and 83.3% for acoustic-tagged fish (Table 10). There were no mortalities until day 12, and mortality was highest during the last 13 d of the trial (Figure 9). Temperature climbed steadily through the 34-d experiment (Figure 9). The lowest temperatures were early in the experiment, when mortality was zero. Mortality increased through time with increasing water temperature (Figure 9).

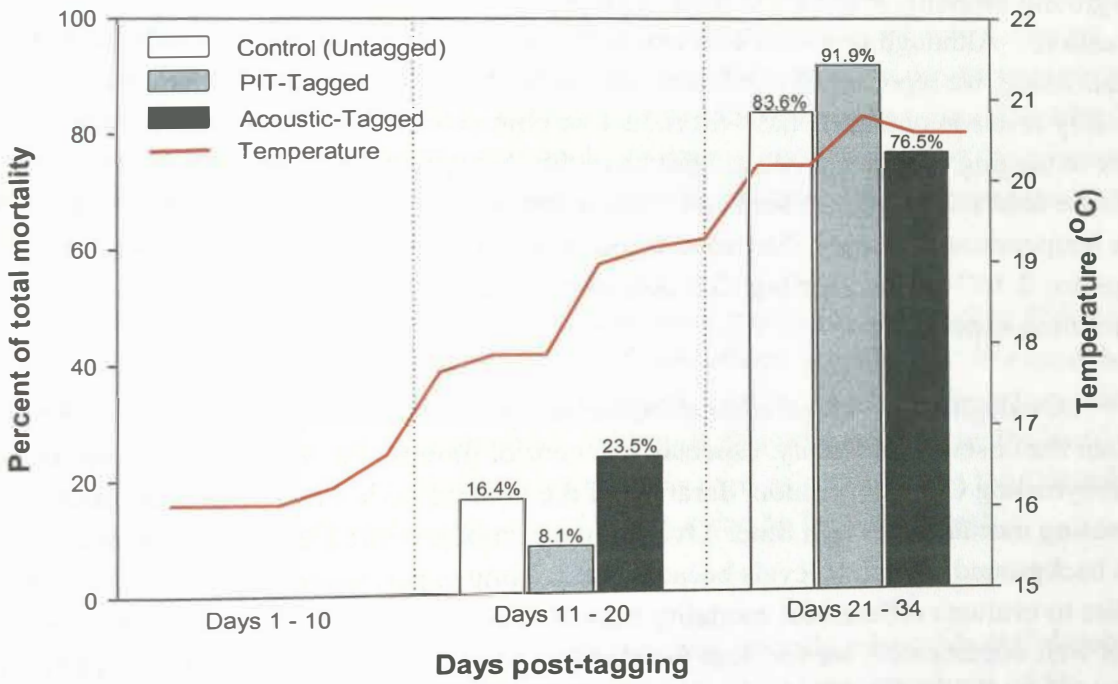


Figure 9. Percent of total mortality and mean daily tank temperatures for river-run subyearling Chinook salmon in three treatment groups, 2006. Mortality is reported for three time periods, based on days post-tagging, through the 34-d experiment. Control fish were minimally handled, PIT-tagged fish were injected with a PIT tag, and acoustic-tagged fish were surgically implanted with both PIT and acoustic tags.

Discussion

We found little evidence that acoustic-tagged fish had reduced performance relative to PIT-tagged fish in either predator avoidance ability, tag loss, or tissue response tests. The predator avoidance tests had good statistical power to detect differences for the yearling Chinook trials (0.94) and somewhat reduced power during the subyearling Chinook trials (0.68). All individual trials and pooled trials for both run types showed the same trend, with random predation. Tag loss and tissue response experiments revealed no grossly observable differences between PIT-tagged and acoustic-tagged fish, but some tissue-level differences in response were noted upon microscopic examination. For example, local fibrous tissue and inflammation were greater in acoustic-tagged fish. There were no indications of processes to initiate transmitter loss in either group, and no transmitters were shed during our 90-d holding period.

Our pilot effort to hold river-run fish for extended periods showed that elevated background mortality will be a complication if active migrants are used for laboratory evaluations. Although river-run fish would theoretically have been ideal study animals for this study, we reported 28-92% mortality in control groups over 34 d. Such high mortality levels in untagged fish will reduce or eliminate the ability to determine any effect of tagging. Certainly, we did not have the ability to tightly control conditions when we held fish at The Dalles Dam. Water temperatures were within 0.5°C of ambient river temperatures, but they fluctuated throughout the study period. The temperature range was 2.3°C for the yearling Chinook salmon experiment and 4.7°C for the subyearling experiment.

Although water temperature affected background mortality rates, it cannot fully explain the observed mortality, especially in control groups. As noted with the findings for subyearling Chinook salmon, duration of the holding period can be a factor, with increasing mortality through time. Overall, we cannot provide a definitive cause for the high background mortality levels because the holding experiments were designed as pilot studies to evaluate differential mortality between the groups. Although the phenomenon is not well documented, we feel that the elevated mortality levels can be at least partially explained by the "frustrated smolt syndrome" described by Carl Schreck. The concept being that captively held active migrants can show reduced condition and physiological stress.

During the subyearling Chinook salmon experiment, we noted that mortality increased with time and temperature. River temperatures climbed steadily throughout the 34-d holding period. Reduced temperatures may have led to reduced background mortality, but the yearling Chinook trials, where temperature was less variable, still had

moderate mortality in the control group. To address questions on transmitter effects, it appears that the best approach is similar to the one used for this work; a combination of both laboratory and field comparisons. If techniques and equipment are standardized between laboratory and field elements, then conclusions can be drawn from analyzing data from both settings.

The JSATS acoustic transmitter and its effects on fish was the primary focus of this work, but the transmitters supplied were highly variable in weight and coating material. This was the first year of large-scale transmitter production, so we anticipated some challenges, but transmitter weights varied by 50%, and differences in tag size,shape, and coating material were visible. We also had concerns that the "dummy" tags produced for this study did not have the same size and shape specifications as the active transmitters used for field studies. As mentioned, close alignment on transmitter specifications is needed to extrapolate from laboratory to field settings.

A final concern related to the JSATS transmitter is based on microscopic examination of acoustic-tagged subyearling Chinook salmon, which showed a foreign body response that may be related to the adhesive used on the tags. Some loose particles were noted in the body cavities of fish, and some transmitters, removed from fish after 21 to 90 d, showed cracking and peeling glue on the surface of the tag. Further histological evaluation of this adhesive may be warranted if the ultimate goal for this transmitter is to evaluate fish over long time periods.

We anticipated potential transmitter effects to be greatest in subyearling Chinook salmon because of their smaller size, but no significant effects were found. We used the same transmitter for yearling and subyearling Chinook salmon; therefore the subyearlings had a higher tag-weight to body-weight ratio. Although this ratio may not be the best indicator of potential transmitter effects, it is currently used as a standard in the Columbia River Basin to determine the minimum size of fish that can be implanted with a transmitter.

Through our experiences in handling and tagging juvenile salmonids at Columbia and Snake River dams and numerous laboratory studies, we set an upper limit of 5% tag-weight to body-weight ratio for telemetry studies (Adams et al. 1998a,b). For subyearling Chinook salmon used in our experiments, the mean ratio for acoustic-tagged fish was 5%, and the maximum ratio was 7.3%. Since most of our subyearling Chinook salmon had a ratio close to the upper limit, and no significant effects were noted, we are confident that larger fish, with lower tag-weight to body-weight ratios, will be less affected by this transmitter.

In summary, this study was part of a larger, collaborative effort to evaluate the effects of the JSATS acoustic transmitter on juvenile salmon in both laboratory and field settings. The combined field and laboratory approach is a powerful way to address questions about transmitter effects. For future work, we recommend continued and increased coordination regarding specifications of the transmitter and techniques used in separate parts of the study, and to continue using hatchery-reared fish for laboratory trials. The best possible interpretive power from our findings will be based on their incorporation with the results of other agencies involved in the larger, overall study.

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CONCLUSIONS AND RECOMMENDATIONS

Field Evaluation of Acoustic Telemetry Tags in Juvenile Salmonids

1. Studies that provide inference from field research to a general population are most appropriately conducted using as much experimental replication as possible. Greater temporal replication would have allowed for comparison between acoustic tagged and PIT-tagged groups using empirical (replicate) variability.

   However, because only two replicate pairs were available, we were constrained to using theoretical sampling variability. Thus the field portion of this study should be considered a pilot study and viewed as a "snapshot in time" of tag effects on hatchery yearling Chinook salmon.

2. An expanded study with much more temporal replication is recommended. This would provide more accurate and unbiased inference to the entire migrating population, as well as more realistic, and possibly narrower statistical bounds.

3. We found no significant difference in PIT tag detection probabilities or survival between the release site and Bonneville Dam (a distance of 460 km) between PIT-tagged fish and fish implanted with both a JSATS acoustic transmitter and PIT tags, except in the first reach evaluated (release to Little Goose Dam). However, more replicates and a larger sample size are needed to accurately assess the influence of tag effects on survival and detection probability.

4. A comparison of travel times between tag treatments failed to show any consistent tag effect. Travel time between release and Little Goose and McNary was significantly longer for acoustic-tagged yearling Chinook salmon released on 6 May. However, no differences were found in among releases on 13 May or among other reaches on that 6 May. Longer travel times may have been an artifact of the smaller sample sizes used on 6 May. Additional replicates are needed to determine if there is a travel time tag effect of the JSATS acoustic tag.

5. Avian predation rates for both acoustic-tagged and PIT-tagged fish were relatively low and were not significantly different.

6. Based on our field study, the JSATS acoustic transmitter may provide unbiased estimates of survival for yearling Chinook salmon through the FCRPS. However, our field study lacked experimental replication due to failed delivery of transmitters from the vendor. Therefore, the study should be repeated using sufficient replication to verify that tag effects of the JSATS acoustic transmitters are minimal.

7. A field study of the JSATS acoustic transmitter tag effects on subyearling Chinook salmon through the FCRPS is needed.

8. During 2006, flow conditions in the Snake and Columbia Rivers were higher than the 10-year average. Juvenile salmonid survival can be poor under low flow conditions, and differences in survival between tag technologies may be observed under lower flow conditions. Further evaluation may provide an opportunity to evaluate tag effects in the field under a normal or low flow condition.

Evaluation of Growth, Survival, Tag Expulsion, and Tissue Reaction of Acoustic-Tagged Juvenile Salmonids

1. We found no negative influence from surgical implantation of JSATS acoustic transmitters on the growth of yearling or subyearling Chinook salmon, and there was no trend of differences in growth among treatments.

2. Histological results suggest that inflammation associated with implantation of an acoustic transmitter can produce fibrous tissue, which can invade and possibly damage internal organs soon after implantation. Reactions severe enough to damage organs were limited to ~20% of subyearling Chinook salmon (all < 101 mm and 12 g at tagging). Infiltration of fibrous tissue into organs was observed most often in fish held for 21 d and appeared to decrease in subsequent holding times.

3. Up to 7.8% of the subyearling Chinook salmon expelled their acoustic transmitters between 5 and 63 days post-surgery (average 27 d). Tag expulsion was limited to fish less than 108 mm. The timing of transmitter expulsion was negatively correlated with tag burden. Tag expulsion may be a problem for smaller subyearling fish which may compromise in-river survival studies. Further research on the cause of this expulsion and the relationship between tag burden and expulsion are needed.

4. We found no difference in growth or survival between fish implanted with an integrated acoustic transmitter and PIT tag vs. those with an acoustic transmitter and PIT tag implanted separately. However, expulsion of PIT tags is generally very low, while acoustic transmitter expulsion occurred in up to 7.8% of subyearling fish studied. Therefore, the use of integrated acoustic and PIT tags is not recommended.

   Integrated transmitters were more often found in the anterior part of the body cavity than non-integrated transmitters; this may lead to expulsion through the surgical incision.

5. Efforts should be made to decrease the size of the JSATS acoustic transmitter, and to improve surgical technique in an effort to decrease the influence of surgical implantation.

Determination of a Minimum Fish Size for Implantation with a Juvenile Salmonid Acoustic Telemetry System (JSATS) Tag

1. The current guideline to limit implantation of JSATS transmitters to fish 95 mm and above appears appropriate. Although growth of implanted fish 88.3 mm and larger was not negatively influenced, survival of fish 95 mm and smaller was negatively influenced.

2. Field research should be conducted to examine survival and migration rates of implanted juvenile Chinook salmon less than 95 mm to those 95 mm or greater. Although laboratory research can provide insight into the survival and behavior of implanted fish, differences exist between holding, feeding, and other conditions in the field vs. laboratory environment.

3. We recommend that research be conducted on the dynamics of fibrous tissue generation (fibrosis) to minimize its volume and extent, especially in smaller fish. This could decrease the tag effect observed in smaller fish, allowing a larger range of subyearling fish to be studied in the field. A review of human medical and mammalian veterinary research could aid in understanding the mechanisms of tissue reaction in Chinook salmon and provide methods for minimizing these reactions.

Laboratory Evaluation of Predator Avoidance Ability, Tag Loss, and Tissue Response of Acoustic-Tagged Juvenile Salmonids

1. Predator avoidance ability of acoustic-tagged yearling Chinook salmon was similar to that of PIT-tagged fish 30 d after tagging. All individual and pooled trials of predator avoidance tests showed the same trend, with random predation for both yearling and subyearling Chinook salmon.

2. Although tag loss and tissue response experiments revealed no grossly observable differences between PIT-tagged and acoustic-tagged fish, some tissue-level differences in response were noted upon microscopic examination. For example, local fibrous tissue and inflammation were greater in acoustic-tagged fish.

3. There were no indications of processes to initiate transmitter loss in either group, and no transmitters were shed during our 90-d holding period.

4. Our pilot effort to hold run-of-the-river fish for extended periods showed that elevated background mortality will be a complication if active migrants are used for laboratory evaluations. Although run-of-the-river fish would theoretically have been ideal study animals for this study, we observed 28-92% mortality in control groups over 34 d. Such high mortality levels in untagged fish will reduce or eliminate the

ability to determine any effect of tagging. Hatchery-reared fish should continue to be used for laboratory trials.

5. We are concerned that the "dummy" tags produced for this study did not have the same size and shape specifications as the active transmitters used for field studies. As mentioned, close alignment on transmitter specifications is needed to extrapolate from laboratory to field settings. For future work we recommend continued and increased coordination regarding specifications of the transmitter and techniques used in separate parts of the study.

6. Microscopic examination of JSATS acoustic-tagged subyearling Chinook salmon showed a foreign body response that may be related to the adhesive used on the tags. Some loose glue particles were noted in the body cavities of fish, and some transmitters, removed from fish after 21 to 90 d, showed cracking and peeling glue on the surface of the tag. Further histological evaluation of this adhesive may be warranted if the ultimate goal for this transmitter is to evaluate fish over long periods.

Comparison between Laboratory Studies

Expulsion of acoustic transmitters was examined by both PNNL and USGS. The USGS did not find any expulsion of acoustic transmitters among either yearling (N = 110; mean weight 16 g, burden range 2.0-5.4%) or subyearling fish (N = 59; mean weight 12 g; burden 3.6-6.8%). Similarly, PNNL did not find any expulsion of yearling Chinook salmon (N = 840; length 98-152 mm; tag burden 1.5-7.3%). However, PNNL researchers did see 1.5-7.8% expulsion in subyearling Chinook salmon (N = 947; length 93-126 mm, tag burden 2.9-8.8%). All tag expulsion was from fish less than 108 mm.

Subyearling Chinook salmon were held by PNNL in water temperatures as high as 21°C, while fish were held by USGS in 17°C water. Subyearling fish studied at PNNL had a tag burden up to 8.8%, while the maximum tag burden at USGS was 6.8%. In addition, much larger numbers of fish were studied at PNNL. Thus, the difference in tag expulsion observed between the two laboratories may have been due to water temperatures at which fish were held, differences in tag burden, or differences in sample sizes.

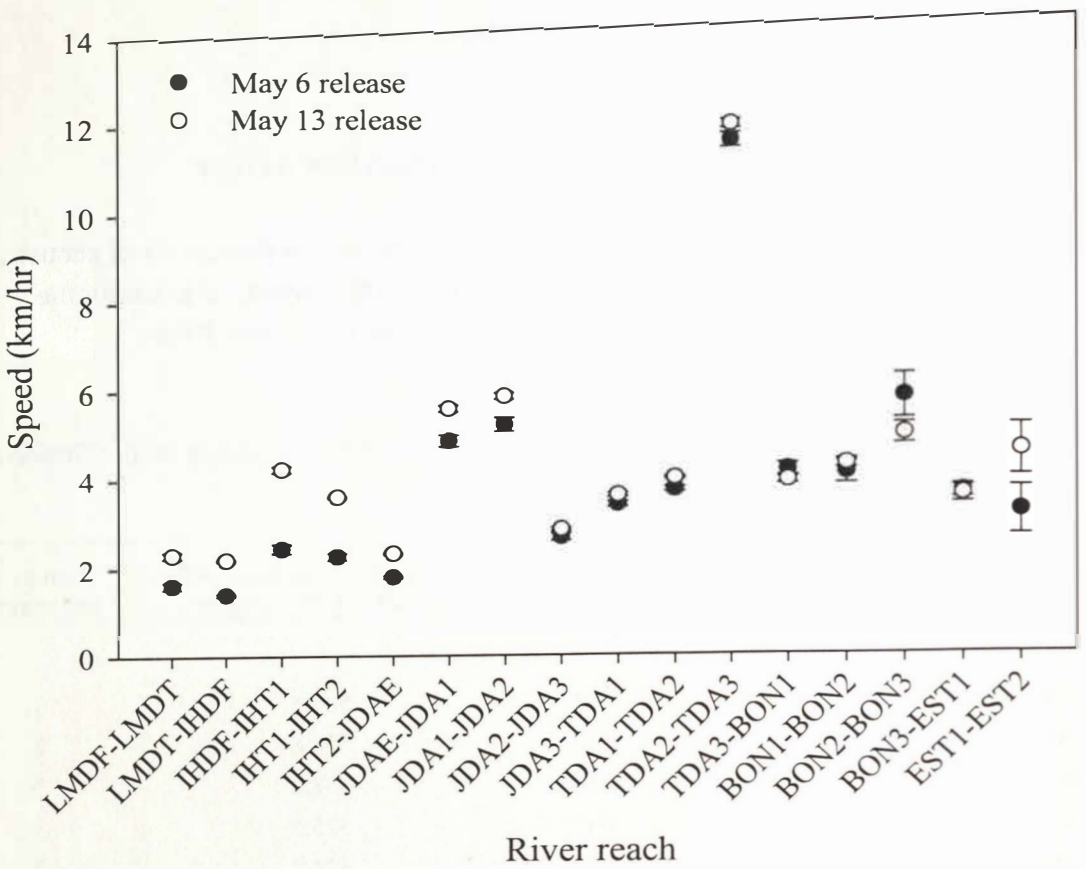
APPENDIX A

Ancillary Data from Acoustic Detection Arrays

The following section provides ancillary information on detections of acoustic tagged fish in our field study collected by 17 acoustic arrays deployed between the forebay of Lower Monumental Dam and the mouth of the Columbia River.

Appendix Table A1. Locations and names of acoustic receiving arrays on the Snake and Columbia Rivers during 2006.

Array description	Array code	Distance above the mouth of the Columbia Rive (rkm)r	Number of nodes/array
Snake River			
Lower Monumental Forebay	LMDF	589.5	3
Lower Monumental Tailrace	LMDT	578.8	2
Ice Harbor Forebay	IHDF	538.4	3
Ice Harbor Tailrace Primary	IHT1	525.5	3
Ice Harbor Tailrace Secondary	IHT2	524.3	2
Columbia River			
John Day Egress	JDAE	339.2	3
John Day Primary	JDA1	325.6	5
John Day Secondary	JDA2	324.2	3
John Day Tertiary	JDA3	312.4	3
The Dalles Primary	TDA1	275.6	5
The Dalles Secondary	TDA2	238.4	3
The Dalles Tertiary	TDA3	236.4	3
Bonneville Primary	BON1	208.8	6
Bonneville Secondary	BON2	204.0	4
Bonneville Tertiary	BON3	193.8	4
Estuary Primary	EST1	8.3	22
Estuary Secondary	EST2	2.8	25



Abbreviations for acoustic receiver array locations:			
LMDF	Lower Monumental Dam Forebay	TDA1	The Dalles Primary
LMDT	Lower Monumental Dam Tailrace	TDA2	The Dalles Secondary
IHDF	Ice Harbor Dam Forebay	TDA3	The Dalles Tertiary
IHT1	Ice Harbor Dam Tailrace Primary	BON1	Bonneville Primary
IHT2	Ice Harbor Dam Tailrace Secondary	BON2	Bonneville Secondary
JDAE	John Day Egress	BON3	Bonneville Tertiary
JDA1	John Day Primary	EST1	Estuary Primary
JDA2	John Day Secondary	EST2	Estuary Secondary
JDA3	John Day Tertiary		

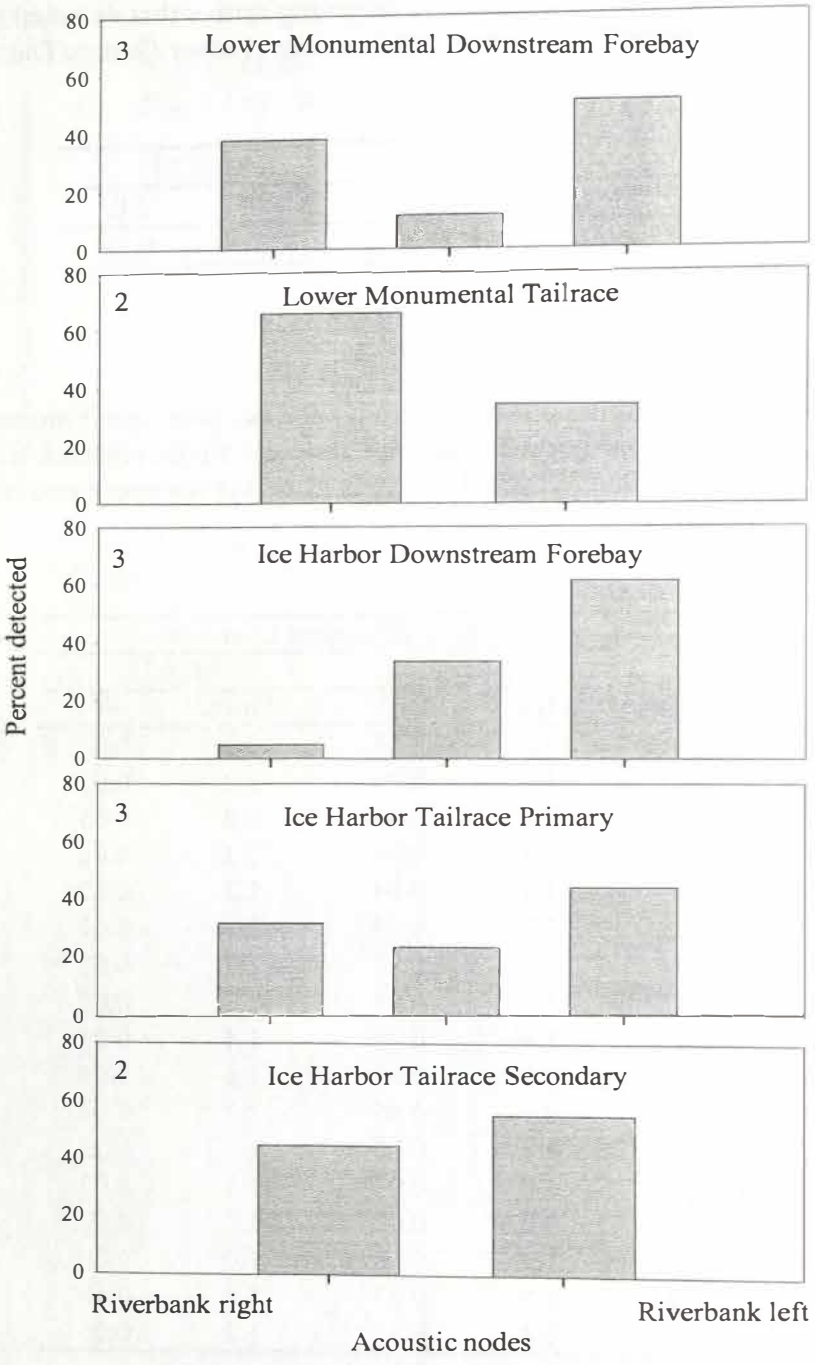
Appendix Figure A1. Mean speed (km/hr) by river reach and release date of yearling Chinook salmon tagged with both acoustic- and PIT-tags, 2006. Fish were released at Lower Granite Dam and detected at acoustic receiving arrays on the Snake and Columbia Rivers. Whiskers represent standard errors.

Appendix Table A2. Mean number of acoustic receiving arrays that detected individual yearling Chinook salmon released at Lower Granite Dam, 2006.

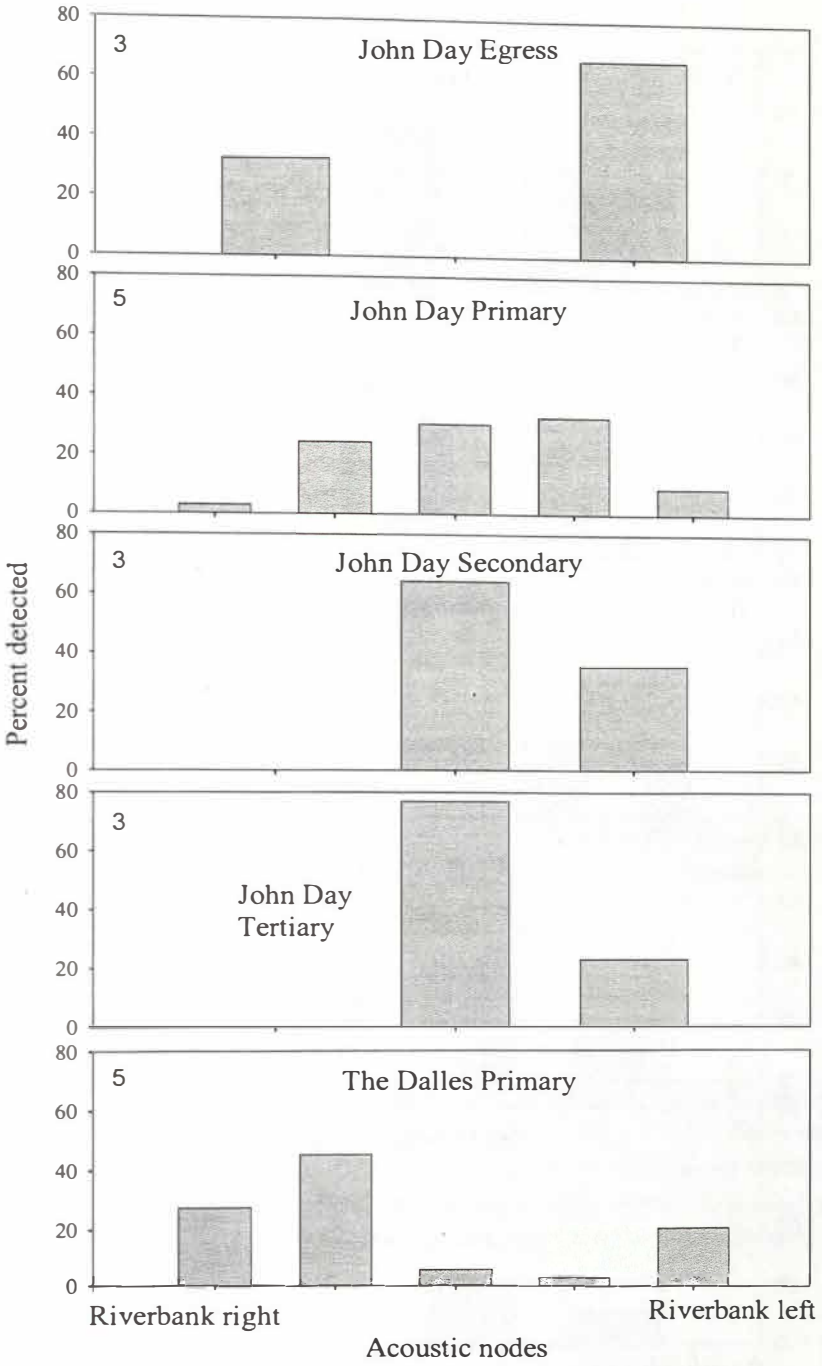
	May 6		May 13	
# of arrays	Mean	SE	Mean	SE
17	8.0	0.30	7.8	0.18

Appendix Table A3. Mean number of nodes within each acoustic receiving array that detected individual yearling Chinook salmon released from Lower Granite Dam, 2006. Locations of arrays are presented in Appendix Figure 1

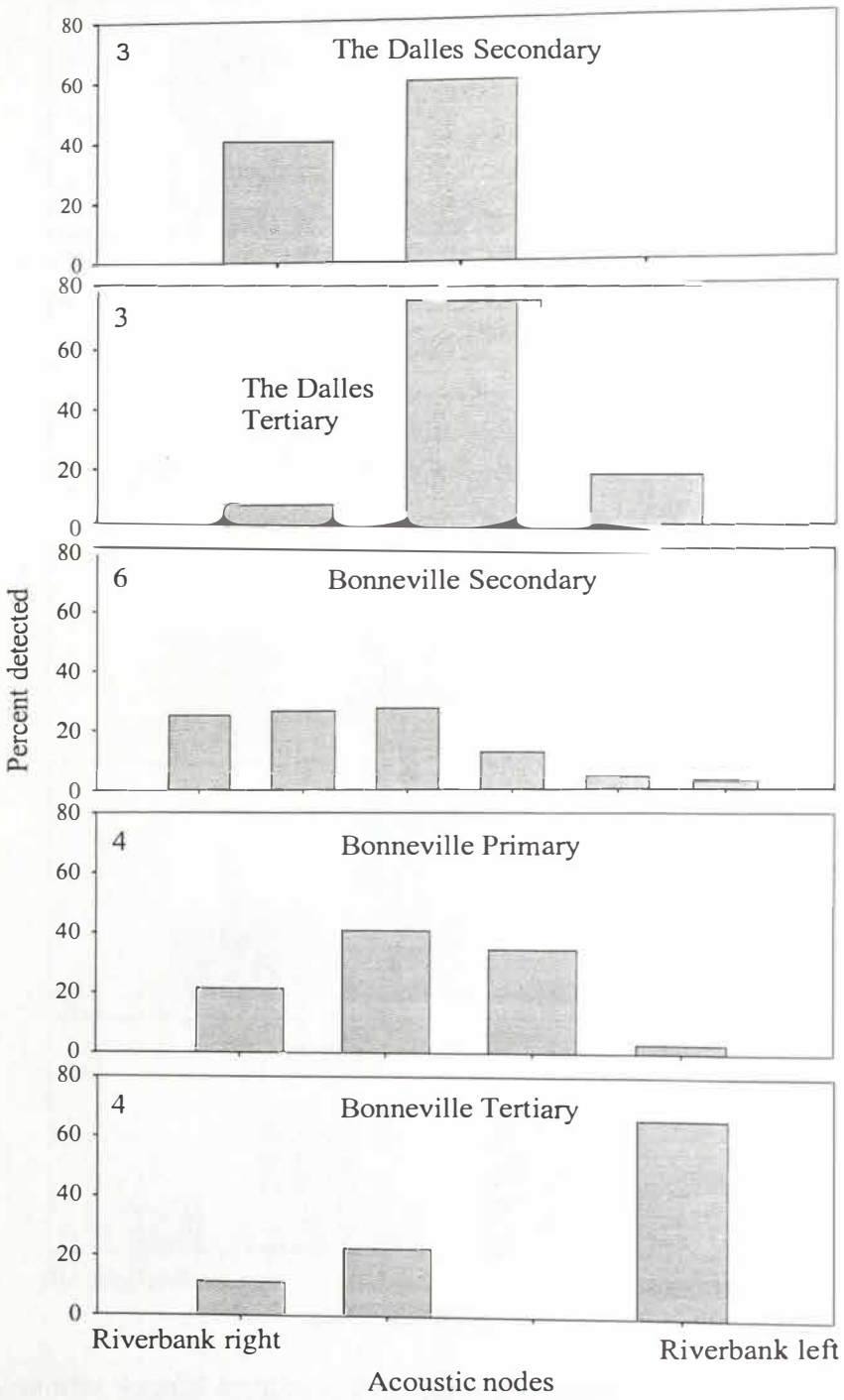
		Node detections by release			
		May 6		May 13	
Array	# of nodes	Mean	SE	Mean	SE
LMDF	3	2.4	0.05	2.4	0.03
LMDT	2	1.4	0.04	1.2	0.02
IHDF	3	2.2	0.05	2.0	0.03
IHT1	3	1.4	0.04	1.3	0.02
IHT2	2	1.4	0.04	1.2	0.02
JDAE	3	1.1	0.03	1.1	0.01
JDA1	5	1.8	0.07	1.7	0.03
JDA2	3	1.2	0.04	1.2	0.02
JDA3	3	1.4	0.05	1.4	0.03
TDA1	5	1.7	0.07	1.6	0.03
TDA2	3	2.1	0.05	2.1	0.04
TDA3	3	1.7	0.06	1.7	0.03
BON1	6	1.0	0.03	1.1	0.02
BON2	4	1.0	0.00	1.0	0.00
BON3	4	1.0	0.05	1.0	0.00
EST1	24	1.7	0.17	1.6	0.10
EST2	25	1.4	0.17	1.2	0.06



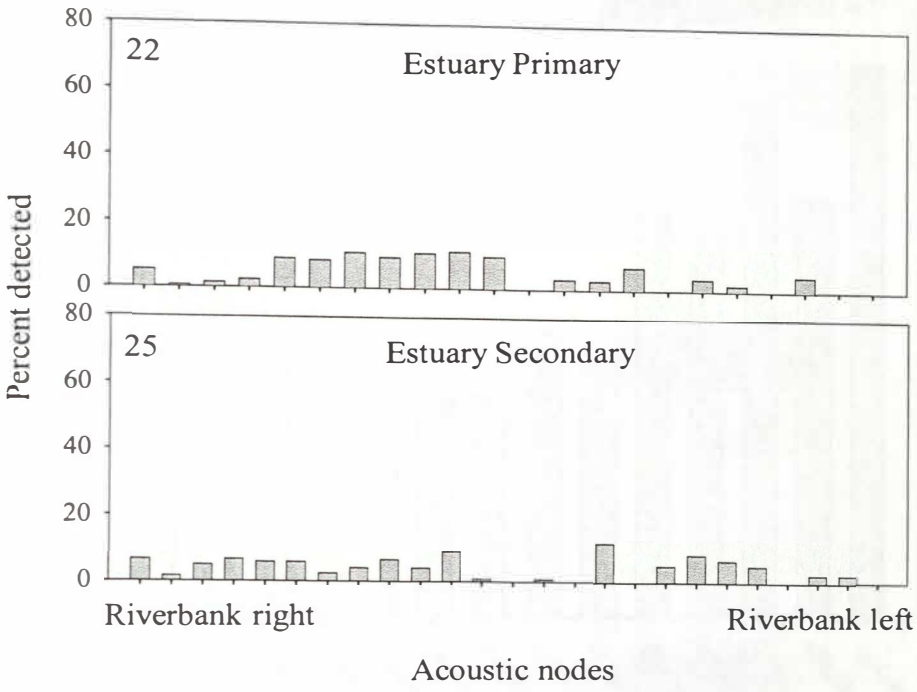
Appendix Figure A2. Cross-channel distribution of yearling Chinook salmon from the Lower Monumental downstream forebay array to the Ice Harbor tailrace secondary array, 2006. The number of nodes in each array is included in the upper left hand corner of each panel. The farthest left node in each panel was located closest to riverbank right (looking downstream) in the respective array.



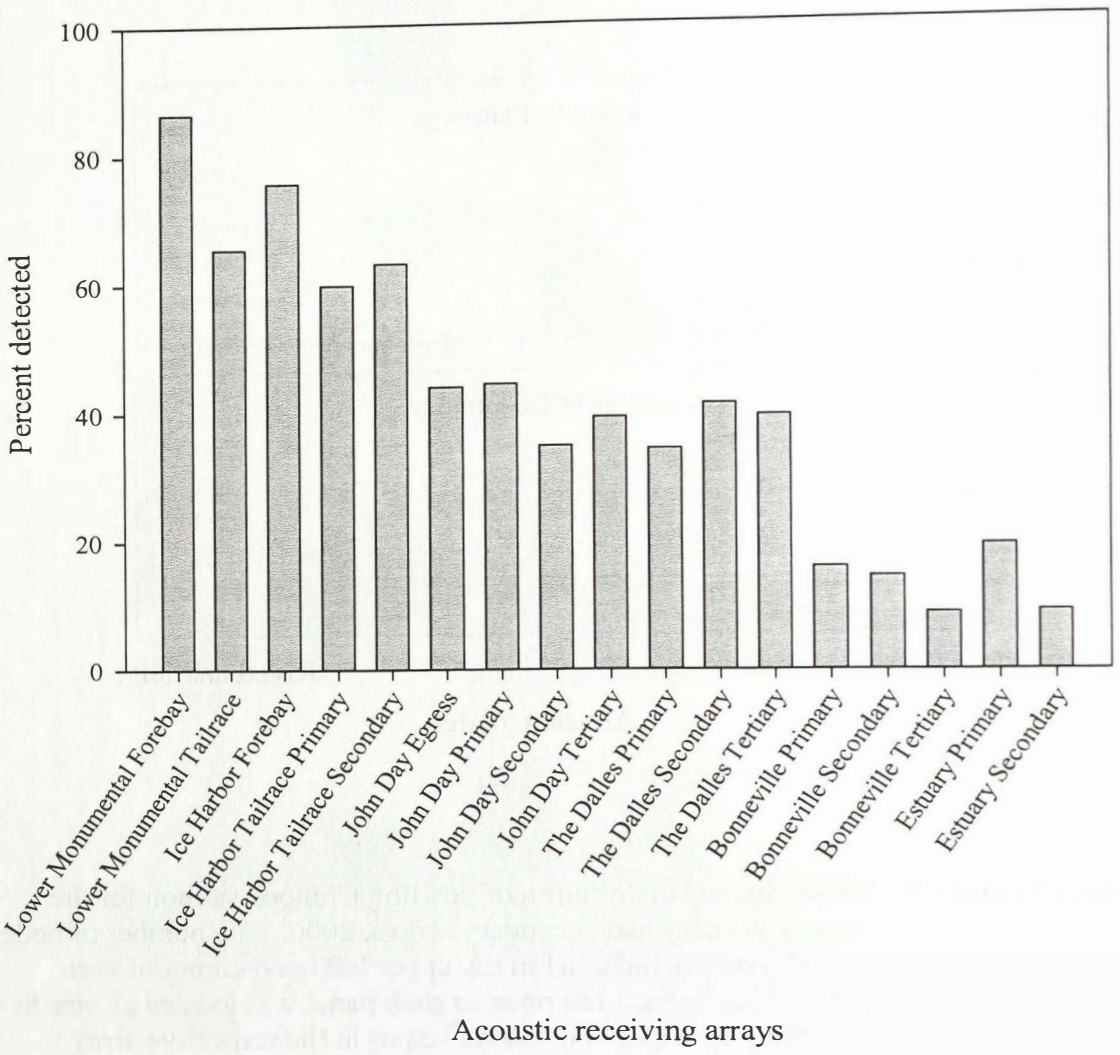
Appendix Figure A3. Cross-channel distribution of yearling Chinook salmon from the Cohn Day egress array to The Dalles primary array, 2006. The number of nodes in each array is included in the upper left hand corner of each panel. The farthest left node in each panel was located closest to riverbank right (looking downstream) in the respective array.



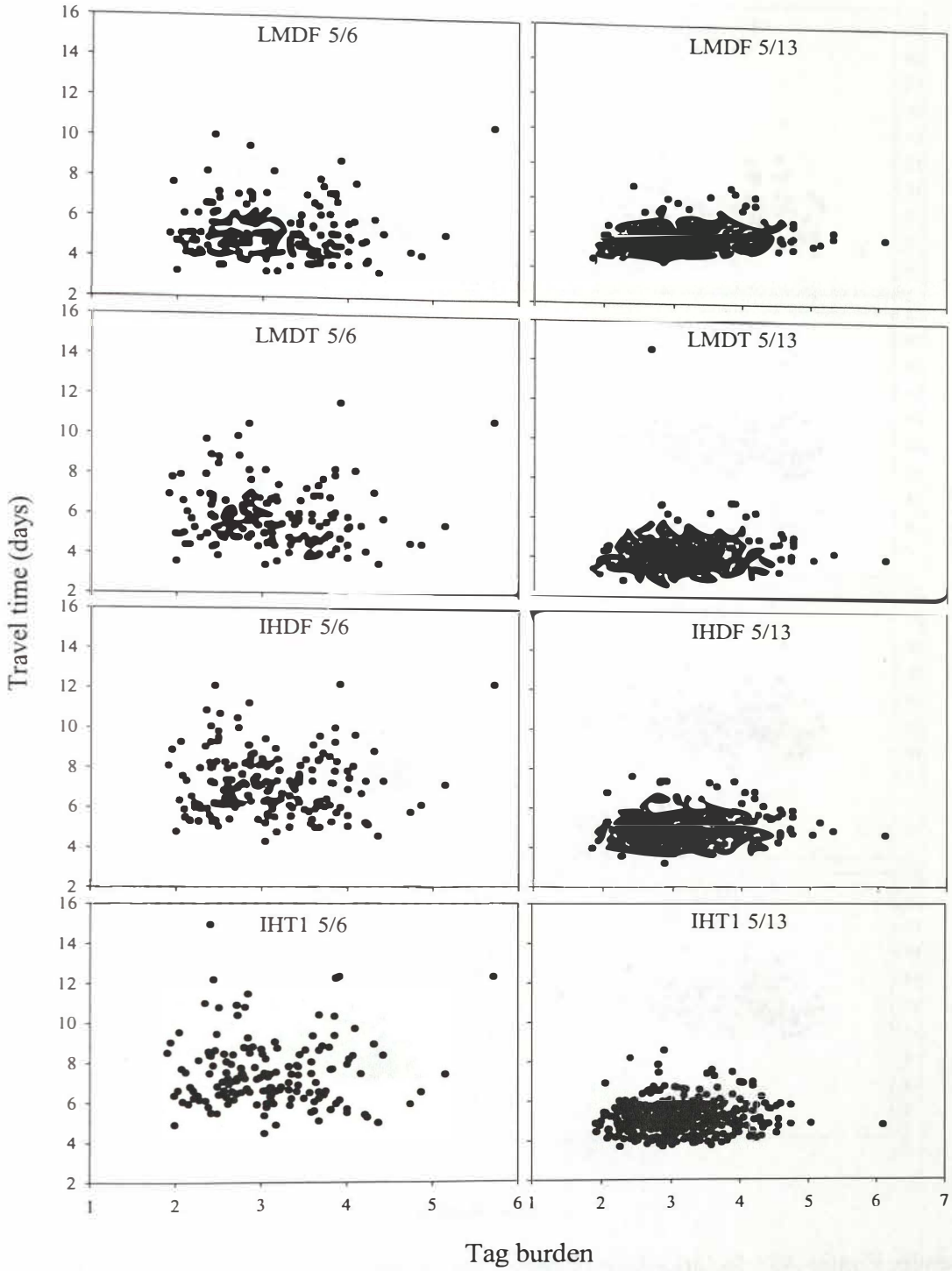
Appendix Figure A4. Cross-channel distribution of yearling Chinook salmon from The Dalles secondary array to the Bonneville tertiary array, 2006. The number of nodes in each array is included in the upper left hand corner of each panel. The farthest left node in each panel was located closest to riverbank right (looking downstream) in the respective array.



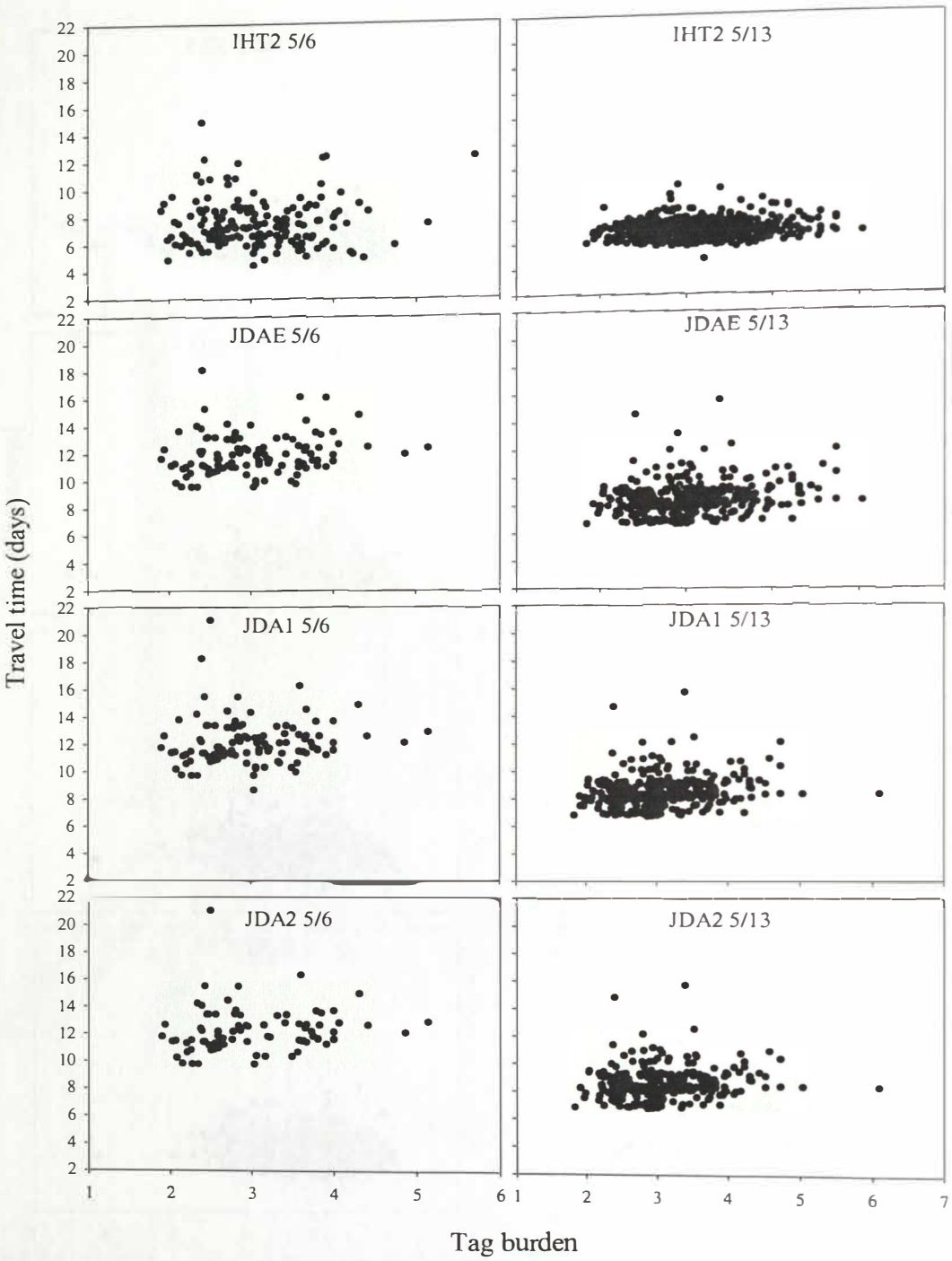
Appendix Figure A5. Cross-channel distribution of yearling Chinook salmon for the estuary primary and secondary arrays, 2006. The number of nodes in each array is included in the upper left hand corner of each panel. The farthest left node in each panel was located closest to riverbank right (looking downstream) in the respective array.



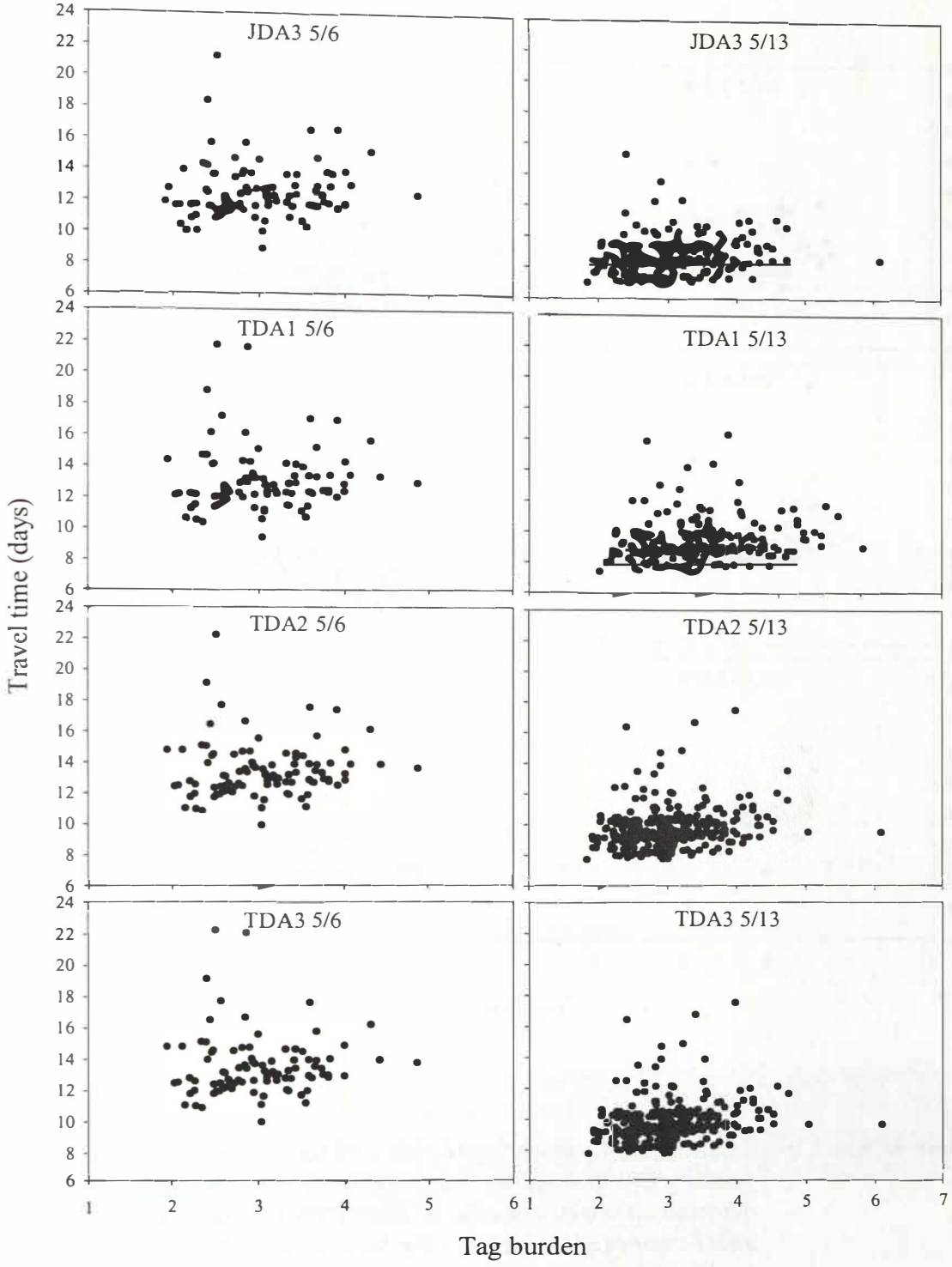
Appendix Figure A6. Percent of yearling Chinook salmon released at Lower Granite Dam and detected at downstream acoustic receiving arrays on the Snake and Columbia Rivers, 2006.



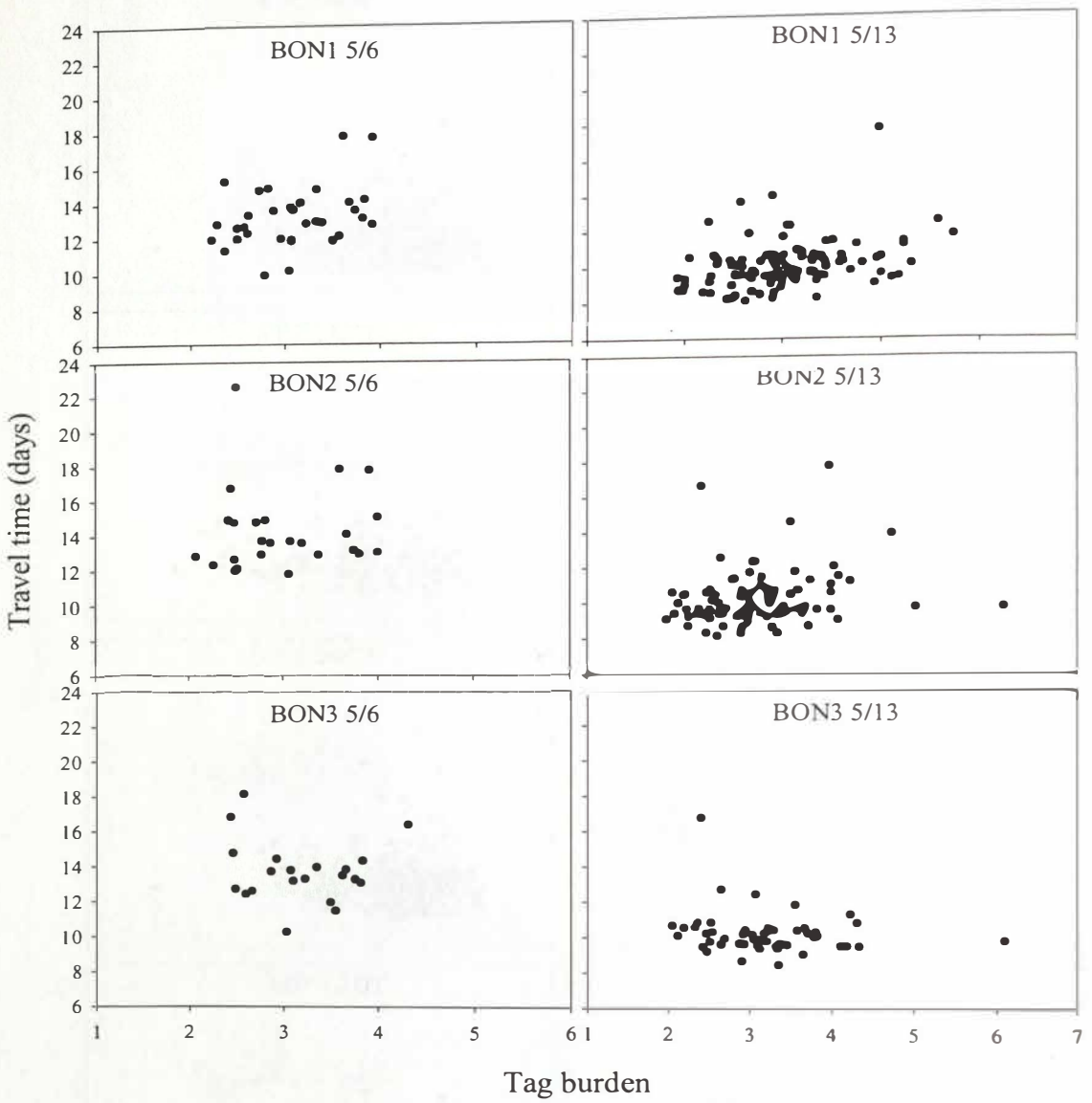
Appendix Figure A7. Relationship between tag burden and travel time (days) for yearling Chinook salmon released from Lower Granite Dam and detected on the Lower Monumental Forebay (LMDF) and Tailrace (LMDT) and Ice Harbor Forebay (IHDF) and Tailrace Primary (IHT1) arrays for May 6 (left column) and May 13 (right column) release groups, 2006.



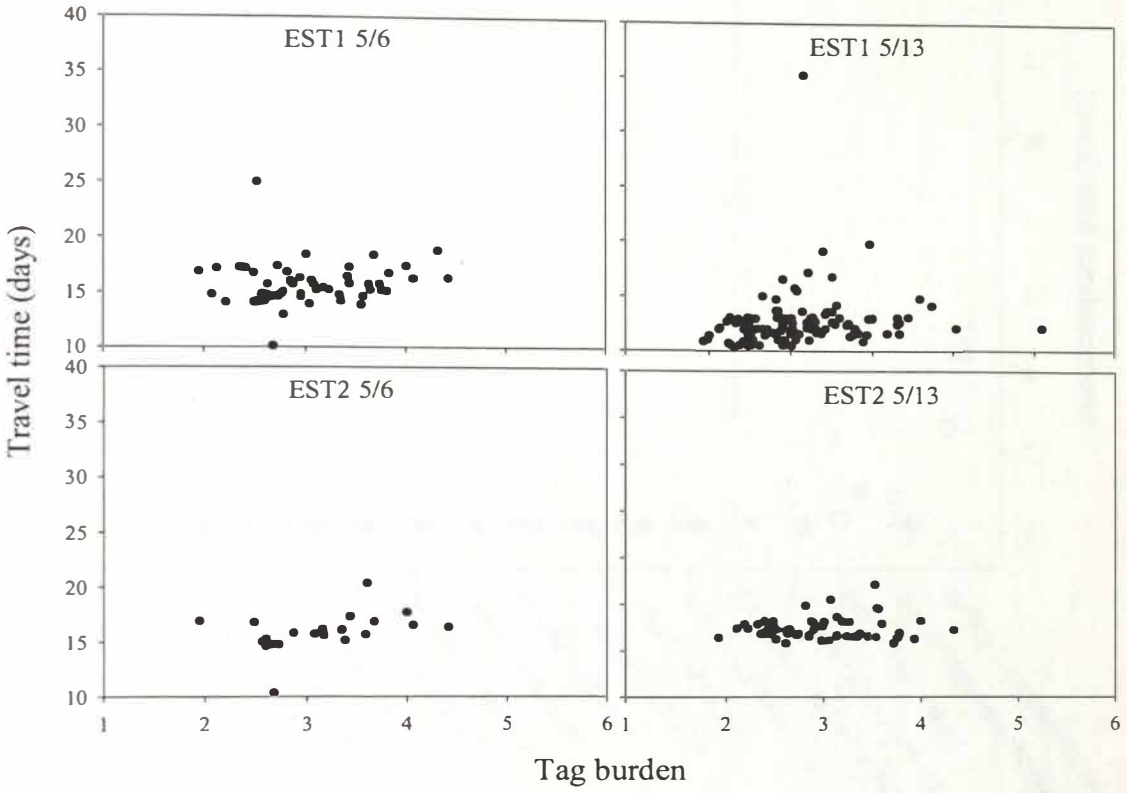
Appendix Figure A8. Relationship between tag burden and travel time (days) for yearling Chinook salmon released from Lower Granite Dam and detected on the Ice Harbor Tailrace Secondary (IHT2), John Day Egress (JDAE), John Day Primary (JDA1), and John Day Secondary (JDA2) arrays for May 6 (left column) and May 13 (right column) release groups, 2006.



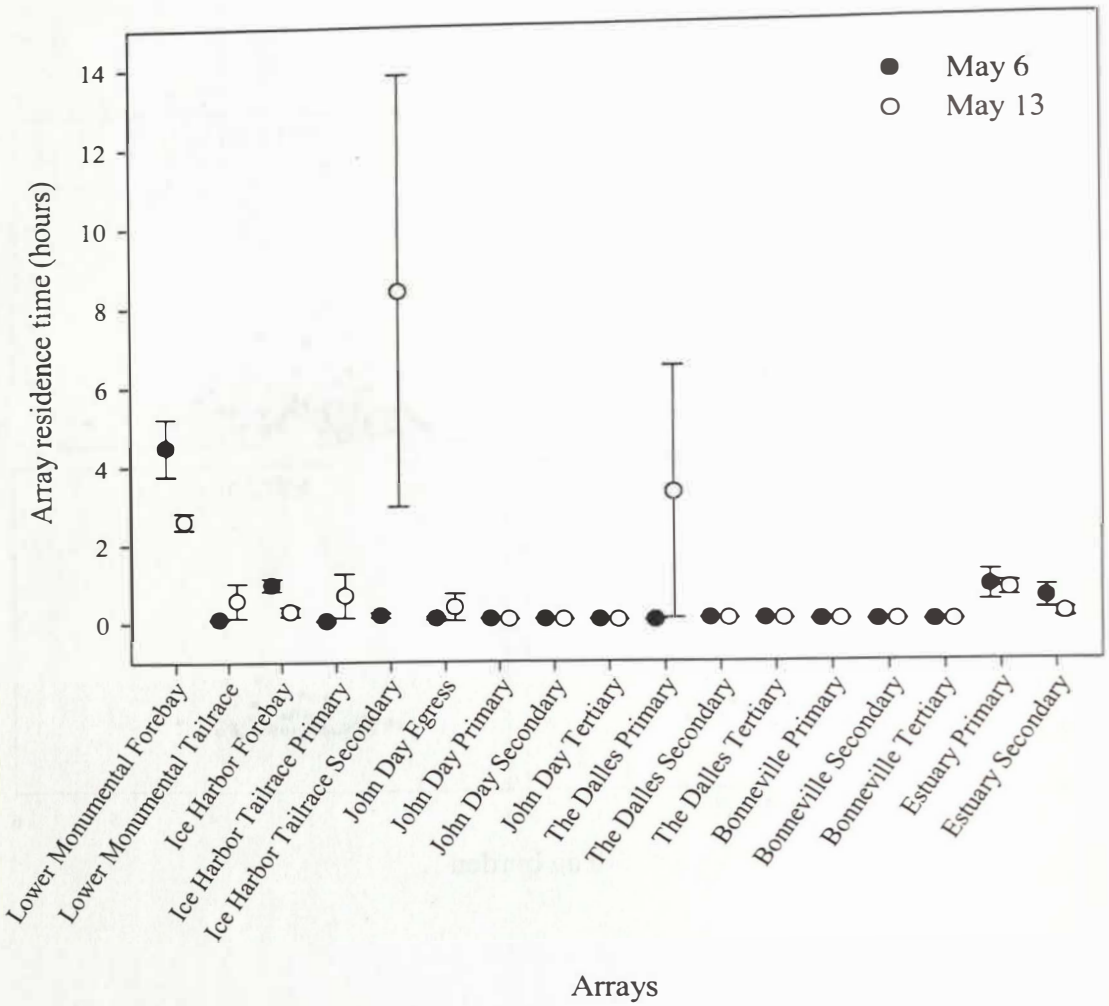
Appendix Figure A9. Relationship between tag burden and travel time (days) for yearling Chinook salmon released from Lower Granite Dam and detected on the John Day Tertiary (JDA3), and The Dalles Primary (TDA1), Secondary (TDA2), and Tertiary (TDA3) arrays for May 6 (left column) and May 13 (right column) release groups, 2006.



Appendix Figure A10. Relationship between tag burden and travel time (days) for yearling Chinook salmon released from Lower Granite Dam and detected on the Bonneville Primary (BON1), Secondary (BON2), and Tertiary (BON3) arrays for May 6 (left column) and May 13 (right column) release groups, 2006.



Appendix Figure A11. Relationship between tag burden and travel time (days) for yearling Chinook salmon released from Lower Granite Dam and detected on the Estuary Primary (EST1) and Secondary (EST2) arrays for May 6 (left column) and May 13 (right column) release groups, 2006.



Appendix Figure A12. Mean residence time by acoustic receiving array and release date of yearling Chinook salmon tagged and released at Lower Granite Dam, 2006. Whiskers represent standard errors. Ice Harbor Tailrace Secondary and The Dalles Primary residence times on May 13 were highly influenced by outliers that represented fish remaining at these arrays for extended periods of time.

APPENDIX B

Statistical Analyses of Survival, Growth, Tag Retention, and Tissue Reaction

Mortality

Logistic regression was used to assess differences in mortality rates and tag expulsion rates among three treatment groups and one control group of juvenile Chinook salmon. The three treatment groups had one group implanted with integrated tags, a second implanted with non-integrated tags, and a third implanted with PIT tags only. The control group was untagged.

Four separate studies followed these four treatment groups for holding times of 21, 30, 60, and 90 d, with each holding time group analyzed separately. The two binary endpoints (mortality and tag expulsion) also were analyzed separately; the control group was omitted from the tag expulsion analysis. All studies followed the same basic methodology that used a logistic regression model with a binary response variable (i.e., mortality or tag expulsion) and was fitted to an independent factor variable that classified fish into their respective groups.

Coefficient estimates and their standard errors from the fitted logistic regression model were used to compute pairwise statistical tests for differences in rates. Each tagged treatment group was compared directly with the control group, and the integrated tag group was compared directly to the non-integrated tags in the analysis of mortality rates. Integrated and non-integrated tagged groups were each compared with the PIT-tagged group and with each other on the incidence of expelled tags. All pairwise comparisons were constructed from the regression coefficient estimates and standard errors used to form Wald chi-square statistics to test for significant differences.

In each analysis, the data were aggregated on holding time group, with the number of mortalities or dropped tags counted along with the total count of fish (N) for that group. In many cases, there were no incidences of mortality or dropped tags for one or more treatment groups, which created very poor estimates of the regression coefficients with largely inflated standard errors. In these cases, a small bias value (e.g., 0.1) was substituted for the 0 before the model was fit. By relaxing the requirement for unbiased estimators, a better estimate of the variance was achieved, which facilitated a more reliable statistical test for differences. This technique falls in the category of *Ridge Regression* and is detailed in Montgomery and Peck (1992).

Appendix Table B1. Analysis of mortality rates of hatchery-reared yearling Chinook salmon, 2006.

Holding time (d)	Treatment 1	Treatment 2	Estimate	SE	df	X^2	P
21	Integrated	Non-integrated	0.00	4.48	1	0.00	1.00
21	Integrated	Control	0.00	4.48	1	0.00	1.00
21	PIT	Control	0.00	4.48	1	0.00	1.00
21	Non-integrated	Control	0.00	4.48	1	0.00	1.00
30	Integrated	Non-integrated	-0.72	1.24	1	0.33	0.56
30	Integrated	Control	2.32	3.32	1	0.49	0.48
30	PIT	Control	0.00	4.48	1	0.00	1.00
30	Non-integrated	Control	3.04	3.25	1	0.88	0.35
60	Integrated	Non-integrated	-2.37	3.32	1	0.51	0.48
60	Integrated	Control	0.00	4.48	1	0.00	1.00
60	PIT	Control	0.00	4.48	1	0.00	1.00
60	Non-integrated	Control	2.37	3.32	1	0.51	0.48
90	Integrated	Non-integrated	0.02	1.43	1	0.00	0.99
90	Integrated	Control	2.33	3.32	1	0.49	0.48
90	PIT	Control	0.02	4.48	1	0.00	1.00
90	Non-integrated	Control	2.32	3.32	1	0.49	0.49

Appendix Table B2. Analysis of mortality rates of hatchery-reared subyearling Chinook salmon, 2006.

Holding time (d)	Treatment I	Treatment 2	Estimate	SE	df	X^2	P
21	Non-integrated	Control	0.76	0.89	1	0.72	0.40
30	Integrated	Non-integrated	0.44	0.93	1	0.23	0.63
30	Integrated	Control	3.40	3.22	1	1.11	0.29
30	PIT	Control	-0.06	4.48	1	0.00	0.99
30	Non-integrated	Control	2.95	3.25	1	0.83	0.36
60	Integrated	Non-integrated	0.61	0.75	1	0.66	0.42
60	Integrated	Control	4.06	3.20	1	1.61	0.20
60	PIT	Control	0.03	4.48	1	0.00	0.99
60	Non-integrated	Control	3.45	3.22	1	1.15	0.28
90	Integrated	Non-integrated	0.07	0.66	1	0.01	0.92
90	Integrated	Control	1.76	1.11	1	2.51	0.11
90	PIT	Control	-2.32	3.32	1	0.49	0.49
90	Non-integrated	Control	1.69	1.11	1	2.32	0.13

Growth

Appendix Table B3. Analysis of growth rates of hatchery-reared yearling and subyearling Chinook salmon, 2006.

Age	Holding time (d)	Effect	tx1	tx1	Estimate	SE	df	X^2	P
0	21	tx1	Control	Integrated	-0.326	0.276	1	1.400	0.237
0	21	tx1	Control	PIT	-0.414	0.271	1	2.330	0.127
0	21	tx1	Control	Non-integrated	-0.471	0.273	1	2.980	0.084
0	21	tx1	Integrated	PIT	-0.088	0.250	1	0.120	0.725
0	21	tx1	Integrated	Non-integrated	-0.145	0.249	1	0.340	0.561
0	21	tx1	PIT	Non-integrated	-0.057	0.246	1	0.050	0.818
0	30	tx1	Control	Integrated	0.533	0.329	1	2.640	0.105
0	30	tx1	Control	PIT	-0.099	0.323	1	0.090	0.760
0	30	tx1	Control	Non-integrated	0.696	0.328	1	4.500	0.034
0	30	tx1	Integrated	PIT	-0.632	0.310	1	4.170	0.041
0	30	tx1	Integrated	Non-integrated	0.163	0.315	1	0.270	0.605
0	30	tx1	PIT	Non-integrated	0.795	0.310	1	6.590	0.010
0	60	tx1	Control	Integrated	-0.867	0.743	1	1.360	0.243
0	60	tx1	Control	PIT	-0.004	0.718	1	0.000	0.995
0	60	tx1	Control	Non-integrated	-0.351	0.718	1	0.240	0.625
0	60	tx1	Integrated	PIT	0.863	0.739	1	1.360	0.243
0	60	tx1	Integrated	Non-integrated	0.516	0.742	1	0.480	0.487
0	60	tx1	PIT	Non-integrated	-0.347	0.717	1	0.230	0.629
0	90	tx1	Control	Integrated	-0.485	1.398	1	0.120	0.729
0	90	tx1	Control	PIT	-0.794	1.310	1	0.370	0.544
0	90	tx1	Control	Non-integrated	-2.454	1.349	1	3.310	0.069
0	90	tx1	Integrated	PIT	-0.310	1.364	1	0.050	0.820
0	90	tx1	Integrated	Non-integrated	-1.970	1.395	1	1.990	0.158
0	90	tx1	PIT	Non-integrated	-1.660	1.316	1	1.590	0.207
1	21	tx1	Control	Integrated	-0.282	0.467	1	0.360	0.547
1	21	tx1	Control	PIT	-0.524	0.484	1	1.170	0.279
1	21	tx1	Control	Non-integrated	-0.582	0.468	1	1.550	0.214
1	21	tx1	Integrated	PIT	-0.242	0.480	1	0.250	0.614
1	21	tx1	Integrated	Non-integrated	-0.301	0.467	1	0.410	0.520
1	21	tx1	PIT	Non-integrated	-0.059	0.475	1	0.020	0.902
1	30	tx1	Control	Integrated	-0.237	0.559	1	0.180	0.672
1	30	tx1	Control	PIT	0.334	0.555	1	0.360	0.547
1	30	tx1	Control	Non-integrated	0.232	0.560	1	0.170	0.679
1	30	tx1	Integrated	PIT	0.570	0.556	1	1.050	0.305
1	30	tx1	Integrated	Non-integrated	0.469	0.563	1	0.690	0.405
1	30	tx1	PIT	Non-integrated	-0.102	0.560	1	0.030	0.856

Appendix Table B3. Continued.

Age	Holding time (d)	Effect	tx1	_tx1	Estimate	SE	df	X^2	P
1	60	tx1	Control	Integrated	2.488	0.890	1	7.810	0.005
1	60	tx1	Control	PIT	2.067	0.883	1	5.480	0.019
1	60	tx1	Control	Non-integrated	0.487	0.895	1	0.300	0.586
1	60	tx1	Integrated	PIT	-0.421	0.853	1	0.240	0.622
1	60	tx1	Integrated	Non-integrated	-2.001	0.870	1	5.300	0.021
1	60	tx1	PIT	Non-integrated	-1.580	0.867	1	3.320	0.069
1	90	tx1	Control	Integrated	1.329	1.778	1	0.560	0.455
1	90	tx1	Control	PIT	0.092	1.768	1	0.000	0.958
1	90	tx1	Control	Non-integrated	-0.005	1.759	1	0.000	0.998
1	90	tx1	Integrated	PIT	-1.236	1.689	1	0.540	0.464
1	90	tx1	Integrated	Non-integrated	-1.333	1.666	1	0.640	0.423
1	90	tx1	PIT	Non-integrated	-0.097	1.660	1	0.000	0.953

Tag Expulsion

Appendix Table B4. Analysis of tag expulsion rates of hatchery-reared yearling Chinook salmon, 2006.

Holding time (d)	Tag 1	Tag 2	Estimate	SE	df	X^2	P
21	Integrated	PIT	0.00	4.48	1	0.00	1.00
21	Integrated	Non-integrated	0.00	4.48	1	0.00	1.00
21	PIT	Non-integrated	0.00	4.48	1	0.00	1.00
30	Integrated	PIT	0.00	4.48	1	0.00	1.00
30	Integrated	Non-integrated	0.00	4.48	1	0.00	1.00
30	PIT	Non-integrated	0.00	4.48	1	0.00	1.00
60	Integrated	PIT	0.00	4.48	1	0.00	1.00
60	Integrated	Non-integrated	-0.05	4.48	1	0.00	0.99
60	PIT	Non-integrated	-0.05	4.48	1	0.00	0.99
90	Integrated	PIT	-2.32	3.32	1	0.49	0.49
90	Integrated	Non-integrated	0.02	4.48	1	0.00	1.00
90	PIT	Non-integrated	2.33	3.32	1	0.49	0.48

Appendix Table B5. Analysis of tag expulsion rates of hatchery-reared subyearling Chinook salmon, 2006.

Holding time (d)	Tag 1	Tag 2	Estimate	SE	df	X^2	P
21	Integrated	PIT	3.77	3.21	1	1.38	0.24
21	Integrated	Non-integrated	1.43	1.14	1	1.58	0.21
21	PIT	Non-integrated	-2.34	3.32	1	0.50	0.48
30	Integrated	PIT	2.32	3.32	1	0.49	0.49
30	Integrated	Non-integrated	-1.12	1.17	1	0.91	0.34
30	PIT	Non-integrated	-3.44	3.22	1	1.14	0.29
60	Integrated	PIT	3.96	3.20	1	1.53	0.22
60	Integrated	Non-integrated	1.67	1.11	1	2.27	0.13
60	PIT	Non-integrated	-2.29	3.32	1	0.47	0.49
90	Integrated	PIT	3.53	3.22	1	1.20	0.27
90	Integrated	Non-integrated	-0.48	0.75	1	0.40	0.53
90	PIT	Non-integrated	-4.01	3.20	1	1.57	0.21

Necropsy

Fish were numerically scored for health index (HI) on 10 health indices (described elsewhere) following completion of their holding period (21, 30, 60 or 90 d). The total HI score was the sum of these 10 indices. Higher HI scores indicated lower health status of fish. Only fish with non-missing values on all 10 indices were used in this analysis.

Health scores had skewed distributions: most HI scores were at lower values, including many scores of 0. The skew and presence of multiple-zero HI scores in these distributions precluded the use of log transformation and normal based parametric statistical tests, while the presence of multiple ties in HI scores between holding time and treatment groups precluded the use of rank-based nonparametric methods. The alternative approach was to compare count distributions across HI scores among treatment groups. Count distributions were statistically compared using a log-linear model with counts as the response variable taken as Poisson distributed.

Because tagging and holding-time studies were conducted as separate experiments, treatments were compared within each holding time. Specific comparisons were made between each tag treatment and the untagged control group, and between the integrated and non-integrated PIT and acoustic tags.

Appendix Table B6. Health index analysis for hatchery-reared yearling Chinook salmon (C = control; I = integrated transmitter; U = non-integrated transmitter; P = PIT tag).

Holding time (d)	Treatment 1	Treatment 2	Estimate	SE	df	X^2	P
21	C	I	0.000	0.386	1	0.000	1.000
21	C	P	0.000	0.386	1	0.000	1.000
21	C	U	0.000	0.386	1	0.000	1.000
21	I	U	0.000	0.386	1	0.000	1.000
30	C	I	0.023	0.388	1	0.000	0.953
30	C	P	0.000	0.386	1	0.000	1.000
30	C	U	0.095	0.395	1	0.060	0.810
30	I	U	0.072	0.398	1	0.030	0.856
60	C	I	-0.052	0.338	1	0.020	0.877
60	C	P	-0.069	0.336	1	0.040	0.837
60	C	U	0.018	0.344	1	0.000	0.958
60	I	U	0.070	0.339	1	0.040	0.836
90	C	I	-0.249	0.356	1	0.490	0.484
90	C	P	-0.249	0.356	1	0.490	0.484
90	C	U	-0.214	0.359	1	0.360	0.550
90	I	U	0.035	0.336	1	0.010	0.918

Appendix Table B7. Health index analysis for hatchery-reared subyearling Chinook salmon (C = control; I= integrated transmitter; U = non-integrated transmitter; P= PIT tag), 2006.

Holding time (d)	Treatment 1	Treatment 2	Estimate	SE	df	χ^2	P
21	C	I	-0.251	0.356	1	0.500	0.480
21	C	P	-0.295	0.353	1	0.700	0.403
21	C	U	-0.056	0.373	1	0.020	0.881
21	I	U	0.196	0.351	1	0.310	0.577
30	C	I	-0.178	0.335	1	0.280	0.594
30	C	P	-0.257	0.329	1	0.610	0.435
30	C	U	0.025	0.351	1	0.000	0.944
30	I	U	0.203	0.337	1	0.360	0.547
60	C	I	0.138	0.294	1	0.220	0.639
60	C	P	-0.016	0.283	1	0.000	0.955
60	C	U	0.299	0.308	1	0.940	0.332
60	I	U	0.160	0.317	1	0.260	0.613
90	C	I	0.109	0.302	1	0.130	0.718
90	C	P	-0.114	0.285	1	0.160	0.690
90	C	U	0.232	0.312	1	0.550	0.458
90	I	U	0.123	0.320	1	0.150	0.702

The classification results for suture retention, capsule appearance, and capsule adhesion defined in the main text of this chapter were analyzed using multinomial response models. Transmitter locations for acoustic and PIT tags were defined as binary outcomes and were analyzed using logistic regression. Statistical results reported in the results section for these classification results are from the fitted multinomial regression models based on the likelihood ratio chi-square tests on the null hypothesis of all groups being equal versus the alternative of at least one group being not equal to the others.

Appendix Table B8. Suture retention analysis for hatchery-reared yearling Chinook salmon (I = integrated transmitter, U = on-integrated transmitter), 2006.

Holding time (d)	Treatment	n0	n1	n2	ntotal	n0p (%)	n1p (%)	n2p (%)
21	I	1	0	34	35	3	0	97
30	I	0	2	41	43	0	5	95
60	I	26	19	16	61	43	31	26
90	I	37	9	13	59	63	15	22
21	U	0	2	28	30	0	7	93
30	U	1	7	34	42	2	17	81
60	U	33	11	12	56	59	20	21
90	U	48	7	5	60	80	12	8

Source	df	X^2	P
H time	3	246.83	<0.0001
Treatment	1	10.75	0.001

Appendix Table B9. Suture retention analysis for hatchery-reared subyearling Chinook salmon (I = integrated transmitter, U = non-integrated transmitter), 2006.

Holding time (d)	Treatment	n0	n1	n2	ntotal	n0p (%)	n1p (%)	n2p (%)
21	I	33	9	3	45	73	20	7
30	I	29	12	8	49	59	24	16
60	I	51	4	0	55	93	7	0
90	I	50	2	0	52	96	4	0
21	U	34	12	2	48	71	25	4
30	U	39	9	3	51	76	18	6
60	U	59	3	0	62	95	5	0
90	U	58	0	0	58	100	0	0

Source	df	X^2	P
H time	3	61.89	<0.0001
Treatment	1	3.09	0.079

Appendix Table B10. Acoustic transmitter location analysis for hatchery-reared yearling Chinook salmon (I = integrated transmitter, U = non-integrated transmitter), 2006.

Holding time (d)	Treatment	n0	n1	ntotal	n0p (%)	n1p (%)
21	I	5	1	6	83	17
30	I	31	6	37	84	16
60	I	36	21	57	63	37
90	I	34	21	55	62	38
21	U	34	1	35	97	3
30	U	33	7	40	83	18
60	U	31	23	54	57	43
90	U	41	17	58	71	29

Source	df	X^2	P
H time	3	27.26	<0.0001
Treatment	1	0.12	0.7309

Appendix Table B11. Acoustic location analysis for hatchery-reared subyearling Chinook salmon (I = integrated transmitter, U = non-integrated transmitter), 2006.

Holding time (d)	Treatment	n0	n1	ntotal	n0p (%)	n1p (%)
21	I	31	14	45	69	31
30	I	12	37	49	24	76
60	I	20	32	52	38	62
90	I	22	28	50	44	56
21	U	23	23	46	50	50
30	U	15	34	49	31	69
60	U	12	49	61	20	80
90	U	14	41	55	25	75

Source	df	X^2	P
htime	3	26.81	<0.0001
tx	1	7.62	0.0058

Appendix Table B12. PIT tag location analysis for hatchery-reared yearling Chinook salmon (P = PIT tag, U = non-integrated transmitter), 2006.

Holding time (d)	Treatment	n0	n1	ntotal	n0p (%)	n1p (%)
21	P	7	33	40	18	83
30	P	24	16	40	60	40
60	P	7	54	61	11	89
90	P	12	47	59	20	80
21	U	16	16	32	50	50
30	U	20	19	39	51	49
60	U	28	23	51	55	45
90	U	30	24	54	56	44

Source	df	X^2	P
htime	3	13.41	0.0038
tx	1	32.25	<0.0001

Appendix Table B13. PIT tag location analysis for hatchery-reared subyearling Chinook salmon (P = PIT tag, U = non-integrated transmitter), 2006.

Holding time (d)	Treatment	n0	n1	ntotal	n0p (%)	n1p (%)
21	P	0	48	48	0%	100%
30	P	5	48	53	9%	91%
60	P	5	58	63	8%	92%
90	P	9	56	65	14%	86%
21	U	31	10	41	76%	24%
30	U	13	33	46	28%	72%
60	U	17	45	62	27%	73%
90	U	33	22	55	60%	40%

Source	df	X^2	P
htime	3	22.67	<0.0001
tx	1	90.79	<0.0001

Appendix Table B14. Acoustic capsule appearance analysis for hatchery-reared yearling Chinook salmon (I = integrated transmitter, U = non-integrated transmitter), 2006.

Holding time (d)	Treatment	n0	n1	n2	n3	ntotal	n0p (%)	n1p (%)	n2p (%)	n3p (%)
21	I	1	17	0	0	18	6	94	0	0
30	I	0	37	6	0	43	0	86	14	0
60	I	1	46	12	0	59	2	78	20	0
90	I	2	49	7	0	58	3	84	12	0
21	U	0	10	0	0	10	0	100	0	0
30	U	0	34	5	1	40	0	85	13	3
60	U	1	43	11	0	55	2	78	20	0
90	U	2	49	6	0	57	4	86	11	0

Source	df	X^2	P
htime	3	7.16	0.0671
tx	1	0.05	0.819

Appendix Table B15. Acoustic capsule appearance analysis for hatchery-reared subyearling Chinook salmon (I = integrated transmitter, U=non-integrated transmitter), 2006.

Holding time (d)	Treatment	n0	n1	n2	n3	ntotal	n0p (%)	n1p (%)	n2p (%)	n3p (%)
21	I	0	5	18	0	23	0	22	78	0
30	I	0	26	15	0	41	0	63	37	0
60	I	2	17	28	0	47	4	36	60	0
90	I	0	22	24	0	46	0	48	52	0
21	U	0	7	18	19	44	0	16	41	43
30	U	0	24	16	7	47	0	51	34	15
60	U	1	17	31	12	61	2	28	51	20
90	U	0	7	31	17	55	0	13	56	31

Source	df	X^2	P
htime	3	44.1	<0.0001
tx	1	4.51	0.0337

Appendix Table B16. Acoustic capsule adhesion analysis for hatchery-reared yearling Chinook salmon (I = integrated transmitter, U = non-integrated transmitter), 2006. N0 = adhesion to body wall not containing incision; N1 = adhesion to body wall containing incision; N2 = adhesion to organs but not the body wall.

Holding time (d)	Treatment	n0	n1	n2	ntotal	n0p (%)	n1p (%)	n2p (%)
21	I	8	18	3	29	28	62	10
30	I	16	0	26	42	38	0	62
60	I	24	1	20	45	53	2	44
90	I	17	6	33	56	30	11	59
21	U	2	19	2	23	9	83	9
30	U	26	0	13	39	67	0	33
60	U	32	2	10	44	73	5	23
90	U	15	3	36	54	28	6	67

Source	df	X^2	P
htime	3	26.36	<0.0001
tx	1	3.35	0.0671

Appendix Table B17. Acoustic capsule adhesion analysis for hatchery-reared subyearling Chinook salmon (I = integrated transmitter, U = non-integrated transmitter), 2006. N0 = adhesion to body wall not containing incision; N1 = adhesion to body wall containing incision; N2 = adhesion to organs but not the body wall.

Holding time (d)	Treatment	n0	n1	n2	ntotal	n0p (%)	n1p (%)	n2p (%)
21	I	7	17	20	44	16	39	45
30	I	9	37	3	49	18	76	6
60	I	16	22	14	52	31	42	27
90	I	9	27	16	52	17	52	31
21	U	9	18	17	44	20	41	39
30	U	5	41	2	48	10	85	4
60	U	17	21	21	59	29	36	36
90	U	8	24	22	54	15	44	41

Source	df	X^2	P
htime	3	15.53	0.0014
tx	1	0.65	0.4201

Size Limit

Growth—Linear regression models were used to assess trends in growth patterns between surgically tagged (treatment) and untagged (control) juvenile Chinook salmon and to find evidence of adverse growth effects among surgically implanted fish. Each model took the change in fish weight (g) after 30 d following surgery as the dependent variable, with independent variables of fork length at time of tagging, and a dichotomous indicator variable (tx) categorizing fish as either in the treatment or control groups. An interaction term between tx and fork length was also included in the model. Significance of both coefficient estimates for fork length and the interaction term would suggest a significant difference in slopes between two groups and thus a real difference in growth trend lines (Neter et al. 1990). Groups showing a significant difference in growth trends are shown in a figure with fitted regression lines overlaid for the treatment and control groups. The fork length at the intersection point of the two regression lines was calculated from the fitted regression equation, and an approximate 95% confidence interval around this fork length was calculated using the inverse regression estimation procedure (Neter et al. 1990).

Mortality—Estimation of differential mortality rates between surgically tagged and untagged fish was carried out in three separate tasks using fish in 10-mm size groups of 80 to 89, 90 to 99, and 100 to 109 mm. For each group, Fisher's exact test was applied on a 2 × 2 contingency table formed by cross-tabulating dichotomous variables for mortality and surgically tagged or untagged fish.

The influence of fork length at time of tagging on mortality was examined to approximate the minimum length at which surgical implantation of an acoustic transmitter and PIT tag would have minimal adverse effects on mortality in juvenile Chinook salmon. The examination was done by comparing the observed mortality rates of surgically tagged and untagged fish. The mortality rates were computed across an interval of fork lengths for surgical (treatment) and control fish, with upper 95% confidence bounds estimated using the binomial variance and assuming the normal approximation to the binomial distribution.

Appendix Table B18. Summary of linear regression parameter estimates used to determine the minimum size at which implantation of acoustic transmitters and PIT tags will not influence growth, 2006. The results shown below were the first phase in this analysis.

Size class (mm)	Parameter	Estimate	SE	*t* value	Pr(>|t|)	
80-89	(Intercept)	-7.87	4.29	-1.84	0.068	
	length	0.19	0.05	3.71	<.001	***
	treatment (tx)	-16.38	6.02	-2.72	0.007	**
	length:tx	0.19	0.07	2.60	0.010	**
90-99	(Intercept)	-9.87	5.74	-1.72	0.087	
	length	0.17	0.06	2.81	0.005	**
	treatment (tx)	-9.22	7.53	-1.22	0.222	
	length:tx	0.10	0.08	1.24	0.216	
100-109	(Intercept)	-9.50	6.77	-1.40	0.162	
	length	0.15	0.06	2.32	0.021	*
	treatment (tx)	10.64	9.44	1.13	0.261	
	length:tx	-0.10	0.09	-1.08	0.282	

*** <0.001; ** <0.01; * <0.05