TROPHIC INTERACTIONS OF BATHYRAJA TRACHURA AND SYMPATRIC FISHES

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TROPHIC RELATIONSHIPS OF BATHYRAJA TRACHURA

AND SYMPATRIC FISHES

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ABSTRACT

Quantifying deep-sea food webs can be resource intensive due to the difficulties of sampling fishes from the deep sea. The diet of fishes is often quantified through stomach content analysis, through this method has many sampling constraints, and it can be difficult to obtain sufficient samples for an in-depth study. This study attempts to fill a critical data gap by determining the diet and trophic level of the deep-sea Roughtail Skate, *Bathyraja trachura*, using traditional stomach content analysis. This study also attempts to determine the validity and accuracy of stable isotope analysis in the continental slope fishes of the deep-sea of the eastern North Pacific, as an alternative method to determine trophic level in fishes.

The Roughtail Skate is an abundant deep-sea skate in the eastern North Pacific. Little is known about the diet of this skate, which is landed as by-catch in commercial bottom trawls. Skates were collected between 2005 and 2008 from fishery-independent trawl surveys of the continental slope and outer shelf. Geometric Index of Importance (GII) values indicated that crustaceans (71.4%), fishes (17.8%), polychaetes (4.3%), and cephalopods (3.7%) were the most important prey groups in the diet. Diet differed significantly with total length, but not with sex. Larger individuals (by total length) had significantly higher trophic level values, and year and latitude explained variation in the diet for three prey categories.

In this study, fishes and invertebrates collected from the continental slope (1,000 m depth) of the eastern North Pacific were analyzed using stable isotope analysis (SIA). The carbon and nitrogen stable isotope results were used to construct dual isotope plots to

investigate the trophic relationships of this deep-sea community. The plots indicated a decoupling of the benthic and pelagic food webs, with the benthic food web being isotopically enriched. Stomach and isotope samples were collected from 32 Roughtail Skates (*Bathyraja trachura*) to determine the validity and accuracy of SIA in determining the trophic levels of the skates. A linear regression analysis indicated that nitrogen values from SIA and trophic levels calculated from stomach content analysis, when plotted against skate total length, exhibited similar variation and patterns, although only the stomach content analysis yielded significant results (stomach content: $p=0.020$, $r^2=0.168$; stable isotope: $p=0.077$, $r^2=0.101$).

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CHAPTER I

Food habits of the Roughtail Skate, *Bathyraja trachura* (Gilbert, 1892), in the eastern North Pacific

ABSTRACT

I determined the diet and trophic level of the Roughtail Skate, *Bathyraja trachura*, an abundant deep-sea skate in the eastern North Pacific. Little is known about the diet of this skate, which is landed as by-catch in commercial bottom trawls. Skates were collected between 2005 and 2008 from fishery-independent trawl surveys of the continental slope and outer shelf. Geometric Index of Importance (GII) values indicated that crustaceans (71.4%), fishes (17.8%), polychaetes (4.3%), and cephalopods (3.7%) were the most important prey groups in the diet. Larger individuals (by total length) had a significantly higher trophic level, and year and latitude explained variation in the diet for three of the higher prey categories. There was no apparent difference in diet between males and females.

INTRODUCTION

The deep sea, typically defined as ocean depths greater than 200 meters, is a vast environment that has been poorly studied compared with the nearshore environment (Herring 2002). The upper continental slope habitat (200-1,500 m) is now receiving increased attention because large commercial fisheries have begun to exploit these deeper waters as nearshore coastal fish stocks have been serially depleted (Haedrich 2007). For these new deep-sea fisheries to be properly managed, information is required on age, growth, reproduction, habitat utilization, and diet of fishes within the assemblage.

Kyne and Simpfendorfer (2007) noted that studies of deep-water chondrichthyans were infrequent in the scientific literature, with a paucity of basic biological information available for many species. Skates (Rajiformes: Rajoidei) are cartilaginous fishes that constitute about 43% of the batoids (Ebert and Compagno 2007) within three families: Anacanthobatidae, Arhynchobatidae, and Rajidae (Nelson 2006). Skates occur in all oceans, although they are more abundant in temperate waters and in the deep sea. With over 126 new species described in the past 60 years, few data exist on species-specific life history traits (Compagno 1990; Ebert and Compagno 2007). This lack of knowledge is amplified by the fact that skates are taken as commercial fishery bycatch, with an estimated peak of 3 million pounds landed in California in 1997, with an average of 284,000 lbs of skate and rays landed each year from 2002 to 2009, due to a decreased Asian market (Haas 2010). Because of their similar morphology, lack of defining characteristics, and lack of stock assessments, 98% of skate landings are listed as "unidentified skate" (Zorzi et al. 2001). Due to new management regulations, starting in

2009 the Longnose Skate, *Raja rhina,* must be separated upon landing, but all other skates will likely remain in the "unidentified skate" category (Haas 2010).

Bathyraja trachura (Gilbert 1892), is a member of the soft-nose skate family Arhynchobatidae. It is characterized by a disc width greater than disc length, thorns along the length of the tail, prickles on the dark plum colored dorsal surface, and a smooth and mostly dark ventral surface (Ebert 2003). The range of *B. trachura* extends from San Diego, California to the Bering Sea and across the Northern Pacific Ocean to the Sea of Okhotsk (Green 1975; Cailliet et al. 1999; Dolganov 1999; Orlov and Tkranov 2005). This skate species is mentioned infrequently in the literature, and when mentioned is often listed as rare, probably because it occurs at depths of 200 to 2,550 meters (Ebert 2003). Ebert (2005) described the smallest specimen, a female measuring 19.6 cm total length, and egg cases from the Eastern Bering Sea. Davis et al. (2007) reported a new maximum total length of 91 cm and maximum ages of 20 years for males and 17 years for females. Although some recent life history information has been collected, trophic information is extremely limited for this species and consists of only four stomach samples (Yang 2003).

The Magnuson-Stevens Fishery Conservation and Management Reauthorization Act of 2006 required the National Marine Fisheries Service (NMFS) and the Fishery Management Councils to conduct a study on the "state of the science for advancing the concepts and integration of ecosystem considerations in regional fishery management." In early 2009, NMFS released the report which stated that to successfully conduct ecosystem-based management more survey data is needed for modelers, stakeholder

participation on the councils should be expanded, and inter-agency communication needs improvement to allow for similar management across jurisdictional areas (NMFS 2009). Dietary information for all marine organisms is essential for such ecosystem-based management (EBM), as this type of management shifts the ultimate goal of maximizing the catch of one stock, to the holistic management of entire ecosystems that contain fisheries, taking into account the predators, prey, and habitat, along with the targeted fishery (Pikitch et al. 2004; Curtin and Prellezo 2010; Fletcher et al. 2010). The Pacific Fishery Management Council, which governs the fishery and fish assemblage in this study, aims to create a Fishery Ecosystem Management Plan (FEMP), which may take into account dietary information such as species-specific diets, trophic interactions and food web complexity such as those investigated in this study. The council voted to go forward with a FEMP in 2006, but the request for funding to support the plan was not granted in 2008 (PFMC 2010).

This study attempts to fill a critical data gap by determining the diet of *Bathyraja trachura*. The specific objectives are to: 1) characterize the diet and trophic level of *B. trachura* based on stomach content analysis; 2) determine environmental and biological sources of variation in the diet; 3) determine if trophic level changes with skate total length; and 4) determine the feeding niche of *B. trachura* based upon the diet. Based on the diet of other sympatric skates species, it was hypothesized that this skate would be a generalist predator on benthic organisms including crustaceans, fishes, cephalopods, and polychaetes (Bizzarro et al. 2007). It was expected that trophic level increased with skate size, as larger skates could consume larger and higher trophic level prey (Alonso et al.

2001; Rinewalt et al. 2007; Robinson et al. 2007; Treloar et al. 2007). A difference between the sexes was not expected as most researchers have found no difference between the diet of males and females (Martin et al. 2007; Robinson et al. 2007). Environmental variables were expected to explain little dietary variation due to the largely homogenous nature of continental slope, and large scale of this study (Tolimieri and Levin 2006).

METHODS

Bathyraja trachura specimens were collected between May to October during 2005 to 2008 NMFS Fishery Resource Analysis and Monitoring (FRAM) Division groundfish surveys. Fishing vessels were contracted by the NMFS FRAM Division to target commercially important groundfishes with bottom trawls in depths of 55 to 1,280 meters. Surveys were conducted from Cape Flattery, Washington to the U.S.-Mexico border with a stratified random sampling scheme considering latitude and depth (Keller et al. 2008).

When recovered in a haul, whole skates were frozen on board survey vessels and returned to the lab for processing. Skates were partially thawed and weighed to the nearest 0.1 kg and total length was measured (+/- 0.1 mm). Sexual maturity of the specimen was determined by examination of claspers (for males) and an internal examination of the reproductive tract for males and females and then categorized on a scale of 1-5 following Ebert (2005). Buccal cavities were observed for evidence of regurgitation and net feeding, and samples removed from analysis if prey were found in

the buccal cavity. The stomach, from the esophagus to the pyloric sphincter, was then excised and re-frozen for later analysis. Tissue samples for stable isotope analysis also were collected from a subsample of skates (see Chapter 2). Stomachs were later thawed at room temperature and dissected over a 500 μm sieve. Prey items were identified to lowest possible taxonomic level, enumerated, weighed wet after excess moisture was blotted away, and the standard length for fishes and carapace width of crustaceans was measured following Bowen (1996) and Lance et al. (2001). Counts of prey were determined by the minimum number of individual prey items represented by the body parts present (Bowen 1996; Lance et al. 2001). The state of digestion for each individual prey item also was recorded on a scale of 1 to 5 (where 1 indicated undigested prey).

The standard parameters for stomach content analysis, percent number (%N) and percent mass (%M), were calculated following Hyslop (1980) for each stomach and were then averaged for a population estimate. The metric %N was calculated as:

$$\%N_i = 100 \bullet \frac{N_i}{\sum_i^j N}$$

where the number of prey in a stomach (N_i) was divided by the sum of all prey, *i* to *j*, and then multiplied by 100 to yield a percentage. The same formula was applied to the mass of the prey to calculate %M. These values were averaged across all stomachs to calculate a population level metric for each prey category (Cailliet et al. 1986). The indices %N and %M were then, themselves, averaged to calculate the Geometric Index of Importance (GII) value:

$$\%GII_i = \left(\frac{\%N_i + \%M_i}{2} \right)$$

on a 0-100% scale (Assis 1996). The GII index was chosen because it treats each dietary metric equally and some prey items were better represented by %N (e.g. eyes of euphausiids) whereas others were better represented by %M (e.g. tanner crabs, fishes). Frequency of occurrence (FO) also was calculated by dividing the number of stomachs containing prey *i* by all stomachs containing prey, multiplied by 100 to yield a percentage. The Index of Relative Importance (IRI) value:

$$IRI_i = \left(\%N_i + \%M_i \right) * FO_i$$

was presented in the dietary table to facilitate comparison with other species, as the majority of dietary studies use this metric for quantifying diet of fishes (Pinkas et al. 1971; Cortés 1997).

A species accumulation curve (cumulative prey curve) was plotted using EstimateS (Colwell 2009) to determine if enough samples had been examined to characterize the diet adequately (Ferry and Cailliet 1996). The curve was generated by calculating the analytical mean and standard deviation for the number of prey categories per stomach, or species richness (Mao et al. 2005). When this curve reached an asymptote and no longer increased, the corresponding sample on the x-axis reflected the number of stomachs needed to have encountered all major prey categories.

For statistical analyses, prey were grouped into the following general categories: Euphausiidae, other crustaceans, Benthesicymidae, fishes, polychaetes, and cephalopods. These groups were chosen as their %GII values were greater than 4% GII of the diet and

skewness was less than 2.0 for statistical testing. All other prey groups were combined into their respective higher taxonomic grouping to ensure all prey items were included in the statistical analyses.

Major prey categories were used for testing the stability of %GII estimates to ensure enough samples were collected to not only adequately capture the variation in number of prey categories (species richness) but also the relative importance (%GII). Mean and standard deviation of the metric %GII was plotted as a function of sample size (randomly sampled from 1 to *n*) to visualize the variation around the mean for each higher prey category to be used in statistical testing.

The relationship between diet, depth, year, haul, and latitude was determined using a canonical correlation analysis. This method tested for linear correlations between two sets of continuous variables. Similar to a Principal Components Analysis (PCA) the multivariate data was transformed into a fewer number of variables, known as canonical variates, with the number of roots (correlations between canonical variates) being equal to the number of variables in the smallest variable set. The variation between the sets was then represented in a biplot, where coefficients and significance values were assigned to each variable.

Multiple regression was used to investigate the relationship between the diet composition and sex and total length of the skates. The major prey categories (%GII) were entered into a forward-stepping regression model ($p>0.05$) to explain total length. A final linear equation was calculated to include all dietary variables that explained a significant amount of the variation in total length.

The trophic level (TL) of each sample was determined from the diet data collected in this study following Cortés (1999) and Ebert and Bizzarro (2007):

$$TL_k = 1 + \left(\sum_{j=1}^{n} P_j * TL_j \right)$$

where TL_k is the trophic level of species k, P_j = proportion of prey category *j* in the diet, *n* = total number of prey categories, and TL_j = trophic level of prey category *j*. The same prey categories and associated trophic levels from Ebert and Bizzarro (2007) were used in this study to facilitate comparisons among skate species. Lastly, trophic level for each sex was regressed against skate total length to determine if trophic level increased with skate size.

RESULTS

A total of 522 stomachs was collected for dietary analysis. These stomachs covered the entire size range of *B. trachura* from size at hatching to the known maximum size and were evenly dispersed between sexes (180-894 mm females; 172-910 mm males; Figure 1). Samples were collected along the entire west coast of the United States from 2005 to 2008 (Figure 2). When present, the average number of *B. trachura* per bottom trawl tow was 2.7 (+/-2.0 SD) with a maximum of 10 individuals within a single tow. Nearly all skates were recovered from the continental slope, with 70% of those caught in waters deeper than 900 meters. Samples were not evenly dispersed throughout the four years, with a greater number of samples collected in 2007 (n=180, all other years ~50). Skates were collected along the entire west coast, although fewer were caught between Cape

Mendocino and Monterey Bay (Figure 2).

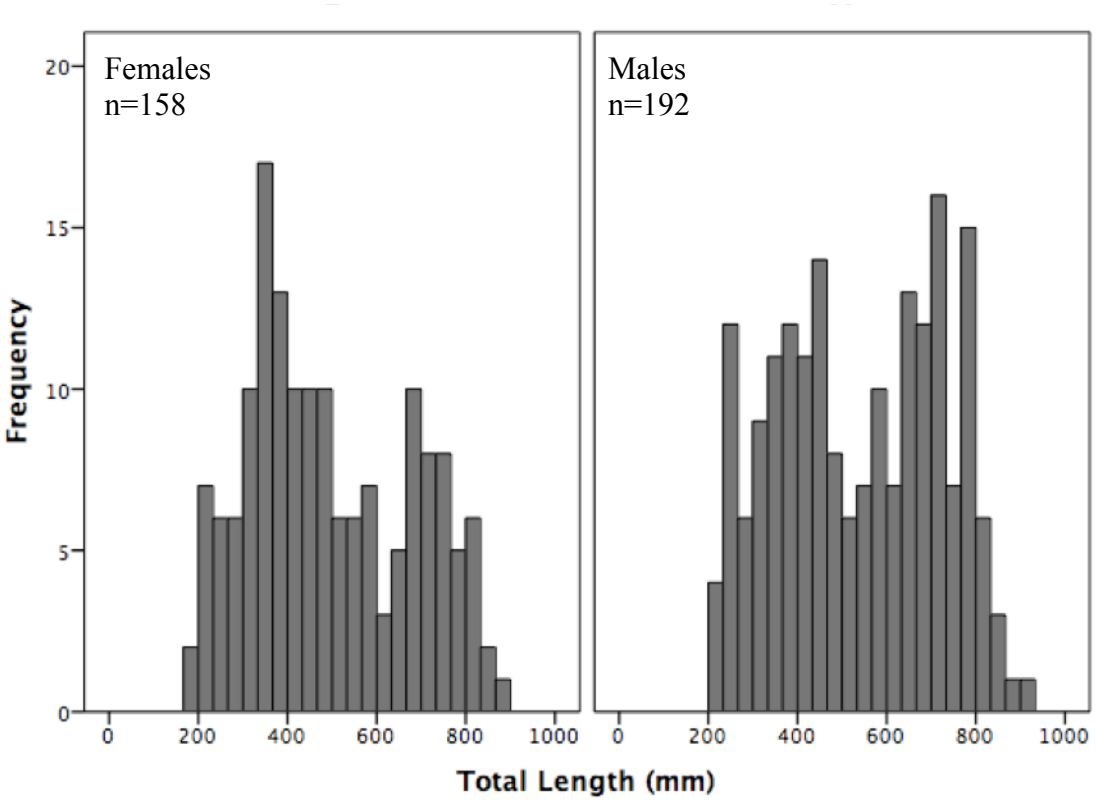


Figure 1: Length frequency histogram for *Bathyraja trachura* collected from 2005 to 2008. These data represent the skates analyzed in the multivariate analyses (n=350). Samples are separated by sex.

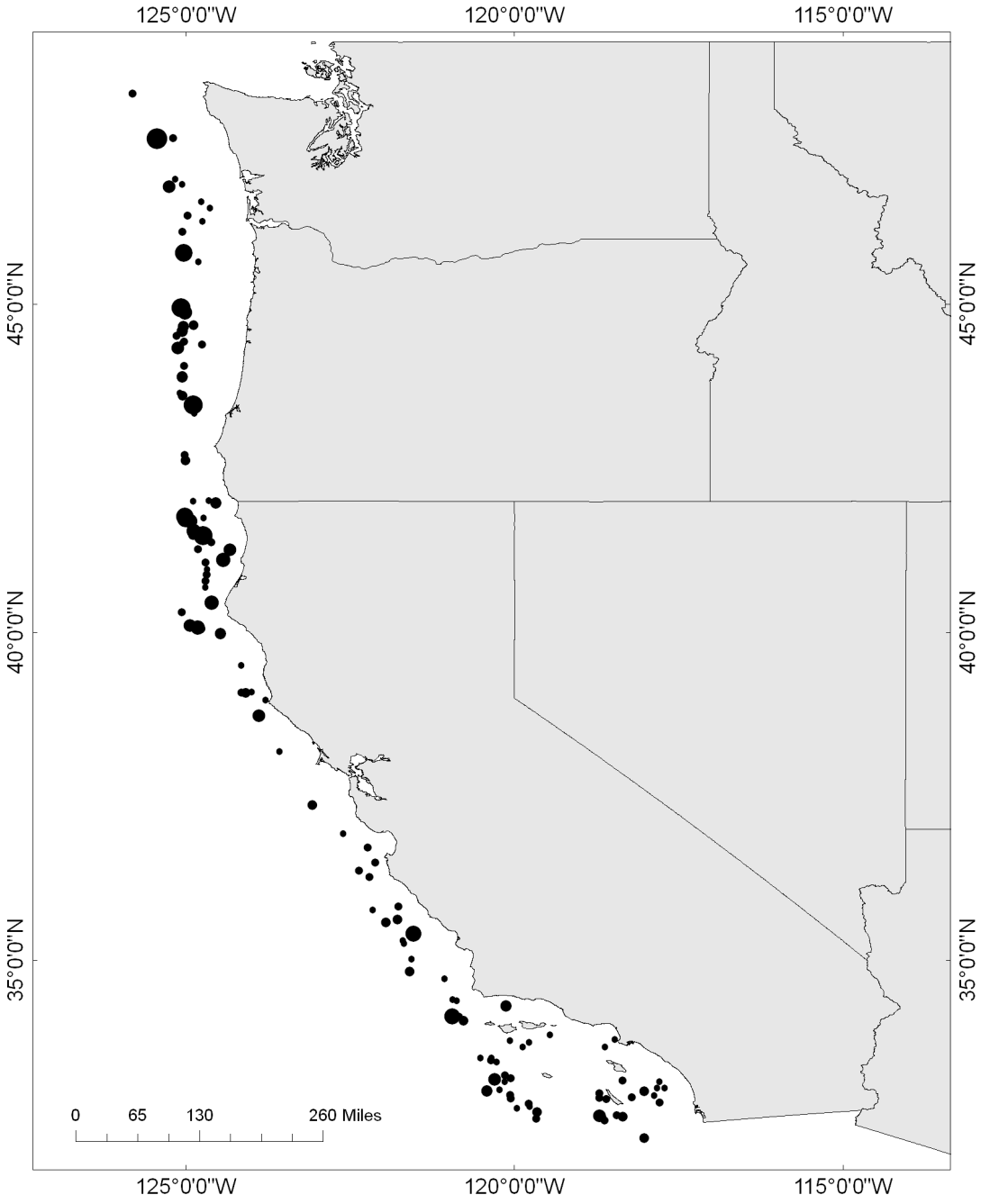


Figure 2: Capture locations for the 350 skates used in statistical analyses. Size of dots corresponds to the number of skates collected, ranging from 1-10, in hauls conducted from 2005 to 2008.

Of the total number collected (n = 522), 61 stomachs (11.7%) contained no prey items. More than 4,000 individual prey items within eighty-six taxonomic groups comprised the diet of *B. trachura* (Table 1). Of the 461 stomachs processed, only 350 had all the associated environmental (e.g. depth, latitude, year, haul) and biological (e.g. total length, sex) data required to run statistical analyses. Therefore the detailed description of the diet (Table 1) and the corresponding graph of the diet (Figure 3) are the only analyses conducted on the entire sample of 461 stomachs. The sample size for all subsequent analyses is 350 stomachs.

Table 1: Prey taxa and importance values for *B. trachura* diet. Mean percent number (%N), mean percent mass (%M), frequency of occurrence (FO), mean Geometric Index of Importance (%GII), and mean Index of Relative Importance (IRI) values calculated for 461 *B. trachura* stomach samples, listed in order of importance.

Class	Lowest Identification	%N	%M	%FO	%GII	IRI
Malacostraca		**82.46**	**60.23**		**71.35**	
	Euphausiidae	34.03	9.20	5.02	21.61	217.01
	Malacostraca (Unid.)	29.18	3.28	21.69	16.23	704.06
	Chionoectes tanneri	1.74	19.49	3.12	10.62	66.24
	Chionoectes spp.	0.51	10.64	1.60	5.57	17.84
	Benthesicymidae	4.48	4.58	5.86	4.53	53.09
	Decapoda	1.53	1.73	4.26	1.63	13.89
	Euphausia pacifica	0.90	1.11	1.22	1.01	2.45
	Eusergestes similis	0.79	0.94	1.83	0.87	3.17
	Pasiphaea tarda	0.12	1.54	0.38	0.83	0.63
	Bathymedon nepos	1.41	0.14	0.61	0.78	0.95
	Metacrangon variabilis	0.79	0.61	0.91	0.70	1.27
	Eualus spp.	0.60	0.48	0.38	0.54	0.41
	Bathymedon covilhani	0.93	0.05	0.53	0.49	0.52
	Crangonidae	0.60	0.32	1.14	0.46	1.05
	Lophogastridae	0.23	0.61	0.30	0.42	0.25
	Lophaxius rathbunae	0.09	0.75	0.15	0.42	0.13
	Eualus biunguis	0.39	0.40	0.76	0.40	0.60
	Rocinela angustata	0.26	0.46	0.46	0.36	0.33
	Neognathophausia ingens	0.16	0.52	0.53	0.34	0.36
	Eualus macropthalmus	0.39	0.25	0.84	0.32	0.54
	?Pseudomma truncatum	0.58	0.04	0.61	0.31	0.38
	Pasiphaea chacei	0.14	0.40	0.46	0.27	0.25
	Neognathophausia spp.	0.12	0.42	0.38	0.27	0.21
	Chorilia longipes	0.12	0.30	0.30	0.21	0.13
	Parapagurus benedicti	0.07	0.33	0.23	0.20	0.09
	Pleocyemata	0.21	0.09	0.38	0.15	0.11
	Pandalopsis spp.	0.05	0.24	0.15	0.15	0.04
	Thysanoessa spp.	0.21	0.06	0.30	0.14	0.08
	Idotea rufescens	0.16	0.05	0.15	0.11	0.03
	Pasiphaea spp.	0.05	0.16	0.15	0.11	0.03
	Pagurus sp.	0.02	0.18	0.08	0.10	0.02
	Isopoda	0.14	0.06	0.46	0.10	0.09
	Ampelisca pugetica	0.19	0.01	0.08	0.10	0.02
	Munida quadrispina	0.12	0.06	0.23	0.09	0.04
	Rhacotropis cervus	0.14	0.01	0.30	0.07	0.05
	Pandalus sp.	0.02	0.12	0.08	0.07	0.01
	Benthogennema spp.	0.02	0.11	0.08	0.07	0.01
	Neognathophausia gigas	0.05	0.08	0.15	0.07	0.02
	Heterophoxus sp.	0.12	0.01	0.08	0.06	0.01
	Ampelisca unsocalae	0.12	0.00	0.08	0.06	0.01
	Munidopsis sp.	0.02	0.09	0.08	0.05	0.01
	Grennadas incertus	0.07	0.04	0.08	0.05	0.01
	Melita lignophila cf	0.09	0.01	0.15	0.05	0.02

	Grennadas sp.	0.05	0.05	0.08	0.05	0.01
	Calocarididae	0.02	0.06	0.08	0.04	0.01
	Pasiphaeaidae	0.02	0.06	0.08	0.04	0.01
	Primno macropa	0.07	0.01	0.15	0.04	0.01
	Byblis veleronis	0.07	0.01	0.08	0.04	0.01
	Lysianassidae	0.07	0.00	0.23	0.04	0.02
	Thysanoessa spinifera	0.05	0.02	0.15	0.03	0.01
	Thoridae	0.02	0.02	0.08	0.02	0.00
	Benthogennema burkenroadi	0.02	0.02	0.08	0.02	0.00
	Cirolana californiensis	0.02	0.00	0.08	0.01	0.00
	Eusiroides longipes	0.02	0.00	0.08	0.01	0.00
	Oedicerotidae	0.02	0.00	0.08	0.01	0.00
	Syrrhoe longifrons	0.02	0.00	0.08	0.01	0.00
	Oediceropsis elsula	0.02	0.00	0.08	0.01	0.00
Actinopterygii		**5.29**	**30.29**		**17.79**	
	Sebastolobus altivelis	2.02	18.55	4.49	10.28	92.36
	Actinopterygii (Unid.)	2.92	4.65	8.52	3.79	64.50
	Merluccius productus	0.16	2.91	0.53	1.54	1.63
	Serrivomer sector	0.02	2.61	0.08	1.32	0.21
	Cololabis saira	0.02	1.21	0.08	0.62	0.10
	Myctophidae	0.07	0.21	0.23	0.14	0.06
	Sebastes sp.	0.02	0.13	0.08	0.07	0.01
	Sternoptychidae	0.02	0.01	0.08	0.02	0.00
	Clupediae	0.02	0.01	0.08	0.02	0.00
Polychaeta		**5.66**	**2.93**		**4.29**	
	Onuphidae	3.71	1.96	4.87	2.83	27.61
	Polychaeta (Unid.)	1.60	0.81	3.95	1.21	9.52
	Polynoidae	0.30	0.07	0.46	0.19	0.17
	Errano bicirrata	0.05	0.09	0.08	0.07	0.01
Cephalopoda		**3.34**	**4.08**		**3.71**	
	Cephalopoda (Unid.)	1.83	2.65	5.40	2.24	24.19
	Abraliopsis felis	0.42	0.39	0.91	0.40	0.74
	Oegopsida	0.32	0.42	0.91	0.37	0.67
	Gonatidae	0.21	0.10	0.53	0.16	0.16
	Loligo opalescens	0.02	0.24	0.08	0.13	0.02
	Gonatus spp.	0.12	0.06	0.15	0.09	0.03
	Gonatus berryi	0.14	0.01	0.38	0.07	0.06
	Gonatus borealis	0.07	0.05	0.23	0.06	0.03
	Octopus sp.	0.07	0.03	0.23	0.05	0.02
	Grandeledone boreopacifica	0.02	0.06	0.08	0.04	0.01
	Helicocranchia sp.	0.02	0.05	0.08	0.04	0.01
	Gonatus pyros	0.07	0.00	0.15	0.04	0.01
	Teuthoidea	0.02	0.01	0.08	0.02	0.00
Unidentified Organic Matter (UOM)		**1.55**	**1.40**		**1.48**	
	UOM (black tissue)	1.04	0.63	2.66	0.84	4.44
	UOM	0.51	0.77	2.13	0.64	2.73
Maxillopoda	Calanoida	**0.05**	**0.00**	**0.15**	**0.02**	**0.01**

Crustaceans were the most important prey group (GII = 72.6%) with euphausiids (21.6%), unidentified crustaceans (16.2%), and *Chionoecetes tanneri* (10.6%) contributing most among identified prey taxa. Less important crustaceans included shrimps, mysids, other crabs, hermit crabs, isopods, and amphipods. Teleost fishes were the second most important group (17.2%) with *Sebastolobus altivelis* (10.3%) and unidentified fishes (3.79%) being most important; other less important groups included myctophids, juvenile *Sebastes* sp., Sternoptychidae, Clupeidae, *Merluccius productus*, and *Serrivomer sector*. The third most important group were polychaetes (4.3%) with the majority being onuphids (2.8%), unidentified polychaetes (1.2%), and polynoid worms (0.2%). Cephalopods were the fourth most important group (3.7%) and included unidentified cephalopods (2.2%), *Abraliopsis felis* (0.4%), and unidentified squids (0.4%). The less important cephalopod taxa included many species of gonatid squid and *Octopus* spp. Calanoid copepods were found in two stomachs (0.02%). Unidentified organic matter (UOM) was less important (1.48%) but noted because of its unique nature. Most UOM was red organic matter that was likely well-digested crustaceans (0.6%). However, a gray to black colored tissue was found in a surprising number of stomachs (n=49) and was of minor dietary importance (0.88%). The results of further investigation into this prey category are presented in the discussion.

Prey were not equally represented in number and mass in the diet; euphausiids were represented by %N more than %M, and fishes were represented by %M more than %N. Other crustaceans, benthesicymid shrimps, polychaetes, and cephalopods were represented nearly equally by mass and number (Figure 3). When prey were grouped by

habitat type (infaunal, benthic, benthopelagic, and pelagic), the diet of *B. trachura* consisted mainly of organisms from benthic (44%) and benthopelagic (39%) habitats, with smaller contributions from pelagic (9%) and infaunal (8%) organisms. Of the groups of prey, crustaceans were mostly benthic and benthopelagic, fishes were benthic, polychaetes were infaunal, and cephalopods were benthopelagic.

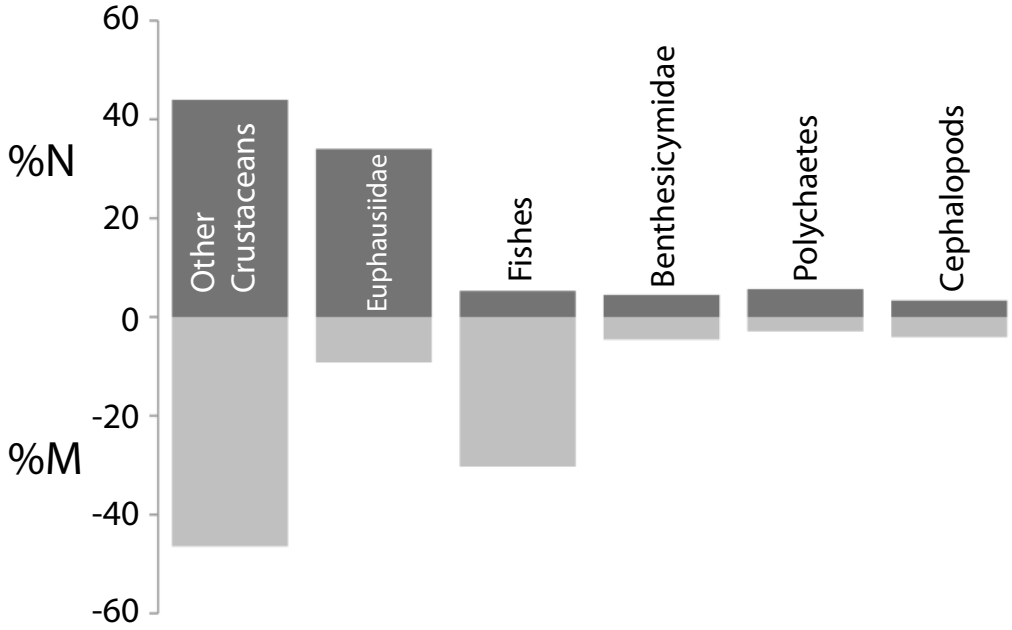


Figure 3: Graphical representation of the major prey categories in the diet of *B. trachura.* The metric of %GII is an average of %N and %M, therefore the area made by these boxes represents the relative dietary importance of each prey group for the 350 skate stomachs sampled.

Species accumulation curves indicated that the species richness of the six higher prey groupings was accurately characterized by the 27th sample, well within the total number of samples collected (n= 350 total; Figure 4). The curve clearly reaches an asymptote; therefore testing the endpoints of the curve to ensure the slope was zero was not needed.

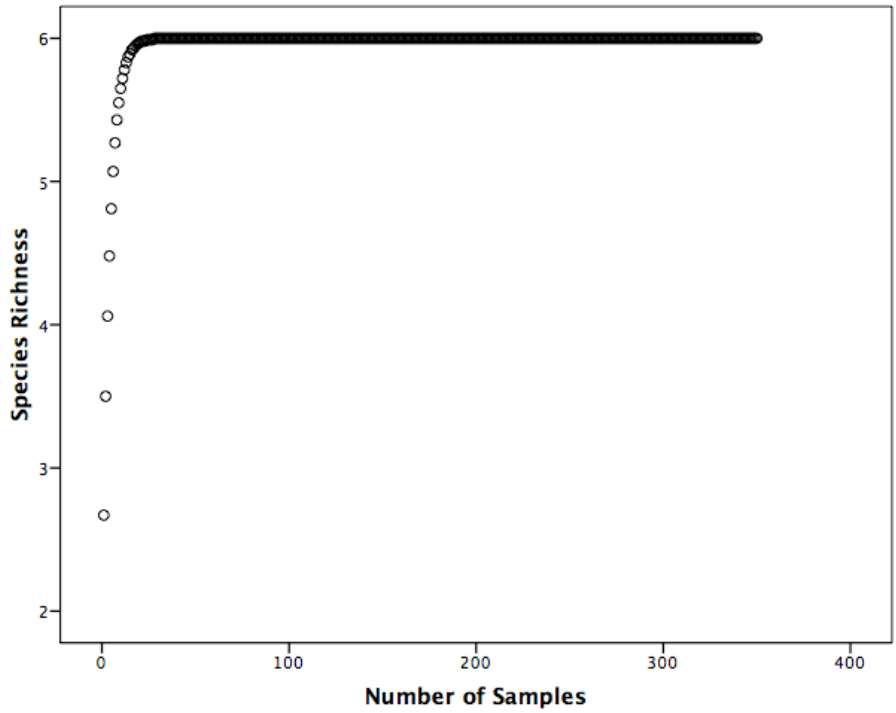
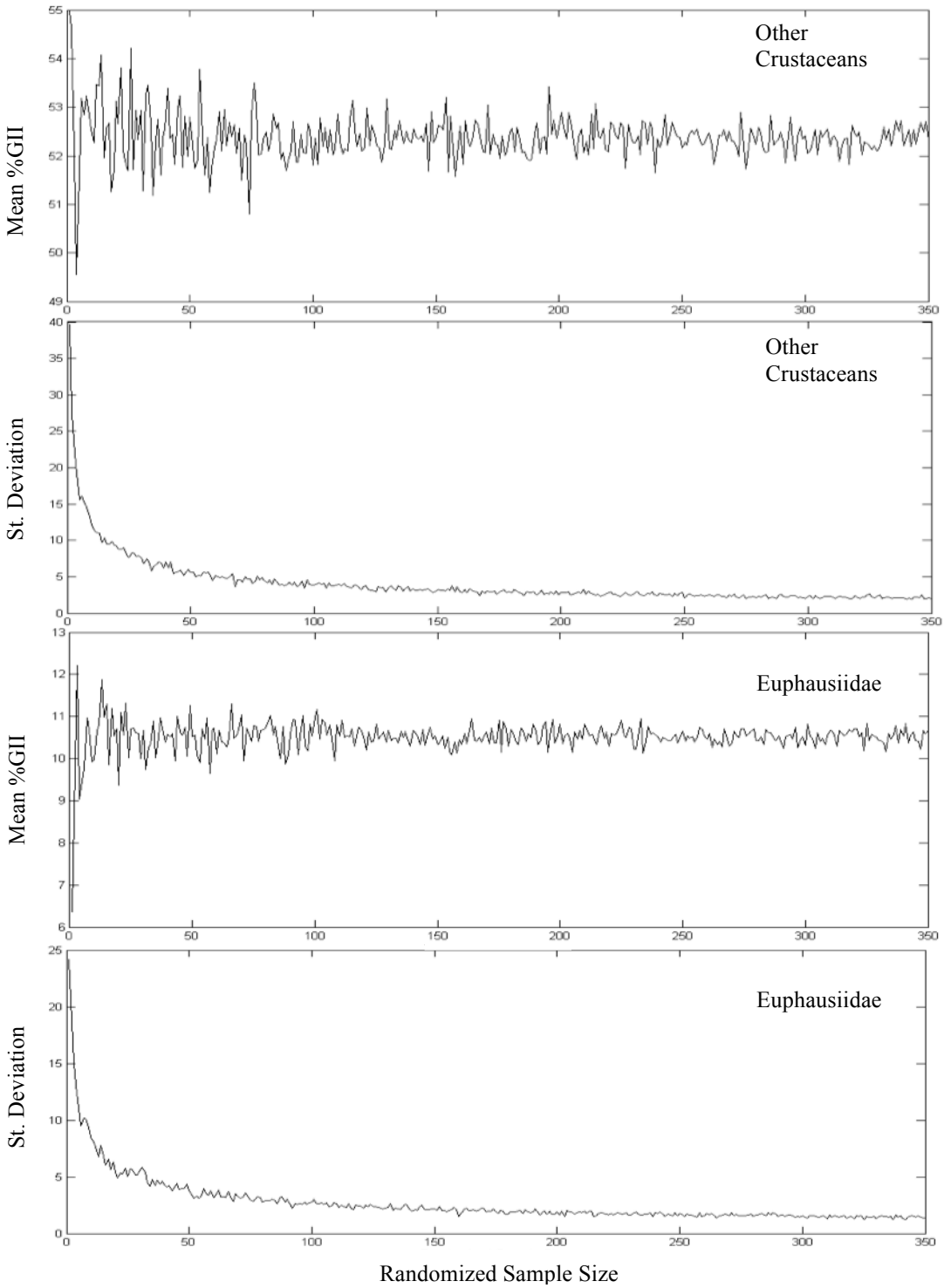
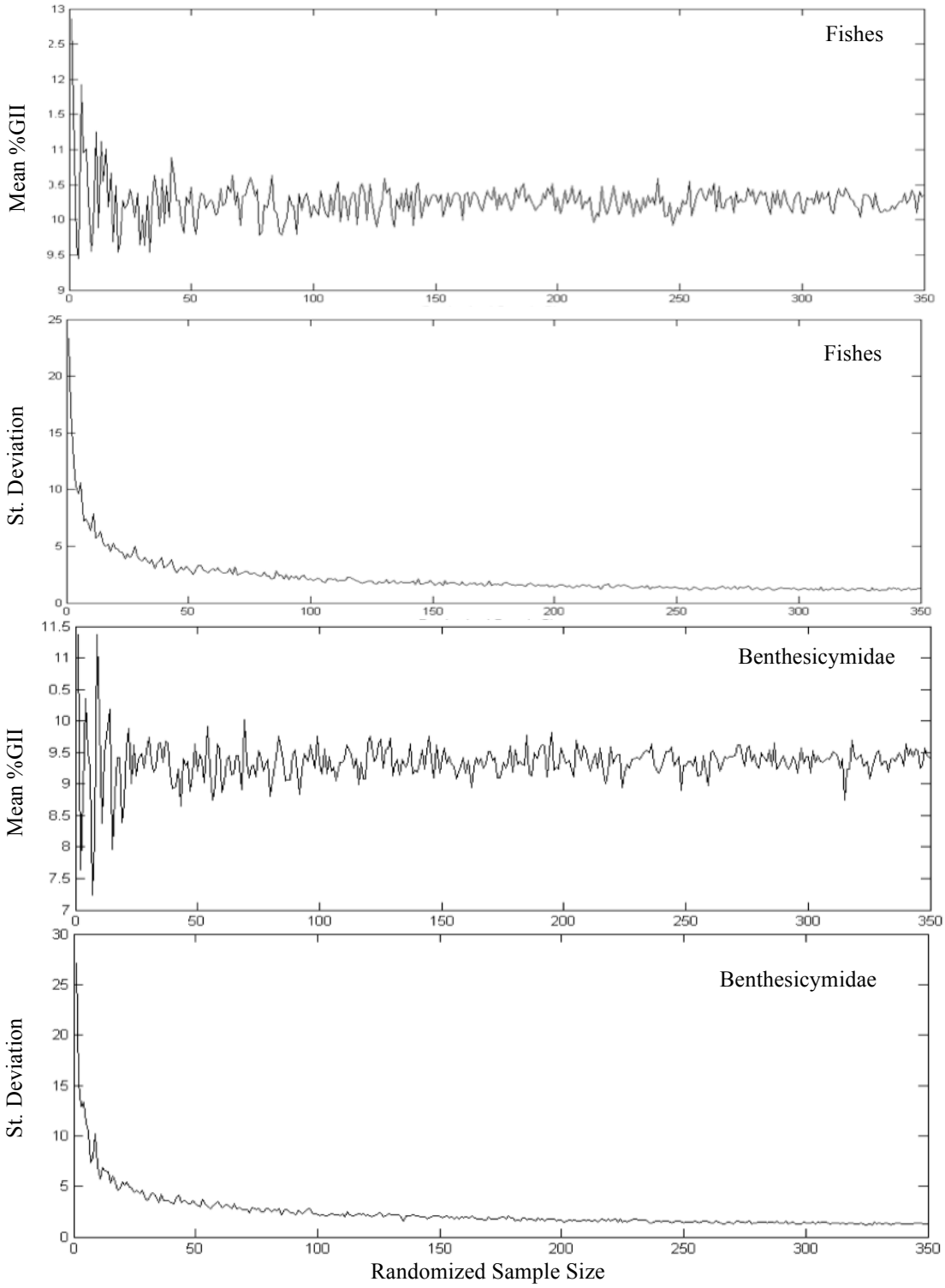


Figure 4: Species accumulation curve of the 6 higher prey categories used in statistical analyses (n=350). Standard deviation is not shown on the graph as there is little deviation in these points, and none once the curve reaches the asymptote.

For all prey categories used in analysis (n=6) a funnel-shaped pattern appeared as variation in the mean of the %GII for each group stabilized with increasing sample size. In addition, a graph of the standard deviation of the mean exhibited a decrease as sample size increased. Although this is simply a visualization technique, it does appear that enough samples were collected to accurately characterize the mean %GII for all prey

groups (Figure 5). While all species richness for the 6 prey categories was characterized accurately within 27 samples, it appears that a sample size of 150 better characterizes the variation in the %GII metric for the 6 higher prey categories. The standard deviation is lower at sample 150 than at smaller sample sizes; however after this point, standard deviation changes little with additional samples.


Other Crustaceans
Mean %GII
Other Crustaceans
St. Deviation
Euphausiidae
Mean %GII
Euphausiidae
St. Deviation
Randomized Sample Size


Fishes
Mean %GII
Fishes
St. Deviation
Benthesicymidae
Mean %GII
Benthesicymidae
St. Deviation
Randomized Sample Size

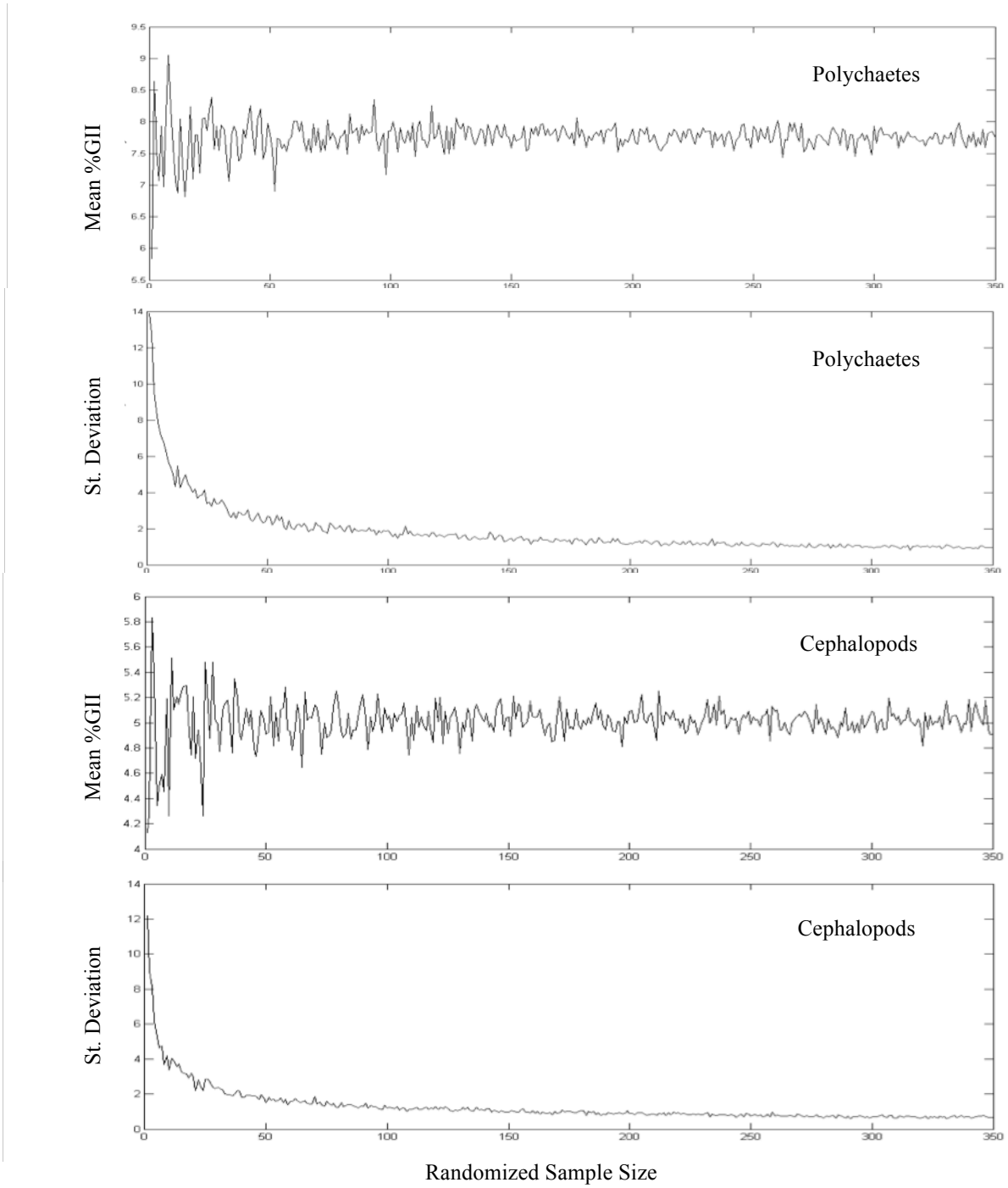


Figure 5: Mean and SD of %GII randomly sampled with replacement for the all stomachs used in multivariate analyses (n = 350). Groups are listed in order of importance.

The average trophic level was 3.53 (standard deviation=0.22) with variation over an entire trophic level. There was a significant relationship between trophic level and total length (F= 56.497, p=0.000); however, there was no difference between the sexes sexes (sex*total length interaction, P=0.256), so they were pooled for this analysis. A slight positive relationship was found and the total length explained 14% of the variation in the trophic level (r^2=0.140), indicating that larger skates had higher trophic levels (Figure 6).

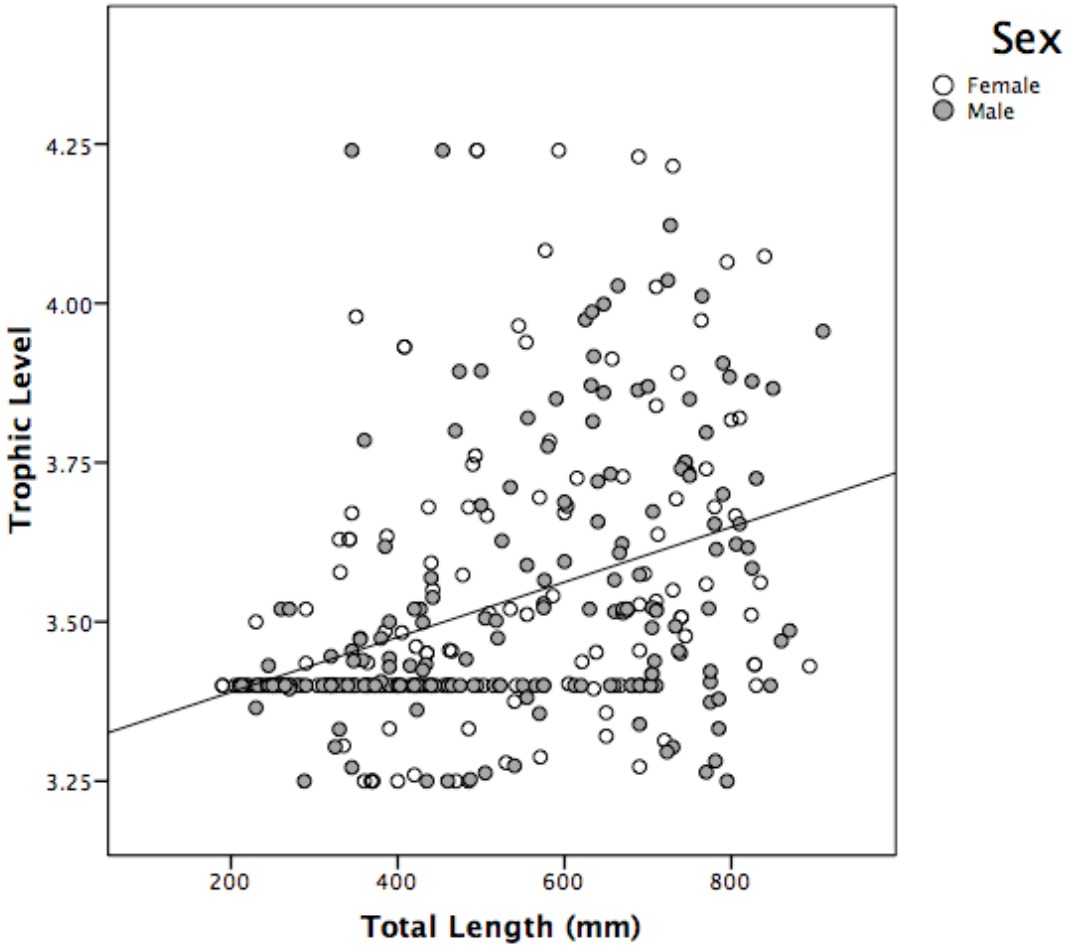


Figure 6: Linear regression of trophic level, determined from individual stomach contents, against skate total length (males= ●, females= ○, n=350).

The dietary variables (%GII for 6 higher prey groups) used in the canonical correlation analysis were not normally distributed, but the absolute value of the skewness, when log transformed, was less than 2.0 for all groups. The absolute value of the skewness for all environmental variables was <1.6. Because canonical correlations are fairly robust to skewed data, the analysis was still conducted. The environmental variable "haul" was highly correlated with year (0.4836) and latitude (0.9205), as haul numbers were unique to location and time. The high correlation was cause to remove the variable "haul" from the analysis. The remaining variables in the two sets (environmental and diet variables) were not highly correlated to other variables within or between the sets (all values < r=0.38; Table 2). A canonical correlation found two weak correlations (r=0.352 and 0.271) between the six higher prey categories and environmental variables (p=0.000 and 0.000; Table 2). Because sex was a discrete variable it could not be included in the analysis, but points were labeled by sex to allow for any patterns based on sex to be visualized.

Table 2: Summary of canonical correlation analysis. Coefficients and significance values for the three canonical variates (CV1, CV2, and CV3) analyzed and the canonical loadings for the two significant roots.

Canonical Correlation Summary

Canonical Variates/Roots	Canonical Correlation	*P*-value
CV1	0.352	0.000
CV2	0.271	0.000
CV3	0.143	0.130

CV1 Canonical Loadings

Environmental Variables	Year	-1.013
	Depth	0.274
	Latitude	0.264
Diet Variables	Fishes	0.438
	Polychaetes	-0.300
	Other Crustaceans	-0.110
	Cephalopods	0.169
	Benthesicymidae	-0.341
	Euphausiidae	-0.758
CV2 Canonical Loadings		
Environmental Variables	Year	-0.143
	Depth	-0.961
	Latitude	-0.096
Diet Variables	Fishes	-0.262
	Polychaetes	0.140
	Other Crustaceans	0.094
	Cephalopods	-0.120
	Benthesicymidae	0.744
	Euphausiidae	-0.605

The first and second root of the canonical correlation were significant. As year increased benthesicymid shrimp increased and fishes decreased in the diet (Figure 7). Sex is a discrete variable and could not be included in the analysis, but when overlaid onto the biplot of root 1, no pattern was evident. The second root was also significant (p=0.000);

at greater latitudes euphausiids decreased and benthesicymid shrimp increased in the diet (Figure 8). When sex overlaid onto the biplot of root 2, no pattern was evident.

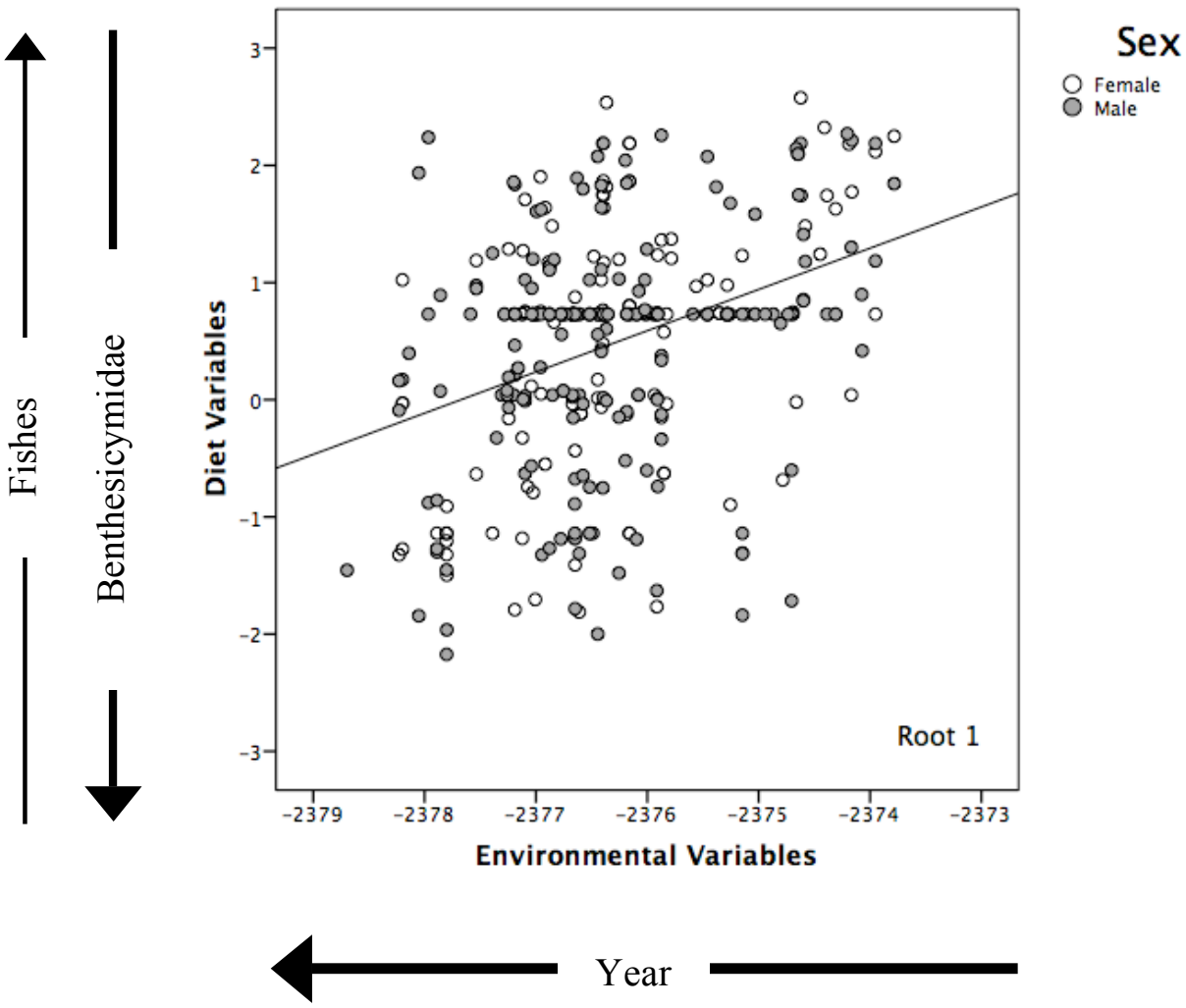


Figure 7: Canonical correlation biplot of diet variables versus environmental variables for Root 1. Each point represents one of the 350 skates sampled. The weight of the lines indicates the strength of the correlation for each of the variables with a correlation coefficient >0.4, males= ●, females= ○.

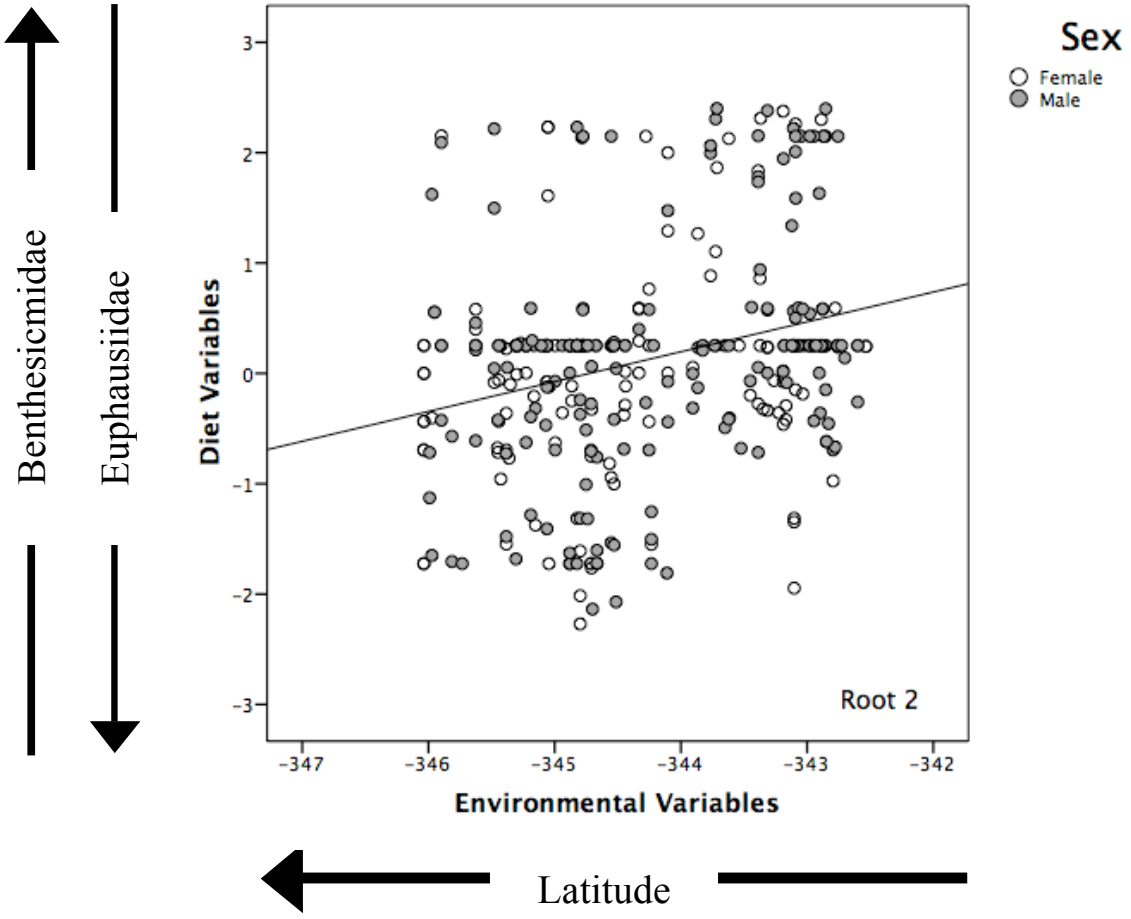


Figure 8: Canonical correlation biplot of diet variables versus environmental variables for Root 2. Each point represents one of the 350 skates sampled. The weight of the lines indicates the strength of the correlation for each of the variables with a correlation coefficient >0.4, males= ●, females= ○.

A multiple regression of the diet variables against skate total length found a significant positive relationship (Table 3). The final model included all variables except "other crustaceans", with fishes, cephalopods, and Benthesicymidae being the most

significant with the highest regression coefficients. Euphausiidae and polychaetes were also included in the model, but were less significant and had lesser coefficients. Although the relationship was highly significant, the linear regression explained only a modest part of overall dietary variation (r^2=0.316).

Table 3: Summary of Final Multiple Regression Model between the independent log-transformed (dietary) variables (n=6; by %GII) and dependent (skate total length) variable.

Final Model	**Sum of Squares**	**df**	**Mean Square**	***F***	***P***
Regression	3818073.608	5	763614.722	33.093	0.000
Residual	7914709.923	343	23074.956		
Total	1.173E7	348			

Final Model	**Unstandardized Coefficients (beta)**	**Unstandardized Coefficients (std. error)**	***t***	***P***
Constant	631.059	12.961	48.689	.000
Fish	54.180	6.790	7.979	.000
Cephalopod	46.511	7.902	5.886	.000
Benthesicymidae	31.734	7.142	4.443	.000
Euphausiidae	19.516	7.046	2.770	.006
Polychaete	17.286	7.097	2.436	.015
Final model: Total Length= $631.059 + 54.180X_{fish} + 46.511X_{ceph} + 31.734X_{benthe} + 19.516X_{euph} + 17.286X_{poly}$				

DISCUSSION

Skates feed more frequently than other chondricthyans (Wetherbee and Cortés 2004) and the percentage of empty stomachs in this study (11.7%) was slightly greater than that found for other skate species in California (Bizzarro et al. 2007; Rinewalt et al. 2007; Robinson et al. 2007). This figure does not include the stomachs removed from the analysis because of evidence of regurgitation. Although skates do not possess a swim bladder that causes stomach eversion and regurgitation problems as in other fishes (Drazen et al. 2001), skates often were found with prey in their mouths, which may have been evidence of net feeding or regurgitation. Often (~10%) euphausiids were found in the mouths of the skates, which probably was not evidence of net feeding as the large mesh size of the nets allowed euphausiids to escape the net easily. It is presumed that barotrauma or visceral trauma associated with recovering skates from >1,000 m is to blame for the loss of samples because of regurgitation.

Many of the prey consumed were not robust in form and structure, making identification and quantification difficult. Hard parts (eyes, otoliths, and beaks) were more readily identified than other prey in the stomachs. This led to many higher-level and unidentified groupings of prey items. As a result of the small percentage of empty stomachs and range of digested prey per stomach, it appears that *B. trachura* is feeding in a largely continuous manner.

The diet of the skate could have been more accurately characterized to lower taxonomic levels if the stomachs had been fixed in formalin instead of frozen. However, for this study the increased effort needed to remove and preserve the stomachs was not

possible. The well-digested state of most prey items and lack of comprehensive identification guides to deep-sea invertebrates required that many specimens be sent to taxonomic experts to verify their identification. It was confirmed by these experts that many of the prey items were simply too well-digested to be identified lower than family in most cases. However, the identification that these experts were able to provide have led to a comprehensive data set for deep sea invertebrates including location and depth, using the skate as a passive sampling unit (Link 2004). Little is known about some of the prey identified (e.g. benthesicymid shrimps) and photos and preserved specimens have been catalogued for future reference and deposited in Moss Landing Marine Laboratories' reference catalog.

The overall diet of *B. trachura* consisted mostly of crustaceans (at least 40 species), accounting for 71% of the %GII of the diet (82% by number and 60% by mass) with %N being greater because of the preservation of eyeballs and small parts. Fishes were the next most important group (18% GII) and contributed more mass (30%) than number (5%). Fishes were mostly juvenile *S. altivelis* about seven centimeters in length, about the size they settle from the water column to the seafloor (Wakefield and Smith 1990). Unidentified fishes were the next most important group, and were mostly comprised of fish eyeballs and degraded otoliths. Only a few other fishes were found in the stomachs, including a hake maxilla that, when compared with bones from hake of known sizes, came from a large Pacific Hake (probably >0.5m long) that would be far greater in volume than that of an adult *B. trachura* stomach. Given the small size of the stomachs of *B. trachura*, this bone is evidence of scavenging. Digested clupeid bones

also may be evidence for scavenging, as they are typically epipelagic species. A few of the fishes were recently ingested and atypical of the habitats in which *B. trachura* feeds (e.g. epipelagic or midwater), and could be evidence of net feeding (*S. sector, Cololabis saira,* and *Sebastes* sp.*)*.

Ingested polychaetes (4% GII) belonged mostly to the onuphid and polynoid families. Onuphid polychaetes are tube-dwelling and when eaten by the skates, the tubes also were ingested. Cephalopods (3.7% GII) were largely unidentified, as tissue or eyes often were found without complete and intact beaks in the stomachs. When complete beaks were present, the cephalopods could often be identified to family. Only a few octopus were found, with the majority of the identified cephalopods being squid.

The types of prey consumed in this study were similar to that of the only other dietary analysis of 4 stomachs of *B. trachura,* but differed in the relative quantities (Yang 2003). According to Yang (2003), *B. trachura* collected in Alaska ate mostly polychaetes, with cephalopods, copepods, mysids, isopods, amphipods, decapods and fishes making smaller contributions to the diet. The trophic level determined in this study (3.53 +/- 0.22 SD) was lower than that calculated for the Yang (2003) samples (3.78; Ebert and Bizzarro 2007). The difference in relative prey quantity and trophic level is probably due to location and sample size. Eight stomachs samples of *B. trachura* from the Bering Sea found prey taxa including tanner crabs (62.5% FO), myctophids (37.5% FO), mysids, shrimps, cephalopods, and a king crab (Ebert, pers. comm.).

The unidentified organic matter (UOM) found in the stomachs was unexpected as it was present in two distinct forms. The first was trace red crustacean parts that were too

small to identify and quantify. The second type of UOM was a layer of tissue-like substance that was gray to black in color and found in small pieces usually composed many layers of tissue. This gray tissue was found in 49 stomachs. It was originally presumed that the tissue could be fish or cephalopod in origin, however, the tissue did not often co-occur with these prey items.

This prompted a small subsample of tissue to be genetically tested in partnership with researchers at the Monterey Bay Aquarium Research Institute (MBARI). Of the 8 samples tested, two were found to be marine polychaetes, four *B. trachura,* one crab, and one marine mammal (with 92% match). *Bathyraja trachura* feeds on marine polychaetes and crabs, and it is not surprising that some of the skate's DNA would be amplified in the testing of the tissue removed from its stomach. However, the presence of marine mammal was unexpected. It is unlikely that *B. trachura* and marine mammals come into contact often; however, it is possible that a marine mammal died and sank to the seafloor, and the skate fed upon the decaying carcass. Many scavengers have been attracted to baited cameras, sunken carcasses, and whale falls in the deep sea (Buckley et al. 1999; Drazen et al. 2001; Goffredi et al. 2004) Although scavenging in not documented frequently in skate literature, two species (*Raja erinacea* and *Raja ocellata*) scavenge on scallop viscera from fishery discards near Georges Bank (Link and Almeida 2002). Skates in Alaska also have been found with large, partially digested salmon in their stomachs, which is either evidence of scavenging fishery offal or opportunistically preying upon weak/injured fish. In a few instances, skates off California have been observed at baited

camera stations and whale falls on deep-sea video footage, though there was no direct evidence of feeding (Ebert, pers. comm; Boyle, pers. obs.).

Bathyraja trachura fed upon prey typical of mostly benthic (44%) and benthopelagic (39%) habitats. Of the groups of prey, crustaceans were mostly benthic and benthopelagic, fishes were benthic, polychaetes were infaunal and cephalopods were benthopelagic. Several studies have found a shift in diet from more benthic sources to more pelagic or benthopelagic sources as skates increased in size (Alonso et al. 2001; Orlov 2003; Farias et al. 2006; Scenna et al. 2006). This study's poor taxonomic resolution makes it difficult to analyze such a trend. However, conversations with MBARI researchers and reviewing some of their deep sea video has shown that many prey items that appear in the literature as pelagic (e.g. euphausiids) can be found in large concentrations only a few meters above the seafloor in the deep sea. *Bathyraja trachura,* when filmed *in situ* is always on or within a few meters of the seafloor, further supporting the benthic/benthopelagic feeding niche determined by my study.

Many researchers of skates have found an increase in trophic level with size, whether statistically significant (Alonso et al. 2001; Rinewalt et al. 2007; Robinson et al. 2007 Treolar et al. 2007) or qualitative (McEachran et al. 1976; Pedersen 1995; Farias et al. 2006; Orlov 2003) while a few have not found such an increase (Martin et al. 2007). Few researchers have found a difference in the diets between the sexes (Scenna et al. 2006; Rinewalt et al. 2007), with no difference found most often (Martin et al. 2007; Robinson et al. 2007), or sexual dimorphism confounding the results (Orlov 1998; Orlov 2003). In this study larger *B. trachura* were found to have higher trophic levels than the

smaller individuals. As evident from the graph of this relationship (Figure 6), although the relationship was significant, the variability in the data around the trendline was great. It is important to note that only the smallest specimens did not fall along the entire range of trophic levels. Once skates are greater than 300 mm in total length, they begin to consume fishes and cephalopods, increasing their trophic level. Similarly, larger skates can consume larger prey, though that difference was not taken into account in these calculations. A large number of individuals had a trophic level of 3.4, which is the trophic level assigned to unidentified crustacean in this study. Unidentified crustaceans were consumed more often in smaller individuals, and less frequently in larger individuals, possibly driving the significant relationship between trophic level and size. The smallest skates sampled in Chapter 1 and 2, a male 172 and 150 mm in total length, respectively, are two new records of the smallest free-swimming *B. trachura* individuals. In the multiple regression, higher trophic level prey (fishes and cephalopods) were the most significant and most positively correlated with total length. Other crustaceans were the only variable removed from the model, probably because this broad prey category was consumed in great numbers across all sizes of skates.

In the canonical correlation, year and latitude were the environmental variables significantly correlated with the dietary variables. Benthesicymid shrimps were negatively correlated with year, whereas fishes had a weaker positive relationship. Unfortunately, samples were not evenly dispersed among the four years, and the large number of zeros in the diet data precluded further investigation into this relationship. In the second root of the analysis, benthesicymid shrimps were more important in the south

whereas euphausiids were more important in the north. Benthesicymid shrimps are infrequent in the literature; however the few shrimps found within this family that were identified to species typically ranged from British Columbia or Oregon southward, across almost the entire latitudinal gradient of this study. Euphausiids, specifically the two genera found in this study, *Euphausia* and *Thysanoessa,* also have been documented throughout the range of this study. Based upon the documented ranges of these prey, it appears that the annual and latitudinal pattern found here cannot be explained by prey distribution alone, indicating that other factors may be driving this pattern (e.g. seasonality, prey availability).

The diet of *B. trachura* is more similar to *Bathyraja kincaidii* than *Raja rhina, Raja binoculata,* and *Raja inornata* in Californian waters (Bizzarro et al. 2007). *Bathyraja kincaidii* is a smaller skate that lives in the deeper waters of the continental slope, much like *B. trachura.* Whereas the depth distribution of *B. kincaidii* from previous studies overlaps little with *B. trachura*, they consume prey taxa in similar quantities. Both skates consume a large amount of shrimp-like crustaceans, including euphausiids, followed by polychaetes, fishes, and cephalopods in varying amounts (Bizzarro et al. 2007; Rinewalt et al. 2007). This is in contrast to another smaller-sized skate, *Raja inornata* that lives on the continental shelf, which consumes mostly crabs, shrimp-like crustaceans, and fishes. The two largest skate species, *Raja binoculata* from the shelf, and *Raja rhina* from the shelf and upper slope consumed shrimp-like crustaceans and fishes in great amounts, whereas *R. binoculata* also consumed a large amount of crabs. It appears that the shared habitat and size of *B. trachura* and *B. kincaidii*

is what allows their dietary niches to be so similar, as large skates in deeper water, and similarly-sized skates in shallower water, do not share less-important prey such as polychaetes.

Bathyraja trachura is a generalist predator on crustaceans, fishes, and polychaetes. Prey were typical of benthic and benthopelagic habitats. Scavenging was apparent, ranging from well-digested pelagic fishes to a marine mammal. The diet of males and females of the skate did not differ; however, there was a significant but modest shift in the diet composition and trophic level of the skates as they increased in size. Latitude and year explained some of the variation in the diet, as benthesicymid shrimps were more important in the diet of skates collected from the south, and euphausiids from the north, though the biological relevance of this statistically significant result is unknown. My study is the first thorough study of the diet of this abundant deep-sea skate, and along with stable isotope analysis (Chapter 2), will improve out understanding of the trophic interactions of the fishes in the food web of the continental slope.

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Chapter II

Stable isotope analysis of continental slope fishes: Evidence of an isotopically enriched benthic food web

ABSTRACT

Stomach content analysis (SCA) is a popular, yet resource intensive, method to quantify the trophic ecology of fishes. Recently marine scientists have begun to utilize stable isotope analysis (SIA) as an alternative method to resolve the trophic relationships of fishes as it avoids some of the obstacles associated with SCA. In this study, fishes and invertebrates collected from the continental slope (1,000 m) of the eastern North Pacific were analyzed using stable isotope analysis. The carbon and nitrogen stable isotope results were used to construct dual isotope plots to investigate the trophic relationships of this deep-sea community. Stomach and isotope samples were collected from 32 Roughtail Skates (*Bathyraja trachura*) to determine the validity and accuracy of SIA in determining the trophic levels of the skates. Dual isotope plots indicated that most species groups (invertebrates and fishes) sorted well along the two axes, with less intraspecific variability than interspecific variability for most species. The results also indicated an isotopically-distinct benthic and pelagic food web, as the benthic food web was more enriched than the pelagic food web. Both SIA and SCA supported this finding, resulting in the assignment of fishes to different trophic levels than those expected based on published dietary information, depending on the habitat of their prey. A linear regression analysis indicated that nitrogen values from SIA and trophic levels calculated from SCA, when plotted against skate total length, exhibited similar variation and patterns, although only the stomach content analysis yielded significant results (stomach content: $P=0.020$, $r^2=0.168$; stable isotope: $P=0.077$, $r^2=0.101$).

INTRODUCTION

It is important to quantify the interactions, especially trophic assemblages, of entire communities for the development and application of management plans (Botsford et al. 1997). Traditional stomach content analysis, used to quantify diet and trophic relationships, can be time consuming and resource intensive. Conducting such studies in the deep sea requires extensive sampling time and sophisticated equipment. In addition, it is difficult to collect an adequate sample size of fish from the deep sea as deep-water teleost fishes possess gas-filled swim bladders that frequently expand upon ascent, everting the stomach, and yielding a nonviable sample. Regurgitation also is common in the recovery of deeper-dwelling teleost and elasmobranch fishes, and further limits sample sizes. Additionally, the prey in the deep sea are often fragile, and species are difficult to identify. To overcome diminished sample sizes of fishes collected from the deep sea, thousands of fish may need to be collected to obtain enough viable samples for a diet study (Drazen et al. 2001). Although time consuming, many researchers have quantified the diet of deep-sea fishes using stomach content analysis, which has provided important information on the trophic ecology of these fishes (Ebeling and Cailliet 1974; Buckley et al. 1999; Drazen et al. 2001;). An apparent solution to this problem is another method of quantifying food web position that does not require a complete stomach sample.

In the past few decades a technique that uses the isotopic chemistry of an organism's tissue to determine its trophic level has become popular in marine studies (Peterson and Fry 1987). This method of nitrogen and carbon stable isotope analysis uses

the theory that isotopic fractionation (retention of the heavier isotope) occurs at a constant, known percentage per trophic level (Post 2002). As a result of normal metabolic functions (respiration, metabolism, and excretion) the heavier isotope is retained within the animal, and the lighter isotope is excreted. Analysis of the tissue can determine the amount of isotopic fractionation within that organism, and comparison of stable isotopes in tissue with other parts of the trophic system, the position of the organism in the food web can be resolved, typically both in trophic level and type and habitat of food source (Peterson and Fry 1987; Michener and Schell 1994 Post 2002).

Although many researchers have used stable isotope analysis to understand the tropic levels and food webs of ecosystems, relatively few have compared the results of stable isotope analysis to stomach content analysis to determine its validity (Michener and Schell 1994). Of the studies that have made such a comparison, the results are mixed. Even fewer researchers have investigated the use of stable isotope analysis in elasmobranchs (Fisk et al. 2002; Estrada et al. 2003; MacNeil et al. 2005; Estrada et al. 2006; Kerr et al. 2006). There have been conflicting thoughts on the accuracy of stable isotope analysis due to the metabolism of elasmobranchs, namely the presence of trimethyl amine oxide (TMAO) and urea retention, as urea is depleted (contains more lighter isotopes). Although a few studies of elasmobranchs have indicated encouraging and mixed results, the usefulness of this method requires more testing to realize its full potential.

The goals of this study were to determine the validity and accuracy of nitrogen and carbon stable isotope analysis (SIA) in an abundant demersal skate, *Bathyraja*

trachura, and broadly analyze the trophic positions of sympatric deep-sea fishes using SIA. The community assemblage along the 1,000 meter isobar on the continental slope of the eastern Pacific includes fishes that are commercially targeted and caught as by-catch. The objectives of this study were to: 1) determine the accuracy of nitrogen and carbon stable isotope analysis in a deep-sea species of skate; 2) compare the trophic level and prey composition from stomach content analysis to the carbon and nitrogen stable isotope results; 3) determine the accuracy of SIA in the deep sea for sympatric fishes; and 4) create a food web for the fish assemblage of the upper continental slope. I hypothesized that nitrogen and carbon stable isotope analysis would accurately reflect the trophic position of the fishes and invertebrates in the system when compared to the habitat of its prey and dietary information from the published literature. It was expected that the trophic level determined from SIA and SCA would not be equal due to their different dietary timeframes, but would indicate similar feeding position in the food web (Fisk et al. 2002; Estrada et al. 2003; MacNeil et al. 2005; Estrada et al. 2006).

METHODS

Tissue samples for stable isotope analysis were collected during groundfish surveys by the National Marine Fisheries Service (NMFS) Fishery Resource Analysis and Monitoring (FRAM) Division in 2007 and 2008. Fishing vessels were contracted by the NMFS FRAM Division to target commercially important groundfishes with bottom trawls at depths of about 55 to 1,280 meters. Surveys were conducted from Cape Flattery,

Washington to the US-Mexico border with a stratified random sampling scheme designed to sample latitude and depth (Keller et al. 2008).

An analysis of NMFS survey data from 1999 to 2002 found that a deep-water fish assemblage was composed of Pacific grenadier, *Coryphaenoides acrolepis*; Giant Grenadier, *Albatrossia pectoralis*; Deepsea Sole, *Embassichthys bathybius*; Longspine Thornyhead, *Sebastolobus altivelis;* California Slickhead, *Alepocephalus tenebrosus*; and the Roughtail Skate, *Bathyraja trachura* (Tolimieri and Levin 2006). These fishes were targeted for collection, as were any prey or benthic organisms recovered in hauls from 1,000 to 1,200 meters of depth. Samples collected during 2008 were collected from specimens within a few hours of capture on board the vessels. In 2007, targeted animals were frozen on board the fishing vessels and returned to the lab for sampling and analysis. Stomachs and tissue from *B. trachura* also were sampled from 2005 to 2008 (Chapter 1).

For each haul that contained *B. trachura*, targeted fishes were set aside for tissue sampling and a sample of invertebrates were frozen whole. Five adult fishes of similar size from each species were chosen haphazardly for tissue sampling. These fishes were identified, sex determined, and total length and weight measured. A small section of clean dorsal muscle tissue was then excised from each fish and stored in a sterile cryovial for subsequent analysis. Tissue was rinsed in seawater and care was taken to collect a muscle sample free of skin, scales, blood, and foreign tissue types. It the laboratory, invertebrate samples from 2007 and 2008 were thawed, and muscle tissue was removed from the animals and stored in cryovials. All tissue samples were kept frozen until

analysis, and care was taken to never allow tissue to thaw for more than 30 minutes before re-freezing.

Most fish and invertebrate samples analyzed in this study were collected from two bottom trawl tows in two grid cells totaling 6.44 kilometers high and 2.4 kilometers wide just south of Newport, Oregon on 7 September 2008 in about 1,100 meters of water, as the author was on the cruise and able to collect a great number of samples free of variation in time and space. Samples were supplemented with individuals collected from other locations in 2007 and 2008 as needed. Twelve different types of invertebrates were analyzed in this study (Appendix I). All samples suspected of containing calcium carbonate in their tissue were decalcified using 0.5N HCl and all samples were treated with ether to remove lipids (see Chemical Preparation in Appendix I). Care was taken to sample tissue free of exoskeleton or viscera that might have different isotopic properties; tissue types sampled are listed in Appendix I. All samples were collected between northern California and northern Washington, with the exception of *Euphausia pacifica* which was from an isotope study in Monterey Bay, California (Brown, pers. comm.). All targeted adult fishes were collected on the same day from the grid cell south of Newport, Oregon. Three juvenile *Sebastolobus altivelis* were recovered from the stomachs of Roughtail Skates collected in 2005. Additionally, four *Stenobrachius leucopsarus* also were from the isotope study is Monterey Bay (Brown, pers. comm.). Care was taken to sample the largest fishes recovered and that all fishes sampled were of relatively the same size and evenly dispersed across the sexes, when possible, to avoid sampling individuals

with different habitats or diets due to differing sizes. Details for the fishes sampled for stable isotope analysis are listed in Appendix II.

Frozen tissue samples were chemically treated before SIA was conducted. A section of tissue the size of a pencil eraser was cut from the tissue sample using a scalpel, and placed into a clean glass scintillation vial. Clean lab techniques were used to prevent contamination. Between samples the scalpel, forceps, and work surface were rinsed with methanol and dried using Kim Wipes.

All invertebrate, skate, and shark tissue was then decalcified using 0.5N HCl. This step was designed to remove all inorganic calcium carbonate from the sample without removing the organic carbon to be tested. To decalcify the samples, 10 mL of 0.5N HCl was added to each glass vial containing the tissue samples. After 24 hours, the HCl was decanted and another 10 mL of HCl was added to each vial. After an additional 24 hours, the samples were visually inspected for signs of remaining calcium carbonate in the form of bubbles rising from the samples. If no bubbles remained, the acid was decanted and the samples were de-lipified.

Lipids were removed from all samples, as deep-sea organisms often contain great amounts lipids and oils. About 10 mL of petroleum ether was added to each glass vial and the vials were then lightly capped and placed in an ultrasonic water bath for 10 minutes. The petroleum ether was then decanted and another 10 mL of petroleum ether added, and again samples were placed in the water bath. If at this time the sample was still bubbling or it appeared that not all of the lipids had been removed, another 10 mL of ether was added to the sample until all the lipids had been removed (typically needed for

teleost samples). The above steps were repeated two times with 10 mL of water and 10 minutes in the water bath for each glass vial. At this time the lipids removed from the sample floated in the water and were decanted with the liquid. The water rinse was used to remove urea from chondrichthyan tissue, but was found to be useful in removing lipids from the vials in all other tissue types. The samples were then frozen in the glass vials until further processing.

Samples were transported to the University of California at Santa Cruz they were freeze-dried, weighed, and analyzed. Samples were freeze-dried in a Labconco Freeze Dry System / Lyph Lock 4.5 for about 24 hours, or until completely dry. Samples were then homogenized and a subsample of the powder weighing 0.5 +/- .05 mg was placed into a Costech tin capsule (3.5x5m). Standards of Pugel and Acetanilidide also were weighed into tin capsules per UCSC procedures, to check the calibration and drift of the machine during sampling. The tin capsules were then placed into a Carlo Erba Instruments CHNS-O EA1108 elemental analyzer coupled in continuous flow to a Finnegan Delta Plus XP mass spectrometer where they were combusted, and carbon and nitrogen were separated by gas chromatography. The weight of the C and N in each sample was used to calculate a C:N ratio. The C:N ratio was used to determine if samples were properly prepared, if the C:N ratios were >5.0 the samples were removed from the analysis, with the exception of *Anoplopoma fimbria* whose C:N ranged from 6.3-8.8 and were included in the dual isotope plots.

The isotope ratios ($\delta^{15}N$, $\delta^{13}C$) determined from SIA followed Post (2002) and were calculated for each individual fish:

$$\delta^n x = \frac{(\delta^n x_s - \delta^n x_{std})}{\delta x_{std}} * 1000$$

where *s*= sample, $\delta^n x$= ratio of $^{15}N:^{14}N$ or $^{13}C:^{12}C$, *std*= standard (PeeDee limestone for carbon and air for nitrogen). These values were then plotted on a dual isotope plot with nitrogen on the y-axis and carbon on the x-axis. Initially, samples were grouped into three higher categories: fishes, invertebrates, and sea cucumbers (detritivores) and plotted on a dual isotope plot.

The trophic level determined from SIA also followed Post (2002):

$$TL_{sc} = \lambda_{base} + \frac{(\delta^{15}N_{sc} - \delta^{15}N_{base})}{\Delta_n}$$

where λ= trophic level of baseline organism, *sc*= secondary consumer, *base*=baseline organism (e.g. detritivore) or organism of known *TL* used as a reference, and $\triangle_n$= known trophic enrichment of element from published literature. An average trophic enrichment value ($\triangle_n$) from the literature of 3.4‰ was used for all teleosts (Post 2002). These values were then compared with the TL determined by the stomach content analysis. The trophic level (TL) for each fish species was determined from dietary data following Cortés (1999) and Ebert and Bizzarro (2007):

$$TL_k = 1 + \left(\sum_{j=1}^{n} P_j * TL_j \right)$$

where TL_k is the trophic level of species *k*, P_j = proportion of prey category *j* in the diet, *n* = total number of prey categories, and TL_j = trophic level of prey category *j*. The trophic levels for *C. acrolepis* and *A. pectoralis* were calculated from the diet study by Drazen et al. (2001), for *S. altivelis, E. bathybius, A. fimbria,* and *S. alascanus* from the diet study

by Buckley et al. (1999), and the diet for *B. trachura* was from this study (Chapter 1). To facilitate comparison, percent weight (mass) was used as the diet metric as it was presented in all studies, and theoretically provides the best estimate of assimilated prey. To ensure the most accurate comparison, the sizes of fishes sampled in this study for SIA were compared only with the most similar size class(es) from the published dietary data, unless otherwise noted. A sensitivity analysis was conducted to explore the effects of enrichment factors and secondary consumers on the calculation of TL. This analysis was conducted by using different enrichment factors and secondary consumers in trophic level calculations, to quantify the difference between resulting trophic levels using different inputs.

Stomachs and tissue from 32 skates were sampled for TL comparisons. Trophic level was calculated from the diet of each individual skate, which was regressed against total length. The nitrogen stable isotope value was used as a proxy for tropic level and converted into a trophic level estimate using tanner crabs as a secondary consumer. This allowed for a comparison of the pattern and variation of the results of the two techniques.

RESULTS

Twelve different types of invertebrates (Appendix I) and 11 fish species were sampled for SIA (Appendix II) for a total of sixty-eight invertebrate and fish samples. Individuals in these categories separated into distinct groups on a dual isotope plot with fishes being highest in trophic level and across the entire range of carbon values, invertebrates lower in trophic level, and cucumbers lowest in trophic level and the most enriched in carbon signal (indicating a benthic habitat).

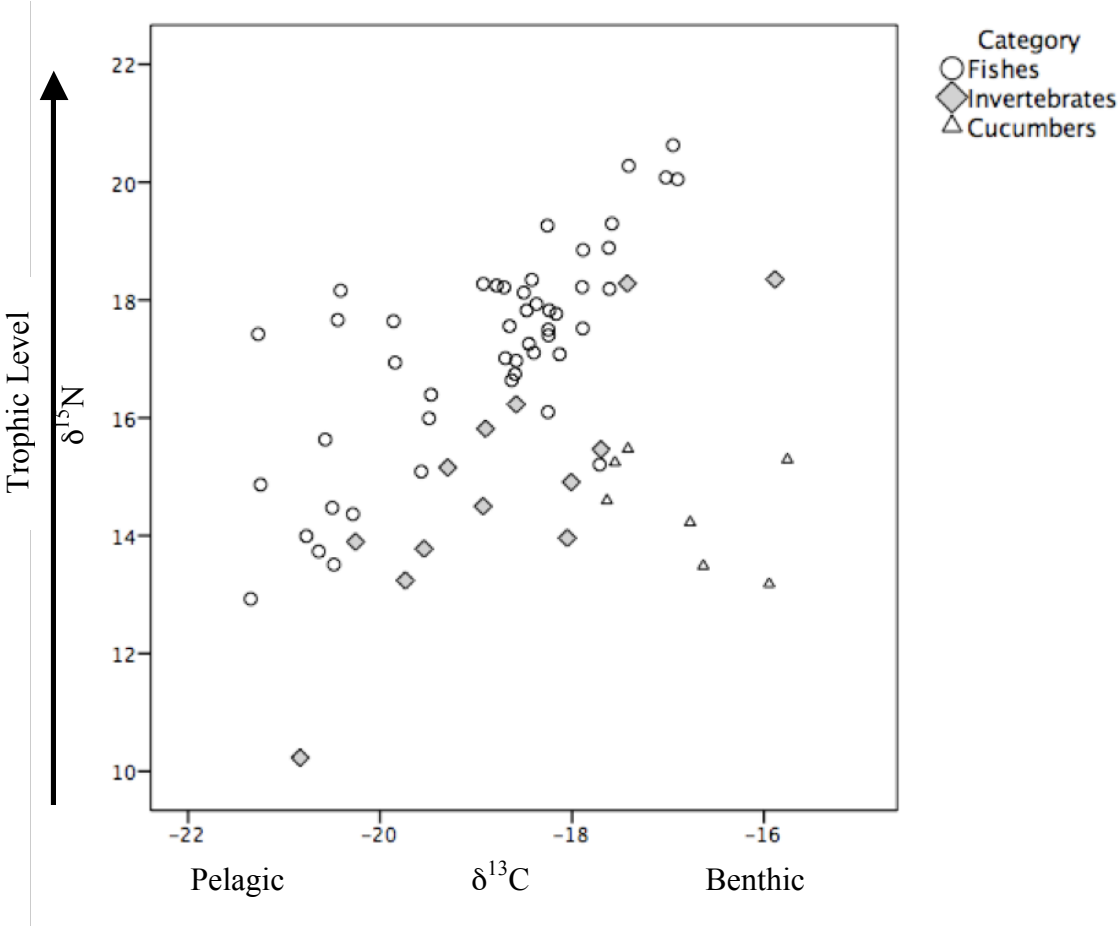

Figure 1: Dual isotope plot of all organisms collected, grouped into three categories. In this plot, $\delta^{15}N$ is a proxy for trophic level, with larger $\delta^{15}N$ values indicating a higher trophic level. Carbon is a proxy for food source, namely benthic or pelagic sources. Less negative values on the $\delta^{13}C$ axis (to the right) indicate more benthic signals, and more negative values indicate pelagic signals.

The dual isotope plot of all invertebrates shows definite sorting along the benthic and pelagic carbon gradient (Figure 2). The intraspecific variation is great but still smaller than interspecific variation among most of the groups. The three species of sea cucumbers *Scotoplanes* sp., *Pseudostichopus mollis* and *Parastichopus leokothele* had enriched, or more benthic, $\delta^{13}C$ values as did the sea star *Crossaster borealis.* The other starfish (*Luidia* spp.), snail *Neptunea* sp.*,* onuphid polychaetes and hormathiid anemone all fell on the middle of the carbon range. The euphausiid *E. pacifica*, and decapods *Eualus biunguis, Eualus macropthalmus,* and *Chionoecetes tanneri* had the strongest pelagic signals. Two different species of starfish had the highest $\delta^{15}N$ values, whereas most invertebrates ranged between 13 and 16‰. One *E. pacifica* sample was low in nitrogen, and it is unknown if this is an outlier or representative of the true $\delta^{15}N$ content of the sample.

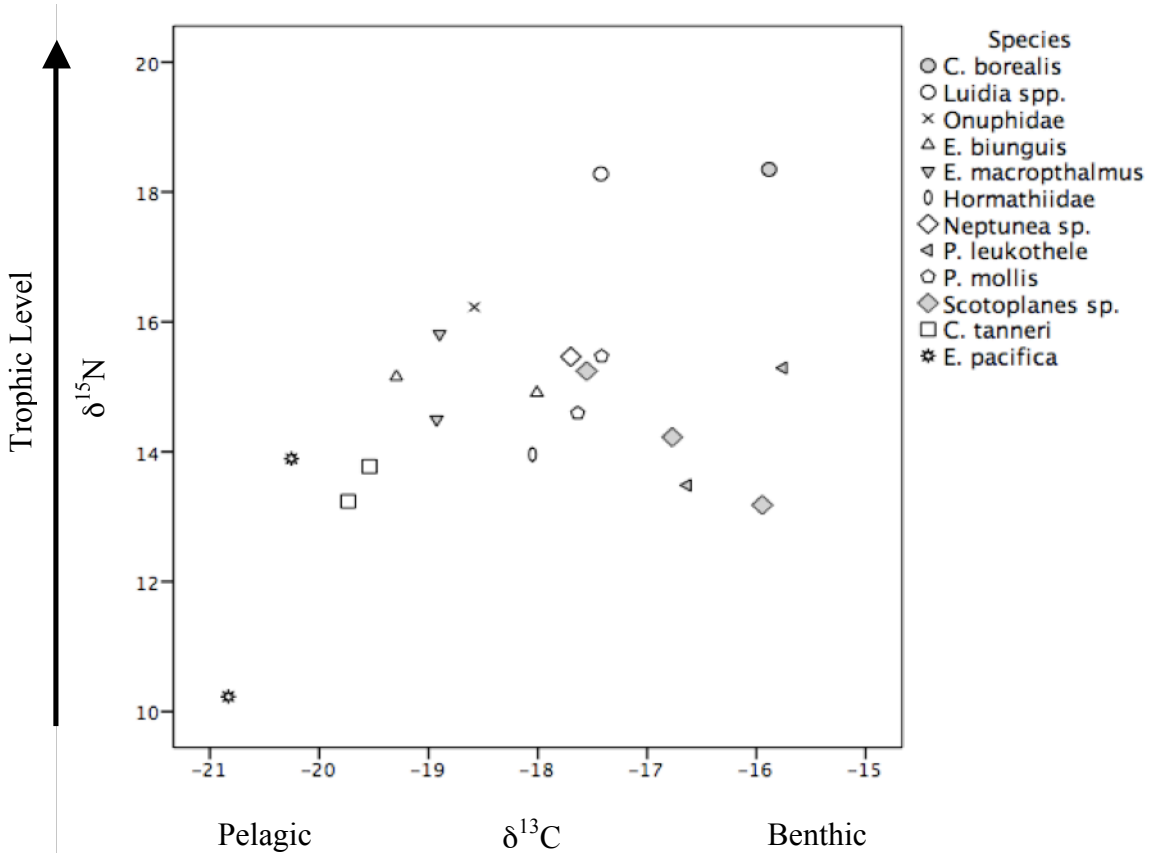

Figure 2: Dual isotope plot of all invertebrates. In this plot, $\delta^{15}N$ is a proxy for trophic level, with larger $\delta^{15}N$ values indicating a higher trophic level. Carbon is a proxy for food source, namely benthic or pelagic sources. Less negative values on the $\delta^{13}C$ axis (to the right) indicate more benthic signals, and more negative values indicate pelagic signals.

The dual isotope plot of fishes shows a distinct, almost linear pattern where benthic feeders are the highest in trophic level, and pelagic feeders are the lowest (Figure 3). Overall, the intraspecific variation is less than the interspecific variation, allowing the fishes to fall into distinct species groups. The dual isotope plot indicates that *S. alascanus, E. bathybius*, *B. trachura*, and juvenile *S. altivelis* were the most benthic feeders. *Stenobrachius leucopsarus*, *A. pectoralis*, *A. fimbria*, *C. acrolepis*, and *Aleopcephalus tenebrosus* were the most pelagic feeders. *Sebastolobus alascanus, E. bathybius, C. acrolepis, S. altivelis,* and *B. trachura* were the highest in the food web, whereas *S. leucopsarus, A. pectoralis,* juvenile *S. altivelis* and *A. fimbria* were the lowest in the food web. In both instances where juveniles and adults were sampled for a species (*S. altivelis* and *B. trachura)* all juveniles were lower in $\delta^{15}N$ than adults.

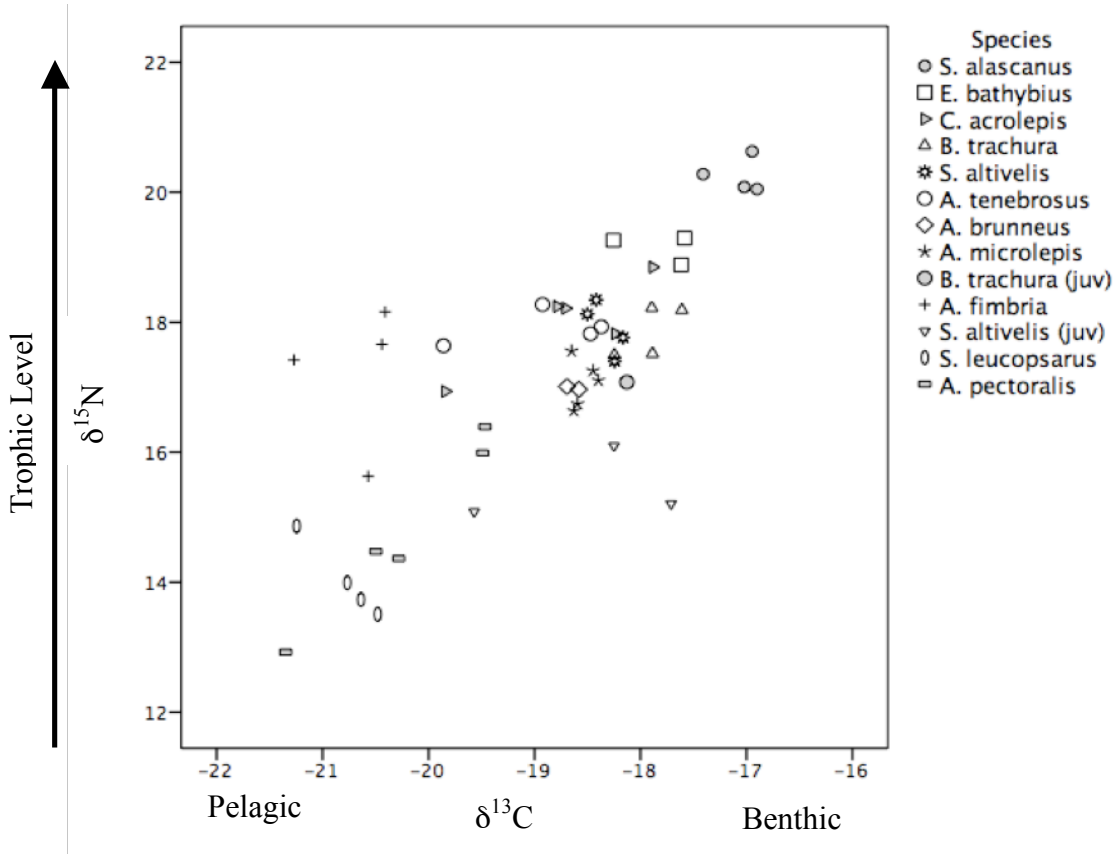

Figure 3: Dual isotope plot of all fishes. All samples are all similar-sized adults unless otherwise noted (juv=juvenile). In this plot, $\delta^{15}N$ is a proxy for trophic level and carbon is a proxy for benthic or pelagic food sources.

A plot of the roughtail skate, *B. trachura*, and its known prey allows for a visual depiction of the isotopic feeding niche of the skate highlighted in gray (Figure 4). All prey items of the skate fell below it in nitrogen values, and almost all prey are less enriched in carbon. Whereas organisms from the benthic and pelagic habitats sort on the carbon axis, carbon also can indicate trophic level with only an increase of 1‰ $\delta^{13}C$ per trophic level. This enrichment, along with a diet of mostly benthic and benthopelagic

organisms (Chapter 1), are probably the reasons why *B. trachura* had more enriched $\delta^{13}C$ values than most of its prey, even though many benthic organisms were sampled.

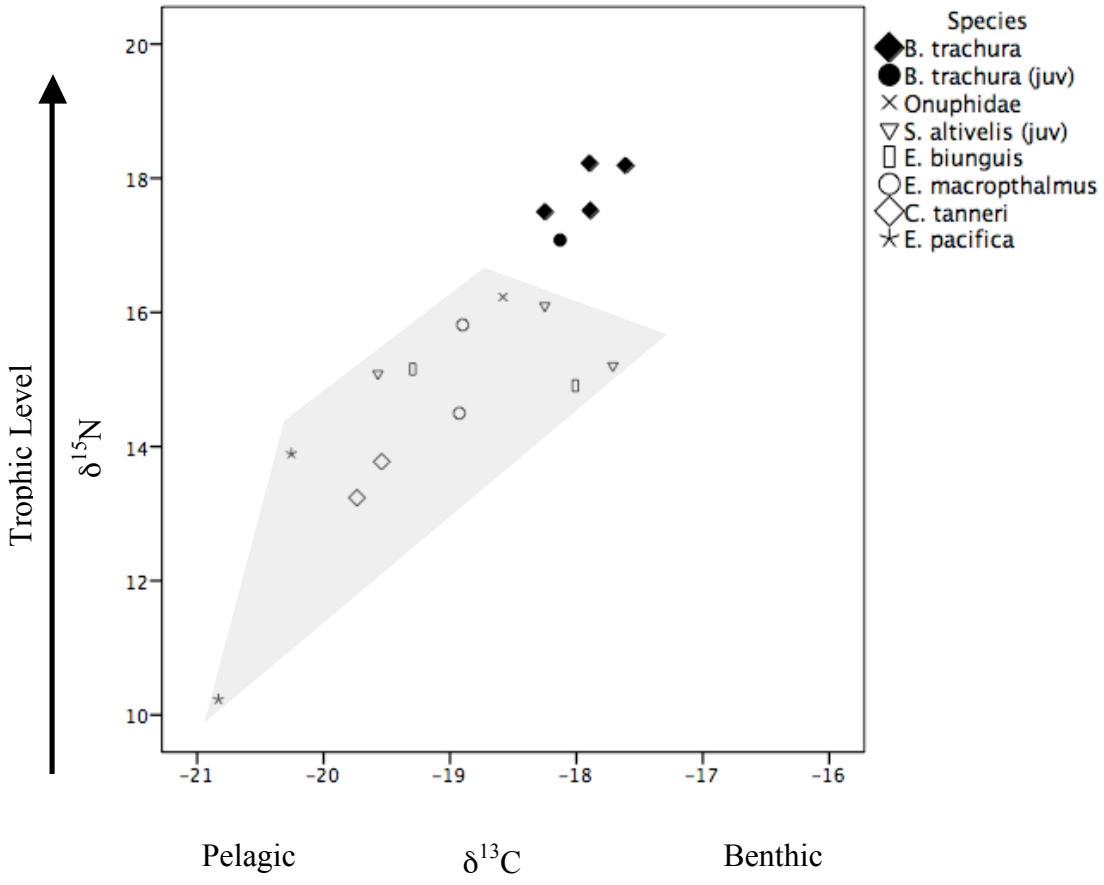

Figure 4: Dual isotope plot of the roughtail skate, *B. trachura*, and its prey. In this plot, $\delta^{15}N$ is a proxy for trophic level and $\delta^{13}C$ is a proxy for benthic or pelagic food sources (juv=juvenile). The gray box indicates the isotopic feeding niche of *B. trachura*, based on the prey sampled.

Adding the 32 skate SIA samples to the graph of *B. trachura* and its prey introduced greater variability in the isotopic values of the skates (Figure 5). However, the overall pattern held true that the skates were greater in $\delta^{15}N$ and $\delta^{13}C$ values than their prey. Interestingly, the juvenile in this graph was an individual of 150 mm in total length,

the smallest specimen collected to date. This juvenile, which had yet to absorb all of its yolk sac, appeared greater in $\delta^{15}N$ than eight other skates, which all were greater in total length. This indicates that skates still metabolizing yolk sac may be more enriched in nitrogen than other individuals that are free swimming and beginning to feed.

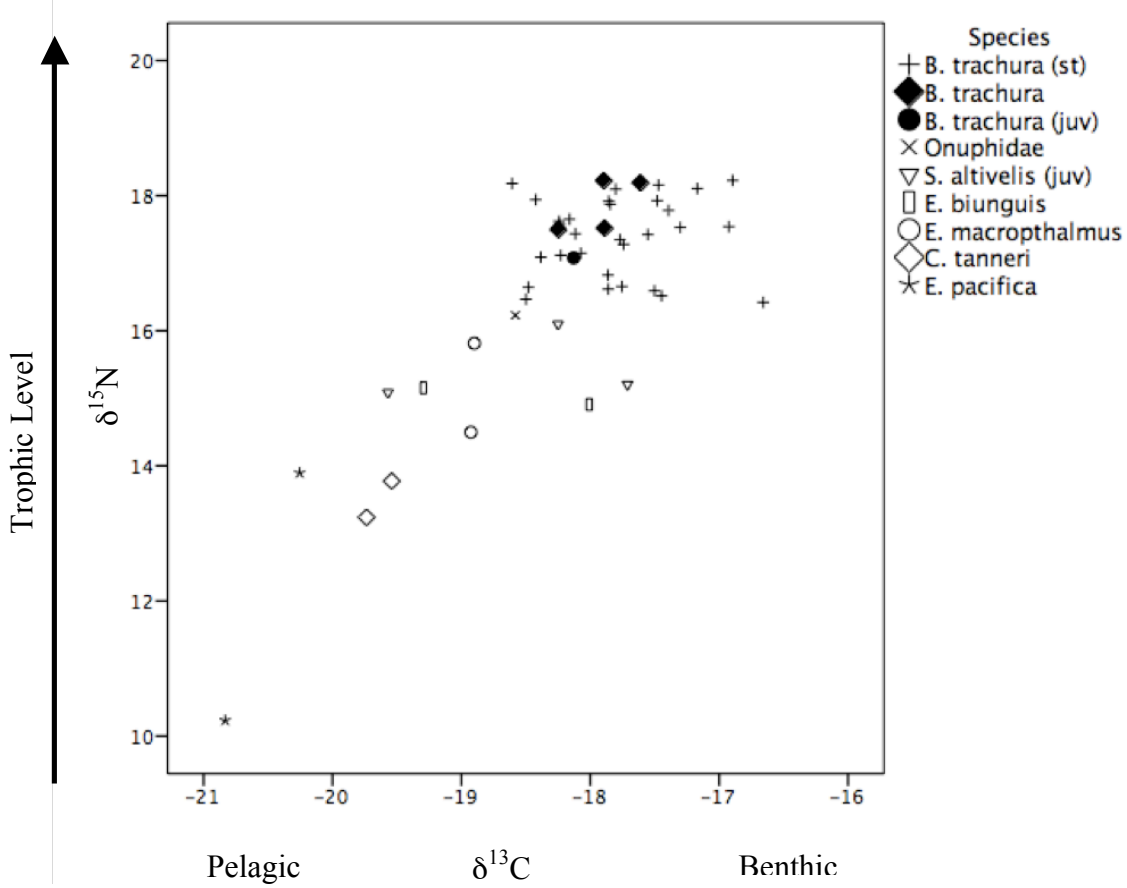

Figure 5: Dual isotope plot of the skate, 32 additional isotope samples, and its prey. The *B. trachura* (st) are the 32 samples used in the length/trophic level comparison (Figure 6), collected from several years various locations along the west coast. In this plot, $\delta^{15}N$ is a proxy for trophic level and $\delta^{13}C$is a proxy for benthic or pelagic food sources (juv=juvenile).

Stable isotope values varied greatly among species. For fishes, the nitrogen values varied as much as 3.47‰ in *A. pectoralis* and carbon varied 1.96‰ in *C. acrolepis* (Table

1). Some fishes exhibited little variation in trophic level (i.e. *B. trachura, S. alascanus*), whereas others varied by more than one trophic level (i.e. *A. pectoralis).*

Table 1: Variability in SI values for seven species of fishes. The difference between the most extreme nitrogen and carbon values are given in the table for each species, and the maximum trophic level difference was calculated by taking the difference in $\delta^{15}N$ and dividing by the enrichment factor of 3.4‰ .

Species	Maximum $\Delta\delta^{15}N$	Maximum ΔTL	Maximum $\Delta\delta^{13}C$
B. trachura	0.72	0.21	0.64
E. bathybius	0.41	0.12	0.67
A. pectoralis	3.47	1.02	1.88
S. altivelis	0.94	0.28	0.34
C. acrolepis	1.91	0.56	1.96
S. alascanus	0.58	0.17	0.51
A. fimbria	2.54	0.75	0.70
A. tenebrosus	0.63	0.19	1.49

A sensitivity analysis for all 32 *B. trachura* nitrogen values (results shown in Figure 6) found that using the enrichment factor of 3.4‰, from the literature, or 3.7‰, from a prey switching experiment on leopard shark (Kim, pers. comm.), changed the resulting TL value by less than 0.1 TL (Table 2). Given the high inherent variability of $\delta^{15}N$ in the fishes sampled (Table 1), the TL calculations are not very sensitive to different enrichment factors. Different secondary consumers, however, do change the final TL value of the skates. Trophic level ranged from 3.76 when using fishes (juvenile *S. altivelis* from the stomachs) as a secondary consumer in TL calculations, to 3.25 when cucumbers were the secondary consumer. This difference in trophic level of 0.55 is greater than that of some of the variability in $\delta^{15}N$ within the species, therefore the TL calculations were sensitive to changes in the secondary consumer. For all subsequent TL calculations, 3.4‰ was the enrichment factor used, and tanner crabs (*C. tanneri*), were chosen as the secondary consumer because they were collected in the same hauls as the

fish samples were collected in 2008, therefore, eliminating possible variation due to time and space.

Table 2: Sensitivity analysis for 32 *B. trachura* samples. Average trophic level values are listed for two different enrichment factors (3.4 and 3.7) and five different groups of secondary consumers. Δ_n is the enrichment factor used in the trophic level calculated in each row of the table.

Δ_n	Fishes TL=3.24	Tanner Crabs TL=2.52	Decapods TL=2.52	Euphausiids TL=2.25	Cucumbers TL=2.4
3.4	3.80	3.66	3.35	3.28	3.25
3.7	3.76	3.57	3.28	3.37	3.18

When trophic level was calculated from the diet of 32 *B. trachura* individuals and plotted against the total length of the skate, no difference in trophic level was detected between sexes (sex*total length interaction, P=0.499) but larger skates had greater trophic levels than smaller skates (P=0.020, r^2=0.168). However, there was a large amount of variation around the trendline (Figure 6). The nitrogen values from the same skates were plotted against skate total length. Again, no difference between the sexes was detected (sex*total length interaction, P=0.526). The relationship between TL calculated from $\delta^{15}N$ and total length was just greater than the significance level of 0.05, indicating the relationship was not statistically significant (P=0.077, r^2=0.101). The TL determined from the diet ranged 0.74 levels, and the isotope-derived TL ranges 1.33 levels.

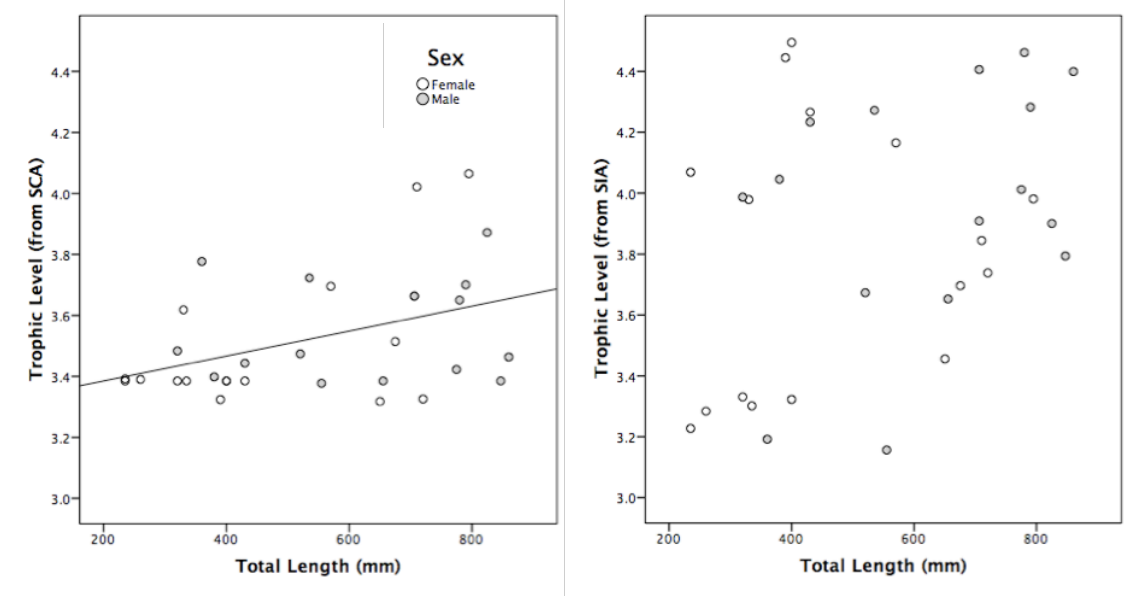

Figure 6: Trophic level calculated from stomach content analysis (left) and nitrogen isotope-derived TL (right) plotted against total length for the same sample of 32 skates. Trendline represents significant linear regression, males= ●, females= ○.

The comparison of the mean TL calculated from the nitrogen isotopes and the diet found that for all fishes that consume benthic prey the TL from diet was greater than the TL from isotope values. The opposite was true for fishes that fed ob benthic and pelagic prey. The variation in the TL from isotope values was high, considering that only 5 fish were sampled for each average TL value.

Table 3: Trophic level values for fishes calculated from the diet and nitrogen stable isotope values. Standard deviation is presented to indicate the variability in the isotope-derived trophic levels.

Species	$\bar{x}_{TL(diet)}$		$\bar{x}_{TL(SIA)}$	**Stdev$_{TL(SIA)}$**	**Typical Prey Habitat**
B. trachura	3.53[1]	<	3.80	(0.12)	Benthic
E. bathybius	3.54[2]	<	4.18	(0.07)	Benthic
S. altivelis	3.72[2]	<	3.81	(0.12)	Benthic
S. alacanus	4.06[2]	<	4.51	(0.08)	Benthic
A. fimbria	4.01[2]	>	3.61	(0.33)	Benthic & Pelagic
C. acrolepis	4.30[3]	>	3.85	(0.21)	Benthic & Pelagic
A. pectoralis	4.25[3]	>	2.91	(0.41)	Benthic & Pelagic

[1] this study, [2] Buckley et al. 1999, [3] Drazen et al. 2008

DISCUSSION

The dual isotope plot of carbon and nitrogen labeled by higher categories found that groups do sort into distinct clusters according to their isotopic signature (Figure 1). Once broken down into their species-specific groups, however, more patterns emerged. Inherent variability was great in some of the invertebrates and fishes (Figures 2 and 3). Despite intraspecific variation, sorting along the carbon axis into benthic (starfish, cucumber, crab) and pelagic (euphausiid, shrimp) groups did occur. An example of large and unexplained inherent variability was the variation between the 3 sea pig samples, *Scotoplanes* sp., which varied greatly on both axes (Figure 2).

The dual isotope plot of fishes was linear, as it appears that the benthic food sources were more enriched than the pelagic food sources (Figure 3). This is evident in Figure 3 as *A. pectoralis, C. acrolepis, A. fimbria*, and *S. alascanus* were all expected to be the top predators in the system. However, only the benthic feeder, *S. alascanus,* was

among the greatest in trophic level, with the other fishes that fed in part on pelagic prey having lesser $\delta^{15}N$ values. This can be explained almost entirely by prey habitat, as the smaller, benthic flatfish, *E. bathybius,* was second greatest in trophic level, presumably because its diet of benthic, carnivorous starfish was more enriched than other higher trophic level fishes that fed on more pelagic food sources. This discrepancy can almost be entirely explained by the fishery offal and scavenging by these species. For the sizes of fishes analyzed in this study, *A. pectoralis* and *A. fimbria* scavenge for 16-19% of their diet, by mass (Buckley et al. 1999; Drazen et al. 2001). This scavenged material has sunk to the seafloor as isotopically-depleted organic matter, bypassing the benthic food web. The benthic food web is typically more enriched than the pelagic food web as it originates from dissolved or particulate organic matter that sank to the seafloor and was consumed by benthic invertebrates, thereby becoming more enriched through the additional trophic level transfers. A separtion between these pathways also was found in a similar study of grenadiers of the abyssal plain (4,000 m) off the California coast (Drazen et al. 2008). These researchers concluded that the abyssal food web was enriched compared with the epipelagic food web, and that the phytodetritus pathway was isotopically distinct from the scavenging pathway as unexpectedly low stable isotope values were found for some fishes. This pattern confirms the findings of other studies in the deep sea that the benthic and pelagic food webs are istopically-distinct as the benthic food web is typically more enriched than the pelagic (Peterson and Fry 1987; Drazen et al. 2008; Nilsen et al. 2008).

The great intraspecific variability in isotope values for the organisms sampled could be due to many factors. Other researchers of stable isotopes have found variation can be due to poor food quality, starvation, isotopic routing and temporal and spatial sampling variation (DeNiro and Epstein 1981; Michener and Schell 1994; Gannes et al. 1997; Peterson 1999; Olbermann and Scheu 2002; Voigt et al. 2008). This study was carefully designed to sample at the same location and within the same day to account for those possible sources of variation. However, food quality and starvation could be factors driving the large variability in some samples. Within the fishes, it appears that food source accounts for some of the variability, where pelagic food sources are more variable in nitrogen and carbon. For some invertebrates with fewer samples, it is difficult to determine if the variability captured was representative of natural variability, was an outlier, or a result of incomplete inorganic carbon or lipid extraction. While isotopic routing is most evident in omnivores, the routing of protein and fats to different tissues within the predator may play a part in the variability of these isotope values (Gannes et al. 1997; Voigt et al. 2008).

Bathyraja trachura was higher in trophic level than all of its prey, and adults were higher in $\delta^{15}N$ than the juvenile *B. trachura* that were collected (Figure 4). The young *B. trachura* that was still absorbing its yolk sac and still possessed a filamentous tail from its time in an egg case was higher in nitrogen than some other skates collected, either indicating that the skate was more enriched due to the yolk it was metabolizing (Olbermann and Scheu 2002) or that the variation in space and time confounded the comparison between specimens. Values for *B. trachura* prey indicated they were slightly

more depleted in $\delta^{13}C$ than *B. trachura*, although it should be noted that only some prey of *B. trachura* were represented; therefore precluding any trophic niche or mixing model analysis (Figure 5).

The comparison of *B. trachura* TL from the diet and $\delta^{15}N$ from 32 samples yielded a significant trend from the diet, and a non-significant trend from the nitrogen isotopes. While statistically these are two different patterns, they do not indicate that SIA and SCA do not yield similar results. While it would be ideal to plot trophic level from SIA and SCA on each axis and hypothesize that the points should fall on a 1:1 trendline, this is an invalid exercise due to the two different time scales of the metrics. Stomach content analysis provides insight only into the last meal of a fish, whereas muscle turnover is on the scale of several months (Michener and Schell 1994). Therefore, it cannot be expected that these two values would yield the exact same result. Instead, the pattern and trends of the results must be compared qualitatively. Furthermore, dietary analysis is of ingested material, whereas stable isotope analysis is of assimilated material. In the graph of trophic level (Figure 6), the data ranged over 0.74 trophic levels. In the isotope graph, the data ranges over 1.33 trophic levels. It is not surprising for the isotope results to be more variable as they incorporate more dietary information along with more factors (e.g. latitude, food source, distance from shore) than simply the last meal. The general patterns of variation and of trophic level increase in larger skates for the two graphs were similar, though their significance was different.

Additionally, both of these analyses found no difference between sexes. Studies of skate diet rarely find dissimilar diets between the sexes (Morato et al. 2003; Robinson

et al. 2007) with the exception of a few studies finding either a qualitative difference based on frequency of occurrence (Orlov, 1998) or statistically significant differences (Rinewalt et al. 2007). The total length, gape, and range of *B. trachura* does not differ between the sexes, so a dietary difference was not expected. An analysis of the diet of 350 skates found that trophic level was greater in larger skates (Chapter 1), a trend frequently found in studies of skates and other fishes.

More isotope studies are needed in the deep sea to help understand the benthic and pelagic food web pathways and interactions, and to understand how various deep sea properties affect stable isotope analysis (e.g. oxygen minimum zone). In addition, the mechanism causing intraspecific variability needs greater understanding, as the great variability in some isotope studies precludes detailed hypothesis testing, including deciding the best way to prepare and treat tissue samples. Many researchers that have investigated the effects of different tissue preparation methods have found differences, though most of those differences are less than the inherent variability in many of the species within this study (Carabel et al. 2006; Barnes et al. 2008; Cherel et al. 2009).

Whereas studies in the past have indicated variable results within elasmobranchs, this study found that stable isotope analysis results were similar, though not equal, to the results from dietary analysis. Most shark researchers have found stable isotope values that corroborate the trophic level determined by stomach content analysis (Estrada et al. 2003; MacNeil et al. 2005; Estrada et al. 2006) whereas other researchers (usually studying the deeper-dwelling species) reported trophic level data that did not correlate with diet studies (Fisk et al. 2002; Kerr et al. 2006). A comparison of the trophic level

calculated from SCA and SIA of 32 skates had trophic level values that were similar in range, average, and overall pattern, but not exactly the same. The trophic level calculated from stable isotope analysis was often higher than that calculated from stomach content analysis. Due to the different tissue timeframes of these two methods (last meal vs. several months) a direct comparison was not statistically tested. Stable isotope analysis also found *B. trachura* was more enriched in both nitrogen and carbon than its known prey items. Therefore, SIA not only appears to be a viable method in this deep-sea skate, but also an accurate method when compared with its known diet.

I suggest that more dual studies of SIA and dietary analysis be conducted for marine species in all habitats. Until researchers agree on the type of chemical preparation to conduct, it is best to collect samples from the entire study assemblage, as the samples are then directly comparable with each other within the study, as other studies may have utilized different chemical preparation techniques, prohibiting comparison. Researchers should also conduct at least a small amount of dietary sampling from the organisms collected for SIA, as a shift in SIA may be due to many environmental factors like changing ocean conditions, or changes in diet. SIA should be considered an important compliment to traditional dietary analysis that will allow for a faster and more efficient method of monitoring food web changes over long periods of time.

Due to the benthic and pelagic pathways that comprise the food web in the deep sea, trophic level must be thought of in two different scenarios when comparing dietary and stable isotope analysis results. The typical trophic pyramid with 5 levels holds true in the nearshore and epipelagic environment, but becomes more complicated in the deep

sea. This becomes important when trophic levels are calculated from stable isotope values, and compared with dietary studies to determine accuracy. Choosing a baseline with the proper benthic or pelagic signal can change the conclusions of an analysis. More studies are needed to understand the properties of these two deep-sea trophic pathways, and to determine the best way to quantify the trophic level of organisms that feed upon prey in both pathways. As we continue to exploit the deep sea via fishing, resource extraction, and sequestration experiments, along with the predicted impacts of climate change and ocean acidification, it is important to quantify the food webs of the deep sea, and monitor them as this ecosystem changes.

Carbon and nitrogen stable isotope analysis is a viable method for determining trophic relationships (relative position in the food web and habitat of prey) of deep-sea organisms. This method can use fewer samples, non-intact stomachs, and can be less resource intensive than stomach content analysis. When these two methods are combined to study an assemblage, they become powerful and complementary tools to resolve detailed trophic relationships. When conducting such a comparison, care should be taken to quantify the inherent variability of the methods and when comparing the results with other studies. If more studies are conducted in this manner, a coupled stable isotope and dietary approach could help quantify many more food webs and allow for improved and more efficient long-term monitoring.

APPENDIX I

Appendix 1: Summary of invertebrate samples collected for stable isotope analysis. Organisms sampled represent the number of total individuals sampled, as sometimes multiple individuals from the same species were combined into one sample. Location indicates the general area where the samples were collected, the * indicates the sampling area off of Newport, Oregon, and year is the year in which the organism was collected. Chemical preparation indicates the used of acid (a) or ether (e) and the tissue type sampled for SIA is listed.

Species	Group	Organisms Sampled	Location	Year	Chemical Preparation	Tissue Type
Hormathiidae	Anemone	3	*	2008	a + e	body tissue
Parastichopus leokothele	Cucumber	3	N. CA	2007	a + e	body tissue (not GI tract)
Pseudostichopus mollis	Cucumber	4	N. CA	2007	a + e	body tissue (not GI tract)
Scotoplanes sp.	Cucumber	6	*	2008	a + e	body tissue (not GI tract)
Neptunea sp.	Snail	2	*	2008	a + e	muscle tissue
Crossaster borealis	Starfish	3	*	2008	a + e	disc meat
Luidia spp.	Starfish	2	*	2008	a + e	disc meat
Chionoecetes tanneri	Crab	3	* & N. WA	2008	a + e	gills & meat
Euphausia pacifica	Euphausiid	2	Cen. CA	4/7/08; 11/15/07	a + e	homogenized
Onuphidae	Polychaete	1	N. WA	2008	e	piece of whole worm
Eualus biunguis	Shrimp	2	N. WA	2008	some a + e	tail meat
Eualus macropthalmus	Shrimp	4	N. WA & N. CA	2008	a + e	tail meat & whole

* = *grid cell coordinates:* 44 23.7926 -125 08.4791; 44 23.7781 -125 06.3719; 44 19.8074 -125 06.4276; 44 19.821 -125 08.5327

APPENDIX II

Table 2: Fishes sampled for stable isotope analysis; where j = juvenile, N=number of fishes sampled, TL= total length, SL= standard length, PAFL=pre-anal fin length, FL=fork length, u=unknown, a=acid, and e=ether. Location indicates the general area where the samples were collected, the * indicates the sampling area off of Newport, Oregon, and year is the year in which the organism was collected. Chemical preparation indicates the used of acid (a) or ether (e).

Species	Common Name	N	Location	Year	Length (mm)	Measurement Type	Sex	Weight (kg)	Chemical Prep
Bathyraja trachura (j)	Roughtail Skate	1	*	2008	150	TL	M	u	a + e
Bathyraja trachura	Roughtail Skate	5	*	2008	820	TL	F	3	a + e
					740	TL	F	2.08	
					820	TL	M	3.44	
					800	TL	M	3.2	
					690	TL	M	1.96	
Embassichthys bathybius	Deepsea Sole	3	*	2008	370	TL	F	0.58	e
					360	TL	F	0.54	
					360	TL	F	0.68	
Antimora microlepis	Flatnose	5	*	2008	380	SL	M	0.32	e
					380	SL	M	0.39	
					400	SL	u	0.484	
					395	SL	u	0.366	
					380	SL	u	0.322	
Albatrossia pectoralis	Giant Grenadier	5	*	2008	210	PAFL	M	1.26	e
					210	PAFL	M	1.4	
					190	PAFL	M	1	
					180	PAFL	M	0.86	
					200	PAFL	F	1.18	
Sebastolobus altivelis	Longspine Thornyhead	4	*	2008	230	SL	M	0.16	e
					230	SL	F	0.16	
					240	SL	M	0.16	
					230	SL	F	0.12	
Sebastolobus altivelis (j)	Longspine Thornyhead	3	skate stomachs	2005	61.5	SL	u	0.005	e
					97	SL	u	u	
					100	SL	u	u	
Coryphaenoides acrolepis	Pacific Grenadier	5	*	2008	220	PAFL	M	0.8	e
					200	PAFL	M	0.88	
					190	PAFL	M	0.68	
					170	PAFL	M	0.58	
					180	PAFL	M	0.64	
Alepocephalus tenebrosus	Slickhead	4	*	2008	400	SL	F	0.68	e
					480	SL	F	0.96	
					410	SL	u	0.46	
					360	SL	u	0.26	

Species	Common Name	N	Location	Year	Length (mm)	Measurement Type	Sex	Weight (kg)	Chemical Prep
Sebastolobus alascanus	Shortpine Thornyhead	4	*	2008	620	SL	F	3.76	e
					540	SL	M	2.26	
					560	SL	F	2.34	
					600	SL	F	3	
A. brunneus	Brown Catshark	2	*	2008	520	FL	F	0.457	a + e
					520	FL	F	0.512	
Anoplopoma fimbria	Sablefish	4	*	2008	630	FL	F	3.18	e
					610	FL	M	2.5	
					630	FL	F	2.5	
					590	FL	F	2.08	
Stenobrachius. leucopsarus	Northern Lampfish	4	Monterey Bay	2007	62	SL	u	u	e
					56	SL			
					Several sm. individuals	SL			

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CONCLUSION

This study is an example of the importance and benefits of collaborative research that can be conducted with government surveys. The skates and fishes in this study were collected from surveys conducted annually to quantify fish abundance, and no additional fishing effort or mortality occurred by collecting the organisms from the trawls. Furthermore, collaborative research allows the researcher to better understand the fishery and needs of government modelers by interacting with all the parties in the fishery and management sectors.

Research on this assemblage is timely, as the West Coast trawl fishery is currently under full Marine Stewardship Council assessment to receive the MSC ecolabel certification. The fishery is also undergoing a rationalization that will shift the management from a limited entry to a quota system starting in January 2011. As management changes and the deep sea is impacted by more anthropogenic (e.g. carbon sequestration, alternative energy experiments, oil drilling) and global (e.g. ocean acidification and warming) factors, it is important to gather information about this assemblage before the system is altered further, without baseline information.

Bathyraja trachura is a generalist predator on crustaceans, fishes, and polychaetes, feeding in benthic and benthopelagic habitats. Scavenging was apparent, ranging from digested pelagic fishes to a marine mammal. The diet of males and females of the skate did not differ; however, there was a significant shift in both the diet composition and trophic level of the skates as they increased in size. Latitude and year were found to explain some of the variation in the diet, as benthesicymid shrimps were more important

in the diet of skates collected from the south, and euphausiids from the north, though the biological relevance of this statistically significant result is unknown. This study found that carbon and nitrogen stable isotope analysis is a viable method for determining trophic relationships (relative position in the food web and habitat of prey) of deep-sea organisms. This method requires fewer samples and can be less resource intensive than stomach content analysis. When these two methods are combined to study an assemblage, they become very powerful and complementary tools to resolve detailed trophic relationships. This study is the first thorough study of the diet of this abundant deep-sea skate, and along with stable isotope analysis, has quantified trophic interactions of the dominant fishes in the food web of the continental slope.