

Multivariate analysis of stable-isotope ratios to infer movements and utilization of estuarine organic matter by juvenile weakfish (*Cynoscion regalis*)

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Abstract: Canonical discriminant analysis of the natural carbon, nitrogen, and sulfur stable isotopes in tissues of young weakfish (*Cynoscion regalis*) captured in Delaware Bay was used to infer movements of juveniles and to estimate the utilization of estuarine organic matter in this marine transient species. Two gradients emerged in the data. The first was associated with contributions from tidal salt marsh macrophytes, *Spartina alterniflora* in the polyhaline lower Bay and *Phragmites australis* in the brackish upper Bay. The second was associated with the expected change in phytoplankton carbon-isotope values along the salinity gradient in addition to the availability of macrophyte-derived organic matter within the Bay. The separation of the gradients reflected differences in the relative contribution of phytoplankton to weakfish secondary production in open waters versus marsh habitats. As they grew, discrepancies between the anticipated isotopic signatures of juvenile weakfish collected in a specific habitat and their actual signatures were interpreted as down-bay movements based on known life-history patterns. The size-specific differences in the isotopic signatures of weakfish suggested that stable-isotope data can be used to gauge the relative magnitude of marsh-derived organic matter exported from Delaware Bay via movements of juveniles out of the estuary.

Résumé : Une analyse discriminante canonique des isotopes stables naturels de carbone, d'azote et de soufre dans les tissus de jeunes acoupas royaux (*Cynoscion regalis*) capturés dans la baie de la Delaware nous a servi à retracer les déplacements des jeunes et à estimer l'utilisation de la matière organique estuarienne chez cette espèce marine de passage. Deux gradients apparaissent dans les données, un premier associé aux contributions des macrophytes des marais salés affectés par la marée, *Spartina alternifolia*, dans la baie inférieure polyhaline et *Phragmites australis*, dans la baie supérieure saumâtre. Le second gradient est associé au changement attendu des valeurs isotopiques du carbone phytoplanctonique en fonction du gradient de salinité, en plus de la disponibilité de la matière organique provenant des macrophytes à l'intérieur de la baie. La séparation des gradients est le reflet de différences dans les contributions relatives du phytoplancton à la production secondaire des acoupas dans les eaux du large et dans les habitats de marais. Durant la croissance, les écarts entre les signatures isotopiques anticipées des jeunes acoupas récoltés dans un habitat particulier et les signatures réelles s'interprètent comme des déplacements vers la baie inférieure basés sur des patterns connus du cycle biologique. Les différences de signatures isotopiques des acoupas reliées à la taille indiquent que les données sur les isotopes stables pourraient servir à évaluer l'importance relative de la matière organique provenant des marais qui est exportée de la baie de la Delaware par les déplacements des acoupas hors de l'estuaire.

[Traduit par la Rédaction]

Introduction

Among the taxa thought to benefit from the primary production of tidal salt marshes are marine transients, nekton adapted to life on a "coastal conveyor belt", often spawning far out on the continental shelf and producing estuary-

dependent young that are recruited into coastal embayments year-round (Deegan et al. 2000). The life cycle of marine transients is completed when juveniles (young-of-the-year) accompany the adults offshore during their migration to wintering areas, which is sometimes repeated over several years until maturation.

Though salt marshes are believed to contribute to the success of juvenile marine transient finfishes (Boesch and Turner 1984), the extent to which they fuel the high rate of secondary production in these habitats, and in the greater estuary, has been a source of conjecture for more than four decades (Weinstein and Kreeger 2000). In the 1960s several investigators suggested that organic matter derived from salt-marsh macrophytes, especially *Spartina* spp., drives much of the secondary production of nearshore waters (Teal 1962; Darnell 1967; Odum 1968). However, as demonstrated in subsequent research, the relative rates of production and the availability of organic matter in estuaries are often mediated

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by geomorphology, spatial complexity, tidal magnitude, freshwater input, and the intermediate compounds through which organic matter is transferred to higher trophic levels (Deegan et al. 2000). In an attempt to unravel this complexity, Childers et al. (2000) posited a framework of estuarine subsystems — tidal marsh, macrofaunal beds, seagrass meadows, subtidal flats, and adjacent uplands — linked by an overlying water column that mediates and integrates processes across all subsystems. Nutrient and organic-matter flux associated with the movements of animals, especially marine transients, was also recognized as an important vector transcending system boundaries. However, attempts to link life-history stages and movements of marine transients to the nexus between primary and secondary production becomes problematic because their behavior and habitat preferences are often very flexible (Deegan et al. 2000).

Here we use stable-isotope analysis ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$) to trace the movements of juveniles of a marine transient, the weakfish (*Cynoscion regalis*), in Delaware Bay, USA, and to gauge the extent of marsh primary production supporting their secondary production. The use of stable isotopes to determine the relative contribution of nutrient sources is predicated upon the observation that stable-isotope ratios of consumers reflect their diet at specific locales (Peterson and Fry 1987; Deegan and Garritt 1997). Contribution is inferred from a comparison of consumer stable-isotope signatures with those of isotopically distinct local organic matter sources while accounting for isotopic shifts associated with trophic fractionation (Peterson et al. 1986; Peterson and Fry 1987; Deegan and Garritt 1997). Spatial variability in sources of organic matter within habitats along the estuarine salinity gradient and geochemical influences (Fry 2002) create a mosaic of isotopic signatures that are characteristic of the local food webs. Nekton that move among habitat patches within the mosaic will retain the isotopic signatures commensurate with their most recent period of residency in a specific habitat, for a period that will be determined largely by growth (i.e., dilution; Hesslein et al. 1993; Herzka and Holt 2000; MacAvoy et al. 2001). Thus, movements and site fidelity can also be inferred by comparing stable-isotopic signatures of nekton captured in a specific habitat patch with the expected range of isotope values at that location (Fry 1983; Hesslein et al. 1991; Klein et al. 1998).

Previously, Litvin and Weinstein (2003) described the distribution of stable isotopes of carbon, nitrogen, and sulfur in post-settlement weakfish collected from open water and tidal-marsh creeks in Delaware Bay. The isotope content of young weakfish (<60 mm standard length (SL)) varied significantly with collection location as a function of (i) the change in dominant marsh vegetation from *Spartina* spp. to *Phragmites australis* along the estuarine salinity gradient; (ii) differences in the relative contributions of phytoplankton-derived organic matter between open-Bay and tidal-marsh habitats; and (iii) the anticipated change in phytoplankton $\delta^{13}\text{C}$ values with salinity (Fry 2002). By examining the stable-isotope content of larger juvenile weakfish (>60 mm SL) collected throughout the saline portion of Delaware Bay, including additional sites not reported on previously, we expand upon the previous study by (i) examining the spatial variability in the isotopic signatures of young-of-the-year weakfish and the corresponding contribution of estuarine or-

ganic sources using a multivariate approach (canonical discriminant analysis); (ii) utilizing the classification functions derived from the isotopic signatures of post-settlement individuals to assign larger, more mobile juveniles to habitats based on their isotopic signatures in order to infer movement patterns (Hobson 1999); and (iii) estimating the interaction of movements and the local availability of organic matter to gauge the relative magnitude of marsh-derived organic matter exported from Delaware Bay via movements of juveniles out of the estuary.

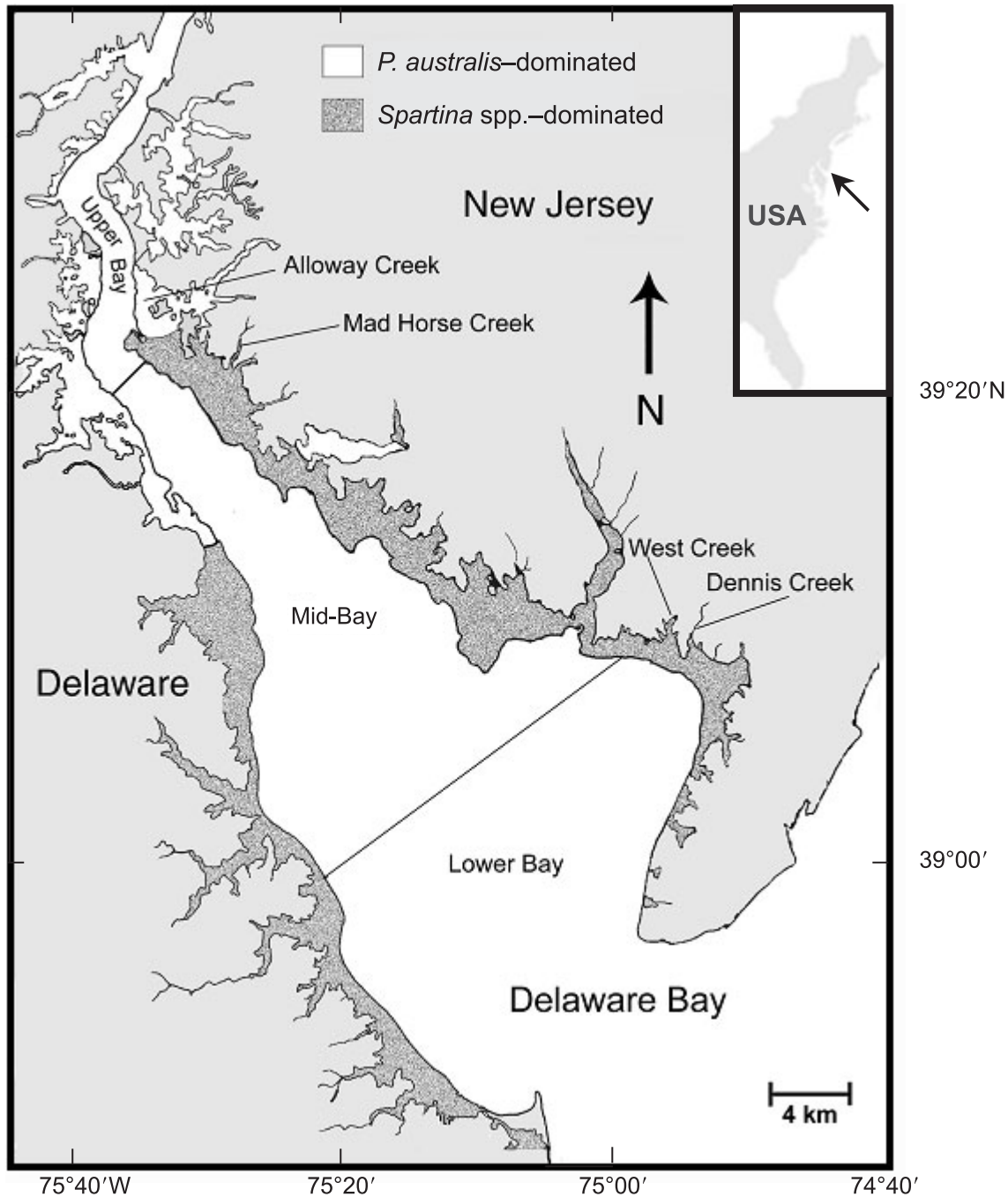
Materials and methods

Study area and sample collection

Tidal salt marsh composes about 73 000 ha of the Delaware Bay shorefront, but a substantial portion of brackish wetlands, at least 16 000 ha in Delaware and probably an area of similar magnitude on the New Jersey side, has been invaded by an aggressive variety of *P. australis* (Fig. 1). Sampling was conducted within three regions designated lower, mid-, and upper (Delaware) Bay, respectively (Fig. 1). Within each region fish were collected from open waters and adjacent tidal creeks: *P. australis*-dominated Alloway Creek, a 1253-ha meso-oligohaline marsh, and Mad Horse Creek, a relatively undisturbed 1558-ha mesohaline marsh, in the upper Bay and mid-Bay regions, respectively, and Dennis Creek, an undisturbed 1100-ha marsh, and West Creek, a nearby 234-ha formerly diked salt-hay farm, were located in polyhaline waters adjacent to the lower Bay (Fig. 1). In contrast to the virtual monocultures of *P. australis* in Alloway Creek, the intertidal marsh surface at both Mad Horse Creek and Dennis Creek was dominated by a more than 90% coverage of smooth cordgrass (*Spartina alterniflora*), but big cordgrass (*Spartina cynosuroides*) was also common on creek banks at Mad Horse Creek (Weinstein et al. 1997). Details of vegetation-sample collection and isotopic analysis (macrophytes, benthic microalgae, and phytoplankton measured as suspended particulate matter (SPM)) appear in Wainright et al. (2000), Weinstein et al. (2000), and Litvin and Weinstein (2003) and are not repeated here. Because whole water samples collected in tidal creeks may contain substantial pyrite, isotope values from the literature for SPM sulfur (Peterson et al. 1986) were substituted (Chanton and Lewis 1999; for a detailed discussion see Wainright et al. 2000).

Juvenile weakfish were collected from June through early October of 1998 and 1999 in tidal salt marsh creeks and the upper, middle, and lower reaches of the Bay (Fig. 1) with otter trawls towed near low tide for 10 min during daytime and (or) nighttime hours ($n = 450$). Fish were measured for SL to the nearest millimetre and preserved on dry ice for transport to storage at $-80\text{ }^{\circ}\text{C}$. On each sampling date and location we attempted to collect individuals from three size classes: post-settlement <60 mm SL (“small”), 60–100 mm SL (“medium”), and >100 mm SL (“large”). These size classes reflect ontogenetic shifts in the juvenile weakfish diet from specialization on *Neomysis americana* when small, to an increasing proportion of fish when medium-sized, to roughly equal proportions of crustaceans and fish when large (Chao and Musick 1977; Nemerson 2001). In addition, juvenile weakfish were collected at the mouth of Delaware Bay prior

Fig. 1. Locations of the three subregions and affiliated marsh creeks that formed part of this study in Delaware Bay, USA (lower, mid- and upper Bay, Alloway Creek, Mad Horse Creek, West Creek, and Dennis Creek) and the extent of tidal salt marshes dominated by *Spartina alterniflora* and *Phragmites australis*.



to emigration in late October and early November of 1998 and 1999 ($n = 141$).

Sample processing and isotope determination

In 1998, samples of muscle tissue were excised from weak-fish >100 mm SL after skin and scales were removed, while smaller fish were used whole. Samples were dried to constant weight at 60 °C, then ground to a fine powder in a Spex/CertiPrep 5100 mill. In 1999, all fish were freeze-dried whole subsequent to removal of gut contents. Freeze-drying

facilitated the simultaneous analysis of lipids and protein for a concurrent biochemical condition study (S.Y. Litvin, M.P. Weinstein, and V. Guida, unpublished data). In 1998, fish tissue samples were analyzed at both the Institute of Marine and Coastal Sciences, Rutgers University, New Brunswick, N.J., USA, on a Europa Scientific continuous-flow elemental analyzer (ANCA-GSL) – mass-spectrometer stable-isotope system and at the Environmental Isotope Laboratory, University of Waterloo, Ontario, Canada, on a Micromass Isochrom Continuous Flow Stable Isotope Mass Spectrome-

ter coupled to a Carla Erba Elemental Analyzer (CHNS-O EA1 108). All fish sampled in 1999 were analyzed at the University of Waterloo. Isotopic composition is expressed in δ notation that indicates depletion (–) or enrichment (+) of the heavy isotope relative to the lighter isotope as

$$\delta X = [(R_{\text{sample}} \cdot R_{\text{standard}}^{-1}) - 1] \times 10^3$$

where X is ^{13}C , ^{34}S , or ^{15}N and R is $^{13}\text{C} \cdot ^{12}\text{C}^{-1}$, $^{34}\text{S} \cdot ^{32}\text{S}^{-1}$, or $^{15}\text{N} \cdot ^{14}\text{N}^{-1}$ for the samples and the standards. The standards were Peedee Belemnite for carbon, air for nitrogen, and Canyon Diablo triolites for sulfur. Instrument precision was ± 0.1 – 0.2‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and $\pm 0.5\text{‰}$ for $\delta^{34}\text{S}$ at the Rutgers University laboratory and $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$, $\pm 0.3\text{‰}$ for $\delta^{15}\text{N}$, and $\pm 0.5\text{‰}$ for $\delta^{34}\text{S}$ at the University of Waterloo laboratory.

Prior to statistical comparison of stable-isotope signatures among juvenile weakfish from different size classes, normalization for ontogenetic shifts was required because of the expected enrichment of stable-isotope values, 1.0‰ $\delta^{13}\text{C}$, 3.4‰ $\delta^{15}\text{N}$, and 0.5‰ $\delta^{34}\text{S}$, associated with trophic transfer (Peterson and Fry 1987). The estimated trophic level for the smallest size class was approximately 0.15 and 0.30 lower than the medium and larger size classes, respectively (Nemerson 2001). To account for these differences, stable-isotope values were reduced by 0.15‰ $\delta^{13}\text{C}$, 0.51‰ $\delta^{15}\text{N}$, and 0.08‰ $\delta^{34}\text{S}$ for medium-sized and 0.30‰ $\delta^{13}\text{C}$, 1.02‰ $\delta^{15}\text{N}$, and 0.15‰ $\delta^{34}\text{S}$ for large juvenile weakfish in the subsequent statistical comparisons.

Data analysis

We used small (least mobile; Greco and Targett 1996) weakfish to establish a post-settlement baseline for isotopic comparisons with larger juveniles captured throughout Delaware Bay. To minimize the confounding effects of collection location and recent movements of individuals, we conjectured that small fish would exhibit the greatest site fidelity and this would be reflected in their tissue isotopic signatures (Deegan and Garritt 1997). This was true in all cases except one (Litvin and Weinstein 2003): the isotopic signatures of small weakfish collected in Alloway Creek were not readily distinguishable from those of individuals captured in open waters of the nearby mid-Bay. We anticipated that these fish would be depleted in both $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$; this is typical of individuals captured in *P. australis*-dominated systems. However, the majority of these fish exhibited the isotopic signatures, intermediate $\delta^{13}\text{C}$ values, and enriched $\delta^{34}\text{S}$ values that are typical of individuals collected in the open reach of the Bay (see Results; Weinstein et al. 2000; Litvin and Weinstein 2003).

Because small fish from Alloway Creek were collected on a single date, and may have been recent recruits (not having had sufficient time to take on the characteristic isotopic signatures of this habitat), they were removed from the pool of fish used to establish the post-settlement baseline and replaced by medium-sized fish from Alloway Creek that exhibited the anticipated isotopic signatures (Litvin and Weinstein 2003). These fish (<60 mm SL from all sites with the exception of medium-sized fish from Alloway Creek; $n = 282$) were analyzed by multiple analysis of variance (MANOVA) with $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ as dependent variables and capture location as the independent factor (McGarigal et

al. 2000). Canonical discriminant analysis was also employed to extract patterns of isotopic signatures as a function of capture location. The ability of the functions to correctly classify post-settlement-baseline weakfish (hereinafter referred to as baseline weakfish) to their collection location was tested by reclassification and jackknife validation where a single fish was removed from the total constituting the baseline, the classification results were re-derived, and the omitted fish were classified to a location, with the process repeated for each remaining fish. Jackknife classification results were then compared with the reclassification results to measure the reliability of the canonical functions (McGarigal et al. 2000). To discern their recent habitat-utilization patterns, we predicted the collection location of the remaining medium-sized and large weakfish ($n = 299$) by applying the classification functions to their isotopic signatures. We reasoned that the discrepancy between the isotopic signature of a medium-sized or large juvenile and the range of values expected for the particular area in which the individual was collected (i.e., the post-settlement baseline) indicated recent utilization of an alternative habitat within the estuary (Hobson 1999).

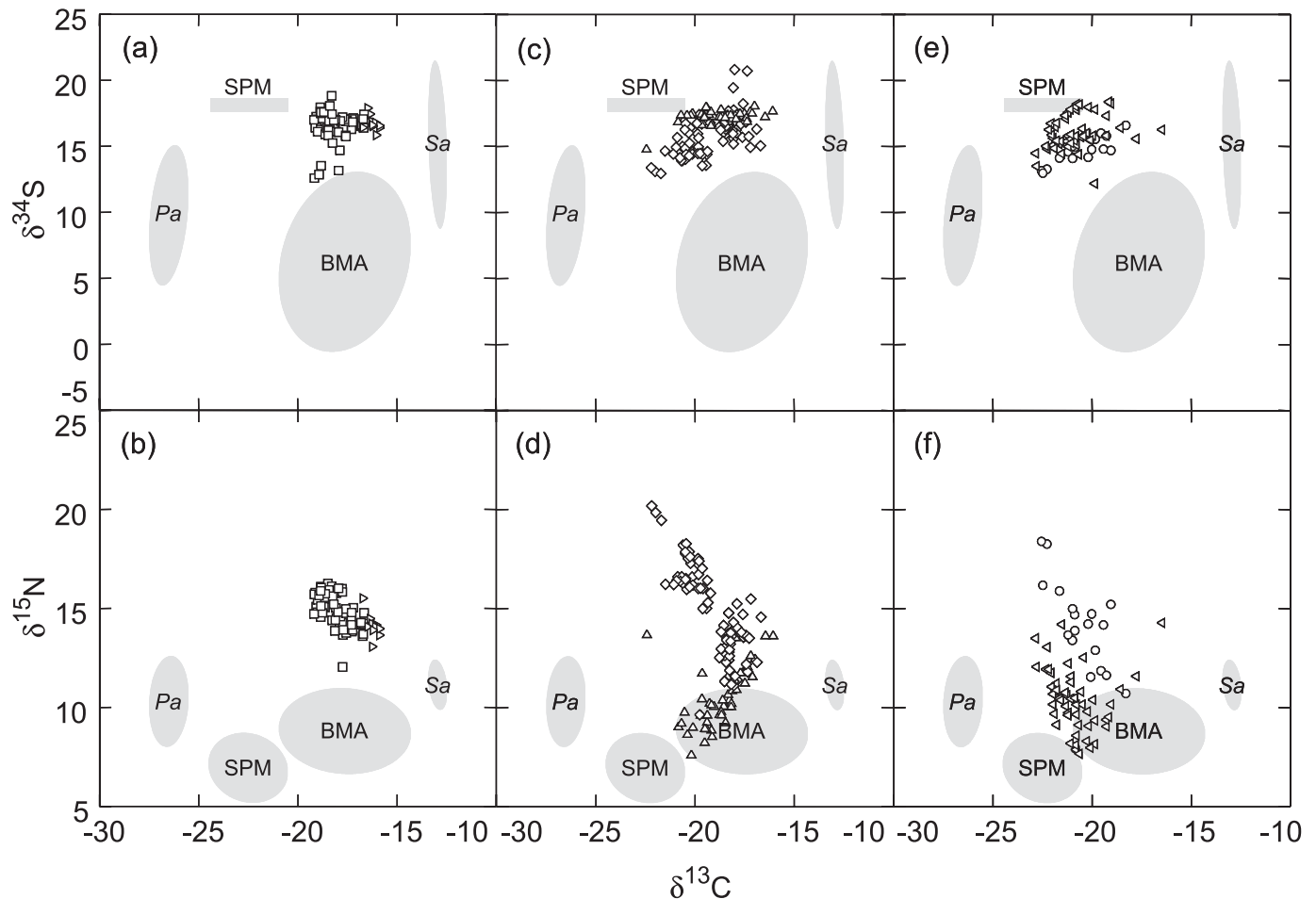
The lack of normality in the $\delta^{34}\text{S}$ data of the baseline weakfish (Kolmogorov–Smirnov one-sample tests; $\alpha = 0.05$) and lack of homogeneity within covariance matrices ($p < 0.001$; Morrison 1976) in the MANOVA resulted in the use of untransformed data and a nonparametric K – nearest neighbor ($K = 10$; $n_{\text{min}} - 1$; SAS Institute Inc. 2000) approach for the canonical discriminant analysis. SYSTAT[®] (SPSS Inc. 1998) and SAS[®] (SAS Institute Inc. 2000) for Windows[®] were used for all statistical comparisons.

Results

Weakfish constituting the post-settlement baseline

Overall, the isotopic signatures of baseline weakfish varied widely across the estuary but were generally bounded by the $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values of primary producers (Fig. 2). In lower Delaware Bay, fish from open waters and *Spartina* spp.-dominated tidal creeks displayed overlapping stable-isotope signatures, with $\delta^{13}\text{C}$ intermediate between *Spartina* spp. and SPM and $\delta^{34}\text{S}$ intermediate between benthic microalgae and SPM (Figs. 2a and 2b). Fish collected in the mid-Bay, where a transition from *Spartina* spp. to *P. australis*-dominated tidal marshes occurred, had $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values intermediate to those of the four organic sources (Fig. 2c). The $\delta^{13}\text{C}$ values of fish collected in this region and lower Bay habitats overlapped; however, the lowest $\delta^{13}\text{C}$ values exhibited by mid-Bay fish showed that they were depleted relative to fish collected in the lower Bay. The $\delta^{34}\text{S}$ contents of fish collected in mid-Bay open waters varied little and were slightly depleted relative to SPM, while the widest range of $\delta^{34}\text{S}$ values in post-settlement individuals was displayed by those in Mad Horse Creek. The $\delta^{15}\text{N}$ values of fish varied widely in the mid-Bay, becoming either enriched, in Mad Horse Creek, or depleted, in the open Bay, with decreasing $\delta^{13}\text{C}$ (Fig. 2d). The $\delta^{13}\text{C}$ values of fish collected in the upper Bay (Fig. 2e), where *P. australis* dominated the tidal-marsh macrophyte community, overlapped little with those from the lower Bay and included the most depleted values in baseline individuals. In fish from both Alloway Creek and upper Bay open

Fig. 2. Dual-isotope plots of $\delta^{15}\text{N}$ on $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ on $\delta^{13}\text{C}$ comparing weakfish, *Cynoscion regalis*, constituting the post-settlement baseline (<60 mm standard length (SL) except those in Alloway Creek, 60–100 mm SL; see the text) collected in lower Bay creeks (\square) (a), lower Bay open waters (\triangleright) (b), Mad Horse Creek (\diamond) (b), mid-Bay open waters (\triangle) (d), Alloway Creek (\circ) (e), and upper Bay open waters (\triangleleft) (f), with those of primary producers (phytoplankton measured as suspended particulate matter (SPM), benthic microalgae (BMA), *Phragmites australis* (Pa), and *Spartina alterniflora* (Sa)). Ellipses represent mean values ± 1 standard deviation.



waters, the $\delta^{34}\text{S}$ content became depleted (Fig. 2e) and the $\delta^{15}\text{N}$ content became enriched (Fig. 2f) with decreasing $\delta^{13}\text{C}$. In addition, fish collected in Alloway Creek were generally depleted in $\delta^{34}\text{S}$ and enriched in $\delta^{15}\text{N}$ relative to individuals collected in the adjacent open Bay.

Multivariate analysis

The isotopic signatures of weakfish constituting the post-settlement baseline were significant for capture location, with $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ all contributing to the differences among groups (MANOVA; Table 1A). The associated canonical discriminant analysis produced two significant canonical functions (Table 1B), the first accounting for 88% and the second for 11% of the variation among isotopic signatures of fish captured at different locations. Contributions by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to the first canonical function were dominant and approximately equal (Table 1B). Although the magnitude of the contribution of the stable isotopes was roughly equal to the second canonical function, the contribution of $\delta^{15}\text{N}$ was negative relative to that of $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ (Table 1B).

Two gradients emerged in the canonical plot comparisons (Fig. 3a). Both originated in fish from the lower Bay that ex-

hibited enriched $\delta^{13}\text{C}$ values combined with intermediate $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values (Fig. 2a). In open waters of the lower and mid-Bay, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values declined, while $\delta^{34}\text{S}$ values remained constant as distance from the Bay mouth increased. Similarly, $\delta^{13}\text{C}$ was further depleted at the transition from mid to upper Bay, while $\delta^{15}\text{N}$ ratios began to increase and $\delta^{34}\text{S}$ ratios to decrease (Fig. 2). This open-water gradient was reflected in the canonical function scores as a steady decrease in the values of the first function coupled with slightly decreasing values of the second function through the mid-Bay, followed by a pronounced decrease in the second function proceeding from mid- to upper Bay (Fig. 3a). A similar pattern emerged for tidal creeks in the form of decreases in $\delta^{13}\text{C}$ values with distance from the Bay mouth coupled with enriched $\delta^{15}\text{N}$ and depleted $\delta^{34}\text{S}$ in fish from the upper (Alloway Creek) and mid-Bay (Mad Horse Creek) marshes relative to those in the lower Bay tidal creeks (Fig. 2). The tidal-marsh gradient resulted in canonical function scores that approached a 1:1 ratio (Fig. 3a).

The canonical functions classified baseline weakfish to their location of collection at an average rate of 85% (reclassification; Table 2). All locations exhibited reclassification rates >79% with the exception of Mad Horse Creek. The

Table 1. Results from the multiple analysis of variance (A) and canonical discriminant analysis (B) utilizing a nonparametric $K - \text{nearest neighbor } (n_{\min} - 1)$ approach for the effect of collection location on the carbon, nitrogen, and sulfur isotopic signatures of juvenile weakfish, *Cynoscion regalis*, constituting the post-settlement baseline (<60 mm standard length (SL), except those in Alloway Creek, 60–100 mm SL).

(A) Multiple analysis of variance: contribution of individual isotopes to group differences. ^a						
Isotope	$F_{[5,276]}$	p				
$\delta^{13}\text{C}$	57.32	<0.0001				
$\delta^{15}\text{N}$	83.32	<0.0001				
$\delta^{34}\text{S}$	15.52	<0.0001				
(B) Canonical discriminant analysis.						
	Squared canonical correlation	Porportion of variance explained	Eigenvalue	F	p	
Canonical function 1	0.78	0.88	3.04	$F_{[115,757]} = 50.25$	<0.0001	
Canonical function 2	0.30	0.11	0.44	$F_{[8,550]} = 15.62$	<0.0001	
Canonical structure						
Isotope	Function 1	Function 2				
$\delta^{13}\text{C}$	0.68	0.69				
$\delta^{15}\text{N}$	0.80	-0.60				
$\delta^{34}\text{S}$	0.02	0.82				

^aOverall effect of collection location on isotopic signature: Wilks' $\lambda = 0.14839$, $F_{[115,757]} = 50.25$, $p < 0.0001$.

jackknife validation success rate was 80% and differed from the reclassification rate for the same location by 9% or less (Table 2).

By applying the canonical functions developed for post-settlement weakfish constituting the baseline, medium-sized and large individuals were classified to location according to their adjusted $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ values (Table 3). In general, the stable-isotope signatures of juvenile weakfish >60 mm SL, and therefore their position on the canonical function plots, fell within the previously reported boundaries of the open-water and marsh-habitat gradients (Figs. 3b, 3c, and 3d). The majority of small fish collected in Alloway Creek ($n = 10$), which were not used to construct the post-settlement baseline, were classified to the mid-Bay (Table 3), supporting the earlier conclusion that they were recent arrivals from other habitats. At most locations large fish displayed a greater range of canonical-function scores than medium-sized fish (Figs. 3b and 3c), with larger individuals from the lower Bay and the Bay mouth exhibiting the widest range (Fig. 3d). However, 102 of 141 fish collected at the Bay mouth were classified into the lower Bay category (Fig. 4).

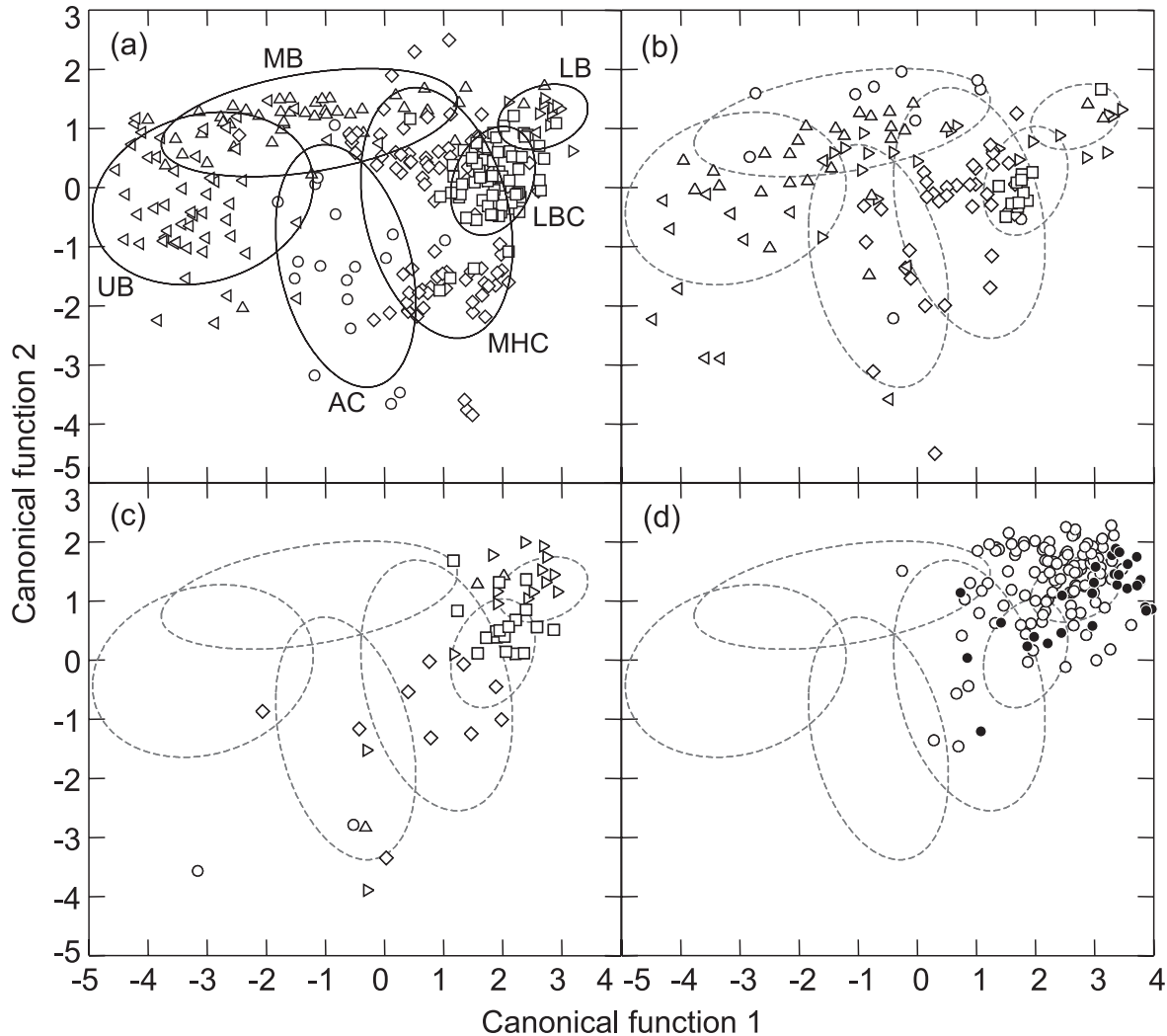
Discussion

Isotopic gradients in post-settlement weakfish

The tidal-marsh and open-water gradients depicted by canonical discriminant analysis underscore the importance of locally produced organic matter in the trophic spectrum of Delaware Bay (Wainright et al. 2000; Weinstein et al. 2000; Litvin and Weinstein 2003) and other estuarine systems (Peterson et al. 1986; Deegan and Garritt 1997; Deegan et al. 2000). Moreover, the stable-isotope composition and resulting canonical scores of juvenile weakfish captured in tidal salt marshes of Delaware Bay reflected their underlying de-

pendence on phytoplankton and the local dominant vegetation, either *P. australis* or *S. alterniflora*. Together, the isotopic signatures of the macrophytes defined the end-members of what we described earlier as the marsh gradient. Baseline juveniles collected from polyhaline lower Bay marsh creeks displayed $\delta^{13}\text{C}$ values intermediate to those of SPM and *S. alterniflora* and $\delta^{34}\text{S}$ values intermediate to those of SPM and benthic microalgae, suggesting that *S. alterniflora* was an important source of organic matter. At the opposite end of the gradient, weakfish collected in Alloway Creek were characterized by $\delta^{13}\text{C}$ isotopic signatures intermediate to those of SPM and *P. australis* and $\delta^{34}\text{S}$ signatures intermediate to those of SPM and benthic microalgae, both characteristic of reliance on *P. australis* organic matter, and perhaps a proximate source of anthropogenic nitrogen as indicated by enriched $\delta^{15}\text{N}$ (McClelland and Valiela 1998; Weinstein et al. 2000; Litvin and Weinstein 2003). Although Mad Horse Creek is *Spartina* spp.-dominated (>90% coverage of the marsh plain), it is located in the *Spartina* spp. – *P. australis* transition zone in Delaware Bay. The canonical scores of young weakfish captured here, which were intermediate to those of individuals from tidal marshes in the upper and lower Bay, appeared to reflect the availability of both dominant macrophytes, which suggests that tidal action and (or) the local movements of individuals were responsible for the observed pattern. The overlap of canonical scores with those of individuals collected in Alloway and the lower Bay creeks was likely a result of the availability of organic matter from both marsh macrophytes in Mad Horse Creek. Interestingly, few post-settlement weakfish captured in tidal marshes exhibited stable-isotope signatures that were consistent with those of organic matter derived from benthic microalgae (intermediate $\delta^{13}\text{C}$ values coupled with depleted $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$). This does not preclude a role for benthic microalgae as an organic matter source; rather it suggests that benthic micro-

Fig. 3. Plot of standardized canonical function scores based on the carbon, nitrogen, and sulfur isotopic signatures of weakfish collected in Alloway Creek (AC; ○), Mad Horse Creek (MHC; ◇), lower Bay creeks (LBC; □), upper Bay open waters (UB; ◁), mid-Bay open waters (MB; △), and lower Bay open waters (LB; ▷). (a) Fish constituting the post-settlement baseline (<60 mm SL, except those in Alloway Creek, 60–100 mm SL; see the text). (b) Fish 60–100 mm SL (except those in Alloway Creek, <60 mm SL; see the text). (c) Fish >100 mm SL. (d) Fish 60–100 mm SL (●) and >100 mm SL (○) collected at the mouth of Delaware Bay prior to emigration. Ellipses represent mean values ± 1 standard deviation of the standardized canonical function scores for fish constituting the post-settlement baseline and are repeated in b, c, and d for reference.



algae may be of less importance than phytoplankton and marsh macrophytes in contributing to the nutrition of this semipelagic species (Litvin and Weinstein 2003).

A similar pattern of spatial heterogeneity was observed in the open-water gradient, where, as in the marsh gradient, the isotopic signatures of the macrophytes defined end-members of the trophic spectrum. However, enriched S and depleted N isotopes reflected the increased reliance on phytoplankton that is represented in the separation of the two gradients on the second canonical axis.

The enriched carbon-isotope values (and the highest mean value for the first canonical function) of small weakfish collected in lower Delaware Bay compared with those found in other collection locations implies that organic matter derived from *S. alterniflora* was a substantial contributor to weakfish nutrition. While the decrease in scores for the first canonical function with distance from the Bay mouth could be solely

attributed to the anticipated change in phytoplankton $\delta^{13}\text{C}$ values with salinity (Fry 2002), the depleted $\delta^{34}\text{S}$ values relative to SPM values observed in most small weakfish captured in the open Bay (combined with the significant change in $\delta^{34}\text{S}$ values with collection location; Litvin and Weinstein 2003) suggest that organic matter from marsh macrophytes was being exported to the open Bay. Additionally, the decrease in the first canonical function also suggests a change in contribution from *S. alterniflora* to *P. australis* organic matter along the estuarine salinity gradient, mirroring the changes seen in the marsh gradient.

Stable-isotope signatures as a measure of juvenile weakfish movements

The ability of canonical discriminant analysis to correctly identify the collection location of post-settlement weakfish validated the pattern of unique isotopic signatures associated

Table 2. Canonical discriminant function classification results for juvenile weakfish constituting the post-settlement baseline (<60 mm SL, except those in Alloway Creek, 60–100 mm SL), based on their carbon, nitrogen, and sulfur isotope signatures.

Collection location	Number of fish classified as:						Correctly classified (%)
	AC	MHC	LBC	UB	MB	LB	
Alloway Creek (AC) (<i>n</i> = 18)							
Reclassification	17	0	0	0	1	0	94
Jackknife	16	1	0	0	1	0	89
Mad Horse Creek (MHC) (<i>n</i> = 86)							
Reclassification	10	51	12	0	6	7	59
Jackknife	10	50	13	0	6	7	58
Lower Bay creeks (LBC) (<i>n</i> = 69)							
Reclassification	0	2	62	0	1	4	90
Jackknife	0	2	60	0	1	6	87
Upper Bay (UB) (<i>n</i> = 53)							
Reclassification	4	1	0	42	5	1	79
Jackknife	4	1	0	41	6	1	77
Mid-Bay (MB) (<i>n</i> = 45)							
Reclassification	1	0	0	1	40	3	89
Jackknife	1	1	0	4	36	3	80
Lower Bay (LB) (<i>n</i> = 11)							
Reclassification	0	0	0	0	0	11	100
Jackknife	0	0	1	0	0	10	91

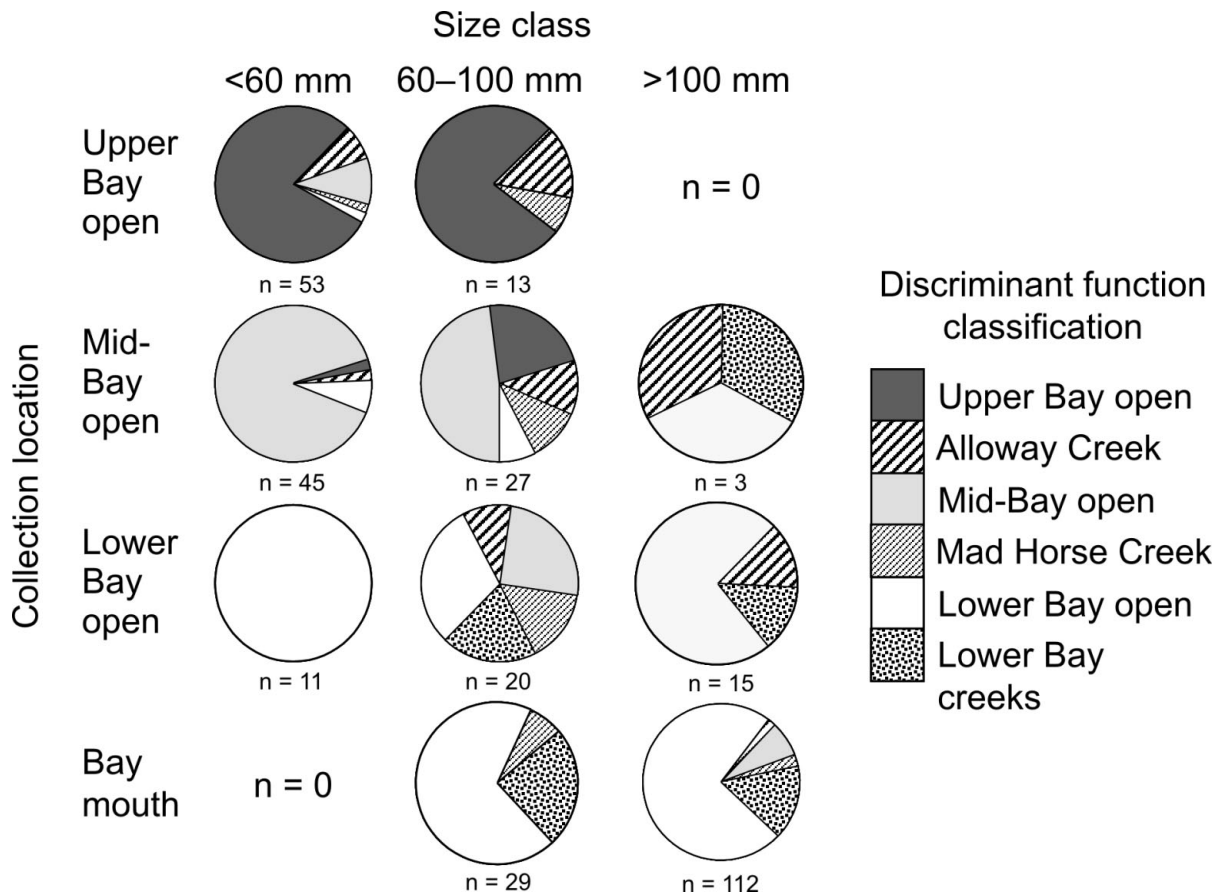
Note: The number of individuals correctly classified to their collection location via the canonical discriminant function is indicated in boldface font (the percentage correctly classified is also shown); *n* is the number of individuals.

Table 3. Canonical discriminant function classification results for medium-sized (60–100 mm SL) and large (>100 mm SL) juvenile weakfish, and small (<60 mm SL) individuals collected in Alloway Creek, based on their carbon, nitrogen, and sulfur isotope signatures.

Collection location	<i>n</i>	Number of fish classified as:					
		AC	MHC	LBC	UB	MB	LB
Alloway Creek (AC)							
<60 mm SL	10	1	0	1	0	8	0
>100 mm SL	2	1	0	0	1	0	0
Mad Horse Creek (MHC)							
60–100 mm SL	38	11	16	10	0	0	1
>100 mm SL	10	4	4	2	0	0	0
Lower Bay creeks (LBC)							
60–100 mm SL	12	0	1	10	0	0	1
>100 mm SL	18	0	1	11	0	1	5
Upper Bay (UB)							
60–100 mm SL	13	2	1	0	10	0	0
>100 mm SL	0	—	—	—	—	—	—
Mid-Bay (MB)							
60–100 mm SL	27	3	3	0	6	13	2
>100 mm SL	3	1	0	1	0	0	1
Lower Bay (LB)							
60–100 mm SL	20	2	3	4	0	5	6
>100 mm SL	15	2	0	2	0	0	11
Bay mouth (BM)							
60–100 mm SL	29	0	2	7	0	0	20
>100 mm SL	112	2	3	17	0	8	82

Note: *n* is the number of individuals.

Fig. 4. Canonical discriminant function classification results based on the carbon, nitrogen, and sulfur isotopic signatures of small (<60 mm SL), medium-sized (60–100 mm SL), and large (>100 mm SL) weakfish collected in the open waters and at the mouth of Delaware Bay prior to emigration.



with short-term residency in open-water and tidal-marsh habitats and allowed us to infer the movements of larger, more mobile individuals within Delaware Bay (Hobson 1999). The classification results for medium-sized fish collected in the open waters of the upper Bay implied the same pattern of site fidelity found in small individuals. Conversely, for mid- and lower Bay open waters, greater than half of medium-sized individuals collected appeared to be recent arrivals from up-Bay habitats or adjacent tidal marshes, while in tidal marshes there was little evidence of emigration. With the exception of Mad Horse Creek, we collected few large juveniles in the mid- and upper Bay, indicating that they had previously emigrated from these habitats. Large juveniles collected in the lower Bay open waters, like medium-sized individuals, appeared to be a mixture of lower Bay residents and recent arrivals from other habitats, although a higher proportion of large individuals, 73% versus 30%, exhibited the stable-isotope signatures characteristic of extended residency in the lower Bay. These results indicate that a proportion of the large juveniles classified as “lower Bay open” arrived as medium-sized individuals and acquired the stable-isotope signature of the lower Bay as they grew. Our premise is further supported by the high growth rates exhibited by juvenile weakfish in Delaware Bay. An individual arriving in the lower Bay at 75 mm SL will increase its biomass by a factor of nearly 5 at 125 mm SL (S.Y. Litvin,

unpublished data). At this new biomass, fish will have an isotopic signature that reflects in-situ trophic transfers and will dilute any previously acquired isotopic signature even in the absence of metabolic turnover (Hesslein et al. 1993; Herzka and Holt 2000; MacAvoy et al. 2001). A similar proportion of weakfish collected at the mouth of Delaware Bay in late October and early November accumulated the bulk of their biomass in lower Bay open waters, leading us to conclude that movements from up-bay habitats and the adjacent tidal marshes followed by continued growth in the lower Bay continued through the time of emigration. Of note were the few (6 of 176) medium-sized or large juveniles collected in the lower Bay open waters or at the mouth that had directly emigrated from upper Bay habitats.

Our observations are consistent with the expected behavior of young marine transients (Chao and Musick 1977; Weinstein and Brooks 1983; Szedlmayer et al. 1990); small juveniles recruit throughout the estuary early in the season and spend sufficient time in a given open-water region or marsh creek to acquire a distinct isotopic signature, i.e., they exhibit substantial site fidelity (Deegan and Garritt 1997). As they grow, they begin to move out of the marsh creeks and (or) upper Delaware Bay, sometimes moving directly to lower Bay habitats, but more often exhibiting “saltatory” behavior, i.e., moving partially down-Bay while spending sufficient time in the intermediate regions to acquire the

dominant isotopic signature of that locality. Juvenile weakfish ultimately arrive in lower Delaware Bay, though apparently not in unison. Early arrivals are resident for a period that allows accumulation of sufficient biomass in the lower Bay to be reclassified as having originated there, while others retain the signature of other locations until the time they emigrate from Delaware Bay.

Implications of juvenile weakfish movements on the export of tidal-marsh production

Although early investigators proposed important modifications to the role of tidal-marsh organic matter in estuarine secondary production, changes to Teal's (1962) original model have largely been refinements rather than outright rejection or dismissal. Organic matter from tidal salt marshes is being made available to juvenile marine transients in open waters of the estuary, and probably in the coastal zone (Turner et al. 1979), through the movement of organisms (Haines 1979; Deegan 1993), phytoplankton and benthic microalgal production (Haines 1979; Sullivan and Montcrieff 1990), trophic relays (Kneib 1997), pulsed events (Odum 2000), export of dissolved organic matter (Eldridge and Cifuentes 2000), or microbial processing (Newell and Porter 2000), or by some as yet undefined route(s).

The isotopic signatures of juvenile weakfish collected just prior to emigration are telling in two ways. First, as suggested by Haines (1979), the presence of juvenile weakfish that are classified as recent inhabitants of salt marshes indicates not only that marsh macrophyte production is exported for utilization in open-estuary food webs (Eldridge and Cifuentes 2000) but that additional export occurs via the juvenile weakfish themselves. Secondly, the majority of juvenile weakfish collected just prior to emigration are reclassified as being from either marsh creeks or the open lower Bay (133 of 141), all locations where marsh macrophytes account for a significant proportion of the organic matter entering the food web. Therefore, a large proportion of the organic matter exported from Delaware Bay via juvenile weakfish in 1998 and 1999 had its origins in salt-marsh macrophyte production.

While it is clear that the most appropriate interpretation of the stable-isotope data requires an understanding of the seasonal movements of larger and more mobile weakfish, ultimately Delaware Bay salt marshes function as important sources of organic matter driving a significant portion of the production of juvenile weakfish both in the marsh and in the Bay as a whole (Litvin and Weinstein 2003). Furthermore, juvenile weakfish emigrating from the estuary in fall leave with a significant proportion of their biomass derived from salt-marsh organic-matter sources. Though our results do not quantify the various pathways that make nutrients available to consumers, including young weakfish, they support the notion that marine transients act as conduits of organic-matter export in estuaries.

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