

Exxon Valdez Oil Spill
Restoration Project Annual Report

SEA: Confirming Food Web Dependencies in the Prince William Sound
Ecosystem Using Stable Isotope Tracers.

Restoration Project 95320I
Annual Report

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

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Study History: This project was initiated under Restoration Project 94320I(2). An annual report (draft final report) was issued in 1994 by Kline, T. under the title SEA: Confirming Food Web Dependencies in the Prince William Sound Ecosystem Using Stable Isotope Tracers - Food Webs of Fishes. This project effort was continued under Restoration Project 95320I, the subject of this annual report. FY 95 is the second field season for this project that will be closed out with a Final Report prepared in FY 99.

Abstract: It is hypothesized that the availability of macrozooplankton forage for salmon and herring varies in space and time because of changes in physical processes in Prince William Sound (PWS). Under the hypotheses promulgated in the SEA project, it can be postulated that natural stable isotope abundance of PWS biota will shift because of changes in trophic level, food web structure, and primary producer. Thus natural stable isotope abundance can be used to assess these changes.

In project 95320I, biotic samples (including bulk zooplankton, individual macrozooplankters/micronekters and fishes) were analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, conventional expressions for $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios, respectively. All samples except for smaller macrozooplankters were analyzed in replicate. Data collected during the FY 94 pilot project suggested the existence of carbon sources postulated to correspond to pelagic and neritic organic production sources. Analysis in FY 95 expanded on the FY 94 results with emphasis on verification of the above postulate.

The large herbivorous copepod *Neocalanus cristatus* and net zooplankton samples from the Northern Gulf of Alaska and the area around Montague Island had distinguishable $\delta^{13}\text{C}$ values compared to analogous samples collected within Prince William Sound thus confirming the postulated production sources. Isotopic signatures of these sources were conserved in copepods undergoing diapause in PWS and fishes sampled from PWS.

Key Words: carbon, carbon sources, $^{13}\text{C}/^{12}\text{C}$, copepods, *Clupea pallasii*, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, ecosystem process, *Exxon Valdez*, food webs, Gulf of Alaska, Herring Overwintering, juvenile fishes, Lake/River, *Neocalanus cristatus*, $^{15}\text{N}/^{14}\text{N}$, *Oncorhynchus gorbuscha*, Pacific herring, pink salmon, plankton, predators, Predator/Prey, Prince William Sound, stable isotopes

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Executive Summary The failure of several Prince William Sound (PWS), Alaska vertebrate species to recover from population crashes following the 1989 *T/V Exxon Valdez* oil spill, has raised concerns that shifts in food web structure may have occurred. Of particular concern are post-spill declines in the abundance of *Clupea pallasii* (Pacific herring), and *Oncorhynchus gorbuscha* (pink salmon). It is hypothesized that abundance of large herbivorous copepods of the genus *Neocalanus* and other macrozooplankton, primary sources of food for these fishes and their predators, are controlled by oceanographic processes. When planktonic prey are unavailable, predators switch feeding mode from planktivory to piscivory. Confirmation of these hypotheses are being tested in a large-scale multi-disciplinary project known as Sound Ecosystem Assessment (SEA).

Because of their predictable nature, stable isotope ratios of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) are providing an effective method for testing the SEA hypotheses in Restoration Project 320I. Natural stable isotope ratios are useful

for providing empirical evidence of trophic relationships in marine food webs. Stable Isotopes are providing evidence in three SEA hypotheses: (1) "prey switching" through observations of seasonal isotope shifts in predators in combination with a prey database; (2) "river-lake processes" through measurement of related temporal and spatial isotopic effects within the plankton community (with the goal to trace food web carbon sources through to fishes); (3) "herring overwintering" through comparisons of energetic condition with food web carbon source as determined by stable isotope signatures.

In project 320I, biotic samples (including bulk zooplankton, individual macrozooplankters/micronekters and fishes) were analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, conventional expressions for $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios, respectively. All samples except for smaller macrozooplankters were analyzed in replicate. Data collected during the FY 94 pilot project suggested the existence of carbon sources postulated to correspond to pelagic and neritic organic production sources. Analysis in FY 95 expanded on the FY 94 results with emphasis on verification of the above postulate.

The large herbivorous copepod *Neocalanus cristatus* and net zooplankton samples from the Northern Gulf of Alaska and the area around Montague Island had distinguishable $\delta^{13}\text{C}$ values compared to analogous samples collected within Prince William Sound confirming the existence of the isotopic gradient. Isotopic analysis of the copepods undergoing their diapause phase during the winter (1994-95) suggests that half of this population fed exclusively in PWS. 17% fed exclusively in the northern Gulf of Alaska. These proportions are expected to vary between years as a function of river-lake processes. Isotopic analysis of the diapausing copepods can be used to assess river-lake variability and validate model predictions of these processes.

Variability in $\delta^{13}\text{C}$ of fishes consistent with shifting from PWS and Gulf of Alaska carbon sources were found to vary spatially and in relation to pre-winter condition of herring. Isotopic shifts in pink salmon fry corresponded to shifts from hatchery diet to carbon from the Gulf of Alaska. $\delta^{15}\text{N}$ values were used to assess trophic levels of forage and predator species. These trophic levels are expected to shift in response to ecosystem shifts resulting from river-lake processes that reduce the availability of macrozooplankton forage. Trophic level shifts monitored with $\delta^{15}\text{N}$ will enable one to detect these changes.

This study is demonstrating the applicability isotopic chemistry shifts in biota to SEA processes that will be useful in the development of model validation tools obligatory for SEA project ecosystem modeling products.

SEA-FOOD: Confirming Fish Food Web Dependencies in the Prince William Sound Ecosystem Using Natural Stable Isotope Tracers

Stable isotope ratios of carbon serve as effective tracers of energy supply in the study area due to conservative transfer of carbon isotope ratios between the lower trophic 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, birds, and fishes 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 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, other forage fish species, and predators vary in space and time because of changes in physical processes in PWS. In the SEA context, the latter is known as the river-lake hypothesis. When macrozooplankton are not available, macrozooplankton consumers are forced to switch prey, thus the predator-prey SEA hypothesis. Mortality of herring during the winters of their early life stages is thought to be the major factor affecting recruitment (overwintering hypothesis). That river-lake processes may affect overwinter survival is being tested by comparison of carbon source (this project) in relation to energetics (95320U). Food web shifts represent fundamental changes in the way the PWS ecosystem produces commercial species injured by the oil spill, 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 SEA hypotheses. Stable isotope ratios are thus be used as a biomonitor of ocean circulation, salmon and herring production, and shifts in predation as tests of the SEA hypotheses.

OBJECTIVES

Hypotheses

The following hypotheses to be tested were specified in the DPDs:

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.

Goals

The 95320I 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. These models are being developed as restoration tools enabling a predictive capability for resource managers. As these models, are "spun up," *model validation tools* will become necessary in order to test models against field data. The development of natural stable isotope model validation tools will be the principal restoration products of this project.

Specific objectives

Specific objectives of the 95320I project were:

1. To determine the $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ 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 are matched with regional isotope abundances in prey species (zooplankton, forage fishes) to allocate food sources and to assess carbon source utilization and trophic transfer efficiencies in specific areas of the sound.
2. Determine isotope ratios on prey species favored by marine mammals in different regions of Prince William Sound. These data will support other Trustee projects (see 95170) concerned with marine mammals and will enable the estimation of seasonal importance of various prey species and the trophic levels of various seal species in the ecosystem. Past data have shown that there are considerable differences between individual animals of a given age and also changes in trophic level over the life span.
3. Synthesize the data obtained in context with conceptual food webs to validate feeding models and expand the natural history information.
4. 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.

METHODS

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. Because stable 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 is 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 "bottom up" shifts (food supply) in consumers such as herring and salmon.

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 food chains and will employ the stable isotope ratios of carbon and nitrogen to trace trophic transfers of carbon and nitrogen between levels. In cases where regional gradients in isotope ratios exist, it may also be possible to identify critical habitats used by marine biota.

Basis for application of the stable isotope methodology

The natural abundance of stable isotopes, e.g., $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$, 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 well understood, the virtual universality of its occurrence 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 $^{15}\text{N}/^{14}\text{N}$ ratio in consumer biota relative to primary food sources over time will reflect change in trophic level (TL). Additionally, shifts in carbon source will be reflected in consumer $^{13}\text{C}/^{12}\text{C}$ when switching from diets of differing $^{13}\text{C}/^{12}\text{C}$. For example, shifts in herring and salmon diets that normally consist of macrozooplankton (largely reflecting allochthonous production having been advected into PWS) to autochthonous production (i.e., PWS production) will be evidenced by stable isotope ratios because (1) a greater proportion of PWS production (enriched in ^{13}C) will be needed to make up the deficit and (2) extension of the food web is expected to cause concomitant shifts in $^{15}\text{N}/^{14}\text{N}$ (reflecting TL shift) with $^{13}\text{C}/^{12}\text{C}$ (reflecting alternate prey). The shift in $^{15}\text{N}/^{14}\text{N}$ will be especially notable in predators because of predicted TL shifts. 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 are thus expected to be useful as modeling validation tools.

Sampling design

The sampling effort was broad-scale and consisted of collecting samples at sampling site-times established by the pink salmon and herring projects. These were determined by pink salmon and herring lead investigators and are reported in their respective chapters. Sample sizes consisted of 20 to 50 organisms per taxon (fishes and prey), when available, as these amounts are

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). Sampled taxa included: (1) pink salmon juveniles (principally from CWT recoveries), (2) herring (principally in conjunction with energetics sampling), (3) macrozooplankton (these include bulk samples and samples of individual zooplankters, principally *Neocalanus cristatus*, and (4) predatory and competitive fish of pink salmon and herring (e.g., pollock, Pacific cod, tom cod, black cod, rockfish, sculpins, sandlance, eulachon, and capelin).

Sample and data integration with other SEA projects

To the extent possible, multiple analyses were made on the same individual organisms. Priority was given to samples for which these other analyses are made. 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, spawning condition, and energetics. This sample protocol can delay the acquisition of some samples until other laboratory processing or analysis is complete. Samples delayed because of post-season laboratory needs and alternate acquisition processes, are presently in process. These samples include herring from which calorimetric analysis was made and coded wire tag (in the future: thermal marked otoliths) recovery pink salmon. Preliminary results from these paired studies are given in this document.

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, allows for more replication and smaller samples, and has greater precision (± 0.1 delta units). This instrument calculates $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\%C$, and $\%N$ from each sample per analysis.

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

Sample preparation

Removal of non-dietary carbon from bulk plankton samples was effected by removal of shelled pteropods. This approach was used instead of acid treatment in order to avoid potential artifacts (Goering et al. 90, Bunn et al. 95). Shelled pteropods were removed from bulk plankton samples by (1) forcepicking, (2) screening with a 2 mm mesh sieve after selective removal of macrozooplankton and (3) decanting after allowing the sample to settle in a beaker.

The gastro-intestinal tract was removed from whole fish samples to remove dietary material from samples.

All samples were stored frozen until freeze dried (Labconco) and ground to a fine powder with a dental amalgamator (Crescent Dental Wig-L-Bug). Replicate (except for individual or composite samples of zooplankters too small for more than one analysis) aliquots of ~1.5 mg were weighed to the nearest μg and placed in combustion boats for loading into the mass spectrometer sample preparation unit.

Isotopic determination

A Europa Scientific model 20/20 stable isotope analyzer equipped with a Europa Scientific Roboprep sample preparation and purification unit was used. Analytical results include $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios in standard delta units, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively, and $\%C$ and $\%N$.

Standard delta notation is used to express stable isotope ratios, which are reported relative to international standards (air for N and Vienna Peedee belemnite (VPDB) for C) and defined by the following expression:

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

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

Samples were rerun when replication was poor (difference in delta units > 0.6). Typically, replication is < 0.2 delta units. The $\%C$ and $\%N$ data were used to calculate C/N. Mean of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C/N replicates were used for data modeling and interpretation.

Lipid normalization

Normalization for lipid composition was by the method of McConnaughey and McRoy(1979) using the C/N ratios derived during mass spectrometry. The C/N atomic ratio is used as a proxy for lipid:

$$(2) \quad L = \left(\frac{93}{1 + \frac{1}{0.246\text{C/N} - 0.775}} \right)$$

that is then used to calculate lipid-normalized $\delta^{13}\text{C}$ (expressed as $\delta^{13}\text{C}'$):

$$(3) \quad \delta^{13}\text{C}' = \delta^{13}\text{C} + 6 \left(\frac{3.9}{1 + \frac{287}{L}} - 1 \right)$$

Estimation of trophic level

The enrichment of ^{15}N that results from a feeding process (Minagawa and Wada 1984) enables one to use $\delta^{15}\text{N}$ as a good proxy for trophic level (Fry 88, Cabana and Rasmussen 1994). The trophic level reference organism used here is *Neocalanus cristatus*. Trophic level relative to the reference organism based

on the $^{15}\text{N}/^{14}\text{N}$ trophic enrichment factor, ϵ_{N} , was determined using the following expression:

$$(4) \quad \Pi = \frac{\delta_{\lambda} - \delta_{\text{Neocalanus}}}{\epsilon_{\text{N}}}$$

where δ_{λ} is the $\delta^{15}\text{N}$ of λ , and Π is the number of trophic steps between *Neocalanus cristatus* and λ ; $\epsilon_{\text{N}} = 3.4$ (Minagawa and Wada 1984).

Neocalanus spp. are the dominant herbivores in the plankton community of the north Pacific (Miller et al. 1984). A canonical trophic level for *Neocalanus cristatus* of 2.1 was based on their trophic level being synonymous with the macrozooplankton component in the carbon flux box model of Parsons (1987), i.e., 90% herbivorous and 10% carnivorous (on microzooplankton). *Neocalanus cristatus* are facultatively carnivorous on planktonic Protozoa, but cannot be sustained on such a diet (Gifford 1993). Thus, the *a priori* canonical trophic level (TL) of 2.1 was used as a reference for estimation of TL of other taxa. The *Neocalanus* $\delta^{15}\text{N}$ reference value (the mean of the measurements) is applied to (4) which is used in the following expression to calculate absolute TL of λ :

$$(5) \quad \text{TL}_{\lambda} = 2.1 + \Pi$$

Trophic level normalization for carbon source assessment

Each $\delta^{13}\text{C}'$ value is trophic level normalized so that the residual value will reflect carbon source. Normalization for trophic enrichment of ^{13}C using the trophic enrichment factor, ϵ_{C} , to the reference trophic level (the TL of *Neocalanus cristatus*) was made using the following relationship:

$$(6) \quad \delta^{13}\text{C}'_{\text{TL}} = \delta^{13}\text{C}' - \epsilon_{\text{C}} \Pi$$

where $\delta^{13}\text{C}'_{\text{TL}}$ is the trophic level normalized $^{13}\text{C}/^{12}\text{C}$ value of $\delta^{13}\text{C}'$ and $\epsilon = 1$ (DeNiro and Epstein 1978, Fry and Sherr 1984). Trophic level normalized $\delta^{13}\text{C}$ without lipid normalization is represented by $\delta^{13}\text{C}_{\text{TL}}$. $\delta^{13}\text{C}_{\text{TL}}$ is generally shown as a function of C/N to account for lipid isotope effects (DeNiro and Epstein 1977).

RESULTS

A. Validation of natural stable isotope techniques in the PWS context

1. Net Plankton

The isotopic composition of net plankton from the upper 50m histogrammed in Fig. 1 was extremely variable suggesting the occurrence of isotopic fractionation processes. The lowest 10% of each plot (indicated by differences in shading) tended to occur around Montague Island and in the northern Gulf of Alaska. Low isotopic values suggest minimal fractionation by phytoplankton

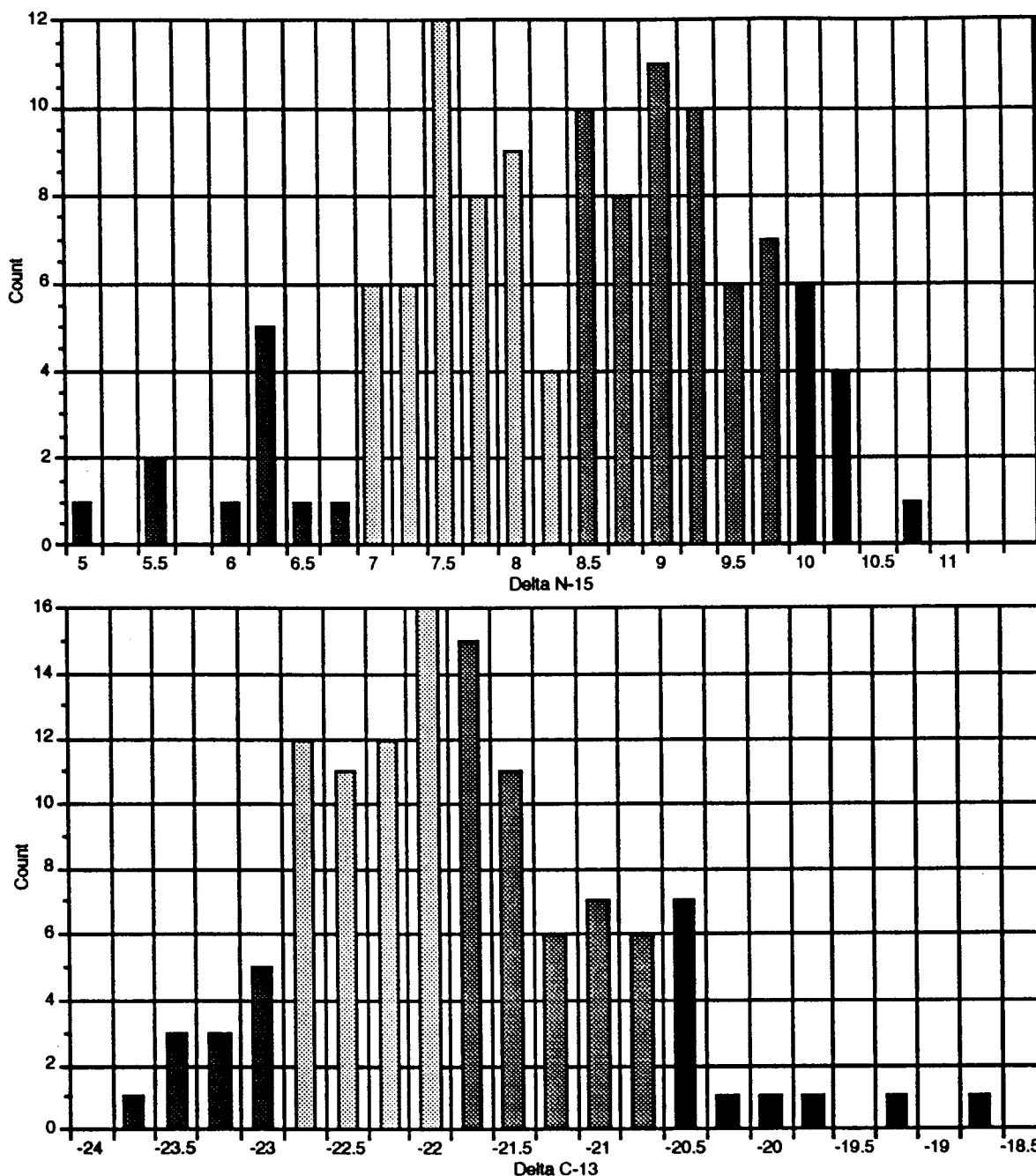


Figure 1. $\delta^{15}\text{N}$ (upper panel) and $\delta^{13}\text{C}$ (lower panel) of upper 50m net plankton sampled from April 1994 to May 1995 in PWS. Upper and lower 10% indicated by dark shading. Medial value occurred at break in intermediate shading.

because of slow growth, lack of nutrient depletion, or input of isotopically depleted C and N. A portion of the variability might also be explained if differences in zooplankton species composition in the samples reflected differences in trophic level and consumption of different phytoplankton species with differing isotopic discrimination. Differences in species composition of net plankton samples (95320H - this volume - samples taken alongside those for isotopic analysis) cannot account for the isotopic distribution. Differences in isotopic fractionation because of low nutrient

depletion in the northern Gulf (suggesting a high degree of isotopic discrimination by phytoplankton) compared to a high degree of nutrient depletion within PWS (resulting in negligible isotopic discrimination by phytoplankton) was probably a significant factor effecting the isotopic composition of zooplankton (see 95320G - this volume - for nutrient data). However, differential growth rates of phytoplankton or existence of isotopically-depleted nutrients (e.g., upwelled CO_2 consisting of respired carbon is expected to have very low $\delta^{13}\text{C}$) are not eliminated as possible factors contributing to the gradient. The significance of the isotopic gradient is that productivity derived from the northern Gulf of Alaska is distinguishable from PWS. The cause of the gradient is beyond the scope of the SEA project. It will be necessary, however to verify the existence of the gradient and to determine $\delta^{13}\text{C}$ values associated with GOA and PWS carbon sources. Verification that this source signature is not dependent on zooplankton species was made by examination of a single herbivore of sufficient size allowing for analysis of individual organisms.

2. *Neocalanus cristatus*

Trophic level

Neocalanus cristatus is a common herbivore of the north Pacific (Miller et al. 1984) and stage C5 (see 95320H - this volume - for life history information) is large enough (~2 mg dry weight) to allow isotopic analysis of individuals. *Neocalanus cristatus* stage C5 was found to be an herbivore (TL ~ 2), based on $\delta^{15}\text{N}$, when compared with other macrozooplankters large enough for individual isotopic analysis (Fig. 2). $\delta^{15}\text{N}$ data collected from 600 C5 and C6 *Neocalanus cristatus* thus far in this project confirms that this organism is virtually a dedicated herbivore (Gifford 1983). Only during March 1995 was there evidence that this species deviates from herbivory (Fig. 3). At this time feeding *Neocalanus cristatus* stage C5 were very rare (n = 7 from 16 stations sampled throughout PWS). The trophic shift in March probably reflects the lack of normal food as this was prior to the phytoplankton bloom (see 95320G - this volume). The value in consistency of TL is (1) that shifts in isotopic composition can be attributed to changes in the isotopic composition of phytoplankton and (2) that the $\delta^{15}\text{N}$ value can be used as a reference for TL determination of other taxa using $\delta^{15}\text{N}$. TL=2.1 (see methods) was used as the average TL for *Neocalanus cristatus* C5. This is a good estimation as this species had TL=2.0 during the end of the phytoplankton bloom (May, Fig. 3)

The small inter-site variation in $\delta^{15}\text{N}$ (~ 2 delta units) of *Neocalanus cristatus* C5 during May 1995 can be attributed to down-draw of inorganic nitrogen (see 95320G - this volume). The lowest $\delta^{15}\text{N}$ values were observed in the northern Gulf of Alaska station (GOA6 - see 95320M, this volume, for station locations) in both *Neocalanus* and net plankton (Fig. 3). Conversely, the highest $\delta^{15}\text{N}$ values were observed at station SEA11 in the deep basin located in western PWS. Intermediate values of both *Neocalanus* and net plankton were observed in the central sound (CFOSBY and CS3). Shift in $\delta^{15}\text{N}$ resulting from nutrient depletion is relatively small compared to trophic enrichment; note box and whisker plot ranges relative to horizontal trophic level lines (Fig. 3), enabling the use of $\delta^{15}\text{N}$ for accurate estimation of trophic level.

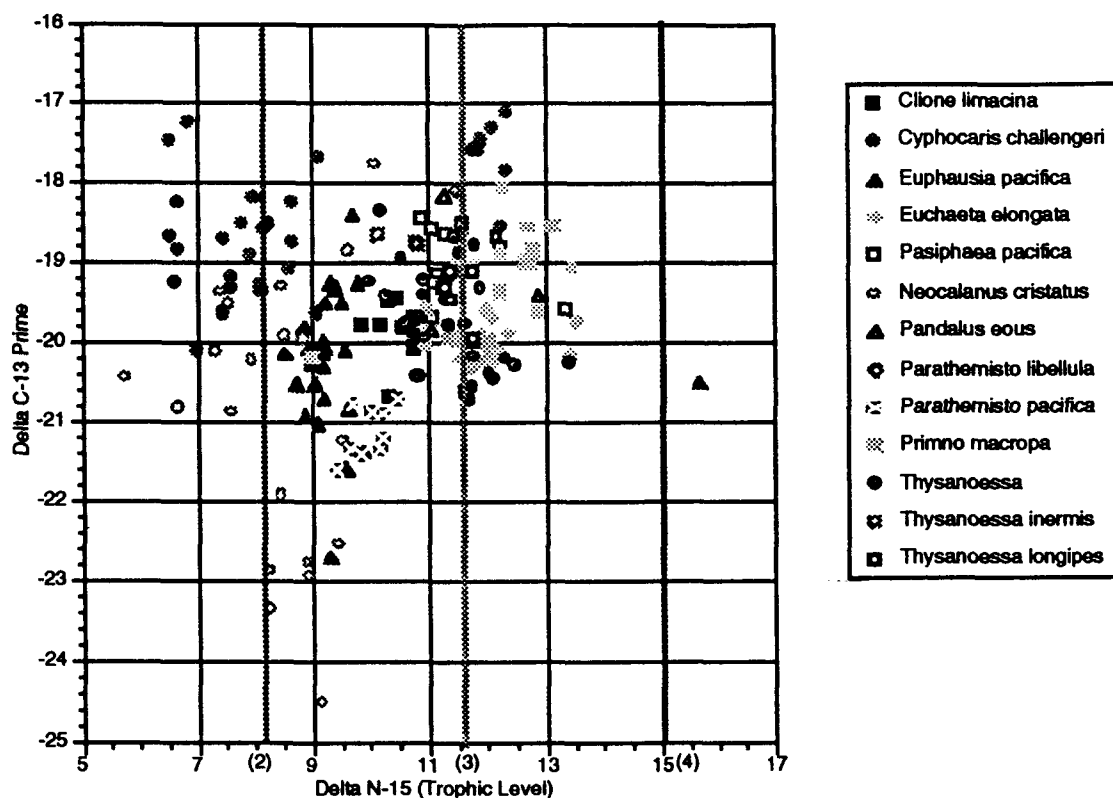


Figure 2. Dual isotope plot ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}'$) of macrozooplankton collected from September to November 1994 in PWS. Trophic levels are indicated by the thick vertical lines. Many macrozooplankton expected to be herbivorous had shifted to TL~ 3 at this time except *Neocalanus cristatus* that was most consistently an herbivore.

Source effects

The isotopic shifts between northern Gulf of Alaska (GOA) and PWS were greatly magnified in $\delta^{13}\text{C}$ compared to $\delta^{15}\text{N}$. This probably occurred because the isotopically depleted signatures in GOA plankton are due to factors in addition to inorganic nutrient depletion in PWS (note that isotopic and nutrient depletion are inversely related due to the preference of phytoplankton for the lighter isotopes of N and C during uptake; isotopically depleted = less of the heavy isotope, nutrient depleted = less nutrients), see 95320G - this volume. Determination of the causal factors is beyond the scope of SEA. However, the existence of the isotopic gradient is of supreme importance. Comparison of PWS and GOA $\delta^{13}\text{C}$ is shown as a function of C/N in Fig. 4 since C/N correlates to lipid content (McConnaughey and McRoy 1979) and lipids are $\delta^{13}\text{C}$ - depleted (DeNiro and Epstein 1977). *Neocalanus cristatus* in diapause show clear isotopic relation to possible feeding in both the GOA and PWS (Fig. 4). This distinction in $\delta^{13}\text{C}$ signature allows one to trace carbon originating from these two sources into PWS biota.

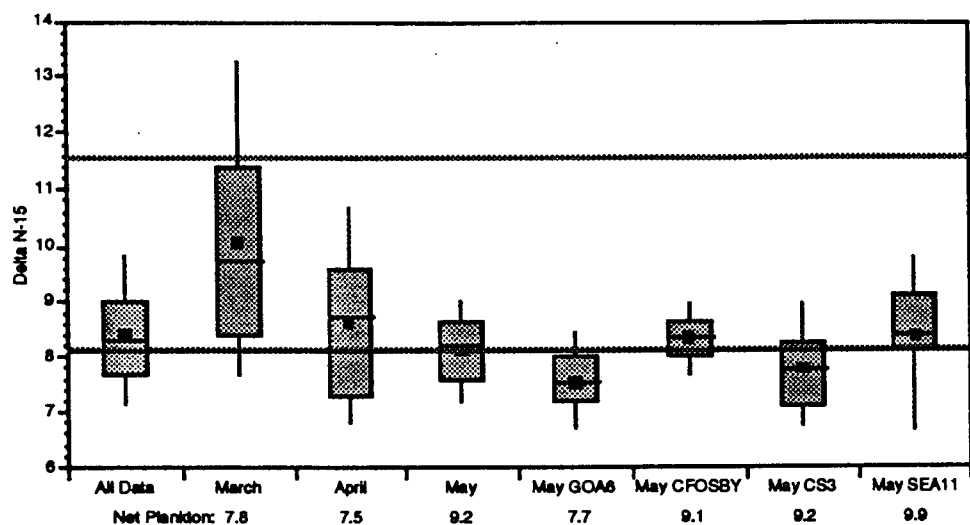


Figure 3. Box and whisker plots *Neocalanus cristatus* $\delta^{15}\text{N}$. Plots include: (1) all data from 1994 to 1995, (2) data from upper 50 m collections in March, April, and May 1995, and (3) data from may 1995 stations with $n \geq 10$ in upper 50 m collections. Synoptic net plankton data $\delta^{15}\text{N}$ listed below plot. Suggested trophic level 2 and 3 positions are suggested by lower and upper, respectively, thick horizontal lines.

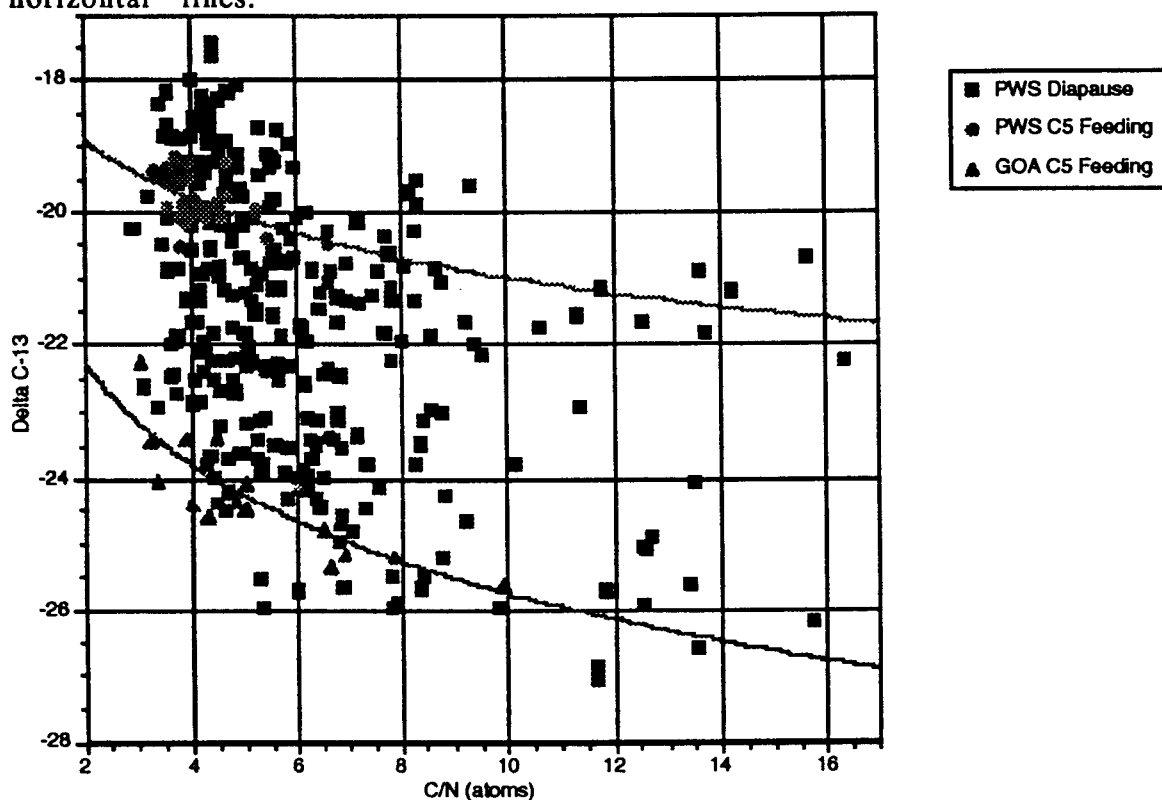


Figure 4. $\delta^{13}\text{C}$ of feeding *Neocalanus cristatus* C5 in PWS and GOA (excluding 3 outliers) compared with diapausing *Neocalanus cristatus* C5 collected throughout PWS as a function of C/N. Differences in $\delta^{13}\text{C}$ between PWS and GOA conserved in diapause stage, as expected, suggest recruitment of copepods from the GOA as well as PWS into diapausing population.

B. Application of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in SEA

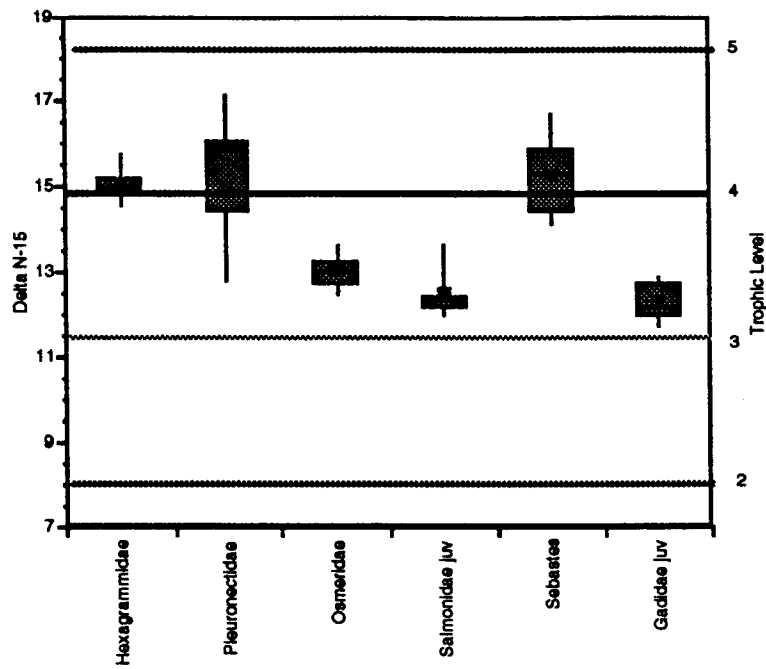
Stable isotope chemistry was applied to SEA problems by estimation of trophic level and identification of carbon sources.

1. Predation: trophic levels

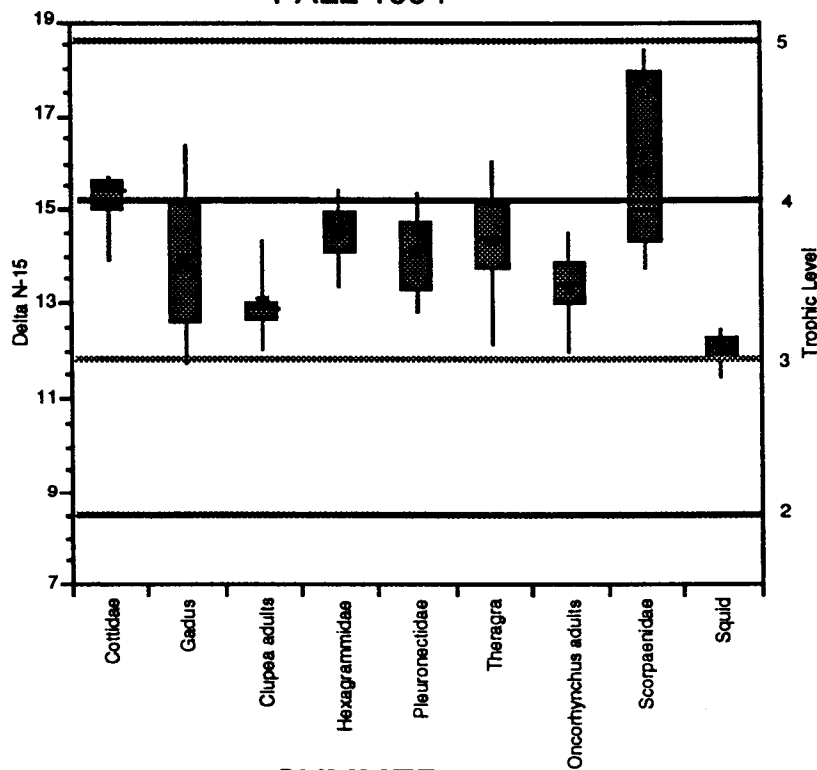
The high mortality of pink salmon from hatcheries suggested that predation is an important process in PWS (see 95320E - this volume). Pollock and other potential predators were compared to potential forage to determine whether estimated trophic levels (number of feeding steps in a food chain, primary producers are TL=1) using $\delta^{15}\text{N}$ is consistent with the predation hypothesis. Piscivorous species are expected to be at TL~4 and those feeding on *Neocalanus* at TL~3. Macrozooplankters can range up from TL~2 (Fig. 2). Thus TL>3 are for mixed TL feeding (including zooplankton and fish, probably more of the later if approaching TL=4). Trophic level estimates are given for several fish taxa and squid comparing Fall 1994 (September to November) with Summer 1995 (late May to July) in Fig. 5.

2. Pink salmon

Shift in pink salmon $\delta^{13}\text{C}$ (Fig. 6) concomitant to decrease in C/N (Fig. 7) and increase in weight (Fig. 8) was tracked in CWT (coded wire tag recovery) samples obtained through the pink salmon project (95320A - this volume). These data suggest a rapid turnover from carbon derived from a hatchery diet that was ~-20 (the value of adult salmon (Kline et al. 1993) as hatchery diet consists largely of the carcasses of adult salmon) to a $\delta^{13}\text{C}$ diet very similar to the GOA signature. Post-hatchery fish had higher C/N compared to later attesting to the good condition of fry at time of release from the hatchery. Because high C/N is associated with low $\delta^{13}\text{C}$, the reverse relationship seen here contraindicates that the $\delta^{13}\text{C}$ shift is due to the C/N shift. These chemical composition shifts coincide with the attainment of the critical size that enhances survival from predation (Fig. 8).



FALL 1994



SUMMER 1995

Figure 5. Estimations of trophic level of predator and forage fish species collected in PWS in FY 95 using $\delta^{15}N$. Upper panel shows collections from fall of 1994 while the lower panel shows collections made in late spring to summer of 1995.

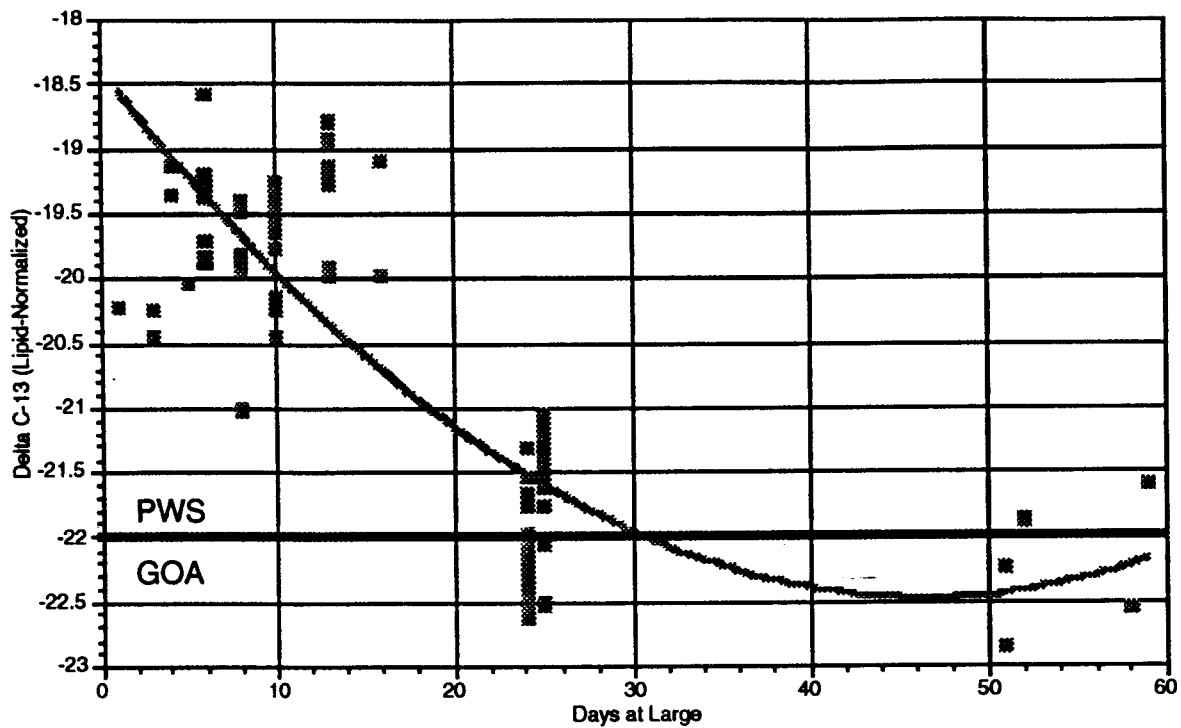


Figure 6. $\delta^{13}\text{C}$ of CWT pink salmon as a function of time since release from the hatchery. The approximate boundary between PWS and GOA carbon signatures indicated.

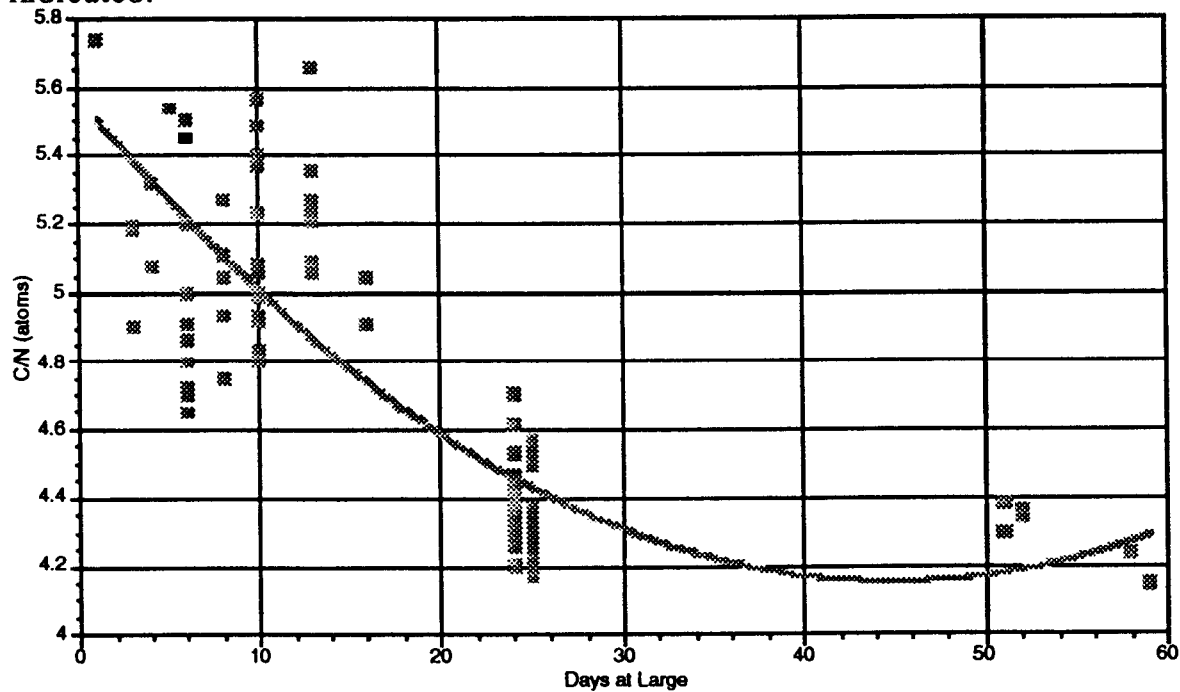


Figure 7. C/N of CWT pink salmon as a function of time since release from the hatchery.

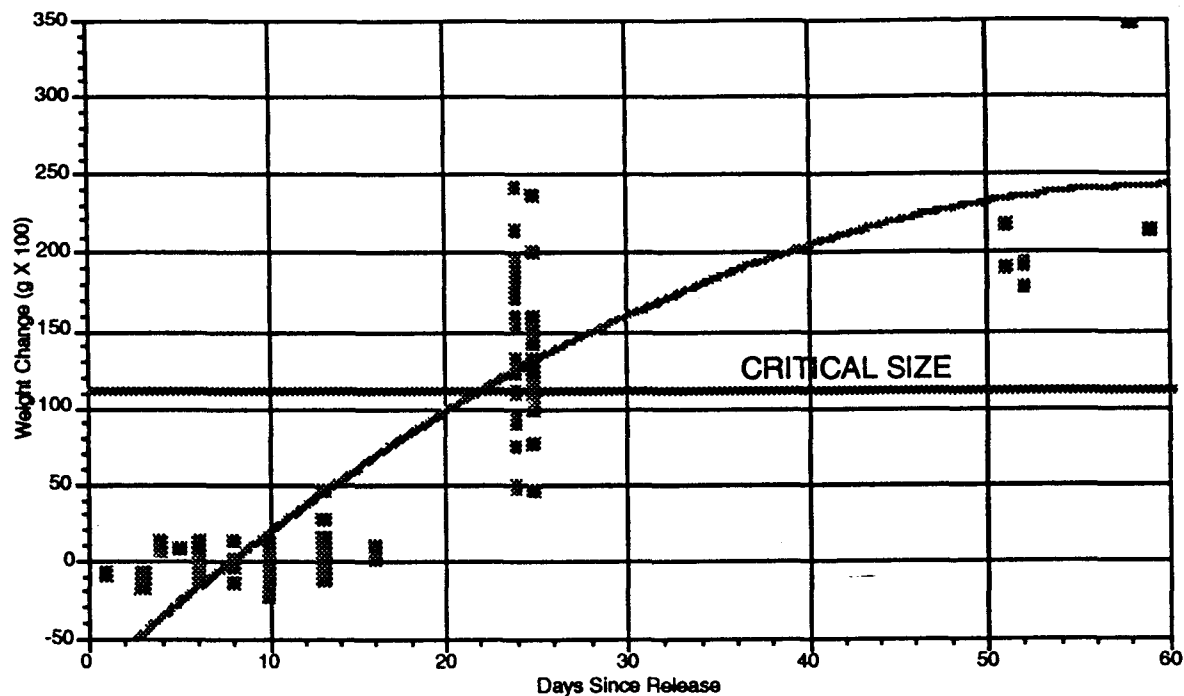


Figure 8. Wet weight of CWT pink salmon (in g x 10) as a function of time since release from the hatchery (data courtesy of 95320A - this volume). The critical size that forms a refugium from predation indicated.

3. Herring

The principal hypothesis vis a vis herring production in PWS is the herring overwintering hypothesis (see 95320T - this volume). Measurement of energetic content addresses this hypothesis (see 95320U - this volume). The isotope technique is being used for parallel analyses. The question being asked is: are there are measurable differences in isotopic signatures of juvenile herring that suggest differences in feeding regime related to winter survival? Maximum benefit of the application of stable isotopes is being attained through paired sampling with the energetics project in order to relate energetic status with past feeding via isotopic signature. This was begun in FY 95 with the analysis of herring juveniles collected in the fall of 1994 (Fig. 9). Two sites in PWS were compared that had different energetic content, Orca Inlet and Port Gravina (see 95320U - this volume). A stratified sample was selected for initial isotope analysis to determine the range in variability. Since Orca Inlet had significantly lower energetic content than Port Gravina, the lower values are more representative of the population whereas the higher values are more representative of Port Gravina (cf. 95320U - this volume). The lower energetic content herring had more positive $\delta^{13}\text{C}$ consistent with a decrease in GOA-derived carbon that is corroborated when overlaying the herring data with *Neocalanus* (Fig. 10). The Port Gravina herring appear to consist largely of GOA-derived carbon. The Orca Inlet population was towards the PWS carbon signature although not completely concordant with it (Fig. 10). $\delta^{15}\text{N}$ and thus trophic level was very similar in both groups of juvenile herring (Fig. 9) and but slightly lower than adult herring (Fig. 5).

DISCUSSION

Results from FY 95 confirm that stable isotope methods are appropriate to studying processes hypothesized in SEA to be important in affecting fisheries recruitment and thus restoration to those fisheries resources injured by the spill. The emphasis in FY 95 was in validating the relationship between $\delta^{13}\text{C}$ and GOA vs. PWS carbon, a requirement prior to exploitation of this natural tracer.

1. Validation of isotopic techniques

Stable isotope applicability hypotheses. The following postulates were confirmed using the data acquired in 1995:

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.

- $\delta^{13}\text{C}$ values relate to GOA vs. PWS production sources.
- $\delta^{15}\text{N}$ values relate to trophic level, i.e., number of feeding steps from production sources to consumers, in the study area.

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.

- $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data of representative samples (N=20 to 50 per sampling stratum) of individual biota sampled integrated with results of other SEA projects.

Utilization of an isotopic source effect is contingent on there being a measurable difference in isotopic signature between production sources of concern. This was suggested primarily through comparison of *Neocalanus cristatus* collected during the C5 feeding stage (Fig. 4). It was also determined that this species is principally an herbivore thus eliminating trophic isotope effects (Fig. 3). This species exists at this stage for a very narrow window of time (~2-3 weeks, see 95320H - this volume), thus providing a narrow temporal perspective which is concentrated during the brief phytoplankton bloom (95320G - this volume) thus ideally integrating principal sources of primary production. The signatures appear to be conserved during diapause as expected since energy needs to be conserved for reproduction, which follows the post-diapause molt to the C6 adult stage that, like the diapause period, is non-feeding. The conservativeness of the signature allows one to use stable isotope analysis of diapausing *Neocalanus* to determine where feeding took place. The majority of diapausing *Neocalanus cristatus* appear to have acquired their carbon in PWS (Fig. 4). In a more river-like year (SEA river-lake hypothesis), it is likely that a greater proportion of diapausing *Neocalanus* will have the GOA signature. It will need to be verified that the $\delta^{13}\text{C}$ signatures of feeding C5 *Neocalanus* for each of the areas are consistent from year to year, particularly during a more river-like year. It is expected that carbon derived from the GOA whether in the Gulf per se or from carbon advected into PWS will have a similar signature.

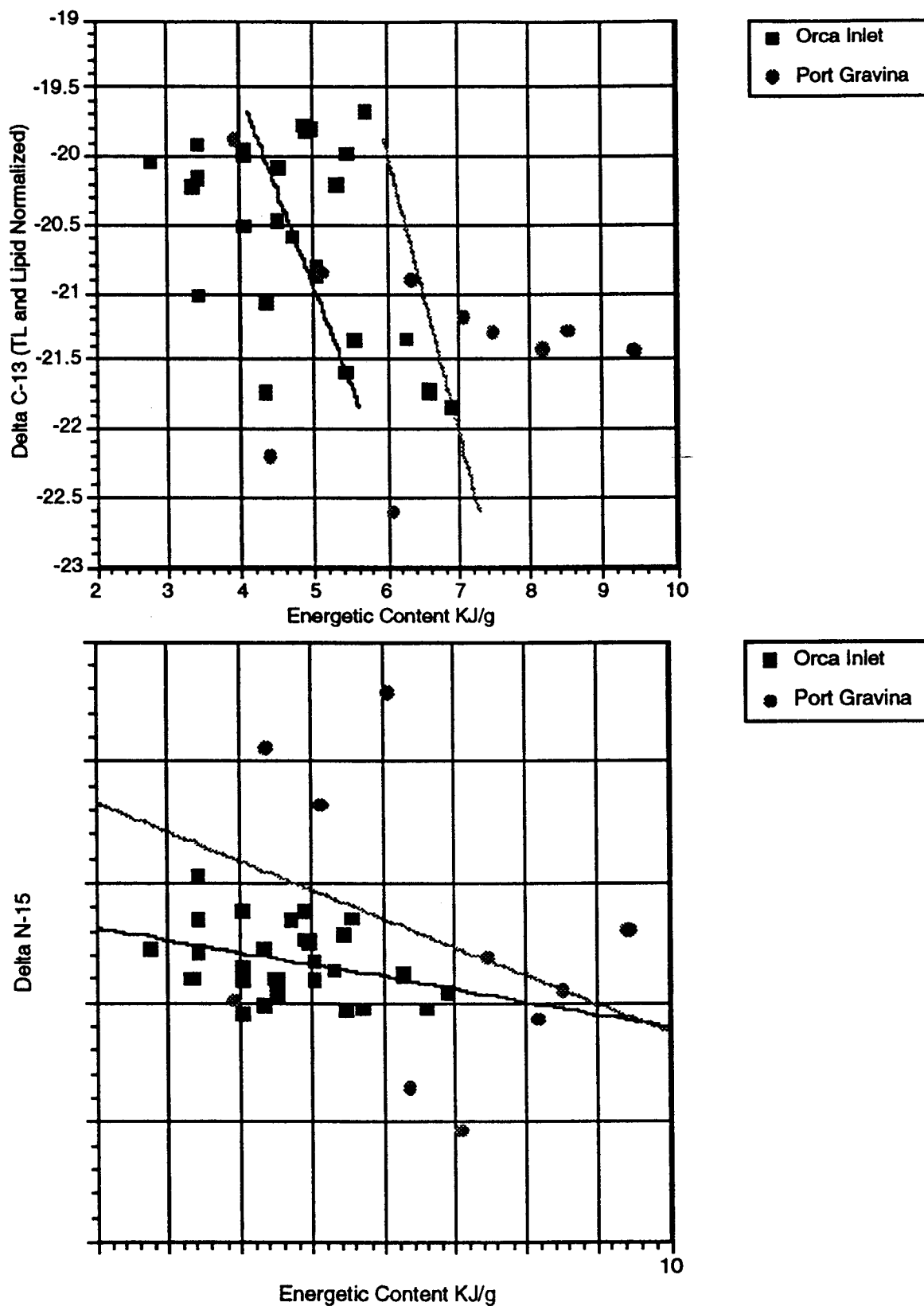


Figure 9. 0-age Pacific herring $\delta^{13}\text{C}$ (upper panel) and $\delta^{15}\text{N}$ (lower panel) as a function of energetic content (see 95320U - this volume).

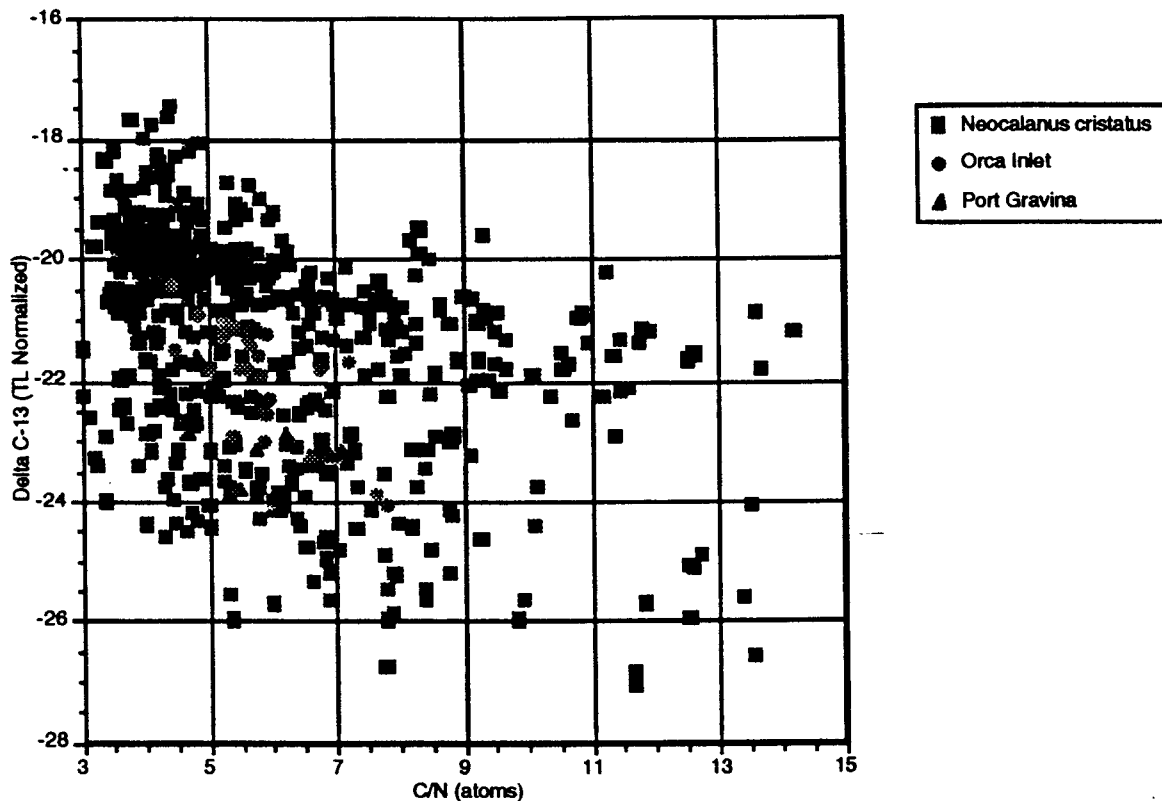


Figure 10. 0-age Pacific herring $\delta^{13}\text{C}$ in relation to possible carbon sources.

The boundary where the PWS signature is likely to be found will most likely be within the area subject to nutrient depletion. Oceanographically, The regime between the nutrient deplete and nutrient replete areas (see 95320G - this volume) reflects oceanographic processes thus directly effecting "bottom-up" processes on food webs and indirectly, inducing "top-down" effects through prey-switching on pink salmon and other alternate prey of predators that otherwise consume zooplankton.

2. Isotopic techniques application: SEA hypotheses

The data suggest that stable isotope abundance relate directly to river-lake processes, thus the technique has direct application to SEA hypothesis testing. The evidence from diapausing *Neocalanus cristatus* suggests that PWS has been more lake-like during this investigation. It is predicted that a more river-like year will result in a greater preponderance of diapausing copepods with the GOA signature. If so, the sampling of diapausing copepods would appear to be a useful tool for assessing river vs. lake conditions. SEA also predicts that more lake-like conditions will result in a greater rate of predation on juvenile fishes. In 1992, there were very few *Neocalanus* in PWS (M. Willette, pers. comm.). The reoccurrence of conditions similar to 1992 will be needed to test whether large-scale prey-switching takes place. The predation project (see 95320E - this volume) has determined that prey switching occurs at the seasonal level. A systematic positive shift in predator $\delta^{15}\text{N}$ values will suggest that large scale (interannual variability) prey-switching takes place. The

trophic level analysis conducted in this project (Fig. 5) and similar data collected in FY 94 will serve as a baseline for comparison should another low-zooplankton, and presumably lake-like, year such as 1992 occur again. Results of the energetics project (see 95320U - this volume) suggest that there are significant differences in pre-winter energetic condition of herring. These pre-winter conditions appear to be related to feeding on PWS vs. GOA, thus river-lake processes may be influencing pre-winter condition in herring and ultimately their over-winter survival. Differences in the availability of GOA occurring between sites and shifts in the availability of GOA occurring between years may modulate recruitment success through over-wintering mortality differential. Since GOA occurrence in herring can be detected in herring with stable isotope abundance, this tool will enable assessment of this phenomenon (i.e., relation between over-winter survival via pre-winter condition and carbon source) in the development of the herring mortality model.

3. Isotopic techniques application: model validation tools

Model validation tools using natural stable isotope abundance are being developed as the primary restoration tool product of the 320I project. The SEA project is focused on generating models that couple ocean physics to biological productivity, and models that can forecast juvenile pink salmon and herring mortality. A first cut at an ocean physics model dealing with circulation in PWS (see 95320J - this volume) has already been created. This circulation model can be used to predict planktonic trajectories such as macrozooplankton forage. The model will need to be validated using field data. The flux of *Neocalanus cristatus* during diapause recruitment can be model-simulated based on the physical input parameters the model is based on and compared with nature. The recruitment of *Neocalanus cristatus* C5s observed in 1995 resulted from processes taking place in 1994. These processes resulted in ~17% of the population consisting of exclusively GOA carbon compared to ~half consisting exclusively of PWS carbon (Fig. 11). Model simulations need to be able to generate these same results. Interannual variability in circulation is likely to affect diapause recruitment by varying the flux of copepods entering PWS from the GOA. The model should be able to predict such differences while isotopic composition can be used to assess them.

The carbon flow from the GOA into PWS biota other than copepods has also been demonstrated using $\delta^{13}\text{C}$ in this project. These biota include net plankton, pink salmon fry, herring, other forage species, and predator species. River-lake processes are hypothesized to modulate the flow of carbon from the GOA into PWS. Differential response in biota to GOA carbon input shifts can be detected by examining the concordance in $\delta^{13}\text{C}$ shifts among species between years. Differential response will suggest which species are most sensitive to bottom-up effects of river-lake processes. It is expected that some species will be less sensitive to these shifts with consequence including a diminished degree of predictability based on carbon input. However it is expected that salmon and herring models predicting juvenile mortality will need to predict effects of GOA carbon input shift based on data collected thus far (Fig. 12).

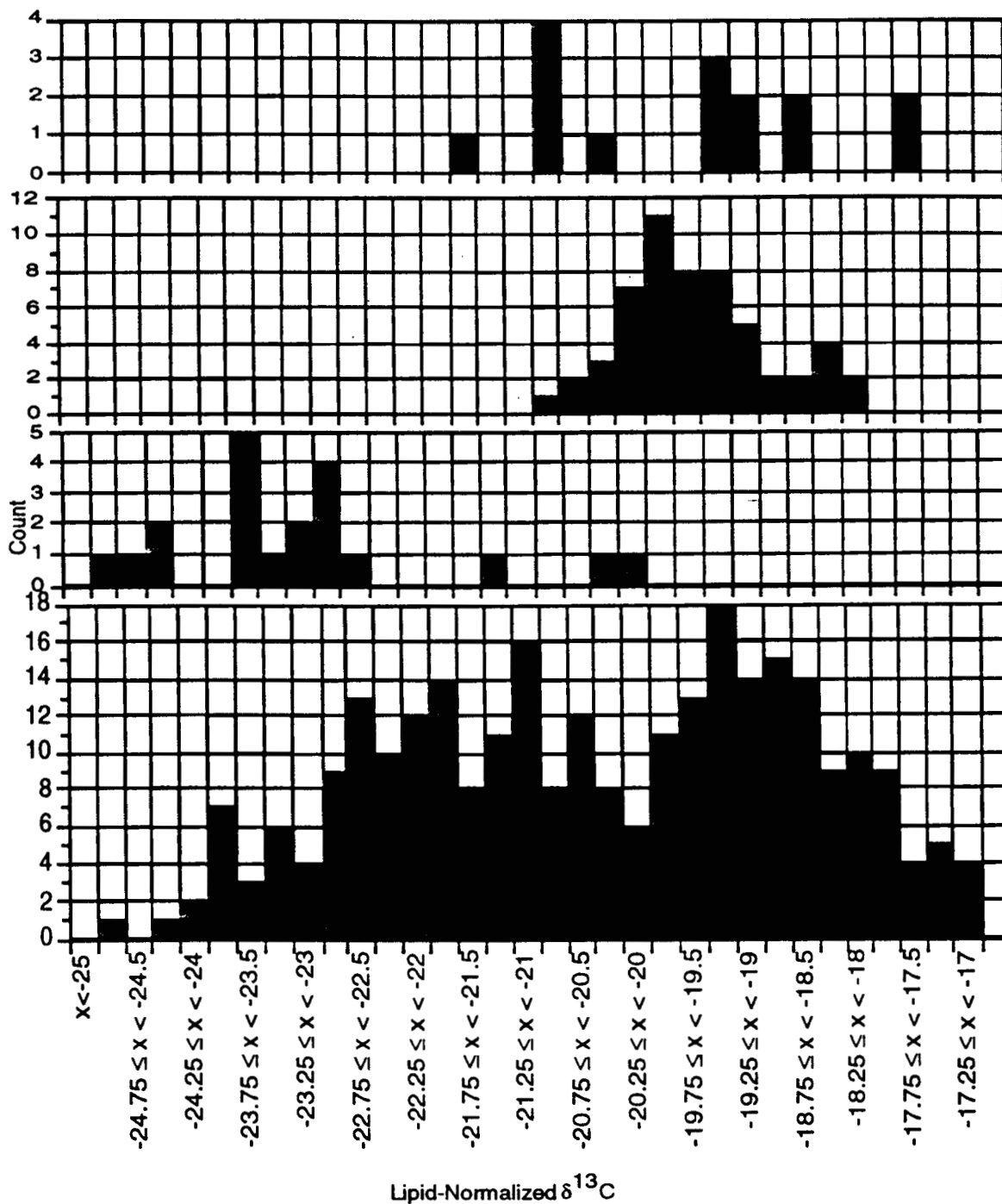


Figure 11. Histograms of $\delta^{13}C'$ of feeding C5 *Neocalanus cristatus* sampled in April and May 1995 in PWS (upper two panels), at station GOA6 in May 1995 (third panel), and diapausing C5 *Neocalanus cristatus* sampled in PWS in March and April. 46 of 277 diapausing C5 *Neocalanus cristatus* had $\delta^{13}C' < -22.5$, i.e., 16.6 % consisting of GOA carbon. 91 of 277 diapausing C5 *Neocalanus cristatus* had $\delta^{13}C' > -22.5$ and < -20.5 , i.e., 32.9 % consisting of carbon of mixed origin i.e., these copepods were being advected into PWS during feeding. 140 of 277 diapausing C5 *Neocalanus cristatus* had $\delta^{13}C' > -20.5$, i.e., 50.5 % consisting of PWS-derived carbon.

Positive trophic level shifts are expected in upper trophic levels if low trophic level prey are less available (expected as a consequence of river-lake). Predicted trophic level shifts can be validated using $\delta^{15}\text{N}$ since $\delta^{15}\text{N}$ relates to trophic level (Fig. 5). Pauly and Christensen (1995) or other Eltonian pyramid models can also be validated by comparing isotopically-determined trophic levels with those predicted by the model (D. Pauly, pers. comm.). Food web reconstruction can include plankton, pink salmon, herring, and other forage species as well as predator species.

Restoration

The goal of the SEA project is to aid in the post-spill 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 have been the ways stable isotopes have been used thus far. These relationships can be used as for model validation that will become paramount as models are spun up.

Economical monitoring framework using stable isotopes

Large-scale field efforts are expensive so ways of providing model validation without great costs should be sought out. It is expected that a certain amount of ship time will be needed to collect oceanographic data. However, "samples of opportunity" can allow a low cost sampling approach, for example commercial species arriving at processing plants. Given that catch location can be had, commercial fishery operations could be a source for some data. We are presently seeking out these channels by acquiring predator samples from processors in Cordova. We have been able to obtain intact pollock allowing us to obtain physical dimensions as well as tissue samples for isotopic analysis. We remove otoliths as well for aging.

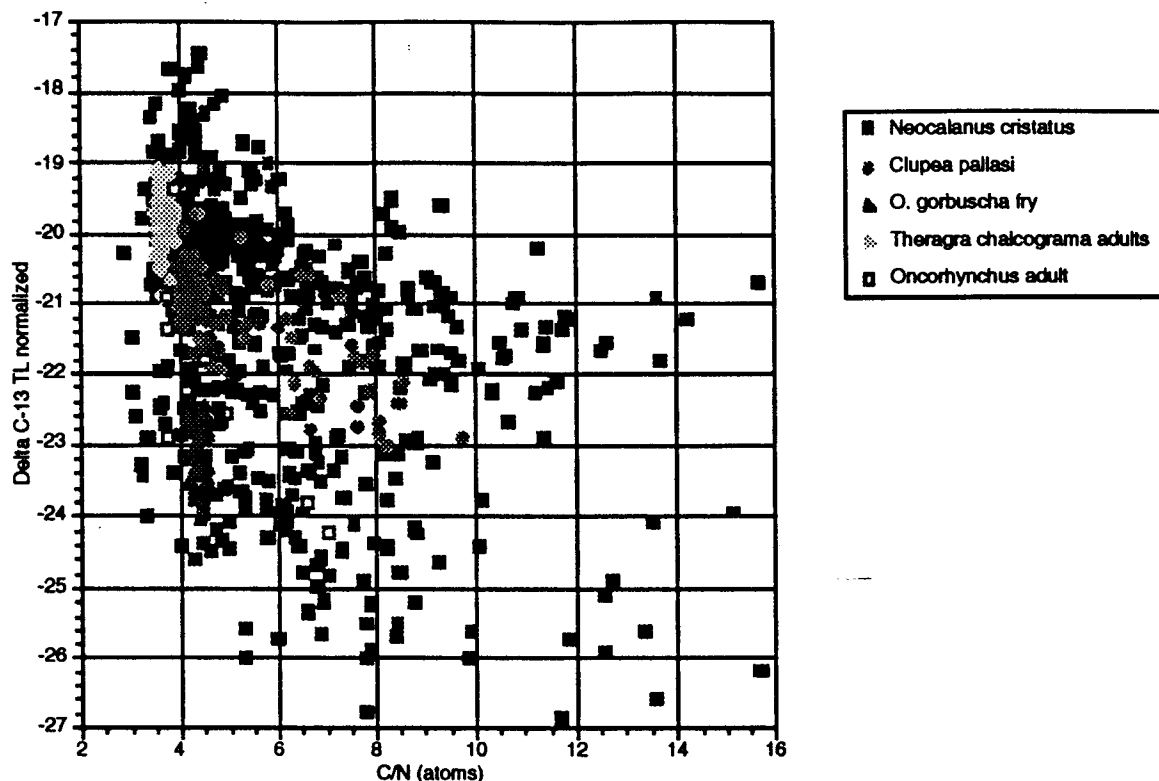


Figure 12. Differential dependence on GOA and PWS carbon sources in principal species suggested by their $\delta^{13}\text{C}$. Pink salmon fry are most dependent on GOA carbon (comparable to returning adults), Adult pollock are most dependent on PWS carbon. Herring are intermediate, utilizing carbon derived from both the GOA and PWS. Shifts in the utilization of these carbon sources expected to occur as a result of varying oceanographic conditions can thus be monitored in these and other fishes.

ACKNOWLEDGMENTS

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