

**NORTH PACIFIC RESEARCH BOARD PROJECT FINAL REPORT**

Analysis of fall, winter, and spring predation of key Bering Sea and Gulf of Alaska  
groundfish through food habits and stable isotope analysis

**NPRB Project 622 Final Report**

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## **Abstract**

Diet studies are a key component required for the success of multispecies and ecosystem modeling. In the Bering Sea and Gulf of Alaska, current multispecies and ecosystem models focusing on fisheries rely on food habits data collected from survey vessels between May and September. However, key ecological processes during fall, winter, and spring may have a strong impact on fish populations and model results. To address the data shortfall, this project shall have four components: (1) The laboratory analysis of historical samples of predator stomach contents collected by observers during non-survey months from the period 2000-2005; (2) Collection and laboratory analysis of predator stomach contents by observers during 2006, with collection focusing on data gaps; (3) extensive collection and analysis of stable isotopes (nitrogen and carbon) for predator and prey species collected from observers and from surveys; and (4) Synthesis of the resulting data on a monthly and geographical scale to provide inputs for future modeling efforts. Particular emphasis shall be placed on stable isotope analysis from different tissue types of both predator and prey species, in order to (a) develop an integrative, quantitative view of shifts from planktivory to piscivory in predators over the course of a year, and (b) estimate the impacts of predators on key, harvested prey species such as pollock, crabs, and other forage fish during non-summer months.

**Key Words:** Bering Sea, food habits, groundfish, walleye pollock, Pacific cod, arrowtooth flounder, stable isotopes, seasonal fish diets

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## Study Chronology

May 2006: NPRB grant approved as requested.

January 2007-January 2009: The following stomach samples (10,954 out of originally-projected 11,091) were analyzed in the laboratory (year and quarter indicates date of collection). Collection years of analyzed stomachs were 2003-2007, rather than 2001-2005 as originally planned:

	Quarter	Year					Grand Total
		2003	2004	2005	2006	2007	
Pacific Cod	1	66	610	625			1301
	2		169	73			242
	3	37	88	91	8		224
	4	5	9	3			17
Pacific Cod Total		108	876	792	8		1784
Walleye Pollock	1		72	227	148	129	576
	2			60	10	2	72
	3		6	1316	1346	918	3586
	4	13	50	121	14	156	354
Walleye Pollock Total		13	128	1724	1518	1205	4588
Arrowtooth Flounder	1	427	373	293	262	542	1897
	2	195	344	88	213	28	868
	3	426	488	334	185	302	1735
	4	8	54	15	5		82
Arrowtooth Flounder Total		1056	1259	730	665	872	4582
Grand Total		1177	2263	3246	2191	2077	10954

January 2006-January 2009: The following stable isotope samples were collected, processed, and analyzed (2,094 out of originally-projected 3,845; funds for balance returned to NPRB).

Species	2005	2006	2007	2008	Grand Total
Arrowtooth Flounder	20	62	359		441
Atka mackerel	10				10
Baythylagus sp.		5			5
C. bairdi		21	5		26
C. opilio			13		13
Capelin		29	30		59
Copepod		4			4
Crangonidae		5	7		12
Diaphus theta			7		7
Eulachon			15		15
Euphausiid		32	8		40
Flathead Sole	10				10
Giant grenadier			20		20
Hippolytidae		1			1
Kamchatka Flounder			34		34
Lampanyctus jordani		4			4
Lampanyctus regalis			1		1
Leuroglossus schmidti			4	10	14
Mysidacea		2			2
N. Rock Sole	10				10
Nannobrachium regale			1		1
Nothern Rockfish	10				10
P. Ocean Perch	10				10
Pacific cod	20	106	279		405
Pacific sandlance		20	25		45
Pandalidae		1	1		2
Pollock	40	172	596		808
Protomyctophum thompsoni			7		7
Red King Crab		18	5		23
Rossia pacifica			1		1
Stenobrachius leucopsarus			10	19	29
Wattled Eelpout			15		15
Yellowfin Sole	10				10
Grand Total	140	482	1443	29	2094

January 2009 Oral presentation at Alaska Marine Science Symposium by Mary Hunsicker: “A three-tiered approach for evaluating the predatory role of the commander squid in the eastern Bering Sea”

December 2009 Summarized diet data for 1982-2006 (including mapped data in GIS format as described in milestones) made available at:

<http://access.afsc.noaa.gov/REEM/WebDietData/DietTableIntro.php> and  
<http://www.afsc.noaa.gov/REFM/REEM/map/dietmap.php>.

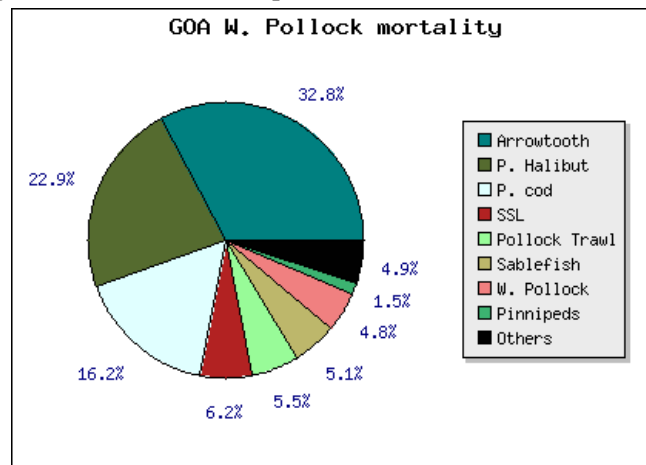
January 2010 presentation at Alaska Marine Science Symposium by Kerim Aydin: “A web for all seasons: an analysis of 30 years of seasonal and geographic variability in marine food webs through fish food habits and stable isotope analyses”.

May 2010 Paper accepted by Marine Ecology Progress Series (Chapter 1 of current report): “The predatory role of the commander squid, *Beryteuthis magister*, in the eastern Bering Sea: insights from stable isotopes and food habits”.

Sept 2010 Final report submitted.

## Introduction

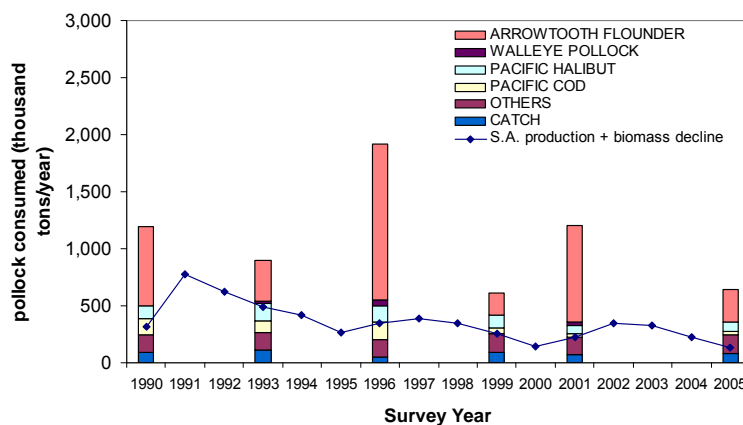
Groundfish predators in the Bering Sea and Gulf of Alaska represent a major source of mortality for key commercial species, especially for walleye pollock (*Theragra chalcogramma*) and commercial crab species. To that end, the North Pacific Fisheries Management Council (NPFMC) Scientific and Statistical Committee has recognized multispecies and ecosystem-level predator/prey models as an important tool for developing ecosystem approaches to management. A critical component for the construction and operation of these models is adequate diet data for predatory fishes. The Resource Ecology and Ecosystem Modeling (REEM) Program's Trophic Interactions Laboratory of the Alaska Fisheries Science Center has examined over 240,000 predator stomachs from the Alaska region since 1981 and these data, available in a fully-operational database, form the basis of many current predator/prey modeling efforts. Models currently in operation (Aydin et al. 2002; Jurado-Molina and Livingston 2002) have been used by REEM scientists as part of the NPFMC groundfish stock assessment process, to evaluate the potential for competition between predators and fisheries, and the effects that shifts in predator abundance might have on prey resources (e.g. Lowe et al. 2004; Dorn et al. 2005). The need for predator assessment in stock assessment processes may be seen in Fig. 1. While fishing is a noticeable component of walleye pollock mortality in the Gulf of Alaska, estimates of predation from current food habits data indicate that groundfish predators, notably arrowtooth flounder (*Atheresthes stomias*) Pacific halibut (*Hippoglossus stenolepis*) and Pacific cod (*Gadus macrocephalus*) are considerably larger sources of pollock mortality, accounting for almost 75% of the total mortality for the modeled time period.



**Figure 1.** Sources of mortality for Gulf of Alaska walleye pollock, averaged over survey periods 1990-1993, as calculated from food habits data and ecosystem models (Dorn et al. 2005).

As predation is a major source of pollock mortality, assessing variation in predator consumption should be an important part of ecosystem considerations for management. However, due to limited data, it is difficult to compare predator consumption rates with production rates of prey resources, and develop an integrated assessment. Fig. 2 shows current best estimates of predator consumption as calculated from NMFS groundfish survey biomass estimates combined with food habits data (diet composition and ration) of major predators in survey years (Dorn et al. 2005). The most recent single-species stock assessment estimate of total walleye pollock production plus measured declines is shown on the same scale. With perfect information, the height of the lines and the bars should match.

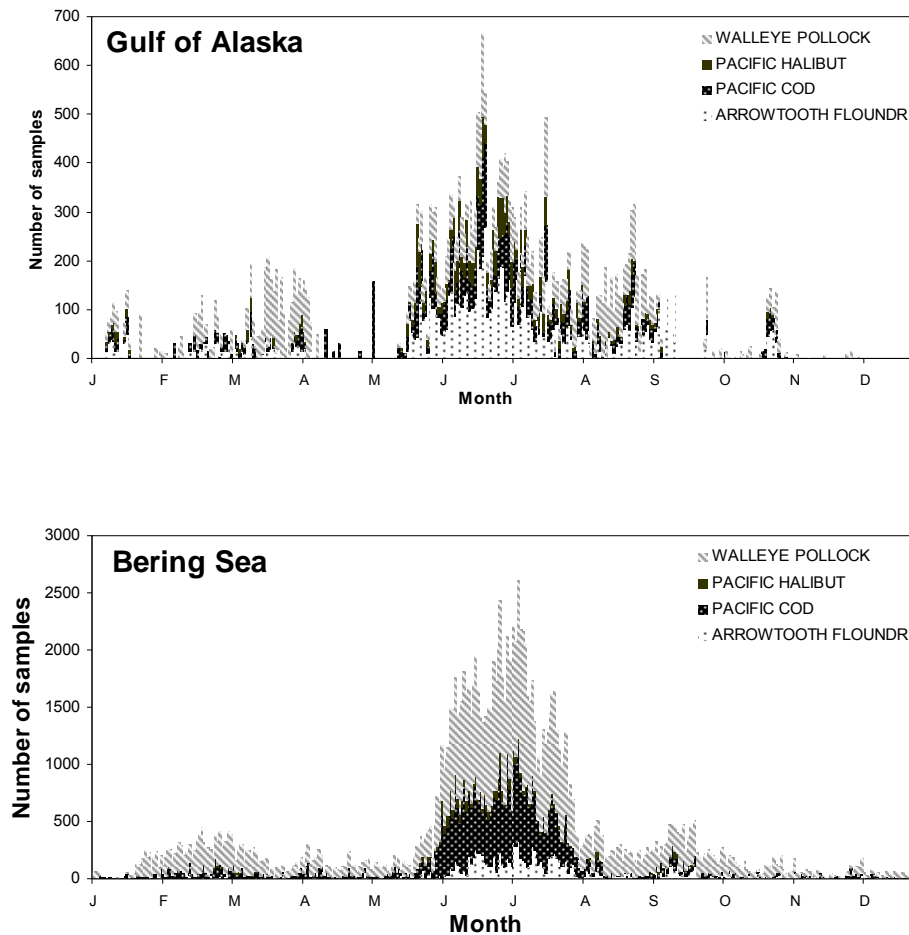
The mismatch between production and consumption estimates in Fig. 2 indicates possible mismatches in methodology between the single-species and multispecies models of the Gulf of Alaska. As shown in Fig. 2, estimates of consumption greatly surpass estimates of production. Overestimates in consumption rates could arise through seasonal differences in diets—while ration is seasonally adjusted, diet proportions are based on summer data. Also, better energetic estimates of consumption would improve these estimates. In terms of the stock assessment, underestimates of production could result from underestimating natural mortality, especially at ages 2-3, underestimating the rate of decline which occurred between 1990-present, or underestimates of the total biomass of pollock.





**Figure 2.** Total production of Gulf of Alaska walleye pollock (lines and points) 1990-2005, according to the most recent stock assessment, compared with independent consumption estimates of major predators on pollock (bars; Dorn et al. 2005).

This project focuses on resolving a specific missing piece: there is little food habits data outside of the May – September survey period for either the Gulf of Alaska or Bering Sea (Fig. 3). This lack of data results in consumption estimates which extrapolate across seasons and may lead to biases in consumption estimates of a particular prey type that is prevalent in a particular season.



**Figure 3.** Number of stomach records for four major groundfish in the Trophic Interactions Lab food habits database, shown by Julian Day for the period 1980-2005.

Currently available food habits data indicates that arrowtooth flounder, Pacific halibut, Pacific cod, and walleye pollock become more piscivorous in non-summer months with the decreased availability of zooplankton. However, the lower levels of prey and colder temperatures during non-summer may lead to considerably lower feeding rates; the net effect of these two opposing changes has not been determined. This project aims to resolve this question, in part through analysis of predator stomachs collected by fisheries observers during non-summer months, and in part through an extensive analysis of stable isotope fractionation in groundfish predators and prey species from the Bering Sea and the Gulf of Alaska.

### *Methodology*

This project shall have three components; (1) The laboratory analysis of historical samples of predator stomach contents collected by observers during non-survey months from the period 2001-2005; (2) Collection and laboratory analysis of predator stomach contents by observers during 2006, with collection focusing on data gaps; (3) extensive collection and analysis of stable isotopes (nitrogen and carbon) for predator and prey species collected from observers and from surveys. The results of these studies will be submitted for publication to peer-reviewed articles as described in the Products and Milestones section, below, and placed in database and GIS format to be made available to current and future ecosystem modeling efforts.

### *Component #1: Historical Analysis of food habits*

Food habits samples collected by observers on fisheries vessels in the Bering Sea and Gulf of Alaska groundfish fisheries represent an important resource for non-summer diet data. Currently, the Trophic Interactions Lab has a total of 11,091 unanalyzed formalin-preserved stomach samples collected by observers between 2001 and 2005, primarily collected in winter and spring months (Table 1). Component #1 will be conducted by contracting a biologist with expertise in groundfish food habits to analyze these stomachs.

**Table 1.** Stomach samples currently preserved in formalin in the Trophic Interactions Lab.

<b>Species</b>	<b>2001</b>	<b>2002</b>	<b>2003</b>	<b>2004</b>	<b>2005</b>
Arrowtooth					
Flounder	504	715	847	1,051	145
Pacific Cod	775	122	739	712	482
Walleye Pollock	1,111	1,005	1,576	1,307	0
<b>TOTAL</b>					<b>11,091</b>

Stomach samples will be transferred to ethanol before being analyzed. Each stomach sample will be rinsed of ethanol and the contents removed. Excess liquid from the stomach will be blotted dry and the contents weighed to the milligram. Each prey item in the sample will be identified to the lowest practical taxon. Each taxon, or taxonomic group, will be weighed to the nearest milligram, counted and assigned a state of digestion. When possible, lengths will be taken for fish and commercially important species of crabs. These data along with associated haul information will then be entered into a database using MS Access.

The overall diet data will be summarized in diet tables showing percent frequency of occurrence (%FO), percent count (%C) and percent weight (%W) for each identified taxon. Ontogenetic shifts in diet are well documented for many fish species (Lang et al. 1991, Yang and Nelson 2000). To examine the variation of diet with size, the diet data will be summarized by four 20 cm length groups. The prey for each size group may be assembled into broad taxonomic assemblages to facilitate clear comparison between size groups. Figures and tables will be produced to illustrate ontogenetic dietary trends. Consumption maps by month will also be created for commercially important prey species showing the geographic position of each haul sampled and indicate the percent weight in the diet of the given species, using data from the time period 1981-2005.

*Component #2: Collection and analysis of 2006 food habits samples*

Currently, the Trophic Interactions Lab collects 1,000-1,200 predator stomachs per year from fisheries observers; while these collections have been during non-summer months, they have not to date targeted specific missing time windows or predator size classes critical to models. To this end, collection of samples for 2006 and 2007 will be targeted at specific data gaps from past

sampling. In addition, a protocol will be developed for 2007 that will include the collection of frozen samples by observers for use in stable isotope analysis as described in component #3, below.

*Component #3: Stable isotope analysis of predators and prey*

Direct diet analysis can be problematic, as it is sometimes difficult to identify well digested stomach contents. Additionally, different prey are digested and evacuated at various rates, which may result in biases in the diet compositions (Davenport and Bax 2002; Michener and Schnell 1994). Stable isotope analysis has been used as a complementary tool for many diet analysis studies in marine ecosystems (Davenport and Bax 2002; Michener and Schnell 1994). The ratio of the light and heavy isotopes of nitrogen ( $^{14}\text{N}$ ,  $^{15}\text{N}$ ) provides an indication of trophic level because  $^{15}\text{N}$  fractionates as it moves up food webs. Isotopic ratios are typically expressed as  $\delta$  units, which indicate the per mil deviation from a standard (for nitrogen, it is atmospheric N). In general,  $\delta^{15}\text{N}$  increases 3-3.5 units per trophic level. In contrast, the ratio of light to heavy carbon ( $^{12}\text{C}$  and  $^{13}\text{C}$ ) is more conservative through trophic transfers, and indicates the photosynthetic pathway used to fix carbon.

Taken together, and with data from direct stomach samples, it is possible to generate more precise estimates of the percent contribution of particular prey items to predator diets. For this project, a samples from 14 classes of predators or prey will be targeted (Table 2), this represents a majority of prey components for arrowtooth flounder, Pacific cod, walleye pollock, and Pacific halibut.

Samples for stable isotope analysis will be collected from several sources. We will rely primarily on samples collected during the 2006 AFSC summer survey of the eastern Bering Sea shelf and slope regions; collections during these cruises shall be conducted by the Trophic Interactions Lab Manager (time in-kind contribution). Additional samples will be collected during the 2007 AFSC Gulf of Alaska survey. A protocol will be developed and implemented for frozen collections by fisheries observers in other seasons in 2007. For all samples, tissue, bone, otolith, and whole full stomachs of Pacific cod, walleye pollock, arrowtooth flounder, Pacific halibut, and squid species will be collected and frozen shipboard and returned to the Trophic Interactions Laboratory.

For fish samples, muscle, bone, and otolith samples will be prepared for analysis. Initially, whole otoliths will be ground to produce an integrated stable isotope signature for the life of the fish; as

these structures represent cumulative lifetime growth, summer collections should provide reasonable samples for analyzing food habits for previous non-summer months. Prey will be taken from frozen stomachs, cleaned, and undigested prey will be ground to produce material for further stable isotope analysis.

**Table 2.** Isotope sample size targets over the course of the project, assuming each sample provides results for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Several samples may be taken from a single fish as described in the text.

<b>Species Group</b>	<b>Sample Size</b>
Copepods	275
Euphausiids	275
Squid	275
Myctophids	274
Capelin	275
Sandlance	275
Pollock	275
Cod	275
Halibut	275
Arrowtooth Flounder	275
King crab	274
Bairdi	274
Opilio	274
Other benthos	274
<b>TOTAL</b>	<b>3845</b>

Particular emphasis will be placed on squid species, which are both an important predator and prey in the Bering Sea and Gulf of Alaska. The method for squid will take advantage of the chronological deposition and layering of eye lens material which preserves a record of past food consumption. Thus, by measuring  $^{15}\text{N}$  in these layers, and relating lens layers to particular body sizes (regression of eye lens radius and squid body size), it is possible to gain valuable insight

into the changes in trophic level over ontogeny. This method was recently developed and successfully applied in the study of *Ommastrephes bartramii* and *Sthenoteuthis oualaniensis* in the sub-tropical Pacific (Parry, M.P. 2003. The trophic ecology of two ommastrephid squid species, *Ommastrephes bartramii* and *Sthenoteuthis oualaniensis*, in the north Pacific sub-tropical gyre. Ph.D. thesis. University of Hawai'i at Manoa). Both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures will be measured from squid muscle tissue and from five to ten layers of the squid eye lens.

Stable isotope analysis will be conducted through a laboratory contracted by the Alaska Fisheries Science Center. For a pilot study, currently being conducted by the P.I., an analysis contract was awarded by competitive bidding in August 2005 to the Colorado Plateau Stable Isotope Laboratory (<http://www4.nau.edu/cpsil/>); for the purposes of this proposal, it is assumed that current (November 2005) costs and protocols of this particular laboratory are valid. Trophic Interactions Lab personnel will perform preliminary grinding and preparation of samples which will be shipped to the laboratory for final preparation and analysis, with electronic tables of results provided approximately 8 weeks from shipping.

The combination of food habits and stable isotope results will be summarized as shifts in trophic level throughout a single year in the Gulf of Alaska and Bering Sea for the four major groundfish predators: arrowtooth flounder, Pacific halibut, Pacific cod, and walleye pollock. By comparing the results of both carbon and nitrogen signatures in predators with measured stomach contents, consumption by prey taxa may be triangulated using these data. These combined diet estimates will be compared to current estimates of consumption and stock assessment estimates of prey production; in addition, they shall be presented as a combined seasonal/spatial map of foraging in a GIS format suitable for future modeling efforts. Additional effort shall be placed into coordinating results with those of lower trophic levels, especially production estimates related to sea ice as described in Lovvorn et al. (2005). Finally, these results shall be published in a NOAA Technical Memorandum (peer-reviewed) and 1 or more articles will be submitted to peer-reviewed journals throughout the course of this project.

## **Overall Objectives**

- Milestone #1:* The completed laboratory analysis of observer-collected stomach samples for five years of samples, including quality control and entering into the Trophic Interaction Lab food habits database.
- Milestone #2:* Collection of stable isotope samples from June 2006 through May 2007 (actual dates January 2007-December 2008).
- Milestone #3:* Processing and analysis of stable isotope samples.
- Milestone #4:* Production of seasonal maps of food consumption and stable isotope values in GIS and table format suitable for inclusion in multispecies and ecosystem models.
- Milestone #5:* Submission of document to peer-review, summarizing results produced in Milestone #4

## CHAPTER 1

The predatory role of the commander squid, *Berryteuthis magister*, in the eastern Bering  
Sea: insights from stable isotopes and food habits

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## ABSTRACT

Squid are an important component of many marine food webs and they can impact other species through predation and competition. However, quantifying their influence on other food web components requires knowledge of their trophic position and trophic ontogeny, which are unknown in many ecosystems. The eastern Bering Sea (EBS) is a highly productive region that supports large commercial fisheries, and a modicum of knowledge exists on the ecological role of squid in this region. Here, we combine stomach content and stable isotope analyses of muscle tissue ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) to identify the feeding ecology of the commander squid, *Berryteuthis magister*, in the EBS continental slope ecosystem. We also use a novel methodology to elucidate potential finer scale variation in squid trophic ecology by reconstructing feeding chronologies of individual *B. magister* from concentric eye lens layers. Our analyses indicate that the position of *B. magister* in the EBS food web increases by approximately one trophic level between juvenile and adult stages. Also, in contrast to many squid species, we found that predation by *B. magister* is not constrained by prey body size and that *B. magister* are more likely to share prey resources with commercially valuable fishes, particularly walleye pollock (*Theragra chalcogramma*), than to prey upon their juvenile stages. Further, the reconstructed feeding chronologies indicate that there is substantial variability in squid feeding patterns that are not captured on the time scales of the conventional analyses. Together, the findings of this study contribute to a better understanding of the ecological role of *B. magister* and the trophic linkages and energy flow within the EBS food web.

Keywords: Squid, trophic interactions, stable isotopes, prey size spectra, feeding chronology, *Berryteuthis magister*, eastern Bering Sea

## INTRODUCTION

Squid occupy a central position in marine food webs. They are a valuable prey resource for fishes, seabirds, and marine mammals and are also voracious predators of crustaceans, squid, and fishes (Clarke 1996, Croxall & Prince 1996, Smale 1996, Klages 1996). Because of their high feeding rates and generalist feeding strategy, squid can potentially exert trophodynamic control on the recruitment of the early life stages of fishes (Dawe 1988, Rodhouse & Nigmatullin 1996, Hunsicker & Essington 2008). Moreover, their importance in food webs may be changing in response to fisheries-induced alterations to trophic structures and climate change (e.g., Humboldt squid (*Dosidicus gigas*); Field et al. 2007, Zeidberg & Robinson 2007). An

understanding of the potential impacts of these changes on marine systems is presently hampered by serious gaps in our understanding of the ecological role of squid.

Similar to many ecosystems, we have a limited understanding of the trophic roles of squid in the eastern Bering Sea (EBS). The EBS is a highly productive ecosystem that supports some of the world's largest commercial fisheries as well as the production of species of conservation interest (NRC; National Research Council 1996). Over the past few decades the structure of this ecosystem has shifted in response to human activities and changing environmental conditions (NRC 1996, Alexander 1999, Springer 1999). To understand how anthropogenic and natural forcing may impact the trophic linkages and energy flow within this system, much effort has focused on identifying the trophic relationships of commercial and conservation species. However, the ecological roles of non-target species, such as mesopelagic squid, have been understudied.

The goal of our work is to provide a better understanding of the trophic ecology of the commander squid, *Berryteuthis magister* (Cephalopoda, Gonatidae), by examining its predatory role in the EBS continental slope ecosystem. *Berryteuthis magister* is the most abundant squid species in the EBS (Radchenko 1992, Sinclair & Stabeno 2002) where it inhabits the outer edge of the continental shelf and slope, reaching depths of 1500 m (Nesis 1998). Juveniles mostly occur in midwater at mesopelagic depths while adults mainly inhabit the bottom layers in the upper bathyal zone (Nesis 1997, 1998, Arkhipkin 1998): both life stages undertake diel vertical migrations with night ascent (Fedorets 1987, Gorbatenko et al. 1995; Arkhipkin 1998). Previous work indicates that *B. magister* is a key component in the food webs of the western and central Bering Sea (Frost & Lowry 1981, Lowry et al. 1982, Radchenko 1992, Nesis 1997, 1998, Aydin et al. 2007). Food web models of EBS also indicate that squid are an important link in the transfer of energy from lower to higher trophic levels (Aydin et al. 2007). Further, squid can be important predators or competitors of juvenile stages of gadoids and other high trophic level fishes (Hunsicker & Essington 2006, 2008, Field et al. 2007) and commercially valuable gadoid fishes, particularly walleye pollock (*Theragra chalcogramma*) dominate the fish biomass in the EBS.

Knowledge of the trophic ontogeny of *Berryteuthis magister* and the size-structure of their feeding relationships is paramount for determining their ecological role. Body size dictates predator-prey relationships in many marine systems (Lundvall et al. 1999, Jennings et al. 2001, Shin & Cury 2004), and squid exhibit ontogenetic, size dependent patterns in predation (Collins & Pierce 1996, Lordan et al. 1998, Hunsicker & Essington 2006). By identifying the prey size spectra of *B. magister* we can determine the prey species and sizes that are vulnerable to squid predation. This information is valuable for assessing the potential consequences of *B. magister*

feeding habits and for exploring how the strength of their trophic interactions may change if predator and (or) prey body sizes are altered by ecosystem perturbations (Woodward et al. 2005).

Identifying the feeding habits of squid is challenging owing to their feeding behavior and rapid digestion of prey (Nixon 1987; Dawe et al. 1997). Squid masticate their food and therefore may only consume a portion of their prey at feeding. Here, we attempted to overcome those challenges by using multiple pathways to characterize the trophic role of *Berryteuthis magister*. We used traditional stomach content analyses to identify prey taxon, estimate the prey size spectra of *B. magister* and determine how prey composition changes with squid ontogeny. Stomach content analysis provides high detail on prey contents; however, due to squid feeding attributes it does not provide a good assessment of their trophic positions (i.e., the average level, relative to primary producers, at which an organism feeds). Also, this analysis only provides information on an animal's last feeding event and reveals no information on the feeding habits of sampled predators with empty or unidentifiable stomach contents. In contrast, stable isotope ratios ( $^{15}\text{N}:$  $^{14}\text{N}$  and  $^{13}\text{C}:$  $^{12}\text{C}$ ) of animal tissues can provide more precise estimates of long term average trophic position. These ratios provide less detail on digested prey items, but they provide a measure of feeding that is integrated over several weeks or months (Ruiz-Cooley et al. 2006). Stable isotope analyses have been a valuable tool for investigating the trophic ecology of squid (Takai et al. 2000, Cherel & Hobson 2005, Ruiz-Cooley et al. 2006, 2010, Parry 2008, Cherel et al. 2009). We used stable isotopes as a complementary method to identify ontogenetic shifts in *B. magister* trophic position and to evaluate their trophic position with respect to marine fishes inhabiting the EBS.

In addition to these two conventional approaches, we further explored whether reconstructed feeding chronologies from concentric eye lens layers can be used as an alternative method to evaluate the ecological role of *Berryteuthis magister*. Feeding chronologies from squid hard parts, such as eye lenses (Parry 2003) and gladii (Ruiz-Cooley et al. 2010) are a valuable tool because they offer several measures of individual feeding events that are integrated over time scales intermediate to stomach content and stable isotope analysis of muscle tissue. For instance, fish lens cells are nearly ametabolic because most of the cells (all but those in the youngest, differentiating layer) lack the cellular functions of protein synthesis and catabolism (Wistow & Piatigorsky 1988, Nicol 1989, Horwitz 1992, Dove 1999). The same is likely to be true for squid eye lenses (see West et al. 1995); thus, with little to no protein turnover in the lens cells isotope signatures laid down in the lens layers may essentially be locked in place during their formation (Parry 2003). From a sampling standpoint, reconstructed feeding chronologies could improve our ability to evaluate the feeding ecology of squid life stages or species that are difficult to capture

with traditional sampling gear. Similarly, feeding chronologies would allow us to explore the trophic ontogeny of squid with far fewer samples than would be needed for stomach content analysis or muscle tissue stable isotope analysis.

In this study, we address the following questions to better understand the role of *Berryteuthis magister* in the EBS continental slope ecosystem. Do *B. magister* exhibit ontogenetic shifts in prey composition and trophic position? Are the predation patterns of *B. magister* dictated by size-constraints? Is there evidence of predation by *B. magister* on higher trophic level fishes, including species of commercial value? Are there variations in *B. magister* trophic patterns revealed by feeding chronologies that are not captured by the conventional analyses of predator food habits? Our findings provide a strong foundation for identifying the potential influence of *B. magister* on other nekton through direct and indirect food web pathways.

## METHODS

### Sample collection

*Berryteuthis magister* were collected near the edge of the continental shelf and slope in the eastern Bering Sea (Figure 1). Squid were sampled during summer and winter seasons with the intention of collecting a wide size range to account for seasonal and ontogenetic shifts in diet composition. Summer samples were collected through research surveys conducted by NOAA Fisheries, Alaska Fisheries Science Center (NOAA - AFSC; June - July 2004) and the University of Washington (August 2007). The summer samples were collected mainly from daytime bottom trawls (cod end mesh size 13 mm) at discrete depths ranging from 330 to 755 meters in summer 2004 and 200 to 400 meters in summer 2007 (Table 1). The winter samples were collected through the North Pacific Groundfish Observer Monitoring Program (January - March 2007). These squid were randomly sampled by observers from pelagic trawls primarily targeting pollock (cod end mesh size ~75-115 mm) and fishing at discrete depths ranging from 350 to 985 meters (Table 1). All samples were frozen and stored at -20°C after collection. Once defrosted, squid dorsal mantle lengths were recorded to the nearest 1 mm and total wet weights were measured to the nearest 0.1 g.

### General Diet Analysis

Approximately 700 *Berryteuthis magister* collected during summer 2004, summer 2007 and winter 2007 were dissected for stomach content analysis (Table 1). Prey items of *B. magister* were identified from undigested hard parts found in squid stomachs. Digested fish were identified from otoliths, scales, and bones and squid prey were identified from beaks, gladii,

hooks, and statoliths (Vinogradov & Noskov 1979, Santos & Haimovici 1998, Hunsicker & Essington 2006). Crustaceans were identified by eyes, appendages, mandibles, and exoskeletons (Karpov & Cailliet 1978, Santos & Haimovici 1998). Prey items were identified using a dissection microscope and were initially grouped by visual identification of hard parts and soft tissues as fish, squid, crustaceans, or unknown. We recorded the wet weights of squid stomach masses and individual prey items. We also noted freshly digested prey items and excluded them from our analyses to omit bias due to predation inside trawl nets. Prey hard parts were stored dry or in 80% ethanol for subsequent taxonomic classification. The diet composition of *B. magister* was quantified by calculating the percent frequency of occurrence of each prey item in the squid stomach contents. Squid masticate their food, thus it is nearly impossible to quantify their prey composition by percent number or mass.

We used logistic regression analysis to detect ontogenetic shifts in the diet of *Berryteuthis magister*. Separate analyses were conducted to gauge the influence of body size on the frequency of occurrence of prey categorized as fish, squid, or crustacean. This analysis relates the probability of occurrence of a specific prey item (fish, squid or crustaceans) to the mantle lengths of *B. magister* collected in the summer and winter seasons. We evaluated the ontogenetic shifts in diets during both seasons by calculating the 95% confidence intervals of the slope. We then repeated this analysis to examine whether *B. magister* might also exhibit dietary shifts related to capture depth or location. We simply replaced the explanatory variable with capture depth and latitude.

### **Prey Size Spectrum**

Fish and squid consumed by *Berryteuthis magister* during summers 2004 and 2007 and winter 2007 were identified to lowest possible taxon by comparing the morphological features and size ratios of digested fish otoliths and squid beaks to those in the reference collection of the National Marine Mammal Laboratory (NMML / NOAA / AFSC). The sizes of fish and squid prey were then estimated using species - specific otolith length to body length relationships and lower rostral beak length (LRL) to mantle length relationships, respectively (W. Walker unpubl. data, Zeppelin et al. 2004, Gudmundson et al. 2006). We note that the beaks of *B. magister* and *Gonatopsis borealis* with LRL of < 1 mm are nearly indistinguishable. Similarly, the small beaks (LRL < 1 mm) of *Gonatus* species as well as *Gonatus middendorffi* and *Gonatus madokai* are difficult to discern from each other. Thus, beaks with LRLs < 1 mm were identified to coarser taxonomic groups, i.e., *B. magister* / *G. borealis*, *Gonatus* sp., or *G. middendorffi*/*G. madoakai*.

We used quantile regression to analyze the relationship between *Berryteuthis magister* mantle lengths and their prey sizes. The 10th and 90th quantiles were used as the upper and lower bound regression quantiles in this analysis (Rogers 1992, Scharf et al. 1998). This technique has been used previously to estimate the relationship between squid body size and the minimum or maximum sizes of their prey (Hunsicker & Essington 2006). Further, we used the Student's *t* test to determine if there was a significant difference in upper bound regression lines of fish and squid prey. A difference in slope values may indicate that maximum prey size is dependent upon the prey type (Scharf et al. 2000, Juanes 2003).

### Stable Isotope Analyses

To determine the trophic position of *Berryteuthis magister* in the EBS, we analyzed  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures of squid mantle muscle tissue. The muscle tissue of squid reflects predator feeding habits (Stowasser et al. 2006) and mantle tissue has been used to quantify the relative trophic position of squid in past studies (Takai et al. 2000, Parry 2008). A small portion (~13 cubic mm) of tissue was excised from the mantles of 50 squid caught in summer 2004 and 50 squid collected in winter 2007. We did not analyze the isotope signatures of squid collected during summer 2007. The tissue samples were dried at 64°C, ground with mortar and pestle into a homogeneous powder, and weighed according to the guidelines of the Colorado Plateau Stable Isotope Laboratory (CPISL). Lipids were not removed from squid tissues prior to the stable isotope analysis. Lipids do not contain nitrogen and thus do not affect nitrogen isotope signatures, though variations in the lipid content within tissues can significantly influence  $\delta^{13}\text{C}$  values and hence bias the interpretation of the carbon isotope results (Sweeting et al. 2006, Kiljunen et al. 2006). However, the C:N ratios of the squid tissues analyzed in this study ranged between 3 to 3.5, indicating that the lipid content in these tissues was quite low (Kiljunen et al. 2006). For that reason, we expect that the  $\delta^{13}\text{C}$  values of *B. magister* muscle tissue would be not be confounded by individual-variation in lipid densities. The isotope analyses were conducted at the CPISL and were carried out in continuous - flow mode using a Thermo - Finnigan Delta<sup>plus</sup> Advantage gas isotope - ratio mass spectrometer interfaced with a Costech Analytical ECS4010 elemental analyzer. Isotopic ratios are expressed using standard  $\delta$  - notation where  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$  and R is the ratio of  $^{15}\text{N}:^{14}\text{N}$  or  $^{13}\text{C}:^{12}\text{C}$  in the sample and standards, respectively (atmospheric nitrogen and Pee Dee Belemnite for carbon). The data were normalized using four internationally accepted isotope reference standards (IAEA CH6, CH7, N1, and N2). External precision on laboratory standards was  $\pm 0.20\text{‰}$  or better for  $\delta^{15}\text{N}$  and  $\pm 0.10\text{‰}$  or better for  $\delta^{13}\text{C}$ . We used the Spearman rank correlation test to determine whether the  $\delta^{15}\text{N}$  and

$\delta^{13}\text{C}$  signatures of *B. magister* muscle tissue differed by capture depth or location. Regression analysis was also done to evaluate the relationship between squid mantle lengths and the isotopic signatures of muscle tissues.

To evaluate the trophic level of *Berryteuthis magister* within a broader, ecosystem context, we compared  $\delta^{15}\text{N}$  signatures of juvenile (<120 mm) and adult squid ( $\geq 120$  mm, i.e. size at maturation; Nesis 1997) collected in summer 2004 to the  $\delta^{15}\text{N}$  signatures of the dorsal white muscle tissue of commercially important fishes inhabiting the eastern Bering Sea (Kurle CM, Sinclair ES, Edwards AE, and Gudmundson CJ unpubl. data). The fishes were collected in summer 2005 through the NOAA Fisheries Research Survey and the North Pacific Groundfish Observer Monitoring Program. The fishes were captured at different times and locations than the *B. magister* samples analyzed in this study. The species include juvenile and adult walleye pollock (*Theragra chalcogramma*), adult yellowfin sole (*Limanda aspera*), adult northern rock sole (*Lepidopsetta polyxystra*), adult flathead sole (*Hippoglossoides elassodon*), adult arrowtooth flounder (*Atheresthes stomias*), and adult Pacific cod (*Gadus macrocephalus*). Again, lipids were not removed from fish tissues prior to stable isotope analysis, thus comparisons between squid and fish  $\delta^{13}\text{C}$  signatures could be biased due to differences in lipid content. For that reason, we limited our comparisons between squid and fishes to  $\delta^{15}\text{N}$  signatures, which are not affected by lipids (Kiljunen et al. 2006). Our comparisons were also restricted to the summer season because at the time of our study the only available estimates of fish  $\delta^{15}\text{N}$  signatures were from fishes captured during this period. With the exception of pollock, we were not able to stratify the  $\delta^{15}\text{N}$  signatures of the fishes by size or life stage because the fishes were of similar sizes. Box plot graphs of the summer  $\delta^{15}\text{N}$  values of *B. magister* and fishes were created to compare their trophic positions.

### **Feeding chronology through eye lens analysis**

Following the methods of Parry (2003), we evaluated the squid feeding chronology by examining changes in isotopic signatures ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) as a function of eye lens radius. The squid eye is a spherical structure comprised of distal and proximal lenses and has a recognizable layering of thin tissue. Similar to many fishes, squid exhibit chronological deposition of concentric layers to the outer surface of their eye lens as they grow (Nicol 1989, West et al. 1995, Dove 1999). The center of the lens contains the oldest layer whereas the youngest layer lies on the outer rim (Figure 2). The proximal lens is the larger of the two lenses and we analyzed the isotopic signatures of this tissue to reconstruct the feeding chronologies of 20 summer squid (year 2004 only) and 21 winter squid (year 2007). Preparations for isotope analysis proceeded in three

steps. First, we cleaved the eyeball of an adult squid while it was thawing to isolate the proximal lens and measured the longest width of this lens using an ocular micrometer on a dissecting microscope at 8X power. Mantle lengths of the squid ranged from 220 to 310 mm and total widths of their proximal lens ranged from 6.5 to 10.5 mm. Second, a segment of tissue layers was excised from the outermost edge of the lens, rinsed with distilled water, and set aside for subsequent preparation. It was nearly impossible to remove single layers of lens tissue, thus we removed relatively precise fixed groups of lens layers to get an approximation of intermediate feeding chronology (Figure 2). To ensure that there was a sufficient amount of tissue for the isotope analyses each segment was  $> 0.3$  mm in width. The number of segments extracted from an individual eye lens ranged between six and eleven among squid samples due to size differences of the squid eyes. Third, upon removal of each segment, the width of the proximal lens was re-measured. To avoid contamination, the scalpels and forceps used to separate and measure the individual lens layers were cleaned with ethanol following each excision. Also, if the proximal lens began to dry, making it more difficult to excise lens segment, we rinsed the eye lens with distilled water. The second and third steps were repeated until the final lens segment at the center of the squid eye was removed. All lens segments were dried, ground, weighed (0.6-1.2 mg), and analyzed for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signature in the same manner as the *B. magister* mantle muscle tissue.

To evaluate the trophic record of individual *Berryteuthis magister*, we related the isotopic signatures of the lens segments to particular squid body sizes. First, a regression for squid eye lens width to squid mantle length was developed from the proximal lenses of 50 summer squid (year 2004 only) and 50 winter squid (year 2007) of various body sizes. Squid mantle lengths ranged from approximately 65mm to 135mm and total widths of the proximal lenses ranged from 2.5 to 10.5 mm. For each lens segment that was analyzed for isotopic signatures, we used these regressions to predict the approximate length range of the squid at the time a lens segment was laid down. Next, we generated coarse estimates of the time scales over which the lens segments were laid down. Length at age was estimated for individual *B. magister* captured during summer 2004 (Drobny 2008), however it was not possible to fit a growth model to the data because the estimates were highly variable. This precluded us from using growth curves to back-calculate the age of squid to the time when the tissues were formed. Thus, we instead calculated the time over which the prey isotope signatures were integrated within the lens segment by dividing the estimated age of an individual squid (Drobny 2008; ~120 to 180 days) by the number of lens segments examined. Age estimates of *B. magister* were only available for the summer 2004 samples, therefore we used the average integration time estimated from these samples to back-



calculate, using a constant growth assumption, the approximate time at which the first lens segments of winter- and summer-caught squid were laid down.

We used a simplified approach to classify the observed patterns of squid feeding chronologies. Our intent was to determine if the  $\delta^{15}\text{N}$  signatures revealed by the individual chronologies exhibited the monotonic increase with mantle length that is often observed from isotopic signatures of squid muscle tissue (e.g., Parry 2008, Cherel et al. 2009). We regressed the  $\delta^{15}\text{N}$  values of the lens segments on the predicted mantle lengths and fit linear models to the data. We then quantified the frequency of individual records that demonstrated positive slope values that were at least marginally statistically significant ( $p < 0.10$ ) and those that did not. Further, we estimated the average slopes of individual eye lens  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values among and within seasons by fitting linear mixed effects models to the isotope data. The  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  value was the response variable and squid mantle length and individual squid ID were the fixed and random effects, respectively. We also determined if the average relationship of lens  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values and estimated squid mantle lengths differed between seasons by adding season and season\*mantle length as fixed effects terms to the linear mixed effects models. The interaction term is useful for identifying whether the average slopes differed between winter and summer seasons. We fit the models using the nlme package in R version 2.10.2 (R Development Core Team 2010). Lastly, we examined whether the average slopes of eye lens  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values among the individuals' were similar to the slopes estimated from muscle tissue analysis.

## RESULTS

### General Diet Analysis

The mantle lengths of the summer 2004 and summer 2007 squid samples ranged from 62 mm to 334 mm and 90 mm to 335 mm, respectively, and the winter 2007 samples ranged from 100 mm to 335 mm (Table 1). Of all of the stomachs analyzed, 56% contained contents that were identifiable to coarse taxonomic groupings (i.e. fish, squid, or crustacean), 13% contained only unidentifiable prey items, and 31% were empty (Table 1). The percent occurrence of fish in summer and winter *Berryteuthis magister* stomach contents was the highest of all the prey items followed by squid and crustaceans. The percent occurrence of fish in squid diets was as high as 47%. The frequencies of empty *B. magister* stomachs or those with only unidentifiable prey items were relatively similar across summer and winter seasons (Table 1).

Results of the logistic regression analysis indicated that *Berryteuthis magister* underwent an ontogenetic shift in diet composition. The consumption of fish and squid by *B. magister* increased with mantle length, while the consumption of crustaceans decreased. This ontogenetic

shift was evident during the summer (years 2004 and 2007 combined) and winter (year 2007) seasons (Figure 3). The regression analysis highlighted the substantial frequency ( $> 0.10$ ) of piscivory by *B. magister* over all body sizes. In the summer, the median estimates of the probability of piscivory increased from 10% to 65% from the smallest to largest observed squid body sizes. For winter, the median estimates ranged from 31% for squid at mantle lengths of 130 mm to 51% for the largest size squid. The regression analysis further revealed a seasonal difference in the pattern of piscivory by *B. magister* with capture depth (data not shown). The median estimates of the probability of piscivory for summer *B. magister* rapidly decreased from 36% to 4% over the primary capture depths (i.e., mean  $\pm$  1 SD; 250-450 m). In contrast, the frequency of piscivory by winter *B. magister* was higher at deeper capture depths and gradually declined with increasing depth: the median estimates of the probability of piscivory ranged from 54% to 40% over the primary capture depths (i.e., mean  $\pm$  1 SD; 350-550 m). The consumption of squid by *B. magister* in relation to capture depth followed the same trends as those described for fish. The median estimate of the probability of squid consumption by *B. magister* declined with capture depth from 42% to 8% in the summer and from 23% to 11% in the winter. The frequency of consumption of crustaceans increased slightly from 10% to 16% with summer capture depths and was  $< 4\%$  across all winter capture depths. We did not observe any meaningful shifts in the diets of *B. magister* with respect to latitude at capture.

Identification of digested hard parts revealed that *Berryteuthis magister* specimens preyed upon deepwater slope fishes and a variety of squid species, including conspecifics. The percent occurrence of otoliths and statoliths in non-empty *B. magister* stomachs was 16% and 9%, respectively. We identified a total of 323 otoliths and 118 statoliths, but the northern smooth-tongue (*Leuroglossus schmidti*) was by far the most common prey identified from the stomach contents (Table 1), representing 95% of the total number of identified otoliths. The remaining otoliths belonged to northern lampfish (*Stenobranchius leucopsaurus*), brokenline lanternfish (*Lampanyctus jordani*), northern pearleye (*Benthabella dentata*), lumpsuckers (*Cyclopteridae* sp.), snailfishes (Liparids), and pollock (*Theragra chalcogramma*). In contrast to the fish otoliths, the squid beaks consumed by *B. magister* were not dominated by a single taxonomic group (Table 1). Approximately 14% of the lower beaks were identified as *B. magister*, 7% were *Gonatus onyx*, and 3% were *Gonatus borealis*. The coarser taxonomic grouping of *Gonatus* sp. represented 40% of the digested beaks whereas the *B. magister* / *G. borealis* and *G. madokiai* / *G. middendorffi* groups represented 31% and 5% of the beaks, respectively.

### Prey Size Spectrum

Quantile regression analysis of all prey sizes consumed by *Berryteuthis magister* during summers 2004 and 2007 and winter 2007 revealed a non-significant slope of the upper bound (90<sup>th</sup> quantile;  $p=1.000$ ) and lower bound regression lines (10<sup>th</sup> quantile,  $p=0.339$ ; Figure 4). However, the regression analysis of *B. magister* prey sizes differed among seasons. We found non-significant upper (90<sup>th</sup> quantile,  $p=0.610$ ) and lower bound regression lines (10<sup>th</sup> quantile,  $p=0.071$ ) of summer prey sizes, whereas the winter prey sizes showed a positive, significant slope of the upper bound regression line (90<sup>th</sup> quantile,  $p<0.001$ ) and a non-significant lower bound (10<sup>th</sup> quantile,  $p=0.228$ ; Figure 4). Also, non-significant trends were observed when we analyzed the prey size spectra of fish prey alone across both seasons (90<sup>th</sup> quantile,  $p=1.000$ ; 10<sup>th</sup> quantile,  $p=0.578$ ) whereas the size spectra of squid prey had positive, significant slopes for the upper (90<sup>th</sup> quantile,  $p<0.001$ ) and lower (10<sup>th</sup> quantile,  $p<0.001$ ) bound regression lines. Statistical comparisons of the regression lines for different prey types indicated that slope of the upper bound of the squid prey size – predator size scatter plot was significantly greater than the slope for fish prey ( $t=25.830$ ,  $df=415$ ,  $p < 0.001$ ). The minimum, maximum and average sizes of squid consumed by *B. magister* were 15 mm, 95 mm, and  $42 \pm 19$  mm ( $\pm$  SD) and the fish prey sizes typically ranged between 30 mm and 112 mm with an average size of  $72 \pm 18$  mm.

### Stable Isotope Analyses

The  $\delta^{15}\text{N}$  signatures of squid muscle tissue indicated that the food web position of *Berryteuthis magister* spanned roughly one trophic level over the sampled size range (winter 2007: 105-310 mm, summer 2004: 72-334 mm). The  $\delta^{15}\text{N}$  signatures of winter 2007 squid samples increased from approximately 11‰ to 15‰ over increasing squid mantle length (Figure 5a). This range in  $\delta^{15}\text{N}$  falls within the range of trophic fractionation (3 - 4‰; Minagawa & Wada 1984, Peterson & Fry 1986). The  $\delta^{15}\text{N}$  signatures of summer 2004 samples followed the same trend as the winter samples, ranging from approximately 10.50‰ for small squid to 13.45‰ for large squid. In contrast to the  $\delta^{15}\text{N}$  signatures, the  $\delta^{13}\text{C}$  of winter 2007 and summer 2004 squid samples did not exhibit any distinguishable trends with body size (Figure 5b). The  $\delta^{13}\text{C}$  values of winter samples ranged from -19.50‰ to -21.20‰ and the summer samples ranged from -18.90‰ to -21.25‰.

Overall, we found that there was a significant, negative correlation between the  $\delta^{13}\text{C}$  signatures of *Berryteuthis magister* muscle tissue and latitude and depth at which they were captured (Table 2). This was found for both summer 2004 and winter 2007 samples. We did not find a significant correlation between the  $\delta^{15}\text{N}$  of *B. magister* muscle tissue and the latitude at which squid were captured (Table 2). However, there was an overall significant, positive

correlation between the  $\delta^{15}\text{N}$  signatures of *B. magister* and capture depths (Table 2). The average  $\delta^{13}\text{C}$  value of winter samples was significantly higher than the average summer  $\delta^{13}\text{C}$  value. The opposite trend was found for the  $\delta^{15}\text{N}$  signatures of *B. magister* muscle tissue. The average C:N mass ratios for summer 2004 and winter 2007 samples did not differ significantly among the seasons (Table 2).

Comparisons between the isotopic signatures of squid and fish muscle tissues indicated that the  $\delta^{15}\text{N}$  values of *Berryteuthis magister* and walleye pollock were similar. The median  $\delta^{15}\text{N}$  value of summer 2004 *B. magister* samples was 11.96‰ (n=50). When grouped by size, the median  $\delta^{15}\text{N}$  values of juvenile (<120 mm, n=11) and adult ( $\geq 120$  mm, n=39) squid were 10.90‰ and 12.19‰, respectively. The median  $\delta^{15}\text{N}$  values of juvenile and adult pollock captured in summer 2005 were 11.66‰ (n=10) and 12.03‰ (n=10), respectively (Figure 6). The  $\delta^{15}\text{N}$  values of the remaining fishes collected in summer 2005 were substantially greater than *B. magister*, ranging between 14.16‰ and 16.09‰ (Figure 6).

### Isotope analysis of squid eye lenses

The number of individual lens segments analyzed per squid eye (proximal lens) varied between six and eleven, and the average width of these lens segments was 0.79 mm  $\pm$  0.41 (SD). To approximate the mantle lengths of *Berryteuthis magister* at the time the segments were laid down, we developed squid proximal eye lens width to squid mantle length regressions. We applied linear regression to summer 2004 *B. magister* data and found a strong, positive relationship ( $R^2 = 0.94$ ,  $a = 0.026$ ,  $b = 1.075$ ,  $df = 46$ ,  $F = 757.7$ ,  $p < 0.001$ ) between squid eye lens width and body size. The winter 2007 samples did not exhibit a linear relationship, thus we fit the data using a non-linear Gompertz function ( $\text{Asym} = 8.380$  ( $p < 0.001$ ),  $b_2 = 11.342$  ( $p = 0.117$ ),  $b_3 = 0.977$  ( $p < 0.001$ )). The Gompertz function was not chosen based on any presumed mechanistic basis to relate eye lens to body size, rather because it is a flexible function that could fit the observed relationship. The relationship between squid eye lens width and body size differed among seasons. The widths of the summer lenses continued to increase over increasing body sizes while the widths of the winter eye lenses appeared to have leveled off at squid mantle lengths of  $>200$  mm.

The eye lens segments revealed variations in the trophic relations of squid at fine temporal scales. We estimated that on average the individual eye lens segments represented approximately a 16-day period ( $\pm 2.0$  SD). Back calculations based on this estimate indicated that the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures of winter 2007 squid lenses reflected prey consumed by squid over lifetimes that spanned early to mid autumn through early to mid winter (Figure 7a,b). In a

similar manner, we found that the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures of summer 2004 squid eye lenses reflected prey consumed by *Berryteuthis magister* from early winter through early to mid summer (Figure 7a,b). From the reconstructed trophic records we were able to detect increases and declines in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of individual *B. magister* at short time intervals over their entire life span.

The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures of the squid eye lenses also revealed that the trophic record of *Berryteuthis magister* varied in notable ways among individuals (Figure 7a,b). We classified the squid  $\delta^{15}\text{N}$  trophic records as two trends: (1) a “conventional” monotonic increase in  $\delta^{15}\text{N}$  with increasing body size or (2) a non-monotonic trend with body size (i.e. an initial increase in  $\delta^{15}\text{N}$  followed by a decrease or vice versa). Based on estimated slopes values, we found that 45% of the total squid samples demonstrated a significant, positive slope ( $p < 0.10$ ) or a “conventional” pattern. When we quantified the occurrence of significant, positive slope for each sampling season we found that the variability in feeding chronologies may be season-dependent: 70% of the winter samples exhibited the “conventional” pattern, whereas only 20% of the summer samples demonstrated a significant linear increase in  $\delta^{15}\text{N}$ . We also found seasonal differences in the variation of  $\delta^{15}\text{N}$  as a function of mantle length (e.g.,  $\sim 3\text{‰}$  for Nov 06 and  $\sim 5\text{-}6\text{‰}$  for July 04; figure 7a) as well as within season sub-groupings (e.g., early July 04; figure 7a).

Overall, the slope of eye lens  $\delta^{15}\text{N}$  was positive ( $t=4.12$ ,  $df=305$ ,  $p < 0.001$ ). The degree to which lens  $\delta^{15}\text{N}$  values increased with increasing mantle length differed between summer 2004 and winter 2007 samples ( $t=3.14$ ,  $df=304$ ,  $p=0.002$ ). The average lens  $\delta^{15}\text{N}$  value did not significantly differ between the two periods ( $t=0.58$ ,  $df=37$ ,  $p=0.560$ ). The slope of lens  $\delta^{13}\text{C}$  values across all individuals was not significantly different from zero ( $t=0.57$   $df=305$ ,  $p=0.573$ ); however, the slope and intercept of lens  $\delta^{13}\text{C}$  values differed between summer 2004 and winter 2007 samples ( $t=4.60$   $df=304$ ,  $p=0.000$  and  $t=7.01$ ,  $df=37$ ,  $p=0.000$ ). Lastly, the average slope of eye lens  $\delta^{15}\text{N}$  values was lower than the slope of  $\delta^{15}\text{N}$  values estimated from muscle tissue analysis (0.004 and 0.009, respectively). The slopes of winter eye lens  $\delta^{15}\text{N}$  values were similar to winter muscle tissue signatures (0.007 and 0.009), but the average slope of summer eye lens  $\delta^{15}\text{N}$  values was lower than the slope of summer muscle tissue signatures (0.001 and 0.010). The slopes of eye lens and muscle tissue  $\delta^{13}\text{C}$  values showed opposite trends in both winter ( $-0.004$  and 0.004) and summer (0.004 and  $-0.003$ ) squid samples.

## DISCUSSION

Our study on the trophic role of *Berryteuthis magister* in the eastern Bering Sea continental slope ecosystem produced three main findings. First, stomach content and stable isotope analyses revealed that *B. magister* exhibits an overall increase in trophic position with ontogeny. The incidence of fish and squid prey in *B. magister* stomach contents gradually increased with increasing squid mantle length and the muscle  $\delta^{15}\text{N}$  signatures revealed that the food web position of *B. magister* increased by approximately one trophic level between juvenile and adult life stages. Second, *B. magister* did not exhibit size-dependent patterns of piscivory. The body sizes of fish prey consumed by *B. magister* remained constant with increasing predator mantle length. Third, in our attempt to reconstruct the feeding chronologies of individual *B. magister*, we found substantial variability in squid trophic records. The feeding chronologies did not consistently reflect a monotonic increase in squid trophic position -- non-monotonic patterns were relatively common. Our results also indicate that the variability in feeding chronologies may be season dependent. These findings suggest that there may be finer scale spatiotemporal variations in squid trophic records, possibly related to seasonal prey availability, that are not captured by traditional isotope analyses of squid muscle tissue.

### **General Diet Analysis**

The stomach content analyses indicate that *Berryteuthis magister* does not demonstrate feeding habits typified by squid species that are potentially important predators of higher trophic level fishes. For example, in the northwest Atlantic Ocean, the longfin inshore squid (*Loligo pealeii*) exhibits a rapid onset of piscivory, preys on the juvenile stages of their predators, including commercially valuable species, and demonstrates size-related patterns of piscivory (Hunsicker & Essington 2006). *Loligo pealeii* and other squid species exhibiting similar feeding traits have been described as voracious predators that may constrain the recruitment success of commercially targeted fishes (Dawe et al. 1997, Rodhouse & Nigmatullin 1996, Hunsicker & Essington 2006, 2008, Field et al. 2007). However, the diets contents of *B. magister* revealed a slower ontogenetic shift to piscivory, and this species preyed mostly upon deepwater forage fishes and showed little evidence of size-related feeding constraints. These findings suggest that *B. magister* may interact with higher trophic level fishes through indirect food web pathways (i.e., sharing or competing for prey resources) rather than directly as a predator. The discrepancy in the types of fish prey consumed by *B. magister* and other well-studied squid may be explained by the differences in their migratory behaviors. Many squid species, including *L. pealeii*, undergo seasonal onshore and offshore migrations (Summers 1969, Serchuk & Rathjen 1974, Ogawa et al. 1983, Hatfield & Rodhouse 1991, 1994, Augustyn 1990, Roberts & Sauer 1994). As a result,

they likely experience high spatiotemporal overlap with the early life stages of high trophic level fishes at spawning and (or) nursery grounds. In contrast, *B. magister* inhabit the edge of the continental shelf and slope over most of their lifetime and do not undergo shelf migrations (Kubodera et al. 1983, Nesis 1985, Okutani et al. 1988). Thus, *B. magister* probably encounters a higher abundance of deepwater slope fishes (i.e., myctophids) than the juvenile stages of many commercially harvested fish species that inhabit shelf waters.

### **Prey Size Spectrum**

The lack of evidence for size-related constraints on piscivory by *Berryteuthis magister* could be due to prey availability. Previous studies of squid feeding ecology have shown that squid predation is dictated by predator and prey body sizes (Rocha et al. 1994, Collins & Pierce 1996, Phillips et al. 2003, Hunsicker & Essington 2006). Prey body sizes typically increase with increasing squid mantle length because squid feeding becomes less constrained by morphological limitations as squid grow. However, our results show that body size constraints may not be an important agent dictating the frequency of piscivory by *B. magister*. We observed an increase in the occurrence of fish in *B. magister* stomach contents over ontogeny, but the sizes of fish consumed by *B. magister* were independent of squid mantle length. A potential explanation is that *B. magister* encountered a limited size range of fish prey in their habitat, and thus our analysis reflects prey availability rather than size constraints. We expect quantile regression to be an effective technique for identifying size constraints when squid are feeding opportunistically on prey items of any size. However, in this study a large majority of the fishes consumed by *B. magister* were northern smooth-tongue (*Leuroglossus schmidti*); suggesting that *B. magister* mainly encountered schools of this fish species. Size-related constraints may have been more evident if we had prey size estimates for a wider size range of *B. magister*. Identifiable otoliths were only found in the stomachs of squid with large body sizes.

### **Stable Isotope Analyses**

Stable isotope analysis of squid muscle tissue has been used successfully as a tool for investigating the role of squid as predator and prey (Hobson et al. 1994, Takai et al. 2000, Cherel and Hobson 2005, Ruiz-Cooley et al. 2006, Stowasser et al. 2006, Parry 2008, Cherel et al. 2009). Our results are consistent with many of these studies. For example, the ontogenetic increase in  $\delta^{15}\text{N}$  observed from *B. magister* muscle tissue has been found for many squid species (Ruiz-Cooley et al. 2004; Parry 2008, Cherel et al. 2009). Similar to *B. magister*, the food web position of three oceanic squids increases by at least one trophic level with ontogeny: the  $\delta^{15}\text{N}$  values of

*Todarodes filippovae*, *Dosidicus gigas*, and *Ommastrephes bartramii* muscle tissues were shown to increase by ~3‰, ~4‰, and >5‰, respectively (Parry 2008, Cherel et al. 2009, see Ruiz-Cooley et al. 2010). Also, consistent with a recent study (Cherel et al. 2009), we found little variation in the  $\delta^{13}\text{C}$  signatures of squid muscle tissue with increasing mantle length. This was expected because the enrichment of carbon isotopes per trophic level is low (i.e., ~1‰; Deniro & Epstein 1978, Tieszen et al. 1983) compared to nitrogen isotopes (i.e., ~3-4‰; Minagawa & Waga 1984). Further, similar to Takai et al. (2000), we found a significant negative correlation between the  $\delta^{13}\text{C}$  signatures of *B. magister* muscle tissue and intra-specific capture location, but did not find a significant correlation between  $\delta^{15}\text{N}$  and latitude. The  $\delta^{13}\text{C}$  signatures likely reflect the latitudinal characteristics of phytoplankton carbon isotopes whereas the large enrichment in  $\delta^{15}\text{N}$  per trophic level may have weakened any correlation between squid  $\delta^{15}\text{N}$  values and latitudinal variation in phytoplankton  $\delta^{15}\text{N}$  signatures (Takai et al. 2000). We note that our findings on squid carbon isotopes need to be interpreted with caution, particularly when making comparisons between species or taxa because lipids ( $^{13}\text{C}$ -depleted) were not extracted from *B. magister* muscle tissues prior to analysis.

One unexpected finding in our study was the correlation between squid isotope signatures and capture depth. It is possible that the capture location (i.e., latitudinal effect) confounds the negative relationship between  $\delta^{13}\text{C}$  and depth while the positive relationship between  $\delta^{15}\text{N}$  and capture depth is related to squid body size. For instance, the adult stages of *Berryteuthis magister* are known to inhabit deeper depths than the juveniles (Nesis 1997, 1998, Arkhipkin 1998), thus we may expect to observe higher  $\delta^{15}\text{N}$  signatures with increasing depths. The positive correlation between  $\delta^{15}\text{N}$  and capture depth could also be attributed to differences in the  $\delta^{15}\text{N}$  values of deep- and surface-water prey items. Several authors have found that the  $\delta^{15}\text{N}$  values of marine predators feeding on mesopelagic prey resources are higher than the  $\delta^{15}\text{N}$  of predators feeding on epipelagic prey (Graham et al. 2007, Ménard et al. 2007, Lorrain et al. 2009). Graham et al. (2007) hypothesize that this trend reflects the higher  $\delta^{15}\text{N}$  values of deep-water prey species, which is an effect of nutrient cycling. The nitrogen isotopes and stomach contents of *B. magister* indicate that squid consumed mostly deep-water prey in the winter period, whereas epipelagic and mesopelagic prey were of similar importance in squid diets during the summer. The summer samples (year 2004) exhibited a positive correlation between  $\delta^{15}\text{N}$  values and capture depth and no correlation was found for winter samples. Also, fish dominated the diets of winter squid over all capture depths while the occurrence of fish and squid in summer squid diets was similar across depths. Together, these findings suggest that the summer squid fed across a strong  $\delta^{15}\text{N}$  depth gradient whereas the winter squid fed more consistently on mesopelagic fishes (e.g., northern



smooth-tongue) with similar  $\delta^{15}\text{N}$  signatures, although water mass mixing in winter may also homogenize the signal. Standardized sampling of *B. magister* and prey isotope signatures is needed to better examine the influences of body size, capture depth, and latitude on squid  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values. Analyses of squid and prey isotope signatures from multiple years and seasons are also needed to determine if our findings are indicative of a season or year effect. Interpretations of our findings are limited by the opportunistic manner in which *B. magister* samples were collected (i.e., different years, depths, and latitudes).

Comparisons of  $\delta^{15}\text{N}$  values from squid and fish muscle tissues revealed that *Berryteuthis magister* might occupy a trophic position similar to or lower than several commercial fishes inhabiting the shelf and slope regions. Specifically, the isotope signatures indicate that adult *B. magister* could occupy a similar trophic level as adult and juvenile walleye pollock (>100 mm and  $\leq$ 100 mm, respectively). The  $\delta^{15}\text{N}$  values of these two species are not directly comparable because the squid and fish samples were collected at different sampling locations and during consecutive years (years 2004 and 2005, respectively). However, diet analyses also show that these species consume similar prey items, such as northern smooth-tongue, lampfish, and lanternfish (Lang et al. 2005). Further, *B. magister* and walleye pollock are known to overlap spatially and temporally in the eastern Bering Sea (<http://www.afsc.noaa.gov/FMA> accessed 01/05/2010). These findings suggest that these species share prey resources and that there could be potential for competition if resources became limited. Quantifying the extent of the dietary overlap of *B. magister* and walleye pollock should be a focus of future work.

### **Isotope analysis of squid eye lenses**

Stable isotope analyses of the *Berryteuthis magister* eye lenses provide a richer picture of squid trophic ontogeny. Our results indicate that squid diets may not simply increase from lower to higher trophic level prey items with increasing body size, but that there can be substantial and sustained deviations over time or body size. The slopes of  $\delta^{15}\text{N}$  in squid muscle tissue and eye lenses in relation to squid mantle length suggest that there is higher variation among the  $\delta^{15}\text{N}$  signatures estimated from squid eye lens layers. Also, the individual feeding chronologies revealed finer temporal variations in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures that were not evident from analysis of muscle tissues. Short-term changes in squid  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  ratios could be due to changes in individuals' feeding habits, prey availability, or localized differences in prey isotope signatures. Temporal variation in prey  $\delta^{15}\text{N}$  levels can be quite pronounced among short-lived, high turnover species (e.g. zooplankton), but would generally not be revealed in the  $\delta^{15}\text{N}$  tissues of predators if

taken from tissues with slower turnover times (Jennings et al. 2008). Indeed, our analysis of muscle tissue suggested a monotonic increase in  $\delta^{15}\text{N}$  values with increasing squid mantle length: the turnover time of squid muscle tissue varies from a few weeks to a few months (Ruiz-Cooley et al. 2006). While we cannot discern whether feeding habits or the underlying prey  $^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures are ultimately responsible for the observed trends in feeding chronologies, our results do point to the presence of intermediate-scale variability in the food webs that support squid.

The variability in *Beryteuthis magister* trophic records revealed by squid eye lenses is supported by earlier studies. Parry (2003) developed the eye lens method to reconstruct the feeding chronologies of two squid species in the north Pacific sub-tropical gyre, *Ommastrephes bartramii* and *Sthenoteuthis oualaniensis*. These squid also exhibited individual variability and non-monotonic trends of lens  $\delta^{15}\text{N}$  values as a function of mantle length. However, these species also demonstrated greater shifts in lens  $\delta^{15}\text{N}$  values over their lifetimes than *B. magister*. For instance, the  $\delta^{15}\text{N}$  values for individual *S. oualaniensis* and *O. bartramii* varied by as much 6.67‰ (avg.  $4.25 \pm 1.91$  SD) and 13‰ (avg.  $8.85 \pm 3.27$  SD) across mantle lengths, respectively. The maximum range of  $\delta^{15}\text{N}$  across *B. magister* across body size was 3.6‰ (avg.  $2.08 \pm 0.63$ ). These differences among species could reflect differences in species' trophic ecology and (or) migration patterns. For example, *O. bartramii* exhibit large, seasonal migrations between spawning and feeding grounds (Murata and Nakamura 1998), whereas *S. oualaniensis* and *B. magister* do not undergo any known seasonal migrations. As a result, *Ommastrephes bartramii* is likely to encounter greater latitudinal shifts in isotope signatures over their lifetime and assimilate a wider range of  $\delta^{15}\text{N}$  in their tissues (Parry 2003). The work of Ruiz-Cooley et al. (2010) provide further corroborate our findings of *B. magister* trophic records. These authors reconstructed the feeding chronologies of individual Humboldt squid (*Dosidicus gigas*) using the  $\delta^{15}\text{N}$  signatures of proostracum sections sub-sampled from squid gladii. Consistent with the *B. magister* eye lenses, the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures of *D. gigas* gladii were highly variable over squid body size and there was an overall increase in the  $\delta^{15}\text{N}$  values of sampled sections as squid became larger. The squid gladii exhibited great differences in the mean isotope values of young and old segments than the *B. magister* eye lenses. For example, based on estimated regression models, the mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of squid gladii differed by 1.6‰ and 0.6‰, respectively, between new (6 cm) and old sections (40 cm), whereas the mean isotope differences of *B. magister* lens layers laid down at juvenile (6 cm) and adult (30 cm) stages differed by 0.96‰ for  $\delta^{15}\text{N}$  and 0.24‰ for  $\delta^{13}\text{C}$ . Again, this finding could be an effect of differences in species' trophic ecology and (or) their lifetime migration patterns. In contrast to *B. magister*, *D. gigas* are large, voracious predators and are highly mobile (Nigmatullin et al. 2001). However, this finding could also

reflect the differences in the molecular structure and isotope fractionation rates of squid eye lenses and gladii. Our results and those of Parry (2003) show that  $\delta^{15}\text{N}$  signatures of squid eye lenses are often lower than the  $\delta^{15}\text{N}$  of squid muscle tissue. The ontogenetic shift in isotope values measured from squid eye lenses was also lower than those measured from squid muscle tissue (present study, Parry 2003). Similarly, the findings of Cherel et al. (2009) and Ruiz-Cooley et al. (2006, 2010) indicate that the isotopic signatures of squid hard parts (beaks and gladii) had lower  $\delta^{15}\text{N}$  signatures and demonstrated smaller ontogenetic shifts in isotope values than soft tissue. These authors suggest that the differences are due to the presence of chitin in squid hard parts, which is a  $^{15}\text{N}$ -depleted molecule. A comprehensive study that examines how squid isotope signatures vary among eye lenses, beaks, gladii, and muscle tissue is needed to make better comparisons between squid isotope studies.

The reconstructed feeding chronologies of *Berryteuthis magister* might reflect a shift in seasonal foraging strategies. The variation among individual trophic records was higher among squid caught in summer than those captured in winter. Also, the winter squid more often exhibited conventional trophic patterns; squid trophic level increased with increasing body size. These differences might be attributed to the seasonal availability of prey to *B. magister* over their short lifetime. For instance, the maximum age (post-embryonic growth) of squid captured in the EBS was estimated to be ~180 days (Drobny 2008). Thus, it is possible that *B. magister* collected in the EBS during summer months were exposed to highly productive ocean conditions (spring & summer) over much of their life span. The high abundance of different prey types available to the summer-caught would have allowed them to feed opportunistically, potentially resulting in more variation among the trophic history of individual squid. In contrast, *B. magister* caught during the winter months were likely to encounter less productive conditions (autumn & winter) and would have been more limited by prey choice. Under such conditions, squid may need to search for additional food items in deeper waters where prey  $\delta^{15}\text{N}$  values may be inherently higher. They might also shift toward more specialized feeding strategies when prey abundance is low to reduce intra-specific competition and maximize energetic return. Previous authors have documented similar shifts in the seasonal foraging patterns of demersal fishes (Reum & Essington 2008), and others suggest that spatial or temporal variation in prey availability and the phenotypic differences among individuals that influence foraging success could promote the divergence of a generalist population into alternate foraging specialists (Partridge & Green 1985; Magurran 1986; Beauchamp et al. 1997; Estes et al. 2003). It's plausible that squid also shift their foraging strategy in response to changes in seasonal and (or) annual prey availability. This notion is supported by the findings Ruiz-Cooley et al. (2010), which suggest that the variable, but

increasing  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  along the proostracum of *D. gigas gladii* is an effect of prey availability and optimal foraging strategy. Feeding chronologies of squid captured in multiple seasons and years are needed to evaluate if the *B. magister* trophic records reflect seasonal environmental conditions or are instead an effect of sampling year (e.g., 2004 vs. 2007).

Our study demonstrates that squid eye lenses can provide a chronology of trophic position over time. We chose to analyze squid eye lenses because the tissue layers are readily distinguishable over the entire lens structure and the numerous segments of the lens tissue laid down over a squid's lifetime can be sampled with ease. Feeding chronologies reconstructed from the squid eye lenses as well as gladii are a valuable tool as they could improve our ability to evaluate the trophic ecology of squid life stages or species that are difficult to capture with traditional sampling gear. For example, adult *B. magister* are frequently captured in bottom trawl surveys or as by-catch in commercial fisheries but the juvenile stages of *B. magister* are more widely dispersed in pelagic habitats (Arkhipkin et al. 1998, Nesis 1998) and are not often captured by traditional sampling methods. To be able to apply the eye lens method in a more rigorous fashion, direct experimental work is needed to validate the assumptions we made here. Also, future work should attempt to verify how often layers of eye lenses are laid down and to relate the age of squid to the time when the tissues were formed. This information can be used to develop more precise estimates of the time scales over which prey isotope signatures are measured and to determine whether there is any synchrony among the age of individuals and their trophic signatures. We could not use squid growth models to back-calculate the age of squid to the time when the tissues were formed due to the high variability surrounding the estimated ages of *Berryteuthis magister* inhabiting the EBS (Drobny 2008). However, we did use these estimates to generate coarse approximations of the time at which the first eye lens segments were laid down. We emphasize that these results need to be interpreted with caution because there is some discrepancy between the estimates used in our study and those from an earlier study. Arkhipkin et al. (1996) aged *B. magister* from the western Bering Sea and found that they live longer than one year.

In summary, our study used three complementary methods to provide valuable information on the trophic position of *Berryteuthis magister* in the EBS and to reveal the individual variation in trophic ontogeny that is masked by traditional stomach content and stable isotope analyses. Future work should aim to concurrently sample and identify the isotope signatures of *B. magister* and their prey over multiple seasons and years. This would allow one to better evaluate the seemingly important effects of body size, depth, latitude and season on squid trophic ecology. Such work would build on the present study and further improve our knowledge

of the role of *B. magister* in the EBS food web and their potential to impact juvenile fishes through indirect food web pathways. Better knowledge of *B. magister* trophic interactions is important for broadening our understanding of how human and environmental forcing can affect the EBS ecosystem.

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#### FIGURES

Figure 1: Capture locations of *Berryteuthis magister* in the eastern Bering Sea. Summer 2004 (black squares), winter 2007 (gray circles), and summer 2007 (black diamonds).

Figure 2: Diagram of the squid proximal eye lens. The youngest tissue lies on the outer edge of the lens and the oldest tissue is in the center of the lens. The width of the eye lenses examined in our study ranged between 6.5 and 10.5mm. Six to eleven lens segments, each greater than 0.3 mm in width, were extracted from the eye lenses for stable isotope analysis. Each segment contained multiple individual lens layers.

Figure 3: The predicted probability of consumption of fish (black lines), squid (dark gray lines), and crustaceans (light gray lines) by *Berryteuthis magister* at different mantle lengths. Logistic regression analyses were conducted for squid collected in summer (2004 and 2007 combined) and winter (2007). The 5th and 95th confidence intervals (dotted lines) are shown.

Figure 4: Relationship between *Berryteuthis magister* mantle length and estimated body sizes of consumed fish (black triangles) and squid (gray circles). Estimated quantile regression lines  $t = 0.10$  (solid lines) and  $0.90$  (dotted lines) are superimposed on the scatterplot. Quantile regression

analyses were conducted for squid collected in summer (2004 and 2007 combined) and winter (2007).

Figure 5: The  $\delta^{15}\text{N}$  (a) and  $\delta^{13}\text{C}$  (b) signatures of *Berryteuthis magister* mantle muscle tissue. The isotopic signatures were quantified from squid collected in summer 2004 (gray triangles) and winter 2007 (black circles). Equations of regression lines are:  $y = 10.071 + 0.010x$  ( $r = 0.79$ ,  $F = 89.26$ ,  $p < 0.001$ ) and  $y = 10.69 + 0.009x$  ( $r = 0.55$ ,  $F = 25.18$ ,  $p < 0.001$ ) for  $\delta^{15}\text{N}$  values of summer and winter muscle tissue versus mantle length, respectively, and  $y = -19.40 - 0.003x$  ( $r = 0.50$ ,  $F = 8.53$ ,  $p = 0.005$ ) and  $y = -21.16 + 0.004x$  ( $r = 0.55$ ,  $F = 20.38$ ,  $p < 0.001$ ) for  $\delta^{13}\text{C}$  values of summer and winter muscle tissue versus mantle length, respectively.

Figure 6: The  $\delta^{15}\text{N}$  signatures of *Berryteuthis magister* and commercial fishes (Kurle CM, Sinclair ES, Edwards AE, and Gudmundson CJ unpubl. data) captured in the eastern Bering Sea during summer 2004 and summer 2005, respectively. The isotopic signatures were quantified from squid and fish muscle tissue. The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles.

Figure 7: The reconstructed feeding chronologies of individual *Berryteuthis magister* captured in summer 2004 (right-hand columns) and winter 2007 (left-hand columns). The lines reflect the relationship between the (a)  $\delta^{15}\text{N}$  signatures and (b)  $\delta^{13}\text{C}$  signatures of *B. magister* eye lens segments and the estimated mantle lengths of squid when the segments were deposited. Each line represents an individual squid. The squid are grouped by capture date (month and year; upper right-hand corner of box) and approximate time at which the first lens segment was laid down (month and year; upper left-hand corner of box). The regression equations are  $y = 9.50 + 0.001x$ ,  $y = 9.28 + 0.007x$ , and  $y = 9.38 + 0.004x$  for summer, winter, and all squid eye lens  $\delta^{15}\text{N}$  values versus mantle length, respectively. The regression equations are  $y = -20.36 - 0.004x$ ,  $y = -18.31 - 0.005x$ , and  $y = -19.31 - 0.001x$  for summer, winter, and all squid eye lens  $\delta^{13}\text{C}$  values versus mantle length, respectively.

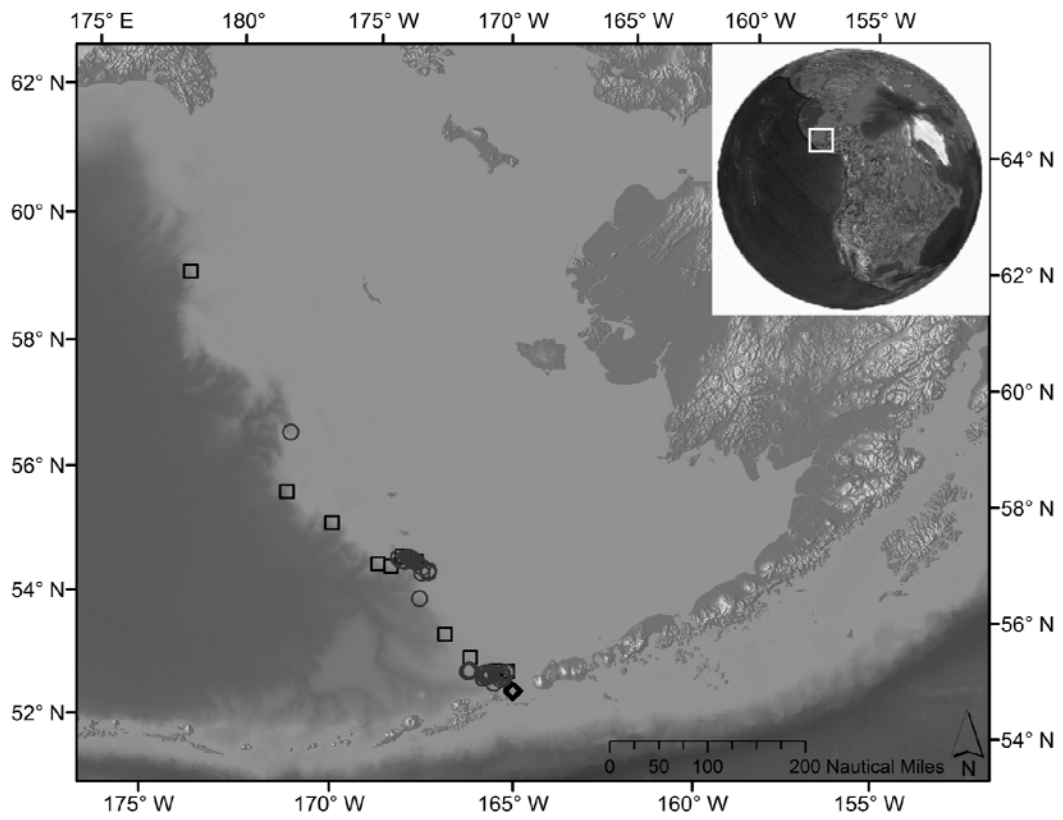


Figure 1

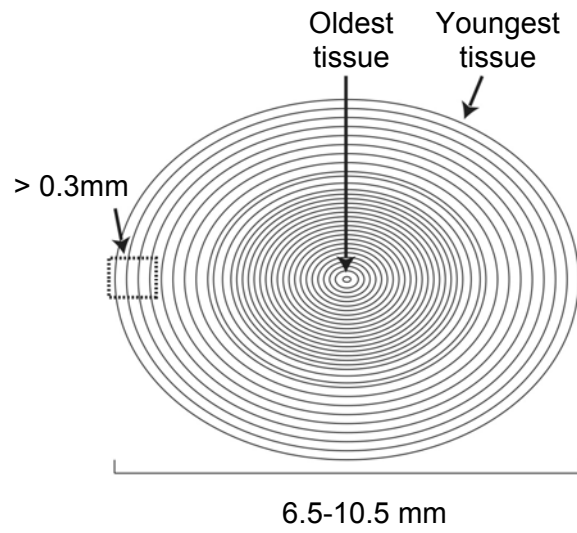


Figure 2



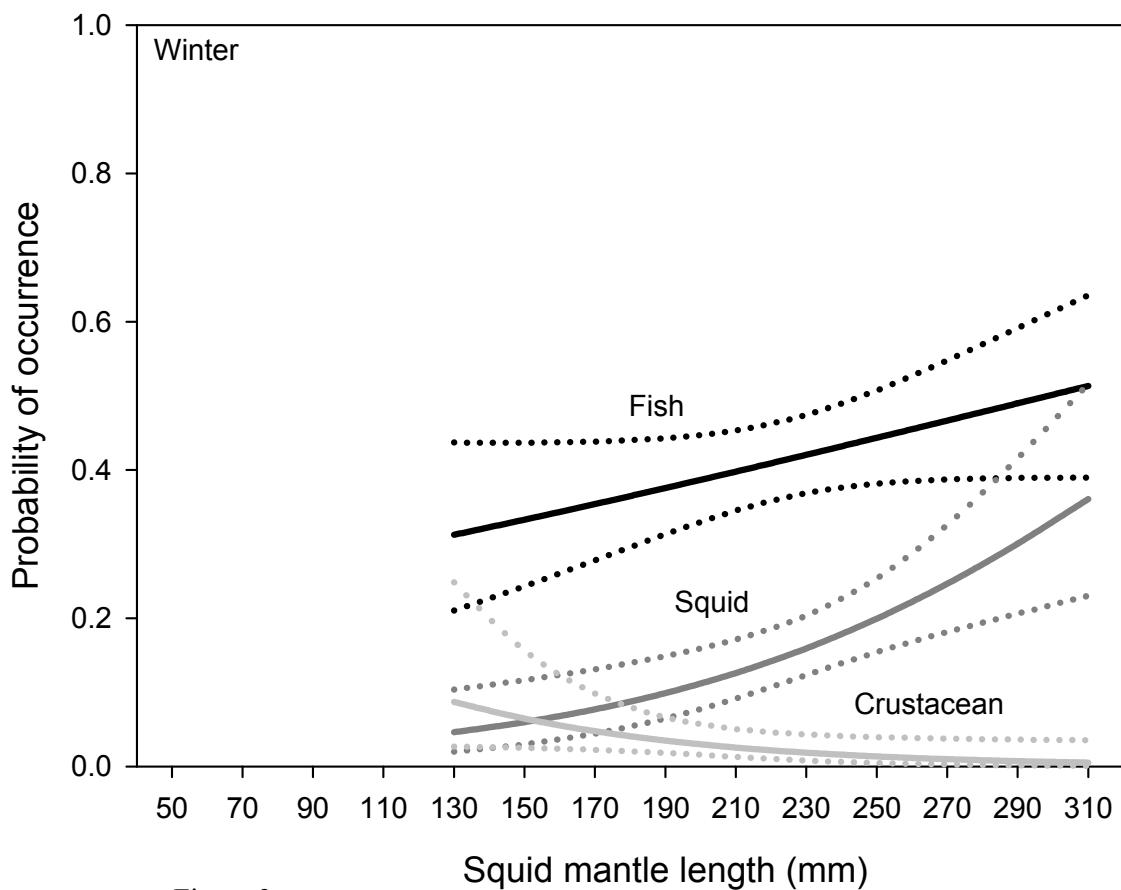
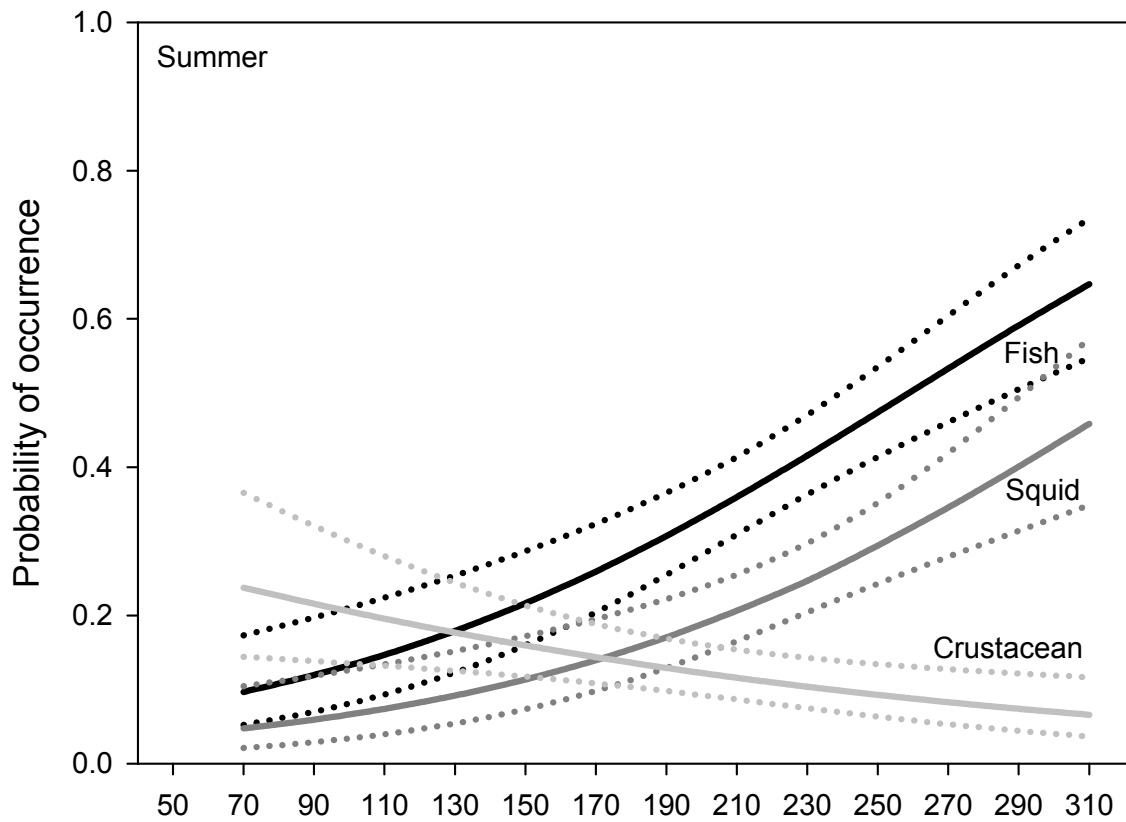


Figure 3

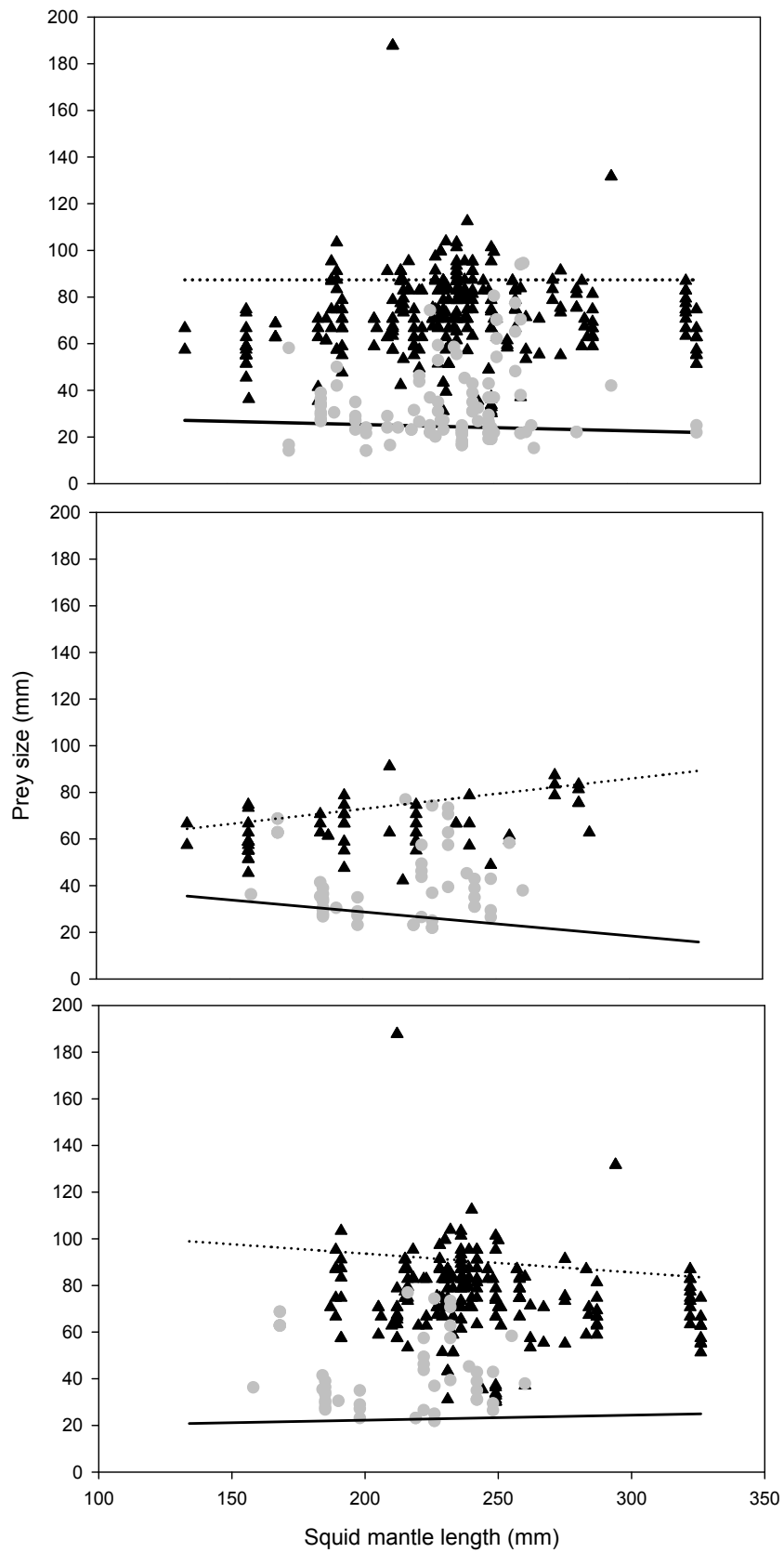


Figure 4

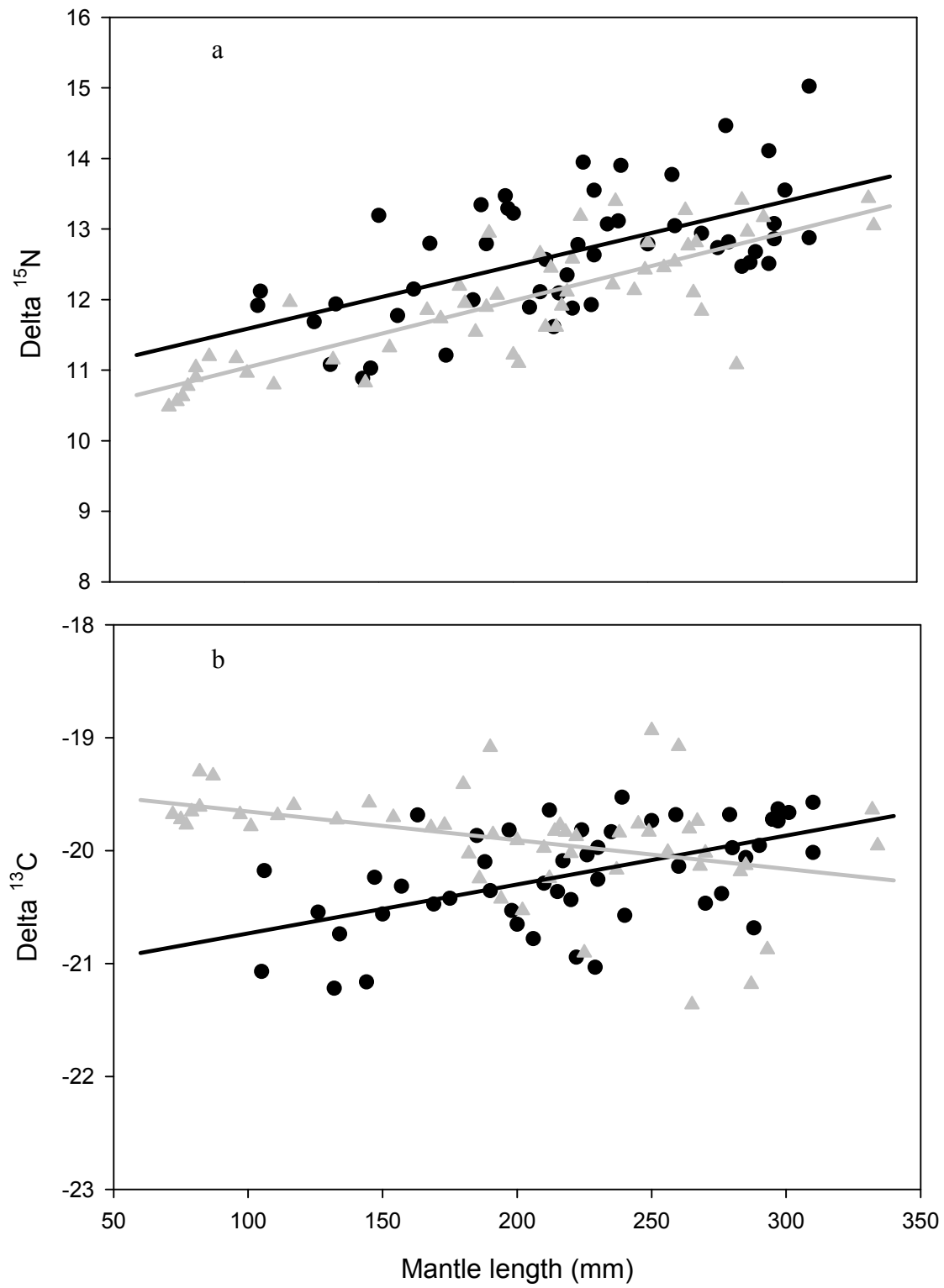


Figure 5

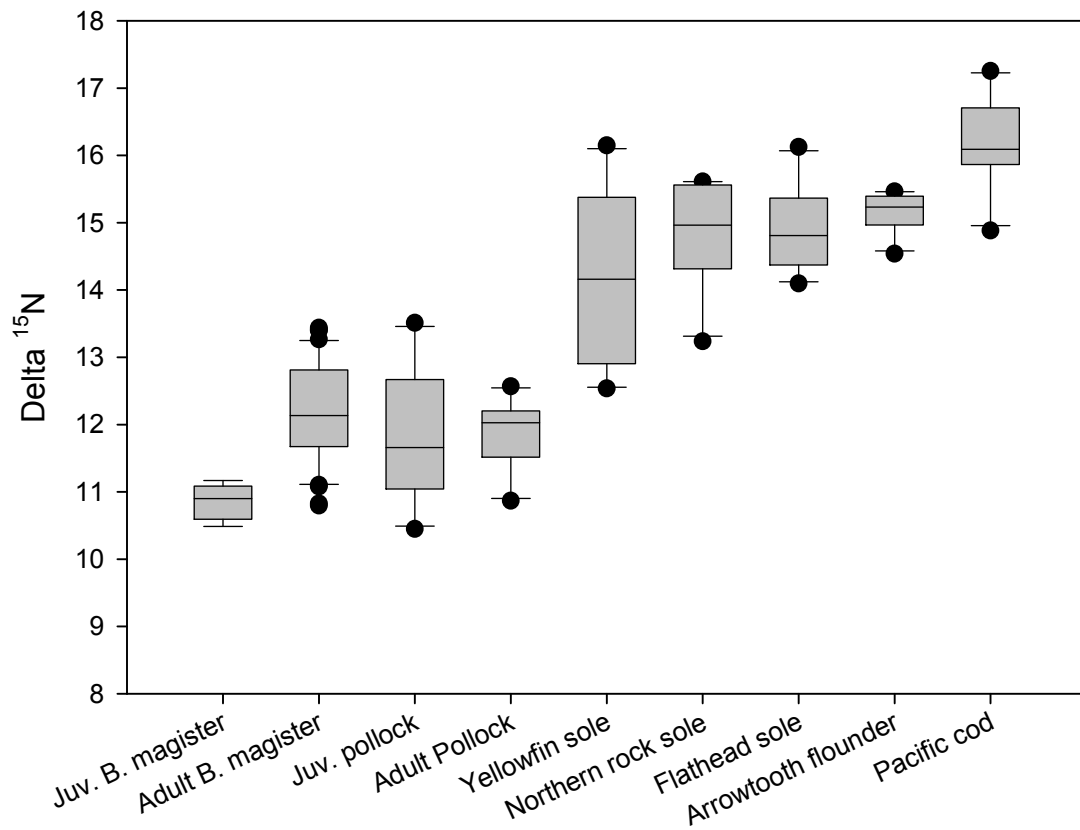


Figure 6

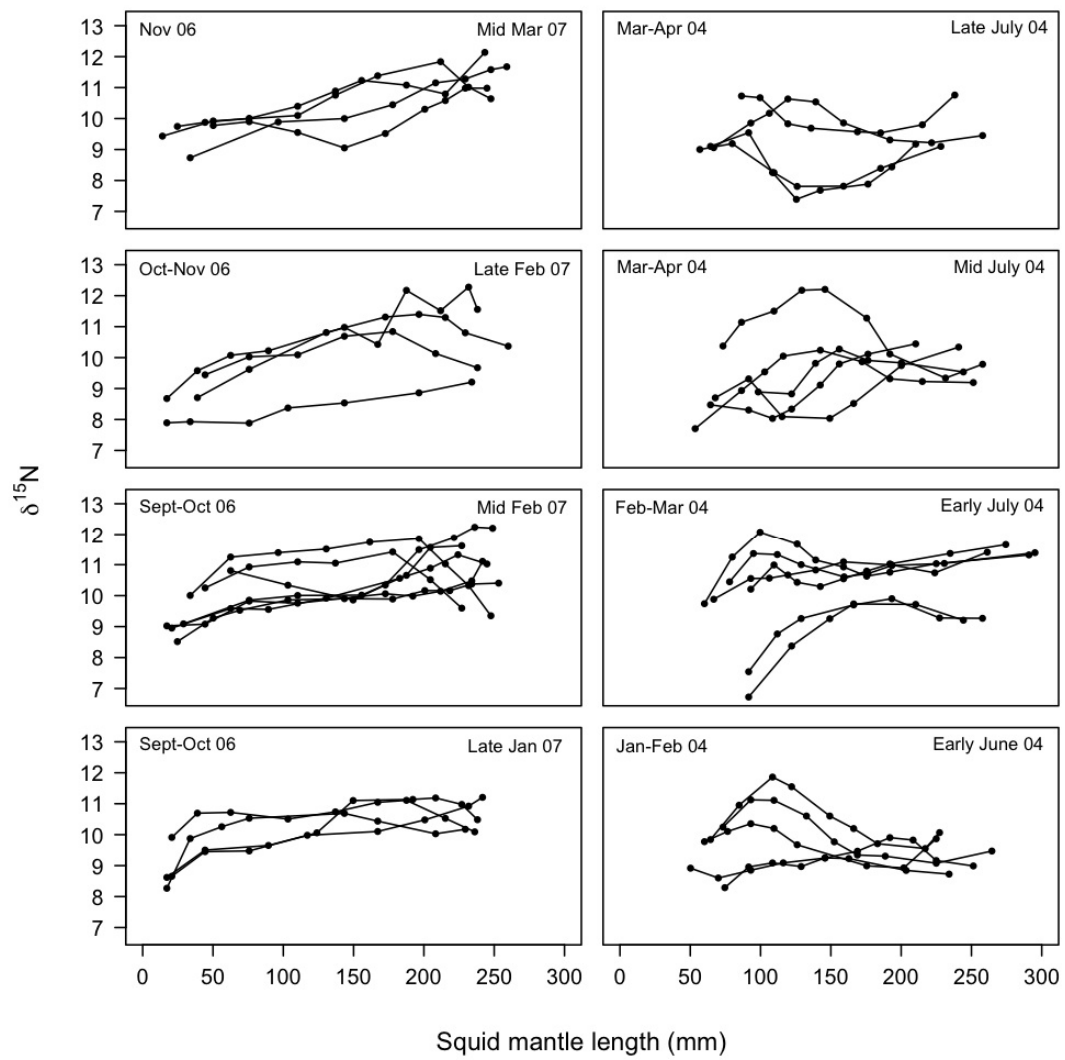


Figure 7a

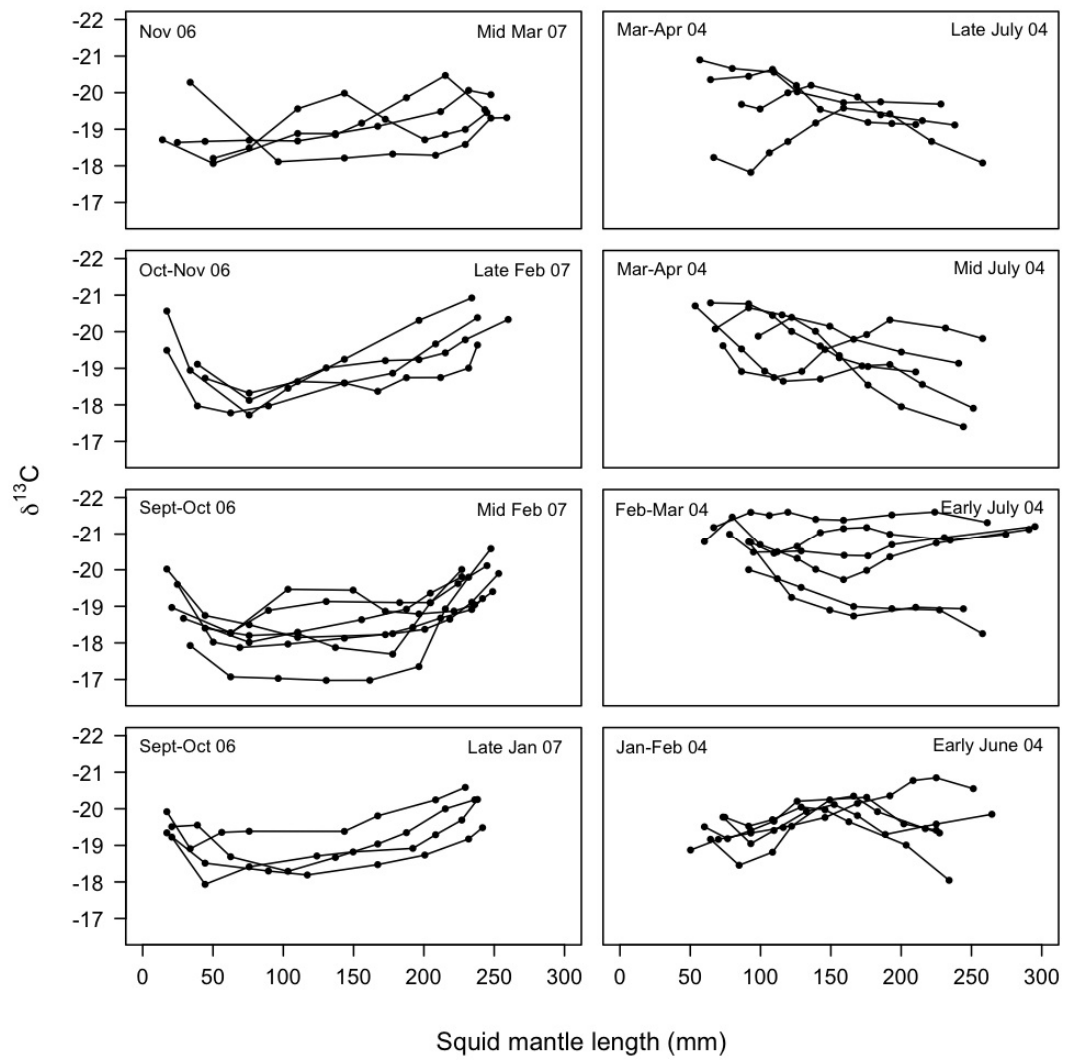


Figure 7b

Table 1: Mean capture depths and body sizes of *Berryteuthis magister* analyzed for stomachs contents (n = sample size). The percent frequency of occurrence of *B. magister* prey items are shown by season and year, and all years combined. The scientific names of prey identified from the stomach contents of *B. magister* and the numbers of otoliths and beaks identified to each prey are also shown by seasons and years.

	All Years n=697	Summer 2004 n=107	Winter 2007 n=336	Summer 2007 n=254
Capture depth (m)	394 ± 103	393 ± 92	466 ± 90	321 ± 61
Dorsal mantle length (mm)	219 ± 97	172 ± 74	227 ± 127	230 ± 47
Body mass (g)	360 ± 216	243 ± 249	338 ± 210	389 ± 192
Frequency of occurrence (%):				
Fish	40.75	20.56	42.56	46.85
Squid	19.94	17.76	16.37	25.59
Crustacean	7.32	14.95	2.68	10.24
Empty	30.70	34.58	34.52	24.02
Unidentifiable prey only	13.20	19.63	8.63	16.54
Number of identified hard parts:				
Fishes (otoliths)				
<i>Leuroglossus schmidti</i>	309	3	214	92
<i>Stenobranchius leucopsarus</i>	7	2	0	5
<i>Lampanyctus jordani</i>	2	0	2	0
<i>Benthabella dentata</i>	2	2	0	0
<i>Post-larval liparid</i>	1	1	0	0
<i>Cyclopteridae sp.</i>	1	0	0	1
<i>Theragra chalcogramma</i>	1	1	0	0
Squid (beaks)				
<i>Berryteuthis magister</i>	17	2	12	3
<i>Gonatopsis borealis</i>	4	0	4	0
<i>B. magister</i> / <i>G. borealis</i>	36	0	17	19
<i>Gonatus sp.</i>	46	30	11	5
<i>Gonatus onyx</i>	9	0	0	9
<i>Gonatus madokai</i> / <i>Gonatus middendorffi</i>	6	1	4	1

Table 2: Capture depths and body sizes of *Berryteuthis magister* analyzed for seasonal  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures of mantle muscle tissue (n = sample size). Student t-tests were performed to identify potential seasonal differences between capture depths, squid body sizes,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures and C:N ratios. The Spearman correlation test was used to identify significant correlations between *B. magister* isotope signatures and latitude and capture depth.

	All Seasons	Summer 2004	Winter 2007	Statistics	
	n=100	n=50	n=50	t	p
Capture depth (m)	438 ± 113	390 ± 62	488 ± 132	-4.68	<0.001
Dorsal mantle length (mm)	210 ± 66	198 ± 72	222 ± 58	-1.80	0.075
Body mass (g)	371 ± 304	336 ± 304	409 ± 302	1.18	0.242
<i>Mantle tissue</i>					
$\delta^{15}\text{N}$ (‰)	12.32 ± 0.93	12.67 ± 0.88	11.96 ± 0.86	4.07	<0.001
$\delta^{13}\text{C}$ (‰)	-20.05 ± 0.48	20.20 ± 0.45	-19.89 ± 0.50	3.31	0.001
C:N mass ratio	3.50 ± 0.10	3.51 ± 0.12	3.50 ± 0.07	-0.22	0.820
<i>Spearman correlation test</i>					
	$\rho$ (p)	$\rho$ (p)	$\rho$ (p)		
Latitude, $\delta^{15}\text{N}$ (‰)	-0.073 (>0.05)	0.255 (>0.05)	-0.278 (>0.05)		
Latitude, $\delta^{13}\text{C}$ (‰)	-0.315 (<0.01)	-0.323 (<0.05)	-0.419 (<0.01)		
Depth, $\delta^{15}\text{N}$ (‰)	0.366 (<0.001)	0.588 (<0.001)	-0.150 (>0.05)		
Depth, $\delta^{13}\text{C}$ (‰)	-0.471 (0.001)	-0.464 (<0.001)	-0.362 (<0.05)		



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## CHAPTER 2

### **A comparison of the trophic levels of eastern Bering Sea groundfish predators by food habits and stable isotope analysis**

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## ABSTRACT

Measurements of trophic level through stable isotope analysis ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) is generally considered complementary/reflective of trophic level analysis performed through analysis of prey identification and stomach content weight. However, depending on the relative baselines and time/space scales to use to analyze each type of data, the two methods may measure different ecosystem processes. Here, we compare trophic levels of three major groundfish predators, walleye pollock (*Theragra chalcogramma*), arrowtooth flounder (*Atheresthes stomias*), and Pacific cod (*Gadus macrocephalus*) in the eastern Bering Sea, as measured in the comparison between 158,327 stomach samples collected during the period 1982-2008, and 2,084 stable isotope samples taken between 2005-2007. The results show a dichotomy; that across the Bering Sea, areas with a higher trophic level as calculated by stomach samples show a lower trophic level as measured by  $\delta^{15}\text{N}$ . This result highlights that the two methods can measure wholly different but complementary processes; with stomach samples monitoring shifts between piscivory and planktivory that may track top-down control exerted on prey species, and  $\delta^{15}\text{N}$  measuring geographic and temporal variation in the length of lower trophic-level (planktonic) processes that may be reflective of bottom-up (climate induced) changes in ecosystem structure and function.

## INTRODUCTION

The trophic level of fished species in marine ecosystems has been an area of recent concern in ecosystem-based approaches to fisheries management. In particular, it has been suggested that shifts in the trophic level of fish in an ecosystem over time may represent the degradation of the ecosystem in response to fishing (Pauly et al. 1998; Pauly and Palomares 2005). Food web modeling has often focused on tracking trophic levels of catch under increased fishing pressure, especially if fisheries include catch of tightly-linked predator and prey species.

However, in order to draw such conclusions, it's important to understand how trophic level may naturally vary throughout an ecosystem over time. Ecosystem-based fisheries models that track trophic level are most often constructed on a "whole-stock" level without regard to geographic or temporal changes in the underlying ecosystem processes. This can lead to the use of ecosystem management indicators which purport to measure, for example, collapse of an ecosystem due to increasing trophic level of the catch, while in reality geographic shifts in populations may lead to different climate-driven exploitation patterns (Essington et al. 2006). Incorporating such dynamics into ecosystem-based indicators will be critical to building appropriate tools.

Most trophic studies to date that underlie predator/prey models have focused on diet analysis; measurement of stomach contents to determine trophic level of species building up from phytoplankton

by solving a set of linear equations based on fixed reference levels of lower trophic levels (Ulanowicz and Kemp 1979). In the marine ecosystems of Alaska, this type of analysis has been most recently performed for the eastern Bering Sea, Gulf of Alaska, and Aleutian Islands by Aydin et al. (2007). However, this fixed method may hide a range of changes and diversity within species that may lead to poor conclusions about trophic level monitoring.

Stable isotope analysis has been used as a complementary tool for many diet analysis studies in marine ecosystems (Davenport and Bax 2002; Michener and Schnell 1994). The ratio of the light and heavy isotopes of nitrogen ( $^{14}\text{N}$ ,  $^{15}\text{N}$ ) provides an indication of trophic level because  $^{15}\text{N}$  fractionates as it moves up food webs. Isotopic ratios are typically expressed as  $\delta$  units, which indicate the per mil deviation from a standard (for nitrogen, it is atmospheric N). In general,  $\delta^{15}\text{N}$  increases 3-3.5 units per trophic level. In contrast, the ratio of light to heavy carbon ( $^{12}\text{C}$  and  $^{13}\text{C}$ ) is more conservative through trophic transfers, and indicates the photosynthetic pathway used to fix carbon. This integration method allows a single fish to be used as a measure of the energy processes leading up to its feeding, and therefore may be a more sensitive instrument for modeling ecosystem variation.

Here, the combination of food habits and stable isotope results are summarized and compared with respects to the shifts that each method measures in trophic level throughout the year in the Gulf of Alaska and Bering Sea. I focus on three major groundfish predators: arrowtooth flounder, Pacific cod, and walleye pollock. By comparing the results of carbon and nitrogen signatures in predators with measured stomach contents, the relationship between food habits analysis and stable isotope analysis may be determined to see under what circumstances one method, or both, may provide the most appropriate results.

## **METHODS**

### **Stomach samples**

Scientists on board chartered bottom trawl vessels collected stomachs from fish captured in NOAA Alaska Fisheries Science Center survey trawls during June-July of each sample year in the Bering Sea, Gulf of Alaska, and Aleutian Islands; fisheries observers collected stomachs from fisheries-caught fish during other seasons. These samples were spread out over the survey area in order to derive food habits information that is representative of each geographic subarea. At each sampling station or observed fisheries haul, 2-3 species were selected for sampling and stomach samples were taken from about 10-20 specimens from each species, spread out over the length groups represented within each species.

Before removing the stomach, fish were examined for evidence of regurgitation or net feeding. If a fish had food in its mouth or around the gills, or if the stomach was inverted or flaccid, the fish was categorized as having regurgitated food and the specimen was discarded. If a predator had fresh food

(usually fish) sticking out of the mouth or the throat, it was categorized as a net-feeding fish and was also discarded. When a sampled stomach was retained, it was put in a cloth bag. A specimen tag containing predator species name, fork length (FL) of the predator, and haul data (including vessel, cruise, haul number, specimen number) was also put in the bag. All of the samples collected were then preserved in buckets containing a buffered 10% formalin solution. When the samples arrived at the laboratory, they were transferred into 70% ethanol before the stomach contents were analyzed. In the laboratory, the stomach was cut open, the contents were removed, and then blotted with a paper towel. The wet weight was then recorded to the nearest 0.01 g. After obtaining the total weight for a stomach's contents, the contents were placed in a Petri dish and examined under a microscope. Each prey item was classified to the lowest practical taxonomic level. Prey weights and numbers of commercially important crabs and fish were recorded. If walleye pollock otoliths were found, otolith lengths were measured and the walleye pollock's standard length (SL) was derived through an otolith length-fish length regression table. Standard lengths of prey fish, carapace widths (CW) of crabs of the genera *Chionoecetes*, *Cancer*, and *Erimacrus*, and the carapace length of king crabs (Family Lithodidae) were also recorded.

During this study, discarded fish parts from commercial fish processing operations were also found quite frequently in the stomachs of some marine fishes. Fishery offal that has been scavenged was distinguished from other prey by characteristics that indicate fisheries processing of the carcass. Fish heads that are relatively undigested and have been cleanly severed from the carcass are one of the most common types of offal encountered. Sometimes wadded up skin, large vertebrae or tails indicate the consumption of fishery offal especially if knife-cuts are still visible. Occasionally the length, of a fish-prey is estimated based on the fish otoliths found in the stomach, may be much larger than can be swallowed by the predator which suggests consumption of only a fish-head and likely scavenging of fishery offal.

### **Stable Isotope Samples**

A small portion (~13 cubic mm) of tissue was excised from muscle and liver tissue of fish sampled collected in Alaskan waters between 2005-2007. To determine the trophic position of groundfish species the EBS, we analyzed  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures of these tissues. Tissue samples were frozen on board ship and returned to the laboratory for processing. Additionally, whole prey samples were preserved from frozen fish stomachs returned to the laboratory; a total of 2,084 samples were collected and analyzed.

The tissue samples were dried at 64°C, ground with mortar and pestle into a homogeneous powder, and weighed according to the guidelines of the Colorado Plateau Stable Isotope Laboratory (CPISL). LipidThe isotope analyses were conducted at the CPISL and were carried out in continuous -

flow mode using a Thermo - Finnigan Delta<sup>plus</sup> Advantage gas isotope - ratio mass spectrometer interfaced with a Costech Analytical ECS4010 elemental analyzer. Isotopic ratios are expressed using standard  $\delta$  - notation where  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$  and R is the ratio of  $^{15}\text{N}:^{14}\text{N}$  or  $^{13}\text{C}:^{12}\text{C}$  in the sample and standards, respectively (atmospheric nitrogen and Pee Dee Belemnite for carbon). The data were normalized using four internationally accepted isotope reference standards (IAEA CH6, CH7, N1, and N2). External precision on laboratory standards was  $\pm 0.20\text{‰}$  or better for  $\delta^{15}\text{N}$  and  $\pm 0.10\text{‰}$  or better for  $\delta^{13}\text{C}$ .

Lipids were not removed from tissues prior to the stable isotope analysis. Lipids do not contain nitrogen and thus do not affect nitrogen isotope signatures, though variations in the lipid content within tissues can significantly influence  $\delta^{13}\text{C}$  values and hence bias the interpretation of the carbon isotope results (Sweeting et al. 2006, Kiljunen et al. 2006). Raw  $\delta^{13}\text{C}$  results from the laboratory were therefore corrected for lipid content using the formula:

$$\delta^{13}\text{C}(\text{corrected}) = \delta^{13}\text{C}(\text{raw}) + \frac{a\text{CN} + b}{\text{CN} + c}$$

where CN is the Carbon/Nitrogen ratio of the sample and a,b, and c are tissue-specific parameters estimated by comparing lipid-extracted samples with non-lipid-extracted samples from the same fish and tissue type; parameters and formula were used from Logan et al. (2008). For muscle samples, the C/N ratio was low (<3) and the correction was minor; for liver samples the ratio ranged from 3 to 61 and thus the correction substantially affected the results.

### Statistical Analyses

Food habits data from stomachs collected during this survey were pooled with data from samples collected and processed using the same methodology from the period 1982-2008 in the Bering Sea (158,327 total samples). To determine the long-term biogeographic pattern of groundfish foraging, samples were pooled by species (walleye pollock, arrowtooth flounder, and Pacific cod) and predator length class (0-20cm, 20-40cm, and 40-80cm, and 80+cm fork lengths), by “season” (Jan-Mar, Apr-May, Jun-Jul, Aug-Sep, and Oct-Dec) and geographic location bins; each geographic bin was approximately 30x30 km. Seasonal and geographic resolution was chosen to balance sample size with geographic coverage and to separate major seasonal processes. For each grouping in which 10 or more fish were sampled, a 1,000-draw bootstrap analysis was performed to determine the mean and standard deviation of stomach fullness (prey weight/predator weight) for each major prey type.

The trophic level (TL) of fish at each pooled location was determined by using the formula  $TL(\text{predator}) = 1 + \sum_{\text{prey}} DC_{\text{prey}} TL_{\text{prey}}$  where DC mean diet composition (wet weight) of that prey type in that pooled group, and  $TL_{\text{prey}}$  is the trophic level of the prey as reported in Aydin et al. (2007). This

was then compared with the nitrogen and carbon isotopic ratios of fish collected during this study in the same geographic area.

## RESULTS

While several hundred samples were collected between 1982-2008 for every size class of groundfish in this study (pollock, cod, and flounder), year-round samples only existed for the 40-80cm size class of each fish; as the fall and winter samples, collected by observers on fishing vessels, almost entirely consist of commercial-sized fish. Therefore, the remainder of this analysis will focus on the 40-80cm size range. In this size range, all three species can show varying degrees of piscivory or planktivory.

Figs. 1-3 show the total stomach content weight by 30x30km geographic cell for each species and each season (all years combined). The June-July samples, consisting primarily of survey rather than commercially caught samples, have the greatest geographic spread.

Overall, pollock show highest stomach content weights in inner Bristol Bay (northeast corner of sample area) and the northwest area (fig. 1). In all seasons, pollock have mean stomach contents weight that are consistently in the 3-5% of body weight range; however, as digestion rate may vary with temperature (colder waters leading to slower digestion; thus less consumption for the same stomach content weight), the difference in ration across seasons cannot be absolutely determined. Pacific cod (fig. 2) and arrowtooth flounder (fig. 3) tend to have the highest stomach content weight towards the outer continental shelf in all seasons.

Trophic level by species and season (for 40-80cm fish) is shown in figs. 4-6 as seasonal anomalies (+/- one trophic level) from the location mean across all years shown in each subfigure. 40-80cm pollock (fig. 4) have a mean trophic level that is lowest in June-July (3.67) and highest in October-December (4.10). In June-July, high trophic level anomalies occur towards the outer shelf, and low trophic level anomalies occur in the east, on the Alaska peninsula and in Bristol Bay. In other seasons, trophic level anomalies have fewer obvious geographic patterns.

Pacific cod show a similar range of seasonal mean trophic level (3.69-4.07) but show a more consistent geographic breakdown of anomalies in all seasons, with a higher trophic level on the outer shelf and lower trophic level on the inner and middle shelf (fig. 5). Arrowtooth flounder have the highest overall mean trophic level (4.32-4.40 depending on season) and show much less difference geographically, with a range that varies in general less than +/-0.2 trophic levels by individual location (fig. 6).

Fig. 7 shows  $\delta^{15}\text{N}$  ratio by location and month in the eastern Bering Sea for all three species, all collected sizes of fish (0-120cm fork length). The strongest pattern is geographical rather than temporal,

with a lower  $\delta^{15}\text{N}$  on the slope versus the shelf for all species. A separate analysis (not shown) confirmed that depth or distance offshore were more strongly correlated with  $\delta^{15}\text{N}$  than was predator size or month.

Fig. 8 shows  $\delta^{13}\text{C}$  ratio for all three species. For pollock and arrowtooth, there is a reasonably clear east/west divide, with lower  $\delta^{13}\text{C}$  ratios in the west. Pacific cod do not seem to show a strong geographic pattern in  $\delta^{13}\text{C}$  ratios.

Fig. 9 shows the median, 25-75% quantiles, and outliers for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  for all species sampled in this study (muscle or whole-tissue samples), sorted by median.  $\delta^{15}\text{N}$  median values range from 8 (for copepods) to 18 (for Pacific cod).  $\delta^{13}\text{C}$  values range from -20 (for copepods) to -15 (for king crab).

Finally, Fig. 10 plots the  $\delta^{15}\text{N}$  ratio of individual whole-body euphausiid samples (N=40) taken from predator stomachs. A clear divide is evident, with samples in Bristol Bay showing a higher  $\delta^{15}\text{N}$  ratio than those further to the southwest along the Alaska Peninsula.

## DISCUSSION

In general, measurements of diet-weighted trophic level and measurements of  $\delta^{15}\text{N}$  ratio are considered corroborative, with higher (diet-weighted) trophic levels corresponding to higher  $\delta^{15}\text{N}$  ratios. However, geographically, this study shows the opposite pattern, with higher diet-weighted trophic levels in the west corresponding with lower  $\delta^{15}\text{N}$  ratios.

An examination of actual prey composition underlying Figs. 4-6 shows that the strong splits between positive and negative anomalies are driven primarily by pollock presence in diets. Pollock (Aydin et al. 2007 trophic level 3.6) are high in the diets of all three species on the outer shelf and in the west; while in the east, the diets of pollock are primarily macrozooplankton (Aydin et al. 2007 trophic level 2.5-2.7) while the diets of cod are primarily benthic such as crabs and brittle stars (Aydin et al. 2007 trophic level 2.5-3.0).

On the other hand, geographic differences in  $\delta^{15}\text{N}$ -implied trophic level seem to correlate with differences in  $\delta^{15}\text{N}$  in the prey themselves; and thus relate to lower-trophic level processes. For example, myctophids and P. Ocean perch, which are deepwater/slope species, have a lower  $\delta^{15}\text{N}$ -implied trophic level than capelin and eulachon (Fig. 9), despite both species feeding on similar clades of zooplankton. Mysiids, as an inshore zooplankton (appearing in diets primarily in shallow waters of Bristol Bay) have a  $\delta^{15}\text{N}$  level higher than the deepwater forage fish. This is emphasized by seeing the geographic trend of  $\delta^{15}\text{N}$  ratios in euphausiids themselves (Fig. 10). The nearshore/offshore patterns in  $\delta^{13}\text{C}$  reflect this geographic difference (Figs. 8, 9).

Overall, this dichotomy shows that trophic analysis based on fisheries network analysis may measure fundamentally different processes than do stable isotope trophic levels. The network-based

analyses show variation in fish predators which is largely based on rates of piscivory versus planktivory (or benthic organisms). Shifts in network-based trophic level, measured over time, may represent top-down effects of prey switching between foraging modes, either due to geography or fishing-induced shifts in prey or predator base. These type of shifts may be most relevant to monitoring by fisheries modelers and managers concerned with strongly-interacting predator/prey pairs that are both subject to fishing (e.g. cod and pollock).

On the other hand, changes appearing in ecosystem-integrated  $\delta^{15}\text{N}$  levels may reflect changes in the bottom-up pathway leading to fisheries resources; in particular plankton production as driven by climate. Changes over time of  $\delta^{15}\text{N}$  ratios may separate climate-induced processes into shelf and shelf-edge components; this deserves continued and expanded monitoring to improve models of the effects of climate change on fish production.

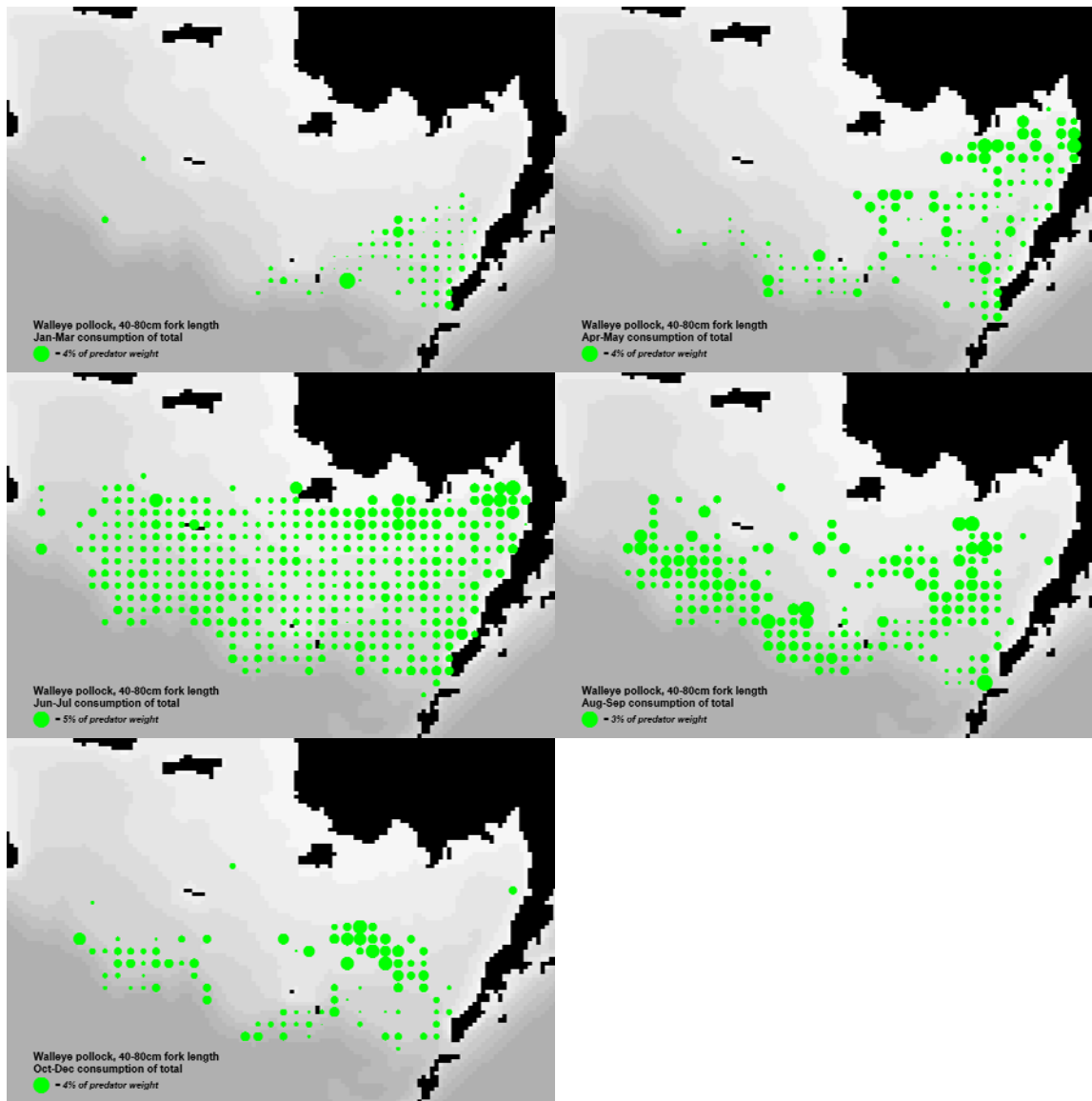


Figure 1. Total mean stomach content weight as percentage of predator weight (bootstrap estimate), for eastern Bering Sea walleye pollock 40-80cm fork length by season (Jan-Mar, Apr-May, Jun-Jul, Aug-Sep, and Oct-Dec), all years 1982-2008 combined for each 30x30km cell. Only cells with more than 10 samples (across all years) are shown. Circle area is proportional to stomach contents weight; size corresponding to maximum average cell weight is shown. Note that scale changes for each seasonal figure.



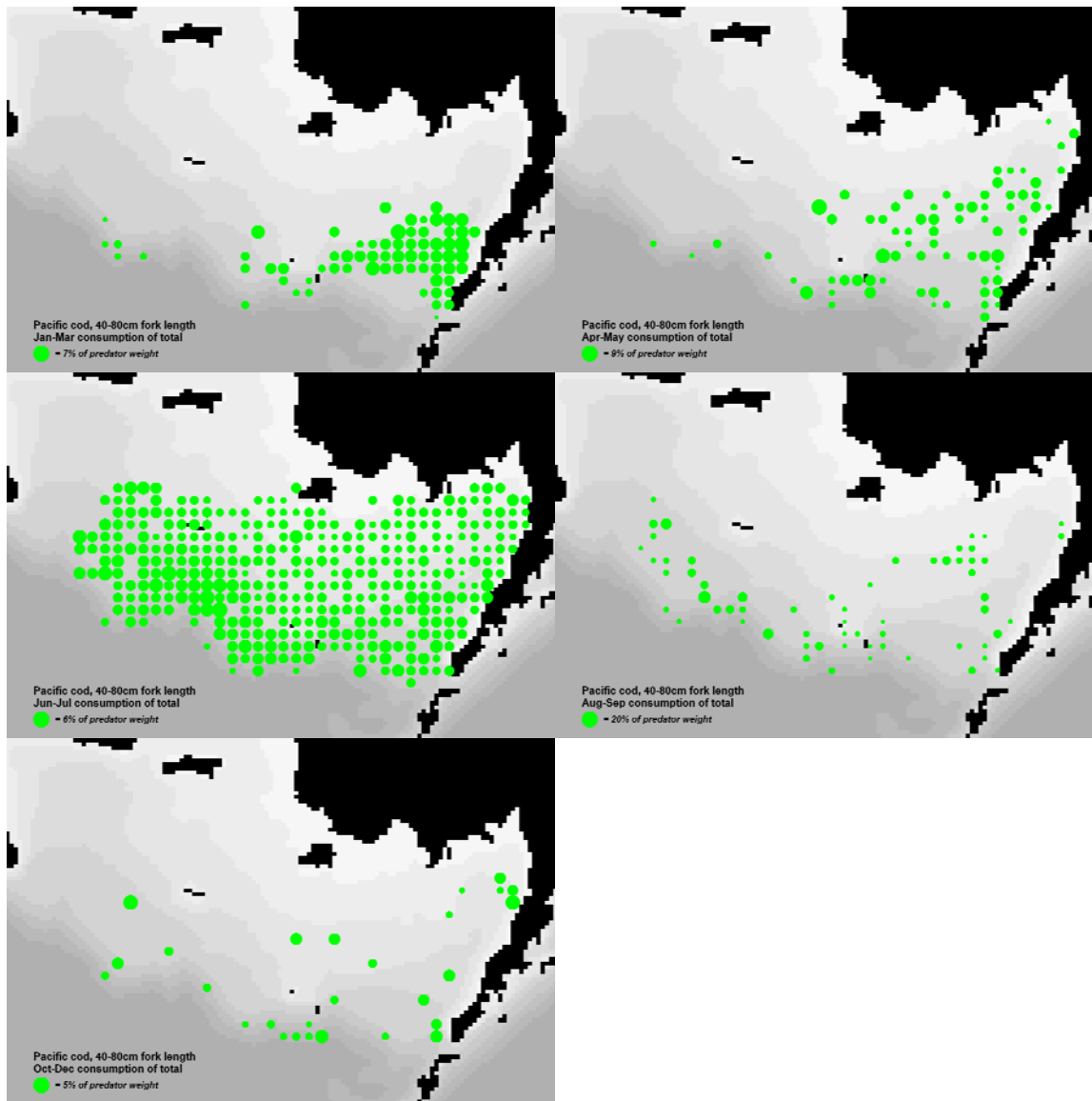


Figure 2. Total mean stomach content weight as percentage of predator weight (bootstrap estimate), for eastern Bering Sea Pacific cod 40-80cm fork length by season (Jan-Mar, Apr-May, Jun-Jul, Aug-Sep, and Oct-Dec), all years 1982-2008 combined for each 30x30km cell. Only cells with more than 10 samples (across all years) are shown. Circle area is proportional to stomach contents weight; size corresponding to maximum average cell weight is shown. Note that scale changes for each seasonal figure.

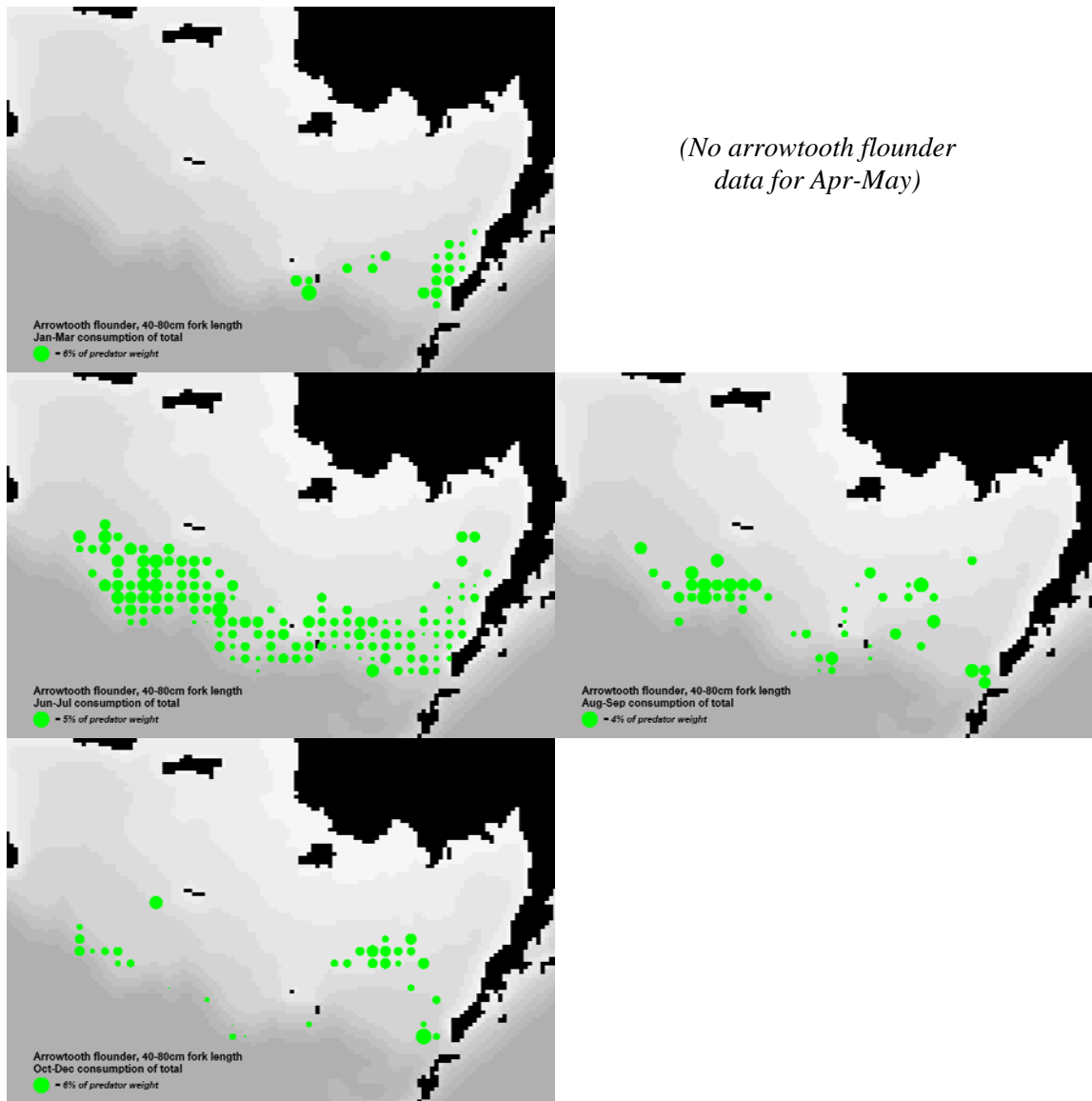


Figure 3. Total mean stomach content weight as percentage of predator weight (bootstrap estimate), for eastern Bering Sea arrowtooth flounder 40-80cm fork length by season (Jan-Mar, Apr-May, Jun-Jul, Aug-Sep, and Oct-Dec), all years 1982-2008 combined for each 30x30km cell. Only cells with more than 10 samples (across all years) are shown. Circle area is proportional to stomach contents weight; size corresponding to maximum average cell weight is shown. Note that scale changes for each seasonal figure.

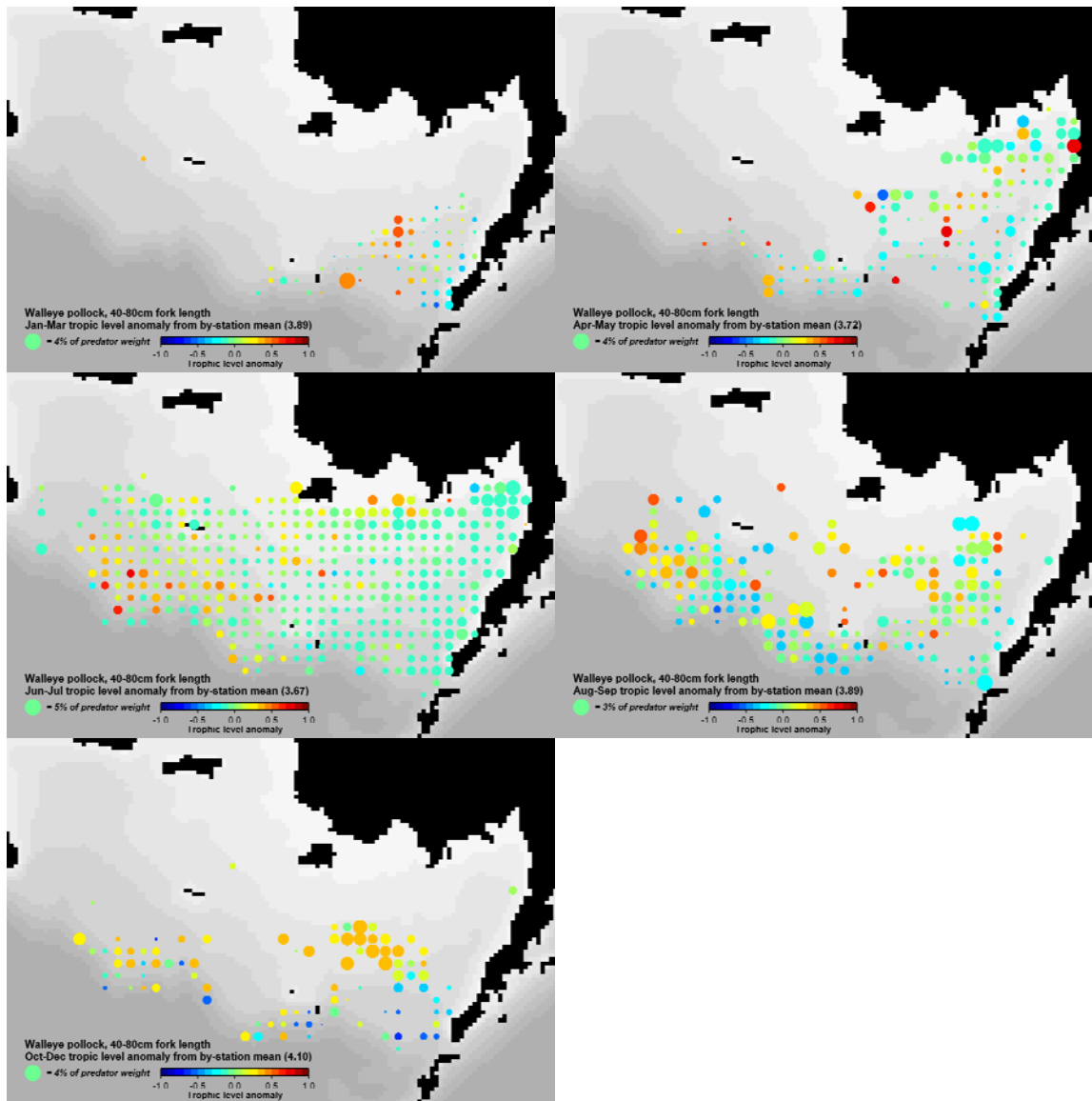


Figure 4. Trophic level seasonal anomaly ( $\pm 1$  trophic level from mean cell trophic level for all samples at that location recorded on each graph), for eastern Bering Sea walleye pollock 40-80cm fork length by season (Jan-Mar, Apr-May, Jun-Jul, Aug-Sep, and Oct-Dec), all years 1982-2008 combined for each 30x30km cell. Only cells with more than 10 samples (across all years) are shown. Circle area is proportional to stomach contents weight; size corresponding to maximum average cell weight is shown. Note that scale changes for each seasonal figure.

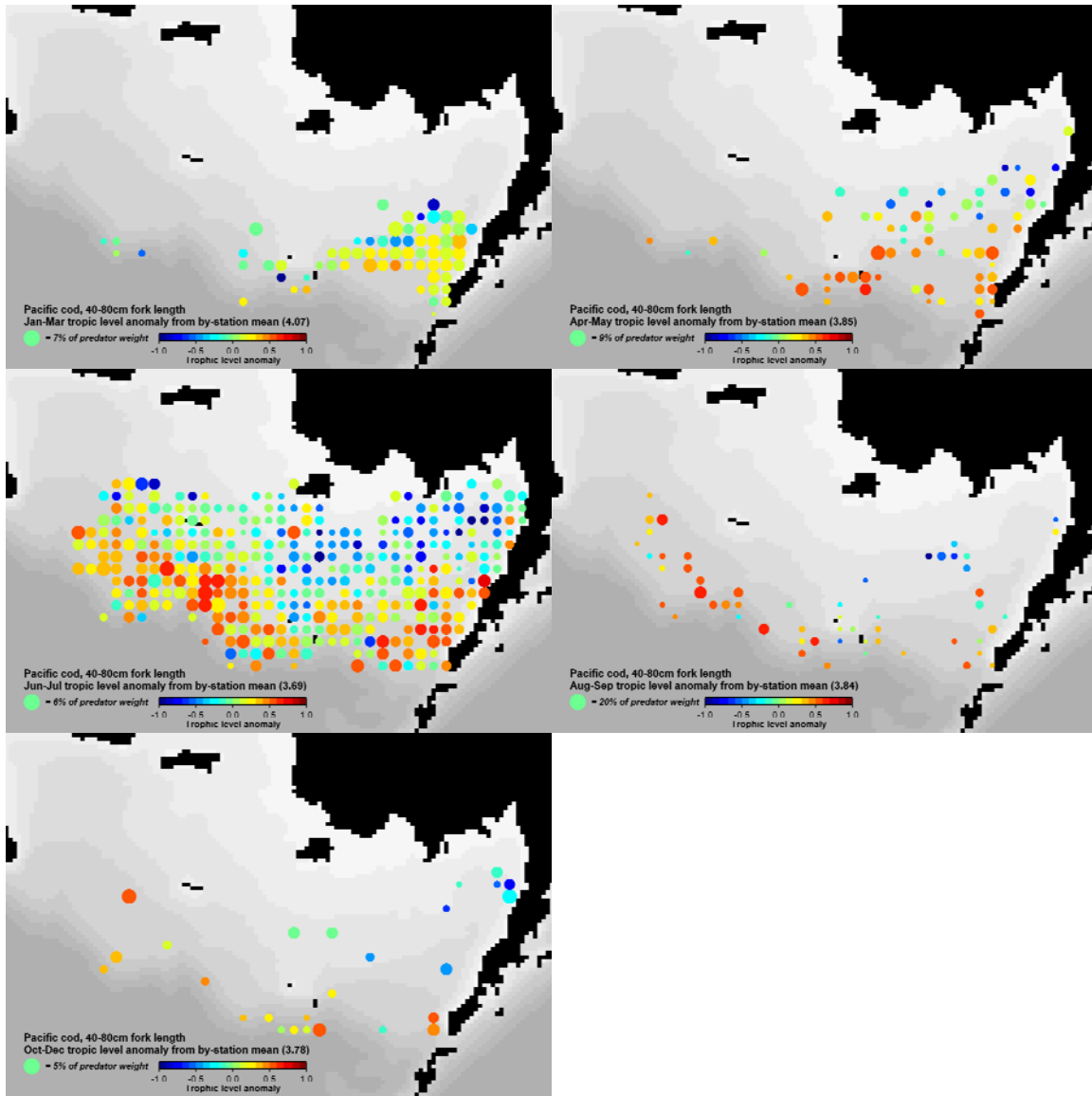


Figure 5. Trophic level anomaly ( $\pm 1$  trophic level from mean cell trophic level recorded on each graph), for eastern Bering Sea Pacific cod 40-80cm fork length by season (Jan-Mar, Apr-May, Jun-Jul, Aug-Sep, and Oct-Dec), all years 1982-2008 combined for each 30x30km cell. Only cells with more than 10 samples (across all years) are shown. Circle area is proportional to stomach contents weight; size corresponding to maximum average cell weight is shown. Note that scale changes for each seasonal figure.

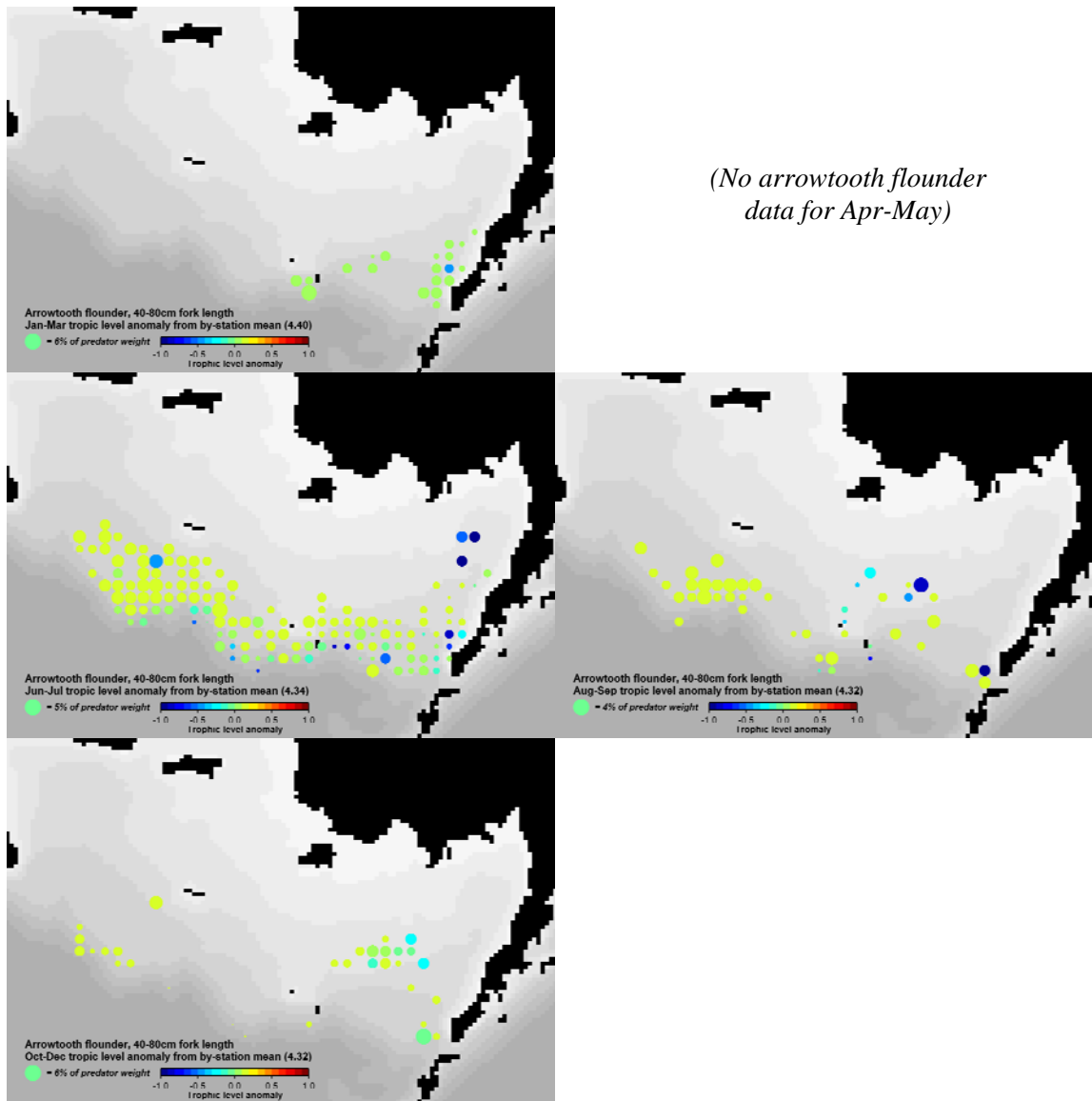


Figure 6. Trophic level anomaly ( $\pm 1$  trophic level from mean cell trophic level recorded on each graph), for eastern Bering Sea arrowtooth flounder 40-80cm fork length by season (Jan-Mar, Apr-May, Jun-Jul, Aug-Sep, and Oct-Dec), all years 1982-2008 combined for each 30x30km cell. Only cells with more than 10 samples (across all years) are shown. Circle area is proportional to stomach contents weight; size corresponding to maximum average cell weight is shown.

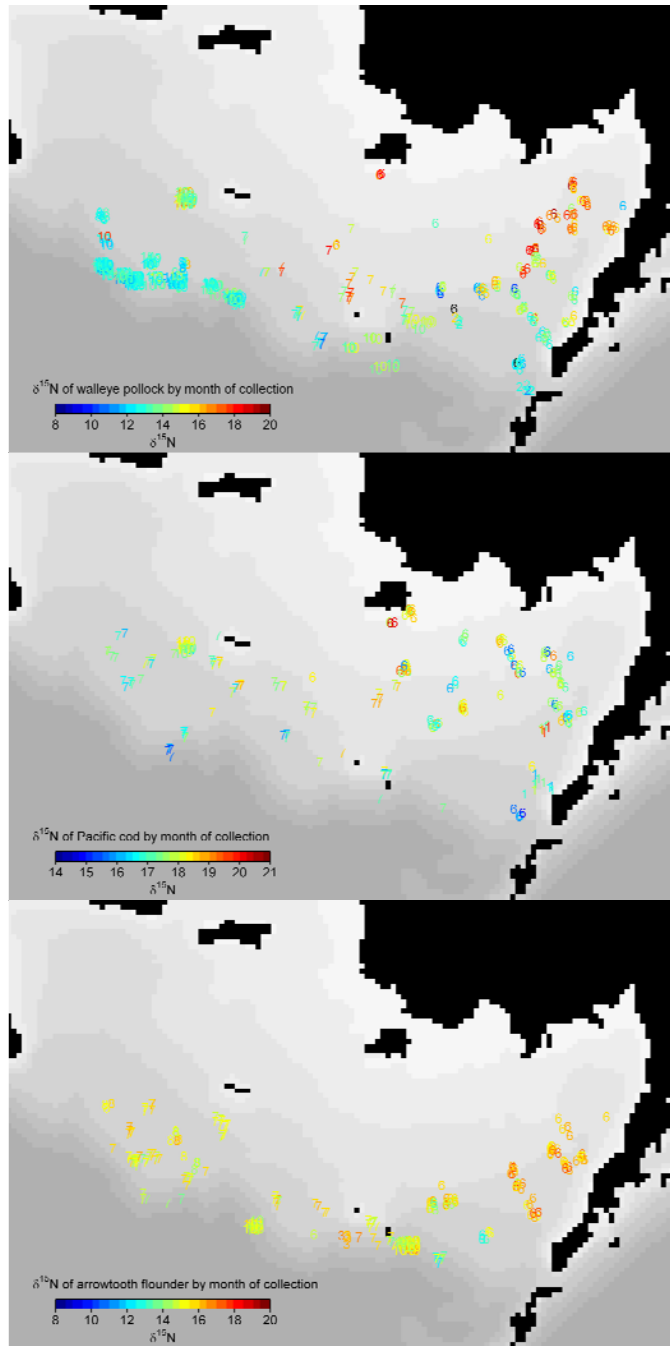


Figure 7.  $\delta^{15}\text{N}$  ratio (colors) and month of collection (numbers) of muscle tissue samples from eastern Bering Sea walleye pollock (top graph), Pacific cod (center graph) and arrowtooth flounder (bottom graph). Collection locations are jittered slightly ( $\pm 10\text{km}$ ) to show sample sizes.

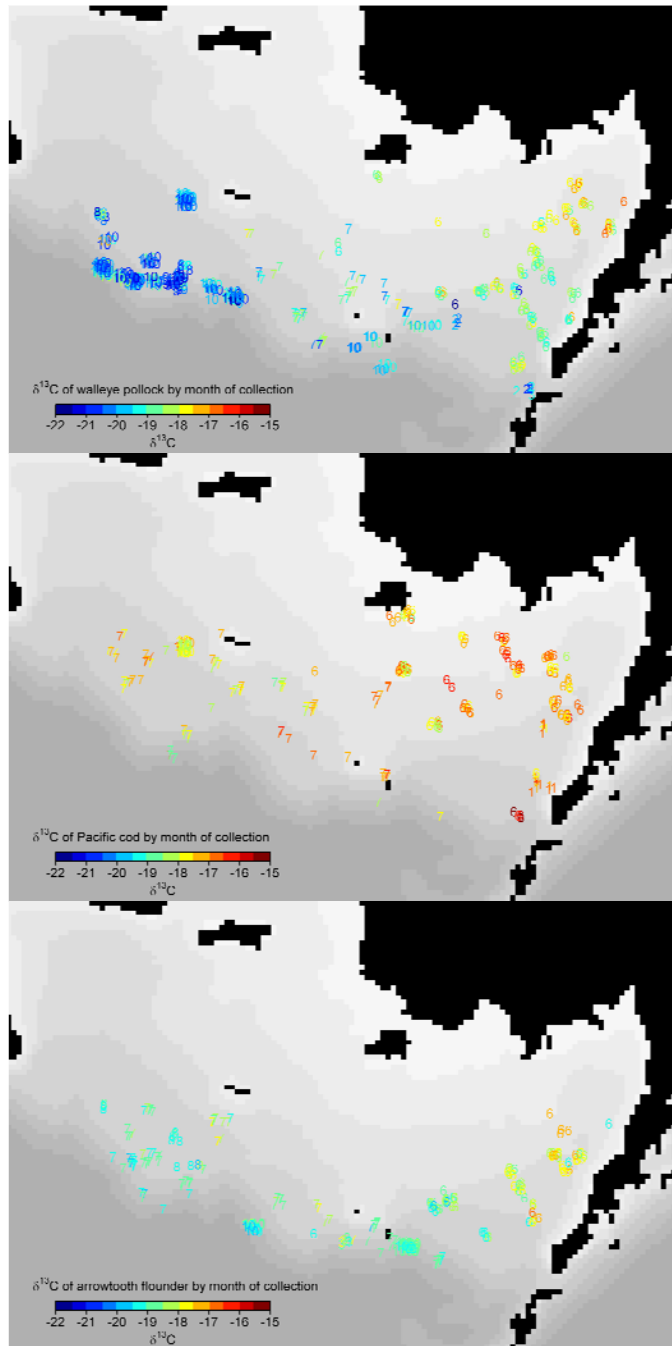


Figure 8.  $\delta^{13}\text{C}$  ratio (colors) and month of collection (numbers) of muscle tissue samples from eastern Bering Sea walleye pollock (top graph), Pacific cod (center graph) and arrowtooth flounder (bottom graph). Collection locations are jittered slightly ( $\pm 10\text{km}$ ) to show sample sizes.

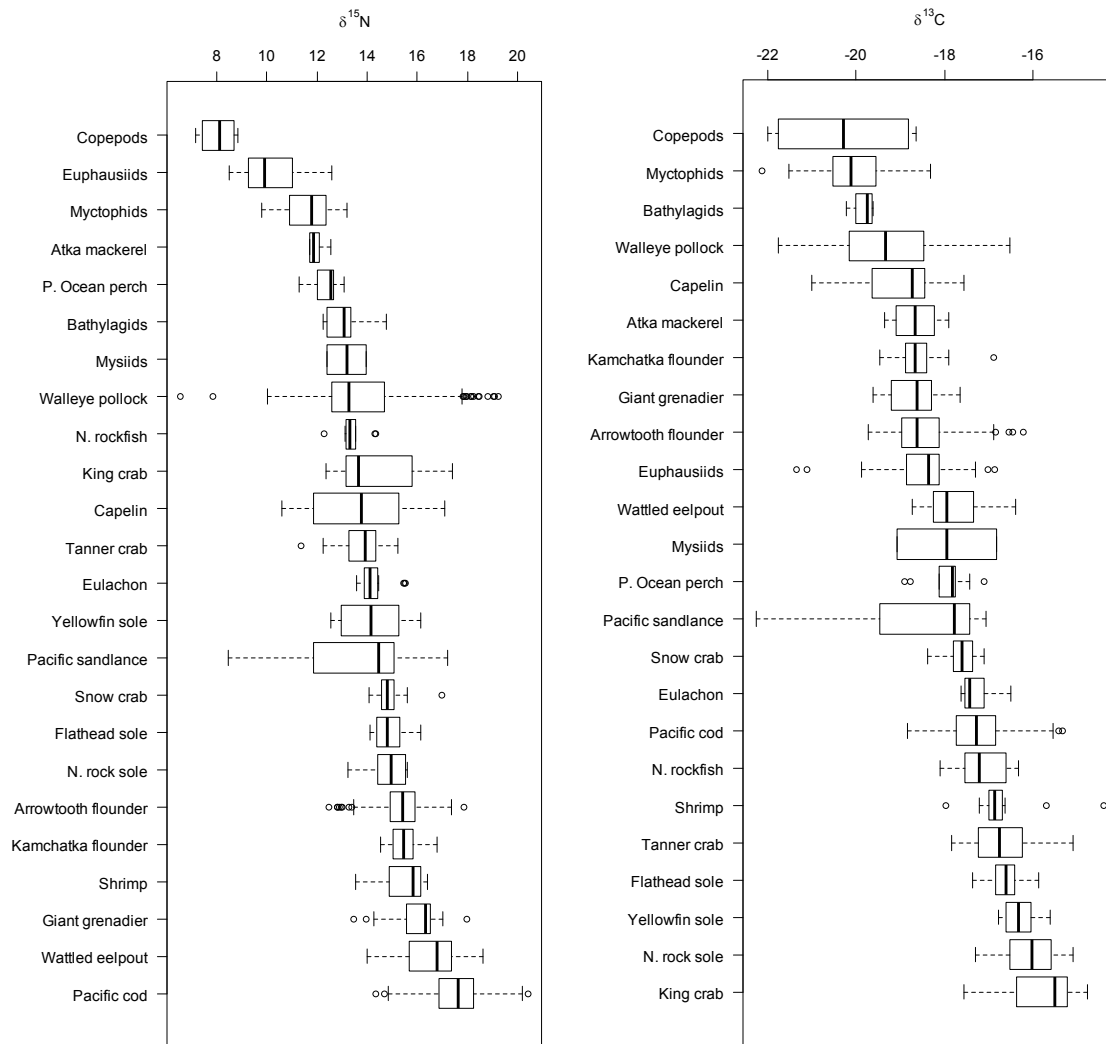


Figure 9. Median (center line), 25-75% range (box) and 5-95% range (whiskers) and outliers of  $\delta^{15}\text{N}$  ratio (left figure) and  $\delta^{13}\text{C}$  ratio (right figure) for all samples collected during this study, 2005-2008 in the eastern Bering Sea, Gulf of Alaska, and Aleutian Islands. Species are sorted by increasing median level of ratio.



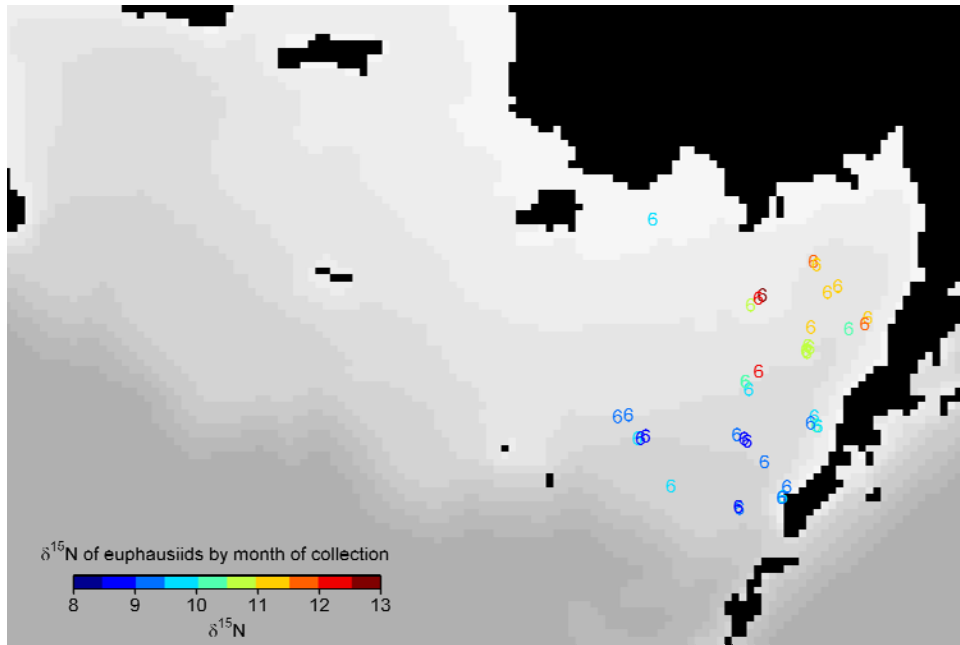


Figure 10.  $\delta^{15}\text{N}$  ratio (colors) and month of collection (numbers) of whole body tissue samples from euphausiids removed from groundfish stomach samples collected in the eastern Bering Sea.

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## **Project Conclusions**

Overall, this project greatly increased our understanding of stable isotope patterns of carbon and nitrogen by the addition of over 2,000 samples taken from Alaskan groundfish to our knowledge base. With the addition of data from nearly 11,000 stomach samples collected during falls and winters 2003-2007 this provides a substantial ability to further examine time trends in trophic level and food web structure as it changes across seasons. However, of particular interest is how our study diverged from overall expectations.

In the first study (Chapter 1), we were able to show, through eye lens chronology of *Berryteuthis magister*, that isotopic signatures showed a seasonal signal that could possibly be attributable to shifts in prey base. It was hoped, going into the second study (Chapter 2), that this would allow stable isotopes to serve as a proxy for dietary shifts that are important to multispecies modeling and the prediction of predation mortality on small fish. For example, if stable isotopes could establish the timing between summer and fall of predatory fish switching from zooplankton to fish, it would greatly aid in estimating fish survival rates in late summer and early fall and increase the accuracy of predictions of recruitment.

However, the results of the second study showed that variation in stable isotopes typically attributed to providing measurements of trophic level, particularly nitrogen, have a signal that may be unrelated to the relative degree of piscivory and plantivory in the fish diets (as measured through stomach samples). Rather, the stable isotope signatures reflected either water properties or shifts in processes occurring in the plankton. This highlights a key finding that “trophic level” is itself an imprecise term and only has meaning relative to potentially unknown baselines. Overall, studies should carefully delimit the processes they are examining (e.g. bottom-up through stable isotopes or top-down through stomach contents analysis) when attempting to develop “holistic” measures of ecosystem function such as trophic-level based indicators or results.

## **Publications**

Hunsicker ME, Essington TE, Aydin KY, and Ishida B. 2010. Predatory role of the commander squid *Berryteuthis magister* in the eastern Bering Sea: insights from stable isotopes and food habits. *Mar Ecol Prog Ser* 415:91-108.

## **Outreach**

January 2009 Oral presentation at Alaska Marine Science Symposium by Mary Hunsicker: “A three-tiered approach for evaluating the predatory role of the commander squid in the eastern Bering Sea”

December 2009 Summarized diet data for 1982-2006 (including mapped data in GIS format as described in milestones) made available at: <http://access.afsc.noaa.gov/REEM/WebDietData/DietTableIntro.php> and <http://www.afsc.noaa.gov/REFM/REEM/map/dietmap.php>.

January 2010 presentation at Alaska Marine Science Symposium by Kerim Aydin: “A web for all seasons: an analysis of 30 years of seasonal and geographic variability in marine food webs through fish food habits and stable isotope analyses”.

October 2010 (funded in-kind) presentation accepted for the North Pacific Marine Science Organization (PICES) annual meeting: “A web for all seasons: an analysis of 30 years of seasonal and geographic variability in marine food webs through fish food habits and stable isotope analyses”.

### **Acknowledgements**

Please see individual chapters for acknowledgements.

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