EVOSTC FY17-FY21 INVITATION FOR PROPOSALS
FY19 CONTINUING PROJECT PROPOSAL SUMMARY PAGE

Project Number and Title

19170111-D
Studies of Reproductive Maturity among Age Cohorts of Pacific Herring (*Clupea pallasii*) in Prince William Sound, Alaska

Primary Investigator(s) and Affiliation(s)

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Date Proposal Submitted

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Project Abstract

To address the lack of recovery of Pacific herring (*Clupea pallasii*, hereafter herring) in Prince William Sound (PWS), Alaska, research by the Herring Research and Monitoring (HRM) Program has been focused on improving predictive models of PWS herring stocks through observations and research. To this end, the goal of the project described here is ***to test the PWS herring Bayesian Age-Structured Assessment model's age at maturity schedule with empirical data***. 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 two through five) in PWS. The proportion of immature and mature herring per age cohort of interest can then be determined 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 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; and 4) Assess annual variation in herring age at maturity schedules before and after 1997 using Alaska Department of Fish & Game's (ADF&G) PWS herring scale image library, which allows for understanding maturity schedules of past cohorts. The work to be conducted in FY19 is focused on Objectives 2-4, namely (2) 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 will follow those employed in FY17 and FY18. The work on Objective 3 couples 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 will focus on evaluating the potential of the scale technique for estimating past maturity schedules using ADF&G's PWS herring scale image library. This analysis will examine the progression of bimodal distributions in scale growth as a cohort of herring passes through time. The prediction is 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. Methods for conducting the scale image library analysis have been included in this proposal.

EVOSTC Funding Requested* ***(must include 9% GA)***					
FY17	**FY18**	**FY19**	**FY20**	**FY21**	**TOTAL**
Auth:$170,000	**Auth:$172,000**	**$165,100**	**$169,600**	**$173,300**	**$850,000**

Non-EVOSTC Funds to be used, please include source and amount per source:					
FY17	**FY18**	**FY19**	**FY20**	**FY21**	**TOTAL**
0	**0**	**0**	**0**	**0**	**0**

**If the amount requested here does not match the amount on the budget form, the request on the budget form will considered to be correct.*

1. PROJECT EXECUTIVE SUMMARY

Provide a summary of the program including key hypotheses and overall goals, as submitted in your original proposal. Please include a summary and highlights since your last annual report: preliminary results with figures and tables. If there are no preliminary results to present, please explain why (i.e., lab analysis is still in progress). List any publications that have been submitted and/or accepted since you submitted your last proposal and other products in *Section 7*. Prior annual reports will be appended to remind reviewers of progress in previous years.

Project Background, Key Hypotheses and Overall Goals

Background

The overall goal of the Prince William Sound Herring Research and Monitoring (PWS HRM) program continues to focus on *improving predictive models of herring stocks through observations and research*. During the last five-year program (2012-2016), the Age-Structure-Analysis (ASA) model was updated in a Bayesian framework, which can more objectively weight different datasets and provide estimates of uncertainty in model parameters and output (Pegau et al. 2014, Muradian et al. 2017). Key ASA model parameter estimates include age at reproductive maturity, i.e., the proportion of mature herring among fish aged 3-5, which is used to estimate the total population biomass based on the age composition of the spawning population. Validating the estimated proportions of immature and mature individuals in each age class is the key objective of the study proposed here as the former and current ASA models' reproductive maturity schedule (Table 1) is not currently based on empirical data, but estimates values that allow the model to best fit the data (Hulson et al. 2008, Muradian et al. 2017).

Table 1. Reproductive maturity parameterization used in former and current (Bayesian) ASA models for Pacific herring in Prince William Sound, Alaska. Values for Muradian et al. (2017) are median percent spawning and associated credible intervals.

Age	*% Spawning*	*% Spawning*	*Reference*
	1980-1996	*1997-2004*	
3	27	48	Hulson et al. 2008
4	89	75	Hulson et al. 2008
5	100	100	Hulson et al. 2008
	1980-1996	*1997-2012*	
3	39 (28, 56)	49 (37, 66)	Muradian et al. 2017
4	80 (62, 97)	90	Muradian et al. 2017
5	100 (assumed)	100 (assumed)	Muradian et al. 2017

Age at reproductive maturity is a key demographic parameter included in stock assessment models such as the ASA. At the individual level, age at maturity can shape overall lifetime reproductive success (Stearns 1992, Bernardo 1993), and therefore, contribute to stock productivity. There is some evidence that age at maturity might vary with population size. For example, Engelhard and Heino (2004a) showed that at 50% maturity Norwegian spring-spawning herring age was reduced and length increased during a period of low stock abundance in comparison with periods of higher stock abundance. The selective factors that likely contributed to this phenomenon include 1) reduced intraspecific competition for food during low abundance that resulted in enhanced growth and early onset of maturity or 2) early reproducing fish were selected for by fishing pressure - the former appears to be a more likely explanation (Engelhard and Heino 2004b).

Within the context of Pacific herring, it is generally understood that age at first maturity increases with latitude from about age two off California, to age four or five in the Bering Sea, western Alaska (Barton and Wespestad 1980, Hay 1985). Given this regional variability in age at maturity, it is unclear whether the proportions of fish spawning for the first time estimated by PWS ASA models, as shown in Table 1, are valid. Vollenweider and coworkers (NOAA-Auke Bay Labs) conducted preliminary studies of age at maturity among PWS herring during the previous HRM program (2012-2016) focusing on the validity of using scale growth to assess age at maturation and skipped spawning among PWS herring. There is considerable debate regarding the use of scale growth measurements as an accurate proxy of reproductive investment by herring (e.g., Kennedy et al. 2011). For example, Engelhard et al. (2003) employed this technique for Norwegian spring-spawn herring (*Clupea harengus*), which have been shown to undergo changes in the width and microstructure of annual scale growth layers in relation to the formation of gonads (see Lea 1928 and Runnstrom 1936 in Engelhard et al. 2003). Engelhard et al. (2003) demonstrated high classification success using two different techniques. However, other researchers have noted that reading errors of scales in age at maturity studies of Norwegian spring-spawn herring may have contributed to over estimation of skipped spawning by second time spawners (Kennedy et al. 2011). A similar approach has been tested in Pacific herring. Vollenweider et al. (2017 EVOS Final Report 13120111-J) examined the scale growth technique commonly used for Norwegian spring-spawn herring in discriminating spawners from non-spawners and concluded that the approach may be extended to Pacific herring in PWS. However, aspects of the work by Vollenweider et al. (2017) was based on relatively small sample sizes.

Research by Vollenweider and coworkers included a lab study of southeast Alaska herring to determine the seasonal timing when spawned female herring could be identified using histological characteristics of ovaries. They confirmed that histology of ovaries is an accurate method for discerning previously spawned females within three months after spawning (Vollenweider et al. 2017). Vollenweider et al. (2017) also conducted a summer only field study (2012) of female PWS herring coupling ovary maturation indices with scale growth patterns. They hypothesized that high energetic provisioning of gonads before the first reproductive effort results in reduced growth that is reflected in the width of scale annuli (Roff 1983, Scott and Heikkonen 2012). Vollenweider et al. (2017) found that during July 2012, immature three-year-old PWS herring not developing for spawning based on gonad maturation and ovary histology had significantly elevated scale growth relative to three-year-old herring preparing to spawn for the first time (n = 17 for age three fish, n = 166 for ages three through 11). Finally, Vollenweider et al. (2017) also examined ADF&G's long-term (1986-2013) herring scale library regarding bi-modal variability in annuli widths for specific age cohorts. With over 1,700 scale samples, analyses revealed that male and female PWS herring ages one and two showed unimodal scale growth, indicating that these fish were non-spawning, immature fish. However, male and female PWS herring ages three through six showed differentiation in scale growth patterns, which was interpreted as evidence of variable spawning activity.

Several aspects of the work reported by Vollenweider et al. (2017) warrant further investigation:

- First, Vollenweider and coworkers never reported estimates of the proportion of immature and mature PWS herring for age cohorts of interest to the ASA models, namely ages two through five (ages that bracket those that are estimated).
- Secondly, inter-annual variability in the proportion of immature and mature PWS herring for age cohorts of interest to the ASA model was not addressed.
- Finally, although Vollenweider and coworkers demonstrated that scale growth patterns reflect gonad maturation, for three-year-old PWS herring and using the ADF&G PWS herring scale library (1986-2013), analyses were unable to retrospectively assess age at maturity before and after 1997 as all data across years were pooled for analysis. Of note, 1997 is the year Hulson et al. (2008) determined maturity schedules changed for PWS herring.

Here, proposed studies aim to build from previous research reported by Vollenweider et al. (2017). Studies are designed to test the estimated age at maturity function in the ASA models primarily using direct estimates of gonad maturity of PWS adult herring, and secondarily through the continued validation of the scale growth technique examined by Vollenweider et al. (2017). Importantly, if the scale growth technique is feasible, it would allow for the determination of age at maturity for the population once individuals have recruited.

Key Hypotheses

H1 (NULL). There is no seasonal variability (spring, summer, fall, and winter) in the determination of both previously spawned and maturing female herring, and maturing male herring, (ages two through five) based on direct measures of gonad maturation (gonad somatic index (GSI) and Hjort indices, ovary histology). Mature female and male herring will have higher GSI and Hjort indices and be heavier and longer than immature herring for a given age cohort. ***Addressed during FY17 and FY18***.

H2 (NULL). For herring collected at the optimal seasonal sampling time (based on H1), there is no inter-annual variability in the proportion of immature and mature individuals. ***Addressed in FY17-FY21***.

H3. Maturation status, within a given age cohort (ages two through five), will correspond to scale growth patterns. Maturation criteria (GSI and Hjort indices, ovary histology) will show a corresponding reduction in the width of the annual scale growth layer for that year because energy is invested into reproduction and away from somatic growth. ***Samples collected in FY17 & FY18, to be further addressed in FY19-FY21***.

H4. Age at maturity at the individual level (following H3) can be used to assess annual variation in maturity schedules before and after 1997 using ADF&G's PWS herring scale library. ***Initial analysis addressed in FY19.Further analysis planned for FY 20-21.***

Importantly, the key aspect of the scale growth technique, if validated as a measure of reproductive investment, is that by assessing scale growth of older, recruited fish, a complete history of spawning effort can be understood. Such histories are especially critical during ages 2 through 4 when fish are spawning for the first time and might be more difficult to capture to assess direct measures of gonad maturation due to possibly being located outside the spawning population or in little-known areas. Thus, although development of the scale technique as a proxy of reproductive investment might be considered a risky research endeavor, should it prove valid (as preliminary work by Vollenweider et al. (2017) suggests), the technique offers a more powerful approach for sampling the entire population, unlike basing the proportion of mature and immature fish at age on sampling methods, since it is impossible to know if sampling has been representative of the entire population. Further, if the scale technique is validated, and ADF& G's herring scale image library shows the potential for evaluating past maturity, the project can further assess herring maturity schedules before and after 1997, the year Hulson et al. (2008) determined maturity schedules changed for PWS herring.

Overall Program and Project-Specific Goals

The overall goal of the proposed Herring Research and Monitoring Program (2017-2021) is *to improve predictive models of PWS herring stocks through observations and research*. To this end, the goal of the project described here is ***to test the PWS herring Bayesian Age-Structured Assessment model's age at maturity schedule with empirical data.***

Proposed research will focus on adult female and male herring caught in PWS to provide annual estimates of the proportion of immature and mature herring for age cohorts of interest to the ASA model (ages two through five) using simple and direct measures of gonad maturation such as a gonadosomatic index (GSI) following Hay and Outram (1981), the Hjort criteria as outlined by Hay (1985), and ovary histology of females (Bucholtz et al. 2008, Brown-Peterson et al. 2011).

As a secondary effort, studies will continue to validate the use of scale growth measures as a technique for discerning age at maturation for both female and male herring in PWS. The advantage of using scale growth as a measure of age at maturity for herring, if accurate, is that it allows for sampling the entire population after individuals have recruited, as opposed to direct measures of gonad maturity that require sampling of younger fish that may be located differently in time or space from the spawning population.

Finally, over the five-year program between FY17-FY21, the project will assess inter-annual variability in the proportion of immature and mature individuals by examining field samples collected during the current five-year program, and by further examining herring maturity schedules before and after 1997 using ADF&G's PWS herring scale image library. An initial effort in working with ADF&G's scale library will begin in FY19 to assess the potential for the scale library to estimate past herring maturity.

Summary of Progress (since submission of FY 17 Annual Report in February 2018)

Significant progress has been made since February 2018 when the EVOS FY17 annual report was submitted for this project. The project was able to obtain structural size, weight, and gonad maturity measurements (GSI and Hjort criteria), but not histology samples, from adult herring, collected in March 2018 (n = 53) by a NOAA cruise led by John Moran that operated in PWS. These samples will be interesting as they were obtained ahead of spawning and will contrast with the previous samples collected in November 2017.

Between April 8-11, 2018, significant numbers of herring were captured during the spring spawn by an ADF&G cruise using a purse seine, and also by a PWSSC cruise using jigging gear. Overall 769 adult herring were processed from this sampling event following the full suite of measurements. The scales from these fish have been mounted for aging and scale growth measurements. Data have been entered into our Access database for this project and are stored on the HRM workspace. Histology samples obtained have not yet been sent for analysis as we would like to send the summer and fall 2018 samples all at once due to the cost of hazardous material shipping to the east coast where the pathology lab is located. The aging of these fish is currently underway by a PWSSC lab assistant. Of note, it is excellent that we were able to sample this many fish during spring 2018 spawning as it was the lowest miles day of spawn in ADF&G's record since 1974.

Between July 5-8, 2018, we deployed a field team in PWS to collect our summer samples of adult herring. We were able to collect and process 850 adult herring for this project following the full suite of measurements. Unlike during 2017, we found fish mainly in the Montague Entrance area of PWS. A local salmon seiner provided us with herring that was caught in their purse seine. An additional 150 fish were obtained via jigging, the methods we proposed in our revised FY18 proposal as being a more efficient method than using trawl gear. The scales from these samples are currently being mounted and therefore aging and scale growth measurement results are not yet available.

The project will deploy personnel on the NOAA/USGS (Moran/Arimitsu) EVOS predator/prey cruise in September 2018. We are hopeful with larger numbers of herring apparent in PWS during July 2018 that more fish will be available for collection as part of this study during fall 2018 as in September 2017 we were only able to collect ~20 adult herring during this time period.

During spring 2018 the project made significant progress in working towards measuring scale growth for fish collected in FY17. We initially aimed to use ADG&G's scale scanner and imaging software, however, the imaging software was obsolete and unable to run on their computer. This resulted in PWSSC purchasing an upgraded version of the imaging software for just under $5000 and a new laptop to run the updated software. The project has hired an experienced assistant to measure adult herring scale growth. In early July 2018, we were finally able to connect with ADF&G staff in Juneau regarding the macro they use with the imaging software to measure fish scale growth. Thus, scale measurements for the fish (n = 157) collected in FY 17 that were sent for histological analysis was recently completed (early August 2018). We are pleased with our progress in setting up the imaging software and macro needed to do the measurements. Lorna Wilson (ADF&G Juneau) was instrumental in assisting us as we learned the new program. A recent communication (early August 2018) with the lab conducting our histological analysis of ovary samples indicated that the samples sent in January 2018 have been mounted on slides and will soon be evaluated for maturation states by a veterinary pathologist. I anticipate these results will be available by September/October 2018.

In spring 2018, the project purchased a new marine scale for $8000, which will significantly help with weighing fish on vessels while they are fresh rather than having to hold them and process them later in the lab.

Preliminary Analysis of Past Samples (Fall 2017 – Spring 2018)

Fall 2017

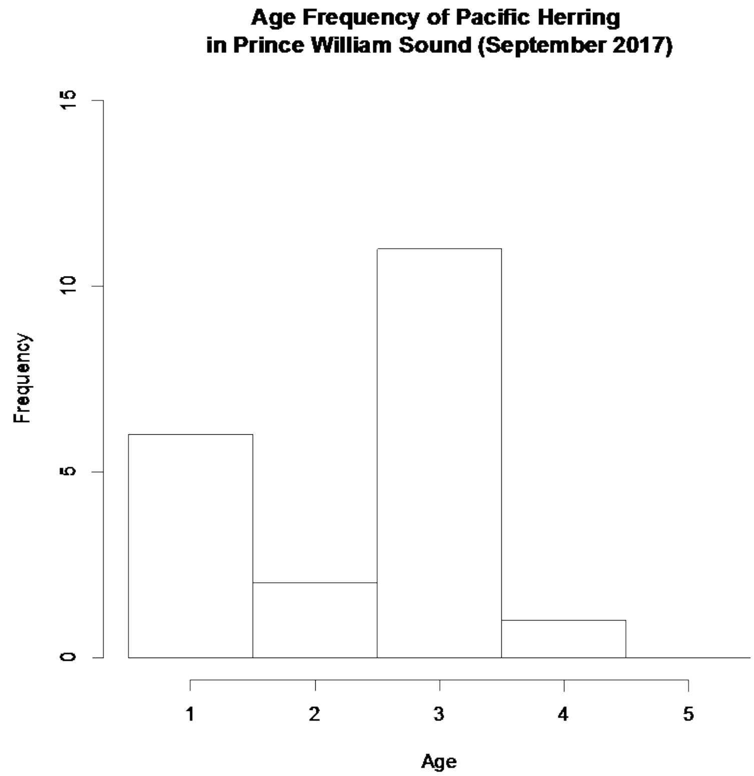

Figure 1. Age frequency distribution of adult herring caught in September 2017.

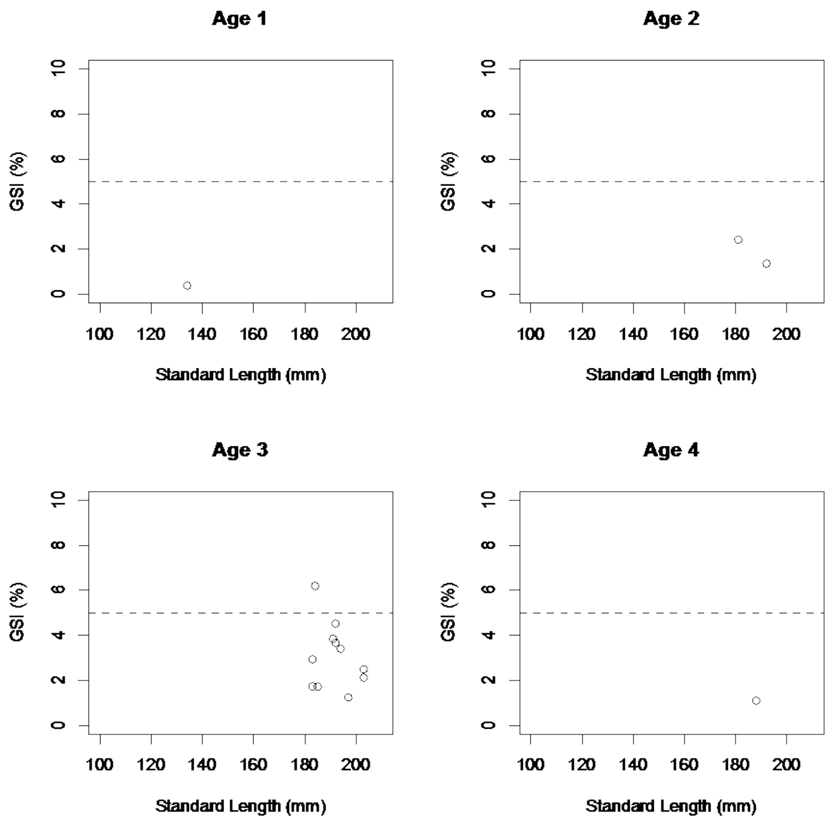

Figure 2. Size and gonadosomatic index data for age cohorts of adult pacific herring caught in September 2017. Dashed line indicates the gonadosomatic index threshold between reproductive immaturity (<5%) and maturity (>5%) following criteria outlined by Hay and Outram (1981) and Hay (1985).

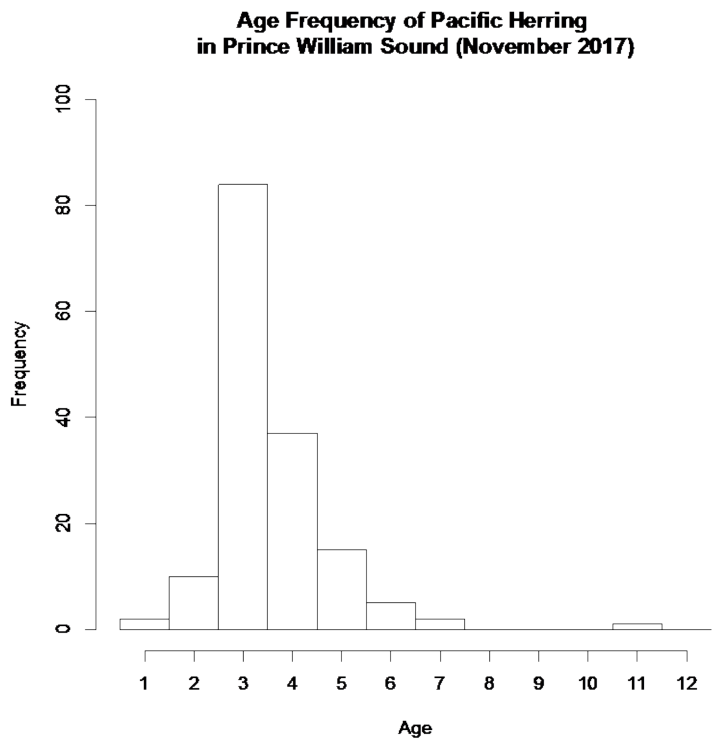

Figure 3. Age frequency distribution of adult herring caught in November 2017.

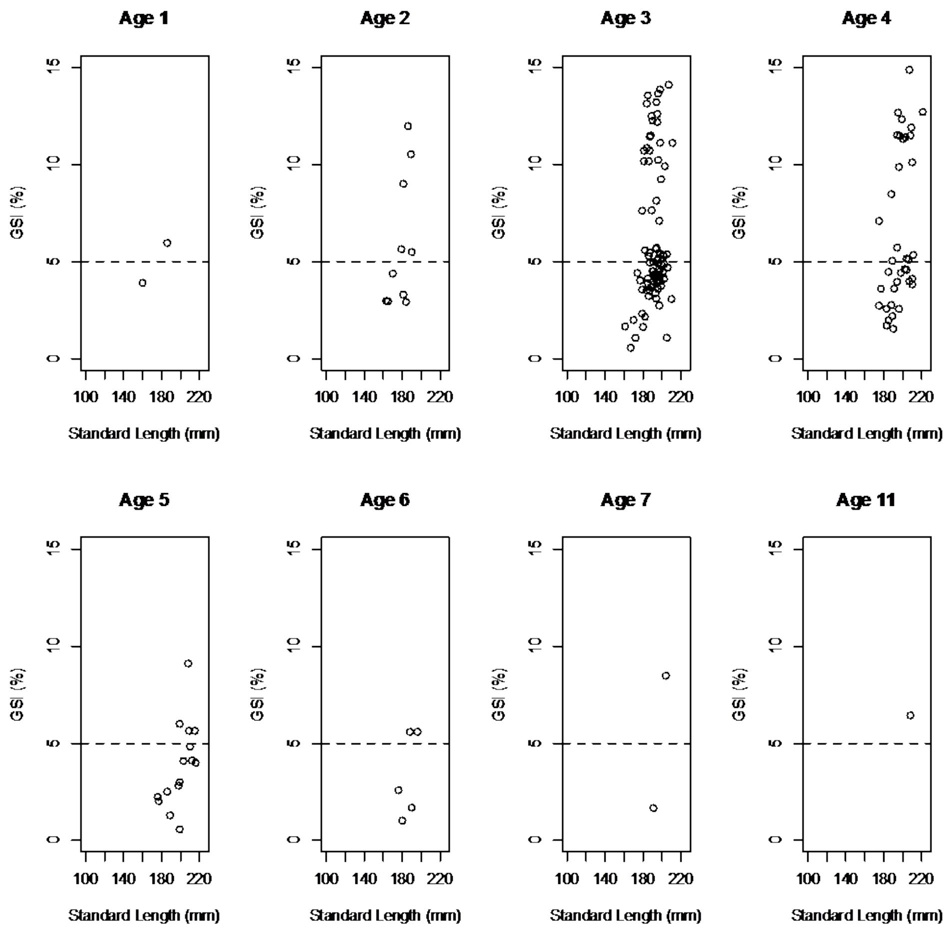

Figure 4. Size and gonadosomatic index data for age cohorts of adult pacific herring caught in November 2017. Dashed line indicates the gonadosomatic index threshold between reproductive immaturity (<5%) and maturity (>5%) following criteria outlined by Hay and Outram (1981) and Hay (1985).

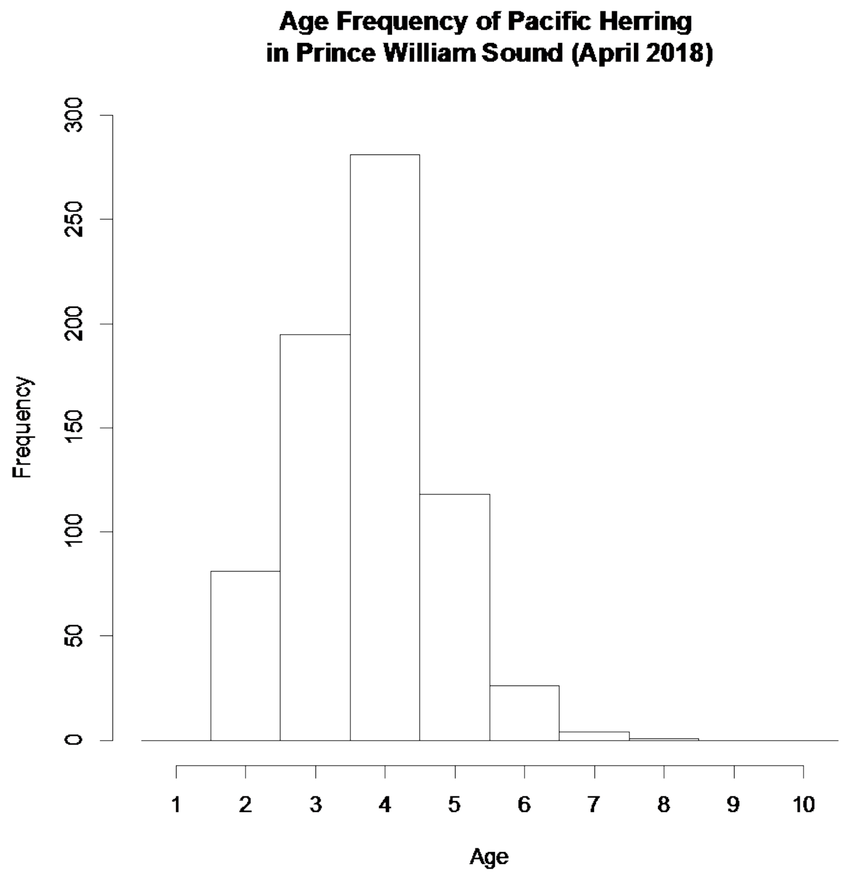

Figure 5. Age frequency distribution of adult herring caught in April 2018.

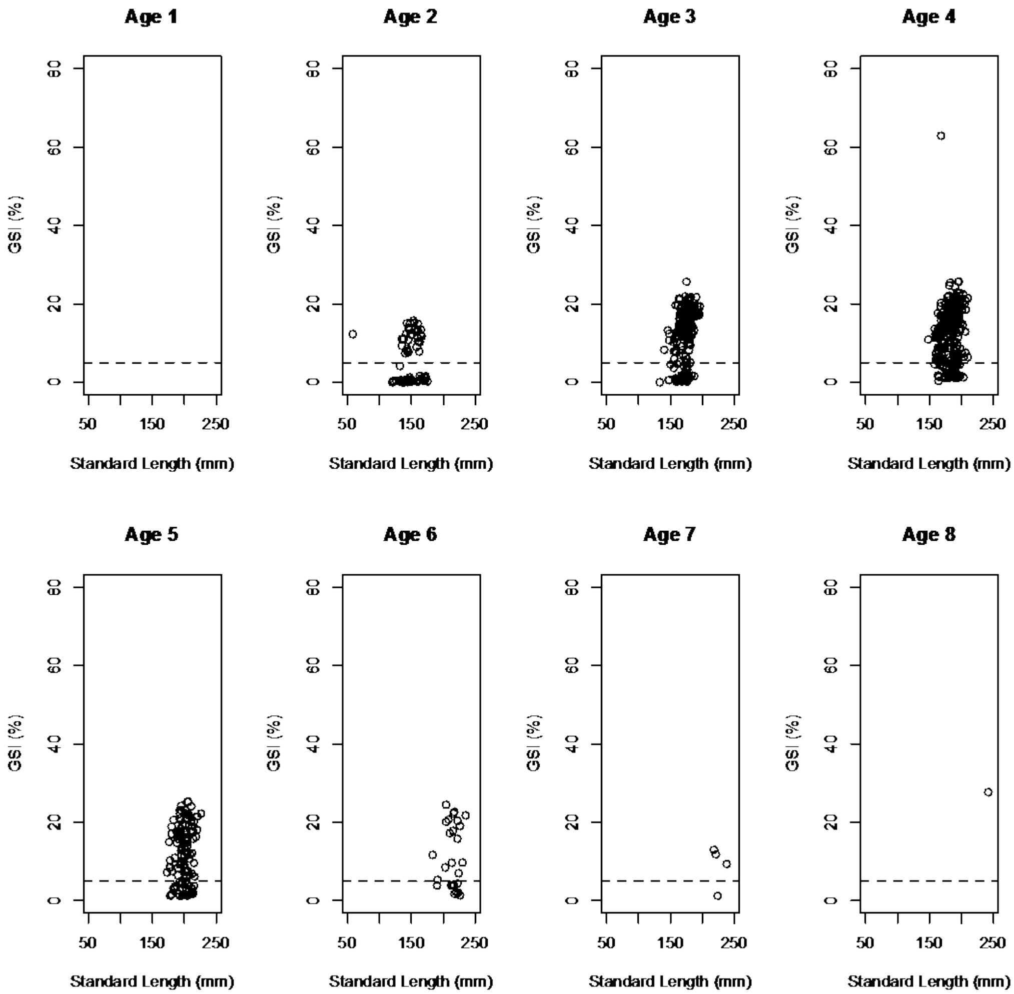

Figure 6. Size and gonadosomatic index data for age cohorts of adult pacific herring caught in April 2018. Dashed line indicates the gonadosomatic index threshold between reproductive immaturity (<5%) and maturity (>5%) following criteria outlined by Hay and Outram (1981) and Hay (1985).

<u>Discussion of Preliminary Analysis of Past Samples (Fall 2017 – Spring 2018)</u>

The age frequency distribution of adult herring caught in September 2017 indicated that three-year-olds were the dominant age class represented by the few samples collected (Fig. 1). In November 2017, age three herring were clearly the dominant age class (Fig. 3), confirming observations from the fall. Age 4 herring were also dominant during November 2017 collections, with ages two and five representing smaller numbers of individuals (Fig. 3). During spring 2018, age four herring were the dominant age class, followed by age three herring (Fig. 5). Age two and five herring also contributed larger numbers of individuals in the spawning population. Of note, the presence of age two herring among fish caught during the spring spawn (Fig. 5) suggests that age two fish are contributing to the spawning population, particularly because a large proportion of age two herring had developed gonads (Fig. 6).

Regarding gonad development, interestingly, age three fish had primarily undeveloped gonads in September 2017 (Fig. 2). However, by November 2017, a larger percentage of age three fish had developed gonads (Fig. 4). Based on data analysis for the FY2017 annual report, the majority of fish with developed gonads during November 2017 were male herring (see Fig. 13b in 17170111-D Gorman FY17 annual report). Interestingly, in comparison with herring sampled during spring 2017, the spring 2018 fish had a higher percentage of fish with undeveloped gonads, or less than 5% GSI. I believe this is because the ADF&G collections in spring 2018 initially caught adults right after the spring spawn, they were unable to catch fish ahead of the main spawning event. This this result is likely an artifact of the timing of sampling. However, histology results will confirm whether or not these with less developed gonads show evidence of spawning given the presence of post-ovulatory follicles.

More final analysis of data will be completed for the FY18 annual report due in February 2019.

2. PROJECT STATUS OF SCHEDULED ACCOMPLISHMENTS

A. Project Milestones and Tasks

Milestones are annual steps to meet overall project objectives. For each milestone listed, specify the status (completed, not completed) when each was completed and if they are on schedule, as submitted in your most current proposal.

Tasks are annual steps to meet milestones. Specify, by each quarter of each fiscal year, when critical tasks (for example, sample collection, data analysis, manuscript submittal, etc.) were and will be completed.

Please identify any substantive changes and the reason for the changes. *Reviewers will use this information in conjunction with annual program reports to assess whether the program is meeting its objectives and is suitable for continued funding.*

B. Explanation for not completing any planned milestones and tasks

Please identify any substantive changes and the reason for the changes. If tasks were not completed as scheduled or delayed, please explain why and the anticipated completion date.

C. Justification for new milestones and tasks

Please identify any new milestones and tasks and the reason why they have been added.

A. Project Milestones and Tasks

Project milestone and task progress by fiscal year and quarter, beginning February 1, 2017. Yellow highlight indicates proposed fiscal year Work Plan. Additional milestones and tasks may be added. C = completed, X = not completed or planned. Fiscal Year Quarters: 1= Feb. 1-April 30; 2= May 1-July 31; 3= Aug. 1-Oct. 31; 4= Nov. 1-Jan 31.

	FY17				FY18				FY19				FY20				FY21			
Milestone/Task	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Fieldwork																				
Spring field collection	C	C			C	C														
Summer field collection		C				C	C													
Fall field collection			C	C			X	X			X				X				X	
Lab work																				
Ship spring histology samples for analysis	X	X			X															
Ship summer histology samples for analysis		X	X			X	X													
Ship fall histology samples for analysis			X	X			X	X			X				X				X	
Scale readings				X				X				X				X				X

Data																				
Determine proportions of spring immature and mature herring per age cohort of interest	X	X			X															
Determine proportions of summer immature and mature herring per age cohort of interest		X				X														
Determine proportions of fall immature and mature herring per age cohort of interest			X	X			X	X												
Upload data and metadata to AOOS portal	X	X	X	X	C	X	C	X	X	X	X	X	X	X	X	X	X	X	X	X
Reporting																				
Annual reports					C				X				X							
FY work plan (DPD)			C				C				X				X				X	
Draft FY17-21 Final Report																				X
Publications																				
Draft Manuscript 1*								X												
Submit Manuscript 1																				
Conferences and Meetings																				
Annual PI meeting				C				X				X				X				X
Attend/present at AMSS				C				X				X				X				X
New Milestone																				
PWS Herring Scale Image Library Preliminary Analysis												X								

*Manuscript 1: Seasonal timing of collections and results concerning the proportions of immature and mature herring

B. Explanation for not completing any planned milestones and tasks

FY17 tasks not completed: Although the project completed field campaigns in summer and fall 2017, no fish were collected during our June 2017 fieldwork (summer) and only a small number of fish were collected during September 2017 (fall) fieldwork. As noted in the extensive response to reviewer comments during fall 2017 regarding the FY18 work plan, the consensus among researchers in 2017 was that herring were mainly absent from PWS during summer and early fall. Thus, our lack of samples did not reflect a lack of effort to collect samples, but the fact that the herring population in PWS is reaching levels where they appear to absent from PWS for some periods of the year.

The shipping of histology samples for those collected in FY17 did not occur until January 2018 (n = 157) as until November 2017 we only had significant samples from Spring 2017. Further, the cost of shipping these samples was high given that they must be shipped as hazardous material to the east coast where the histology/pathology labs are located, thus sending them all together in one shipment was the most cost-effective way to manage this part of the project. Going forward, histology samples will be sent for analysis once the final ageing for processed fish has been conducted, which will be completed as soon as possible after sampling.

Scale growth measurements were completed in August 2018 for sampled FY17 herring that were also sent for histological analysis (n = 157). Measuring scale growth took some time to complete as ADF&G’s imaging software and computer were obsolete. This required PWSSC to purchase new software, and laptop, and work with

ADF&G in Juneau during spring and summer 2018 to understand the macro needed to conduct this analysis. Going forward, we will now be much for efficient in producing these data.

Determining the proportions of FY17 spring, summer and fall immature and mature herring was completed as part of the FY17 annual report that was submitted in February 2018. Additional information on the age class distribution of the samples collected during FY17 and spring FY18 is present as part of this work plan, in Section 1.

Data were submitted to the AOOS workspace as the time the FY17 annual report was submitted (February 2018). The Access database will be updated to include currently available data in association with the submission of this work plan in August 2018.

FY18 tasks not completed: The aging of herring collected in spring 2018 was just recently completed (July 2018). We are currently mounting scales and aging the fish collected during summer 2018. The histology samples for fish collected in spring, summer, and fall 2018 will be sent as soon as possible. Scale measurements for spring, summer, and fall 2018 will be conducted after histology samples have been selected. The proportions of immature and mature herring for each collection period will be conducted once aging data are available.

C. Justification for new milestones and tasks

Following initial Science Panel review of the FY19 proposal, a new milestone has been added to the FY19 project. This task will focus on evaluating the potential for ADF&G's herring scale library to estimate past herring maturity, such as before and after 1997 - the year Hulson et al. (2008) determined maturity schedules changed for PWS herring. The details of this analysis are provided below in Changes to Project Design and Objectives.

3. PROJECT COORDINATION AND COLLABORATION

A. Within an EVOTC-Funded Program

Provide a list and clearly describe the functional and operational relationships with any EVOSTC-funded Program (Herring Research and Monitoring, Long-Term Research and Monitoring or Data Management Programs). This includes any coordination that has taken or will take place and what form the coordination will take (shared field sites or researchers, research platforms, sample collection, data management, equipment purchases, etc.).

B. With Other EVOSTC-funded Projects

Indicate how your proposed project relates to, complements or includes collaborative efforts with other proposed or existing projects funded by the EVOSTC that are not part of a EVOSTC-funded program.

C. With Trustee or Management Agencies

Please discuss if there are any areas which may support EVOSTC trust or other agency work or which have received EVOSTC trust or other agency feedback or direction, including the contact name of the agency staff. Please include specific information as to how the subject area may assist EVOSTC trust or other agency work. If the proposed project requires or includes collaboration with other agencies, organizations or scientists to accomplish the work, such arrangements should be fully explained and the names of agency or organization representatives involved in the project should be provided. If your proposal is in conflict with another project, note this and explain why.

Coordination within the program

The proposed work will couple sampling during the first two years of the study with another HRM investigator leading adult herring acoustics (P. Rand, PWSSC). Further, the data will be used to validate the ASA model maturity schedule estimates (T. Branch, UW).

Coordination with other EVOSTC-funded programs

The work described here plans to build off relationships established with GWA including maximizing the forage fish (M. Arimitsu and J. Piatt, USGS) and whale survey (J. Moran and R. Heintz, NOAA) efforts to collect samples for this study.

Coordination with Management Agencies

The scale aging and growth measurement work is collaborative with ADF&G (S. Haught, ADF&G Cordova).

4. PROJECT DESIGN

A. Overall Project Objectives

Identify the overall project objectives for your project as submitted in your original proposal.

B. Changes to Project Design and Objectives

If the project design and objectives have changed from your original proposal, please identify any substantive changes and the reason for the changes. Please include the revised objectives in this section. Include any information on problems encountered with the research or methods, if any. This may include logistic or weather challenges, budget problems, personnel issues, etc. Please also include information as to how any problem has been or will be resolved. This may also include new insights or hypotheses that develop and prompt adjustment to the project.

Overall Project Objectives

The objectives of the proposed research follow:

1) Assess the seasonal timing (spring, summer, fall, and winter) that allows for accurate determination of both previously spawned and maturing female herring, and maturing male herring, based on direct measures of gonad development to determine maturation states and the proportion of immature and mature herring among ages cohorts of interest (ages two through five) in PWS (FY17-18).

Although a laboratory study by Vollenweider et al. (2017) of post-spawn female herring collected in Southeast Alaska indicated that the presence of post-ovulatory follicles were a reliable indicator of past spawning activity within three months, I continue to test the null hypothesis so that these relationships can be established specifically for PWS herring. This proposal assumes that age 6+ fish are 100% mature and therefore are not considered in the sampling design. The proposal also assumes that evidence of gonad maturation indicates that an adult herring will, in fact, spawn in the spring. For some herring systems (i.e., Norwegian Spring-Spawn herring) there is evidence that females may not abandon ovary development until very close to the spawning event (Kennedy et al. 2011). There is no indication that late abandonment of gonad development occurs in PWS herring. Therefore, this proposal assumes that gonad maturation indicates that spawning will occur in the spring. Once the optimal seasonal sampling time is determined that best identifies past and future spawning effort (Objective 1), subsequent years (through FY21) will continue to sample at this time only to address inter-annual variability in the proportion of immature and mature herring per age cohort (see Objective 2).

2) Assess inter-annual variability (FY19-21) in the proportion of immature and mature herring in PWS collected at the optimal seasonal time as determined by Objective 1 per age cohort of interest.

3) Couple histological assessment of maturity with annual scale growth data at the individual level, within specific age cohorts, to understand if scale growth patterns reflect reproductive investment (FY19-21).

4) Assess annual variation in herring age at maturity schedules before and after 1997 using ADF&G's PWS herring scale library given results from Objectives 1 through 3 (FY21). A preliminary analysis related to the PWS herring scale library has been added to the FY19 proposal, which aims to begin evaluating the potential of ADF&G's PWS herring scale image library to estimate past herring maturity by first detecting bimodal distributions in herring scale growth as a cohort of fish ages over time.

The data produced by these studies will test the former and current (Bayesian) ASA models' age at maturity schedule. The proposed work builds on previous work by HRM investigators regarding age at maturity of PWS herring (Vollenweider et al. 2017). However, there are some clear differences from the past work supported by EVOSTC. First, the proposed research will primarily focus on direct measures of gonad maturation to produce annual estimates of the proportion of immature and mature herring in PWS for age cohorts of interest. The proposed research will cautiously continue to validate scale growth as a technique for discerning age at reproductive maturity among Pacific herring by increasing sample sizes of fish processed for both gonad maturation and scale growth patterns within age cohorts. Finally, should the scale growth technique prove to be reliable, future work will aim to use the approach to assign an age at maturity schedule for fish sampled before and after 1997 as part of the ADF&G scale library. An important initial step in working with ADF&G's PWS herring scale image library will begin in FY19 to understand the potential for the scale image library to detect past herring maturity.

The anticipated final products from this work will be a series of papers that address each of the main hypotheses outlined above, in addition to validating the age at maturity function estimated by PWS herring ASA models (Hulson et al. 2008, Muradian et al. 2017).

Procedural and Scientific Methods (specifically for FY19)

Objective 2. Once the optimal seasonal timing of sampling is determined during the first two years of the project, the following three years will focus collections on this one time period and increase the sample sizes for age 2+ through 5+ fish. In years 3-5, samples sizes for age 2+ through 5+ fish during one collection period only will be n = 460 each for a total of 1840/year. Data will be collected in the field and at the PWSSC lab following procedures previously outlined, gonad maturation will be determined by GSI and Hjort indices, and a professional veterinary pathologist will determine female maturation states based on ovary histology.

Objective 3. This study will build from the first two years of data collected where gonad maturation characteristics based on GSI and Hjort criteria, as well as histology, will be compared with annual scale growth data at the individual level within age cohorts. Fish will be processed in the field and lab as outlined above, and the individual scale annuli of both females and males will be measured using ADF&G imaging software. The key to the success of this part of the project is to be able to compare scale growth patterns from individuals of the same age cohort that have differing gonad characteristics. For example, scale growth patterns of age 3+ females that have evidence of POFs and developing follicles will be compared with other age 3+ females that have no evidence of POFs and either developing or non-developing ovaries in preparation for the following spring spawning event. The prediction is that age 3+ females with POFs would have a smaller year 3 summer growth layer in comparison with age 3+ females with no POFs. This would determine if scale growth patterns could help resolve age at reproductive maturity. This analysis will also be applied to herring age 2+ through 5+ females and males. Previous work funded by EVOSTC examined the precision of herring scale measurements (Moffitt 2017). This project will also conduct similar precision tests of scales collected from fish by either multiple reads of the same scales or measures of multiple scales from the same fish.

Statistical analyses will use traditional statistics such as regression or ANOVA in an information-theoretic context (AIC, Burnham and Anderson 2002) to explore questions of interest related to age, ovary characteristics, scale growth and year. This approach would allow for determination of most parsimonious models and weighted parameter estimation, which is particularly well suited for observational studies such as the work described here.

Changes to Project Design and Objectives

Objective 4. A key final product of the overall project is to assess annual variation in herring age at maturity schedules before and after 1997 using ADF&G's PWS herring scale image library given results from Objectives 1 through 3. An important first step in this analysis is to evaluate the PWS herring scale image library's potential for this work by examining the dataset for the presence of bimodal distributions in herring scale growth as a cohort of fish ages through time. This cohort-specific approach is an improvement over the work reported by Vollenweider et al. (2017) that pooled data across years to examine the presence of bimodal distributions in herring scale growth by normalizing data to account for inter-annual variability in scale growth.

This analysis will rely on ADF&G's PWS herring scale image library that includes over 8000 scale images for fish sampled between 1985-2015. The details of these herring scale measurements are reported by Batten et al. (2016) and Moffitt (2017). Importantly, scales from approximately 30 male and 30 female herring from each of three age classes (4, 5, and 6) were imaged, resulting in a total of 180 scales measured from individual fish for every year between 1985-2015. The resulting samples for a given cohort are as follows: age 6 (n = 60), age 5 (n = 120), age 4 (n = 180), age 3 (n = 180), age 2 (n = 180), age 1 (n = 180). The analysis will test the prediction that as a cohort of herring matures over time, the frequency distribution of individual fish showing either large (presumably non-spawning individuals) or small (presumably spawning individuals) scale growth will change such that at young ages (1-2) a unimodal distribution in scale growth should be detected, followed by bimodal distributions for older ages (3-4) as fish begin maturing reproductively to spawn. By age 5-6, it is expected that unimodal distributions should be detected if herring of these ages have all recruited to the spawning population.

A key first step in this exercise is to conduct a power analysis using information on the expected variance of each mode (i.e., larger or smaller scale growth), which can be obtained from the preliminary work by Vollenweider et al. (2017). PI Gorman will work on a power analysis with a biometrician at NOAA Auke Bay Labs, Jacek Maselko, who assisted Vollenweider and Heintz in their initial analysis of bimodal distributions among herring scales imaged for ADF&G's library. Pending results from the power analysis, it may be necessary to increase sample sizes for each cohort of interest, which would require further measurements of scale growth. Once adequate samples sizes have been determined, it is proposed to examine bimodal distributions for the following years: 1984 (measured in 1990), 1988 (measured in 1994), 1999 (measured in 2005), and 2005 (measured in 2011). These years (1990, 1994, 2005, and 2011) were the strongest cohorts of age 6 herring in ADF&G's age, sex, length time series, and therefore, offer the best possibility for obtaining the required data. By selecting age 6 fish for scale measurements it is possible to obtain measurements for the greatest number of younger age classes. Further, these years also represent two sampling years before and after 1997, which allows for some replication before and after the year Hulson et al. (2008) determined maturity schedules changed for PWS herring. Once the additional scale growth data are available, analysis will proceed following Vollenweider et al. (2017) by using a Gaussian mixture model employed in the R language environment (R Core Team 2018) with the mixtools package (Benaglia et al. 2009). Data will be analyzed for each age class separately. We will further evaluate the possibility of conducting analysis for males and females, separately, for each age class (J. Vollenweider, pers. comm.).

5. PROJECT PERSONNEL – CHANGES AND UPDATES
If there are any staffing changes to Primary Investigators or other senior personnel please provide CV's for any new personnel and describe their role on the project.

No major staffing changes are anticipated for FY19. PI Gorman will continue to lead the project.

6. PROJECT BUDGET FOR FY19
A. Budget Forms (Attached)
Provide completed budget forms.

B. Changes from Original Proposal
If your FY19 funding request differs from your original proposal, provide a detailed list of the changes and discuss the reason for each change.

C. Sources of Additional Funding
Identify non-EVOSTC funds or in-kind contributions used as cost-share for the work in this proposal. List the amount of funds, the source of funds, and the purpose for which the funds will be used. Do not include funds that are not directly and specifically related to the work being proposed in this proposal.

Budget Forms

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	$82.6	$85.2	$393.0	
Travel	$1.1	$1.1	$1.1	$1.1	$1.1	$5.5	$ -
Contractual	$45.0	$44.2	$34.2	$34.2	$34.2	$191.8	
Commodities	$2.4	$1.8	$1.8	$1.8	$1.8	$9.6	$ -
Equipment	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$ -
Indirect Costs (*will vary by proposer*)	$36.0	$36.4	$35.0	$35.9	$36.7	$180.0	
SUBTOTAL	$156.0	$157.8	$151.5	$155.6	$159.0	$779.8	$0.0
General Administration (9% of	$14.0	$14.2	$13.6	$14.0	$14.3	$70.2	N/A
PROJECT TOTAL	$170.0	$172.0	$165.1	$169.6	$173.3	$850.0	
Other Resources (Cost Share Funds)	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	

COMMENTS:
This summary page provides an five-year overview of proposed project funding and actual cumulative spending. The column titled 'Actual Cumulative' must be updated each fiscal year as part of the annual reporting requirements. Provide information on the total amount actually spent for all completed years of the project. On the Project Annual Report Form, if any line item exceeds a 10% deviation from the originally-proposed amount; provide detail regarding the reason for the deviation.

Changes from Original Proposal
No changes in budget are requested.

Sources of Additional Funding
No additional sources of funding.

7. FY18 PUBLICATIONS AND PRODUCTS

Products include publications (include *in prep* and *in review*), published and updated datasets, presentations, and outreach during FY18.

Gorman, K.B., M.E. Roberts, T.C. Kline Jr., and W.S. Pegau. Comparing calorimetric and stable isotope-derived measures of energy density among juvenile Pacific herring (*Clupea pallasii*). *In prep for Fisheries Research.*

Gorman, K.B., T.C. Kline Jr., F.F. Sewall, R.A. Heintz, and W.S. Pegau. Changes in condition and of Pacific Herring (*Clupea pallasii*) during their first overwinter period. *In prep, EVOS juvenile herring intensives project.*

Gorman, K.B., F.F. Sewall, R.A. Heintz. Winter foraging among juvenile Pacific herring in Prince William Sound, Alaska: Stable isotope ellipses and diet composition. *In prep, EVOS juvenile herring condition monitoring project.*

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