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Trophic Ecology of Summer Flounder in Lower Chesapeake Bay Inferred from Stomach Content and Stable Isotope Analyses

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Abstract

Trophic studies of summer flounder *Paralichthys dentatus* have relied on traditional stomach content analyses to infer contributions of prey to species productivity. We applied both stable isotope and stomach content analyses to identify prey groups that are responsible for summer flounder productivity in lower Chesapeake Bay and to explore ontogenetic patterns in prey utilization. Summer flounder (total length = 138–682 mm; age = 0–11 years) were collected for stomach and tissue samples (liver, blood, and muscle) during spring–summer (May–July) and fall (November) of 2006 and 2007. Commonly consumed crustacean and fish prey species were also collected: mysid shrimp *Neomysis americana*, sevenspine bay shrimp *Crangon septemspinosa*, mantis shrimp *Squilla empusa*, bay anchovy *Anchoa mitchilli*, spotted hake *Urophycis regia*, and juvenile sciaenids. Analysis of the nitrogen stable isotope ratio ($\delta^{15}\text{N}$; ratio of ^{15}N to ^{14}N relative to a standard) revealed that crustaceans comprised the majority (72–100% on average) of the summer flounder diet except in spring 2006, when fish consumption was more dominant. Analysis of corresponding stomach contents indicated a lower contribution of crustacean prey. Based on isotopes, summer flounder tended to occupy the same trophic level as the prey fishes. The $\delta^{15}\text{N}$ in all tissues exhibited a positive trend with body length, indicating that larger summer flounder fed at approximately one trophic level above smaller individuals; the positive trend also corresponded with increasing proportions of fish in summer flounder stomachs. Our stable isotope analysis indicates that growth and production of summer flounder in lower Chesapeake Bay are highly dependent on assimilation of mysid shrimp, sevenspine bay shrimp, and mantis shrimp—more so than previously expected based on stomach content research.

Along the U.S. eastern seaboard, the Chesapeake Bay acts as a primary juvenile nursery and crucial foraging habitat for a broad fauna of coastal migratory fishes (Murdy et al. 1997), but the prey resources that support fishery production in this region have not been thoroughly examined with methods other than traditional stomach content analysis. The summer flounder *Paralichthys dentatus* is a seasonal inhabitant of Chesapeake Bay that resides in the estuary from spring to fall (Able et al. 1990; Szedlmayer et al. 1992; Bonzek et al. 2008) before migrating to spawning regions on the continental shelf (Packer et al. 1999; Terceiro 2002; Able and Fahay 2010). Owing to the economic and ecological importance of summer flounder along

the U.S. Atlantic coast, this species has been strictly managed (Terceiro 2002). To support management needs and multispecies modeling efforts, summer flounder and other bay fishes have been monitored by the Chesapeake Bay Multispecies Monitoring and Assessment Program (ChesMMAAP) since 2002 (Latour et al. 2003). To date, summer flounder diet assessments from this program (Latour et al. 2008) and trophic studies conducted in similar environments have relied solely on stomach content analyses (e.g., Burke 1995; Rountree and Able 1992; Link et al. 2002). These studies have documented that summer flounder primarily consume crustaceans (e.g., mysid shrimp *Neomysis americana*, sevenspine bay shrimp *Crangon septemspinosa*, and

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mantis shrimp *Squilla empusa*) and small fishes (e.g., bay anchovy *Anchoa mitchilli* and sciaenids), but it remains unclear how each prey group contributes to actual somatic growth and production.

Stable isotopes have emerged as a valuable complement to traditional stomach content analyses by contributing to the characterization of trophic relationships and aiding in food web modeling. Dietary studies involving stable isotope ratios (e.g., $\delta^{15}\text{N}$; the ratio of ^{15}N to ^{14}N relative to a standard) rely on the fact that an organism's tissue is synthesized from assimilated organic matter and reflects the isotopic signatures of the consumed prey (Fry 2006). The consumer's tissue will be a time-integrated dietary representation on a scale of weeks to months (e.g., Buchheister and Latour 2010), unlike stomach contents, which document feeding habits on the order of hours. Thus, stable isotopes can elucidate the prey groups that are directly responsible for driving tissue growth and production of consumer species (Fry 2006). This information is important for ecosystem modeling efforts requiring quantitative information on food web relationships over longer time periods that are more relevant to ecosystem processes. Stable isotope analyses do not attain the same level of taxonomic resolution afforded by stomach content analysis, but isotope techniques are useful for identifying broader sources of production and for differentiating between benthic and pelagic trophic pathways (Fry and Sherr 1989).

The goal of this study was to use stable isotopes in multiple tissues of summer flounder to build upon previous ChesMMAp stomach content analyses by providing a broader understanding of the prey groups driving summer flounder production in lower Chesapeake Bay. The specific objectives were to (1) characterize and compare summer flounder food habits in the bay's main stem by means of both stomach content analysis and stable isotope methods and (2) explore ontogenetic patterns in resource use. Diet analyses conducted by Latour et al. (2008) are expanded in the present paper by examining another year of ChesMMAp data, but here we focus primarily on stable isotope analyses of summer flounder diets. Despite the prominent role of Chesapeake Bay in the life histories of many fishes, few studies have applied stable isotope techniques to address diets of species at higher trophic levels in this estuary (Hoffman et al. 2007). To our knowledge, this is the first study to use stable isotopes from multiple tissues to assess summer flounder food habits. We demonstrate the utility of isotopic approaches while also highlighting the assumptions and complications associated with applying stable isotopes to migratory fish species that inhabit dynamic estuarine environments.

METHODS

Sample collection.—Samples of summer flounder and prey species for stable isotope analysis were primarily obtained by using the ChesMMAp bottom trawl survey to complement the

routine analysis of stomach contents by the monitoring program. The ChesMMAp survey, operated by the Virginia Institute of Marine Science (VIMS), fishes a four-seam balloon trawl with 7.6-cm stretch mesh and monitors the main stem of Chesapeake Bay (for a detailed description of trawl procedures and gear, see Hoffman et al. 2009). Samples of summer flounder and common prey species were collected from the Virginia portion of Chesapeake Bay, where summer flounder distribution tends to be concentrated (Latour et al. 2008; Figure 1). Targeted prey items included mysid shrimp (primarily *N. americana*), sevenspine bay shrimp, mantis shrimp, bay anchovy, weakfish *Cynoscion regalis*, spot *Leiostomus xanthurus*, Atlantic croaker *Micropogonias undulatus*, and spotted hake *Urophycis regia*. Sampled prey were restricted to sizes found in the stomach contents of summer flounder (Table 1; Latour et al. 2008). Fishes and mantis shrimp were collected by the ChesMMAp trawl, whereas mysid shrimp and sevenspine bay shrimp were picked from habitat (e.g., hydroids) caught in the net. When possible, samples of summer flounder and prey species were collected concurrently at the same stations, but species distributions and gear selectivity often prevented this. Samples of smaller fishes and invertebrates were augmented with collections from the VIMS Juvenile Finfish and Blue Crab Trawl Survey, which uses a smaller, finer-mesh net with a cod-end liner (for a description of trawl procedures and gear, see Fabrizio and Tuckey 2008). In cases where samples from the VIMS trawl survey were required, they were obtained from similar locations and time periods as the ChesMMAp collections. Because of difficulties in obtaining mysid shrimp during November 2007, freshly consumed specimens were collected from the stomachs of summer flounder captured in September and October of that year (Grey et al. 2002). Any temporal or spatial discrepancies between predator and prey samples were acceptable given that (1) the goal of the study was to look at broad-scale patterns of resource use by summer flounder within lower Chesapeake Bay and (2) isotopic signatures integrate consumed material over weeks to months, incorporating similar temporal and spatial isotopic variability.

Upon capture of summer flounder on the ChesMMAp survey, the fish were processed to determine total length (TL) and wet weight, and otoliths were removed for later sectioning and age determination. When multiple distinct size-classes of summer flounder were captured, subsamples from each size-group were collected. Stomachs (up to 10 per station) were excised and preserved in formalin for future diet analysis in the laboratory. Samples of blood, muscle, and liver from a portion of summer flounder were collected for isotopic determination of diets to be compared with the standard stomach content analyses. Whole blood (~1 mL) was withdrawn from the caudal vein and placed in a sterile vial, and small samples of white muscle (from the ocular side above the pectoral fin) and liver were excised and bagged individually. Samples of blood, muscle, and liver from summer flounder were immediately frozen for later isotope analysis. Samples of prey with smaller body sizes

TABLE 1. Summary description of summer flounder samples and other taxa collected for stable isotope analyses, including number of samples, mean \pm SD C:N ratio, and mean \pm SD length (summer flounder length-classes: small [S] < 225 mm total length [TL]; medium [M] = 225–374 mm TL; large [L] \geq 375 mm TL). Lengths were measured as the total length (Atlantic croaker, spotted hake, weakfish, and summer flounder), fork length (bay anchovy and spot), or carapace length (mantis shrimp, mysid shrimp, and sevenspine bay shrimp).

Group	Species	Tissue	Length-class	Number of samples				C:N	Length (mm)	
				Spring 2006	Fall 2006	Spring 2007	Fall 2007			
Fish prey	Atlantic croaker	Muscle			6		8	3.2 \pm 0.2	62 \pm 15	
	Bay anchovy	Muscle		9	8	8	9	3.4 \pm 0.3	61 \pm 8	
	Spot	Muscle		8	8	6		3.4 \pm 0.4	133 \pm 16	
	Spotted hake	Muscle		4				3.8 \pm 0.2	155 \pm 12	
	Weakfish	Muscle			4	6	6	3.2 \pm 0.2	97 \pm 34	
Crustacean prey	Mantis shrimp	Whole body		6	4	6	6	3.9 \pm 0.3	100 \pm 20	
	Mysid shrimp	Whole body			2	9	9	3.7 \pm 0.2	— ^a	
	Sevenspine bay shrimp	Whole body		9	8	6	4	3.7 \pm 0.2	29 \pm 7	
Predator	Summer flounder	Muscle	S	1	3	8		3.1 \pm 0.2	181 \pm 27	
			M	6	6	5	14	3.1 \pm 0.2	275 \pm 34	
			L	7	6		3	3.1 \pm 0.4	460 \pm 78	
		Blood	S			6			3.9 \pm 0.1	161 \pm 19
			M			5	14		3.7 \pm 0.1	275 \pm 31
			L				3		3.7 \pm 0.1	424 \pm 37
		Liver	S			7			5.1 \pm 0.3	165 \pm 20
			M			5	14		6.3 \pm 1.7	275 \pm 31
			L				3		10.0 \pm 3.8	424 \pm 37

^aIndividual lengths were not measured for mysid shrimp, but sizes ranged from approximately 5 to 15 mm.

(e.g., juvenile fishes and invertebrates) were frozen whole. For larger fish prey, pieces of white muscle were excised from the musculature below the first dorsal fin and were frozen. Preservation by freezing was selected for isotope samples because freezing has a minimal effect on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Kaehler and Pakhomov 2001; Sweeting et al. 2004). All collection protocols were approved by the Institutional Animal Care and Use Committee at the College of William and Mary.

Collections were made during different time periods to characterize seasonal dietary patterns and account for temporal variability in isotopic signatures resulting from migration or fluctuations in prey isotopic signatures. Stable isotope samples were collected in 2006 and 2007 during two seasonal periods. Samples collected in May, June, or July represented the spring and early summer (hereafter, “spring”), while samples collected in November represented the fall season. Largely owing to the availability of summer flounder and their prey, more southern stations were represented in 2006 samples than in 2007 samples (Figure 1). Blood and liver samples were not collected from summer flounder in 2006, and individual prey species were not represented in every season \times year combination, mostly because of seasonal trends in species abundances (Table 1). Summer flounder stomachs (regularly sampled by the ChesMMAP survey) were collected during all ChesMMAP cruises conducted in March, May, July, September, and November of 2006 and 2007 (except September 2007, when a cruise was cancelled).

Laboratory processing.—Stomach contents were sorted and analyzed in the laboratory by following the methods of Latour et al. (2008). Briefly, prey items found in the stomachs were identified to the lowest possible taxon, and wet weight was recorded to the nearest 0.001 g. For age determination, otoliths of summer flounder were sectioned with cuts made perpendicular to the sulcal groove through the nucleus by following the method of Sipe and Chittenden (2001), and annuli were examined by two readers to assign ages.

Samples for stable isotope analysis were processed in the laboratory. Smaller samples (~1–2 g) of white dorsal muscle were taken from each summer flounder and prey fish collected in the field; for any fish prey that was collected whole, the muscle sample was removed after fish length was measured. Invertebrate samples were measured for carapace length and processed whole. For mysid shrimp, multiple individuals were aggregated to obtain sufficient mass for stable isotope analysis. All muscle, liver, and whole-body samples were rinsed with deionized water, dried at 50°C, and ground with a mortar and pestle. Blood samples were dried and ground in their storage vials. Carbonates found in exoskeletons of the crustacean prey were acidified with added drops of 10% HCl (Pinnegar and Polunin 1999). Dry subsamples (1.0 \pm 0.2 mg) of all tissues were packaged in tin capsules and analyzed at the Stable Isotope Facility, University of California–Davis, by use of a Europa Hydra 20/20 continuous-flow isotope ratio mass spectrometer.

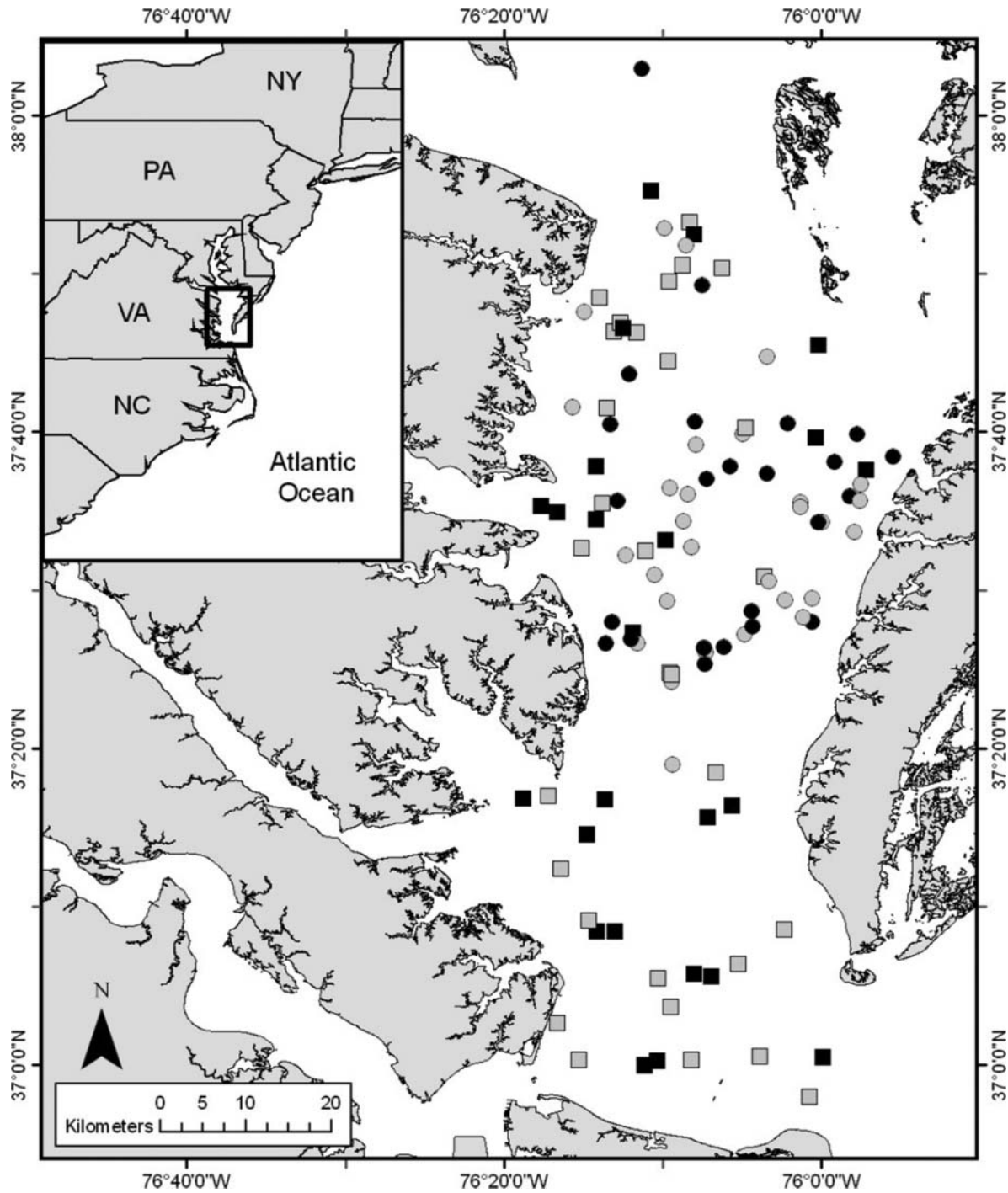


FIGURE 1. Sampling locations within lower Chesapeake Bay, where summer flounder and their common prey types were collected. Symbol shape indicates sampling year (squares = 2006; circles = 2007); symbol color represents season of capture (gray = spring; black = fall).

Stable isotope ratios are reported in relation to conventional standards, that is,

$$\delta X = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1,000, \quad (1)$$

where X is either ^{13}C or ^{15}N , and R is the mass ratio of the heavy to light stable isotopes ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) for either the sample or the standard. The conventional standards used for the analyses were Pee Dee Belemnite for carbon and air for nitrogen. The value for δX is reported in per

mille (‰) units. Repeated measurements of a calibration standard indicated that instrument precision (SD) was 0.29‰ for $\delta^{13}\text{C}$ and 0.12‰ for $\delta^{15}\text{N}$. The mass ratio of elemental carbon and nitrogen (C:N) was also obtained for each analyzed sample.

Because of the high lipid concentrations in liver, which are known to bias $\delta^{13}\text{C}$ values, a mathematical correction was used to normalize the liver $\delta^{13}\text{C}$ values because of the high lipid concentrations in liver, which are known to bias $\delta^{13}\text{C}$ values (DeNiro and Epstein 1977; McConnaughey and McRoy 1979). Each raw value of liver $\delta^{13}\text{C}$ was converted to a lipid-adjusted value based on measured C:N (a proxy for lipid content) by using an empirically derived conversion equation (Buchheister and Latour 2010). Lipid corrections were not applied to $\delta^{13}\text{C}$ for muscle and blood due to their relatively low C:N values (Table 1; Post et al. 2007), limited variability across individuals, and presumably small effects on $\delta^{13}\text{C}$ (Kiljunen et al. 2006).

Data analysis.—Diet compositions based on stomach content analyses were calculated as percent contribution by weight; based on the methods of Latour et al. (2008), a cluster sampling estimator was used to account for dietary similarity among fish caught at the same location. Summer flounder were classified into three size-classes (small < 225 mm TL; medium = 225–374 mm TL; large \geq 375 mm TL) to capture broad ontogenetic changes in diet as documented in previous research (Latour et al. 2008). Diets were calculated by year, month, and size-class; we present only those results that were determined with a minimum of five stomachs. For a closer correspondence to summer flounder isotopic samples, analysis of ChesMAP stomach content data were restricted to individuals that were captured within Virginia waters of the lower Chesapeake Bay.

Isotopic contributions of prey to summer flounder diets were assessed graphically and were calculated by use of mixing models. Prey species were separated into two broad taxonomic groups (crustaceans and fishes) to capture the major differences in stable isotope signatures (Phillips et al. 2005; McIntyre et al. 2006). Prey were assigned to the two taxonomic groups based on similarity among prey isotopic signatures; this was done to reduce the number of sources and to calculate unique solutions in the mixing model. IsoError, a two-source mixing model that accounts for variability in both prey and predator isotope values, was used to calculate the mean contribution (\pm SE) of each prey group to summer flounder diets (Phillips and Gregg 2001). To apply the mixing models, we first adjusted the summer flounder $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values to account for isotopic fractionation (i.e., changes in isotopic values caused by physiological processing of consumed material). Tissue- and isotope-specific fractionations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (represented as $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$, respectively) were derived from growth-based turnover models of wild summer flounder that were subjected to a dietary shift experiment in the laboratory (Buchheister and Latour 2010). Applied values of $\Delta^{13}\text{C}$ were +0.71‰ for muscle, +3.27‰ for blood, and +3.05‰ for liver; applied values of $\Delta^{15}\text{N}$ were +2.53‰ for

muscle, +2.80‰ for blood, and +2.28‰ for liver. The mixing model was applied to each summer flounder tissue from each year, season, and length-class by using the corresponding prey isotope values for the season and year.

A multivariate analysis of variance (MANOVA) was used to test for significant year and season effects on the mean stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of each prey group. Assumptions of normality and homogeneity of variance for the MANOVA were upheld based on residual analysis, Kolmogorov–Smirnov tests, and Levene's tests. For each prey group, the following a priori comparisons were made with the Wilks' lambda statistic at an α -level of 0.05: season effects were tested within each year, and year effects were tested within each season. The analyses were conducted in the Statistical Analysis System version 9.1 (SAS Institute, Cary, North Carolina).

Regression analyses were used to test for significant ontogenetic trends in summer flounder isotopic signatures and to examine dietary shifts inferred from tissue comparisons. Ontogenetic trends in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were assessed by regressing the isotopic values on fish TL. Dietary shifts that occur within the time frame of the tissue with the slowest turnover rate can be reflected by differences in isotopic values between tissues (MacNeil et al. 2005; Fry 2006). For each individual, fractionation-adjusted $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for muscle were subtracted from fractionation-adjusted blood and liver values to examine recent shifts in summer flounder feeding and potential ontogenetic trends. For example, a recent dietary shift toward feeding on prey with enriched (i.e., more-positive) $\delta^{15}\text{N}$ would manifest as a positive difference between liver and muscle tissues owing to the faster turnover of the liver (Buchheister and Latour 2010). Differences between tissue pairs were calculated and plotted against fish length for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and regression analysis was used to test for significant relationships.

RESULTS

Stomach Contents

In total, 563 fish (with nonempty stomachs; TL = 138–682 mm; age = 0–11 years) from 301 stations were analyzed for stomach contents during the 2-year study period. The prey species that were sampled for stable isotopes represented the majority of consumed material in the stomachs of all length-classes (86–96% in 2006; 71–91% in 2007), thus confirming the importance of the prey species that were selected for sampling. Overall, summer flounder stomach contents from both years showed a consistent shift from crustacean prey to fish prey with increasing predator length (Figure 2). Mysid shrimp were the dominant prey item (contributing up to 86% of the diet by weight) for small summer flounder, whereas larger individuals had diverse diets that included greater percentages of bay anchovy, weakfish, spot, and other fishes. Fish prey (mostly bay anchovy) comprised a greater proportion of the diet in 2006 than in 2007 by an average of 21% (range = 7–40%) for each month and size-class but most notably for medium-sized

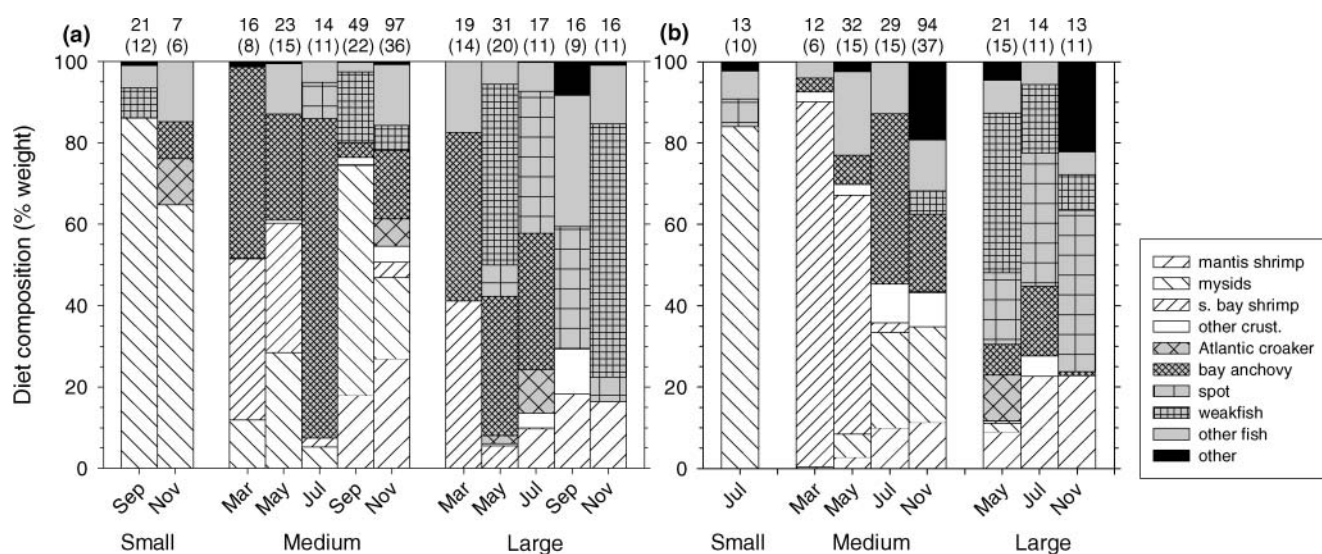


FIGURE 2. Monthly mean percent diet composition (by weight) based on stomach contents of small (<225 mm total length [TL]), medium (225–374 mm TL), and large (≥ 375 mm TL) summer flounder collected in (a) 2006 and (b) 2007. Fill patterns represent different prey species (s. bay shrimp = seven-spine bay shrimp; other crust. = other crustaceans), and colors indicate prey groups (white = crustaceans; gray = fishes; black = other). Values above each bar are the number of analyzed fish and the number of represented stations (in parentheses). Months in which fewer than five stomachs were sampled were omitted.

summer flounder (Figure 2). The contributions of individual prey species to the diets varied by month. For example, seven-spine bay shrimp were particularly important in the diets of medium-sized summer flounder in late winter and spring (March and May), whereas mysid shrimp increased in the diets of medium-sized summer flounder during summer and fall (Figure 2).

Stable Isotopes

Fifty-nine summer flounder were analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Table 1); most of these individuals ranged from 138 to 478 mm TL and from 0 to 3 years of age. However, there were three outliers in spring 2006 (TL = 599–624 mm; age = 4–7 years). Sampled fish included 32 females, 26 males, and 1 fish of unknown sex. Larger individuals were predominantly females, thus reflecting the skewed sex ratio of age-1 and older summer flounder in the Middle Atlantic Bight and lower Chesapeake Bay (Morse 1981; Bonzek et al. 2008).

Aggregation of summer flounder prey into two prey groups (crustaceans and fishes) was supported by the isotopic separation between the groups, particularly for $\delta^{15}\text{N}$ (Figure 3). Fishes generally exhibited $\delta^{15}\text{N}$ values that were approximately 3‰ greater than those for crustaceans; this result conforms to the traditional assumption of an approximately 3.4‰ shift per trophic level (Post 2002; Sweeting et al. 2007). The $\delta^{13}\text{C}$ was not a useful dietary indicator because of (1) the similarity and overlap of prey $\delta^{13}\text{C}$ values and (2) the high variability in summer flounder $\delta^{13}\text{C}$ values, which often extended beyond the constraints of prey $\delta^{13}\text{C}$ (e.g., Figure 3a). Consequently, the mixing models in this study were applied by using $\delta^{15}\text{N}$ values only.

Isotopic values of summer flounder resembled the values of the fish prey (Figure 3), which suggests a shared nutritional reliance on the lower prey group (i.e., crustaceans). Summer flounder predation on fishes would have been indicated by tissue $\delta^{15}\text{N}$ values greater than those of prey fishes, but the average $\delta^{15}\text{N}$ values for small-, medium-, and large-sized summer flounder were not significantly greater than the $\delta^{15}\text{N}$ of prey fishes (ANOVA: $P > 0.05$) except in spring 2006. For fall samples in both years, the mixing model results (based on $\delta^{15}\text{N}$ only) indicated that crustaceans dominated the diets of summer flounder, reaching 100% for some sizes (Table 2). On average, fishes accounted for 35% or less of the total diet during fall, but SE values for the estimates ranged from 9% to 24%, indicating that some individuals may rely more heavily on fish prey than others. During spring 2006, summer flounder appeared to assimilate a greater amount of fish (54–68%). Highly depleted $\delta^{15}\text{N}$ values of tissues in spring 2007 prevented the use of the mixing model for this season (Table 2), but the raw $\delta^{15}\text{N}$ values placed small summer flounder at a trophic level similar to that of the crustaceans and placed medium summer flounder at a trophic level similar to that of the sampled fish prey (Figure 3c).

Temporal Variability in Prey Stable Isotopes

Isotopic values of prey groups were not temporally consistent; rather, they exhibited seasonal patterns that differed by year. From spring to fall in 2006, crustacean $\delta^{13}\text{C}$ became more depleted while $\delta^{15}\text{N}$ became more enriched, but the opposite pattern was observed in 2007 (Table 3). Prey fishes experienced a similar depletion of $\delta^{13}\text{C}$ and enrichment of $\delta^{15}\text{N}$ from spring to fall during 2006, but seasonal differences in isotope signatures of fishes were not significant in 2007 ($F = 2.69$,

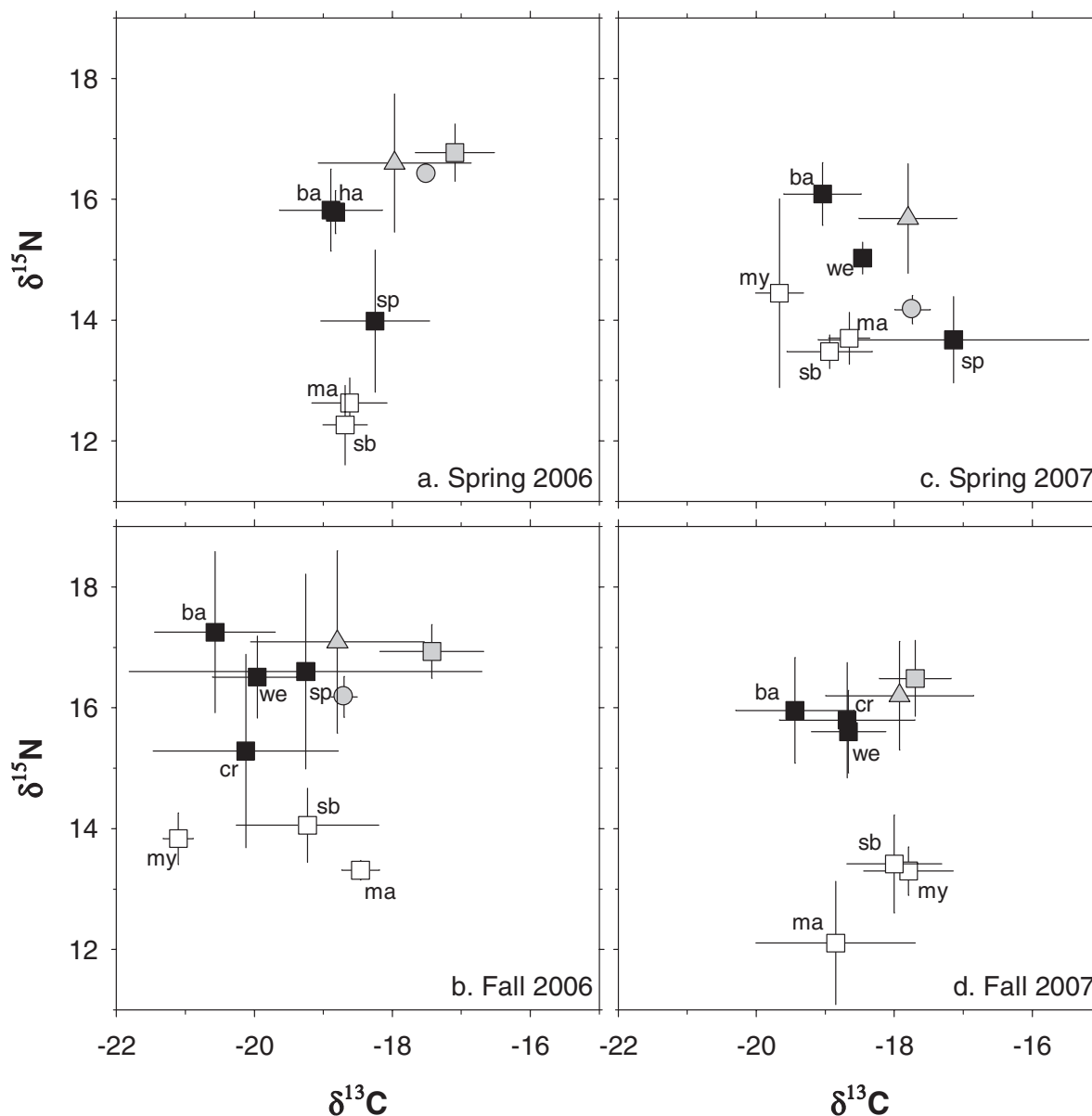


FIGURE 3. Biplots of carbon and nitrogen stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$; mean \pm SD) for summer flounder and other sampled species collected in (a) spring 2006, (b) fall 2006, (c) spring 2007, and (d) fall 2007. Values for muscle are plotted for three summer flounder length-classes (gray circles = small [<225 mm total length]; gray triangles = medium [225–374 mm]; gray squares = large [≥ 375 mm]). For figure clarity, blood and liver values from summer flounder are not plotted. Values for fish prey (black squares) and crustacean prey (white squares) are labeled by species (ba = bay anchovy; cr = Atlantic croaker; ha = spotted hake; ma = mantis shrimp; my = mysid shrimp; sb = sevenspine bay shrimp; sp = spot; we = weakfish).

$P > 0.05$). Isotopic differences between seasons within a prey group were relatively small and typically less than 1.4‰ in magnitude; however, the SDs for group means reached 1.66‰ due to interspecies and intraspecies variability (Table 3). Isotope means of prey groups also tended to vary by year, as most multivariate ANOVA tests of interannual differences by season yielded significant results ($P < 0.05$; Table 3). Only the isotopic means of prey fishes in spring were found to be similar between 2006 and 2007.

Ontogenetic Trends in Stable Isotope Signatures

Positive relationships between $\delta^{15}\text{N}$ and summer flounder TL indicated an ontogenetic shift toward feeding at higher trophic levels as the fish grew (Figure 4). This effect was most pronounced in 2007, manifesting in all sampled tissues, and was most evident for the spring sampling. Based on the assumed $\delta^{15}\text{N}$ fractionations of 2.3–2.8‰ for the sampled tissues (Buchheister and Latour 2010), relatively large summer flounder were feeding at approximately one trophic level above small

TABLE 2. Results of mixing models examining the contribution of fish and crustacean prey to summer flounder diets (%; mean \pm SE) based on the nitrogen stable isotope ratio ($\delta^{15}\text{N}$; mean \pm SD). Contribution to the diet was calculated for each combination of summer flounder total length (small [S] < 225 mm; medium [M] = 225–374 mm; large [L] \geq 375 mm) and tissue. Results for spring 2007 are not shown because all mean values were not constrained by 0% and 100%. Note that summer flounder $\delta^{15}\text{N}$ values were adjusted for fractionation (see Methods).

Time period	Length-class	Tissue	Summer flounder		Fish prey			Crustacean prey		
			$\delta^{15}\text{N}$	<i>n</i>	$\delta^{15}\text{N}$	<i>n</i>	% in diet	$\delta^{15}\text{N}$	<i>n</i>	% in diet
Spring 2006	S	Muscle	13.9 \pm 0	1	15.1 \pm 1.2	21	54 \pm 6	12.4 \pm 0.6	15	46 \pm 6
	M	Muscle	14.1 \pm 1.1	6	15.1 \pm 1.2	21	61 \pm 18	12.4 \pm 0.6	15	39 \pm 18
	L	Muscle	14.2 \pm 0.5	7	15.1 \pm 1.2	21	68 \pm 10	12.4 \pm 0.6	15	32 \pm 10
Fall 2006	S	Muscle	13.6 \pm 0.3	3	16.5 \pm 1.5	26	-6 \pm 10	13.8 \pm 0.6	14	106 \pm 10
	M	Muscle	14.6 \pm 1.5	6	16.5 \pm 1.5	26	28 \pm 24	13.8 \pm 0.6	14	72 \pm 24
	L	Muscle	14.4 \pm 0.4	6	16.5 \pm 1.5	26	22 \pm 9	13.8 \pm 0.6	14	78 \pm 9
Fall 2007	M	Muscle	13.7 \pm 0.9	14	15.8 \pm 0.8	23	25 \pm 10	12.9 \pm 0.9	21	75 \pm 10
		Liver	12.9 \pm 0.9	14	15.8 \pm 0.8	23	2 \pm 10	12.9 \pm 0.9	21	98 \pm 10
		Blood	13.0 \pm 0.7	14	15.8 \pm 0.8	23	-1 \pm 11	12.9 \pm 0.9	21	101 \pm 11
	L	Muscle	14.0 \pm 0.6	3	15.8 \pm 0.8	23	35 \pm 14	12.9 \pm 0.9	21	65 \pm 14
		Liver	13.4 \pm 0.6	3	15.8 \pm 0.8	23	-28 \pm 16	12.9 \pm 0.9	19	128 \pm 16
		Blood	12.1 \pm 0.6	3	15.8 \pm 0.8	23	16 \pm 14	12.9 \pm 0.9	19	84 \pm 14

summer flounder. Larger summer flounder exhibited $\delta^{15}\text{N}$ values that were similar to or slightly greater than those of prey fishes, and the $\delta^{15}\text{N}$ values of some individual summer flounder were more than 1 SD greater than the mean for fish prey (Figure 4). For each tissue, linear regressions of $\delta^{15}\text{N}$ on summer flounder TL were significant ($P < 0.05$) when 2007 data were pooled. When the three fish exceeding 350 mm were excluded from the analysis, slopes were not significantly different among tissues (estimated slope [mean \pm SE], muscle: 0.016 \pm 0.003; blood: 0.020 \pm 0.002; liver: 0.015 \pm 0.003). Although not presented, strong relationships between $\delta^{15}\text{N}$ and individual mass were also observed for fish captured in 2007. In 2006, however, significant relationships were not observed between $\delta^{15}\text{N}$ and TL (or mass), possibly because small, age-0 individuals were lacking in samples collected during spring, a season when size and dietary differences between length-classes may be most

pronounced. Relationships between $\delta^{13}\text{C}$ and summer flounder TL were not observed except in 2006, when there was a slight trend of more-enriched $\delta^{13}\text{C}$ in the muscle tissue of larger fish (Figure 5).

Summer Flounder Tissue Differences

Isotopic values for blood and liver were mostly depleted relative to muscle and showed some significant relationships ($P < 0.05$) with fish TL (Figure 6). For $\delta^{15}\text{N}$, blood samples were most depleted relative to muscle for small summer flounder, and tissue differences were diminished with increasing length (Figure 6a). Unlike blood $\delta^{15}\text{N}$, liver $\delta^{15}\text{N}$ showed a negative relationship with fish length, although the fit of the linear regression model was poor ($r^2 = 0.19$) and largely driven by the four largest individuals (Figure 6a). Although most of the tissue $\delta^{15}\text{N}$ differences were negative (as low as -2.4‰), these values were

TABLE 3. Mean carbon and nitrogen stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$; with SDs) for samples of summer flounder prey groups collected in the lower Chesapeake Bay main stem. For each prey group, significant multivariate ANOVA (MANOVA) results (for the season effect tested within each year and the year effect tested within each season; $P < 0.05$) are indicated by different letters.

Prey group	Time period	<i>n</i>	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		MANOVA
			Mean	SD	Mean	SD	
Crustaceans	Spring 2006	15	-18.66	0.41	12.41	0.59	a
	Fall 2006	14	-19.28	1.15	13.81	0.58	b
	Spring 2007	21	-19.17	0.61	13.95	1.11	c
	Fall 2007	19	-18.17	0.93	12.95	0.90	d
Fishes	Spring 2006	21	-18.63	0.73	15.11	1.23	a
	Fall 2006	26	-19.97	1.66	16.48	1.53	b
	Spring 2007	20	-18.29	1.34	15.04	1.14	ac
	Fall 2007	23	-18.97	0.89	15.81	0.83	c

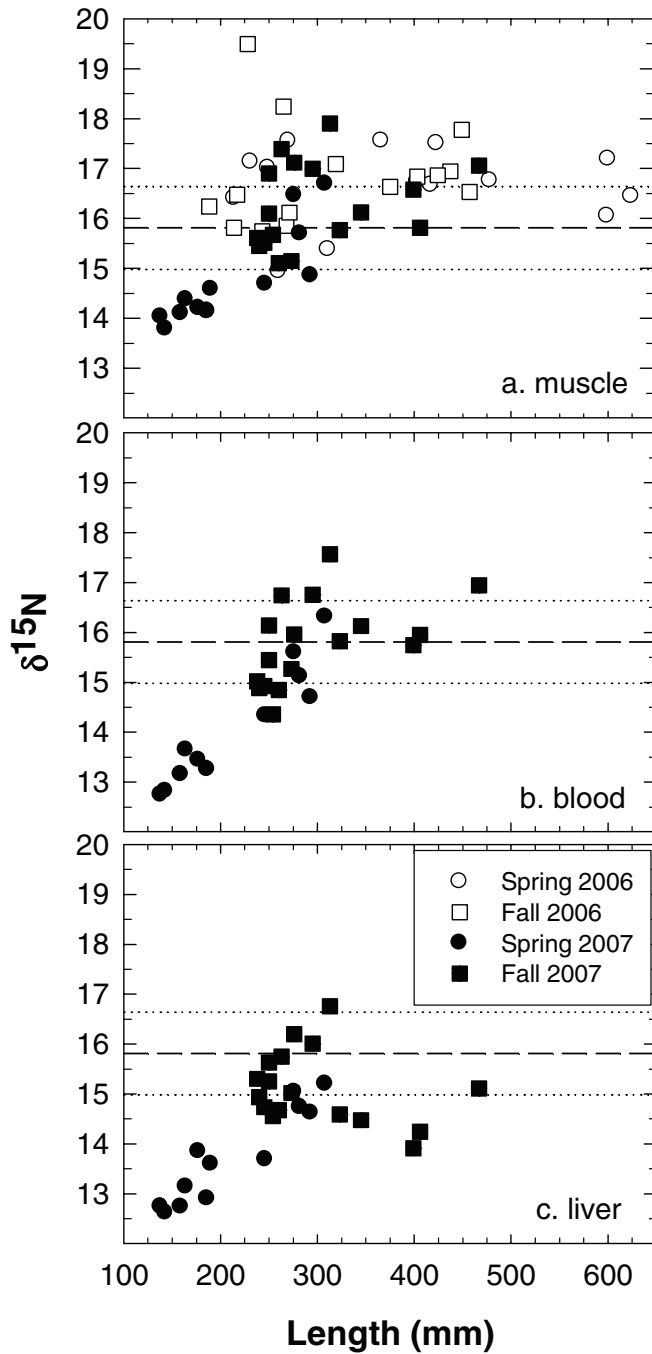


FIGURE 4. Nitrogen stable isotope ratios ($\delta^{15}\text{N}$) for (a) muscle, (b) blood, and (c) liver plotted in relation to total length (mm) for individual summer flounder. Symbol shape represents season of capture (circles = spring; squares = fall), and symbol color indicates year (white = 2006; black = 2007). For reference, the mean $\delta^{15}\text{N}$ (dashed line; with SD, dotted lines) of prey fishes collected in fall 2007 is plotted.

particularly sensitive to the choice of applied fractionation value. If a muscle $\Delta^{15}\text{N}$ of 3.4‰ (Vander Zanden and Rasmussen 2001) had been applied instead of 2.53‰ (Buchheister and Latour 2010) as described in Methods, the tissue $\delta^{15}\text{N}$ differences would have been mostly centered around zero (Figure 6a).

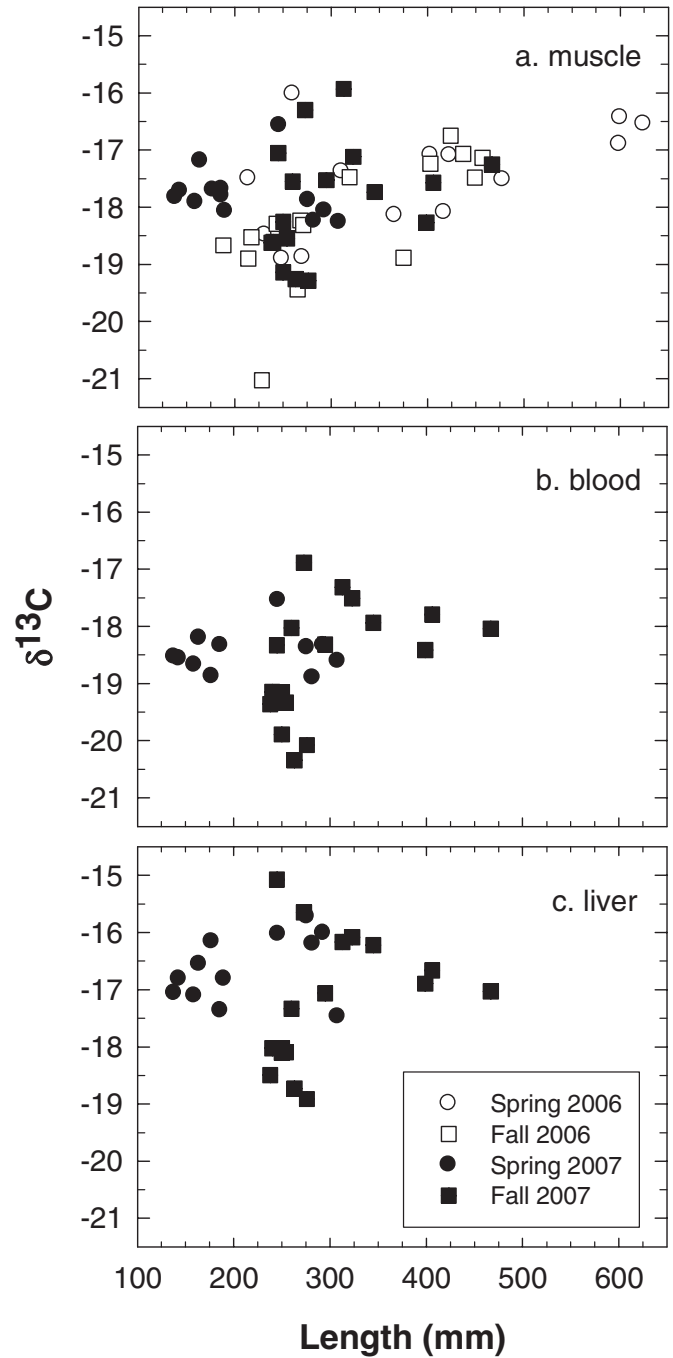


FIGURE 5. Carbon stable isotope ratios ($\delta^{13}\text{C}$) for (a) muscle, (b) blood, and (c) liver plotted in relation to total length (mm) for individual summer flounder. Symbol shape represents season of capture (circles = spring; squares = fall), and symbol color indicates year (white = 2006; black = 2007).

For $\delta^{13}\text{C}$, blood values were consistently more than 2.9‰ depleted relative to muscle values but showed a significant positive relationship with summer flounder TL (Figure 6b). Liver $\delta^{13}\text{C}$ values were typically 0–2.5‰ lower than muscle $\delta^{13}\text{C}$ values and showed no significant relationship with length.

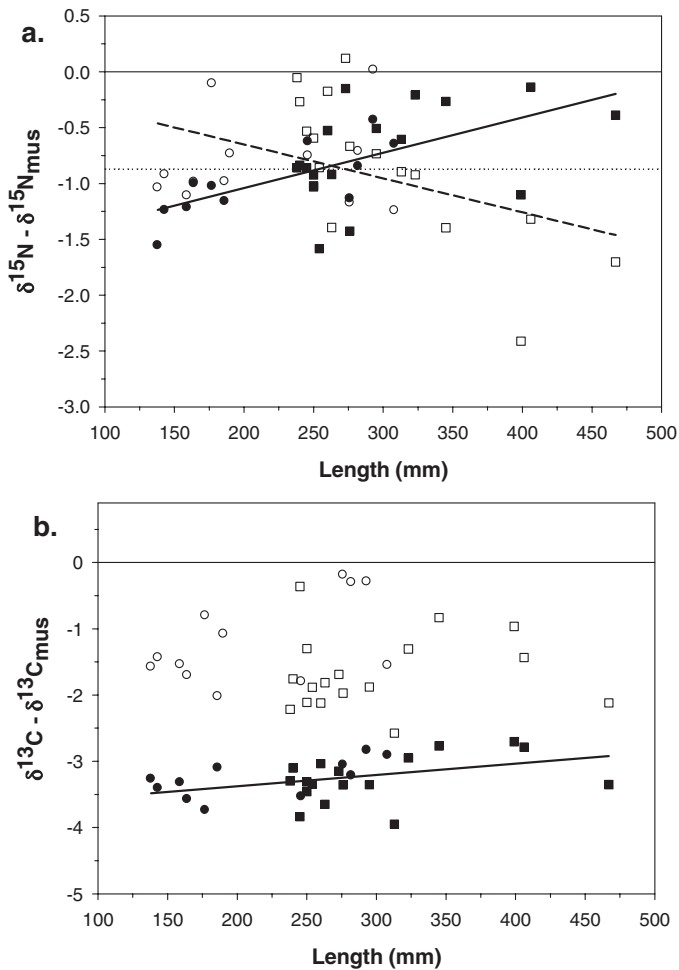


FIGURE 6. Differences in nitrogen or carbon stable isotope ratio ($\delta^{15}\text{N}$ or $\delta^{13}\text{C}$) values between tissues of summer flounder as a function of fish total length. After all tissues were adjusted for fractionation effects, the (a) $\delta^{15}\text{N}$ of muscle ($\delta^{15}\text{N}_{\text{mus}}$) or (b) $\delta^{13}\text{C}$ of muscle ($\delta^{13}\text{C}_{\text{mus}}$) was subtracted from the value obtained from the blood (shaded symbols) or liver (open symbols) of each fish collected in spring 2007 (circles) and fall 2007 (squares). Significant regression lines are plotted for blood (solid line) and liver (dashed line) tissues (data pooled across seasons). The horizontal dotted line represents a difference of zero if the $\delta^{15}\text{N}$ fractionation value ($\Delta^{15}\text{N}$) of 3.4‰ is applied for muscle instead of the $\Delta^{15}\text{N}$ measured for summer flounder (see Methods).

DISCUSSION

Stable Isotopes versus Stomach Contents

Stable isotope and stomach content analyses presented differing results on the relative importance of crustacean and fish prey in the diets of summer flounder; stable isotope analysis suggested higher levels of crustacean consumption than did stomach content analysis. Although this discrepancy is reduced when the SEs from mixing model results are considered (SE for dietary contribution based on $\delta^{15}\text{N}$ was as high as $\pm 24\%$), dietary differences were also probably influenced by the different time periods represented by the two methodologies. Stomach content analysis identifies prey that were recently consumed

(i.e., on the order of hours; Hyslop 1980), whereas stable isotopes provide a more time-integrated dietary representation on a scale of weeks to months (MacNeil et al. 2006; Buchheister and Latour 2010). Consequently, even if diet characterizations by the two methods differ, they can both be accurate. For example, higher levels of presumed fish consumption in the late summer and fall (based on stomach contents) may be reflected weakly in isotopic signatures because juvenile growth rates (and thus isotopic assimilation) are reduced by October and November relative to spring and summer (Powell 1982; Rountree and Able 1992).

The discrepancy between methodologies could also result from underrepresentation of mysid shrimp in stomach contents because of differences in prey digestibility, evacuation rates, and periodicity in prey availability (Hyslop 1980). Relative to fish prey or to crustacean prey with more-robust exoskeletons (e.g., sevenspine bay shrimp and mantis shrimp), mysid shrimp possess a small size, a high surface-to-volume ratio, and a thin exoskeleton, all of which can facilitate digestion and evacuation (Lankford and Targett 1997; Andersen 1999). In addition, consumption of mysid shrimp by summer flounder, whose eyes are well adapted for visual foraging in low light (Horodysky et al. 2010), is probably greater at night, when mysid shrimp migrate out of benthic refuges and into the water column (Hulburt 1957; Herman 1963). Consequently, the daytime sampling by ChesMMAAP may have captured summer flounder at times when crustacean prey were not as available to the predators or when a large proportion of prey consumed at dusk and night had already been evacuated from the gut.

Ontogenetic Patterns in Summer Flounder Feeding

Both the stable isotope and stomach content analyses confirmed that summer flounder undergo an ontogenetic transition to prey resources at higher trophic levels as predator size increases. In accordance with general size-based predation theories (Werner and Hall 1974; Scharf et al. 2000), ontogenetic changes in summer flounder feeding have previously been demonstrated for different life stages by using stomach contents (Burke 1995; Link et al. 2002; Latour et al. 2008); however, ours is the first study to show such trends for summer flounder by use of stable isotope analysis. The size-based trend in $\delta^{15}\text{N}$ was strongly driven by small fish (<225 mm TL) collected during spring 2007, supporting the size cutoffs presented by Latour et al. (2008). The 225-mm size may represent a morphological point of transition at which gape width or swimming speed aids in capture of the piscine prey that occupy higher trophic levels.

For spring 2007, the use of the mixing model to corroborate the strong relationship between summer flounder size and $\delta^{15}\text{N}$ was prevented by $\delta^{15}\text{N}$ values that were depleted relative to prey samples. According to $\delta^{15}\text{N}$ values in this season, small individuals occupied a trophic level that was more consistent with the sampled invertebrates and did not feed on fishes. However, contrary to the implications of this information, stomach content analyses provide no evidence that summer flounder (of

the sizes examined here) feed on smaller zooplankton (e.g., copepods; Grimes et al. 1989; Rountree and Able 1992; Latour et al. 2008). Consequently, the prey sampled in spring 2007 may not have been isotopically representative of consumed organisms because of regional or temporal variability in $\delta^{15}\text{N}$. For example, small shifts in basal organic matter sources or small changes in the $\delta^{15}\text{N}$ of primary producers can be transferred up the food web, altering the isotopic signatures of organisms at lower trophic levels more strongly (Horrigan et al. 1990; Montoya et al. 1990; Perga and Gerdeaux 2005; Hoffman et al. 2007). This possibility was supported by the unusually small separation (1.09‰) in $\delta^{15}\text{N}$ between the crustacean and fish prey groups and the higher variability in crustacean $\delta^{15}\text{N}$ during spring 2007.

Stable Isotopes of Multiple Tissues

Analysis of multiple tissues with varying turnover rates was important for this study given the life history of summer flounder and the temporal variability in prey stable isotopes. Age-0 and adult summer flounder migrate into lower Chesapeake Bay from offshore spawning locations in the spring (March–April) to utilize the productive estuarine habitat (Able et al. 1990; Packer et al. 1999). From late spring to fall, juveniles use a variety of habitats (e.g., estuarine marsh creeks, seagrass beds, mud flats, and open bay areas; Packer et al. 1999), and growth rates remain high through the summer (Powell 1982; Malloy and Targett 1994). During this time, summer flounder can show a high degree of site fidelity or can move several kilometers among estuarine locations (Szedlmayer and Able 1993; Able and Fahay 2010; M. Henderson, Virginia Institute of Marine Science, unpublished data). Adults and juveniles migrate out of Chesapeake Bay in the fall (approximately October–December). Muscle tissue has a slow turnover rate and would equilibrate to a fish's diet after about 5 months (Buchheister and Latour 2010). Consequently, the muscle isotopic values measured in spring for medium- and large-sized fish (age > 0) could reflect prey consumption that occurred while the fish were offshore. In addition, both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of primary producers and lower trophic levels can vary spatially and temporally in Chesapeake Bay (Montoya et al. 1990; Zimmerman and Canuel 2001; Hagy 2002). Such variability can complicate interpretation of isotopic data for muscle tissue, especially for any summer flounder that are more mobile within the bay. Unlike muscle, liver and blood would approach equilibrium with a dietary signal in approximately 20 d (liver) or 75 d (blood; Buchheister and Latour 2010). In our study, analysis of tissues with faster turnover rates helped to minimize any bias resulting from fish movements or from isotopic heterogeneity of prey. The relatively consistent conclusions drawn from the different tissues analyzed indicate that the fish did not move between habitats with drastically different isotopic characteristics.

All three tissues captured the same ontogenetic trend of increasing $\delta^{15}\text{N}$ with increasing size while also producing diet estimates that emphasized crustacean assimilation. Given the

positive relationship between $\delta^{15}\text{N}$ and length, we expected to see enriched $\delta^{15}\text{N}$ values for the tissues with faster turnover rates (blood and liver) relative to muscle, which would better represent an individual's more recent consumption at higher trophic levels (Fry 2006). Instead, the $\delta^{15}\text{N}$ values for blood and liver were depleted relative to muscle. This pattern could indicate recent nutritional augmentation from lower-trophic-level crustacean prey (opposing the general trend from stomach contents) or perhaps an overall depletion in $\delta^{15}\text{N}$ across the local prey community (O'Reilly et al. 2002). However, we argue that the expected positive ontogenetic signal within an individual's tissues was probably obscured by fractionation assumptions and by isotopic variability of the sampled organisms. This possibility was supported by the sensitivity of the $\delta^{15}\text{N}$ tissue differences to the choice of applied fractionation values, especially considering the small isotopic differences that we were trying to detect between tissues (Figure 6). However, the positive trend for blood $\delta^{15}\text{N}$ (irrespective of the absolute values) suggests that relatively large summer flounder have recently shifted to prey types that are less depleted than the summer flounder's long-term diet, which would be consistent with increased consumption of fish. Higher variability in liver $\delta^{15}\text{N}$ may have masked such a signal in that tissue.

Tissue differences in $\delta^{13}\text{C}$ were probably also influenced by fractionation assumptions. Although the depleted blood and liver $\delta^{13}\text{C}$ values could potentially indicate more recent feeding on $\delta^{13}\text{C}$ -depleted prey, this pattern was not supported as strongly by $\delta^{13}\text{C}$ values for liver tissue, which has the fastest turnover rate (Figure 6b). The relative consistency in the phenomenon of depleted blood $\delta^{13}\text{C}$ across seasons and sizes—in conjunction with the weaker signal in the liver tissue—suggests that the applied fractionation for blood may have contributed some bias to these $\delta^{13}\text{C}$ results.

Sources of Error

The fractionation values applied here contributed to some error in the results, but the major conclusions were not altered. As recommended for stable isotope applications (Gannes et al. 1997), we applied $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ fractionations that were empirically determined (Buchheister and Latour 2010) for the same species, same tissues, and similar body sizes as were used in this study. However, fractionation values can also vary by diet (Gorokhova and Hansson 1999), dietary protein content (Kelly and Martinez del Rio 2010), feeding mode (Vander Zanden and Rasmussen 2001), growth rate (Trueman et al. 2005), and tissue composition (Kelly and Martinez del Rio 2010; Pecquerie et al. 2010). Additionally, laboratory fractionations tend to be larger than field-derived values (Vander Zanden and Rasmussen 2001), as appeared to be the case with the $\delta^{13}\text{C}$ in summer flounder blood. Errors in the applied fractionations would also affect mixing model estimates, particularly since isotopic separation between prey groups was small (Vander Zanden and Rasmussen 2001). However, unlike $\delta^{13}\text{C}$ fractionations, the $\delta^{15}\text{N}$ fractionation estimates used in the mixing models did not

deviate as greatly from previous research (Post 2002; Sweeting et al. 2007), and mixing model results for $\delta^{15}\text{N}$ were relatively similar across tissues. More importantly, the fractionations did not change the major conclusions because the unadjusted summer flounder $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values coincided with those of the other sampled fishes, indicating that summer flounder and other fishes fed at a similar trophic level and utilized similar prey.

Isotopic routing is another potential source of error in mixing model estimates (Martinez del Rio et al. 2009). Dietary components that are isotopically distinct can be allocated or "routed" unequally among a consumer's tissues such that mixing model results from individual tissues may provide biased results (e.g., Kelly and Martinez del Rio 2010). However, biases introduced by isotopic routing would be minimal for summer flounder given their carnivorous diets; isotopic routing is more problematic for omnivores, which consume materials that differ markedly with respect to protein composition (Martinez del Rio et al. 2009).

Isotopic signatures of the crustacean and fish groups exhibited temporal variability, as can be expected in large, dynamic estuaries such as Chesapeake Bay (Cifuentes et al. 1988; Montoya et al. 1990; Cloern et al. 2002). Seasonal and annual differences (typically $< 1.4\text{‰}$) within prey groups were probably mediated through bottom-up effects (Peterson and Fry 1987; Cloern et al. 2002) and were also influenced by natural fluctuations of species abundances in the bay, which generated seasonal differences in the representative species that were actually sampled (Murdy et al. 1997; Able and Fahay 2010). However, the similarity in dietary evaluations among tissues with fast and slow turnover rates suggests that such variability would not compromise the documented importance of crustaceans in the diets of summer flounder.

Lower Chesapeake Bay Food Web

Carbon isotope signatures for the sampled species in this study corresponded with mixed organic matter sources to the food web of lower Chesapeake Bay. The value of $\delta^{13}\text{C}$, which is commonly used to identify sources of primary production (Peterson and Fry 1987), ranged from approximately -22‰ to -16‰ at our main-stem stations. These values could result from mixed consumption of carbon from marine phytoplankton ($\sim -21\text{‰}$), benthic microalgae ($\sim -17\text{‰}$), and possibly C_4 marsh grasses ($\sim -13\text{‰}$) or seagrasses ($\sim -10\text{‰}$; Fry and Sherr 1989). The $\delta^{13}\text{C}$ values of summer flounder were intermediate between these organic matter sources, indicating that both pelagic (i.e., phytoplankton) and benthic organic matter sources are important for summer flounder production. Although many sampling locations were several kilometers away from coastal wetlands and samples were collected at depths where benthic photosynthesis would be light limited (Kemp et al. 2005), summer flounder can be highly mobile and are known to use shallow habitats where benthic primary production is greater (Rountree and Able 1992; Packer et al. 1999; Fabrizio et al. 2007). Incorporation of dead phytoplankton and other detritus into a longer benthic food chain may also help to explain some of the more-

enriched $\delta^{13}\text{C}$ values that were measured for summer flounder and other species (e.g., spot and mantis shrimp) that are more strongly coupled to benthic food sources, in contrast to pelagic species (e.g., bay anchovy; Fry and Sherr 1989). Generally, the sampled species demonstrated a high degree of overlap in $\delta^{13}\text{C}$ values and relied on mixed organic matter sources, making carbon a poor dietary tracer for summer flounder in the main stem of the lower Chesapeake Bay.

Compared with $\delta^{13}\text{C}$, values of $\delta^{15}\text{N}$ yielded better isotopic differentiation among sampled species in this study and supported the relative trophic positions of these groups in a generalized Chesapeake Bay food web (Baird and Ulanowicz 1989; Hagy 2002). Excluding the data from spring 2007, prey fishes exhibited $\delta^{15}\text{N}$ values that were on average 2.7–2.9‰ greater than those of the crustacean group, which equates to a difference of approximately one trophic level (Sweeting et al. 2007). These results corroborate stomach content studies showing the predominance of mesozooplankton (particularly mysid shrimp) in the diets of bay anchovy (Hartman et al. 2004), juvenile weakfish (R.J.L., unpublished data), and spotted hake (Steimle et al. 2000). Spot and Atlantic croakers are more strongly associated with benthic habitats, and a greater contribution of amphipods, copepods, and annelids to their diets may explain their tendency to exhibit $\delta^{15}\text{N}$ values that were intermediate between those of crustaceans and the other, more-pelagic fishes (Stickney et al. 1975; Nemerson and Able 2004). The $\delta^{15}\text{N}$ values for our sampled prey (and their corresponding trophic level; A.B., unpublished data) also agreed with available isotopic and trophic level data for these species in Chesapeake Bay and similar environments (Hagy 2002; Litvin and Weinstein 2003; Hoffman et al. 2007; Douglass 2008). This agreement helps to validate the accuracy of the summer flounder results, for which comparable stable isotope data do not exist.

CONCLUSIONS

Within the lower Chesapeake Bay main stem, stable isotope analysis of multiple summer flounder tissues revealed a strong dietary reliance on crustacean prey, including mysid shrimp, mantis shrimp, and sevenspine bay shrimp. Although some individual summer flounder appeared to assimilate appreciable amounts of fish prey, calculated mean contributions (based on $\delta^{15}\text{N}$) of the crustacean group to summer flounder diets ranged from 65% to over 100% during the fall, indicating that the majority of assimilated tissue was generated from crustacean food sources. Consequently, the crustacean prey can play an important role in energy transfer as a critical food web link for juvenile and young adult summer flounder. Given their preponderance in summer flounder stomachs, mysid shrimp in particular may be the most important prey driving summer flounder productivity (Latour et al. 2008; this study). Fish prey also contributed to tissue growth and metabolism in summer flounder, especially larger individuals, but dietary contributions by fish exhibited annual variability. Summer flounder $\delta^{15}\text{N}$ values generally

mirrored the values for other small fishes (bay anchovy and juvenile sciaenids), which suggests that these species occupied a similar trophic level in the food web of lower Chesapeake Bay. Isotopic results were in general agreement with the results of stomach content analysis, although the importance of crustaceans may have been underrepresented in stomach contents, particularly those of larger summer flounder. Overall, we support recommendations that stable isotope methods are best when applied in conjunction with additional techniques in diet studies (Cloern et al. 2002; Fry 2006). The use of stomach content and stable isotope analyses combined provided both taxonomic specificity and integrative information on assimilation. Together, these methods characterized the diets of summer flounder more comprehensively and can be of greater benefit to resource managers, ecosystem modelers, and other researchers.

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REFERENCES

- Able, K. W., and M. P. Fahay. 2010. Ecology of estuarine fishes: temperate waters of the western North Atlantic. Johns Hopkins University Press, Baltimore, Maryland.
- Able, K. W., R. E. Matheson, W. W. Morse, M. P. Fahay, and G. P. Shepherd. 1990. Patterns of summer flounder (*Paralichthys dentatus*) early life history in the Mid-Atlantic Bight and New Jersey estuaries. U.S. National Marine Fisheries Service Fishery Bulletin 88:1–12.
- Andersen, N. G. 1999. The effects of predator size, temperature, and prey characteristics on gastric evacuation in whiting. *Journal of Fish Biology* 54:287–301.
- Baird, D., and R. E. Ulanowicz. 1989. The seasonal dynamics of the Chesapeake Bay ecosystem. *Ecological Modelling* 59:329–364.
- Bonzek, C. F., R. J. Latour, and J. Gartland. 2008. Data collection and analysis in support of single and multispecies stock assessments in Chesapeake Bay: the Chesapeake Bay Multispecies Monitoring and Assessment Program. Virginia Institute of Marine Science, Gloucester Point.
- Buchheister, A., and R. J. Latour. 2010. Turnover and fractionation of carbon and nitrogen stable isotopes in tissues of a migratory coastal predator, summer flounder (*Paralichthys dentatus*). *Canadian Journal of Fisheries and Aquatic Sciences* 67:445–461.
- Burke, J. S. 1995. Role of feeding and prey distribution of summer and southern flounder in selection of estuarine nursery habitats. *Journal of Fish Biology* 47:355–366.
- Cifuentes, L. A., J. H. Sharp, and M. L. Fogel. 1988. Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary. *Limnology and Oceanography* 33:1102–1115.
- Cloern, J. E., E. A. Canuel, and D. Harris. 2002. Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. *Limnology and Oceanography* 47:713–729.
- DeNiro, M. J., and S. Epstein. 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science (Washington, D.C.)* 197:261–263.
- Dougllass, J. G. 2008. Community dynamics in submersed aquatic vegetation: intermediate consumers as mediators of environmental change. College of William and Mary, Gloucester Point, Virginia.
- Fabrizio, M. C., M. J. Henderson, and J. A. Lucy. 2007. Understanding localized movements and habitat associations of summer flounder in Chesapeake Bay using passive acoustic arrays. Virginia Marine Resources Commission, Final Report, Richmond. Available: www.mrc.state.va.us/vsrfdt/pdf/RF06-11_Dec07.pdf. (October 2010).
- Fabrizio, M. C., and T. D. Tuckey. 2008. Estimating relative juvenile abundance of ecologically important finfish in the Virginia portion of Chesapeake Bay. Virginia Marine Resources Commission, Federal Aid in Sport Fish Restoration, Project F-104-R-12, Annual Report, Richmond.
- Fry, B. 2006. Stable isotope ecology. Springer-Verlag, New York.
- Fry, B., and E. B. Sherr. 1989. $\delta^{13}\text{C}$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Pages 196–229 in P. W. Rundel, J. R. Ehleringer, and K. A. Nagy, editors. Stable isotopes in ecological research. Springer-Verlag, New York.
- Gannes, L. Z., D. M. O'Brien, and C. M. del Rio. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecology (Washington, D.C.)* 78:1271–1276.
- Gorokhova, E., and S. Hansson. 1999. An experimental study on variations in stable carbon and nitrogen isotope fractionation during growth of *Mysis mixta* and *Neomysis integer*. *Canadian Journal of Fisheries and Aquatic Sciences* 56:2203–2210.
- Grey, J., S. J. Thackeray, R. I. Jones, and A. Shine. 2002. Ferox trout (*Salmo trutta*) as 'Russian dolls': complementary gut content and stable isotope analyses of the Loch Ness food web. *Freshwater Biology* 47:1235–1243.
- Grimes, B. H., M. T. Huish, and J. H. Kerby. 1989. Species profile: life histories and environmental requirements of coastal fishes and invertebrates (Mid-Atlantic)—summer and winter flounder. U.S. Fish and Wildlife Service Biological Report 82 (11.112).
- Hagy, J. D. 2002. Eutrophication, hypoxia and trophic transfer efficiency in Chesapeake Bay. Doctoral dissertation. University of Maryland, College Park.
- Hartman, K. J., J. Howell, and J. A. Sweka. 2004. Diet and daily ration of bay anchovy in the Hudson River, New York. *Transactions of the American Fisheries Society* 133:762–771.
- Herman, S. S. 1963. Vertical migration of the opossum shrimp *Neomysis americana* Smith. *Limnology and Oceanography* 8:228–238.
- Hoffman, J. C., C. F. Bonzek, and R. J. Latour. 2009. Estimation of bottom trawl catch efficiency for two demersal fishes, the Atlantic croaker and white perch, in Chesapeake Bay. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* [online serial] 1:255–269.
- Hoffman, J. C., D. A. Bronk, and J. E. Olney. 2007. Tracking nursery habitat use in the York River estuary, Virginia, by young American shad using stable isotopes. *Transactions of the American Fisheries Society* 136:1285–1297.
- Horodysky, A. Z., R. W. Brill, E. J. Warrant, J. A. Musick, and R. J. Latour. 2010. Comparative visual function in four piscivorous fishes inhabiting Chesapeake Bay. *Journal of Experimental Biology* 213:1751–1761.
- Horrigan, S. G., J. P. Montoya, J. L. Nevins, and J. J. McCarthy. 1990. Natural isotopic composition of dissolved inorganic nitrogen in the Chesapeake Bay. *Estuarine, Coastal, and Shelf Science* 30:393–410.
- Hulbert, E. 1957. The distribution of *Neomysis americana* in the estuary of the Delaware River. *Limnology and Oceanography* 2:1–11.
- Hyslop, E. J. 1980. Stomach contents analysis: a review of methods and their application. *Journal of Fish Biology* 17:411–429.

- Kaehler, S., and E. A. Pakhomov. 2001. Effects of storage and preservation on the delta C-13 and delta N-15 signatures of selected marine organisms. *Marine Ecology Progress Series* 219:299–304.
- Kelly, L. J., and C. Martinez del Rio. 2010. The fate of carbon in growing fish: an experimental study of isotopic routing. *Physiological and Biochemical Zoology* 83:473–480.
- Kemp, W. M., W. R. Boynton, J. E. Andolf, D. F. Boesch, W. C. Boicourt, G. Brush, J. C. Cornwell, T. R. Fisher, P. M. Glibert, J. D. Hagy, L. W. Harding, E. D. Houde, D. G. Kimmel, W. D. Miller, R. I. E. Newell, M. R. Roman, E. M. Smith, and J. C. Stevenson. 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. *Marine Ecology Progress Series* 303:1–29.
- Kiljunen, M., J. Grey, T. Sinisalo, C. Harrod, H. Immonen, and R. I. Jones. 2006. A revised model for lipid-normalizing delta C-13 values from aquatic organisms, with implications for isotope mixing models. *Journal of Applied Ecology* 43:1213–1222.
- Lankford, T. E., and T. E. Targett. 1997. Selective predation by juvenile weakfish: post-consumptive constraints on energy maximization and growth. *Ecology* (Washington, D.C.) 78:1049–1061.
- Latour, R. J., M. J. Brush, and C. F. Bonzek. 2003. Toward ecosystem-based fisheries management: strategies for multispecies modeling and associated data requirements. *Fisheries* 28(9):10–22.
- Latour, R. J., J. Gartland, C. F. Bonzek, and R. A. Johnson. 2008. The trophic dynamics of summer flounder (*Paralichthys dentatus*) in Chesapeake Bay. *U.S. National Marine Fisheries Service Fishery Bulletin* 106:47–57.
- Link, J. S., K. Bolles, and C. G. Milliken. 2002. The feeding ecology of flatfish in the Northwest Atlantic. *Journal of Northwest Atlantic Fishery Science* 30:1–17.
- Litvin, S. Y., and M. P. Weinstein. 2003. Life history strategies of estuarine nekton: the role of marsh macrophytes, benthic microalgae, and phytoplankton in the trophic spectrum. *Estuaries* 26:552–562.
- MacNeil, M. A., K. G. Drouillard, and A. T. Fisk. 2006. Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. *Canadian Journal of Fisheries and Aquatic Sciences* 63:345–353.
- MacNeil, M. A., G. B. Skomal, and A. T. Fisk. 2005. Stable isotopes from multiple tissues reveal diet switching in sharks. *Marine Ecology Progress Series* 302:199–206.
- Malloy, K. D., and T. E. Targett. 1994. The use of RNA: DNA ratios to predict growth limitation of juvenile summer flounder (*Paralichthys dentatus*) from Delaware and North Carolina estuaries. *Marine Biology* 118:367–375.
- Martinez del Rio, C., N. Wolf, S. A. Carleton, and L. Z. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews* 84:91–111.
- McConnaughey, T., and C. P. McRoy. 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. *Marine Biology* 53:257–262.
- McIntyre, J. K., D. A. Beauchamp, M. M. Mazur, and N. C. Overman. 2006. Ontogenetic trophic interactions and benthopelagic coupling in Lake Washington: evidence from stable isotopes and diet analysis. *Transactions of the American Fisheries Society* 135:1312–1328.
- Montoya, J. P., S. G. Horrigan, and J. J. McCarthy. 1990. Natural abundance of ^{15}N in particulate nitrogen and zooplankton in the Chesapeake Bay. *Marine Ecology Progress Series* 65:35–61.
- Morse, W. W. 1981. Reproduction of the summer flounder, *Paralichthys dentatus* (L.). *Journal of Fish Biology* 19:189–203.
- Murdy, E. O., R. S. Birdsong, and J. A. Musick. 1997. *Fishes of Chesapeake Bay*. Smithsonian Institution Press, Washington, D.C.
- Nemerson, D. M., and K. W. Able. 2004. Spatial patterns in diet and distribution of juveniles of four fish species in Delaware Bay marsh creeks: factors influencing fish abundance. *Marine Ecology Progress Series* 276:249–262.
- O'Reilly, C. M., R. E. Hecky, A. S. Cohen, and P. D. Plisnier. 2002. Interpreting stable isotopes in food webs: recognizing the role of time averaging at different trophic levels. *Limnology and Oceanography* 47:306–309.
- Packer, D. B., S. J. Griesbach, P. L. Berrien, C. A. Zetlin, D. L. Johnson, and W. W. Morse. 1999. Essential fish habitat document: summer flounder, *Paralichthys dentatus*, life history and habitat characteristics. NOAA Technical Memorandum NMFS-NE-151.
- Pecquerie, L., R. M. Nisbet, R. Fablet, A. Lorrain, and S. A. L. M. Kooijman. 2010. The impact of metabolism on stable isotope dynamics: a theoretical framework. *Philosophical Transactions of the Royal Society of London B* 365:3455–3468.
- Perga, M. E., and D. Gerdeaux. 2005. 'Are fish what they eat' all year round? *Oecologia* (Heidelberg) 144:598–606.
- Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18:293–320.
- Phillips, D. L., and J. W. Gregg. 2001. Uncertainty in source partitioning using stable isotopes. *Oecologia* (Heidelberg) 127:171–179.
- Phillips, D. L., S. D. Newsome, and J. W. Gregg. 2005. Combining sources in stable isotope mixing models: alternative methods. *Oecologia* (Heidelberg) 144:520–527.
- Pinnegar, J. K., and N. V. C. Polunin. 1999. Differential fractionation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among fish tissues: implications for the study of trophic interactions. *Functional Ecology* 13:225–231.
- Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* (Washington, D.C.) 83:703–718.
- Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi, and C. G. Montana. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* (Heidelberg) 152:179–189.
- Powell, A. B. 1982. Annulus formation on otoliths and growth of young summer flounder from Pamlico Sound, North Carolina. *Transactions of the American Fisheries Society* 111:688–693.
- Rountree, R. A., and K. W. Able. 1992. Foraging habits, growth, and temporal patterns of salt-marsh creek habitat use by young-of-year summer flounder in New Jersey. *Transactions of the American Fisheries Society* 121:765–776.
- Scharf, F. S., F. Juanes, and R. A. Rountree. 2000. Predator size-prey size relationships of marine fish predators: interspecific variation and effects of ontogeny and body size on trophic-niche breadth. *Marine Ecology Progress Series* 208:229–248.
- Sipe, A. M., and M. E. Chittenden. 2001. A comparison of calcified structures for aging summer flounder, *Paralichthys dentatus*. *U.S. National Marine Fisheries Service Fishery Bulletin* 99:628–640.
- Steimle, F. W., R. A. Pikanowski, D. G. McMillan, C. A. Zetlin, and S. J. Wilk. 2000. Demersal fish and American lobster diets in the lower Hudson-Raritan estuary, volume 161. *National Marine Fisheries Service, Woods Hole, Massachusetts*.
- Stickney, R. R., G. L. Taylor, and D. B. White. 1975. Food habits of five species of young southeastern United States estuarine Sciaenidae. *Chesapeake Science* 16:104–114.
- Sweeting, C. J., J. Barry, C. Barnes, N. V. C. Polunin, and S. Jennings. 2007. Effects of body size and environment on diet-tissue $\delta^{15}\text{N}$ fractionation in fishes. *Journal of Experimental Marine Biology and Ecology* 340:1–10.
- Sweeting, C. J., N. V. C. Polunin, and S. Jennings. 2004. Tissue and fixative dependent shifts of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in preserved ecological material. *Rapid Communications in Mass Spectrometry* 18:2587–2592.
- Szedlmayer, S. T., and K. W. Able. 1993. Ultrasonic telemetry of age-0 summer flounder, *Paralichthys dentatus*, movements in a southern New Jersey estuary. *Copeia* 1993:728–736.
- Szedlmayer, S. T., K. W. Able, and R. A. Rountree. 1992. Growth and temperature-induced mortality of young-of-the-year summer flounder (*Paralichthys dentatus*) in southern New Jersey. *Copeia* 1992:120–128.
- Terceiro, M. 2002. The summer flounder chronicles: science, politics, and litigation, 1975–2000. *Reviews in Fish Biology and Fisheries* 11:125–168.

- Trueman, C. N., R. A. R. McGill, and P. H. Guyard. 2005. The effect of growth rate on tissue-diet isotopic spacing in rapidly growing animals: an experimental study with Atlantic salmon (*Salmo salar*). *Rapid Communications in Mass Spectrometry* 19:3239–3247.
- Vander Zanden, M. J., and J. B. Rasmussen. 2001. Variation in delta N-15 and delta C-13 trophic fractionation: implications for aquatic food web studies. *Limnology and Oceanography* 46:2061–2066.
- Werner, E. E., and D. J. Hall. 1974. Optimal foraging and the size selection of prey by the bluegill sunfish (*Lepomis macrochirus*). *Ecology* (Washington, D.C.) 55:1042–1052.
- Zimmerman, A. R., and E. A. Canuel. 2001. Bulk organic matter and lipid biomarker composition of Chesapeake Bay surficial sediments as indicators of environmental processes. *Estuarine, Coastal, and Shelf Science* 53:319–341.