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

What is the Age at First Spawning for Female Herring in PWS?

Exxon Valdez Oil Spill Trustee Council Project 13120111-J
Final Report

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Study History: This project originated in response to an invitation for proposals from the *Exxon Valdez* Oil Spill Trustee Council for the request to improve predictive models of herring stocks through observation and research. One highlighted need was to test assumptions and inform the age-structure-analysis model, which is currently used by the Alaska Department of Fish and Game for estimating herring biomass. The goal of this study was to determine the age when herring first spawn in Prince William Sound. The goals of this study support the Herring Research and Monitoring Program goals by evaluating the assumption of the age-structure-analysis model that herring first spawn at age three. The predictive capabilities of current population models of herring in Prince William Sound may be improved by knowing the proportions of primiparous individuals in each age class. This number provides a means for adjusting estimates of the total post-spawning biomass in the age-structure-analysis model by estimating the proportion of each age class that was not on the spawning grounds in the previous year. Data regarding the proportions of spawners by age class would improve the accuracy of model estimates of spawning stock biomass. This multi-year project was conducted at Auke Bay Labs, Alaska Fishery Science Center, National Oceanic and Atmospheric Administration Fisheries in Juneau, Alaska between spring 2011 and 2013.

Abstract: The age at maturation and proportion of fish in each age class that spawn are integral ecological components in fish productivity and consequently key parameters in stock assessment models. The age-structure-analysis stock assessment model used by the Alaska Department of Fish and Game to manage Pacific herring (*Clupea pallasii*) in Prince William Sound includes these parameters, though to date derived estimates have not been validated in the field. We confirmed that the commonly-used technique for Norwegian spring-spawning Atlantic herring (*Clupea harengus*) of using herring scale growth to discriminate spawners from non-spawners can be applied to Pacific herring. The basis for this methodology is that herring lay down annual growth rings on their scales in proportion to their body growth and spawning fish would experience reduced growth resulting from their elevated energetic demands. We used a multi-faceted approach in which we monitored histological characteristics of post-spawning herring sequentially sampled in a laboratory setting and found that post-ovulatory follicles are the clearest evidence of spawning for 3 months after spawning. Using this schedule, we sampled herring in Prince William Sound in July 2012, 3 months after spawning to allow for maximal scale growth. Comparison of maturation from histology to scale growth showed that 3 year old herring preparing to spawn for the first time had significantly diminished growth relative to immature 3 year olds not preparing to spawn. Finally we sought to quantify the age at first spawn and subsequent frequency of skip-spawning of herring in Prince William Sound. Using a Gaussian modeling approach to detect bimodal distributions of scale growth representing spawners (low growth) versus non-spawners (high growth) in a sample of female herring from Prince William Sound spawning aggregations in 2013, we failed to detect bimodal growth distributions in any age of fish resulting from low sample size (n=234). We used the same approach with the dataset from *Exxon Valdez* Oil Spill Trustee Council project 13120111-N

(Scales as growth history records) with over 1,700 measurements of scale annuli growth from herring ages 4-6 collected from Prince William Sound spawning aggregations between 1986-2013. Scale growth of herring ages 1 and 2 were unimodal, indicating that all fish of these ages were immature and not spawning while ages 3-6 showed differentiation in growth patterns and consequently spawning activity. Results indicate a difference in skip-spawn probabilities between with females having a higher overall probability of skip-spawn (13 to >50%) than males (10-15%). Annual variability in skip-spawning probabilities was highest in ages 3 and 6. Future efforts include examination of interannual variability in spawning probability by age in relation to environmental parameters.

Key Words: age at first spawn, ovary histology, Pacific herring, primiparous, scale growth increments

Project Data: Whole, live herring were collected in Lynn Canal Southeast Alaska and cultured at Auke Bay Labs, Juneau. Fish were sacrificed and samples of ovaries were sent to external laboratories for histological characterization. In addition, wild herring were collected in Prince William Sound and similar ovary histological characterizations were made. Also from these fish, scales were mounted and assessed for age and growth increments. All data are available in Excel spreadsheets. These data have also been provided to the Alaska Ocean Observing System for dissemination and archiving.

Current Data Custodian:
Johanna Vollenweider, Auke Bay Labs, Alaska Fishery Science Center, NOAA Fisheries, 17109 Point Lena Loop Road, Juneau, Alaska 99801.

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:
https://portal.aoos.org/old/gulf-of-alaska.php#metadata/bf83ddf9-9579-4e73-99ac-f16feb744b08/project

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.

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EXECUTIVE SUMMARY

The age of maturation and proportion of fish spawning of older age classes are important ecological components in fish productivity. Additionally, these parameters in fishery stock assessment models heavily influence the estimation of biomass and consequently sustainable harvest rates. The age-structure-analysis (ASA) stock assessment model used by the Alaska Department of Fish and Game (ADF&G) to manage Pacific herring (*Clupea pallasii*) in Prince William Sound (PWS) includes age at maturity as a parameter. Currently, however, the ASA model produces estimates of maturation that have little to no validation through field sampling. The objective of this study was to determine if a method used to assess age at maturation and skip-spawning of older ages for Norwegian spring-spawning Atlantic herring (*Clupea harengus*) is valid for use with Pacific herring. The basis for this methodology is that herring lay down annual growth rings on their scales in proportion to their body growth. Reproductive investment in herring is high resulting in reduced growth of spawning fish relative to non-spawners. Therefore scales provide a spawning history for individual fish across their lifetime. \

We used a multi-faceted approach to validate the use of the scale growth method for discriminating spawners from non-spawners in Pacific herring. In a laboratory study, we sequentially sampled post-spawn herring and found that the most reliable histological characteristic of spawning was the presence of post-ovulatory follicles, which were a clear indication of recent spawning for three months after spawning. Accordingly, we sampled herring in PWS in July 2012, 3 months after the spawn. This allowed for maximal scale growth for comparison to histology. Immature 3 year old herring not developing for spawning had significantly elevated scale growth relative to 3 year olds preparing to spawn for the first time.

We also sought to quantify the age at first spawn and subsequent frequency of skip-spawning of herring in PWS. We used a Gaussian modeling approach to detect bimodal distributions of scale growth representing spawners (low growth) versus non-spawners (high growth) in a sample of female herring from PWS spawning aggregations in 2013. Examination of the 234 female herring collected from the spawn failed to detect bimodal growth distributions in any age of fish, likely resulting from low sample size. A secondary attempt to boost sample size was to use the same approach with the scale dataset from the *Exxon Valdez* Oil Spill Trustee Council project 13120111-N by S. Moffitt and R. Anderson which provided over 1,700 measurements of scale annuli growth from herring ages 4-6 collected from PWS spawning aggregations between 1986-2013. Scale growth of herring ages 1 and 2 were unimodal, indicating that all fish of these ages were immature and not spawning. In contrast, scale growth of fish ages 3-6 showed differentiation in growth patterns and consequently spawning activity. Results indicate a difference in skip-spawn probabilities between genders for ages 3 and 6, but not 4 and 5. Females had a higher overall probability of skip-spawn, ranging from 13% to upwards of 50% for ages 3, 4, 5, and 6, while males ranged from 10% to 15%. Additionally, the annual variability in skip-spawning probabilities was highest in ages 3 and 6. Future efforts include examination of interannual variability in spawning probability by age in relation to environmental parameters.

INTRODUCTION

The age of maturation is an important component of fishery stock assessment models as it heavily influences the estimation of biomass and consequently sustainable harvest rates (Reynolds et al. 2005, Clark 1991). Age at maturation incorporates the number of times an individual reproduces as well a pattern of higher fecundity and larger eggs in older fish (Hay 1985) with cascading effects on productivity. The age-structure-analysis (ASA) stock assessment model used by the Alaska Department of Fish and Game (ADF&G) to manage Pacific herring (*Clupea pallasii*) in Prince William Sound (PWS) includes age at maturity as a parameter. However, the ASA model produces estimates of maturation that have little to no validation through field sampling. Large changes in maturation have coincided with a collapse of Norwegian spring-spawning Atlantic herring (*Clupea harengus*) in the late 1960's during which time the age at maturity was considerably reduced relative to periods before and after the collapse (Engelhard & Heino 2004). Therefore, it is essential to obtain empirical values of age at maturity to refine the ASA model to improve model performance.

Literature assessing age at maturity for Pacific herring has not been updated for over a decade, but clearly indicates that maturation can be variable. Across broad spatial scales, herring tend to mature at older ages with increasing latitude, with most fish maturing at age 2 at the southern extent of their range in California and the Yellow Sea (Spratt 1981, Cheng 1980), while Alaskan stocks may mature later at age 4 to 5 (Barton 1978). This latitudinal trend is apparent within Alaska as well, with fish reaching sexual maturity at older ages in the Chukchi Sea than the Bering Sea (Barton and Steinhoff 1980). Despite the general trend, age at maturity can vary significantly at a given location. For example in Bristol Bay, Alaska, 50% of all age 3 fish and 78% of all age 4 fish were sexually mature in 1964 (Rumyantsev & Darda 1970), but 15 years later maturation was accelerated and ~74% of all age 2 herring were sexually mature (Barton & Steinhoff 1980). Localized changes in maturation reflect body growth and fish nutritional status, particularly fat content (Engelhard & Heino 2004, Rajasilta 1991, Hay 1985). Consequently changes in maturation have been attributed to a variety of variables affecting fish condition, including stock density, competition, and environmental conditions such as water temperature (Ware and Tanasichuk 1988, Toresen 1986, Hay 1985)

Methods for assessing age at maturation depend on ecological characteristics of the target species. For example, age at maturation for some groundfish species are assessed via visual observation of gonad maturity from collections of samples in schools comprised of individuals of mixed maturities. In contrast, estimates for age at maturity for herring are derived from samples from spawning schools. Sampling from spawning aggregations has multiple drawbacks for maturation assessments. Primarily, only the fish that are going to spawn are included while non-spawners are not accounted for. Another complicating factor of sampling from herring spawns is spawning waves in which older herring arrive earlier than younger herring, a representative sample requiring sampling throughout the duration of the spawn (Ware and Tanasichuk 1988, Hay 1985, Barton and Steinhoff 1980). Additionally, smaller spawns of different fish may be missed entirely. For example, Hay and McCarter (1999) found that age at maturity estimates derived from spawning schools provide contradictory results from other methods such as

assessment of overwintering schools consisting of a different age composition. The overarching problem is resolving how to obtain a representative sample of all fish.

Herring collections during non-spawning periods introduce the complication of assessing the maturation state of fish. Determination of age at first spawn can be accomplished via histological analysis of ovaries. The presence of post-ovulatory follicles (POV) after spawning indicates an individual fish has recently spawned while oocyte maturation identifies individuals about to spawn. By sampling at a time when both POV and maturing oocytes could be present it is possible to discern immature, primiparous and repeat spawning individuals. While the histological method provides direct observation of the spawning history of individuals it is unlikely that developing oocytes can be observed among spawners. Hence the histological analysis must occur some months after spawning (Saborido-Rey & Junquera 1998). The difficulty of using histological analysis for determining age at maturation is that the process requires intensive time and effort and is relatively expensive. Additionally, the need to obtain a representative sample of all fish remains.

A more rapid-screening approach has been used for Norwegian spring-spawning herring, the largest population of Atlantic herring. Since the late 1920's, age at maturation has been inferred from differential growth increments on scales (Engelhard et al. 2003). The basis for this method is that herring lay down annual growth rings on their scales in proportion to their body growth. Because reproductive investment in herring is high, energetic provisioning of gonads manifests in reduced growth, starting some time before the first reproductive event (Scott and Heikkonen 2012, Lester et al. 2004, Schweigert et al. 2001, Roff 1983). Consequently, growth ring width on scales have been used to differentiate years in which the fish did or did not spawn, where growth rings are relatively wider prior to their first spawning event (and during years of skip-spawn), and are narrower for years in which they spawn (Engelhard and Heino 2005, Engelhard et al. 2003). As such, scales provide a spawning history for an individual fish across their lifetime, eliminating the need to find a representative sample of all fish that occupy different habitats during their various ontogenetic stages.

Our goal was to assess the applicability of the scale technique to determine age of maturation of Pacific herring. Validation of the method would provide a relatively inexpensive monitoring tool that could be used to adjust the ASA model in real time with empirically-derived values for maturity.

OBJECTIVES

1. Determine the maximum time window in which histological analysis can be employed as evidence of prior spawning and allow for maximum scale growth of Pacific herring under laboratory conditions.
2. Sample fish for scale and histological analysis at the optimal time window (determined in objective 1) to compare estimates of spawning history obtained from scales and histology.
3. Field test scale analysis to identify the age at first spawn of herring caught in PWS.
4. Use historical collections of herring scales to identify age at first spawn of herring in PWS.

METHODS

Lab study

To determine the maximum time histological evidence of prior spawning lingers in female herring, we maintained post-spawn herring in the laboratory and resampled them for 3 months after spawning. On April 21, 2011, field scouting surveys aboard a 7-m skiff were initiated in Lynn Canal, Southeast Alaska, using visual observation to find pre-spawning herring along the shoreline (Fig. 1). Five total surveys occurred within 2 hours prior-to and following low tide over a 21-day period (April 29, May 3, 4, 12) until large schools of pre-spawning fish were encountered on May 12. From these schools, live adult herring were collected using a 39-m variable mesh beach seine set in round-haul style according to methods of Thedinga et al. (2013). Approximately 1,000 live herring were transported in an insulated, aerated tote back to Auke Bay Laboratories wet lab where they were maintained for 4 months under natural photoperiod in holding tanks with through-flow ambient seawater drawn from 30m water depth. During this time, herring were fed to satiation daily using Bio-Oregon[1] commercial fish food (Longview, WA).

Captive herring spawned in the tanks on multiple occasions in the next several weeks in late May subsequent to their capture. For the purposes of analysis the spawn date was set as May 24 which was half way through the spawning period. Prior to commencing collections, we waited approximately a month to elapse from the mean spawn timing in order for ovaries to commence recovery from spawning. Starting on June 24, collections of post-spawning females were taken for histological examination of ovaries and scale measurements on approximately a weekly basis over the next four months (Table 1). At time of harvest, herring were carefully cut open for visual inspection of gender and spawning history. Post-spawning females were retained, measured for length (fork length, mm), weighed (g wet mass), and ovaries were removed and weighed (g wet mass). Gonadosomatic index (GSI) was calculated as (ovary wet mass/total body wet mass) * 100. A section of ovary was dissected from the forward-most end of one ovary and preserved in labeled megacassettes in 10% neutral buffered formalin.

After 24 hours of preservation in formalin, histological samples were changed to a water bath and shipped to Dr. Gary Marty (Fish Pathology Services, University of California Davis, Davis, California) for histological analysis. Ovaries were processed into paraffin and sectioned according to routine procedures. Ovaries were examined using magnification. Multiple characteristics of ovarian development were scored using terms for staging following Bucholtz et al. (2008). Gender and post-spawning status were verified histologically. Examination of seven histological characteristics included:

1) Presence of post-ovulatory follicles. Post-ovulatory follicles are what remain of the follicle after the egg is released at the time of ovulation. Post-ovulatory follicles are present in spawning and post-spawn Pacific herring (Koya et al. 2003) and are frequently used as an indicator of prior spawning in other fish species (Brown-Peterson et al. 2011). All ovary sections were scored for having the presence or absence of post-ovulatory follicles.

2) Number of post-ovulatory eggs. Post-ovulatory eggs are those that have been released from their follicle but not spawned. The number of post-ovulatory eggs were enumerated in each ovary section and categorized as 0 eggs, 1-5 eggs, 6-10 eggs, or 10+ eggs.

[1] References to trade names, brands or companies does not imply endorsement by NOAA

3) Degree of inflammation. Post-spawning ovaries commonly have a range of inflammatory changes that an expert can distinguish from other causes of inflammation (*Ichthyophonus* infection, Anisakis infection, gonadal granulomas, and eosinophilicgranular cells). Inflammation was categorized as <5%, 5-20%, or >20% of the volume of the histological section.
4) Presence of gonadal pigmented macrophage aggregates (GMAs). GMAs are a marker of previous follicle maturating and spawning but can also be a result of massive atresia (degeneration) of immature follicles. Histological sections were scored as having no GMA or ≤2 GMAs > 60 µm in diameter per 10x objective-lens field.
5) Atresia of immature (unyolked) follicles. The number of immature follicles in a histological section undergoing atresia was counted and categorized as 0, <1, 1-3, or >3 follicles.
6) Atresia of mature or maturing (yolked) follicles. The number of mature follicles in a histological section undergoing atresia was counted and categorized as 0, <1, or 1-3 follicles.
7) Presence of hyalinized vessel walls. Hyalinization (deterioration) of vessel walls is usually limited to ovaries immediately post-spawning. Wall thickness of hyalinized vessels were measured and counted.

Field collections: summer 2012

In order to validate the use of scales for detecting age at first spawn, the age of fish was identified and compared with the results of a histological analysis of their ovaries. Wild adult herring were collected from PWS approximately 3 months after spawning according to the timeline of lingering evidence of prior spawning determined in the lab study (post-ovulatory follicles). Fish were collected in collaboration with the Prince William Sound Science Center (PWSSC) aboard *the R/V New Wave* on July 16-20, 2012 using jigging and gillnets (Fig. 2). Vessel acoustics and presence of herring predators such as humpback whales (*Megaptera novaeangliae)*, Steller sea lions (*Eumetopias jubatus*) and seabirds were used to look for herring. Samples were additionally collected and preserved from the concurrent *Exxon Valdez* Oil Spill Trustee Council (EVOSTC) Long-term Monitoring Program "Gulf Watch Alaska" project 12120114-O forage fish survey which surveyed the entire Sound over a longer time period. Aboard the vessels, herring length was measured and several scales were collected consistent with methodology of Otis and Heintz (2003) from the preferred area on the left side of the herring approximately 2 rows below the lateral line and 3 scales back from the operculum. In the case that scales were not in the preferred area on the left side of the fish, they were pulled from the right side of the fish, which can also produce clear scales. Scales were cleaned and mounted following ADF&G protocols by gently wiping them with moist Kim wipes and then affixed to glass slides using Elmers glue diluted with water. Gonads were dissected and preserved according to the same protocol in the lab study, with the exception that gonad mass was not measured due to ship-board imprecision with small masses. Preserved gonads were sent to Histologistics, Inc. (Worcester, MA)[1] for sectioning and characterization of maturation state.

Individual fish were classified according to their current maturation state using standard terminology (Brown-Peterson 2011): never spawned - immature, never spawned - developing, developing, spawning capable, regressing or regenerating. An immature fish is regarded as a fish that has never spawned with no indication to do so. A fish that has never spawned but was

developing gonads was a primiparous fish spawning for the first time. A developing fish was a repeat spawner provisioning their gonads for future spawning. A spawning capable fish was ready to spawn. Regressing fish were spent and beginning to recover post-spawn. Regenerating fish were sexually mature but reproductively inactive and recovering their gonads.

Herring scales were aged by counting the light colored annuli representative of winter periods according to standard methods. Widths of the outermost annuli were measured using a 25X magnification by microfiche.

Spawning histories from histological analysis were compared to scale growth patterns. Based on the developmental state ascertained with histological analysis, females were identified as being mature or immature the previous spawning event. Age-specific scale growth patterns were examined to determine if the proportion of fish that were mature or immature during the last spawning event could be discriminated from each other. If scales reliably identify individuals that have spawned then it should be possible to use the growth pattern to project entire spawning history of the fish.

Field collections: spring 2013
Upon the development of the tool for using herring scales to discriminate spawners from non-spawners, we used a statistical approach to quantify the ages of primiparous fish and the proportion of skip-spawners at older ages. Pre-spawning herring were collected in the spring of 2013 in PWS for scale analysis. Spawning history of individual fish was constructed from the historical catalog of growth on scales.

Samples were collected during the ADF&G vessel-based spawning survey in 2013 and frozen. In the lab, all female fish were measured for length and mass as described previously. Ovaries were dissected and weighed to calculate GSI. Scales were preserved according to previously sited methods.

To identify spawners from non-spawners, we used a Gaussian mixture model using the *mixtools* package (Benaglia et al. 2009) in R (R Core Team 2016) to detect bimodal distributions of annulus ring growth. Significant bimodal distributions of annuli growth would indicate a group of spawners (low growth) and non-spawners (high growth).

Statistical analysis of historical collections of PWS herring scales
In a collaborative effort, we examined herring scale measurements made from the ADF&G's PWS herring scale library to examine for historical indications of age at first spawn and skip-spawning in scale growth patterns. Scale measurements were provided by EVOSTC project 13120111-N following methods detailed in their final report (Moffit and Anderson 2017). The dataset included scale annulus measurements of PWS herring ages 4-6 collected from herring in spawning aggregations in 1986-2013. By measuring all annuli we were able to determine growth at ages younger than when the fish were collected. We used the same Gaussian mixture model technique as described above to test for bimodal distributions of growth, differentiating spawners from non-spawners. We controlled for year effect by subtracting the mean growth for a given year from each observation in that year. We then fit the Gaussian distributions to each age group for each gender.

RESULTS

Lab study

While in captivity in the wet lab, ambient seawater temperatures in which herring were reared averaged 7.8 °C, ranging from 5.6 °C in May to a peak of 9.4 °C in August (Fig. 3). Ambient photoperiod varied by 5.5 hours over the course of study, with 15.8 hours of daylight at the time of herring capture in May, increasing to 18.3 hours on summer solstice, and declining to 12.8 hours in mid-September at the conclusion of the study (Fig. 4).

The GSI of post-spawn herring did not change significantly over the 100 days after spawning (ANOVA, p=0.276, Fig.5). In general, average GSI of post-spawn female herring on sampling dates did not exceed 1.9. GSI of individual fish was less than 4.2 with the exception of one individual with a GSI of 6.5, 87 days after spawning.

Histological characteristics of herring ovaries had variable utility as indicators of prior spawning. The clearest evidence of previous spawning was observed in the presence of post-ovulatory follicles. Post-ovulatory follicles were present in 100% of ovaries for nearly 2 months after spawning (Fig. 6). During the third month after spawning, post-ovulatory follicles were present in 93% of ovaries, followed by a significant drop to only 67% of ovaries with post-ovulatary follicles a week later (ANOVA, p = 0.008).

Other histological characteristics were less clear-cut indications of prior spawning. Three histological indicators showed cyclical trends after spawning, all sharing an inflection point in late July, 2 months after spawning. The number of post-ovulatory eggs and ovary inflammation both increased after spawning and peaked by late July, followed by subsequent decreases (Figs. 7 and 8). Conversely, the presence of GMAs decreased until late July, followed by a subsequent increase (Fig. 9). Two months after spawning when histological characteristics made this distinct change, the majority of ovaries had more than 10 post-ovulatory eggs per section (71%), were the most inflamed (70% had >5% inflammation), and had no GMAs (93%). Throughout the entire 3 month period post-spawn, only 1 ovary had hyalinized vessel walls ($\leq$ 40 μm thick), which was on June 25 approximately a month after spawning.

Atresia of both immature and mature follicles occurred after spawning. Immature follicles generally became more atretic as time progressed after spawning (Fig.10), while mature follicles became less atretic over time and were absent three months after spawning (Fig. 11).

Field collections: summer 2012

Finding age 2+ herring in PWS in July proved to be very difficult. In total we caught 169 herring, 105 of which were females and 64 which were males. Age was determined for 166 of the herring, with 3 herring having unreadable scales (Fig. 12). In the July collection, ages ranged from 3 to 11 years old, with females being predominantly ages 4-7 (75%) and the majority of males (73%) were ages 4-6. Typical for herring, fish length increased with age with significant overlap between ages (Fig. 13).

Histological analysis of ovaries revealed that female herring within PWS in July were in various states of maturity. A little over half (58%) of females across all ages had spawned before and were developing their ovaries in preparation for spawning (ages 3-11) (Table 2). Another 6% were spawning capable, while 23% were regressing or regenerating from earlier spawning. 7% (n=7) of the females were primiparous and preparing to spawn for the first time. Three of the primiparous females were age 3, 2 were age 4, 1 was age 6, and another was not aged due to poor scale condition. Another 7% of the females had never spawned and were not developing their ovaries for spawning. These fish were all age 3, meaning they would spawn for the first time at age 4. Only one 3 year old caught in the July collection had spawned previously, indicating it had likely spawned for the first time at age 2. Of the males that could be characterized for maturity (59%), the majority of fish were developing for spawning. No males were identified as primiparous, however 1 male (3% of the males that could be characterized) had never spawned and was not developing, suggesting it would be primiparous at age 5 or older.

Annual growth on herring scales diminishes with age and does not vary by gender (Fig. 14). Therefore, to determine if scale growth is an indicator of spawning status requires fish of the same age to be collected in both non-spawning and spawning states. Age 3 herring were the only age class for which there was a viable comparison of non-spawners to spawners (Fig. 15). Herring developing their gonads in preparation for spawning had lower scale growth (0.3210 mm) than fish that were not going to spawn and could allocate energy toward growth (0.3836 mm) (ANOVA GLM, log-transformed, $p = 0.041$). Growth in previous years during the first and second year of life had no influence on maturation state of the three year olds ($p \geq 0.379$).

Field collections: spring 2013

Of the herring collected from the 2013 pre-spawning aggregation, 234 individuals (47%) were females (Table 3). Females from the spawning collection in 2013 ranged from ages 3-13, with similar age-at-length to females caught in July 2012 (Fig.16). GSI's were > 15 and generally increased with age (Fig. 17).

Bimodal distributions of scale growth were not detected using Gaussian mixture models for any age of fish, indicating samples sizes were too small to differentiate spawning herring from non-spawning herring.

Statistical analysis of historical collections of PWS herring scales

Scale growth of herring ages 1 and 2 were unimodal, indicating that all fish of these ages were immature and not spawning. In contrast, scale growth of fish ages 3-6 showed differentiation in growth patterns and consequently spawning activity (Fig. 18). Results indicate a difference in skip-spawn probabilities between genders for ages 3 and 6, but not 4 and 5. Females had a higher overall probability of skip-spawn, ranging from 13% to upwards of 50% for ages 3, 4, 5, and 6, while males ranged from 10% to 15%. Additionally, the annual variability in skip-spawning probabilities was highest in ages 3 and 6.

DISCUSSION

This study demonstrated that the technique of using differential scale growth to discriminate spawning history commonly used for Norwegian spring-spawning Atlantic herring can be applied to Pacific herring. We found that 3 year old herring preparing to spawn for the first time

had significantly diminished scale growth compared to immature 3 year olds that were not going to spawn. We were forced to make this comparison only 3 months after spawning in order to maintain histological integrity to compare to scale growth. Continued growth and ovary provisioning over additional summer months would result in further differentiation between spawners and non-spawners. Consequently, statistical separation between spawners and non-spawners is likely greater than observed in this study.

Using the validated methodology, we sought to quantify the age at first spawn and subsequent frequency of skip-spawning of herring in PWS. Examination of sufficient numbers of historical herring scale measurements from EVOSTC project 13120111-N detected bimodal distributions of scale growth indicating spawners and non-spawners in fish aged 3-6. Females and males had similar incidence of skip-spawners for ages 4 and 5. Females had substantially higher rates of skip-spawning than males for ages 3 and 6, which could likely be a factor of greater energetic reproductive investment in ovaries than sperm (Slotte 1996). High incidence of skip-spawning in females ~50% is in agreement with Norwegian spring-spawning herring, which undergo extensive migrations (Engelhard and Heino 2006). PWS herring may also undergo extensive migrations out of PWS during the summer, as evidenced by the lack of herring encountered in the July sampling effort and results as well as tagging (EVOTSC project 14120111-B, Bishop et al. 2017) and genetics studies (EVOSTC project 16120111-P, Wildes et al. 2011). Elevated rates of skip-spawning in age 3 females is to be expected as age 3 is the youngest age at which herring start spawning in PWS, as determined here. Incidence of skip-spawning decreases for ages 4-5, as expected, but increased for age 6 fish. The increase in skip-spawning of females aged 6 may be a result of senescence (Tanasichuk 2000).

As a note of caution, the proportion of fish in the July 2012 sample caught in PWS should not be considered representative of PWS herring. In total we were only able to obtain 165 herring during this time. The lack of abundance of age 2+ herring in summer is further evidence of fish moving to the continental shelf to feed in the summer. Age 2 fish were completely elusive in PWS in July, as were herring in general. This is not surprising as one of the least understood things about Pacific herring is when and where recruits join adults (Hay 1985).

Of the multitude of histological characteristics assessed as indicators of previous spawning in herring, the presence of post-ovulatory follicles was the most clear-cut, enduring indication. All other histological characteristics fluctuated in non-consistent manners, making them less desirable as clear indicators of prior spawning. Presence of post-ovulatory follicles were 100% indicators of previous spawning activity for over 2 months after spawn timing, and continued to be the most accurate histological indication for as long as 3 months after spawning. In a similar laboratory study in Japan, post-ovulatory follicles were only present in post-spawn herring ovaries for 1 month after spawning and not present 2 months after spawning (Koya et al. 2003). In that study, however, herring were examined on a more coarse frequency of once a month. Other aspects of the Koya et al. (2003) study that could contribute to a shorter time frame for the presence of post-ovulatory follicles compared to what we observed was that 1) herring were reared in a lab an entire year prior to spawning rather than obtaining fish from recent spawn aggregations, and 2) Japan is over 20 degrees south in latitude from Southeast Alaska, resulting in warmer ambient water temperatures and different photoperiod to which the fish were subjected.

In conclusion, this study validated the use of scale growth increments to determine spawning history of Pacific herring. Application of the methodology to ADF&G's time series of scale measurements indicates that a significant portion of age 3 herring have not spawned yet. Furthermore, older ages of herring skip-spawn, particularly age 6's. Future work should include estimation of probability of skip-spawning by year for different aged fish, correlation of skip-spawning to environmental variables, and a sensitivity analysis of the ASA model for the proportion of skip-spawners by age class.

ACKNOWLEDGMENTS

We thank the *Exxon Valdez* Oil Spill Trustee Council for the opportunity to participate in the Long-Term Herring Research and Monitoring Program and the funding to conduct this study. Many thanks to Ryan Bare, NOAA Affiliate, who conducted dissections, scale mounting and measuring of the 2013 spawning herring. Other appreciated sample handling was provided by Ann Robertson and Matt Callahan, NOAA Affiliates. Many thanks to Gary Marty for his thorough histological analysis of the laboratory-derived samples, and Histologics, Inc. for their analysis of the 2012 field-derived samples. We thank Rob Campbell and the Prince William Sound Science Center for their collaboration and use of their vessel, *R/V New Wave*, for summer herring collections in PWS. We also thank Mayumi Arimitsu (USGS) and crew aboard the R/V *Gyre* for sampling herring. In addition, we thank ADF&G, particularly Steve Moffit and Rich Brenner, for their collection, preservation and shipment of spawning herring to us for analysis. We also thank Steve Moffit for his enthusiastic collaboration and sharing of scale measurements. Many thanks additionally go to Scott Pegau for his organization of the Long-Term Herring Research and Monitoring Program. 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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TABLES

Table 1. Post-spawn female herring harvested from laboratory tanks for analysis of progression of histological maturation and scale growth. Mean ± 1SE.

Collection date (Year = 2011)	n	Fork length (mm)	Body mass (g)
June 24	5	212.4 ± 3.5	86.18 ± 4.76
July 8	6	209.7 ± 4.3	83.58 ± 6.19
15	14	208.6 ± 3.4	79.89 ± 3.33
22	15	209.4 ± 3.2	83.21 ± 5.07
29	14	204.1 ± 2.7	72.63 ± 3.09
Aug 5	14	210.1 ± 3.6	78.51 ± 5.01
19	15	208.4 ± 4.8	76.55 ± 5.05
26	15	211.5 ± 2.2	81.66 ± 2.63
Sept 2	15	207.5 ± 3.1	74.44 ± 4.03
TOTAL	113	208.8 ± 1.2	78.80 ± 1.46

Table 2. Maturation state by age and gender of Pacific herring (*Clupea pallasii)* collected in Prince William Sound, Alaska in July 2012. Numbers are expressed as % of the fish caught and (n) by gender. Female statistics are listed in the top half of cells and males are below.

Age Maturity	3	4	5	6	7	8	9	10	11	Unknown Age	TOTAL
Never Spawned – Immature	6.7% (7)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	6.7% (7)
	1.6% (1)	1.6% (1)	0.0% (0)	1.6% (1)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	4.7% (3)
Never Spawned – Developing	2.9% (3)	1.9% (2)	0.0% (0)	1.0% (1)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	1.0% (1)	6.7% (7)
	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)
Developing	1.0% (1)	14.3% (15)	13.3% (14)	10.5% (11)	11.4% (12)	4.8% (5)	1.0% (1)	0.0% (0)	1.0% (1)	1.0% (1)	58.1% (61)
	1.6% (1)	15.6% (10)	7.8% (5)	7.8% (5)	1.6% (1)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	34.4% (22)
Spawning Capable	0.0% (0)	1.9% (2)	0.0% (0)	1.0% (1)	1.0% (1)	1.0% (1)	1.0% (1)	0.0% (0)	0.0% (0)	0.0% (0)	5.7% (6)
	0.0% (0)	0.0% (0)	1.6% (1)	1.6% (1)	0.0% (0)	0.0% (0)	0.0% (0)	1.6% (1)	0.0% (0)	0.0% (0)	4.7% (3)
Regressing	0.0% (0)	0.0% (0)	1.9% (2)	1.9% (2)	2.9% (3)	1.0% (1)	0.0% (0)	0.0% (0)	0.0% (0)	1.0% (1)	8.6% (9)
	0.0% (0)	0.0% (0)	1.6% (1)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	1.6% (1)
Regenerating	0.0% (0)	2.9% (3)	3.8% (4)	4.8% (5)	0.0% (0)	1.0% (1)	1.9% (2)	0.0% (0)	0.0% (0)	0.0% (0)	14.3% (15)
	0.0% (0)	4.7% (3)	4.7% (3)	3.1% (2)	0.0% (0)	0.0% (0)	1.6% (1)	0.0% (0)	0.0% (0)	0.0% (0)	14.1% (9)
Unknown	0.0% (0)	0.0% (0)	0.0% (0)	1.6% (1)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)
	6.3% (4)	6.3% (4)	9.4% (6)	6.3% (4)	6.3% (4)	6.3% (4)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	40.6% (26)

Table 3. Female herring harvested in 2013 from Prince William Sound spawning aggregations. Mean ± 1SE.

Age	n	Fork length (mm)	Body mass (g)	GSI
3	11	182.9 ± 3.3	71.80 ± 3.27	21.6 ± 1.7
4	92	200.1 ± 1.1	98.12 ± 1.82	25.2 ± 0.5
5	15	205.0 ± 3.1	116.77 ± 5.51	28.1 ± 0.8
6	43	216.4 ± 1.9	129.24 ± 3.72	28.3 ± 0.8
7	21	221.0 ± 2.7	142.62 ± 6.15	28.9 ± 0.8
8	32	231.6 ± 1.8	161.73 ± 5.24	28.9 ± 0.7
9	14	231.3 ± 3.2	168.60 ± 5.09	31.6 ± 0.9
10	2	235.5 ± 3.5	160.30 ± 13.20	26.2 ± 0.7
11	2	246.0	233.93	n/a
12	1	251.0	174.72	28.4
13	1	242.0	167.27	29.0
TOTAL	234	211.8 ± 1.2	123.08 ± 2.36	27.2 ± 0.3

FIGURES

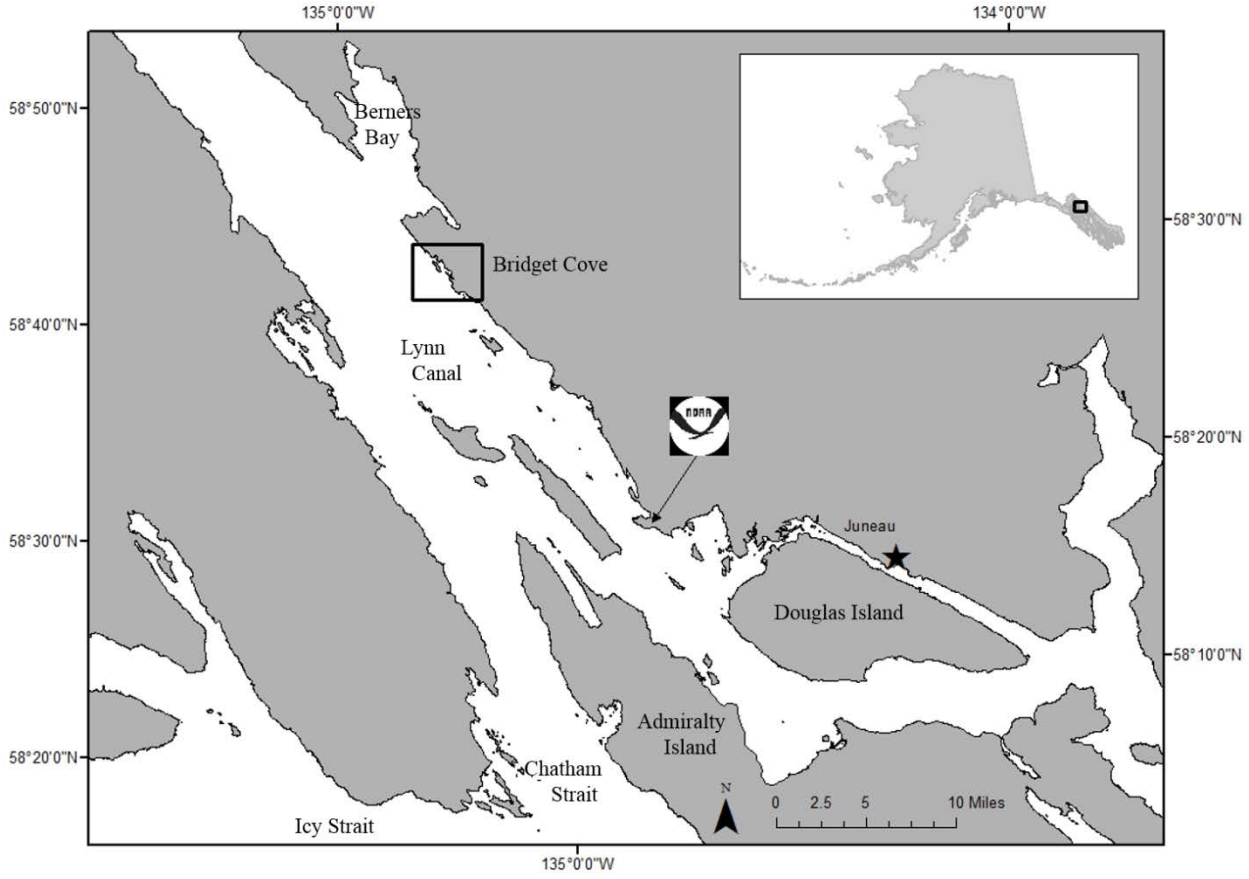

Figure 1. Map of collection site for pre-spawning Pacific herring (*Clupea pallasii)* in Lynn Canal and the NOAA lab facility where herring were reared.

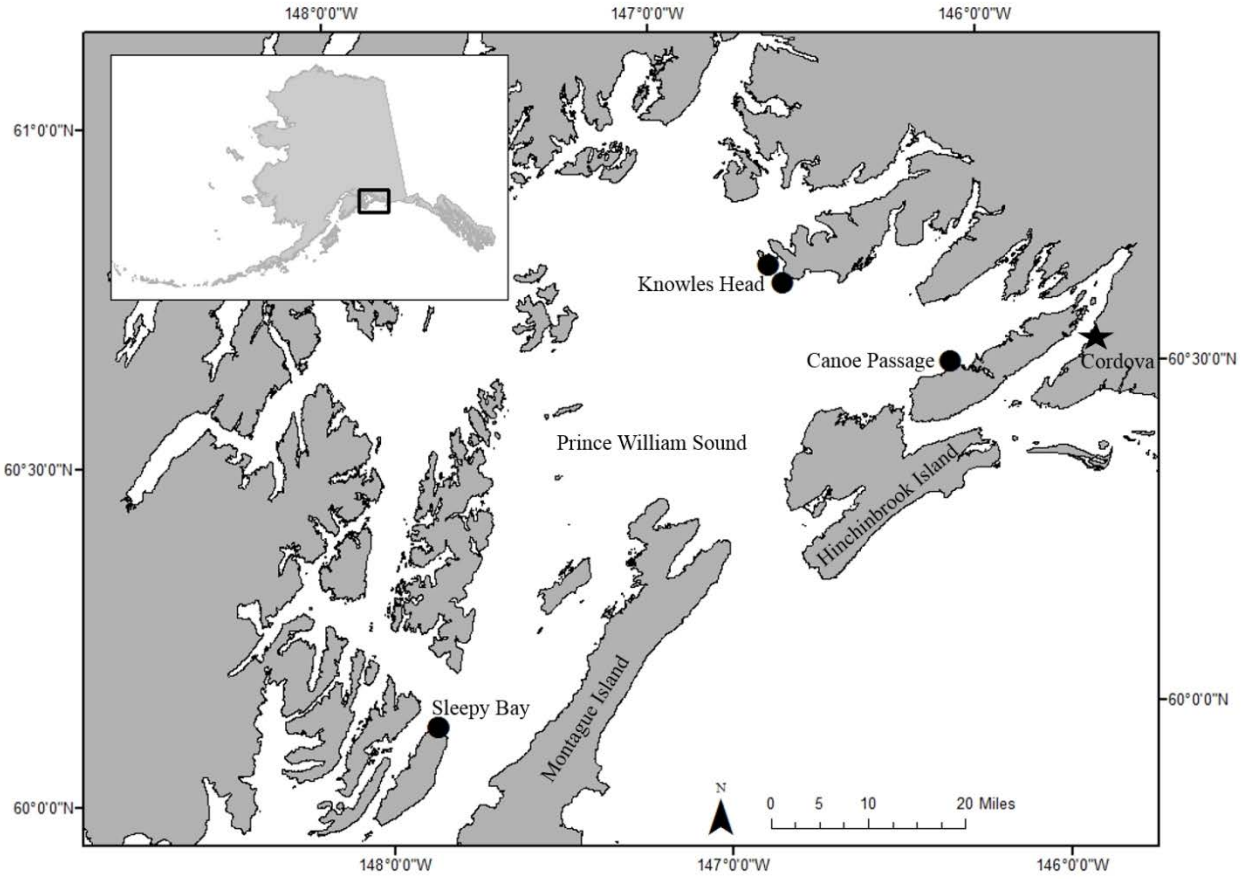

Figure 2. Map of collection sites for Pacific herring (*Clupea pallasii)* in Prince William Sound in July 2012 for histological comparisons of ovaries to scale growth.

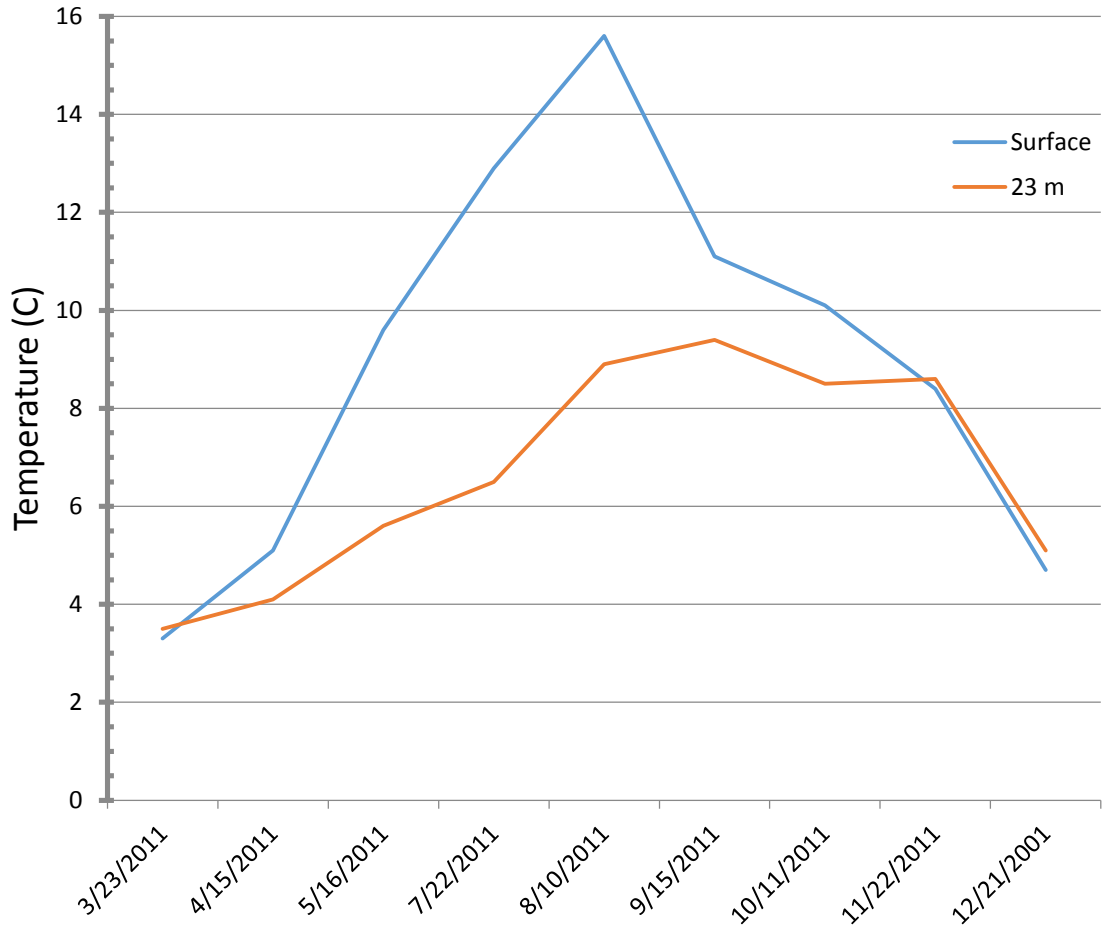

Figure 3. Ambient sea water temperature near the 30m salt-water intake for NOAA's wet laboratory facility where Pacific herring (*Clupea pallasii)* were reared.

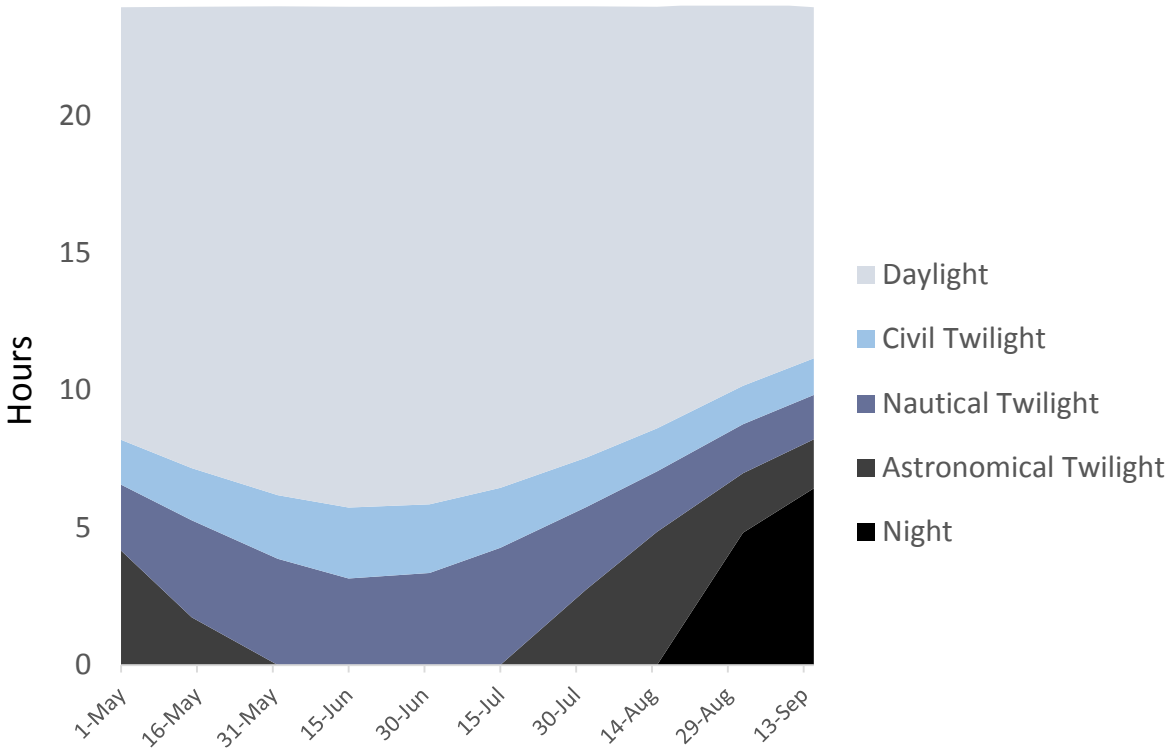

Figure 4. Ambient photoperiod during laboratory rearing of Pacific herring (*Clupea pallasii).*

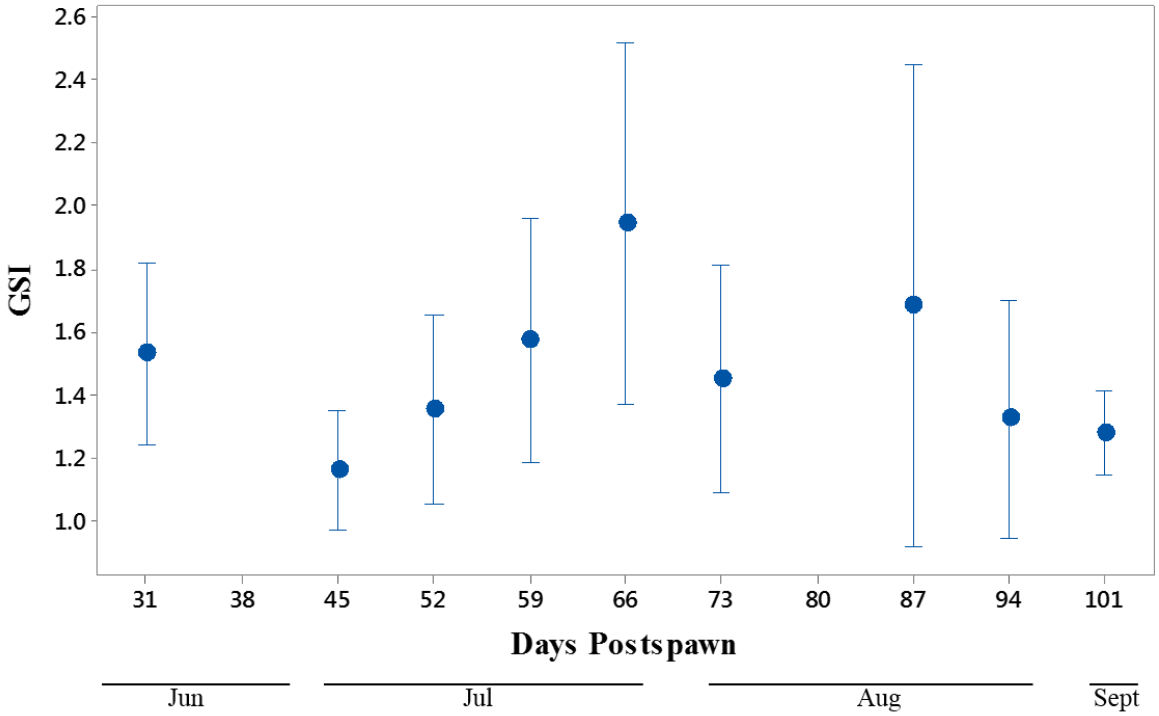

Figure 5. Gonadosomatic index (GSI) of female Pacific herring (*Clupea pallasii)* sampled weekly after spawning. Mean ± 95% CI.

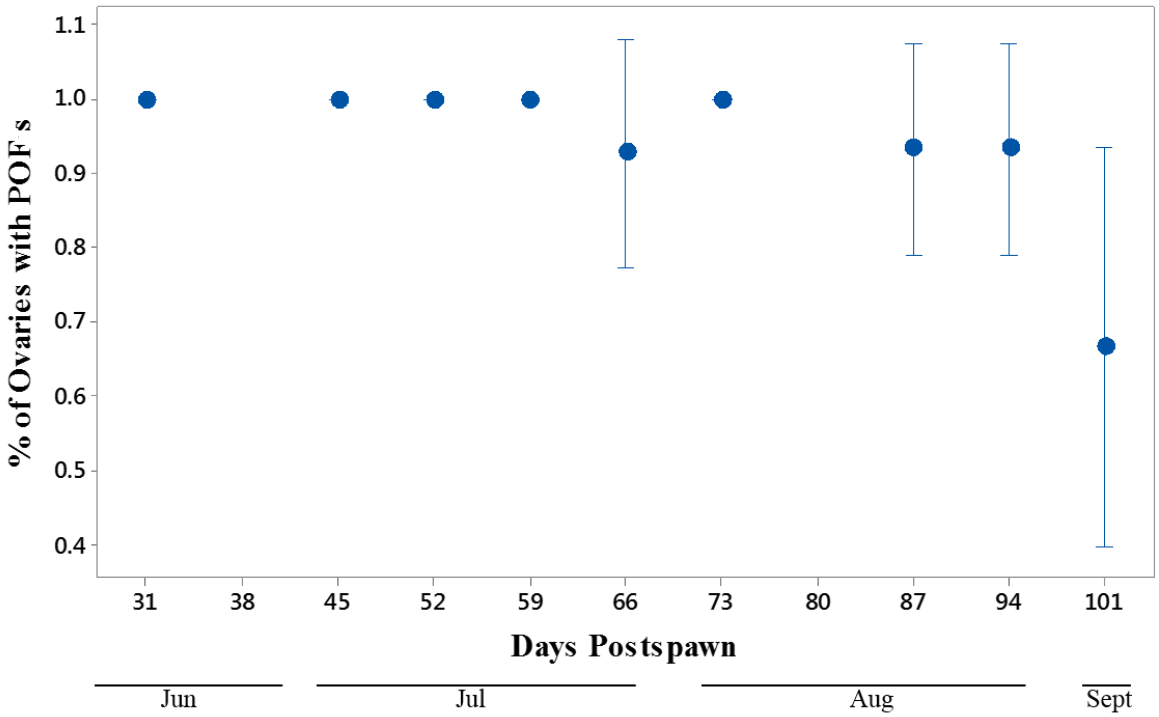

Figure 6. Percentage of ovaries with post-ovulatory follicles (POF) in female Pacific herring (*Clupea pallasii)* sampled weekly after spawning. Mean ± 95% CI.

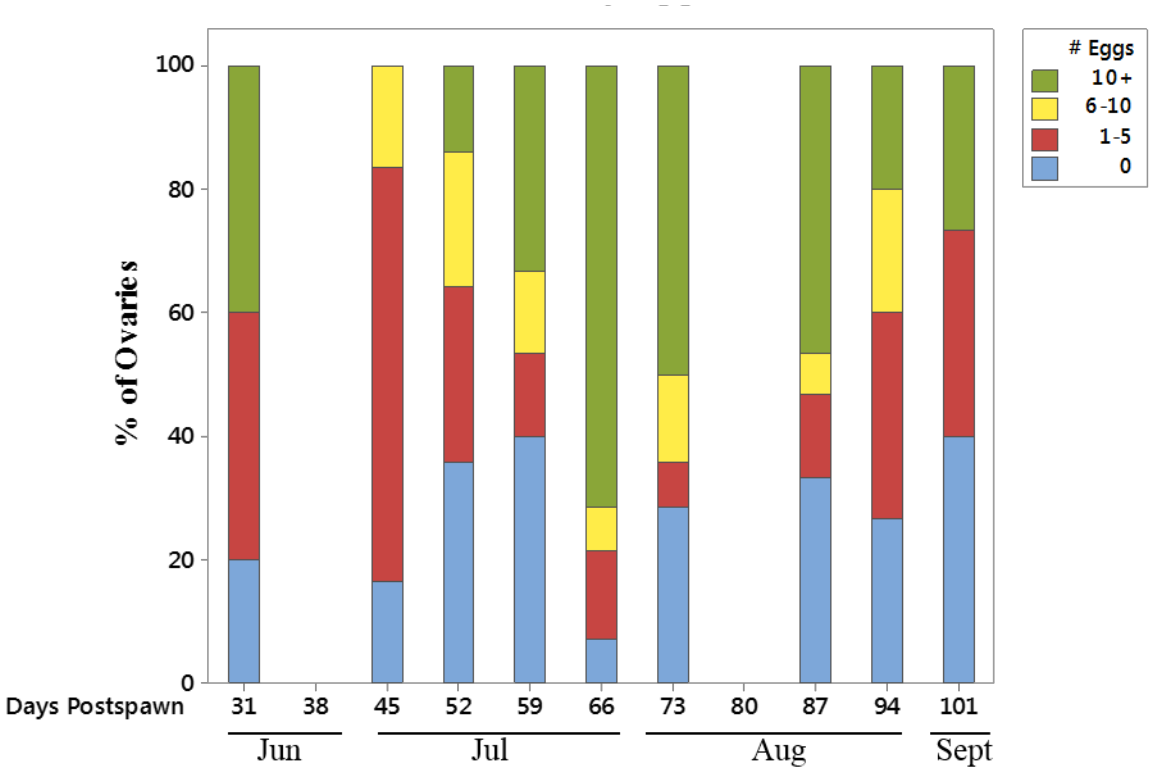

Figure 7. Number of post-ovulatory eggs per histological section of ovaries from female Pacific herring (*Clupea pallasii)* sampled weekly after spawning.

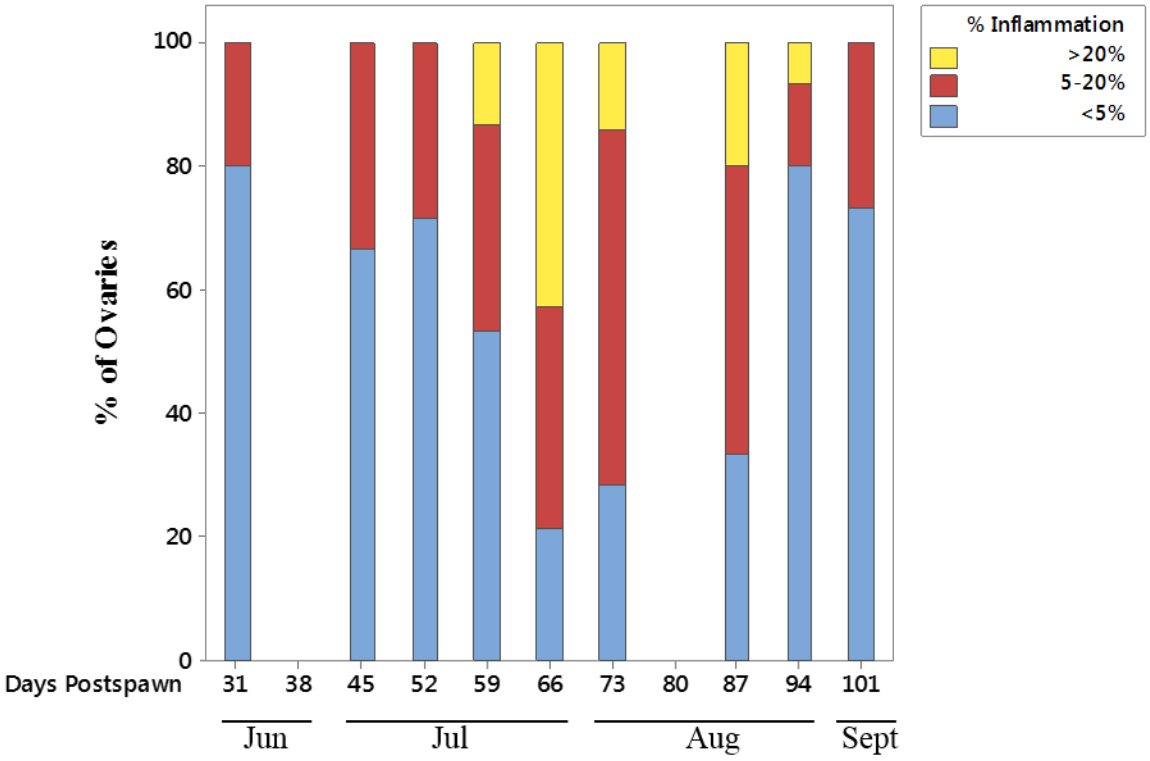

Figure 8. Amount of inflammation in ovaries of Pacific herring (*Clupea pallasii)* sampled weekly after spawning. Inflammation was categorized into 3 levels and is expressed as % volume per histological section. No ovaries had 0% inflammation.

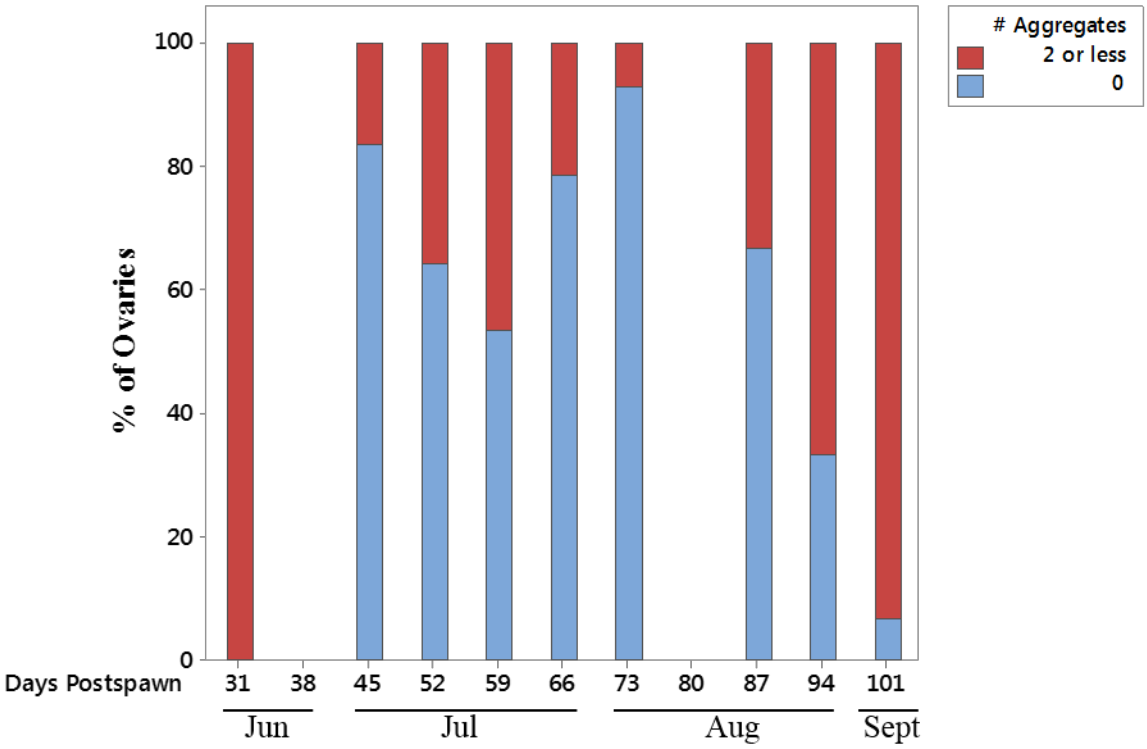

Figure 9. Number of gonadal pigmented macrophage aggregates (GMAs) in ovaries of Pacific herring (*Clupea pallasii)* sampled weekly after spawning. Aggregates were categorized into 2 levels and are expressed as number of aggregates > 60µm in diameter per 10x objective lens field. No ovaries had more than 2 aggregates.

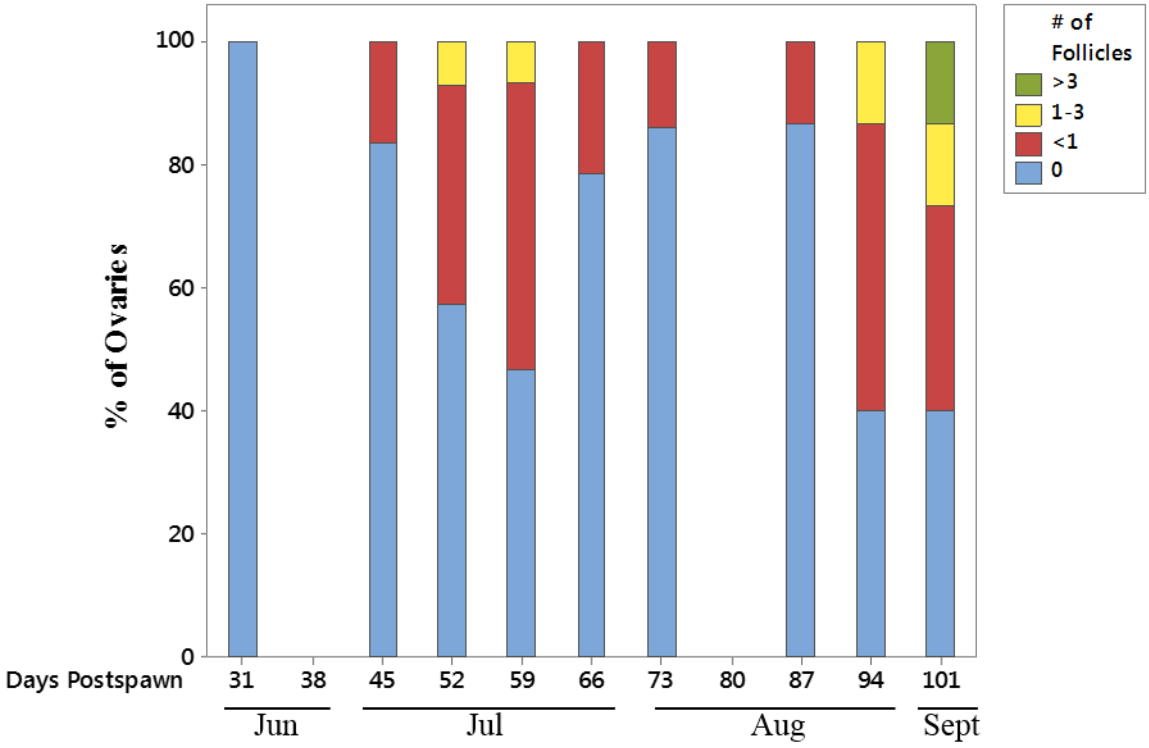

Figure 10. Atresia of immature follicles in ovaries of Pacific herring (*Clupea pallasii)* sampled weekly after spawning. Number of immature follicles per 10x objective-lens field were categorized into 4 levels.

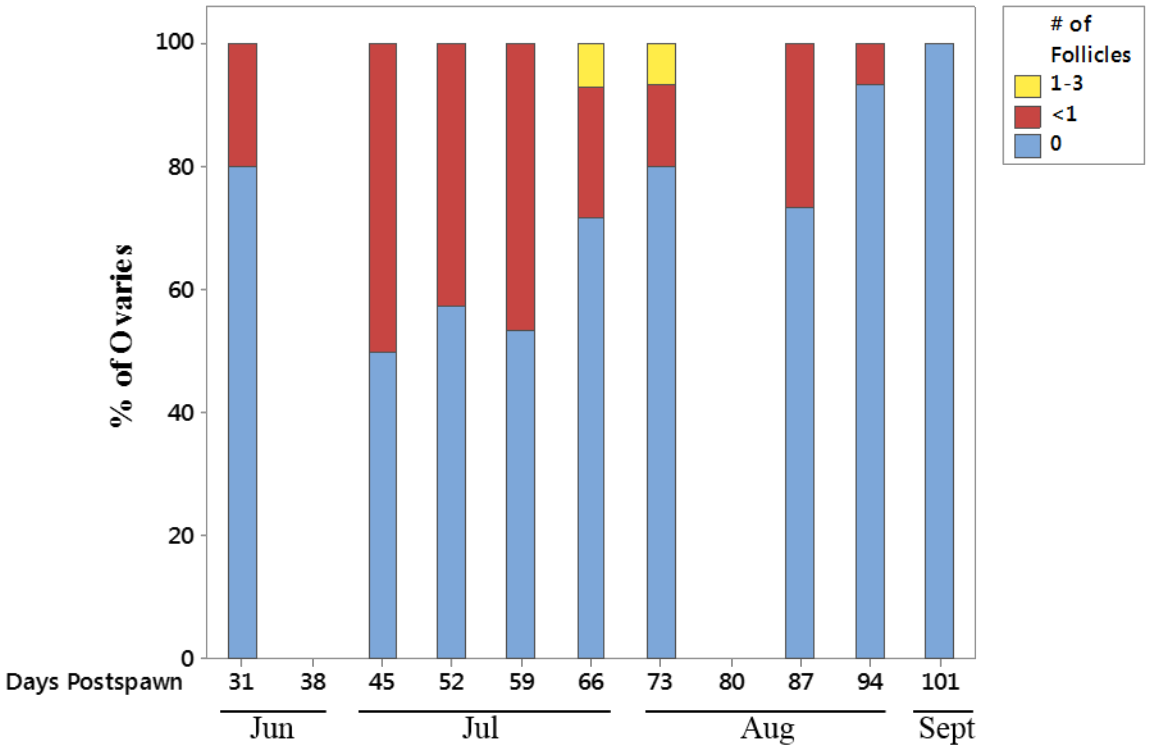

Figure 11. Atresia of mature follicles in ovaries of Pacific herring (*Clupea pallasii)* sampled weekly after spawning. Number of immature follicles per 4x objective-lens field were categorized into 3 levels.

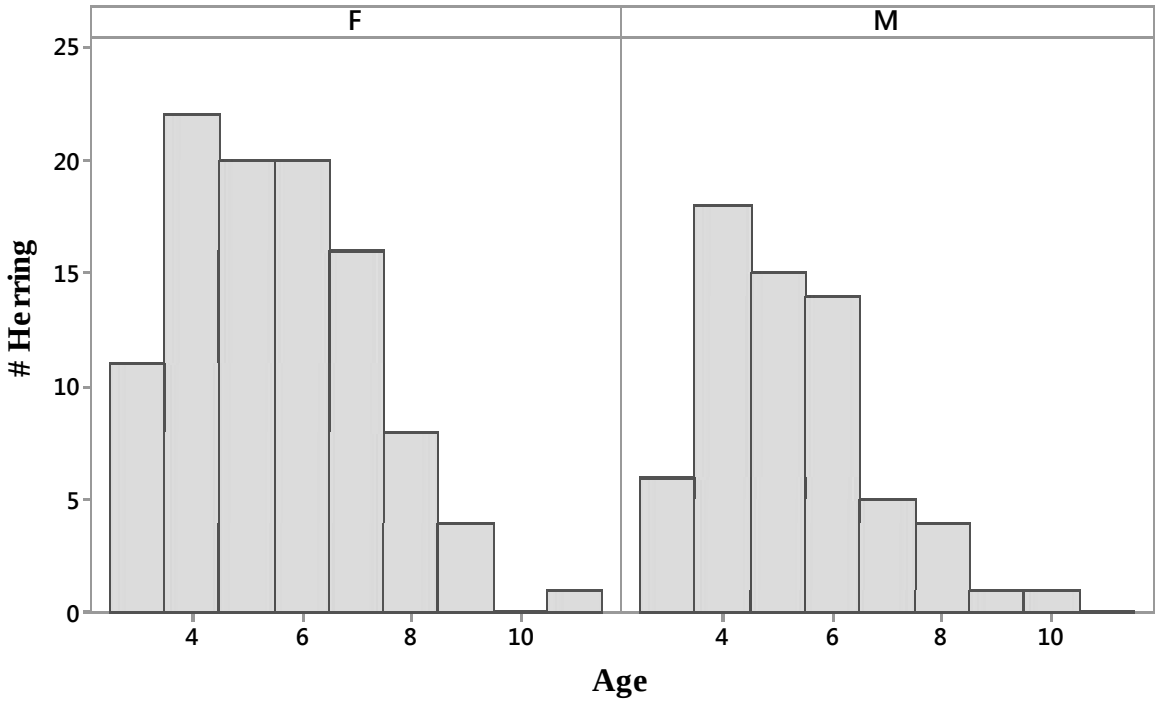

Figure 12. Age distribution of Pacific herring (*Clupea pallasii)* by gender (female n = 105, male n = 64) collected in Prince William Sound in July 2012 for comparisons of ovary histology to scale growth.

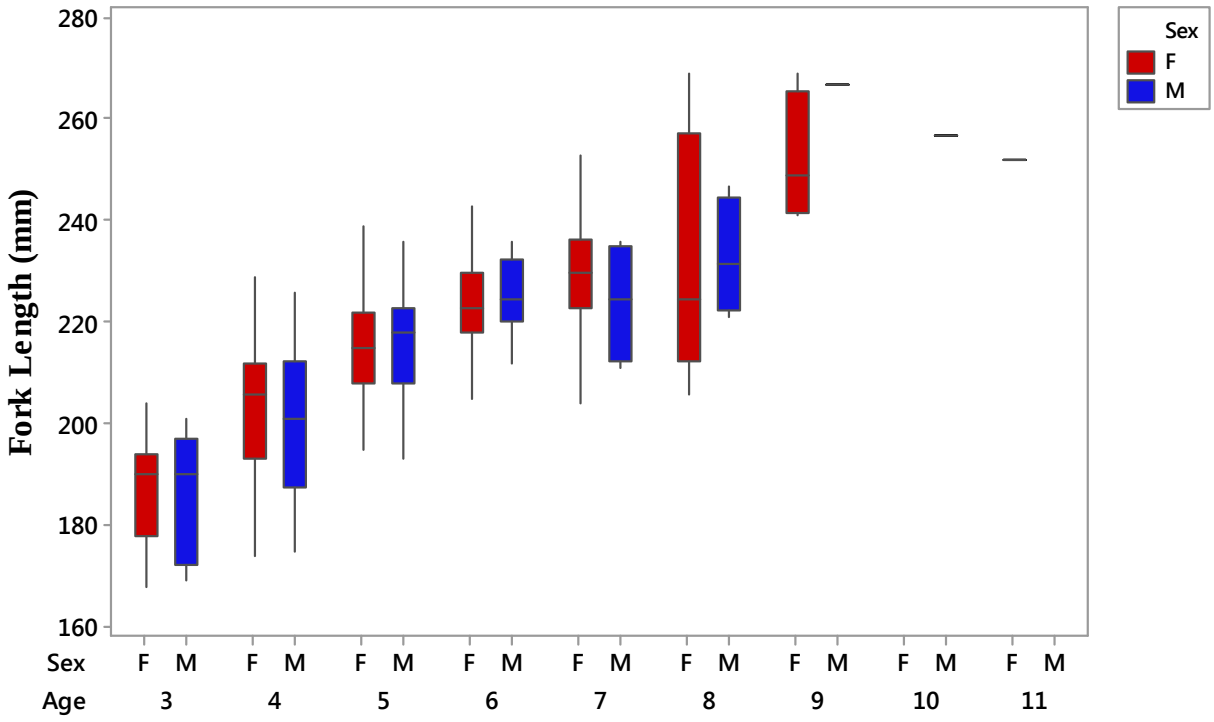

Figure 13. Fork length at age of Pacific herring (*Clupea pallasii)* collected in Prince William Sound in July 2012 for comparisons of ovary histology to scale growth. Colored boxes depict the middle 50% of the data with the horizontal line in the box indicating the median value. Whiskers extending from the boxes indicate the upper and lower 25% of the data. For herring ages 9 through 11 where only horizontal lines are shown, sample sizes are 1. Sample sizes are given in Table 2.

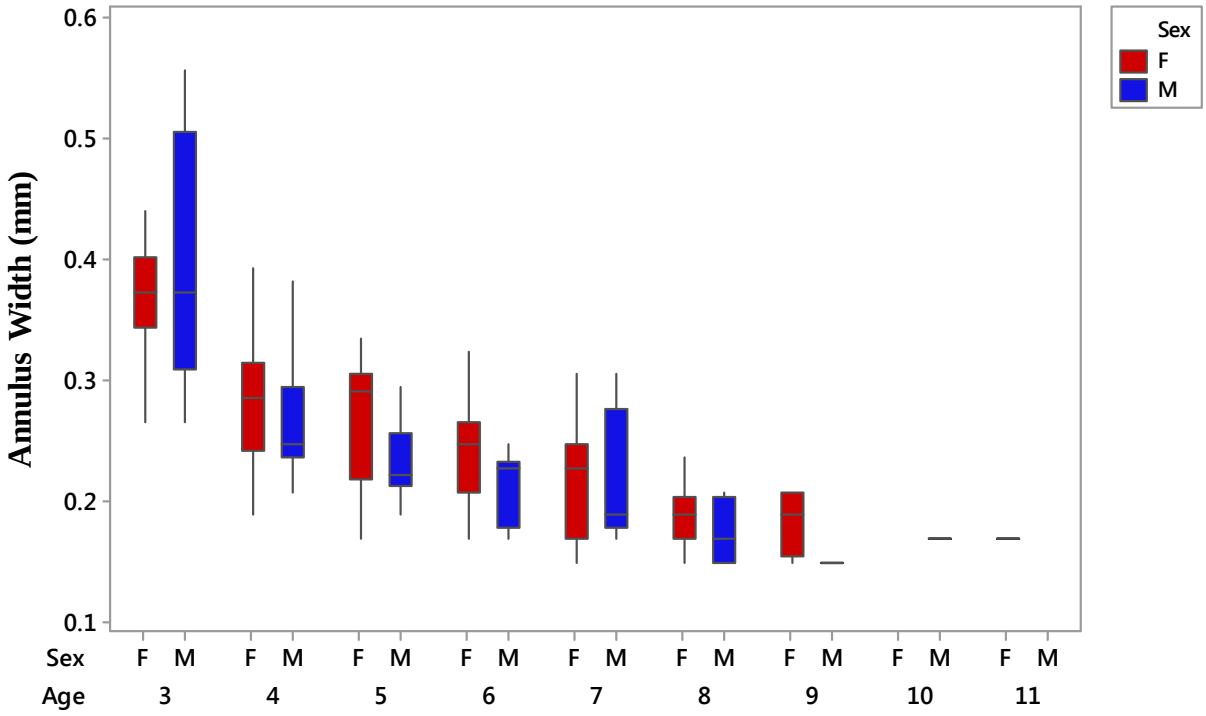

Figure 14. Width of annuli attributed to growth in 2012 on scales of Pacific herring (*Clupea pallasii)* collected in Prince William Sound in July 2012. Colored boxes depict the middle 50% of the data with the horizontal line in the box indicating the median value. Whiskers extending from the boxes indicate the upper and lower 25% of the data. For herring ages 9 through 11 where only horizontal lines are shown, sample sizes are 1. Sample sizes are given in Table 2.

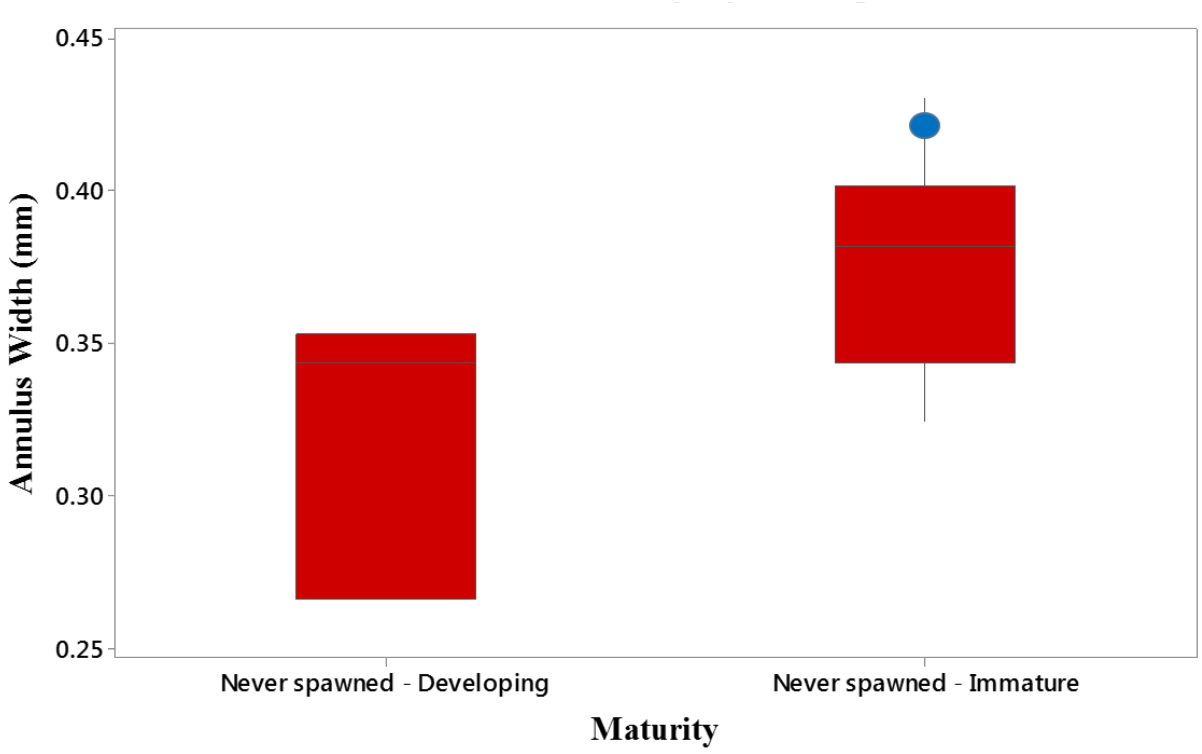

Figure 15. Scale annulus width comparing spawning status of age 3 female Pacific herring (*Clupea pallasii)* collected in Prince William Sound in July 2012. The blue dot indicates the one 3 year old male that had never spawned before shown for comparison. Colored boxes depict the middle 50% of the data with the horizontal line in the box indicating the median value. Whiskers extending from the boxes indicate the upper and lower 25% of the data. Sample sizes are given in Table 2.

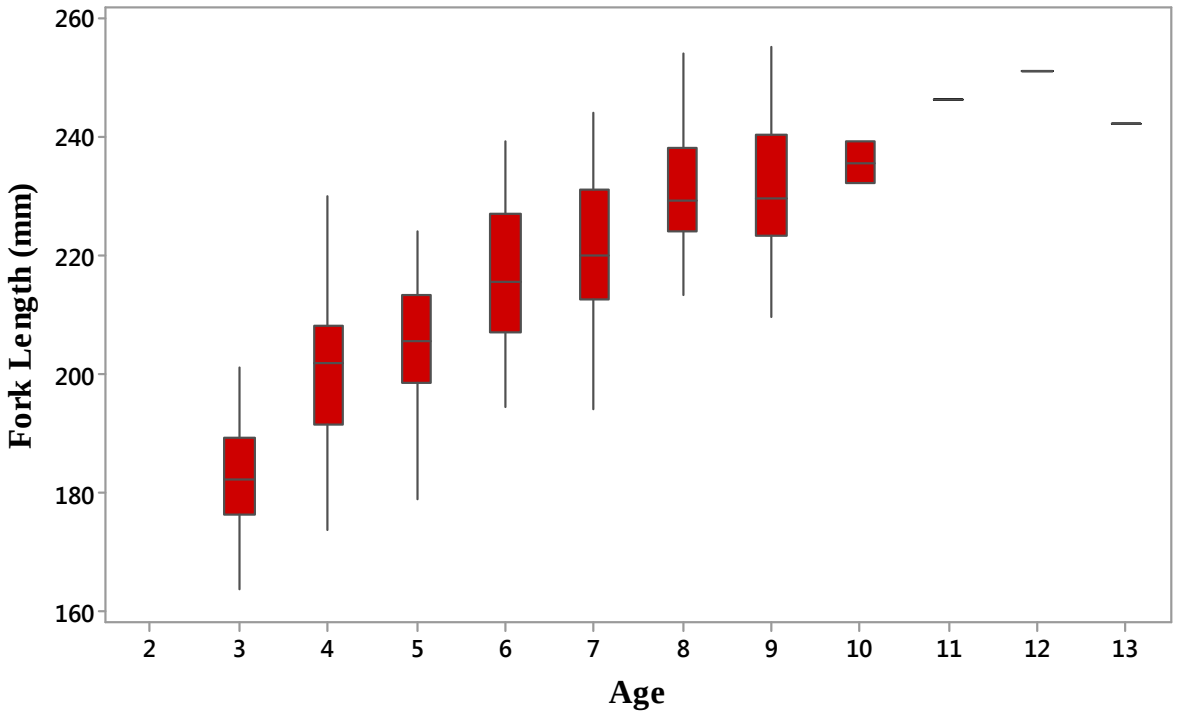

Figure 16. Fork length at age of female Pacific herring (*Clupea pallasii)* collected in Prince William Sound from spawning aggregations in 2013. Colored boxes depict the middle 50% of the data with the horizontal line in the box indicating the median value. Whiskers extending from the boxes indicate the upper and lower 25% of the data. For herring ages 11 through 13 where only horizontal lines are shown, sample sizes are 1. Sample sizes are given in Table 3.

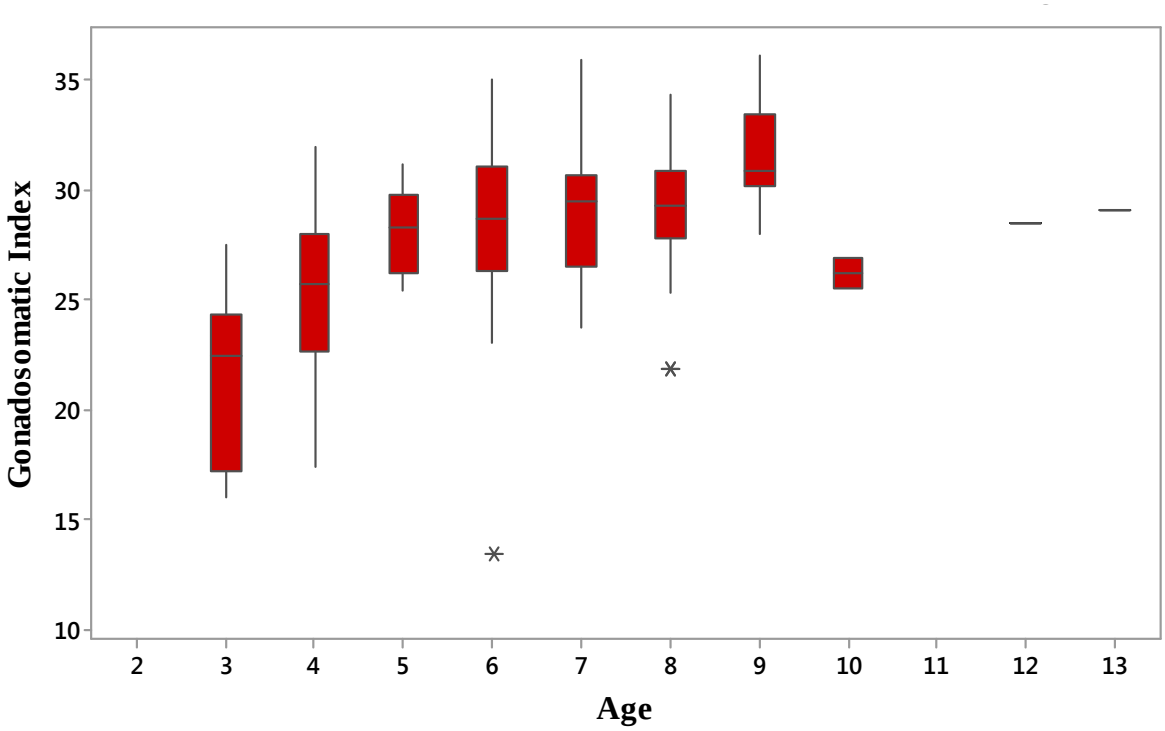

Figure 17. Gonadosomatic index (GSI) of female Pacific herring (*Clupea pallasii)* collected in Prince William Sound from spawning aggregations in 2013. Colored boxes depict the middle 50% of the data with the horizontal line in the box indicating the median value. Whiskers extending from the boxes indicate the upper and lower 25% of the data. For herring ages 12 and 13 where only horizontal lines are shown, sample sizes are 1. Asterisks indicate outliers. Sample sizes are given in Table 3.

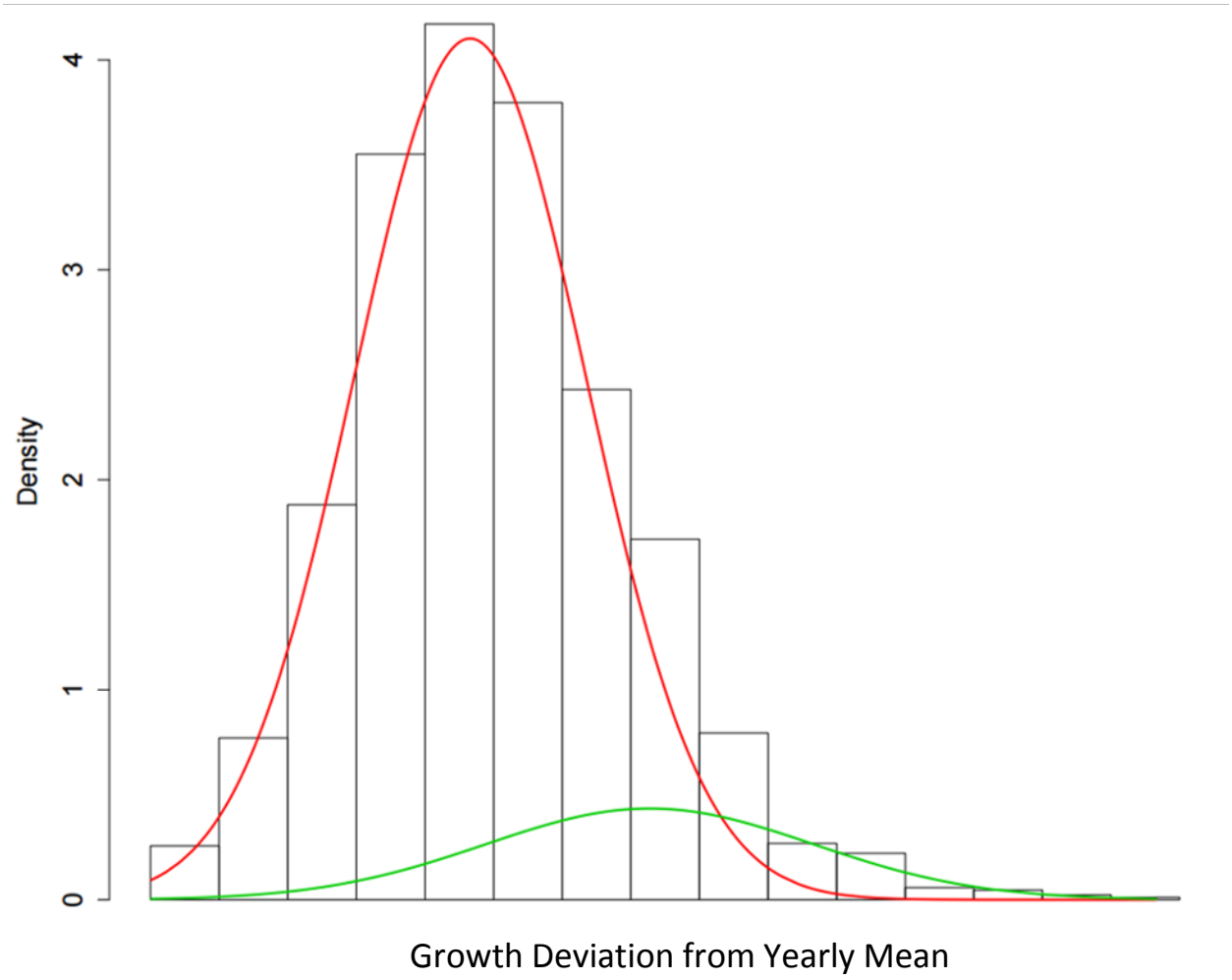

Figure 18. Example of bimodal distribution of scale annulus widths representative of growth of 5 year old female Pacific herring (*Clupea pallasii)* from ADF&G's herring scale library measured for EVOSTC project 13120111-N (Moffitt and Andersen 2017). The red curve represents growth of 5 year old spawning herring and the green curve represents 5 year old non-spawning herring.