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Michael Baffrey
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July 25, 2007

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

Dear Michael:

Enclosed is our revised Detailed Project Description (DPD) including updated budget sheets and a summary of our progress. In this proposal, we request support to continue Year 1's field collections of herring to examine energetic limitations that may be inhibiting herring recruitment in PWS. An additional component added to this year's proposal is a series of laboratory trials to complete a bioenergetic analysis of herring. The energetic data provided by the lab component (different temperatures, different age classes) will provide a secure foundation for weighing the evidence for or against energy limitations contributing to the population decline in PWS. We anticipate that these data will be invaluable for future bioenergetic models describing herring growth, consumption, reproduction and response to disease. To accomplish the laboratory component, Dr. Paul Hershberger of USGS/Marrowstone Marine Field Station has been added as a co-PI. Herring capture, culture and disease challenges are routine practices at the Marrowstone facility.

The proposed budget includes an additional $50 K to fund the laboratory component. We have consolidated vessel charter and analytical costs by close collaboration with other studies, particularly the whale predation study (Rice et al.). This has allowed us to redirect charter costs to chemical analysis of fish cultured by Hershberger at Marrowstone.

We realize this invitation for a Detailed Project Description requests application for FY08 funds only. However, we have included objectives that we will propose for FY09 funding in order to place this year's proposal in context of a more comprehensive study. For year 2 (FY08 funds), we will continue the field energetic assessment of juvenile and adult herring before and after winter, and add an energetics modeling effort based on laboratory measurements with this species including early life history stages. The FY09 proposal will continue the field energetic assessment for the third and final field year and will incorporate disease challenges on herring energetics in the laboratory tests so that the energetics model will contain a disease component. However, the proposal for FY08 funds is a stand-alone product with the assumption that the pending herring restoration plan will request this type of study in the future for FYO9.

Sincerely,

Johanna Vollenweider

Progress to Date

Project: 070806/Are Herring Energetics in PWS a Limiting Factor?

Summary: This project was approved in late fall of 2006, precluding initial sampling planned for the fall. However, an early winter collection period was accomplished and the study is otherwise generally on track to meet the projected deadlines.

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

Objective 1. Measure caloric content and proximate composition in YOY and age-1 herring in the fall and the subsequent spring. Field measurements to be met by May 2007; Chemical analysis to be met by July 2007.

Juvenile herring were collected from all 3 study sites during early winter and spring sampling periods. Exact ages of specimens are unknown until scales have been aged. Bioelectrical impedance analysis was performed at the time of capture. We are in the processes of finishing laboratory dissections and measurements of herring and have commenced the chemical analysis of proximate composition and energy content of voucher specimens.

Objective 2. Measure caloric content and proximate composition in adult herring in the fall and in the subsequent spring, before and after the spawning event. Gonads will be separated and measured separate from the rest of the carcass. Field measurements to be met by May 2007; Chemical analysis to be met by July 2007.

Adult herring were collected from all 3 study sites during early winter and spring sampling periods. Bioelectrical impedance analysis was performed at the time of capture. We are in the processes of finishing laboratory dissections and measurements of herring and have commenced the chemical analysis of proximate composition and energy content of voucher specimens.

Objective 3. Compare the measurements from 3 regions and interpret the regional differences and similarities with implications for management. Statistical analysis and interpretation to be met by September 2007.

Upon completion of the chemical analysis of the voucher specimens, we will convert bio-electrical impedance measurements to proximate composition and energy values based on our herring model. Statistical analysis and interpretation will follow.

Trustee Council Use Only Project No.______________ Date Received: _________

FY08 INVITATION
PROPOSAL SUMMARY PAGE

Project Title: Are herring (*Clupea pallasi*) energetics in PWS a limiting factor in successful recruitment of juveniles or reproduction investment of adults? Part II: Weighing the evidence for population effects of energy limitations using a bioenergetics model.

Project Period: October 1, 2007 – September 30, 2008

Proposer(s): Johanna Vollenweider[1*], Ron Heintz[1*], Stan Rice[1**], and Paul Hershberger[2**]
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* Proposer on original proposal ** Additional proposer for revised project description

Study Location: Prince William Sound, Sitka Sound, Lynn Canal

Abstract: We propose to determine if the availability of energy is limiting production of PWS herring. In year 1 of the study, we made field collections of Pacific herring to examine two energetic mechanisms that could potentially inhibit herring recruitment in Prince William Sound (PWS). These were (1) overwinter mortality of juveniles, and (2) low reproductive energy investments by adults. These processes were compared among thriving (Sitka Sound) and depressed (Lynn Canal) herring stocks to calibrate PWS observations. Differences among stocks would suggest site-specific conditions that may translate into recruitment success. We propose extending these analyses over two more years to better estimate interannual variability. Collection costs can be decreased because of sampling efficiency with other projects. However, it is necessary to develop bioenergetic parameters for Pacific herring so energy consumption rates among herring from different locations can be directly compared. Energy consumption is a function of size, temperature and physiological condition. In order to compare the energy consumption rates of herring from different locations it is necessary to know how metabolic rates vary with respect to the temperatures in those locations. Therefore, we propose to supplement the field observations with a detailed bioenergetic analysis of YOY, juvenile and adult herring. The physiological parameters to be monitored (food intake, assimilation efficiency, growth, and resting metabolic rate), will be supplemented with 2 commonly used proxies for growth (RNA/DNA and enzyme analysis) to determine their suitability for measuring growth in the field. The additional data provided by the lab component will provide a secure foundation for weighing the evidence for or against energy limitations contributing to the population decline in PWS. Currently the data we seek are unavailable, however recent advances in culturing herring will allow us to make the necessary laboratory manipulations to obtain the data. While fulfilling our immediate needs for comparing herring populations, we anticipate that these data will be invaluable for future bioenergetic models describing herring growth, consumption, reproduction and response to disease. In year 3 (FY 2009) we propose to apply these data by examining the energetic cost of overwintering among healthy and disease challenged herring. This examination specifically tests the hypothesis that low levels of disease in PWS stocks are inhibiting recruitment. All of the herring culturing will be conducted at the USGS facility at Marrowstone Harbor, Washington, where herring capture, culture, and disease challenges are routine. The energetics measurements will be conducted over a range of temperatures, encapsulating the temperatures of Alaska, and will focus on three developmental stages of herring (age 0, age 1, and adults).

KeyWords: Pacific herring, *Clupea pallasi*, energy phenology, energetic stress, winter, size-selective mortality, reproductive investment, spatial variation, energy content, proximate composition, bioenergetics model
Funding: - EVOS Funding Requested: FY07 $ 187.3 (must include GA) - Non-EVOS Funds to be used: FY07 $ 54.2 (In the form of in-kind donation of personnel salaries from Ted Stevens Marine Research Institute) GRAND TOTAL: $ 241.5
Date: July 25, 2007

PROJECT PLAN

PROJECT TITLE: Are herring (*Clupea pallasi*) energetics in PWS a limiting factor in successful recruitment of juveniles or reproduction investment of adults? Part II: Weighing the evidence for population effects of energy limitations using a bioenergetics model.

I. NEED FOR THE PROJECT

A. Abstract

We propose to determine if the availability of energy is limiting production of PWS herring. In year 1 of the study, we made field collections of Pacific herring to examine two energetic mechanisms that could potentially inhibit herring recruitment in Prince William Sound (PWS). These were (1) overwinter mortality of juveniles, and (2) low reproductive energy investments by adults. These processes were compared among thriving (Sitka Sound) and depressed (Lynn Canal) herring stocks to calibrate PWS observations. Differences among stocks would suggest site-specific conditions that may translate into recruitment success. We propose extending these analyses over two more years to better estimate interannual variability. Collection costs can be decreased because of sampling efficiency with other projects. However, it is necessary to develop bioenergetic parameters for Pacific herring so energy consumption rates among herring from different locations can be directly compared. Energy consumption is a function of size, temperature and physiological condition. In order to compare the energy consumption rates of herring from different locations it is necessary to know how metabolic rates vary with respect to the temperatures in those locations. Therefore, we propose to supplement the field observations with a detailed bioenergetic analysis of YOY, juvenile and adult herring. The physiological parameters to be monitored (food intake, assimilation efficiency, growth, and resting metabolic rate), will be supplemented with 2 commonly used proxies for growth (RNA/DNA and enzyme analysis) to determine their suitability for measuring growth in the field. The additional data provided by the lab component will provide a secure foundation for weighing the evidence for or against energy limitations contributing to the population decline in PWS. Currently the data we seek are unavailable, however recent advances in culturing herring will allow us to make the necessary laboratory manipulations to obtain the data. While fulfilling our immediate needs for comparing herring populations, we anticipate that these data will be invaluable for future bioenergetic models describing herring growth, consumption, reproduction and response to disease. In year 3 (FY 2009) we propose to apply these data by examining the energetic cost of overwintering among healthy and disease challenged herring. This examination specifically tests the hypothesis that low levels of disease in PWS stocks are inhibiting recruitment. All of the herring culturing will be conducted at the USGS facility at Marrowstone Harbor, Washington, where herring capture, culture, and disease challenges are routine. The energetics measurements will be conducted over a range of temperatures, encapsulating the temperatures of Alaska, and will focus on three developmental stages of herring (age 0, age 1, and adults).

B. 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 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 in the absence of 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 fished. 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, energetics measurements will be made in the laboratory for different life stages (and temperatures) to generate an energetics model. In year 3, the last of the field assessments will be made, and the energetics measurements in the lab will be replicated with disease challenges.

C. Relevance to Restoration Goals and 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.

III. PROJECT DESIGN

A. Objectives

We realize this invitation for a Detailed Project Description requests application for FY08 funds only. However, we have included objectives that we will propose for FY09 funding in order to place this year's proposal in context of a more comprehensive study. For year 2 (FY08 funds), we will continue the field assessment of juvenile and adult herring before and after winter, and add an energetics modeling effort based on laboratory measurements with this species at early life history stages. The FY09 proposal will build upon knowledge attained in years 1 and 2 and incorporate disease effects on herring energetics, but the proposal for FY08 funds is a stand alone product in the case that FY09 is not funded. Specific objectives are outlined below:

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 year 2.
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.***
   a. Measure bioenergetic parameters to determine resting metabolic rate, maximum consumption rate, and assimilation efficiency at 3 temperatures and rations. Life history stages will be age 0, age 1, and adults.
   b. Relate increases in herring mass and length to biochemical measures 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 year 3.
2. ***Laboratory-based studies – Measure influence of disease on herring energetics.***
   a. Measure bioenergetic parameters similar to those in year 2, but using disease challenges. Life history stages will be age 0, age 1, and adults.
   b. Determine the sensitivity to disease following the winter fasting period.

B. Procedural Methods and Scientific Design

Year 2: Objective 1 - Winter Energy Expenditure of Herring

Winter energy expenditures of herring will be measured following the same procedures used in Year 1 of the study. 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. Analysis of body composition will be performed using bioelectrical impedance analysis (BIA) when possible and chemical analysis otherwise. Temperature measurements will accompany the herring collections. Statistical analyses detailed in Year 1 will be employed.

Year 2: Objective 2a – Parameterize the Wisconsin Bioenergetics Model

To understand the basis for differences among the three study regions, we need to know how the observed energy loss relates to metabolic demand. If observed winter energy losses are less than that required by resting metabolic demand, then feeding must occur in winter. Conversely, if observed winter energy losses exceed those required by resting metabolic demand, additional costs are imposed by disease or increased activity such as predator avoidance or foraging. Thus, knowing the *in-situ* winter energy loss in combination with the basic energetic parameters will allow us to determine if activity costs are the same amongst the three study regions by using the following logic.

Our data (Heintz and Vollenweider *In Review*, Vollenweider and Heintz *In Prep*) and that of Paul and Brown (1998) indicate that length adjusted changes in energy content is the result of consumptive losses of energy reserves due to reduced feeding in winter. Thus, these changes represent the net of:

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

Where ΔB is the change in energy content, C is the amount of energy consumed, R_t is the respiration rate and W is amount of ingested energy lost as waste products (i.e. feces, ammonia and urea). R_t is total metabolic cost and incorporates resting metabolism (R_s), locomotion (R_a), and specific dynamic action (SDA) (R_d).

In the laboratory, we can directly observe ΔB. We assume that W is equal to 1- pC 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,

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

Assuming the temperature functions for R_s and p are constant across regions, then differences in energy loss between regions is due to differences in amount of food consumed, C, and the amount of energy expended in activities such as foraging or predator avoidance, R_a. Knowing p and R_s we can model the trade-off between C and R_a in each location by continuously varying C and determining the value of R_a that accounts for the observed ΔB. These empirical relationships will be related to observed ΔB at the three study sites, and thus we will determine the relative activity cost associated with a fixed value of C in each location.

Finally we need to determine the functional relationship between temperature and C_{max}, which is the estimated maximum consumption rate. If comparisons between ΔB and R_s for wild fish indicate that herring are consuming energy over winter, we can apply the Wisconsin model to determine the apparent value for C. By comparing the ratio of C to C_{max} for different regions we can assess differences in the availability of winter forage.

Specific procedures for measuring these parameters are outlined below. Sample sizes are given in Table 1.

1. Feeding trials will be used to estimate of C and *p*. We propose to weigh the food fed to fish and collect and weigh the uneaten food. The difference is equal to C and the proportion ΔB to C estimates *p*. Note that C and ΔB will be expressed in kJoules. Energy values will be determined from the proximate composition of food and fish. This procedure will be repeated for each life stage (YOY, age-1, adult) at three different temperatures, using 3 replicate populations. A total of 10 fish per replicate will be sampled before and after the trial to determined ΔB.

2. Estimation of C_{max} will be accomplished during the feeding trials. Four times during the feeding trial fish will be fed repeatedly over a 24 hour period. Throughout each 24 hour trial, the mass of food consumed over each 2 hour interval will be measured. The sum of consumed masses is equal to C_{max}.

3. W (equation 1) will be determined by filtering the water in fish culturing tanks and collecting the suspended organic material each day and weighing it. Fish will be fed frozen euphausids, so food will be readily discerned from feces.

4. Estimation of R_s is accomplished by withholding food from fish that are resting for a 30 d period and monitoring the change in their energy content. This procedure will be repeated at 3 different temperatures and commence following the feeding trial. Initial energy content of each fish will be determined from allometric relationships describing the length and energy content of a sample of 10 fish collected at the end of the feeding trial. In addition, we will determine initial energy levels by BIA to provide an independent assessment of energy initial energy content.

These parameters can only be obtained by precise manipulation of herring diets and ambient temperatures in the laboratory. During the first laboratory trials, culturing tank effects will be examined by ANOVA. In order to keep costs down, a sample of five fish from each tank will be processed for the initial ANOVA, if no tank effects are observed, then the fish will be pooled across tanks to increase the sample size to 15 and therefore increase the precision of our estimates. If tank effects are evident, then the number of fish processed per tank will be doubled to decrease between tank variability.

As a source of experimental animals for these laboratory-based studies, wild Pacific herring (age 0+, 1+ and 2+ yr) from Puget Sound will be collected by dip net and/or purse seine and transported to flow-through seawater tanks at the USGS-Marrowstone Marine Field Station, where empirical manipulations will be performed. Briefly, groups of captured herring will be maintained at each of three temperatures, (ambient temperature at the Marrowstone facility, low and high temperatures) using appropriate chillers and heaters. Additionally, feed rations will be precisely metered by administering known quantities (mass) of commercially available, lyophilized euphausids. The proximate compositions of the euphausids will also be determined in order to estimate the lipid, protein and energy masses supplied to the fish.

We anticipate that the functions relating temperature and the measured parameters will depend on life history stage because the demands and resources for age 0, age 1, and reproductive adults are all different. An overview of treatments, sampling times and sample sizes is given in Table 1. Allometries between size and energy consumption indicate that juvenile herring should have different strategies for surviving winter (Schultz and Conover 1999). Age 0 fish are likely to be maximizing growth in order to maximize survival, while age 1 entering winter should be maximizing energy storage (Post and Parkinson 2001). Condition prior to the winter for these age classes is critical for survival, and the different age classes will have different masses and respond differently to the winter. Hence, we are focused on these life stages. Reproductive adults have energy reserves and their survival is less of a problem; however, their reproductive success depends directly on their condition entering and exiting the winter. Adult herring time spawning to coincide with spring blooms, hence there is no time prior to spawning to take in energy to invest in spawning. Adult condition prior to winter may therefore have a great impact on their reproductive quality and success.

Table 1. Sample size of laboratory fish for bioenergetics model parameterization. Top panel shows numbers of samples to be collected in order to estimate growth and change in energy content. Bottom panel indicates the proposed number to process in the chemistry lab.

	Time	After Feeding									After Fasting									After Spawning									
	0	Low Temp			Ambient Temp			High Temp			Low Temp			Ambient Temp			High Temp			Low Temp			Ambient Temp			High Temp			Total
Age	Rep	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10										190
1	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10										190
Adult	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	280
																												Total	660

	Time	After Feeding									After Fasting									After Spawning									
	0	Low Temp			Ambient Temp			High Temp			Low Temp			Ambient Temp			High Temp			Low Temp			Ambient Temp			High Temp			Total
Age	Rep	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5										95
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5										95
Adult	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	140
																												Total	330

Year 2: Objective 2b – Growth Measurements

To better understand how survival strategies vary between age classes it is necessary to measure the growth rates of the different age classes. Measuring growth in the field is problematic because of the low probability of re-sampling a given population. We propose to use RNA/DNA and enzymatic measures to estimate relative growth in the field. We also propose to calibrate these measures using captive fish. We will be able to monitor growth in terms of length, wet and dry mass in these latter individuals and relate those measures to RNA/DNA and enzyme activities. These data are necessary because Weber et al. (2003) determined that responses of RNA/DNA to different nutritional conditions varied across species. In addition, RNA/DNA is likely to be influenced by temperature (Calderone et al. 2003) and life stage (unpublished data).

We also propose to include measures of specific metabolic enzyme activity which is another tool that can be used to examine growth response and condition in juveniles across different taxa of fish. Specific metabolic parameters such as activity of glycolytic (pyruvate kinase (PK)), aerobic (citrate synthase (CS)), and anaerobic (lactate dehydrogenase (LDH)) enzymes, involved in growth and energy production, can be used as indicators of physiological status.

White muscle RNA/DNA ratios for all samples will be determined using the 2-dye procedure modified from Calderone and Buckley (1991). Enzyme assays for citrate synthase (CS; E.C. 4.1.3.7), pyruvate kinase (PK; E.C. 2.7.1.40), and lactate dehydrogenase (LDH; E.C. 1.1.1.27) for all fish samples will be performed according to procedures modified from Leonard et al. (2002).

Year3: Objective 1 - Winter Energy Expenditure of Herring

Winter energy expenditures of herring will be measured following the same procedures used in Years 1 and 2 of the study.

Year 3: Objective 2a - Parameterize the Wisconsin Bioenergetics Model using Disease-Challenged Herring

Variables inherent to wild herring including their unknown exposure, infection, disease, and immunological competence histories severely limit their suitability as experimental animals to address the confounding effects of disease to bioenergetic parameters. Therefore, in Year 3, we propose to tease out the effects of disease on the Wisconsin Bioenergetic parameters using specific pathogen-free (SPF), immunologically naïve Pacific herring as the test animal. Briefly, groups of laboratory-reared, SPF herring (currently maintained at the USGS-Marrowstone Marine Field Station) will be challenged with *Ichthyophonus hoferi*, by interperitoneal injection of primary *Ichthyophonus* spores isolated from wild Pacific herring. Bioenergetic parameters will be compared between infected and uninfected cohorts, using techniques described for wild herring in Year 2 (FY 08) of this proposal.

Year 3: Objective 2b - Sensitivity to Disease Following the Winter Fasting Period

Previous studies speculated that poor condition of herring after a period of winter fasting leads to increased susceptibility to disease (Marty et al. 2003). However, exiting data to support this hypothesis is circumstantial and based on uncontrolled observations of wild herring populations. We propose to empirically investigate this hypothesis by maintaining groups of SPF herring on different feed rations throughout the winter, then comparing their susceptibilities to ichthyophoniasis via IP injection challenge. Body composition (condition) of the SPF herring maintained on the different feed rations will be measured by bioelectrical impedance.

Susceptibility of the herring groups to ichthyophoniasis will be based on prevalence of infection, intensity of infection, and disease kinetics of all experimental herring, including dead/moribund individuals and euthanized survivors at the end of the study. Prevalence of infection will be determined by explant culture of cardiac tissue in Eagles Minimum Essential Medium; cultures will be incubated at 15˚C and screened at 40× magnification for the presence of *Ichthyophonus* after 14d. Intensity of infection will be determined by examination of challenged individuals for gross signs of ichthyophoniasis, and screening of stained histological sections (periodic acid-Schiff and hematoxylin and eosin stains) for parasite load and cellular immune response. Disease kinetics will be reported as the mean day-to-death among the groups.

IV. COORDINATION AND COLLABORATION WITH OTHER EFFORTS

Field collections: Herring will be collected in collaboration with multiple parties to reduce cost and effort. Sampling in PWS will rely heavily on collaboration with the whale predation study (Rice et al.), which can overlap in study platforms, personnel, collaborators, and study sites. Data from this study (energy content) will support the modeling data used in the whale predation study. Additional sampling collaboration in PWS will be with the Alaska Department of Fish and Game in Cordova. Sampling in Sitka Sound will be accomplished through collaboration with Dr. Keith Cox.

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. 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. These specific measurements will be on the same samples collected for regular energetic assessments, and will likely aid in the interpretation of the measurements.

V. SCHEDULE

A. Project Milestones

Milestone 1
(Objective 1) Measure caloric content and proximate composition of field-collected herring from the 3 study regions in the fall and the subsequent spring (pre- and post-spawning). Field collections to be complete by June 2008; Chemical analysis to be complete by September 2008; Data analysis to be complete by November 2008; Report to be complete by February 2009.

Milestone 2
(Objective 2a) Measure bioenergetic parameters to determine resting metabolic rate, maximum consumption rate, and assimilation efficiency at 3 temperatures and rations. Laboratory component to be complete by September 2008; Chemical analysis to be complete by November 2008. Data analysis to be complete by January 2009; Report to be complete by April 2009.

Milestone 3
(Objective 2b) Relate increases in herring mass and length to biochemical measures of growth including RNA/DNA and enzyme activities. Laboratory component to be complete by July 2008; Chemical analysis to be complete by September 2008. Data analysis to be complete by November 2008; Report to be complete by February 2009.

B. Measurable Project Tasks

Measurable tasks are detailed according to the field or laboratory component they are associated with.

FY 08, 1st quarter (October 1, 2007-December 31, 2007)

October — Laboratory Component: Collect wild samples of ages 0 and 1 for laboratory work from Nordland, WA
November — Field Component: Sample collection at all 3 sites, all age classes
December — Laboratory Component: 1st laboratory measurements on ages 0 and 1

FY 08, 2nd quarter (January 1, 2008-March 31, 2008)

January — Laboratory Component: 2nd laboratory measurements on ages 0 and 1
February — Laboratory Component: 3rd laboratory measurements on ages 0 and 1
February — Laboratory Component: Collect wild samples of spawning herring (age 2+) for laboratory work from Nordland, WA
February — Laboratory Component: 1st laboratory measurements on spawners (Age 2+)
March — Laboratory Component: 2nd laboratory measurements on spawners (Age 2+)
March — Field Component: Sample collection at all 3 study sites, all age classes

FY 08, 3rd quarter (April 1, 2008-June 30, 2008)

April-May — Field Component: Sample collection at all 3 study sites, adult herring only
April — Laboratory Component: 3rd laboratory measurements on spawners (Age 2+)
June — Field Component: Proximate analysis and bomb calorimetry of voucher specimens

FY 08, 4th quarter (July 1, 2008-September 30, 2008)

July-August — Field Component: Proximate analysis and bomb calorimetry of voucher specimens
September — Field Component: Statistical summary & analysis

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

October — Field Component: Statistical summary & analysis
October-Nov — Laboratory Component: Chemical analysis (Proximate analysis, bomb calorimetry RNA/DNA and enzyme activity analyses)
Nov-Dec — Field Component: Report drafting
December — Laboratory Component: Statistical summary & analysis

FY 09, 2nd quarter (January 1, 2009-March 31, 2009)

January — Field Component: Report Drafting

January-Mar Laboratory Component: Statistical summary & analysis, report drafting
January Annual Marine Science Symposium

FY 09, 3n4d quarter (April 1, 2009-June 30, 2009)
April Laboratory Component: Report complete

VI. RESPONSIVENESS TO KEY TRUSTEE STRATEGIES

A. Community Involvement and Traditional 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), Chenega Environmental, Sitka Sound Tribal, Dr. Keith Cox 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.

VII. 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.

VIII. PROFESSIONAL CONFERENCES

- Alaska Marine Science Symposium

Literature Cited

Calderone EM, St.Onge-Burns JM and Buckley LJ (2003) Relationship of RNA/DNA ratio and temperature to growth in larvae of Atlantic cod Gadus morhua. Mar. Ecol. Prog. Ser. 262:229-240.

Caldarone EM, and Buckley LJ (1991) Quantification of DNA and RNA in crude tissue extracts by flow injection analysis. Analytical Biochemistry 199: 137-141.

Heintz RA, Vollenweider JJ (*In review*) Seasonal and ontogenetic changes in the energy allocation strategies of walleye pollock. Canadian Journal of Fisheries and Aquatic Sciences.

Hillgruber N, Vollenweider JJ, Fournier W (2006) Distribution, composition and energy density of zooplankton in the southeastern Bering Sea *In*: Survey strategies for assessment of Bering Sea forage species. NPRB Final Report.

Leonard JBK, Iwata M, Ueda H (2002) Seasonal changes of hormones and muscle enzymes in adult lacustrine masu (*Oncorhyncus masou*) and sockeye salmon (*O. nerka*). Fish Physiology and Biochemistry 25: 153-163.

Marty GD, Quinn TJII, Carpenter G, Meyers TR, Willits NH (2003) Role of disease in abundance of a Pacific herring (*Clupea pallasi*) population. Canadian Journal of Fisheries and Aquatic Sciences 60(10): 1258-1265.

Norcross BL, Brown ED, Foy RJ, Frandsen , Gay SM, Kline Jr TC, Mason DM, Patrick EV, Paul AJ and Stokesbury KDE. 2001. A synthesis of the life history and ecology of juvenile Pacific herring in Prince William Sound, Alaska. Fisheries Oceanography 10(Suppl. 1):42-47.

Paul AJ, Paul JM, Brown ED (1998) Fall and spring somatic energy content for Alaska Pacific herring (*Clupea pallasi* Valenciennes 1847) relative to age, size and sex. Journal of Experimental Marine Biology and Ecology 223: 133-142

Post JR, Parkinson EA (2001) Energy allocation strategy in young fish: allometry and survival. Ecology 82(4):1040-1051.

Schultz ET, Conover DO (1999) The allometry of energy reserve depletion: test of a mechanism for size-dependent winter mortality. Oecologia 119:474-483.

Vollenweider JJ (2005) Variability in Steller sea lion (*Eumetopias jubatus*) prey quality in southeastern Alaska. Juneau Center, School of Fisheries and Ocean Sciences, Fairbanks.

Vollenweider JJ, Heintz RA (*In prep*) Seasonal and annual energy phenology of Pacific herring (*Clupea pallasi*) in Lynn Canal, southeastern Alaska.

Vollenweider JJ, Heintz RA, Kelly BP (*Submitted*) Seasonal variation in whole-body proximate composition and energy content of forage fish in southeastern Alaska. Marine Ecology Progress Series.

Weber LP, Higgins PS, Carlson RI, Janz DM (2003) Development and validation of methods for measuring multiple biochemical indices of condition in juvenile fishes. Journal of Fish Biology 63(3):637-658.

Budget Justification

Total Budget Request: $187.3 K

***Personnel:* $6.4 K ($54.2 K in-kind)**
Overtime costs for Vollenweider ($6.4 K) are requested for herring collection trips in the field.

In-kind contribution for personnel salaries include ($54.2 K):
1. In-kind contribution for PI Vollenweider (5 months @ $26.5 K) for project organization, study design, sample collections, data summarization and manuscript writing
2. In-kind contribution for PI Heintz (2 months @ $19.2 K) to assist in study design, report & manuscript writing
3. In-kind contribution for chemist Bradshaw (1 month @ $8.5 K) for chemical analysis of energetic samples

***Travel*: $27.4 K**
Vollenweider and Heintz will present results at the annual Marine Science Symposium in Anchorage, Alaska in 2008 ($3.4 K).

Travel for field work ($11.4 K):
PWS ($7.0 K): Vollenweider will travel from Juneau to Prince William Sound to conduct field work for herring collections. Per diem rate reflects vessel days and town days.
(2 people * 4 sampling cruises * 5 days per cruise)
Sitka ($4.4 K): A total of 4 trips between Juneau and Sitka will occur to coordinate field sampling methods with Sitka personnel
Lynn Canal: No travel required

A total of 8 trips between Juneau and Marrowstone Marine Field Station in Nordland, Washington are required for the laboratory component of the study, including laboratory set-up and collection of physiological measurements (Per diem reflects reduced rate due to collaboration with Marrowstone Marine Field Station). These trips will be conducted by Vollenweider, Heintz, Hershberger and the contractor performing the growth analyses. ($12.6 K)

***Contractual:* $117.5 K**
Vessel contracts ($36.0 K)
Vessel contracts to collect herring samples are required for each region, though costs are reduced due to vessel sharing with the whale study. Lynn Canal charters will be supplemented with in-kind contributions of the NOAA vessels R/V Quest and R/V John N. Cobb. Charter costs in Sitka Sound are particularly low due to project leveraging with collaborator, Keith Cox.
- PWS - $20 K
- Lynn Canal - $8.5 K
- Sitka Sound - $7.5 K

Contract costs for the chemical analysis (proximate composition and bomb calorimetry) of energetic samples from field and laboratory collections ($40 K).

A 3/4 time GS-7 Biological Fisheries technician ($37 K) will be hired at the USGS-Marrowstone Marine Field Station to perform duties associated with the empirical manipulations in the laboratory. The GS-7 technician will also be involved in experimental set up, laboratory sampling, and assistance with herring collections. This position will be under the direct supervision of Dr. Hershberger and under the remote supervision of Mr. Heintz and Dr. Rice.

Contract costs for the growth analyses (RNA/DNA and enzyme activities) of laboratory collections ($4.5 K).

***Commodities*: $20.5 K**
Fuel for the Lynn Canal vessel, NOAA R/V Quest ($2.5 K)

Shipment of supplies/samples to and from field sites and the laboratory ($2.0 K)

Supplies for growth analyses (solvents…) ($4.5 K)

Supplies for sample preservation (zip-lock bags, boxes, other containers & packaging) ($2.5 K)

Funding is requested for fish food, nets, disinfectants, and other laboratory supplies needed to maintain herring in the laboratory. ($9 K)

Existing Equipment Usage:
Accelerated Solvent Extractor
Leco Protein Analyzer
Thermogravimetric Analyzer
Parr Semi-micro Bomb Calorimeter
Bioelectrical Impedance Analyzers (2)
Frame Trawls
R/V Quest, R/V John N. Cobb

***General Administration:* $15.5 K**

DATA MANAGEMENT STATEMENT

This project involves a field component and a laboratory component. The field component includes collection of biological specimens, measurement of morphological characteristics, chemical analyses for energetic parameters (lipid, protein, calories), and analyzing and interpreting results. The laboratory component includes manipulation of cultured fish, measurements of energetic status, chemical analyses for energetic parameters and growth parameters, and analyzing and interpreting results. Data management and quality control will be the responsibility of J. Vollenweider and R. Heintz of the Auke Bay Lab, using established scientific protocols.

Study design, study sites and statistical analyses are given elsewhere in this proposal.

1. Standard scientific protocols will be used for field studies and hypothesis testing. Chemistry QA/QC measures are standardized for our lab and are published in Vollenweider 2005, Vollenweider et al. *Submitted*, Heintz and Vollenweider *In Review*, and Hillgruber et al. 2006 (see literature cited).
2. Data characteristics:
    a. If funded, we will use MetaLite, freeware created by USGS for collecting and validating Federal Geographic Data Committee (FGDC)-compliant metadata, as requested.
    b. Quantitative datasets obtained in this proposal include:
        1. Measurements of field-collected fish:
            i. seasonal body composition of herring (energy, lipid and protein content) in 3 locations:
            ii. Prince William Sound, Sitka Sound, and Lynn Canal; length, mass, gender and age of herring from the 3 locations,
            iii. associated temperature measurements at the 3 study locations
        2. Laboratory-derived bioenergetic model parameters
3. Handling and custody of samples will follow standard ABL and protocols, which follows a dataflow from sample custody sheets, through chemical analysis to final archival in a standard database. Samples for chemical analysis will be maintained in a frozen state to prevent degradation prior to chemical analyses.
4. Calibration and evaluation of analytical instruments are routinely performed at ABL and the University of Alaska. These evaluations include analysis of standard QA/QC samples of known composition (Vollenweider 2005, Vollenweider et al. *Submitted*, and Hillgruber et al. 2006).
5. Data will be reported as described earlier in this proposal (see Publications and Reports, Professional Conferences). In addition, raw data & accompanying metadata will be published on the internet in a publicly-available form once peer-reviewed manuscripts have been published. This is standard protocol for the Nutritional Ecology Lab. Standard software will be used (Microsoft Office). Minitab will be used to perform statistical analyses.

2008 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2007 - September 30, 2008

Budget Category:	Authorized FY 2007	Proposed FY 2008						
Personnel	$11.4	$6.4						
Travel	$12.2	$27.4						
Contractual	$90.1	$117.5						
Commodities	$8.6	$20.5						
Equipment	$5.3	$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$127.6	$171.8						
General Administration	$11.5	$15.5						
Project Total	$139.1	$187.3						
Full-time Equivalents (FTE)	0.2	0.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

Corrected FY within the DPD on the Schedule; Corrected Authorized budget for FY 07 (travel was reduced to $12.2)

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

FY08

Project Number: 080806
Project Title: Are herring (Clupea pallasi) energetics in PWS a limiting factor in successful recruitment of juveniles or reproduction investment of adults? Part II: Weighing the evidence for population effects of energy limitations using a bioenergetics model.

Prepared:

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, Johanna	Fisheries Research Biologist	ZPII-1			6.4	
	Subtotal		0.0	0.0	6.4	
					Personnel Total	

Travel Costs:	Ticket	Round	Total	Daily	
Description	Price	Trips	Days	Per Diem	
Vollenweider & Heintz to AK Marine Science Symposium	0.7	2	10	0.2	
Travel for field collections of herring:					
PWS	0.6	5	40	0.1	
Sitka Sound	0.3	4	16	0.2	
Lynn Canal	0.0	0	0	0.0	
Travel for laboratory work @ Marrowstone Marine Field Station	0.7	8	70	0.1	
				Travel Total	

FY08

Prepared:

Project Number: 080806
Project Title: Are herring (Clupea pallasi) energetics in PWS a limiting factor in successful recruitment of juveniles or reproduction investment of adults? Part II: Weighing the evidence for population effects of energy limitations using a bioenergetics model.

Contractual Costs:	
Description	
Vessel charters for field collections of herring: PWS Lynn Canal Sitka Sound Chemical analysis of energetic (field & laboratory) specimens Fish culture technician Chemical analysis of growth 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) Shipment of supplies/samples to & from field sites/laboratory Supplies for growth analysis Supplies for specimen preservation Supplies for herring culture	
Commodities Total	

FY08

Prepared:

Project Number: 080806
Project Title: Are herring (Clupea pallasi) energetics in PWS a limiting factor in successful recruitment of juveniles or reproduction investment of adults? Part II: Weighing the evidence for population effects of energy limitations using a bioenergetics model.

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

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

Existing Equipment Usage: Description	Number 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	2	
R/V Mel	1	
Trawl Nets	2	
Herring culturing facility	1	

FY08

Prepared:

Project Number: 080806
Project Title: Are herring (Clupea pallasi) energetics in PWS a limiting factor in successful recruitment of juveniles or reproduction investment of adults? Part II: Weighing the evidence for population effects of energy limitations using a bioenergetics model.