Exxon Valdez Oil Spill
Long-Term Herring Research and Monitoring Program Final Report

Scales as Growth History Records

Exxon Valdez Oil Spill Trustee Council Project 13120111-N

Final Report

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Study History: This final report summarizes work conducted from 2012-2015 and work that contributed to the December 1, 2014 Herring Research and Monitoring program Science Synthesis Report (project 14120111).

Abstract: Pacific herring (*Clupea pallasii*) scales have been collected in Prince William Sound, Alaska and archived since 1973. These scales contain a retrospective, longitudinal growth history that this project worked to capture. Using a spatially-calibrated scanning microfiche this project collected digital images of randomly selected herring scales. Annual scale growth increments were measured from the images using image analysis software. Data were subject to quality control analysis and precision of the scale measurements was evaluated by a blind second measurement of random selected scales. Correlation analyses of these scale growth history data and environmental and biological data have helped explain some of the growth drivers for Pacific herring in Prince William Sound.

Key words: Alaska, *Clupea pallasii*, growth, longitudinal study, Pacific herring, Prince William Sound, scales

Project Data: Data include Prince William Sound, Alaska, Pacific herring scale annual increment measurements for fish aged 4, 5, and 6 by sex and year (1982–1983, and 1985–2015). These data are paired with sex, size, approximate location, sample date, harvest type, and sampling gear. Data are available in Excel at the Alaska Department of Fish and Game office in Cordova. These data have also been provided to the Alaska Ocean Observing System for dissemination and archiving.

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Data collected for the Herring Research and Monitoring Program projects that contributed to this report are available through the Alaska Ocean Observing System (AOOS) Gulf of Alaska data portal: http://portal.aoos.org/gulf-of-alaska#module-metadata/ad7118be-ea24-11e0-b488-0019b9dae22b/ee8a692c-ea24-11e0-b73c-0019b9dae22b.

The data may also be found through the DataONE earth and environmental data archive at https://search.dataone.org/#data and by selecting the Gulf of Alaska Data Portal under the Member Node filter. The Alaska Ocean Observing System data custodian is Carol Janzen, Alaska Ocean Observing System, 1007 W. 3rd Ave. #100, Anchorage, AK 99501, 907-644-6703, janzen@aoos.org.

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EXECUTIVE SUMMARY

Analysis of annular growth increments on scales provide a retrospective, longitudinal growth history that can assist in evaluating environmental and density-dependent causes of growth variation. Determining the underlying distribution of individual growth patterns can provide improved inputs into population dynamics models used to establish harvest guidelines.
Two main objectives were outlined for this project. The first objective included several items: (a) standardize scale age interpretive criteria for Pacific herring (*Clupea pallasii*), (b) evaluate alternative measurement methods for scale growth increments, and (c) develop semi-automated methods to measure scales. The second objective was to image and measure scale growth increments on as many fish as possible. The first objective was met starting in the fall of 2012 after travel to Juneau, Alaska to meet staff at the statewide mark, tag, and age lab to learn about the equipment and methods. Equipment was then purchased to take digital images of scales and software to measure the scale increments. A random selection of herring scales of ages 4, 5, and 6 at collection, equally split between sexes, were chosen to image and measure. The second objective was adjusted to measure scales from 30 fish of each sex for ages 4–6 for as many years as possible beginning with the recent years. A total of 7,270 scales were imaged and measured and an additional 3,500 scales were imaged but not measured because the reader did not agree with the assigned age or the scale had morphological problems (e.g., torn or folded edges, or asymmetrical shape) that would not allow measurements. The data were evaluated for outliers using scatter plots and box plots. Statistical outliers (N=57) were imaged and measured a second time. Wilcoxon signed-rank tests indicated only 1 of the 14 combinations of age and scale growth increment was significantly different ($P=0.046$) at $P<0.05$, from the first set considered outliers. A test of the precision of the measurements was conducted by randomly selecting 101 scales to be imaged and measured a second time. Linear regression analysis of paired measurements of scale annuli to test the precision were highly significant for all increments ($P<.001$) and indicated there was no significant difference between the two sets of data when measurements of scale increments (e.g., first year growth) were combined across ages and sexes. This project generated a retrospective, longitudinal growth history of scale growth for Prince William Sound Pacific herring. These data have been and will be useful to help understand how environmental and biological factors affect herring scale growth.

INTRODUCTION

Fish grow in response to environmental conditions and are constrained by intrinsic influences of genetic predisposition for growth and current size (Weatherley and Gill 1987, Weisberg 1993). Understanding how growth is influenced by the environmental and genetic sources of variability is important for several reasons. Effects of stock size and environmental conditions on growth have been studied by many investigators (Anthony and Fogarty 1985, Hagen and Quinn 1991, Martinson et al. 2009, Peterman and Bradford 1987, Stocker et al. 1985), because growth variation can affect reproductive potential through its influences on fecundity and spawn timing (Ware and Tanasichuk 1989), natural mortality, recruitment, and age at maturity (Haist and Stocker 1985, Schmitt and Skud 1978). Haist and Stocker 1985 stated that factors affecting growth rates can be of fundamental importance to understanding the dynamics of exploited populations and responses of natural populations to abundance and environmental influences have remained a central issue in population biology (Tanasichuk 1997). Variation in growth affects the appropriate harvest policies based on demographic models that reflect natural processes (Methot 1997, Tanasichuk 1997).

The underlying mechanisms for changes in annular growth for herring in the northern Gulf of Alaska are still not completely understood. A period with the lowest observed average body sizes for Prince William Sound (PWS) Pacific herring (*Clupea pallasii*) coincided with a period of historically high abundance followed by a large population decline associated with outbreaks of viral hemorrhagic septicemia virus (VHSV) and the parasite *Ichthyophonus hoferi* (Marty et al. 1998, Marty et al. 2003, Marty et al. 2010); but see Elston and Meyers (2009) for a critique of the hypothesis that disease was a root cause of the 1993 PWS herring collapse . Although the links between herring growth and disease susceptibility are not yet well understood, it is hypothesized that the observed population decline resulted from density dependent growth causing decreased body condition and susceptibility to disease (Rice and Carls 2007). Analysis of annular growth increments on scales provide a retrospective, longitudinal growth history that will assist in evaluating environmental and density-dependent causes of growth variation.

Herring growth is currently understood based on cross-sectional size at age data. In contrast, growth increment information incorporates a longitudinal history of growth that increases the effective degrees of freedom and can be used in modeling changes in growth in relationship to environmental and population indices (Chambers and Miller 1995, Kreuz et al. 1982, Tanaischuk 1997, Weisberg 1993). Determining the underlying distribution of individual growth patterns can provide improved inputs into population dynamics models used to establish harvest guidelines.

Age, sex, and size data from PWS Pacific herring have been collected by the Alaska Department of Fish and Game (ADF&G) from commercial fisheries, test fisheries, and fishery independent research projects since the early 1970s. The archive currently contains approximately 210,000 scales paired with size and sex data (most collected since 1979). Summaries of age, sex, and size data have been published for a portion of the time series (e.g., Sandone 1988*a*, *b*; Willette et al. 1999). Age, sex, and size processing methods are similar those described by Baker et al. (1991); however, electronic fish measuring boards were used to enter sample summary data and individual fish data (standard length in mm, whole body weight in grams, and sex) at the time of processing since 1989.

This project imaged and measured Pacific herring scales from the ADF&G archive in Cordova, Alaska using methods described in Batten et al. (2016). The methods used for this project were similar to those described by Hagen et al. (2001) to image and measure annular growth increments of Pacific salmon (*Oncorhynchus* sp.) scales. The first year of this project was focused on developing the image processing methods and criteria. The second year of the project was primarily focused on measuring growth increments of randomly selected scales from the archived collection.

OBJECTIVES

1. Fiscal Year 2012:
    a. Standardize scale interpretive criteria, evaluate alternative measurement techniques, and develop semi-automated procedures for measuring scale increments of PWS herring.
    b. Measure scale growth increments on scales subsampled from archived collections.
2. Fiscal Year 2013:
    a. Finish measurements of scale growth increments on subsampled scales.

METHODS

Study Area

No field work was conducted for this project. A large existing Pacific herring scale archive maintained at the ADF&G office in Cordova, Alaska provided samples for the project. The scale archive is almost exclusively from the Prince William Sound Management Area which includes all state waters west of Cape Suckling (approximately -143.872° West Long.) and east of Cape Fairfield (approximately -148.844° West Long.). One purse seine test set collection is from Resurrection Bay near Seward, Alaska in 1996 and 12 scales were used for this project.

Sample Archive

The PWS scale archive collections start in 1973, but more consistent and larger collections began in 1979. The archive contains approximately 210,000 scales classified by commercial harvest type (spring sac roe, pound, or fall food and bait fisheries) or research project type (acoustics, disease assessment, or spawning collection) by collection gear type (commercial purse seine, gillnet, or trawl gear; and research purse seine, cast net, variable mesh gillnet, or jigs).

Sample Size

The original project proposal indicated the number of scales to measure would be based on a power analysis with a preliminary goal of 50 scales from six to seven age classes per year. However, a delay in hiring staff at the beginning of the project combined with the time required to acquire and setup equipment, modify the equipment for use with large Pacific herring scales, and calibrate the scanner, caused a later than anticipated start to production processing. Additionally, an examination of the number of scales available by age indicated that 60 scales per year (30 for each sex) for three age classes would be more likely to be completed with the available funds. The three age classes selected for this project were ages 4, 5, and 6 because an examination of the scale archive indicated the sample goal could be achieved more often than for the other age classes.

Pre-processing Setup

Prior to starting the project, project personnel met with staff at the ADF&G Mark-Tag-Age (MTA) Laboratory in Juneau, Alaska to consult on standardizing scale interpretive criteria, evaluating alternative measurement techniques, and to develop semi-automated procedures for measuring scale increments. The MTA laboratory staff also provided guidance on the appropriate equipment, equipment setup and calibration, image analysis software, and data processing. MTA Laboratory staff members have extensive experience with fish scale image capture, annular measurements, and processing so using similar equipment and procedures saved a significant amount of development time and knowledgeable source for help.

Age interpretation criteria for this project were the same as shown by the pictorial guide of Lebida (1986) for Bristol Bay herring scales. Because an annulus forms on the scale margin in the spring, this project standardized on spring collections of scales from March, April, or May.

Prior to starting production scale imaging work, test scales were imaged with our Screenscan® microfiche scanner attached to an Indus® microfiche reader (Indus International, Inc. West Salem, Wisconsin). Adjustments to the magnification were necessary to get all of the larger herring scales to fit in the image and a neutral density filter was added to increase contrast of the image. Additionally, the Image-Pro® Plus Software (Media Cybernetics, Inc. Rockville, Maryland) was spatially calibrated using a stage micrometer for the magnification objective used to measure growth increments.

Scale Processing

When available, 100 scales were randomly selected for each age (4, 5, and 6), sex, and year (1982–1983 and 1985–2015). This pool of 100 scales was necessary because not all scales were useable for this project. Scales were selected for imaging and measuring if they met the following criteria: (1) the scale reader agreed with the age previously assigned by ADF&G staff, (2) the scale appeared to be from the preferred area, and (3) the scale annuli were defined sufficiently to allow discrete measurements. Scales from the preferred areas 1–4 (Fig. 1) were much more likely to be symmetrical with clearly defined annuli and baseline.

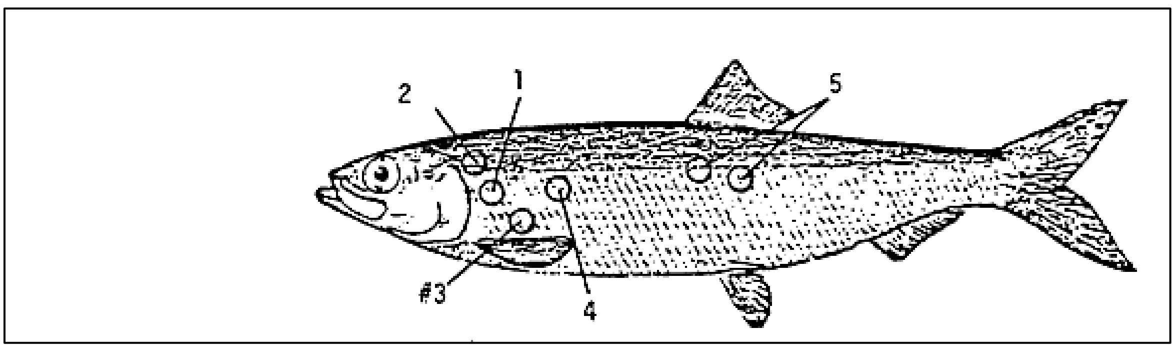

FIGURE 1–PREFERRED BODY AREAS TO COLLECT PACIFIC HERRING SCALES FOR AGE INTERPRETATION.

Scales were rejected for measuring if (1) the scale was asymmetrical such that the appropriate axis to measure along was unclear, (2) the scale quality would make measuring difficult or impossible. Most scales were acceptable for determination of total age, but some were unsuitable for measuring growth increments because the scale was torn, folded, or had unusual growth patterns along the measurement axis of the scale.

Each randomly selected scale was imaged with a Screenscan® microfiche scanner attached to a microfiche reader. The scanned images are saved in a tagged image file format (.tiff) with 8-bit depth sized at 3352 x 4425 pixels (14.5 Mb files). Images were saved to the ADF&G local area network and backed up to a remote server daily. Each image was given a unique file name that includes the age, sex, and size sample file name, scale slide or page number, scale fish number, sex, and age as originally assigned. File names include the year, harvest type, gear, and a location code.

Scale images were measured using Image-Pro® Plus Software and a fish scale measurement macro. Measurements were made on a measurement axis perpendicular to the reference line approximately through the scale focus (Fig. 2). Each annulus was marked on the scale by the reader in the image analysis macro.

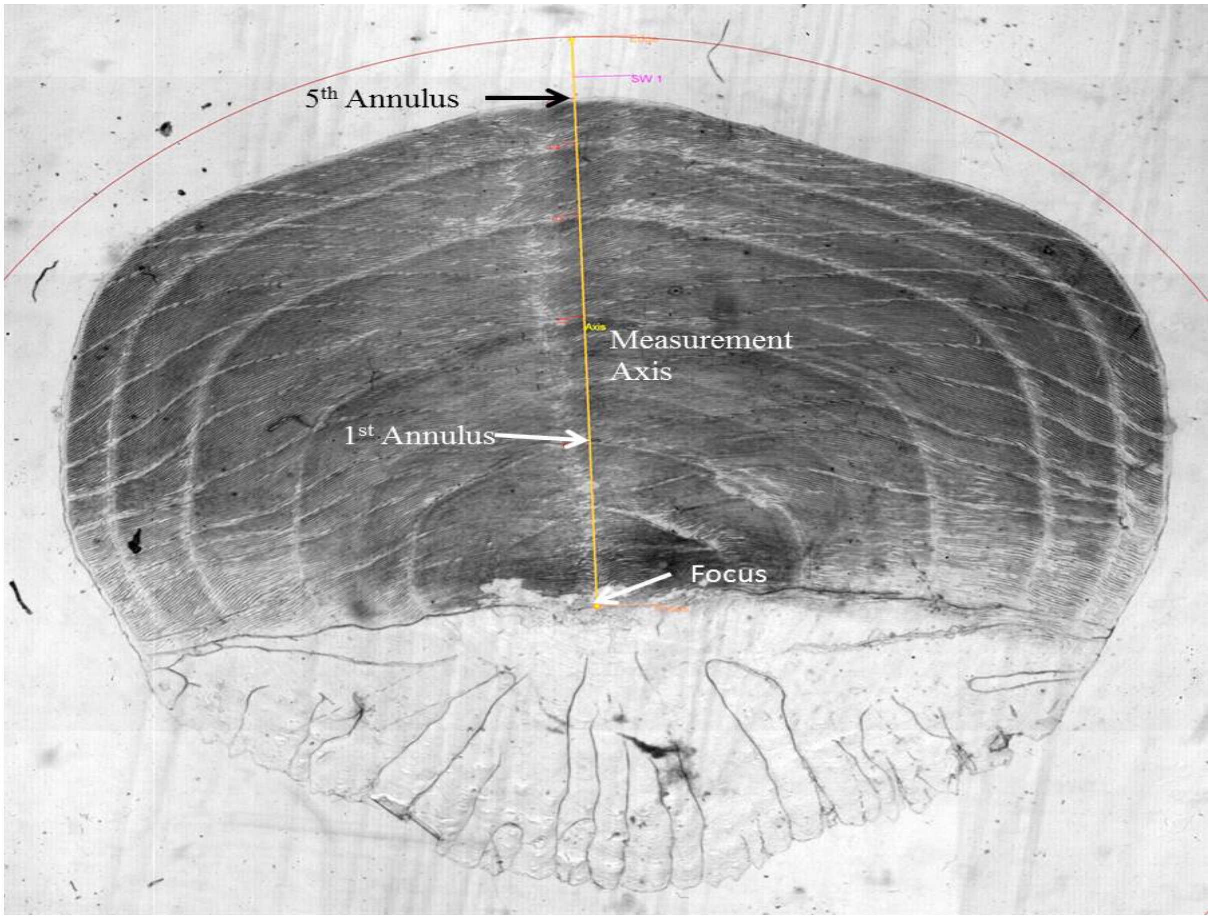

FIGURE 2–EXAMPLE OF A PACIFIC HERRING SCALE WITH MEASUREMENT OVERLAY FROM IMAGE ANALYSIS SOFTWARE

The focus, measurement axis, and each annulus are marked the scale. The number of annuli and the spacing between annuli were exported to a text file with the unique image name, date and time the measurement occurred, and reader initials. The text file data were imported into Excel (Microsoft Corporation, Redmond, WA) for analysis in Excel and SAS (SAS Institute, Inc., Cary North Carolina; Environmental Systems Research Institute, Inc., Redlands, California).

Quality Control

Scale measurements were examined for outliers by (1) filtering in Excel to examine the range of measurements and (2) using SAS Proc SGPlot to make scatter plots and box plots of each annular measurement by age, sex, and year. Scales identified as outliers in box plots were measured a second time. The reader was not informed that these scales had been measured previously to reduce the possibility of a different process being followed for the second measurements. Paired measurements by age and scale annulus (14 combinations) were examined with SAS Proc Univariate and quantile-quantile plots. Because the differences between paired data for each age and scale annulus were mostly non-normally distributed, a Wilcoxon signed-rank test (Zar 1996) was used to test the null hypothesis that the median difference between paired observations is zero.

Precision Test

The precision of measurements was evaluated by randomly selecting 101 scales from fish aged 4, 5, and 6 to measure a second time. As previously discussed for the outliers that were re-measured, the reader was not informed that these scales had been measured previously to reduce the possibility of a different process being followed for the second measurements. Paired measurements were examined with SAS Proc Reg to calculate a linear regression model for each scale growth annulus (1 through 6) with ages and sexes combined. Regression models were also examined for individual ages (4, 5, or 6) with the sexes combined.

Growth Correlations

Herring scale annular measurements, historical herring biomass, and historical herring recruitment estimates were tested for normality with quantile-quantile plots in SAS Proc Univariate. Pearson product-moment correlation coefficients (Zar 1996) were calculated between herring scale annular measurements for ages 4–6 (scale growth annuli in the same growth year) and the natural log of herring biomass using SAS Proc Corr. Historical adult herring biomass (biomass recruited to the spawning population) and recruitment data are from an ADF&G age structured model (Steve Moffitt, Alaska Department of Fish and Game, unpublished data). Adult herring biomass data were natural log transformed to normalize the data prior to using it in correlation analysis.

RESULTS

Scale Images and Measurements

A total of 7,270 Pacific herring scales were imaged and measured (Table 1). The sample size averaged >35 fish by age, sex, and year and >30 fish for 88% of the 136 age, sex, and year combinations (Appendix A.1).

TABLE 1–COUNT OF PACIFIC HERRING SCALES IMAGED AND MEASURED FOR ANNULAR GROWTH INCREMENTS FOR HERRING AGED 4, 5, AND 6 CAPTURED IN 1982, 1983, AND 1986–2015. F=FEMALE, M=MALE.

	Age 4			Age 5			Age 6			Totals		
	F	M	Total	F	M	Total	F	M	Total	F	M	Total
Total	1,212	1,231	2,443	1,224	1,203	2,427	1,183	1,217	2,400	3,619	3,651	7,270
Average	37	37	74	37	36	74	36	37	73	110	111	220
Minimum	8	7	20	16	18	42	12	20	33	83	88	179
Maximum	43	43	83	47	46	87	42	43	85	123	126	249

An additional 3,300 scales were imaged, but not measured because either (1) the reader did not agree with the originally assigned age or (2) the scale had morphological problems that made it unlikely to work for measuring.

Quality Control

Examination of scatter plots and box plots located 57 scales with measurements that were outliers for one or more annular measurement. All of these scales were imaged and measured a second time. Examination of the paired measurements with SAS Proc Univariate quantile-quantile plots indicated the differences between the measurements were not normally distributed. Only 1 of the 14 combinations of age and scale growth increment was significantly different at a $P<0.05$ (Table 2).

TABLE 2–WILCOXON SIGNED-RANK TESTS OF PAIRED MEASUREMENTS OF SCALES MARKED AS OUTLIERS.

Scale		Age		
Increment		4	5	6
First	N	22	22	13
	P	0.7181	0.1256	0.8926
Second	N	22	22	13
	P	0.7897	0.8385	0.1226
Third	N	22	22	13
	P	0.4172	**0.0466**	0.8926
Fourth	N	22	22	13
	P	0.0544	0.3669	0.2104
Fifth	N	NA	21	13
	P	NA	0.920	0.4973

N = sample size and P= probability that the median of the difference between paired measurements is zero. Bold notes significance at P < 0.05.

Precision Test

All linear regression models of paired measurements of scale annuli (ages 4–6 combined) to test the precision were highly significant (Table 3).

TABLE 3–RESULTS OF LINEAR REGRESSION MODELS OF PAIRED MEASUREMENTS OF RANDOMLY SELECTED HERRING SCALES AGED 4-6 COMBINED.

Annular Increment	Observations	R^2	F Value	Pr > F
First	101	0.94	1443	<.0001
Second	101	0.96	2661	<.0001
Third	101	0.91	1039	<.0001
Fourth	101	0.94	1510	<.0001
Fifth	81	0.91	778	<.0001
Sixth	40	0.92	434	<.0001

Growth Correlations

For herring aged 4–6 the first-year scale growth was significantly positively correlated within the same growth year (Table 4). However, the natural log of adult herring biomass estimates and first-year herring scale growth were negatively correlated, but the correlations were not significant (Fig. 3). Herring recruitment data were not used in this analysis because they were not sufficiently normalized by a log transformation.

TABLE 4–PEARSON CORRELATION COEFFICIENTS OF THE FIRST YEAR OF HERRING SCALE GROWTH BY AGE (SEXES COMBINED) AND THE LOG OF AGE STRUCTURED MODEL ESTIMATES OF ADULT HERRING BIOMASS FOR GROWTH YEARS 1976-2011.

	Prob > |r| under H_0: Rho=0			
	Age 4 Growth	Age 5 Growth	Age 6 Growth	Log(Biomass) metric tons
Age 4 Growth	1.0			
Age 5 Growth	0.893 <.0001	1.0		
Age 6 Growth	0.820 <.0001	0.796 <.0001	1.0	
Log(Biomass) metric tons	-0.244 0.201	-0.229 0.233	-0.165 0.391	1.0

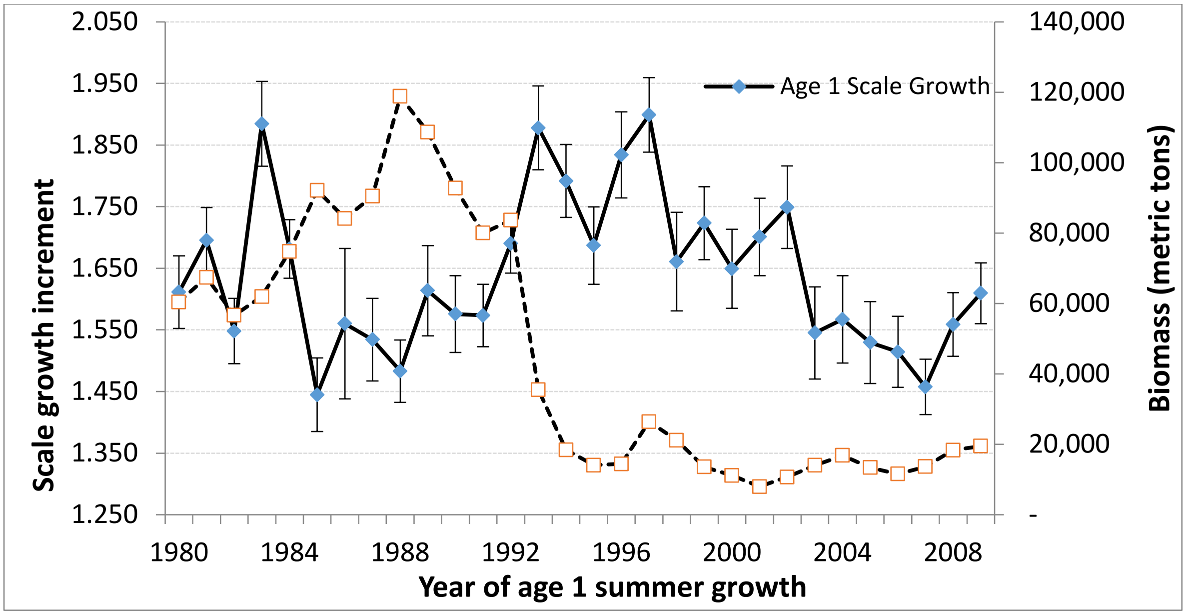

FIGURE 3–BIOMASS, AVERAGE SCALE GROWTH ANNULI (MM) AND 95% CONFIDENCE INTERVALS FOR THE FIRST YEAR OF GROWTH OF AGE-6 PACIFIC HERRING CAPTURED IN EARLY SPRING IN PRINCE WILLIAM SOUND, ALASKA FOR GROWTH YEARS 1980–2009.

DISCUSSION

Scale Images and Measurements

The original objectives of this study were focused on developing appropriate criteria and techniques to image and measure as many herring scales as possible in a short time period. The program also limited its scope to three age classes to help ensure measuring a sufficient number of scales each year to allow meaningful assessment of changes in growth among years. A much smaller sample size could be used to generate a defensible age composition for a season, but the ability to measure 30 or more useable scales for multiple age classes within a year requires much larger sample sizes. In this project ~31% of the randomly selected scales were not measured because the reader did not agree with the original age assigned or the scale had morphological issues that made it unusable. Many of these scales may still be useable after reassessment of the age. However, the high rejection rate suggests that a review of scale collection and age training and in-season assessment should be conducted.

Quality Control

Examination of the statistical outliers paired with data from a second measurement showed no significant difference when tested with combined age and sex data for each scale annulus. This suggests that most of the outliers were natural biological outliers and not an indication of differences in the way the paired measurements were generated, e.g., choosing a different axis line to measure along. However, testing by individual ages may have detected differences in future assessments. Because no differences were found in 13 of 14 age-scale annuli combinations, the statistical outliers were not removed from the production data.

Precision Test

It was reassuring that the precision assessments provided highly significant relationship between the paired measurements. Only one reader worked on this project, so no between reader comparisons were possible. In future programs to measure significant numbers of scales, the precision assessment should be part of the initial training and conducted with smaller sample sizes (30 to 50) multiple times during the project; however, it will be difficult to make second measurements blind to the reader.

Growth Correlations

The scale growth correlations for fish collected at different ages from the same brood year is again reassuring. The variability in growth shown in Figure 3 would suggest there should be some environmental variable that could at least act as a proxy to the sum of variables that affect scale growth for larval fish. Batten et al. (2016) found that July and August water temperature has a significant positive correlation with first-year scale growth (except in the warmest years) using scale growth data from this study. They also found a strong relationship between Continuous Plankton Recorder diatom anomalies and first-year herring scale growth that extended into the warmest years. These results provide more evidence that longitudinal growth histories can provide value in untangling the way that herring scale growth is influenced by environmental conditions.

CONCLUSIONS

The major objective of this study was to build a defensible, retrospective, longitudinal history of scale growth for Pacific herring in the Prince William Sound Management Area. The scale growth history data could then be used along with other environmental and biological data to determine possible influences on herring scale growth. Significant progress was made in developing the growth history dataset, conducting quality control, and assessing the precision of the scale increment measurements. Additionally, the dataset has already been used to evaluate some environmental and biological data.

ACKNOWLEDGEMENTS

Ron Andersen helped develop the procedures used in Cordova and completed all the scale imaging and measuring work. The project would not have been possible without Ron's diligent, hard work. I would also like to thank staff at the Statewide Mark Tag and Age Lab (MTA) in Juneau. Tim Frawley helped us with the process to use the measurement macro tool designed for salmon to get the measurements we needed from herring. Lorna Wilson and Detlef Buettner shared their expertise about the equipment and procedures developed at the MTA Lab and knowledge about herring scale age interpretation. The views expressed here are our own and do not necessarily represent those of the *Exxon Valdez* Oil Spill Trustee Council.

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APPENDIX A–COUNT OF PRINCE WILLIAM SOUND PACIFIC HERRING SCALES THAT WERE IMAGED AND GROWTH ANNULI MEASURED BY YEAR. NO SCALES WERE MEASURED FOR 1984.

	Age 4			Age 5			Age 6			Totals		
Year	F	M	Total	F	M	Total	F	M	Total	F	M	Total
1982	26	35	61	34	35	69	34	35	69	94	105	199
1983	29	37	66	16	26	42	38	35	73	83	98	181
1985	40	39	79	40	38	78	40	31	71	120	108	228
1986	39	41	80	40	40	80	40	41	81	119	122	241
1987	41	40	81	41	40	81	39	41	80	121	121	242
1988	40	39	79	41	40	81	40	40	80	121	119	240
1989	41	39	80	40	40	80	40	38	78	121	117	238
1990	8	12	20	47	37	84	40	39	79	95	88	183
1991	40	43	83	29	18	47	40	41	81	109	102	211
1992	41	40	81	33	32	65	12	21	33	86	93	179
1993	43	40	83	40	39	79	25	40	65	108	119	227
1994	39	40	79	31	34	65	39	41	80	109	115	224
1995	31	40	71	41	40	81	32	36	68	104	116	220
1996	33	39	72	33	29	62	39	37	76	105	105	210
1997	40	40	80	41	37	78	37	38	75	118	115	233
1998	42	38	80	40	40	80	31	42	73	113	120	233
1999	38	41	79	39	41	80	36	42	78	113	124	237
2000	35	37	72	40	40	80	40	41	81	115	118	233
2001	39	39	78	36	39	75	34	38	72	109	116	225
2002	39	39	78	41	42	83	36	35	71	116	116	232
2003	41	40	81	39	33	72	36	40	76	116	113	229
2004	35	37	72	41	40	81	20	20	40	96	97	193
2005	41	39	80	34	34	68	42	43	85	117	116	233
2006	39	34	73	35	36	71	36	29	65	110	99	209
2007	41	40	81	42	40	82	33	31	64	116	111	227
2008	40	40	80	41	46	87	42	40	82	123	126	249
2009	35	37	72	44	34	78	28	32	60	107	103	210
2010	41	38	79	36	35	71	38	38	76	115	111	226
2011	41	41	82	29	27	56	41	31	72	111	99	210
2012	39	40	79	30	30	60	40	41	81	109	111	220
2013	40	42	82	25	36	61	40	41	81	105	119	224
2014	39	38	77	43	44	87	36	39	75	118	121	239
2015	16	7	23	42	41	83	39	40	79	97	88	185
Total	1,212	1,231	2,443	1,224	1,203	2,427	1,183	1,217	2,400	3,619	3,651	7,270
Average	37	37	74	37	36	74	36	37	73	110	111	220