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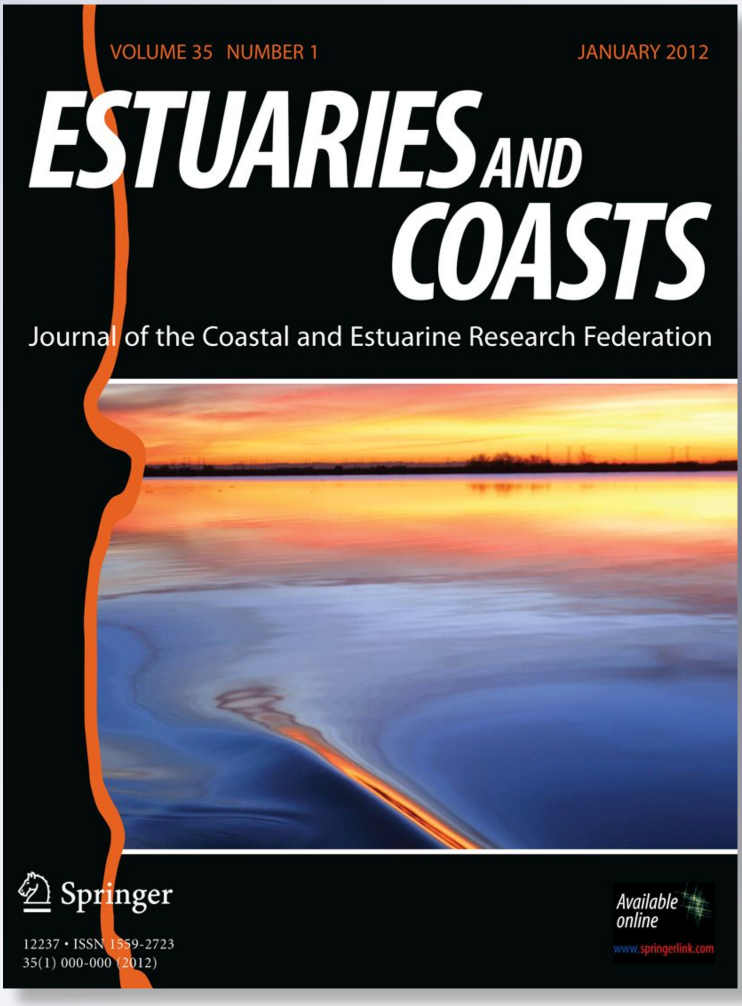
Estuaries and Coasts
Journal of the Coastal and Estuarine Research Federation

ISSN 1559-2723

Estuaries and Coasts
DOI 10.1007/s12237-011-9467-6



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Isotopic Ratios Reveal Mixed Seasonal Variation Among Fishes from Two Subtropical Estuarine Systems

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Received: 26 February 2011 / Revised: 24 November 2011 / Accepted: 4 December 2011

Abstract Characterizing dietary resources and species interactions in estuaries is challenging, particularly when considering the dynamic nature of these ecosystems, the ranges in body sizes of species, and the potential for trophic roles to vary with ontogeny. We examined the influence of season and location on relationships between body size and δ^{15}N, δ^{13}C, and δ^{34}S values across a range of fishes from two subtropical estuaries. The results suggest that isotopic values of estuarine fishes are independent of body size. However, seasonal variation propagated throughout the assemblage as the majority of fishes integrated different δ^{15}N, δ^{13}C, or δ^{34}S values. The absence of δ^{15}N–, δ^{13}C–, and δ^{34}S–body size relationships suggests that either (1) dietary preference of these fishes do not shift within the range of body sizes sampled, (2) these fishes shift to an alternate diet that is not isotopically distinct, or (3) that spatial and temporal variation in isotopic signatures of prey negate any size-based relationships. Seasonal variability in the isotopic values of these fishes suggests either movement to an alternative habitat or a shift in organic matter source associated with the transition of dry to wet seasons. Moreover, variance distributions of the best-fit models indicate that seasonal dietary preferences of conspecifics do not vary over moderate spatial scales. Seasonal variability among fishes in these estuaries suggests plasticity in feeding strategies that may afford greater adaptive flexibility to these species in response to changes in food availability resulting from variable environmental conditions.

Keywords Body size · δ^{15}N · δ^{13}C · δ^{34}S · Spatial variation · Caloosahatchee River · Myakka River

Introduction

Estuaries are highly productive and complex ecosystems that derive organic carbon from a combination of sources (Bouillon et al. 2004; Peterson and Howarth 1987). As a result, estuaries serve as nursery, rearing and feeding grounds for a diverse assemblage of both resident and transient fish and invertebrate species (e.g., Beck et al. 2001). This complexity makes characterizing feeding relationships and dietary resource partitioning in these systems especially challenging, particularly when considering that body sizes of some individual consumer species can range over an order of magnitude (Rountree and Able 1992) and that trophic roles can vary with ontogeny (Wilson and Sheaves 2001).

The use of stable isotopes of nitrogen (δ^{15}N), carbon (δ^{13}C), and sulfur (δ^{34}S) to characterize dietary resources has become commonplace in studies of feeding ecology as they provide a time-integrated perspective of a consumer's

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Electronic supplementary material The online version of this article (doi:10.1007/s12237-011-9467-6) contains supplementary material, which is available to authorized users.

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diet (Peterson and Fry 1987). Specifically, $\delta^{15}N$ values are used in determining the relative trophic position of a consumer (Minagawa and Wada 1984), and $\delta^{13}C$ and $\delta^{34}S$ values have found application in determining basal organic matter sources incorporated into a consumer's diet (Peterson and Fry 1987). Changes in $\delta^{15}N$ in particular can be attributed to either a trophic level shift (i.e., feeding on more ^{15}N-enriched or ^{15}N-depleted prey) or to a change in organic matter sources supplementing the diet (i.e., pelagic- to terrestrial-derived organic matter) or both (Peterson and Howarth 1987). Thus, applying $\delta^{13}C$ and $\delta^{34}S$ with $\delta^{15}N$ in combination can help distinguish the potentially wide range of dietary resources available to consumers (Connolly et al. 2004; Peterson and Howarth 1987).

Body size has long been recognized as influential on the structural and functional complexity of aquatic food webs (Elton 1927). Size-based shifts in dietary resources, reflecting the changing role of an organism within its community, are widespread in aquatic species, including invertebrates (Cherel et al. 2009; Hoeinghaus and Davis 2007), teleosts (Deudero et al. 2004; Greenwood et al. 2010; Kolasinski et al. 2009), marine turtles (Godley et al. 1998), and marine mammals (Newsome et al. 2009). Such size-based differences often explain variation in stable isotope composition between species (Akin and Winemiller 2008) and among conspecifics within a population (Davenport and Bax 2002; Jennings et al. 2002). However, the ability to detect size-based isotopic variation is often limited (Galván et al. 2010) as sampling the range of body sizes needed to account for ontogenetic differences in the feeding ecology of consumers can be difficult. This is particularly relevant in estuarine ecosystems as high levels of spatial and temporal variability in the physical and chemical properties (Deegan and Garritt 1997; Abrantes and Sheaves 2010) influence the age class composition of species at any particular time.

Size-dependent temporal variation in $\delta^{15}N$ and $\delta^{13}C$ has been observed in coastal and open-water marine organisms (Goering et al. 1990; Jennings et al. 2008). Although these observations were largely noted in lower trophic levels, such as zooplankton, body size-related temporal variation has been identified in fishes (Vizzini and Mazzola 2003). However, evidence against size- and temporal-based isotopic shifts has been reported within estuarine consumers that indicated a dietary shift with size, based on stomach content analyses (Wilson et al. 2009). Detection of temporal variation in a consumer's isotopic values, however, is in part dependent on the lag associated with processing alternative dietary resources (i.e., growth rates, tissue turnover rates, or both; Fry and Arnold 1982; Hesslein et al. 1993). Temporal shifts in isotopic values would therefore more likely be detected in species or individuals (e.g., smaller fish) with fast growth and tissue turnover rates (MacNeil et al. 2006).

Using the estuarine reaches of two subtropical tidal rivers located in southwestern Florida, USA (the Caloosahatchee and the Myakka), we examine temporal and spatial relationships between body size and $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ values for fish species across multiple trophic levels. Because riverine systems undergo periods of increased freshwater flow, which provides terrestrial organic matter and nutrients to the receiving estuary (e.g., Chanton and Lewis 2002), we hypothesize that small-bodied, relative to larger bodied, fishes will reflect the seasonal variability of the two estuaries via their $\delta^{13}C$ and $\delta^{34}S$ values. An additional hypothesis is that $\delta^{15}N$ will scale with body size within each fish species. Our objectives were to (1) determine whether body size or season influence the isotopic values of individual fish species; (2) determine whether these relationships are consistent for multiple fish species; and (3) determine whether body size/seasonal–isotopic relationships were consistent across estuarine systems.

Methods

Sample Collection

The Caloosahatchee (26°30′ N, 81°54′ W) and Myakka (82°12′ W, 26°57′ N) Rivers are major tributaries of Charlotte Harbor, a large relatively shallow estuary on the southwest coast of Florida (Fig. 1). The study was completed in the estuarine reach of the two rivers, encompassing ~27 km of habitat in the Caloosahatchee and ~32 km in the Myakka (Fig. 1, inset). The upper reaches of the Caloosahatchee and the shoreline areas of the Myakka are characterized by mangroves and salt marsh, principally red mangrove (*Rhizophora mangle*), black mangrove (*Avicennia germinans*), salt marsh cordgrass (*Spartina alterniflora*), and black needlerush (*Juncus roemerianus*). The shoreline habitats closer to the Caloosahatchee River mouth have been largely altered by urbanization, as evidenced by extensive canal developments and shoreline modifications.

From 2006 to 2008, fishes were collected in spring (i.e., May–June) and autumn (i.e., September–October) from the Caloosahatchee and Myakka estuaries as a component of a larger study aimed at characterizing the food web dynamics of the two estuaries using a shallow water (<10 m), longline (800 m), seine (21.3 × 1.8 m at the center bag, 3-mm-stretch mesh), gillnet (50 m), and otter trawl (6.1 m with 38-mm-stretch mesh and 3-mm mesh liner). Upon collection, individuals were measured (standard length, to the nearest centimeter) and white muscle tissue was excised from the dorsal area anterior to the first dorsal fin. Muscle samples were stored on ice in the field and then stored frozen upon return to the laboratory (−20°C).

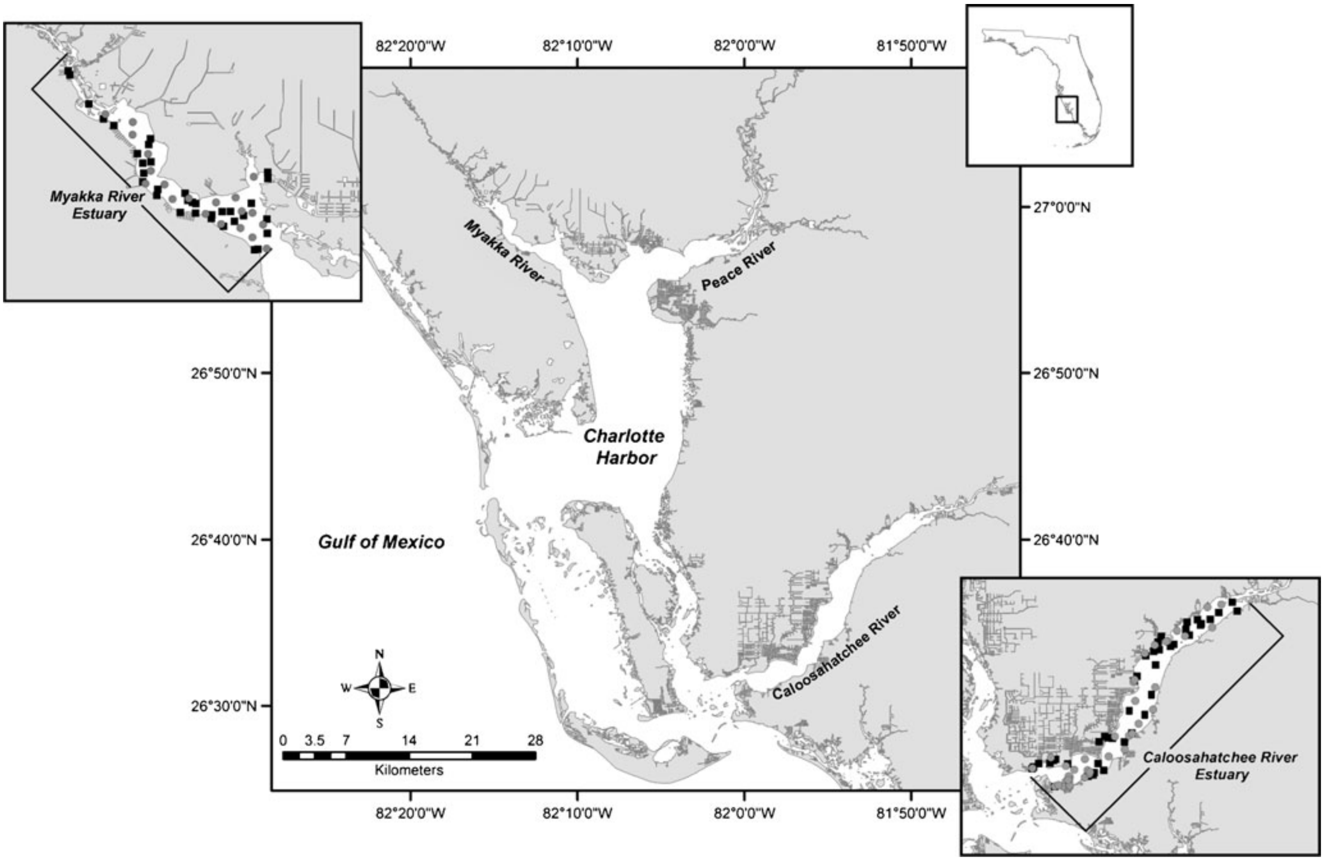

Fig. 1 Map of the study site showing the locations of the Caloosahatchee and Myakka Rivers with respect to the southwestern coast of Florida. *Insets* Locations of the estuarine portions of the two rivers from which fishes were sampled (*black squares* represent spring sample locations; *gray circles* represent autumn sample locations)

Stable Isotope Analysis

Muscle tissues were subsampled (~1.0 g), freeze-dried for 48 h, and homogenized in a SPEX CertiPrep 8000-D ball milling unit (SPEX CertiPrep, Metuchen, NJ). Lipids are depleted in ^{13}C relative to other major tissue components (i.e., proteins and carbohydrates; DeNiro and Epstein 1977), and their presence in muscle tissue samples can negatively skew the observed δ^{13}C values (Post et al. 2007). Thus, to standardize within and among species, lipids were removed from all samples prior to isotopic analysis using a modified method outlined by Bligh and Dyer (1959): twice vortexing the pulverized tissue in 5 ml of 2:1 chloroform/methanol solution for 24 h and decanting the solvent through filter paper (Whatman™ Grade 1, 125 mm) to isolate the muscle tissue sample.

Relative abundances of nitrogen (^{15}N/^{14}N) and carbon (^{13}C/^{12}C) were determined on ~0.5 mg subsamples sealed in tin capsules on a Thermo Finnigan Delta$^{\text{Plus}}$ mass spectrometer (Thermo Finnigan, San Jose, CA, USA) coupled with an elemental analyzer (Costech, Valencia, CA, USA) at the Chemical Tracers Laboratory, Great Lakes Institute for Environmental Research, University of Windsor. Relative abundance of sulfur (^{34}S/^{32}S) was determined on ~2 and ~6 mg subsamples sealed in tin capsules on an Isochrom Continuous Flow IRMS (GV Instruments/Micromass, UK) coupled with an elemental analyzer (Costech) at the Environmental Isotope Laboratory, University of Waterloo, and by a Thermo-Electron Delta$^{\text{Plus}}$ Advantage IRMS at the Colorado Plateau Stable Isotope Laboratory, Northern Arizona University, respectively.

Stable isotope results are expressed in standard delta notation (δ), which are parts per thousand differences from a standard as follows: $\delta = \left[\left(R_{\text{sample}}/R_{\text{standard}}\right) - 1\right] \times 10^3$ (Peterson and Fry 1987), where R is the ratio of heavy to light isotopes in the sample and a standard reference material (atmospheric nitrogen for nitrogen, Pee Dee Belemnite carbonate for carbon, and Canyon Diablo Troilite for sulfur). The analytical precision based on the standard deviation of two standards (NIST 8414 and internal lab standard, n=76) ranged from 0.10‰ to 0.21‰ in δ^{15}N, 0.06‰ to 0.09‰ in δ^{13}C, and 0.3‰ for δ^{34}S based on three sulfide standards (NBS-123, EIl-40, and EIL-43). Accuracy of analysis based on the analysis of NIST standards, performed with muscle tissue sample analysis, sucrose (NIST 8542), ammonium sulfate (NIST 8547), and bovine liver and mussel (n=3 for each), were within 0.07‰ for δ^{15}N, 0.01‰ for δ^{13}C, and 0.5‰ for δ^{34}S of certified values.

Data Analysis

Seven common estuarine fish species representing a range of trophic guilds, i.e., primary, secondary, and tertiary consumers, were chosen for this analysis (for species names and descriptions, see Table 1). These fishes were collected from a number of locations throughout each estuary. The authors recognize that consumers occupying different locations within an estuary often differ in their isotopic values (e.g., Chanton and Lewis 2002), specifically those sampled upriver relative to those sampled near the mouth. However, Wilson et al. (2009) and Chanton and Lewis (2002) observed no significant differences in the δ^{15}N and δ^{34}S values, respectively, of consumers sampled from the upper and middle reaches of the Apalachicola Bay. Therefore, because of sample size consideration in this study, we elected to group all individuals of each species regardless of sampling location. Because fishes were sampled from the two estuaries during the same time periods annually (i.e., 2006–2008), using the same sampling techniques, isotopic data were pooled from all years for each river (following Layman et al. 2005) to examine whether body size or environmental (i.e., seasonal) factors influence δ^{15}N, δ^{13}C, and δ^{34}S muscle tissue values of individual species and whether evidence exists for size-based seasonal variability in isotopic values.

Body size and seasonal relationships were analyzed using linear mixed-effects models fit using restricted maximum likelihood in the *lme4* package in R (R Core Development Team 2009; Bates and Maechler 2010). Prior to analysis, all stable isotope data were tested for normality using quantile–quantile probability plots and log-transformed where appropriate. We developed a set of three candidate models with estuary as the random effect and body size and season as fixed effects: a model with no predictors (null model; isotope$=\gamma_0+\beta_0+\varepsilon$) and models including the body size (isotope$=\gamma_0+\gamma_1$Body Size$+\beta_0+\varepsilon$) and seasonal (isotope$=\gamma_0+\gamma_2$Season$+\beta_0+\varepsilon$) predictors suspected of influencing isotopic values of the fishes collected during the sampling period. All candidate models were implemented for each species. An examination of the probability plots of residuals from all candidate models relating site-specific species isotopic values to species body size and season sampled indicated that candidate models fit adequately, and quantile–quantile plots showed data to be generally described by normally distributed errors for all fishes.

Model selection was based on Akaike's information criterion (AIC; Akaike 1973) with small-sample bias adjustment (AIC$_c$; Hurvich and Tsai 1989). In determining model AIC$_c$ values, both random (i.e., estuary) and fixed (i.e., body size and season) effects, were counted as unique parameters and the number of observations used to compute the log-likelihood were used in calculating AIC$_c$. Models were ranked and compared using AIC$_c$ weights and ΔAIC$_c$, where AIC$_c$ weights measure the weight in support of the model given the data and ΔAIC$_c$ is the relative difference between the top-ranked model and each alternative model. In most cases, the model with the lowest AIC$_c$ value was considered the best-supported model. However, when the AIC$_c$ of several models differed by $\leq$2, we considered these models to be equally parsimonious. Additionally, if the number of parameters (K) in comparative models differed by 1, then model selection was based on the log-likelihood, with the best-supported model having the lower log-likelihood (Burnham and Anderson 2002). Akaike weights (w_i) were calculated to interpret the weight of evidence for the best-fitting model with evidence ratios used to compare among models (Johnson and Omland 2004). For the best-supported model, parameter estimates and associated 95% confidence intervals (CIs) were determined using the *HPDinterval* function provided in the *lme4* package in R. For each estimated parameter, predictors were considered significant if the confidence interval did not contain zero. To test the effect of body size and season among estuaries, we calculated the intraclass correlation coefficients (ICC), reflecting the proportion of variance attributable to each level of the model (see Raudenbush and Bryk 2002; Elgee et al. 2010). The ICC approaches 1 when the between-estuary variation is large relative to the within-estuary variation, and this coefficient has a 0 value when the within-estuary variation equals the between-estuary variation.

Results

Results from the candidate models used to describe the relationships between δ^{15}N and season–body size effects in the fishes sampled from both the Caloosahatchee and Myakka estuaries indicated that the null model was the top-ranked model for five out of seven species (i.e., there was no effect of season or body size, Table 2; see Electronic Supplementary Material (ESM) Table S1 for full model comparisons for δ^{15}N). However, evidence based on model comparisons indicated that season was the most plausible model describing the δ^{15}N values of two species: *Mugil cephalus* and *Chaetodipterus faber* (Table 2). The parameter estimates for season were significant for both species (i.e., zero was not included within the CI; Fig. 2a), and evidence ratios estimated for these species indicate that the model that included season was 48.5 and 47 times more likely than the model that included body size, respectively (see ESM Table S1). Model comparisons indicated depletion in ^{15}N between spring and autumn in *C. faber* (Table 1), whereas *M. cephalus* enriched in ^{15}N between spring and autumn.

Relationships between season and body size and δ^{13}C favored the null model for four of the seven species in this study (Table 2; see ESM Table S2 for full model

Table 1 Maximum recorded standard lengths (in centimeters), length (FL, mean±SE, range, in centimeters), sample size (*n*), and δ^{15}N, δ^{13}C, and δ^{34}S values for muscle tissue (‰ mean±SE) of fish species sampled seasonally (i.e., spring, May–June; autumn, September–October) from the Caloosahatchee and Myakka estuaries

Species	Season	MSL[a]	Caloosahatchee					Myakka				
			n	Length	δ^{15}N	δ^{13}C	δ^{34}S	*n*	Length	δ^{15}N	δ^{13}C	δ^{34}S
Striped mullet												
Mugil cephalus	Spring	90	5	22.8±0.3 (21–24)	7.6±0.3	−14.1±0.8	8.3±0.7	1	19.0	5.74	−14.57	5.23
	Autumn		6	19.0±7.5 (2–43)	9.4±0.2	−21.9±1.8	9.0±1.1	4	69.3±11 (37–86)	7.4±1.0	−19.26±1.5	10.1±2.3
Striped mojarra												
Eugerres plumieri	Spring	34	10	15.5±1.7 (9–23)	10.2±0.3	−18.4±1.3	5.3±1.7	1	9.5	10.0	−22.8	10.6
	Autumn		31	9.0±1.0 (3–24)	10.6±0.3	−21.6±0.8	9.0±0.7	17	4.6±0.5 (1–8)	9.0±0.1	−22.3±0.5	10.6±0.3
Pinfish												
Lagodon rhomboides	Spring	37	12	9.4±0.6 (6–12)	11.2±0.4	−19.4±0.8	13.7±0.8	11	9.7±0.4 (7–12)	9.6±0.2	−21.8±0.5	13.4±0.3
	Autumn		5	12±1.9 (8–18)	10.6±0.6	−18.9±1.0	11.4 ±1.1	3	12.3±0.9 (11–14)	10.0±0.3	−22.2±0.9	13.4±0.4
Atlantic spadefish												
Chaetodipterus faber	Spring	85	10	12.1±1.2 (7–19)	12.2±0.2	−20.6±0.4	13.4±0.4	3	10.5±4.0 (6–19)	11.3±0.2	−21.3±0.6	11.8±0.7
	Autumn		9	17.9±0.9 (11–21)	10.2±0.6	−20.0±0.6	10.9±0.9	2	7.0, 20.0	10.7, 11.5	−22.0,−24.1	12.9, 13.5
Hardhead catfish												
Ariopsis felis	Spring	62	12	30.4±0.8 (25–33)	11.8±0.3	−21.1±0.4	13.5±0.4	10	29.7±1.5 (23–38)	11.0±0.3	−21.4±0.5	12.7±0.6
	Autumn		28	23.5±1.8 (5–35)	12.1±0.3	−21.0±0.4	12.6±0.5	13	24.4±4.0 (6–40)	10.5±0.6	−20.9±0.8	11.0±0.4
Gafftopsail catfish												
Bagre marinus	Spring	60	13	30.0±2.3 (20–50)	12.4±0.7	−20.5±0.7	14.0±0.5	6	42.2±3.8 (29–57)	12.1±0.1	−18.9±0.5	13.6±0.2
	Autumn		10	24.4±4.6 (10–47)	11.1±1.0	−19.1±1.0	12.9±0.6	16	38.3±2.3 (13–46)	11.1±0.3	−18.9±0.6	12.2±0.3
Bull shark[b]												
Carcharhinus leucas	Spring	180	12	91.3±2.9 (81–106)	13.1±0.1	−18.0±0.4	11.5±0.5	3	102.5±3.0 (87–98)	12.6±0.3	−17.8±0.4	11.1±0.6
	Autumn		3	127.1±18.4 (95–159)	14.1±0.7	−17.7±0.6	13.4±0.5	3	91.6±13.8 (78–126)	12.7±0.2	−18.5±0.7	12.0±0.1

For $n<3$, all values are presented

MSL maximum standard lengths

[a] Maximum recorded standard lengths derived from FishBase (Froese and Pauly 2010). Maximum length recorded for *C. leucas* presented here represents size at maturity as only individuals ranging from neonate to juvenile age classes are common to these estuaries

[b] Only bull sharks with healed umbilical scars (approx. ≥1 year old) were included in this study to eliminate any potential for maternal isotopic influence (Olin et al. 2011)

Table 2 Model selection results for top-ranked models for $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ values of each fish species pooled across both estuaries

Species		Model	K	n	LogLik	AIC	AIC_c	w_i
Mugil cephalus	$\delta^{15}N$	Season	4	16	−24.21	56.41	60.06	0.97
	$\delta^{13}C$	Season	4		−39.44	86.89	90.52	0.99
	$\delta^{34}S$	Null	3		−39.34	84.69	86.68	0.37
Eugerres plumieri	$\delta^{15}N$	Null	3	59	−98.83	203.70	204.10	0.69
	$\delta^{13}C$	Season	4		−161.00	330.00	330.74	0.71
	$\delta^{34}S$	Season	4		−152.40	312.90	313.54	0.96
Lagodon rhomboides	$\delta^{15}N$	Null	3	31	−49.12	104.20	105.13	0.68
	$\delta^{13}C$	Null	3		−68.04	142.10	142.97	0.57
	$\delta^{34}S$	Null	3		−66.40	138.80	139.69	0.29
Chaetodipterus faber	$\delta^{15}N$	Season	4	25	−40.47	88.93	90.94	0.93
	$\delta^{13}C$	Null	3		−45.61	97.21	98.36	0.70
	$\delta^{34}S$	Season	4		−50.59	109.20	111.18	0.77
Ariopsis felis	$\delta^{15}N$	Null	3	63	−113.00	231.90	232.41	0.73
	$\delta^{13}C$	Null	3		−138.5	283.00	283.41	0.65
	$\delta^{34}S$	Season	4		−127.10	262.10	262.89	0.80
Bagre marinus	$\delta^{15}N$	Null	3	45	−99.38	204.80	205.35	0.28
	$\delta^{13}C$	Season	4		−103.6	215.10	216.20	0.70
	$\delta^{34}S$	Season	4		−81.15	170.30	171.30	0.92
Carcharhinus leucas	$\delta^{15}N$	Null	4	21	−22.18	50.36	51.77	0.34
	$\delta^{13}C$	Null	3		−31.68	69.36	70.77	0.76
	$\delta^{34}S$	Null	3		−37.72	81.45	90.62	0.33

K number of model parameters, *n* sample size, *logLik* model log-likelihood, *AIC* Akaike's information criterion, *AIC_c* AIC with small-sample bias adjustment, w_i Akaike's weight

comparisons for $\delta^{13}C$), suggesting limited evidence for size or seasonal effects in the data. The most plausible model describing the $\delta^{13}C$ values of *Bagre marinus*, *M. cephalus*, and *Eugerres plumieri* included season (Table 2). However, confidence intervals that overlapped zero suggest that there is only weak evidence of a seasonal effect on the $\delta^{13}C$ values of *B. marinus* (Fig. 2b). Carbon isotope values of *E. plumieri* were generally lower in the autumn relative to the spring, and evidence ratios indicated that the model which included season was 2.8 times more likely than the

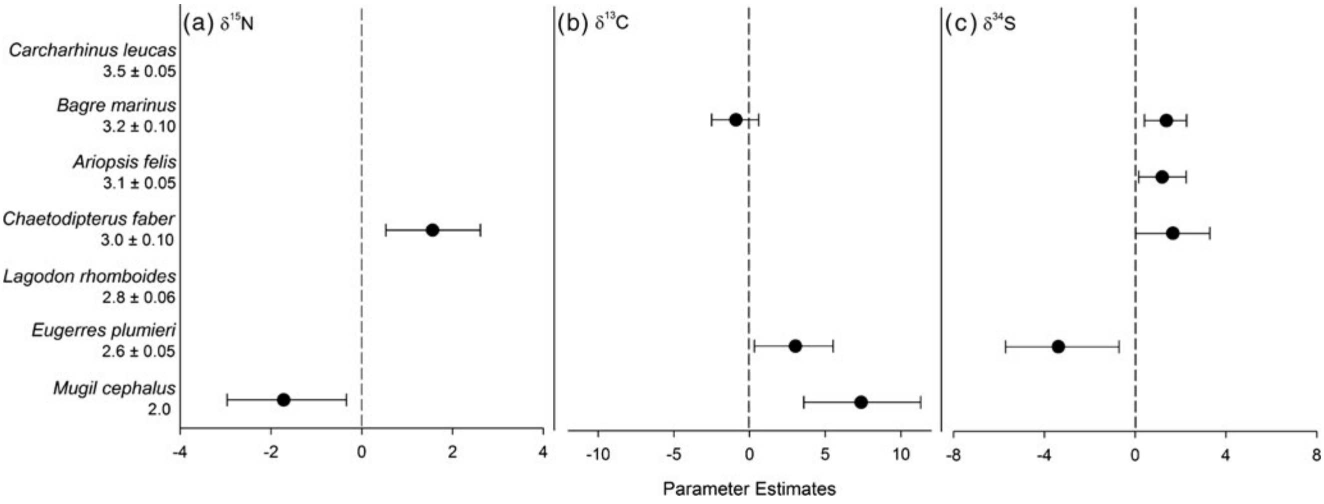

Fig. 2 Parameter estimate results with 95% confidence intervals for the best-fit models for $\delta^{15}N$ (**a**), $\delta^{13}C$ (**b**), and $\delta^{34}S$ (**c**) values for each fish species sampled from the Caloosahatchee and Myakka estuaries. *Symbols* indicate species isotopic relationships were best described by season where AIC_c supported such an effect (Table 2). Negative parameter estimates represent enriched isotopic values in autumn and positive parameter estimates represent depleted isotopic values in autumn. Trophic position is indicated along the *y*-axis for each species. Trophic position (*TP*) was estimated for all fishes using $\delta^{15}N$ as follows: $TP = TP_{baseline} + (\delta^{15}N_{consumer} - \delta^{15}N_{baseline})/\Delta^{15}N$, where $TP_{baseline}$ is the estimated TP of the baseline organism, $\delta^{15}N_{consumer}$ and $\delta^{15}N_{baseline}$ are the mean $\delta^{15}N$ of the consumer of interest and of the baseline organism, respectively, and 3.4‰ was used as the $\Delta^{15}N$ (Post 2002). Mean $\delta^{15}N$ of *M. cephalus*, designated as TP 2.0, was used as the baseline for all fishes as this species is characterized as a primary consumer over the size range sampled here (Platell et al. 2006)

model which included body size. This was also the case for the $\delta^{13}C$ values of *M. cephalus*, a clear depletion in ^{13}C in the autumn (Table 1 and Fig. 2b).

Seasonal variability was identified in four of the seven species using $\delta^{34}S$ (Table 2; see ESM Table S3 for full model comparisons for $\delta^{34}S$). The support for *C. faber*, *B. marinus*, and *Ariopsis felis* was strong (Fig. 2c) with the model that included season, being 30, 4.1, and 32 times more likely than the model containing body size, respectively (see ESM Table S3). Moreover, depletion in ^{34}S from spring to autumn was evident for the three species (Table 1), further supporting a seasonal effect in both estuaries (Fig. 2c). Alternatively, the best model describing the sulfur isotopes of *E. plumieri* indicated a general enrichment in ^{34}S in autumn relative to spring (Fig. 2c).

Intraclass correlation coefficients indicated that the proportion of variance attributable to the seasonal variation within-estuary (61–100%) was greater than the proportion attributable to season between-estuary (0–39%) in all isotopic comparisons in all species, suggesting that seasonal variability was similar between our study locations.

Discussion

Our results provide evidence that for most species examined, season is the dominant influence on isotopic values within the Caloosahatchee and Myakka estuaries relative to the body sizes of the fishes sampled here. Our results are in accordance with those of Wilson et al. (2009), supporting the fact that body size is not an important determinant of isotopic enrichment in estuarine fishes. However, there was evidence for seasonal variability in isotopic values in fish species that spanned several trophic levels and across spatially distinct systems. It is well known that many fishes undergo size-based or ontogenetic changes in diet and thereby occupy a number of trophic levels in the course of their life history (Winemiller 1990). The absence of intraspecific association between $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$, and body size suggests that these estuarine fish species do not undergo size-based dietary changes within the size ranges sampled here. However, the seasonal shift in isotopic values supports the finding of Polis and Strong (1996) in that the relative trophic positions of species, whether attributable to a change in diet or a shift in isotopic values of organic matter sources in food webs, are dynamic rather than fixed. Differing $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ values between seasons suggest that seasonal variability influences the isotopic values of estuarine fishes and, thus, the interpretation of species interactions and food web structure of these estuarine systems.

Body Size Variability

The absence of body size-based $\delta^{15}N$ relationships in the fishes sampled likely result from (1) dietary preferences of these fishes not shifting within the range of body sizes sampled; (2) the fishes do shift to alternative diets with size, yet the isotope ratios of the new diet are similar to the former and are not reflected in isotopic distinctions; or (3) that spatial and temporal variations in isotopic signatures of prey negate any size-based relationships in higher trophic level species (Vander Zanden et al. 2000). Deudero et al. (2004) observed no size-based $\delta^{15}N$ changes in fishes that fed primarily on small benthic invertebrates, suggesting that although these fishes possess very diverse diets throughout their lives, they likely select prey of a relatively similar trophic level. Given the trend of increasing body size–trophic level relationships in large predatory (Scharf et al. 2000) and piscivorous fishes (Deudero et al. 2004), the lack of size-based $\delta^{15}N$ relationships in the fishes included in our study may be a consequence of the fact they are predominantly secondary and tertiary consumers. As such, early life stages (i.e., larvae and young-of-year) generally feed in the pelagic environment on zooplankton and switch to benthic macroinvertebrates in later stages. Thus, significant size-based $\delta^{15}N$ relationships would likely have been evident from a broader range of sizes that includes larval individuals (Mittelbach and Persson 1998). Nonetheless, similar results for estuarine species of the Apalachicola Bay, an estuary in northern Florida, have been observed (Wilson et al. 2009).

Galván et al. (2010) raised the point that the absence of a size-based relationship with $\delta^{15}N$ often resulted from the statistical power being too low to detect a significant relationship. This may be the case here as both the sample size and the range of sizes sampled were low for a number of species. Yet, given the assumptions for estimating the minimal sample size required to analyze size-based feeding relationships using $\delta^{15}N$ (Galván et al. 2010), the body size-independent $\delta^{15}N$ results for 57% of the focal species (4/7) were sampled across size ranges that exceeded Galvan's suggested cutoff. Although we are confident in our relationships for the majority of species sampled, limited statistical power suggests that further sampling may be required for some species. For species that did not meet the sample size minimum for each season, i.e., *M. cephalus*, *Lagodon rhomboids*, and *C. faber*, improvement in power can be achieved by sampling a greater number of individuals over a broader size range to confirm the absence of size relationships with $\delta^{15}N$ and seasonal shifts in isotopic values. However, it is important to note that the use of estuaries by fishes is often seasonally based (Sheaves et al. 2010), and therefore sampling the entire size range of an individual species may not be possible.

Body size-dependent shifts in isotope ratios that reflect a shift in a consumer's diet can be attributed to either a trophic level shift and/or changes in organic matter source available to a consumer. However, in complex ecosystems, such as tropical floodplain rivers, size-related isotopic shifts are less common than in temperate aquatic habitats (Jennings et al. 2002) as multiple primary production sources support highly variable trophic assemblages whose interactions may favor a diversification of size across trophic levels (Layman et al. 2005). Our finding that neither $\delta^{13}C$ nor $\delta^{34}S$ was associated with body size suggests the potential for the absence of systematic shifts in organic matter source use that could obscure the $\delta^{15}N$ trends with body size, lending support to the lack of evidence of size-based isotopic shifts within our study systems.

Seasonal Variability

Body size-dependent diet shifts have been shown to influence temporal variation in aquatic food webs, particularly in highly seasonal systems (Winemiller 1990). Goering et al. (1990) suggested that aside from primary producers, seasonal isotopic variability is confined to relatively short-lived primary consumers because of relatively fast growth and associated tissue turnover rates. This has been supported by studies examining the influence of seasonal variation on producers and consumers (Jennings et al. 2008), attributing the lack of evidence in secondary and tertiary consumers to the weak seasonal variability of the system under examination and to the relatively slow rate of muscle turnover in vertebrate species (MacAvoy et al. 2001). Despite these potential limitations, seasonal variability was evident for all three isotopes employed in our study, a result similar to those reported by Vizzini and Mazzola (2003) from a Mediterranean coastal lagoon and by Chanton and Lewis (2002) from the Apalachicola Bay.

Generally, with respect to $\delta^{13}C$ and $\delta^{34}S$, the most depleted values were observed in autumn. Although we did not characterize the primary producers of either estuary, overall seasonal variability in $\delta^{13}C$ (mean±SE: spring, −19.6±0.3‰ and −20.8±0.4‰; autumn, −20.6±0.4‰ and −20.6±0.3‰) and $\delta^{34}S$ (mean±SE: spring, 12.9±0.3‰ and 12.7±0.3‰; autumn, 10.3±0.4‰ and 11.1±0.3‰) of all fishes combined in the Caloosahatchee and the Myakka, respectively, was relatively low. Shifts in $\delta^{13}C$ and $\delta^{34}S$ are, however, reflected in the fishes' tissues, likely indicating either movement to new habitats or a shift in organic matter source associated with the transition of dry to wet seasons in these estuaries. With the onset of the wet season, both rivers experience increased freshwater flow from natural sources such as rain and subsequent watershed drainage. This source of freshwater into the system could lead to consumers assimilating a more mangrove/upland carbon and sulfur source. The autumnal shift in the sulfur isotope ratios potentially reflects the input of upland/mangrove organic matters sources into the estuaries. The fact that this shift was more evident in $\delta^{34}S$ as opposed to $\delta^{13}C$ may be a consequence of sulfur sources being more distinguishable (i.e., sulfide vs. sulfate). Interpreting $\delta^{13}C$ values in estuarine organisms can often be difficult because a mixture of terrestrial (~27‰) and salt marsh (~13‰) organic matter sources can yield a $\delta^{13}C$ value similar to marine phytoplankton (~21‰; Connolly et al. 2004; Peterson and Fry 1987).

Seasonal variation in isotopic values was prevalent in the majority of fishes regardless of trophic position. This result has implications for the trophic roles of species in estuarine food webs and the tools we use to identify these relationships within the food web. One way that seasonal variation can influence our conceptual understanding of trophic relationships within estuaries relates to the use of stable isotopes. Because tissue turnover is related to growth and metabolism, rates can vary by species, tissue type, and body size. For instance, generally accepted estimates of isotopic turnover in muscle range from less than a week for larval red drum (*Sciaenops ocellatus*; Herzka and Holt 2000) to >400 days in juvenile catfish (*Ictalurus punctatus*; MacAvoy et al. 2001) to >500 days for $\delta^{13}C$ and >300 days for $\delta^{15}N$ in muscle tissues of juvenile sandbar sharks (*Carcharhinus plumbeus*; Logan and Lutcavage 2010). Consideration of temporal variability in isotope values must be taken into account in all species of the community despite the expected lag in tissue turnover rates as shifts in prey resources or environmental conditions can greatly alter isotopic signals.

Spatial Variability

The seasonally driven isotopic trends were similar among conspecifics of the Caloosahatchee and Myakka estuaries as the proportion of variance attributable to seasonal effects within-estuary was greater than that attributable to seasonal effects between-estuary despite the limitation of small sample size for some species. Arguably, there is the potential that the similar seasonal trends observed here among the estuaries is a result of small sample sizes and that more focused sampling would result in different results. Estuarine consumers, however, are known to exhibit omnivory and have the ability to exploit peaks of prey abundance. Isotopic differences among conspecifics have been identified at multiple spatial scales: among habitats within an estuary (Deegan and Garritt 1997) and among neighboring estuaries (Griffin and Valiela 2001). Spatial differences in isotopic values would indicate that fishes adopt site-specific feeding strategies or the variability in the isotopic composition of prey resources. Similar trends between conspecifics of the two rivers therefore suggest that the seasonal factors driving the isotopic dynamics of these fish species are of similar magnitude and that the fishes are responding to environmental factors in a comparable fashion.

We expected that the seasonal isotopic trends of the fishes examined here would have differed over these moderate spatial scales. However, within the southeastern USA, the magnitude of nutrient input entering into estuarine systems depends strongly on riverine discharge and can vary seasonally (Dardeu et al. 1992). In southwest Florida, many rivers are categorized as having the southern river flow pattern, i.e., a significant proportion of riverine annual flow (~60%) is concentrated in the rainy season, which generally occurs in the months of June–September (Kelly and Gore 2008). This is particularly relevant to the Caloosahatchee and Myakka Rivers and provides a rationale for the similar seasonal trends exhibited between the two estuaries.

Conclusions

We have established that isotopic variation in the Caloosahatchee and Myakka estuaries is influenced by seasonal differences as opposed to size-based structuring within fish species. Evidence of seasonal variability among fishes, across a range of trophic levels, suggests that these fishes exhibit plasticity in feeding strategies that may afford greater adaptive flexibility in response to specific changes in food availability resulting from changes in environmental conditions. Likewise, the response of conspecifics between the two estuaries is similar, suggesting that the environmental influence on the isotopic composition (δ^{13}C and δ^{34}S) of these estuarine fishes is of comparable magnitude. These results further suggest that the trophic structure of these estuarine food webs, as indicated by δ^{15}N, is variable among seasons, a result that may be attributable to the alteration in organic matter and/or nutrient sources associated with changes to hydrological regime.

Acknowledgments The authors thank the staff of Mote Marine Laboratory's Center for Shark Research and of Florida Fish and Wildlife Conservation Commission's, Charlotte Harbor Field Laboratory, especially Michelle Heupel, Colin Simpfendorfer, Beau Yeiser, Tonya Wiley, Gregg Poulakis, Philip Stevens, and Amy Timmers for their assistance with sample collection and logistics. We are grateful to William Mark and Richard Doucette for their assistance with sulfur isotope processing and to Alice Grgicak-Mannion for her advice with ArcGIS. This research was funded through University of Windsor scholarships to J.A. Olin and Natural Sciences and Engineering Research Council (NSERC) of Canada Discovery grant funds to A.T. Fisk.

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