Exxon Valdez Oil Spill
Restoration Project Final Report

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

Restoration Project 00347
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Study History:

This study was initially funded as a three year project in 1998. The first year of the project (Restoration study 98347), samples of young-of-the-year sand lance and herring were collected for lipid analysis. These samples were collected from various locations in Prince William Sound on a cruise operated by the University of Alaska as part of their participation in the Alaska Predator Experiment (APEX) program. During the second year of the project (Restoration study 99347) samples of sand lance were collected bi-weekly from Kachemak Bay by the U.S. Fish and Wildlife Service's component of the APEX program. The enclosed report presents those data.

Abstract:

We examined spatial and temporal variation in the lipid content of young-of-the-year (YOY) Pacific sand lance (*Ammodytes hexapterus*) and spatial variation in YOY Pacific herring (*Clupea pallasi*). Spatial variation of YOY herring and sand lance was evaluated by collecting fish from six locations in Prince William Sound, Alaska between July 15 and August 2, 1997. This allowed us to remove the effects of season and age as alternative sources of variation. Lipid content of herring and sand lance varied between sites, and fish from sites in southwestern PWS generally had the highest lipid contents. After accounting for size differences between sites, herring ranged between 8% and 10% lipid (dry weight) and sand lance between 9% and 16%. To examine temporal variation, we sampled age-0 and age-1 sand lance from a single location in Cook Inlet, Alaska between May 22 and August 28, 1998. Temporal variation in lipid content of age-0 and age-1 sand lance followed different patterns. Lipid content of age-0 sand lance declined by 50% between June 1 and July 9. In contrast, age-1 had increased their lipid content during the same period. These differences in pattern are believed to result from differing energy allocation strategies that relate to the differences in their life histories.

Key Words:

lipid, sand lance, herring, life history

Project Data:

Data collected for this project include the lengths, weights, sampling locations, lipid content and water content of juvenile herring and sand lance. In addition, quality control data accompany the lipid analyses. The data are organized in two spreadsheets, one for the spatial variation component and the other for the temporal variation component. A copy of all the data described in this report is found at the end in Appendices A and B. Custodian : Ron A. Heintz, Auke Bay Laboratory, 11305 Glacier Hwy, Juneau, Alaska 99801 (voice: 907-789-6058; fax 907-789-6094).

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Executive Summary

This report demonstrates that lipid levels in forage fish species vary spatially and temporally. These variations result from differences in the availability and quality of prey and differences in the strategies of energy allocation of different life stages. Thus, lipid analysis can be a powerful tool for comparing the potential success of populations about to undergo important life history events.

We analyzed the lipid content of young-of-the-year (YOY) sand lance and herring collected from six locations in Prince William Sound (PWS). Collections were made within a two week period, consequently confounding factors such as age and time were removed from our analysis of spatial differences in lipid content. Our identification of differences demonstrate that lipid contents of recruiting forage fish varied between southwestern and central PWS. These differences likely result from differences in the quality of their forage or from differences in the time since hatching. Previous reports describe a relationship between whole body energy density of YOY herring in October and their over winter survival. These reports also note that energy densities vary among locations in PWS. The data described here support those reports by demonstrating spatial variation in the lipid content of YOY herring and further suggest spatial variation in the whole body energy content of YOY herring observed in October may arise much earlier in the year.

Temporal variation in the lipid content of age-0 sand lance from Kachemak Bay indicates that the lipid content of recruiting sand lance is depleted to a minimum as they complete metamorphosis. After this time, they slowly begin increasing lipid content. Completion of metamorphosis was indicated by the appearance of pigment in the skin of sand lance less than 100mm in length. This occurred in early July when food supplies and quality have been shown to begin declining. Thus, YOY sand lance must provision themselves with lipid to forestall winter starvation during a time window when lipid supplies are diminishing. The data we collected for age-1 sand lance were consistent with previous reports which noted a peak in lipid content immediately prior to gonadal recrudesence.

These data have been used to develop a model which describes the temporal pattern of lipid dynamics in sand lance. This model suggests lipid levels signal critical periods in their life history. If food supplies during the end of metamorphosis are insufficient, then YOY sand lance may not provision themselves with adequate supplies of lipid to survive over winter. If food supplies during the spring bloom are inadequate, then the availability of energy to maturing sand lance may reduce the quality of their offspring.

INTRODUCTION

Examination of lipid dynamics holds promise for identifying critical periods in the development of forage fish that might affect their recruitment. For example Marshall et al. (1999) described a close relationship between the energy available as lipid in arctic cod and their total egg production, demonstrating that lipid content predicts recruitment better than the stock size does. Relationships such as this may prove valuable as efforts are focused on understanding the population dynamics of forage species such as Pacific herring (*Clupea pallasi*) and Pacific sand lance (*Ammodytes hexapterus*). These species are important prey to protected and commercially valuable species, but little is known about the processes which influence their production. Understanding fluctuations in the lipid content of forage fish populations may therefore prove to be important in understanding spatial and temporal variation in forage species abundance.

Recent work in Prince William Sound (PWS) provides a valuable example of how examination of variation in energy density can identify sensitive stages in the life history of Pacifc herring *(Clupea pallasi*). Reports reviewed by Norcross et al. (2001) show that the energy density of young-of-the-year (YOY) herring prior to their first winter has a profound influence on their survival, and herring energy densities vary spatially and temporally. Consequently, the recruitment of age-1 herring in spring is influenced by the availability of food the previous year. While these conclusions are based on energy density measurements from calorimetry, they largely derive from fluctuations in lipid content because the caloric value of a unit mass of lipid is more than twice that of protein (Anthony et al. 2000). For example Robards et al. (1999a) traced seasonal variation in the proximate composition of adult Pacific sand lance (*Ammodytes hexapterus*) in Kachemak Bay, Alaska. Energy densities of adults decreased by 25% between

July and November while lipid content decreased by 50% during the same period.

These data suggest that systematic evaluations of the seasonal and spatial variation of lipid content are likely to reveal sensitive periods that presage critical life history events such as survival to maturity or reproductive output. Evaluation of the mean energy content or nutritional state at the beginning of these events is likely to reflect the relative success of a population facing these events. This report describes an initial evaluation of the spatial and temporal variation in the lipid content of these species. We report on a spatial comparison of the lipid content of juvenile sand lance and herring collected from various locations around Prince William Sound during a single two week period in mid-summer 1997. This evaluation tests the hypothesis that spatial differences in lipid content of YOY herring and sand lance can be detected in the summer. In addition, we describe temporal variation in the lipid content of Age-0 and Age-1 sand lance collected from a single location throughout the summer of 1998. This examination tests the hypothesis that seasonal variation in lipid content of juvenile sand lance is similar to that of adults. These evaluations scope the potential for identifying causal relationships between lipid content and the success at transiting through life history phases in forage species.

METHODS

Samples collected to examine spatial variation

Samples were opportunistically collected in the field by investigators from the University of Alaska and transferred to the Auke Bay Laboratory. Sample collections were made by beach seining at several locations in Prince William Sound between July 19 and August 8, 1997. Fish collected at each sample station were bagged together and frozen. They were held at -20 ° C between collection and processing for lipids 8 months later. A total of 6 stations were sampled, 3 in central Prince William Sound (PWS) and three in southwestern PWS (Figure 1), providing the opportunity to determine if the variation between sites in a given region of PWS was as great as the variation among regions. . The central PWS stations are referred to as Cabin Bay, northwestern Naked Island (NW Naked) and western Naked Island (W Naked). Only sand lance were sampled at West Naked Island and only herring were found at NW Naked. Both species were collected in Cabin Bay, but only sand lance were available for lipid analysis. The southwestern stations are referred to as Shelter Bay, Bainbridge Pass, and Whale Bay. Both herring and sand lance were collected at the Shelter Bay site, while only herring were collected at Whale Bay and sand lance at Bainbridge Pass. See Mabry (2000) for details on the sampling procedures.

Prior to lipid extraction the lengths and weights were recorded. Lengths of all fish were from the tip of the snout to the fork of the tail. The lipids were extracted from a total of 15 fish of each species for each site. Fish were randomly drawn from the mass of frozen fish found in a given bag.

Samples collected to examine temporal variation

Samples of age-1 and age-0 sand lance were collected bi-weekly between May 30 and August 22, 1998 in Kachemak Bay. Samples were collected by beach seining and digging, and fish were individually bagged and frozen for lipid analysis. Samples were collected as part of the routine sampling for a much larger project described by Robards et al. (1999a) and are representative of the fish caught during a given sampling period. Sampling often occurred over a multiple number of days, so samples were assigned to two week time periods beginning with May 22, 1998. Differences between sampling dates within a period were less than four days. Fish were stored at -20 ° C until they were transferred to the Auke Bay Laboratory and processed beginning in March 1999. The lengths and wet weights of each fish were recorded prior to lipid extraction. Three age-0 and age-1 sand lance were evaluated from each sampling period. The presence of pigment was used to discriminate age-0 and age-1 sand lance. Age-1 fish were dissected and inspected to verify that they were not sexually mature.

Homogenization and lipid extraction

Lipids were extracted using a modification of Folch's method outlined by Christie (1982). Whole fish were extracted by mixing them with a solution of 33% methanol and 66% chloroform and homogenizing them with a tissuemiser. The homogenate was vacuum filtered and the solid residue re-extracted and filtered. The two filtrates were combined in a separatory funnel and a liquid-liquid extraction was performed using an aqueous solution of 0.88% potassium chloride (KCl) at one quarter of the total volume of filtrate. The bottom layer of the resulting bi-phasic solution was collected and re-extracted with a fresh KCl solution. After this second liquid-liquid extraction, the purified lipid was collected and it's volume reduced to 5 ml with a rotary evaporator. A 2 ml aliquot was withdrawn to gravimetrically determine the lipid mass. .

Statistical analysis

Lipid contents of fish collected from different locations in PWS are expressed on a dry weight basis and compared by analysis of covariance (ANCOVA). Fish sampled for lipid content were generally too small to establish dry weights, so dry weights were estimated from fish sampled from the same locations. Samples were dried by in an oven at 60°C until and repeatedly weighed until they no longer changed. For herring, no relationship between fish size and dry weight was observed so dry weights were estimated by multiplying the average proportion of dry mass of six herring from a given location by the observed wet weight. We observed a linear relationship between fish length and dry weight for the sand lance. Consequently, we estimated dry weights by fitting lengths to a linear model derived by regression. The single factor ANCOVA model used to compare lipid content of fish from different locations used length as a covariate to account for size differences among fish from different locations. Pairwise comparisons among locations were made using Tukey's method.

Lipid contents of sand lance collected from Kachemak Bay during the summer of 1998 are expressed on a wet weight basis and compared by a single factor ANCOVA with time as the main factor and length as a covariate. Lipid is expressed on wet weight basis because there were insufficient numbers of fish collected from Kachemak Bay to support estimation of dry weights. Lengths of Age-0 and juvenile sand lance were compared by single factor ANOVA with period as the main factor. Age-0 and juvenile sand lance were tested separately, and pairwise comparisons between period means were made using Tukey's method.

RESULTS

Spatial variation in lipid content of herring and sand lance

Herring sampled in different locations were similar in size ($P = 0.414$), but varied in their lipid content ($P < 0.012$). Herring lengths ranged between 62 and 66 mm (Figure 2), consistent with the expected size of herring of age-0 herring in August (Norcross et al. 2001). Size corrected estimates of mean lipid content ranged between 7.8% and 10.4% of the whole dry mass. Herring from Shelter Bay had significantly more lipid than those from NW Naked ($P < 0.01$). Spatial data were limited to the three locations where we obtained herring, but pairwise testing indicated that lipid contents of herring from the two southwestern sites had greater similarity to each other than the central site ($P = 0.028$) (Figure 2). It is not clear if this represents a regional difference or a difference between bays and corridors because the two southwestern sites were collected in bays, while the site on NW Naked Island was an unprotected point.

Sampling location was an important factor in the estimated size of sand lance ($P < 0.001$). Sand lance lengths differed widely with the largest fish, 94.7 ± 1.5 mm (mean ± 1 standard error), coming from Shelter Bay and smallest, 70.5 ± 2.0 mm from nearby Bainbridge Pass (Figure 3). Fish from collections made from bays were longer than those collected from passages ($P < 0.001$), but there was no difference in the size relative to sites in southwestern and central PWS ($P = 0.616$).

Lipid contents also differed between sampling locations ($P<0.001$), but the differences did not follow the same pattern as the size differences (Figure 3). Pairwise testing indicated that lipid contents of sand lance were higher in the two locations sampled in southwestern PWS than those from central PWS ($P < 0.001$), but there were no differences with respect to bays and passes ($P=0.479$). No difference was detected between samples collected within each of these regions ($P = 1.00$). The lowest lipid content, 8.9 ± 0.7% of the whole dry mass, was observed in fish collected from Cabin Bay. The highest lipid concentration, observed in Shelter Bay, was more 67% greater than the lowest level, averaging 14.9 ± 1.0% of the whole dry mass.

Seasonal variation in sand lance lipid content

Size of age-0 sand lance increased during the sampling period (P = 0.014). Larvae collected on May 30 were smallest averaging 44.7 ± 2.6 mm (Figure 4) while those sampled on July 9 were larger (P = 0.0085), averaging 61.3 ± 2.6 mm. No fish without pigment were caught between July 9 and August 22. Age-0 fish obtained on August 22 were slightly smaller than those collected on July 9.

Despite increasing size, the lipid content of age-0 sand lance declined during the summer (P = 0.006), decreasing from a high of 7.3 ± 0.8 % of the whole wet mass on May 30 to a minimum of 2.1 ± 0.6% on August 22 (Figure 5). The greatest difference between periods occurred between May 30 and June 11 when lipid content declined by 50% (P = 0.02). After June 11, lipid levels moderated displaying no detectable change (P > 0.315). No sand lance without pigment were caught between July 9 and August 22.

Changes in the size and lipid content of age-1 sand lance contrasted with those of age-0 fish. The mean length of age-1 sand lance also initially increased from a mean of 81.0 ± 3.5 mm on May 30 to 96 ± 5.4 mm on July 11, but then declined afterwards (Figure 4), but no statistical significance could be attributed to this trend (P = 0.476). In contrast to the age-0 fish, age-1 sand lance increased their lipid content during the first half of their summer (P = 0.011). Lipid content more than doubled from a low of 2.3 ± 0.6% of the whole wet mass on May 30 to a peak value of 5.7 ± 0.6% on July 9 (Figure 5). Thereafter, lipid content declined to approximately 3% of the wet mass and stabilized for the rest of the sampling time.

DISCUSSION

Spatial variation in lipid content

Data provided here demonstrate the existence of a spatial component to the total variation in lipid content of age-0 sand lance and herring in late July. Other factors that have been cited as contributing to the variation in mean lipid content of these species include sex, age, size and time (Anthony et al. 2000). Elimination of these factors reveals a strong influence of location on estimates of mean lipid content. Moreover, statistically significant differences in lipid content were observed over a range of approximately 75 km, which is much finer than previously considered. Previous reports of spatial differences in lipid content of sand lance (Payne et al. 1999) and herring (Perez 1994) contrasted fish collected in the Bering Sea and Gulf of Alaska. On a finer scale, age-1 herring from northeastern PWS were found to have higher lipid contents than those from other parts of PWS (Anthony et al. 2000). Similarly, Iverson et al. (1997) described regional variation in the fatty acid composition of age-0 and age-1 herring from PWS, but did not discuss variation in the total lipid content.

Our observations of regional scale variation are consistent with other reports that described spatial differences in energy content of age-0 herring and sand lance in 1997. Mabry (2000) reported spatial differences in the caloric content of sand lance using fish collected from the same beach seine sets used to collect fish reported here. Both studies found less energy in the sand lance sampled near Naked Island relative to those sampled in southwestern PWS. Similarly, Paul and Paul (1999) described regional differences in the caloric content of age-0 herring from three sites in PWS in October, 1997, but found the highest energy densities in herring from northwestern PWS. However, their locations differed from those described here, despite their attempts to sample at Whale Bay. They also describe differences in two closely located sites (20 km) in eastern PWS in 1994, indicating variation can exist on finer spatial scales than observed here.

Regional differences in the lipid content of YOY herring and sandlance were likely driven by regional differences in the quality of forage. Zooplankton biomass and species compositions differed between central and southwestern PWS in late July 1998 (Mabry 2000). Zooplankton densities in southwestern PWS were greater than those in central PWS, as a result of increased numbers of copepods. These observations are consistent with the differences in lipid content of sand lance collected from the Naked Island sites and those in southwestern PWS. Further evidence of regional differences in the quality of forage is reviewed by Norcross et al. (2001) who describe transport mechanisms for delivering carbon to herring nursery areas, and also report local differences in the energy content and composition of herring diets in October, 1995.

The spatial variation in average lipid content of herring in July suggests the potential for spatial variation in the survivability of these populations during the following winter. Norcross et al. (2001) reviewed the importance of autumn energy reserves in guaranteeing the overwinter survival of age-0 herring, and most of this energy is probably stored as lipid (Anthony et al. 2000). Energy density of age-0 herring increases between July and October (Paul and Paul 1998; Norcross et al. 2001)indicating continued feeding, presumably with a concomitant increase in lipid content. However, it is not clear if continued feeding would ameliorate the spatial disparities in lipid content we observed in late July, because the availability of energy in herring diets decreases after early August, as does the number of feeding YOY herring (Norcross et al.

2001). For example, energy density of herring diets was significantly higher in Whale Bay in October, 1995 relative to Simpson Bay in Prince William Sound (Foy and Norcross 1999), but the energy density of herring did not differ (Paul and Paul 1999). This suggests that lipid contents of Simpson Bay herring were initially higher than those from Whale Bay. Consequently, if the spatial differences observed in late July were conserved, then over winter survival might be predicted by the lipid content of age-0 herring after assimilation of the spring zooplankton bloom.

Alternatively, differences in lipid content in age-0 fish such as those reported here might result from differences in the timing of metamorphosis and recruitment. For example, Gatten et al. (1983) demonstrated the lipid content of Atlantic herring larvae began increasing 40 d post-hatch. This coincided with the onset of feeding. Assuming an average hatching date of May 15 (Norcross et al. 2001) then herring sampled in PWS were approximately 90 d post-hatch. Thus, small differences in the amount of time the fish had been feeding may have contributed to differences in the amount of lipid they identified in herring sampled in late July.

Seasonal Variation in Lipid Content

The changes in size and lipid content displayed by both age classes of sand lance indicated differences in their life histories. Age-0 sand lance increased their size more rapidly than the age-1 fish during the early summer. This may be consistent with the need for juvenile fish to grow rapidly to avoid predation (Parker 1971). In contrast, age-1 fish increased their lipid content during the same period, reflecting a need to store energy to fuel reproduction (Robards et al. 1999a).

Paradoxically, sand lance classified as age-1 demonstrated an upward trajectory in size and lipid content until early July when the length and lipid content declined dramatically. This decrease in both lipid and size is more likely the result of age-0 fish completing metamorphosis and taking on the physical appearance of age-1 fish. Fish ages were determined by the presence of skin pigments. The age-0 fish apparently increased their length by approximately 0.8% • day^{-1} between May 30 and July 9, suggesting an average length of 70 mm in length on July 24, which is consistent with the lengths observed for the fish initially classified as age-1. In contrast, the age-1 fish increased their length by an average 0.4 % • day^{-1} between May 30 and July 9, suggesting that they should have been more than 100 mm by July 24 (Robards et al. 1999b). Blackburn and Anderson (1997) reported a similar pattern to the change in size-at-age of sand lance collected in Kachemak Bay during monthly surveys in 1976. Samples collected by beach seine in May revealed a population with a unimodal length distribution averaging 82mm. In late July, the population became bimodally distributed and averaged 65mm. This apparent shrinkage was attributed to the offshore movement of age-1 fish, and appearance of the smaller age-0 recruits.

Further evidence that age-1 fish were not sampled after July 9 is presented by the changes in lipid content shown in Figure 6. The pattern of increasing lipid content of age-1 sand lance prior to July 9 is consistent with observations reported by Robards et al. (1999a). However, after July, lipid content of adult sand lance declines until the following spring (Robards et al. 1999a) in response to increased energy demands for gonadal recrudesence (Robards et al. 1999b) and decreased feeding (Robards et al. 1999a).

In contrast, the lipid content in fish initially identified as age-1 decreased to levels near those of age-0 fish and began increasing in late summer. If the pigmented fish we sampled after July 9 were age-0 sand lance that had metamorphosed into pigmented juveniles then seasonal variation in the lipid content of age-0 sand lance follows a markedly different pattern than that of age-1 and older. This is the same pattern for age -0 sand lance in the northern GOA described by Anthony et al. (2000). The appearance of unpigmented age-0 sand lance in late August likely represents a second cohort of recruits as described by McGurk and Wharburton (1992).

It is not clear from these data when lipid content in the age-0 fish reached a maximum. Sand lance feed shortly after hatching when they still maintain yolk reserves (Yamashita and Aoyama 1985), and hatching likely occurs prior to the spring plankton bloom (Haldorson et al. 1993). Thus, age-0 sand lance are able to actively forage during the peak of zooplankton production suggesting the decline began sometime after the spring zooplankton bloom. However, sand lance absorb their yolk and oil globules shortly after hatching (Smigielski et al. 1984), but hatched larvae have incompletely formed mouths and guts (Pinto 1984) suggesting that lipid declines may begin with hatching.

Decreases in lipid content of age-0 sand lance occurred during a period of rapid growth (Blackburn and Anderson 1997), suggesting that the energy gained through foraging was insufficient to meet physiological demands. Allocating energy entirely to growth without any storage is believed to be a predator avoidance mechanism employed by many juvenile fish (Parker 1971) including pink salmon (Azuma et al. 1998). However, during this period, age-0 sand lance are also undergoing morphological changes as indicated by the appearance of pigment in fish greater than 70 mm long. Thus, age-0 fish faced energetic demands associated with maximizing growth and completing larval development. In August, lipid contents appear to begin increasing which was consistent with Robards et al. (1999a) report of increased lipid content of age-0 sand lance whose lengths exceeded 80 mm. The levels reported here for early August are similar to those we report for southwestern Prince William Sound if we assume the dry mass averages 21.7% (Robards et al. 1999a). It is not clear if these increases in lipid content observed resulted from a change in their energy allocation strategy or from increased food supplies. However, Blackburn and Anderson (1997) reported the proportion of empty stomachs among sand lance in Cook Inlet increased between April and October. Changes in energy allocation strategies might result from diminished energetic demands associated with metamorphosis and reallocation of that demand to energy storage. Such a strategy might provide YOY sand lance with lipid stores just as food availability and quality begin diminishing (Norcross et al. 2001).

Increased energy reserves in fall are likely to be as important for juvenile sand lance as they are for juvenile herring. While sand lance may undergo a winter dormancy buried in the substrate (Winslade 1974; Ciannelli 1997) entering such a period with more lipid is likely to improve prospects for survival and future reproduction once feeding re-commences in the spring. These points are illustrated in Figure 6, where the lipid data have been combined with those reported by Robards et al. (1999a) and re-plotted to model seasonal changes in lipid content of sand lance over their first 2+ years of life. Newly recruited age-0 sand lance lose lipid until late summer when their lipid content begins increasing. The rate of loss is likely to be dependent on the amount of prey available during the zooplankton bloom. Therefore, the strength of the bloom also determines the amount of lipid retained by age-0 recruits when they begin increasing lipid content in August.

The lipid content of older age classes of sand lance continues to be tightly coupled to the productivity of the spring zooplankton bloom (Figure 6). Lipid levels in adult sand lance diminish rapidly in late summer during maturation, reaching relative low points in fall. Lipid levels continue declining throughout the winter until the spring zooplankton bloom commences, at which point lipid levels begin increasing rapidly (Robards et al. 1999a). It is not clear if the relatively low loss of lipid overwinter results from the increased rates of protein metabolism, or from diminished metabolic costs resulting from dormancy. However, reproductively active sand lance apparently are dependent on the amount of lipid retained at the end of winter and the amount obtained during the spring zooplankton bloom for much of the energy they expend during maturation (Robards et al. 1999a). Previously described relationships between the maternal lipid content and offspring survival for other species (Izquierdo et a. 2001; Marshall et al. 2000; Adams 1999; Rainuzzo et al. 1997) suggest that the reproductive success of sand lance is therefore dependent on the amount of lipid retained after winter and the supplementary amounts obtained from the spring bloom.

These data demonstrate the value of systematic evaluations of the seasonal and spatial variation of lipid content. Evaluations of spatial variation demonstrate fine scale spatial structuring to the overall variability in the lipid content of a species. This suggests energetic differences that may influence overwinter survival may manifest themselves by the end of the spring zooplankton bloom. It also suggests the existence of fine scale structuring to both the forage available to these fish and the quality of forage available to their predators. Evaluation of the seasonal variation in the lipid content of sand lance demonstrates how fluctuations in the lipid content of sand lance signal important life history events, such as the end of metamorphosis and the beginning of gonadal recurdesence. Thus, the data presented here demonstrate periods in which recruiting populations are apt to be especially vulnerable to the effects of low food availability or poor quality. Coupling the temporal and spatial variation suggests the direct influence of energy produced by the spring zooplankton bloom on the successful recruitment and propagation of forage fish populations in the northern Gulf of Alaska.

LITERATURE CITED

Adams, S.M. 1999. Ecological role of lipids in the health and success of fish populations. in: Lipids in Freshwater Ecosystems. M.T. Arts and B.C. Wainman eds. Springer-Verlag New York. pp132-160.

Anthony, J.A. , Roby, D.D., and Turco, K.R. 2000. Lipid content and energy density of forage fishes from the northern Gulf of Alaska. Journal of Experimental Marine Biology and Ecology **248**: 53-78.

Azuma, T., Yada, T., Ueno, Y. and Iwata, M. 1998. Biochemical approach to assessing growth characteristics in salmonids. North Pacific Anadromous Fish Commission Bulletin **1**:103-111.

Blackburn, J.E., and Anderson, P.J. 1997. Pacific sand lance growth, seasonal availability, movements, catch variability and foold in the Kodiak-Cook Inlet area of Alaska. *in:* Forage Fishes in Marine Ecosystems. Proceedings of the International Symposium on the Role of Forage Fishes in Marine Ecosystems. Alaska Sea Grant College Program Report No. 97-01. University of Alaska Fairbanks. pp 409-426.

Christie, W. W. 1982. Lipid analysis. Pergamon Press, Oxford, UK.

Ciannelli, L. 1997. Winter dormancy in the Pacific sand lance (Ammodytes hexapterus) in relation to gut evacuation time. *in:* Forage Fishes in Marine Ecosystems. Proceedings of the International Symposium on the Role of Forage Fishes in Marine Ecosystems. Alaska Sea Grant College Program Report No. 97-01. University of Alaska Fairbanks. pp 95-104.

Foy, R.J. and Norcross, B.L. 1999. Spatial and temporal variability in the diet of juvenile Pacific herring (*Clupea pallasi*) in Prince William Sound, Alaska. Canadian Journal of Zoology 77:697-706.

Gatten, R.R., Sargent, J.R. and Gamble, J.C. 1983. Diet-induced changes in fatty acid composition of herring larvae reared in enclosed ecosystems. Journal of the Marine Biological Association of the United Kingdom. 63:575-584.

Haldorson, L., Pritchett, M., Sterrit, D. and Watts. J. 1993. Abundance patterns of marine fish larvae during spring in a southeastern Alaskan bay. Fishery Bulletin. 91:36-44.

Iverson, S.J., Frost, K.J. and L.J. Lowry. 1997. Fatty acid signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. Marine Ecology Progress Series. 151:255-271.

Izquierdo, M.S., Fernandez-Palcios, H. and Tacon, A.G.J. 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture. 197:25-42.

Mabry, J. 2000. Condition and food availability to Pacific sand lance (*Ammodytes hexapterus*) in Prince William Sound, Alaska. Masters Thesis. University of Alaska. Fairbanks, Alaska.88 pp.

Marshall, C.T., Yaragina, N.A., Lambert, Y.K., and Kjesbu, O.S. 1999. Total lipid energy as a proxy for total egg production by fish stocks. Nature **402**: 288-290.

McGurk, M.D. and Whartburton, H.D. 1992. Relationships of growth, dispresion and mortality of sand lance larvae to environmental conditions in the Port Moller estuary. U.S. Department of Interior, Minerals Management Service. OCS Study MMS-92-0019. Anchorage, Alaska, USA.39 pp.

Norcross BL, Brown, E.D., Foy, R.J., Frandsen, M., Gay, S.M., Kline, T.C., Mason, D.M., Patrick, E.V., Paul, A. J., and Stokesbury, K. D. E. 2001. A synthesis of the life history and ecology of juvenie Pacific herring in Prince William Sound, Alaska.. Fisheries Oceanography **10**: 42-57.

Paul, A.J. and Paul, J.M. 1998. Spring and summer whole-body energy content of Alaskan juvenile Pacific herring. Alaska Fishery Research Bulletin. 5(2):131-136.

Paul, A.J. and Paul, J.M. 1999. Interannual and regional variations in body length, weight and energy content of age-0 Padcific herrring from Prince William Sound, Alaska. Journal of Fish Biology. 54:996-1001.

Parker, R.R. 1971. Size selective predation among juvenile salmonid fishes in a British Columbia inlet. J. Fisheries Research Board of Canada. 25:757-794.

Payne, S.A., Johnson B.A. and Otto, R.S. 1999. Proximate composition of some north-eastern Pacific forage fish species. Fisheries Oceanography 8:159-177.

Perez, M.A. 1994. Calorimetry measurements of energy value of some Alaskan fishes and squids. NOAA Technical Memorandum NMFS-AFSC-32. U.S. Department of Commerce. Seattle Washington. 18 pp.

Pinto, J.M. 1984. Laboratory spawning of *Ammodytes hexapterus* from the Pacific coast of North America with a description of its eggs and early larvae. Copeia 1:242-244.

Rainuzzo, J.R., Reitan, K.I. and Olsen, Y. 1997. The significance of lipids at early stages of marine fish: a review. Aquaculture 155:103-115.

Robards, M.D., Anthony, J.A., Rose, G.A., and Piatt, J.F. 1999a. Changes in proxtimate composition and somatic energy content for Pacific sand lance (*Ammodytes hexapterus*) from Kachemak Bay, Alaska relative to maturity and season. Journal of Experimental Marine Biology and Ecology **241**: 245-258.

Robards, M.D., Piatt, J.F. and Rose, G.A. 1999b. Maturation, fecundity, and intertidal spawning of Pacific sand lance in the northern Gulf of Alaska. Journal of Fish Biology. 54:1050-1068.

Smigielski, A.S., Halavik, T.A., Buckley, L.J., Drew, S.M., and Laurence, G.C. 1984. Spawning, embryo development and growth of the American sand lance *Ammodytes americanus* in the laboratory. Marine Ecology Progress Series 14:287-292.

Winslade, P.R. 1974. Behavioral studies on the lesser sandeel, Ammodytes marinus Raitt. III: The effect of temperature on activity and environmental control of the annual cycle of activity. Journal of Fish Biology. 6:587-599.

Yamashita, Y. and Aoyama, T. 1985. Hatching time, yolk sac absorption, onset of feeding and early growth of the Japanese sand eel *Ammodytes personatus*. Bulletin of the Japanese Society of Scientific Fisheries. 51:1777-1780.

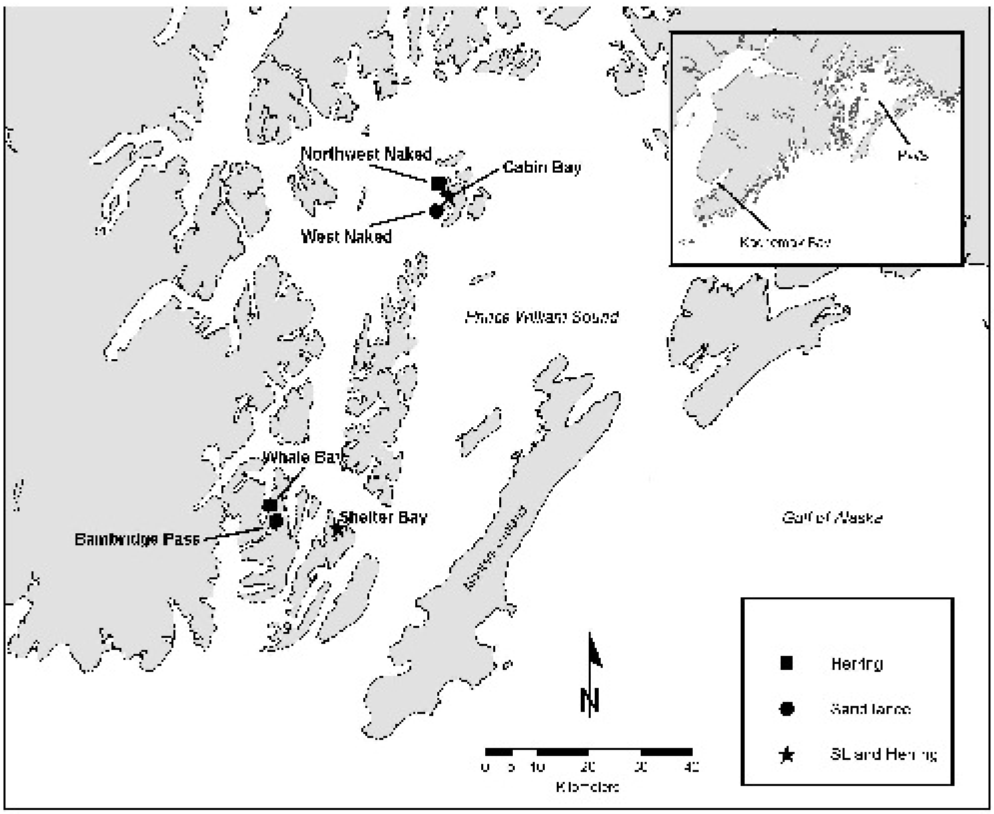

Figure 1.-- Locations where sand lance and herring were sampled in Prince William Sound (PWS) during a two week period in 1997. Inset shows location where sand lance were sampled during the summer of 1998.

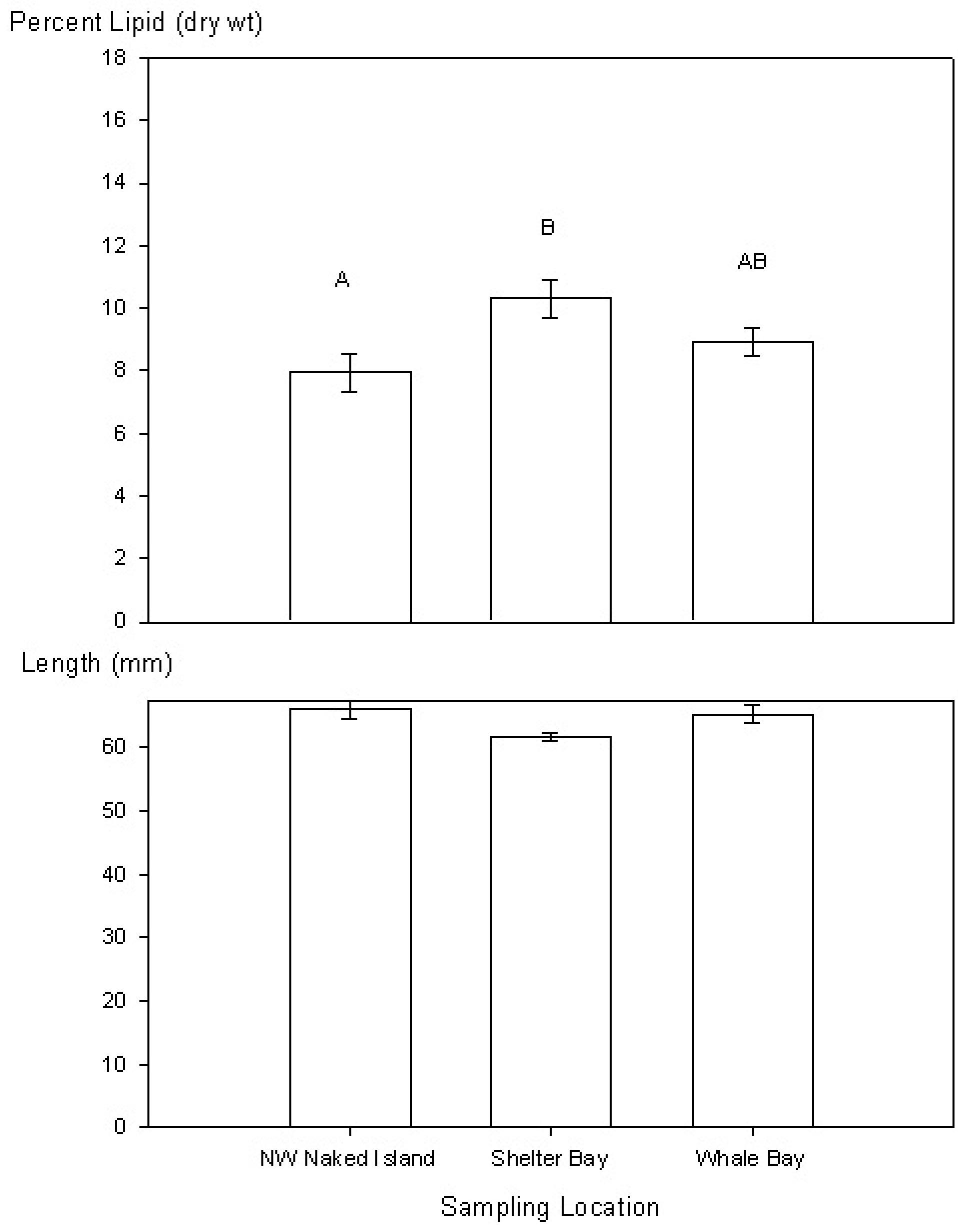

Figure 2.-- Lipid content and standard length (mean ± 1 s.e.) of YOY herring in different locations of PWS during August 1997. Letters show locations where lipid contents were statistically similar.

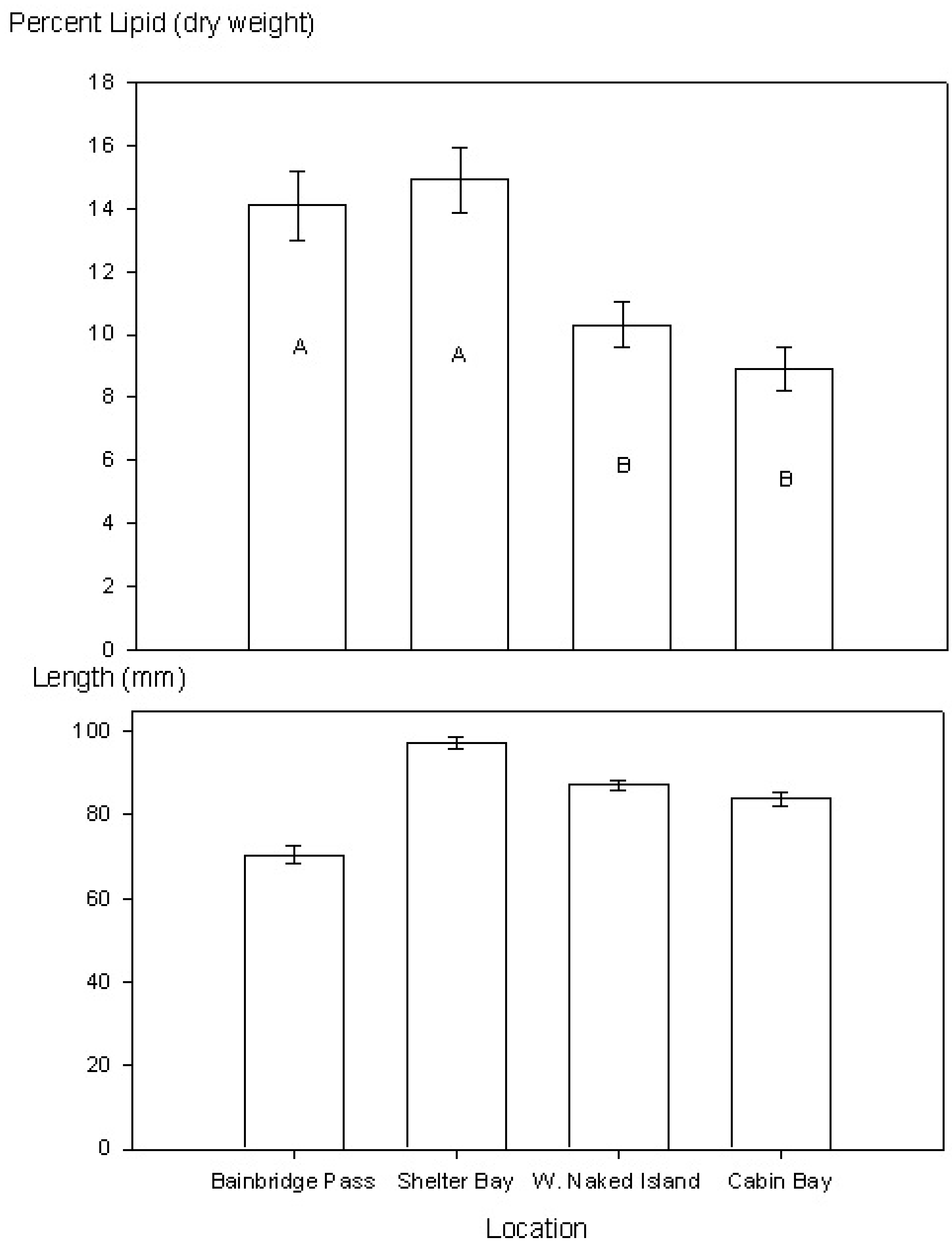

Figure 3.-- Lipid content and standard length (mean ± 1 s.e.) of YOY sand lance in different locations of PWS during August 1997. Letters how locations where lipid contents were statistically similar.

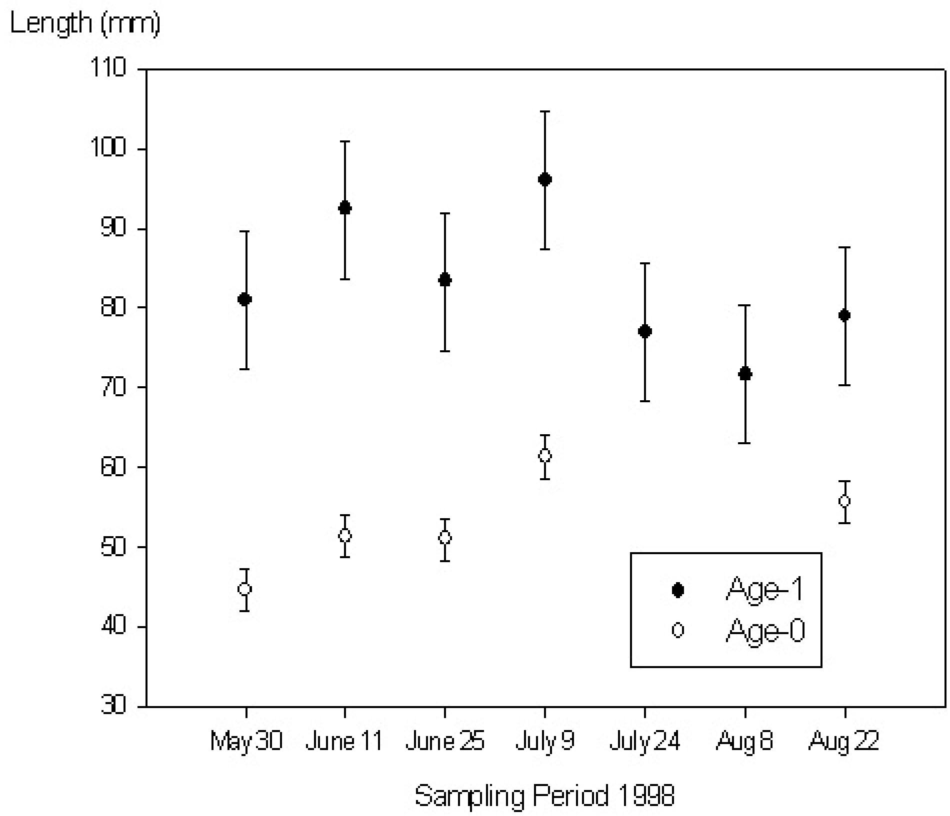

Figure 4.-- Seasonal changes in the total lengths of age-0 and age-1 sand lance sampled in Kachemak Bay, Alaska during the summer of 1998.

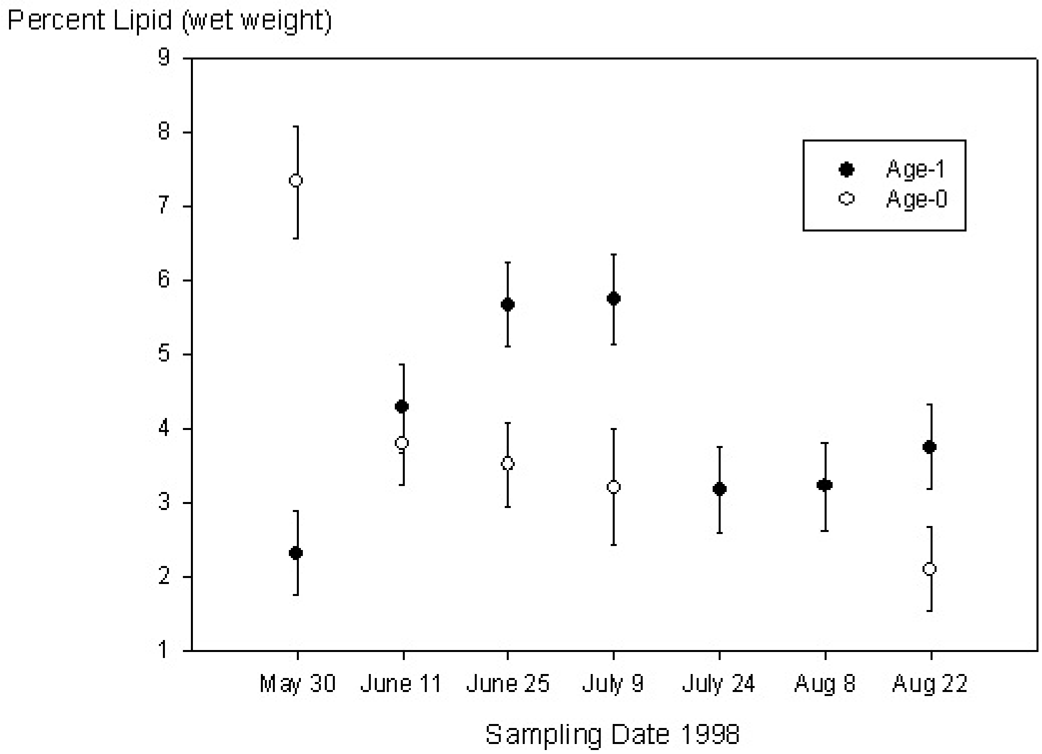

Figure 5.-- Seasonal changes in the lipid content of age-0 and age-1 sand lance sampled in Kachemak Bay, Alaska during the summer of 1998. Symbols depict mean ± 1 s.e. (wet weight) after correcting for differences in length.

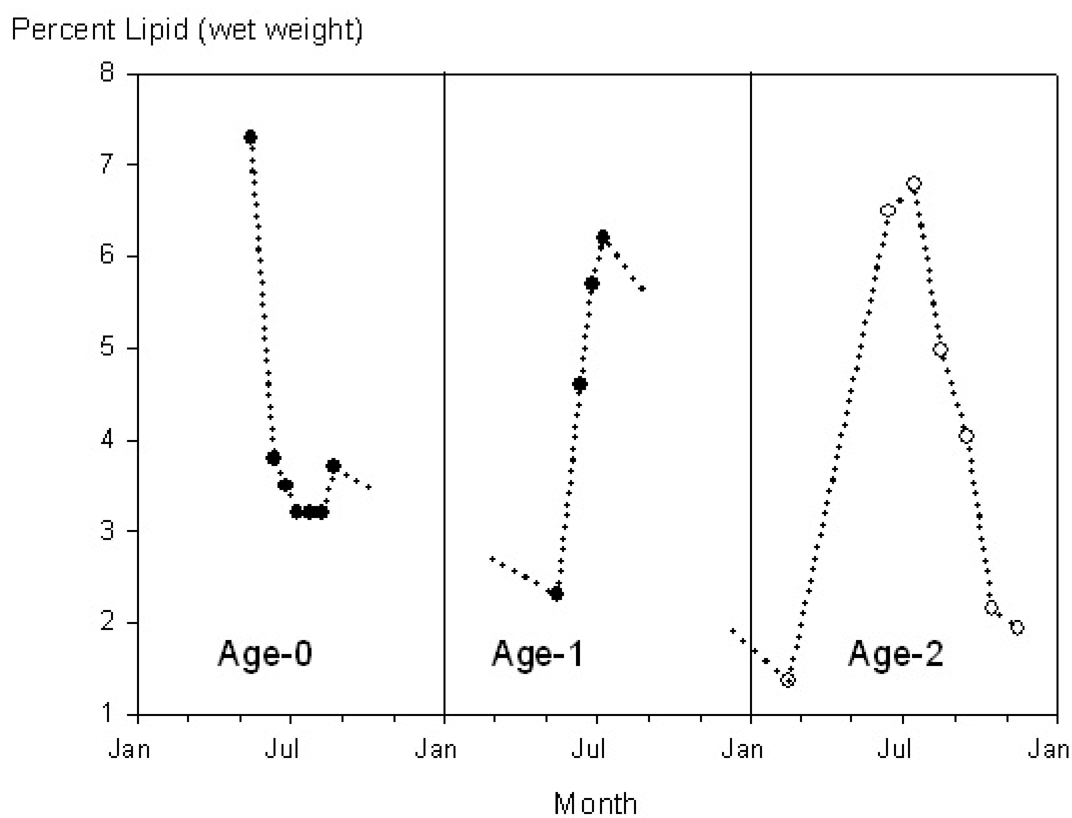

Figure 6.-- Lipid phenology for sand lance in Kachemak Bay, Alaska. Filled circles represent data described in this report, open circles taken from Robards et al. (1999a). Breaks in the line indicate uncertainties in trajectory.

Appendix 1.– Collection dates, lipid content and length of juvenile herring and sand lance collected from various locations in Prince William Sound during the summer of 1997.

Location	Collection Date	Length (mm)	Herring weight (g)	% Lipid (wet weight)	% Water	% Lipid (Dry weight)
NW Naked	8/2/1997	74	2.9	1.7	77.0	7.4
NW Naked	8/2/1997	61	1.5	1.3	77.0	5.7
NW Naked	8/2/1997	67	2.2	1.8	77.0	7.9
NW Naked	8/2/1997	64	2.0	2.0	77.0	8.8
NW Naked	8/2/1997	66	2.5	1.4	77.0	6.2
NW Naked	8/2/1997	65	1.7	1.8	77.0	7.6
NW Naked	8/2/1997	70	2.1	3.2	77.0	13.7
NW Naked	8/2/1997	55	1.0	1.5	77.0	6.5
NW Naked	8/2/1997	66	2.1	2.4	77.0	10.2
NW Naked	8/2/1997	71	2.3	2.0	77.0	8.7
NW Naked	8/2/1997	66	1.9	0.8	77.0	3.5
NW Naked	8/2/1997	62	1.6	1.5	77.0	6.7
NW Naked	8/2/1997	70	2.3	2.2	77.0	9.4
NW Naked	8/2/1997	57	1.3	2.0	77.0	8.7
NW Naked	8/2/1997	78	3.4	1.9	77.0	8.2
Whale Bay	8/3/1997	67	2.0	1.8	81.0	9.3
Whale Bay	8/3/1997	68	2.2	1.4	81.0	7.1
Whale Bay	8/3/1997	67	2.1	1.9	81.0	9.8
Whale Bay	8/3/1997	59	1.4	1.8	81.0	9.7
Whale Bay	8/3/1997	61	1.5	1.7	81.0	8.8
Whale Bay	8/3/1997	63	1.8	1.4	81.0	7.4
Whale Bay	8/3/1997	63	1.7	1.5	81.0	7.7
Whale Bay	8/3/1997	78	3.4	2.1	81.0	11.0
Whale Bay	8/3/1997	70	2.6	2.3	81.0	12.2
Whale Bay	8/3/1997	67	2.2	1.1	81.0	6.0
Whale Bay	8/3/1997	62	1.8	1.9	81.0	10.1
Whale Bay	8/3/1997	71	2.6	1.2	81.0	6.2
Whale Bay	8/3/1997	65	2.0	1.7	81.0	9.0
Whale Bay	8/3/1997	55	1.2	1.7	81.0	8.8
Whale Bay	8/3/1997	62	1.8	2.0	81.0	10.4
Shelter Bay	8/4/1997	60	1.3	1.6	79.0	7.4
Shelter Bay	8/4/1997	59	1.2	2.1	79.0	10.0
Shelter Bay	8/4/1997	68	2.1	1.4	79.0	6.9
Shelter Bay	8/4/1997	60	1.3	2.3	79.0	10.9
Shelter Bay	8/4/1997	63	1.5	1.7	79.0	8.2
Shelter Bay	8/4/1997	59	1.1	2.3	79.0	10.8
Shelter Bay	8/4/1997	67	2.1	2.4	79.0	11.4
Shelter Bay	8/4/1997	60	1.5	2.7	79.0	13.0
Shelter Bay	8/4/1997	62	1.5	2.4	79.0	11.2
Shelter Bay	8/4/1997	61	1.8	1.4	79.0	6.5
Shelter Bay	8/4/1997	61	1.4	2.8	79.0	13.4
Shelter Bay	8/4/1997	64	1.9	2.3	79.0	11.0
Shelter Bay	8/4/1997	59	1.6	1.9	79.0	9.2
Shelter Bay	8/4/1997	60	1.5	2.3	79.0	10.8
Shelter Bay	8/4/1997	63	1.7	2.9	79.0	13.9

Appendix 1–continued.

Sand Lance						
Location	Collection Date	Length (mm)	Weight (g)	% Lipid (Wet weight)	% Water	% Lipid (Dry weight)
Cabin Bay	8/2/1997	76	1.4	3.3	78.5	15.4
Cabin Bay	8/2/1997	77	1.6	2.0	78.4	9.4
Cabin Bay	8/2/1997	82	2.2	1.3	77.9	5.8
Cabin Bay	8/2/1997	83	2.2	1.7	77.8	7.7
Cabin Bay	8/2/1997	90	2.3	1.2	77.0	5.1
Cabin Bay	8/2/1997	87	2.2	2.5	77.3	10.9
Cabin Bay	8/2/1997	86	2.1	1.5	77.4	6.8
Cabin Bay	8/2/1997	90	2.6	2.3	77.0	10.1
Cabin Bay	8/2/1997	74	1.4	1.6	78.7	7.4
Cabin Bay	8/2/1997	84	2.1	1.8	77.7	8.1
Cabin Bay	8/2/1997	92	2.7	2.8	76.8	11.8
Cabin Bay	8/2/1997	93	2.9	2.6	76.7	11.1
Cabin Bay	8/2/1997	83	2.0	1.9	77.8	8.4
Cabin Bay	8/2/1997	82	1.7	1.6	77.9	7.3
Cabin Bay	8/2/1997	78	1.5	1.5	78.3	6.9
W Naked	7/24/1997	83	1.6	2.5	77.8	11.0
W Naked	7/24/1997	91	2.6	2.9	76.9	12.5
W Naked	7/24/1997	86	2.0	3.1	77.4	13.6
W Naked	7/24/1997	86	2.0	2.4	77.4	10.4
W Naked	7/24/1997	91	2.5	3.3	76.9	14.4
W Naked	7/24/1997	86	2.0	2.3	77.4	10.2
W Naked	7/24/1997	87	2.1	2.3	77.3	10.0
W Naked	7/24/1997	87	2.3	2.0	77.3	8.7
W Naked	7/24/1997	93	2.7	2.6	76.7	11.0
W Naked	7/24/1997	86	2.0	1.7	77.4	7.6
W Naked	7/24/1997	93	2.9	2.6	76.7	10.9
W Naked	7/24/1997	76	1.4	1.3	78.5	6.2
W Naked	7/24/1997	91	2.5	2.8	76.9	11.9
W Naked	7/24/1997	81	1.9	1.8	78.0	8.0
W Naked	7/24/1997	89	2.2	2.5	77.1	10.7
Shelter Bay	8/4/1997	103	3.9	3.8	75.6	15.7
Shelter Bay	8/4/1997	94	2.9	3.4	76.6	14.6
Shelter Bay	8/4/1997	92	2.6	5.8	76.8	25.0
Shelter Bay	8/4/1997	95	3.3	4.6	76.5	19.6
Shelter Bay	8/4/1997	93	3.7	4.0	76.7	17.3
Shelter Bay	8/4/1997	105	4.5	3.4	75.4	13.6
Shelter Bay	8/4/1997	96	3.2	3.1	76.3	13.3
Shelter Bay	8/4/1997	97	3.2	3.1	76.2	13.1
Shelter Bay	8/4/1997	92	2.7	3.7	76.8	16.1
Shelter Bay	8/4/1997	103	3.8	3.9	75.6	16.1
Shelter Bay	8/4/1997	96	3.2	3.1	76.3	13.2
Shelter Bay	8/4/1997	110	4.3	3.5	74.8	13.9
Shelter Bay	8/4/1997	97	3.5	2.9	76.2	12.0
Shelter Bay	8/4/1997	89	2.4	4.2	77.1	18.3
Shelter Bay	8/4/1997	99	3.5	4.3	76.0	17.8
Bainbridge	7/19/1997	66	1.7	1.5	79.6	7.4

Appendix 1–continued.

Sand Lance						
Location	Collection Date	Length (mm)	Weight (g)	% Lipid (Wet weight)	% Water	% Lipid (Dry weight)
Bainbridge	7/19/1997	70	1.0	2.5	79.2	12.2
Bainbridge	7/19/1997	59	1.0	2.0	80.4	10.1
Bainbridge	7/19/1997	60	1.5	2.3	80.3	11.7
Bainbridge	7/19/1997	68	0.9	2.1	79.4	10.3
Bainbridge	7/19/1997	56	1.2	2.1	80.7	11.1
Bainbridge	7/19/1997	76	1.3	3.0	78.5	14.2
Bainbridge	7/19/1997	74	1.2	3.7	78.7	17.3
Bainbridge	7/19/1997	83	1.9	2.9	77.8	13.1
Bainbridge	7/19/1997	80	1.7	3.4	78.1	15.7
Bainbridge	7/19/1997	75	1.2	3.3	78.6	15.3
Bainbridge	7/19/1997	72	1.3	2.4	79.0	11.2
Bainbridge	7/19/1997	75	1.4	2.8	78.6	13.2
Bainbridge	7/19/1997	66	1.0	2.9	79.6	14.2
Bainbridge	7/19/1997	77	1.4	3.6	78.4	16.4

Appendix 2.– Size and lipid data from data from the temporal variation study of age-0 and age-1 sand lance in Kachemak Bay, Alaska during the summer of 1998. Lipid content is the percentage of the whole wet weight. Age-0 individuals were identified by the lack of pigment on their bodies.

	Sample Date	Length (mm)	Weight (g)	% Lipid
Age-0	30-May-98	45	0.18	6.87
Age-0	30-May-98	46	0.2	6.41
Age-0	30-May-98	43	0.11	9.17
Age-0	11-Jun-98	50	0.29	2.6
Age-0	11-Jun-98	52	0.18	4.12
Age-0	13-Jun-98	52	0.32	4.73
Age-0	25-Jun-98	54	0.41	3.06
Age-0	25-Jun-98	54	0.4	3.72
Age-0	26-Jun-98	45	0.19	3.87
Age-0	9-Jul-98	63	0.66	3.77
Age-0	9-Jul-98	61	0.7	2.51
Age-0	9-Jul-98	60	0.61	2.86
Age-0	22-Aug-98	47	0.25	2.02
Age-0	22-Aug-98	64	0.78	1.92
Age-0	22-Aug-98	56	0.56	2.22
Age-1	30-May-98	78	1.5	2.33
Age-1	30-May-98	88	1.97	2.67
Age-1	30-May-98	77	1.55	1.78
Age-1	11-Jun-98	90	2.45	3.78
Age-1	11-Jun-98	92	2.57	3.98
Age-1	11-Jun-98	95	2	5.99
Age-1	25-Jun-98	108	4.06	5.85
Age-1	25-Jun-98	100	3.47	5.84
Age-1	25-Jun-98	42	2.8	5.36
Age-1	9-Jul-98	103	4.29	6.82
Age-1	9-Jul-98	90	2.64	5.11
Age-1	9-Jul-98	95	3.51	6.55
Age-1	24-Jul-98	91	2.83	5.03
Age-1	24-Jul-98	73	1.04	2.4
Age-1	24-Jul-98	67	0.83	1.51
Age-1	8-Aug-98	71	1.17	3
Age-1	8-Aug-98	71	0.83	2.41
Age-1	8-Aug-98	73	0.95	3.15
Age-1	22-Aug-98	82	1.77	3.97
Age-1	22-Aug-98	83	1.79	4.76
Age-1	22-Aug-98	72	1.17	2.13