Temporal and spatial variation in the $\delta^{15}N$ and $\delta^{13}C$ values of fish and squid from Alaskan waters

Carolyn M. Kurle, Elizabeth H. Sinclair, Ann E. Edwards & Carolyn J. Gudmundson

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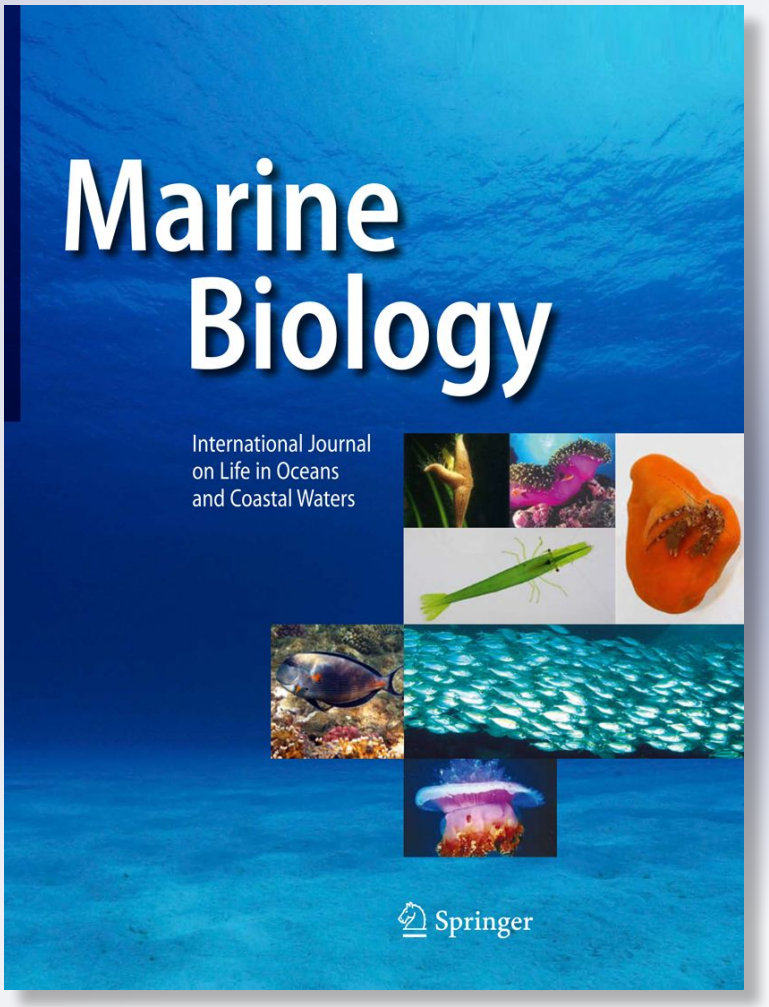

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ORIGINAL PAPER

Temporal and spatial variation in the δ^{15}N and δ^{13}C values of fish and squid from Alaskan waters

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Abstract To test the hypothesis that stable isotope ratios from marine organisms vary, the δ^{15}N and δ^{13}C values from fish and squid collected in Alaskan waters were measured across years (1997, 2000, and 2005), seasons, geographic locations, and different size/age classes, and between muscle tissue and whole animals. Temporal, geographic, and ontogenetic differences in stable isotope ratios ranged from 0.5–2.5‰ (δ^{15}N) to 0.5–2.0‰ (δ^{13}C). Twenty-one comparisons of stable isotope values between whole organisms and muscle tissue revealed only four small differences each for δ^{15}N and δ^{13}C, making costly and space prohibitive collection of whole animals unnecessary. The data from this study indicate that significant variations of stable isotope values from animals in marine systems necessitates collection of prey and predator tissues from the same time and place for best interpretation of stable isotope analysis in foraging ecology studies.

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C. M. Kurle (✉)
Division of Biological Sciences, Ecology, Behavior, and Evolution Section, University of California San Diego, 9500 Gilman Drive, #0116, La Jolla, CA 92093, USA
e-mail: ckurle@ucsd.edu

E. H. Sinclair · C. J. Gudmundson
National Marine Mammal Laboratory, Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA, 7600 Sand Point Way NE, Seattle, WA 98115, USA

A. E. Edwards
Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA, 7600 Sand Point Way NE, Seattle, WA 98115, USA

A. E. Edwards
School of Aquatic and Fisheries Sciences, University of Washington, Seattle, WA 98195, USA

Introduction

The ratios of stable nitrogen (^{15}N/^{14}N, reported as δ^{15}N) and carbon (^{13}C/^{12}C, reported as δ^{13}C) isotopes have proven useful in describing the foraging ecology and movement patterns of marine apex predators (Kurle and Worthy 2002; Herman et al. 2005; Kurle and Gudmundson 2007; Newsome et al. 2010). Predictable enrichment of ^{15}N with increasing trophic level allows for the estimation of an animal's relative trophic position (Vanderklift and Ponsard 2003), whereas stable carbon isotopes can be strong indicators of an animal's habitat use and are valuable for estimating an animal's foraging region (Rubenstein and Hobson 2004). Variations in carbon isotope ratios reflect sources of primary productivity which, in turn, reflect foraging area, making it possible to track movement and migration patterns between isotopically distinct geographic regions (Hobson 2005).

Carbon isotope ratios of organisms in a marine trophic system are influenced by the phytoplankton and marine algae at the base of the food web which are influenced primarily by phytoplankton size, geometry, and growth rate (Popp et al. 1998), the occurrence of phytoplankton blooms (Nakatsuka et al. 1992), the amount and types of primary productivity (Descolas-Gros and Fontugne 1990), CO_2 concentration and CO_2 uptake mechanisms (Burkhardt et al. 1999; Tortell et al. 2000), and variations in aeration surrounding algae caused by turbulence (France 1995). These influences contribute to geographic patterns of δ^{13}C values in marine environments, including decreasing δ^{13}C values with increasing latitude in the northern hemisphere, higher δ^{13}C values in northern

oceans versus southern oceans, and higher $\delta^{13}C$ values in benthic versus pelagic habitats and in nearshore versus offshore food webs (France 1995; Rubenstein and Hobson 2004; Hobson 1999, 2005).

The use of stable isotope ratios to describe foraging ecology of marine predators typically involves comparing the isotope ratios of both prey and predator tissues through application of known or estimated isotope discrimination (or fractionation) factors that exist between predator tissues and their presumed prey (DeNiro and Epstein 1978, 1981). The spatial variation in stable isotope assimilation in marine food webs suggests that, when estimating the foraging ecology of marine predators, it is best to collect and analyze both predator and prey from the same location (Schell et al. 1998; Baduini et al. 2006; Kurle and Gudmundson 2007). However, the potential for variation in stable isotope values not only across marine regions but among different years, seasons, and size/age classes of prey is relatively unknown (but see Baduini et al. 2006; Revill et al. 2009; Karnovsky et al. 2008).

It is increasingly important to understand these variations if we are to successfully use stable isotopes to track and estimate foraging ecology of marine animals. For example, there has been increasing interest in the development of "isoscapes" or maps of regional variation in isotope values (Bowen 2010; Graham et al. 2010). These may be an important tool for studying the foraging ecology and movement patterns of animals without the high cost and time intensive field study required with the use of satellite tracking devices (Graham et al. 2008; Jaeger et al. 2010). However, data for building marine isoscapes are greatly lacking (but see Schell et al. 1998) and, unless we have some understanding of the potential for variability in the stable isotope values of marine organisms, isoscapes created from one set of data points may not be applicable over multiple years, seasons, or age classes of organisms.

Our primary objective in this study was to determine the variability of the $\delta^{15}N$ and $\delta^{13}C$ values from marine fish and squid species across time and space and among age/size classes and between muscle tissue and whole animals. Specifically, we compared: (1) annual, seasonal, geographic, and age-/size-related variations in the $\delta^{15}N$ and $\delta^{13}C$ values of fish and squid common in the marine mammal and seabird prey base of the North Pacific Ocean, and (2) the $\delta^{15}N$ and $\delta^{13}C$ values obtained from whole fish and squid versus subsamples of muscle tissue.

Methods

Sample collection and processing

The selected forage species are fundamental in the diets of cetaceans and depleted or endangered pinnipeds and seabirds that breed in Alaskan waters including: killer whales (*Orcinus orca*) (Herman et al. 2005), Dall's porpoise (*Phocoenoides dalli*) (Sinclair et al. 2005; Herman et al. 2005; Beamish et al. 1999), northern fur seals (*Callorhinus ursinus*) (Antonelis et al. 1997; Sinclair et al. 1994; Kurle and Worthy 2001), Steller sea lions (*Eumetopias jubatus*) (Kurle and Gudmundson 2007; Sinclair and Zeppelin 2002), common murres (*Uria aalge*), thick-billed murres (*U. lomvia*), black-legged kittiwakes (*Rissa tridactyla*), and red-legged kittiwakes (*R. brevirostris*) (Sinclair et al. 2008; Springer et al. 1999). The species selected for the evaluation are also critical to large endangered cetaceans dependent on Alaskan waters as summer foraging grounds including: fin whales (*Balaenoptera physalus*), minke whales (*Balaenoptera acutorostrata*), and humpback whales (*Megaptera novaeangliae*) (Sinclair et al. 2005).

Whole fish or squid were collected opportunistically from the southeastern Bering Sea (BS), Gulf of Alaska (GOA), and Shelikof Strait (SS) in Alaska (Fig. 1) and stored frozen at sea and in the laboratory until analysis. A total of 28 fish from three species collected in summer 1997 from the BS were used in this study. They were collected opportunistically in trawls by National Marine Fisheries Service (NMFS) research cruises (see Kurle and Worthy 2001). Species collected in 1997 and used for this study (with sample sizes in parentheses) were eulachon (*Thleichthys pacificus*) (10), Pacific herring (*Clupea pallasii pallasii*) (8), and walleye pollock (*Theragra chalcogramma*) (10).

A total of 188 individuals from 12 species of fish and squid were collected during winter (February and March) and summer (June through September) 2000 in midwater trawls by NMFS research cruises (see complete list in Supplementary Table S1). Species collected (with sample sizes in parentheses) in 2000 were arrowtooth flounder (*Atheresthes stomias*) (8), eulachon (20), Pacific cod (*Gadus macrocephalus*) (27), Pacific herring (25), salmon (Pink: *Oncorhynchus gorbuscha,* Coho*: O. Kisutch*, and

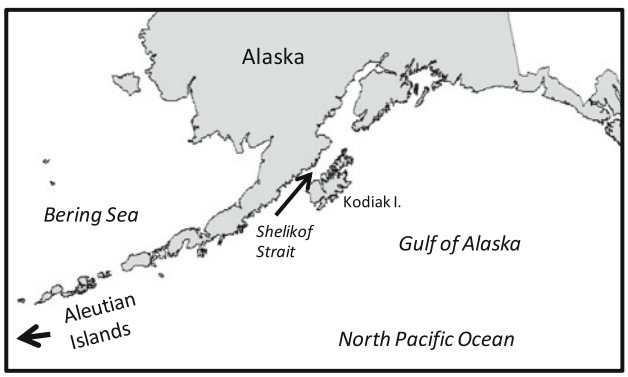

Fig. 1 Fish and squid were collected opportunistically from the southeastern Bering Sea, Gulf of Alaska, and Shelikof Strait in Alaska

Chinook: *O. Tshawytscha*) (17), sandlance (*Ammodytes hexapterus*) (1), squid (*Berryteuthis magister* and *Gonatopsis borealis*) (18), walleye pollock (68), and yellow Irish lord (*Hemilepidotus jordani*) (4). Age approximation data were only available for pollock, so size categories were used for all remaining species. Species were classified by size (total length for fish; dorsal mantle length for squid; cm) as small, medium or large as follows: arrowtooth flounder (0–10, 11–30, 31+), atka mackerel (large: 31+), eulachon (medium: 11–25), P. cod (0–30, 31–70, 71+), P. herring (0–15, 16–30, 31+), salmon (small: 0–30, medium: 31–75), sandlance (small: 0–6), squid (small: 0–10, medium: 11–25), yellow Irish lord (medium: 20–35, large: 35+) (G. Thompson and K. Aydin, NOAA Fisheries, Alaska Fisheries Science Center, personal communication). Walleye pollock were classified by age according to size (total length; cm) (Incze et al. 1988; Sinclair et al. 1994).

We analyzed both whole fish and muscle subsamples from most fish collected in 2000 (see Supplementary Table S2). Muscle samples (1.5 × 2.5 cm) were subsampled at the laboratory and cut from fish at the center of the body below the dorsal fin and above the lateral line and skin removed. Muscle samples were cut from squid at the dorsal posterior portion of the mantle just forward of the fin base.

Muscle tissue from 90 individuals representing four species of fish was collected during summer (June and July) 2005 in midwater trawls by a NMFS research cruise (see Supplementary Table S3). Species analyzed from 2005 (with sample sizes in parentheses) were arrowtooth flounder (20), atka mackerel (*Pleurogrammus monopterygius*) (10), Pacific cod (20), and walleye pollock (40). Sizes were classified in the same methods described above. Muscle samples (2.5 × 2.5 cm) were collected from the dorsal side of the fish with skin attached and frozen until processed for analysis. Whole fish were not saved for samples collected in 2005, and so could not be analyzed for comparison with muscle tissue.

Stable isotope analyses

We homogenized whole individuals collected in 2000 using blenders or grinders and subsampled approximately 5–10 grams of the resulting homogenate. The muscle tissue and subsamples of homogenate were freeze-dried for 24 h, and all lipids removed using petroleum ether (Dobush et al. 1985) in a Soxhlet extractor for 2 h. The solvent was evaporated under a fume hood, and samples ground to a powder by hand. Approximately 1.0–1.5 mg of powdered sample from each specimen was sealed into tin capsules and analyzed using a Carlo Erba NA 1500 CHN Combustion Analyzer interfaced to a Finnigan Delta C mass spectrometer at the Stable Isotope/Soil Biology Laboratory, University of Georgia Institute of Ecology. Measurements of commercially available reference materials across all runs were accurate, and the average precision was 0.10‰ for nitrogen and 0.16‰ for carbon. Samples from 1997 (from Kurle and Worthy 2001) were prepared in the same manner as those collected in 2000, and the average precision was 0.08‰ for both δ^{15}N and δ^{13}C.

We thawed the muscle samples from fish collected in 2005, removed the skin, and placed them in a drying oven for 24 h. Dried samples were ground, weighed, and stable C and N isotope ratios determined using a Costech Analytical Technologies ECS4010 elemental analyzer interfaced via a CONFLO II device to a Thermo-Electron Delta Plus XL mass spectrometer at the Colorado Plateau Stable Isotope Laboratory, Northern Arizona University, Department of Biological Sciences. Measurements of commercially available reference materials across all runs were accurate, and the average precision was 0.14‰ for nitrogen and 0.04‰ for carbon.

The muscle samples from fish collected in 2005 were not lipid extracted. We do not think this is problematic for comparison of stable isotope values from fish muscle among years for several reasons. First, we compare samples collected in 2005 with those collected in 1997 and 2000 in just three interannual comparisons (medium cod, and 1–2 and 6+ year pollock); all other comparisons were made between tissues that were identical in their lipid extraction status. Second, lipids were removed using petroleum ether. Petroleum ether does not extract non-lipid material such as amino acids which may impact protein levels in the lipid-extracted tissue (Dobush et al. 1985). Therefore, it has been demonstrated that the δ^{15}N values from muscle tissues that have had lipids extracted with petroleum ether are the same as those that have not undergone lipid extraction (Dobush et al. 1985; Doucette et al. 2010). This is in contrast to the more commonly employed chloroform/methanol mixtures that remove both lipid and non-lipid material and result in significant elevations of the δ^{15}N values from lipid-extracted tissues (Dobush et al. 1985; Sweeting et al. 2006; Logan et al. 2008). In addition, seabird and tilapia muscle tissues that have been lipid extracted with petroleum ether have δ^{13}C values that are closer to bulk tissue δ^{13}C values than muscle extracted using chloroform/methanol (Schlechtriem et al. 2003; Doucette et al. 2010). Finally, Post et al. (2007) indicate that lipids in a particular tissue need not be accounted for when lipid content is below 5% or C:N ratios are less than 3.5 in aquatic animals. The mean (±SE) C:N ratios for the muscle tissue that was not lipid extracted from the medium cod, and 1–2 and 6+ year pollock collected in 2005 were 3.2 ± 0.01, 3.3 ± 0.03, and 3.2 ± 0.01, respectively, all below the 3.5 value judged necessary to warrant lipid extraction. For these reasons, we are confident that the lipid-extracted fish samples are comparable with those that were not lipid extracted.

All comparisons of stable isotope values from fish collected in 2000 were conducted using whole fish subsamples except for two of the interannual comparisons. For these two cases (medium cod and 6+ year pollock), we compared fish muscle collected in 2000 and 2005 because only muscle was available for comparison from 2005. We contrasted stable isotope values from whole fish collected in 1997 (from Kurle and Worthy 2001) with muscle tissue collected in 2005 for only one interannual comparison (1–2 year pollock). We did this because only whole fish were available from 1997, and only muscle was collected in 2005. We compared stable isotope values from whole animals and muscle tissue from 21 fish and squid and found only four differences each in the δ^{15}N and δ^{13}C values (see "Results" below and Supplementary Table S2). Therefore, we feel that that our one interannual comparison between whole fish and muscle was warranted.

Statistical analyses

We used *t* tests to determine whether δ^{15}N and δ^{13}C values from a single species differed between two age/size classes, and whether the δ^{15}N and δ^{13}C values from a single species were different between regions, seasons, or years. Analysis of variance (ANOVA) and subsequent Tukey's pairwise comparison tests were used to determine differences in carbon and nitrogen isotope ratios when multiple comparisons between age/size classes were possible. We used paired *t* tests to compare the δ^{15}N and δ^{13}C values between muscle and whole-body samples from fish and squid collected in 2000 to determine whether analysis of an entire specimen is required for accurate representation of stable isotope values. All statistical tests were generated using SYSTAT v. 10.2 (SYSTAT Software, Inc., 2002), and significance was determined at the $\alpha = 0.05$ level.

Results

All tests outlined below contrasted groups that were identical save for the characteristic(s) being compared (year, season, region, or age/size class).

Interannual comparisons

We conducted five interannual comparisons of the δ^{15}N and δ^{13}C values from fish collected in summer in different years from the BS (Table 1; Fig. 2a). The δ^{15}N and δ^{13}C values from eulachon were higher in 1997 versus 2000, whereas the δ^{15}N and δ^{13}C values from herring were lower and higher, respectively, in 1997 versus 2000. The δ^{15}N values from cod and 6+ year pollock were higher in 2000 versus 2005, whereas the δ^{13}C values were not different. The δ^{15}N and δ^{13}C values from age 1–2 year pollock were higher in 1997 versus 2005.

Seasonal comparisons

We conducted three seasonal comparisons of the δ^{15}N and δ^{13}C values from fish collected in 2000 in either winter or summer from the BS (Table 2; Fig. 2b). The δ^{15}N values from medium cod and 6+ year pollock were lower for fish caught in the winter versus summer, but not different between seasons for small cod. The δ^{13}C values from small cod and 6+ year pollock were higher in winter versus summer, whereas they were lower in winter versus summer for medium cod.

Geographic comparisons

We conducted five geographic comparisons of the δ^{15}N and δ^{13}C values from whole fish (collected in winter 2000) or fish muscle (collected in summer 2005) from either the BS or GOA (Table 3; Fig. 2c). The δ^{15}N values from large flounder and medium cod muscle tissue collected in 2005 were lower in the GOA versus the BS, but not different between regions for age 1–2 or 6+ year pollock. The δ^{13}C values from large flounder and age 6+ year pollock muscle tissue collected in 2005 were higher in the GOA versus the BS, but not different between regions for medium cod or age 1–2 year pollock. The δ^{15}N values from whole, age 6+ year pollock caught in 2000 were higher in the GOA versus the BS, but the δ^{13}C values were not different.

Geographic and seasonal comparisons

We conducted three comparisons of the δ^{15}N and δ^{13}C values from whole fish collected in either winter or summer 2000 from the BS, GOA, or SS (Table 4; Fig. 2d). The δ^{13}C values were all higher for fish caught in winter in SS (medium eulachon and herring) or GOA (age 6 + pollock) versus in the summer from the BS. However, the δ^{15}N values did not follow this pattern. Eulachon caught in winter from SS had higher δ^{15}N values than those caught in summer in the BS, whereas herring caught in winter from SS had lower δ^{15}N values versus those collected in summer in the BS. The δ^{15}N values from pollock were not different between regions or seasons.

Age/size class comparisons

We conducted comparisons of the δ^{15}N and δ^{13}C values among different age/size classes from eight species caught in 2000 (Table 5; Fig. 2e). Four species showed no differences in stable isotope values between size classes (small versus medium Coho salmon, Chinook salmon, and squid

Table 1 Interannual comparisons

Species	*N*	Mean length (cm)	Estimated age (years) or size class	Collection season/year	Tissue	δ^{15}N	δ^{13}C
Eulachon	10	19.2	Medium	Summer 1997	Whole	**14.0 ± 0.2[a]**	**−18.4 ± 0.1[b]**
Eulachon	10	20.9	Medium	Summer 2000	Whole	**13.5 ± 0.1[a]**	**−19.4 ± 0.1[b]**
Pacific cod	5	45.5	Medium	Summer 2000	Muscle	**16.9 ± 0.2[c]**	−17.0 ± 0.2[d]
Pacific cod	10	58.5	Medium	Summer 2005	Muscle	**16.2 ± 0.2[c]**	−17.3 ± 0.2[d]
Pacific herring	8	28.9	Medium	Summer 1997	Whole	**13.5 ± 0.1[e]**	**−17.6 ± 0.1[f]**
Pacific herring	5	25.0	Medium	Summer 2000	Whole	**14.5 ± 0.1[e]**	**−19.9 ± 0.3[f]**
Walleye pollock	10	14.6	1–2	Summer 1997	Whole	**12.7 ± 0.2 [g]**	**−18.3 ± 0.1 [h]**
Walleye pollock	10	15.7	1–2	Summer 2005	Muscle	**11.8 ± 0.4 [g]**	**−19.6 ± 0.2 [h]**
Walleye pollock	5	48.3	6+	Summer 2000	Muscle	**13.1 ± 0.2[i]**	−19.6 ± 0.2[j]
Walleye pollock	10	49.1	6+	Summer 2005	Muscle	**11.9 ± 0.2[i]**	−19.4 ± 0.1[j]

Letter	*t* value	*df*	*P* value
a	−2.21	18	0.04
b	−5.55	18	<0.01
c	−2.11	13	0.05
d	−0.90	13	0.38
e	5.37	11	<0.01
f	−8.46	11	<0.01
g	−2.21	18	0.04
h	−6.98	18	<0.01
i	−4.17	13	<0.01
j	1.29	13	0.22

The mean (±SE) δ^{15}N and δ^{13}C values from fish collected in the Bering Sea and compared between years. Where possible, we compared muscle tissue or whole, subsampled fish with same. One muscle/whole fish comparison is included as muscle tissue was not available from fish collected in 1997. All fish were lipid extracted except those collected in 2005 (see "Methods" and "Discussion"). Superscripts refer to the results of the *t* tests listed below the table, and numbers in bold indicate a significant difference between pairs

(*G. borealis*) and medium versus large yellow Irish lord), whereas the remaining comparisons (flounder, cod, herring, and pollock) demonstrated at least one isotopic difference (δ^{15}N, δ^{13}C, or both) between various age/size classes.

Larger fish demonstrated higher δ^{15}N values over smaller fish for herring and pollock caught in the winter from SS. Conversely, smaller cod and herring caught in winter and summer, respectively, from the BS had higher δ^{15}N values than their larger counterparts. All remaining comparisons were not different in their δ^{15}N values.

Larger fish demonstrated higher δ^{13}C values over smaller fish for flounder caught in winter from SS and for cod and herring caught in summer from the BS. Conversely, small cod caught in winter from the BS had higher δ^{13}C values than medium cod, and 1–2 year pollock caught in winter from SS had higher δ^{13}C values than 5–6 year pollock. All remaining comparisons were not different in their δ^{13}C values.

Whole animal versus muscle tissue

We compared the δ^{15}N and δ^{13}C values from subsamples of whole fish and squid versus muscle tissue from animals caught in 2000 and found very few significant differences (4 of 21 comparisons each for δ^{15}N and δ^{13}C values; Supplementary Table S2). Muscle tissue had higher δ^{15}N values than whole fish for small cod and medium herring caught in summer from the BS, whereas muscle had lower δ^{15}N values for medium cod caught in summer from the BS and for 3–4 year pollock caught in winter from the GOA. Muscle tissue had higher δ^{13}C values than whole fish for small Coho salmon and medium Coho and Chinook salmon caught in winter from SS, whereas muscle had lower δ^{13}C values for medium cod caught in summer from the BS. All differences were very small and ranged from 0.2 to 0.5‰ for δ^{15}N and 0.3 to 0.4‰ for δ^{13}C.

Discussion

Stable isotope ratios from animal tissues reflect an amalgamation of nutrients incorporated some time previous and up to tissue collection. The speed at which dietary isotopes are incorporated into a particular tissue depends on the growth, metabolism, protein turnover, and synthesis of that

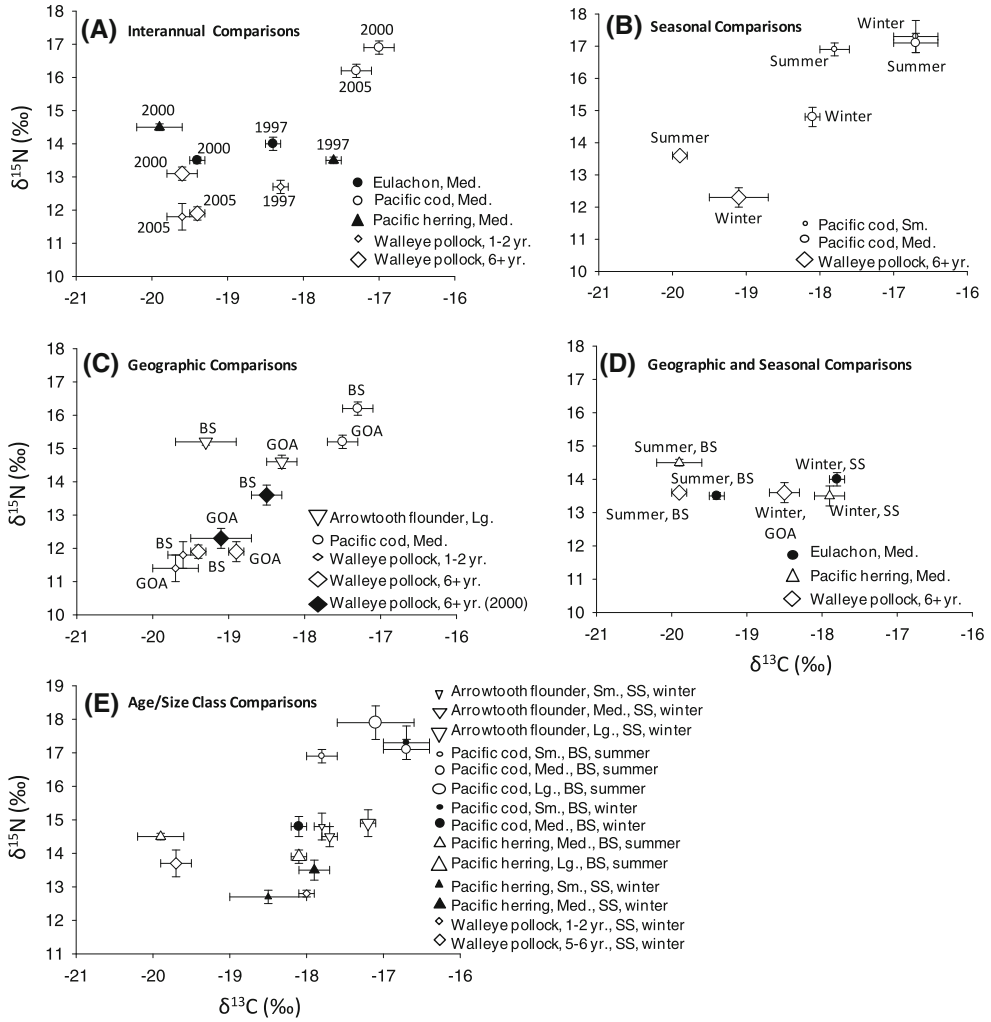

Fig. 2 Stable nitrogen and carbon isotope values (±SE) from different size or age-class fish (*sm* small, *med* medium, *lg* large; *yr* years) collected in various years (1997, 2000, or 2005) and seasons (summer or winter) from Alaskan waters (*BS* Bering Sea, *GOA* Gulf of Alaska, *SS* Shelikof Strait). Tests outlined below contrast groups that are identical save for the characteristic(s) being compared. All statistical results are listed in Tables 1, 2, 3, 4, and 5. **a** Interannual comparisons: All fish were collected in summer from the BS, and the year of collection is marked. Isotope values were significantly different between years for all comparisons except the δ^{13}C values from cod and 6+ year pollock (Table 1). **b** Seasonal comparisons: All fish were collected in 2000 from the BS. Seasons are marked. Isotope values were significantly different between seasons except for the δ^{15}N values from the small cod (Table 2). **c** Geographic comparisons: All fish were collected in summer 2005 except for the 6+ year pollock collected in 2000 (see legend) from either the BS or GOA. All comparisons were significantly different except the δ^{15}N values from all pollock from 2005 and the δ^{13}C values from the cod and 1–2 year pollock (Table 3). **d** Geographic and seasonal comparisons: All fish were collected in 2000. All comparisons were significantly different except the δ^{15}N values from the pollock (Table 4). **e** Age/size class comparisons: All fish were collected in 2000. All comparisons shown are significantly different except the δ^{15}N values from the flounder and the cod collected in summer and the δ^{13}C values from herring collected in winter (Table 5). Fish with no differences in either δ^{15}N or δ^{13}C values are not shown on the graph but appear in Table 5

tissue (Kurle 2009; MacAvoy et al. 2005; MacNeil et al. 2006). Fish muscle has an isotopic turnover that reflects growth and metabolic tissue replacement and varies depending upon the species and age of the fish (Perga and Gerdeaux 2005; Buchheister and Latour 2010; Tarboush et al. 2006; Weidel et al. 2011). Younger fish with higher growth rates have faster isotope turnover times than do adult fish who grow slowly and whose isotopic turnover is due mostly to metabolic tissue replacement (Weidel et al. 2011). Stable isotope residence times (the amount of time the stable isotope signature from a particular diet remains within an animal's tissues; see Supplemental Appendix in Kurle 2009) for muscle from adult fish ranges from 71 to 250 days for carbon (see review in Weidel et al. 2011) and 135–196 days for nitrogen (Buchheister and Latour 2010; Tarboush et al. 2006; Church et al. 2009). Isotope residence times are much shorter for young, rapidly growing fish, and range from 3 to 42 days for carbon (see review in Weidel et al. 2011) and 6–20 days for nitrogen (Sweeting et al. 2005; Bosley et al. 2002). Therefore, although tissues for

Table 2 Seasonal comparisons

Species	*N*	Mean length (cm)	Estimated age (years) or size class	Collection season	δ^{15}N	δ^{13}C
Pacific cod	5	30.6	Small	Winter	17.3 ± 0.5[a]	**−16.7 ± 0.3[b]**
Pacific cod	9	20.6	Small	Summer	16.9 ± 0.2[a]	**−17.8 ± 0.2[b]**
Pacific cod	5	65.4	Medium	Winter	**14.8 ± 0.3[c]**	**−18.1 ± 0.1[d]**
Pacific cod	5	45.5	Medium	Summer	**17.1 ± 0.3[c]**	**−16.7 ± 0.3[d]**
Walleye pollock	3	58.3	6+	Winter	**12.3 ± 0.3[e]**	**−19.1 ± 0.4[f]**
Walleye pollock	5	48.3	6+	Summer	**13.6 ± 0.0[e]**	**−19.9 ± 0.1[f]**

Letter	*t* value	*df*	*P* value
a	−0.94	12	0.37
b	−2.87	12	0.01
c	6.40	8	<0.01
d	6.45	8	<0.01
e	5.91	6	<0.01
f	−2.39	6	0.05

The mean (±SE) δ^{15}N and δ^{13}C values from whole, subsampled fish collected in 2000 from the Bering Sea and compared between seasons. Superscripts refer to the results of the *t* tests listed below the table, and numbers in bold indicate a significant difference between pairs

Table 3 Geographic comparisons

Species	*N*	Mean length (cm)	Estimated age (years) or size class	Collection location	Collection season/year	δ^{15}N	δ^{13}C
Arrowtooth flounder	10	52.1	Large	GOA	Summer 2005	**14.6 ± 0.2[a]**	**−18.3 ± 0.2[b]**
Arrowtooth flounder	10	47.3	Large	BS	Summer 2005	**15.2 ± 0.1[a]**	**−19.3 ± 0.2[b]**
Pacific cod	10	62.5	Medium	GOA	Summer 2005	**15.2 ± 0.2[c]**	−17.5 ± 0.2[d]
Pacific cod	10	58.5	Medium	BS	Summer 2005	**16.2 ± 0.2[c]**	−17.3 ± 0.2[d]
Walleye pollock	10	14.6	1–2	GOA	Summer 2005	11.4 ± 0.4[e]	−19.7 ± 0.3[f]
Walleye pollock	10	15.7	1–2	BS	Summer 2005	11.8 ± 0.4[e]	−19.6 ± 0.2[f]
Walleye pollock	10	48.4	6+	GOA	Summer 2005	11.9 ± 0.3[g]	**−18.9 ± 0.1[h]**
Walleye pollock	10	49.1	6+	BS	Summer 2005	11.9 ± 0.2[g]	**−19.4 ± 0.1[h]**
Walleye pollock	4	49.0	6+	GOA	Winter 2000	**13.6 ± 0.3[i]**	−18.5 ± 0.2[j]
Walleye pollock	3	58.3	6+	BS	Winter 2000	**12.3 ± 0.3[i]**	−19.1 ± 0.4[j]

Letter	*t* value	*df*	*P* value
a	2.38	18	0.03
b	−3.27	18	<0.01
c	3.43	18	<0.01
d	0.98	18	0.34
e	0.99	18	0.33
f	0.40	18	0.70
g	−0.17	18	0.87
h	−2.43	18	0.03
i	3.50	5	0.01
j	1.45	5	0.21

The mean (±SE) δ^{15}N and δ^{13}C values from muscle tissue (fish collected in 2005) or whole, subsampled fish (2000) and compared between the Gulf of Alaska (GOA) and Bering Sea (BS). Superscripts refer to the results of the *t* tests listed below the table, and numbers in bold indicate a significant difference between pairs

Table 4 Geographic and seasonal comparisons

Species	*N*	Mean length (cm)	Estimated age (years) or size class	Collection location	Collection season	δ^{15}N	δ^{13}C
Eulachon	10	19.4	Medium	SS	Winter	**14.0 ± 0.2[a]**	**−17.8 ± 0.1[b]**
Eulachon	10	20.9	Medium	BS	Summer	**13.5 ± 0.1[a]**	**−19.4 ± 0.1[b]**
Pacific herring	3	18.8	Medium	SS	Winter	**13.5 ± 0.3[c]**	**−17.9 ± 0.2[d]**
Pacific herring	5	25.0	Medium	BS	Summer	**14.5 ± 0.1[c]**	**−19.9 ± 0.3[d]**
Walleye pollock	4	49.0	6+	GOA	Winter	13.6 ± 0.3[e]	**−18.5 ± 0.2[f]**
Walleye pollock	5	48.3	6+	BS	Summer	13.6 ± 0.0[e]	**−19.9 ± 0.1[f]**

Letter	*t* value	*df*	*P* value
a	−2.59	18	0.02
b	−8.95	18	<0.01
c	6.90	6	<0.01
d	−4.61	6	<0.01
e	−0.06	8	0.95
f	−6.86	6	<0.01

The mean (±SE) δ^{15}N and δ^{13}C values from whole, subsampled fish collected in 2000 and compared between regions (*BS* Bering Sea, *GOA* Gulf of Alaska, and *SS* Shelikof Strait) and seasons. Superscripts refer to the results of the *t* tests listed below the table, and numbers in bold indicate a significant difference between pairs

this study were collected during either summer or winter, they reflect fish dietary choices that can span from days (younger, growing fish) to months (older fish) previous and up to their collection times.

Interannual variability

The result with perhaps the greatest potential to impact studies employing stable isotope analyses in marine systems is the interannual variability observed in the δ^{15}N and δ^{13}C values from fish. Our results echo those found by Baduini et al. (2006). They demonstrated annual variation in the δ^{15}N and δ^{13}C values from seabird prey items (euphausiid species and pollock) collected in the southeastern BS. Variability in the δ^{15}N and δ^{13}C values from fish among years could be due to several factors. Fish may be foraging at different trophic levels or on different species whose availability may shift with differing conditions from year to year (e.g., see variation in pollock distributions among years in Bailey et al. 1999a). In addition, fish may move through different geographic areas from year to year, thereby acquiring isotopic signatures that do not directly reflect the area in which they were caught.

Interannual variability may also be due to atmospheric changes in carbon composition. All three of the significantly different interannual comparisons of δ^{13}C values from fish were lower in later years (Table 1). These results are consistent with those from studies that found decreases in δ^{13}C values over ~50 years from the middle to the end of the twentieth century in northern fur seal teeth and whale baleen (Cullen et al. 2001; Newsome et al. 2007; Schell 2000, 2001). These changes may be due to increases in isotopically light, anthropogenically created carbon in surface ocean carbon reservoirs (Newsome et al. 2007). However, our data only span 8 years which may not be enough time to reflect atmospheric changes.

In any case, reliance on past studies that categorize marine geographic isotope trends to inform current research on foraging ecology of apex marine predators may be misleading as stable isotope values can vary annually. This type of variability should be considered for future studies. For example, the creation and use of an accurate isoscape, or map, of stable isotope values from fish in Alaskan waters for general use in foraging studies may require annual updating.

As mentioned previously in the Methods, comparison of stable isotope values from lipid- and non-lipid-extracted tissue for three interannual comparisons (medium cod and 1–2 and 6+ year pollock) is not considered problematic. The δ^{15}N values should not be different between treatments because we extracted lipids using the solvent petroleum ether which has been shown to have no effect on the δ^{15}N values of tissue (Dobush et al. 1985; Doucette et al. 2010). The δ^{13}C values may be very slightly different between treatments, but the impact would be extremely minimal as the muscle tissue from the three fish in question all had C:N ratios of 3.2 or 3.3, indicating that the percent lipid in those tissues was ~3% (Post et al. 2007) which is well below the amount required to justify lipid extraction in fish (Post et al. 2007). For example, Post et al. (2007) showed that

Table 5 Age/size class comparisons

Species	*N*	Mean length (cm)	Estimated age (years) or size class	Collection location	Collection season	$\delta^{15}N$	$\delta^{13}C$
Arrowtooth flounder	2	9.3	Small	SS	Winter	14.8 ± 0.4^{a}	-17.8 ± 0.1^{bcd}
Arrowtooth flounder	3	20.2	Medium	SS	Winter	14.5 ± 0.3^{a}	-17.7 ± 0.1^{bce}
Arrowtooth flounder	2	61.5	Large	SS	Winter	14.9 ± 0.4^{a}	-17.2 ± 0.1^{bde}
Pacific cod	9	20.6	Small	BS	Summer	16.9 ± 0.2^{f}	-17.8 ± 0.2^{ghi}
Pacific cod	5	45.5	Medium	BS	Summer	17.1 ± 0.3^{f}	-16.7 ± 0.3^{ghj}
Pacific cod	2	75.0	Large	BS	Summer	17.9 ± 0.5^{f}	-17.1 ± 0.5^{gij}
Pacific cod	5	30.6	Small	BS	Winter	17.3 ± 0.5^{k}	-16.7 ± 0.3^{l}
Pacific cod	5	65.4	Medium	BS	Winter	14.8 ± 0.3^{k}	-18.1 ± 0.1^{l}
Pacific herring	5	25.0	Medium	BS	Summer	14.5 ± 0.1^{m}	-19.9 ± 0.3^{n}
Pacific herring	9	33.8	Large	BS	Summer	13.9 ± 0.2^{m}	-18.1 ± 0.1^{n}
Pacific herring	8	11.2	Small	SS	Winter	12.7 ± 0.2^{o}	-18.5 ± 0.3^{p}
Pacific herring	3	18.8	Medium	SS	Winter	13.5 ± 0.3^{o}	-17.9 ± 0.1^{p}
Salmon, Coho	3	26.0	Small	SS	Winter	15.2 ± 0.2^{q}	-18.0 ± 0.2^{r}
Salmon, Coho	7	55.9	Medium	SS	Winter	15.0 ± 0.2^{q}	-17.6 ± 0.2^{r}
Salmon, Chinook	2	26.8	Small	SS	Winter	15.3 ± 0.2^{s}	-17.7 ± 0.0^{t}
Salmon, Chinook	3	50.7	Medium	SS	Winter	14.9 ± 0.1^{s}	-17.5 ± 0.0^{t}
Squid (*G. borealis*)	2	9.5	Small	BS	Winter	10.0 ± 0.3^{u}	-20.2 ± 0.4^{v}
Squid (*G. borealis*)	9	12.0	Medium	BS	Winter	9.7 ± 0.4^{u}	-20.4 ± 0.2^{v}
Walleye pollock	41	11.6	1–2	SS	Winter	12.8 ± 0.1^{w}	-18.0 ± 0.1^{x}
Walleye pollock	3	42.7	5–6	SS	Winter	13.7 ± 0.4^{w}	-19.7 ± 0.2^{x}
Yellow Irish lord	2	31.0	Medium	BS	Winter	14.9 ± 0.2^{y}	-16.8 ± 0.8^{z}
Yellow Irish lord	2	39.5	Large	BS	Winter	15.1 ± 0.1^{y}	-17.8 ± 0.3^{z}

Superscript	ANOVA and tukey tests	*t* value	*df*	*P* value
a	ANOVA, $F_{2,4} = 0.3$, $P = 0.78$			
b	ANOVA, $F_{2,4} = 12.5$, $P = 0.02$			
c	Tukey's, $P = 0.82$			
d	Tukey's $P = 0.02$			
e	Tukey's $P = 0.03$			
f	ANOVA, $F_{2,13} = 2.0$, $P = 0.18$			
g	ANOVA, $F_{2,13} = 5.3$, $P = 0.02$			
h	Tukey's $P = 0.02$			
i	Tukey's $P = 0.74$			
j	Tukey's $P = 0.34$			
k		−4.48	8	<0.01
l		−4.84	8	<0.01
m		−2.37	12	0.04
n		6.63	12	<0.01
o		4.85	9	<0.01
p		1.85	9	0.10
q		−0.79	8	0.45
r		1.28	8	0.24
s		−1.92	3	0.15
t		2.95	3	0.06
u		−0.32	9	0.76
v		−0.39	9	0.70
w		−3.67	42	<0.01
x		8.59	42	<0.01

Table 5 continued

Superscript	ANOVA and tukey tests	*t* value	*df*	*P* value
y		1.81	2	0.21
z		−1.17	2	0.36

The mean (±SE) δ^{15}N and δ^{13}C values from whole, subsampled fish collected in 2000 and compared between age/size classes. Superscripts refer to the results of the ANOVA, Tukey, and *t* tests listed below the table, and underlined superscripts indicate a significant difference among age or size classes. *BS* Bering Sea, *GOA* Gulf of Alaska, and *SS* Shelikof Strait

fish with C:N ratios of 3.2 and 3.3 demonstrated a decrease of 0.3‰ and an increase of 0.5‰, respectively, in their δ^{13}C values after they were lipid extracted with chloroform/methanol. These are very small differences which would likely be even smaller in our study as we used petroleum ether which is known to yield δ^{13}C values that are closer between lipid- and non-lipid-extracted tissue samples than when chloroform/methanol is used (Doucette et al. 2010; Schlechtriem et al. 2003).

Fish used for these three comparisons were also analyzed for stable isotope values in different laboratories (see "Methods"). Both laboratories are standardized relative to IAEA standards, and the significant differences in stable isotope values between the fish were well over 0.5‰ for both δ^{15}N and δ^{13}C. Therefore, we are confident that any potential calibration difference between laboratories would be so small as to be inconsequential given the large isotopic disparities between samples.

Seasonal variability

While our fish were collected in either summer or winter, stable isotope turnover times in muscle tissue from adult fish are such that the isotope values reflect an amalgamation of dietary choices that include prey from months previous and up to the time of collection (see above). Therefore, stable isotope values from adult fish tissue collected in summer reflect diet items incorporated starting in early spring and extending to the time of collection, whereas isotope values from adult fish collected in winter reflect diet items incorporated in early fall to the time of collection. Stable isotope values from juvenile fish with rapid growth rates reflect dietary items incorporated weeks to days previous and up to collection and so represent the season in which they were collected.

We recognize that true seasonal variation in stable isotope values should be measured from individuals collected over multiple years to determine whether seasonal patterns exist. Our data were collected opportunistically, and we were unable to conduct such rigorous comparisons. However, we still think it is interesting to consider the potential seasonal variations suggested by our data and the possible ramifications for using stable isotopes in marine systems. For example, medium-sized Pacific cod caught in the summer from the BS had δ^{15}N and δ^{13}C values that were 2.3 and 1.4‰ higher, respectively, than those from cod caught in the winter. Substantial differences such as these could be ecologically significant when trying to reconstruct diet and movement patterns of marine animals and should be considered.

The δ^{13}C values from all three seasonally compared fish were different, but with no consistent pattern. The δ^{13}C values were higher in winter for small cod and adult pollock, and in summer for medium cod. Seasonal variation in the stable carbon isotope values from fish is likely due to their movement patterns. For example, in winter, adult pollock in the eastern BS move onto the shelf from the upper slope. Starting in spring (March), they move to the outer shelf to spawn and, in late summer and fall, are widely distributed (Mito et al. 1999). The stable isotope values from adult pollock collected in winter in this study would reflect dietary items incorporated starting in late fall and continuing into winter when they are on the inner BS shelf, whereas values from fish collected in summer would reflect late spring/early summer when they are on the outer shelf spawning. This could lead to the higher δ^{13}C values we observed from adult pollock in winter as animals foraging more nearshore would have higher δ^{13}C values than those foraging more offshore (see "Introduction"). A similar pattern is likely influencing cod δ^{13}C values. We observed higher δ^{13}C values from adult cod in summer over those caught in winter. Cod in the eastern BS move off-shelf toward the shelf break starting in late September, whereas they move from the deep off-shelf water to shallower depths on the shelf in early summer during their post-spawning dispersal (Shimada and Kimura 1994). This would lead to higher δ^{13}C values in spring/summer (on-shelf, more nearshore) than in fall/winter (deeper water, off-shelf). Cod do not mature until approximately 2 years of age (~41–70 cm) (Coonradt 2002; Gustafson et al. 2000), so the small cod in our study are juveniles. They demonstrate an isotopic pattern opposite that shown by the mature cod which is likely influenced by movement patterns that are entirely different from mature cod as juveniles are not motivated by spawning. In addition, the isotope turnover is faster for growing juveniles, so their

tissues are more reflective of the waters in which they were caught (see "Discussion" below).

Many seasonal factors influence the $\delta^{13}C$ values from phytoplankton at the base of the marine food web which would impact organisms higher in the trophic chain. For example, seasonal differences in availability of light, nutrients, and speed of cellular growth all influence algal blooms in the marine environments of Alaska and could contribute to the seasonal variations observed in the $\delta^{13}C$ values from fish in this study (see "Introduction").

Seasonal variation in movement patterns may also lead fish to encounter different prey, resulting in changing $\delta^{15}N$ values throughout the year. Juvenile cod appear to be foraging at the same trophic level even if they are located in different areas (as indicated by their $\delta^{13}C$ values). Mature cod and pollock appear to be foraging at higher trophic levels in spring/summer which may be related to prey availability in the different waters in which they reside in the different seasons.

Geographic variability

Regional trends of higher $\delta^{13}C$ values from copepods, herring, pacific cod, and walleye pollock in the GOA over those from the BS have been demonstrated in other studies (Kurle and Gudmundson 2007; Hirons et al. 1998). In this study, higher $\delta^{13}C$ values from the GOA were indicated in two (6+ year pollock and large flounder caught in summer 2005) of five comparisons, whereas the remaining fish showed no difference between regions. It is difficult to determine why we saw geographic differences in $\delta^{13}C$ values for only two comparisons, but it may be due to variations in site fidelity and movement patterns.

For example, mature pollock (>3–4 year) appear to demonstrate spawning site fidelity (Ciannelli et al. 2007; Bailey et al. 1999a, b), implying that older pollock throughout the GOA and BS regions remain in their distinct bodies of water just before and during spawning which occurs from approximately mid-March to early May in the GOA (Hinckley et al. 2001) and from March to June in the southeastern BS (Hinckley 1987). Indeed, several studies have shown that walleye pollock demonstrate moderate amounts of genetic differentiation between GOA and eastern BS populations (Bailey et al. 1999a, b; O'Reilly et al. 2004), suggesting an isolation-by-distance pattern in their stock structures. Therefore, stable isotope signatures from adult pollock collected in summer from the GOA and BS may reflect the $\delta^{13}C$ values representative of their respective spawning grounds. Site fidelity may also explain the distinct $\delta^{13}C$ signatures observed from mature arrowtooth flounder. For example, mature flounder ($\sim$46 cm) (Stark 2008) in the GOA spawn from January to April and remain within the GOA throughout this time (Bailey et al. 2008).

We found no difference in the $\delta^{13}C$ values from medium Pacific cod caught in the GOA and BS despite evidence for genetically distinct stock structures that follow an isolation-by-distance pattern between these two regions (Cunningham et al. 2009). There is evidence for long-range movement in Pacific cod (Shi et al. 2007; Shimada and Kimura 1994; Conners and Munro 2008), especially following the peak of spawning in March when they disperse widely on their feeding migration (Shimada and Kimura 1994; Conners and Munro 2008). Widespread movement between the southeastern BS and GOA would result in similar $\delta^{13}C$ values from fish caught in both regions.

The $\delta^{13}C$ values from age 6+ year pollock were also not different from fish collected in both regions in the winter. Mature pollock are not spawning in late fall/early winter, and therefore do not demonstrate the site fidelity observed in spring/summer discussed above. In fact, adult pollock move long distances during their fall feeding season and could be moving between the GOA and the southeastern BS during this time (Bailey et al. 1999a, b). Tissues from adult pollock collected in winter from the BS and GOA could reflect an amalgamation of the $\delta^{13}C$ signatures of both areas which likely accounts for the similarity in the $\delta^{13}C$ values between regions. This is also likely why the $\delta^{13}C$ values were the same from age 1–2 year pollock collected in the summer from the GOA and BS. Juvenile pollock are also known to disperse widely and could easily move between the Shumagin Islands in the GOA and the southeastern BS through Unimak Pass (Personal Communication, K. Bailey, Senior Scientist, Alaska Fisheries Science Center, National Marine Fisheries Service, Seattle, WA), making them isotopically indistinct between regions.

There were three differences in the $\delta^{15}N$ values from fish observed between the GOA and BS (large flounder and medium P. cod caught in summer and adult pollock caught in winter). The values were higher for fish caught in the BS in summer which follows the same pattern observed in Hirons et al. (1998) for adult pollock and cod (higher $\delta^{15}N$ values from fish in the eastern BS and lower values in the GOA). In winter, the fish caught in the GOA had higher $\delta^{15}N$ values than those caught in the BS which is the pattern observed by Hirons et al. (1998) for copepods and adult herring. Their study did not indicate the season in which they were collected.

As mentioned previously, $\delta^{15}N$ values reflect animal diets and therefore reflect similarities or differences in the trophic level at which fish are foraging regardless of their location. For example, spawning and genetic data from other studies, and the differential carbon stable isotope signatures between regions from this study, all indicate that adult pollock have strong site fidelity during spring/early

summer, but the δ^{15}N values are identical between regions for fish caught in the summer indicating that adult pollock are foraging at a similar trophic position in spring/early summer regardless of location. Alternatively, medium cod had nearly identical δ^{13}C values between regions, indicating that mixing occurred between regions. However, their δ^{15}N values were 1.0‰ different, indicating that fish caught in BS were feeding on slightly higher trophic level prey.

Geographic and seasonal variability

Our data allowed us to compare the stable isotope values between different regions and seasons for three species of fish. All δ^{15}N and δ^{13}C values were different except for the δ^{15}N values from age 6+ year pollock caught in winter from the GOA and in summer from the BS. As explained above, adult pollock move extensively in the fall feeding season and fish caught in winter in the GOA could easily be reflecting the δ^{13}C values from southeastern BS waters. We included these comparisons to underscore the point that geographic and seasonal differences in stable isotope values exist and should be considered when interpreting stable isotope data from marine species.

Age/size class variability

Whereas we found more differences than similarities in the δ^{15}N and δ^{13}C values from fish among years, seasons, and from different regions, the differences were minimal when comparing isotope ratios among size/age groups of the same species. For example, the δ^{15}N value from arrowtooth flounder was equal among all size classes. This is expected as their diet contains a mix of mostly euphausiids and walleye pollock that varies little with age (Yang and Livingston 1986).

The δ^{15}N values of some fish and squid are thought to increase with size/age because they accumulate heavier isotopes with increasing body size (Hobson et al. 2004; Kurle and Worthy 2001; Revill et al. 2009) due to foraging at higher trophic levels. The trend observed in this study of higher δ^{15}N values with age between 1- to 2-year-old and 5- to 6-year-old pollock from SS is consistent with this scenario and with observations that age 1–2 year pollock eat at a lower trophic level than age 5–6 pollock (Incze et al. 1988), and older pollock are cannibalistic on younger pollock (Wespestad et al. 2000).

Small to medium cod from winter and medium to large herring collected in summer from the BS show small decreases in δ^{15}N values with increasing size. This is the same pattern observed for herring by Kurle and Worthy (2001), sardines (*Sardina pilchardus*) by Bode et al. (2003), and albacore tuna (*Thunnus alalunga*) by Revill et al. (2009). Both Revill et al. (2009) and Bode et al. (2003) attribute this pattern to a dietary shift to lower trophic levels with increasing age which could also be occurring with the cod and herring in this study. Pacific cod diet ranges from invertebrates to fish (Albers and Anderson 1985), and data indicate that piscivory does not correlate with increasing body size in cod (Knoth and Laurel 2009). Therefore, older cod in this study may have been foraging on a higher amount of lower trophic level invertebrates than their younger counterparts. Pacific herring reach maturity at 17 cm (Paul et al. 1996), so both medium and large herring in this study were adults. The small increase in the δ^{15}N value from medium over large herring caught in summer from the BS may reflect some small variation in the trophic level at which they were foraging as adult herring eat a mix of lower trophic level zooplankton and higher trophic level fish (Brodeur and Livingston 1988). Finally, our findings could be an artifact of small sample sizes or may reflect sampling bias due to size categorization rather than reflecting a biological pattern.

Our data demonstrated increasing δ^{13}C values with size in four comparisons (small to large and medium to large arrowtooth flounder, small to medium cod caught in the summer from the BS, and medium to large herring caught in summer from the BS), whereas two demonstrated the opposite pattern (small to medium cod caught in winter from the BS and 1–2 year versus 5–6 year pollock caught in winter from SS) and nine demonstrated no differences (see Table 5).

Reasons for discrepancies in δ^{13}C values with size are likely a reflection of variation in the distribution of each species in different life-history stages, further influenced by seasonal variation in distribution at size. For example, animals foraging nearshore typically have higher δ^{13}C values than those foraging offshore (McConnaughey and McRoy 1979). Life-history information on many species of fish and especially squid is limited, but it is known that Pacific herring move inshore as adults to lay eggs (Mito et al. 1999) and that 1- to 2-year-old pollock tend to be closer inshore over the continental shelf in the eastern BS than adults (Brodeur et al. 1999, 2002).

Compounding this, adult fish have much slower isotopic turnover times than faster growing juveniles, so differences in δ^{13}C values between fish size classes caught in the same area and at the same time may simply be reflecting differences in geographic location. As mentioned above, the difference in stable isotope turnover times between adult and juvenile fish means that isotope values from adults reflect a time period up to several months previous to their winter collection, whereas the juvenile tissues reflect a time period that is only weeks to days previous to their collection. For example, the adult pollock (5–6 year) have higher δ^{15}N values than the juveniles (1–2 year), indicating that

they are foraging at a higher trophic level which follows published accounts of pollock diets (e.g., Wespestad et al. 2000). However, the adult pollock δ^{13}C values are lower than those from the juveniles, which does not follow the expected pattern given their higher trophic position. The δ^{13}C values from adults are probably reflecting a location other than SS where they were caught which would account for the difference in δ^{13}C values between age/sex classes. This variation in the time periods reflected in the fish tissue could account for the differences in the δ^{13}C values between juvenile and adult fish for all comparisons in this study and should be considered.

Whole animal versus muscle tissue

Our data indicate that stable isotope analysis of fish or squid muscle is comparable with that of the entire homogenized animal (Table S2), thus saving preparation time and storage space when collecting marine species for isotopic analyses. However, there may be conditions under which signatures could differ. For example, whole-body chemistry of gravid females or especially oily fish may have different isotope signatures than a subsample of their muscle tissue. Future comparisons considering such life-history differences would be useful for isotope studies.

Conclusions

The δ^{15}N differences in this study ranged from 0.5 to 2.5‰ which could be ecologically significant as the accepted stable nitrogen isotope discrimination value between prey and predator in marine environments is 3–5‰ (Minagawa and Wada 1984; Kurle 2002). Significant differences in δ^{13}C values as small as 0.7‰ have been shown from serum collected from Steller sea lions foraging in different regions (Kurle and Gudmundson 2007). Therefore, even small differences in δ^{13}C values from species caught at different times or in different seasons or from different age/size classes may be enough to misinterpret marine predator foraging ecology.

Our research supports previous studies indicating that isotopic variations in marine organisms occur with changes in environment and life-history stage (i.e., Barnes et al. 2008, 2009; Jennings and Warr 2003), but our work is expansive in that we analyzed 13 species over multiple years, seasons, geographic locations, and size/age classes. We found more significant differences than similarities in our comparisons of δ^{15}N and δ^{13}C values from fish and squid between years, seasons, regions, and size/age classes, indicating that coherency is critical for accurate interpretation of marine predator foraging ecology.

Our samples were primarily collected opportunistically, resulting in very small sample sizes for some species. Small samples sizes can lead to a reduction in statistical power and an increased probability of failing to detect a statistical difference when one exists. Therefore, our results may underestimate the variability that exists in stable isotope values of fish and squid and this caveat should be considered when interpreting our results. We strongly recommend further study of temporal and spatial patterns in stable isotope values from animals in marine systems.

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