1. Project Number:

19170111-D

2. Project Title:

Studies of Reproductive Maturity among Age Cohorts of Pacific Herring (*Clupea pallasii*) in Prince William Sound, Alaska

3. Principal Investigator(s) Names:

Kristen B. Gorman, Prince William Sound Science Center

4. Time Period Covered by the Report:

February 1, 2019-January 31, 2020

5. Date of Report:

March 2020

6. Project Website (if applicable):

https://pwssc.org/herring/

7. Summary of Work Performed:

Overview FY19

This is the third annual report for this project, *Studies of Reproductive Maturity among Age Cohorts of Pacific Herring (Clupea pallasii) in Prince William Sound, Alaska*, which is a component of the *Exxon Valdez* Oil Spill Trustee Council's (EVOSTC's) Prince William Sound (PWS) Herring Research and Monitoring (HRM) program. This project was the only new project that HRM initiated at the start of the current five-year funding cycle as all other projects were extensions of previous projects. The goal of this report is to provide a summary of the research accomplished over the last year (FY19).

The FY19 project began in February 2019 and was focused on continuing the progress that had been made by the project in FY18 during the second year of the study. During this second year of the study in 2018, the project had undergone significant revision from the first year of the study in FY17 based on comments by the EVOSTC Science Panel. These revisions were extensively outlined in the project's FY17 annual report and FY18 workplan. Feedback from the EVOSTC Science Panel primarily requested that the project 1) focus on direct methods of gonad maturation as the primary

test of the Bayesian Age-Structured-Assessment (BASA) model's maturity schedule, as opposed to relying on a scale growth technique, and 2) that the project focus on males as well as females in FY18 and in subsequent years. For FY19, the Science Panel requested that initial work on the scale growth library be initiated. Unlike the first two years, we refined our sampling to slightly different times of year than had been sampled in FY17 and FY18 based on what we learned in the first two years of the project.

Following the FY19 workplan, the main objectives of the study originally proposed in FY17 are fourfold: 1) Assess the seasonal timing (spring, summer, fall, and winter) that allows for determination of both previously spawned and maturing female herring, and maturing male herring, based on direct measures of gonad development to assess reproductive maturation states per age cohort of interest (ages 2-5) in PWS. The proportion of immature and mature herring per age cohort of interest can then be determined by using the information obtained on maturation states. 2) Assess inter-annual variability in the proportion of immature and mature herring per age cohort of interest in PWS collected at the optimal seasonal time as determined by Objective 1 by using direct measures of gonad maturation. 3) Couple histological analysis of gonad maturity with annual scale growth information at the individual level, within specific age cohorts of interest, to understand if scale growth patterns reflect reproductive investment. 4) Assess annual variation in herring age at maturity schedules before and after 1997 by using Alaska Department of Fish & Game's (ADF&G's) PWS herring scale image library, which allows for understanding maturity schedules of past cohorts.

The work conducted in FY19 focused on Objectives 2-4, namely assessing inter-annual variability in the proportion of immature and mature PWS herring per age cohort of interest (ages 2-5) collected at the optimal seasonal time as determined by Objective 1. Methods for determining the proportion of immature and mature herring at the optimal seasonal time in FY19 followed those employed in FY17 and FY18. The work on Objective 3 is coupling histology results with annual scale growth information at the individual level, within specific age cohorts of interest, to understand if scale growth patterns reflect reproductive investment. The work on Objective 4 focused on evaluating the potential of the scale technique for estimating past maturity schedules using ADF&G's PWS herring scale image library. This analysis examined the progression of bimodal distributions in scale growth as a cohort of herring passes through time. The prediction being that if scale growth is related to investment in reproduction, then the frequency of fish showing reduced scale growth should increase as a cohort of fish matures over time from age-1 through age-6. This analysis was presented as part of the HRM synthesis report submitted in December 2019. The results for this analysis are presented below.

Progress FY19

For FY19 field work, the project obtained an annual collection permit through ADF&G (CF-19-036). The Prince William Sound Science Center's (PWSSC's) Institutional Animal Care and Use Committee protocol (PWSSC2018-02) remained in effect during FY19. Collection reports for FY19 research were submitted to ADF&G in January 2020.

In FY19, the third year of this project, one of the main goals was to begin annual sampling during an "optimal" time (i.e., spring, summer, fall, or early winter) when evidence of both past reproduction (based on the presence of post-ovulatory follicles [POFs]) and future reproduction (based on elevated gonadosomatic indices (GSI) and Hjort criteria) would be discernable based on results from

FY17 and FY18. Results from FY17-FY18 indicate that detecting past and future spawning effort cannot be determined at the same time period (see FY17 and FY18 annual reports). Post-ovulatory follicles (POFs) were observed among female herring collected as part of this project in July 2018, but POFs were not observed among females collected in September 2017/18, or November 2017 (no histology was conducted on herring ovaries collected in November 2018). Elevated GSI and Hjort indices were only observed among herring collected in November 2017/18 and April 2017/18. Thus, for FY19, sample collection was planned only for early summer (June) to evaluate evidence of past spawning, and also early winter (November) and early spring (February/March) to evaluate future reproduction. To this end, between late May and early July 2019, this project conducted three separate field trips to collect herring in PWS to obtain direct measurements of gonad maturation, age and annual growth information from scales. During this time, the project collected and processed more than 600 fish. On the first cruise between May 28-31, 2019, the field crew was unable to collect any herring even after surveying significant areas between Gravina, Montague Strait, Chenega Island, Main Bay, Glacier Island, Valdez Arm, Port Fidalgo, and Simpson Bay. The incredible lack of herring in PWS at this time was surprising. However, an earlier collection effort by PWSSC researchers to collect adult herring in spring 2019 proved extremely difficult due to a general lack of fish (M.A. Bishop, project 19160111-B, pers. comm.). This is not the first time this project has had trouble finding adult herring in PWS during early summer. During the first year of the project, FY17, field crews were unable to find adult herring to collect in June. The current extremely low biomass of PWS herring, and the fact that acoustic tracking data suggest adults move out of PWS during the summer (M.A. Bishop, project 19160111-B, pers. comm.), makes it difficult to find fish during this time period. That said, this project did collect a significant number of adults in June and July 2018. Fortunately, in mid-June 2019 (June 13-14), field crews collected 300 adult (older than age-1) herring off of Gravina Point (Red Head/Knowles Head) using a gillnet with aerial survey support. Additionally, 345 adult herring were caught in early July (July 4-8) off Double Bay/Middle Ground Shoal, Port Etches, and Zaikoff Bay. Gulf Watch Alaska investigator, Mayumi Arimitsu (U.S. Geological Survey), provided some of these fish (~60 fish from Middle Ground Shoal and Port Etches, and ~130 fish from Zaikoff Bay) collected during her group's acoustic survey of forage fish in July 2019. An additional 155 fish were collected by this project's field crew in early July at both Middle Ground Shoal and Port Etches given the success in catching fish at these locations by M. Arimitsu's crew. All weight, length, gonad weight, and ovary biopsies for histology were processed immediately after collection. The project's research assistant (K. Jurica, PWSSC) completed mounting scales for aging and growth measurements from these fish in fall 2019. The age and scale growth data take several months to produce from collections of several hundred fish. These age data are complete, but scale growth measurements for fish collected in summer 2019 is ongoing in the lab. Field collections proposed in the FY19 workplan for November 2019 were not completed, see response to Science Panel comments in Section 10 below. In FY19, the project focused in completing the summer 2019 dataset for aging, scale growth, and histology, as well as the preliminary assessment of the scale growth library data.

Results

The purpose of the analysis is to assess if there is evidence for bimodal distributions of herring scale growth as individual cohorts of herring move through time from age-1 through age-6. The analysis reported here considers two cohorts of PWS herring before and after the 1997 break point as

estimated by the BASA. The basic prediction is that unimodal scale growth distributions would be expected for age-1 and age-2 herring. As herring mature, bimodal distributions are expected for ages 3-5. By age-6, unimodal scale growth distributions are expected as all fish should be recruited by this age. This idea was tested using ADF&G's scale growth library (Moffitt 2017). Because the analysis does not include any information on scale growth changes directly in relation to known spawning activity, i.e., histology, the analysis here is meant to explore the scale growth library to understand bimodal patterns in scale growth as a single cohort of fish matures over time. At this point, results cannot be used to say anything specific about direct spawning activity, but simply the presence or absence of bimodal patterns in scale growth for specific cohorts of PWS herring.

Four cohorts of PWS Pacific herring were considered in analyses – 1984, 1988, 1999, and 2005 representing two cohorts before and after the 1997 breakpoint as estimated by the BASA. Scale growth information for these cohorts from age-1 through age-6 were obtained from ADF&G's scale growth library (Moffitt 2017). Data were collated from age-4, age-5, and age-6 fish, respectively, from the years 1988-1990, 1992-1994, 2003-2005, and 2009-2011 to produce each cohort's dataset. Histogram and density plots were created for males and females separately from each cohort to help identify which age group might be predicted to show bimodal distributions in scale growth. Additionally, a Gaussian mixture model was used to detect bimodal distributions in annual scale growth using the *mixtools* package (Benaglia et al. 2009) in the R language environment (R Core Team 2018). The *mclust* package (Scrucca et al. 2016), also implemented in R, was used to conduct likelihood ratio tests and Bayesian clustering analysis of the mixture models produced by the *mixtools* package.

Histograms, density plots, and mixture model results are show in Figs. 1-8 for female and male cohorts of Pacific herring for 1984, 1988, 1999, and 2005, respectively. Sample sizes for each age group are noted in histogram and density plots. Sample sizes specific to the Bayesian clustering analysis are noted for each Gaussian mixture model plot.

Density plots suggest that age-5 female herring in 1988 might be composed of two groups based on scale growth due to the apparent bimodal distribution (Fig. 1). Gaussian mixture model results indicate that females age-4 to age-6 show bimodal distributions in scale growth based on likelihood ratio tests (LRT: $p \leq 0.05$). Bayesian clustering analysis indicated that only age-5 females in this cohort can be distinguished into two groups based on scale growth (Fig. 1).

Density plots suggested that age-3 male herring in 1986 might be composed of two groups based on bimodal scale growth (Fig. 2). Likelihood ratio tests were significant for male herring age-3 to age-5 in the 1984 cohort (LRT: $p \leq 0.05$). Bayesian clustering analysis only distinguished age-4 and age-5 males as having bimodal scale growth. However, the sample sizes in each group show only a small number of males belonging to groups with larger scale growth (i.e., 1 or 2 individuals) (Fig. 2).

Density plots did not show any obvious sign of bimodal distributions in scale growth, with the possible exception of age-4 females from the 1988 cohort (Fig. 3). Likelihood ratio tests were all non-significant except for age-4 females (LRT: $p = 0.5$). However, the Bayesian clustering analysis detected no bimodal distributions for any age class in the 1988 female cohort.

Density plots suggested that age-2 male herring from the 1988 cohort might show bimodal distributions in scale growth (Fig. 4). Likelihood ratio tests and Bayesian clustering analysis did not detect bimodal distributions in scale growth for any age class of males from the 1988 cohort (Fig. 3).

Density plots for female Pacific herring from the 1999 cohort did not obviously suggest any group might show bimodal scale growth distributions, possibly with the exception of age-6 female herring from 2004 (Fig. 5). The likelihood ratio test for age-6 female herring was significant, indicating bimodal scale growth. However, the Bayesian cluster analysis grouped all ages into one group for females of the 1999 cohort (Fig. 5).

Density plots did not obviously suggest any age group of male herring from the 1999 cohort might show bimodal scale growth distributions (Fig. 6). Likelihood ratio tests were significant ($p < 0.05$) for age-1 and age-6 male herring, which was also confirmed by the Bayesian clustering analysis (Fig. 6).

Density plots suggested that age-3 and age-4 female herring from the 2005 cohort might show bimodal distributions in scale growth (Fig. 7). Likelihood ratio tests and Bayesian clustering analysis were only significant for age-6 female herring in 2010 (Fig. 7).

Density plots appeared to indicate that age-2 male herring from the 2005 cohort might show bimodal distributions in scale growth (Fig. 8). The likelihood ratio test was significant ($p< 0.05$) for age-1 male herring in 2005 only, but this result was not supported by the Bayesian clustering analysis. All other age groups showed unimodal scale growth patterns (Fig. 8).

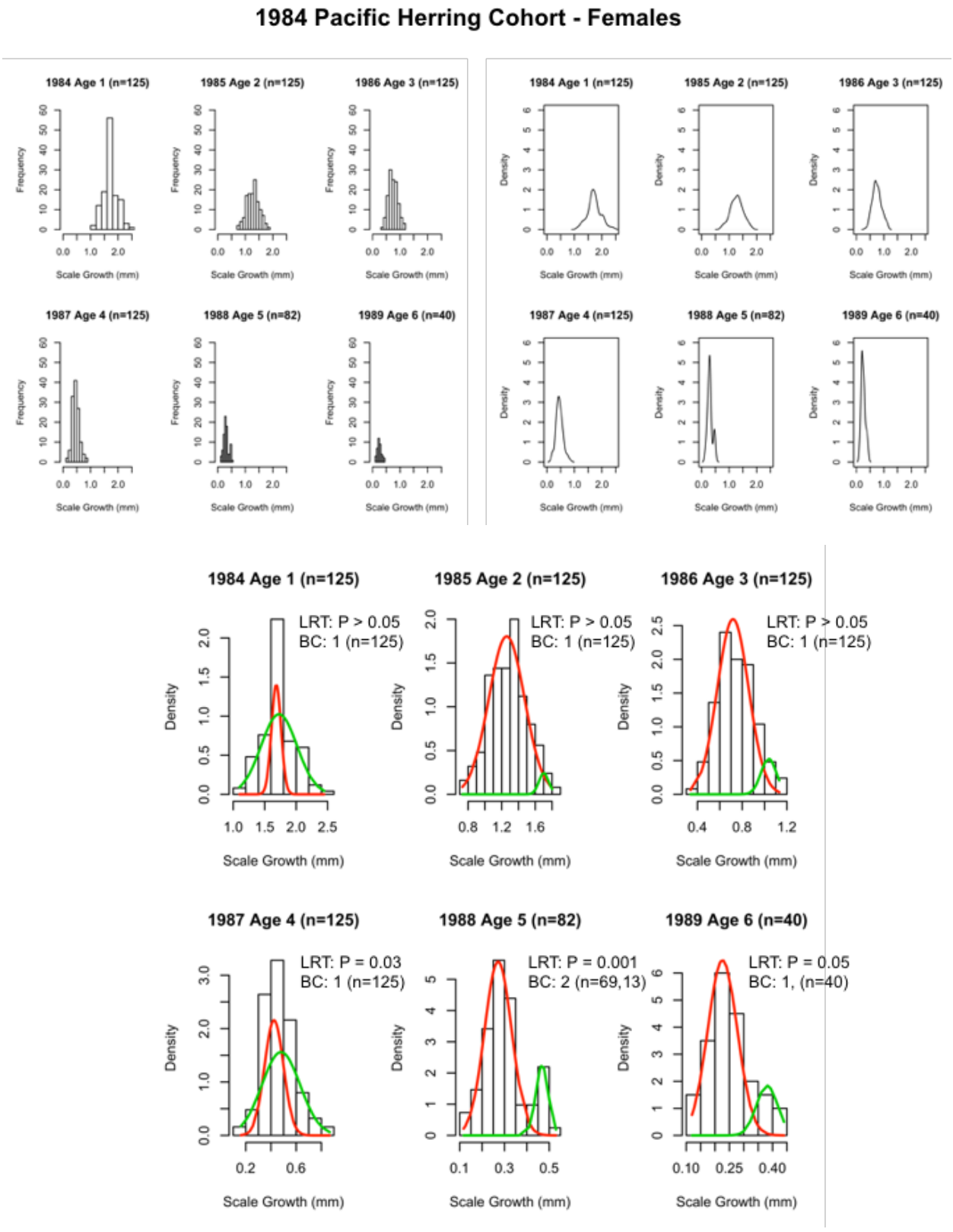

Figure 1. Scale growth histograms, density plots, and Gaussian mixture models results for female Pacific herring of the 1984 cohort in Prince William Sound, AK.

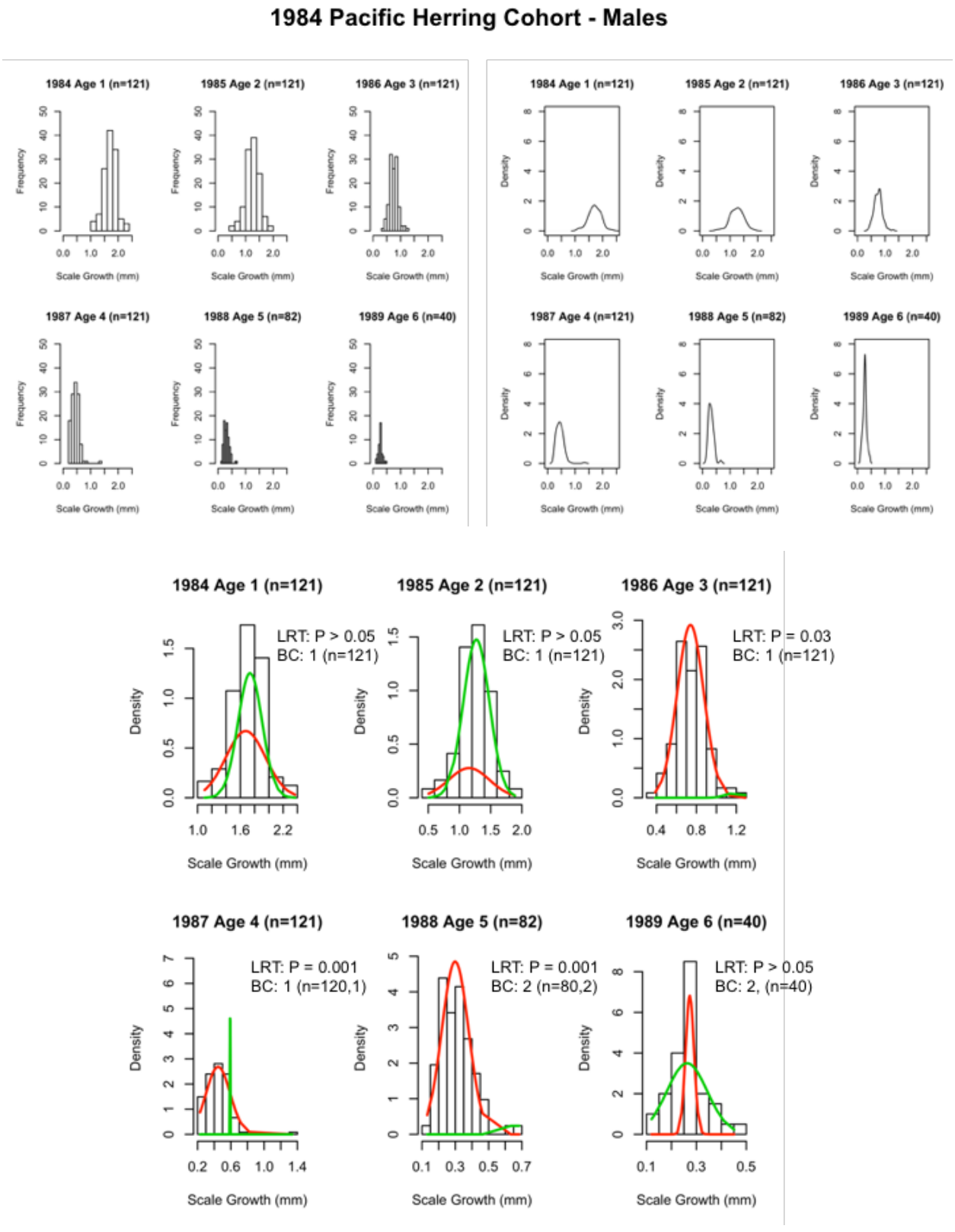

Figure 2. Scale growth histograms, density plots, and Gaussian mixture models results for male Pacific herring of the 1984 cohort in Prince William Sound, AK.

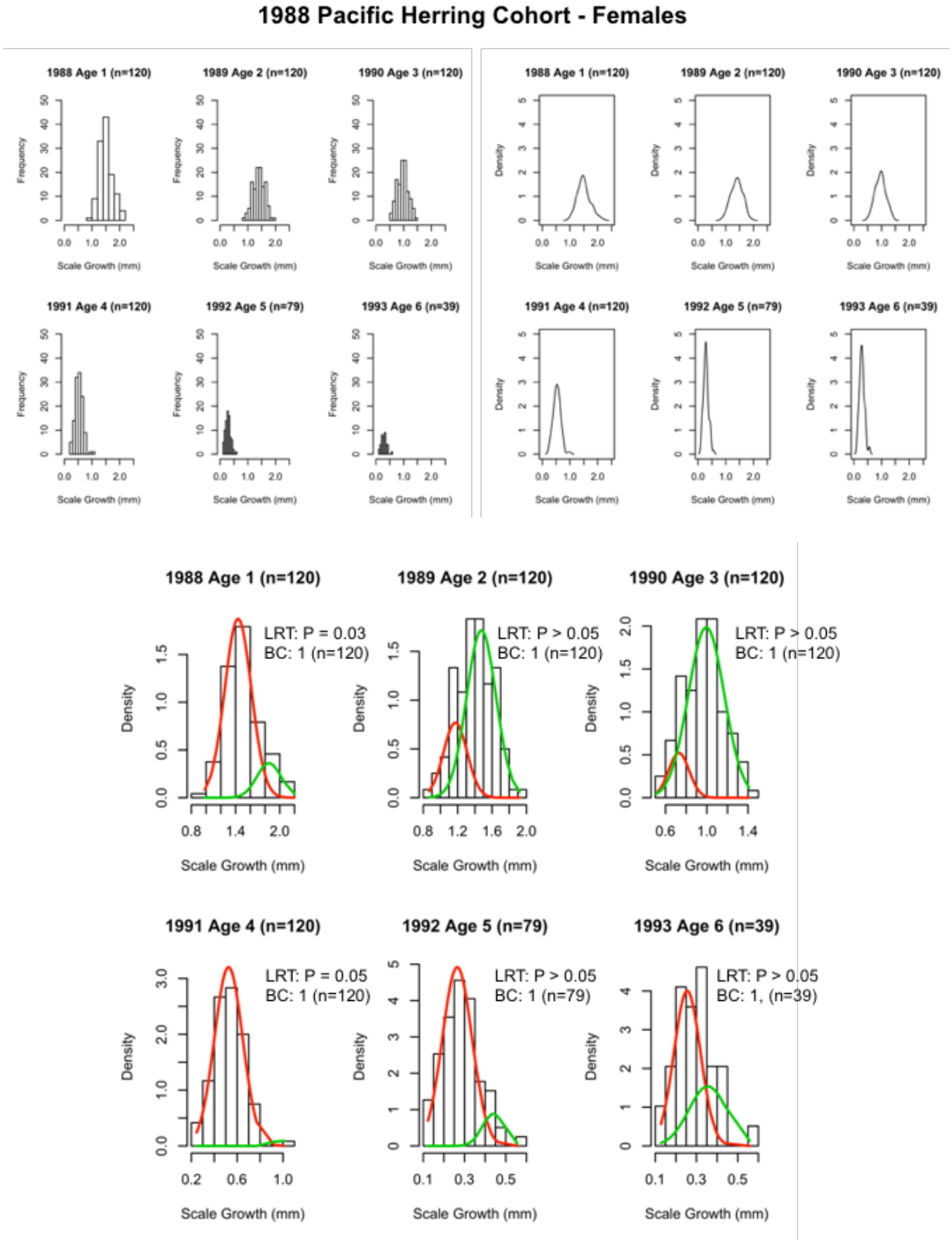

Figure 3. Scale growth histograms, density plots, and Gaussian mixture models results for female Pacific herring of the 1988 cohort in Prince William Sound, AK.

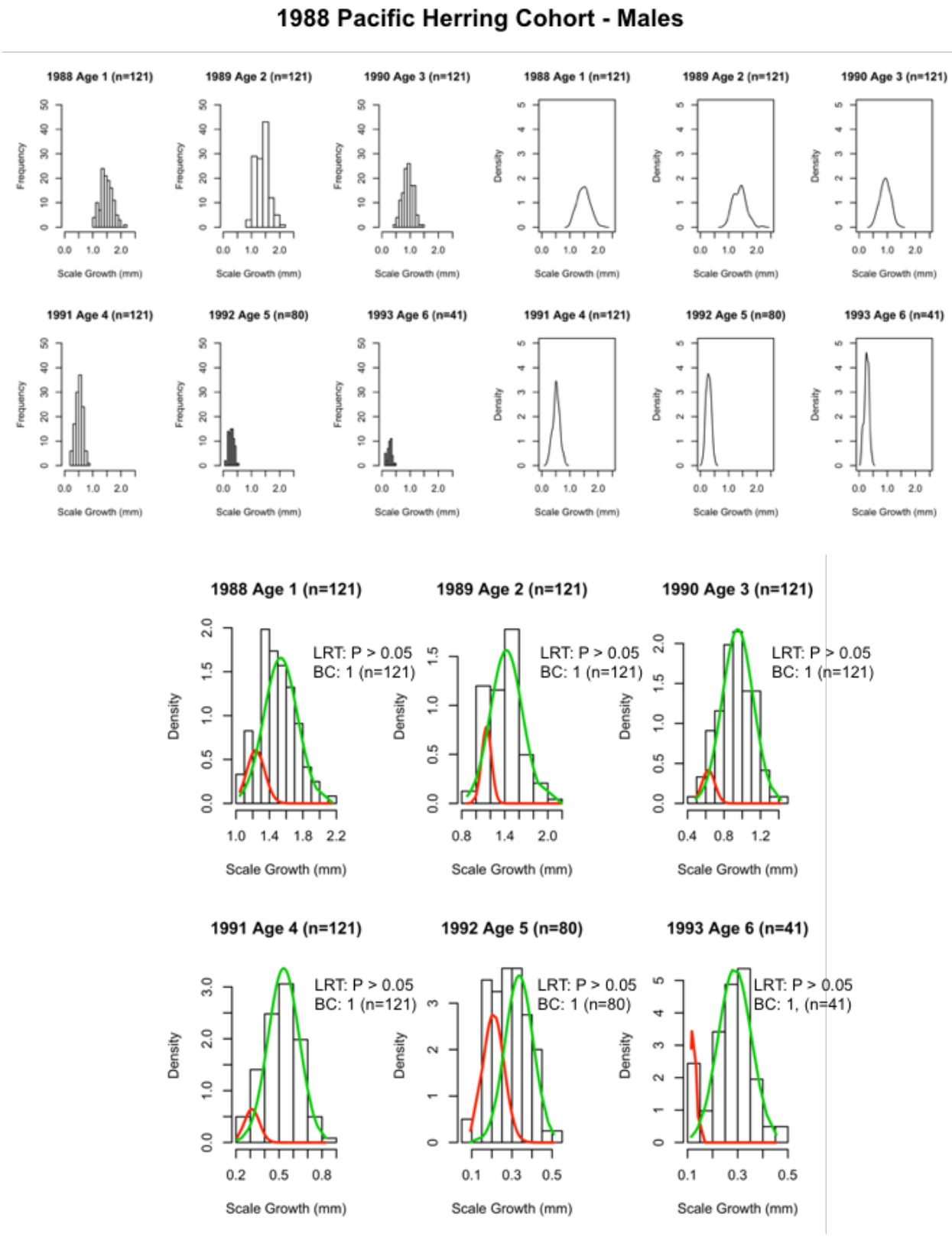

Figure 4. Scale growth histograms, density plots, and Gaussian mixture models results for male Pacific herring of the 1988 cohort in Prince William Sound, AK.

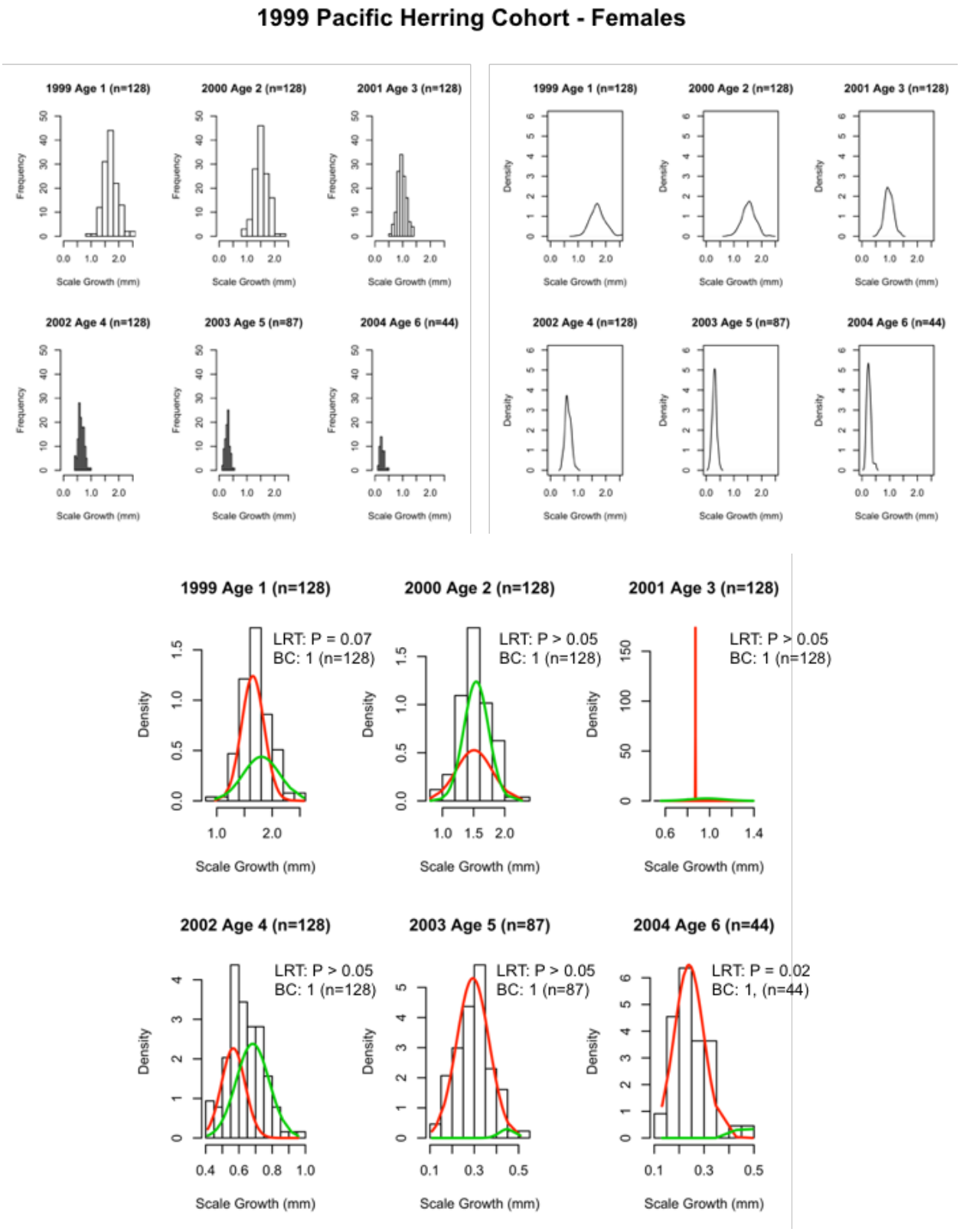

Figure 5. Scale growth histograms, density plots, and Gaussian mixture models results for female Pacific herring of the 1999 cohort in Prince William Sound, AK.

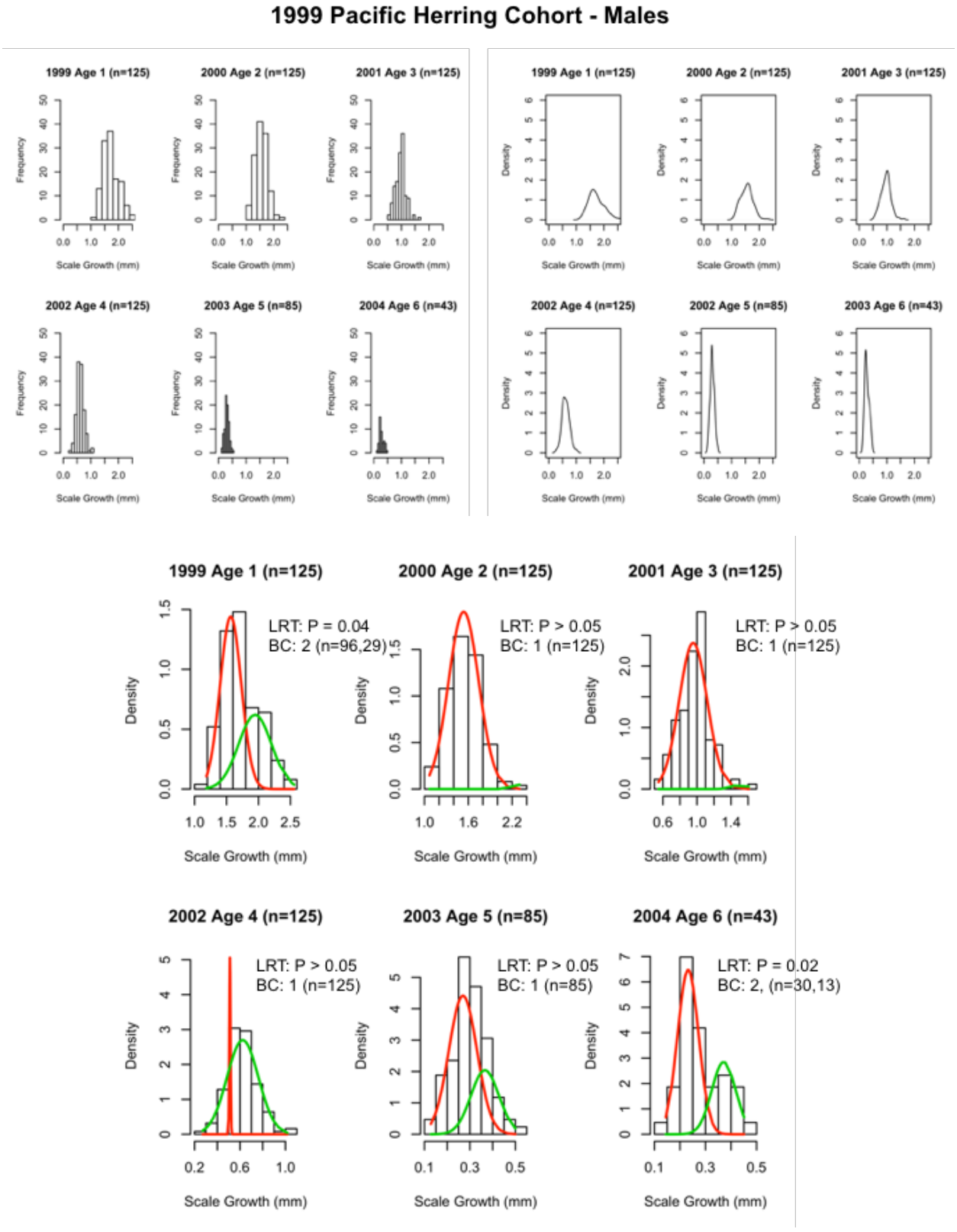

Figure 6. Scale growth histograms, density plots, and Gaussian mixture models results for male Pacific herring of the 1999 cohort in Prince William Sound, AK.

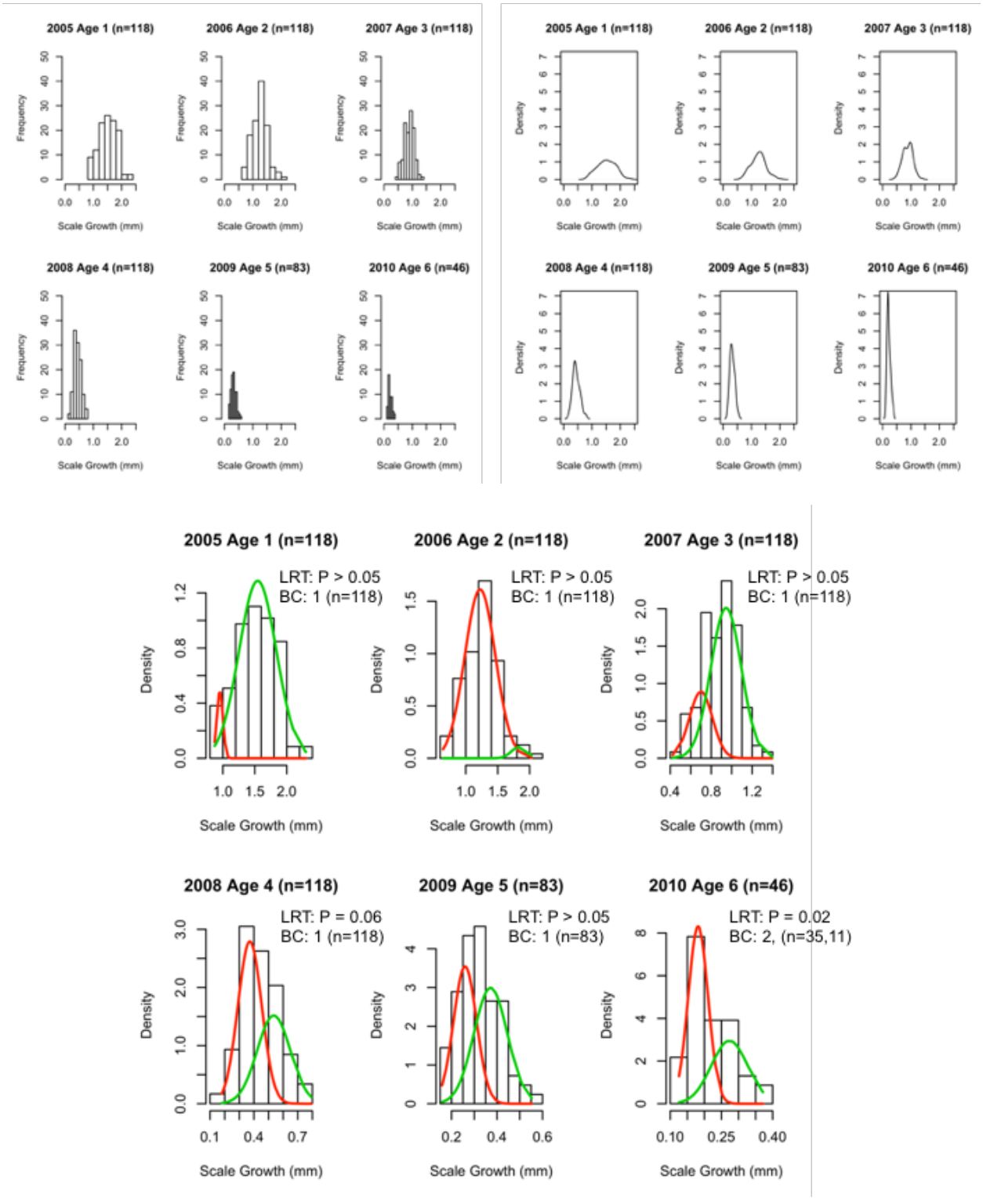

Figure 7. Scale growth histograms, density plots, and Gaussian mixture models results for female Pacific herring of the 2005 cohort in Prince William Sound, AK.

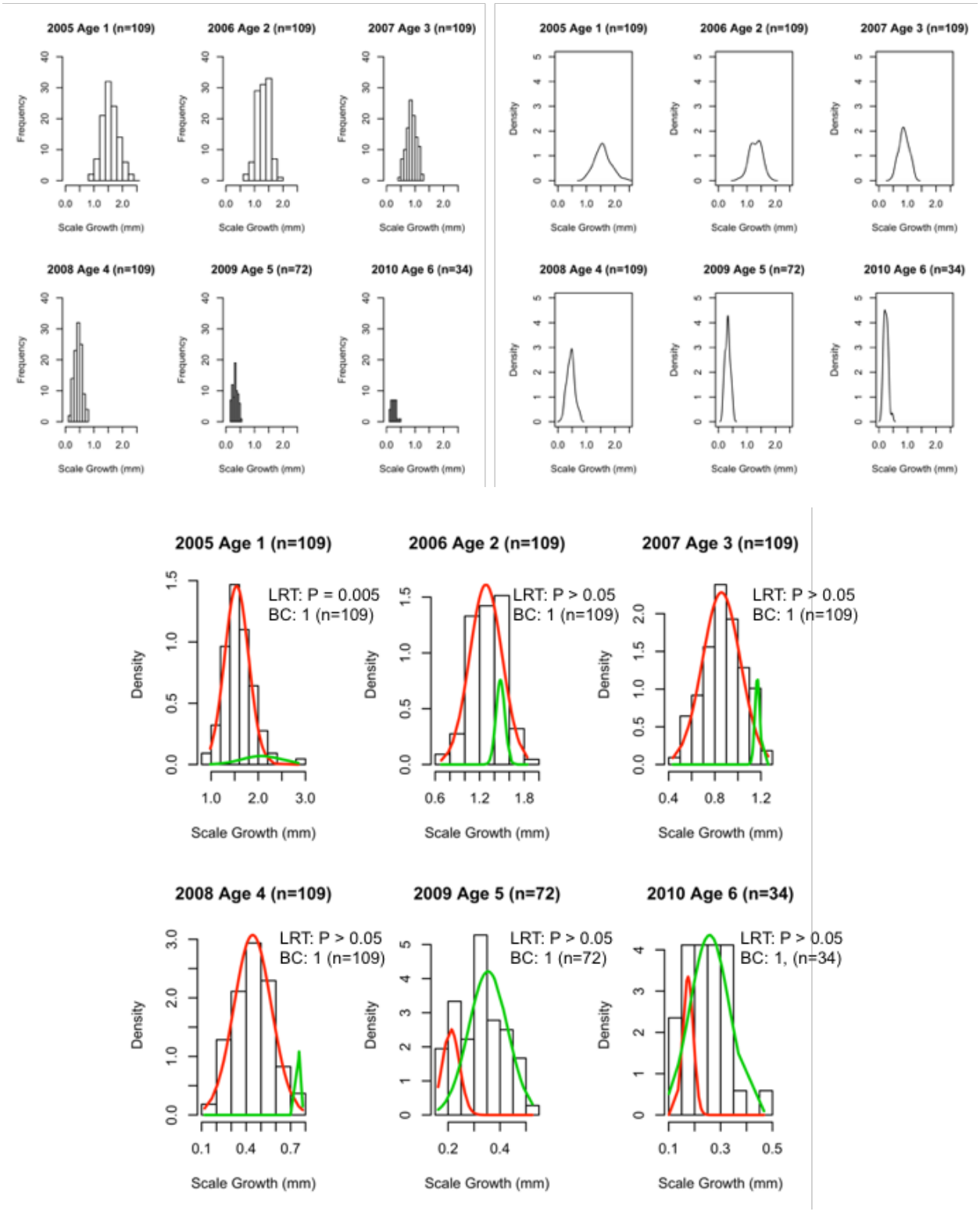

Figure 8. Scale growth histograms, density plots, and Gaussian mixture models results for male Pacific herring of the 2005 cohort in Prince William Sound, AK.

This preliminary analysis demonstrates some evidence for bimodal distributions in scale growth of specific cohorts of PWS herring as they mature over time. Of interest is the fact that bimodal distributions were detected for both female and male herring. There was no strong evidence that younger fish show unimodal distributions in scale growth that then diverge into bimodal distributions in older fish as bimodal distributions in scale growth were detected in several age-1 groups (1988 females, 1999 males, and 2005 males). Age-2 groups did not exhibit any evidence of bimodal distributions in the dataset. Only one age-3 group had evidence of bimodal scale growth distributions (1986 males). Several age-4 groups showed bimodal distributions for scale growth (1987 females and males, and 1991 females), as well as age-5 and age-6 groups (age-5: 1988 females and males; age-6: 1989 females, 2004 females and males, 2010 females). Clearly, this analysis indicates the presence of bimodal distributions in scale growth for PWS Pacific herring, which confirms the conclusion by Vollenweider et al. (2017). It would be useful to increase the sample sizes of scale growth measurements for future analysis for all age groups.

8. Coordination/Collaboration:

A. Long-term Monitoring and Research Program Projects

1. Within the Program

In FY 2019, the project coordinated with ADF&G's age, sex, and length project (19160111-F) to obtain scale growth library data. In previous years since FY17, the project coordinated with HRM projects 19160111-F (herring age, sex, length project), 19160111-B (herring tagging project), and 19120111-C (BASA modeling project).

2. Across Programs

a. Gulf Watch Alaska

In FY19, the project contributed to the Gulf Watch Alaska synthesis report chapter on the synchronous collapse of forage species during the marine heatwave. In past years this project has coordinated with several Gulf Watch Alaska Pelagic component projects (18120114-C [forage fish], 18120114-E [wintering marine birds], and 18120114-O [humpback whales]).

b. Data Management

PI Gorman met with data coordinator Stacey Buckelew at the October 2019 PI meeting in Homer. Data management is on schedule.

B. Individual Projects

None

C. With Trustee or Management Agencies

The project has coordinated with ADF&G regarding fieldwork and existing data for analysis.

9. Information and Data Transfer:

A. Publications Produced During the Reporting Period

1. Peer-reviewed Publications

None. Two in preparation for spring 2020 (juvenile energetics, and first 2 years of age at maturity data).

2. Reports

Arimitsu, M., J. Piatt, R.M. Suryan, S. Batten, M.A. Bishop, R.W. Campbell, H. Coletti, D. Cushing, K. Gorman, S. Hatch, S. Haught, R.R. Hopcroft, K.J. Kuletz, C. Marsteller, C. McKinstry, D. McGowan, J. Moran, R.S. Pegau, A. Schaefer, S. Schoen, J. Straley, and V.R. von Biela. 2019. Chapter 3 Synchronous collapse of forage species disrupts trophic transfer during a prolonged marine heatwave. In M.R. Suryan, M.R. Lindeberg, and D.R. Aderhold, eds. The Pacific Marine Heatwave: Monitoring During a Major Perturbation in the Gulf of Alaska. Gulf Watch Alaska Long-Term Monitoring Program Draft Synthesis Report (*Exxon Valdez* Oil Spill Trustee Council Program 19120114). *Exxon Valdez* Oil Spill Trustee Council, Anchorage, Alaska.

Gorman, K. 2019. Studies of reproductive maturity among age cohorts of Pacific herring (*Clupea pallasii*) in Prince William Sound, Alaska. FY18 annual report to the *Exxon Valdez* Oil Spill Trustee Council, project 18170111-D. *Exxon Valdez* Oil Spill Trustee Council, Anchorage, AK.

Gorman, K., and R. Heintz. 2020. A high temporal and spatial resolution study to validate the juvenile herring condition monitoring project. Final report for *Exxon Valdez* Oil Spill Restoration Project 12120111-M. *Exxon Valdez* Oil Spill Trustee Council, Anchorage, Alaska.

Pegau, W.S. J. Trochta, K. Gorman, and T. Branch. 2019. Chapter 2 Maturity. In, W.S. Pegau and D.R. Aderhold, editors. Herring Research and Monitoring Science Synthesis. Herring Research and Monitoring Synthesis Report, (*Exxon Valdez* Oil Spill Trustee Council Program 20120111). *Exxon Valdez* Oil Spill Trustee Council, Anchorage, Alaska.

3. Popular articles

Gorman, K. 2019. Inter-annual variability in seasonal gonad development of Pacific Herring (*Clupea pallasii*) in Prince William Sound, Alaska. Delta Sound Connections. Prince William Sound Science Center (https://pwssc.org/wp-content/uploads/2019/05/DSC-2019_WEB.pdf).

B. Dates and Locations of any Conference or Workshop Presentations where EVOSTC-funded Work was Presented

1. Conferences and Workshops

None

2. Public presentations

None

C. Data and/or Information Products Developed During the Reporting Period, if Applicable

Data collected in 2019 available on the Research Workspace.

D. Data Sets and Associated Metadata that have been Uploaded to the Program's Data Portal

PWS Herring Age at Maturity dataset.

10. Response to EVOSTC Review, Recommendations and Comments:

EVOSTC Science Panel Comment. The project submitted a proposal renewal in August 2019 and despite our progress in FY18 and success in summer collections in FY19 the Science Panel decided to not fund this project further. Specifically, the Science Panel stated "Although this project has provided some useful data on herring maturity from recently samples, overall project progress has been slow. Work reported in the FY18 annual report focused on the first of four objectives, namely, assess the seasonal timing (spring, summer, fall, and winter) that allows for determination of both previously spawned and maturing female herring, and maturing male herring, based on direct measures of gonad development to assess reproductive maturation states in each age cohort of interest (ages two through five) in PWS. The annual report provides summarized data on age frequency, GSI, and a modified Hjort Index for fish collected during 2018 (only). Histology data from FY17 were received, but no analysis was presented and histology results from FY18 were not yet available. Thus, validation has not been conducted and objective 1 is not yet complete. Additionally, this project, now in its third year, does not appear to have made any progress to definitively determine whether archived scale growth measurements can be used as a proxy for reproductive status of herring".

PI Response. In response to the Science Panel's recommendation, the project PI notes:

- The project was designed as a five-year study with the first two years focused on establishing the optimal sampling time (Objective 1). There were issues with being able to collect fish in the first year as the population dropped to record low levels in response to the unprecedented Gulf of Alaska marine heatwave, which was noted extensively in the 2019 synthesis reports submitted. Further, in FY17 and FY18 we were refining our sampling approach. Thus, at the time the Science Panel decided to no longer fund the project we were effectively 1.5 years into the study, not 3 as the Science Panel noted above.
- As collection techniques were refined, we had success in collecting samples in 2018 allowing us to examine the optimal timing of histology. A second setback occurred in that the histology lab that we were using had a technician become injured and sample processing was delayed significantly. Despite that delay, we were able to establish that we could not use histology to look at past spawning beginning by late July and set our sampling effort to May/June in 2019. Using the gonad maturation state, we recognized that sampling in November could allow us to discern if a fish was likely to mature by the next year. Therefore, we feel that we were able to establish the optimal sampling timing and completed Objective one as reported in the 2019 proposal renewal. Despite the two setbacks, this objective was completed on the schedule we had originally proposed, which is not reflected by the comments provided by the Science Panel above.

- The Science Panel's comment regarding progress associated with using scales is perplexing since that work was delayed by an earlier recommendation from them in response to the FY18 renewal proposal but was entirely within the schedule we proposed in the FY19 work plan. We proposed to begin working on examining scales in FY18, and at the Science Panel's recommendations associated with the FY18 work plan we delayed that effort to focus on direct measures of maturity. The Science Panel recommended that we begin the work with scales in their comments on the FY19 work plan. In fact, this analysis was completed and reported in the HRM synthesis document submitted in December 2019. Those results are also reported below.
- Overall, the project has been very responsive to the recommendations of the Science Panel. Despite setbacks associated with very low herring populations and delays at the histology lab we have achieved our objectives on time.

This project was not funded for FY20; thus, the planned fall 2019 collection in November was not completed.

11. Budget:

EXXON VALDEZ **OIL SPILL TRUSTEE COUNCIL**
PROGRAM PROJECT BUDGET PROPOSAL AND REPORTING FORM

Budget Category:	Proposed FY 17	Proposed FY 18	Proposed FY 19	Proposed FY 20	Proposed FY 21	TOTAL PROPOSED	ACTUAL CUMULATIVE
Personnel	$71.5	$74.3	$79.4			$225.2	$ 112.7
Travel	$1.1	$1.1	$1.1			$3.3	$ 3.2
Contractual	$45.0	$44.2	$34.2			$123.4	$ 83.2
Commodities	$2.4	$1.8	$1.8			$6.0	$ 8.0
Equipment	$0.0	$0.0	$0.0			$0.0	$ 8.2
Indirect Costs (*will vary by proposer*)	$36.0	$36.4	$35.0			$107.4	$ 62.1
SUBTOTAL	$156.0	$157.8	$151.5			$465.2	$277.4
General Administration (9% of	$14.0	$14.2	$13.6	$0.0	$0.0	$41.9	N/A
PROJECT TOTAL	$170.0	$172.0	$165.1	$0.0	$0.0	$507.1	
Other Resources (Cost Share Funds)	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	

LITERATURE CITED

Benaglia, T., D. Chauveau, D. Hunter, and D. Young. 2009. mixtools: An R Package for Analyzing Finite Mixture Models. Journal of Statistical Software, University of California, Los Angeles 32:1-29.

Moffitt, S.D. 2017. Exon Valdez Lomg-Term Herring Restoration and Monitoring Program: Scales as growth history records. Exxon Valdez Long-Term Herring Research and Monitoring Final Report (Restoration Project 13120111-N), Alaska Department of Fish & Game, Cordova, Alaska.

R Core Team. 2018. R Foundation for Statistical Computing, Vienna, Austria., Vienna, Austria.

Scrucca, L., M. Fop, T.B. Murphy, and A.E. Raftery. 2016. mclust 5: clustering, classification and density estimation using Gaussian finite mixture models. The R Journal 8/1, pp. 205-233.

Vollenweider, J., J. Maselko, and R. Heintz. 2017. *Exxon Valdez* Lomg-Term Herring Restoration and Monitoring Program: What is the age at first spawning for female herring in PWS? *Exxon Valdez* Long-Term Herring Research and Monitoring Final Report (Restoration Project 13120111-J), Auke Bay Labs, Alaska Fisheries Science Center, NOAA Fisheries, Juneau, Alaska.