Chapter 5.

Stable Isotopes as Food Web Tracers (96320I)

Exxon Valdez Oil Spill
Restoration Project Annual Report

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

Restoration Project 97320I
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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April 1997

Isotopic Signature and Somatic Energy Content of Young of the Year Pacific Herring at Two Sites in Prince William Sound, Alaska: Implications for Trophic Studies

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Revised draft resubmitted to:
Canadian Journal of Fisheries and Aquatic Sciences

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Running head: Herring trophics

Key Words: Pacific herring, *Clupea*, somatic, energetics, stable N and C isotopes

Abstract

We compare the somatic energy content and natural stable isotopic signatures of young-of-the-year Pacific herring from 2 sites in Prince William Sound, Alaska, (PWS) in the fall of 1994. The fish at one site had an average standard length of 83 mm and 99 mm at the other. At the site with the smaller fish, the recruiting herring had an average of 5.5 kJg^{-1} dry wt for whole body samples vs. 8.9 kJg^{-1} at the other site. The isotopic signatures of the fish tissues were compared to that of terminal feeding stage (copepodite V) *Neocalanus cristatus* sampled throughout PWS. The copepods, which were collected during the period March through June, were relatively uniform and collectively dichotomus in their $^{13}C/^{12}C$ content compared to those collected at a station located on the continental shelf in the Gulf of Alaska (GOA) south of the entrance to PWS. The $^{15}N/^{14}N$ values of *N. cristatus,* which were relatively uniform throughout the region, were compared with herring to normalize the trophic enrichment of ^{13}C so that $^{13}C/^{12}C$ values from herring tissues could be compared with the copepods. The isotopic analysis showed that the herring with the most stored energy had lower $^{13}C/^{12}C$, which suggested they were utilizing carbon derived from GOA production rather than PWS. The study demonstrates that in Prince William Sound schools of young-of-the-year herring can have markedly different levels of somatic energy stores as they prepare to over-winter and that energy storage may depend on their nutritional sources, and the distribution of planktonic prey by physical processes.

Introduction

In Prince William Sound (PWS) the Pacific herring *Clupea pallasi* has supported important commercial and subsistence fisheries. Since 1993, herring have not been abundant enough to harvest. An ecosystem level study called Sound Ecosystem Assessment (SEA) is examining trophic interactions to see if the flow of energy through the food web is impeding

recovery of herring stocks in PWS. The principal SEA hypothesis, known as the river-lake hypothesis, states that spatial and temporal variability in regional oceanographic conditions control production in PWS. Conditions are postulated to vary from lentic (lakelike) to lotic (riverlike) in relation to the strength and influence of the Alaska Coastal Current (Niebauer et al. 1994) on PWS.

Physical oceanographic processes are further hypothesized to affect both bottom-up and top-down biological processes that in turn affect recruitment of exploited fish stocks. Of concern here is the bottom-up effects on Pacific herring in PWS. Herring are seasonal feeders building up fat stores to sustain them when food is scarce during the winter months (Blaxter and Holiday 1963). In late autumn or winter, feeding either stops (Blaxter and Holiday 1963) or continues at low levels (Hay et al. 1988). Of concern here is the condition of herring prior to the long high-latitude winter. Since there is a wide range in the amount of fat stored for the winter season by individuals, even for similar sized fishes in same school (Blaxter and Holiday 1963), it is hypothesized in SEA that overwintering survival of herring may be dependent on body condition resulting from bottom-up effects. In this study, we began testing this hypothesis by comparing herring from two sites at the end of their first growing season for differences in somatic energy content and stable isotope composition as indicators of condition and bottom-up effects, respectively.

Stable Isotope chemistry is a powerful tool for use in ecological studies because of naturally existing isotope gradients and the fidelity of consumer isotope ratios with their diet (Fry and Sherr 1984, Wada et al. 1991, Michener and Schell 1994). In this study we compare the stable isotopic ratios of carbon and nitrogen in the bodies of young-of-the-year herring (i.e., 0-age) collected at 2 sites that had markedly different fall somatic energy and body size. The comparison was facilitated by using the large interzonal copepod *Neocalanus cristatus* as a proxy for carbon fixed during the spring bloom and as a trophic level reference. *N. cristatus* is one of three congeners which dominate herbivore plankton community of the north Pacific (Miller et al. 1984, 1993, Cooney 1986). Although *N. cristatus* is facultatively carnivorous on planktonic Protozoa, the species cannot be sustained on such a diet (Gifford 1993). *N cristatus* was the ideal low trophic reference organism for this study since they are a priori herbivores with a life history which makes them good integrators of the spring phytoplankton bloom when coupled with their large size (~ 2 mg dry weight), which enables isotopic measurement of individuals.

Methods

Neocalanus cristatus

Monthly oceanographic cruises, each one week in duration, were made from March to June, 1995 to capture conditions prior to, during, and following phyto- and zooplankton blooms in PWS on the *R/V Bering Explorer*. Twenty-two stations were sampled each cruise as conditions permitted. At these stations a 0.5 m-diameter 335μ-mesh ring net was lowered to 50 m and brought to the surface at 1 m^{-1}. The contents of the haul were processed immediately upon recovery. Individual life-history-stage copepodite V (C5) *Neocalanus cristatus* were forcepted

out, species identity verified under a dissecting microscope (25X), placed into polyethlene vials (Wheaton Omni-vials), frozen (- 20 °C), and stored frozen (-20 °C) until freeze dried. Samples were freeze-dried (Labconco) in their vials and then sent to the University of Alaska Stable Isotope Facility where they were removed, weighed to the nearest µg and placed into combustion boats for mass spectrometric analysis.

Herring

In 1994 fine mesh purse seines were used to capture herring at one site in Windy Bay (60° 30.5' N and 146 ° 0.6' W), on the North side of Hawkins Island in eastern Prince William Sound, and another in Port Gravina Bay (60 ° 40.0' N and 146° 20.0' W), located about 10 km away in northern Prince William Sound (Fig. 1), for chemical analyses. The fish were collected on 26 and 29 October, respectively.

A subsample of the fish caught (Table 1) were immediately frozen in sea water aboard ship and kept frozen until processed. The length and weight of the remaining fish were determined by biologists from the Alaska Department of Fish and Game (Table 1). In the laboratory the fish were partially thawed, just enough to handle, but not enough so fluids were lost. All fish were measured for standard length (SL) to the nearest mm then weighed to the nearest 0.1 g using an electronic balance. Scales were removed from each fish for aging by the Alaska Department of Fish and Game. The scale-age data were used to select (randomly) only 0-age herring for this study.

Whole carcasses were freeze dried for 48 hours and then placed in a convection oven at 60°C until they reached a constant dry weight. The difference between individual wet and dry weight values were used to calculate the moisture content of each fish. Dried bodies were ground in a mill and somatic energy content (SEC) for each individual was estimated by bomb calorimetry. All calorimetric samples were weighed to the nearest mg. Somatic energy content is based on a single sample burned per fish and reported in kJ per g dry weight. A representative 88 fish from each site were used for energetic analysis (Table 1).

After bomb calorimetric analysis, 32 fish from Windy Bay and 28 fish from Port Gravina were randomly selected for natural stable isotopic analysis. The comparable size range of these fish with the calorimetric and field samples suggests that this was a representational sample (Table 1). The samples were ground to a fine powder with a dental amalgamator (Crescent Dental Wig-L-Bug). Ground freeze-dried samples stored in LSC vials were sent to the Stable Isotope Facility at the University of Alaska Fairbanks where replicate aliquots of ~2 mg were weighed to the nearest µg and loaded into combustion boats for mass spectrometric analysis.

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 $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in standard delta units, $\delta^{13}C$ and $\delta^{15}N$, respectively, and %C and %N.

The 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:

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

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 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 $\delta^{13}C$, $\delta^{15}N$ and C/N replicates were used for further analysis.

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 (proxy for lipid, L):

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

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

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

Trophic level normalization for carbon source assessment

$\delta^{13}C'$ values were normalized for trophic level so that the residual values reflect carbon source isotopic effects (Fry and Sherr 1984). Normalization for trophic enrichment of ${}^{13}C$ was madeusing:

$$(6) \qquad \delta^{13}C'_{TL} = \delta^{13}C' - \varepsilon_C \Pi$$

where $\delta^{13}C'_{TL}$ is the trophic level normalized ${}^{13}C/{}^{12}C$ value of $\delta^{13}C'$, the trophic $\delta^{13}C$ enrichment factor, $\varepsilon_C = 1$ (DeNiro and Epstein 1978, Rau et al. 1983, Fry and Sherr 1984, McConnaughey

and McRoy 1979), and Π is the trophic level difference with respect the reference organism (Cabana and Rasmussen 1994, 1996) and is calculated by:

$$(4) \qquad \Pi = \frac{\delta - \delta_F}{\varepsilon_N}$$

where δ is the $\delta^{15}N$ of the sample, δ_F is the $\delta^{15}N$ of the reference species, which here is *Neocalanus cristatus*, and the trophic $\delta^{15}N$ enrichment factor, ε_N = 3.4 (Minagawa and Wada 1984, Owens 1987).

Thus the expressions $\delta^{13}C$, $\delta^{13}C'$, $\delta^{13}C_{TL}$, or $\delta^{13}C'_{TL}$ are used to denote ^{13}C abundance with respect to the international standard, with normalization for lipid content, with normalization for trophic level, and with normalization for lipid content and trophic level, respectively. Whether or not lipid or trophic level normalization is used depends on the context of the data analysis. "^{13}C" is used to reflect generic $^{13}C/^{12}C$ isotopic trends irrespective of normalization.

Results and Discussion

Neocalanus cristatus

Neocalanus cristatus were found at least once in 16 of the zooplankton stations (Table 2). The peak occurrence in May corresponded to the peak in zooplankton biomass in PWS in 1995 (R. T. Cooney, University of Alaska, pers. comm.). The $\delta^{13}C$ of feeding stage C5 *N. cristatus*) in the northern GOA south of the entrance to PWS were found to be consistently dichotomous when compared to those from within PWS (Table 2). Feeding C5 *N. cristatus* from the GOA had $\delta^{13}C$ = -24.3 (SD = 1.4, N = 33) and $\delta^{13}C'$ = -23.2 (SD = 1.1, N = 33) whereas those from PWS had $\delta^{13}C$ = -20.2 (SD = 1.1, N = 99) and $\delta^{13}C'$ = -19.7 (SD = 0.9, N = 99). The relative uniformity of $\delta^{13}C'$ within PWS and strength of the $\delta^{13}C'$ gradient between PWS and the GOA is obvious when contours of the mean $\delta^{13}C'$ values observed for each station throughout the sampling period (Table 2) are potted (Fig. 2). Thus organic carbon in the form of this herbivorous zooplankter from the northern GOA is ^{13}C-depleted by ~ 4 per mil compared with PWS. This gradient is similar to the ~2.5 per mil gradient in $\delta^{13}C$ between the Bering Sea and the GOA at the Aleutian passes (Schell 1996) and the 7 per mil $\delta^{13}C$ gradient found across Drake Passage (Rau et al. 1991).

Herring

Somatic energy content (SEC)

The mean SEC for the young-of-the-year herring were 5.5 ± 1.0 (SD) and 8.9 ±1.3 (SD) kJg^{-1} dry wt at Windy Bay and Port Gravina, respectively (Fig. 3). The mean SL values for these two samples were 83 and 99 mm, respectively, very similar to the large collections made at the

time of capture reported in Table 1. The SEC and SL values for the Windy Bay young-of-the-year herring were both significantly lower (Mann-Whitney Rank Sum test, $p < 0.0001$) than Port Gravina fish. The positive relationship between SEC to SL (Fig. 4), suggests accumulation of SEC with growth. Note, however, that where the size range of herring from the two sites overlapped with a fair number, i.e., from ~90 to 100 mm, there was a SL-independent difference in SEC of ~ 1 kJg g^{-1} dry wt (Fig. 4) suggesting site-specific effects on energy accumulation, thus inferring significant bottom-up effects.

Isotopic analysis

Windy Bay and Port Gravina young-of-the-year herring also differed in ^{13}C content (Fig. 5). The difference in $\delta^{13}C$ was partially due to lipid isotope effects (DeNiro and Epstein 1977) and thus related to energetic content. However, normalization for lipid effect reduced the ^{13}C difference between the two sites only slightly (Fig. 5). The difference in $\delta^{15}N$ between Windy Bay and Port Gravina young-of-the-year herring (Fig. 6) suggested a slight difference of ~ 0.1 trophic levels. Following normalization of $\delta^{13}C'$ for trophic level, the two sites continued to maintain a difference in ^{13}C (ANOVA, $p = 0.0001$, Fig. 5). Comparison of the copepod $\delta^{13}C'$ proxy for PWS and GOA production may explain the large range of herring $\delta^{13}C'_{TL}$ (Fig. 3). In comparison, the large ^{13}C range (Fig. 5) observed in PWS herring is >> the 1.2 per mil $\delta^{13}C$ gradient observed across the Georges Bank (Fry 1988). The mean $\delta^{13}C'$ of copepods sampled in PWS and GOA are denoted by the solid reference lines for PWS and GOA carbon in Figs. 7 and 8. A $\delta^{13}C'$ of -21.5 corresponded to half way between the distributions (Fig. 5) and is shown in Figs. 7 and 8 as a dashed reference line to suggest ~ 50 % from each carbon source.

The difference in $\delta^{15}N$ of ~0.3 per mil between the two sites is much less than the $\delta^{15}N$ trophic enrichment factor of 3.4 per mil (Minagawa and Wada 1984) and thus differ by no more than ~ 0.1 trophic levels. Given that $\delta^{15}N$ is a better indicator of trophic level than $\delta^{13}C$ (Fry 1988), the ~ 0.8 per mil difference in $\delta^{13}C'_{TL}$ means between the sites, which approximates the $\delta^{13}C$ trophic enrichment factor of 1 per mil (DeNiro and Epstein 1978, McConnaughey and McRoy 1979, Fry and Sherr 1984), most likely reflects differences in $\delta^{13}C'_{TL}$ of prey. Thus, herring from these two sites appear to have fed at a similar trophic level (number of trophic steps from primary producer) based on their $\delta^{15}N$ values (Cabana and Rasmussen 1994, 1996), but on different carbon sources based on their ^{13}C content.

Size

Comparison of the relation of herring ^{13}C to length suggests that the carbon source shift occurs relative to SL and is independent of site (Fig. 7). Collectively (both sites pooled), the regression of the $\delta^{13}C'_{TL}$ to SL had an $r^2 = 0.31$, $p = 0.0001$. Thus, the difference in ^{13}C between Windy Bay and Port Gravina reflects, in part, site-specific differences in fish size. The Port Gravina young-of-the-year herring had already shifted to the carbon source with less ^{13}C at the time the Windy Bay young-of-the-year herring were still feeding on the carbon with more ^{13}C. The shift to different carbon sources concomitant with growth is consistent with ontogenic shifts in diet of herring known since the work of Hardy (1924). The abundance of ^{13}C in consumers

usually does not reflect specific taxa in the diet, but reflects isotopic signatures generated by primary producers which is passed conservatively (with a known trophic-step enrichment) on to higher trophic levels (Michener and Schell 1994). The observed shift in ^{13}C as a function of size (and indirectly, location) suggests ontogenic changes in the primary producer and thus, carbon source of herring food webs during their first summer in Prince William Sound.

The rapid growth rates of juvenile fishes result in isotopic composition change in proportion to addition of new tissue (Hesslein et al. 1993). Isotopic differences between individuals in groups of fast-growing fish changing diet to a different isotopic signature reflect different growth rates (Hesslein et al. 1993). Our observations are consistent with this notion. The change in $\delta^{13}C'_{TL}$ occurring with growth (Fig. 7) and the site-specific variability of $\delta^{13}C'_{TL}$ reflects the differences in fish size at the two sites and suggests that the faster growing Port Gravina herring had a better food source. Herring 90 - 100 mm at the two sites differed by ~ 1Kj/g dry weight (Fig. 2) inferring site-specific differences in available energy. The Port Gravina herring with SEC < ~7 kJ/g dry weight that overlaped the Windy Bay data distribution may have been recent immigrants to the area since some of them had $\delta^{13}C'_{TL}$ that was similar to Windy Bay (Fig. 8). SEC was generally proportional to a decrease in $\delta^{13}C'_{TL}$ ($r^2 = 0.37$, $p = 0.0001$, Fig. 8) suggesting a trend to a ^{13}C-depleted carbon source as energy is accumulated. The Fig. 8 plot demonstrates via the $\delta^{13}C'$ of *Neocalanus cristatus* a diet shift that results in a different SEC and shows the typical variation in these parameters encountered at different capture sites. Both the SEC and isotopic data represent an integration of carbon over time, unlike stomach samples that only reveal the most recent meal and cannot resolve effects of recent fish or plankton movement. Both SEC and isotopic values integrate a number of factors that affect assimilation of carbon and are difficult to assess in the wild, such as the effects of prey density, prey SEC, costs related to predator avoidance, and prey-density independent foraging costs.

Potential pelagic carbon source differentiation suggested by the $\delta^{13}C$ of *Neocalanus cristatus* sampled in PWS and the GOA south of Hinchinbrook Entrance, where the Alaska Coastal Current enters PWS (Niebauer et al. 1994) make it a good reference organism (Cabana and Rasmussen 1996) (Figs. 5, 7, and 8). The isotope gradient found in this zooplankter when used as a proxy inferred that PWS herbivores have $\delta^{13}C' > -21.5$ whereas those from the northern GOA are <-21.5. Larger young-of-the-year herring shift to more ^{13}C-depleted values (i.e., $\delta^{13}C'_{TL} < -21.5$) consistent with a shift toward oceanic carbon (Fig. 8). This shift of herring to more ^{13}C-depleted carbon and a higher SEC is consistent with the ontogenic shift of switching from neritic prey to eating larger oceanic calanoid copepods as they grow (Hardy 1924). Attainment of differential size and SEC levels at the end of the first summer's growth could have resulted from differences in growth rate or the timing of metamorphosis.

Typically there is a wide variety of lipid content seen within all age classes of herring (Blaxter and Holiday 1963. This survey suggests that food source plays a role in delimiting growth and energy storage in first-year herring. We have no way of knowing where our test fishes were foraging prior to capture, but clearly those captured at Windy Bay were not storing as much

energy as those from Port Gravina. The measurements of natural stable isotopic ratios as energy supply tracers showed historical differences in prey type. The better fed fish in Port Gravina had a greater affinity for Gulf of Alaska carbon than the Windy Bay fish (Fig. 8) which increased as a function of size for both samples. This project has demonstrated the usefulness of combining energetic and isotopic techniques to measure nutritional status and looking back-wards at prey types consumed. Since the 0-age herring reflect the input of differing carbon sources, our combined tools could be the basis for surveys of herring in space and time to identify areas of prime habitat linking oceanographic factors to inshore pelagic habitat quality, providing a means to measure interannual offshore influences on the nearshore.

Acknowledgments: This project was funded by the Exxon Valdez Oil Spill Trustee Council through the Sound Ecosystem Assessment project. However the findings presented by the authors are their own and not necessarily the Trustee Council position. SEA colleagues collected the fish. Evelyn Brown of the Alaska Department of Fish and Game Cordova office oversaw aging of the fish. J. M. Paul and P. Shoemaker did the calorimetric analysis. Captain Mike Gaegel and crew of the *R/V Bering Explorer*, E. Stockmar, J. Murphy, B. Barnett, and T. Cooney assisted with *Neocalanus cristatus* sampling. Kim Antonucci did the laboratory processing of samples for stable isotope analysis. Bruce Barnett, University of Alaska Fairbanks Stable Isotope Facility performed the stable isotope analysis. John Williams made the contour plot (Fig. 2). Nancy Bird and three anonymous reviewers, through their constructive comments, aided the completion of this manuscript.

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Figure captions (tables and figures follow)

1. Map of eastern Prince William Sound, Alaska indicating sampling sites at Port Gravina and Windy Bay.

2. Contoured $\delta^{13}C'$ data (from Table 2) of *Neocalanus cristatus* sampled as feeding stage copepodite V throughout Prince William Sound and adjacent Gulf of Alaska.

3. Box and whisker plots of young-of-the-year herring somatic energy content (SEC) in kJg^{-1} dry weight sampled from Windy Bay and Port Gravina in October, 1994. The upper, lower, and line through the middle of the box correspond to the 75th, 25th, and 50th percentiles, respectively. The "whiskers" indicate the 10th and 90th percentiles. The mean value is shown as a square symbol.

4. Relationship of young-of-the-year herring somatic energy content (SEC) in kJg^{-1} dry weight sampled from Windy Bay and Port Gravina in October, 1994 with standard length. Windy Bay $r^2 = 0.36$, $p = 0.0001$; Port Gravina $r^2 = 0.12$, $p = 0.0009$.

5. Box and whisker plots of young-of-the-year herring $\delta^{13}C$, $\delta^{13}C'$, and $\delta^{13}C'_{TL}$ sampled from Windy Bay and Port Gravina in October, 1994 (left three panels) compared with $\delta^{13}C'$ of the herbivorous copepod *Neocalanus cristatus* (data from Kline 1996, 1997) sampled in PWS and the Gulf of Alaska (GOA). The TL normalization makes the herring and copepods comparable (two right-hand panels). Box and whisker plots percentiles shown same as Fig. 2. The dashed lines correspond with the mean $\delta^{13}C'$ of copepods from PWS and GOA, and the midpoint between PWS and GOA.

6. Box and whisker plot of young-of-the-year herring $\delta^{15}N$ sampled from Windy Bay and Port

Gravina in October, 1994. Box and whisker plots percentiles shown same as Fig. 2.

7. $\delta^{13}C'_{TL}$ in young-of-the-year herring as a function of standard length. PWS and GOA reference lines as described in text. Data suggest ontogenic shift to more ^{13}C-depleted GOA-carbon based diet. Synoptic samples suggest heterogeneity in distribution of young-of-the-year herring with respect to this diet shift.

8. $\delta^{13}C_{TL}$ in young-of-the-year herring versus SEC in kJ/g dry weight sampled from Windy Bay and Port Gravina in October, 1994. PWS and GOA reference lines as described in text.

Table 1. Comparison of sizes and standard lengths of young-of-the-year herring samples used in this paper.

	All fish caught	Energetics sample	Isotope sample
Windy Bay			
Number of fish	99	88	32
Mean standard length	83.6 mm	82.9 mm	83.2 mm
Standard deviation	9.1	6.7	7.7
Mean wet weight	7.6 g		
Standard deviation	3.6		
Port Gravina			
Number of fish	381	88	28
Mean standard length	95.3 mm	98.6 mm	98.3 mm
Standard deviation	7.2	6.1	7.0
Mean wet weight	12.2 g		
Standard deviation	4.4		

Table 2. Number of copepodite V *Neocalanus cristatus* sampled from upper 50 m at indicated oceanographic stations in Prince William Sound and Gulf of Alaska where at least one individual was found in March to June, 1995. Zeros indicate that none were found and dashes indicate that station was not made. Mean $\delta^{13}C$' and SD of total sample from each station given.

				Number Sampled				$\delta^{13}C$'	
Name	Latitude N	Longitude W	Depth (m)	March	April	May	June	Mean	SD
Prince William Sound stations									
CFOS13	60 35.1	146 55.7	441	0	0	1	0	-19.3	
CFOSBY	60 36.3	147 12.2	205	0	0	21	0	-19.7	0.4
CS3	60 28.7	147 05.5	300	-	0	11	0	-19.9	0.6
CS9	60 35.1	146 44.4	400	0	0	0	14	-20.3	0.6
HE12	60 15.7	146 49.4	254	0	0	2	0	-19.4	
NS1	60 46.8	146 55.8	289	0	1	2	0	-19.5	
NWS4	60 46.8	147 22.2	430	1	0	4	0	-19.1	1.1
OB1	60 36.6	145 55.8	180	-	1	0	0	-18.7	
OB2	60 35.2	146 24.6	120	0	1	0	8	-20.0	0.8
PV1	60 55.9	146 50.0	330	-	0	2	0	-18.9	
PW1	60 50.4	148 12.3	405	6	1	0	0	-19.8	1.5
SEA4	60 46.2	148 04.9	330	-	0	3	0	-19.4	
SEA11	60 37.0	148 00.0	496	0	0	9	0	-19.4	0.6
SEA22	60 40.5	147 41.0	753	-	3	0	0	-19.3	1.6
SEA25	60 18.1	147 58.0	558	0	8	0	0	-19.4	1.3
Gulf of Alaska station									
GOA6	60 00.0	146 40.0	100	0	-	20	13	-23.2	1.1

FIGURE 1

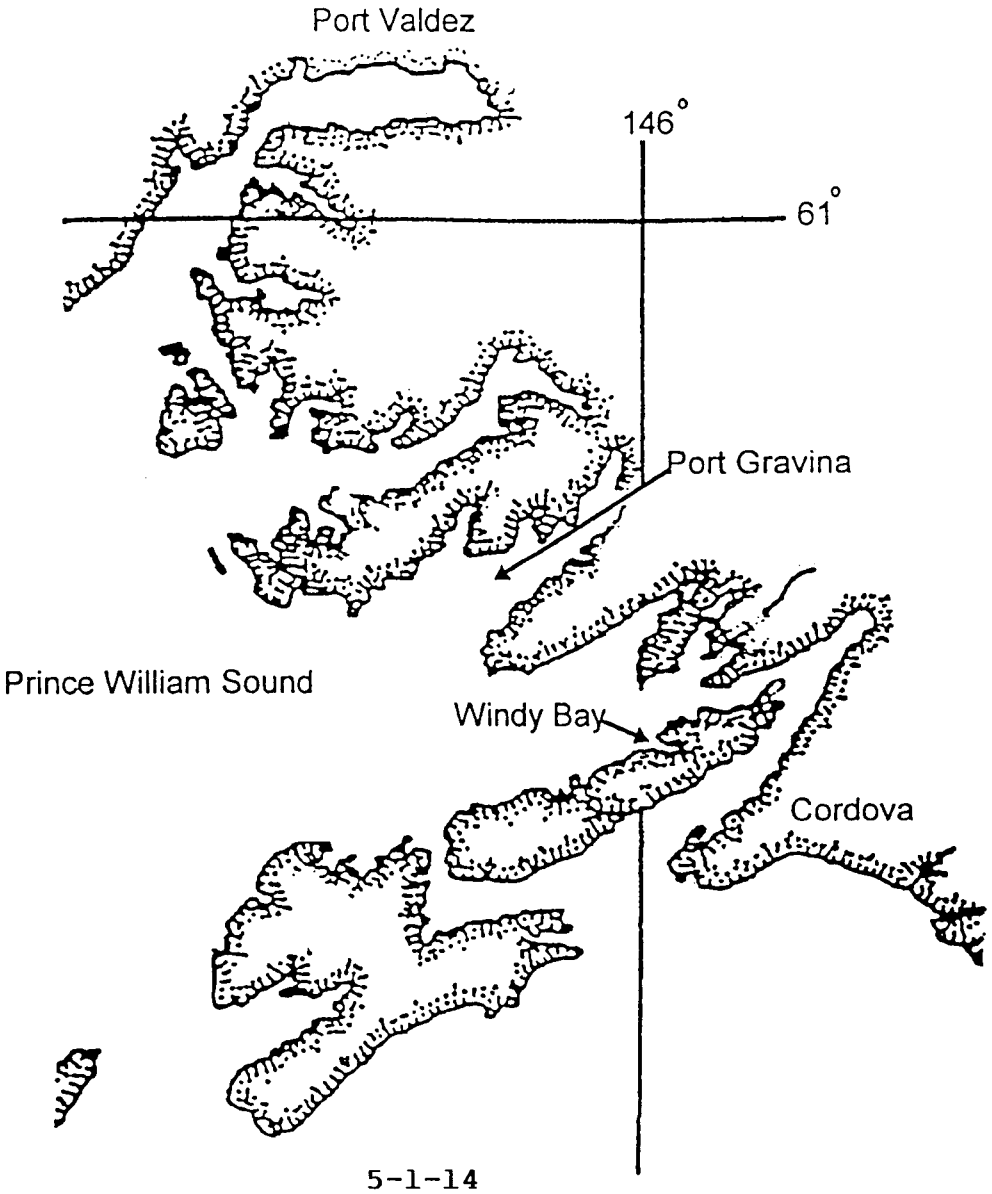

Port Valdez
146°
61°
Port Gravina
Prince William Sound
Windy Bay
Cordova

FIGURE 2

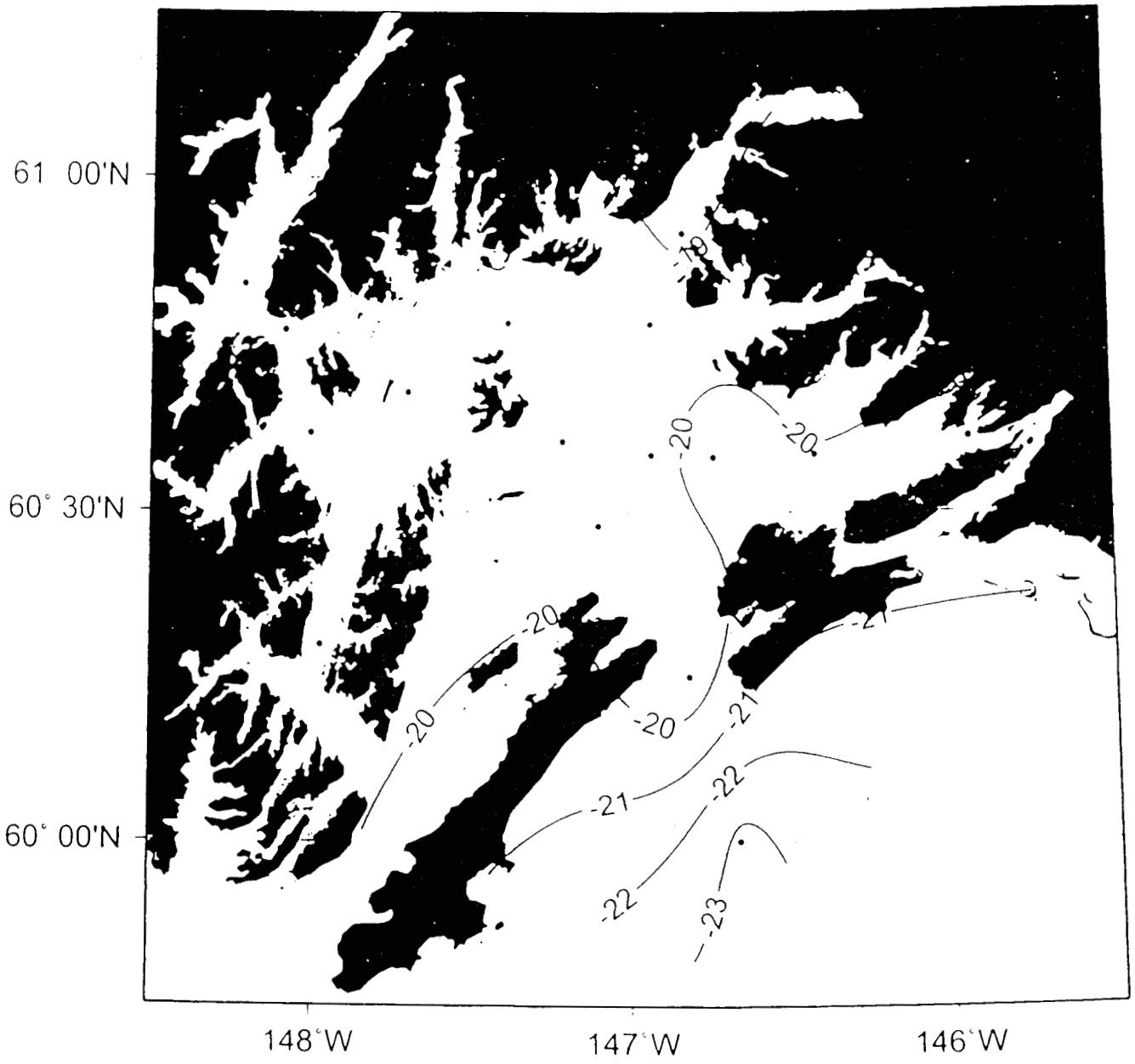
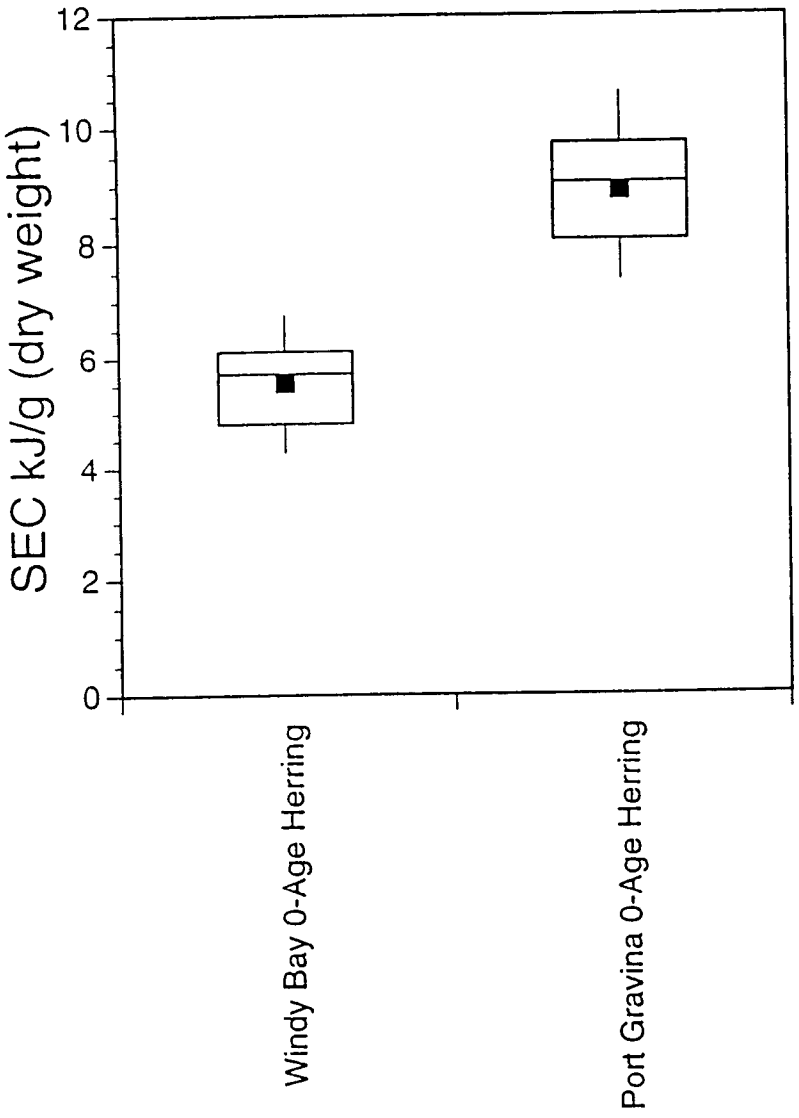


FIGURE 3


SEC kJ/g (dry weight)
12
10
8
6
4
2
0
Windy Bay 0-Age Herring
Port Gravina 0-Age Herring

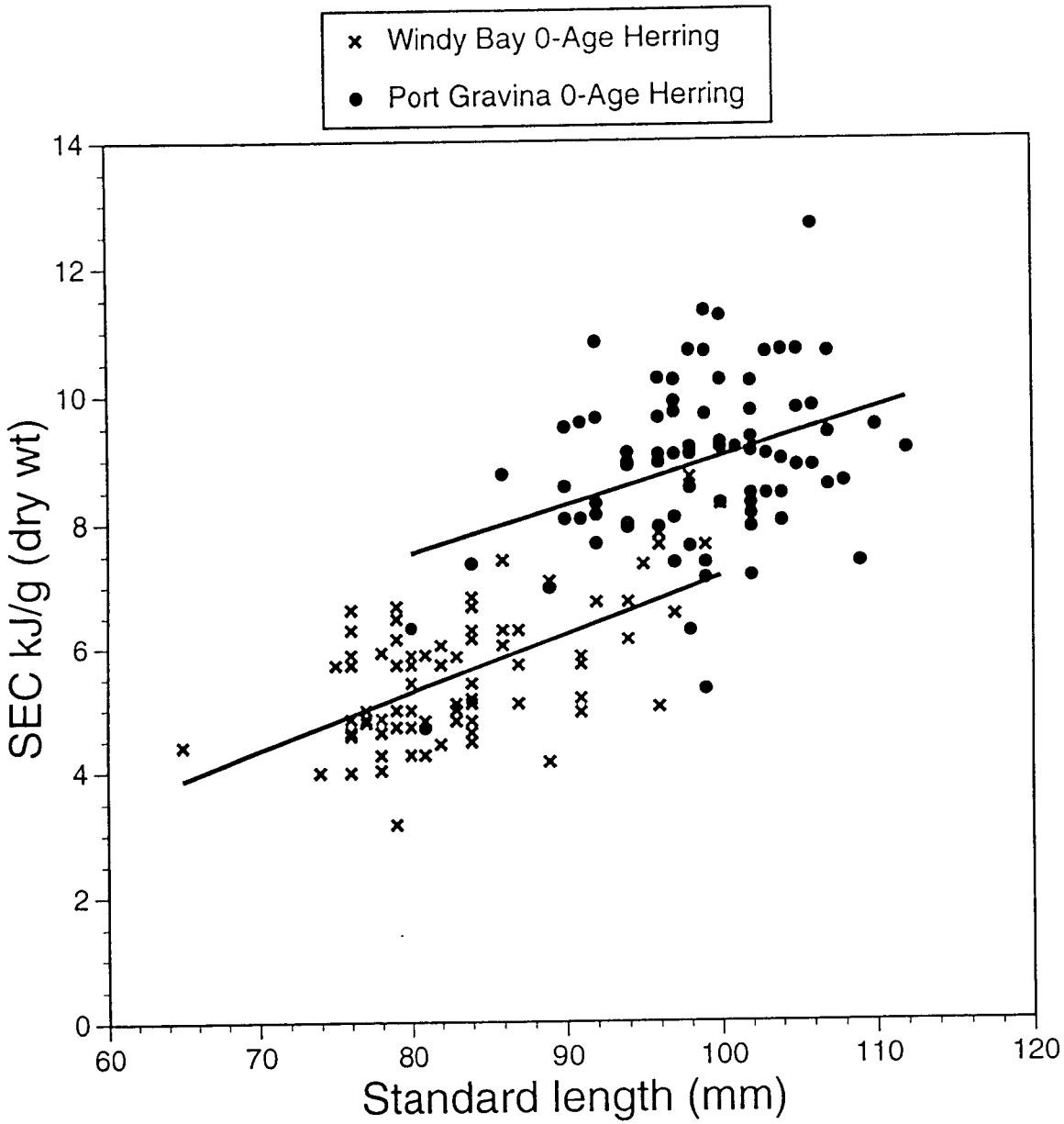

Windy Bay 0-Age Herring
Port Gravina 0-Age Herring
SEC kJ/g (dry wt)
Standard length (mm)
0
2
4
6
8
10
12
14
60
70
80
90
100
110
120

Fig 4

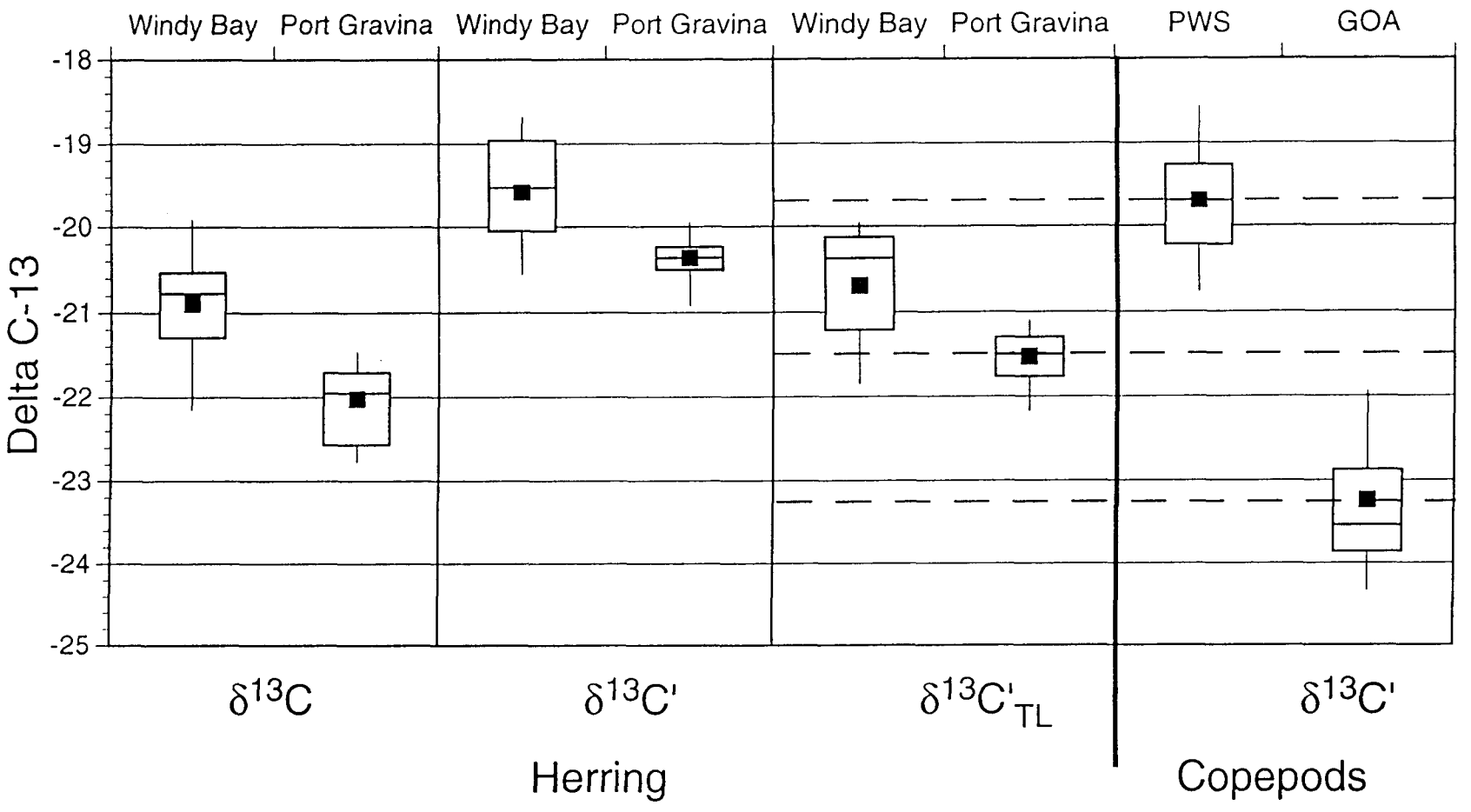
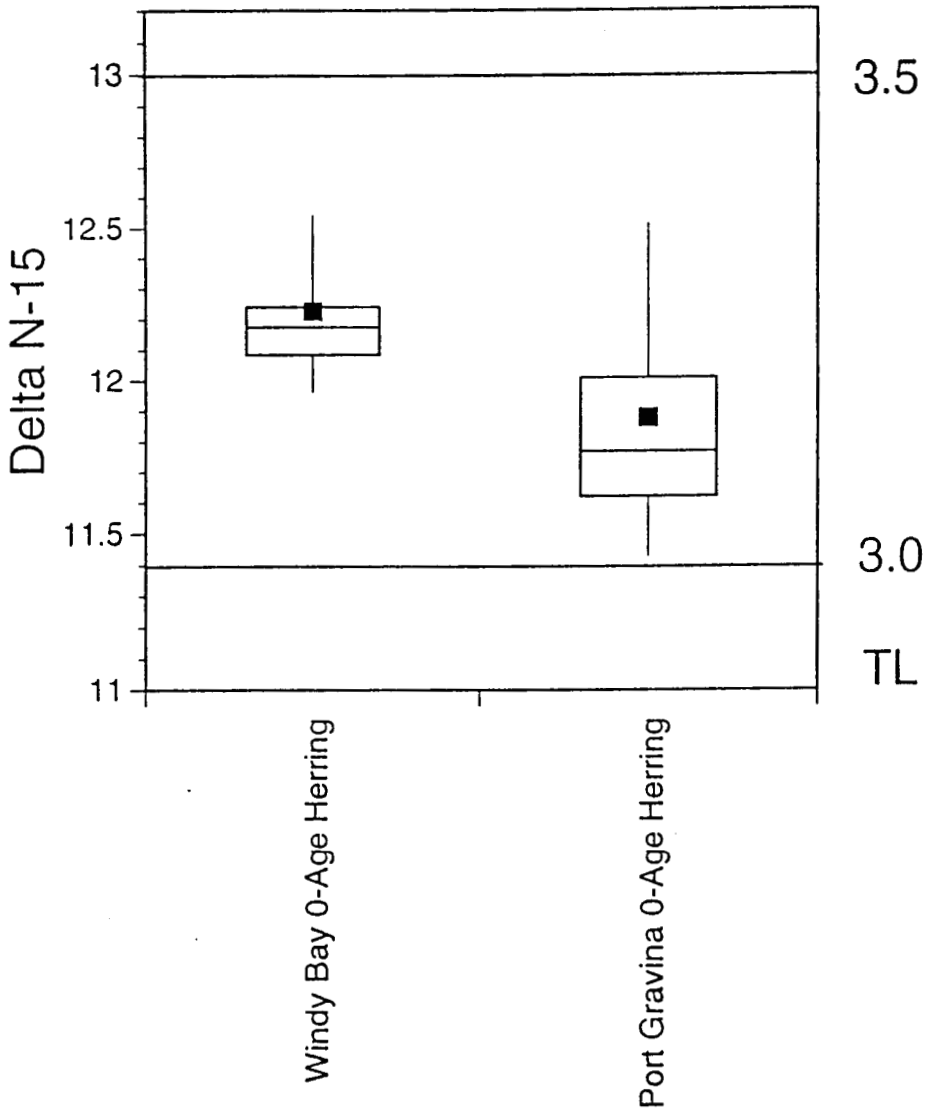

Windy Bay
Port Gravina
Windy Bay
Port Gravina
Windy Bay
Port Gravina
PWS
GOA
-18
-19
-20
-21
-22
-23
-24
-25
Delta C-13
δ13C
δ13C'
δ13C'TL
δ13C'
Herring
Copepods

Fig 5

FIGURE 6


Delta N-15
13
12.5
12
11.5
11
3.5
3.0
TL
Windy Bay 0-Age Herring
Port Gravina 0-Age Herring

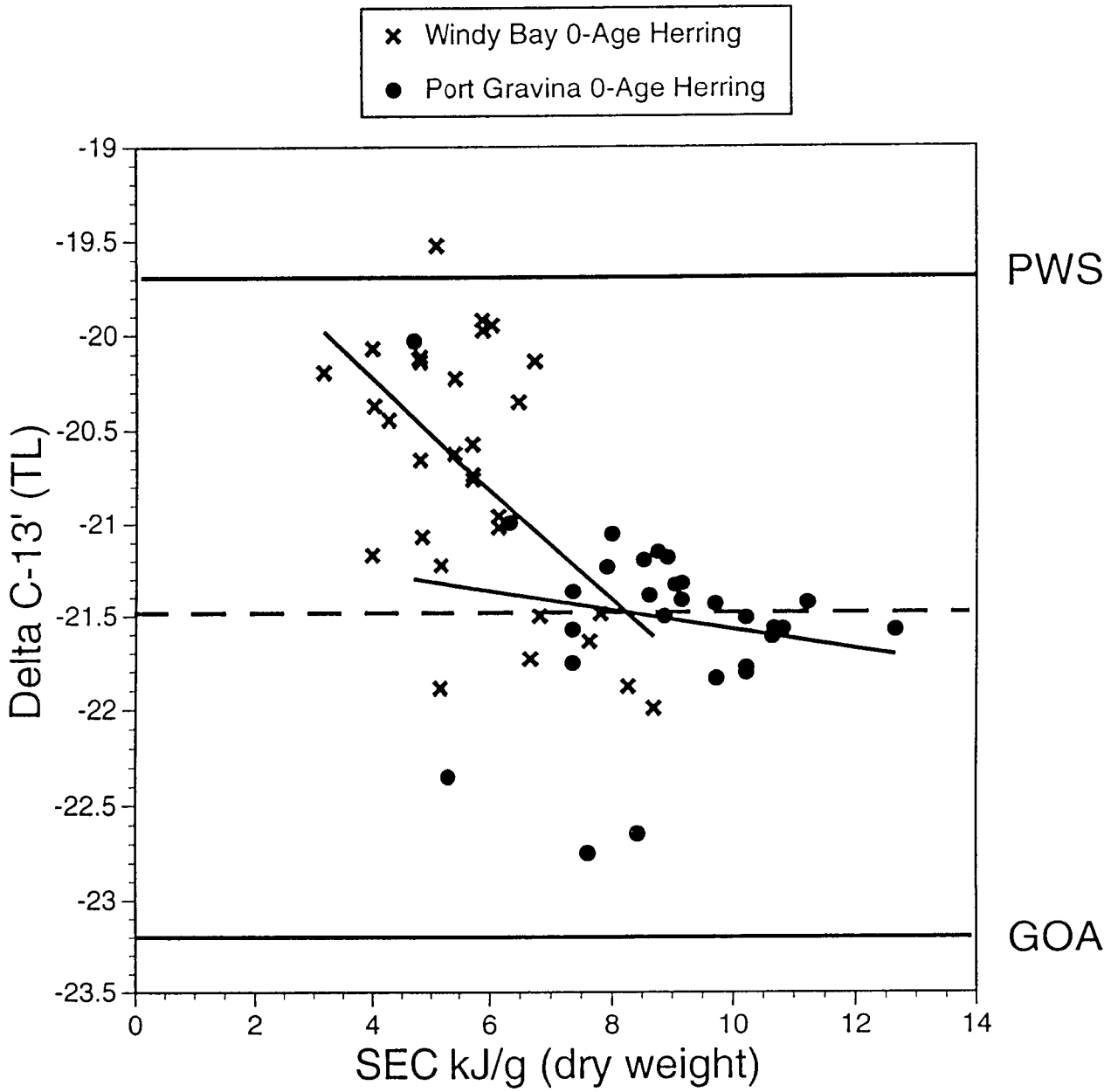

Windy Bay 0-Age Herring
Port Gravina 0-Age Herring
-19
-19.5
-20
-20.5
-21
-21.5
-22
-22.5
-23
-23.5
Delta C-13' (TL)
PWS
GOA
0
2
4
6
8
10
12
14
SEC kJ/g (dry weight)

Fig 7

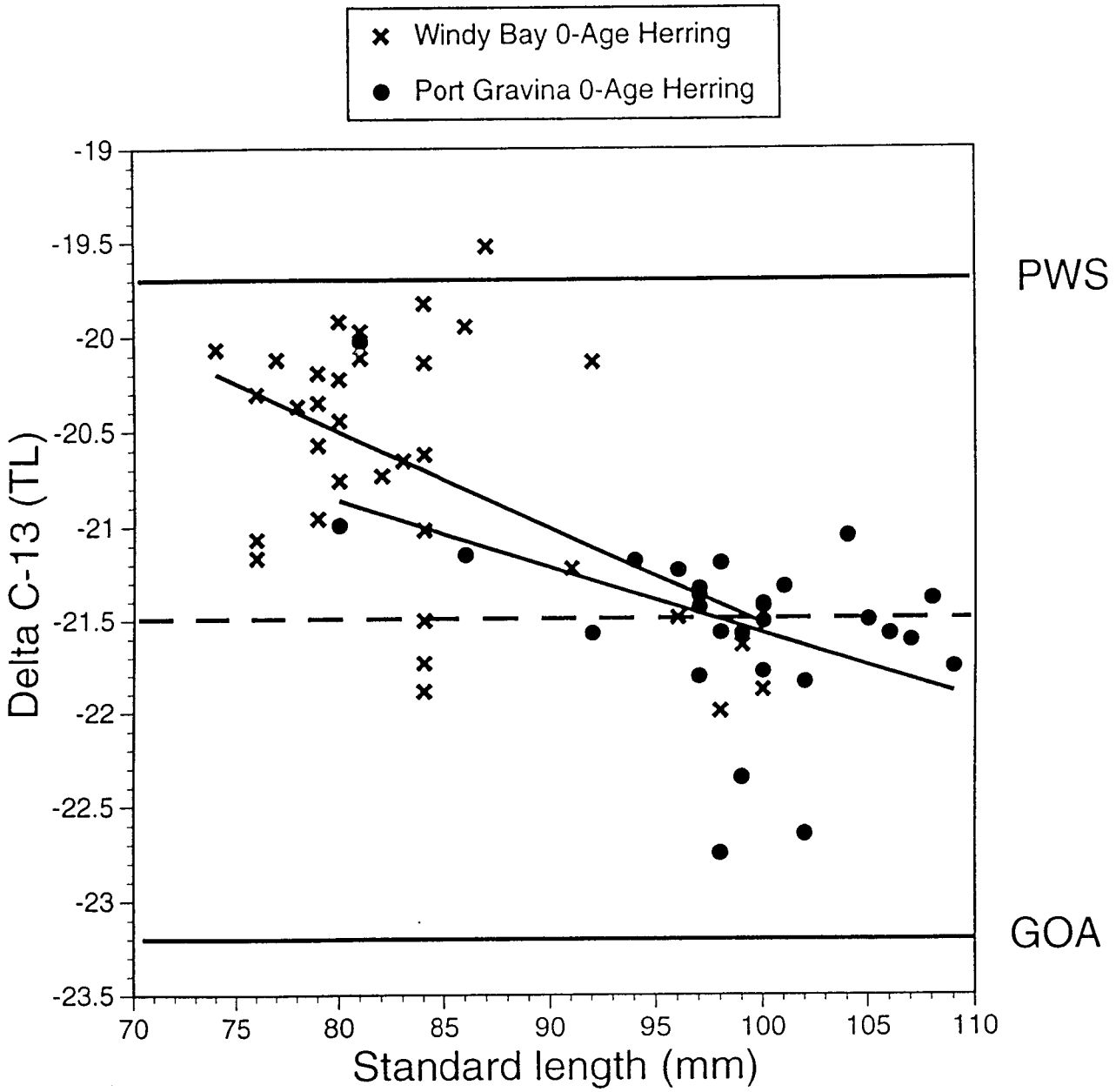

Windy Bay 0-Age Herring
Port Gravina 0-Age Herring
-19
-19.5
-20
-20.5
-21
-21.5
-22
-22.5
-23
-23.5
Delta C-13 (TL)
70
75
80
85
90
95
100
105
110
Standard length (mm)
PWS
GOA

Fig 8

International Symposium on the Role of Forage Fishes in Marine Ecosystems
B. Baxter (ed), Alaska Sea Grant College Program

Confirming Forage Fish Food Web Dependencies in the Prince William Sound Ecosystem Using Natural Stable Isotope Tracers

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Confirming Forage Fish Food Web Dependencies in the Prince William Sound Ecosystem Using Natural Stable Isotope Tracers.

Thomas C. Kline, Jr.

***Abstract**

$^{15}N/^{14}N$ and $^{13}C/^{12}C$ natural abundances were measured in plankton and nekton samples collected in 1994 and 1995 throughout Prince William Sound, Alaska (PWS) and selected locations in the northern Gulf of Alaska (GOA).

$\delta^{15}N$ values were used to determine realized trophic levels (TL) of potential forage and predator nekton species using the copepod *Neocalanus cristatus* as a reference.

A $\delta^{13}C$ gradient is suggested for carbon in the study area plankton, with high ^{13}C in PWS and low ^{13}C in the GOA. The interplay of these carbon sources is hypothesized to play a role in PWS food web nutrition. The initial focus of analytical work was addressed at resolving the relationship of $\delta^{13}C$ to GOA versus PWS carbon sources. These analyses consisted of extensive isotopic analyses of individuals of the herbivorous copepod *Neocalanus cristatus* taken from bulk net plankton samples collected during oceanographic surveys in 1994 and 1995. Copepods feeding in the GOA were significantly ^{13}C depleted compared to those feeding in PWS consistent with a source isotope effect.

After removing lipid- and trophic level-isotope-effects from nekton $\delta^{13}C$, it was possible to assess significance of GOA and PWS carbon sources. This approach was used to determine the relative importance of GOA-origin carbon, which was found to vary among "forage-fish" species. By combining isotopic with energetic analyses, it is possible to ascertain which locations in PWS are most dynamic with respect to transfer of energy into food webs via forage fishes.

***Introduction**

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 (EVOS), has raised concerns that shifts in food web structure may have occurred. Of particular concern is recruitment of *Clupea pallasi* (Pacific herring), presently at a historical low in abundance in PWS, a fjordlike inland sea that receives oceanic water from the Gulf of Alaska via the Alaska coastal current (Niebauer et al. 1994). The emergent hypothesis is that when large herbivorous copepods of the genus *Neocalanus* and other macrozooplankton, primary sources of food for predatory fishes (Parsons 1987), are in low abundance, these fishes resort to piscivory. Prey include *Clupea*, *Oncorhynchus* spp. fry including *O. gorbuscha* (pink salmon, also impacted by the oil spill) and other age 0+ fishes that can be regarded as "forage-fish" species. Predator species were expected to be dominated by gadid species in the pelagic system. The switch to piscivory is hypothesized to be a factor in recruitment of fishes, many of which are also important as forage for birds, and mammal species that were also affected by EVOS in PWS.

Confirmation of the hypothesis that macrozooplankton availability and related processes control fisheries recruitment are being tested in a large-scale multidisciplinary project known as Sound Ecosystem Assessment (SEA). Because of their predictable nature, stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) are providing an effective method for testing this

hypothesis. Natural stable isotope ratio analysis of fishes, their prey and their predators serve as effective tracers of energy supply thus providing insight into both habitat usage and assist in quantifying amount of carbon and by extension, energy, derived from various areas of production. Nitrogen stable isotope ratios provide excellent definition of relative trophic level (Fry 1988, Wada et al. 1991, Hobson and Welch 1992, Kiriluk et al. 1995). The heavy isotope of nitrogen, ^{15}N, is enriched by about 0.34 % (or 3.4 per mil in conventional delta units, see materials and methods) with each trophic level (Minagawa and Wada 1984) and has been shown to accurately indicate the realized trophic level of species within an ecosystem (Kling et al. 1992, Cabana and Rasmussen 1994). Carbon isotope signatures can effectively be used to trace multiple sources of carbon into food webs once it can be established that these sources have distinctive isotopic signatures (Fry and Sherr 1984, Wada et al. 1991). The data obtained from stable isotope measurements are unique in that they trace assimilated material and thus can be used for accurate ecosystem process modeling (e.g., Wada et al. 1991, Nadelhofeffer and Fry 1994, Macko and Ostrom 1994, Michener and Schell 1994, and Conway et al. 1994).

Because of their predictable relationship when comparing consumers to diet, stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) effectively provide empirical evidence of trophic relationships in marine food webs. Natural abundance of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ of Prince William Sound Alaska pelagic biota were measured in samples collected from 1994 to 1995. $\delta^{15}N$ values were used to determine trophic level (TL) relative to the large herbivorous copepod *Neocalanus cristatus* whereas $\delta^{13}C$ values were used to differentiate carbon derived from Gulf of Alaska production versus carbon produced within Prince William Sound.

***Materials and Methods**

**Sampling

Nekton and zooplankton were sampled from a variety of vessels ranging from a 25m trawler, *F/V Alaska Beauty*, equipped with a 40 x 28m mid-water wing trawl (2.0 cm stretch-mesh web codend) to small fry skiffs and seine boats that participated as part of the SEA project. Zooplankton samples were collected with a 335μ-mesh 0.5 m-diameter ring net towed vertically to the surface from station depth and 50 m at designated SEA project stations. Life-history-stage copepodid-V (C5) *Neocalanus cristatus* were picked from zooplankton samples and analyzed as individuals. Sampling of nekton for stable isotope analysis consisted of a section (~ 1 g) of epaxial muscle (fishes) or mantle (squid) for those with lengths > ~ 100 mm or the whole organism for those < 100 mm. Samples where frozen (-20°C) on board the vessel for later laboratory preparation for natural stable isotope abundance analysis.

**Laboratory preparation

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 aliquots of ~1.5 mg (except for individual samples of *Neocalanus* which were too small for more than one analysis) were weighed to the nearest μg and then loaded into combustion boats for mass spectrometric analysis.

**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 $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in standard delta units, $\delta^{13}C$ and $\delta^{15}N$, 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 for C) and defined by the following expression:

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

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 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 $\delta^{13}C$, $\delta^{15}N$ and C/N replicates were used for further analysis.

**Lipid normalization

Normalization for lipid composition was by the method of McConnaughey and McRoy (1979) using their C/N lipid proxy:

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

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

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

**Trophic level and normalization

The enrichment of ^{15}N that results from a feeding process (Minagawa and Wada 1984) enables one to use $\delta^{15}N$ as a good proxy for trophic level (Fry 1988, Kling et al. 1992, Cabana and Rasmussen 1994). *Neocalanus* spp. are the dominant herbivores in the plankton community of the north Pacific (Miller et al. 1984). Although *Neocalanus cristatus* are facultatively carnivorous on planktonic Protozoa, they cannot be sustained on such a diet (Gifford 1993). Thus, the a priori trophic level (TL) of 2 (i.e., herbivores) was applied to the $\delta^{15}N$ of *Neocalanus cristatus* as a baseline for estimation of TL of other taxa. The $\delta^{15}N$ values corresponding to higher TLs was estimated by adding the ^{15}N trophic fractionation factor, $\varepsilon_N = 3.4$, to the value obtained for next lower TL, e.g., 3.4 was added to the $\delta^{15}N$ of *Neocalanus cristatus* to estimate the $\delta^{15}N$ of TL = 3.

$\delta^{13}C$ values were normalized for TL as well as lipid content so as to have the residual variation reflect $\delta^{13}C$ of the carbon source. Normalization for trophic enrichment of ^{13}C using the trophic enrichment factor, $\varepsilon_C = 1$, to the reference TL (the TL of *Neocalanus cristatus*) was made using the following relationship:

(4) $\delta^{13}C'_{TL} = \delta^{13}C' - \varepsilon_C(\delta^{15}N_{sample} - \delta^{15}N_{Neocalanus})/\varepsilon_N$

where $\delta^{13}C'_{TL}$ is the TL normalized $^{13}C/^{12}C$ value of $\delta^{13}C'$, $\varepsilon_C = 1$ (DeNiro and Epstein 1978, McConnaughey and McRoy 1979, Rau et al. 1983, Hobson and Welch 1992, Fry and Sherr 1984), the $\delta^{15}N$ values are those of the sample and *Neocalanus*, and $\varepsilon_N = 3.4$ (Minagawa and Wada 1984, Owens 1987). This normalization makes it possible to make direct $\delta^{13}C$ comparisons between the sample in question with *Neocalanus*, which is being used as representative of the organic carbon pool at the herbivore level.

***Results and Discussion**

***Neocalanus* herbivore (TL = 2) reference

938 individual *Neocalanus cristatus* sampled from 1994 through 1995 had a mean $\delta^{15}N$ = 8.0 (SD = 1.8). This $\delta^{15}N$ value of 8.0 was used as the TL = 2 (herbivore) reference value. The $\delta^{15}N$ values for TL = 3 and 4 calculated using (4) were 11.4 and 14.8, respectively. $\delta^{13}C$ of stage C5 *Neocalanus cristatus* feeding (only those sampled from upper 50m during March to June, 1995) in the northern GOA just south of the entrance to PWS were found to be consistently dichotomous when compared to those from within PWS (Kline 1996). Feeding C5 *Neocalanus cristatus* from the GOA had $\delta^{13}C$ = -24.4 (SD = 1.4, N = 33) and $\delta^{13}C'$ = -23.1 (SD = 1.1, N = 33) whereas those from PWS had $\delta^{13}C$ = -20.2 (SD = 1.1, N = 101) and $\delta^{13}C'$ = -19.7 (SD = 0.9, N = 101). Thus organic carbon in the form of zooplankton from the northern GOA is ^{13}C-depleted by ~ 4 per mil compared with PWS. This gradient is similar to the ~2.5 per mil gradient in $\delta^{13}C$ between the Bering Sea and the GOA at the Aleutian passes (Schell 1996) and the 7 per mil $\delta^{13}C$ gradient found across Drake Passage (Rau et al. 1991).

**Nekton database

Data from 1592 nekton samples, consisting principally of fishes, collected in 1994 and 1995 from PWS, and analyzed for $\delta^{15}N$ and $\delta^{13}C$ are presented in this paper. Invertebrate nekton included squid (*Berryteuthis magister*) and glass shrimp (*Pasiphaea pacifica*). Nekton $\delta^{15}N$ values were plotted against length to suggest how nekton shift in TL as a function of size (Fig. 1A). This plot also provides an indication of the nekton size distribution in the database. The large cluster of nekton ≤ 180 mm in Fig. 1A have a $\delta^{15}N$ of ~ +12 suggesting a TL ~ 3 consistent with the concept of a forage class TL. Higher TLs are indicated by higher $\delta^{15}N$. Thus $\delta^{15}N$ defines a forage class by realized TL (Kling et al. 1992) and size class. Larger nekton, more likely to be predators, show considerable TL variability (from ~ TL = 2 to TL > 4) consistent with some zooplankton foraging which is expected to vary according the SEA hypotheses. $\delta^{13}C$ and $\delta^{15}N$ are expected to increase with TL (DeNiro and Epstein 1978, McConnaughey and McRoy 1979, Rau et al. 1983, Fry and Sherr 1984, Minagawa and Wada 1984, Owens 1987, Hobson and Welch 1992, Kiriluk et al. 1995) at a ratio of 1/3.4 (the ratio of $\varepsilon_C/\varepsilon_N$, the trophic fractionation factors). The scatterplot and linear regression of the nekton database (Fig. 1B) has a slope inconsistent with this ratio. This fact, the low correlation coefficient of r^2= 0.37, and the wide scatter of $\delta^{13}C$ suggests that a significant source of variation is independent of TL. Note, however, that normalization for TL (Fig. 1C) resulted in a decrease in the correlation (r^2 = 0.14). TL-normalized $\delta^{13}C$ values when plotted against C/N (Fig. 1D) show the effect of increased C/N on $\delta^{13}C$ (McConnaughey and McRoy 1979, Rau et al. 1992). When C/N was used to normalize for lipid content, in addition to TL normalization, the slopes of the regressions of the net result,

$\delta^{13}C'_{TL}$, with $\delta^{15}N$ (Fig. 1E) and C/N (Fig. 1F) was eliminated, validating the use of the C/N correction for lipid content (Rau et al. 1992). The TL- and lipid-normalized values when replotted vs. length (Fig. 1G) also had no slope. The variance in $\delta^{13}C'_{TL}$ shown in Fig. 1G thus reflects isotope effects other than lipid content or TL.

The coincidence of the large *Neocalanus* $\delta^{13}C'$ gradient between the GOA and PWS with the Nekton $\delta^{13}C'_{TL}$ (recall that the TL normalization normalizes the $\delta^{13}C'$ value to the same TL as *Neocalanus* making the $\delta^{13}C'$ values comparable) value distribution suggest that GOA-derived productivity is important for nekton. Periodic flow reversals at Hinchinbrook Entrance (Niebauer et al. 1994), downward diapause migration of C5 *Neocalanus* spp. during the late spring (Miller 1993) coinciding with deep water renewal in PWS (Niebauer et al. 1994), and simultaneous transport of zooplankton by the landward movement of coastal waters (Cooney 1986) suggest mechanisms that could transport secondary productivity into PWS. Low $\delta^{13}C'_{TL}$ values (e.g. values < ~ -21) measured in the nekton are consistent with the flux of carbon from outside PWS making its way into food chains there. Thus $\delta^{13}C'_{TL}$ values of nekton provide direct evidence for the hypothesis of plankton flow into PWS influencing nekton production. From the $\delta^{15}N$-based TL determination (Fig. 1A), nekton < ~180 mm can be defined as "forage fishes". Fig. 1H shows the data from Fig. 1G, but restricted to nekton ≤ 180 mm, where it can be seen that a substantial portion of the forage class nekton has $\delta^{13}C'_{TL}$ consistent with utilization of carbon derived from the GOA. However, most data points in Fig. 1G appear between $\delta^{13}C'_{TL}$ = -20 and -21 suggesting a significant overlap in use of both PWS as well as GOA carbon. Further analysis examines the forage fishes (and other nekton) by species (Fig. 2).

**Forage class nekton by species

Box and whisker plots of $\delta^{15}N$ data of nekton ≤180 mm (Fig. 2A) suggest species-level differences in realized TL (Kling et al. 1992). Whereas most species had $\delta^{15}N$ consistent with TL = 3, i.e., primary carnivores, northern lanternfish were about 0.5 TL higher. Conversely walleye pollock (these consisted of < 100 mm, young-of-the-year) were consistently lower in TL. Glass shrimp had the greatest range in $\delta^{15}N$ consistent with facultative herbivory although principally carnivorous.

Box and whisker plots of $\delta^{13}C'_{TL}$ data of nekton ≤180 mm (Fig. 2B) suggest some variance in carbon source dependencies. There was spatial variability in the degree to which GOA-derived carbon is found in young-of-the-year herring which was concordant with energetic content (Kline and Paul 1997). Those fishes with higher GOA content (i.e. more negative $\delta^{13}C'_{TL}$ values), such as capelin and pink salmon fry, are more likely to have been affected by variance in flux of zooplankton from the GOA to PWS. Conversely, those species with high $\delta^{13}C'_{TL}$, such as juvenile gadids and sandlance, are likely not to be directly affected zooplankton inputs. These results thus provide evidence of bottom-up effects with an inherent source of environmental variability. Particularly noteworthy are potential species-level differential effects that could result in the advantage of one species over another as a function of oceanographic conditions that moderate zooplankton flux from the GOA to PWS.

*Summary

1. $\delta^{15}N$ relates to trophic level and can be used to delineate forage-class species.

2. $\delta^{13}C'_{TL}$ can be related to carbon source which can be related to oceanographic processes affected nekton production.
3. Species-level differences in dependencies on GOA carbon exist within the forage fish class nekton suggesting bottom-up effects in community structure.

***Acknowledgements**

The research described in this paper was part of the Exxon Valdez Oil Spill Trustee Council - funded Sound Ecosystem Assessment (SEA) project. However, the findings and conclusions presented by the author are his own and do not necessarily reflect the views or position of the Trustee Council. The author acknowledges Captain Mike Geagel, the crew of the R/V *Bering Explorer*, E. Stockmar and B. Barnett for field and laboratory assistance in zooplankton sampling. The author acknowledges the captains and crews of the F/V *Alaska Beauty* and other fishing vessels used in this study. M. Powell, K. Antonucci, and D. Salmon collected the majority of the nekton samples. E. Brown and M. Willette coordinated the nekton sampling. Preparation of samples for mass spectrometry was done by R. Sundown, K. Antonucci, and B. Barnett. B. Barnett was responsible for the mass spectrometry. Discussions with D. Schell and R. Spies on isotope gradients, with T. Cooney and L. Tuttle on *Neocalanus* life histories, and with D. Salmon and S. Vaughan on regional physical oceanography provided the author with much insight.

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***Figure Captions (figures follow)**

Figure 1A to H. Isotopic analysis of Prince William Sound nekton.

A. Nekton $\delta^{15}N$ values suggesting trophic level (TLs indicated on right axis) change as a function of length; based on the herbivorous copepod, *Neocalanus cristatus*, reference ($r^2 = 0.56$, P = 0 .0001; β coefficient X, P = 0.0001, β coefficient X^2, P = 0.0001, β coefficient X^3, p = 0.0001).

B. Nekton $\delta^{13}C$ as a function of $\delta^{15}N$. Regression line ($r^2 = 0.37$, P = 0.0001) slope = 0.62 (SE = 0.02, P = 0.0001) which differed from the ratio of the trophic fractionation factors $\varepsilon_C/\varepsilon_N$ = 1.0/3.4 = 0.29.

C. Data in Fig. B replotted following normalization of $\delta^{13}C$ for trophic level which reduced the correlation, r^2, to 0.14 (P = 0.0001) and slope to 0.32 (SE = 0.02, P = 0.0001).

D. Trophic level normalized $\delta^{13}C$ varied as a function of C/N as expected because of lipid isotope effects ($r^2 = 0.60$, P = 0.0001; β coefficient X, P = 0.0001, β coefficient X^2, P = 0.0001, β coefficient X^3, P = 0.0001).

E. Normalization for trophic level and C/N eliminated the relationship of $\delta^{13}C$ with $\delta^{15}N$ (r^2 = 0.04, P = 0.0001; slope = 0.12, SE = 0.01,P = 0.0001; compare with Fig. 1B).

F. Normalization for trophic level and C/N eliminated relationship of $\delta^{13}C$ with C/N ($r^2 = 0.07$, P = 0.0001; β coefficient X, P = 0.0001, β coefficient X^2, P = 0.0072, β coefficient X^3, P = 0.072; compare with Fig. 1D).

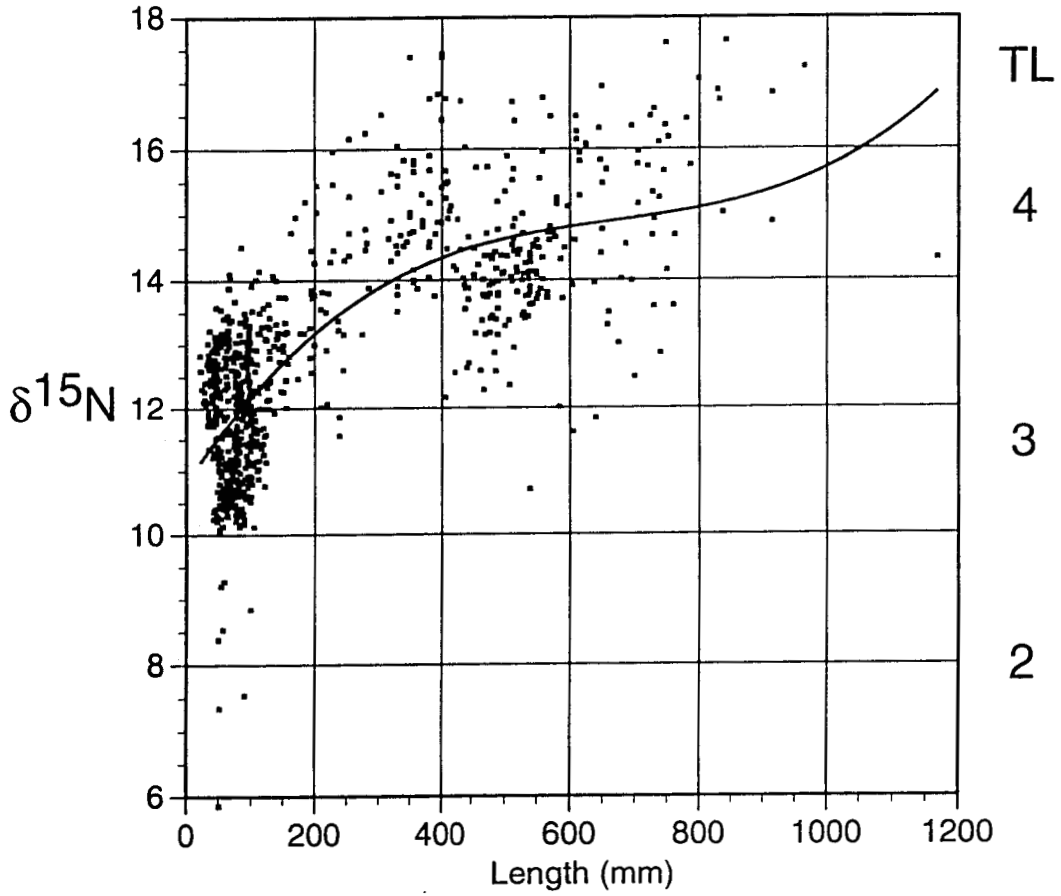
G. Relationship of $\delta^{13}C'_{TL}$ with length suggesting carbon source variation throughout size range ($r^2 = 0.04$, P = 0.0001; slope = 0.001, P =0.0001). The SD of *Neocalanus cristatus* $\delta^{13}C'$ values from PWS and the GOA indicated by bars on right axis.

H. Data from Fig. 1G expanded to show only "forage-fish", i.e. those nekton ≤ 180mm.

Figure 2A and 2B. Isotopic analysis of Prince William Sound forage-class nekton by species.

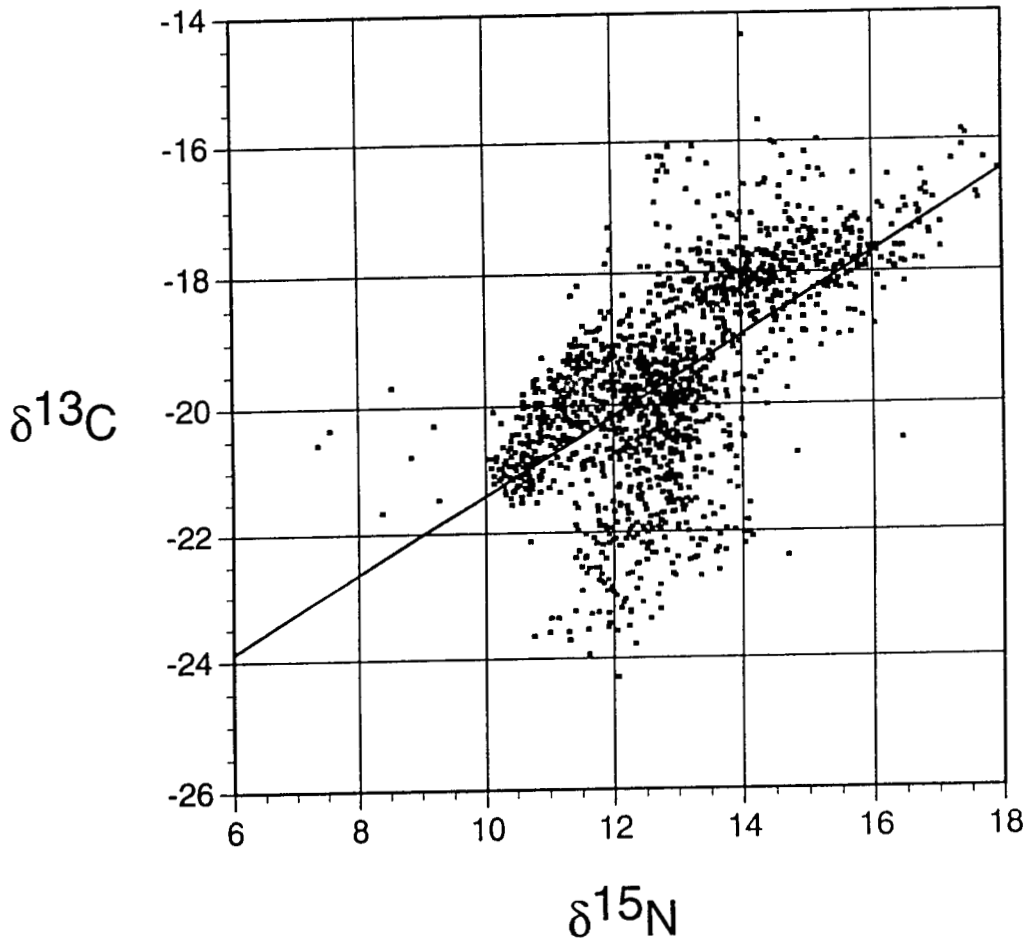
A. "Forage-fish" $\delta^{15}N$ box and whisker plots (10, 25, 50, 75, and 90th percentiles and means are indicated) and interpreted TLs suggesting species-level differences in realized trophic level.

B. "Forage-fish" (box and whisker plots as in Fig. 2A) and *Neocalanus cristatus* (SD range of samples from the GOA and PWS) $\delta^{13}C'_{TL}$ suggesting species-level differences in dependency on carbon source.



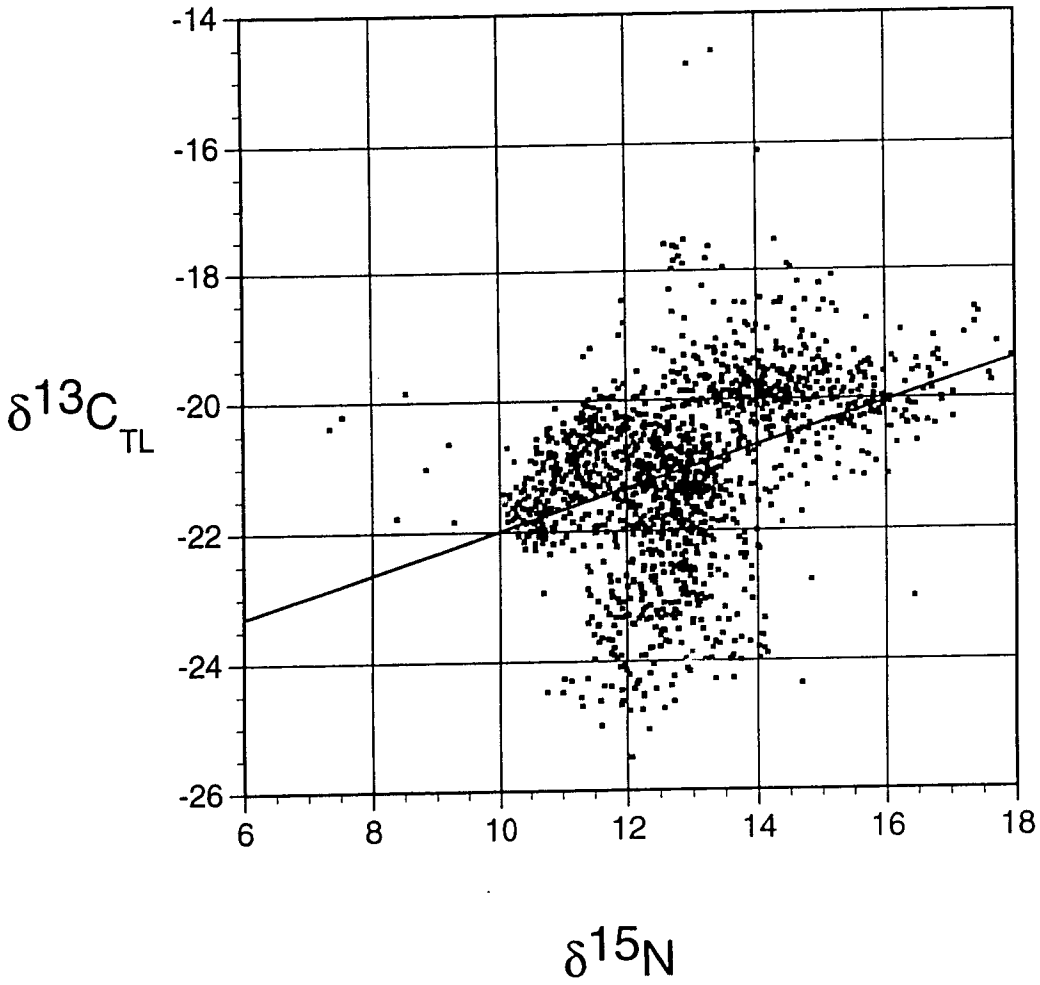
5-2-10

FIGURE 1A



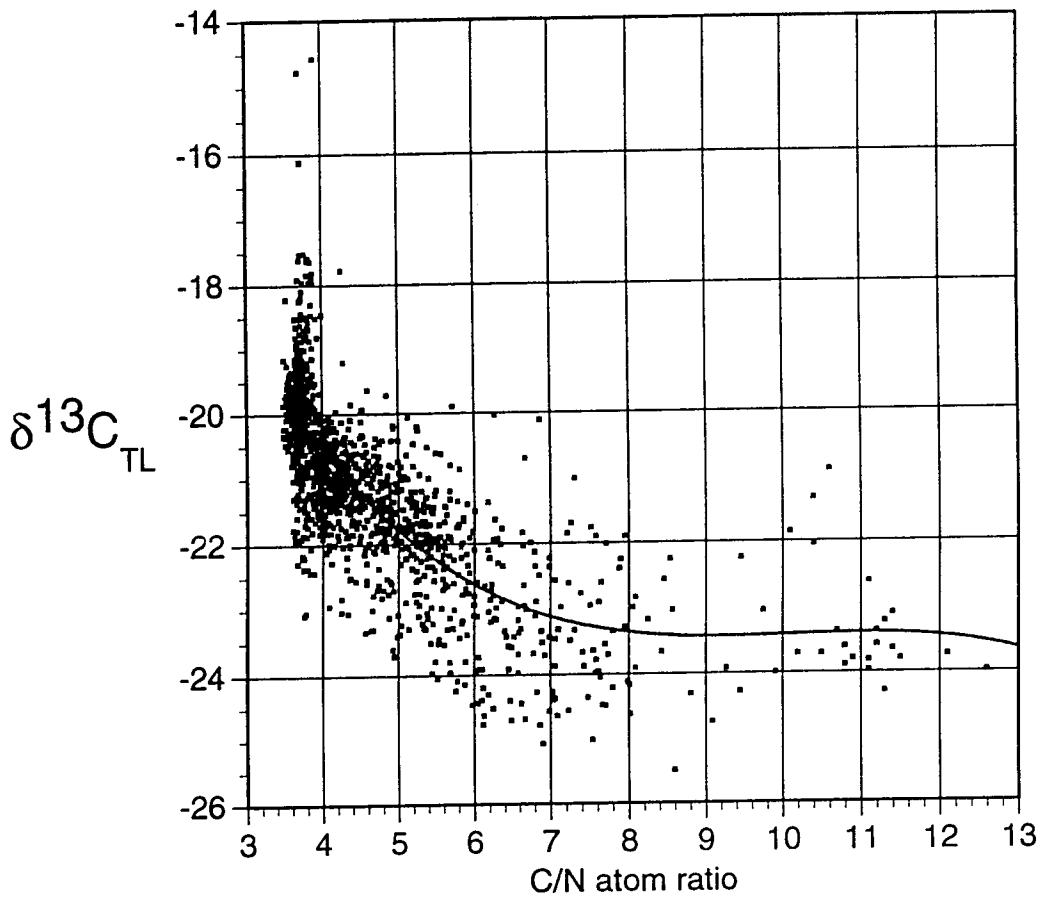
5-2-11

FIGURE 1B



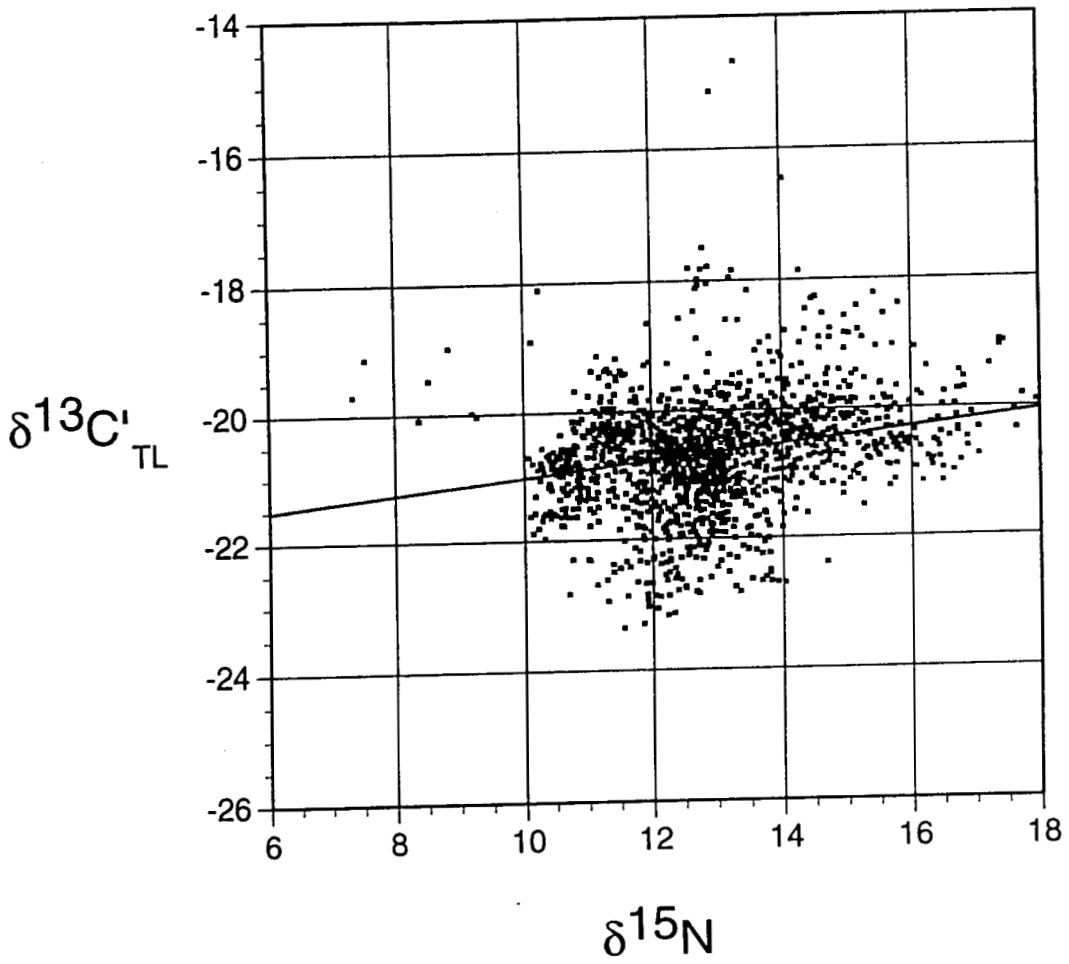
5-2-12

FIGURE 1C



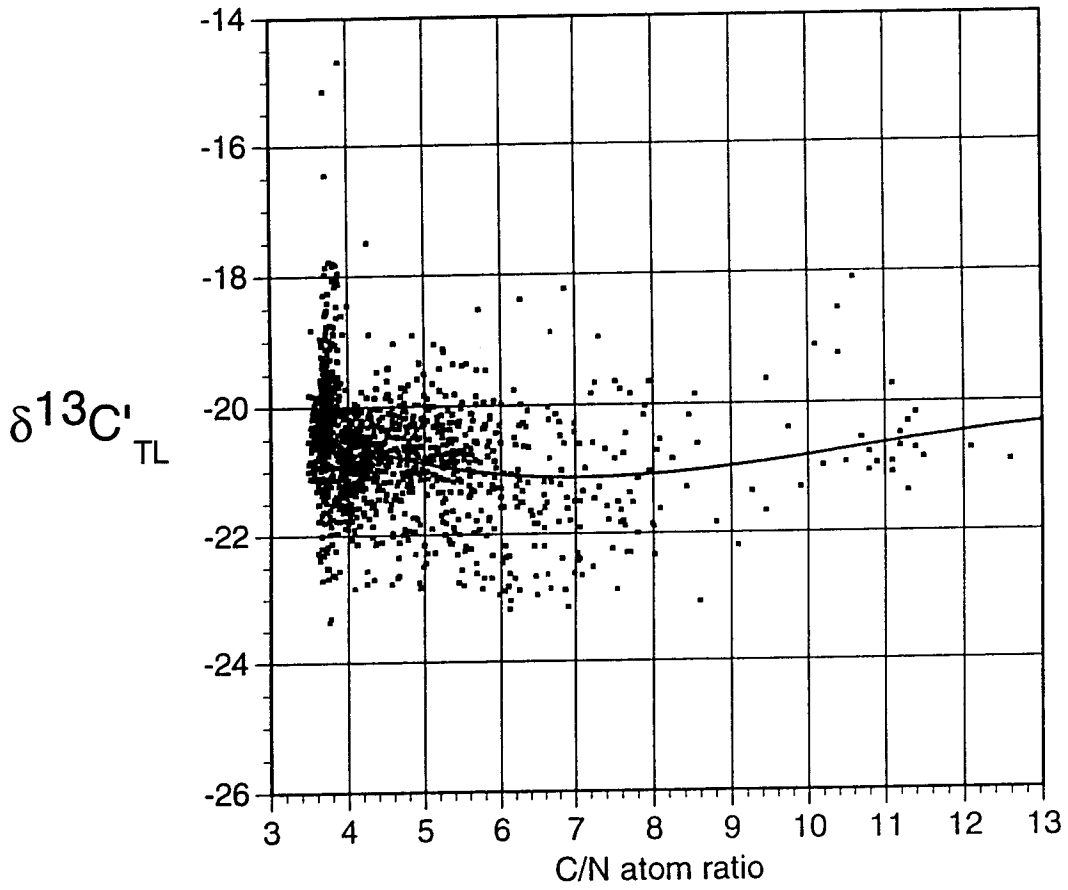
5-2-13

FIGURE 1D



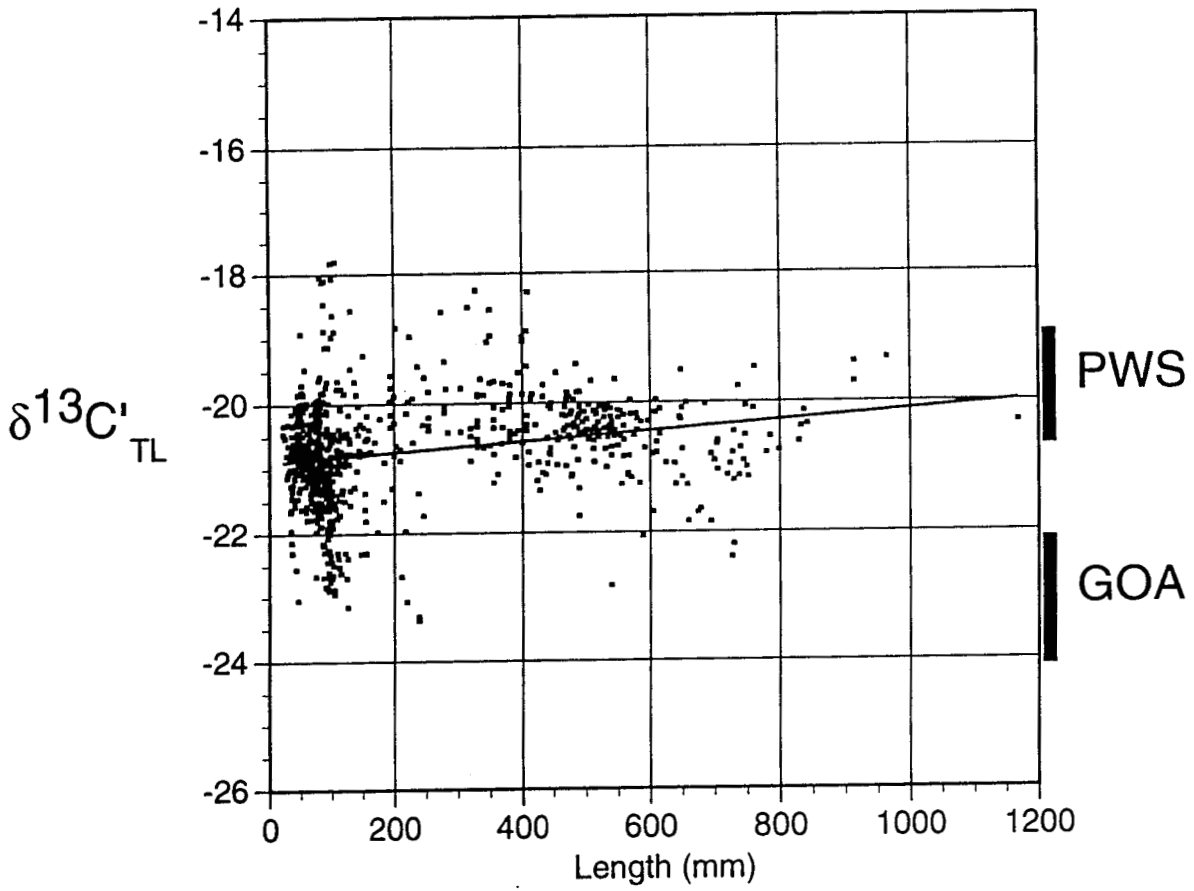
5-2-14

FIGURE 1E



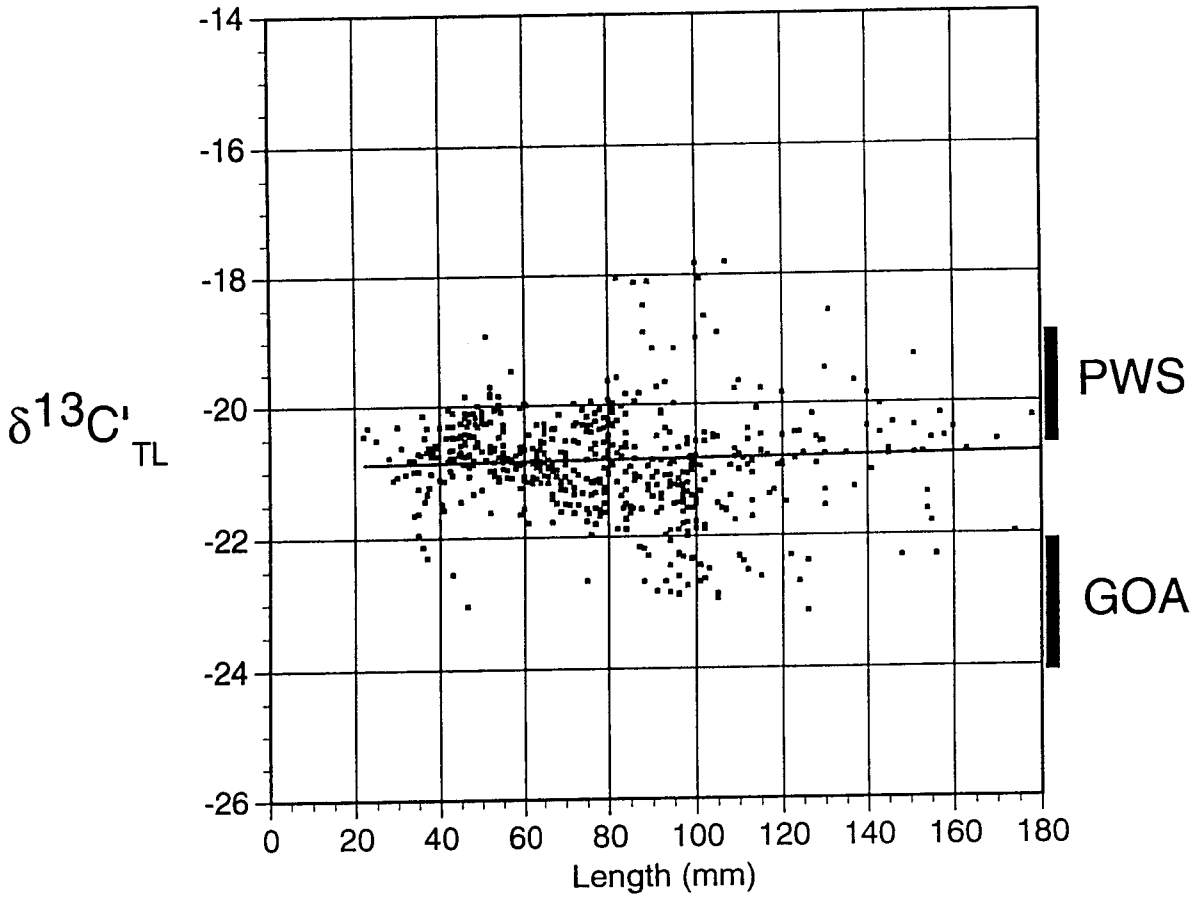
5-2-15

FIGURE 1F



5-2-16

FIGURE 1G



5-2-17

FIGURE 1H

FIGURE 2A

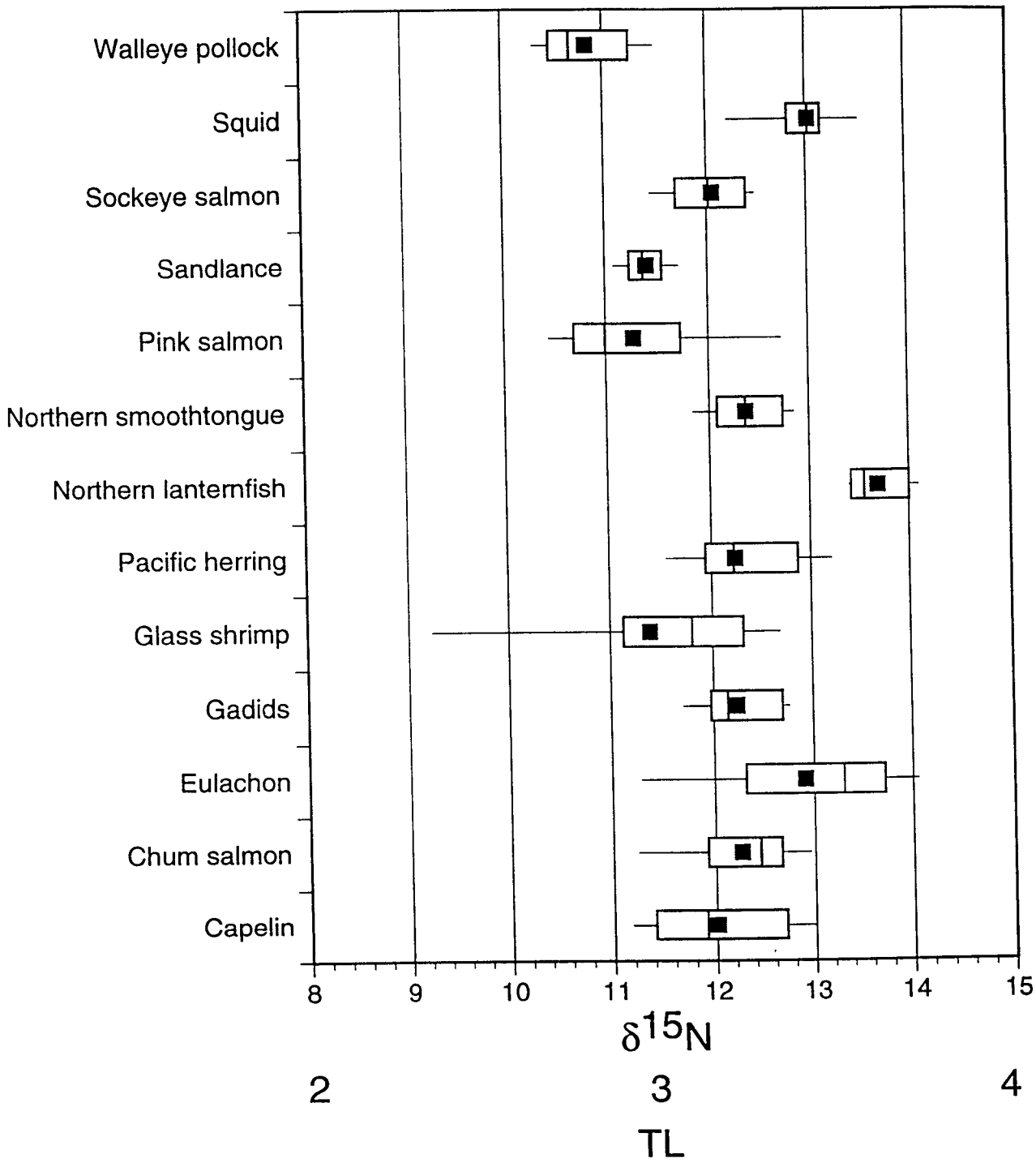
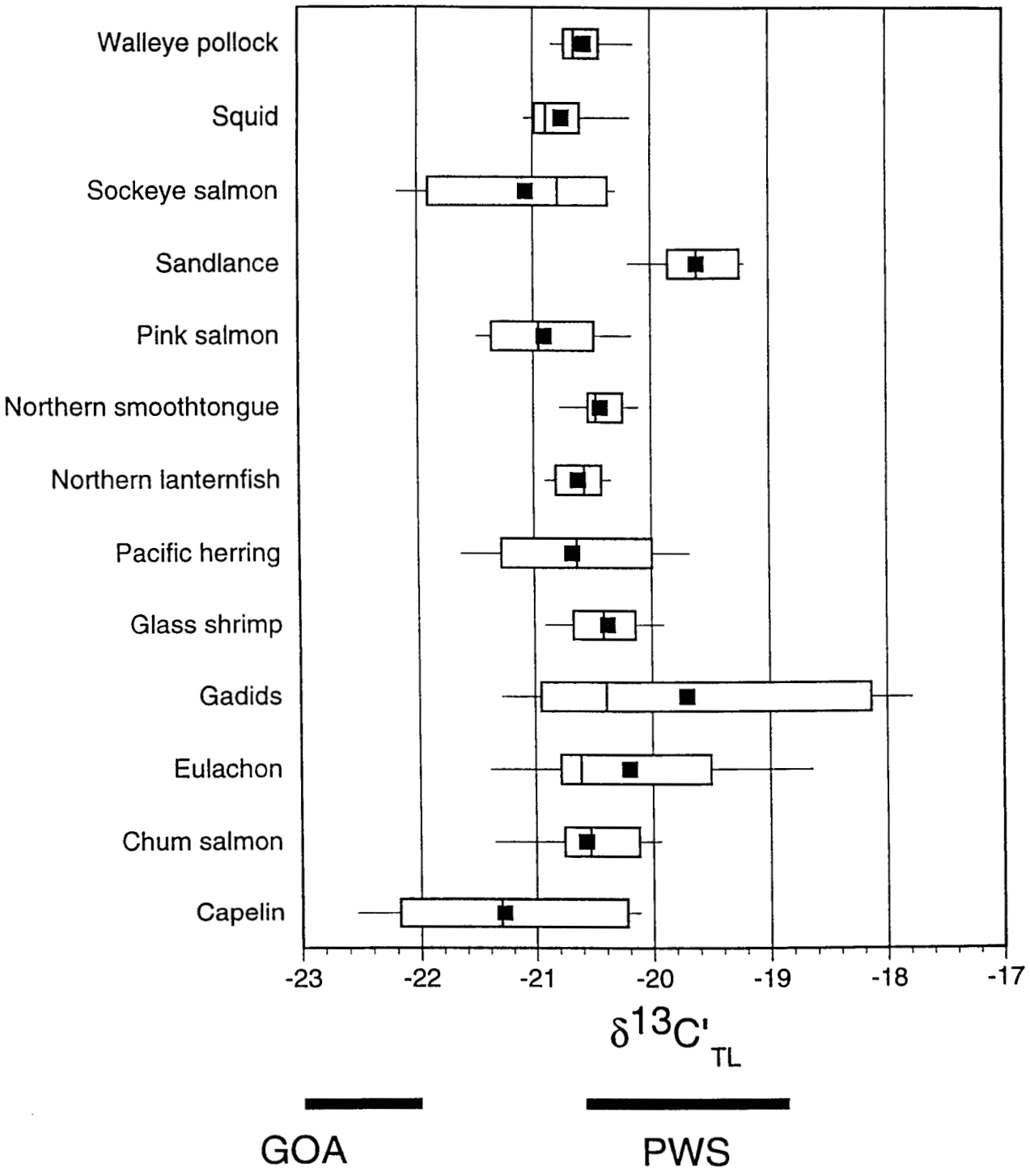

Walleye pollock
Squid
Sockeye salmon
Sandlance
Pink salmon
Northern smoothtongue
Northern lanternfish
Pacific herring
Glass shrimp
Gadids
Eulachon
Chum salmon
Capelin
8
9
10
11
12
13
14
15
δ15N
2
3
4
TL

FIGURE 2B


Walleye pollock
Squid
Sockeye salmon
Sandlance
Pink salmon
Northern smoothtongue
Northern lanternfish
Pacific herring
Glass shrimp
Gadids
Eulachon
Chum salmon
Capelin
-23
-22
-21
-20
-19
-18
-17
δ13C'TL
GOA
PWS

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Contribution to:

Pollock predation synthesis paper
by Willette et al. *(In Prep.)*

Abstract

$^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios were measured in epaxial muscle samples from adult pollock collected in 1994 and 1995 throughout Prince William Sound, Alaska (PWS). $^{15}N/^{14}N$ values were used to determine trophic level (TL) and $^{13}C/^{12}C$ values, after removal of lipid- and trophic level-isotope-effects, were used to determine source of carbon. Adult walleye pollock from bays in southwestern PWS had similar $^{13}C/^{12}C$ as those from within PWS. The $^{13}C/^{12}C$ values were consistent with PWS or hatchery salmon carbon signatures. A switch in diet to increased piscivory in the Spring of 1995 was indicated by $^{15}N/^{14}N$ from pollock sampled in May. The $^{15}N/^{14}N$ values were consistent with consuming subadult and adult herring or salmon fry following release from hatcheries.

Introduction

Because of their predictable nature, stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) provide an effective method for testing the prey-swiching hypothesis. Natural stable isotope ratio analysis of consumers, their prey and their predators serve as effective tracers of energy supply thus providing insight into both habitat usage and assist in quantifying amount of carbon and by extension, energy, derived from various areas of production (Michener and Schell 1994). Nitrogen stable isotope ratios provide excellent definition of relative trophic level (Fry 1988, Wada et al. 1991, Hobson and Welch 1992, Kiriluk et al. 1995). The heavy isotope of nitrogen, ^{15}N, is enriched by about 0.34 % (or 3.4 per mil in conventional delta units, see materials and methods) with each trophic level (Minagawa and Wada 1984) and has been shown to accurately indicate the "realized" trophic level of species within an ecosystem (Kling et al. 1992, Cabana and Rasmussen 1994). Carbon isotope signatures can effectively be used to trace multiple sources of carbon into food webs once it can be established that these sources have distinctive isotopic signatures (Fry and Sherr 1984, Wada et al. 1991). The data obtained from stable isotope measurements are unique in that they trace assimilated material and thus can be used for accurate ecosystem process modeling (e.g., Wada et al. 1991, Nadelhofeffer and Fry 1994, Macko and Ostrom 1994, Michener and Schell 1994, and Conway et al. 1994).

Natural abundance of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios of Prince William Sound Alaska pelagic biota measured in samples collected from 1994 to 1995 were used to determine trophic level (TL) relative to the large herbivorous copepod *Neocalanus cristatus* and distinguish carbon generated in the Gulf of Alaska (GOA) on the continental shelf outside Prince William Sound (PWS) from carbon generated in PWS (Kline and Paul 1997). The approach is extended here by comparing $^{15}N/^{14}N$ and $^{13}C/^{12}C$ of Prince William Sound adult pollock with herring and salmon fry to assess their TL and carbon source as a means of testing the prey-switching hypothesis.

Materials and Methods

Sampling for stable isotope analysis

Pollock, herring, and salmon fry were sampled from a variety of vessels ranging from a 25m trawler, *F/V Alaska Beauty*, equipped with a 40 x 28m mid-water wing trawl (2.0 cm stretch-mesh web codend) to small fry skiffs and seine boats that participated as part of the SEA project. Additional pollock were sampled from a pollock fishery that occured in the southwest area of PWS early 1995. Pollock and herring were sampled for stable isotope analysis by excising a section (~ 1 g) of epaxial muscle either on board the sampling or from whole carcasses brought ashore. Salmon fry samples consisted of coded wire tag recovery salmon that had been stored frozen following tag removal. All samples were stored frozen until freeze dried and ground to a fine powder.

The laboratory and data analysis procedures were as described by Kline and Paul (1997). In brief; conventional delta notation is used to express stable isotope ratios, which are reported relative to international standards (air for N and Vienna Peedee belemnite for C) and defined by the followingexpression:

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

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. Normalization of $^{13}C/^{12}C$ for lipid composition and trophic level (TL) was by the method of McConnaughey and McRoy (1979) and Kline and Paul (1997) that used C/N as lipid proxy and $\delta^{15}N$ to normalize to the TL of the herbivorous copepod *Neocalanus cristatus*. TL was estimated using $\delta^{15}N$ of the samples in relation to the *Neocalanus cristatus* reference (Kline and Paul 1997).

Results and Discussion

Herring and salmon fry $\delta^{15}N$

The $\delta^{15}N$ of herring was ~ +12 to +13 corresponding to a TL slightly greater than 3 (Fig iso-1). The lowest values came from herring > 250mm in length which were adult herring. Herring in the 150 mm to 200mm size class from Green Is. and Redhead were very similar wheras the intermediate size class, 200 to 250mm fell in between. The data suggest a progressive shift to

lower $\delta^{15}N$ consistent with a TL lowering. The herring > 250mm also had the most negative $\delta^{13}C'_{TL}$, (Kline unpublished data) consistent with a greater untilization of GOA carbon (Kline and Paul 1997) than the smaller size classes. The data suggest a switch to increased consumption of herbivores dependent on GOA carbon with size. Higher $\delta^{15}N$ suggests a greater proportion of carnivorous zooplankton in younger herring.

Hatchery salmon fry in PWS had highly elevated $\delta^{15}N$ shortly after release compared to 3 to 4 weeks later (Fig. iso2). The initial salmon $\delta^{15}N$ reflects the trophic enrichment (Minagawa and Wada 1984) relative to an animal protein based hatchery diet (PWSAC- ?). Rapid replacement of protein in proportion to growth (Hesslein et al. 1993) is confirmed here by the rapid change in $\delta^{15}N$ (Fig iso2). Salmon fry shortly after release as well as herring 150 to 200mm have a $\delta^{15}N \sim +12.8$. Since their predators were expected to have a $\delta^{15}N \sim 16.2$ (Minagawa and Wada 1984, this value was used in evaluating adult pollock $\delta^{15}N$ for evidence of predation on fishes. Unlike the $\delta^{15}N$ value dichotomy of alternate salmon fry nitrogen, hatchery salmon fry shortly after release and salmon fry utilzing PWS carbon had the same $\delta^{13}C'_{TL}$ values (Kline unpublished data). Thus pollock feeding on recently released hatchery salmon were expected have a similar $\delta^{13}C'_{TL}$ as pollock feeding on PWS production.

Adult pollock stable isotopes

Pollock had a very consistent $\delta^{13}C'_{TL}$ (Fig. iso3) that was very similar to the PWS carbon isotope reference value of -19.7 from Kline and Paul (1997). The $\delta^{15}N$ of adult pollock varied by ~ 1.5 per mil, equivalent to ~ 0.5 TL range (Fig. iso4). The in $\delta^{15}N$ declined ~ 1 per mil during 1994 inferred a ~1/3 TL decline. The $\delta^{15}N$ values increased in 1995 between winter and May. The May $\delta^{15}N$ value, +14.7, corresponds to 2/3 the diference between an initial value of 13.7 measured in PWS pollock in Jan and Feb (Fig iso4) and +16.2, the predicted value after consuming recently released hatchery fry or herring. Pollock could have obtained a value of +14.7 by replacing 2/3 of their protein with 100% consumption of herring or fry. A lower consumption rate of herring or fry (or other prey combination with a mean $\delta^{15}N$ similar to herring and fry) would be explained by a higher protein turnover, which, however is, unlikely (Hesslein et al. 1993). Separation of the May, 1995 pollock sample by collection site (Fig. iso5) suggests that the switch to higher $\delta^{15}N$ was localized within PWS to Port Gravina, an important herring habitat area (E. Brown - ?). The second most switched site is Esther Island in the vicinity of the Wally Nuernburg salmon hatchery. In comparison, Perry Is. pollock were more like those sampled the previous winter (Fig iso5). The stable data suggest that prey switching is likely to be localized and intense near important prey sites.

Acknowledgements

The research described in this paper was part of the Exxon Valdez Oil Spill Trustee Council - funded Sound Ecosystem Assessment (SEA) project. However, the findings and conclusions presented by the author are his own and do not necessarily reflect the views or position of the

Trustee Council. North Pacific Processors, Inc. provided the winter 1995 pollock samples obtained during the fishery. Preparation of samples for mass spectrometry was done by Kim Antonucci. Bruce Barnett, University of Alaska Stable Isotope Facility performed the mass spectrometry.

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Figure Captions (figures follow)

iso1. Herring > 150 mm $\delta^{15}N$ box and whisker plots (10, 25, 50, 75, and 90th percentiles and means are indicated) and interpreted TLs by size group and sampling location. Size group 2 = fish > 150mm up 200 mm, size group 3 = fish > 200 mm up 250 mm, and size group 3 = fish > 250 mm. N(Green-2) = 31, N(Green-3) = 41, N(Green-4) = 14, N(Redhead-2) = 51.

iso2. Hatchery salmon fry $\delta^{15}N$ by release date, hatchery and species as a function of capture date. Individual fish inidcated by points were identified by implanted coded wire tags. Hatchery codes as follow Wally Nuernburg Hatchery (WN), Main Bay Hatchery (MB) and AFK Hatchery (AFK). Species codes as follows pink salmon(P), chum salmon (C), and red salmon (R). Regression lines fitted to WN 25 April and 7 May pink salmon, WN 3 June chum salmon, and MB 28 to 31 May release cohorts suggest replacement of protein obtained fom hatchery in three to four weeks. Fry imediately after release had $\delta^{15}N \sim +12.8$

iso3 Pollock $\delta^{13}C'_{TL}$ from PWS by sample arranged by calendar month. Samples consisted of N = 25 each from Port Bainbridge and Day Harbor and N = 47, 40, 99, 19, and 17 each from western and northern PWS sampled in April 1994, May 1994, May 1995, June 1994, and July, 1994, respectively. Data showed strong consistency to carbon derived from PWS based on Kline and Paul (1997).

iso4 Pollock $\delta^{15}N$ of same samples in Fig. iso3 and interpreted TL based on Kline and Paul (1997). A large shift in $\delta^{15}N$ between early 1995 "A" to values approaching "B" which is the estimated $\delta^{15}N$ of pollock feeding on herring < 250 mm or hatchery salmon fry within a few weeks after release.

iso5 May 1995 pollock shown in Fig. iso4 by capture site suggesting spatial variability in switching from "A" to "B" as in Fig iso4. Samples consisted of N = 34, 25, 39, and 2 from Perry Is., Port Gravina, Esther Is., and Zaikof Bay, respectively.

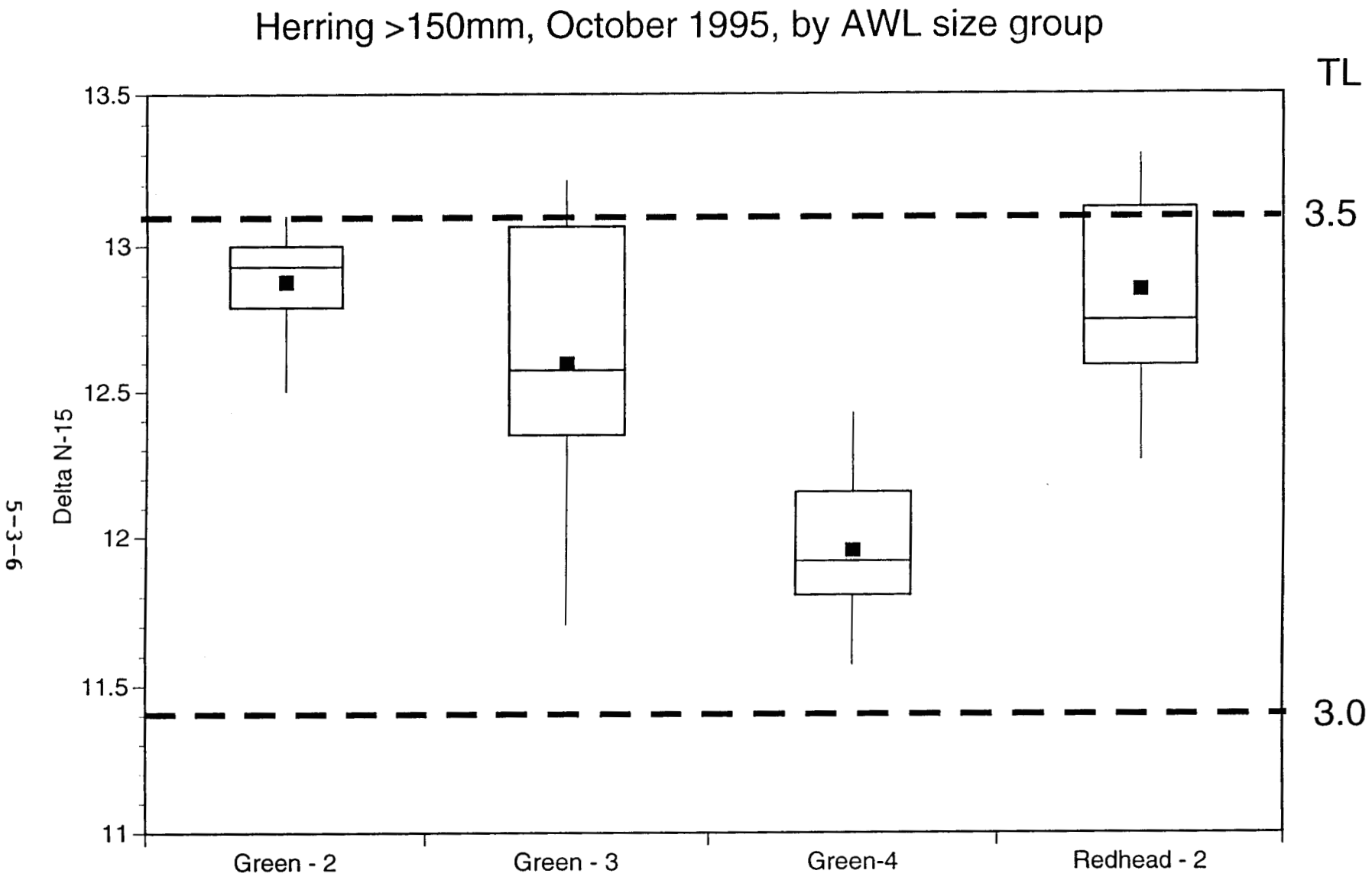
Herring >150mm, October 1995, by AWL size group
TL
13.5
13
12.5
12
11.5
11
Delta N-15
3.5
3.0
Green - 2
Green - 3
Green-4
Redhead - 2

Figure 1.

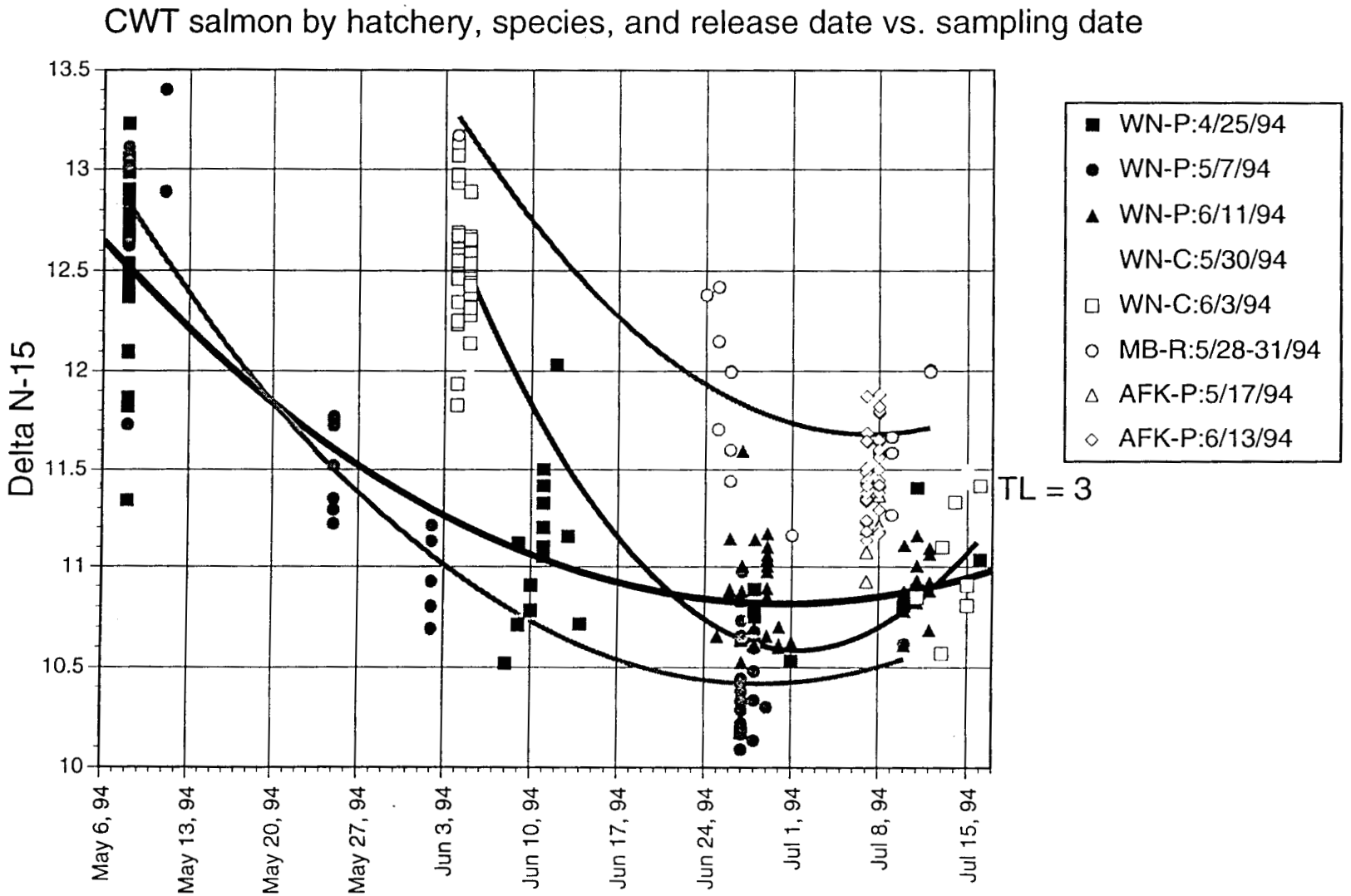
CWT salmon by hatchery, species, and release date vs. sampling date
Delta N-15
13.5
13
12.5
12
11.5
11
10.5
10
May 6, 94
May 13, 94
May 20, 94
May 27, 94
Jun 3, 94
Jun 10, 94
Jun 17, 94
Jun 24, 94
Jul 1, 94
Jul 8, 94
Jul 15, 94
TL = 3
WN-P:4/25/94
WN-P:5/7/94
WN-P:6/11/94
WN-C:5/30/94
WN-C:6/3/94
MB-R:5/28-31/94
AFK-P:5/17/94
AFK-P:6/13/94

Figure 2.

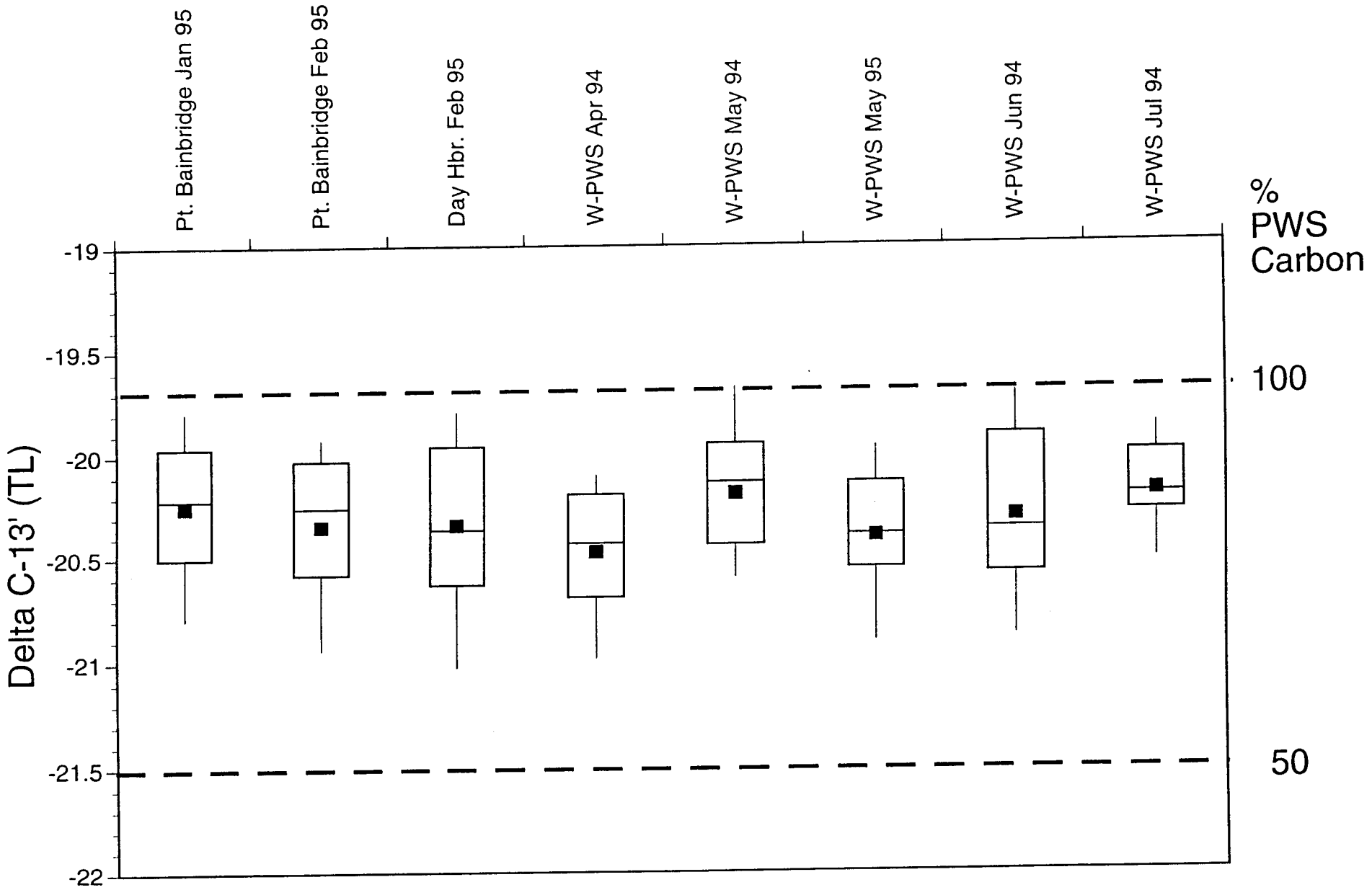
Pt. Bainbridge Jan 95
Pt. Bainbridge Feb 95
Day Hbr. Feb 95
W-PWS Apr 94
W-PWS May 94
W-PWS May 95
W-PWS Jun 94
W-PWS Jul 94
% PWS Carbon
100
50
Delta C-13' (TL)
δ-13-8
-19
-19.5
-20
-20.5
-21
-21.5
-22

Figure 3.

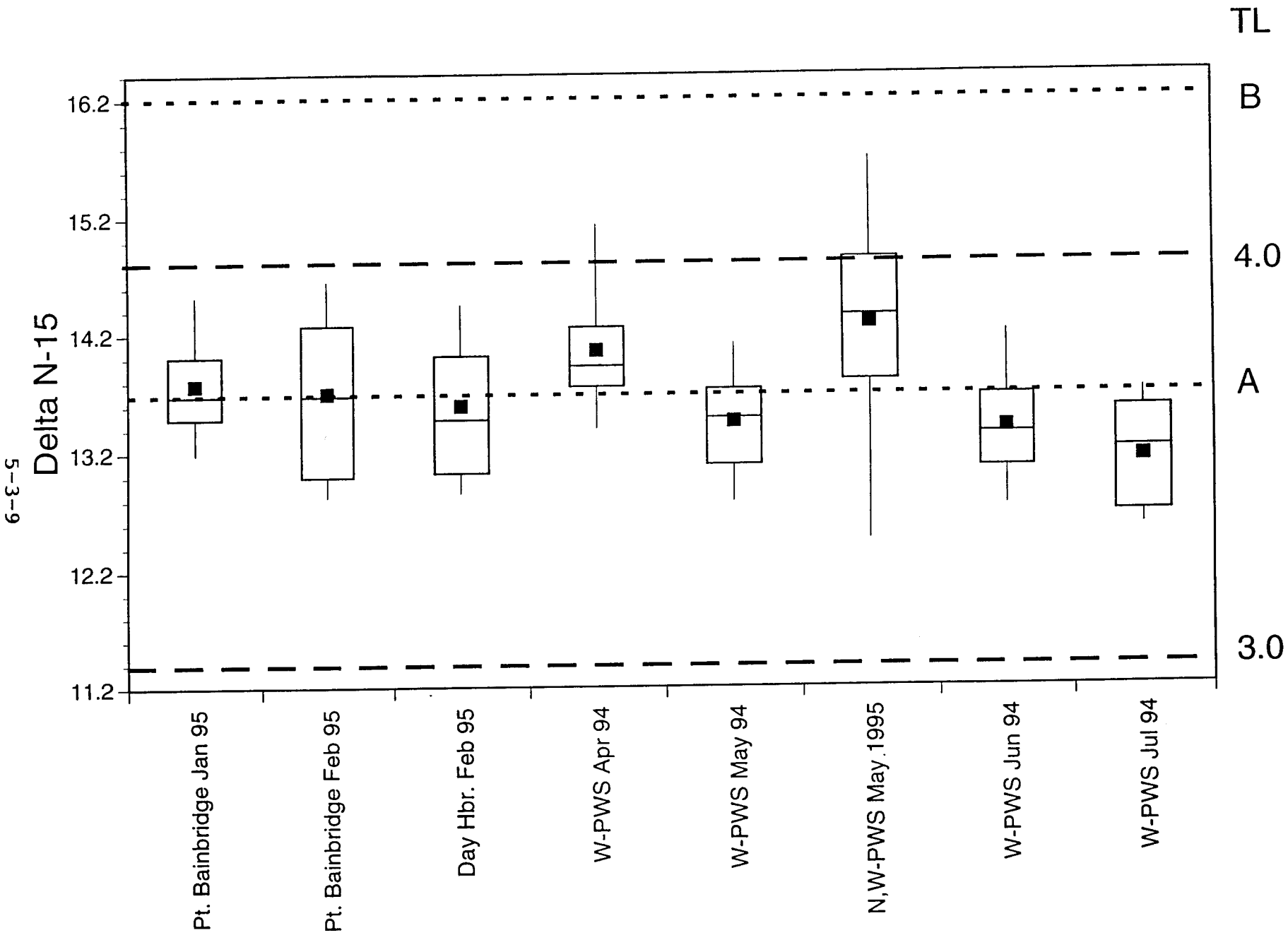
TL
B
4.0
A
3.0
Delta N-15
16.2
15.2
14.2
13.2
12.2
11.2
Pt. Bainbridge Jan 95
Pt. Bainbridge Feb 95
Day Hbr. Feb 95
W-PWS Apr 94
W-PWS May 94
N,W-PWS May 1995
W-PWS Jun 94
W-PWS Jul 94

Figure 4.

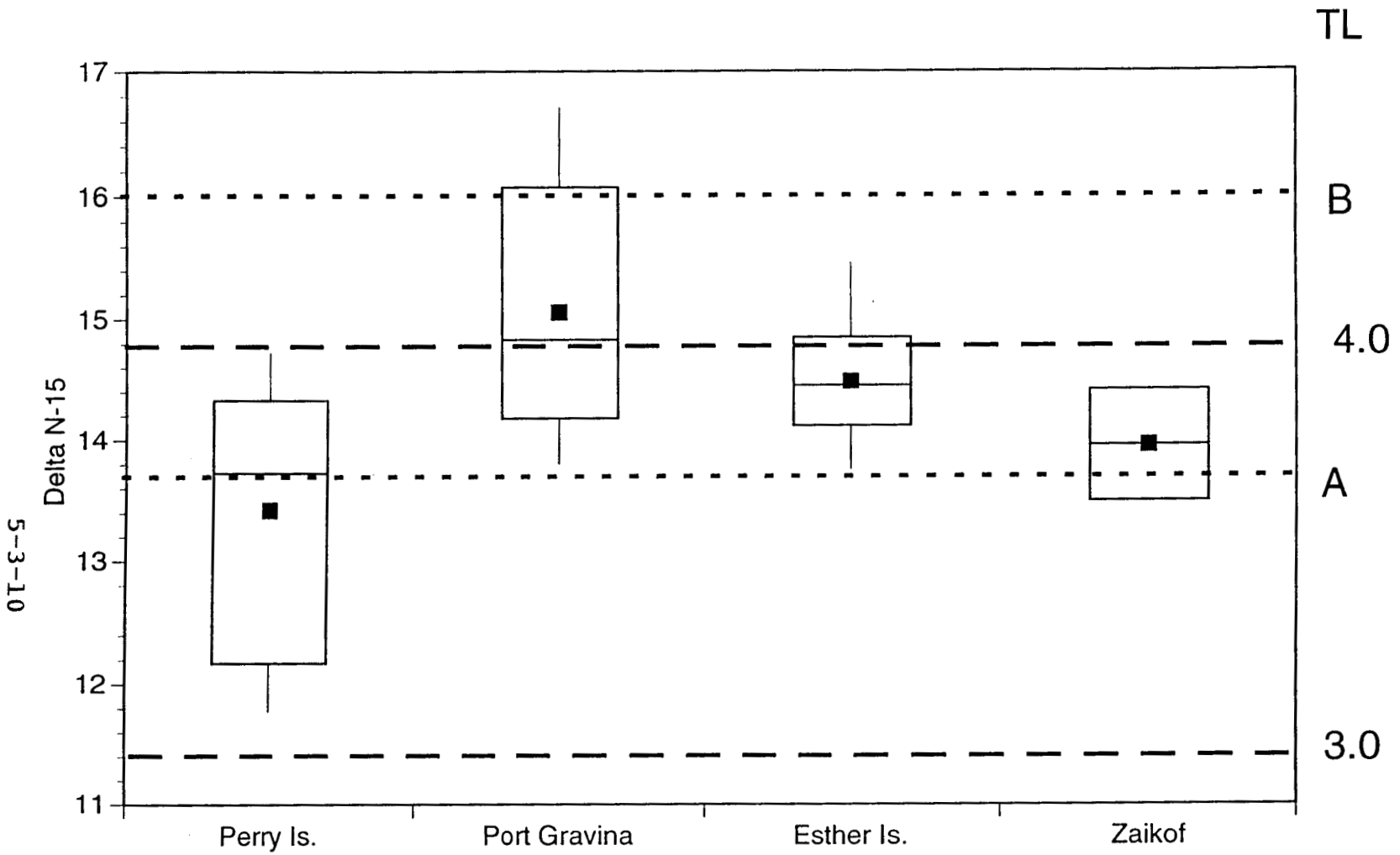
TL
B
4.0
A
3.0
Delta N-15
17
16
15
14
13
12
11
Perry Is.
Port Gravina
Esther Is.
Zaikof
5-3-10

Figure 5.