EXXON VALDEZ Oil Spill Trustee Council

FY 01 Detailed Project Description

Evaluation of two methods to discriminate Pacific herring (Clupea pallasi) stocks along the northern Gulf of Alaska

Project Number:	01538
Restoration Category:	Research
Proposer:	Ted Otis (ADF&G) Ron Heintz (NMFS-Auke Bay)
Lead Agency:	ADF&G
Cooperating Agencies:	ADF&G, NMFS-Auke Bay
Alaska SeaLife Center:	No
Duration:	Closeout in FY02
Cost FY 2001:	$ 10.1K
Cost FY 2002:	$ 47.1K
Cost FY 2003:	$ 0.0K
Cost FY 2004:	$ 0.0K
Geographic Area:	PWS, Kodiak, Lower Cook Inlet
Injured Resource/Service:	Pacific herring/commercial fishing

ABSTRACT

Pacific herring within the spill area, and particularly within Prince William Sound, were injured by the 1989 *Exxon Valdez* oil spill and have not yet fully recovered. Because herring are important prey for many marine species, as well as humans, their stock health is relevant to the recovery of other injured resources and services. To increase our understanding of the distribution and mixing of Northwest Gulf of Alaska (NWGA) herring stocks and to help identify important habitats and rearing areas for individual populations, it is relevant to be able to determine the stock of origin for herring sampled during field investigations. This 1-year pilot study will perform a comparative investigation of two promising stock identification techniques (elemental analysis of otoliths and fatty acid profile analysis of select soft tissues). Limited samples from Sitka Sound, PWS, Kamishak Bay, Kodiak Island, and Togiak will be collected and analyzed to determine if stock differences are detectable by each procedure, and at what scale. Successful results from this pilot study should be followed up with future evaluations of the temporal and structural (i.e., sex, age, maturity) stability of these biomarkers.

INTRODUCTION

Pacific herring *Clupea pallasi* within the spill area, and particularly within Prince William Sound (PWS), were injured by the 1989 *Exxon Valdez* oil spill (Brown 1995) and have not yet fully recovered (EVOS Restoration Plan 1998). Elevated levels of physical and genetic abnormalities in newly hatched larvae and reduced hatching success of embryos were documented in 1989 (Brown 1995). Significant histopathological damage was measured in adults collected in oiled areas in both 1989 and 1990 confirming exposure of the fish to toxins (Brown 1995). In 1993, the herring population in PWS collapsed. The total observed spawning population was less than one third of preseason predictions and the average sizes of herring in each age class were some of the smallest on record. The total commercial harvest for that year was also one of the lowest on record. In 1994, the total observed spawning population was below the threshold biomass required to conduct a commercial harvest and no fishing occurred. Pathology studies indicated that viral hemorrhagic septicemia (VHS) and the fungus *Ichthyophonus hoferi* probably contributed most to the population decline (Meyers 1994, Marty et al. 1996, 1998). After rebounding from the 1993 decline, the PWS herring population collapsed again in the winter of 1998-99. Viral hemorrhagic septicemia and *Ichthyophonus hoferi* were found in many herring sampled in 1998 and 1999, respectively, and appear to have once again contributed to their decline (pers. comm. Steve Moffitt, PWS Area Research Project Leader, ADF&G-Cordova).

Herring are an important component of the marine ecosystem providing a trophic pathway for energy flowing from secondary producers to apex predators. Throughout their life, herring are prey to birds (Logerwell and Hargreaves 1997), marine mammals (Iverson et al. 1997), invertebrates (e.g. hydromedusae: Wespestad and Moksness 1989), other fish (Tanasichuk et al. 1991), and humans (Fischer et al. 1997). Understanding the role herring occupy in the food web of marine ecosystems is relevant to sustaining viable populations of herring and the species that prey on them (Schweigert 1997). The ability to define the stock of origin for herring sampled during ecosystem level investigations (e.g., Gulf Ecosystem Monitoring [GEM]) would dramatically improve our understanding of the distribution and ecology of this organism. Researchers would be better able to evaluate cause and effect relationships associated with the population dynamics of NWGA herring stocks and thereby improve the management and recovery of herring, as well as other marine species that feed on them.

Many diverse techniques have been investigated to facilitate discriminating between fish populations including: nuclear and mtDNA analysis (Seeb 1995), enzyme electrophoresis (Schweigert and Withler 1990), parasite markers (Moles et al. 1990), scale pattern (Rowell 1981, Ross and Packard 1990, Barros and Holst 1995), mass marking of otoliths using temperature manipulation (Joyce et al. 1996) and fluorescent markers (Beckman et al 1990), and meristic and morphometric characteristics (Schweigert 1990). While many of these techniques have proven successful for certain applications, each has its own set of limitations that may reduce its effectiveness for specific stock identification situations. For instance, DNA analysis and enzyme electrophoresis are often able to discriminate stocks on a broad geographic scale, however, these techniques can falter when even a small amount of genetic drift occurs between closely distributed populations.

We propose to conduct a pilot study of two promising techniques for herring stock identification. Herring from Prince William Sound, Kamishak Bay, Kodiak Island, Togiak, and Sitka Sound will be collected. Otoliths and heart tissue will be extracted from each specimen to facilitate

elemental analysis (EA) and fatty acid analysis (FAA), respectively. To minimize sample sizes (i.e., costs) for this pilot study, we propose to focus our investigation on age-4 and age-5, pre-spawning female herring. If these procedures prove capable of identifying significant differences between similar cohorts from different stocks, further investigation would be warranted to evaluate the temporal, spatial, and structural (i.e., sex, age, gonad maturity) variability associated with each stock's unique biomarkers. Our principal objective is to determine which of these two stock identification tools is most robust.

NEED FOR THE PROJECT

A. Statement of Problem

Herring populations in PWS and Kamishak Bay are depressed. To better understand factors affecting the dynamics of these populations, and therefore effect their recoveries through potential improvements in management, we propose to evaluate two tools that may facilitate determining the scale at which discrete stocks exist within PWS and the greater NWGA. Herring researchers have long pondered the degree to which herring return to natal areas to spawn and the scale at which population structure exists within large geographic areas (Hourston 1982, Wheeler and Winters 1984, Hay and McCarter 1997, McQuinn 1997). Answers to these fundamental questions are directly relevant to the manner in which herring are assessed and managed. One of the underlying principles of sustainable fisheries management is the ability to monitor the dynamics (environmental, biological, and human induced) of individual populations (Mundy 1996). The inability to accurately apportion the catch from mixed stock fisheries, for example, is a common problem that undermines fishery managers' abilities to manage populations discretely.

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This pilot study proposes to evaluate the potential for elemental analysis (EA), and fatty acid analysis (FAA) to discriminate NWGA herring stocks residing within and between PWS, Kodiak Island, and Kamishak Bay. Our principal objective will be to determine which of these two stock identification tools is most robust. The stock identification technique developed through this project could eventually be applied to identify the stock of origin for juvenile and adult herring collections made during long term monitoring (e.g., GEM) and also to apportion mixed stock harvests during commercial fisheries (e.g., Shelikof Strait food/bait fishery). Finding discernable differences between Kodiak and Kamishak herring is of particular interest to managers of these respective stocks (Otis and Bechtol 1997, Otis et al. 1998). Herring harvested from northwest

Kodiak Island (e.g., Shuyak, Afognak, and Raspberry Is.) during the Shelikof Strait fall food/bait fishery are presumed to include both Kodiak and Kamishak stocks (Johnson et al. 1987). The Department's Kamishak Bay District Herring Management Plan addresses this presumed mixed-stock fishery through allocation of the Kamishak Bay harvestable surplus (5 AAC 27.465). The success of this pilot project may result in the ability of managers to more accurately allocate the harvest of herring taken during the Shelikof fall food/bait fishery.

Fatty acid compositions of fish lipids have been investigated for decades (Ackman et al. 1963). Much of the early lipid research was directed at determining the commercial value of fish oils (e.g. Ackman 1966) and understanding how fat content relates to various life history functions (e.g. Rajasilta 1992). Because the composition of certain lipids can be closely related to the types of food recently ingested (Navarro et al. 1995, Kirsch et al. 1998), recent investigations have been directed at diet analysis and foraging distribution (e.g. Iverson et al 1997). The composition of phospholipid fatty acids prominent in some body tissues (e.g., heart tissue, gills, eggs) have been shown to have a more stable genetic or environmental basis that makes analysis of these tissues appropriate for stock identification studies. As early as the 1930's it was demonstrated that different stocks of fin whale *Balaenoptera physalus* could be distinguished by the degree of unsaturation of their oils (measured as iodine value: Lund 1934, as cited in Grahl-Nielsen et al. 1993). Recently, fatty acid analysis of eggs has been used to discriminate between American lobster *Homarus americanus* populations (Castell et al. 1995), Baltic cod *Gadus morhua* stocks (Pickova et al. 1997), and even the wild/domestic origin of sturgeon ova (Czesny et al. 2000).

Chemometry of fatty acids from heart tissue has been used to discriminate stocks of striped bass *Morone saxatilis* (Grahl-Nielsen and Mjaavatten 1992), Atlantic herring *Clupea harengus harengus* (Grahl-Nielsen and Ulvund 1990), and Atlantic cod *Gadus morhua* (Joensen et. al. 2000). This technique has also been used to distinguish between closely related species of the genus *Sebastes* from the Faroe Islands (Joensen and Grahl-Nielsen 2000). It is often the fatty acid profile (i.e., unique composition of an array of fatty acid levels; also referred to as a 'signature' by Iverson et al. 1997 and Smith et al. 1998) that distinguishes individual stocks, and not a single distinct fatty acid. Considerable variability can naturally occur in the fatty acid profiles (especially lipid profiles) between individual fish (Viga and Grahl-Nielsen 1990). This variability can be influenced by changes in diet, water temperature, salinity, growth, reproductive cycle, and pollution (Viga and Grahl-Nielsen 1990). The fatty acid profiles of certain tissues (e.g., heart) and specific lipids (e.g., phospholipids) are considered more stable, but still exhibit some variability. Recently published research found significant differences in the fatty acid profiles of heart tissue extracted from representatives of 2 cod stocks that had been reared under identical conditions since hatching (Joensen et. al. 2000). This key study demonstrates the potential for fatty acid compositions to discriminate fish stocks, even when they may occupy similar environments during later stages of their life histories (e.g., Kamishak Bay and Northwest Kodiak stocks).

Trace elemental analysis of otoliths has been used to identify stocks of pink snapper, (Edmonds et al. 1989), orange roughy (Edmonds et al. 1991), yellow-eye mullet (Edmonds et al. 1992), Atlantic cod (Campana and Gagne 1995, Campana et al. 1995), and salmonids (Kalish 1990). Thresher (1999) provides a comprehensive review of the use of otolith elemental composition as stock discriminators and offers some cautionary suggestions for researchers interested in employing this promising technique. Of particular concern is the potential for non-standardized

lab equipment and procedures to contribute to differences in otolith elemental composition reported among published studies (Campana et al. 1997).

Otoliths are acellular, so once accreted, the material is not resorbed or reworked (Campana and Nielson 1985). As a result, otolith microchemistry can be used to identify the environments inhabited by fish during their life (Gunn et al. 1992, Radtke and Shafer 1992, Secor et al. 1992). The use of otoliths as records of environmental exposure is based on the premise that otolith microchemistry reflects differences in water chemistry in the environment (Radtke and Shafer 1992, Campana and Gagne 1995). The trace elemental composition of fish otoliths is determined by the elemental composition of the endolymph (Kalish 1989, 1991). The concentration of various trace elements in the environment and the physiology of the fish largely determine the composition of the endolymph. Physiological processes may be modified by temperature (Kalish 1991), or subtle differences in the genetics of the fish affecting the uptake of various elements and their inclusion in the endolymph (Thresher et al. 1994). Controlled laboratory studies have shown that otolith microchemistry is strongly affected by temperature, salinity and ontogeny (Fowler et al. 1995a, 1995b).

Successful application of trace otolith elemental analysis for stock discrimination is likely dependent on the extent of the differences in water chemistry between the environments inhabited by each stock. But, the need to identify stocks often arises when they are exploited in mixed-stock fisheries in the same environment. Three methods are commonly employed for otolith elemental analysis. Solution-based inductively coupled plasma mass spectrometry (ICPMS) is typically used to measure elemental concentrations in whole otolith samples or portions of whole otoliths (Date 1991). Laser-ablation ICPMS is a technique that can be used to analyze trace elements (ppm) at specific loci (30 μm) on the otolith (Gray 1985, Denoyer et al. 1991). Electron microprobes (EM) also allow analysis of specific loci (5-7 μm), albeit at a reduced resolution in the parts per thousand (ppt) range (pers. comm. K. Severin, UAF Dept. of Geology and Geophysics). Solution-based ICPMS may successfully discriminate stocks that inhabit different environments exhibiting different water chemistries during the majority of their life history (Campana et al. 1995). However, techniques that target specific loci, such as EM and LA-ICPMS, may be more appropriate for identifying stocks that spawn in different environments but later reside in similar environments (Coutant and Chen 1993, pers. comm. K. Severin). In this case, the microchemistry of the otolith accreted during the embryo or larval stage may indicate differences between stocks. It is unknown to what extent herring spawning around Kodiak Island, in Kamishak Bay or PWS may inhabit similar environments throughout their life history. Therefore, the proposed project will examine the efficacy of either EA or LA-ICPMS of the primordium of the otolith for discriminating between herring stocks.

B. Rationale/Link to Restoration (Why should work be done)

Pacific herring is a key species in the marine ecosystem affected by the 1989 Exxon Valdez oil spill. Herring is also a primary forage species for other marine fishes, birds and mammals, and is used extensively by subsistence and commercial fishers.

C. Location

Herring will be collected from Sitka Sound, PWS (Montague and NE PWS), Lower Cook Inlet (Kamishak Bay), Kodiak Island (west side), and Bristol Bay (Togiak) waters.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

Lab Study/Not Applicable

PROJECT DESIGN

A. Objectives

To accommodate funding limitations, we propose to restrict our FY01 activities to sample collection and preservation. The bulk of our lab analyses will take place in FY02.

FY01:

1. Collect herring samples from Sitka, PWS, Kodiak, Kamishak, and Togiak; extract lipids for fatty acid analysis to be performed in FY02.

FY02:

1. Determine whether EA or FAA will allow discrimination among Alaska's 3 major herring stocks, and if so;

2. Determine whether EA or FAA can detect finer scale structuring of putative herring stocks inside PWS and elsewhere in the NWGA.

B. Methods

This pilot study has one main objective broken down into two parts: to determine if EA or FAA can distinguish between Pacific herring stocks, and if so, on what scale. To accomplish the first part of this objective we plan to compare the biomarker profiles of herring collected from Alaska's three major stocks- PWS, Sitka Sound and Togiak. Togiak and PWS have already been shown to have disparate genetic profiles (O'Connel et. al. 1998), so they make ideal initial test groups. If EA and FAA are not able to distinguish between these stocks, then they probably have little value as stock discrimination tools for Pacific herring. However, if PWS, Sitka and Togiak samples are distinguishable, we will process our remaining samples to investigate smaller scale population structuring within the NWGA, and PWS specifically.

To minimize the inherent natural variability that may reside within each population, only age-4 and age-5 prespawning female herring will be collected. This will also allow us to minimize our sample sizes (i.e., cost) for this pilot study while still retaining the ability to look for variability in chemical markers across adjacent year classes. Herring will be collected from the north end of Montague Island (e.g., Zaikof Bay) and the northeast corner of PWS (e.g., Galena Bay), locations

believed to represent the focal spawning areas for two putative PWS herring stocks (Pers. Comm., Evelyn Brown, UAF, IMS). We will also collect herring from NWGA spawning aggregations centered on the west side of Kodiak Island (e.g., Uganik Bay) and in Lower Cook Inlet (Kamishak Bay). Processing samples from these collection sites will allow us to resolve the scale at which EA and FAA techniques are able to discriminate between herring stocks in the NWGA.

Collections will be made where significant numbers of herring spawn in areas judged to be the focal spawning area for each respective stock and will target the first groups of returning fish (Table 1). For each specimen, length, wet weight, sex, and gonad maturity will be determined. When pre-spawning female herring between 190-250 mm SL are encountered, a scale will be removed to determine the age of the fish. If determined to be age-4 or age-5, their heads will be removed, individually labeled, and stored frozen in plastic bags for later laboratory processing of the otoliths. Whole hearts will be removed, transferred to labeled vials, placed in liquid nitrogen, and stored at –70° C until analyzed (Ackman et al. 1969, Grahl-Nielsen and Mjaavatten 1992). The remaining body of the fish will be labeled, bagged, and frozen whole for possible whole body fatty acid analysis. EA and FAA will only be conducted on specimens from the same two adjacent age classes from each area (e.g. age 4 and 5). This approach will control for biomarker variability that may occur across cohorts. To reduce project costs, only 30 samples from each area will be processed (180 total samples). However, 50 additional fish of similar age/sex will be collected in case the sample variance dictates more individuals are needed to facilitate robust statistical comparisons (Johnson and Wichern 1992).

Table 1: Dates, locations, and sample sizes for FY01 collections to evaluate the feasibility of EA and FAA to discriminate between northern Gulf of Alaska herring stocks.

		Sample sizes	
Location	Date	EA	FAA
Sitka Sound	3/10-4/10	30	30
PWS, N. Montague	4/10-4/20	30	30
PWS, NE (e.g., Galena Bay)	4/10-4/30	30	30
LCI, Chenik/Amakdedori	4/20-5/5	30	30
KDK, Paramanof/Foul Bay	4/15-4/30	30	30
Togiak	5/1-5/20	30	30
Totals Samples		180	180

Direct methanolysis of the thawed heart tissue and gas chromatography of the resulting fatty acid methyl esters will follow procedures described by Viga and Grahl-Nielsen (1990) and Grahl-Nielsen and Mjaavatten (1992). Representative peaks (i.e. fatty acid levels) from the resulting chromatograms will be selected and quantified. Multivariate techniques such as principal components analysis (PCA), soft independent modeling of class analogy (SIMCA), linear discriminant analysis (LDA), and classification and regression trees (CART) have typically been used to compare fatty acid compositions (Grahl-Nielsen and Mjaavatten 1992, Navarro et al 1995, Castell et al. 1995, Smith et al. 1997). However, there remains some debate over which

multivariate techniques are most robust for this application (Grahl-Nielsen 1999, Smith et al. 1999).

Otoliths will be removed from heads and processed as described by Fowler et al. (1995a). Left and right sagittal otoliths will be dissected from each specimen using glass probes on a glass surface, insuring that the otolith and dissection equipment do not touch metal. Tissue adhering to the otoliths will be removed with glass probes and the sample washed in Super Q water. Otoliths will be air dried in a positive flow flume hood and weighed to the nearest 0.01 mg. Those used for laser-ablation ICPMS will be mounted on glass slides using thermal plastic cement, then ground and polished in the sagittal plane until the otolith primordium is visible. Polished otoliths will be rinsed in super Q water (deionized, purified through reverse osmosis, and millipore filtered) and stored in paper envelopes for later analysis (Fowler et al. 1995b). Methods described by Fowler et al. (1995a) and Fowler et al. (1995b) will be used for the laser-ablation ICPMS analyses of the primordium of each otolith.

Discriminant function analysis (DFA) and principle component analysis (PCA) will be applied to the calibration samples collected from all areas to determine which analytical technique (EA or FAA) is the best stock discriminator (Johnson and Wichern 1992). Each technique produces a biomarker signature (trace elements or fatty acid profiles) that will be evaluated for the level of discrimination (e.g. number of stocks identified) and the accuracy of discrimination. Each multivariate statistical technique will first be applied to the data sets derived from each analytical technique (EA or FAA), separately. The misclassification probabilities associated with each technique will be compared to evaluate the accuracy of each method. DFA and PCA could then be conducted on the data set derived from all analytical techniques combined. This approach would enable us to determine whether a combination of variables from the two analytical techniques provides greater classification accuracy than any of the individual techniques.

A stepwise discriminant analysis will first be applied to the variables derived from each analytical technique to identify any biomarker signatures associated with herring stocks or age classes. All variables found to be poor discriminators will be discarded. DFA will then be applied to the reduced set of variables. DFA produces a probability density function (pdf) for each group identified. If DFA can not discriminate between stocks it will combine all stocks into one pdf. The number of unique stocks identified will indicate the level of discrimination achieved. If DFA can discriminate between stocks, misclassification probabilities (accuracies) will be determined by the number of specimens that incorrectly fall outside the pdf for their respective stock in the calibration sample. Groups may be pooled if misclassification is high. This will reduce detail but increase overall accuracy.

PCA will be used to express the biomarker signatures as a set of principal component variables. Skree plots will be used to determine how many principal components are needed to accurately describe the variation in the biomarker signatures. To reveal relationships that exist within the signatures a varimax rotation of the principle components will be completed and the components will be graphed against each other. If PCA appears to distinguish among stocks, additional PCAs will be conducted for each individual stock and perhaps age-classes within stocks. Cross-validation analysis will be used to determine the number of principal components that best describe the data (Wold 1978). Varimax rotation plots will be used to evaluate misclassification (accuracies).

C. Cooperating Agencies, Contracts, and other Agency Assistance

This project is jointly proposed by the Alaska Department of Fish and Game (ADF&G) and the National Marine Fisheries Service (NMFS), Auke Bay Lab. ADF&G will collect all the necessary samples and Auke Bay Lab will perform the fatty acid analysis of soft tissues. If this project is funded, the department will draft specifications for EA and solicit bids from at least three qualified vendors. This process will follow standard State of Alaska bidding and contract award procedures. The successful bidder will be offered a co-authorship option if publishable findings result from the analyses.

SCHEDULE

A. Measurable Project Tasks for FY01

Feb-Mar:	Contract laboratory for elemental analysis of otoliths.
Apr-May:	Collect otolith and heart samples from spring spawning herring from Sitka Sound, PWS, Kodiak, Kamishak, and Togiak.
Jun-Sep:	Extract lipids from soft tissue, store samples until they can be processed in FY02.

B. Measurable Project Tasks for FY02

Oct-Jan:	Perform fatty acid and elemental analyses of soft tissue and otoliths, respectively.
Feb-Mar:	Analyze results, write project final report.
April:	Submit project final report

C. Project Milestones and Endpoints

Sep 2001	Complete FY01 objective 1
Jan 2002	Complete FY02 objectives 1 and 2
April 2002	Complete project final report.

C. Completion Date

This project will be completed in FY02. A final report will be submitted by April 15, 2002.

PUBLICATIONS AND REPORTS

A project final report will be submitted by April 15, 2002. Selected results from the project may be published in referred journals in FY02 or 03 as appropriate.

PROFESSIONAL CONFERENCES

Travel funds have been requested to present selected project results at one professional conference in FY02, as appropriate (e.g. Lowell Wakefield Symposium).

NORMAL AGENCY MANAGEMENT

The ability to distinguish between and manage stocks discretely is a principal component of sustainable fisheries management. However, this principle cannot be implemented effectively in many cases due to inherent difficulties in distinguishing discrete stocks using methods commonly available to fishery managers. New advances in fisheries stock identification are necessary to fill these gaps. The techniques we propose to evaluate may have broad application towards better understanding the structuring of many marine mammal and fish populations, including those not managed by the proposing agencies. Successfully applying these techniques as stock discriminators could also illuminate pathways for more effective long term monitoring through GEM.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

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 pathways for long term monitoring under GEM.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

This is a new project.

PRINCIPAL INVESTIGATORS

Edward O. Otis
Alaska Dept. Fish and Game
Division of Commercial Fisheries
P.O. Box 1402 Homer, AK 99603
Phone 907-235-1723
FAX 907-235-2448
e-mail: ted_otis@fishgame.state.ak.us

Education: Master of Science, Fisheries Science, University of Arizona, 1994. Bachelor of Science, Environmental Science, University of New Hampshire, 1988.

Professional Experience: April 1996-present: Asst. Area Research Biologist for Lower Cook Inlet, Alaska Department of Fish and Game, DCF, Homer, AK. Supervised by William R. Bechtol. Responsible for assessment and forecasting of Kamishak Bay herring stock, direct

salmon and herring catch and escapement sampling programs, forecast Lower Cook Inlet salmon returns. April 1994-March 1996: Fishery 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): Fisheries 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); evaluating angler effort (Kenai River); investigating run-timing and migration rates of chinook salmon (Kuskokwim River); and inventorying salmon spawning habitat (Ayakulik R., Kodiak).

Selected Publications:

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.

Otis, E.O. and W.R. Bechtol. 1997. Forecast of the Kamishak herring stock in 1997. Alaska Dept. of Fish and Game, Regional Information Report No. 2A97-03.

Otis, E.O. 1997. Lower Cook Inlet pink salmon forecast for 1997. Alaska Department of Fish and Game Regional Information Report No. 2A97-09.

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, ed. F. Funk, T.J. Quinn II, J. Heifetz, J.N. Ianelli, J.E. Powers, J.F. Schweigert, P.J. Sullivan, and C.-I. Zhang, Alaska Sea Grant College Program Report No. AK-SG-98-01, University of Alaska Fairbanks.

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Ron Heintz
National Marine Fisheries Service

Auke Bay Laboratory
11305 Glacier Hwy.
Juneau, AK 99801
Phone: 907-789-6058
FAX : 907-789-6094

email: Ron.heintz@noaa.gov

Education:
Currently enrolled as PhD candidate at University of Alaska
Master of Science, Fisheries Biology, University of Alaska, Fairbanks. 1987.
Bachelor of Science, Ecology Ethology and Evolution, University of Illinois, Champaign. 1979.

Principle Findings involving chemometric techniques:

1. Oil weathers by first-order loss-rate kinetics (Short and Heintz 1997).
2. Most toxic PAHs spilled by *Exxon Valdez* persisted in spawning habitats for at least six years after the spill (Murphy et al. 1999).
3. Marine derived fatty acids provided by returning salmon are an important source of nutrition to fish residing in the natal streams (Wipfli et al. in press).

Current Research:

1. Evaluation of the potential use of fatty acid and lipid class analysis for discriminating diet and diet quality in marine species.
2. Use of lipid class and fatty acid analysis for discriminating populations of northern fur seals.
3. Characterization of the quality of salmon rearing habitats by evaluation of the lipid class and fatty acid composition of overwintering parr.

Cited Publications

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

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

Wipfli, M., , J. P. Hudson, J. P. Caouette, R. A. Heintz, D. T. Chaloner, M. L. Larsen, and L. G. Holland. (In Press). Marine subsidies in freshwater: salmon carcasses increase the body fitness of stream salmonids. Ecology.

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Ackman, R.G., and C.A. Eaton. 1966. Some commercial Atlantic herring oils; fatty acid composition. Journal of the Fisheries Research Board of Canada 23:991-1006.

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2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Budget Category:	Authorized FY 2000	Proposed FY 2001	PROPOSED FY 2001 TRUSTEE AGENCIES TOTALS					
			ADEC	ADF&G	ADNR	USFS	DOI	
				$4.0				
Personnel	$0.0	$5.3						
Travel	$0.0	$0.0						
Contractual	$0.0	$0.0						
Commodities	$0.0	$4.0						
Equipment	$0.0	$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$0.0	$9.3				Estimated		
General Administration	$0.0	$0.8				FY 2002		
Project Total	$0.0	$10.1				$47.1		
Full-time Equivalents (FTE)	0.0	0.1						
	Dollar amounts are shown in thousands of dollars.							
Other Resources	$0.0	$0.0				$0.0		

Comments:
Heart tissue samples for fatty acid analysis will be collected in FY01 by normal ADFG catch sampling in each area. Thus, costs associated with vessel charters and field sampling crews will not be incurred by EVOS.

FY01

Prepared:

Project Number: 01538
Project Title: Northwest Gulf of Alaska Herring Stock Identification
Lead Agency: ADFG

Budget Category:	Authorized FY 2000	Proposed FY 2001

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Personnel		$0.0						
Travel		$0.0						
Contractual		$0.0						
Commodities		$4.0						
Equipment		$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$0.0	$4.0				Estimated FY 2002		
General Administration		$0.0						
Project Total	$0.0	$4.0				$47.1		
Full-time Equivalents (FTE)		0.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

FY01

Prepared:

Project Number: 01538
Project Title: Northwest Gulf of Alaska Herring Stock Identification
Agency: ADFG

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

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

		Subtotal		0.0	0.0	0.0
						Personnel Total

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

FY01

Prepared:

Project Number: 01538
Project Title: Northwest Gulf of Alaska Herring Stock Identification
Agency: ADFG

Contractual Costs:	
Description	

When a non-trustee organization is used, the form 4A is required. **Contractual Total**	
Commodities Costs:	
Description	
Liquid Nitrogen to fill four 35VHC containers (Including cool down and haz. mat. charges) Freight charges for four containers of liquid Nitrogen (Anchorage to field; field to contract lab) 15 ml nalgene cryotubes (200 ct), labels Misc sampling gear,	
Commodities Total	

FY01

Prepared:

Project Number: 01538
Project Title: Northwest Gulf of Alaska Herring Stock Identification
Agency: ADFG

New Equipment Purchases:	Number of Units	Unit Price	
Description			

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Those purchases associated with replacement equipment should be indicated by placement of an R. **New Equipment Total**

Existing Equipment Usage:	Number of Units	
Description		
Vessel charters for sample collection	4	
35VHC liquid nitrogen containers	4	
Personal computers	2	

FY01

Project Number: 01538
Project Title: Northwest Gulf of Alaska Herring Stock Identification
Agency: NMFS

Prepared:

Budget Category:	Authorized FY 2000	Proposed FY 2001
Personnel		$5.3
Travel		$0.0
Contractual		$0.0
Commodities		$0.0
Equipment		$0.0
Subtotal	$0.0	$5.3
General Administration		$0.8

LONG RANGE FUNDING REQUIREMENTS

			Estimated FY 2002		

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Project Total	$0.0	$6.1				$47.1		
Full-time Equivalents (FTE)		0.1						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:
To limit FY01 costs, fatty acid analysis will be delayed until FY02. Only lipid extraction and sample preservation will occur in FY01.

FY01

Prepared:

Project Number: 01538
Project Title: Northwest Gulf of Alaska Herring Stock Identification
Agency: NMFS

Personnel Costs:		GS/Range/	Months	Monthly		
Name	Position Description	Step	Budgeted	Costs	Overtime	
Larry Holland	Chemist (lipid extraction and preservation)	GS/12	0.8	7030.0		

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

		Subtotal		0.8	7030.0	0.0
						Personnel Total

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

FY01

Prepared:

Project Number: 01538
Project Title: Northwest Gulf of Alaska Herring Stock Identification
Agency: NMFS

Contractual Costs:	
Description	

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

When a non-trustee organization is used, the form 4A is required. **Contractual Total**	
Commodities Costs:	
Description	
Commodities Total	

FY01

Prepared:

Project Number: 01538
Project Title: Northwest Gulf of Alaska Herring Stock Identification
Agency: NMFS

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

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Existing Equipment Usage:	Number	
Description	of Units	
GC/MS	1	
HPLC	1	
computers, analytical software	2	

FY01

Prepared:

Project Number: 01538
Project Title: Northwest Gulf of Alaska Herring Stock Identification
Agency: NMFS