The following supplement accompanies the article

Differences in juvenile trophic niche for two coastal fish species that use marine and estuarine nursery habitats

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Supplement. Supporting information and analysis: diet and stable isotope data

S1. METHODS

S1.1. Ancillary *Pomatomus saltatrix* diet analysis

Due to the size difference of age-0 bluefish *Pomatomus saltatrix* collected from the inner continental shelf (shelf) and mainstem Chesapeake Bay (estuary) for the present study, we used an unpublished data set of bluefish stomach contents to test the hypothesis that diet varies significantly between length classes of bluefish pertinent to this study. These data were collected from 570 age-0 bluefish from Maryland's coastal habitats (coastal lagoons, surf zone [0–2 m], shelf [5–20 m], and mainstem Chesapeake Bay [upper: mesohaline, lower: polyhaline]) from June to July (early summer) and August to September (late summer) of 1999–2001. Only bluefish of 75–274 mm total length (TL) were included in the analysis (n = 430 with prey present); these were separated into 50 mm length classes and biomass of prey items aggregated into dominant prey categories. Stomach contents biomass data (g) were $\log_e(x+1)$-transformed, Bray-Curtis similarities were calculated among individuals, and analysis of similarity (ANOSIM; Clarke 1993) was used to compare diet across length classes. Season, year, habitat, and length class were all identified as significant main effects (1-way ANOSIM; Table S1); therefore, a single dummy variable was created from every combination of season, year, and habitat (e.g. early summer 1999 shelf). This dummy variable was used as a block effect to account for external variation in pairwise contrasts of diet between length classes (2-way ANOSIM).

S1.2. Establishing an isotopic baseline

A comparison of planted to wild oysters *Crassostrea virginica* indicated planted oysters had not yet equilibrated to local isotopic conditions after the 70 d period (2-sample t-test, $t = 3.51$, df = 8, p = 0.008; Fig. S1); therefore, we used a 2 end-member mixing model to estimate the equilibration rate of oysters planted at estuary Location 1:

$$R_t = R_f + (R_i - R_f) * e^{-(k+m)t} \quad \text{(Eq. S1)}$$

where R_t is the sample $d^{15}N$ value measured at time t, R_f is the local equilibrium $d^{15}N$ value, R_i is the initial $d^{15}N$ value, $k + m$ represents the isotopic equilibration rate based on somatic growth (k) and metabolic turnover (m), and t is the length of time the oysters were allowed to equilibrate (Hesslein et al. 1993). All parameters were known except for k and m (R_f = wild-caught oyster $d^{15}N$ value). For simplicity, $k + m$ were modeled as a single term km_{est} representing the integrated isotopic equilibration rate. A maximum-likelihood estimate of km_{est} at estuary Location 1 was solved numerically based on our empirical R_i, R_t, and R_f measurements (Fig. S1). This km_{est} was then used at estuary Location 2 in conjunction with empirical R_i and R_t values from this location to estimate R_f. The empirical R_f from estuary Locations 1 and 2 and the modeled R_f from estuary Location 2 were averaged and the mean used as the $d^{15}N$ isotopic baseline ($d^{15}N_{base}$) for the estuary.

Two important assumptions of using stable isotopes to compare trophic niche differences between habitats are: (1) the consumer(s) of interest are actually resident to each of the habitats, and (2) the consumer's tissues have equilibrated to the geochemistry of the local food web (Post 2002, Herzka 2005). We tested the first assumption (local residency) through a pilot study that involved analyzing the $d^{18}O$ and $d^{13}C$ composition of age-0 bluefish otoliths collected from shelf and Chesapeake Bay habitats during August 2005. Otoliths are metabolically inactive, thus the isotopic composition of material deposited on the otolith surface during accretion is conserved through time. The conservative property of otoliths and reflection of ambient water temperature ($d^{18}O$) and dissolved inorganic carbon ($d^{13}C$) conditions in otoliths has been successfully used to discriminate juvenile habitat use across salinity gradients for estuarine fish (e.g. Kerr et al. 2007). Based on known differences in temperature and salinity between shelf and estuary habitats (see Table 1 in the main article), we used $d^{18}O$ and $d^{13}C$ composition of otoliths to test for evidence of spatial mixing among age-0 bluefish (data were not available for age-0 bay anchovy). Whole otoliths were removed from age-0 bluefish (n = 10 per habitat), cleaned of all residual soft tissue, and sent to the University of Arizona Environmental Isotope Laboratory for sample preparation and isotope analysis. Isotope data were tested for significant differences (ANOVA) and visually examined for size-dependent trends.

To address the second assumption (tissue equilibration), we modeled isotopic equilibration time using biomass-specific growth rates and simple exponential growth models (Herzka 2005), making the implicit assumption that dilution was principally responsible for equilibration dynamics (Fry & Arnold 1982). Absolute average daily growth rates $\bar{g}$ of 2.01 mm d^{-1} (bluefish; Callihan 2005) and 0.40 mm d^{-1} (bay anchovy *Anchoa mitchilli*; Able & Fahay 1998) were used to calculate biomass-specific growth rates G (d^{-1}) for bluefish feeding on a novel forage base at 30, 50, 70, and 80 mm total length (endpoint = 150 mm) and bay anchovy at 15, 20, 25, and 30 mm total length (endpoint = 50 mm) using empirically derived length-to-weight conversions:

$$Wt_{\text{bluefish}} = 3 \times 10^{-6} * \text{TL}^{3.2062} \quad \text{(Eq. S2)}$$

$$Wt_{\text{bay anchovy}} = 2 \times 10^{-6} * \text{TL}^{3.2647} \quad \text{(Eq. S3)}$$

where Wt is biomass. We assumed that juveniles equilibrated to the isotopic characteristics of a novel forage base after a 4–6-fold increase in initial biomass (Herzka 2005). The residency period required to achieve isotopic equilibrium t_{equil} in days was estimated for each species and initial size combination as

$$t_{equil} = \frac{Wt_{initial} / Wt_t}{G} \quad \text{(Eq. S4)}$$

where $Wt_{initial}$ is the biomass at $t = 0$ (i.e. first day of foraging within novel food web) and Wt_t is the biomass at day t.

S2. RESULTS

S2.1. Bluefish diet analysis

Small-bodied forage fish (e.g. *Anchoa* spp., *Menidia* spp.) contributed 57–92% of the identifiable prey biomass across bluefish length classes (Fig. S2). The contribution of juvenile moronid species to diet declined from 2% in fish 125–174 mm to 0% in fish 225–274 mm, whereas clupeids (e.g. Atlantic menhaden *Brevoortia tyrannus*) increased from 4% biomass in fish 125–174 mm to 27% in fish 225–274 mm. The 3 dominant invertebrate categories contributed relatively little (<1–6%) to diet across length classes.

Due to unbalanced sample size among habitats, years, and seasons, 1-way tests of length class as a main effect yielded biased tests of length effects (Table S1). For example, bluefish 75–124 mm were captured during early and late summer in the shelf, yet bluefish 125–174 mm were only captured during late summer. A 1-way test of length class as a main effect was therefore confounded with temporal changes in diet between early and late summer. Spatiotemporal changes in diet explained much of the variation present in the 1-way tests of main effects; but importantly, bluefish 75–274 mm in length did not exhibit significant differences among length classes in diet composition after accounting for geographic, seasonal, and annual variability (2-way ANOSIM; Table S1). Specific to the trophic analysis of the present study, there was no difference (2-way ANOSIM, *R*-statistic = 0.20, p = 0.18) between length classes of bluefish 125–174 mm and 225–274 mm in the 1998–2001 data set from mainstem Chesapeake Bay habitats.

S2.2. Isotopic baseline and equilibration estimates

The empirically measured R_f from estuary Location 2 was 10.37 ± 0.71‰ (SD) and the maximum-likelihood estimate of km_{est} was 0.46 ± 0.04 (‰ mo^{-1}; asymptotic SD) (Fig. S1). Based on km_{est} from Location 1, the modeled R_f value for Location 2 was 12.42 ± 0.07‰ (ASD). Estimated equilibrium values of oysters at Location 2 were –20.41 ± 0.23‰ (SD) for $d^{13}C$ and 12.68 ± 0.12‰ for $d^{15}N$. The final isotopic signature of the pelagic estuarine baseline (grand mean across estuarine Locations 1–3) was –20.81 ± 0.54‰ for $d^{13}C$ and 12.41 ± 1.59‰ for $d^{15}N$.

Carbon and oxygen isotope composition of age-0 bluefish otoliths from shelf ($d^{13}C$ = –4.71 ± 0.41‰ [SD], $d^{18}O$ = –2.76 ± 0.20‰) and estuary ($d^{13}C$ = –5.84 ± 0.31‰, $d^{18}O$ = –3.75 ± 0.43‰) habitats were significantly different (1-way ANOVAs, $F_{1,18}$ ³ 43.12, $p < 0.001$). Otoliths from shelf bluefish were more enriched (less negative) in both isotopes relative to otoliths from estuarine bluefish (Fig. S3). Plots of otolith $d^{18}O$ and $d^{13}C$ versus total length (mm) showed no evidence of convergence in otolith composition with increased body size between habitats (Fig. S4). The lack of convergence at larger sizes (and presumably increased movement capabilities) supports the assumption that age-0 bluefish remain resident within Maryland's shelf and Chesapeake Bay habitats during the interval between early summer recruitment and the late summer.

Biomass-specific growth rates for bluefish ranged from 0.058–0.087 d^{-1}, whereas they ranged from 0.044–0.033 d^{-1} for bay anchovy. Equilibration models for bluefish and bay anchovy indicate that a 4–6-fold increase in biomass requires t_{equil} = 16–31 d for bluefish after switching to a novel forage base at 30–80 mm, whereas t_{equil} = 31–54 d for 15–30 mm bay anchovy (Fig. S5). The models were relatively insensitive to changes in absolute growth rate (D $\bar{g}$). This was examined by recalculating t_{equil} for $\bar{g}$ = 1.71 and 2.31 mm d^{-1} for bluefish (i.e. D $\bar{g}$ = ±0.30 mm d^{-1}) and for $\bar{g}$ = 0.35 and 0.45 mm d^{-1} for bay anchovy (i.e. D $\bar{g}$ = ±0.05 mm d^{-1}). Bluefish t_{equil} increased by 2.9–5.7 d (–D $\bar{g}$) and decreased by 2.0–3.9 d (+D $\bar{g}$). Bay anchovy showed changes of similar magnitude: t_{equil} increased by 4.0–7.0 d (–D $\bar{g}$) and decreased by 3.8–6.6 d (+D $\bar{g}$).

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Table S1. Analysis of similarity of age-0 bluefish diet data set from mainstem Chesapeake Bay[a] (estuary), Maryland coastal bays and inner continental shelf[b] (shelf), and all habitats combined. Main effects: Season (June, July: early summer; August, September: late summer), Year (1999–2001), Habitat[a,b], Length-class (Lclass: 50 mm intervals, 75–274 mm total length). Results from 1-way ANOSIM (Main effects), 2-way ANOSIM (Lclass blocked by dummy variable: Season, Year, Habitat; e.g. early summer 1999 shelf) and pair-wise Lclass contrasts from 2-way ANOSIM. Significant effects are in **bold** ($a \leq 0.05$). Sufficient data were not available (na) for 2 length-class contrasts from the estuary

	Estuary (n = 191)		Shelf (n = 239)		All (N = 430)	
Factors/Groups	*R*	p	*R*	p	*R*	p
Main effects (1-way ANOSIM)						
Season	**0.10**	**0.006**	**0.25**	**0.001**	**0.21**	**0.001**
Year	**0.10**	**0.001**	**0.13**	**0.002**	**0.08**	**0.001**
Habitat	**0.09**	**0.004**	**0.26**	**0.001**	**0.22**	**0.001**
Lclass	**0.08**	**0.001**	0.02	0.13	**0.03**	**0.005**
Main effects (2-way ANOSIM)						
Lclass	0.07	0.07	0.02	0.25	0.04	0.09
Block effect	**0.08**	**0.0001**	**0.19**	**0.002**	**0.21**	**0.001**
Lclass contrasts (from 2-way ANOSIM)						
75–124 vs. 125–174	0.07	0.07	–0.01	0.55	0.03	0.23
75–124 vs. 175–224	0.06	0.35	0.001	0.39	0.01	0.32
75–124 vs. 225–274	na	na	0.20	0.12	0.20	0.12
125–174 vs. 175–224	0.001	0.43	0.03	0.23	0.03	0.23
125–174 vs. 225–274	0.20	0.18	0.13	0.22	0.13	0.21
175–224 vs. 225–274	na	na	0.03	0.44	0.03	0.43

[a]Lower (polyhaline) and upper (oligo-mesohaline) mainstem Chesapeake Bay
[b]Chincoteague Bay, Assateague Bay, Assateague Island surf zone, Maryland inner continental shelf (5–20 m)

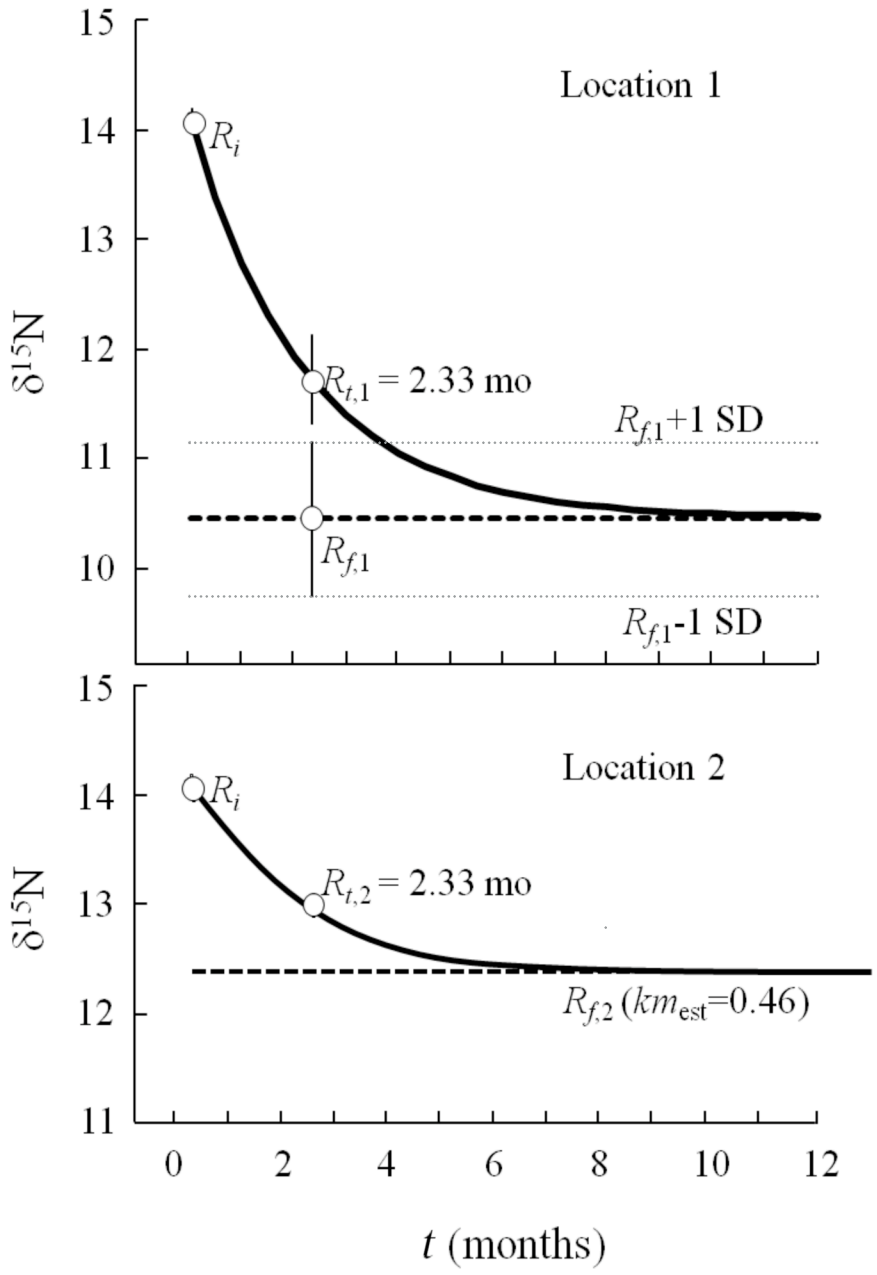

Fig. S1. $d^{15}N$ mixing models (solid lines) for age-1 eastern oysters *Crassostrea virginica* from 2 locations in lower Chesapeake Bay (Location 1: upper panel, Location 2: lower panel; see Fig. 1 in the main article for geographic reference) for a 1 yr equilibration period. Empirical $d^{15}N$ (±SD) values at time = 0 d (R_i, caged oysters) and 70 d (R_t, caged and wild oysters) given as open circles. Local $d^{15}N$ equilibria (R_f) = dashed lines and represent observed (Location 1) and modeled (Location 2) estimates; grey dotted lines (upper panel only) = R_f ± 1 SD. km_{est}: integrated isotopic equilibration rate

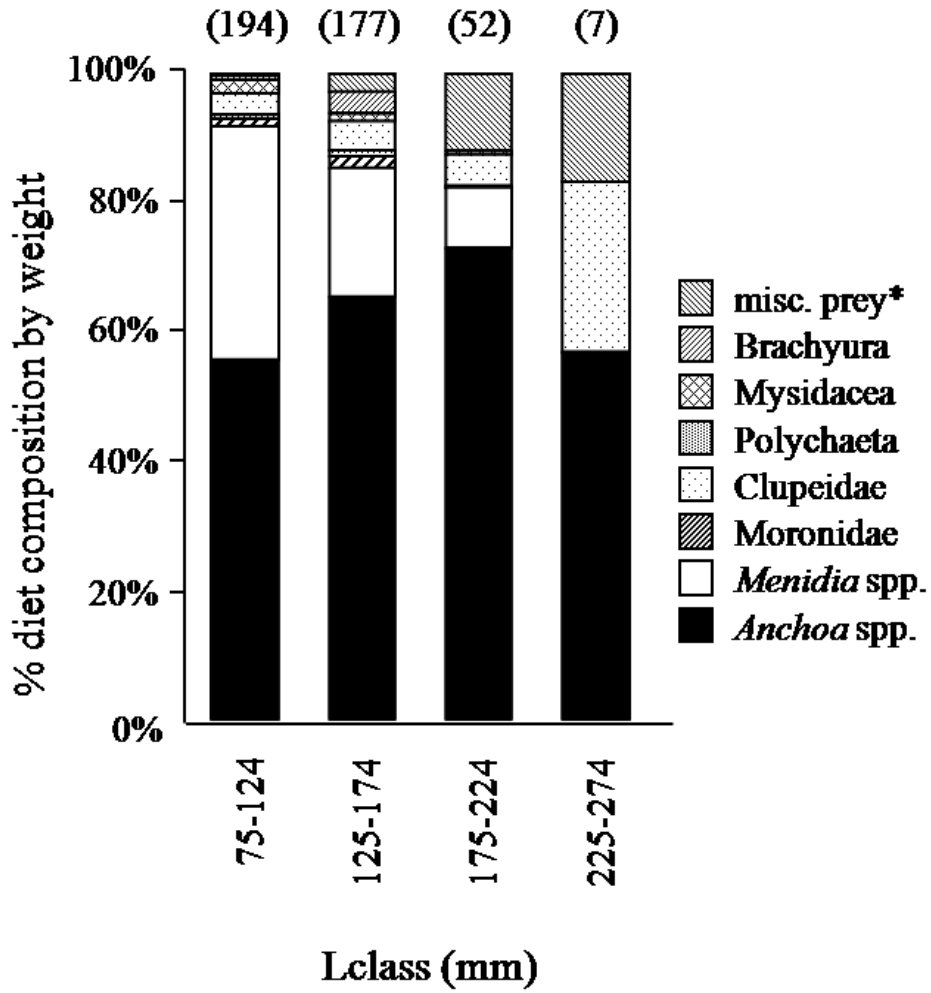

Fig. S2. Percent diet composition by weight for 4 length-classes (Lclass: 50 mm intervals) of age-0 bluefish *Pomatomus saltatrix* (N = 430, n per Lclass given above bars) from mainstem Chesapeake Bay, Maryland's coastal bays and inner continental shelf (see Table 2 in the main article for habitat details) collected from June to September 1999–2001. *Miscellaneous prey category includes rare vertebrate and invertebrate prey types

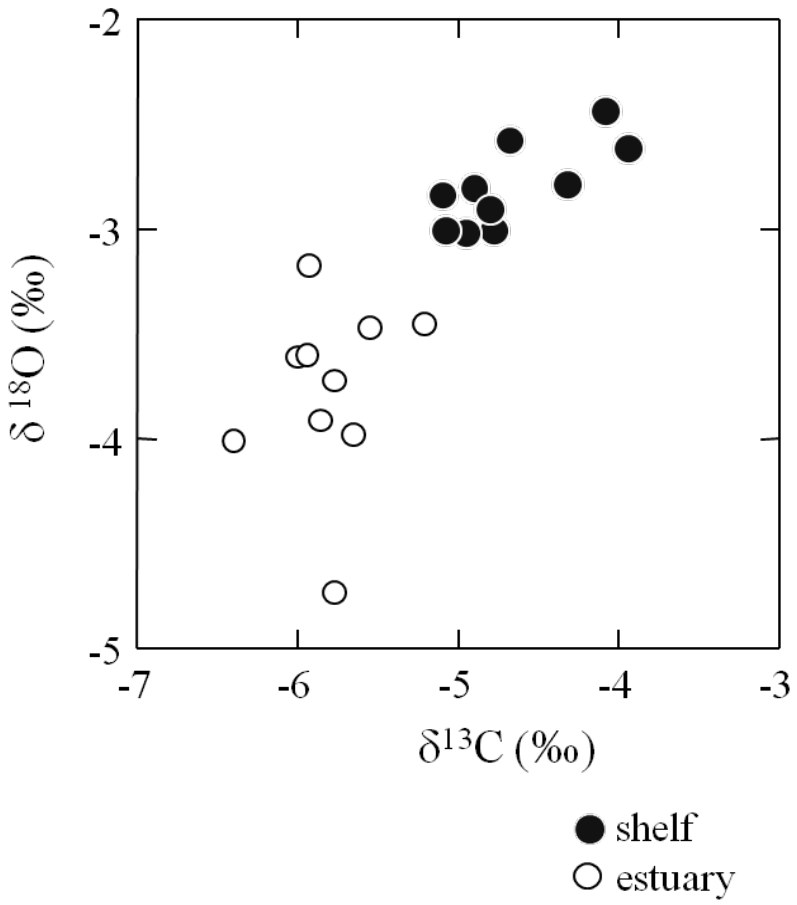

Fig. S3. Whole otolith $d^{18}O$ versus $d^{13}C$ values of age-0 *Pomatomus saltatrix* (n = 10 per habitat) from mainstem Chesapeake Bay (estuary, open circles) and Maryland's inner continental shelf (shelf, filled circles)

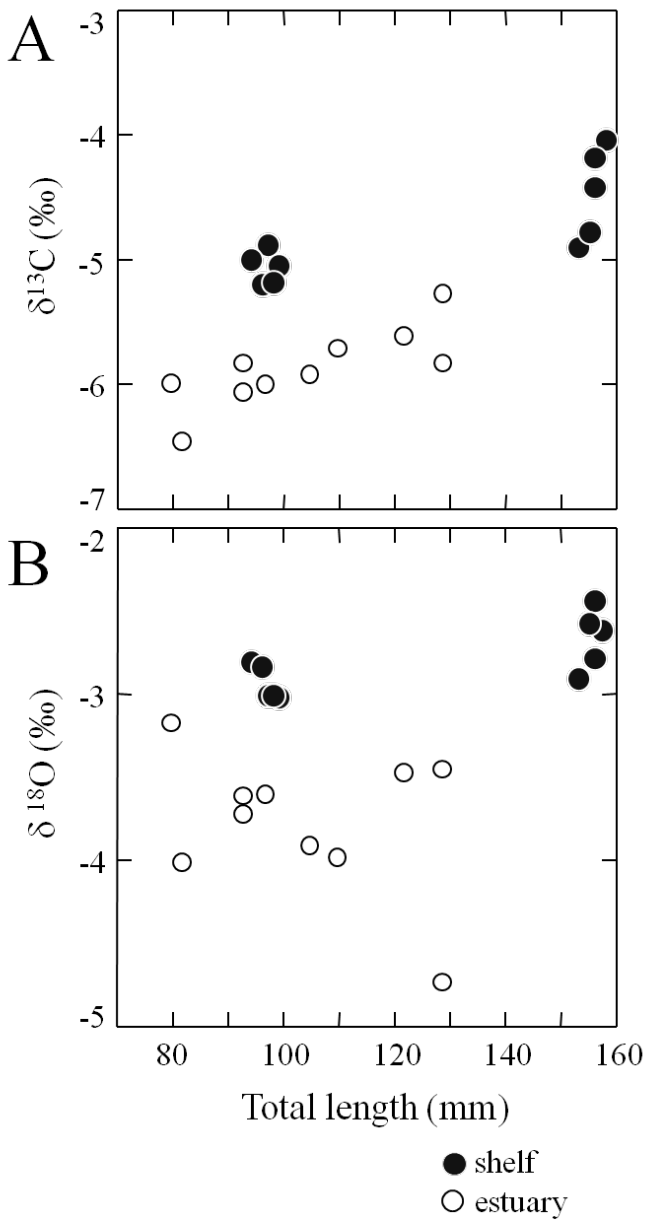

Fig. S4. Whole otolith $d^{13}C$ (A) and $d^{18}O$ (B) values versus body size (total length) of age-0 *Pomatomus saltatrix* (n = 10 per habitat) from mainstem Chesapeake Bay (estuary, open circles) and Maryland's inner continental shelf (shelf, filled circles)

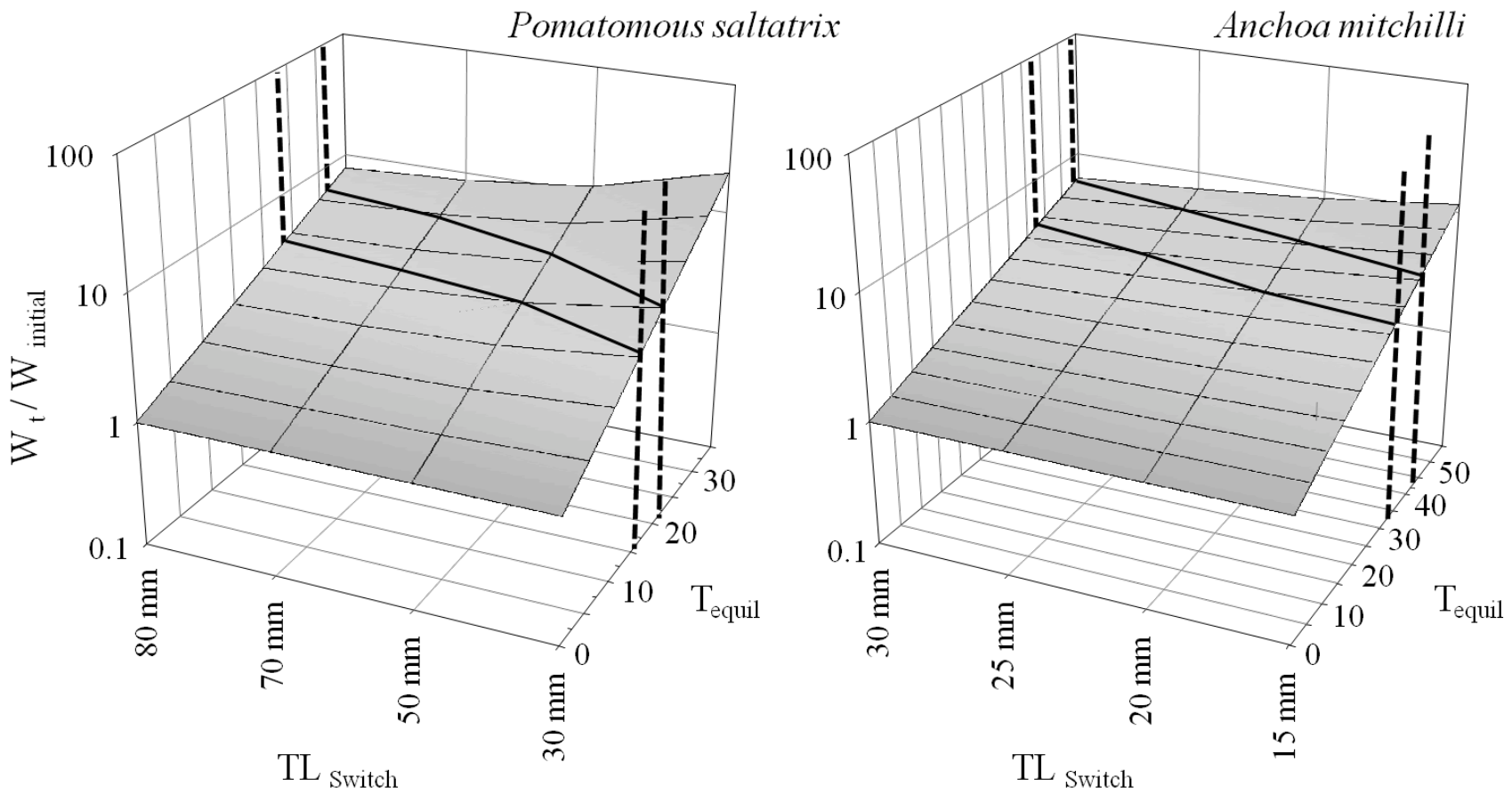

Fig. S5. Isotopic equilibration schedule models for age-0 bluefish *Pomatomus saltatrix* (left panel) and bay anchovy *Anchoa mitchilli* (right panel) based on average individual growth rates in coastal Mid-Atlantic Bight habitats. Time interval (days) corresponding to a 4–6-fold increase in $Wt_t/Wt_{initial}$ (ratio of body weight at time t to initial body weight) for 4 hypothetical sizes at the switch to a novel forage base (TL_{switch}) is shown by surface trajectories (solid lines) and ancillary axis intercepts (dashed lines)