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GEM PROPOSAL SUMMARY PAGE
(To be filled in by proposer)

Project Title:

Temporal stability of fatty acids used to discriminate Pacific herring in Alaska.

Project Period:

October 2005 to September 2007 (FY05-FY07)

Proposer(s):

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Study Location:

Gulf of Alaska (Sitka, Prince William Sound, Kodiak, Cook Inlet) and Bering Sea (Dutch Harbor, Togiak, Kuskokwim Bay)

Abstract:

This project follows up on a promising pilot study that demonstrated the ability to discriminate Alaska herring stocks at relatively fine spatial scales (> 100 km) based on the fatty acid composition of their heart tissue. The investigators propose to assess the temporal stability and biological variability of stock discrimination criteria derived from fatty acid analysis of herring cardiac tissues. Samples will be collected during the spring and fall/winter of 2005 and 2006 from putative herring stocks from Sitka, PWS, Kamishak, Kodiak, Dutch Harbor, Togiak, and Kuskokwim Bay. Results should allow managers to better define ecologically significant stock boundaries, which would likely affect how commercially exploited herring populations are assessed and managed. Results will be published in a peer-reviewed report and may lead to revision of fishery management plans for affected areas.

Keywords: Pacific herring, stock identification, fatty acid analysis, Gulf of Alaska

Funding:	EVOS Funding Requested:	FY05	\$ 67.7	
	(Must include 9% GA)	FY06	\$ 89.4	
		FY07	\$ 25.1	
				TOTAL: \$182,184
	NON-EVOS Funds to be used:	FY05	\$ 99,954	
		FY06	\$ 99,954	
		FY07	\$ 0	
				TOTAL: \$199,908

Date: 14 April 2004

GEM RESEARCH PLAN

I. NEED FOR THE PROJECT

A. Statement of Problem

Despite decades of study and over a hundred years of commercial exploitation targeting Pacific herring (*Clupea pallasii*), considerable uncertainty continues to exist regarding: 1) the scale at which population structure exists within large geographic areas and, 2) the degree to which herring return to natal areas to spawn. These fundamental life history traits are directly relevant to how exploited herring stocks should be assessed and managed (Hourston 1982; Wheeler and Winters 1984; Hay and McCarter 1997; McQuinn 1997). State fishery managers require a tool that can identify ecologically significant population structuring among adjacent spawning aggregations that are exploited during spring sac-roe herring fisheries. They also require a mixed stock analysis tool that allows them to investigate whether winter herring fisheries (e.g., food/bait fisheries) target only the local spawning stock or a mixture of nearby stocks that aggregate during winter. The ability to manage stocks discretely is a principal component of sustainable fisheries management- one that requires the ability to accurately apportion the catch from mixed stock fisheries.

Researchers have attempted to use many different techniques to distinguish among herring stocks, including: scale pattern analysis (Rowell 1981), tagging studies (Hourston 1982), morphometrics and meristics (Schweigert 1990), microsatellite DNA (O'Connell et al. 1998), and otolith microchemistry (Otis and Heintz 2003). However, most techniques have proven to be unreliable at fine spatial scales. For example, O'Connell et al. (1998) found that herring from Prince William Sound (PWS) and the Bering Sea were genetically divergent, but they were unable to find similar divergence among stocks sampled within the north Gulf of Alaska. The difficulty encountered with genetic markers is likely due to the relatively high stray rates exhibited by herring (e.g., Tester 1949; Cushing and Burd 1957; Hourston 1982; Wheeler and Winters 1984). Very little gene flow between populations is necessary to compromise the ability of allozyme markers to discriminate among putative stocks (Smith and Jamieson 1986; Bembo et al. 1996; Waples 1998). In particular, Waples (1998) observed that "because the amount of migration necessary to obscure most genetic evidence of stock structure (only a handful of individuals per generation) is generally inconsequential as a force for rebuilding depleted populations on a time scale of interest to humans, there is no guarantee that genetic methods alone will provide sufficient precision for key management decisions involving marine species". Thus, herring managers have continued to seek a tool that allows them to identify population structure within and among their respective management areas.

In the absence of more definitive tools, many fishery managers have traditionally used spawning timing and location as proxies to roughly define herring stock structure. The logical assumption is, the greater the temporal and spatial separation between spawning aggregates, the greater the likelihood that they are discrete stocks. However, problems can arise when mixing of putative stocks occurs across jurisdictional boundaries. Anecdotal observers have reported examples in which the abundance of one presumptive spawning stock "crashes" while an adjacent area's presumptive stock simultaneously increases by a commensurate amount. Such observations of

“spawner relocation” highlight the behavioral complexity of herring (Overholtz 2002; Hay and McKinnell 2002; Huse et al. 2002) and raises questions regarding stock discreteness and population “sub-units” (Stephenson 1999).

Recently, a new method of stock identification was applied successfully to discriminate known herring stocks and reveal differences among putative stocks at relatively fine spatial scales (≥ 100 km). The method discriminates stocks using differences in the fatty acid composition of cardiac tissue (Otis and Heintz 2003). This method has been tested for other fish species (e.g. Grahl-Nielsen and Mjaavatten 1992, Castell et al. 1995, Pickova et al. 1997, Joensen et al. 2000) but requires further testing before it can be applied to herring. To date, these tests indicate that the fatty acid composition of cardiac tissues are the least influenced by environmental factors (Viga and Grahl-Nielsen 1990), is sensitive at discriminating stocks over small geographic scales (Grahl-Nielsen and Mjaavatten 1992) and has a genetic basis (Joensen et al. 2000).

Whether or not detection of discernable differences in arbitrarily selected variables constitutes ecologically significant, distinct populations is open to debate (Waples 1998). That debate has particular relevance to this proposal since many studies have shown that the fatty acid compositions of some tissues and lipid classes are highly sensitive to changes in diet and the environment (e.g., Hazel 1984, Henderson and Tocher 1987, Cordier 2002). Therefore, demonstrating that the variation in heart tissue fatty acid composition observed between stocks exceeds that imposed by the environment on a given stock will be a key element in the development of this method (Begg et al. 1999). We are proposing to target heart tissues because heart phospholipids are less subject to environmental influences than other tissues or lipid classes (Grahl-Nielsen and Ulvund 1990, Czesny et al. 2000, McKenzie 2001). Several studies have shown that dietary impacts on fatty acid composition are minimized in heart lipids. Viga and Grahl-Nielsen (1990) cultured groups of Atlantic salmon from the same stock for eight months on prescribed diets and found the fatty acid composition of salmon hearts was independent of diet. Grisdale-Helland et al. (2002) found significant differences in the heart phospholipids of Atlantic salmon fed different diets for approximately three months. However, they identified much greater differences in the composition of heart triacylglycerols. Similarly, studies reviewed by McKenzie (2001) reveal the tendency for heart fatty acid composition to respond to diet but at much lower magnitude than muscle or liver. These data indicate that examination of heart fatty acids should minimize the apparent variation imposed on populations due to diet, ration, and temperature (Grisdale-Helland et al. 2001; Kiessling et al. 2001; Jobling et al. 2002).

Three recent laboratory studies reported evidence of genetic control over some fatty acid concentrations, thus supporting the premise that they can be effective stock identifiers. Joensen et al. (2000) found significant differences in the fatty acid profiles of heart tissue extracted from representatives of two cod stocks that had been reared for 44 months under identical diets and environments. Peng et al. (2003) compared the fatty acid compositions of anadromous and landlocked Atlantic salmon (*Salmo salar*) fry, fed identical diets throughout a 44-day feeding trial, and reported significant differences in their phospholipids. In a companion study, Rollin et al. (2003) concluded that differences in the fatty acid composition of different strains of Atlantic salmon resulted from variation in the rates of desaturation and elongation of linolenic and linoleic acids. This suggests that differences in the activities of enzymes that regulate

phospholipid composition might explain the stock differences identified here and in other species examined in field studies (Grahl-Nielsen and Ulvund 1990, Grahl-Nielsen and Mjaavatten 1992).

The concept of genetic control over the composition of heart fatty acids is bolstered by studies demonstrating relationships between cardiac function and fatty acid composition. Bell et al. (1993) reported heart lesions in Atlantic salmon fed diets with high levels of n-6 fatty acids after the fish had been stressed. Agnisola et al. (1996) reported reduced heart rate and cardiac power output in the hearts of sturgeon fed diets high in n-3 fatty acids relative to those fed diets high in n-6 fatty acids. These data demonstrate an influence of heart fatty acid composition on individual fitness, thereby providing a basis for differences among reproductively isolated aggregates. Alternatively, interactions between phospholipid composition, eicosanoid production and cardiac function have rarely been described for fish (Stenslokken et al. 2002) despite their frequently described impacts on mammalian health (Das 2001). These data may account for the conclusion that C22:6n3 in fish heart phospholipids is not strongly influenced by diet (Thomassen and Røsjø 1989, Caballero et al. 2002, Grisdale-Helland 2002), and in fact may be under strong genetic control (Peng et al. 2003).

B. Relevance to GEM Program Goals and Scientific Priorities

The proposed project is intended to address several GEM Program goals outlined under the *Management Applications* section of the 2004 RFP. Specifically, we'll utilize existing ADF&G biological sampling programs and platforms (vessels) to collect the samples we need, and the new stock identification technique we're evaluating has tremendous potential to augment existing ADF&G stock assessment and management strategies. If we achieve the results we expect, ADF&G will gain a valuable tool to help them define ecologically significant stock boundaries for exploited herring spawning aggregations (spring sac roe fisheries) and determine the stock contribution for herring harvested in mixed-stock food/bait fisheries. Ultimately, the ability to identify the stock of origin for herring collected away from their natal spawning areas would provide a basis for better understanding the important role herring play in the marine ecosystem (e.g., GEM Ecosystem Model) by enabling studies directed at: larval dispersal patterns, home ranges of individual populations, locations of stock specific over-wintering areas, and perhaps the degree to which Pacific herring home back to their natal spawning areas. Recipients of the potential benefits resulting from this project include ADF&G (improved stock assessment and fishery management plans), subsistence and commercial herring fisherman (improved management of sac roe and food/bait fisheries), and herring researchers statewide (ability to define stock of origin for herring sampled away from natal spawning areas).

II. PROJECT DESIGN

A. Objectives

The goal of this research is to evaluate the temporal stability and biological variability of the fatty acid compositions that have already been used to discriminate Alaska herring stocks (Otis and Heintz 2003). Accurate knowledge of stock structure is relevant to the manner in which state officials assess and manage this commercially and ecologically important resource. The ability to identify the stock of origin for herring collected away from their natal spawning areas

would also have tremendous utility to managers of fisheries that may be harvesting mixed stocks (e.g. herring food/bait fisheries). For these purposes, we propose the following objectives:

Objective 1) Assess the temporal stability and biological variability of stock discrimination criteria derived from fatty acid analysis of cardiac tissues.

This objective addresses three hypotheses:

- 1). At spawning, the variation in fatty acid composition within a spawning stock is equal to the variation observed between that stock and other spawning stocks.
- 2) At spawning, the variation in fatty acid composition of a spawning aggregation is equal to that of a similar aggregate spawning in the same general area, but later during the spawning period.
- 3) The variation in fatty acid composition of a spawning stock in a given year is equal to the variation between that stock and a stock using the same spawning area in a different year.

The first of these hypotheses is an attempt to re-create the results described in Otis and Heintz (2003), without controlling for age, sex, and gonad maturity, as was done in their pilot study. Evaluation of this hypothesis will establish the extent to which heart fatty acid composition naturally varies across all contributing members (i.e., sexes, cohorts) of a putative spawning stock. The second examines temporal variation within a putative stock over the course of a protracted spawning period, within a given spawning year. The third hypothesis, undertaken in year two of the proposed study, examines the temporal variation in heart fatty acid composition across successive years. In addition, since we are proposing to resample the stocks examined in Otis and Heintz (2003), hypothesis 3 can be examined over a 6-year period (i.e. 2001 – 2006).

Objective 2) Assess whether the stock(s) of origin for herring harvested in winter food/fisheries can be determined by comparing their heart fatty acid composition to those of local area spawning aggregations.

This objective addresses one additional hypothesis:

- 4) The variation in fatty acid composition within herring schools aggregating during winter is equal to the variation observed between herring schools using the same general area for spring spawning.

This final hypothesis evaluates whether or not fatty acid compositions from spawning herring can be used to determine the stock(s) of origin for herring harvested during winter food/bait fisheries.

B. Procedural and Scientific Methods

To facilitate the most robust evaluation of hypothesis 3, we intend to resample the putative spawning stocks examined in Otis and Heintz's (2003) pilot study: Togiak, Kodiak, Kamishak,

Prince William Sound, and Sitka. At least two spawning samples will be collected in each of these principal areas, targeting the early and late season spawning waves, in order to best evaluate hypothesis 2. A third temporal sample will be collected during the fall/winter food/bait fisheries scheduled to occur in Kodiak and Dutch Harbor to evaluate hypothesis 4. The Dutch Harbor food/bait fishery is suspected to include herring from the Nelson Island, Togiak, and possibly Goodnews Bay stocks. All of the spring spawning collections outlined in Table 1 will be repeated in FY06 to facilitate evaluation of hypothesis 3. The winter food/bait fishery samples will only be collected in FY05, resulting in a total sample of approximately 840 herring hearts for the entire study.

Table 1. Proposed sampling locations, dates, and sample sizes for herring to be collected for heart tissue fatty acid analysis.

Region	Sample location(s)	Sample date(s)	Sample Type	Sample size (2005)	Sample size (2006)
Sitka	Sitka Sound	Mar 25-Apr 5	Spring (spawning)	30	30
	Hoonah	Apr 10-20	Spring (spawning)	30	30
Prince William Sound	Montague Island	Apr 15-20	Spring (spawning)	30	30
	NE (Gravina Bay)	Apr 5-10	Spring (spawning)	30	30
	N (Fairmont Bay)	Apr 15-20	Spring (spawning)	30	30
Westward (Kodiak)	Paramanof Bay	Apr 15	Spring (spawning)	30	30
	Uganik Bay	Apr 15-30	Spring (spawning)	30	30
	Uganik Bay	Nov-Jan	Winter (food/bait fishery)	30	0
Kamishak Bay	Dutch Harbor	July 15	Summer (food/bait fishery)	30	0
	Chenik Head	April 25-May 5	Spring (spawning)	30	30
	Iniskin Bay	May 15-25	Spring (spawning)	30	30
Togiak	Nunavachak	May 1-10	Spring (spawning)	30	30
	Hagemeister Is.	May 10-15	Spring (spawning)	30	30
Bering Sea	Nelson Island	May 15-30	Spring (spawning)	30	30
	Goodnews Bay	May 25-Jun 5	Spring (spawning)	30	30
Total samples				450	390

At collection, the lengths and sexes of the sampled fish will be recorded and scale samples will be removed for aging. In contrast to Otis and Heintz (2003), the proposed study will target all age classes, maturity stages and sexes. Herring hearts will be removed and placed immediately in liquid nitrogen for shipment to Juneau (NMFS-Auke Bay Lab), where they will be stored at -80°C until analyzed.

Fatty acid analysis will be performed on the lipids of whole hearts, extracted using the Folch method. Before extraction, 50 μL of extraction surrogate (C23:0) and BHT will be added to the homogenized cardiac tissue. The homogenates will be extracted with on a Dionex 200 Accelerated Solvent Extractor (ASE), followed by rotary evaporation and volumetric dilution to a final volume of 1 ml. A 30 mg sample of the purified lipid will be suspended in toluene, mixed with 50 μL of transesterification surrogate (C21:0) and transesterified in methanol and sulfuric acid for two hours at 80°C . The resulting solution of fatty acid methyl esters will be extracted into hexane and dried by passing through a column packed with Na_2SO_4 . The eluant will be spiked with 50 μL of internal standard (C19:0).

Fatty acids will be identified by injecting 1.0 μL of each transesterified sample into a Varian CP3800 gas chromatograph equipped with a Saturn model 2200 mass-detector. Fatty acids will be separated with a 30 m Omegawax 250 fused silica column operating under a ramped temperature program. Mass detection will be in single ion mode and 39 fatty acid peaks will be identified. These will be quantified relative to five- point calibration curves for each of the fatty acids and normalized to the recovery of the internal standard. Calibration curves will be developed for each batch of 15-20 tissue samples. Analytical accuracy for each batch of samples will be determined by examining the fatty acid composition of an in-house standard reference material (SRM). The composition of the SRM was initially calibrated against the National Institute for Standards and Technology SRM-1946, whose fatty acid composition has been certified. Precision of the estimated fatty acid concentrations will be evaluated by examining the variation observed in a duplicated sample. Sample purity will be examined by processing blank samples.

C. Data Analysis and Statistical Methods

Differences in the fatty acid composition of hearts collected from spawning aggregates at a given site will be determined by multivariate analysis of variance (MANOVA). Evaluation of our first two hypotheses will employ a one-way nested MANOVA with stock as the main factor and sampling period nested within stock. Response variables will be the percentages of analytes transformed following the method of Aitchison (1992). Wilk's lambda with α set to 0.05 will be used to test the hypothesis that sampling period has no effect on fatty acid composition and that stock has no effect on fatty acid composition. If the first hypothesis is accepted and the second rejected, the same approach will be repeated for samples collected in the second year.

Assuming no effect of spawn timing is found, then comparisons across years will be made to test the third hypothesis. Data described by Otis and Heintz (2003) will be used for one of the years. Data from the different sampling times will be pooled into a common stock in a given year, and a two-way MANOVA with years and stocks as the main factors will be evaluated by calculating

Wilk's lambda with α set to 0.05. If significant differences are found between spawning events within regions, sample events will not be pooled and the above analysis will be conducted for each unique spawning stock identified during evaluation of hypothesis 2.

Differences detected among groups under each hypothesis will be further examined by descriptive discriminant analysis (DA) to identify which groups differ. DA resolves differences among groups by identifying a series of canonical functions, each of which is a linear combination of the response variables. These functions progressively reduce the error in the data set. The number of functions that account for the error represents the dimensionality of the data set, which is determined by iteratively fitting a function and testing the hypothesis that the residual error is equal to zero (Huberty 1994). Bi-plots for each function will be constructed to examine how the functions separate the data. We will also examine the pooled within group canonical structure to identify which fatty acids exert the most influence on the separating functions. The results of the MANOVAs will be further examined by predictive discriminant analysis to examine the robustness of the conclusions. The analysis will employ the leave-one-out method to determine how frequently the discriminant functions accurately identify "unknown" samples. Results of these tests will be expressed as the probability of correctly identifying members of a test group to the appropriate stock or aggregate. The MANOVAs and discriminant analyses will be performed in SAS release number 6.12 using the non-parametric DISCRIM procedure. Prior to analysis, the homogeneity of the covariance matrices will be examined. If they are found to be not homogenous then correlation matrices will be used.

D. Description of Study Area

Our proposed study area includes sampling locations extending from Sitka Sound (~57° N Latitude, 136° W Longitude), north to Prince William Sound (~61° N Latitude) and west to Dutch Harbor (~54° N Latitude, 167° W Longitude; Figure 1). Except for Dutch Harbor, Togiak and Kuskokwim Bay (Goodnews Bay, Nelson Island), which are in the Bering Sea, all sampling locations are within the Gulf of Alaska (GOA), with most of the proposed samples coming from locations in the Northern Gulf of Alaska (NGA). Pacific herring can be found spawning at many locations along Alaska's ubiquitous coastline with commercially viable populations of interest to this study being located in Sitka Sound, Prince William Sound, Kamishak Bay (Lower Cook Inlet), Kodiak/Afognak Island (Paramanof Bay), Togiak Bay, and Kuskokwim Bay.

E. Coordination and Collaboration with Other Efforts

This collaborative project (ADF&G-Commercial Fisheries, NMFS-Auke Bay Lab) builds on the EVOS funded pilot study (Project 02538) conducted by Principal Investigators Otis and Heintz, which demonstrated the potential for using fatty acid analysis of herring hearts to discriminate among spawning aggregates sampled from Sitka, Cook Inlet, Kodiak, Togiak, and two locations in Prince William Sound. This follow-up study will rely on close coordination with existing ADF&G herring stock assessment projects in order to save on vessel charter costs to obtain samples. The following ADF&G collaborators will facilitate sample collections from their respective areas: Marc Pritchett (Sitka), Steve Moffitt (PWS), Mark Witteveen (Kodiak/Dutch Harbor), Ted Otis (Kamishak), Lowell Fair (Togiak), and Craig Whitmore (Bering Sea). Finally, this project proposes to develop stock discrimination tools that may help resolve questions

concerning the scale at which discrete herring stocks exist in PWS and the greater Gulf of Alaska. Information gained by this project could help put the results of other EVOS projects into context and illuminate new directions for long term monitoring under GEM.

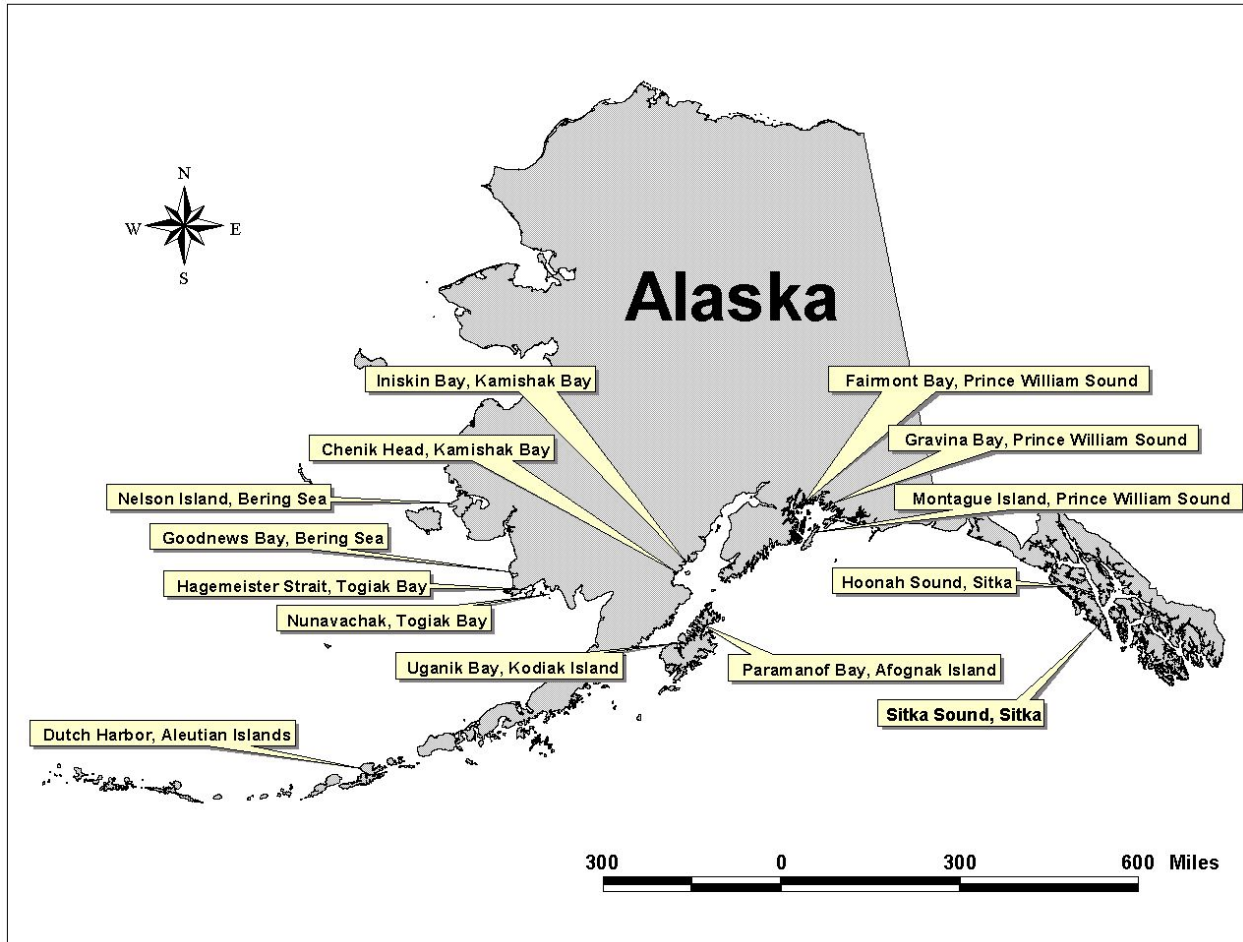


Figure 1. Map of Alaska illustrating the 14 locations from which Pacific herring will be sampled to evaluate the temporal stability of heart tissue fatty acid markers used to discriminate stock structure.

III. SCHEDULE

A. Project Milestones

- Objective 1. Assess the temporal stability and biological variability of stock discrimination criteria derived from fatty acid analysis of cardiac tissues.
Preliminary assessment based on Year 1 data to be met by September 1, 2006
Final assessment based on two years data to be met by September 30, 2007

Objective 2. Assess whether the stock(s) of origin for herring harvested in winter food/fisheries can be determined by comparing their heart fatty acid composition to those of local area spawning aggregations.
To be met by September 1, 2006

B. Measurable Project Tasks

FY 05, 1st quarter (October 1, 2004-December 31, 2004)

October: Project funding approved by Trustee Council

FY 05, 2nd quarter (January 1, 2005-March 31, 2005)

January 12-16 (tentative): Annual GEM Workshop

March: Collect Sitka spawning samples

FY 05, 3rd quarter (April 1, 2005-June 30, 2005)

April - June: Collect PWS, Kodiak, Kamishak, Togiak, and Bering Sea spawning samples

FY 05, 4th quarter (July 1, 2005-September 30, 2005)

July: Collect Dutch Harbor food/bait samples

August-September: Begin chemical analysis of hearts

September 1: Submit Annual Report

FY 06, 1st quarter (October 1, 2005-December 31, 2005)

November-December: Collect samples from Kodiak food/bait fishery
Continue chemical analysis of hearts

FY 06, 2nd quarter (January 1, 2006-March 31, 2006)

(dates not yet known) Annual GEM Workshop

January-March: Complete chemical analysis/begin statistical analysis of year 1 data

March: Collect Sitka spawning samples (year 2)

FY 06, 3rd quarter (April 1, 2006-June 30, 2006)

April-June: Collect PWS, Kodiak, Kamishak, Togiak, and Bering Sea spawning samples (year 2)

FY 06, 4th quarter (July 1, 2006-September 30, 2006)

August-September: Begin chemical analysis of year 2 heart samples

September 1: Submit Annual Report

FY 07, 1st quarter (October 1, 2006-December 31, 2006)

October-December: Complete chemical analysis/begin statistical analysis of year 2 data

FY 07, 2nd quarter (January 1, 2007-March 31, 2007)

(dates not yet known) Annual GEM Workshop

January-March: Complete statistical analysis

FY 07, 3rd quarter (April 1, 2007-June 30, 2007)
April-June: Begin writing final report/manuscript for publication

FY 07, 4th quarter (July 1, 2007-September 30, 2007)
September 30: Submit final report/manuscript for publication.

IV. RESPONSIVENESS TO KEY TRUSTEE STRATEGIES

A. Community Involvement and Traditional Ecological Knowledge (TEK)

Because this is a lab study evaluating a developing technology, there is not much room for incorporating TEK at this stage. However, we do envision opportunities to engage interested subsistence/commercial fisherman and coastal community members through various outreach endeavors. Along with presenting project results at at least one professional meeting in 2006/2007, we've identified two potential outlets that will reach a more general audience. The newly completed Alaska Islands and Oceans Visitor Center (AIOVC) in Homer should make an excellent outlet to reach up to 10,000+ visitors per year, including K-12 children attending AIOVC environmental education programs. We have contacted Carmen Field, the Kachemak Bay Research Reserve's (KBRR) Environmental Education Coordinator at AIOVC and begun developing potential outreach projects to be implemented in cooperation with the KBRR at the AIOVC in Homer. Because our project spans so much of coastal Alaska, we've also contacted Alan Parks, with the Alaska Marine Conservation Council (AMCC), to develop an appropriate strategy to outreach our project to the many fishing/coastal communities represented by AMCC's active membership (e.g., Sitka, Homer, Kodiak, Dutch Harbor). These partnerships will be further developed if our proposal is funded, however, we anticipate that our outreach efforts will take the form of visitor center displays, newsletter articles, project web site creation/maintenance, and using local AMCC representatives to outreach our project to their coastal communities.

B. Resource Management Applications

This project has tremendous resource management potential. A tool that is able to discriminate herring stocks over fine spatial scales would have great value to fishery managers. In the near term, this method could be used to resolve a number of pressing commercial fishery management questions regarding stock structure in the Bering Sea (e.g., What is the stock composition of herring harvested in Dutch Harbor's food/bait fishery) and Gulf of Alaska (e.g., Do spatially/temporally isolated spawning aggregations in Kamishak Bay [or Prince William Sound, Kodiak, or Sitka] represent discrete stocks?). Ultimately, the ability to identify the stock of origin for herring collected away from their natal spawning areas would provide a basis for better understanding the important role herring play in the marine ecosystem by enabling studies directed at: larval dispersal patterns, home ranges of individual populations, locations of stock specific over-wintering areas, and perhaps the degree to which Pacific herring home back to their natal spawning areas. This proposal has broad support from ADF&G Management/Research

staff, as demonstrated by their commitments to help collect samples from their respective areas (e.g., Sitka [Marc Pritchett], PWS [Steve Moffitt], Lower Cook Inlet [Lee Hammarstrom], Kodiak [Mark Witteveen, Kevin Brennan], Togiak [Lowell Fair], and Kuskokwim Bay [Craig Whitmore]).

V. PUBLICATIONS AND REPORTS

This project will provide a peer-reviewed final report on the identification of Alaska herring stocks based on free fatty acid composition of heart tissue, as well as an evaluation of the temporal stability of the fatty acid compositions used to discriminate among herring stocks. Annual Progress reports will be submitted by September 1 in Fiscal Years 2005 and 2006. We also intend to seek publication of an article tentatively entitled “Evaluation of the temporal stability of heart fatty acid compositions used to discriminate among Pacific herring stocks in Alaska“ in the refereed journal *Transactions of the American Fisheries Society* (to be submitted in September 2007).

VI. PROFESSIONAL CONFERENCES

Along with presenting project updates (posters) at the annual GEM workshop, we intend to give an oral presentation at the Alaska Chapter Meeting of the American Fisheries Society in November 2006 (location to be determined). Travel funds have been requested to meet each of these obligations.

Literature Cited

- Aitchison, J. 1992. On criteria for measures of compositional difference. *Math. Geol.* 24:4 365-379.
- Agnisola, C., D.J. McKenzie, E.W. Taylor, C.L. Bolis and B. Tota. 1996. Cardiac performance in relation to oxygen supply varies with dietary lipid composition in sturgeon. *Am. J. Physiol.* 271 R:417-425.
- Begg, G.A., K.D. Friedland, and J.B. Pearce. 1999. Stock identification and its role in stock assessment and fisheries management: an overview. *Fisheries Research* 43:1-8.
- Bell, J.G., J.R. Dick, A.H. McVicar, J.R. Sargent and K.D. Thompson. 1993. Dietary sunflower, linseed and fish oils affect phospholipid fatty acid composition, development of cardiac lesions, phospholipase activity and eicosanoid production in Atlantic salmon (*Salmo salar*). *Prostaglandins, Leukotrienes, Essential Fatty Acids* 49:665-673.
- Bembo, D.G., G.R. Carvalho, N. Cingolani, and T.J. Pitcher. 1996. Electrophoretic analysis of stock structure in Northern Mediterranean anchovies, *Engraulis encrasicolus*. *ICES Journal of Marine Science* 53:115-128.
- Caballero, M.J., A. Obach, G. Rosenlund, D. Montero, M. Gisvold, M.S. Izquierdo. 2002. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 214:253-271.

- Castell, J.D., L.D. Boston, R.J. Miller, and T. Kenchington. 1995. The potential identification of the geographic origin of lobster eggs from various wild stocks based on fatty acid composition. *Can. J. Fish. Aquat. Sci.* 52:1135-1140.
- Cordier, M., G. Brichon, J-M. Weber, and G. Zwingelstein. 2002. Changes in the fatty acid composition of phospholipids in tissues of farmed sea bass (*Dicentrarchus labrax*) during an annual cycle. Roles of environmental temperature and salinity. *Comparative Biochemistry and Physiology Part B* 133:281-288.
- Cushing, D.H. and Burd, A.C. 1957. On herring of the southern North Sea. III. *Fish. Invest. Ser. II, Vol. 10*, 31 pp.
- Czesny, S., K. Dabrowski, J.E. Christensen, J. Van Eenennaam, and S. Doroshov. 2000. Discrimination of wild and domestic origin of sturgeon ova based on lipids and fatty acid analysis. *Aquaculture* 189:145-153.
- Das, U.N. 2001. Beneficial effect(s) of n-3 fatty acids in cardiovascular diseases: but, why and how? *Prostaglandins Leukot Essent Fatty Acids*.63:351-62.
- Grahl-Nielsen, O. and K.A. Ulvund. 1990. Distinguishing populations of herring by chemometry of fatty acids. *American Fisheries Society Symposium* 7:566-571.
- Grahl-Nielsen, O. and O. Mjaavatten. 1992. Discrimination of striped bass stocks: A new method based on chemometry of the fatty acid profile in heart tissue. *Trans. Amer. Fish. Soc.* 121:307-314.
- Grisdale-Helland, B., B. Ruyter, G. Rosenlund, A. Obach, S.J. Helland, M.G. Sandberg, H. Standal and C. Rosjo. 2002. Influence of high contents of dietary soybean oil on growth, feed utilization, tissue fatty acid composition, heart histology and standard oxygen consumption of Atlantic salmon (*Salmo salar*) raised at two temperatures. *Aquaculture* 207:311-329.
- Hay, D. and P. McCarter. 1997. Larval distribution, abundance, and stock structure of British Columbia herring. *Journal of Fish Biology* 51(Suppl. A): 155-175.
- Hay, D.E. and S.M. McKinnell. 2002. Tagging along: association among individual Pacific herring (*Clupea pallasii*) revealed by tagging. *Can. J. Fish. Aquat. Sci.* 59:1960-1968.
- Hazel, J.R. 1984. Effects of temperature on the structure and metabolism of cell membranes in fish. *American Journal of Physiology* 246:460-470.
- Henderson, R.J., and D.R. Tocher. 1987. The lipid composition and biochemistry of freshwater fish. *Progress in lipid research*: 281-347.
- Hourston, A.S. 1982. Homing by Canada's west coast herring to management units and divisions as indicated by tag recoveries. *Can. J. Fish. Aquatic Sci* 39(10): 1414-1422.
- Huberty, C. J. 1994. *Applied Discriminant Analysis*. Wiley Series in Probability and Mathematical Statistics. John Wiley and Sons, Inc. New York.
- Huse, G., S. Railsback, and A. Ferno. 2002. Modeling changes in migration pattern of herring: collective behavior and numerical domination. *Journal of Fish Biology* 60:571-582.

- Jobling, M., A.V. Larsen, B. Andreassen, T. Sigholt, and R.L. Olsen. 2002. Influence of dietary shift on temporal changes in fat deposition and fatty acid composition of Atlantic salmon post-smolt during the early phase of seawater rearing. *Aquaculture Research* 33:875-889.
- Joensen, H., P. Steingrund, I. Fjallstein, and O. Grahl-Nielsen. 2000. Discrimination between two reared stocks of cod (*Gadus morhua*) from the Faroe Islands by chemometry of the fatty acid composition in the heart tissue. *Marine Biology* 136: 573-580.
- Kiessling, A., J. Pickova, L. Johansson, T. Åsgård, T. Storebakken, K-H. Kiessling. 2001. Changes in fatty acid composition in muscle and adipose tissue of farmed rainbow trout (*Oncorhynchus mykiss*) in relation to ration and age. *Food Chemistry* 73:271-284.
- McKenzie, D.J. 2001. Effects of dietary fatty acids on the respiratory and cardiovascular physiology of fish. *Comp. Bio. Physio. Pt A.* 128:607-627.
- McQuinn, I.H. 1997. Metapopulations and the Atlantic herring. *Reviews in Fish Biology and Fisheries* 7(3):297-329.
- O'Connell, M., M.C. Dillon, J.M. Wright, P. Bentzen, S. Merkouris, and J. Seeb. 1998. Genetic structuring among Alaskan Pacific herring populations identified using microsatellite variation. *Journal of Fish Biology* 53:150-163.
- Otis, E.O., and R. Heintz. 2003. Evaluation of two methods to discriminate Pacific herring (*Clupea pallasii*) stocks along the northern Gulf of Alaska. Exxon Valdez Oil Spill Restoration Project Draft Final Report (Restoration Project 02538), Alaska Department of Fish and Game, Division of Commercial Fisheries, Homer, Alaska. 48 pp.
- Overholtz, W.J. 2002. The Gulf of Maine-Georges Bank Atlantic herring (*Clupea harengus*): spatial pattern analysis of the collapse and recovery of a large marine fish complex. *Fisheries Research* 57:237-254.
- Peng, J., Y. Larondelle, D. Pham, R.G. Ackman, and X. Rollin. 2003. Polyunsaturated fatty acid profiles of whole body phospholipids and triacylglycerols in anadromous and landlocked Atlantic salmon (*Salmo salar* L.) fry. *Comparative Biochemistry and Physiology Part B* 134:335-348.
- Pickova, J., D.C. Paresh, P. Larsson, and A. Kiessling. 1997. Early embryonic cleavage pattern, hatching success, and egg-lipid fatty acid composition: comparison between two cod (*Gadus morhua*) stocks. *Can. J. Fish. Aquat. Sci.* 54:2410-2416.
- Rollin, X., J. Peng, D. Pham, R. Ackman, and Y. Larondelle. 2003. The effects of dietary lipid and strain difference on polyunsaturated fatty acid composition and conversion in anadromous and landlocked salmon. *Comparative Biochemistry and Physiology Part B* 134:349-366.
- Rowell, K. A. 1981. Separation of spawning stocks of Bering Sea herring based on scale growth patterns. In B.R. Melteff and V.G. Westpestad (ed.) *Proceedings Alaska herring symposium*. Alaska Sea Grant Report No. 80-4. Fairbanks, Alaska.

- Schweigert, J.F. 1990. Comparison of morphometric and meristic data against truss networks for describing Pacific herring stocks. In N.C. Parker, A.E. Giorgi, R.C. Heidinger, D.B. Jester, Jr., E.D. Prince, and G.A. Winans (eds.) Fish Marking Techniques, American Fisheries Society Symposium 7. 879 pp.
- Smith, P.J., and A. Jamieson. 1986. Stock discreteness in herrings: A conceptual revolution. Fisheries Research 4:223-234.
- Stenslokken K.O., L. Sundin, and G.E. Nilsson. Cardiovascular effects of prostaglandin F(2 alpha) and prostaglandin E(2) in Atlantic cod (*Gadus morhua*). J Comp Physiol [B] 172:363-369.
- Stephenson, R.L. 1999. Stock complexity in fisheries management: a perspective of emerging issues related to populations subunits. Fisheries Research 43:247-249.
- Tester, A.L. 1949. Populations of herring (*Clupea pallasii*) in the coastal waters of British Columbia. J. Fish. Res. Board Can. 7:403-420.
- Thomassen, M., and C. Røsjø. 1989. Different fats in feed for salmon: influence on sensory parameters, growth rate and fatty acids in muscle and heart. Aquaculture 79:129-135.
- Viga, A., and O. Grahl-Nielsen. 1990. Genotypic and phenotypic fatty acid composition in the tissues of salmon, *Salmo salar*. Comp. Biochem. Physiol. 96B: 721-727.
- Waples, R. S. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. Journal of Heredity 89:438-450.

RESUME

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Education:

Master of Science, Fisheries Science, University of Arizona, 1994.
Bachelor's of Science, Environmental Science, University of New Hampshire, 1988.

Professional Experience: *April 1996-present:* Area Finfish Research Biologist for Lower Cook Inlet, Alaska Department of Fish and Game- Comm. Fish., Homer, AK. Supervised by Jim Edmundson. Responsible for assessment and forecasting of Kamishak Bay herring stock; directs salmon and herring catch/escapement-sampling programs; forecasts Lower Cook Inlet salmon returns; develops new approaches to monitoring salmon escapement (e.g., remote video and time-lapse recording). Writes grants to secure outside funding for research projects, acts as principal investigator. *April 1994-March 1996:* Fishery Bio-technician, Kenai Fishery Resources Office, U.S. Fish and Wildlife Service, Kenai, AK. Supervised by Gary Sonnevil. Project leader for Andreafsky River (Yukon) adult salmon enumeration project: constructed and deployed resistance board/floating weir to count adult salmon; project leader for Kenai River rainbow trout radio-telemetry project: surgically implanted radio transmitters and tracked fish using mobile receivers and remote data loggers. *June 1991-March 1994:* Graduate Research Asst., Univ. of Arizona, Dept. of Renewable Natural Resources, Tucson, AZ. Supervised by Dr. O. Eugene Maughan. Designed and implemented field studies to assess the composition, abundance, and distribution of fishes in streams tributary to the Colorado River in Grand Canyon. Designed and implemented field study to inventory aquatic habitat available to stream fishes in Grand Canyon. *August 1987-June 1991 (intermittent):* Fishery Bio-technician, Kenai Fishery Resources Office, U.S. Fish and Wildlife Service, Kenai, AK. Supervised by Gary Sonnevil. Project Leader or team member on various field projects including: assessing adult salmon returns using weirs (Uganik R, Kodiak); developing new approaches to aging Dolly Varden and lake trout otoliths; enumerating emergent salmon fry (Tustumena Lake); investigating steelhead distribution and angler effort (Cold Bay); investigating run-timing and migration rates of chinook salmon (Kuskokwim River); and inventorying salmon spawning habitat (Ayakulik R., Kodiak).

Publications and Reports:

Otis, E.O., and R. Heintz. 2003. Evaluation of two methods to discriminate Pacific herring (*Clupea pallasii*) stocks along the northern Gulf of Alaska. Exxon Valdez Oil Spill Restoration Project *Draft* Final Report (Restoration Project 02538), Alaska Department of Fish and Game, Division of Commercial Fisheries, Homer, Alaska. 37 pp.

Otis Resume Cont'd

Publications and Reports (cont'd):

- Otis, E.O., and M. Spahn. 2003. Improving Access to ADF&G's Lower Cook Inlet Pacific Herring Stock Assessment and Commercial Fishery Databases, Including Observations of Steller Sea Lions. Final Report Submitted to: United States Department of Commerce, National Oceanic and Atmospheric Administration National Marine Fisheries Service (NOAA Award NA16FX1411).
- Otis, E.O., and M. Dickson. 2002. Improved salmon escapement enumeration using remote video and time-lapse recording technology. Exxon Valdez Oil Spill Restoration Project Final Report (Restoration Project 00366), Alaska Department of Fish and Game, Division of Commercial Fisheries, Homer, Alaska. 29 pp.
- Otis, E.O., W.R. Bechtol, and W.A. Bucher. 1998. Coping with a challenging stock assessment situation: the Kamishak Bay sac-roe herring fishery. Pages 557-573 In Fishery Stock Assessment Models: Proceedings of the International Symposium on Fishery Stock Assessment Models for the 21st Century, October 8-11, 1997, Anchorage, Alaska. Eds. F. Funk, T.J. Quinn, J. Heifetz, J.N. Ianelli, J.E. Powers, J.F. Schweigert, P.J. Sullivan, and C.-I. Zhang. University of Alaska Sea Grant College Program AK-SG-98-01.
- Weiss, S.J., E.O. Otis, and O.E. Maughan. 1998. Spawning ecology of flannelmouth sucker *Catostomus latipinnis* (Catostomidae) in two small tributaries of the lower Colorado River. Environmental Biology of Fishes 52:419-433.

Recent Project Collaborators (≤ 4 years)

William Bechtol, ADF&G-Commercial Fisheries, Homer
Wes Bucher, ADF&G-Commercial Fisheries, Homer (Retired)
Mark Dickson, ADF&G-Commercial Fisheries, Homer
Lee Hammarstrom, ADF&G-Commercial Fisheries, Homer
Ron Heintz, NMFS-Auke Bay Lab
Joe Meehan, ADF&G-Wildlife Conservation, Anchorage
Steve Moffitt, ADF&G-Commercial Fisheries, Cordova
Ken Severin, University of Alaska-Fairbanks
Margaret Spahn, ADF&G-Commercial Fisheries, Homer
Mark Witteveen, ADF&G-Commercial Fisheries, Kodiak

RESUME

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EMAIL: Ron.Heintz@NOAA.GOV

Education:

B.S. Ecology Ethology and Evolution, June 1979, University of Illinois, Urbana Illinois
M.S. Fisheries Biology, May 1987, University of Alaska, Juneau Alaska

Employment and Study Focus:

U.S. Department of Commerce, National Oceanic and Atmospheric Administration,
National Marine Fisheries Service, Auke Bay Laboratory since 1985.

Prior to 2000

Examined the effects of crude oil exposure during embryogenesis on the life history of fish.

Since 2000

Lead laboratory program investigating the nutritional status and trophic relationships of marine forage species.

Principle Findings:

Embryonic exposure to crude oil results in life long effects in pink salmon .

The components of oil that persist longest in the environment are those that are also the most toxic.

Fatty acids are better at discriminating herring stocks than elemental analysis of otoliths.

The benefits provided to juvenile salmon by decaying salmon carcasses include substantial increases in reserve energy.

Relevant Publications:

Barron, M. G., R. Heintz, M. M. Kran. 2003. *Sci. Tot. Env.* 311:111-133.

Heintz, R. A., J. W. Short, S. D. Rice. 1999. *Env. Tox. and Chem.* 18:3.

Heintz, R.A., S. D. Rice, A. C. Wertheimer, R. F. Bradshaw, F. P Thrower, J. E. Joyce and J. W. Short. (2000). *Mar. Ecol. Prog. Ser.* 208:205-218.

Heintz, R.A., B. D. Nelson, M. Larsen, L. Holland, M. Wipfli, and J. Hudson. In Press. *Trans. Am. Fish. Soc.* Accepted Oct. 12, 2003.

Heintz Resume Cont'd

Relevant Publications (cont'd):

Marty, G. D., J. W. Short, D. M. Dambach, N. H. Willits, R. A. Heintz, S. D. Rice, J. J. Stegeman, and D. E. Hinton. 1997. *Can. J. Zoology*. 75:989_1007.

Murphy, M. L., R. A. Heintz, J. W. Short, M. L. Larsen, and S. D. Rice. 1999. *Trans. Am Fish Soc.* 128:909-918.

Otis, E.O., and R. Heintz. 2003. Exxon Valdez Oil Spill Restoration Project . Draft Final Report (Restoration Project 02538), Alaska Department of Fish and Game, Division of Commercial Fisheries, Homer, Alaska. 48 pp.

Short J. W and R. A. Heintz. 1997. *Environmental Science Technology*. 31:2375-2384.

Recent Project Collaborators (≤ 4 years)

Dr. Mace Barron	US EPA GED
Mark Carls	NOAA Fisheries AFSC
Dr. Scott Gende	National Park Service
Dr. Margaret Krahn	NOAA Fisheries NWFSC
Dr. Gary Marty	University of California Davis
Julie Meka	US Geological Survey ASC
Dr. Frank Morado	NOAA Fisheries AFSC
James Murphy	NOAA Fisheries AFSC
Ted Otis	ADFG
Dr. Ken Severin	University of Alaska Fairbanks
Jeff Short	NOAA Fisheries AFSC
Dr. Mike Sigler	NOAA Fisheries AFSC
Dr. Mike Stekoll	University of Alaska Southeast
Frank Thrower	NOAA Fisheries AFSC
Dr. Mark Wipfli	University of Alaska Fairbanks
Jamie Womble	University of Alaska Fairbanks

Budget Justification

Otis/Heintz: Temporal stability of fatty acids used to discriminate Pacific herring in Alaska

Note: Non-agency funding is required for this project because ADF&G does not have the lab facilities/expertise to conduct this type of chemical analysis, nor does ADF&G have in-house funding to develop new technologies at the present time.

Fiscal Year 2005

Personnel: \$36.8 K

Otis will organize logistics for sample collections, attend meetings, write annual report.
Cope will assist in collecting field samples and inputting/summarizing field data
Heintz will manage sample inventory, attend meetings and conduct statistical analysis.
Schaufler will conduct the fatty acid analysis.

Travel: \$1.6 K

Otis and Heintz will attend the Annual GEM meeting.

Contractual: \$14.5 K

\$3,000: for freight hauling charges to ship heavy liquid nitrogen containers (hazmat) to and from remote sampling locations.

\$3,500: Contracting for Public Outreach of project results (e.g., Alaska Marine Conservation Council, Web site design/construction, etc.)

\$8,000: ABL will contract out sample preparation (sample homogenization, lipid extraction, and esterification).

In-Kind Match: ADF&G providing ocean going research vessels and skiffs to provide sampling platforms (\$58,500), and air charters to transport samples (\$7,800).

Commodities: \$7.3 K

\$3,500: Expendible sampling equipment (e.g., liquid nitrogen, shipping containers, cryovials, etc.) and office supplies (e.g., waterproof paper, labels, photocopying, etc.)

\$3,750: Items expended during chemical processing (e.g., gases, solvents, reagents).

Equipment: \$0

No new equipment needed; desktop/laptop computers provided by NMFS-ABL and ADF&G.

In-Kind Match: NMFS-ABL providing Accelerated Solvent Extractor (\$51.3 K), Varian GC/MS (\$72.5 K), LN containers (\$2,400 K).

Fiscal Year 2006

Personnel: \$54.5 K

Otis will organize logistics for sample collections, attend meetings, co-author annual report.
Heintz will attend meetings, conduct statistical analysis, and co-author annual report.
Schaufler will conduct the fatty acid analysis.
Cope will assist in collecting field samples and inputting/summarizing field data.

Fiscal Year 2006 cont'd

Travel: \$1.6 K

Otis and Heintz will attend the Annual GEM meeting.

Contractual: \$17.5 K

\$3,000: for freight hauling charges to ship heavy liquid nitrogen containers (hazmat) to and from remote sampling locations.

\$2,500: Contracting for Public Outreach of project results (e.g., Alaska Marine Conservation Council, Web site maintenance, etc.)

\$12,000: ABL will contract sample preparation (sample homogenization, lipid extraction, and esterification).

In-Kind Match: ADF&G providing ocean going research vessels and skiffs to provide sampling platforms (\$58,500), and air charters to transport samples (\$7,800).

Commodities: \$8.4K

\$3,500: Expendible sampling equipment (e.g., liquid nitrogen, shipping containers, cryovials, etc.) and office supplies (e.g., waterproof paper, labels, photocopying, etc.)

\$4,875: Items expended during chemical processing (e.g., gases, solvents, reagents).

Equipment: \$0

No new equipment needed; desktop/laptop computers provided by NMFS-ABL and ADF&G.

In-Kind Match: NMFS-ABL providing Accelerated Solvent Extractor (\$51.3 K), Varian GC/MS (\$72.5 K), LN containers (\$2,400 K).

Fiscal Year 2007

Personnel: \$17.7 K

Otis will attend meetings, co-author final report and manuscript for publication.

Heintz will attend meetings, conduct statistical analysis, co-author final report and manuscript for publication.

Travel: \$ 2.3 K

Otis and Heintz will attend the Annual GEM meeting; Otis or Heintz will present project results at the Alaska Chapter Meeting of the American Fisheries Society.

Contractual: \$2.5 K

\$2,500: Contracting for Public Outreach of project results (e.g., Alaska Marine Conservation Council, Web site maintenance, etc.)

Commodities: \$0.5 K

Misc. office supplies

Equipment: \$0.0

No new equipment needed; desktop/laptop computers provided by NMFS-ABL and ADF&G.

Data Management and Quality Assurance/Quality Control Statement

NOAA Sec. 515 - Data Quality Act Guidelines will be followed. These guidelines can be found at <http://www.noaanews.noaa.gov/stories/iq.htm>

1. **Study design.** The study design is a stratified random sample. The sampling strata include stocks and times. Sample sizes are based on the results of previous studies.
2. **Acceptable data.** Acceptable chemistry data will need to conform to standard QA criteria employed by our laboratory.
3. **Data characteristics.**
 - a. Fish data include lengths, weights, ages and sex of herring, which can be mapped onto ADF&G stock assessments based on spawning surveys.
 - b. In addition, we will provide data on the fatty acid composition of the lipids extracted from the hearts of selected individuals. Fatty acids to be measured are listed in Table1.
4. **Algorithms to convert signals from sensors to observations.** Concentrations of specific fatty acids will be determined by gas chromatography and mass spectrometry. A Saturn model 2200 mass spectrometer operating in simulated ion mode will be used to identify specific peaks separated by a Varian Model CP3800 gas chromatograph equipped with a 100 m Chrompak Select fused silica capillary column for FAMES. Peak heights will be converted to concentrations using a 5-point calibration curve and an internal standard (C19:0) added immediately before esterification.
5. **Sample handling and custody.** Biological information for each sample will be entered onto a custody sheet. The custody sheet has columns for sample identification number (SIN), fish length, weight, age, sex, date of processing, processor's sample identification number, the processor's name, and a column for any comments that might be important in interpretation. Examples of commentary would be any noticeable evidence of disease or parasites. Processors will be issued custody sheets, which will be shipped to ABL with the samples. The sample numbers will be assigned in the field and correlated with the processor's sample identification number. Sample identification numbers on custody sheets will be used to track the progress of samples through the analytical process and to correlate those results with the initially collected biological information. Fatty acid and biological data will be maintained in ABL's fatty acid database, after completion of the report, a copy of the data will be issued to ADF&G.
6. **Calibration and performance evaluation of analytical instrumentation.** For gravimetric determination of lipid content, a duplicate sample will be analyzed per group of 15-20 fish along with a standard reference sample (SRM) and blank. A second set of samples will be re-analyzed if the coefficient of variation for the duplicates was greater than 25% or if the reference sample value not within 15% of the established value or if detectable lipid is found in the blank. A whole herring homogenate is used as the SRM. The lipid content of the herring homogenate was initially determined in a group of samples that included the National Institute of Standards and Technology (NIST) SRM-1946, which has a certified value for lipid. The NIST SRM-1946 is processed monthly, along with the herring SRM to ensure consistency. The same group of samples will be analyzed to determine their fatty acid composition. Fatty acid concentrations for specific samples will be discarded if the observed value of the SRM is not within pre-specified tolerances. Similarly, samples will be reanalyzed if the recovery of an internal standard (C23:0)

applied at the time of lipid extraction or an internal standard (C21:0) added immediately before injection in the GC falls outside a prescribed range.

7. **Data reduction and reporting.** All data will be tabulated by stock and time. Stocks will be compared using MANOVA, to test for differences within stocks with respect to time and differences among stocks regardless of time. See the proposal for details. Differences will be further evaluated by discriminate function analysis. The statistical software will be SAS.

Table 1. Fatty Acids to be Quantified

14:0
 14:1 (n-5)
 15:0
 15:1(n-5)
 16:0
 16:1(n-9)
 16:1(n-7)
 16:1(n-5)
 17:0
 17:1(n-7)
 18:0
 18:1(n-11)
 18:1(n-9)c&t
 18:1(n-7)
 18:2(n-6)c
 18:2(n-6)t
 18:3(n-6)
 18:3(n-3)
 18:4(n-3)
 20:0
 20:1(n-11)
 20:1(n-9)
 20:1(n-7)
 20:2(n-9)
 20:2(n-6)
 20:3(n-6)
 20:4(n-6)
 20:3(n-3)
 20:4(n-3)
 20:5(n-3)
 22:0
 22:1(n-11)
 22:1(n-9)
 22:2(n-6)
 22:5(n-3)
 22:4(n-6)
 22:5(n-6)
 24:0
 22:6(n-3)
 24:1(n-9)
