Exxon Valdez Oil Spill
Restoration Project Annual Report

Fatty Acid Profile and Lipid Class Analysis for Estimating Diet Composition and Quality at Different Trophic Levels

Restoration Project 98347
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in the annual report.

Ron A Heintz
Marie Larsen
Larry Holland

National Marine Fisheries Service
Auke Bay Laboratory
11305 Glacier Hwy.
Juneau, AK 99801

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Study History: This is the first report submitted for Restoration Study 347, therefore it contains previously unreported data on the lipid class and fatty acid composition of juvenile sandlance and prey. Fish samples described here were collected by APEX investigators from several locations in Prince William Sound (PWS) during a two week period in July 1997. These samples provide the basis for evaluating the spatial variation in fatty acid composition of fishes found in different parts of PWS and examining how these differences relate to the animal's nutritional condition at the time of capture. The second component of this study will examine the fatty acid composition of the prey for these species and evaluate of how developmental stage and seasonal progression influence the fatty acid composition of sandlance. Those data will be provided in a later report.

Abstract: The analysis of lipid class and fatty acid (FA) composition in juvenile herrring and sandlance demonstrates a high degree of localized variation in the availability of energy that may be related to the abundance of specific prey. Herring and sandlance juveniles were sampled from several locations around Prince William Sound during a two week period in late July, 1997. Analysis of their lipid class and FA composition revealed sandlance and herring had signficant differences in the relative amount of triglyceride (TAG) in their lipids depending on the location where they were sampled. These data indicates that the ability of these species to acquire energy varied between sampling sites, even when they were 20 km apart. Fish with high levels of TAG tended to have relatively invariant FA compositions suggesting their acquisition of energy resulted from the consumption of a limited number prey types. These data, while still preliminary, indicate that the combined analysis of lipid class and FA composition may be extremely powerful tool for identifying localized groups of foragers and ranking the relative success of those groups.

Key Words: Fatty Acid Composition, Sandlance, Herring, Lipid Class, Nutritional Condition

Project Data: This report contains fatty acid data collected from juvenile sandlance and herring collected under APEX 163A in July 1997. In addition to sample collection information and quality assurance data, the concentrations of 29 fatty acids and 10 lipid classes have been determined for 45 juvenile herring, and 60 juvenile sandlance. These data are maintained in a Microsoft Access database at the National Marine Fisheries Service Auke Bay Laboratory. The data will be available for public inspection once this project is complete.

Contents

Executive Summary The analysis of lipid class and fatty acid (FA) composition in juvenile herrring and sandlance demonstrates a high degree of localized variation in the availability of energy that may be related to the abundance of specific prey. Herring and sandlance juveniles were sampled from several locations around Prince William Sound during a two week period in late July, 1997. Analysis of their lipid class and FA composition revealed sandlance and herring had signficant differences in the relative amount of triglyceride (TAG) in their lipids depending on the location where they were sampled. These data indicates that the ability of these species to acquire energy varied between sampling sites, even when they were 20 km apart. Fish with high levels of TAG tended to have relatively invariant FA compositions suggesting their acquisition of energy resulted from the consumption of a limited number prey types. These data, while still preliminary, indicate that the combined analysis of lipid class and FA composition may be extremely powerful tool for identifying localized groups of foragers and ranking the relative success of those groups.

This is the first year of a two year project. In the second year we will examine 1) how the FA compositions of the fish reported here relate to that of their prey, 2) how FA compositions change with time for a given year class, and 3) how developmental changes influence FA composition. We have already processed 21 composited prey samples representing 8 taxa through our chemistry lab, and statistical evaluation of those data are currently underway. APEX investigators have provided us with juvenile, immature and mature sandlance samples from Kachemak Bay collected between May and September, 1998. These samples have all been processed to determine their lipid class composition, and analysis of their FA composition is nearly complete.

Introduction

Analysis of lipid class and fatty acid (FA) composition in fish may potentially provide a method for identifying localized groups of foragers and provide a basis for ranking the relative success of those groups. Recently, the analysis of the FA composition of predators has been proposed as a method for distinguishing localized groups of seals (Iverson et al. 1998) in Prince William Sound (PWS). These observations rely on the assumption that the FA composition of a predator reflects that of its diet, indicating the existence of localized variation in the FA composition of seal prey. Analysis of FA composition of seal prey may therefore provide a reliable tool for discriminating groups of fish foraging on different prey fields. The consequences of dietary differences, as inferred from the FA composition, can be determined by analysis of the lipid class composition. The relative concentration of triacyglycerides (TAG) provides a measure of the amount of surplus energy reserved by an organism. Comparison of the relative amount of TAG in different foraging groups provides a measure of how successful those groups are at acquiring energy. These analytical methods further complement each other because FA acquired from prey are usually stored without modification as TAG (Stryer 1978). Consequently, analyzing a predator's TAG for its FA composition may potentially provide a powerful tool for discriminating local foraging groups and evaluating their relative success.

The utility of these methods has been demonstrated under laboratory conditions, but field evaluations are rare. Usually discrimination of foraging groups under laboratory conditions relies on relatively large dietary differences that may overstate the differences likely to be encountered in the field (Viga and Grahl-Nielsen 1990). Lipid class analysis has been demonstrated to have large potential for ordering groups with different success at obtaining energy, but field based examples are rare. Testing of these methods in the field requires examination of species known to forage in localized areas with varying amounts of success at obtaining energy.

Juvenile herring and sandlance in PWS provide an excellent field test of the potential for these analyses as tools for discriminating subtle diet differences between localized foraging groups, and ranking their success. Local populations of juvenile herring and sandlance, in Prince William Sound (PWS), are thought to be isolated because of their propensity to rear in those bays where they metamorphose from larvae (Paul and Paul 1999), and they select prey in proportion to their abundance (Sturdevant et al. 1998). Apparently, their diets vary spatially in response to random variation in the composition of local prey fields, with the result that some populations acquire lipids at higher rates than others (Paul and Paul 1999).

We present data collected to evaluate the utility of (1) the analysis of FA composition for discriminating spatially segregated populations of juvenile sandlance and herring and (2) the analysis of lipid class composition for examining the nutritional consequences for these predators. In particular, we report the analytical methods used to determine the FA and lipid class composition in fish samples collected from different parts of PWS in late July 1997. We also present tables summarizing these observations for each species. Statistical evaluation of these data completed thus far include examination of the spatial variation in TAG content that of FA compositions among fish collected from different parts of PWS is nearly complete.

In this report we rely on the assumption that FA composition in predators is determined by their prey to infer that differences in FA composition are the result of dietary differences. In FY99 when FA composition information is available for the prey of these species we can begin examining this assumption directly.

Objectives

In FY98 we evaluated the following 5 hypotheses that are derived from the underlying assumption that a predator's FA composition is largely determined by that of its prey.

(1) TAG content of herring and sandlance depends on the location where these fish are sampled.

(2) Local populations of forage fish can be discriminated by their FA compositions.

(3) FA compositions in sandlance and herring foraging in the same location are similar.

(4) The populations discriminated on the basis of their FA compositions have acquired different amounts of TAG.

(5) Spatial patterns in TAG content are identical to those for lipid content in sandlance and herring.

The first two hypotheses derive from the suggestion that local differences in forage should lead to concomitant differences in the FA compositions and amount of energy acquired by different predators. The third hypothesis examines the idea that different predators who consume the same prey should have similar FA compositions if their FA compositions are determined by their prey. However, if the variation in FA composition among fish collected from a single location is as great as the variation observed between locations, then it can be concluded the there are either no detectable dietary differences between locations, or the underlying assumption requires re-evaluation. Given the existence of dietary differences between local populations the fourth hypothesis asks if these diets result in differing probabilities of survival. The final hypothesis is designed to determine if lipid class analysis provides a more sensitive measure of local variation in energy content than proximate analysis.

Testing these hypotheses required meeting these specific objectives in FY98.

1. Acquire samples of herring, sandlance and their prey collected simultaneously from different locations around PWS.

2. Develop analytical methods for characterizing the FA and lipid class compositions of forage fish and their predators.

3. Identify differences in the TAG content in of herring and sandlance collected from different locations.

4. Identify differences the FA composition of the TAG component of the lipids in sandlance and herring collected from different locations.

5. Compare the FA composition of TAG taken from herring and sandlance sampled from the same location.

This is the first year of a two year project. In FY99 we will examine 1) how the FA compositions of the fish reported here relate to that of their prey, 2) how FA compositions change with time for a given year class, and 3) how developmental changes influence FA composition. We have already processed 21 composited prey samples representing 8 taxa through our chemistry lab, and statistical evaluation of those data is currently underway. APEX investigators have provided us with juvenile, immature and mature sandlance samples from Kachemak Bay collected between May and September, 1998. These samples have all been processed to determine their lipid class composition, and analysis of their FA composition is nearly complete.

Methods

Sample Acquisition

Juvenile sandlance and herring were collected with beach seines between July 19 and August 4, 1997 from sites near Naked Island and Bainbridge Passage by APEX investigators. Fish were sorted by species and each collection was stored in a separate polyethylene bag. Samples were immediately frozen and shipped to Juneau by APEX investigators for long term storage at -20° C.

Fish collections were selected for lipid analysis from the APEX inventory on the basis of the composition of the catch at a site, and the site's location. Sites located near Naked Island are termed "central" and sites near Bainbridge Passage "southwestern" PWS. In each of these general areas we attempted to examine fish collected from sites where herring and sandlance either occurred alone or together. The selected samples permitted comparison of groups distributed over relatively small (≈20 km) and large(≈90 km) distances.

Table 1 summarizes the collection dates and times, latitudes and longitudes of specific locations, provides the set numbers used by APEX to identify the sites, and proviides our code for the site name. In the rest of this report we will refer to the sites with a species abbreviation (SL for sandlance and H for Herring) followed by an abbreviation for the general location of the site (S for southwestern or C for central) and the number of competitors simultaneously sampled there (0 or 1). Thus, SL-S0 refers to the site in southwestern PWS where sandlance were the only species caught in the beach seine. All the site locations are shown in Figure 1, and 15 fish were selected for analysis from each site. Although we identified a site in central PWS where both sandlance and herring were sampled, we only examined sandlance from that site because APEX investigators had insufficient numbers of herring to permit our analysis.

Individual fish from each collection were randomly selected, weighed, and measured. Herring stomachs and sandlance viscera were removed to ensure that the analysis would not be influenced by recently consumed prey. Samples were immediately placed in individual vials, covered with a chloroform/butylated hydroxy toluene (BHT) solution, an antioxidant, and labeled

with unique sample identification numbers (SIN).

Analytical Methods for determining Lipid Class Composition

Lipids from sandlance, and herring were extracted by methods developed by Bligh and Dyer, modified by Folch, and outlined in Christie (1982). Wet tissue was homogenized and extracted with a tissuemizer probe in a solution of 33% methanol and 66% chloroform. Each mixture was vacuum filtered and the solid residue re-extracted in a solvent solution of the same proportions. The mixture was re-filtered and the combined filtrates underwent a liquid-liquid extraction with an aqueous solution of 0.88% potassium chloride (KCl) at one quarter of the total volume of filtrate. The bottom layer of the resultant biphasic solution was withdrawn and underwent a second liquid-liquid extraction with one quarter of its volume of a fresh aqueous KCl solution. The bottom layer which contained the purified lipid was withdrawn and its volume reduced with a rotovap concentrator. This method of extraction has been documented by Christie to yield 95-99 percent recovery of lipids.

The purified lipid was separated into lipid classes on a high pressure liquid chromatograph (HPLC) and quantified with an evaporative light scattering detector (ELSD). Representative samples of each species were initially processed to determine sample dilution factors required to keep chromatographic signals within calibration range. Samples were diluted, spiked with internal standard, phosphatidyldimethylethanolamine (PDME), and processed by HPLC. The HPLC method employed is based on Christie (1985), i.e., used a silica column and a tertiary solvent gradient system, but has been modified slightly to optimize separation of lipid components. Lipid classes were quantified by processing a series of lipid calibration standards by the identical instrumental parameters and from the chromatographic data, establishing calibration curves for individual lipid components. The chromatographic data from the samples were used along with the mathematical definitions of the calibration curves to quantitate the amount of each lipid class in the samples. Reference samples and method blanks were also processed as quality assurance checks for accuracy, precision, and method cleanliness.

The HPLC system was equipped with a stream splitter and a fraction collector. The stream splitter allowed one fraction of the HPLC eluant to flow to the ELSD for quantitation as describe above, and the other fraction flowed to the fraction collector where the eluant was collected for FA analysis. The TAG fraction of sandlance and herring samples were collected for fatty acid (FA) analysis to maximize the potential for discriminating samples on the basis of dietary lipids.

Analytical Methods for determining FA Composition

FA profiles for sandlance and herring were determined on the fraction isolated by HPLC as described above. Lipids were transesterified to fatty acid methyl esters (FAME) as outlined in Christie (1982). Samples were spiked with surrogate FA, C19:0 and C23:0, acidic methanol was added, and the mixture was heated at 80^{E} C for two hours. The esterification reaction was stopped by removing the sample from the heat and adding an aqueous solution of sodium chloride (NaCl). The biphasic solution was extracted twice with hexane and the extract washed with

aqueous solution of potassium bicarbonate. The hexane layer was isolated, dried over anhydrous sodium sulfate, and concentrated to 1 ml under a stream of nitrogen. The sample is spiked with internal standard, C21:0 methyl ester, and stored in a refrigerator until analysis.

FA were separated on a gas chromatograph equipped with a mass selective detector (GC/MS) operated in selected ion monitoring mode (SIM). FAMEs were separated on a 30m Omegawax 250 fused silica capillary column with the sample injected at a temperature of 80 C then ramped to 260 C. FA were quantified by processing a series of FAME calibration standards using identical instrumental parameters, and from the chromatographic data, establishing calibration curves for individual FA components. The chromatographic data from the samples are then used, along with the mathematical definitions of the calibration curves to quantitate the amount of each FAME component in the samples. Reference samples and method blanks are processed as quality assurance checks for accuracy, precision, and method cleanliness.

Statistical Analysis

ANOVA was used to test the hypothesis that length, percent lipid and relative amount of TAG in the lipids did not vary between locations in PWS for each species. For herring, these tests were constructed as a one-way ANOVA testing the 3 hypotheses that length, lipid content and TAG content were similar at all three sites where fish were sampled. Differences between samples collected at southwestern and central parts of PWS were examined with t-tests. For sandlance, the same hypotheses were tested with a heirarchical design with sampling sites nested in central or southwestern PWS. For all the ANOVA's the assumptions of normality and homogeneity of variance were examined prior to analysis and appropriate transformations were made. The relationship between relative TAG concentration and fish size was examined by regression analysis.

Spatial differences in the fatty acid compositions of herring and sandlance were examined by principle components analysis (PCA) of the covariance matrix derived from the normalized raw data matrix. PCA models, 1 for each species, were used to evaluate differences in the fatty acid compositions of fish collected from different sampling sites. The number of components that best described each of the 2 data sets was determined by the factor indicator function (Malinowski 1991). Factor scores were calculated for each observation in the data set and plotted to examine their relative positions in the space described by the PCA model. The mean square error (MSE) for each observation was calculated as the sum of the squared residuals between the normalized data matrix and the data matrix predicted by the PCA. The mean MSE was calculated for each sampling location and these means were tested by a one-way ANOVA to determine if they were equal.Only 13 FA were used to generate the PCA models (Table 1), however these 13 FA account for at least 98% of the FA observed in any fish. Observed FA concentrations were normalized by transforming them to standard normal deviates prior to analysis by PCA.
Sandlance data were fit to the herring model for those samples collected in the same location, and plotted to examine the similarity in the FA compositions. More detailed analysis of relative distances between group centroids is pending.

Results

Spatial differences in size, lipid and TAG content of herring

The relative amount of TAG in herring lipids depended on the location where fish were sampled. The mean relative concentrations differed among all three sampling sites, ($P = 0.004$) (Figure 2A) and the highest mean relative concentration, 9.5± 6.4% (mean±1 s.d.), was observed among those herring that were caught along with sandlance at H-S1. The lowest mean relative concentration was 3.0 ± 2.8% for fish collected at H-C0. This meant that the mean relative concentration of TAG was signficantly higher in the southwestern part of PWS ($P = 0.003$) with those fish having more than twice the relative amount of TAG as those from the central sampling site. These observations were independent of fish size ($P = 0.26$) (Figure 3A), and the highest TAG content was observed among the smallest fish (figure 2A), suggesting that local variation in TAG content was independent of food availability. Further, the August sampling dates (Table 1) suggest that the influence of residual yolk from the larval stage on TAG content was nonexistent or minimal.

Spatial trends in TAG content were more conspicuous than those for total lipid content. Mean lipid content was greatest for those fish sampled at site H-S1 as was TAG, but no differences were observed in the lipid content of fish collected at sites H-S0 and H-C0 (Figure 2A). Consequently, no differences in the mean lipid content of fish from the southwestern and central parts of PWS were detected ($P = 0.529$).

Herring sizes also depended on where they were sampled ($P = 0.05$), and the smallest herring were those collected along with sandlance at site H-S1. However, despite the presence of competitor and their small size these fish had the highest TAG and lipid content (Figure 2). Fish from the remaining two sites were similar in size, but those from H-C0 which were on the average the largest, had the lowest TAG content.

Spatial differences in size, lipid and TAG content of sandlance

The relative amount of TAG in sandlance lipids was greater than that of herring, but also depended on the location where fish were sampled. Variability between sampling sites within the southwestern region (Figure 2B) was greater than variability between the central and southwestern regions. Thus, the mean relative concentrations differed among sampling sites ($P < 0.001$) but not between the southwestern and central parts of PWS ($P= 0.137$). The highest mean relative concentration, 38.6 ± 8.4%, was observed at site SL-S1, the same site where herring TAG was greatest. The lowest mean relative concentration, 9.9 ± 7.9% for fish collected at SL-S0, was greater than the maximum value observed for herring. The wide variability in TAG content observed in southwestern PWS was not seen in central PWS (Figure 2B).

As with herring, lipid content depended on where sandlance were sampled, but predicted a spatial pattern to energy acquisition different from that of TAG content. While lipid content differed among sampling sites ($P < 0.001$) the local differences were less than those between southwestern and central PWS ($P < 0.001$). As with TAG, the mean lipid content was greatest in southwestern PWS averaging 3.2 ± 0.9% compared 2.3 ± 0.6% for central PWS. However, in contrast to the pattern observed for TAG content, lipid content of sandlance collected from site

SL-S0 had the second highest lipid content.

Unlike herring, TAG content in sandlance was highly dependent on fish size ($P < 0.001$) (Figure 3B) suggesting that sandlance grow best when they are acquiring surplus energy. The spatial distribution of sandlance size was similar to that of their TAG content with significant differences existing between sampling sites ($P = 0.001$), but not between southwestern and central PWS ($P = 0.349$). As with TAG, the largest difference in length was for fish from sites SL-S1 and SL-S0, and those collected along with herring had the greatest average size, 97.4 ± 5.7 mm. Size of fish from the central part of PWS seemed to be relatively unaffected by the presence of herring (Figure 3B).

Differences in Fatty acid composition

Both herring and sandlance had relatively large amounts of essential FA in their TAG (Table 1). The combined concentrations of C20:5n3 and C22:6n3 accounted for more than 30% of the TAG in herring and sandlance. Overall, polyunsatruated FA with 18 or more carbons accounted for nearly two thirds of the TAG fatty acids. The most abundant FA in both species was C16:0 with mean relative concentrations of 27 ± 4.0 % and 23 ± 3.0% for herring and sandlance, respectively.

Examination of variation in the FA composition of herring collected from different locations with PCA indicated two primary components accounted for 83% of the error in the data set. Examination of the loads associated with each of the FA used to generate the model did not indicate any particular FA formed the basis of the factors. The modeled data had an average mean squared error (MSE) of 0.09. However, the average MSEs differed among the sampling locations meaning that the FA compositions of herring from site H-C0 (average MSE = .17) were not as well explained by the model as those from the southwestern hauls with average MSE=s of .05 and .06 for sites H-S0 and H-S1, respectively. However, these differences were barely signficant ($P = 0.07$).

The better fit of the two southwestern sites is evident on plots of the scores of samples taken from these locations when they are plotted on the first two component axes (Figure 4). Samples from the two southwestern hauls cluster tightly near the origin, while samples from the central haul vary widely. This plot suggests the FA composition of the herring from central PWS may differ from that of herring collected in southwestern PWS.

Similar examination of variation in the FA composition of sandlance collected from different locations indicate two primary factors account for a total of 79% of the total error. Examination of the loads indicates that the first factor is largely influenced by the two essential FA; C20:5n3 and C22:6n3. Much of the variation associated with the second factor is explained by variation in C16:1. The modeled data have an average MSE of 0.404, and each of the different locations had a disparate influence in the overall mean ($P = 0.014$). As with the herring the average MSE for samples collected in southwestern site SL-S1 had the lowest average MSE, .213, which was less than a third of the average MSE of the other sample collected in the southwestern part of PWS.

Plots of the the first two component scores demonstrate that FA compositions in sandlance collected at site SL-S1 were much less variable than those collected in the central part of PWS at SL-C0 and SL-C1 (Figure 5). Samples collected at SL-S1, which also contained herring, cluster

tightly near the origin while those from the two central locations are spread over a larger area. Samples collected at site SL-S0 are coincident with those of SL-S1, suggesting the southwestern FA compositions may be more similar to each other than the compositions from the two central sites. The positions of the samples from central PWS relative to those of southwestern PWS are reminiscent of the herring plot, suggesting that there may more similarity in FA composition between species within a region, than between regions for a given species.
This is further seen in Figure 6 where sandlance collected from site SL-S1 are plotted on the herring plot, after the sandlance were fit to the herring model. The similarity in the distribution of samples collected from the site where herring and sandlance co-occur suggest a high degree of similarity in their FA compositions.

Discussion

TAG content is a sensitive measure of the differences in the quality of the prey fields encountered by fish in different locations. Measurements of TAG reported here indicate that energy availability is highly variable between locations. However, a clinal distribution of energy content inferred from the observations of sandlance lipid content was inconsistent with the spatial distribution of TAG content. While lipid content is useful for inferring the whole body energy content of a prey item, it apparently provides less information about the nutritional condition of a predator. This distinction is important because spatial variability in whole body energy content has been used to describe the energy density of herrring (Paul and Paul 1999) and infer their probability of overwinter survival. Values reported here for total lipid are for eviscerated fish and therefore may be lower than those reported by Paul and Paul (1999a), but retaining the viscera is unlikely to alter the improved precision affored by lipid class analysis.

The differences in the relation between lipid content and TAG content may point to differences in the way herring and sandlance convert their food into tissue. In herring, fish from H-C0 and H-S0 were similarly sized, had similar lipid contents but had disparate amounts of TAG. The PCA model suggested that they had different FA compositions which sugggests their diets may differ. One possible explanation for these observations is that one diet has high lipid content, but little protein so energy acquisition is high, but growth is slow. The alternative diet may have high protein content and consequently growth is high, but lipid content is low. This does not appear to be the case for the sandlance. Sandlance have a size related potential to acquire energy from their prey. Thus the smallest and largest sandlance appear to have similar FA compositions, hence diets, but disparate relative amounts of TAG. This suggests larger sandlance convert a greater proportion of their diets into tissue which is consistent with observations reported for cod larvae (Olsen et al. 1991).

Other important metabolic differences between sandlance and herring are apparent from the TAG data. Sandlance demonstrated a significant relationship between size and TAG suggesting simultaneous partitioning of energy between growth and storage. Acquisition of surplus energy is important for sandlance because their foraging is linked with specific benthic habitats where they burrow in the substrate to avoid predation (Hobson 1986). Consequently, sandlance may not forage as frequently as herring, and may have to wait for prey patches to transit their preferred substrates. Alternatively, herring can freely follow their prey and avoid predation by growing quickly. Thus, acquired energy is shunted primarily into growth resulting in an independence

between size and TAG content. This explanation also accounts for the overall lower TAG content of herring compared to sandlance. However, lipid content of herring prior to the onset of winter fasting periods indicates a need for juveniles to acquire surplus energy. It therefore, seems likely that herring may partition some energy into TAG as their growth reduces the probability of predation (Paul and Paul 1998).

The TAG data also indicate that the mutual presence of these two species does not inhibit either's ability to acquire energy. Herring and sandlance both acheived the greatest mean TAG content at site H-S1. This same lack of inhibition is seen in sandlance collected along with herring in haul SL-C1 which had similar TAG content to similarly size sandlance caught alone in haul number SL-C0. This contrasts with observations reported by Sturdevandt et al. (1999) who reported an increase in the number of empty stomachs in both herring and sandlance when they co-occurred. However, our observations of energy acquisition suggest a minimal effect of co-occurrence on energy acquisition. Rather, the relatively small size of the herring at H-S1 suggests that the mutual presence may be inhibiting acquisition of some other resource such as nitrogen (Short and Harris 1999).

One interpretation of the observation of elevated TAG accumulation when both species co-occur is their presence represents a numerical response to the increased availability of prey. PCA results for both species indicated the least amount of variation in the FA compositions occurred in fish collected at SL-S1, suggesting a single factor could explain the variation observed in that set. Scores for the sandlance observations from SL-S1 after they were fit to the herring model, demonstrated a high degree of fidelity to the scores for herring from the same location. This indicates that the same factor was explaining the variation in their FA compositions which is consistent with observations of herring and sandlance consuming similar prey (Sturdevant et al. 1999).

This concept that individuals from both species profit simultaneously from the presence of abundant prey is supported by the observation that individuals in the locations with the highest TAG had the least variable FA compositions. The error in fitting the PCA models for both herring and sandlance depended on where the samples were located so that those individuals with the greatest tag also had the lowest MSE (Figure 7). The converse, individuals with low TAG had highly variable FA compositions, suggests those individuals consumed more diverse diets with less success at obtaining energy. Perhaps the small size and high TAG content of herring at site H-S1 indicates that the prey were a poor source of nitrogen thereby resulting in inhibited growth despite the availability of sufficient energy.

The analysis of lipid class and FA composition demonstrates a high degree of localized variation in the availability of energy that may be related to the abundance of specific prey. Consequently, the potential for using FA compositions to identify dietary differences between groups of fish is encouraging. However, identifying the underlying compositions of the different diets remains problematic. While the variation in the FA composition of forage fish species may be dictated by the abundance of their prey, it is unlikely that these compositional differences will be conserved through time. FA compositions for fish collected at SL-S1 as described by the PCAs employed here may have resulted from the availability of a single zooplankton species, or some combination of a multiple number of species. However, the relative abundance of zooplankters varies over time and space, as does the factor space. Without a consistent factor space, identifcation of the prey responsible for the primary factors is analogous to shooting at a moving

target.

Conclusions

1) TAG content of herring and sandlance in Prince William Sound show a high degree of local variation, so that differences between adjacent bays may be greater than the differences between regions.

2) Variation in FA composition is evident from PCA plots for herring and sandlance, these plots suggest that existence of regional differences in FA composition.

3) When sandlance collected along with herring are fit to the herring PCA model for FA composition, the differences in FA composition for a given species sampled in different locations appears to be greater than species differences in a given location. This lends support to the idea that a predator=s FA composition is influenced by its prey.

4) TAG content appears to be related to the specificity of the predator=s diet as a result of the wide availability of a given prey item in a specific location. This is supported by the observation that the mutual presence of sandlance and herring indicates areas where the most energy was acquired.

5) The relative concentration of TAG is a more precise estimator of the amount of surplus energy contained in a predator than total lipid.

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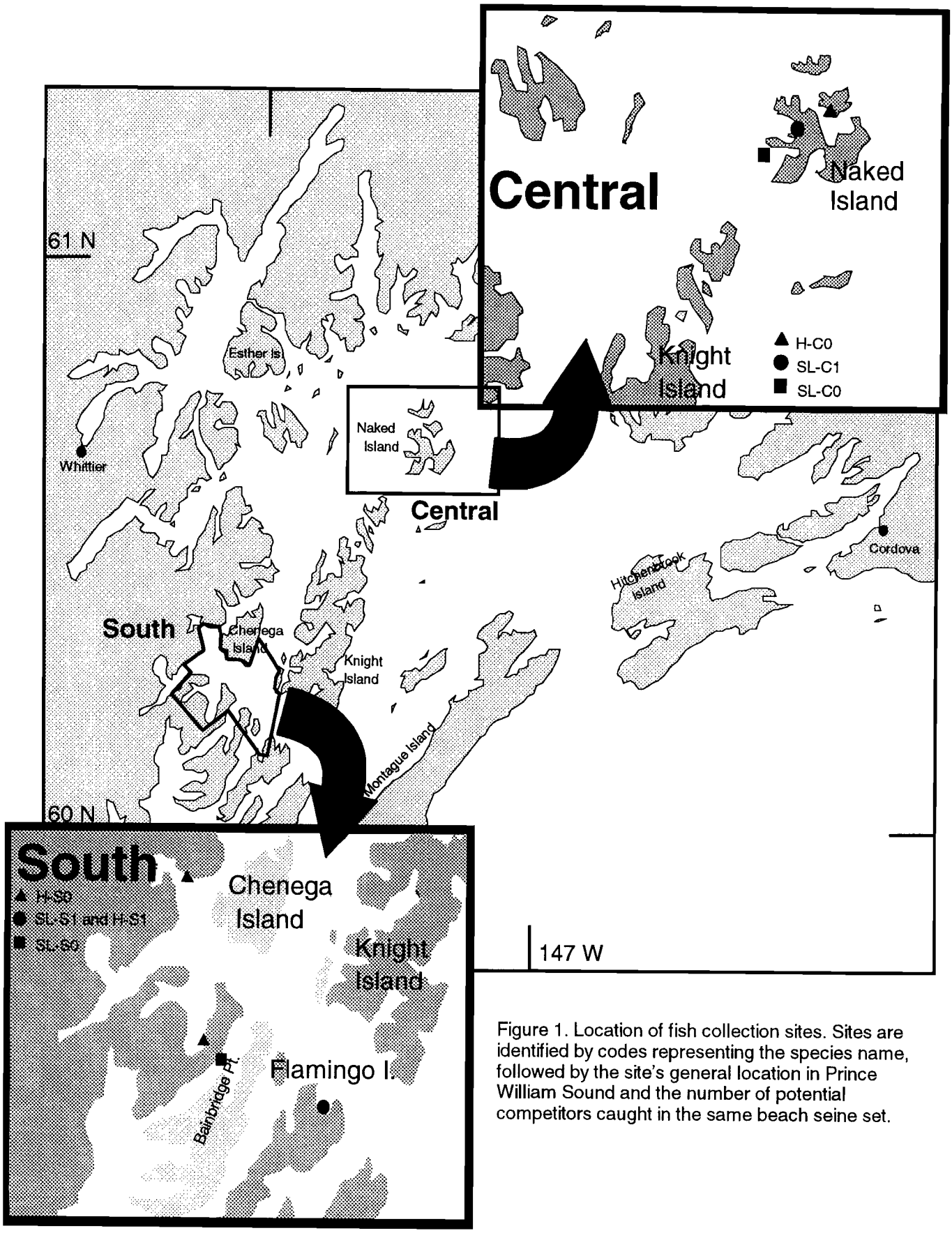

Figure 1. Location of fish collection sites. Sites are identified by codes representing the species name, followed by the site's general location in Prince William Sound and the number of potential competitors caught in the same beach seine set.

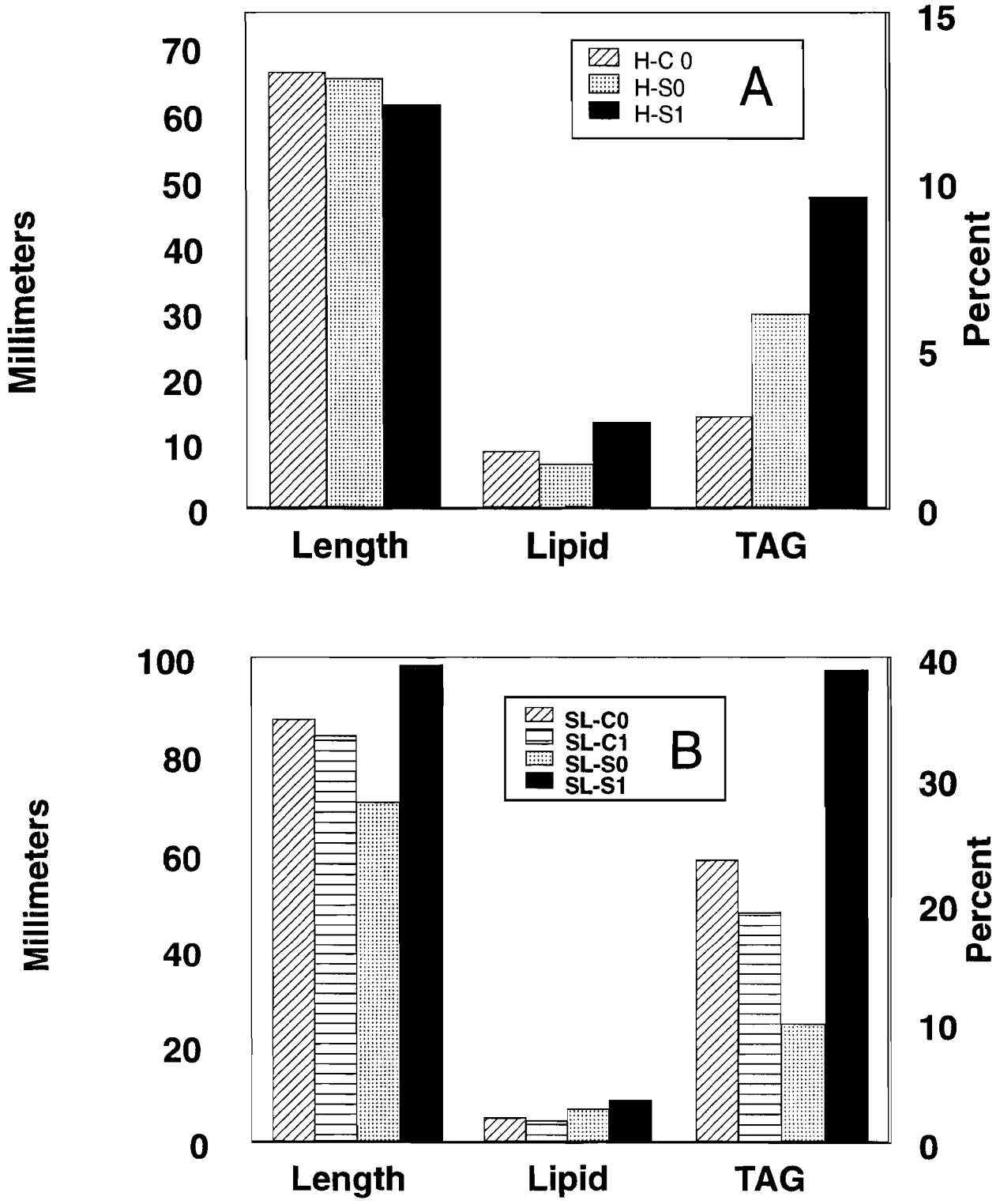

Figure 2. Mean length, percent lipid and triacylglyceride (TAG) of herring (A) and sandlance (B) sampled in different locations of PWS in July, 1997. In each case means were found to be different by ANOVA ($P < 0.05$) . Percent lipid is average proportion of wet weight found to be lipid, TAG content is expressed as the percent of the total lipid content. The scale for percent lipid and TAG is located on the right of each chart.

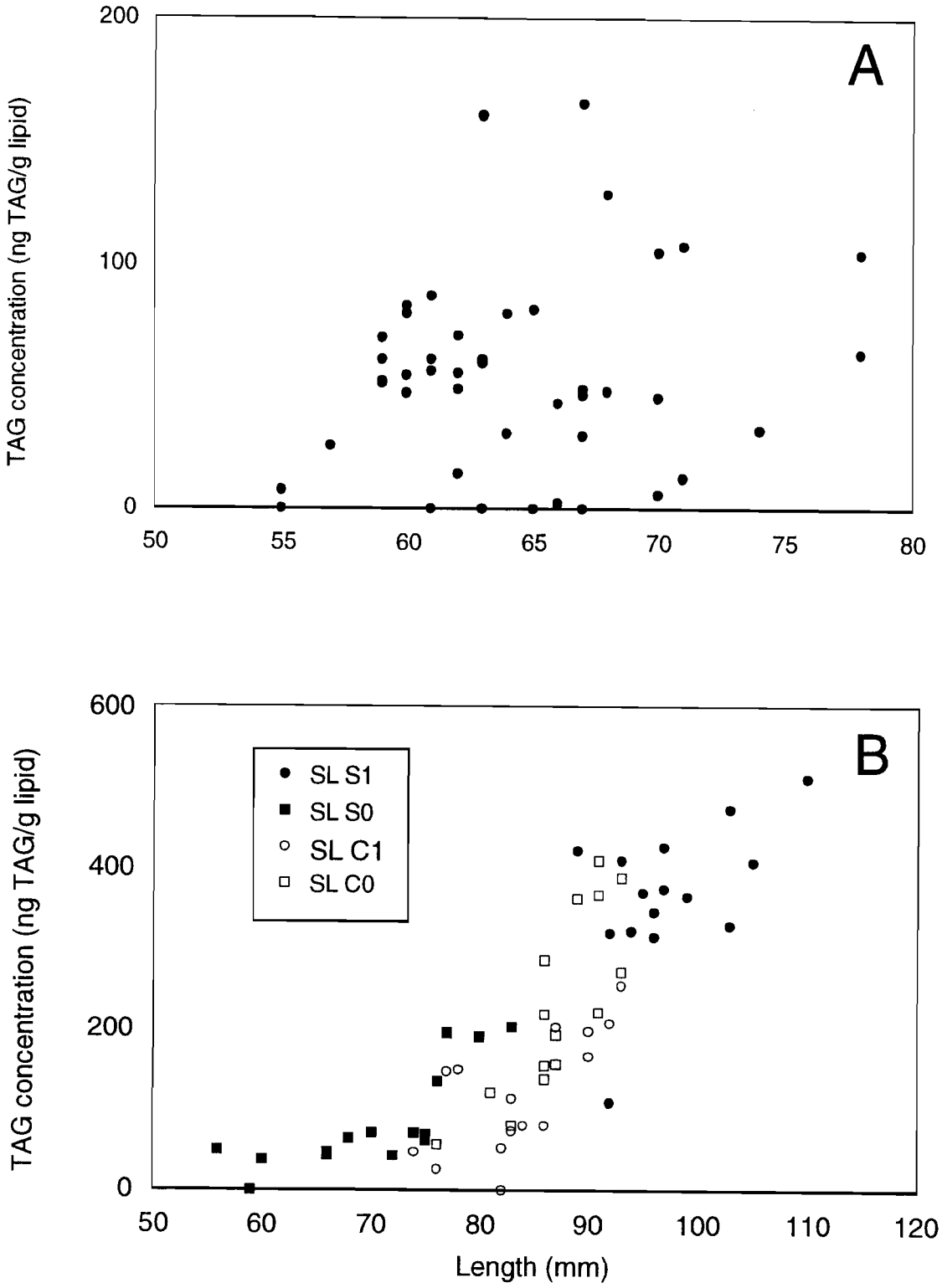

Figure 3. Relationship between TAG content and length in herring (A) and sandlance sampled in Prince William Sound in July, 1997. No relationship was found for herring (P = .940), while in sandlance the relationship is highly signficant (P = 0.001).

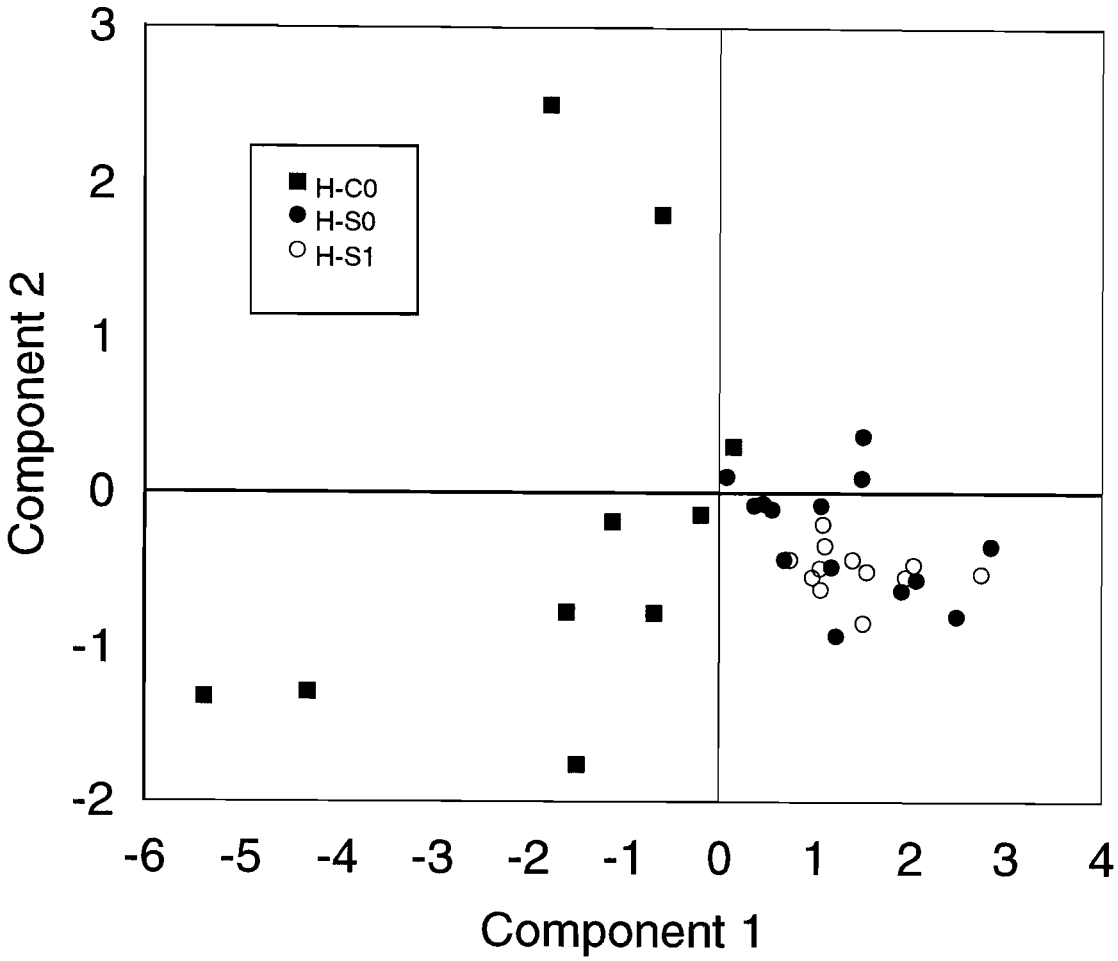

Figure 4. PCA scores for herring samples collected from various locations in Prince William Sound (PWS) in July 1997. Herring are represented from 3 locations, H-S0 is the site in southwestern PWS where only herring were caught, H-S1 the site where herring and sandlance were caught together, and H-C0 the site in central PWS where herring were caught alone.

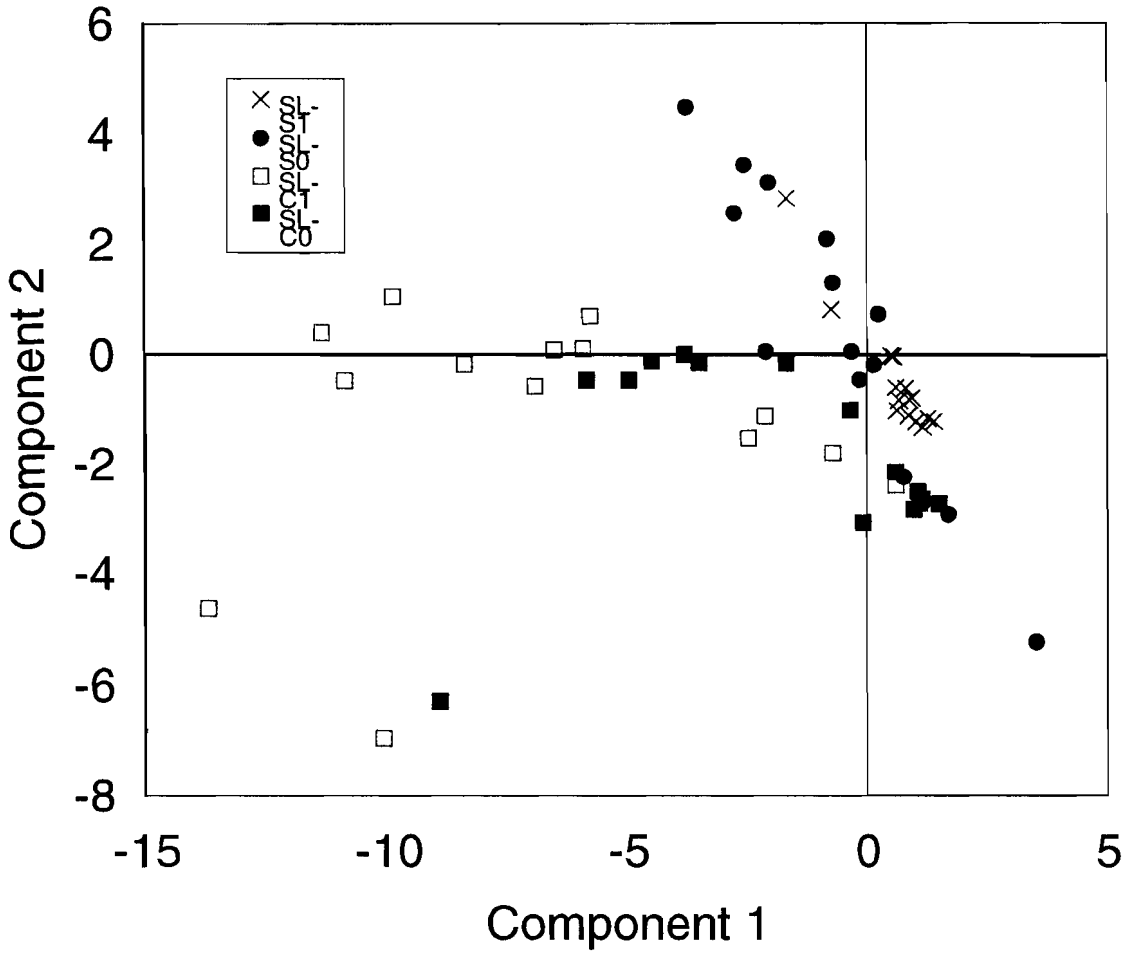

Figure 5. PCA scores for sandlance samples collected from various locations in Prince William Sound (PWS) in July 1997. Sandlance are represented from 4 locations, SL-S0 is the site in southwestern PWS where only sandlance were caught, SL-S1 the southwestern site where herring and sandlance were caught together, and SL-C0 the site in central PWS where sandlance were caught alone, and SL-C1 the site where sandlance and herring were caught together.

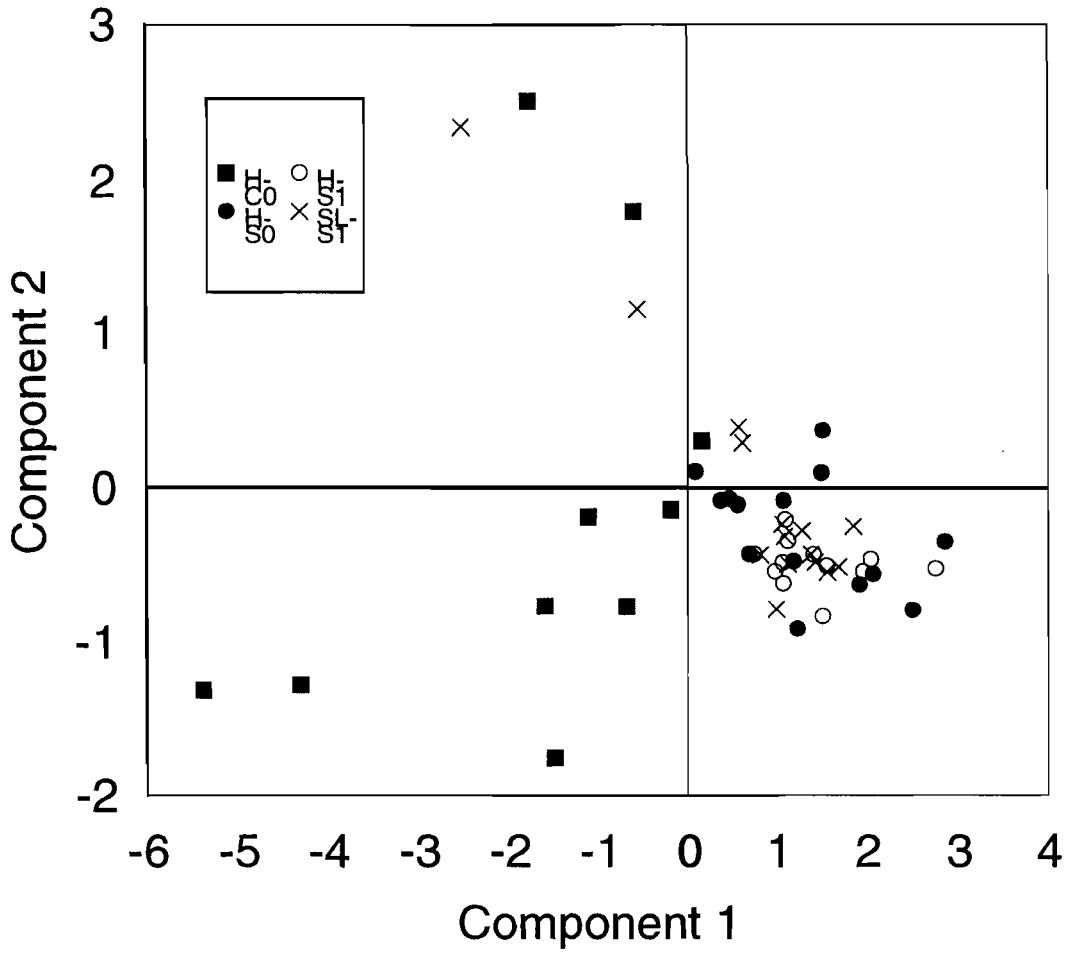

Figure 6. PCA scores for herring and sandlance fit to the herring model. Sandlance samples were collected the site in southwestern Prince William Sound where both species were caught together.

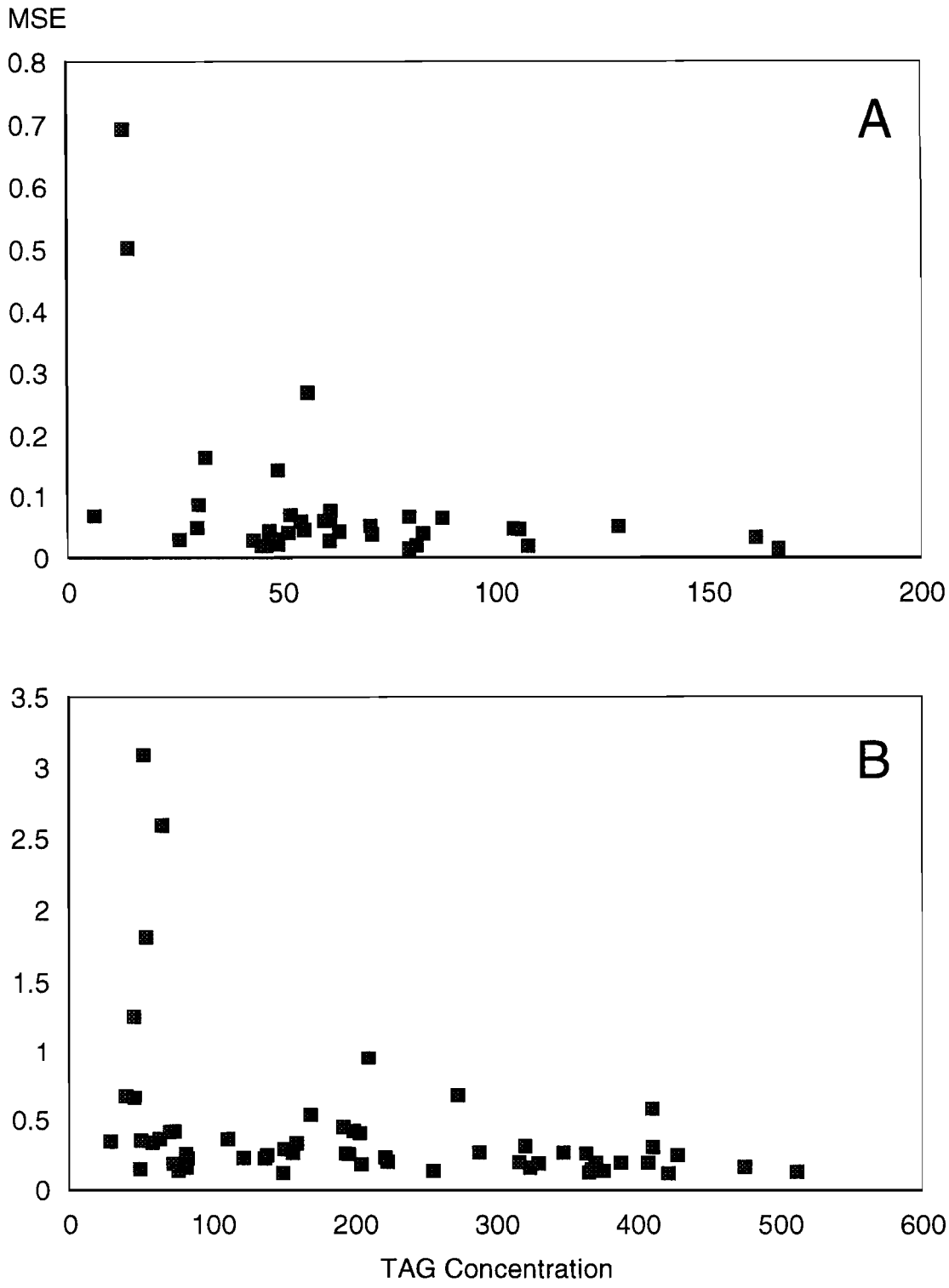

Figure 7. Relationship between the mean squared error (MSE) for the PCA models and the amount of TAG for each of the herring (A) and sandlance (B) sampled.

Table 1. Latitude, longitude APEX identification numbers and location codes for sites where fish were sampled. Fish from each of these locations were analyzed to determine their lipid class and FA compositions.

DATE	TIME IN	STN	DESCRIPTION	LAT IN	LONG IN	CODE
19/07	14:15	14	South PWS Sandlance Only	60 08.94	148 09.46	SL S0
24/07	11:00	51	Central PWS Sandlance Only	60 38.75	14729.71	SL C0
02/08	09:45	135	Central PWS Herring Only	60 41.52	14728.65	H C0
02/08	13:00	136	Central PWS Sandlance with Herring	60 40.15	14726.77	SL C1
03/08	08:45	137	South PWS Herring Only	60 20.69	148 12.08	H S0
03/08	14:00	142	South PWS Herring Only	60 10.36	148 10.25	H S0
04/08	13:45	151	South PWS Herring/Sandlance	60 07.70	147 56.67	H S1/SL S1

Table 2. Mean amounts (± 1 standard deviation) of fatty acids in herring and sandlance tissues expressed as relative and observed concentrations. Fatty acids with an "X" in the PCA column were used to generate the principle components analysis models for both species.

		Relative Concentrations (% of TAG)		Observed Concentrations (ng/μg)	
Fatty Acids	PCA	Herring n = 39	Sandlance n = 58	Herring N = 39	Sandlance N = 58
14:0	X	6.60 ± 1.02	7.03 ± 1.36	47.84 ± 34.41	31.85 ± 8.86
14:1		0.00 ± 0.01	0.02 ± 0.03	0.01 ± 0.04	0.07 ± 0.10
15:0		0.65 ± 0.33	0.74 ± 0.36	4.16 ± 2.41	3.41 ± 2.06
16:0	X	27.35 ± 3.86	22.80 ± 2.72	215.95 ± 217.39	103.27 ± 28.39
16:1	X	7.77 ± 1.78	5.36 ± 2.01	63.43 ± 61.17	23.91 ± 9.48
17:0	X	0.33 ± 0.36	0.53 ± 0.25	1.98 ± 2.70	2.42 ± 1.34
18:0		0.00 ± 0.00	0.11 ± 0.16	16.49 ± 11.52	18.06 ± 15.16
18:1n9 c&t	X	10.41 ± 2.53	9.16 ± 1.55	84.75 ± 87.86	40.90 ± 10.05
18:1n7	X	2.36 ± 0.59	1.80 ± 0.40	17.92 ± 16.61	7.96 ± 1.86
18:2n6 c	X	1.84 ± 1.04	2.49 ± 0.86	12.38 ± 13.52	11.13 ± 4.45
18:2n6 t		0.00 ± 0.00	0.00 ± 0.00	-1.24 ± 3.04	-1.23 ± 5.42
18:3n6		0.04 ± 0.10	0.00 ± 0.00	0.44 ± 1.46	0.25 ± 2.92
18:3n3	X	1.60 ± 0.91	2.15 ± 0.53	13.11 ± 21.43	9.75 ± 3.00
20:0		0.04 ± 0.07	0.06 ± 0.12	0.19 ± 0.30	0.26 ± 0.58
20:1n9	X	0.96 ± 1.00	1.86 ± 0.89	7.08 ± 10.50	8.44 ± 4.21
20:2n6		0.32 ± 0.71	0.31 ± 0.68	1.25 ± 2.43	1.58 ± 2.51
20:3n6		0.01 ± 0.05	0.00 ± 0.00	0.06 ± 0.23	-0.30 ± 1.17
20:4n6		0.52 ± 0.77	1.06 ± 1.35	3.43 ± 5.91	5.13 ± 7.81
20:3n3		0.01 ± 0.06	0.42 ± 1.50	0.05 ± 0.20	2.42 ± 6.97
20:5n3	X	14.65 ± 2.56	16.39 ± 2.59	111.01 ± 104.54	72.64 ± 14.63
22:0		0.03 ± 0.06	0.02 ± 0.02	0.09 ± 0.44	0.08 ± 0.07
22:1n9		0.22 ± 0.55	0.68 ± 0.50	0.82 ± 1.52	3.12 ± 2.47
22:2n6		0.35 ± 1.02	0.13 ± 0.20	7.18 ± 29.81	0.58 ± 0.68
22:5n3		1.28 ± 0.70	0.98 ± 0.62	11.46 ± 16.21	4.46 ± 2.83
22:6n3	X	18.76 ± 3.94	21.04 ± 4.24	141.79 ± 138.97	94.04 ± 26.34
24:0		0.01 ± 0.02	0.03 ± 0.09	0.05 ± 0.10	0.13 ± 0.53
24:1	X	1.74 ± 0.87	1.24 ± 0.33	12.42 ± 11.71	5.53 ± 1.95