FY09 EVOS ANNUAL PROGRESS REPORT

PROJECT NUMBER: 090806

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

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TIME PERIOD COVERED BY REPORT: September 1, 2008 - September 1, 2009

DATE OF REPORT: September 1, 2009

WORK PERFORMED:

Objective 1. Field Collections (3rd replicate year) – Measure overwinter energetic changes in herring to examine juvenile mortality and adult reproductive investment

FY09 was the third consecutive year of field collections to examine overwinter energetic losses in herring. Sampling effort was increased in all 3 study locations (PWS, Sitka Sound, Lynn Canal) in order to secure samples from each prescribed sample period and to ensure that samples were collected concurrently amongst the 3 study sites. In particular, juvenile herring had been difficult to find in previous years. Improved collection techniques (larger gillnets, greater variety of mesh sizes) and additional time dedicated to location and capture of juveniles provided a more complete sample set relative to past years (collection periods, ages,…).

FY09 Sample Collections:

	PWS	**Sitka Sound**	**Lynn Canal**
"Pre-Winter" (November)	ADF&G Adult Survey (11 DAS) PWSSC Juvenile Survey (10 DAS)	Small skiff surveys – no herring herring in Sitka yet (8 DAS)	Small skiff collections (8 DAS)
"Mid-Winter" (Late Jan/ Early Feb)	NOAA Charter *R/V Montague* (5 DAS)	NOAA Charter *R/V Medeia* (4 DAS) ADF&G Adult Survey (4 DAS)	NOAA Charter *R/V Medeia* (4 DAS)
"Pre-Spawn" (March)	ADF&G Adult Survey (6 DAS)	ADF&G Adult Survey (2 DAS) Sitka Tribe Surveys (6 DAS)	NOAA Charter *R/V Medeia* (4 DAS)
"Post-Spawn" (April in PWS/SS, May in LC)	NOAA Charter *R/V Montague* (5 DAS)	ADF&G Adult Survey (2 DAS)	Small skiff collections (6 DAS)
Total Days-At-Sea/ Charter days	37/37	26/4	22/8

Wild-caught samples collected during the winter of 2008/2009 represent the third replicate year to examine interannual variability and consistency amongst sites. All FY09 sample collections (n = 1215) are complete and all bio-processing measurements have been made (length, weight, gonad development, stomach contents) on a subsample of fish (n = 400). During the first two winters, a total of 1040 fish were aged using scales according to the procedure employed by ADF&G. We examined the data to evaluate age correlations with bioenergetic condition and found that length is a superior covariate to age. Thus, we did not age herring collected during the third winter. Rather, we will utilize length/age relationships measured by ADF&G. In many circumstances, we sampled fish from the same schools that were used by ADF&G for their annual age-weight-length assessments (in all 3 locations).

Proximate analysis (lipid, protein, energy content) of the samples is nearing completion (anticipated at the end of September). Mature fish were divided into soma and reproductive organs to assess the energy content of roe and milt for reproductive analyses. Analysis from the first two winters indicated that the chemical composition of milt was relatively stable over the winter and relatively consistent amongst study sites, thus milt was not analyzed for the third winter's collections. This allowed for an increased emphasis on roe samples. 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 indicates 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 are being analyzed.

In total, field collections over the course of the three study years resulted in a total of 3400 herring collected, 1500 subsampled for detailed bioprocessing, and 600 samples analyzed in the chemistry laboratory.

Initial Findings:

1) Adult herring in PWS lose energy at a higher rate (0.42% per day) than in Sitka (0.36% per day) or Lynn Canal (0.25% per day). 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.

2) YOY and juvenile herring from PWS also lose energy at a faster rate overwinter than do their analogous cohorts in southeast Alaska.

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

Two iterations of the YOY fasting bioenergetics lab study have been completed (trial #1 → 100 days, trial #2 → 66 days). We originally proposed to run one trial, however we opted to perform a second iteration because sample numbers were relatively small in the first trial and time and resources permitted a second, though abbreviated iteration. The second trial had nearly twice as many fish as the first (trial #1: 35 fish/starvation tank, 15 fish/control tank; trial #2: 55 fish/starvation tank, 35 fish/control tank), providing more robust results. In addition, the temperatures differed between trials because they were carried out in different seasons; trial #1 was in the spring and had cooler temperatures (5.5, 9, 12.5 ^{o}C), trial #2 was in the fall and had warmer water temperatures (7.5, 11.5, 14 ^{o}C). Thus, we now have 6

experimental temperatures under which herring were fasted until 60% mortality and refed to examine potential for compensatory growth. In addition, we have 6 temperature/energy curves to calculate metabolic rate under various temperatures. Chemical analysis (proximate composition and caloric content) of samples from the first trial is complete while samples from the second trial are currently being analyzed. In addition, maximum consumption rate and assimilation efficiency was determined from these fish. Proximate analysis and bomb calorimetry of these samples is complete.

Samples from the fasting trials were also saved to examine biochemical indices of growth, including RNA/DNA and enzyme activities. The methods development of the RNA/DNA assay is complete, as is the biochemical analyses of the samples. Data analysis is in progress.

In addition to fasting YOY, we had the opportunity to examine the fasting kinetics of age-2 herring, though only at ambient temperature. The larger fish are proficient at storing greater lipid stores which can sustain them for considerably longer duration than the YOY. Thus, we did not encounter any mortalities over the 107-day fasting period, however we hope to detect rates of lipid utilization upon completion of chemical analysis to compare to energy utilization rates from the field.

Initial Findings:

1) We determined that YOY herring in PWS are foraging over winter. In our fasting study we observed average mass loss rates of 0.24%, 0.25% and 0.42% per day in our cold, ambient and warm tanks, respectively. Wild-caught YOY in PWS lost energy at a slower rate than the fasting fish in the lab study, indicating that fish in PWS are forestalling mass loss by foraging. This was consistent with observations of prey in 60% of the stomachs we examined.

2) Laboratory fasting trials determined that YOY herring have an energy threshold of ~9kJ, below which mortality occurs. At the end of winter, PWS YOY's were relatively close to the energy threshold, whereas YOY's in Sitka were in better condition and had an "energetic buffer".

2) Under all experimental temperatures, YOY herring that have been starved to 60% morality are capable of compensatory growth within a matter of months, reaching RNA/DNA levels similar to cohorts that had been fed continuously.

Objective 3. Laboratory-based studies – Disease challenges (3 Trials)

i. Compare the survival of *Icthyophonus*-infected and uninfected herring under food-withheld conditions

During the first food deprivation study, time to starvation among uninfected, specific pathogen-free Pacific herring was temperature-dependent, with the 50% mortality occurring after 46d in the hot groups (8.6 - 12.6 C) and 58d in ambient groups (7.9 - 10.4 C); cumulative mortality in the cold groups (5.4 - 7.9 C) only reached 27% at the end of the experiment (61d). Among analogous groups of herring that were diseased with *Ichthyophonus*, time to mortality was much shorter at all temperatures and was characteristic of disease progression rather than starvation. Within the first 10 d after moving diseased herring to their temperature treatments and withholding food, mortality reached 37.3% in the hot groups, 26.0% in the cold groups, and 21.3% in the ambient groups. Among diseased

herring maintained at ambient temperatures, the kinetics of mortality were similar among starved and fed groups for the first 40 d (49-55% cumulative mortality); after which mortality slowed in the fed groups and continued to increase in the starved groups through the end of the study (58.7% and 76.% cumulative mortalities after 61d, respectively).

ii. Determine bioenergetic parameters among *Ichthyophonus*-infected and uninfected herring under food-withheld conditions.
Trial 2 is complete and data analysis of mortality rates is underway. Samples for energetics have been sent to Auke Bay Laboratories and will undergo chemical analysis (lipid, protein, energy content) beginning in October.

iii. Determine the impact of bioenergetic condition on the ability of herring to survive exposure to *Ichthyophonus*.
Trial 3 is underway and will be terminated when cumulative mortality reaches 90% (anticipated in the next 2 weeks). Energetics samples will be sent to Auke Bay Laboratories upon completion.

Future Work:
We have received funding for FY10 to perform data analysis, write reports, and prepare data synthesis publications from these three years of study. In the future, we will be interested in pursuing other questions that have arisen from our research.

Preliminary findings from this study have shown that YOY and juvenile herring in PWS incur larger overwinter energetic demands than their counterparts in Southeast Alaska and must forage overwinter to stave off starvation. The source of the energetic demands are unknown, but speculated to be related to disease, predation, or prey. Upon completion of our disease challenges, we will know the energetic price YOY pay from infection. The influence of prey quality and quantity on YOY condition will be determined in PWSCC and Heintz's FY10 collaborative study. But the energy toll from predation is poorly understood. This is a critical piece of the puzzle in understanding the failure of the PWS herring population to recover.

In the future, we want to quantify energetic costs of predation to weigh predatory pressure against disease and foraging as causes of the population decline. Specific objectives that must be investigated include:
1) determine which sizes of herring humpback whales (and other predators) consume,
2) quantify the frequency of predatory interactions,
3) quantify energetic costs of predator avoidance as
    i. swimming costs
    ii. lost foraging opportunities while taking refuge from predators

Coordination/Collaboration: 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 were often made from the same platform. Furthermore, each study was a scout for the other study, as where we find herring in the winter, we often find whales, or vice versa.

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 were 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 are testing this hypothesis in the FY09 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 are testing.

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.
8) Auke Bay Labs Herring Tagging Studies – Live herring collected during this study are currently being used for preliminary tagging studies to be expanded on in an NPRB-funded study next summer (J. Eiler).
9) Auke Bay Labs Respirometry Trials – Live herring collected during this study are currently being used in respirometry trials (B. Nelson).
10) Auke Bay Labs Genetic Studies – During field collections, samples were retained for genetic analysis to examine population structure (S. Wildes).

Community Involvement/TEK & Resource Management Applications:
Community Involvement - Multiple components of this study have been used to provide opportunities for students to participate in marine science through internships and dissertations. The laboratory component examining biochemical indices of growth (RNA/DNA analysis and enzyme activities) is one portion of a PhD dissertation. A smaller component of this study examining roe quality was conducted by Alaska Native students during a summer NOAA

internship. Locals were also involved in the collection and reporting of herring. McLaughlin Environmental Services (Chenega Bay, AK) were contracted to collect herring samples from the vicinity of Sawmill Bay. Sitka Tribe members, Sitka Coast Guard, and citizens of Sitka reported herring and whale sightings.

Resource Management - Information collected during this study will be instrumental in understanding the interplay of disease and predation on herring populations through mortality and recruitment processes. This understanding will be key in PWS and Lynn Canal where herring populations are struggling.

Information Transfer:

Attendance at Symposia:

Alaska Marine Science Symposium: Vollenweider, Heintz, Hershberger, Rice, Sreenivasan
American Fisheries Society: Heintz

Presentations

Vollenweider, Heintz. Do Energy Limitations Cause Overwinter Mortality of Young-of-the-Year and Juvenile Pacific herring (*Clupea pallasi*)?

Heintz, Vollenweider. Seasonal Flux in the Energy and Proximate Composition of Maturing Pacific Herring. Alaska Marine Science Symposium, January 2009.

Posters

Vollenweider, Heintz. Is Reproductive Investment of Adult Pacific Herring (*Clupea pallasi*) Contributing to Their Decline? Alaska Marine Science Symposium, January 2009.

Sreenivasan, Heintz, Schaufler, Hurst, Rice, Smoker, Vollenweider. Differences between observed growth and a physiological growth index (RNA/DNA ratio) in larval Pacific cod (*Gadus macrocephalus*) at different temperatures. Alaska Marine Science Symposium, January 2009.

PAPERS in Draft:

Vollenweider, Heintz. Is Reproductive Investment of Adult Pacific Herring (*Clupea pallasi*) Contributing to Their Decline?

Vollenweider, Heintz. Do Energy Limitations Cause Overwinter Mortality of Young-of-the-Year and Juvenile Pacific herring (*Clupea pallasi*)?

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

Vollenweider, Heintz, Hershberger. Energetic demands of *Icthyophonus* infection in Pacific herring.
(Hershberger will have disease kinetic papers from his related disease project)

Budget: Expenditures were close to budgeted values. Overtime estimates were too low ($5.8K) due to increased field time to acquire necessary samples as well as weather delays. Travel expenses were well under budget ($14K) because much of the anticipated help required to perform the disease challenges were met by employees at Marrowstone Marine Research Station;

savings in travel expenditures were used for contract labor to help with the elevated field work, bio-processing and chemical analysis of more samples.

Signature of PI:_____

Project Web Site Address:_____