ATTACHMENT B. Annual Project Report Form (Revised 11.21.19)

1. Project Number:

20120111-A

2. Project Title:

Herring Program – Program Coordination

3. Principal Investigator(s) Names:

W. Scott Pegau, Prince William Sound Science Center

Maya Groner, Prince William Sound Science Center

4. Time Period Covered by the Report:

February 1, 2020-January 31, 2021

5. Date of Report:

March 2021

6. Project Website (if applicable):

https://pwssc.org/herring/

7. Summary of Work Performed:

The work described here outlines the work of the Coordination project of the Herring Research and Monitoring (HRM) program. The Coordination project includes efforts on coordination, outreach efforts, a postdoctoral researcher to analyze the relationships between herring diseases and physical and biological oceanographic conditions, works with principal investigators (PIs) to examine new means of collecting information, and works with PIs to synthesize existing information. The goal of the project is to provide coordination within the HRM program and with the Gulf Watch Alaska (GWA) and Data Management (DM) programs. The objectives of the project are:

1. *Coordinate efforts among the HRM projects to achieve the program objectives, maximize shared resources, ensure timely reporting, and coordinate logistics.*

2. *Provide outreach and community involvement for the program.*

3. *Oversee a postdoctoral researcher.*

Coordination is primarily through e-mail and teleconference. We hold one PI meeting each year to provide more intensive interactions between the investigators. The focus of the HRM PI meeting is in sharing findings and spending time coordinating between projects. To coordinate with the other programs, the management team of GWA and the lead of DM are included in the emails to HRM PIs to ensure they are aware of our activities. We also have joint PI meetings and community involvement activities with the other programs.

Coordination effort

Work and travel restrictions associated with the COVID-19 outbreak began right at the beginning of the field season associated with the herring spawn in Prince William Sound (PWS). This led to extra coordination efforts as we worked with different groups to ensure collection and processing of samples for all groups. We were able to collect all of the samples that were planned. We worked with a local fisherman to collect samples for the age-sex-length analysis. Investigators in Cordova were trained to process samples for the disease project.

Reports and proposals were submitted to the National Oceanic and Atmospheric Administration (NOAA) and the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC) on time. Reports and proposals are being requested from the PIs earlier to allow the HRM Science Oversight Group time to comment on them before submission and to provide additional internal review of the grammar and formatting of the materials being submitted.

The annual PI meeting was held through video conferencing in conjunction with the GWA PI meeting. We were able to provide updates on the work completed by each project and work with GWA PIs to examine current and future opportunities to collaborate.

Outreach effort

Outreach efforts are focused on providing up-to-date information on the projects and their findings. Keeping the HRM section of the Prince William Sound Science Center (PWSSC) website up to date has been a key objective. During the last year we continued to update the HRM program website with new findings from ongoing projects. We began reaching out to the Village of Tatitlek to assess the opportunity for a listening session. Those efforts ended with the COVID-19 outbreak. We recently have reached out again to see if there is an approach to conduct a listening session in the upcoming year. We also contributed six articles to the *Delta Sound Connections* publication in an HRM 2-page spread, updating the spill-affected communities on the year's findings. *Delta Sound Connections* is a newspaper produced annually by the PWSSC and distributed widely throughout Prince William Sound and the Copper River Delta. Lastly, social media outlets were leveraged in the spring. Posts were designed to inform PWSSC's followers on Facebook and Instagram of spawn activity and location as it was happening.

Research effort

Over the past 3 years, research conducted by Maya Groner has been focused on three topics:

quantifying mortality in adult herring from PWS and Sitka Sound due to Ichthyophoniasis, characterizing gene expression in both host and virus during incubation, viral proliferation and viral shedding stages of an experimental challenge of herring with viral erythrocytic necrosis (VEN), and developing a mathematical model for viral hemorrhagic septicemia (VHS) in wild herring.

Research in 2020 has primarily focused on the first topic with the specific goals of (1) quantifying patterns between infection severity, host response and survival using a lab experiment, (2) characterizing patterns of *Ichthyophonus* across age and time in Sitka and PWS, and (3) developing a mathematical model to quantify mortality due to infection in PWS and Sitka.

Goal 1: Identifying correlations between infection severity, host response, and survival

The first topic is being addressed with both a lab experiment and a field study. The goal of the lab study was to quantify the relationship between *Ichthyophonus* sp. pathogen load and mortality of herring. To do this we infected specific pathogen-free herring at the U.S. Geological Survey (USGS) Marrowstone Laboratory. Replicate tanks (3) of age-4 herring were exposed to *Ichthyophonus* via intraperitoneal injection. Mortality, infection status, size, and cardiac tissue samples were collected from all tanks over the course of the experiment. Infection load was quantified by sectioning, staining, and counting all pathogen (schizonts) in ventricle tissue of herring that were sampled during and at the end of the experiment (165 days) (Fig. 1).

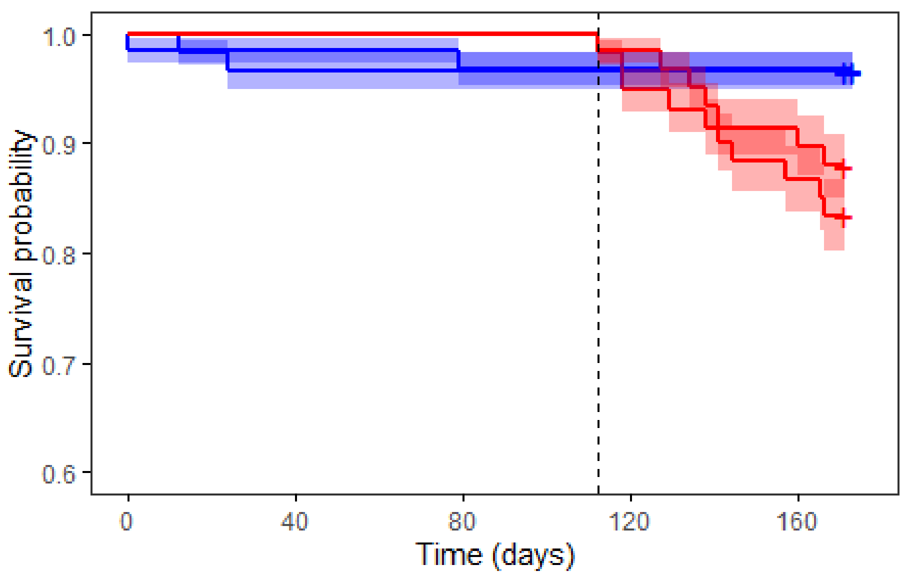


Figure 1. Survival curves for control (blue) and infected (red) herring. Infected herring were exposed to Ichthyophonus sp. via intraperitoneal injection on day 111 after in-feed exposure was unsuccessful.

Pathogen load was quantified in moribund, dead, and subsamples of live fish using quantitative image analysis of histologically sectioned and stained cardiac tissue (specifically ventricle tissue) (Fig. 2). The lethal infectious dose increased with time since exposure. This is likely explained by the host response (Fig. 2), which also increased after time since exposure and indicates an increased ability to tolerate infection. Results of this experiment indicate the infection severity is NOT a good predictor of mortality risk in wild fish unless time since exposure and host response can also be taken into consideration. Understanding the interrelationship between host response, pathogen tolerance and survival is critical for interpreting surveillance data in the wild. Because this relationship is so complex for *Ichthyophonus* infection, modeling efforts to estimate disease mortality (goal 3 below) infection utilized prevalence data as opposed to infection severity data.

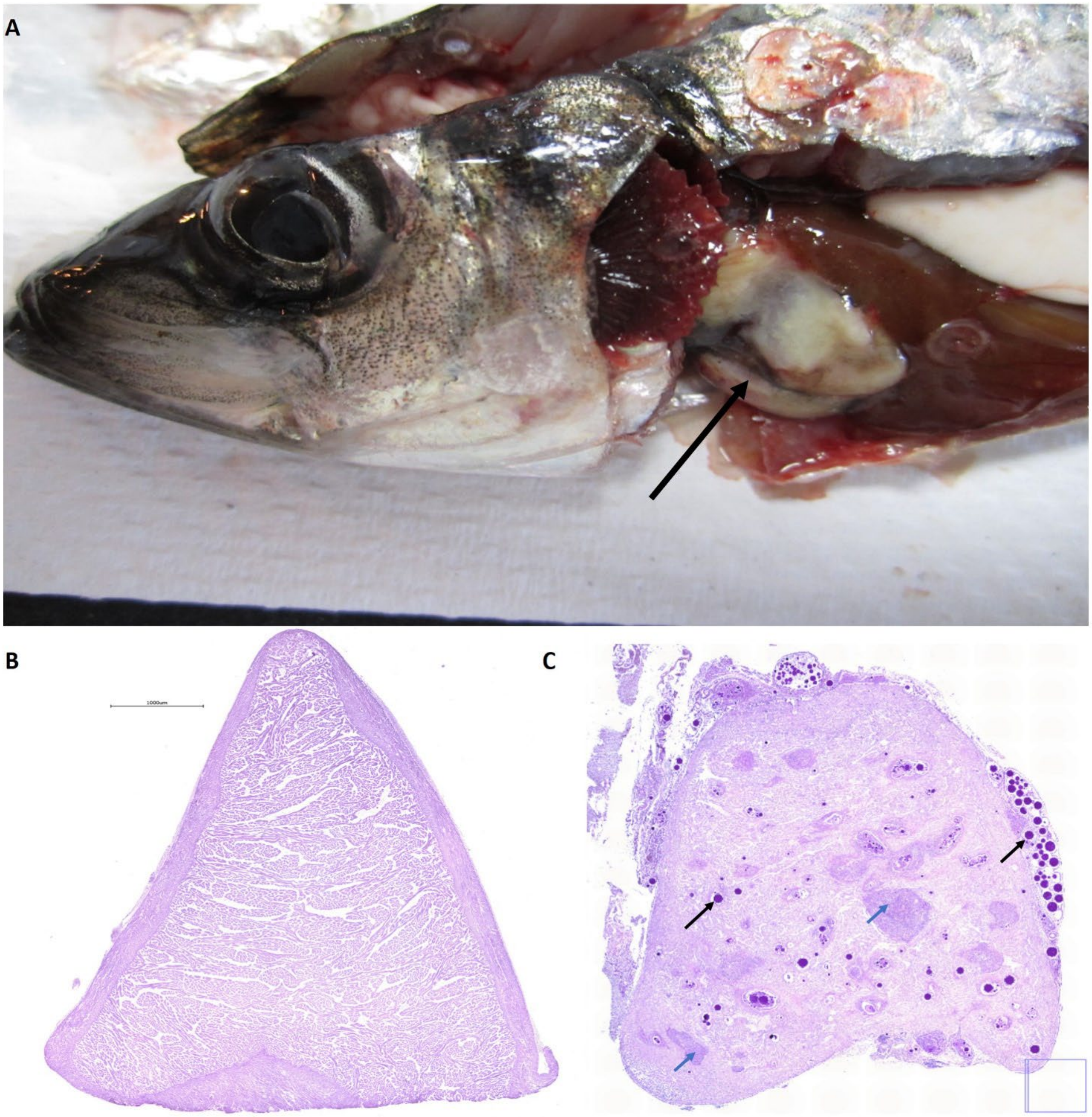


Figure 2. Hearts heavily infected with Ichthyophonus sp. can appear white instead of dark red (arrow) (A). Histological sections of a healthy herring ventricle (B) next to an infected ventricle (C). On the infected ventricle, the dark purple circles (black arrows) indicate Ichthyophonus sp. schizonts. Granulomas of darker staining tissue surround some of the schizonts (blue arrows).

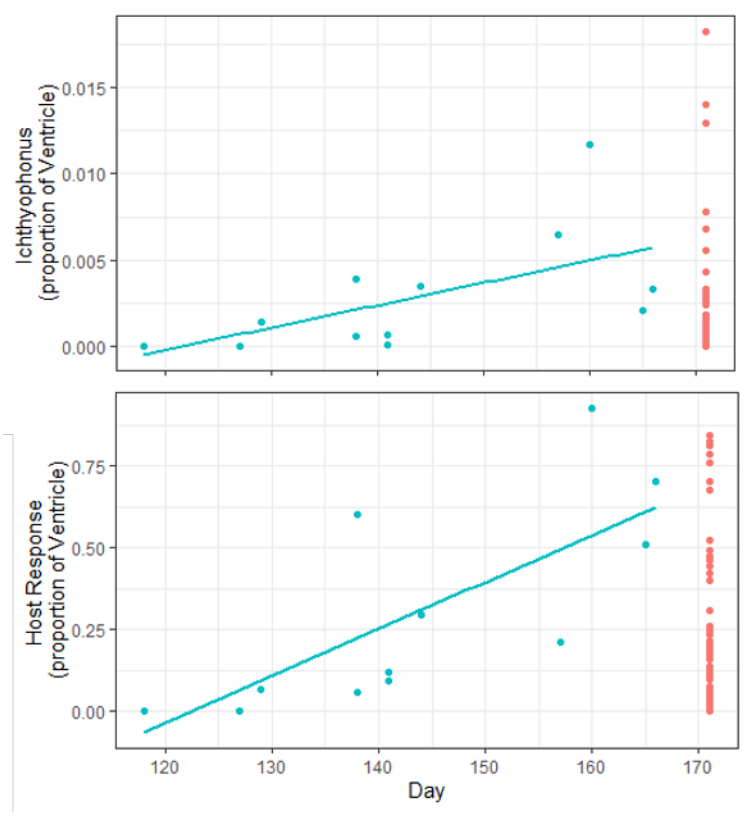


Figure 3. Infection severity (top panel) and host response (bottom panel) after in mortalities (blue) and live herring (red) after IP injection with Ichthyophonus.

Goal 2: Characterization patterns of *Ichthyophonus* across age and time in Sitka and PWS

To characterize patterns of *Ichthyophonus* from wild caught samples, we quantified both measurements of pathogen presence and infection severity across age and time using data from herring sampled each spring at Sitka Sound and PWS between 2007 and 2019 (~ 180 fish sampled per survey, total n=3,769). Sampling was conducted during or slightly before spawning took place. Herring were sampled using a purse seine or cast net in 3 sets of 60 fish. Fork length and sex were recorded for each fish and scales were collected from a subset of PWS fish to determine age. In Pacific herring, *Ichthyophonus* has a strong tropism for cardiac tissue and the heart is the preferred organ for assessing infection prevalence and intensity (Hershberger 2012). For each sampled fish, the heart was aseptically dissected; half of the heart was placed into culture media for explant culture to determine infection prevalence (Hershberger et al. 2016), while the other half was fixed in 10% NB formalin for histology. The *Ichthyophonus* growth medium (>1:5 W:V), consisted of tris-buffered Eagle's Minimum Essential Medium supplemented with 5% foetal bovine serum, 100 IU ml^{-1} penicillin, 100 µg ml^{-1} streptomycin and 100 µg ml^{-1} gentamycin (MEM). Explant cultures were examined microscopically (40x magnification) for the presence of *Ichthyophonus* after 7 and 14 days; cultures without detectable *Ichthyophonus* schizonts or hyphae after 14 days were considered negative.

Histological processing

Infection intensity in archived heart tissues from PWS and Sitka Sound (2009 –2019) were analyzed using standard histopathological techniques. All culture positive samples were processed for histology along with 20 culture-negative samples (to confirm culture negative results). Formalin fixed herring hearts were dehydrated and infiltrated with paraffin wax following standard histological procedures. After tissues were embedded in paraffin wax blocks, they were trimmed and sectioned at 5um with a Leica RM2255 microtome. Tissue sections for each sample were mounted on one charged slide to be stained with Periodic Acid Schiff (PAS) and one uncharged slide to be stained with Gill's Hematoxylin and Eosin (Carson 1997).

Imaging & Analyzing

A Keyence BZ-X710 microscope with automatic stitching was used to capture and stitch the sectioned hearts at 6x magnification. PAS stained hearts were cropped to exclude cardiac tissue that was not the ventricle. Automatic classifiers were created from a group of hearts within the sample set using the hybrid cell count program within the BZ-X Analyzer (version 1.4.0.1). Classifiers were used to produce ventricle area, schizont count and schizont area for all samples with *Ichthyophonus*. Due to uneven staining, classifiers did not always discriminate between ventricle and schizont tissue. All classified images were checked and modified as needed to improve accuracy. Intensity scores for each fish were calculated as the proportion of the observed ventricle area that contained schizonts.

Age-length key

To quantify patterns of disease across cohorts, it was necessary to estimate ages of sampled fish. Age-length relationships vary over time in both Sitka and PWS, so unique keys were needed for each year for each site. The Alaska Department of Fish and Game (ADF&G) provided datasets for 2009-2019 that included both age (determined from scale annuli) and standard lengths for Pacific herring caught in March and April of each year. Sitka samples were caught using cast nets, while PWS samples combination of cast nets, gill nets and purse seines.

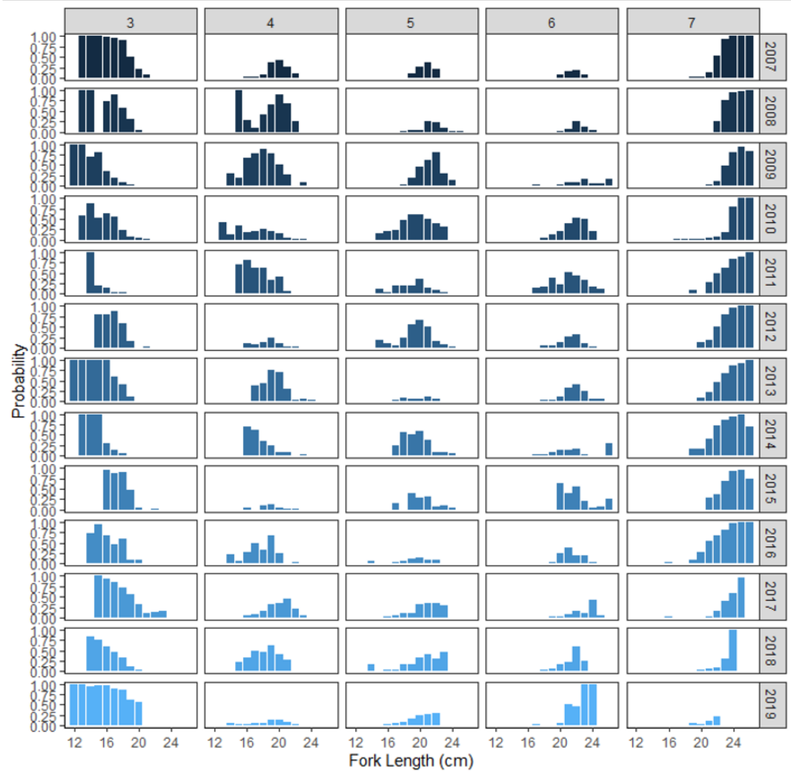
Prior to calculation of the age-length key, standard lengths were converted to fork lengths using the following relationship which was determined from a dataset consisting of fork length and standard length measurements from 2,907 Pacific herring collected in PWS in 2017 and 2018 ($R^2 = 0.98$):

Fork Length = 2.35+1.06*Standard length

Age-length keys were created by binning fork lengths for each 10 mm group. For each year and site combination, 100 datapoints from each age cohort (age-3 through -7+) were randomly sampled with replacement to create a distribution of lengths for each age. Fish under age-3 are not common in pre-spawn aggregations of Pacific herring in either Sitka or PWS and too few of them were caught include in the age-length key. Due to small sample sizes, fish age-7 and older were grouped into a single plus group. Once the age-length keys were created, they were multiplied by the mean percent age composition for the combined pre-fishery mature and immature population and then scaled so that the total probability for each size class summed to one. These age-composition estimates were derived from the 2019-forecast age-structured model for the herring population for Sitka and the

2019 Bayesian age-structured assessment model for PWS (Sherri Dressel, unpublished data, John Trochta, unpublished data) (Fig. 4).

A. Prince William Sound



B. Sitka Sound

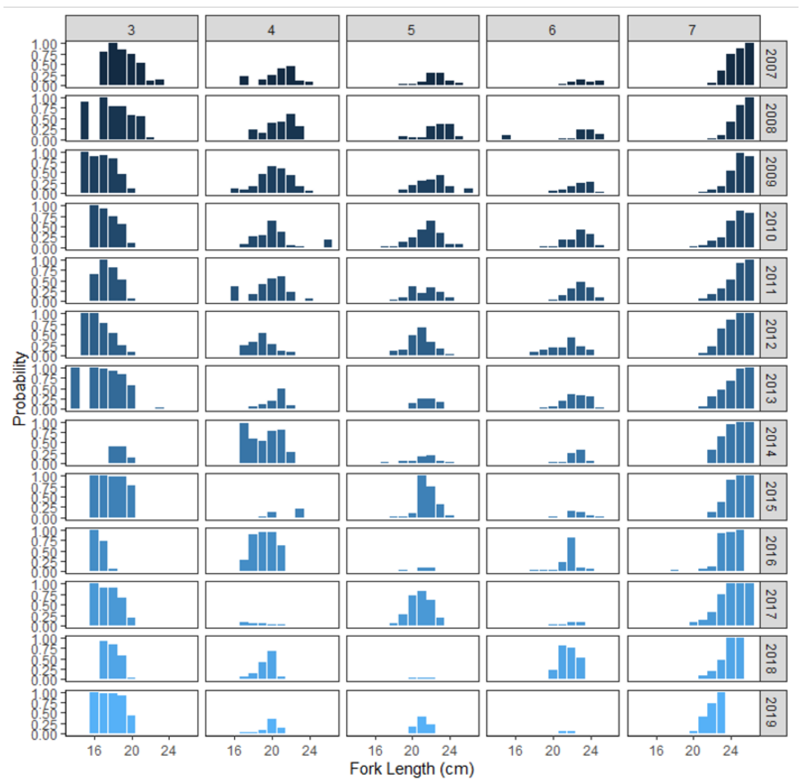


Figure 4. Weighted age-length key for (A) Prince William Sound and (B) Sitka Sound from 2007-2019. Ages evaluated include age 3 through a 7 plus group (indicated by columns). Years are indicated by rows.

Analysis

Logistic regression and zero inflated beta regressions with a logit link function were used to identify factors correlated with *Ichthyophonus* infection presence and severity Pacific herring, respectively (package 'glmmTMB', Brooks et al. 2017). For both models, the sample set (3 sets of ~60 fish per site per year) was included as a random effect. We used model selection based on Akaike Information Criteria adjusted for small sample sizes (AICc) to evaluate the correlation between demographic and environmental variables and our response variables. The severity models were set up as zero-inflated models with asymptomatic fish (culture positive, but no schizonts detected histologically) making up the zeros and the proportion of the ventricle occupied by schizonts making up the non-zero data of the dataset. Therefore, the zero-inflated model predicted the probability that an infection was symptomatic, while the conditional model predicted the severity of infections for symptomatic herring. Demographic variables evaluated include the assigned fish age and the estimated recruited population size (from the respective Sitka Sound and PWS stock assessments). The variables were chosen because disease prevalence is expected to increase with age in the absence of disease mortality and because pulses of new susceptibles (e.g., recruits) can influence epidemiological outcomes. Environmental variables evaluated include summer and winter Pacific Decadal Oscillation (PDO) and North Pacific Gyre Oscillation (NPGO) and summer temperature anomalies (for PWS). We focused our efforts on these specific variables because *Ichthyophonus* proliferation increases with temperature (Sitja-Bobadilla and Alvarez-Pellitero 1990), and swimming speeds of infected rainbow trout are decreased at higher temperature (Kocan et al. 2009). Cooler winters may also interact with infection; in experimental trials, energy storage rates of infected herring were lower than those of healthy herring, particularly at low temperatures (Vollenweider et al. 2011). Both the winter PDO and the winter and summer NPGO were included because they are important covariates for herring mortality in the PWS Bayesian age structured model, suggesting that they have an (as yet unknown) role in shaping population dynamics (Muradian et al. 2017, Trochta et al. in prep). Summer anomalies from 2m depth assembled from a series of available datasets (described in Campbell et al. 2018). Model selection based on AICc was used to compare models (package 'AICcmodavg'). All analysis was done in R (v. 4.0.3).

Results

Patterns of infection prevalence across time and age are distinctly different between PWS and Sitka Sound (Fig. 5). The expectation for incurable infections such as *Ichthyophonus* is a constant or increasing infection prevalence across age. Patterns of decreasing infection prevalence across age can only be explained by heightened mortality of infected individuals relative to healthy individuals within an age class. Linear regressions of infection prevalence across age show similar (nearly parallel) increasing age-prevalence patterns in Sitka Sound. This is consistent with our null expectation of low mortality across age classes. In contrast, PWS shows patterns of both increasing and decreasing infection prevalence with age. **From 2016-2018, there is a clear pattern of decreasing infection prevalence with age in the 3 dominant cohorts, indicative of disease mortality in PWS.**

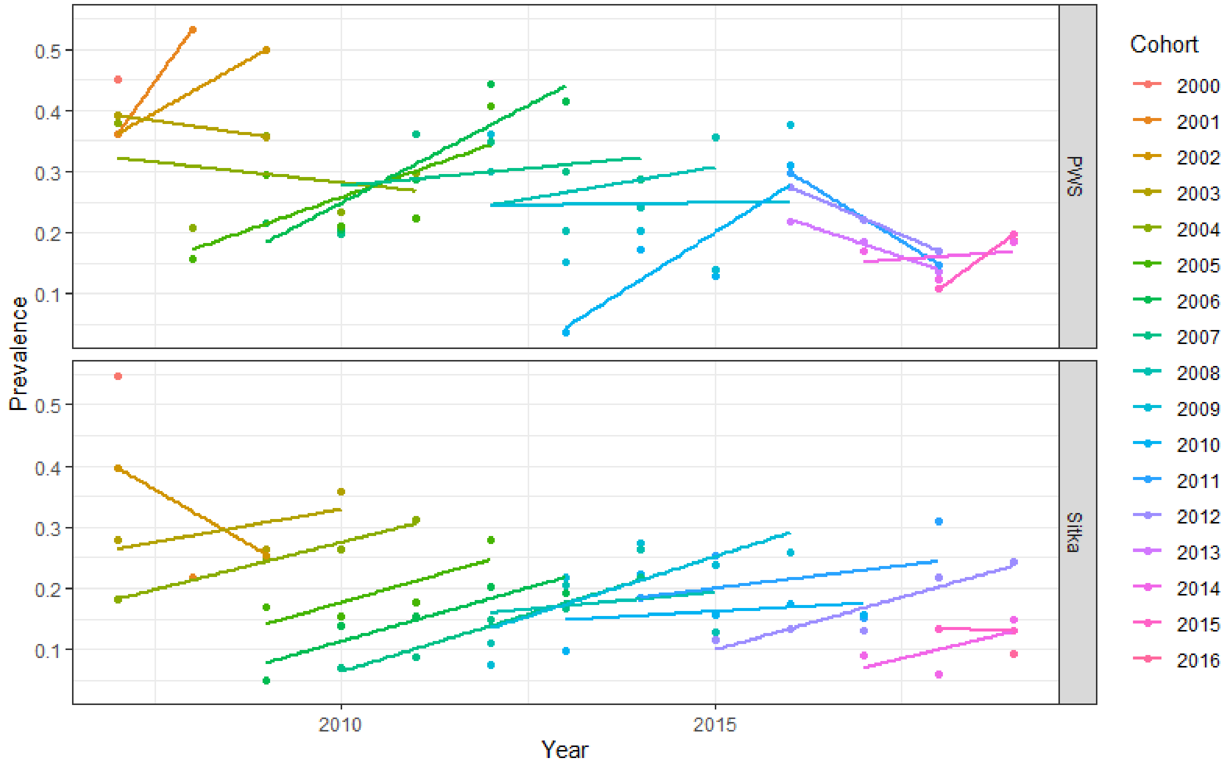


Figure 5. Patterns of infection prevalence across over time for unique cohorts (indicated by color) in Prince William Sound (PWS) and Sitka Sound. Data points indicate infection prevalence values from surveys conducted prior to or during spawning, while lines show linear regressions of cohort-level data.

The best models for infection presence in Sitka Sound and PWS included age; however, in Sitka Sound cohort year was also in the model, while in PWS mature population size was in the best model (Tables 1-3, Fig. 6). At both locations, infection prevalence was positively correlated with age. In Sitka Sound (Table 3), infection prevalence was predicted to increase 22% with additional year of age. Infection prevalence was 12.7 ± 6.7% (mean ± 1sd) for age-3 fish and increased to 27 ± 10% for the age-7+ group. Infection prevalence was significantly lower in the 2006, 2007, 2008, 2014, and 2016 cohorts relative to the baseline case, the 2000 cohort. No other models had a ΔAICc <3 relative to this best model.

In PWS, numerous models had a ΔAICc <3 from the best model (Table 4A, B). Each of these models included age as a predictor and varied in what demographic or environmental parameter they also included (see Table 1). Infection prevalence was 16.5 ± 2.6% (mean ± 1sd) for age-3 fish and increased to 36 ± 4.5% for the age-7+ group. Infection prevalence was predicted to be 60-75% greater in each age class when the mature population was 250 million fish compared to 50 million fish. This span represents the variation in mature population sizes estimated for PWS during the study period.

Table 1. Best-fitting models for infection presence in ventricle tissue of herring from Prince William Sound 2007-2019. Models with ΔAICc < 3 from the best fitting model are shown. NPGO = North Pacific Gyre Oscillation; PDO = Pacific Decadal Oscillation.

Model	**AICc**
Mature Population (Millions) + Assigned Age	2145.309
Year * Assigned Age	2145.632
NPGO (Oct-March) * Assigned Age	2145.852
NPGO (Oct-March) + Assigned Age	2145.874
Assigned Age	2146.033
NPGO (April-Sept) * Assigned Age	2146.545
PDO (Oct-March) + Assigned Age	2146.821
Recruitment Population (Millions) + Assigned Age	2146.987
Mature Population (Millions) * Assigned Age	2147.183
PDO (April-Sept) + Assigned Age	2147.779
NPGO (April-Sept) + Assigned Age	2147.967
Year + Assigned Age	2147.998

Table 2. Best model for predicting infection status of ventricles of herring in Prince William Sound 2009-2019 (n = 1,914). α = 0.95 for this analysis.

	Estimate	**1 Std. Error**	**z value**	**p value**
(Intercept)	-2.530	0.330	-7.7	< 0.001
Estimated Mature Population (Millions)	0.002	0.001	1.7	0.088
Assigned Age	0.219	0.044	5.0	< 0.001

Table 3. Best model for predicting infection status of ventricles of herring in Sitka Sound 2007-2019 (n = 1,855). The baseline case is the year 2000 cohort. α = 0.95 for this analysis.

	Estimate	**1 Std. Error**	**z value**	**p value**
(Intercept)	-1.52	0.89	-1.7	0.09
Cohort2001	-1.14	0.89	-1.3	0.20
Cohort2002	-1.45	0.86	-1.7	0.09
Cohort2003	-0.84	0.82	-1.0	0.30
Cohort2004	-0.73	0.81	-0.9	0.37
Cohort2005	-1.22	0.82	-1.5	0.14
Cohort2006	-1.59	0.82	-1.9	0.05
Cohort2007	-1.59	0.81	-2.0	0.05
Cohort2008	-2.26	0.84	-2.7	0.01
Cohort2009	-0.83	0.85	-1.0	0.33
Cohort2010	-1.47	0.81	-1.8	0.07
Cohort2011	-0.68	0.83	-0.8	0.41

	Estimate	1 Std. Error	z value	p value
Cohort2012	-1.49	0.82	-1.8	0.07
Cohort2013	-15.07	57.24	-0.3	0.79
Cohort2014	-2.10	0.96	-2.2	0.03
Cohort2015	-0.87	0.92	-1.0	0.34
Cohort2016	-1.84	0.91	-2.0	0.04
Assigned Age	0.248	0.054	4.6	<0.00001

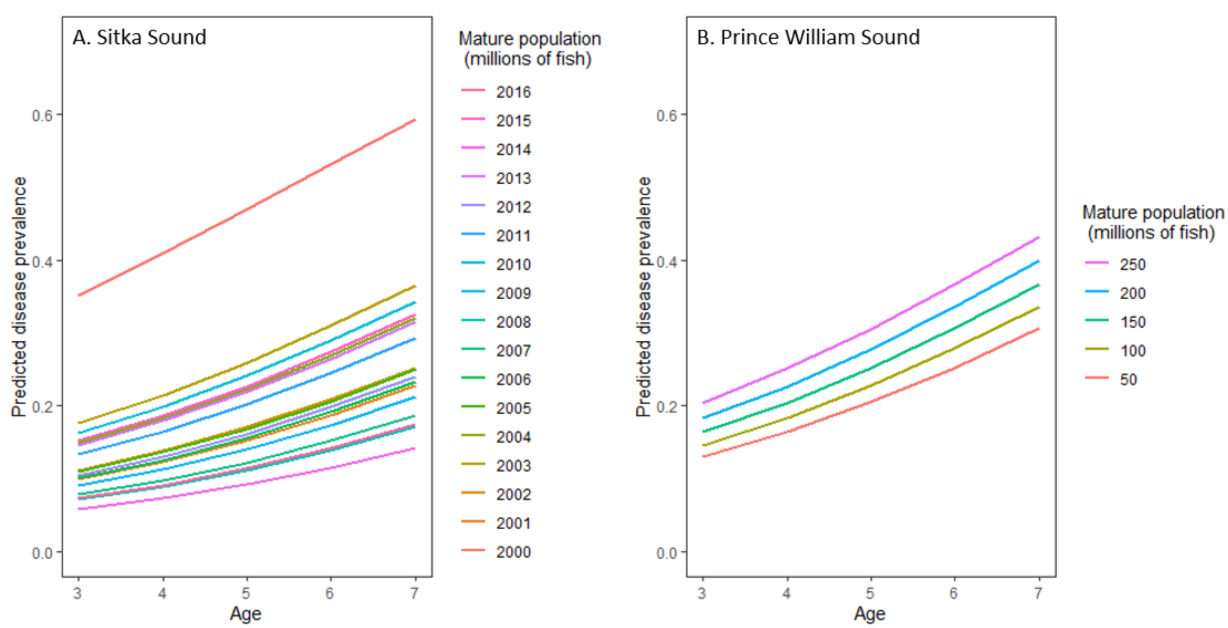


Figure 6. Disease prevalence in Sitka Sound and Prince William Sound as predicted by the best fit logistic regression models.

The best fit predictors for infection level and severity differed between PWS and Sitka Sound (Table 4). In Sitka Sound, mature population size was the best predictor for infection severity, while none of the tested variables explained the probability that an infection would be symptomatic (e.g., the zero-inflated portion of the model. Infection severity was positively correlated with the mature population size (Table 4).

In PWS, age was the best predictor of infection severity and mature population size was the best predictor that an infection would be symptomatic. Age was negatively correlated with infection severity, and mature population size was negatively correlated with the probability that an infection would be symptomatic.

Table 4. Best fit zero-inflated beta models predicting the severity of infection (conditional model) and the probability that an infection is symptomatic (zero-inflation model) in (A) Prince William Sound (n = 378) and (B) Sitka Sound (n = 255).

A. Prince William Sound

Conditional model:

	Estimate	**Std. Error**	**z value**	**p-value**
(Intercept)	-5.94	0.21	-28.3	< 0.001
Assigned Age	-0.074	0.036	-2.1	0.04

Zero-inflation model:

	Estimate	**Std. Error**	**z value**	**p-value**
(Intercept)	0.148	0.267	0.6	0.58
Mature Population (Millions)	-0.0060	0.0021	-2.9	0.004

B. Sitka Sound

Conditional model:

	Estimate	**Std. Error**	**z value**	**p-value**
(Intercept)	-7.30	0.17	-43.5	< 0.001
Mature Population (Millions)	0.00041	0.00019	2.1	0.03

Zero-inflation model:

	Estimate	Std. Error	z value	p-value
(Intercept)	-0.4879	0.129	-3.782	< 0.001

Discussion

Overall, this study shows that age is a strong predictor of infection pressure. Across years, age-prevalence patterns increase at very similar rates in both Sitka Sound and PWS, while PWS has overall higher infection levels after accounting for age. The departure from this pattern in PWS from 2016-2018 suggests that infection-related mortality may be occurring in these years. This is consistent with the substantial decrease in the proportion of fish that are age-6 and older in these years. For the first 9 years of the study (2007-2015), age-6 and older fish comprise 49.4 ± 22.1% (mean ± 1sd) of the population, while for the last 4 years of the study (2016-2019) age-6 and older fish comprise 13.3 ± 10.8% of the population. In PWS, the correlation in infection severity with age may be reflective of mortality of highly infected individuals. This correlation is not found in Sitka Sound. The pattern of decreasing infection prevalence but increasing infection severity with declining mature population sizes in PWS is also consistent with this hypothesis; as the population declined, there were fewer older fish (which we hypothesize were dying due to infection). This would lower the overall infection prevalence. Increased overall infection severity with lower population size may explain why mortality increased in recent years. More investigation as to why infection pressure may have been higher in PWS in recent years is warranted; if fish had lower overall condition, they may not have tolerated infection (e.g., with formation of granulomas) as effectively. If the main source of infection transmission occurs with consumption of infected eggs during the spawning season (currently under investigation by Paul Hershberger, project 20120111-E), overwintering condition may be an important metric to consider. A manuscript with these results has been drafted and is being edited for journal submission.

Goal 3: Develop a mathematical model to quantify mortality due to *Ichthyophonus* infection in PWS and Sitka Sound

The goal of this study is to estimate recent *Ichthyophonus* mortality and transmission rates for PWS and Sitka Sound populations of Pacific herring using partially observed Markov process (POMP) models. For each population we construct and evaluate a series of stochastic state-space models which include variation in sample measurements due both to measurement/sampling error as well as variation in the underlying ecological process (in this case disease). Through replicates of iterated particle filtering, the model is optimized to fit both the disease survey data from each study site (from 2007-2019) as well as acoustic survey data (collected by Pete Rand, project 20120111-G). The basic model structure is shown in Fig. 7.

Methods

We developed a susceptible-infected (SI) epidemiological model for *Ichthyophonus* infection in mature Pacific herring (Fig. 7). Experimental challenge studies suggest that herring do not recover or become immune to this disease, thus the model has a single one-way path from susceptible to infected. The force of infection (λ) was modeled as a frequency-dependent process such that:

$$\lambda(t) = \frac{\beta * I(t)}{S(t) + I(t)}$$

where β is the transmission coefficient, *S(t)* is the number of susceptible individuals at time *t* and *I(t)* is the number of infected individuals at time *t*. While herring populations sizes change over time, they tend to form large, dense schools regardless of population size. The mechanism for transmission of *Ichthyophonus* to planktivorous fish is hypothesized to occur through feed of infected plankton or eggs (Gregg et al. 2012), although this has not been confirmed.

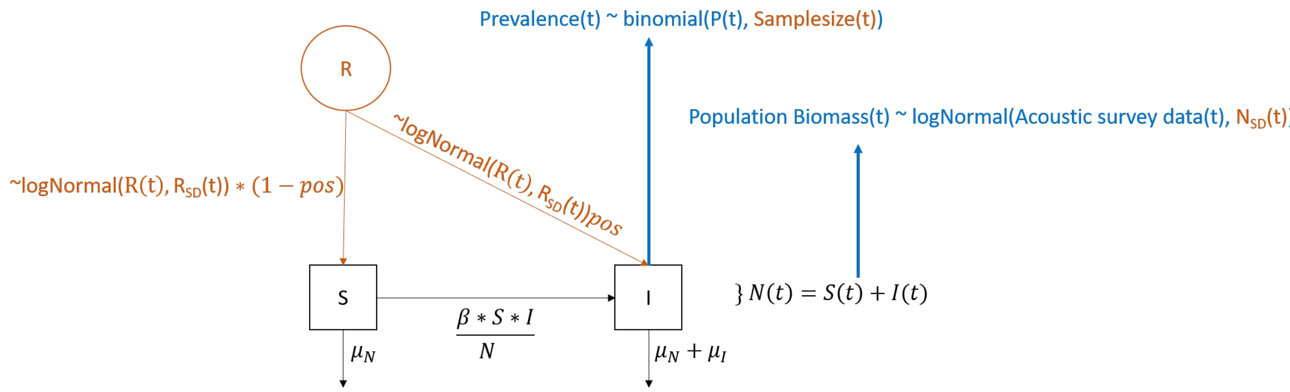


Figure 7. State space model used to quantify disease mortality (μ_I), background mortality (μ_N), and the transmission coefficient (β) for Ichthyophonus in Prince William and Sitka Sounds. The basic disease model is in black and shows transmission from susceptible to infected herring with no recovery. Susceptible herring die at rate (μ_N), while infected herring die at rate ($\mu_N + \mu_I$). Transmission is frequency-dependent and defined by the equation (($\frac{\beta * S * I}{S+I}$). Parameters are optimized to field data on infection prevalence and acoustic survey estimates of the age-3+ population size (in blue). Covariates in the model (orange) are data sources that are inputs to the model. R indicates recruitment. Except for the prevalence data, which follows a binomial distribution, data are lognormally distributed.

Mortality estimates for susceptible fish consist of a time-independent natural mortality rate (μ_N), and, where relevant, a time-dependent fishing mortality rate ($\mu_{F,\,t}$). Commercial fishing has occurred in most study years in Sitka and none of the study years in PWS. Mortality estimates for infected fish include natural mortality and fishing mortality estimates along with a time-independent estimate of disease mortality (μ_D). Disease and natural mortality occur throughout the year, while fishing mortality occurs in month 4.

The population model includes all individuals age-3 and older regardless of maturity. Recruitment is estimated by the age structured models for each site, respectively. Recruited fish are added to the epidemiological model through recruitment during the first month of each year. While >80% of recruiting fish are susceptible, some portion of recruits are infected (R_+).

Inference

We used the POMP model to combine the mechanistic disease process that we hypothesized were driving *Ichthyophonus* epidemiology (the process model) with probabilistic models that linked the observed data to the latent process (the measurement model). Estimates were made using the 'pomp' package in R (v. 4.0.3) (King et al. 2015/2016).

The process model (described above) was implemented as two stochastic differential equations.

$$S(t) = Rec(t) * (1 - pos) - \lambda(t) * S(t) - \mu_N * S(t) - \mu_F(t) * S(t)$$

$$I(t) = Rec(t) * pos + \lambda(t) * I(t) - \mu_{N*} * I(t) - \mu_F(t) * I(t) - \mu_D * I(t)$$

Recruitment was implemented in the first month of each year as a gamma-distributed function with shape and scale parameters as:

$$shape = \frac{R_y^2}{\sigma_{R_y}^2}$$

$$scale = \frac{\sigma_{R_y}^2}{R_y}$$

Where $\sigma_{R_y}^2$ is the variance of the recruitment estimate for year=y and R_y is the estimated number of fish recruited in year=y. The proportion of recruits that are infected (pos) is estimated by the model.

Natural and disease mortality were estimated by the model. Fishing mortality was estimated from data supplied by the Sitka ADF&G. Fishing mortality was implemented in the fourth month of each year as a gamma-distributed function with shape and scale parameters as:

$$shape = \frac{\mu_{F,y}^2}{\sigma_{\mu_{F,y}}^2}$$

$$scale = \frac{\sigma_{\mu_{F,y}}^2}{\mu_{F,y}}$$

Where $\sigma_{\mu_{F,y}}^2$ is the ADF&G estimate of the recruitment variance in year=y and $\mu_{F,y}$ is the estimated number of fish caught in year=y.

State transitions and mortality rates were estimated in the model by randomly choosing from euler-multinomial distributions (Breto and Ionedes 2011).

Process model

We optimized the log likelihood of our model using iterated particle filtering ('mif2' in 'pomp') (King et al. 2018). A broad exploration of parameter space was initiated using 300 sets of parameter values, with each value randomly drawn from a uniform distribution (a hypercube). During iterative filtering ('mif2' in 'pomp'), all parameters were perturbed. For each of 10 replicated model runs, we ran 100 particle filters with 100,000 particles to calculate the log likelihood.

Data that the model was optimized to include the number of Ich positive fish sampled in a given year and the estimated number of age-3+ fish as estimated from the acoustic survey. The age-3+ population size was calculated by first multiplying the biomass by the estimated bias in estimation (parameter q_2 in Muradian et al. 2017) and dividing the biomass by the average fish size per year (as indicated from the weight-at-age estimates multiplied by the spawner survey age composition data).

Preliminary results

Preliminary models have been run for PWS. Fig. 8 depicts parameter estimates for each iteration from a local search of parameter space. Parameter estimates are chosen to maximize the log likelihood of the model given the data. Fig. 9 depicts 20 stochastic simulations of the epidemiological model using the best parameter set from the local parameter search.

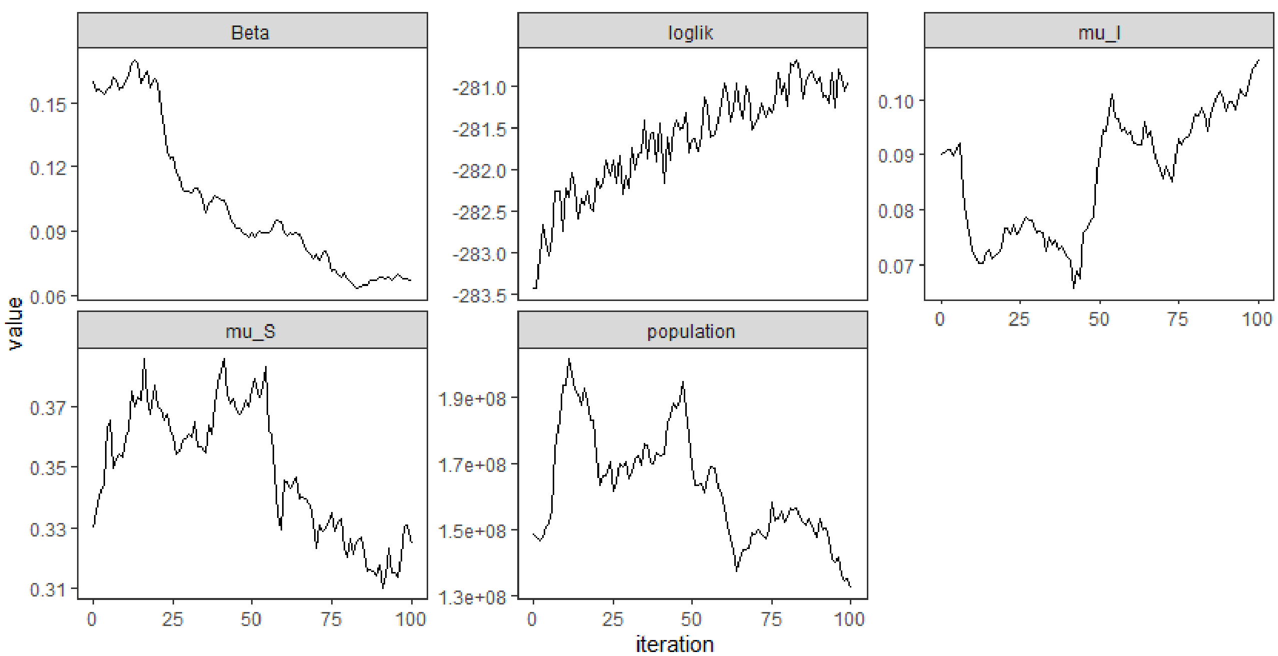


Figure 8. Parameter estimates for epidemiological model of Ichthyophonus in Prince William Sound. Parameters are estimated from a local search of parameter spaces, designed to maximize the log likelihood (loglik) of the model over multiple iterations. The next step in the model is to search

across a wider parameter space. This is a computationally demanding step and a method for parallelizing the parameter search across multiple cores is in development.

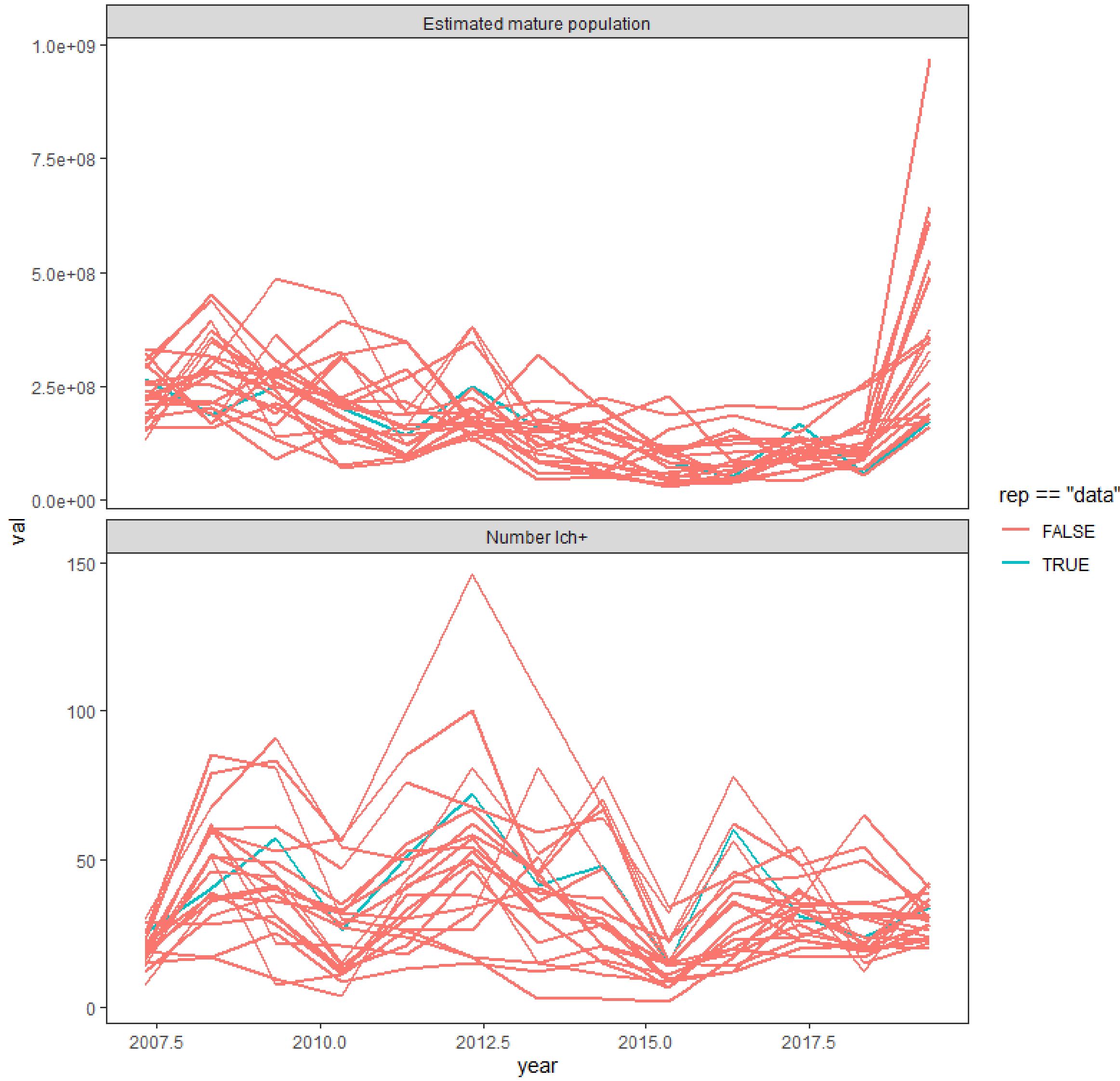


Figure 9. Twenty simulations of the epidemiological model for Ichthyophonus using the best parameters from the local parameter search. The actual data (from the disease surveys (top panel) and the acoustic survey data, converted from fish biomass to numbers of fish (bottom panel)) are shown in blue. Simulations are shown in pink.

Next steps

Subsequent steps are to adapt this model for data in Sitka Sound and to run global parameter searches for both the PWS and Sitka models. Subsequent sensitivity analyses will be performed to quantify the influence of various assumptions affecting recruitment estimates on parameter estimates. We anticipate publishing these results before the end of 2021.

Additional projects

Dr. Groner has participated in several publications that are tangentially related to this project, including (1) a book chapter on marine disease modeling (published 2020, Oxford University Press), (2) a manuscript identifying a hotspot for *Ichthyophonus* infection in Pacific herring in Puget Sound (published in 2019 in Diseases of Aquatic Organisms) and (3) a manuscript describing methods for modeling dispersal of marine pathogens using coupled biophysical-hydrodynamic models (published 2-20 in Trends in Parasitology. In addition, Dr. Groner has been collaborating closely with John Trochta (graduate student in Trevor Branch's lab) to develop mathematical methods for quantifying mortality due to VHS using seroprevalence data. This work is outlined within Trevor Branch's annual report (project 20120111-C).

8. Coordination/Collaboration:

A. Long-term Monitoring and Research Program Projects

1. Within the Program

This project works with all projects within the HRM program. Coordination is primarily through email and the annual PI meeting. Work with projects includes ensuring reporting is completed promptly and assisting the coordination of sampling logistics. Dr. Pegau works with individual projects to coordination of the collection of samples needed and as a source of information about existing data and results. This year required extra effort to accomplish the planned sampling as the COVID-19 travel and work restrictions were put in place right at the beginning of the herring spawn survey time. By cross-training and working with local fishermen we were able to collect all the samples that had been planned for this year.

The research aspects of this project are closely tied to the herring disease research being led by Dr. Hershberger (20120111-E).

2. Across Programs

a. Gulf Watch Alaska

Dr. Pegau serves as the primary contact for the HRM program with the GWA and DM programs. Coordination includes having the leads to all the programs on the HRM mailing list, so everyone is aware of any information going out to the HRM PIs. He also works with the leads to address specific topics of joint interest, such as reporting. The HRM PI meeting was conducted in conjunction with the GWA PI meeting in November.

The Prince William Sound Regional Citizens' Advisory Council is funding the continuation of aerial forage fish surveys conducted by Dr. Pegau. This project is completed in coordination with the GWA forage fish project. The aerial survey data is provided to the GWA project and the GWA project provides validation of aerial observations.

b. Data Management

We work with the DM team to ensure PIs are submitting data and metadata in a timely manner. The DM team works with PIs to ensure they understand how to best get their data into the Workspace.

B. Individual Projects

We do not have connections with any of the projects outside the large programs.

C. With Trustee or Management Agencies

Sherri Dressel of ADF&G is on the HRM scientific oversight group. Sherri, along with Stormy Haught of the Cordova office of ADF&G, are the primary contact points between the HRM program and the Trustee Agency with oversight of herring in PWS. The monitoring work of the HRM program provides the data necessary for ADF&G to monitor the Pacific herring population in PWS and determine if the population is at a fishable threshold. The exchange of information with ADF&G is important for being able to track similar research efforts ongoing at ADF&G and in the HRM program.

A status of PWS herring was provided to the National Oceanographic and Atmospheric Administration for incorporation into their Gulf of Alaska ecosystem status report, which is reviewed by the North Pacific Fisheries Management Council (Ferriss and Zador 2020).

9. Information and Data Transfer:

A. Publications Produced During the Reporting Period

1. Peer-reviewed Publications

Ben-Horin, T., G. Bidegain, G. de Leo, M.L. Groner, E.E. Hofmann, H. McCallum, and E.N. Powell. 2020. Chapter 13. Modeling and forecasting disease dynamics in the sea. In DC Behringer, BR Silliman, KD Lafferty (Eds.), *Marine disease ecology.* Oxford, England: Oxford University Press.

Cantrell, D.L., M.L. Groner, T. Ben-Horin, J. Grant, and C.W. Revie. 2020. Modeling parasite dispersal in marine ecosystems. Trends in Parasitology 36:239-249.

2. Reports

Pegau, W.S., J. Trochta, and S. Haught. 2020. Prince William Sound Herring. In Ferriss, B., and S. Zador (Eds.) Ecosystem Status Report 2020 Gulf of Alaska. North Pacific Fishery Management Council, Anchorage, AK.

3. Popular articles

Hoover, H. 2020. Herring Research and Monitoring. *Delta Sound Connections*. Prince William Sound Science Center (https://pwssc.org/wp-content/uploads/2020/07/DSC-2020-web.pdf).

Pegau, S. 2020. Population Estimates. *Delta Sound Connections*. Prince William Sound Science Center (https://pwssc.org/wp-content/uploads/2020/07/DSC-2020-web.pdf).

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

1. Conferences and Workshops

Groner, M.L., E. Bravo-Mendosa, C. Conway, J. Gregg, A. Mackenzie, and P. Hershberger. 2021. Epidemiology of Ichthyophoniasis in Pacific herring in Sitka Sound and Prince William Sound from 2007-2018. Oral Presentation. Alaska Marine Science Symposium.

2. Public presentations

Groner, M.L. 2020. Lobsters in a pinch and other tails of disease in fished populations. School of Aquatic Fisheries Sciences. University of Washington, USA.

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

Field Notes podcast on *Ichthyophonus* in herring

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

2020 aerial forage fish survey data

Datasets and relevant code for projects conducted by Maya Groner on *Ichthyophonus* in herring will be uploaded by Feb 28, 2021

10. Response to EVOSTC Review, Recommendations and Comments:

Sept 2020: Science Panel Comment – FY21: The SP is very pleased to see the excellent productivity of the postdoctoral researcher. The PI has made a request for 4 months of additional funding. The SP noted that EVOSTC supported her for 3 years during FY18, 19, and 20. We also noted that she has NPRB funding for FY21. Thus, the SP seeks clarification. Is the postdoc not fully covered by NPRB for this final year? The SP wishes to ensure that she is fully funded to fully utilize her talents for the duration of this project but wants to make sure that her time is not already covered by NPRB. Please clarify.

Pegau response to Science Panel:

No, Dr. Groner has some external funding, but it isn't enough to support her through FY21. The additional four months of salary requested is what would be required to support her through the end of this cycle of the Herring Research and Monitoring program and allow her to better combine the work supported by the North Pacific Research Board with the work included in this program.

Sept 2020: Science Panel Comment – FY21: Looking ahead, the SP is very interested in the incorporation of disease into the herring assessment model. In advance, the SP encourages the PIs to work with Trevor Branch. Specifically, can the model be configured to test its sensitivity to transmission rates? As an example, the herring model was recently examined with alternative assumptions about maturity and it was found that the model was not very sensitive to these assumptions. So, one maturity schedule was adopted. Understanding the model's sensitivity to disease transmission could help focus the remaining disease work.

Pegau response to Science Panel:

Maya Groner and Trevor Branch are both interested in continued collaborations aimed at calculating disease parameters that can feed into the BASA and evaluating the role of disease in the BASA. The first step towards this goal is the calculation of realistic parameters for disease mortality that can be used in the BASA. Over the past six months, Groner has been working closely with John Trochta, Trevor's graduate student, as he develops a model that can calculate herring mortality due to VHS (viral hemorrhagic septicemia) using time series data on seroprevalence across age classes. The model has currently been tested on an artificial dataset to test its performance. Because it is performing well, the next step is to calculate mortality using the VHS seroprevalence timeseries dataset collected by Paul Hershberger in PWS. We are also applying a variation of this model to the PWS *Ichthyophonus* timeseries data to calculate mortality due to *Ichthyophonus* as a function of disease prevalence across age classes. Mortality estimates from both models can be used in future iterations of the BASA. Currently, transmission is not directly included in the BASA. However, it is a key parameter for disease models. Epidemiological models that include transmission estimates can be used to project disease spread under different scenarios (e.g., differing population size and structure, with varying levels of immunity) in order to identify scenarios that could lead to high mortality. Building dynamic disease models that can feed data into the BASA is a direction that we would like to pursue in the next funding period. Sensitivity analyses could then be performed on both the disease models and the BASA to determine which parameters most influence disease mortality and population estimates, respectively. The parameter estimation that is being conducted during this funding period is critical to that goal.

Sept 2020: Science Panel Comment – FY21: Given the addition of so much more herring research and observations since the 1980s, the SP encourages the PIs, perhaps in collaboration with other scientists, to develop a new) synthesis on PWS herring that would become the new authoritative reference.

Pegau response to Science Panel:

This comment was taken from the comments provided to project 21120111-F. There are many references that are important to our work. The book Herring Expectations for a New Millennium is a common reference. As suggested, it is time to revisit either a single paper like Norcross et al. (2001) A synthesis of the life history and ecology of juvenile Pacific herring in Prince William Sound, Alaska. A single manuscript may be too limited in scope and it may be better to work on a book with chapters more similar to Herring Expectations for a New Millennium.

Sept 2020: Science Panel Comment – FY21: What are the program and project contingency plans for FY21 in regard to accomplishing goals and field activities?

Pegau response to Science Panel:

The most likely impacts are to the principal investigator meeting and in outreach efforts. We anticipate that the principal investigator meeting will become a virtual meeting. We plan to investigate the appropriate means to hold a listening session with at least one village in Prince William Sound. We anticipate supporting other projects in field sampling if needed. The disease work being conducted by Dr. Groner can be accomplished by working at home.

Sept 2020: Science Panel Comment – FY21: Will any unused funds for FY21 be repurposed for additional lab and/or data analyses?

Pegau response to Science Panel:

We do not anticipate any unused funds. It is more likely that we may have to make small budget modifications within the existing funding to allow contracting sampling assistance if needed.

Sept 2019: Science Panel Comment – FY20: The Science Panel is pleased to see the involvement of an intern; however, we are concerned with the validity of the interpretation of the data by an inexperienced reader. For example, it was pointed out that many scales in recent years have had unusually closely spaced annuli and there was discussion whether these were true annuli or false annuli that shouldn't be considered when aging. The Panel notes that there is a Committee of Age Reading Experts (CARE) that may be able to help address such difficulties. Age readers benefit from years of experience to interpret annuli. If there is agreement about the apparent closely spaced annuli in recent warm years, would it be possible to look at archived samples to see if the same annuli patterns in past warm years?

Pegau response to Science Panel:

There appears to have been a misinterpretation of what was presented in the work plan. The intern imaged and measured the scales. They were not responsible for aging the fish. That is done by the ADF&G led project. The intern's aging efforts were limited to agreeing on age before imaging, as was set up in the original protocols. The image library that the intern was updating provides an easy way to look at scales from previous warm periods. One can either look at the measured growth or the images of the scales.

Thanks to the panel for the reference to the Committee of Age Reading Experts. The discussion noted in the work plan was between the different aging labs within ADF&G to see how difficult scales would be read and why each person interpreted them the way they did.

Sept 2019: Science Panel Comment – FY20: The Science Panel had considerable discussion about the quantification of spawning. The following few paragraphs attempt to capture this discussion. Evidence presented in the Branch proposal indicates that herring spawn has shifted both in time (among years) and space, both within PWS and the adjacent area of Kayak Island. Similar temporal and spatial changes have occurred recently in other regions of the eastern Pacific, such as the Strait of Georgia. Based on the new (but preliminary) spatial-temporal analyses of spawning presented in the Branch proposal (project 20120111-C) the Panel requested clarification about survey effort and if some of the explanation for recent change might be related to limitations of resources for surveys. Additional information was provided by phone by Pegau. See HRM Program 20120111 comments (above). During the phone call, however, the Panel was advised that the relatively recent occurrences of spawning on Kayak Island since about 2010 (see Figure 2 in the Haught proposal (project 20170111-F) and Figures 5-6 in the Branch proposal) are not included as part of the spawn estimates. If so, we would question the validity (or biological justification) for such an exclusion. Further, and echoing previous comments provided by the Panel, we also question the validity of the continued use of 'mile days' as quantitative units of spawn. We suggest two things. First, that the summing of spawn lengths, for two consecutive days in the same location may serve to inflate spawn deposition for certain areas. We strongly advise that this procedure requires re-examination and explanation – but this recommendation should not be interpreted as a criticism of the aerial surveys per se. On the contrary, the Panel applauds the efforts made to locate and measure the spawning. Second, the Panel points out that a linear measure of spawn may vary significantly depending on the location where it occurred. This is self-evident: spawning on steep narrow beaches with patchy macrophytes would not be expected to have equal numbers of eggs as broad beaches with dense macrophytes. These statements are clear from diver surveys conducted in other parts of the coast, including a few years in PWS. In this regard the panel wonders if there would be merit in attempting to calibrate different spawning areas in terms of their egg-rearing capacity. Similar attempts have been made elsewhere.

Pegau response to Science Panel:

There appears to be a couple main points to these comments. The first is the potential for movement of the location of spawn within PWS and movement of spawn to locations outside of PWS. The second is the validity of the survey technique used.

The movement of spawn and the change in spawn timing has long been a topic of discussion among herring researchers working in PWS. Without a doubt there easily could be a shift of spawning to locations outside of PWS that are not observed. There has never been regular surveys of spawn on Kayak Island and that will need to be made clear to the modeling effort. We have tried several methods (volunteer aircraft surveys, remote camera, person on the ground, satellite imagery) to improve our understanding of spawn in that area, but we have not been able to find a reliable means to survey spawn on Kayak Island. The remoteness of the island and weather in the area limits our ability to reach those spawning areas. Regular surveys of Kayak Island are limited to some extent by the funds available; however, access to the area is a greater limitation.

Two indices considered for spawn documented from aerial surveys were 1) discrete miles of milt over the season and 2) the sum of miles of milt for all survey days (mile-days of milt). The advantages of milt observations compared to school biomass observations are 1) herring schools likely spawn a single time e.g., a single day, but a herring school may be observed for several days prior to, or after spawning, 2) milt is relatively easy to observe from the air and observation efficiency is generally not influenced by ocean bathymetry (Brady 1987). Discrete miles of milt do not account for multiple spawning events in the same area, so are unlikely to be a good index of total abundance in areas with multiple days of spawning on the same beach (Brady 1987). Mile-days of milt provide a better index to abundance because they account for multiple spawning days on the same beach (Funk 1994).

Willette et al. (1999) collected paired spawn deposition survey estimates from dive surveys and aerial survey estimates of miles of milt; the short tons (dive survey) per mile of milt (aerial survey) were much larger on Montague Island beaches when compared to short tons per mile of milt in northern or northeastern PWS beaches. Montague Island shoreline typically has large shallow, subtidal areas with complex kelp structure while the northern and northwestern beaches tend to have a steeper gradient to deep waters and less complex kelp structure. Currently, biomass estimates derived from miles days of milt observations are weighted by district according to Willette et al. 1999.

Brady, J.A. 1987. Distribution, timing, and relative biomass indices for Pacific Herring as determined by aerial surveys in Prince William Sound 1978 to 1987. Alaska Department of Fish and Game, Division of Commercial Fisheries, Prince William Sound Data Report 87-14, Anchorage.

Funk, F. 1994. Forecast of the Pacific herring biomass in Prince William Sound, Alaska, 1993. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 5J94-04, Juneau.

Willette, T.M., G.S. Carpenter, K. Hyer, and J.A. Wilcock. 1999. Herring natal habitats, Exxon Valdez Oil Spill Restoration Project Final Report (Restoration Project 97166), Alaska Department of Fish and Game, Division of Commercial Fisheries, Cordova, Alaska.

We will continue to work with ADF&G to review the selection on the survey technique being used. The mile-days measure obtained from the ADF&G surveys could certainly be explained in more detail, and different ways of measuring have been explored. The danger to changing this metric is that it would break the long time series of consistent estimates over time, which would require the model to treat a new approach as a separate time series from the existing time series, and this would not be useful until the new time series included at least 4-5 years of data. Direct diver surveys for eggs would be expensive to start up again since this would require training divers in the methodology required, and again would require sufficient years to be useful. Past diver surveys were very useful because they provided an absolute measure of biomass, which although it was highly uncertain compared to the aerial surveys and the hydroacoustic surveys, provided an anchor point for the stock assessment (Muradian et al. 2019).

Sept 2019: Science Panel Comment – FY20: The Panel welcomed the development of mathematical models of VHS but had concerns with the model in this proposal. One of the stated motivations related to the idea that the benefits of herd immunity might be compromised by harvesting of older fish. Yet the S-E-I-C model presented does not take account of age structure. Despite this, there are stated aims to parameterize the model and publish a paper. What would be the goals of this paper?

Pegau response to Science Panel:

This is a good question and requires further explanation. Age is related to immunity. The older a fish is, the more likely it is to have experienced a VHS epizootic and to have immunity. There are several ways to explicitly or implicitly incorporate age into a model. As the panel suggests, one way to incorporate age into the model structure is to explicitly model it. For every age group modeled, this will add an additional 4 equations to the model, causing a shift from a relatively simple model to a fairly complex model that can be more complicated to solve, analyze and present. Alternatively, we can make some assumptions about the composition of herd immunity in older relative to younger fish. We can assume that older fish are more likely to be in the 'C' or carrier state, while younger fish are more likely to be in the 'S' or susceptible state. Thus, when we apply a fishing pressure to the population, we can adjust target the fishing to affect mostly older fish in state 'C' (i.e., in a gill net fishery), or an even proportion of fish in all states (i.e., a purse net fishery). Young fish can be added to the model as a 'pulse' of susceptible fish each year and the proportion of susceptible fish relative to carrier fish will determine the herd immunity of a population at any time. Because this is the first VHS model we are constructing and we will need to test parameters to calibrate the model, we prefer to keep the model simple. The focus of this paper will be on communicating and demonstrating key concepts that determine VHS epidemiology: immunity, population structure (w/ regards to disease states and population size), the parameterization of the model and the justification of the S-E-I-C design (as opposed to an SIR, or SI model). It is our plan, however, that this baseline model will serve as a template for more complex models that can incorporate additional factors such as age-structure and temperature-dependence, though the exact structure of any model version will be determined by the research question being proposed. We are currently working with John Trochta and Trevor Branch to adapt this model to have an age-structure in a later paper that is focused on how serology (i.e., immunity data) can be used to infer unobserved processes in a VHS outbreak, such as mortality or transmission.

Sept 2019: Science Panel Comment – FY20: The concerns about the modeling were mitigated by the description (page 5 of project 20120111-C) of a simulation study using an age structured model of VHS based on a slightly different epidemiological model (S-I-R). This is potentially very useful indeed and will contribute to integrating the findings from the disease study with the stock assessment modeling.

Pegau response to Science Panel:

The disease team and modeling team are working in close collaboration on disease models, which will include age structure and be incorporated into the stock assessment, should simulations demonstrate this would be a useful addition.

Sept 2019: Science Panel Comment – FY20: Older fish are now spawning in 2019. This means those fish have strong immunity. The panel would be interested to see if juveniles or year 1-2 fish from this older cohort are more resistant as compared to juveniles or years 1-2 from younger spawners in the past. This would suggest that there is a transfer of immunity (transcriptome or genetic) which could be addressed by the Whitehead project (20170115) and certainly could be critical information for model.

Pegau response to Science Panel:

Great observation: we will absolutely be tracking the VHSV antibody status of individual year classes as they get progressively older. We are as excited as you are to see these results, and we expect to have them prepared in the 2020 report. One word of caution however, there is no indication that there is any vertical transfer of VHSV antibodies from the mother to the progeny; therefore, any immunity to VHSV detected in an individual year class would have been achieved via adaptive immunity (i.e., surviving prior virus exposure).

11. Budget:

Budget Category:	Proposed FY 17	Proposed FY 18	Proposed FY 19	Proposed FY 20	Proposed FY 21	TOTAL PROPOSED	ACTUAL CUMULATIVE
Personnel	$57.0	$153.3	$177.4	$171.9	$100.1	$659.5	$ 457.1
Travel	$6.4	$9.9	$6.4	$6.4	$6.7	$35.8	$ 32.1
Contractual	$24.7	$26.0	$26.2	$11.0	$4.4	$92.3	$ 81.6
Commodities	$3.8	$1.5	$3.5	$1.4	$1.5	$11.7	$ 2.6
Equipment	$0.0	$0.0	$0.0	$0.0	$32.1	$32.1	$ -
Indirect Costs (*will vary by proposer*)	$35.1	$57.20	$64.0	$57.2	$33.8	$247.3	$ 179.6
SUBTOTAL	$127.0	$247.8	$277.5	$247.9	$178.6	$1,078.7	$753.0
General Administration (9% of subtotal)	$11.4	$22.3	$25.0	$22.3	$16.1	$97.1	N/A
PROJECT TOTAL	$138.4	$270.2	$302.5	$270.2	$194.6	$1,175.8	
Other Resources (Cost Share Funds)	$26.0	$26.6	$69.7	$90.5	$28.0	$240.8	

LITERATURE CITED

Brooks, M.E., K. Kristensen, K.J. van Benthem, A. Magnusson, C.W. Berg, A. Nielsen, H.J. Skaug, M. Machler, and B.M. Bolker. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal 9:378-400.

Campbell, R.W. 2018. Hydrographic trends in Prince William Sound, Alaska, 1960–2016. Deep Sea Research Part II: Topical Studies in Oceanography 147:43-57.

Ferriss, B, and S. Zador. 2020. Ecosystem Status Report 2020 Gulf of Alaska. North Pacific Fishery Management Council, Anchorage, AK.

King, A.A., D. Nguyen, and E.L. Ionides. 2016. Statistical inference for partially observed Markov processes via the R package pomp. Journal of Statistical Software 69:1-43.

Kocan, R.M., P.K. Hershberger, and J.R. Winton. 2004. Ichthyophoniasis: An emerging disease of Chinook salmon, *Oncorhynchus tshawytscha* in the Yukon River. Journal of Aquatic Animal Health 16:58-72.

Muradian, M.L., T.A. Branch, S.D. Moffitt, and P.J.F. Hulson. 2017. Bayesian stock assessment of Pacific herring in Prince William Sound, Alaska. PloS one 12(2), p.e0172153.

Sitja-Bobadilla, A. and P. Alvarez-Pellitero. 1990. First report of *Ichthyophonus* disease in wild and cultured sea bass *Dicentrarchus labraxfrom* the Spanish Mediterranean area. Diseases of Aquatic Organisms 8:145-150.

Vollenweider, J.J., J.L. Gregg, R.A. Heintz, and P.K. Hershberger. 2011. Energetic cost of *Ichthyophonus* infection in juvenile Pacific herring (*Clupea pallasii*). Journal of Parasitology Research 2011 doi:10.1155/2011/926812.