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Michael Baffrey
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441 W. 5th Ave., Suite 500
Anchorage, AK 99501-2340

August 28, 2008

Re: Project 080806/Are Herring Energetics in PWS a Limiting Factor?

Dear Michael:

Please find enclosed the documents for consideration for an extension of our current FY08 project, including detailed project description, budget, signature page and progress report.

Our request this year exceeds last year's budget by approximately 10% as a result of increased fuel and charter costs. We plan to use these funds to complete the third year of our project as we outlined in last year's request. We are currently on schedule, all of our field samples have been processed through our chemistry lab. We have completed one iteration of our laboratory study at the Marrowstone lab in conjunction with Paul Hershberger and two other trials are underway. Our preliminary results indicate:

1) Age-0 herring in PWS lost wet mass at a lower rate than herring starved under controlled conditions. This indicates that PWS herring are foraging over winter.

2) In PWS, adult herring use energy at a higher rate over winter than herring in southeast Alaska.

Our findings of elevated energy demand among herring in PWS are consistent with other EVOS programs that EVOS is funding. Project 080819, has determined that herring in PWS have a higher prevalence of *Ichthyonphonus* than herring in Sitka Sound on Lynn Canal. We hypothesize that the immune response to the presence of *Ichthyophonus* in PWS adds an additional cost to overwintering. This additional cost could account for the increase rate of energy loss. We plan to test this hypothesis in FY09 in collaboration with Hershberger (Co-PI) at Marrowstone. In addition, Hulson et al. (2008) concluded that infection of fish in low nutritional condition was responsible for the initial decline in herring abundance in 1992. This is another hypothesis we plan to test in FY09. Finally increased energy cost may be the result of increased predator avoidance. Project 090804 has determined that whales are significant predators of herring in PWS and that herring schooling behavior in PWS differs from that in southeastern AK.

We hasten to also point out that this study provides fundamental services to two other current EVOS studies. We provide the seasonal energy content of wild herring from PWS, Sitka Sound, and Lynn Canal for the modeling component of the whale project (Project 090804) in order to estimate the number of herring required to meet the caloric demand of humpback whales throughout the winter. Secondly, this study is tightly coupled to the Project 080819 the Herring Disease Program, by providing reference samples to measure disease prevalence from Sitka Sound and Lynn Canal, as well as energy content in these samples. In addition the laboratory component in FY09 will directly test important hypotheses regarding disease and metabolic rates. Together, the three tightly integrated studies will provide a much more comprehensive picture than any of studies could alone and provide a suite of data for modelers.

Additional collaborative associations include logistical efforts with ADF&G Cordova, Sitka Sound Science Center. In addition, we have been able to leverage support from NOAA's Undersea Research Program (NURP) to obtain imagery of herring behavior following predator attacks using DIDSON sonar. The bioenergetic data we are collecting here can be combined with the NURP – funded observations to estimate the cost of predator avoidance.

Our specific objectives with this third year of study will be to continue collections of wild fish over winter and to specifically test the aforementioned hypotheses regarding the interaction between nutritional status, disease exposure and metabolic rate. We require a third year of field collections because the delay in funding in FY07 impaired out ability to obtain sufficient samples at the beginning of winter in 2007. Although we are field sampling in three areas, the field costs are dominated by the focus in PWS The sampling in Lynn Canal and Sitka Sound is logistically easier and cheaper than the PWS sampling. It should be clear by now that inclusion of these latter sites is greatly improving our understanding of the stresses and costs incurred by PWS herring. .

Thank you for your consideration for a funding extension.

Sincerely,

Johanna Vollenweider & Ron Heintz

Project 080806 - Are Herring Energetics in PWS a Limiting Factor?

I. PROGRESS REPORT

Outlined below are the second year objectives. Specific progress towards these goals is itemized in following remarks.

Objective 1. Field collections (Replication of year 1) - Measure overwinter energetic changes in herring to examine juvenile mortality and adult reproductive investment.

Wild herring were collected from each of the 3 study regions (PWS, Sitka, Lynn Canal) between December 2007 and May 2008. Bioelectrical impedance (BIA) measurements to estimate energy content were performed at the time of capture. In addition, samples were collected for chemical analysis. Collected samples generally fulfilled the study design (collection periods, ages…) with some low sample sizes for YOY fish which were difficult to find. In addition, no pre-winter samples were collected in Sitka Sound this past winter due to their scarcity. Usually herring arrive in Sitka Sound in December or January to form their deep, overwintering schools. This past winter, however, the herring were several months late, as were the humpback whales. In addition to our search efforts, a variety of entities verified the lack of herring in the sound, including the hydroacoustic surveys made by the whale project, ADF&G searching for roe samples, Jan Straley (UAF) searching for humpbacks (whale project), and from local contacts out on the water, including Sitka Sound Science Center, and the US Coast Guard. Thus, herring collections in Sitka were limited to immediately prior to spawning and after spawning this year.

All measurements of the wild-caught herring are complete for both collection years (n=2000). These measurements included length, wet weight, stomach content weight, age and chemical analysis. A total of 1040 were aged using scales and 940 were assessed for stomach contents. Chemical analysis of 300 wild-caught herring has been completed for both years of field collections, including lipid, protein, and energy content of fish. Mature fish were divided into soma and reproductive organs to assess the energy content of roe and milt for reproductive analyses. Analysis of these additional 160 samples is also complete. Initial analysis of voucher specimens indicates that bioelectrical impedance (BIA) measurements are highly correlated with estimates of energy content derived from proximate analysis (R^2=0.91). This suggests that our existing BIA data can be used to significantly expand the number of observations for energy content in our data set. Water temperature data obtained in CTD casts still require analysis.

Objective 2. Laboratory-based studies – Parameterize the Wisconsin bioenergetics model to weigh the evidence for or against energy limitations contributing to the PWS population decline via winter survival or reproduction.

One iteration of the lab study has been completed. After plumbing and laboratory set-up, the trials ran for 4 months. Specifically, 3 replicate tanks of YOY herring (35 per tank) were starved to approximately 50% mortality at 3 temperatures (5.5, 8.5-ambient, 12.5 ^{o}C) Resting metabolic rates will be calculated from energy losses incurred by these fish. After the starvation period was over, the remaining fish were re-fed to examine the potential for compensatory growth to simulate the spring bloom. Concurrently, 3 replicate tanks (15 fish per tank) at each temperature contained fed herring. These fish were sampled for analyses to relate increases in herring mass and length to biochemical measures of growth including RNA/DNA and enzyme activities. In addition, maximum consumption rate and assimilation efficiency was determined from these fish. Proximate analysis and bomb calorimetry of these samples will begin soon. We have completed development of the RNA/DNA assay and those analyses are underway.

A second iteration of the YOY starvation study is underway. We opted to perform a second iteration because sample numbers were relatively small in the first trial. We were able to obtain sufficient numbers of fish earlier this summer to redo the experiment. This second iteration involves nearly twice as many fish as the first and will therefore provide more complete results. In addition, the temperatures for the second iteration will differ from those in the first, providing more observations with which we can relate metabolic rate and temperature. In addition to the YOY herring study we have initiated a starvation trial for age-2 fish.

II. INITIAL RESULTS

Our initial analysis indicates that adult herring in PWS lose energy at a higher rate than herring in southeastern Alaska. We used allometric relations between energy content and fish length to estimate the energy content of fish of a fixed length in each of our sampling strata. We calculated the energy loss rate (*r*) as the percentage lost per day from:

$$r = \frac{Ln(e_f) - Ln(e_0)}{t} \times 100 \qquad (1)$$

Where e_f is the estimated energy content at the end of winter and e_o is the estimated energy content at the beginning of winter and t is the number of days between e_f and e_o.
Energy loss averaged 0.42% per day for herring in PWS sampled over two winters. In contrast, energy loss was 0.36% per day for herring from Sitka in the winter of 2005-2006. In Lynn Canal loss rates averaged 0.25% per day over two winters. It is interesting to note that *Ichtyophonus* prevalence is highest in herring from PWS and lowest in herring from Lynn Canal (Paul Hershberger personal communication). Sitka has an intermediate prevalence. This suggests that disease exposure may impose a higher metabolic cost.

We have also determined that YOY herring in PWS are foraging over winter. In our starvation study we observed we average mass loss rates of 0.24%, 0.25% and 0.42% per day in our cold, ambient and warm tanks, respectively. Using an approach similar to that described in equation (1) we determined that YOY herring lost 0.09% of their wet mass per day during the winter of 2006-2007 and 0.04% per day in the winter of 2007-2008. The lowest temperature in our laboratory study was 5.5° C, we will have temperature for PWS once we complete our analysis of CTD data. It is unlikely that temperature will account for the disparity in mass loss rates, instead it appears that fish in PWS are forestalling mass loss by foraging. This was consistent with observations of prey in 60% of the stomachs we examined.

III. INTEGRATION WITH OTHER PROJECTS

Our project is tightly integrated with two other current EVOS projects, providing fundamental services to both studies. This project is a companion project with the whale project both in terms of data sharing as well as logistical support. We provide the seasonal energy content of wild herring from PWS, Sitka Sound, and Lynn Canal for the modeling component of the whale project (Project 080804) in order to estimate the number of herring required to meet the caloric demand of humpback whales throughout the winter. Additionally, both projects incur costs savings since herring collections and whale observations are often made from the same platform. Furthermore, each study is a scout for the other study, as where we find herring in the winter, we often find whales, or vice versa. For example, the acoustic surveys performed for the whale study are integral for finding herring for sample collections in southeast Alaska. Additionally, whale identification trips in Sitka carried about by Jan Straley (Sitka local) have been instrumental in saving us money and logistical effort by postponing our trips when the herring and humpbacks were late in arriving this past year.

The second current EVOS study we are tightly coupled to is the Herring Disease Program (Project 080819). During field collections, the herring energetics study provides reference samples to measure disease prevalence from Sitka Sound and Lynn Canal. The fact that the two studies are conducted during the same years has implications for both studies. Disease prevalence and energetic condition may have synergistic effects. In addition, measuring both variables in the field simultaneously eliminates any potential confounding effect from interannual variability. Project 080819, has determined that herring in PWS have a higher prevalence of *Ichthyonphonus* than in Sitka Sound and Lynn Canal. We hypothesize that our concurrent observations of increased energy loss of PWS herring results from increased metabolic demand incurred by the presence of *Ichthyophonus*. We plan to test this hypothesis in FY09 in the lab component. In addition, Hulson et al. (2008) concluded that infection of fish in low nutritional condition was responsible for the initial decline in herring abundance in 1992. This is another hypothesis we plan to test in FY09. An additional benefit of the two studies occurring simultaneously is that this has allowed for spontaneous data collections and collaboration when representatives from both studies have been aboard the same vessel (ADF&G's sampling cruises). For example, last year we began measuring bioenergetic parameters on the same individual fish that were being analyzed for disease, providing another comparison of field observations to the controlled conditions of the laboratory component.

Together, this project, the whale and disease studies provide a much more comprehensive picture of herring in PWS than any of studies could alone. Additionally, the collaboration of these three projects provides large cost savings.

Additional collaborative associations include logistical efforts with a multitude of other groups, including:

1) ADF&G Cordova – provided vessel support during their biannual herring collection trips in PWS
2) McLaughlin Environmental Services (Sawmill Bay) – have provided herring samples from Sawmill Bay
3) Prince William Sound Science Center – have provided herring samples from PWS
4) Sitka Sound Science Center – have provided herring samples from Sitka Sound and logistical support during our visits to Sitka (vessel support, in-town transportation, knowledge of herring locations…)
5) ADF&G Sitka – provided vessel support during herring collection trips in Sitka Sound
6) USCG Sitka – provided observations of humpback whales in Sitka Sound in order to find herring
7) NOAA Undersea Research Program program provided funding for us to study herring predator avoidance behavior with DIDSON sonar. This project was leveraged off of an EVOS funded survey. We were able to capture multiple videos of Steller sea lion attacks on herring in winter. From the imagery we can estimate swimming speed. Combining the swimming estimates with bioenergetic data collected under this study will allow us to estimate the cost of predator avoidance.

PROPOSAL SIGNATURE FORM

THIS FORM MUST BE SIGNED BY THE PROPOSED PRINCIPAL INVESTIGATOR AND SUBMITTED ALONG WITH THE PROPOSAL. If the proposal has more than one investigator, this form must be signed by at least one of the investigators, and that investigator will ensure that Trustee Council requirements are followed. Proposals will not be reviewed until this signed form is received by the Trustee Council Office.

By submission of this proposal, I agree to abide by the Trustee Council's data policy (*Trustee Council Data Policy**, adopted March 17, 2008) and reporting requirements *(Procedures for the Preparation and Distribution of Reports***, adopted June 27, 2007).

PROJECT TITLE: Are herring (Clupea pallasi) energetics in PWS a limiting factor in successful recruitment of juveniles or reproduction investment of adults? Part III: Impacts of Ichthyophonus on metabolic rates of fasting herring.

Printed Name of PI: Johanna Vollenweider____________________

Signature of PI: ____________________________Date______________

Printed Name of co-PI: Ron Heintz____________________________

Signature of co-PI: ____________________________Date______________

Printed Name of co-PI: Jeep Rice____________________________

Signature of co-PI: ____________________________Date______________

* Available at www.evostc.state.ak.us/Policies/data.htm
** Available at www.evostc.state.ak.us/Policies/guidelines.htm

Trustee Council Use Only
Project No: _____
Date Received: _____

PROPOSAL SUMMARY PAGE
(To be filled in by proposer)

Project Title: Are herring (*Clupea pallasi*) energetics in PWS a limiting factor in successful recruitment of juveniles or reproduction investment of adults? Part III: Impacts of *Ichthyophonus* on metabolic rates of fasting herring.

Project Period: Dec. 1, 2006 – April 15, 2010

Proposer(s): Johanna Vollenweider[1], Ron Heintz[1], Stan Rice[1], and Paul Hershberger[2]
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Study Location: Prince William Sound, Sitka Sound, Lynn Canal

Abstract: We hypothesize that increased *Ichthyophonus* prevalence in PWS herring results in an increased metabolic cost relative to other locations in the state. We propose a combination of field collections to estimate energy content in juvenile and adult age classes before and after the winter, and a series of controlled laboratory tests at different starvation states to measure energy costs of the disease infection. Field collections to estimate the cost of overwintering and spawning in herring will be from PWS, Sitka Sound and Lynn Canal. The latter two locations are areas in which herring populations are healthy (Sitka Sound) and depressed but not impacted by *Ichthyophonus* (Lynn Canal). This will be the third year of our study. To date we have determined that the average energy loss rate of adult herring in PWS is greater than that of fish in southeast Alaska (predator avoidance? Greater disease challenge?) and age-0 herring in PWS must be foraging during winter. In this last year we will continue field sampling to determine if that trend continues. Our controlled laboratory tests, conducted at Marrowstone Marine Field Station, will determine if exposure to *Ichthyophonus* increases metabolic costs and if fish in poor nutritional condition are more susceptible to *Ichthyophonus*. Together, these data sets will illustrate how disease impacts energy costs.

Funding: EVOS Funding Requested: FY 09 $ 206.4
(must include 9%GA)
Non-EVOS Funds to be Used: FY 09 $ 54.2

TOTAL: 260.6

Date: September 3, 2008

PROJECT PLAN

I. NEED FOR THE PROJECT

A. Statement of Problem

The reasons underlying recruitment failure among Prince William Sound (PWS) herring stocks are unknown. Historically, PWS herring stocks were sustained by the periodic recruitment of strong year classes. These year classes occurred approximately every four years and sustained an important economic base for people living in the region. Significant recruitment events have not happened in the last decade and the population size is severely constrained. The causes underlying these recruitment failures are currently unknown, but they may relate to disease, predation, or reduced forage quality. Surveys conducted in the late 1990's and early 2000's indicate that viral hemmorhagic septicemia (VHS) may be reducing recruitment of early age classes and ichthyophoniasis may be reducing the maximum age at maturity.

In addition to acute effects, disease could affect recruitment by increasing energetic demand. In earlier EVOS funded studies, the SEA project determined overwinter survival was a major limiting factor for young of the year (YOY) and probably age-1 herring (Norcross et al. 2001). Both year classes are highly vulnerable to predation, and must grow rapidly to minimize their exposure to some predators. However, allocation of ingested energy to growth obviates allocation to energy storage. In winter, food availability is severely limited and fish must rely heavily on energy reserves to meet metabolic demand. This means that juvenile herring must successfully negotiate the conflicting demands of growth and energy storage if they are to survive winter. In energetically demanding winters, relatively small herring have little energy reserves and high metabolic demand. Any additional drain on their energy reserves will likely make them vulnerable to mortality. Increased antibody titers resulting from low level infections may cause increases in energetic demand due to the relative high cost of protein synthesis. Increased disease prevalence and intensity therefore could increase vulnerability to mortality through natural causes during periods when exogenous energy supplies are diminished.

A second potential bottleneck to the recruitment process relates to the availability of high quality forage. Adult herring spawn in spring so that hatching coincides with the spring bloom. The paucity of prey available in the months prior to spawning means that adult herring must produce gametes under conditions of extremely limited exogenous energy sources. This accounts for the relative peaks in energy content of adult herring at the onset of winter (Vollenweider 2005). If forage quality in fall is poor, adults will enter winter with reduced nutritional condition and they will have reduced energy available for provisioning offspring. We propose to continue monitoring the energetic cost of over wintering and reproduction in adult herring. In addition, we propose to develop bioenergetic models for adult herring so that field measurements can be compared after accounting for temperature differences.

This study will make energetic assessments of age 0, 1 and adult herring at the beginning and end of winter from three regional populations (PWS, Sitka, Lynn Canal), and will compare the status between these populations over a three year period. Sitka is a healthy population that is currently harvested. Lynn Canal is a depressed stock that may be listed as threatened or endangered. These represent extremes with which the PWS data can be compared. To aid in interpretation of the field data, energetic measurements will be made in the laboratory for different life stages (and temperatures) to generate an energetic model. In year 3, the last of the field assessments will be made, and the energetic measurements in the lab will be replicated with disease challenges.

B. Relevance to 1994 Restoration Plan Goals and Scientific Priorities

This project addresses "Injured Resources and Services: Evaluation and Restoration". In particular, we will examine Pacific herring, an injured resource which has been classified as "not recovered". One indication of recovery has been identified as highly successful recruitment of a year class. We will directly test hypotheses for recruitment failures. Identification of processes contributing to recruitment failures (or conversely, ruling out of these processes) will provide valuable information to managers for remediation.

II. PROJECT DESIGN

A. Objectives

Our objectives are to determine if the costs of overwintering and spawning are higher in PWS herring than those in other parts of Alaska. We theorize that reduced recruitment of herring in PWS relates to increased energy demand resulting from exposure to *Ichthyophonus*. Energy losses in overwintering herring are well documented (Vollenweider 2005, Norcross et al. 2001). We propose comparing spawning and overwintering costs with those experienced by herring in Sitka Sound, a healthy population, and Lynn Canal, a depressed population with low *Ichthyophonus* prevalence. In order to accurately assess differences in cost we require measuring the energy content of herring in each location at the beginning and end of winter and after spawning. Water temperature and disease prevalence are possible confounding factors in our estimation of overwintering costs. Consequently we also propose a series of laboratory studies to measure the relationship between routine metabolism and temperature and the relationship between *Ichthyophonus* exposure and routine metabolism. The first of these two laboratory studies will allow us to account for temperature differences in our assessment of spatial effects on overwintering costs. The second laboratory study allows for an accounting for spatial differences in disease exposure.

In FY07 and FY08 we completed the field collections and the first of the laboratory studies. In FY09 our objectives are to complete another set of field collections and complete the second set of laboratory studies. These objectives are detailed below. Methods for the field collections will be the same as in first two years, methods for the FY09 laboratory study are described below.

YEAR 1 (FY07)

Field collections - Measure overwinter energetic changes in herring to examine juvenile mortality and adult reproductive investment.

a. Field collections of herring from each of the 3 study regions (PWS, Sitka, Lynn Canal).

b. Chemical analysis to determine overwinter changes in lipid, protein and energy content of fish collected in the field.

YEAR 2 (FY08)

1. ***Field collections (Replication of year 1) - Measure overwinter energetic changes in herring to examine juvenile mortality and adult reproductive investment.***
    a. Replicate field collections of herring from each of the 3 study regions (PWS, Sitka, Lynn Canal) sampled in year 1.
    b. Chemical analysis to determine overwinter changes in lipid, protein and energy content of fish collected in the field.

2. ***Laboratory-based studies – Measure herring energetic parameters.***
    a. Measure bioenergetic parameters to determine routine metabolic rate, maximum consumption rate and assimilation efficiency at 3 temperatures. Life stages will be age 0, and age 1+.
    b. Relate changes in herring mass and length to biochemical indices of growth including RNA/DNA and enzyme activities.

YEAR 3 (FY09)

1. ***Field collections (Replication of years 1 & 2) - Measure overwinter energetic changes in herring to examine juvenile mortality and adult reproductive investment.***
    a. Replicate field collections of herring from each of the 3 study regions (PWS, Sitka, Lynn Canal) sampled in years 1 & 2.
    b. Chemical analysis to determine overwinter changes in lipid, protein and energy content of fish collected in the field.

2. ***Laboratory-based studies – Measure influence of disease on herring energetics.***
    a. Compare the survival of *Ichthyophonus*-infected and uninfected herring under food-withheld conditions.
    b. Compare bioenergetic parameters among *Ichthyophonus*-infected and uninfected herring under food-withheld conditions.
    c. Determine the impact of bioenergetic condition on the ability of herring to survive exposure to *Ichthyophonus*.

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B. Procedural and Scientific Methods

YEAR 3 (FY09)

Objective 1. Field collections – Measure overwinter energetic changes in herring to examine juvenile mortality and adult reproductive investment.

Winter energy expenditures of herring will be measured following the same procedures used in Years 1 and 2. In summary, we will compare winter whole-body energy expenditure (lipid, protein and energy) of juvenile and mature herring in PWS, Sitka Sound and Lynn Canal by examining pre-winter, post-winter and post-spawn field collections. Chemical analysis of body composition will follow procedures outlined in Vollenweider (2005). Additional estimates of body composition will be performed using bioelectrical impedance analysis (BIA) following methods outlined by Cox et al. (2005). Temperature measurements will be acquired from CTD casts made in conjunction with each herring collection.

YEAR 3 (FY09)

Objective 2. Laboratory based studies – Measure influence of disease on herring energetics.

Among wild Pacific herring, exposure to *Ichthyophonus* begins during the first summer after larval metamorphosis to juveniles, when 5-12% of age 0 juveniles are infected (Kocan et al 1999, Hershberger et al 2002). The increased metabolic demand of these infections, combined with decreased nutritional condition of overwintering juveniles has led to development of the hypothesis that infected cohorts lack sufficient energy reserves to survive the winter fasting period in Prince William Sound (Marty et al 2003). We propose to test this hypothesis using a series of three experiments under controlled laboratory conditions. Variables inherent to wild herring including unknown histories of exposure, infection, disease, and immunological competence severely limit their suitability as experimental animals to understand the interactions of disease and nutritional condition. Therefore, the controlled experiments will be performed using specific pathogen-free (SPF), immunologically naïve Pacific herring (Holmes Harbor stock) at the Marrowstone Marine Field Station.

Objective 2a - Compare the survival of Ichthyophonus-infected and uninfected herring under food-withheld conditions.

The ability of poikilotherms to respond to pathogens is a function of water temperature and nutritional condition. Extremes in water temperature can result in failed up-regulation of immune response genes, and result in unencumbered disease dissemination within the host. Additionally, *Ichthyophonus* growth is a direct function of water temperature, and the parasite can be more pathogenic (Okomato et al 1987) and progress to terminal disease more quickly at higher temperatures (Kocan et al in preparation). Similarly, animals with low nutritional condition (i.e. low energy reserves) are typically thought to have greater susceptibility to chronic diseases as a result of shifted allocation of energy from immune functions to maintenance of basic metabolic functions.

We propose to study appropriate groups of infected and uninfected Pacific herring to determine how *Ichthyophonus* infections impact the survival of fasting juveniles (Figure 1). Briefly, a group of SPF herring (n=400) will be infected with *Ichthyophonus* by intraperitoneal (IP) injection of parasite resting stages harvested from infected, wild herring; a parallel group of controls (n=400) will be sham injected with phosphate buffered saline. The infected and control groups will be maintained in ambient seawater for 30d to allow the infections to establish. Herring from the infected and uninfected groups will be partitioned into respective, triplicate tanks (n=30 herring/tank at each of three temperatures) where they will be held without feeding. The effect of infection on fasting fish will be determined by recording mortality daily until the cumulative mortality reaches 90%. Additionally, triplicate tanks of infected and uninfected herring will be fed to satiation several days/week will be maintained at one temperature (ambient) (Figure 1). The effects of fasting on the kinetics of ichthyophoniasis will be determined by contrasting the mortality schedules of fasting and fed fish infected with *Ichthyphonus.*

Sampling of fish during this study includes estimation of the prevalence of *Ichthyophonus* and the energy content of the herring. All dead herring from all treatment groups will be necropsied to determine prevalence and intensity of *Ichthyophonus* infection; additionally, survivors at the end of the experiment will be euthanized, necropsied to determine *Ichthyophonus* prevalence and intensity, and processed to determine energy content. Prevalence of *Ichthyophonus* will be determined by primary explant culture of heart and liver tissue in tris-buffered MEM supplemented with 5% fetal bovine serum, 100 IU mL^{-1} penicillin, 100 IU mL^{-1} streptomycin, and 100 IU mL^{-1} gentamycin. Cultures will be incubated at 12 °C and examined microscopically (100 X magnification) for the presence of *Ichthyophonus* spores and/or germinating bodies after days seven and fourteen. Intensity of *Ichthyophonus* infections will be quantified by examining each fish for gross signs of disease, including open ulcers on the skin and presence of white nodules on the heart, liver, and spleen. Additionally, half the heart and liver will be fixed in 5% neutral-buffered Formalin for histological processing and evaluation (hematoxylin-eosin and periodic acid-Schiff stains). The number of *Ichthyophonus* spores and germinating bodies in histological cross section will be used to quantify intensity/severity of infection. Energy content will be determined by bomb calorimetry.

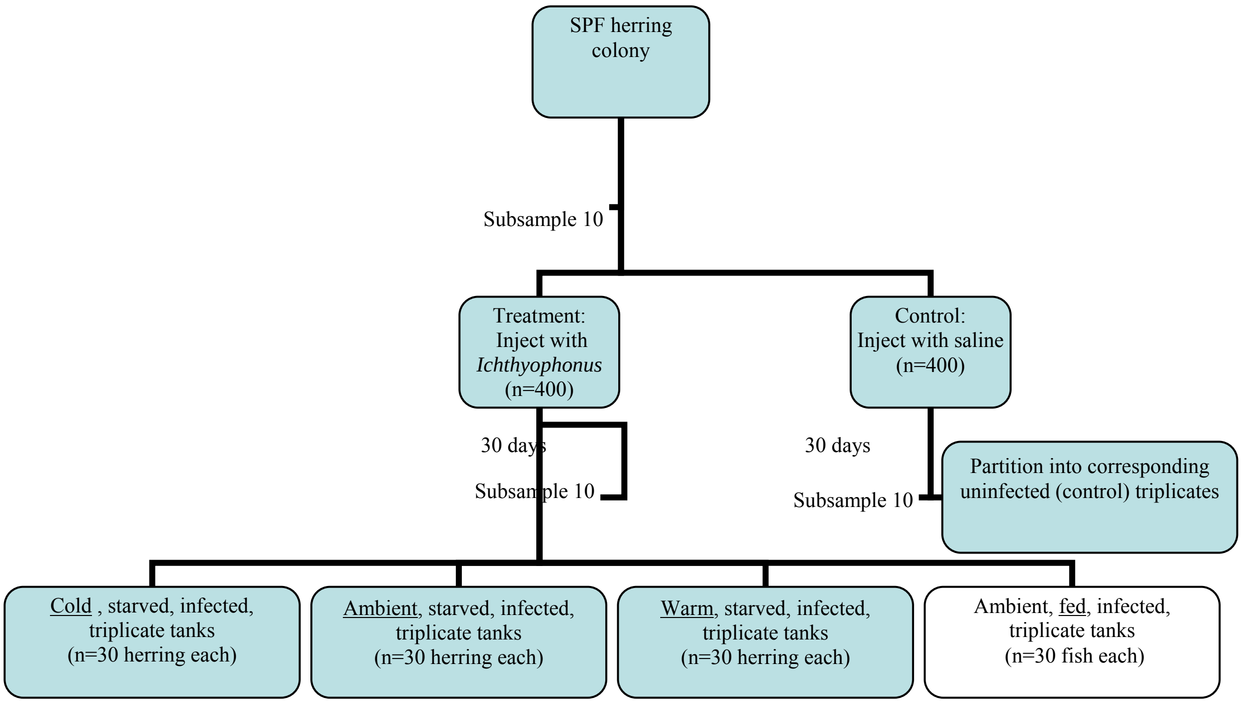

Figure 1. Flow chart depicting experimental layout for Year 3 Objective 2a. Food-withheld treatments will consist of *Ichthyophonus*-infected herring in triplicate tanks (n=30 fish / tank) at each of 3 temperatures (cold, ambient, and warm), with corresponding triplicates containing uninfected controls at each temperature. Additionally, fed treatments will consist of *Ichthyophonus*- infected herring in triplicates tanks (n=30 fish / tank) at ambient temperature, with corresponding triplicates of uninfected controls.

Objective 2b – Determine bioenergetic parameters among Ichthyophonus-infected and uninfected herring under food-withheld conditions.

Routine metabolic costs and other bioenergetic parameters for fish infected with *Ichthyophonus* may not be accurately reflected in estimates derived from fish used in the Year 2 laboratory studies. We propose to repeat that analysis using fish infected with *Ichthyophonus*. Comparison of the two laboratory studies will provide a firm basis for estimating the energetic cost associated with infection. In Year 2 we determined the metabolic cost of overwintering in fasting herring held at three temperatures. In addition we estimated conversion efficiencies and maximum consumption rates. In Year 3 we propose to use the same methods to estimate the metabolic cost associated with *Ichthyophonus* infection. Changes in energy (ΔB) in a fish result from the net effects of food consumption (C), respiratory processes (R_t) and production of feces, ammonia and urea (W).

$$\Delta B = C - R_t - W \quad (1)$$

R_t is total metabolic cost and incorporates routine metabolism (R_r), locomotion (R_a), and specific dynamic action (R_d). We assume that W = 1- *p*C where *p* is the assimilation efficiency determined as the proportion of C that is retained as biomass. Note, that this definition of W also reflects the amount of energy lost to specific dynamic action and therefore incorporates R_d.

Hence,

$$\Delta B = C - R_r - R_a - (1-pC) \quad (2)$$

In FY08, we measured the routine metabolic rate (R_r) of herring. By withholding food and keeping activity to a minimum we were able to directly measure R_r as the change in energy content of herring over a fixed time period. However, an additional variable inherent to Pacific herring in the eastern north Pacific involves *Ichthyophonus* infections, which are very likely to impact the metabolic cost of infected individuals. Therefore, changes in energy need to account for the metabolic cost of *Ichthyophonus* infection (R_i).

$$\Delta B = C - R_r - R_a - R_i - (1-pC) \quad (3)$$

In FY09 we will estimate R_i by withholding food from groups of *Ichthyophonus*-infected and uninfected herring and minimize their activity; the difference in their respective routine metabolic costs will estimate R_i. Briefly, a group of SPF herring (n=600) will be infected with *Ichthyophonus* by IP injection of parasite resting stages harvested from infected, wild herring; a parallel group of controls (n=600) will be sham injected with phosphate buffered saline. The infected and uninfected groups will be split into triplicate tanks (n=60 herring/tank) for each of three different temperatures after a 30 d incubation period in ambient water. Food will be withheld and the fish will be sampled every 2 weeks for 2 months, or until 90% of the fish have succumbed to starvation. Sampled herring will be processed to determine body composition (lipid, protein, ash and water) energy content and prevalence/intensity of Ichthyophonus infection. Starvation periods, subsample size and frequency will be re-evaluated after completion of Objective 2a; hence, this experimental outline serves as a general guide, but sampling details are subject to change upon reviewing the starvation kinetics from Objective 2a. The layout of the experiment is given in Figure 2.

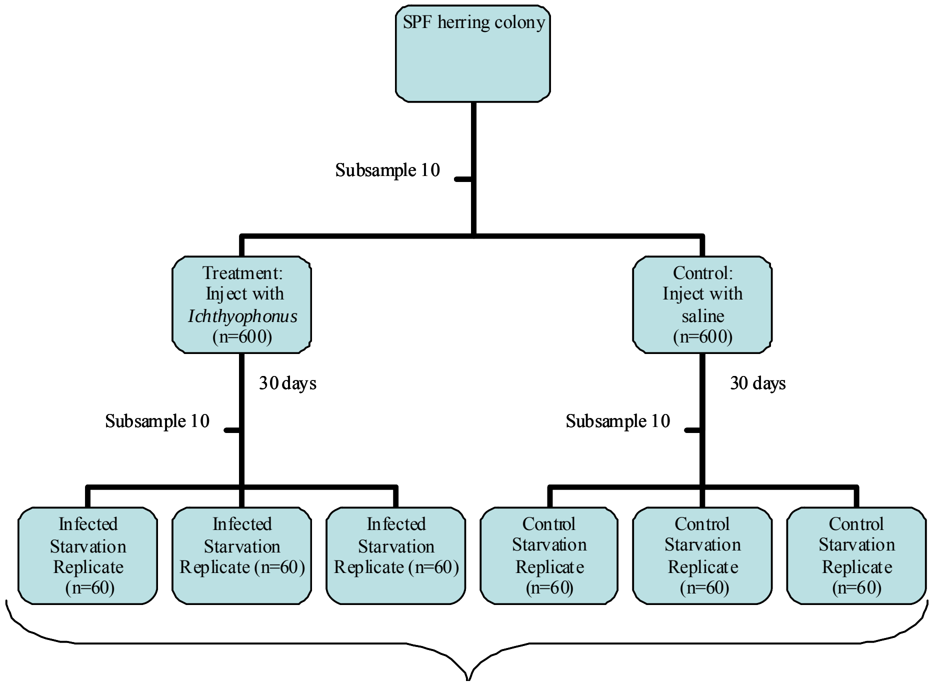

Figure 2. Flowchart depict the experimental layout for Year 3 Objective 2b. Fish will incubate for 30 d in ambient water following initial exposure to *Ichthyophonus*. Next 60 fish will be apportioned to one of three tanks. Each triplicate is repeated at 3 temperatures and the whole design is repeated for unexposed fish. See Table 3 for sample numbers.

Objective 2c – Determine the impact of bioenergetic condition on the ability of herring to survive exposure to Ichthyophonus.

Previous studies speculated that poor condition of herring after a period of winter fasting leads to increased susceptibility to infection and disease (Marty et al. 2003). However, existing data to support this hypothesis are circumstantial and based on uncontrolled observations of wild herring. Unlike objectives 2a&b, where we proposed to investigate the fate of *Ichtyophonus*-infected cohorts that enter the winter starvation period, here we propose to determine whether herring that come out of the winter fasting period in decreased condition are more susceptible to mortality from ichthyophoniasis.

To determine the influence of *Ichthyophonus* on the ability of herring to recover from a fasting period, triplicate treatment tanks, each containing 50 herring, will be set up at each of three temperatures (low, ambient, and high). Food will be withheld from herring in all tanks. When the nutritional condition falls to a level that is consistent with that of Prince William Sound herring coming out of the winter fasting period (estimated by the results of Objective 2b and field observations), herring in all treatment replicates will be challenged with *Ichthyophonus* by IP injection of resting spores. Respective triplicate control tanks, containing starved herring at each temperature will be injected with saline rather than *Ichthyophonus*. After challenge, herring in all tanks (treatment and control) will be fed to satiation daily to simulate feeding on the spring plankton bloom.

To investigate the influence of fasting on the ability of herring to survive exposure to *Ichthyophonus*, herring in six additional tanks (n=60 each) will be fed to satiation daily while fasting is underway in the previously described treatments. Subsamples from each tank (n=10) will be collected on the same day as the fasting treatments to compare metabolic content among fasted and fed groups. Herring in triplicate tanks will then be challenged with *Ichthyophonus* by IP injection; herring in respective triplicate control tanks will be injected with saline. Feeding to satiation will continue in all tanks. Mortality will be compared among previously fasted and continuously fed groups. Due to time and space constraints, the continuously-fed groups will be maintained only at a single temperature (ambient). Mortalities will be collected daily and analyzed for *Ichthyophonus* prevalence and intensity, and the experiment will be terminated after mortality reaches 90% or after 2 months, whichever comes first. Mean day-to-death will be compared among *Ichthyophonus* infected and uninfected (control) groups and among continuously fed and food-withheld groups. Bioenergetic samples are outlined in Table 4. Note that data from unexposed controls will also provide information on the potential for compensatory growth in juvenile herring.

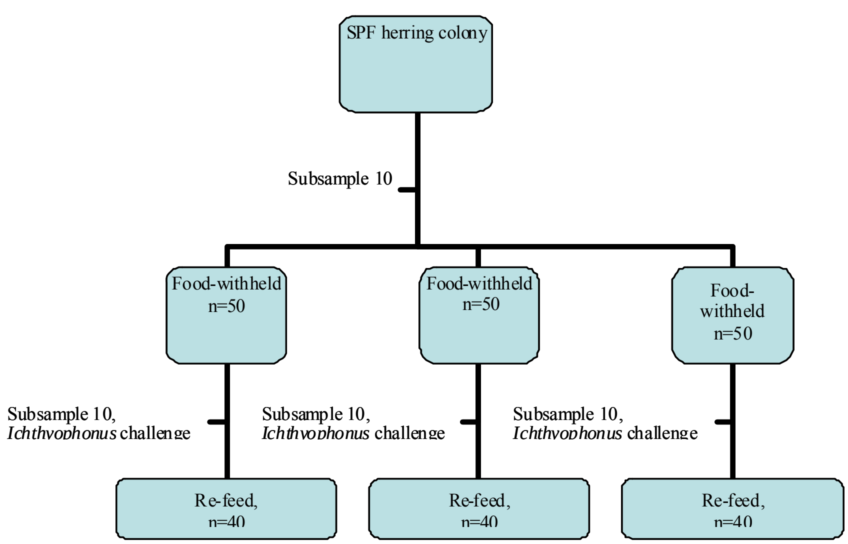

Figure 3. Flowchart depicting the experimental layout for Year 3 Objective 2c. Fish will fast at three different temperatures until energy levels drop to a level consistent with observations made on wild fish from PWS. Fasted fish will be exposed to *Ichthyophonus* and refed. In addition, a set of tanks for ambient temperature will hold fish that are fed while the others are fasting.

C. Data Analysis and Statistical Methods

Year 3 Objective 1 – Field collections.

Field data collected in Year 3 will be combined with data from Years 1 and 2 to determine if there are regional differences in the rate of energy loss, energetic minima, and reproductive costs for herring. Each of these parameters will be compared across regions by a one-way ANOVA with locations as fixed factors and the parameter estimates for each year as the replicates. Parameters will be normalized across regions as follows:

Energy loss (r) expressed as the proportional loss of energy per day will be estimated from equation 3.

$$r = [\ \mathrm{Ln}\,(MAX) - \mathrm{Ln}\,(MIN)] \times t^{-1} \qquad (3)$$

MAX is the maximum energy content observed in a given location and year, MIN is the minimum. The number of days elapsed between MAX and MIN is given by t. MAX and MIN for a given location and year will be estimated as the total energy content of a fish of fixed length using ANCOVA to account for regional and temporal differences in the size of fish.

MIN for juveniles will be estimated as the predicted energy content of juveniles immediately prior to the onset of the spring bloom. Field observations of juveniles will be used to estimate r following equation 3. Bloom dates will be obtained from local hatcheries or monitoring programs. Energy loss will be calculated for the number days between MAX and the onset of the bloom (t) from equation 3 to find MIN. Values for MAX for each location and year will be determined from ANCOVA using length as a covariate to account for regional and temporal variation in fish size. MIN for adults will be compared by ANCOVA using only fish in post-spawning condition.

The energetic cost of reproduction will be estimated separately for males and females and calculated as the proportional change in energy content observed in pre- and post-spawning herring.

Further adjustments to the analysis of loss rates will be made using the data collected following the laboratory studies. Comparisons of loss rates will be evaluated with respect to observed differences in temperature and adjusted using regressions that relate temperature to energy loss rate. Similar adjustments will be possible for populations infected with *Ichthyophonus*.

Year 3 Objective 2 – Laboratory studies.

Mortality schedules from the laboratory studies will be examined by survival analysis. Bioenergetic parameters will be measured as in Year 2.

D. Description of Study Area

The study will be conducted in three locations in order to place PWS energetic processes in context of other herring stocks. Comparison to other stocks is critical, to provide contrast to stocks in variable condition.

1. Prince William Sound

Each data point we collect will consist of fish sampled from multiple areas because habitat diversity within the Sound appears to be influential on fish condition (Foy and Norcross 1999; Paul and Paul 1999b; Stokesbury et al. 1999; Norcross et al. 2001).

2. Sitka Sound

Due to the commercial importance of herring in Sitka Sound, the distribution of adults is well known (Alaska Department of Fish and Game), though juvenile distribution is less documented (Haldorson and Collie 1990).

3. Lynn Canal

We have investigated the seasonal distribution and abundance of herring in Lynn Canal since 2001 and have considerable knowledge regarding nursery areas of juveniles and overwintering areas for adults (Vollenweider 2005; Sigler and Csepp 2005).

E. Coordination and Collaboration with Other Efforts

Field collections: The field collection component of the project will rely heavily on collaboration with existing platforms in PWS operated by ADFG and PWSSC. In addition data provided in this project will be of direct use to the EVOSTC funded project examining effects of whale predation. Furthermore, this project will continue to supply the disease study with samples, and collaborative efforts to compare disease incidence and energy content in individual fish will be pursued.

Laboratory culturing and energetics measurements: The laboratory component of the project will rely heavily on collaboration with the expert personnel and facilities at the USGS Marrowstone Marine Field Station. Cost sharing with the EVOS TC-funded Herring Disease Program will be utilized to minimize budgetary requirements. The Marrowstone facility and staff are unique in that they are amongst a handful of facilities in the world which are proficient at culturing Pacific herring. Some NOAA staff will make periodic trips to aid in specific measurements of the cultured fish.

Energetic assessments and chemical analyses: Proximate and lipid content of field collections and laboratory tests will be conducted by TSMRI. The bioenergetic measurements such as assimilation efficiency, maximum consumption rate and routine metabolic rates will be of direct interest to bioenergetic modelers.

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III. SCHEDULE

Below are tables outlining cruise schedules for collection of field samples (Table 1) and sample collection schedules for the laboratory component (Tables 2-4).

Table 1. Field collection schedule. Note samples of YOY and adults will be collected at each collection time and location for a total of 15 sampling strata. We propose to sample 15 fish from each stratum.

Event	Life stage	Sitka Sound	PWS	Lynn Canal
Begin winter	YOY Adult	December, Jan	November, Jan	November, Jan
End of winter	YOY Adult	March	March	March, May
Post - spawning	Adult	March	March	May

Table 2. Sample numbers for Year 3 Objective 2a. Note fasted groups will be held in 3 replicate tanks at each of 3 temperatures. Fed groups will be held in 3 replicate tanks at one temperature (see Figure 1).

Event	Treatment	Number of samples for necropsy	Number of samples for calorimetry
Initial exposure	All unexposed	10	10
End of incubation - Start fast	Exposed	10	10
	Unexposed	10	10
	Ambient – exposed – fed	10	10
	Ambient – unexposed –fed	10	10
End of fast	Cold – exposed-fasted	9	9
	Cold – unexposed-fasted	9	9
	Ambient – exposed- fasted	9	9
	Ambient –unexposed – fasted	9	9
	Ambient – exposed – fed	9	9
	Ambient – unexposed –fed	9	9
	Warm – exposed – fasted	9	9
	Warm – unexposed – fasted	9	9
	Total	122	122

Table 3. Sample numbers for Year 3 Objective 2b. See Figure 2 for events. Numbers reflect the total number collected for a treatment. Replicates number three tanks per treatment.

Event	Treatment	Number of samples for necropsy	Number of samples for calorimetry	Number of samples for proximate analysis
Initial exposure	All unexposed	10	10	0
End of incubation / Start of fast	Exposed	30	0	30
	Unexposed	30	0	30
Midpoints during fast	Cold-Exposed	60	10	0
	Cold-Unexposed	60	10	0
	Ambient -Exposed	10	10	0
	Ambient -Unexposed	10	10	0
	Warm – Exposed	10	10	0
	Warm-Unexposed	10	10	0
End of fast	Cold-Exposed	10	0	10
	Cold-Unexposed	10	0	10
	Ambient -Exposed	10	0	10
	Ambient -Unexposed	10	0	10
	Warm - Exposed	10	0	10
	Warm-Unexposed	10	0	10
	Total	290	70	120

Table 4. Sample numbers of Year 3 Objective 2c. Fasted groups will be held in ambient water. After fasting, fish will be exposed and moved to triplicate tanks with different temperatures of water (Figure 3).

Event	Treatment	Number of samples for necropsy	Number of samples for calorimetry	Number of samples for proximate analysis
Initial	All unexposed	0	0	15
End of fast	Ambient –starved	10	0	15
	Ambient – fed	10	0	15
End of re-feeding	Cold - exposed	10	5	15
	Cold - unexposed	10	5	15
	Ambient – fasted – exposed	10	5	15
	Ambient – fasted - unexposed	10	5	15
	Ambient – fed - exposed	10	5	15
	Ambient – fed - unexposed	10	5	15
	Warm - exposed	10	5	15
	Warm - unexposed	10	5	15
	Total	100	40	165

A. Project Milestones

Year 3 Objective 1. Field collections

Field collections to be completed by May 2009
Chemical analysis to be completed by October 2009

Year 3. Objective 2a Survival of infected herrring.

Experiment set up by October 2008
Experiment completed by February 2009
Complete energy analysis by March 2009

Year 3. Objective 2b Bioenergetics of infection.

Experiment set up by February 2009
Experiment completed by June 2009
Complete chemical analysis by August 2009

Year 3. Objective 2c Exposure of fasted herring

Experiment set up by May 2009
Experiment completed August 2009
Complete chemical analysis by October 2009

B. Measurable Project Tasks

FY 09, 1st quarter (October 1, 2008-December 31, 2008)

October Infect herring for Objective 2a
November Partition infected herring into appropriate tanks, adjust temperature, and initiate starvation experiment, collect field samples
December Continue sampling for Objective 2a, collect field samples

FY 09, 2nd quarter (January 1, 2009-March 31, 2009)
January Complete sampling for objective 2a and break down the experiment
Infect herring for objective 2b
Annual Marine Science Symposium
February Partition infected herring into appropriate tanks, adjust temperature, and initiate starvation experiment (Objective 2b).
March Continue sampling herring for objective 2b, collect field samples

FY 09, 3rd quarter (April 1, 2009-June 30, 2009)
April Complete sampling for objective 2b and break down the experiment
May Initiate starvation study for objective 2c
June Challenge herring for objective 2c

FY 09, 4th quarter
July Complete sampling for objective 2c and break down the experiment.
August Processing of energetic and disease data; data analysis

FY 10, 3rd quarter
Issue final report

IV. RESPONSIVENESS TO KEY TRUSTEE COUNCIL STRATEGIES

A. Community Involvement and Traditional Ecological Knowledge (TEK)

This project relies heavily on local knowledge and community involvement to locate known locations of herring aggregations. The Alaska Department of Fish and Game (Cordova), McLaughlin Environmental, Sitka Sound Science Center, Sitka Sound Tribal, and colleagues at TSMRI will be instrumental in locating herring aggregations

B. Resource Management Applications

Recruitment failures are preventing recovery of PWS herring stocks. The information learned from this proposal will have two ramifications. First, we will place the energetic status of PWS herring in context with other herring stocks, and determine if recruitment processes in PWS are unique or more limited in comparison to other regions. This will influence the thinking and planning of potential herring enhancement projects. Second, data from this study will provide the critical parameters required by bioenergetic models to examine herring recruitment and reproduction. Furthermore, results may provide a basis for structuring a monitoring program where lipid and energy phenology could possibly be used to index recruitment. Finally, we will examine disease as a potential mechanism of energy limitation. Other assessments such as predation may be limiting the population, but energetic limitations, whatever their source, would provide a basis for population stress. It is important to determination which factors are the major contributors to recruitment limitations, and which are secondary. Restoring a population without understanding the limiting factors is risky.

V. PUBLICATIONS AND REPORTS

Vollenweider JJ, Heintz RA (2009) Regional variation in energy consumption of juvenile Pacific herring (*Clupea pallasi*) overwintering in Alaska. Peer Reviewed Journal.

Vollenweider JJ, Heintz RA (2009) Regional variation in energy expenditure of spawning Pacific herring (*Clupea pallasi*) in Alaska. Peer Reviewed Journal.

Heintz RA, Vollenweider JJ, Hershberger P (2009) Parameterization of the Wisconsin model to determine metabolic costs of activity in Pacific herring (*Clupea pallasi*). Peer Reviewed Journal.

VI. BUDGET JUSTIFICATION

We request a total of $ 206.4K to be shared between USGS and NOAA.

NOAA Budget Justification - $ 160.3K

<u>Personnel Services ($2.5K requested):</u> Funds are requested to cover overtime costs for scientists on surveys. No salaries for permanent personnel are requested, although several years of effort will be committed to the project (Rice, Heintz, Vollenweider, Bradshaw, Schaufler).

<u>Travel: ($21.1K requested):</u> Travel funds are requested for Vollenweider and Heintz to travel from Juneau to the AK Marine Science Symposium ($3.4K). Additional funds ($10.9K) are requested for travel from Juneau to Sitka (1 round trip) and Cordova (5 round trips). Funds are also requested ($6.8K) for travel between Juneau and Nordland, WA, the location of our laboratory studies.

<u>Contractual:</u> ($108.0K requested). Funds cover the cost of vessel charters ($45.0K) and chemical analysis of samples ($63.0K). Vessel charters include 22 sea days in PWS, 8 sea days each in Lynn Canal and Sitka. Contracts for sample analysis include 222 samples from the laboratory study for bomb calorimetry at $40 per sample ($8.9K), 285 samples from the laboratory study for proximate analysis at $100 per sample ($28.5K) and approximately 250 samples for proximate analysis from the field ($25.0K).

<u>Commodities: ($13.5K requested):</u> Requested funds will be used to purchase fuel for Lynn Canal surveys, shipment of samples and gear to and from the field and to purchase laboratory supplies (solvents, gases, glassware, etc).

<u>Equipment: ($2.0K requested).</u> We request funds to purchase a Kodiak FRI trawl for sampling YOY herring. Our current trawl requires extensive repair and was not specifically designed for capturing herring. The requested trawl is designed for herring.

USGS Budget Justification - $ 46.0K

Personnel Services: none are requested

Travel: no travel funds are requested

Contractual: ($33.2K requested). Funds cover the cost of a contracted fish culturist at Marrowstone Marine Laboratory. Cost was estimated as: 40 hrs/wk * 52 wks * $16.0/hr.

Commodities: ($9.0K requested) Funds cover the cost of fish food, plumbing supplies, disinfectants and other lab supplies

General Admin ($3.8K requested) Funds are calculated as 9% of the total direct cost.

2008 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2007 - September 30, 2008

Budget Category:	Authorized FY 2008	Proposed FY 2009	PROPOSED FY 2009 TRUSTEE AGENCIES TOTALS					
			ADEC	ADF&G	ADNR	USFS	DOI	
							$46.1	
Personnel	$6.4	$2.5						
Travel	$27.4	$21.1						
Contractual	$117.5	$141.3						
Commodities	$20.5	$22.5						
Equipment	$0.0	$2.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$171.8	$189.4						
General Administration	$15.5	$17.0						
Project Total	$187.3	$206.4						
Full-time Equivalents (FTE)	0.0	0.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources	$0.0	$0.0				$0.0		

Comments:
The following personnel costs will be provided in-kind:
Vollenweider, Johanna Fisheries Research Biologist ZPII-1 5 months $5.3/month
$26.5
Heintz, Ron Director Nutritional Ecology Lab ZPIII-3 2 months $9.6/month
$19.2
Bradshaw, Robert Chemist ZPIII-1 1 month $8.0/month
$ 8.5

$54.2

FY09

Prepared:

Project Number: 090806
Project Title: Are herring energetics limiting?
Lead Agency: NOAA/TSMRI

2008 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2007 - September 30, 2008

Budget Category:	Authorized FY 2008	Proposed FY 2009						
Personnel	$6.4	$2.5						
Travel	$26.7	$21.1						
Contractual	$157.0	$108.0						
Commodities	$20.5	$13.5						
Equipment		$2.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$210.6	$147.1						
General Administration	$19.0	$13.2						
Project Total	$229.6	$160.3						
Full-time Equivalents (FTE)		0.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

FY09

Prepared:

Project Number: 090806
Project Title: Are herring energetics limiting?
Lead Agency: NOAA/TSMRI

2008 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2007 - September 30, 2008

Personnel Costs:		GS/Range/	Months	Monthly		
Name	Position Description	Step	Budgeted	Costs	Overtime	
Vollenweider, J	Fisheries Research Biologist	ZP II-1			2.5	
	Subtotal		0.0	0.0	2.5	
					Personnel Total	

Travel Costs:	Ticket	Round	Total	Daily	
Description	Price	Trips	Days	Per Diem	
Vollenweider & Heintz attendance @ AK Marine Science Symposium	0.7	2	10	0.2	
Travel for field collections:					
PWS	0.7	5	32	0.2	
Sitka Sound	0.4	1	3	0.2	
Travel for laboratory work @ Marrowstone Marine Field Station	0.7	4	20	0.2	
				Travel Total	

FY09

Prepared:

Project Number: 090806
Project Title: Are herring energetics limiting?
Lead Agency: NOAA/TSMRI

Contractual Costs:	
Description	
Vessel charters	
Chemical analysis of specimens	
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	

Commodities Costs:	
Description	
Fuel for Lynn Canal Field Work (NOAA R/V Quest & other skiffs) Shipment of supplies/samples to & from field sites/laboratory Supplies for chemical anaysis and sample preservation	
Commodities Total	

FY09

Project Number: 090806
Project Title: Are herring energetics limiting?
Lead Agency: NOAA/TSMRI

Prepared:

2008 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2007 - September 30, 2008

New Equipment Purchases:		Number	Unit	
Description		of Units	Price	
1	Kodiak FRI Trawl	1	2.0	
Those purchases associated with replacement equipment should be indicated by placement of an R.			**New Equipment Total**	

Existing Equipment Usage:	Number	
Description	of Units	
Leco Protein Analyzer	1	
Accelerated Solvent Extractor	1	
Leco Thermogravimetric Analyzer	1	
Parr Semi-Micro Bomb Calorimeter	1	
Bio-Impedance Analyzers	2	
NOAA R/V Quest, NOAA R/V John N. Cobb	1	
Trawl nets	2	

FY09

Project Number: 090806
Project Title: Are herring energetics limiting?
Lead Agency: NOAA/TSMRI

Prepared:

2008 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2007 - September 30, 2008

Budget Category:	Authorized FY 2008	Proposed FY 2009						
Personnel		$0.0						
Travel		$0.0						
Contractual		$33.3						
Commodities		$9.0						
Equipment		$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$0.0	$42.3						
General Administration		$3.8						
Project Total	$0.0	$46.1						
Full-time Equivalents (FTE)		0.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

FY09

Prepared:

Project Number: 090806
Project Title: Are herring energetics limiting?
Agency: USGS - Marrowstone Field Station

2008 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2007 - September 30, 2008

Personnel Costs:		GS/Range/	Months	Monthly		
Name	Position Description	Step	Budgeted	Costs	Overtime	
	Subtotal		0.0	0.0	0.0	
					Personnel Total	

Travel Costs:	Ticket	Round	Total	Daily	
Description	Price	Trips	Days	Per Diem	
				Travel Total	

FY09

Prepared:

Project Number: 090806
Project Title: Are herring energetics limiting?
Agency: USGS - Marrowstone Field Station

Contractual Costs:	
Description	
Laboratory technician (40 hrs/wk x $16/hr x 52 weeks)	
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	
Commodities Costs:	
Description	
Fish food and culture supplies	
Commodities Total	

FY09

Prepared:

Project Number: 090806
Project Title: Are herring energetics limiting?
Agency: USGS - Marrowstone Field Station

New Equipment Purchases:	Number	Unit	
Description	of Units	Price	
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	

Existing Equipment Usage:	Number	
Description	of Units	

FY09

Project Number: 090806
Project Title: Are herring energetics limiting?
Agency: USGS - Marrowstone Field Station

Prepared: