CHAPTER 5

94320-I Confirming Food Web Dependencies Using Stable Isotopes

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Project Title: SEA:Confirming Food Web Dependencies Using Stable Isotope Ratios

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1. ABSTRACT

This project consists of two components - provision of analytical services for the stable isotope ratio investigations associated with EVOS projects and an investigation of food web relationships and trophic interactions of harbor seals and other top consumers of Prince William Sound. Through the use of harbor seal tissues collected from native harvested animals and tagging programs, seasonal and migrational information is being obtained with regard to prey utilization and trophic status at differing locations. Preliminary results indicate that within Prince William Sound, harbor seals fall at the top of food chains based on in situ primary and secondary productivity. Isotope ratios along whiskers grown over the past year indicate, however, that some individuals migrate into areas (presumably in the Gulf of Alaska) wherein the food web structure is very different and the seals feed at a full trophic level below that in the sound. An alternative explanation may be that isotope ratios of similar prey are reduced by several parts per thousand in the Gulf of Alaska. Current analyses of potential food items may provide indication of the dietary composition of prey in these locations. Experiments with captive animals to determine whisker growth rates and body tissue turnover times are being conducted to calibrate observed changes in the wild.

2. INTRODUCTION

This project contributes to the Sound Ecosystem Assessment program being conducted by the Prince William Sound Science Center and the Institute of Marine Science, University of Alaska Fairbanks to describe the food chains supporting important commercial fish species that appear to have been impacted by the Exxon Valdez Oil Spill. In addition, it contributes to the studies by the Alaska Department of Fish and Game personnel to determine the reasons for the decline of harbor seal and steller sea lion populations in Prince William Sound. In addition, the project seeks to better describe the trophic interactions and trophic status of marine mammals, birds and their prey species. The integrating methodology for this wide range of tasks is the use of stable isotope ratios as natural tracers of carbon and nitrogen transfers through the food webs. This project was interrupted in October 1994 and no interim funding was provided. Since then the project has resumed but the results described herein are preliminary and will be almost certainly be drastically modified by completion of the study.

Carbon isotope ratios serve as conservative tracers of energy supply between trophic levels (phytoplankton to zooplankton to fishes to top consumers). Seals, cetaceans, birds, etc., acquire the isotope ratios in proportion to the amount of food derived from each differing source. This, in turn, is reflected in the composition of body tissues and in keratinous tissues (claws, feathers, baleen, whiskers) as a temporal record when multiple sources of food are consumed over time and space. This allows the discerning of important habitats and food resources in animals that seasonally migrate or undergo periods of hyper- and hypotrophy.

Nitrogen isotope ratios reflect both the food sources and the trophic status of that animal. As nitrogen in food is consumed and assimilated by a consumer, the heavy isotope is enriched by approximately 3 ‰ with accompanying loss of the lighter isotope through excretion. The enrichment occurs with each trophic step and thus allows the construction of conceptual models and food webs and the assignment of trophic status to species for which dietary data are sparse. The data obtained from these measurements are unique in that they trace materials actually assimilated and thus can be used for more accurate ecosystem modeling.

It can be postulated that the natural stable isotope abundances of PWS biota will shift because of changes in trophic level, food web structure, and primary productivity in the context of the SEA hypotheses, thus providing an independent tool to verify, quantify and model ecosystem processes. The tracer nature of the approach will enable the integration of ecosystem components. It will enable us to monitor both "top down" (predation) and "bottom-up" (food supply) controls on herring and salmon production.

The project is composed of three elements:

1. A research component on marine mammals focusing on the trophic energetics and ecosystem dynamics of harbor seals conducted by Dr. Schell, PI, in cooperation with ADF&G personnel working as part of the marine mammal program. A smaller additional

effort using captive animals to calibrate the responses to changing isotopic composition in diet and to determine vibrissae growth rates is also currently under way

2. A research effort closely tied to the study focusing on lower trophic levels having direct application to the testing of hypotheses regarding fisheries resources. This work is being conducted by Dr. T. Kline of the Prince William Sound Science Center in cooperation with the marine mammal component and is described in the report accompanying this section.

3. As the major isotope ratio analysis facility, we have provided analytical services for carbon and nitrogen isotope ratios to other PI's involved with EVOS studies and assisted with the interpretation of the acquired data. This task has required approximately 20% of the analytical and research effort and is continuing.

The objectives of our section of the isotope study include:

1. Collect samples of harbor seal vibrissae through continued cooperative work with the Alaska Department of Fish and Game in Prince William Sound;

2. Collect samples of harbor seal prey species including forage fishes, salmon and herring in the vicinity of major haul-outs and high population densities. Samples of seal tissues will be collected from native hunters. These samples will be obtained through assistance by ADF&G personnel monitoring harvests and through the efforts of T. Kline.

3. Perform stable isotope ratio analyses on tissues and organisms collected during the sampling program. Through the use of **carbon** isotope data on taxa collected over geographical regions, the presence/absence of **isotopic gradients** useful in sorting out habitat dependencies will be determined.

4. Assist other research programs in the Prince William Sound ecosystem study by conducting stable isotope ratio analyses on samples provided and aid the interpretation of results. This effort will require approximately 20% of the analytical and research effort.

5. Through the use of **nitrogen** isotope ratios in collected taxa, assign **trophic status** to species in each region. Compare trophic status with predictive models based on conceptual food webs.

6. Determine temporal changes in harbor seal trophic status and food dependencies by comparing isotope ratios along the lengths of vibrissae with prey availability and their isotope ratios. Through the use of captive animals being fed known diets, establish the relationships between whisker growth rate and temporal changes and the fractionation factors between the $\delta^{13}C$ and $\delta^{15}N$ values of diet and consumer.

7. Compare the isotope-ratio derived food web models to predictions by the "lake-river" hypothesis and others being tested by the SEA project as an independent means of validation.

3. METHODS

The analytical methodology for stable isotope analysis are described in detail in the accompanying report by T. Kline. Sampling of tissues for stable isotope analysis has been described for both bulk tissues (muscle, blubber) and temporally variable tissues (whiskers, claws, etc.) (Schell, et al. 1989; Michener and Schell, 1994). This report includes only the pertinent sampling protocols and a synopsis of the analytical methods.

Sampling of marine fauna - - The collection of samples for isotope analysis was conducted through several channels. Forage fish, pollock and other commercial species have been obtained through cooperative programs with the National Marine Fisheries Service, the Alaska Dept. of Fish and Game, and from the Prince William Sound Science Center. As part of the cooperative effort with Dr. Kline, samples from both programs were compared and the analyses run on a coordinated suite of specimens collected over the geographic regions of the Sound and over the seasons. This allows "within taxa" comparisons to determine if the observed shifts in isotope ratios arise from changes in trophic levels or from the effects of geographic gradients in the isotope ratios of primary producers growing in differing regions. Isotope ratios changes from the latter source may arise from migrations into the region or the advection of differing source waters into the Prince William Sound.

Samples of marine mammals, birds, etc., have been and will be obtained from archived materials, strandings, native harvests and in some cases, collection in the field. This effort will be closely coordinated with the US Fish and Wildlife Service, ADF&G, and the EVOS-sponsored efforts having field programs. Our experience in 1994 has already produced a wide variety of samples and there is reason to anticipate that 1995 will be even more productive as the requests for materials are communicated to field researchers. The small amounts of sample required for isotopic analyses means that little effort for preservation or transport is required.

Analytical techniques - - The samples obtained are dried and powdered for homogeneity and the isotope ratios of carbon and nitrogen determined with a Europa 20/20 mass spectrometer system. The sample is combusted at high temperature and the nitrogen and carbon dioxide gases separated and purified by gas chromatography. These are subsequently led into the mass spectrometer by capillary and the isotope ratios determined. Results are reported in the standard $\delta^{13}C$ and $\delta^{15}N$ notation.

Captive animal studies - - The application of isotope ratio work with marine mammals is relatively new and the technique is still in a process of calibration. We have been offered the opportunity to conduct captive animal experiments at the Memorial University of Newfoundland and the Mystic Aquarium in Connecticut using harp seals and harbor seals. We plan to conduct measurements of whisker growth rates and correlation experiments between seals and diets of known composition. Seal vibrissae will be marked and growth rates measured over the seasonal cycles to determine if physiological effects are translated into differing isotope ratios. This work will comprise only a limited amount of the total effort but will be essential, given this relatively new field of application. This work will be conducted by Ph. D. student Amy Hirons as part of her dissertation program. This project will support travel costs to Memorial University to establish experiment protocols and to acquire data and information from cooperating investigators.

Synthesis of data - - The plots of isotope ratios of carbon and nitrogen along the lengths of vibrissae from harbor seals show oscillations in isotope ratios in response to dietary changes over the season. As data with supporting natural history information are acquired, the values at specific intervals will be compared with potential prey for likely matches. These will be compared with observational data and known feeding habits. From this information, sampling can be constrained to the most probable food sources and further directed analyses performed to confirm or deny conceptual food web structure. Once the growth rates of whiskers are known for a species of marine mammal it will be possible to estimate the seasons in which marked changes in diet occur. This information will focus future sampling of potential prey and provide insight into the ecological interactions contributing to the decline of populations. During 1995, in cooperation with ADF&G personnel, the stable isotope data will also be compared with fatty acid compositions in seal blubber to determine if individual molecular compounds can also serve as more specific proxies for dietary components.

4. PRELIMINARY RESULTS

Isotope Ratio Variations in Harbor Seals

To date, whiskers from over 60 harbor seals have been analysed. The isotopic data from representative seals from regions within Prince william Sound are shown for illustration of the types of patterns found. Figures 1-3 show the $\delta^{13}C$ and $\delta^{15}N$ values at 0.25 cm intervals along the lengths of harbor seal whiskers collected during tagging operations in Prince William Sound. Male seals show relatively constant values over the time span represented by the length of the whisker whereas the two female animals (Figs. 2 and 3) both showed a marked change in isotope ratios of both carbon and nitrogen during the same length of time. The cause of this shift currently is not known, but data on zooplankton from the Bering Sea indicate that a major geographic gradient exists between on-shelf and deep water regions with samples form deep water having much more depleted values for both $\delta^{13}C$ and $\delta^{15}N$. Whether this is also true of the Gulf of Alaska is not known but the sampling program for 1995 is being designed to test if the same is true south of Montague and Hinchinbrook Islands.

Figure 4 compares the isotope ratios in the seal whiskers with the values found for potential prey and other species resident in Prince William Sound. The values for most of the samples indicate that the seals fit the expected trophic enrichments in $\delta^{15}N$ and closely match the $\delta^{13}C$ of Prince William Sound prey. However, the most enriched values present in the females do not match prey from Prince William Sound but have depleted

$\delta^{15}N$ values that may result from migration into deep water feeding areas outside of the Sound. Determination of the causes for these variations is part of the focus for 1995.

Captive Animal Studies

Through cooperation with Keith Hobson of the Canadian Wildlife Service, we were able to acquire whiskers from two harp seals that had been held in captivity and fed known diets of herring. The whiskers from these animals were analyzed along their lengths and are being compared with the isotopic composition of the diets. This work is almost complete. Preliminary results indicate that the seals closely reflect the diet, remaining within 1.5^{0}/oo in carbon and within approximately the same range in $\delta^{15}N$ but showing the expected 3^{0}/oo trophic enrichment. The data from this experiment will be assembled into a manuscript upon completion of the remaining diet and tissue samples. Other experiments using steller sea lions are being conducted on a related project and the data from those experiments should prove useful in helping us interpret the seal data although the sea lions continue to grow their whiskers over multiple years. harbor seals apparently shed their whiskers during the annual molt.

Isotope Ratios in Potential Prey

The wide spread of potential prey items in Prince William Sound that may be consumed by harbor seals has been collected over the past field season or was archived from previous sampling trips. These data are presented in the section by T. Kline accompanying this report. Samples of harbor seal prey species including forage fishes, salmon and herring in the vicinity of major haul-outs and high population densities have been collected by us and further collections are planned for spring and summer 1995.

Samples of seal tissues collected from native hunters through assistance by ADF&G personnel monitoring harvests are currently being analyzed. These data will be integrated into our growing data base and compared with archived samples. Through the use of isotope data on taxa collected over the Gulf of Alaska and along the Aleutian Islands, we have identified isotopic gradients that may be responsible for the major shifts found in the tissues of some harbor seals (see Fig. 1).

Interactions with Other Studies

The interaction with the modeling component of the SEA program will intensify during the months ahead. As more data are acquired, we will be able to test model assumptions and predictions by independent comparison using the isotopic model as a validation measure. Although similar carbon isotope labels in different members of the marine community may be indistinguishable, the trophic changes predicted will lead to testable shifts in the isotope ratios of nitrogen.

To date the interaction with other studies on top consumers has been limited to the acquisition of whiskers from archived carcasses of sea otters and sea birds. Following the analysis and interpretation of these samples in 1995, further investigations will be planned linking the top consumers of Prince William Sound into an ecosystem trophic model. No data are available for reporting at this time.

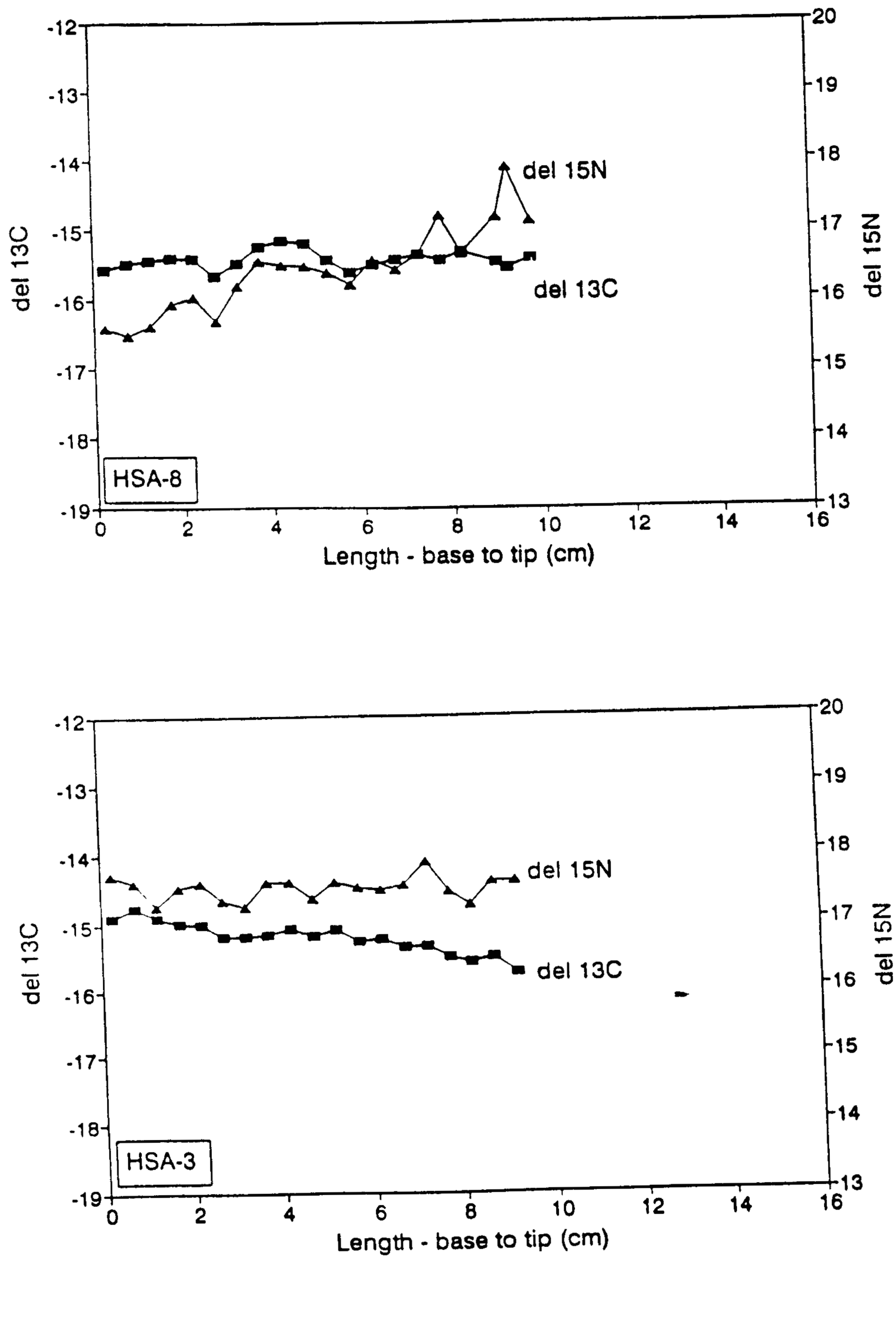

Figure 1. $\delta^{13}C$ and $\delta^{15}N$ values for adult male (upper) and juvenile male (lower) harbor seal vibrissae collected at Seal Island and Applegate Rocks (respectively), Prince William Sound, May 1993.

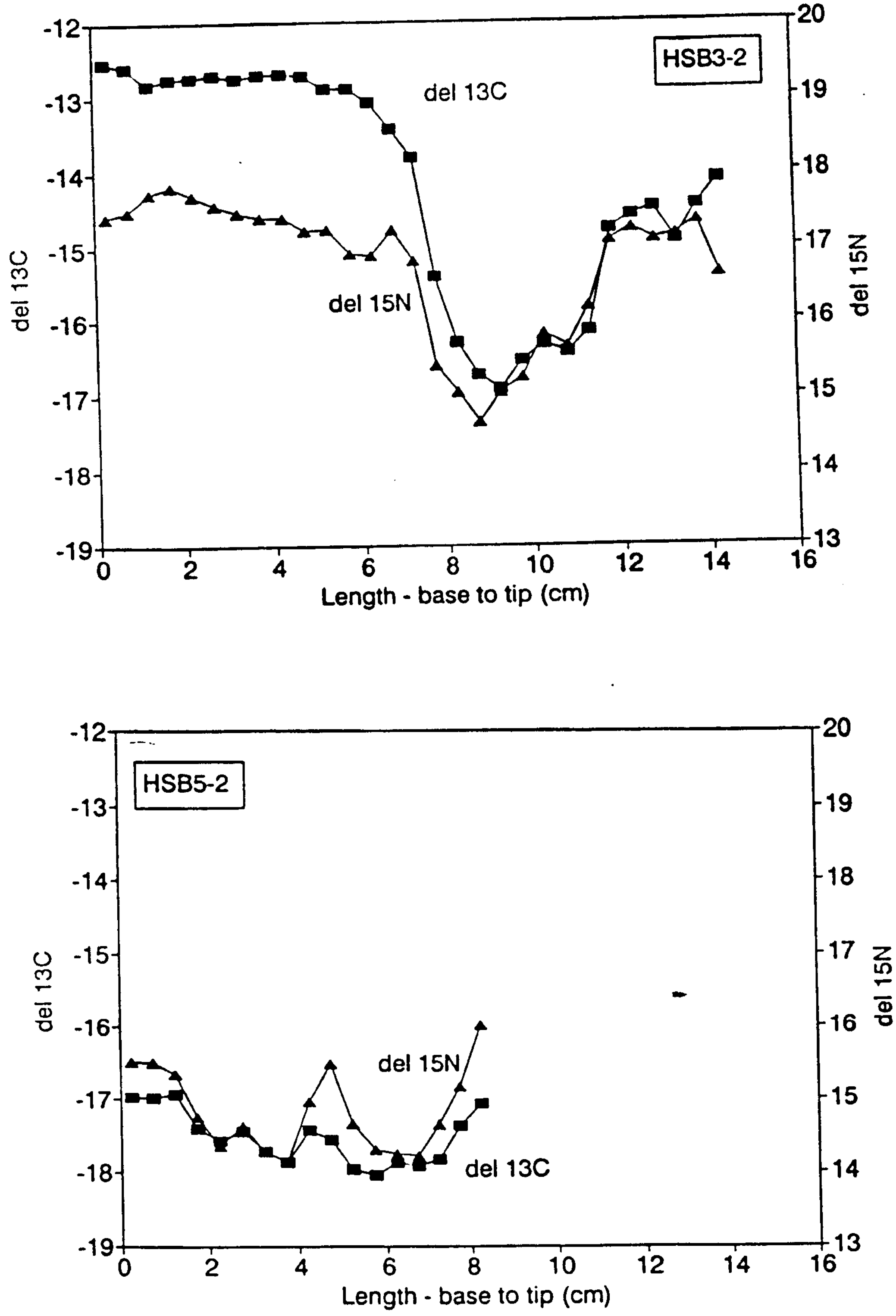

Figure 2. $\delta^{13}C$ and $\delta^{15}N$ values for adult female (upper) and subadult male (lower) harbor seal vibrissae collected at Montague Island, Prince William Sound, April 1994.

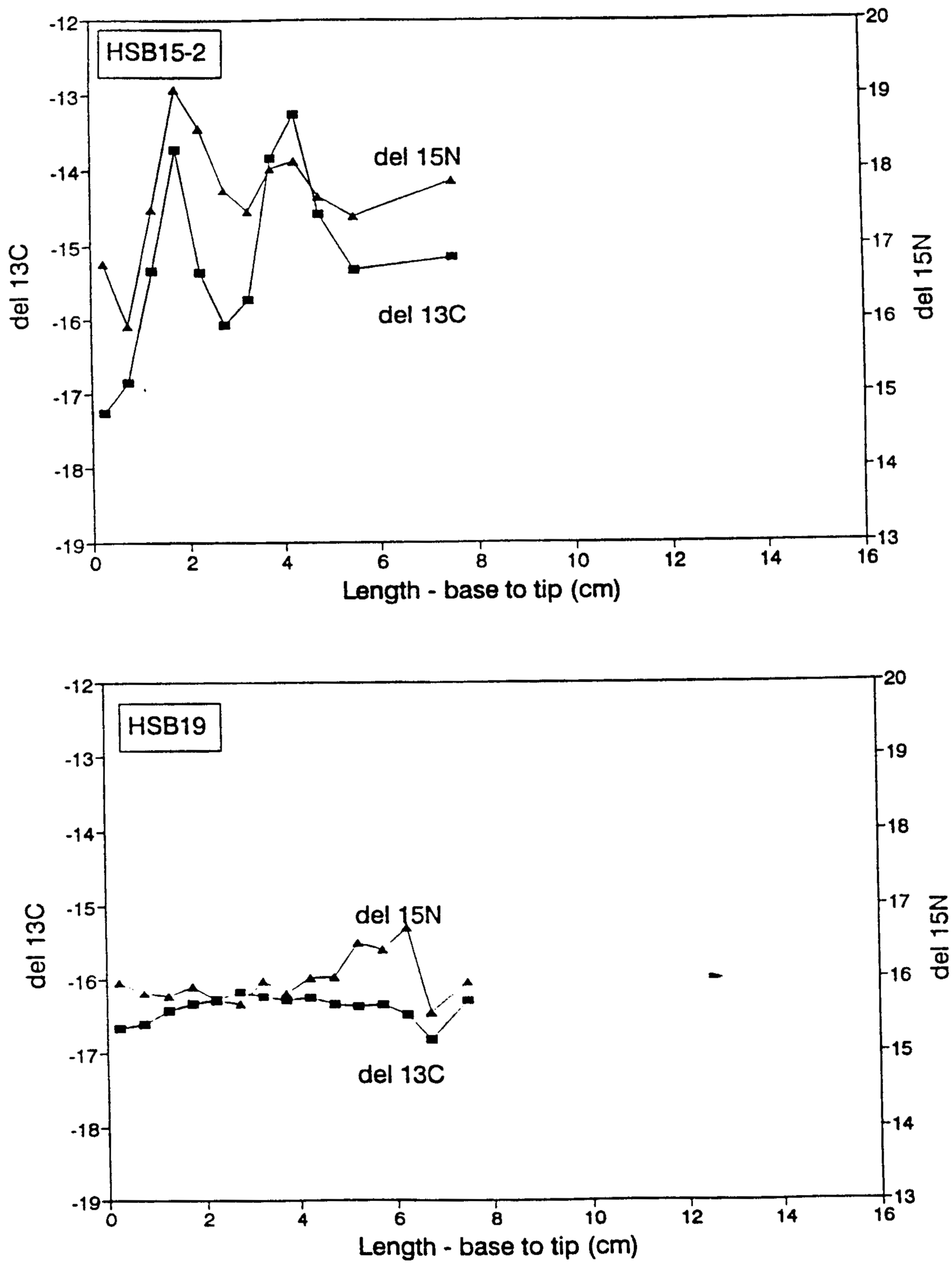

Figure 3. $\delta^{13}C$ and $\delta^{15}N$ values for subadult female (upper) and subadult male (lower) harbor seal vibrissae collected at Channel Island, Prince William Sound, September 1994.

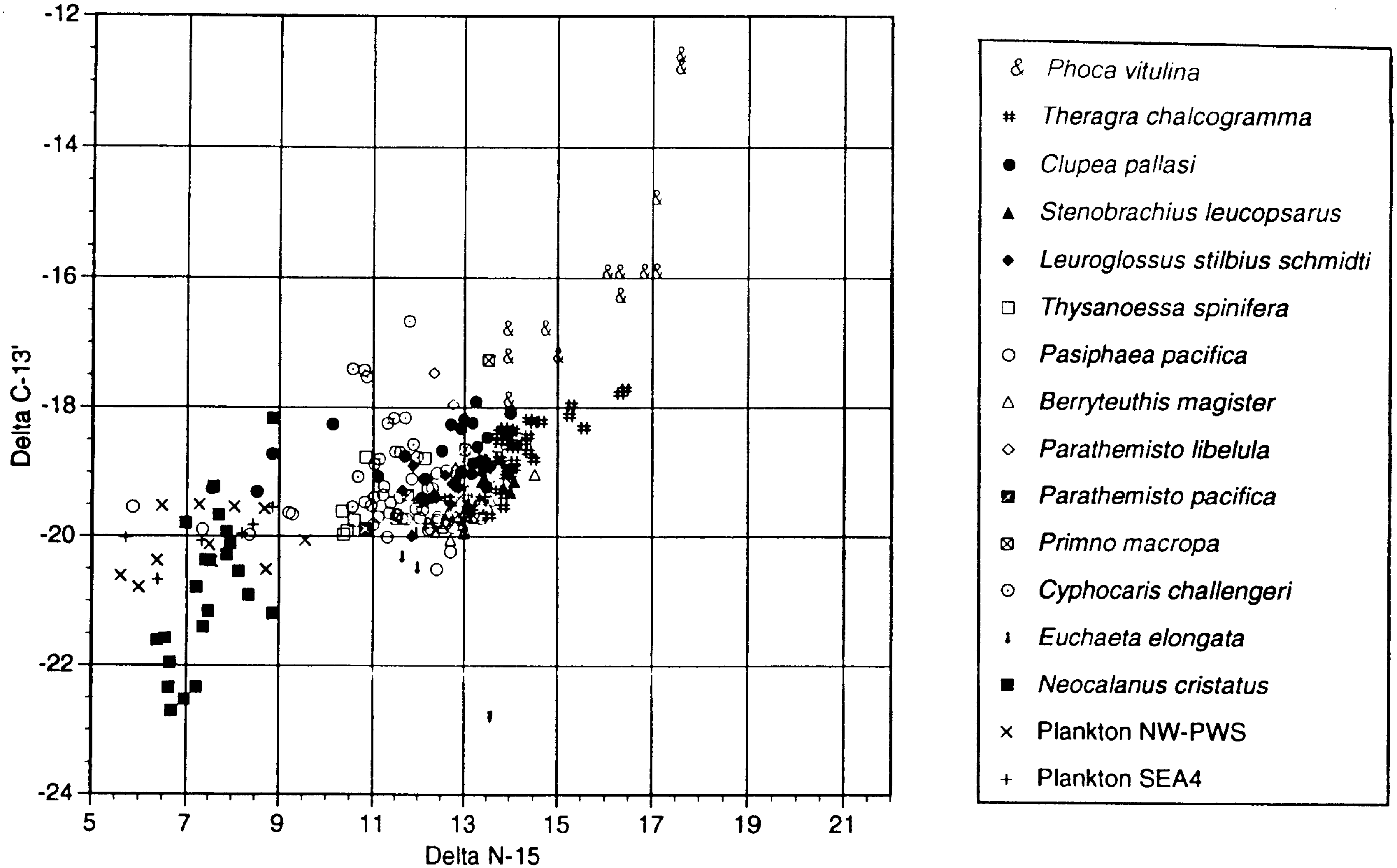

Figure 4. Kline et al. and Schell et al. data; not to be published without written permission.

Exxon Valdez Oil Spill Trustee Council
FY94 DRAFT FINAL REPORT

Project Title: SEA: Confirming Food Web Dependencies in the Prince William Sound Ecosystem Using Stable Isotope Tracers - Food Webs of Fishes.

Project Number: 94320I(2)

Lead Agency: ADF&G

Project Start-up Date: April 1994

Project Duration: 5 Years

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1. ABSTRACT

In project 94320I(2), 500 samples (including bulk zooplankton, individual macrozooplankters/micronekters and fishes) were analyzed for $\delta^{13}C$ and $\delta^{15}N$, conventional expressions for $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios, respectively. All samples except for smaller macrozooplankters were analyzed in replicate. The resultant data were used to reconstruct food webs using dual isotope plots. These plots suggest the existence of multiple carbon sources postulated to correspond to pelagic and neritic organic production sources. The large copepods of the genus *Neocalanus* had distinguishable $\delta^{13}C$ compared to bulk plankton samples. Isotopic shifts in herring were consistent with shifts in feeding regimes hypothesized to occur between overwintering and spring. The pelagic squid *Berryteuthis magister* found to be abundant in trawl sampling had large in-season shifts in $\delta^{13}C$ suggesting that this species might be an ideal biomonitor using stable isotopes. These shifts corresponded to SEA processes related the Lake/River and Predator/Prey hypotheses. Shifts in $\delta^{15}N$ suggest small yet significant changes in relative trophic positions and hence energetic position of major species. A seasonal downshift in $\delta^{15}N$ contraindicates depletion of the dissolved nitrogen pool in Prince William Sound.

2. INTRODUCTION

Stable isotope ratios of carbon can serve as effective tracers of energy supply in the study area due to conservative transfer of carbon isotope ratios between the lower tropic levels (phytoplankton to zooplankton to forage fishes, etc.) of Prince William Sound (PWS) and adjacent Gulf of Alaska waters up to the top consumers. The seals, whales and birds acquire these isotope ratios in response to the importance of the food sources and record temporal signals in keratinous tissues (claws, hair, feather) and reflect the major sources of their food in the bulk body tissues (muscle and fat). Isotope ratio analysis of these tissues can provide insight into both habitat usage and assist in quantifying amounts derived from various areas. Nitrogen isotope ratios, in turn, provide excellent definition of relative trophic level. The heavy isotope of nitrogen is enriched by about 0.3 % with each trophic level and thus can accurately indicate the relative trophic status of species within an ecosystem.

The availability of macrozooplankton forage for salmon, herring, and their predators varies in space and time because of changes in physical processes in PWS. In the SEA context, the latter is known as the Lake/River hypothesis. When macrozooplankton are not available, macrozooplankton consumers are forced to switch prey, thus the Predator/Prey SEA hypothesis. These shifts represent fundamental changes in the way the PWS ecosystem produces commercial species, i.e. herring and salmon. A better understanding, particularly a quantitative understanding, is a prerequisite to determining protocols for restoration and recovery of these species.

Natural stable isotope abundances reflect (1) trophic level and (2) source of assimilated matter and are thus a proxy for the change in diet specified in the Lake/River->Predator/Prey hypotheses. Stable isotope ratios will thus be used as a biomonitor of salmon and herring production and shifts in predation as tests of the SEA hypotheses.

A. Hypotheses

The following hypotheses were specified in the 1994 DPD:

Hypothesis 1. Carbon and nitrogen stable isotope ratios of biota from Prince William Sound can be used to identify major food sources to top trophic levels and to assign trophic positions to specific consumers of given age classes and habitat.

Hypothesis 2. Isotope ratios in consumers provide a means to validate conceptual food web structures, identify trophic variability by individuals within species, and to validate quantified energy flows in ecosystem models.

B. Goals

The 94320I study builds upon the existing stable isotope data base and adds new data to construct and test conceptual food webs supporting injured species (and other species for which samples are, or become available) in Prince William Sound and their prey organisms. The goal is to determine the trophic positions and to define the natural history parameters accessible from isotope ratio data in light of the observed declines in their populations. These include changes in trophic level over natural history stages, habitat dependencies, seasonal energetics and trophic dynamics relative to other community organisms. As part of this goal, this project will integrate analytical results with the field and laboratory studies of other investigators looking at food web structure, productivity of lower trophic levels, and will provide validation data for assessment of conceptual and quantitative models.

Specific objectives of the 94320I(2) project are:

i. To determine the $^{15}N/^{14}N$ and $^{13}C/^{12}C$ of species collected from the Prince William Sound ecosystem with a focus on those components important to man or important in the food webs supporting these species. Herring and salmon collected from PWS will be matched with regional isotope abundances in prey species (zooplankton, forage fishes) to allocate food sources and to assess trophic transfer efficiencies in specific areas of the sound.

ii. Determine isotope ratios on prey species favored by marine mammals in different regions of Prince William Sound. These data will allow estimation of seasonal importance of various prey species and the trophic levels of various seal species in the ecosystem. Past data has shown that there re considerable differences between individual animals of a given age and also changes in trophic level over the life span.

iii. Synthesize the data obtained in context with conceptual food webs to validate feeding models and expand the natural history information.

iv. Contribute stable isotope results to formal tests of the Lake/River-driven prey switching hypothesis developed by SEA to explain pink salmon and herring production trends.

3. METHODS

A. The Stable Isotope Approach

The use of natural abundance ratios of stable isotopes in biological systems has expanded rapidly in recent years and has proved extremely valuable in tracing carbon and nitrogen in both terrestrial and aquatic ecosystems. Most ecosystem studies depend upon two approaches: One is to construct budgets or mass balances of a key element such as carbon and attempt to determine which actions or processes in the natural history of the species of interest dominate these budgets. The second approach is to measure key rates or feeding processes and to relate the findings to the overall goal of assessing energy intake from the habitat. Although the two approaches should ideally coalesce into a coherent and complementary picture, this goal is difficult to attain. There are mismatches between time and space scales of the two approaches and such processes as feeding and isotopes can contribute both source (tracer) information and process information, they are ideally suited for identification and measurement of the movements of carbon and nitrogen in the ecosystem. Since they occur naturally, there are no concerns regarding perturbing the system or the need for experimental manipulations that might alter behavior or ambient conditions.

It was postulated that natural stable isotope abundance of PWS biota will shift because of changes in trophic level, food web structure, and primary producer in the context of the SEA hypotheses, thus providing an independent tool to verify, quantify and model ecosystem processes. The tracer nature of the approach will enable the integration of ecosystem components. It will enable monitoring of both "top down" (predatory) and "bottom up" shifts (food supply) in herring and salmon production.

The stable isotope project is an interdisciplinary effort focused on the food web dynamics supporting top trophic levels in Prince William Sound. The study provides an integrating function to projects focusing on several levels in the food chains and will employ the stable isotope ratios of carbon and nitrogen to trace trophic transfers of carbon and nitrogen between levels. One focus will concern building the data base regarding harbor seals, project 94320I(1), whereas the remaining work will seek to build a comprehensive base of isotopic data for the Prince William Sound region. In cases where regional gradients in isotope ratios exist, it may also be possible to identify critical habitats used by marine biota.

B. Basis for application of the stable isotope studies

The natural abundance of stable isotopes, e.g. $^{15}N/^{14}N$ and $^{13}C/^{12}C$, is a very powerful tool for ecological analysis because of the conservative nature of isotopic signatures in food webs (Wada and Hatori 1991). The most extensively measured process that enriches ^{15}N is the trophic level enrichment phenomenon (e.g., the transfer of material and energy from plants to animals or animals to animals). It is now well-established that consumers are enriched in ^{15}N by 0.34 ± 0.10 % compared to their diet irrespective of taxon or ecosystem (Minagawa and Wada, 1984). Although the consistency of the enrichment is not understood, the universality of it allows one to determine the number of trophic steps in a food chain from a given producer to consumer (Fry, 1988; Wada et al., 1991). Thus change in $^{15}N/^{14}N$ ratio in biota over time will reflect change in trophic level (TL). Shifts in herring and salmon diets that normally consist of macrozooplankton (largely reflecting allochthonous production having been advected into PWS) to autochthonous production (e.g. PWS neritic production) will be evidenced by stable isotope ratios because (1) a greater proportion of neritic production (enriched in ^{13}C) will be needed to make up the deficit and (2) extension of the food web will cause concomitant shifts in $^{15}N/^{14}N$ (reflecting TL shift) with $^{13}C/^{12}C$ (reflecting alternative prey). The shift in $^{15}N/^{14}N$ will be especially notable in predators because of the large TL shift. The numerical nature of stable isotope data lend themselves to modeling, e.g. modeling effects of marine-derived nitrogen using ^{15}N (Kline et al. 1993). The data can thus be used in collaboration with modeling efforts in SEA.

C. Sampling design

A broad-scale sampling effort with sufficient sample sizes at different sampling site-times for post-season analytical selection is required. This is important because one objective is to observe shifts in feeding among the target species, their prey and their predators in relation to zooplankton abundance. Thus a minimal sampling design would consist of stratifying the sampling over space and time corresponding to the sampling interval dictated by natural events. A minimal of 20 to 50 samples per taxon (fishes and their prey) is required for statistical validation to test for variation with respect to size (Kline et al. 1993) and to determine modalities occurring at a sampling site/time (Kline et al. 1990). Assuming monthly sampling intervals (3) in three generalized regions (W. PWS, NW PWS, and SW PWS), yields 9 time-space strata (this project is using the same sampling strata and stations used in the other SEA projects). A total minimal sample size of 50 per taxon thus adds up to 450 per taxon per season. Sampled taxa include: (1) pink salmon (juveniles and adults), (2) herring, (3) macrozooplankton (these include bulk samples and samples of individuals), (4) predatory and competitory fish of target species (pollock, true cod, tom cod, black cod, sand lance, rockfish, sculpins). This idealized sampling design is contingent on funding and availability of specimens for analysis. In 1994, the project was funded at an exploratory scale. It is being expanded in 1995 because of the positive outcome of 1994 analyses. Additional 1994 samples not analyzed in FY94 will be included in the FY95 effort. A final total of >1000 different samples collected in 1994 will have been analyzed for natural stable isotope abundance by the end of 1995.

D. Sample and data integration with other SEA projects

To the extent possible, multiple analyses are made on the same individual organisms. This is accomplished through the integrated field effort as samples must be routed through several procedures such that the all SEA projects needing samples and data from a specific organism can obtain them. In general, tissue collection for stable isotope analysis is done following sampling for length, weight, age (otolith and scale removal), stomach contents, and spawning condition. This sample protocol may delay the acquisition of some samples until laboratory processing is complete. The data presented here reflect samples that were obtainable in the field. Samples delayed because of post-season laboratory needs and alternate acquisition processes are presently in process. These samples include herring from which pathology analysis was made (this will enable the determination of whether infected fish were predisposed to their condition from anomalies in feeding as reflected by proxy analysis: C/N, $^{13}C/^{12}C$, and $^{15}N/^{14}N$) and CWT, coded wire tag (in the future: thermal marked otoliths) recovery pink salmon (these samples have known origins and growth rates that will compared with feeding via proxy analysis).

E. Sampling and Analytical procedures

The methodology used for isotopic sampling, analysis and data interpretation are documented in several publications resulting from prior work (See Kline et al. 1990, 1993). The UAF Stable Isotope Facility, where this project's isotopic analyses were made, has three isotope ratio mass spectrometers including a new automated system which facilitates faster sample processing and allows for more replication in small samples. This instrument calculates $\delta^{13}C$, $\delta^{15}N$, %C, and %N from each sample analysis.

Sampling protocols in the field for zooplankton and fishes are well established and will be used in any future sampling. Where samples of prey species are missing or few, proxy samples from the same area (zooplankton, benthos) which will enable a similar comparison. After the isotopic values are in hand, synthesis of the data with past unpublished data and with other literature isotope ratio values will be used to establish a trophic model.

Stable isotope ratios are reported relative to international standards (air for N and Pee Dee Belemnite (PDB) limestone for C) in standard delta notation:

$$\delta^{15}N \text{ or } \delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \text{ per mil} \qquad (1)$$

where R = $^{15}N/^{14}N$ or $^{13}C/^{12}C$ (after Craig 1957). The isotope standards have delta values of 0 by definition, i.e. $\delta^{15}N = 0$ for atmospheric N_2. Naturally occurring $\delta^{15}N$ and $\delta^{13}C$ values observed in biota, range from ~0 to ~ +20 and from ~ 0 to ~ -50, respectively. The negative $\delta^{13}C$ values reflect the relative enrichment of ^{13}C in limestone compared with biota.

F. Post-mass spectrometry procedures

i. C/N correction. Natural stable isotope data can often be interpreted without further modification. However, the data may reflect the effects of several processes that that when removed prior to interpretation are more effective for a given application. Isotope data can be normalized with respect to trophic and other isotope effects. In this project, certain biota exhibit significant shifts in lipid content. A consequence of change in lipid content is that $\delta^{13}C$ shifts occur because lipids are $\delta^{13}C$ depleted. Normalization for lipid content is made following the procedure of McConnaughey and McRoy (1979) that uses C/N ratio as a proxy for lipid content.

ii. Modeling. Graphical and numerical modeling procedures exist in the literature and new ones will be developed in the course of this project. Data modeling is particularly useful in the context of application of isotope data to specific questions answerable with this technique. Of special interest in the SEA context is the assessment of trophic level shift using $\delta^{15}N$. Stable isotope data will be incorporated into trophic models by providing empirically-determined trophic level assessment of energetics and concordance in use of carbon source among different species. Trophic level based food web energetics is based on the enrichment of $\delta^{15}N$ that occurs at each feeding step (Minagawa and Wada 1984) and the efficiency of energy transfer that occurs at each feeding step (e.g. 15%). The available energy at a given trophic level, λ, expressed as K_{λ}, is determined by the following relationship:

$$K_{\lambda} = B_{\alpha} E^{\Delta\lambda} \qquad (2)$$

where B_{α} is the available energy at a given base trophic level α, Δ is the number of trophic steps between α and λ, and E is the trophic efficiency factor, e.g. 0.15.

Δ is estimated with $\delta^{15}N$ by:

$$\Delta = (\delta_{\lambda} - \delta_{\alpha})\, 3.4^{-1} \qquad (3)$$

where δ_{λ} and δ_{α} are the $\delta^{15}N$ of λ, and α, respectively.

4. RESULTS

A. Effort

Sampling commenced with the first Alaska Beauty cruise in April 1994. Dr. Kline lead the stable isotope sampling effort during this time. ADF&G and other project personnel conducted subsequent sampling for stable isotopes because funds were not available for Kline to participate in other cruises. Approximately half of samples obtained during FY94 and analyzed for $\delta^{15}N$ and $\delta^{13}C$ came from April. Additional samples were obtained from other SEA and EVOS projects at the end of the field season. About 500 samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ in replicate during FY94. Additional samples collected during FY94 and FY95 (calender 1994) are currently in process. Results from these samples are not included here.

B. Data

Data are shown in the accompanying figures. Analysis is in three forms (1) dual isotope scatterplots to show trophic relations among the various species, (2) data plots of individual species to show relations of stable isotope ratios to other parameters and to evaluate distribution of data (histograms), and (3) evaluation of trophic level with $\delta^{15}N$ and application of trophic modeling.

5. DISCUSSION

A. Food Web Reconstruction

Food web reconstruction based on $\delta^{15}N$ and $\delta^{13}C$ shown in dual isotope plots (Peterson et al. 1987, Wada et al. 1991, Kline et al. 1990, 1993) can elucidate the flow of C and N from low to high trophic level organisms. Dual isotope plots allows one to synoptically visualize the relative importance of alternate prey in terms of carbon source to high trophic level consumers. Three such plots are shown here (Figs. 1.2 to 1.3); they are consistent in that >1 source of carbon is suggested for PWS pelagic food webs. In particular, is the differences in $\delta^{13}C$ in the *Neocalanus* species versus other zooplankton, as their abundance is postulated as being the principal determinant in the Predator/Prey hypothesis. The differentiation in $\delta^{13}C$ seen in the plankton is passed up to higher level consumers and is suggested to represent the dichotomy in pelagic and neritic inputs (Cooney 1993). Recent observation that pollock are abundant in late winter in SW PWS bays (G. L. Thomas, PWS Science Center, pers. comm.) is consistent with the more positive $\delta^{13}C$ reflecting neritic production and that the more negative $\delta^{13}C$ of the pelagic squid *Berryteuthis magister* (Nesis 1987) reflecting pelagic production. By extension, other species associated with the pollock $\delta^{13}C$ form a neritic assemblage and similarly, the more negative $\delta^{13}C$ species form a pelagic assemblage. Their appearance in PWS samples reflects the commingling of pelagic and neritic regimes via the Alaska Coastal Current and PWS topography, an aspect of the River/Lake hypothesis. Under this hypothesis, the extent of this mixing is expected to vary from year to year and should thus be detectable in terms of $\delta^{13}C$ in addition to abundance of particular organisms. Expanded discussion on each dual isotope plot is given below.

i. April, 1994. (Fig. 1.1) The ultimate purpose for using stable isotope analysis in connection with the SEA project is to test SEA hypotheses. Under these hypotheses shifts in diet from macrozooplankton to small fishes would result in extension of food chain length that would be resolved in terms of inter-annual $\delta^{15}N$ shifts. The data shown in Figure 1.1 can thus be used as a baseline to test this hypothesis should the postulated increase in food chain length occur. The increase in $\delta^{15}N$ shown in the figure corresponds to the expected enrichment in $\delta^{15}N$ of high-trophic-level taxa expected in food chains. Based on Minagawa and Wada (1984), the data suggest that pollock (mature, post-spawners) are approximately one to two trophic levels above the *Neocalanus* whereas herring (immature, age 0+) are 0 to 1.5 trophic levels above the *Neocalanus*.. Under the prey switching hypothesis, pollock are expected to increase in $\delta^{15}N$. Switching to a diet of herring should result in a pollock $\delta^{15}N$ of ~ +16.

The dual isotope plot also illustrates carbon relationships. There is a large range in $\delta^{13}C$ present in Fig. 1.1 suggesting two or more carbon sources in pelagic food webs. Bulk plankton samples (50 to 0 m vertical tow) were more restricted in $\delta^{13}C$ than *Neocalanus*. The ranging in $\delta^{13}C$ of *Neocalanus* may reflect the mixing processes, i.e. the *Neocalanus*, although sampled at one location (station SEA4, Wells Passage, south of Esther Island), may have been advected in from several natal habitats, each with particular $\delta^{13}C$ signatures. Higher trophic levels separate into different $\delta^{13}C$-based carbon groups. At given $\delta^{15}N$ indicating a single trophic level, several taxa have distinguishing $\delta^{13}C$. E.g. at $\delta^{15}N=13$, herring were -18 to -19, smoothtongues were -19 to -19.5, and squid were -19 to -20. Applying the postulate that herring overwinter in bays, then the more positive $\delta^{13}C$ corresponds to neritic carbon and the more negative carbon (e.g. the pelagic squid, *Berryteuthis*) corresponds to pelagic carbon. Thus $\delta^{13}C$ suggests that the different higher trophic level species sampled acquired their carbon from different sources. The simultaneous sampling of organisms having fed on different carbon sources probably reflects recent migration. The significance of the differing carbon signatures is that estimating fish productivity in PWS based on a single estimate of primary productivity and carbon source (Cooney 1993) may be erroneous. There may be several separate carbon sources that should be budgeted separately. It is quite possible that a significant portion of the pelagic secondary productivity is swept into PWS via the Alaska Coastal Current. Hence this production is allochthonous in nature and supplements secondary production in PWS derived from in-situ primary production. The isotope technique could assist efforts to model secondary productivity by identifying the source of organic carbon. Herring sampled at this time appear to have fed on several trophic levels. Whereas a number of herring had $\delta^{15}N$ ~+13, a few were +7 to 10, ~ 1 trophic level less.

ii. May, 1994. (Fig. 1.2) Samples collected in May and June are amalgamated into Fig. 1.2 which is also lipid corrected like Fig. 1.1. The late-season *Neocalanus* species (*plumchrus and flemingeri*) differed significantly from the *N. cristatus* sampled in April in their $\delta^{13}C$. These late-season *Neocalanus* also differed from bulk plankton sampled from the upper 50m. Euphausids are also more positive in $\delta^{13}C$ than bulk plankton. Herring $\delta^{13}C$ shifted from the more positive $\delta^{13}C$ in April sample to a more typically pelagic isotopic signature, consistent with observations that juvenile herring overwinter in bays and move into the open areas in the summer. Herring and pollock also appear to occupy similar trophic positions which is consistent with lake conditions as specified in the Lake/River SEA hypothesis. During river conditions, pollock are expected to be more positive in $\delta^{15}N$ and $\delta^{13}C$, if they feed on age 1 herring. May-June $\delta^{13}C$ is increasingly stratified in $\delta^{13}C$ - note the separation of most pollock from squid (herring overlap).

iii. July, 1994. (Fig. 1.3) This sample includes 60 young of the year pollock that were abundant at this time. These pollock feed at a slightly higher trophic level as suggested by $\delta^{15}N$ as well as on a more negative $\delta^{13}C$ than most euphausids. Apex trophic positions are indicated for rockfishes. Squid are more positive in carbon as compared with May-June.

B. Autecological Studies - Proxy Analysis

The previous section dealt with food web analysis using stable isotope data. This section deals with individual species. This enables a more clear analysis of stable isotope ratios in relation with other proxy data and for time-series and statistical analyses. Examples of this approach are given below.

i. Pacific Herring. (Fig. 2.1 and 2.2) Our lack of fundamental knowledge coupled with the dire situation of herring in PWS makes any source of information extremely valuable. The variation of stable isotope data with respect to size and lipid content over time suggests that their may be significant bottom-up effects on this species in PWS (Figs 2.1 and 2.2). Larger fish at each time appear to have a higher lipid content although lipid content decreased as the population (single population assumed) grew. It is possible that faster growing fish (larger) allocate a greater portion of their energy to growth and that the smaller fish reflect a greater allocation into lipid storage. There appears to be no relation of carbon source to lipid storage. Also trophic level was independent of lipid storage (Fig 2.2). Note that the low trophic level individuals had both high and low lipid content. Direct measurements of energy planned in 1995 along with a greater emphasis on herring may help elucidate these data. Increased sampling (data points) may elucidate trends not seen in the minimal sampling shown in Figs 2.1 and 2.2 (Additional 1994 samples are available and are presently being analyzed using FY95 funding). This will enable statistical analysis of the data as well.

ii. Pollock. (Fig. 3.1) Pollock were found to be the major pelagic predator in the predation study (94320E). Isotope data of some of the fish sampled in that study were analyzed in 94320I. Fig. 3.1 shows $\delta^{15}N$ and $\delta^{13}C$ as a function of cruise number. Although no shifts in $\delta^{13}C$ were discernable (the 95% C.I. range was about 1 per mil), adult pollock underwent small (compared to the range in the entire food web) yet significant (@ 95% C.I.) shifts during the course of the 1994 season. These shifts were consistent with shifts observed in stomach contents (project 94320E) and the postulated dependence on *Neocalanus plumchrus* (and *flemmingeri*) when these species are present. These data also show that in-season shifts in diet are manifest by shifts in $\delta^{15}N$ suggesting relatively rapid turnover of assimilated protein. Assessment of the 95 % C.I. suggests that a minimal sample size of adult pollock should be 20 in a given sampling stratum. The 95 % C.I. provide a degree of statistical validity that the trends are indeed significant.

iii. Squid. (Fig. 4.1) A large number of the pelagic squid *Berryteuthis magister* were caught during trawler surveys (Kline, pers. observation during cruise 1, and further observations by other SEA personnel). The squid were also observed to feed on pink salmon (D. K. Salmon, pers. comm.). Figure 4.1 shows the distribution of $\delta^{13}C$ in late April, May, and July 1994. April squid are evidently bi-modal consistent with the neritic-pelagic $\delta^{13}C$ dichotomy seen in other biota. The May data are more constricted and are either unimodal with a skew to the more positive values or consist of two closely placed modes. It is likely that the data represents a shifting from the two modes seen in April to a third signature, possibly pink salmon. In July, squid in PWS consisted of only the more positive $\delta^{13}C$, compare with April. This corroborates the postulate that the more positive $\delta^{13}C$ represents the neritic as the late summer is dominated by neritic plankton species (Cooney 1993). Thus the squid may act as a good biomonitor of large-scale trends in PWS as they reflect the dominant carbon sources available to higher trophic levels. Future sampling will help to verify this postulate.

C. Trophic Energetics Studies (Fig. 5.1, Table 1)
Stable isotope data will be incorporated into models being developed in SEA by providing empirically-determined trophic level assessment of energetics and concordance in use of carbon source among different species (Figs. 1.2 to 1.3). Trophic level based food web energetics is based on the enrichment of $\delta^{15}N$ that occurs at each feeding step (Minagawa and Wada 1984) and the efficiency of energy transfer that occurs at each feeding step (e.g. 15%). Assessment of a species' carrying capacity hinges on understanding the transfer of productivity through food chains (e.g. Cooney 1993). Carrying capacity of a high trophic level consumer may be effected if their high trophic level prey are already at their carrying capacity. Because energy is lost at each feeding step in a food chain, assessment of the trophic level (number of steps from a given organic matter source) is needed in order to determine availability of energy for a given species (predators and prey) at given life history stages as they are known to vary in some species (e.g., Brodeur 1990). That is, determination of the available energy within the ecosystem at the specific trophic levels occupied by these organisms. The relationship of high trophic level species with respect to each other and their food sources is also needed to determine how the available energy is partitioned and how it varies in space and time.

Three taxa that were sampled during three periods are plotted to show their relative $\delta^{15}N$ shifts and statistical validation (95 % C.I.). The data plot shows two aspects of $\delta^{15}N$: (1) the relative trophic positions that is further evaluated in Table 1, and (2) the concomitant shift $\delta^{15}N$ in all consumer trophic levels in July compared with earlier. This downshift in $\delta^{15}N$ contraindicates a decrease in dissolved nitrogen such as seen in Auke Bay, Alaska (Goering et al. 1990). Although this is a significant finding, it is beyond the scope of this project but may be examined by the phytoplankton component (9X320G) in the future. The differentials in $\delta^{15}N$ correspond to trophic level differences if we assume that $\delta^{15}N$ is relatively uniform among the postulated multiple carbon sources (this assumption is corroborated by the similarity in $\delta^{15}N$ in bulk plankton and *Neocalanus* samples at a given time (Fig 1.2 and 1.3)). Notable in this analysis is that pollock breaks away from the trend of the other species, which is consistent with a diet consisting primarily of copepods (i.e. low trophic levels) when available in the late spring. Pollock become more energy limited in July when *Neocalanus* are known to lose dominance in the plankton (Cooney 1993).

Table 1. Example of using $\delta^{15}N$ to estimate trophic energetics using data obtained of pollock, squid, and euphausids from Prince William Sound, Alaska in 1994. Energy [K in (2)] available to adult pollock and pelagic squid [λ in (2) and (3)] is expressed as a fraction of euphausid energetics [B and α in (2) and (3)] based on $\delta^{15}N$ and at trophic efficiency of 15%, using relationships (2) and (3).

Month	$\delta^{15}N$		Δ (3)		% of Euphausid Energy (2)	
	S - E	P -E	S - E	P - E	Squid	Pollock
April	2.61	3.61	.767	1.06	23.4%	13.3%
May	2.57	2.93	.755	.863	23.9%	19.4%
July	2.70	3.78	.795	1.11	22.1%	12.1%

S=Squid, E=Euphausid, P=Pollock

D. Summary

The primary goal of 94320I was to demonstrate that natural stable isotope abundance could be useful in the SEA project. This is shown by examination of the data for food web reconstruction, through autecological proxy analysis, by determination of trophic energetics, and ultimately - by hypotheses testing.

i. Stable isotope applicability hypotheses. The following postulates can be stated based on the data acquired in 1994:

Carbon and nitrogen stable isotope ratios of biota from Prince William Sound can be used to identify major food sources to top trophic levels and to assign trophic positions to specific consumers of given age classes and habitat.

Isotope ratios in consumers provide a means to validate conceptual food web structures, identify trophic variability by individuals within species, and to validate quantified energy flows in ecosystem models.

ii. SEA hypotheses. Stable isotope ratios are suggested to be a means of testing the SEA hypotheses because of the concordance of $\delta^{13}C$ with River/Lake processes. The observed $\delta^{15}N$ in 1994 were consistent with the

Predator/Prey situation in a "Lake" year. A "River" year needs to occur to further the hypothesis that $\delta^{15}N$ would shift as a result. Stable isotopes have potential as a monitoring tool to ascertain the existence of River/Lake conditions needed for model forecasts. The squid *Berryteuti is magister* is a likely candidate as a biomonitor species.

iii. Products for modeling. Because of their numerical nature, stable isotope data can be incorporated into models. This can be in the form of raw or modified data (e.g. $\delta^{13}C'$) or it can be in the form of isotope modeling products such as the trophic level energetic assessment.

iv. Products for non-SEA EVOS projects. The natural abundance of stable isotopes in marine mammals 94320I(1) is utilizing data of potential prey being generated in this study as part of their data interpretation.

E. Directions in 95320I

Completion of 1994 samples is anticipated in project 9520I that commenced in January, 1995 when interim FY95 funding was realized.

i. Additional 1994 samples:

a. Herring. The variance in April stable isotope data warrants further sampling (30 additional fish are available). Additional samples have been received from non-SEA ADF&G projects. Although there was no "herring SEA project" in 1994, a reconstruction of some stable isotope aspects are achievable through this sample supplementation. Additional samples available include 212 herring used in EVOSTC-funded pathology studies.

b. CWT recovery pink salmon. These samples have known growth rates since they can be identified as to point of origin to the marine environment. Approximately 200 CWT recovery samples are presently in process for stable isotope analysis.

c. September 1994 R/V Bering Explorer Oceanography cruise. Samples collected consist of bulk net plankton, macrozooplankton/micronekton samples high-graded from bulk plankton samples, and fishes caught by rod and reel by cruise participants. These samples samples are presently in process for stable isotope analysis.

ii. The future:

a. 1995 sampling expanded studies. The successful implementation of stable isotope analysis in 1994 has rationalized an increased effort in 1995 in addition to expanding 1994 studies through analysis of the samples just listed. This will include continuation of large scale variation studies as well as more focussed studies. The variation in *Neocalanus* is being tested for source effects by deep water samples to be collected in the early season. This project will incorporate additional collaborative sampling. These include the new study headed by AJ Paul. Stable isotope analyses will made on the same fish he is analyzing for energetics. This combination has not been done before and we are anticipating exciting results. Because of the new otolith thermal mass marking program we will be able to identify most pink salmon sampled in the wild as to point of origin. We are presently developing a proposal at the PWSSC to obtain pink salmon samples in the Gulf of Alaska where outmigrating salmon are expected to be found. We will use this opportunity in conjunction with the otolith markings to extend pink salmon studies from PWS to early ocean stages just outside PWS. We may thus get a firmer picture of processes occurring with pink salmon as they enter the high seas.

b. New hypotheses based on 1994 results. New hypotheses based on 1994 results were presented in the 95320 combined DPD and are not addressed here. These hypotheses represent applications of stable isotope abundance to questions raised in the major SEA components.

c. Restoration. The goal of the SEA project is to aid in the post-EVOS restoration effort. Stable isotopes have a major role in understanding the processes, a prerequisite in order to achieve restoration. Isotopically definable carbon sources and trophic levels are the principal products established thus far.

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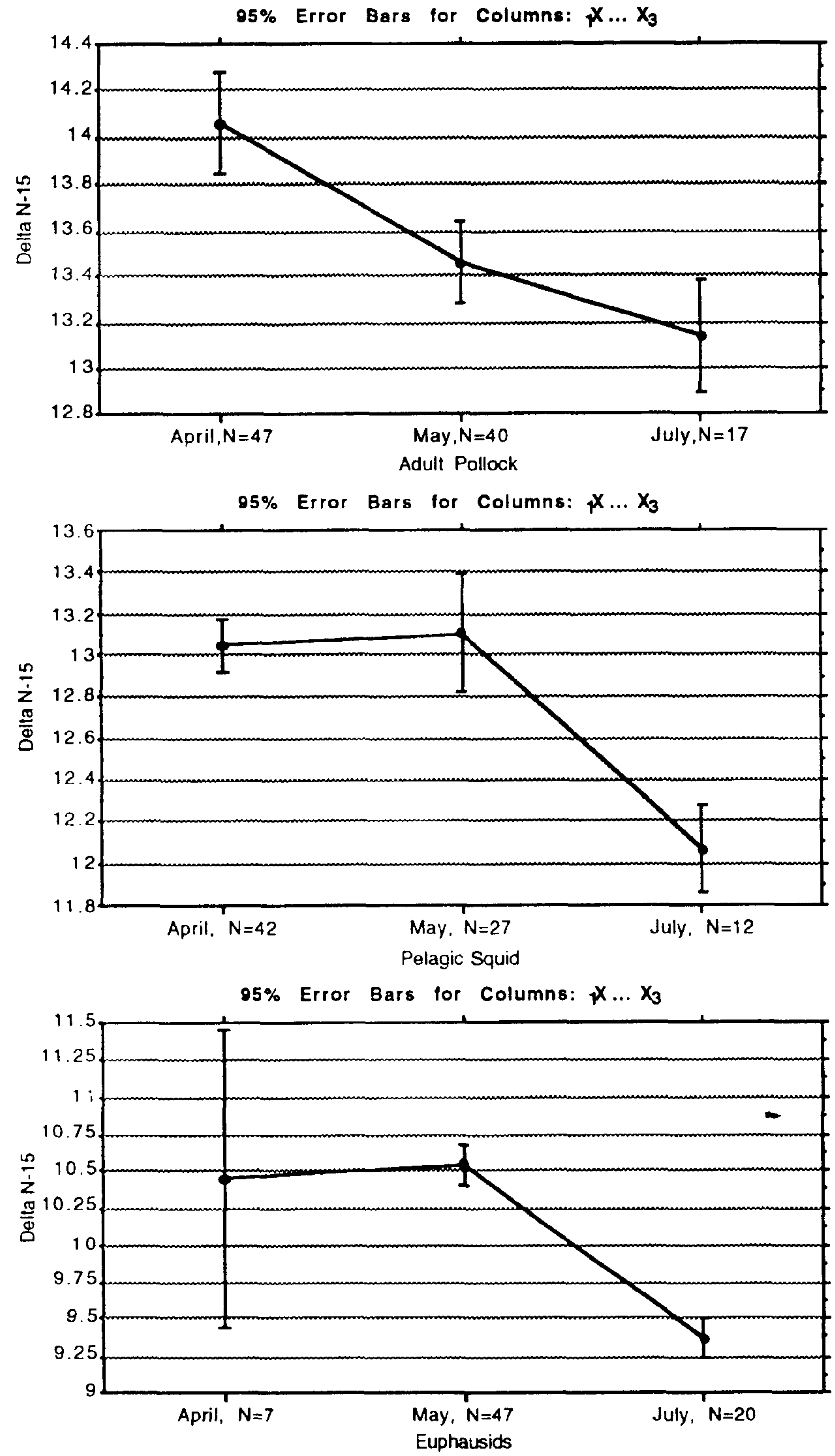


Figure 5.1. Shift in $\delta^{15}N$ seen in three pelagic PWS biota during the course of Spring - Summer, 1994. May data are the collective results from F/V Alaska Beauty Cruise 2 and 3 (May pollock data split by cruise in Fig. 3.1). April and July data from cruise 1 and 6, respectively. Sample sizes were as indicated. Kline et al. data; not to be published without written permission.

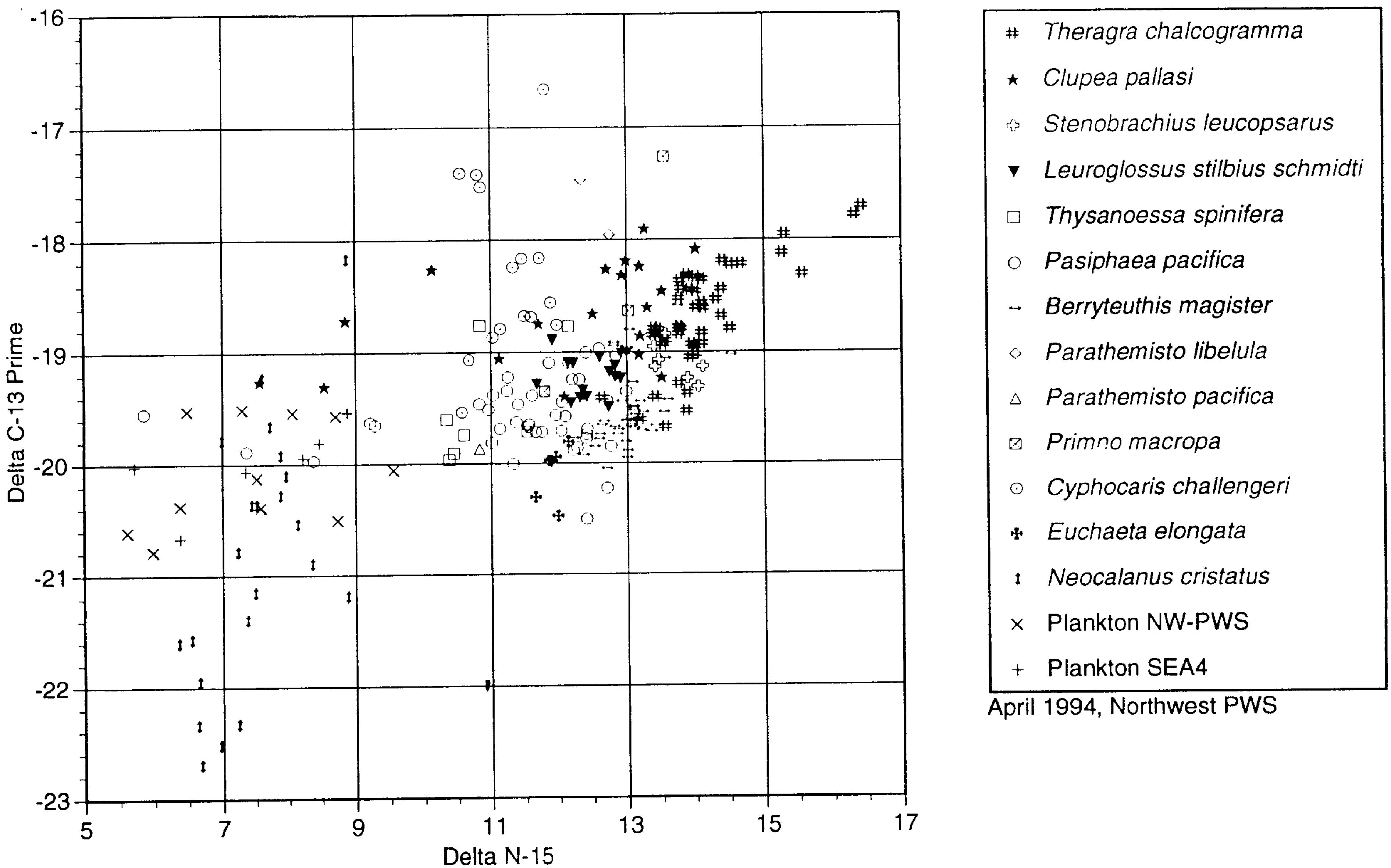


Figure 1.1. Dual stable isotope plot of Prince William Sound pelagic biota collected during late April 1994. Data points are means of replicate analyses of individual animals except *Neocalanus*, which were non-replicated individuals, and plankton, which were replicated bulk samples sampled with a 335µ-mesh 0.5 m diameter ring net. Kline et al. data; not to be published without written permission.

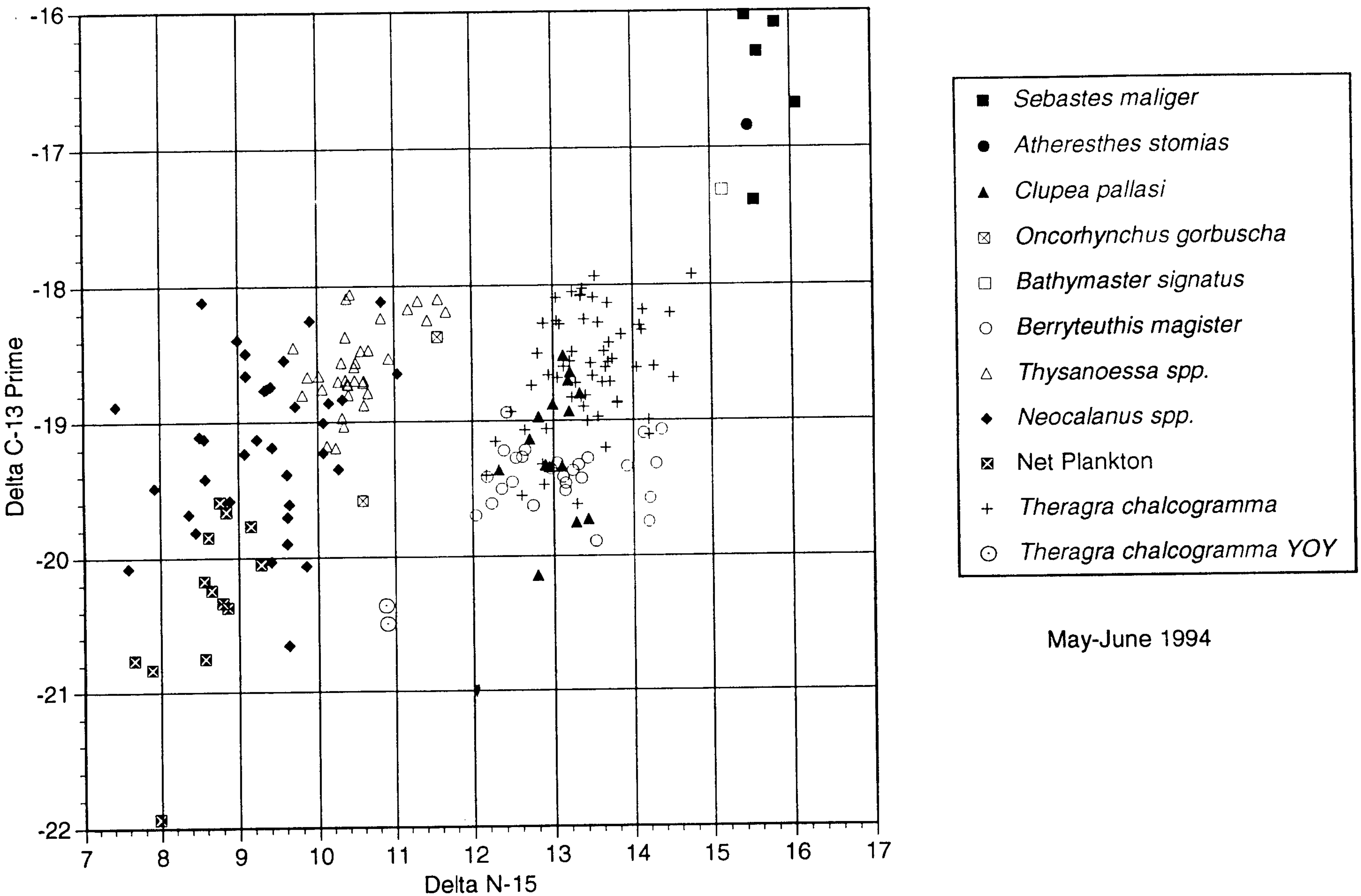


Figure 1.2. Dual stable isotope plot of Prince William Sound pelagic biota collected during May and June 1994. Data points are means of replicate analyses of individual animals except *Neocalanus*, which were non-replicated pools of 2 or 3 individuals, and plankton, which were replicated bulk samples sampled with a 335μ-mesh 0.5 m diameter ring net. Kline et al. data; not to be published without written permission.

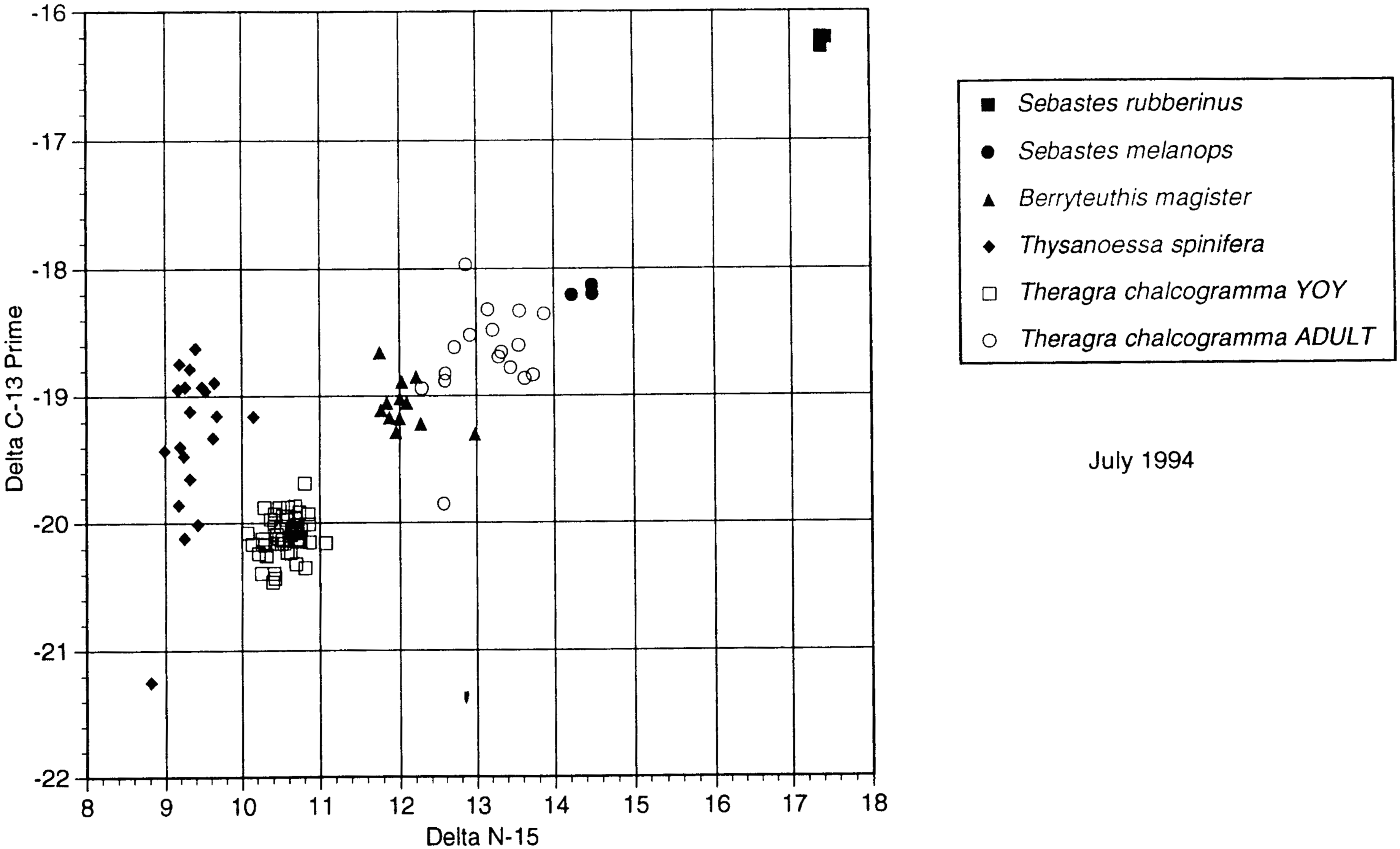


Figure 1.3. Dual stable isotope plot of Prince William Sound pelagic biota collected during July 1994. Data points are means of replicate analyses of individual animals. Kline et al. data; not to be published without written permission.

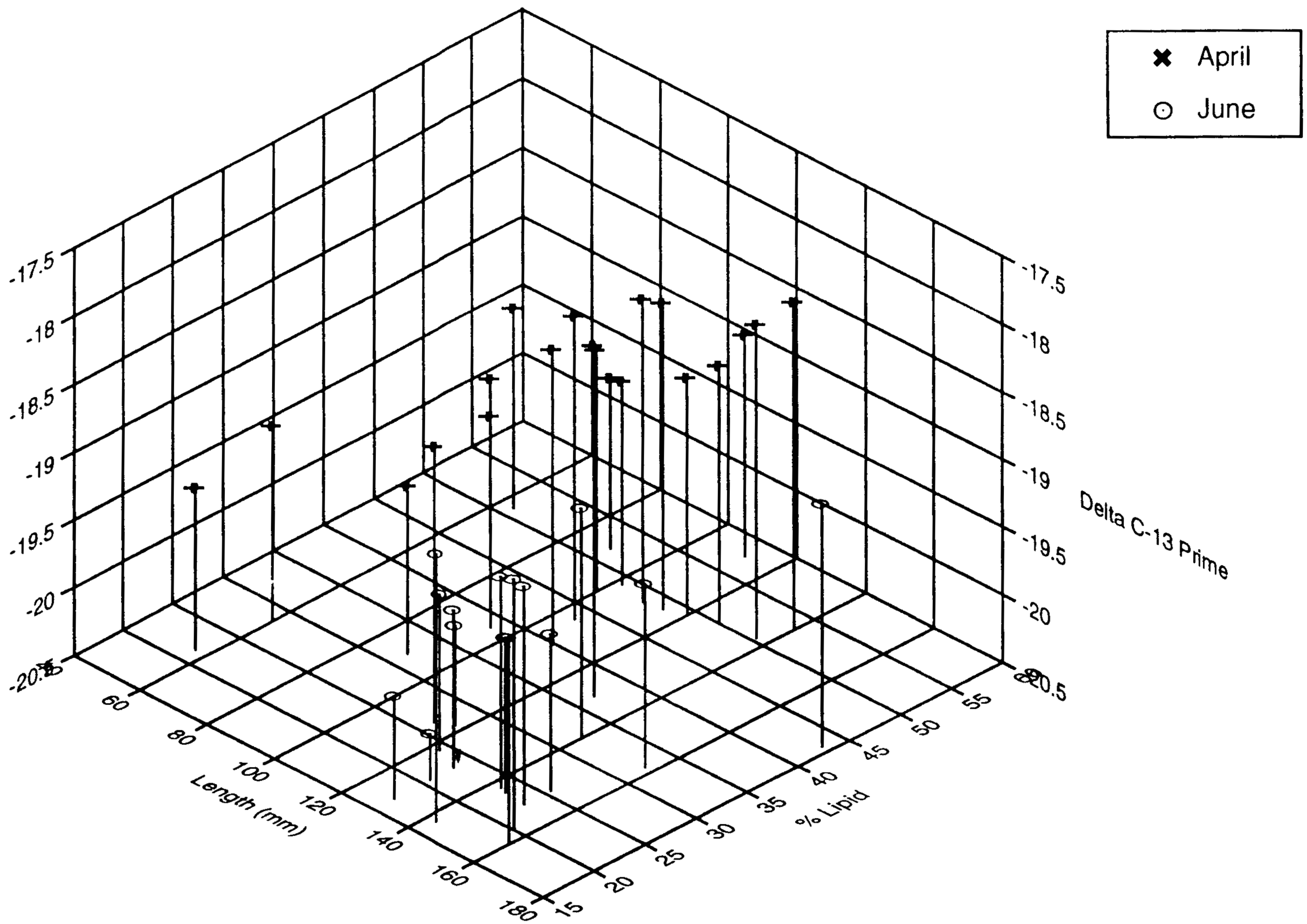


Figure 2.1. δC-13' (vertical axis and droplines) of juvenile Pacific herring sampled in PWS in 1994 as a function of length and % lipid. Kline et al. data; not to be published without written permission.

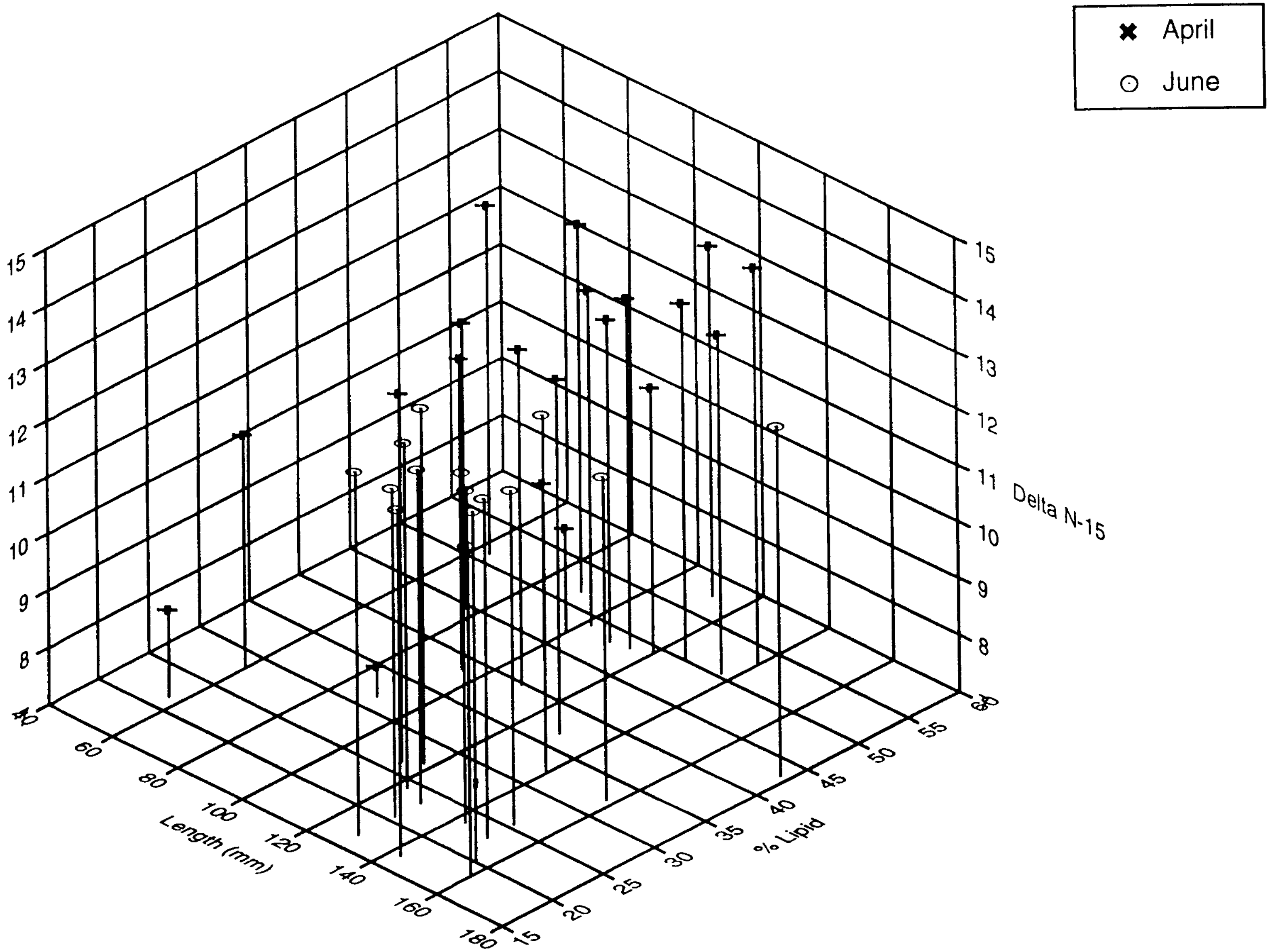
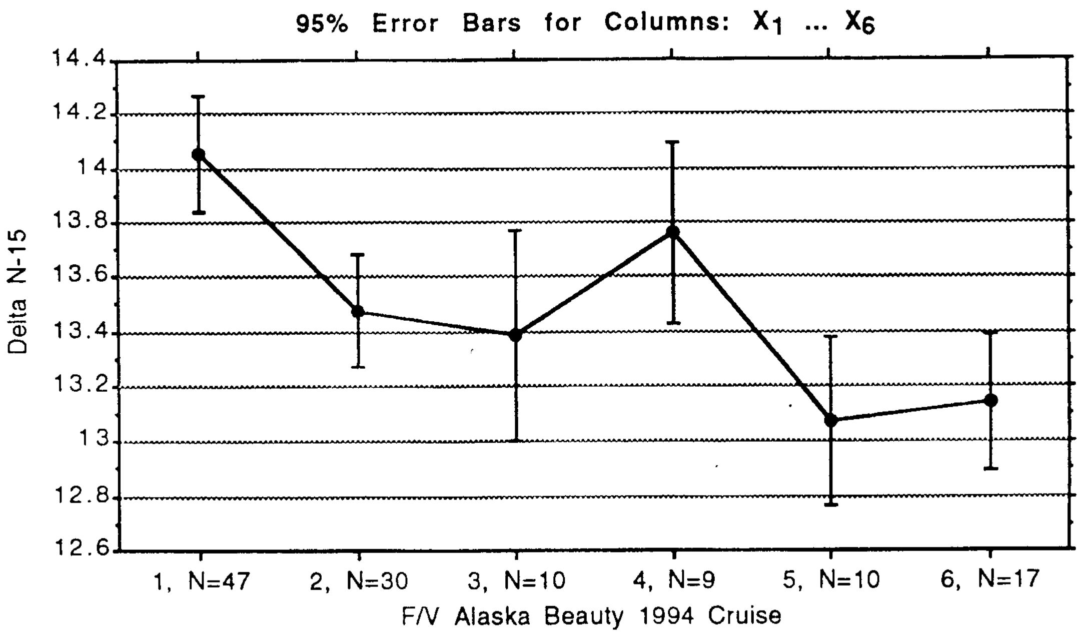


Figure 2.2. δN-15 (vertical axis and drop lines) of juvenile Pacific herring sampled in PWS in 1994 as a function of length and % lipid. Kline et al. data; not to be published without written permission.



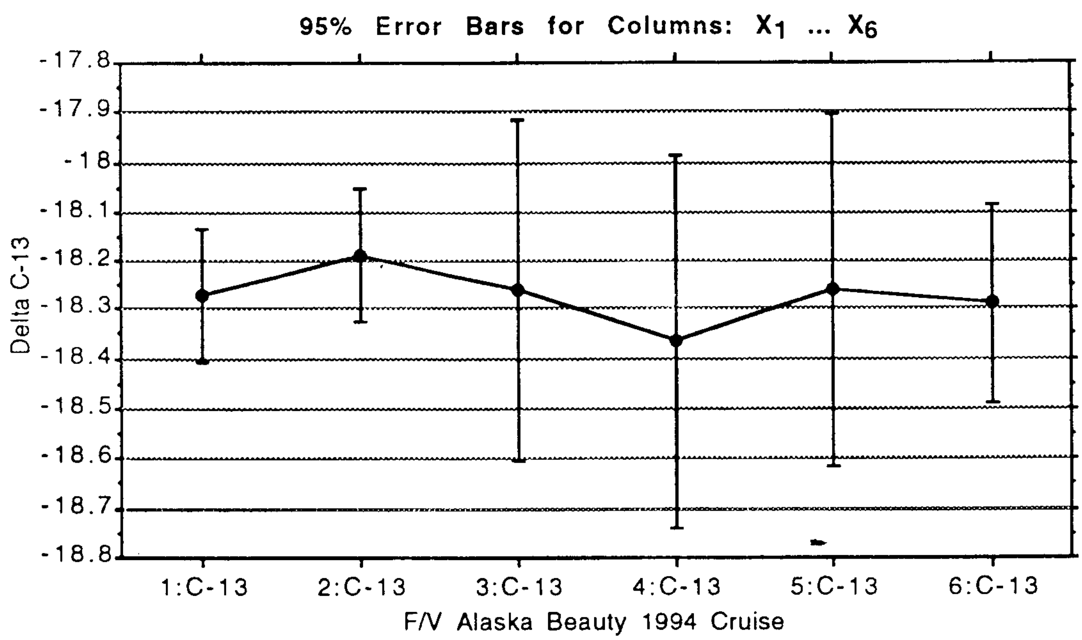


Figure 3.1 Shift in stable isotope chemistry of adult pollock in 1994. Upper panel is $\delta^{15}N$, lower panel is $\delta^{13}C$. Sample sizes indicated in upper panel applies to both. Kline et al. data; not to be published without written permission.

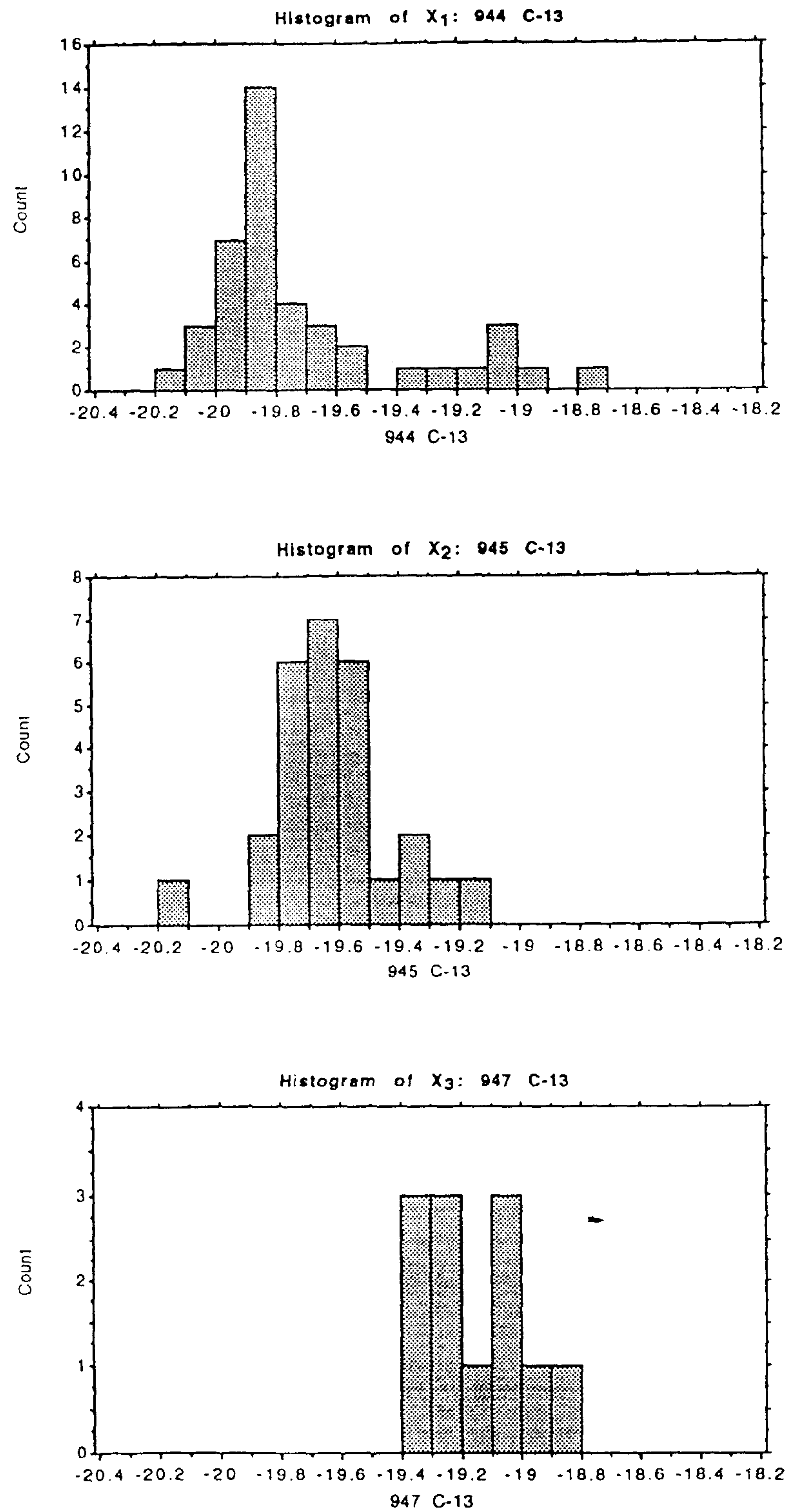


Figure 4.1 Histograms of $\delta^{13}C$ of the pelagic squid, *Berryteuthis magister* sampled in PWS during 1994. Two modes in April are postulated to correspond to pelagic and neritic carbon sources present at the sampling location (Wells Passage). Comfirmation that the shift in May corresponds to a pink salmon diet depends on on-going analytical work. Only the neritic signature was evident in July consistent with our understanding of seasonal regime shifts in PWS (Cooney 1993). Kline et al. data; not to be published without written permission.