Exxon Valdez Oil Spill
Long-Term Herring Research and Monitoring Program Final Report

Fatty Acid Analysis as Evidence for Winter Migration of Age-0 Herring in Prince William Sound

Exxon Valdez Oil Spill Trustee Council Project 13120111-I
Final Report

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Study History: The work described in this report is part of the *Exxon Valdez* Oil Spill Trustee Council's Herring Research and Monitoring Program in Prince William Sound. One of its goals was to develop a program for monitoring the overwinter success of juvenile Pacific herring (*Clupea pallasii*) in Prince William Sound in an effort to understand why recruitment has remained at low levels. The herring population in Prince William Sound has only been fished commercially twice since it crashed in 1993. It is clear that recovery of the population will require recruitment of strong year class, yet that has yet to happen. Previous work under the Sound Ecosystem Assessment program (Project 98320) funded by the *Exxon Valdez* Trustee Council in the mid to late 1990s indicated that winter was a distinct bottleneck to the survival of juvenile herring. As a result the Herring Research and Monitoring Program focused much of its effort at understanding the processes regulating winter survival of juvenile herring.

A theory that developed out of the Sound Ecosystem Assessment Program was that surface currents in Prince William Sound distributed larvae from spawning beaches to nursery bays around the perimeter of the Sound. Larvae settled out of the water column in these bays and remained there for the next year or two. Consequently, the Herring Research and Monitoring Program developed a series of studies aimed at understanding processes within the bays. However, central questions related to developing the program included which bays and how many should be monitored.

This project, initiated in fall 2011, sought to determine if the movements of young-of-the-year herring in bays in Prince William Sound are restricted to the bays in which they are sampled. Determining if fish move between bays is important to developing an appropriate sampling strategy. Prince William Sound is characterized by a series of passages lined by shorelines deeply incised with bays and fjords. Many of these bays and fjords tens of kilometers wide and feature smaller inlets and bays. Therefore any appropriate monitoring program requires knowledge of the spatial scales at which juvenile fish are aggregated. In this work we determine the scale at which their fatty acid compositions can be discriminated. The fatty acid compositions of fish derive from the compositions their diet, hence differences in fatty acid composition reflect differences in diet. Fish foraging over the same spatial scales should encounter the same prey fields and consume similar diets. Thus fatty acids can help elucidate the spatial scales at which juvenile herring are foraging providing a basis for determining the spatial scale at which they should be monitored.

Abstract: Here we describe the fatty acid composition of young-of-the-year Pacific herring (*Clupea pallasii*) in Prince William Sound collected over two winters (2010-2012) and relate differences in their compositions to the results of simulated conditions in controlled laboratory studies. In the controlled studies we developed two groups of fish with distinct fatty acid compositions by feeding them different diets. The groups were subsequently subjected to a variety of conditions for four months. The conditions represented the possible behavior of overwintering herring: reduced rations of the initial diet, fasting, and switching to reduced rations of the alternate diet. Seasonal changes in the fatty acid composition of herring in Prince William Sound over winter indicated that they were switching diets after November. Very few fish were found to fast over winter. Examination of the spatial distribution of fatty acid "types" in Prince William Sound revealed a high degree of specialization among fish. While bays differed in their overall compositions there was also detectable variation between parts of bays and within those parts of bays. This high degree of specialization indicates that populations in bays are not mobile and that variation in prey selection among individuals results in portfolios of diet "types" within bays.

Key words:
Fasting, fatty acids, herring, lipid, spatial variation, starvation, winter, young-of-the-year

Project Data: Project data includes the sample collection information for herring from Prince William Sound and the laboratory study. These data include sampling dates, location names, latitude, longitude, and sampling gear. Data describing individual fish include sample identification numbers, lengths, weights, stomach content weight, lipid, protein, ash, moisture content and fatty acid composition. In addition, quality assurance data for the analyses conducted to determine lipid, protein, moisture, ash and fatty acid data have been retained. All of these data are located in an Access database maintained by the Alaska Fisheries Science Center at the Ted Stevens Marine Research Institute in Juneau, Alaska. The contact for the data is Fletcher Sewall and the database is called Lipid. There are no limitations to accessing these data.

Data collected for the Herring Research and Monitoring Program projects that contributed to this report are available through the Alaska Ocean Observing System (AOOS) Gulf of Alaska data portal: http://portal.aoos.org/gulf-of-alaska#metadata/4e73652c-858f-4a2a-9d0d-de53b62a27db/project

The Alaska Ocean Observing System data custodian is Carol Janzen, Alaska Ocean Observing System, 1007 W. 3rd Ave. #100, Anchorage, AK 99501, 907-644-6703, janzen@aoos.org. The data may also be found through the DataONE earth and environmental data archive at https://search.dataone.org/#data and by selecting the Gulf of Alaska Data Portal under the Member Node filter

Citation:

Heintz, R., F. Sewall, L. Schaufler, and C. Fugate. 2018. Fatty acid analysis as evidence for winter migration of age-0 herring in Prince William Sound. *Exxon Valdez* Oil Spill Long-Term Herring Research and Monitoring Program Final Report (*Exxon Valdez* Oil Spill Trustee Council Project 13120111-I), *Exxon Valdez* Oil Spill Trustee Council, Anchorage, Alaska**.**

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EXECUTIVE SUMMARY

This project, initiated in fall 2011, sought to determine if the movements of young-of-the-year (YOY) Pacific herring (*Clupea pallasii*) in Prince William Sound (PWS) are restricted to the bays in which they are sampled. Determining if fish move between bays is important for developing an appropriate strategy for monitoring juvenile populations. We analyzed the fatty acid compositions of YOY herring to determine the scale (broad vs. fine) at which foraging groups could be discriminated. By monitoring the location of discrete foraging groups seasonally we hypothesized we could determine if YOY herring move between bays over winter. Our approach was based on the idea that the fatty acid compositions of fish are derived from the compositions their diets; hence differences in composition reflect differences in diet. Fish foraging over the same spatial scales should encounter the same prey fields and consume the similar diets. Thus fatty acids may help elucidate the spatial scales at which juvenile herring are feeding and where they should be monitored. Further, we hypothesized that these differences would be conserved in winter when food is scarce permitting the use of fatty acid compositions as natural tags to monitor movement between bays.

Identification of the scale at which discrete groups of fish forage has significant value towards understanding production of those species. Pacific herring, move through a variety of habitats during their early life. As larvae they are transported in pelagic waters from spawning areas to bays located around the perimeter of PWS. In July they settle out of the water column into nearshore habitats within a kilometer of shore (Suryan et al. 2002). They remain in structured eelgrass and kelp habitats until early fall (Johnson et al. 2008). By October YOY herring can be found further off shore (Stokesbury et al. 1999) but they are still oriented towards the head end of bays in late fall and winter (Lewandoski et al. 2018). Eventually older juveniles move into deeper waters and eventually join adults that migrate between foraging habitats over the continental shelf and inshore wintering/spawning habitats (Brown et al. 2002). In order to adequately describe the trophodynamics of herring it is important to quantify the spatial scales at which they forage. This provides a basis for constructing models that can relate environmental conditions to the growth and survival of juvenile fish.

We evaluated the fatty acid compositions of YOY herring from different locations (bays) and season (fall and winter) in PWS to determine if fish are moving among bays. We included a laboratory study to simulate winter conditions so we could better interpret the data from the field. The concept included sampling fish in fall to establish a set of fatty acid "types" associated with different bays and then sampling again in spring to see where the "types" have moved. Several hypotheses required testing in order to accept the idea that the fatty acid compositions of fish in spring represented the movement of fall fatty acid "types". These hypotheses are: 1) fatty acid compositions in YOY herring are driven by diet composition, 2) fatty acid compositions acquired at the end of a feeding period are conserved in fasting fish or those on limited rations of the same diet, 3) prey fields in different bays are sufficiently distinct to cause detectable differences in fatty acid composition in resident herring, and 4) fatty acid compositions observed in fall can be detected in spring.

We accepted each of the first three hypotheses. In the laboratory we found that diets clearly changed the fatty acid compositions of YOY herring. We also observed that fasting fish conserved their fatty acid compositions so that fish initially fed different diets maintain their differences in fatty acid composition. The same held true for fish held on reduced rations of their initial diet. There were, however, large changes in composition for fish that consumed reduced rations of an alternative diet. These observations were consistent with tests designed to compare the fatty acid compositions of fish collected from different bays in Prince William Sound at the end of the growing season. However, a strong seasonal component to the fatty acid compositions of herring sampled in the field indicated that most surviving fish foraged over winter. We were able to detect a few individuals that conserved their fatty acid compositions over winter, but these individuals were rare. Thus, we conclude that it is not feasible to use of fatty acids to discriminate movement of YOY herring over winter.

However, the data were very useful for describing the spatial scales of foraging for YOY herring within a season. It is unlikely that fish are moving between bays within a season. Moreover, we demonstrated that fish are not moving much between areas within bays. Thus the spatial ranges for foraging among YOY herring in PWS are on the order of kilometers. Moreover, there was a detectable hierarchical structure to the fatty acid compositions within bays, demonstrating a high degree of diet specialization. This likely develops out the complex set of factors that influence prey choice for predators. There was some evidence that the degree of specialization may relate to the intensity of competition within a location.

INTRODUCTION

Identification of the scale at which discrete groups of fish forage has significant value towards understanding production of those species. Models that attempt to identify the direct and indirect effects of environmental features on fish growth and productivity necessarily rely on trophic interactions. However, the spatial scales at which growth and production operate are not always clear. If the models involve species, like Pacific herring (*Clupea pallasii*), that move through a variety of habitats during their lifetimes they require an understanding of the spatial scales at which fish can be expected to forage so that they can adequately describe trophodynamics (Aydin et al. 2005). Charactering the spatial scale for foraging can be problematic in rocky nearshore habitats due to their physical complexity. For species rearing in these habitats the difficulty of defining the spatial scale of foraging is exacerbated by a variety of factors including ontogeny, size, predation risk, and competition (Nunn et al. 2012). Consequently, traditional approaches to characterizing habitat use, such as stomach content analysis, limits our ability to adequately characterize the spatial scales (broad vs. fine) at which diets are consumed.

Fatty acid analysis may help elucidate the spatial scales over which diets are consumed. The rationale for conducting fatty acid analysis to evaluate foraging ecology rests with the idea that fatty acids retained in depot lipids are representative of their ingested concentrations. This concept forms the basis of quantitative (Iverson et al. 2004) and qualitative (i.e., Dalsgaard et al. 2003) uses of a fatty acid signature analysis model to estimate diet composition. Qualitative assessments of diet composition based on fatty acid composition typically depend on the identification of markers that can be traced from prey items into the tissues of predators. For example, dramatic shifts in the fatty acid compositions of juvenile coho salmon (*Oncorhynchus kisutch*) were used to demonstrate their consumption of adult salmon carcasses in freshwater streams (Heintz et al. 2010) and newly settled Atlantic cod (*Gadus morhua*) were found to rely on terrestrial energy sources (Copeman et al. 2009). The movement of such markers has been documented in mesocosm studies where fatty acids formed by phytoplankton were shown to appear sequentially in phytoplankton grazers and their predators (Fraser et al. 1989). Similarly, spatial variation in the fatty acid composition of phytoplankton sampled in mixed frontal structures were conserved in fish larvae found foraging near those fronts when compared with larvae sampled in stratified areas (St. John and Lund 1996). Presumably many of the seasonal changes in fatty acid composition reported for marine species are the result of dietary shifts (e.g., Beck et al. 2007).

Existing literature clearly supports the idea of applying fatty acid analysis to understand the spatial scales of foraging. An analysis of the fatty acid composition of harbor seals (*Phoca vitulina*) in the Gulf of Alaska revealed variation in fatty acid composition over both broad (400-800km) and fine spatial scales (9-15 km). These fine scale differences were consistent with the idea that harbor seals are central place foragers with relatively small foraging ranges (Iverson et al. 1997). Similarly, fatty acids were used to determine the use of shelf versus off-shelf habitats by foraging southern elephant seals (*Mirounga leonine*, Bradshaw et al. 2003). The same applications and inferences may be possible for fish. For example, American plaice (*Hippoglossoides platessoides*), yellowtail flounder (*Pleuronectes*

ferruginea) and Atlantic cod sampled from George's Bank, the Scotian Shelf and the southern Gulf of St. Lawrence all differed in fatty acid composition suggesting differences in prey fields for the different stocks (Budge et al. 2002). However discerning differences in foraging on finer scales may be more difficult. Adult herring sampled from different locations in Prince William Sound (PWS), Alaska lacked a detectable difference within an area of 90 x 130 km (Iverson et al. 2002).

One issue with the inability to detect intraspecific variation in fatty acid composition over small spatial scales may relate to differences in the scales surveyed and foraging ranges of the fish. Adult Pacific herring in PWS may forage over a spatial scale that exceeds the area of the Sound. Tagging studies reveal that many post-spawn adults move to forage near entrances to the Sound in Hinchinbrook Entrance and Montague Strait (Bishop et al. 2015), transiting the sound in just a few days. Thus fish collected in a given location may have recently foraged in very different locations obscuring the potential to detect spatial structuring of fatty acid compositions.

In contrast, juvenile fish that use shallow nearshore habitats, such as age-0 herring, may display variation in diet over relatively small spatial scales. For instance, diets inferred from stomach contents of age-0 herring varied among bays in PWS (Foy and Norcross 1999). Similarly, age-0 pink salmon (*Oncorhynchus gorbuscha*) stomach contents varied between stations as close as 19 km (Boldt and Haldorson 2003). To some extent the variation in diets results from analysis of stomach contents, which gives information about the most recent diet rather than using a method like fatty acids, which integrates diets over a period of weeks (Dalsgaard et al. 2003). However, if the diets of these less mobile organisms are truly different then their fatty acid compositions should also vary over fine spatial scales. Comparison of the fatty acid composition of less mobile fish from different bays may therefore reveal the spatial scales over which these fish can be expected to consume different diets; that is the fatty acids can be used to identify discrete foraging groups.

If the fatty acid composition of fish from a specific location was stable in time then the compositions could be used as natural markers to reveal movement. Stability in fatty acid compositions might be obtained at the end of the growing season when prey production is reduced, foraging becomes limited and seasonal starvation commences (Foy and Paul 1999). During starvation triacylglycerols are mobilized after glycogen depletion and consequently the ratio of phospholipids to triacylglycerols increases (McCue 2010). This accounts for the apparent increase the relative concentrations of polyunsaturated fatty acids and decrease in monenes in studies involving whole animals (e.g., Tandler et al. 1989, De Silva et al. 1997, Price and Valencak 2012). Similarly, Bluegills (*Lepomis sp.*) on sub-maintenance rations had altered fatty compositions at the end of winter (Roy et al. 2017). However, most starvation studies have not compared the changes incurred in animals held on different diets. While the effect of diet on triacylglycerols is well known, diet also influences phospholipids to a lesser degree. Thus some of the dietary information is also maintained in cell membranes. Consequently, it is unclear if the variation in fatty acid composition among animals in different nutritional states after eating the same diet is

greater than or less than that of animals fed a different diet and in the same nutritional states.

The purpose of this study is to evaluate the utility of fatty acid analysis as a tool for establishing the spatial scales of discrete foraging groups of young-of-the-year (YOY) herring in PWS and determine if fatty acid compositions can be used as natural markers to monitor winter movement of YOY herring.

OBJECTIVES

The goal of this research is to understand the spatial scales (broad vs. fine) over which YOY herring move during winter. We evaluated the use of fatty acid compositions of YOY herring from different locations as natural markers to trace movement. The concept included sampling fish in fall to identify a set of fatty acid "types" associated with different bays and then sampling again in spring to see if these "types" have moved. Several hypotheses required testing in order to accept the idea that the fatty acid compositions of fish in spring represented the movement of fall fatty acid "types". These hypotheses are: 1) fatty acid compositions in YOY herring are driven by diet composition, 2) fatty acid compositions acquired at the end of a feeding period are conserved in starving fish or those on limited rations of the same diet, 3) prey fields in different bays are sufficiently distinct to cause detectable differences in fatty acid composition in resident herring at the end of the growing season, and 4) the fatty acid compositions observed in fall can be detected in spring. If each of these hypotheses is accepted then the fatty acid compositions observed in spring can be reliably traced to their origin and reveal movement patterns.

The specific objectives of the study were to evaluate each of the four hypotheses through a series of laboratory and field studies. The objectives of the laboratory study were to evaluate the first two hypotheses by conducting a controlled feeding trial followed by examination of the fatty acid compositions under conditions of starvation and reduced ration. The third and fourth hypotheses were evaluated with field data collected from surveys conducted in Prince William Sound during the winters of 2010-2011 and 2011-2012. These surveys were part of the Herring Research and Monitoring Program (Project 16120111).

METHODS

Laboratory study

A controlled feeding study was conducted at the National Oceanic and Atmospheric Administration's Auke Bay Laboratories during the winter of 2011-2012 to assess the behavior of herring fatty acids under a range of treatments simulating winter feeding conditions. YOY herring for the study were collected by beach seine in Auke Bay and approximately 1000 individuals were transported live to nearby wet laboratory facilities on October 27, 2011. Herring were maintained through April 2012 in fourteen 50-gallon (189-liter) oval fiberglass tanks at an initial density of 75 individuals per tank and provided with sand-filtered flow-through seawater. Herring experienced natural variability in ambient sunlight, photoperiod, and temperature of seawater from Auke Bay, typically 4 - 8°C. Water temperature was monitored daily with a digital thermometer and recorded every 6 hours with data loggers in 6 tanks. Salinity and dissolved oxygen were monitored

bi-weekly with handheld YSI salinity meter and Hach DO meter, respectively. Seawater flow rates were manually adjusted daily to maintain flows of 6 liters per minute. Uneaten food particles and feces were removed weekly by siphoning, and tanks were checked for individual mortalities daily.

Herring were allocated to treatment groups according to diet type and ration (Fig. 1), with two replicate tanks per treatment group. Following capture ten fish were retained for

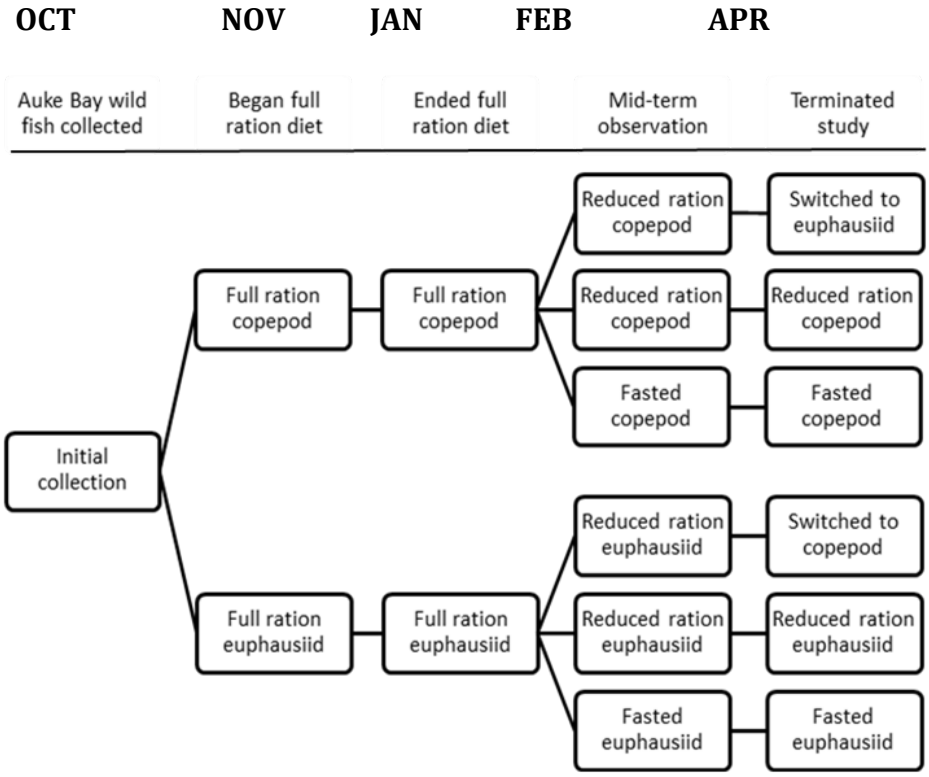

Figure 1. Schematic diagram showing layout for the controlled feeding studies conducted in the Auke Bay Laboratories' wet laboratory. Boxes show treatments applied to the fish and columns indicate when the treatments were applied.

chemical analysis. The rest were placed into tanks and fed with one of two types of commercially-available (San Francisco Bay Brand) frozen food: euphausiids (*Euphausia superba*), or copepods (*Calanus finmarchicus*). Fish were fed to satiation daily between capture and January (70 d) to create groups with two distinct fatty acid compositions. On January 6, tanks from each diet were randomly assigned to ration levels. Food was completely withheld from two tanks of fish for each diet group (starvation groups) and reduced to twice weekly in four tanks representing each diet (reduced ration). Fish sampled in January at the start of trial are referred to as the full ration groups. The starvation groups and full ration groups were maintained until the experiment terminated on April 4. The reduced ration groups were further manipulated on February 6 when the fish in two of the tanks were switched to the alternative diet (switched groups). The diet

offered the fish in the remaining two reduced ration tanks was not changed. By the end of the experiment we could assess the fatty acid compositions of fish that had starved, consumed reduced rations and full rations for both of the diets. In addition, we could observe the fatty compositions of fish that switched reduced rations diets midway through the study.

Samples were collected at the beginning and at the end of the experiment. At collection, herring were euthanized for sampling by immersion in a solution of 0.3g/L tricaine methanesulfonate (MS-222) in seawater. To minimize the influence of undigested food on fatty acid analysis, food was withheld from all herring for a minimum of 24 hours prior to sampling. Morphometric data were collected on herring as they were sampled, and herring were stored at -80 °C until processed for chemical analysis. A minimum of ten individuals were sampled across replicate tanks for each treatment group in January and April. A total of 152 herring were sampled for the study.

Mortalities occurred throughout the study duration, likely due to diseases known to infect wild herring populations. Many of the dead herring showed redness (hemorrhaging) around the jaws, head, and fin bases, consistent with infection by viral hemorrhagic septicemia virus (VHSV). Pathology of fresh mortality samples collected in early December in week 5 of captivity indicated 70% infection with VHSV and 83% infected with viral erythrocytic necrosis (VEN; T. Meyers, ADFG, pers. comm. 12/20/11). Infection rates are not available by treatment.

Field study

Location

A fatty acid analysis of specimens sampled in the field were used to evaluate the hypotheses that YOY herring in different bays differ in fatty acid composition at the end of the growing season and that these compositions will be conserved over time. Juvenile herring were collected from multiple bays in PWS (Fig. 2) over two winters (2010-2011 and 2011-2012). Collections were made at the beginning (November) and end (March) for each of the winters. The November samples represent the end of the growing season and the March samples were collected prior to the onset of the spring bloom. Therefore, the November and March samples respectively represent the beginning and end of the starvation season for each winter. Not all sites were represented in all collection periods due to the inability to capture fish. Sampling effort focused on Eaglek Bay, Lower Herring Bay, Simpson Bay, Zaikof Bay, and Whale Bay due to their distribution across PWS and for continuity with previous herring research (Norcross et al. 2001). Additional bays were sampled opportunistically. Gear types included variable-mesh gillnets and cast nets. Some locations have multiple embayments. (Fig. 2), occasionally collections were made from different embayments within a location (i.e. Whale Bay, Simpson Bay and Eaglek Bay; Fig. 2). Analysis of the fatty acid compositions from these "sub-locations" provided for comparison fatty acid composition on fine spatial scales (i.e., within a bay).

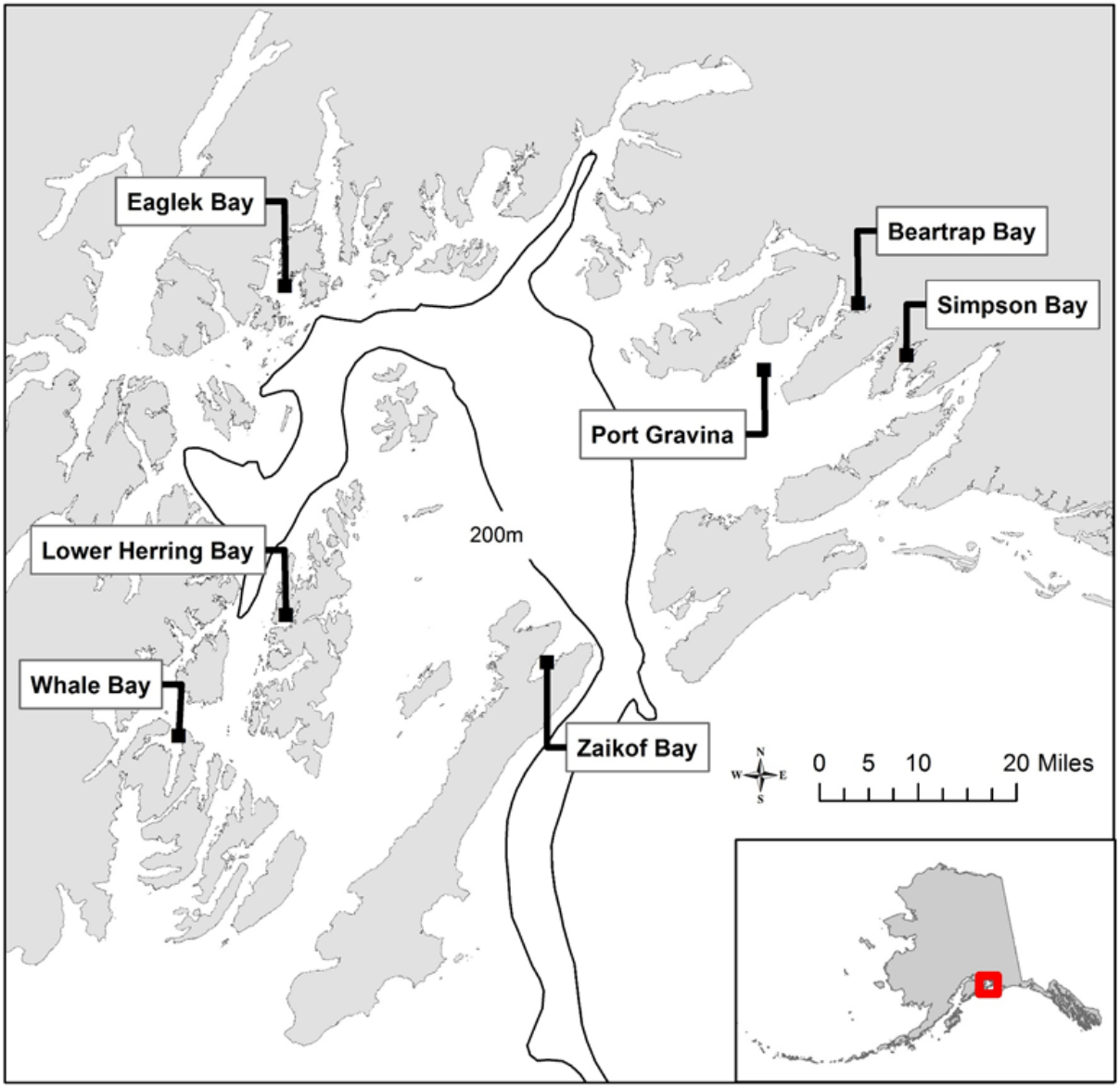

Figure 2. Sampling locations in Prince William Sound.

Collected herring were frozen and transported by air to the Auke Bay Laboratories in Juneau, Alaska for further analysis. Sample sizes for chemical analysis varied by sampling period and location (Table 1). When the number collected per bay exceeded 20, subsamples in proportion to length frequency distributions of the field collections were chosen for analysis. We increased the number analyzed per bay when more than one site within a bay was sampled allowing us to evaluate the third hypothesis on multiple spatial

scales. Prior to preparing the fish for fatty acid analysis they underwent laboratory processing to estimate their average size and remove their stomach contents. Fish were removed from the freezer and measured for fork length and wet mass was recorded. The still frozen fish were dissected to remove their stomachs. The contents were removed, weighed and the stomach tissues returned to the carcass.

Chemical analysis of samples

Lipid analysis was carried out according to a protocol modified from the Folch method, outlined by Christie (2003). Approximately 0.2 g wet sample homogenate was placed in a Dionex Accelerated Solvent Extractor 200, and lipid extracted using 2:1 (v:v) chloroform:methanol. All extracts were then sequentially washed with 0.88% (w/v) potassium chloride and 1:1(v:v) methanol:deionized water in a volume that equaled 25% of extract volume.

Fatty acid composition of lipid extracts was determined by gas chromatography-mass spectrometry (GC-MS) following a procedure adapted from Christie (1998). In brief, 0.3 mg of lipid extract underwent transesterification to fatty acid methyl esters (FAMEs) by the addition of Hilditch reagent (0.5 N sulfuric acid in methanol) held at 55°C for greater than 12 hours. The solution was then washed with 5% (w/v) sodium chloride. FAMEs were extracted into hexane, washed with 2% (w/v) potassium bicarbonate, and passed through an anhydrous sodium sulfate column to remove any co-extractables and water. The FAME containing hexane was reduced in volume to approximately 1 mL and spiked with 2000 ng of C23:0 FAME, which is used as an internal standard. The FAMEs were separated using a temperature gradient on a Varian Saturn gas chromatograph equipped with a 60 meter DB-23 column (J & W Scientific). Five-point calibration curves were created from known concentrations of a GLC-463 standard mix (Nu-Chek Prep, Inc.). An in-house herring reference and a National Institute for Status and Trends standard reference material (SRM 1947) were run along with each set of samples. Quantitated FAMES are listed in Appendix 1.

Data analysis

Fatty acid compositions of fish from the laboratory and field studies were examined using a variety of multivariate methods. Non-parametric multidimensional scaling (NMDS) was used to evaluate the spatial arrangement of observations in a reduced set of dimensions. Differences between groups were evaluated using permutated multivariate analysis of variance (PERMANOVA). Prior to analysis, fatty acid concentrations were expressed as the percent of total fatty acids and transformed by taking the logarithm of the percent plus one. A resemblance matrix was constructed using all the pairwise Bray-Curtis distances between samples in a data set. All multivariate analyses were conducted using Primer-e version 7. We set the α value to 0.05 to determine the significance of the differences.

Laboratory experiments

The size and lipid content of fish in the laboratory experiment was examined by ANOVA in order to verify that rations were insufficient to meet metabolic costs and the fish lengths remained constant. One-way ANOVAs were conducted using ration level as a fixed effect to compare the lengths of the fish sampled at the beginning of the study and at the end. The

ration levels included the fully fed fish sampled in January, fish fed reduced rations, fasted and switched to the alternative diets. Separate tests were conducted for each diet. The total lipid content of the fish was examined by ANCOVA with length as the covariate. Main factors were fixed and included ration and diet. The interactions between diet and length and ration and length were also included. The expectation was that fish fed reduced rations or switched to the alternate diet would have higher lipid levels than fasted fish at the end of the experiment and that all groups would have lower lipid levels than the fully fed fish at the start. All ANOVAs and ANCOVAs were conducted using Minitab version 7 with the GLM option.

For the laboratory data, the NMDS analysis was conducted on all of the sample data including the various treatments, the diets and the composition of the fish at capture prior to any feeding. Fatty acids used in the analysis are shown in the appendices. Confidence intervals for the centroids of each the groups used in the NMDS were constructed by bootstrapping. Graphs showing the areas occupied by the bootstrapped centroids are presented to illustrate the locations of the centroids in the reduced dimensions.

A PERMANOVA was conducted on the laboratory data to evaluate the first two premises. The model evaluated both premises by comparing differences between the fatty acid "types" developed after feeding to the compositions at the end of the winter. It was a two-way design mixed effects model using diet as a random factor and feeding level as a fixed factor. The model used the April observations for the fasted, reduced ration and switched diet groups and the full ration group was represented by the January sample. Pairwise tests were selected to compare rations within a diet.

Table 1. Sample sizes, fish size and lipid content for fish collected during field studies. Mean ± 1 s.e.

	Winter 1							
	November 2010				March 2011			
	n	Length (mm)	Weight (g)	% lipid	n	Length (mm)	Weight (g)	% lipid
Eaglek East					12	86.92 ± 1.86	5.21 ± 0.28	1.33 ± 0.04
Eaglek West	18	90 ± 1.13	6.2 ± 0.25	5.06 ± 0.44	8	83.88 ± 2.36	4.38 ± 0.4	1.36 ± 0.07
Lower Herring South	20	84.4 ± 1.99	5.27 ± 0.32	4.71 ± 0.45	19	92.26 ± 0.93	5.98 ± 0.21	1.7 ± 0.17
Lower Herring West					19	92.26 ± 0.93	5.98 ± 0.21	1.7 ± 0.17
Simpson West	17	62.18 ± 1.82	2.09 ± 0.16	3.43 ± 0.28	19	74.47 ± 1.99	3.53 ± 0.29	2.03 ± 0.2

	Winter 2							
	November 2011				March 2012			
	n	Length (mm)	Weight (g)	% lipid	n	Length (mm)	Weight (g)	% lipid
Beartrap Bay	20	82.9 ± 3.12	5.51 ± 0.58	6.01 ± 0.42				
Eaglek	20	96.7 ± 0.97	8.29 ± 0.27	6.52 ± 0.33	17	94.76 ± 1.7	6.69 ± 0.26	1.68 ± 0.1
Simpson East	20	57.85 ± 0.56	1.65 ± 0.05	3.76 ± 0.15				
Simpson West	20	77.5 ± 4.62	4.66 ± 0.76	5.64 ± 0.42	20	89.95 ± 2.47	5.96 ± 0.43	1.75 ± 0.21
Whale East	6	99.67 ± 1.45	9.13 ± 0.38	10.36 ± 0.35				
Whale West	20	87.05 ± 2.54	6.44 ± 0.61	6.58 ± 0.65				
Zaikof	20	92.6 ± 0.83	6.63 ± 0.2	4.9 ± 0.43				

Field Study

The size, condition and feeding status of fish collected from the field were compared by a two-way mixed effects ANOVA. Winter was a random factor and month was fixed. Three tests were conducted. The first test included fish lengths as the response variable, the second examined the percent lipid of the fish and the third compared stomach fullness. The last of these responses was estimated by dividing the weight of the stomach contents by the wet mass of the fish and expressing the quotient as a percentage.

Fatty acid compositions of the fish in the field study were analyzed using similar procedures to the laboratory study. NMDS analysis was conducted for each winter independently to visualize the relationships between locations and month in each winter. A PERMANOVA was conducted using fish from all the bays sampled in November to test the hypothesis that the fish acquired unique fatty acid compositions in each bay by the end of the growing season. The mixed two-way model included winter as a random term, bays as fixed term and their interaction. Pairwise tests were limited to comparing bays within a given winter. A second set of analyses was conducted on samples collected within sub-locations within a given bay. All collections with two sub-locations collected at the same time were analyzed by a two-way mixed model with bay as the random term and sub-location as the fixed term.

Efforts to determine if fatty acid compositions are conserved over winter by wild populations relied on the use of cluster analysis with similarity profile permutations (SIMPROF). Cluster analysis was used to objectively aggregate fatty acid compositions into classes for a given winter. Cluster analysis produces dendograms consisting of stems that are successively aggregated into clusters at nodes. The length of the stems between nodes relates to the average Bray-Curtis distance between the samples in aggregation. SIMPROF identified nodes below which there is no detectable structure (i.e. the samples are indistinguishable). Spring samples that were in clustered with November samples that could not be differentiated were considered to be conserved over winter.

RESULTS
Laboratory study

Treatment effects on weight and lipid content

There was significant mortality during the laboratory experiment, which influenced the comparisons between groups fed the different diets. Overall, 45% of the fish died during the study and the mortality was size dependent (data not shown). Mortality was likely due, in part, to diseases known to infect wild herring populations. Despite the mortality fish fed aggressively on the euphausiid diet and were more reticent to consume copepods. These apparent differences in palatability influenced mortality rates as the majority of the mortality (52 ± 8%) (mean ± 1s.e.) was observed in the tanks fed copepods.

The size dependence of the mortality obscured monitoring of lipid loss during the experiment. One-way ANOVAs designed to determine if the lengths of the fish changed during the study failed to detect a change ($P > 0.852$). The ANCOVA designed to test for changes in total lipid revealed a significant interaction between length and ration ($F_{4,95} = 4.97$, $P = 0.003$) (Table 2). Inspection of the relationships between total lipid and length for each ration (Fig. 3) revealed the expected relationships among the treatments. As the experiment progressed the relationships changed so that the length-adjusted lipid content of the reduced ration groups was higher than that of the starved fish but less than that of the fish at the start of the study. Fish with switched rations were intermediate.

First Hypothesis – Effect of diet on fatty acid composition

The fatty acid compositions of fish fed full rations of euphausiids differed significantly from both the fish collected from the wild and those fed copepods (Fig. 4A) demonstrating the effect of diet on fatty acid composition (Appendix 1). The PERMANOVA identified a significant effect of diet on the fatty acid compositions of fish from the two diet groups (Table 3). The average dissimilarity between the euphausiid-fed and cope-fed groups was 19.05 and C22:1n11 and C20:1n9 accounted for 24% of the dissimilarity. Concentrations of these fatty acids were highest in the copepod-fed group.

Second Hypothesis – Conservation of fatty acids in low ration or fasting fish

Monenes and saturated fatty acids were lost during the 88 days of starvation but the amounts depended on the diets. For the copepod fed fish the average dissimilarity between the starved group and the fish prior to starvation was 16.14. Losses of 22:1n11, 20:1n9 and 20:1n11 accounted for 20.9% of the dissimilarity while losses of 20:0 accounted for 3.2%. For the euphausiid fed fish the average dissimilarity after starvation was 13.38. The only monene that decreased was 22:1n9 and it accounted for only 3.7% of the dissimilarity. In contrast, losses of the saturates 16:0, 18:0, 20:0, and 22:0 accounted for 20.2% of the dissimilarity. The relative concentrations of the polyunsaturated fatty acids 20:5n3 and 22:6n3 increased for the eupahusiid fed fish accounting for 15.8% of the dissimilarity. In contrast, the copepod fed fish lost these fatty acids and the change accounted for 11.5% of the dissimilarity.

The PERMANOVA identified an interaction between diet type and feeding treatment, but no overall effect of feeding treatment (Table 3). The feeding treatments included the initial composition, starved fish, fish on reduced rations and fish switched to the alternate diet midway during the trial. The interaction resulted from differences in the response between the initial condition and fish on the different reduced diets. Those on the reduced euphausiid diet differed from the initial fish while fish on the reduced copepod diet did not differ (Table 3). Otherwise all ration treatments differed from the initial condition. However, examination of the spatial relationships between the treatments (Fig. 4B) indicates that these differences are small relative to the differences between diets. Also, the switched group differed from all other treatments ($P < 0.002$). Importantly, the pairwise contrasts and NMDS plot revealed that changing diets during the experiment resulted in significantly different fatty acid compositions.

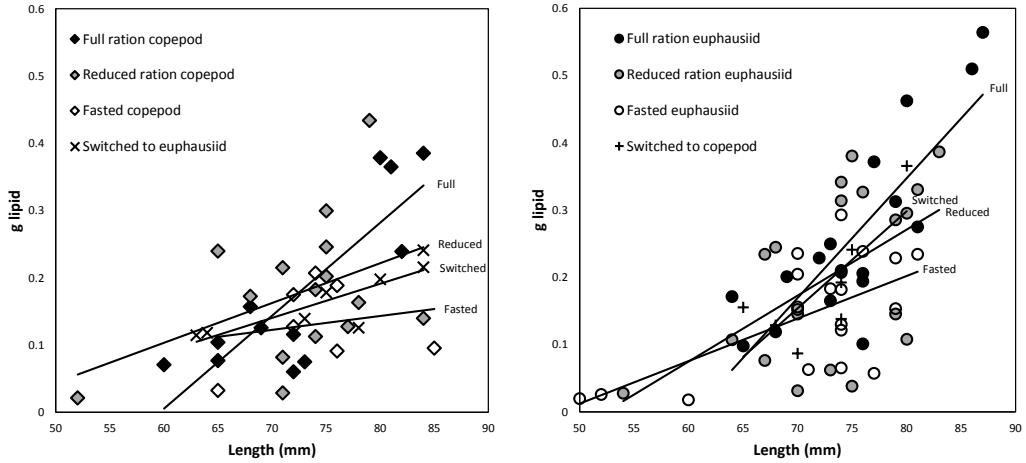

Figure 3. Relationship between the total lipid content of fish and their length for herring in the laboratory study. Left panel shows treatments involving fish fed copepods, right panel shows those fed euphausiids.

Field Study

The fish collected during the winter of 2010-2011 were in poorer nutritional condition than those from 2011-2012. There were no differences in the lengths of the fish when compared by year, season or their interaction (ANOVA $F_{1, 272} < 11.2$, $P > 0.185$). However, a similar test found a significant interaction between season and year ($F_{1, 272} = 7.64$, $P = 0.006$) on the lipid content of the fish. This was likely due to the low lipid levels observed in November 2010 when fish averaged 4.4 ± 0.3% lipid compared to 5.8 ± 0.2% lipid in November 2011. Lipid levels in March were similar in both years averaging about 1.7 ± 0.1%. Stomach fullness also depended on the season and year (ANOVA $F_{1, 272} = 6.49$, $P = 0.011$) likely due to the low level of fullness observed in November 2010. Stomach contents in fall of 2010 averaged 0.68 ± 0.04% of wet body mass, levels that were more similar to those observed in spring of both winters. In contrast, fish were better fed in November 2011, when stomach contents weighed an average 1.50 ± 0.2% of body mass.

Third Hypothesis – Detectability of spatial variation in fatty acids in November

Variability in juvenile herring fatty acid composition was greater between bays than within bays, regardless of month during both winters. Thus there were significant differences in composition among bays in November in a given winter, supporting

Table 2. Results of ANOVA test on lipid content of fish in controlled laboratory study.

Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	*F*	*P*
Length	1	0.36652	0.366521	55.64	0
Ration	3	0.08433	0.028109	4.27	0.007
Food	1	0.01829	0.018287	2.78	0.099
Length x ration	3	0.09825	0.032751	4.97	0.003
Length x food	1	0.02245	0.022454	3.41	0.068
Error	95	0.6258	0.006587		
Lack-of-Fit	77	0.53817	0.006989	1.44	0.196
Pure Error	18	0.08763	0.004868		
Total	104	1.32339			

the idea that fatty acid compositions have a high degree of spatial variability (Appendix 2). Inspection of the bootstrapped two-dimensional NMDS analysis of samples collected in the first winter indicated that samples spread along the first axis according to the month of collection while different bays tended to be spread along the second axis (Fig. 5A). The reverse was true for the second winter. Locations were spread along the first axis while the seasons were spread along the second axis (Fig. 5B). The spread along the first axis was driven by a large difference in the fatty acid composition of fish collected from Zaikof Bay in fall relative to all other groups. Sublocations sampled within bays appeared to differ in composition but tended to be most similar to each other (Figs. 5A and 5B).

A PERMANOVA comparing fatty acid compositions among bays in each November supported the idea that locations differed. There was a significant interaction between winter and bay indicating that fatty acid compositions of juvenile herring depended on the bay and winter in which they are sampled (PERMANOVA winter x bay P=0.001; Table 5). There was no detectable main effect of bay (P =0.211) indicating that bays did not systematically vary. Fatty acid compositions also varied by winter (P = 0.001). Pairwise contrasts indicated that all bays differed in composition in a given November (P < 0.001, Table 4) (P < 0.003).

Fatty acid compositions also varied over relatively small spatial scales (Fig. 5). Fish were collected from the east and west arms of Eaglek Bay in March of 2011 and from the different arms of Simpson Bay and Whale Bay in November 2012. Fatty acid compositions depended on which part of the bays the fish were sampled (PERMANOVA bay x sub-location P = 0.001; Table 5). Fatty acid composition of herring differed between bays (P = 0.001), but the compositions in eastern arms did not differ from the western arms, i.e. there was no effect of sublocation (P = 0.247,).

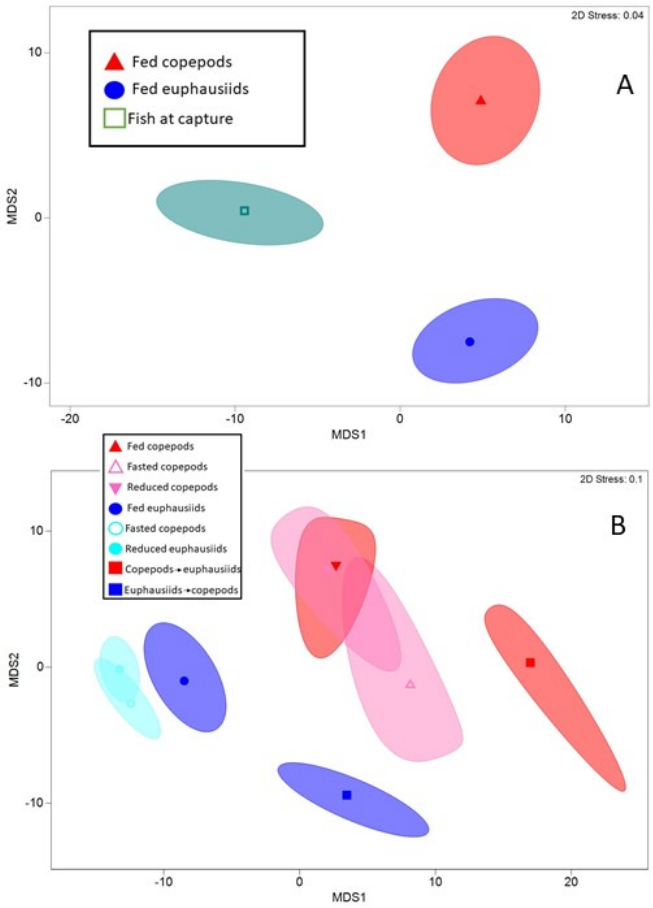

Figure 4. Bootstrapped NMDS plots of the fatty acid compositions of fish from the laboratory experiment. Panel A shows fish at the time of capture and after 70 days feeding. Panel B shows fish after feeding and after starvation.

Table 3. Results of the PERMANOVA testing for differences in fatty acid composition among treatment groups in the laboratory study.

PERMANOVA

Source	df	SS	MS	Pseudo-*F*	*P*	Unique permutations
Diet	1	64.246	64.246	38.675	0.001	999
Ration	3	33.01	11.003	0.81339	0.585	976
Diet x ration	3	40.584	13.528	8.1436	0.001	998
Residual	56	93.025	1.6612			
Total	63	252.65				

Pairwise tests of rations within diets

	Euphausiid Diet			Copepod Diet		
Ration groups	**t**	***P*(perm)**	**Unique permuatations**	**t**	***P***	**Unique permutations**
fasted vs. full	3.2131	0.001	955	2.2634	0.011	990
fasted vs. reduced	0.80157	0.636	978	1.1681	0.272	120
fasted vs. switched	4.9522	0.001	969	2.5583	0.001	916
full vs. reduced	3.495	0.001	979	1.6056	0.075	407
full vs. switched	2.6063	0.002	946	3.122	0.001	995
reduced vs. switched	5.1117	0.001	961	1.8181	0.045	165

Fourth Hypothesis - Fatty acid signature conservation from November until March

Cluster analysis provided some evidence for fish movement between Eaglek Bay and Simpson Bay during the first winter. The fatty acid compositions of 113 samples collected from seven strata during November 2010 and March 2011 (Table 1) clustered into 48 unique groups. Of these 48 clusters, 64% contained only spring samples and 32% contained only fall samples. Five of the clusters (10.4%) contained fish from multiple locations and there were never more than 2 locations in a cluster. Only two clusters (4.1%) contained samples from both seasons (Fig. 6). The first of these contained a sample collected in November from Simpson Bay and 6 samples from March in Eaglek Bay. The other cluster had one sample collected in Eaglek Bay in November, another from March and one collected in Simpson Bay in March. It is worth noting that fish with the conserved fatty acid compositions were among those in the worst condition in November and March. The average lipid content of the November samples in these two clusters averaged 1.5% lipid which was much lower than the overall average lipid content of fish sampled in November (4.4%). The lipid content of fish with conserved fatty acid compositions in March averaged 1.4% compared with an overall average of 1.7%.

There was less evidence of movement observed for the winter of 2011-2012. The procedure identified 69 clusters comprising 163 samples from five bays in fall and two in spring (Table 1). Of these 69 clusters 23% contained samples collected in spring. Two of the clusters (2.9%) contained samples from multiple locations. As in the first winter, there were no more than 2 locations per cluster. None of the clusters contained samples collected in both seasons. Both seasons included samples from Eaglek Bay and Simpson Bay, but there was no evidence of movement between these bays.

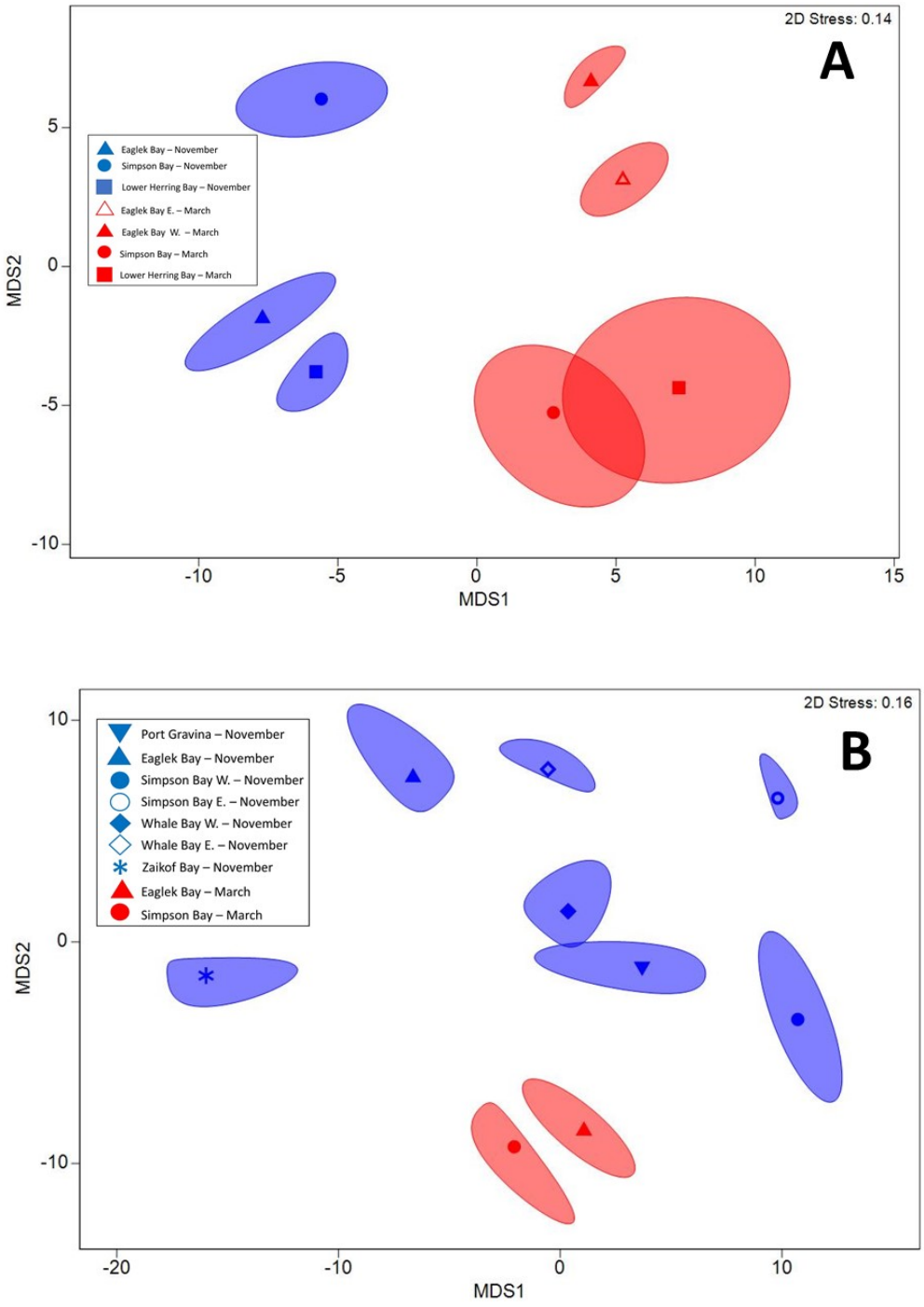

Figure 5. Bootstrapped centroids for the fatty acid compositions of YOY herring collected during the winters of 2010-2011 (A) and 2011-2012 (B). Symbols show centroid location and clouds depict 95% confidence intervals. Sublocations in bays are differentiated by E. or W.

Table 4. Results of PERMANOVA test for differences in the fatty acid compositions of juvenile herring collected from different bays in fall of 2010 and 2011.

PERMANOVA

Source	df	SS	MS	Pseudo-*F*	P	Unique permutations
Winter	1	24.898	24.898	14.359	0.001	999
Bay	5	215.14	43.029	2.775	0.234	970
Winter x Bay	1	14.946	14.946	8.6196	0.001	998
Residual	173	299.97	1.7339			
Total	180	581.54				

Pairwise Comparisons

	Winter 2010-2011			Winter 2011-2012		
Bays	T	P	Unique permutations	t	P	Unique permutations
Eaglek vs. Lower Herring	1.7278	0.022	999			
Eaglek vs. Simpson	3.8615	0.001	999	5.8228	0.001	999
Lower Herring vs. Simpson	4.0075	0.001	998			
Eaglek vs. Port Gravina				3.5011	0.001	999
Eaglek vs. Whale				3.533	0.001	999
Eaglek vs.. Zaikof				4.9562	0.001	999
Simpson vs. Port Gravina				2.7992	0.001	997
Simpson vs. Whale				3.7724	0.001	999
Simpson vs. Zaikof				8.7742	0.001	998
Port Gravina vs. Whale				2.0284	0.007	999
Port Gravina vs. Zaikof				6.0828	0.001	999
Whale vs. Zaikof				7.6982	0.001	997

Table 5. Results of PERMANOVA test for differences in the fatty acid compositions of YOY herring collected from different sub locations within bays in Prince William Sound.

PERMANOVA

Source	df	SS	MS	Pseudo-*F*	*P*	Unique permutations
Bay	2	73.629	36.815	30.859	0.001	998
Sub location	1	12.049	12.049	1.5969	0.362	332
Bay x Sub location	2	15.704	7.8522	6.5819	0.001	999
Residual	80	95.439	1.193			
Total	85	204.6				

Pairwise comparisons

	Eaglek		Simpson		Whale	
	t	*P*	t	*P*	t	*P*
East arm vs. West Arm	2.9103	0.002	3.9406	0.001	2.3108	0.004

DISCUSSION

The fatty acid compositions of YOY herring under controlled laboratory and field conditions were used to evaluate the potential use of fatty acids as natural markers to monitor fish movement in PWS. The observed concentrations were consistent with those from previous studies of YOY herring in PWS (Iverson et al. 2002; Appendix B). In this study four hypotheses were examined: 1) fatty acid compositions in YOY herring are driven by diet composition, 2) fatty acid compositions acquired at the end of a feeding period are conserved in fasting fish or those on limited rations of the same diet, 3) prey fields in different bays are sufficiently distinct to cause detectable differences in fatty acid composition in resident herring, and 4) the fatty acid compositions observed in fall can be detected in spring. The results of those tests indicate that 1) diet alters fatty acid composition, even when consumption levels are insufficient to meet maintenance needs, 2) fatty acid compositions are conserved in starving fish and those consuming reduced rations of their initial diet, 3) fish sampled from different locations at the end of the feeding season have unique fatty acid compositions and 4) while some fish conserve their fatty acid compositions over winter the majority of YOY herring forage on alternative diets. These observations preclude the use of fatty acids to trace movements in winter, but the existence of a hierarchical structure to the compositions in the field samples can be used to assess the spatial scale at which fish are foraging at the time of collection. These conclusions and the results of the laboratory and field studies are discussed in greater detail below.

Laboratory study

The patterns observed in YOY herring lipid and length over the course of the experiment are consistent with the idea that the reduced ration fish lost lipid. This is important because one goal of the experiment was to determine how fatty acids respond to a prolonged period of rations below those required to sustain routine metabolism. The expectation for the ANCOVA, based on other studies involving fasting fish (Kooka et al. 2009, Heintz and Vollenweider 2010) was that the intercepts would decrease from that of the initial condition and the slopes would remain constant or that slopes would increase as small fish lose lipid at a higher rate than larger fish (Miranda and Hubbard 1994). A different pattern was observed here with slopes decreasing, and lipid levels remaining constant for small fish. Regardless of the pattern, the length-adjusted lipid content based on the ANCOVA was in the expected order (Full ration > Reduced ration > Switched > Starved). It is unlikely that small fish maintained their lipid levels. Instead, size dependent mortality may have influenced the response of the slopes. Small fish are expected to starve first (Schultz and Conover 1999, Kooka et al. 2009) and the loss of small fish was likely exacerbated by the disease conditions in the tanks. Infection of YOY herring with *Ichthyophonus* has been shown to increase metabolic costs (Vollenweider et al. 2011). Consequently, small lean fish were likely the most at risk of mortality. Preferential loss of small lean individuals would counteract the effect of lipid loss on the intercepts of the ANCOVA and have the effect of rotating the slope downward while increasing the intercept, as observed in the euphausiid fed groups. Consequently, the disease effect may have obscured the losses that would be expected under routine conditions.

The comparison of the fish at the end of the initial feeding period with those collected from the wild demonstrated the influence of diet on the fatty acid compositions of YOY herring. For the copepod fed fish increases in C20:1n9 and C22:1n11 were especially noteworthy. Similarly, the euphausiid fed fish had conspicuous increases in C16:1n7 and C18:1n9. While it is unclear if the differences observed between the two groups were maximized after the 70 day feeding period it is likely they were near maximum. Saturation of 18:2n6 in muscle tissue of Atlantic croaker (*Micropogonias undulates*) required 44 to 81 days depending on their diet, less time was required for saturation in liver (Mohan et al. 2016). Similar uptake rates were observed in Atlantic cod (Copeman et al. 2013). Our observations are based on whole body homogenates which would be dominated by muscle. Thus transformation may not have been complete. However, the lipid content of our herring more than doubled during the feeding period and saturation of 18:2n6 in croaker muscle was complete before their lipid doubled (Mohan et al. 2016).

The pairwise contrasts made among the various dietary treatments provide insight into how fatty acids are stored and catabolized during winter. Starved fish always differed in composition from those at the start of the study. Also, the starved and reduced ration fish had similar fatty acid compositions. For the euphausiid fed fish, those compositions were statistically different from those at the start of the trial. The difference in composition between the fish fed copepods on a reduced ration and those that the start of trial was harder to detect. Our data suggest that eupahausiid fed fish preferentially catabolize specific fatty acids when assimilated energy is insufficient to meet metabolic costs. The

results for the copepod fed fish are consistent with this conclusion, but less clear. Importantly, the fatty acid composition of fish fed reduced rations and switched to the alternate diets were different from the other treatments. This indicates that dietary fatty acids contribute to energy stores even when fish are at an energy deficit. Moreover, they indicate that if winter foraging involves a shift in diet then fatty acid compositions can also be expected to change. This is what we observed when we compared spring samples to fall samples in both winters in PWS.

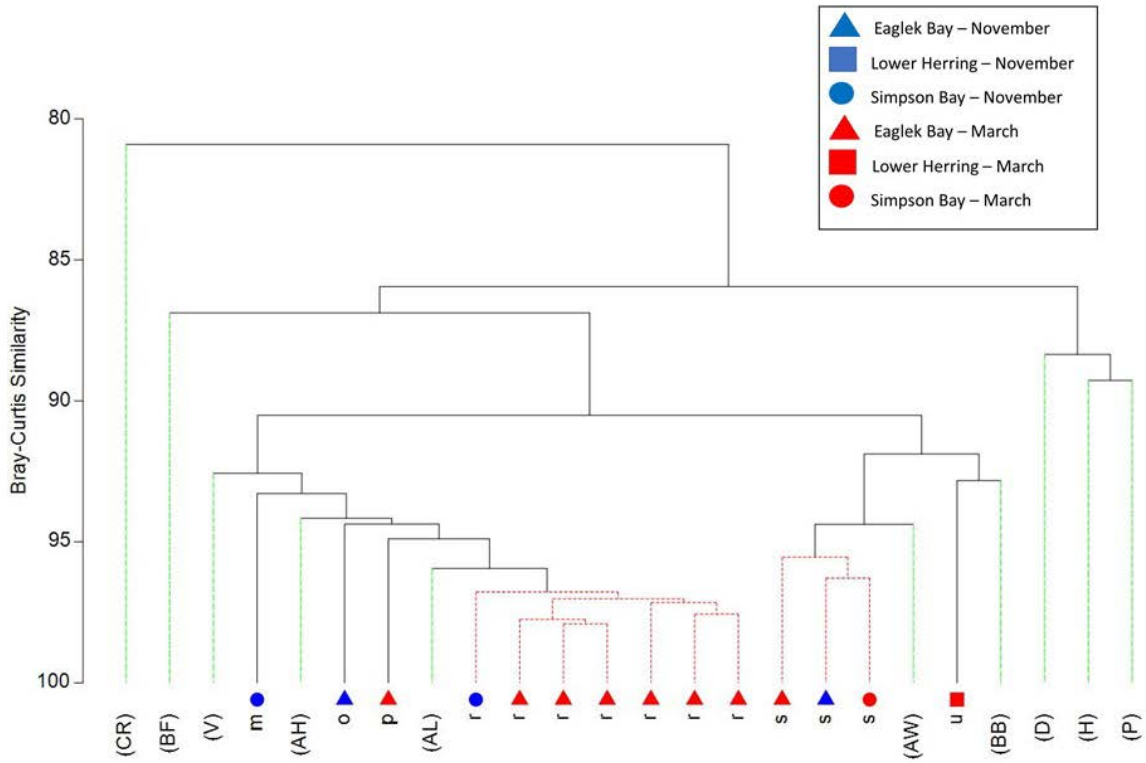

Figure 6. Some of the groups defined by the cluster analysis of samples collected during the winter of 2010-2011. Symbols at the base of the figure show where the samples were sampled and the letters below identify their respective clusters. Note clusters "r" and "s" have members with different locations in fall and spring. Red dashed stems join samples that cannot be distinguished. Green dashed lines show where stems have been collapsed to improve viewability. Horizontal black lines join statistically different clusters.

The differences in fatty acid composition we observed between starved fish and those at the start of the trial were much smaller than those between the dietary groups. Changes within dietary groups were relatively subtle with respect to the differences between dietary groups. Thus, even though there may be preferential catabolism of specific fatty acids in fish experiencing energy deficits the original differences between groups are conserved supporting the idea that in the absence of a diet change fatty acid compositions can be used as natural tags to identify different groups of fish. Differences between diets in the fatty acids lost during starvation relate to differences in the relative abundances of

saturates and monoenes in their tissues at the outset. The copepod fed fish had about one third more monoenes in their tissues after feeding than the euphausiid fed fish. Both groups had similar amounts of saturates. The loss of n3 polyunsaturates among the starved copepod fish combined with their high mortality suggests a loss of phospholipid may have been manifest by the combined effects of starvation and an upregulated immune system.

Field Study

Congruent with the laboratory data, the field study demonstrated that fish in different bays had different fatty acid compositions at the end of the growing season (i.e. November). Laboratory demonstration of the effect of diet on fatty acid composition indicates that the differences between bays are the results of differences in diet. Strong seasonal variation in fatty acid composition of herring diets limits the use of fatty acids as natural markers to monitor their winter movement. The most plausible explanation for the seasonal shift in fatty acid composition is foraging over winter. The prey communities at the head ends of many of these bays varies seasonally and copepod species available to YOY herring in winter include *Calanus pacifica* and *Oithona spinirostris* (McKinstry and Campbell 2017). Our laboratory study demonstrated that sub-maintenance level rations of an alternative diet alters fatty acid compositions. Therefore, fish sampled in spring had likely consuming reduced rations of prey different from those consumed prior to November. This is also consistent with observations of lower lipid levels among fish samples in March.

Our cluster analysis indicated that different types of fatty acid compositions could be detected within bays in a given season indicating the existence of a hierarchical structure to herring foraging. This hierarchical structure is indicated by three observations. First, fatty acid compositions varied among bays, regardless of when they were sampled. Second, differences in fatty acid compositions could be detected between different sub-locations within bays and third, unique clusters of fatty acid compositions were found within the sub-locations in each season. The implication is that not only do fish diets appear to differ among bays but diets can differ within bays and even within sub-locations. This is consistent with observations of differences between the prey fields at the head ends of bays and at their mouths (McKinstry and Cambell 2017).

This spatial structuring demonstrates that movement of YOY herring among bays or even within them is extremely limited. Changes in the fatty acid composition of growing wild fish must reflect the increased mass of fatty acids in the reserve and structural lipid pools. Consequently, fatty acids are typically found to fully integrate dietary levels over periods of weeks under controlled conditions (Copeman et al. 2013, Mohan et al. 2016). In the wild, fatty acid composition differences are likely to be more subtle than those observed in controlled studies where diets are manipulated with purpose of creating differences. At a minimum, the differences in fatty acid composition detected here therefore reflects the effects of consuming different diets over a period of several weeks. Herring larvae in PWS settle out of the water column in nursery bays in early July (Norcross et al. 2001). By November YOY herring have been out of the larval stage for approximately 17 weeks. If fatty acids are integrating diets over 8 to 12 weeks (Copeman et al. 2013, Mohan et al. 2016) then it is unlikely that fish from different locations have consumed a common diet

since settlement. If fish or their prey fields were moving freely among bays or sub-locations then there should be little difference in their fatty acid compositions because their movements would ensure that they are exposed to dietary fatty acids.

The existence of multiple clusters within bays indicates that the diets of individual herring in PWS are specialized. Prey fields and diets were expected to vary across bays based on previous work conducted in many of the same bays studied here (Foy and Norcross 1999). While there are distinct seasonal patterns in zooplankton community composition in PWS (Coony et al. 2001, McKinstry and Campbell 2017) prey fields in bays can be expected to vary by virtue of the interaction between the differing oceanographic processes in the bays (Gay and Vaughn 2001). Variation in fatty acid composition within bays relates to differing prey selection by individuals within a bay. Such variation in prey selection is influenced by a range of factors including size (Arrehnius 1996), ontogeny (Iverson et al. 2002), interspecific interactions and intraspecific interactions (Nunn et al. 2012). In fact, sizes of fish varied between clusters in a given bay and sampling period. Habitat differences within bays may also influence the variety of fatty acid compositions observed. YOY herring in PWS move from shallow nearshore habitats to deeper water habitats in fall (Stokesbury et al. 2000, Lewandoski and Bishop 2017) so it is possible that sampling encountered fish from different habitats and thus, different prey fields that influenced their fatty acid composition.

Notably a few of the fatty acid compositions were shared among bays particularly in the winter 2010-2011. However, this was relatively rare as shared compositions were detected in only 10% of the clusters during the winter of 2010-2011 and 3% in the next winter 2011-2012. When multiple locations were detected within a cluster the locations were typically adjacent to each other and they involved Simpson Bay, Port Gravina, Eaglek Bay and Lower Herring Bay. This may offer some evidence of movement between bays, but it is more likely due to the availability of similar prey in the bays. Kline and Campbell (2010) showed that differences in the zooplankton communities among bays was not due to different species compositions, but due to differences in their relative abundances. Selectivity for specific prey items on the part of individuals could therefore account for the apparent similarity in fatty acid compositions of a few individuals between bays. Notably there was greater overlap between bays in the year with the lowest lipid content.

The strong seasonal component to fatty acid compositions provides evidence that access to forage is important to the YOY herring in winter. The vast majority of the spring fatty acid compositions differed from the fall compositions and few fish were observed to have conserved fall fatty acid compositions. The importance of winter foraging is demonstrated by the low lipid levels encountered among the fish sampled in spring. In both years spring lipid levels were near those identified as critical for survival (1.5% of wet mass, unpublished data). Apparently foraging by these fish likely forestalled starvation. The individuals identified by the cluster analysis as having conserved fatty acid compositions averaged 1.4 ± 0.1% lipid in spring compared with 1.7 ± 0.1%, suggesting that their lack of foraging may have resulted in an increased risk of starvation. However, this same fatty acid "type" was also characteristic of individuals with low lipid levels in fall. Moreover, the ratio of 22:6n3 to 16:0 in November for fish in these clusters was more typical of values

observed in March suggesting their triacylglycerol levels were much lower than other November fish. The more parsimonious explanation is that rather than conserving a fatty acid signature these individuals were characteristic of those in extremely poor nutritional condition. Coincidently, fish were only observed to conserve fatty acid compositions during the winter of 2010-2011, which was the year in which fish started winter with the least amount of lipid.

CONCLUSIONS

This work demonstrated the utility of fatty acid analysis for discerning the spatial scales of foraging for YOY herring in PWS. Diets influence fatty acid composition of YOY herring, fasting conserves prior compositions and the spatial variability of fatty acid compositions in PWS indicate little movement of fish among locations within a season. Additionally, these data show that populations within bays display a portfolio of diet specializations. Presumably these specializations are a response to size, ontogeny, habitat selection, inter- and intraspecific interactions. There was little evidence that fatty acid compositions are conserved over winter indicating that survival of overwintering fish depends on their access to food at some point during winter. It is important to note that the fish we sampled in spring were survivors and their low lipid levels indicate that they were near their physiological limits. This demonstrates that access to forage during winter is an important determinant of their survival. While the question of inter-seasonal movements remains unanswered we have been able to establish that there is little movement of herring within seasons and their foraging is predominately restricted to locations within bays. These data suggest that adult improve their fitness by broadcasting their offspring across a variety of rearing habitats and recruitment is maximized when food is readily accessible to YOY herring across a multiplicity of bays.

ACKNOWLEDGEMENTS

We would like to acknowledge the efforts of staff the Prince William Sound Science Center in collecting samples for this project, Scott Pegau, Kristen Gorman, Michele Buckhorn, Tom Kline, Julia McMahon, Megan Roberts, and Jennifer Todd. We are also indebted to staff at NOAA's Auke Bay Laboratories for sample preparation, Ryan Bare, Sarah Ballard, Robert Bradshaw, Sarah Christiansen, Eamon Conheady, Andrew Eller, Hannah Findlay, Meghan Garrison, Kevin Heffern, Lia Heifetz, Tayler Jarvis, Stella Mosher, Tom Parker, Connor Pihl, Erik Pihl, Ann Robertson, and Wes Strasburger. Thanks are also due to Courtney Weiss and Bonita Nelson for editorial comments on the manuscript. The views expressed here are our own and do not necessarily represent those of the *Exxon Valdez* Trustee Council.

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Appendix 1. Average (± 1 s.e.) relative fatty acid concentrations for wild fish, food offered and fish fed after 70 days.

	Wild herring	Copepod fed	Euphausiid fed	Copepods	Euphausiids
N	8	12	9	3	3
Saturates					
16:0	29.21 ± 1.86	23.93 ± 3.90	38.56 ± 3.61	14.12 ± 3.91	24.79 ± 1.40
17:0	0.80 ± 0.07	0.39 ± 0.05	0.27 ± 0.03	0.16 ± 0.04	0.05 ± 0.00
18:0	5.07 ± 0.55	2.73 ± 0.44	4.17 ± 0.40	1.04 ± 0.29	2.05 ± 0.04
20:0	0.94 ± 0.06	0.81 ± 0.07	0.47 ± 0.03	0.32 ± 0.02	0.11 ± 0.01
22:0	0.18 ± 0.03	0.42 ± 0.13	0.80 ± 0.07	0.10 ± 0.01	0.13 ± 0.01
24:0	1.24 ± 0.13	1.09 ± 0.16	0.82 ± 0.07	0.69 ± 0.46	0.73 ± 0.09
MUFA					
16:1n-7	3.84 ± 0.78	5.37 ± 0.33	8.06 ± 0.50	4.19 ± 0.15	10.02 ± 0.07
18:1n-11	0.43 ± 0.03	0.71 ± 0.12	0.21 ± 0.02	0.43 ± 0.09	0.00 ± 0.00
18:1n-9	7.60 ± 1.80	7.92 ± 1.00	11.19 ± 1.45	2.83 ± 0.12	13.13 ± 0.05
18:1n-7	3.41 ± 0.13	1.99 ± 0.13	5.23 ± 0.53	0.89 ± 0.03	9.93 ± 0.06
20:1n-11	0.31 ± 0.13	0.71 ± 0.16	0.00 ± 0.00	1.47 ± 0.17	0.52 ± 0.02
20:1n-9	0.89 ± 0.08	5.71 ± 1.00	1.40 ± 0.15	5.86 ± 0.18	1.42 ± 0.03
22:1n-11	0.15 ± 0.16	12.36 ± 2.28	0.76 ± 0.35	17.31 ± 0.59	0.00 ± 0.00
22:1n-9	1.29 ± 0.09	2.02 ± 0.17	1.14 ± 0.09	2.72 ± 0.26	1.52 ± 0.06
24:1n-9	3.61 ± 0.24	2.40 ± 0.23	1.69 ± 0.18	2.32 ± 0.58	0.44 ± 0.12
PUFA					
18:2n-6	0.80 ± 0.14	1.20 ± 0.14	1.06 ± 0.21	0.36 ± 0.22	1.62 ± 0.06
18:3n-6	0.37 ± 0.02	0.32 ± 0.03	0.35 ± 0.02	0.40 ± 0.03	0.44 ± 0.01
18:3n-3	2.10 ± 0.22	2.38 ± 0.13	1.72 ± 0.19	2.75 ± 0.02	1.04 ± 0.02
18:4n-3	2.66 ± 0.70	4.71 ± 0.36	3.35 ± 0.46	14.87 ± 1.91	2.22 ± 0.06
20:2n-6	0.18 ± 0.02	0.48 ± 0.12	1.04 ± 0.07	0.07 ± 0.02	0.01 ± 0.00
20:3n-6	0.56 ± 0.06	0.28 ± 0.05	0.30 ± 0.05	0.43 ± 0.06	0.30 ± 0.02
20:3n-3	1.09 ± 0.08	0.81 ± 0.08	0.65 ± 0.04	1.31 ± 0.18	0.59 ± 0.02
20:4n-6	0.95 ± 0.08	0.66 ± 0.05	0.53 ± 0.02	0.29 ± 0.03	0.55 ± 0.04
20:4n-3	1.21 ± 0.04	1.01 ± 0.08	0.81 ± 0.02	1.28 ± 0.06	0.71 ± 0.02
20:5n-3	7.31 ± 0.32	5.98 ± 0.44	6.48 ± 0.89	10.27 ± 1.52	16.00 ± 0.44
22:4n-6	0.01 ± 0.02	0.09 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
22:5n-6	1.04 ± 0.08	0.68 ± 0.10	0.91 ± 0.20	0.20 ± 0.16	0.00 ± 0.00
22:5n-3	1.78 ± 0.09	1.46 ± 0.10	0.82 ± 0.03	2.55 ± 0.35	1.41 ± 0.04
22:6n-3	20.95 ± 2.79	11.40 ± 1.15	7.20 ± 1.33	10.75 ± 2.42	10.28 ± 0.55

Appendix 1 continued.

	Fed copepods	Starved	Reduced rations	Switched to euphauiids
n	12	7	3	8
Saturates				
16:0	23.93 ± 3.90	33.76 ± 3.69	37.09 ± 11.96	45.19 ± 5.76
17:0	0.39 ± 0.05	0.39 ± 0.05	0.34 ± 0.10	0.48 ± 0.07
18:0	2.73 ± 0.44	6.02 ± 0.88	5.12 ± 1.90	5.46 ± 0.82
20:0	0.81 ± 0.07	0.31 ± 0.01	1.25 ± 0.34	0.40 ± 0.03
22:0	0.42 ± 0.13	0.21 ± 0.03	1.30 ± 0.43	0.14 ± 0.02
24:0	1.09 ± 0.16	1.78 ± 0.28	1.68 ± 0.60	1.78 ± 0.16
MUFA				
16:1n-7	5.37 ± 0.33	4.26 ± 0.35	4.67 ± 1.53	3.66 ± 0.64
18:1n-11	0.71 ± 0.12	0.64 ± 0.07	0.81 ± 0.08	1.36 ± 0.08
18:1n-9	7.92 ± 1.00	11.20 ± 1.59	9.31 ± 4.00	4.84 ± 1.20
18:1n-7	1.99 ± 0.13	2.08 ± 0.20	1.91 ± 0.47	1.33 ± 0.24
20:1n-11	0.71 ± 0.16	0.36 ± 0.08	1.13 ± 0.16	0.03 ± 0.03
20:1n-9	5.71 ± 1.00	3.47 ± 0.99	2.41 ± 2.19	7.19 ± 0.59
22:1n-11	12.36 ± 2.28	7.41 ± 2.59	6.27 ± 4.63	6.78 ± 1.96
22:1n-9	2.02 ± 0.17	2.31 ± 0.36	2.00 ± 0.14	2.23 ± 0.17
24:1n-9	2.40 ± 0.23	3.04 ± 0.34	2.03 ± 0.42	0.19 ± 0.18
PUFA				
18:2n-6	1.20 ± 0.14	0.30 ± 0.22	0.47 ± 0.51	0.11 ± 0.12
18:3n-6	0.32 ± 0.03	0.32 ± 0.06	0.50 ± 0.15	0.44 ± 0.04
18:3n-3	2.38 ± 0.13	2.20 ± 0.07	1.88 ± 0.21	1.13 ± 0.19
18:4n-3	4.71 ± 0.36	3.15 ± 0.32	3.22 ± 0.90	2.50 ± 0.51
20:2n-6	0.48 ± 0.12	1.26 ± 0.14	0.53 ± 0.37	0.49 ± 0.12
20:3n-6	0.28 ± 0.05	0.66 ± 0.09	0.22 ± 0.17	0.15 ± 0.08
20:3n-3	0.81 ± 0.08	1.29 ± 0.16	1.31 ± 0.41	0.87 ± 0.07
20:4n-6	0.66 ± 0.05	0.55 ± 0.04	0.81 ± 0.20	0.48 ± 0.02
20:4n-3	1.01 ± 0.08	1.32 ± 0.15	1.34 ± 0.32	0.57 ± 0.09
20:5n-3	5.98 ± 0.44	3.59 ± 0.41	3.68 ± 0.96	3.53 ± 0.57
22:4n-6	0.09 ± 0.05	0.08 ± 0.06	0.00 ± 0.00	0.29 ± 0.02
22:5n-6	0.68 ± 0.10	0.09 ± 0.10	0.61 ± 0.44	0.68 ± 0.18
22:5n-3	1.46 ± 0.10	1.92 ± 0.16	1.91 ± 0.46	1.95 ± 0.11
22:6n-3	11.40 ± 1.15	6.03 ± 1.26	6.22 ± 3.19	5.76 ± 1.25

Appendix 1, continued.

	Euphausiid fed	Starved	Reduced rations	Switched to copepods
n	9	9	9	6
Saturates				
16:0	38.56 ± 3.61	21.30 ± 0.73	20.01 ± 0.91	42.63 ± 3.26
17:0	0.27 ± 0.03	0.09 ± 0.01	0.09 ± 0.01	0.24 ± 0.05
18:0	4.17 ± 0.40	2.60 ± 0.08	2.46 ± 0.09	4.90 ± 0.81
20:0	0.47 ± 0.03	0.18 ± 0.02	0.17 ± 0.02	1.06 ± 0.15
22:0	0.80 ± 0.07	0.34 ± 0.11	0.30 ± 0.09	1.15 ± 0.18
24:0	0.82 ± 0.07	0.59 ± 0.04	0.46 ± 0.03	1.44 ± 0.26
MUFA				
16:1n-7	8.06 ± 0.50	9.88 ± 0.34	9.94 ± 0.44	6.59 ± 1.19
18:1n-11	0.21 ± 0.02	0.07 ± 0.01	0.09 ± 0.02	0.62 ± 0.07
18:1n-9	11.19 ± 1.45	18.11 ± 0.53	16.91 ± 0.36	10.22 ± 1.93
18:1n-7	5.23 ± 0.53	7.65 ± 0.32	8.00 ± 0.53	4.63 ± 1.01
20:1n-11	0.00 ± 0.00	0.04 ± 0.02	0.06 ± 0.03	0.74 ± 0.18
20:1n-9	1.40 ± 0.15	1.38 ± 0.17	1.49 ± 0.21	1.32 ± 0.38
22:1n-11	0.76 ± 0.35	1.13 ± 0.38	1.32 ± 0.56	2.63 ± 0.19
22:1n-9	1.14 ± 0.09	0.68 ± 0.09	0.75 ± 0.11	1.84 ± 0.12
24:1n-9	1.69 ± 0.18	1.45 ± 0.16	1.12 ± 0.15	1.90 ± 0.19
PUFA				
18:2n-6	1.06 ± 0.21	1.61 ± 0.12	1.65 ± 0.09	0.40 ± 0.19
18:3n-6	0.35 ± 0.02	0.25 ± 0.01	0.25 ± 0.01	0.46 ± 0.05
18:3n-3	1.72 ± 0.19	1.35 ± 0.09	1.34 ± 0.13	1.28 ± 0.09
18:4n-3	3.35 ± 0.46	3.21 ± 0.27	3.27 ± 0.27	2.38 ± 0.20
20:2n-6	1.04 ± 0.07	0.43 ± 0.02	0.35 ± 0.01	0.47 ± 0.14
20:3n-6	0.30 ± 0.05	0.22 ± 0.03	0.22 ± 0.04	0.30 ± 0.07
20:3n-3	0.65 ± 0.04	0.45 ± 0.03	0.40 ± 0.04	1.12 ± 0.16
20:4n-6	0.53 ± 0.02	0.61 ± 0.09	0.66 ± 0.02	0.72 ± 0.07
20:4n-3	0.81 ± 0.02	0.64 ± 0.03	0.63 ± 0.03	1.12 ± 0.14
20:5n-3	6.48 ± 0.89	10.44 ± 0.37	12.23 ± 0.45	4.04 ± 0.66
22:4n-6	0.00 ± 0.00	0.11 ± 0.05	0.19 ± 0.03	0.00 ± 0.00
22:5n-6	0.91 ± 0.20	0.55 ± 0.06	0.47 ± 0.08	0.74 ± 0.19
22:5n-3	0.82 ± 0.03	1.20 ± 0.05	1.12 ± 0.04	1.57 ± 0.20
22:6n-3	7.20 ± 1.33	13.43 ± 0.71	14.05 ± 0.76	3.49 ± 0.48

Appendix 2 Average (± 1 s.e.) fatty acid concentrations for fish from Prince William Sound collected over the winters 2010 – 2011 and 2011- 2012. Concentrations are expressed as percent of total fatty acids.

	Winter 2010-2011					
Location	Eaglek Bay	Eaglek Bay	Lower Herring	Lower Herring	Simpson	Simpson
	Nov	Mar	Nov	Mar	Nov	Mar
n	18	20	20	19	17	19
Saturates						
16:0	19.31 ± 0.54	16.79 ± 0.29	18.16 ± 0.39	15.86 ± 0.53	19.96 ± 0.72	15.94 ± 0.57
17:0	0.50 ± 0.02	0.38 ± 0.02	0.50 ± 0.03	0.48 ± 0.02	0.71 ± 0.04	0.39 ± 0.04
18:0	3.63 ± 0.16	4.77 ± 0.08	3.55 ± 0.11	5.16 ± 0.18	4.86 ± 0.14	4.17 ± 0.21
22:0	0.19 ± 0.01	0.39 ± 0.02	0.20 ± 0.01	0.36 ± 0.03	0.38 ± 0.03	0.29 ± 0.03
24:0	0.66 ± 0.04	1.83 ± 0.09	0.51 ± 0.02	1.17 ± 0.14	0.53 ± 0.08	0.76 ± 0.06
MUFA						
16:1n7	9.39 ± 0.70	4.31 ± 0.42	8.03 ± 0.38	6.68 ± 0.73	8.51 ± 0.78	5.96 ± 0.59
18:1n11	0.19 ± 0.01	0.09 ± 0.02	0.19 ± 0.01	0.16 ± 0.03	0.23 ± 0.06	0.26 ± 0.05
18:1n9	11.04 ± 0.47	10.88 ± 0.45	13.24 ± 0.54	16.95 ± 0.91	9.54 ± 0.52	19.81 ± 1.34
18:1n7	4.84 ± 0.14	5.66 ± 0.32	4.29 ± 0.23	1.53 ± 0.49	6.40 ± 0.33	3.32 ± 0.56
20:1n11	0.49 ± 0.03	0.33 ± 0.02	0.57 ± 0.04	0.13 ± 0.04	2.13 ± 0.53	0.34 ± 0.08
20:1n9	2.39 ± 0.40	0.91 ± 0.12	2.02 ± 0.17	1.98 ± 0.34	2.04 ± 0.19	2.51 ± 0.35
22:1n11	3.84 ± 0.90	1.39 ± 0.25	2.55 ± 0.40	1.28 ± 0.29	1.21 ± 0.30	1.86 ± 0.22
22:1n9	1.86 ± 0.17	1.02 ± 0.21	1.17 ± 0.16	1.31 ± 0.33	0.73 ± 0.13	1.86 ± 0.36
24:1n9	2.80 ± 0.12	4.35 ± 0.18	3.19 ± 0.15	4.41 ± 0.25	1.46 ± 0.15	3.29 ± 0.17
PUFA						
18:2n6	1.23 ± 0.07	0.47 ± 0.02	1.24 ± 0.05	0.73 ± 0.08	0.53 ± 0.06	0.56 ± 0.07
18:3n6	0.24 ± 0.01	0.18 ± 0.01	0.20 ± 0.00	0.15 ± 0.01	0.13 ± 0.01	0.13 ± 0.01
18:3n3	1.21 ± 0.08	0.46 ± 0.01	1.21 ± 0.07	0.44 ± 0.05	0.72 ± 0.06	0.19 ± 0.03

Appendix 2 continued.

Location	Eaglek Bay Nov	Eaglek Bay Mar	Lower Herring Nov	Lower Herring Mar	Simpson Nov	Simpson Mar
18:4n3	2.91 ± 0.22	0.67 ± 0.03	3.25 ± 0.27	0.72 ± 0.08	1.77 ± 0.18	0.70 ± 0.06
20:2n6	0.33 ± 0.01	0.10 ± 0.01	0.30 ± 0.02	0.10 ± 0.02	0.50 ± 0.04	0.30 ± 0.03
20:3n6	0.22 ± 0.01	0.20 ± 0.01	0.06 ± 0.01	0.07 ± 0.02	0.16 ± 0.01	0.12 ± 0.02
20:3n3	0.54 ± 0.02	0.67 ± 0.02	0.48 ± 0.01	0.99 ± 0.09	0.66 ± 0.02	0.65 ± 0.07
20:4n6	0.42 ± 0.02	0.68 ± 0.01	0.44 ± 0.02	0.91 ± 0.09	1.01 ± 0.08	0.75 ± 0.09
20:4n3	0.74 ± 0.01	0.67 ± 0.01	0.72 ± 0.03	0.79 ± 0.06	0.72 ± 0.03	0.64 ± 0.03
20:5n3	9.68 ± 0.37	9.14 ± 0.25	10.55 ± 0.39	9.80 ± 0.52	10.56 ± 0.39	8.69 ± 0.40
22:3n3	0.62 ± 0.02	0.59 ± 0.02	0.65 ± 0.02	0.23 ± 0.07	0.17 ± 0.07	0.30 ± 0.06
22:4n6	0.29 ± 0.01	0.29 ± 0.01	0.26 ± 0.01	0.10 ± 0.03	0.09 ± 0.03	0.13 ± 0.03
22:5n6	0.71 ± 0.02	0.92 ± 0.03	0.57 ± 0.04	0.32 ± 0.10	0.55 ± 0.04	0.45 ± 0.08
22:5n3	1.27 ± 0.03	1.56 ± 0.03	1.46 ± 0.05	1.74 ± 0.07	1.92 ± 0.16	1.81 ± 0.08
22:6n3	18.49 ± 1.40	30.28 ± 0.78	20.44 ± 0.87	25.44 ± 1.53	21.83 ± 1.35	23.82 ± 1.62

Appendix 2 continued.

	Winter 2011 - 2012						
Location	Port Gravina Nov 20	Whale Bay Nov 26	Zaikof Bay Nov 20	Eaglek Bay Nov 20	Eaglek Bay Mar 17	Simpson Bay Nov 40	Simpson Bay Mar 20
Saturates							
16:0	18.84 ± 0.57	17.95 ± 0.70	14.60 ± 0.88	17.71 ± 0.53	20.69 ± 0.61	17.72 ± 0.48	15.72 ± 0.44
17:0	0.48 ± 0.04	0.31 ± 0.02	0.25 ± 0.02	0.29 ± 0.01	0.29 ± 0.02	0.37 ± 0.02	0.36 ± 0.03
18:0	3.56 ± 0.15	2.16 ± 0.10	2.02 ± 0.13	3.67 ± 0.13	4.22 ± 0.29	2.68 ± 0.11	2.64 ± 0.19
22:0	0.20 ± 0.06	0.21 ± 0.06	0.04 ± 0.03	0.18 ± 0.01	0.20 ± 0.02	0.45 ± 0.05	0.61 ± 0.08
24:0	0.24 ± 0.08	0.49 ± 0.04	0.03 ± 0.02	0.86 ± 0.04	0.92 ± 0.08	0.36 ± 0.05	0.54 ± 0.12
MUFA							
16:1n7	11.33 ± 0.70	5.85 ± 0.39	4.22 ± 0.46	3.30 ± 0.30	3.99 ± 0.35	7.32 ± 0.48	5.95 ± 0.25
18:1n11	0.94 ± 0.76	0.32 ± 0.03	0.35 ± 0.02	0.54 ± 0.04	0.55 ± 0.05	0.66 ± 0.34	0.24 ± 0.04
18:1n9	16.24 ± 1.33	12.63 ± 0.68	12.47 ± 1.02	12.19 ± 0.67	11.04 ± 0.76	11.69 ± 0.64	10.98 ± 0.63
18:1n7	6.33 ± 0.33	4.22 ± 0.48	3.67 ± 0.34	4.25 ± 0.11	4.23 ± 0.25	3.94 ± 0.20	3.60 ± 0.20
20:1n11	0.87 ± 0.16	0.56 ± 0.04	0.05 ± 0.04	0.38 ± 0.03	0.34 ± 0.05	0.80 ± 0.09	1.16 ± 0.15
20:1n9	1.55 ± 0.43	2.69 ± 0.32	3.15 ± 0.50	3.12 ± 0.40	5.87 ± 1.43	3.68 ± 0.39	3.58 ± 0.41
22:1n11	0.66 ± 0.13	4.61 ± 0.77	9.25 ± 2.02	3.68 ± 0.61	5.56 ± 1.52	4.43 ± 0.93	3.70 ± 1.02
22:1n9	0.74 ± 0.14	2.16 ± 0.19	4.28 ± 0.73	1.27 ± 0.17	1.62 ± 0.25	1.56 ± 0.13	2.07 ± 0.29
24:1n9	2.03 ± 0.25	2.24 ± 0.30	4.65 ± 0.46	3.16 ± 0.13	2.55 ± 0.26	1.96 ± 0.09	2.52 ± 0.22
PUFA							
18:2n6	0.95 ± 0.18	0.73 ± 0.07	0.09 ± 0.07	0.38 ± 0.06	0.30 ± 0.09	0.52 ± 0.06	0.16 ± 0.08
18:3n6	0.18 ± 0.02	0.22 ± 0.01	0.22 ± 0.01	0.16 ± 0.00	0.17 ± 0.01	0.18 ± 0.01	0.27 ± 0.02
18:3n3	1.23 ± 0.10	1.38 ± 0.09	1.39 ± 0.08	0.85 ± 0.07	0.36 ± 0.07	1.17 ± 0.05	1.38 ± 0.07
18:4n3	2.36 ± 0.23	7.59 ± 1.55	3.65 ± 0.16	1.11 ± 0.12	0.75 ± 0.17	6.78 ± 1.14	5.09 ± 1.22
20:2n6	0.23 ± 0.04	0.41 ± 0.03	0.17 ± 0.02	0.43 ± 0.01	0.41 ± 0.04	0.41 ± 0.04	0.40 ± 0.07
20:3n6	0.06 ± 0.01	0.14 ± 0.00	0.02 ± 0.01	0.24 ± 0.01	0.22 ± 0.02	0.15 ± 0.01	0.10 ± 0.02

Location	Port Gravina Nov	Whale Bay Nov	Zaikof Bay Nov	Eaglek Bay Nov	Eaglek Bay Mar	Simpson Bay Nov	Simpson Bay Mar

20:3n3	0.76 ± 0.07	0.55 ± 0.05	0.53 ± 0.02	0.69 ± 0.03	0.49 ± 0.05	0.66 ± 0.06	0.96 ± 0.10
20:4n6	0.57 ± 0.06	0.56 ± 0.03	0.49 ± 0.04	1.07 ± 0.07	0.91 ± 0.08	0.62 ± 0.04	0.71 ± 0.06
20:4n3	0.74 ± 0.07	2.84 ± 0.80	2.43 ± 0.33	0.62 ± 0.04	0.34 ± 0.06	3.19 ± 0.67	1.56 ± 0.67
20:5n3	10.37 ± 0.39	9.34 ± 0.50	9.72 ± 0.40	7.56 ± 0.18	7.87 ± 0.49	9.28 ± 0.48	12.54 ± 0.76
223n3	0.35 ± 0.03	0.61 ± 0.02	0.68 ± 0.02	0.65 ± 0.02	0.65 ± 0.05	0.55 ± 0.03	0.59 ± 0.06
22:4n6	0.06 ± 0.01	0.10 ± 0.04	0.45 ± 0.03	0.05 ± 0.01	0.07 ± 0.01	0.18 ± 0.04	0.30 ± 0.07
22:5n6	0.11 ± 0.02	0.48 ± 0.08	0.77 ± 0.06	0.07 ± 0.04	0.47 ± 0.05	0.24 ± 0.07	0.68 ± 0.14
22:5n3	1.88 ± 0.13	1.65 ± 0.12	1.53 ± 0.07	1.93 ± 0.06	1.47 ± 0.14	1.56 ± 0.12	2.12 ± 0.24
22:6n3	16.13 ± 1.25	16.98 ± 0.99	18.84 ± 0.66	29.58 ± 1.49	23.47 ± 2.32	16.91 ± 0.79	19.45 ± 1.08