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Spatial, Temporal, and Interspecific Differences in Composition of Stable Isotopes in Fishes in Maryland Coastal Bays

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Abstract: Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes were used to evaluate spatial, temporal, and interspecific differences in trophic relationships of four fish species (*Paralichthys dentatus*, *Anchoa mitchilli*, *Leiostomus xanthurus*, and *Bairdiella chrysoura*) in Maryland's coastal bays. The $\delta^{13}C$ values for all species were more enriched in 2017 than in 2018, a year of higher-than-average rainfall that likely caused higher amounts of terrestrial carbon to enter the estuary. There were significant differences among species in the $\delta^{13}C$ values, with *L. xanthurus* being the least depleted (−17.2‰ in 2017; −18.8‰ in 2018). Spatially, the $\delta^{13}C$ values of the species, particularly *P. dentatus* and *B. chrysoura*, were more depleted in the northern bays, which have a higher nutrient content and receive more freshwater inflow directly from tributaries, than the southern bays. The observed $\delta^{13}C$ values (−19.5 ± 0.2‰ to –17.2 ± 0.3‰), however, indicate that marine phytoplankton was the primary carbon source of the fishes. Overall, *A. mitchilli* was the most enriched in $\delta^{15}N$ (13.0‰), and *L. xanthurus* was the most depleted (10.2‰). $\delta^{15}N$ was more enriched in fish from the more human-impacted northern bays than in fish from the southern bays, though this might also have stemmed from the differences in the diet composition of the species in the northern and southern bays. *A. mitchilli* had the highest trophic level, while *L. xanthurus* and *P. dentatus* had the lowest trophic levels. Niche breadth was widest in *L. xanthurus* compared to the other fish species, suggesting a higher variability in diets among *L. xanthurus* individuals, leading to specialized diets. There was a high niche overlap between *B. chrysoura*, *A. mitchilli*, and *L. xanthurus*, which indicates they fed on similar prey resources.

Keywords: food webs; stable isotopes; fishes; coastal bays

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Citation: Richardson, C.; Chigbu, P.; Ishaque, A. Spatial, Temporal, and Interspecific Differences in Composition of Stable Isotopes in Fishes in Maryland Coastal Bays. *Diversity* **2024**, *16*, 331. https://doi.org/10.3390/d16060331

Academic Editor: Michael Wink

Received: 18 March 2024
Revised: 23 May 2024
Accepted: 27 May 2024
Published: 4 June 2024

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1. Introduction

Stable isotopes are useful for analyzing food web structure because they provide time and space integration into trophic relationships among organisms [1]. Stable isotope analysis can be used to determine both source and trophic level information. In particular, carbon and sulfur isotopes are used to determine source information, and nitrogen isotopes are used to determine an organism's trophic position [2,3]. In a predator, the heavier isotope, ^{15}N, is enriched by 3–5‰ compared to its diet [2–4]. The ^{15}N enrichment in animals compared to their diet mainly occurs from fractionation during deamination and transamination that transfers nitrogen from amino acids to urea for excretion of the isotopically lighter nitrogen in the urine [2,4–6]. Due to nitrogen successively increasing with trophic level, $\delta^{15}N$ is useful for determining an organism's trophic position. Stable isotopes of C and N have been widely used to examine trophic relationships in fishes and show that the diet composition of a species may vary spatially between and within estuaries, ontogenetically, and between years [7,8]. However, little information exists on the trophic relationships of fishes in Maryland coastal bays (MCBs), and the few studies [9,10] were based on traditional gut content analysis.

MCBs provide food resources and spawning areas for many fish species, as well as nursery habitats for young-of-year fish [11–13]. The four fish species (*Paralichthys dentatus*, *Anchoa mitchilli*, *Leiostomus xanthurus*, and *Bairdiella chrysoura*) used in this study are among the most abundant species in MCBs. In 2018, *A. mitchilli*, *B. chrysoura*, *L. xanthurus*, and *P. dentatus* ranked 3rd, 4th, 5th, and 18th out of 74 species in overall finfish abundances [14]. These fish species are economically and ecologically important.

A. mitchilli are pelagic and zooplanktivorous. The larvae generally prey on copepod nauplii and rotifers; the juveniles prey on copepods, crab zoea, and other zooplankton; whereas the adult diet includes crustaceans such as mysids and ostracods in addition to small fish and small benthic mollusks [15].

Juvenile *B. chrysoura* tend to be pelagic, feeding mainly on zooplankton [16]. *B. chrysoura* show ontogenetic diet shifts; as they increase in size, they consume larger prey that are more energetically valuable. Their primary diet consists of calanoid copepods and mysid shrimp, with mysids being the predominant prey of choice as *B. chrysoura* increase in size [17].

L. xanthurus larvae act as selective plankton feeders that consume ciliates, invertebrate eggs, and copepod nauplii. *L. xanthurus* juveniles and adults are benthic feeders that consume ostracods, amphipods, mysids, gastropods, nematodes, and polychaetes [18].

Due to *P. dentatus* searching, stalking, active eye motion, and visual fixation on prey, they are primarily considered visual feeders on prey found on the bottom and in the water column [19]. Studies have also shown that *P. dentatus* are opportunistic feeders [20]. At the juvenile stage, *P. dentatus* tend to feed on mysid shrimp and small fish, and when they reach adulthood, they shift their diet to decapod crustaceans and larger fish [20,21].

In this study, we used $\delta^{13}C$ and $\delta^{15}N$ stable isotope values to compare the feeding ecology of *A. mitchilli*, *B. chrysoura*, *L. xanthurus*, and *P. dentatus* between the northern and southern MCBs and between 2018, a year of higher-than-normal precipitation, and 2017, a year of relatively lower precipitation [22]. We hypothesized that the $\delta^{13}C$ of the fish species would be more depleted in the more eutrophic northern part of the MCBs, which receives freshwater from St. Martin River, than in the southern bays and more depleted in 2018 than in 2017.

2. Materials and Methods

2.1. Study Area

MCBs occupy an area of 453.2 km² that includes Berlin, Ocean City, Snow Hill, and Pocomoke (Figure 1). They consist of more than 117,000 acres of land, 71,000 acres of water, and 450.6 km of shoreline [12]. The system has a uniform depth < 10 ft [23] and is divided into the northern coastal bays that include Isle of Wight Bay, Assawoman Bay, and St. Martin River and the southern bays including Sinepuxent Bay, Newport Bay, and Chincoteague Bay. The bays connect to the Atlantic Ocean through the Ocean City inlet in the north and the Chincoteague inlet in the south [23].

Groundwater is the main pathway through which freshwater and nutrients enter the system, while winds and tidal exchange at the Ocean City and Chincoteague inlets regulate the system [23]. Flushing times of the bays are slow, causing the system to accumulate contaminants and nutrients [24].

Land use for the coastal bays includes agricultural land or cropland, forestry, pasture, and urban area [25]. The primary economic operations include intensive poultry production, crop production (corn, soybeans, and barley), fishing, and tourism [25]. Land use in the northern bays is predominantly residential and urban development, leading to more anthropogenic eutrophication from wastewater [13]. The southern bays have more agricultural nitrogen inputs [13]. Freshwater inflow directly into the MCBs is higher in the northern bays because of the location of the St. Martin River. Phytoplankton biomass, macroalgae abundance and the associated amphipods, as well as fish abundance, are higher in the northern than southern bays [13,26,27].

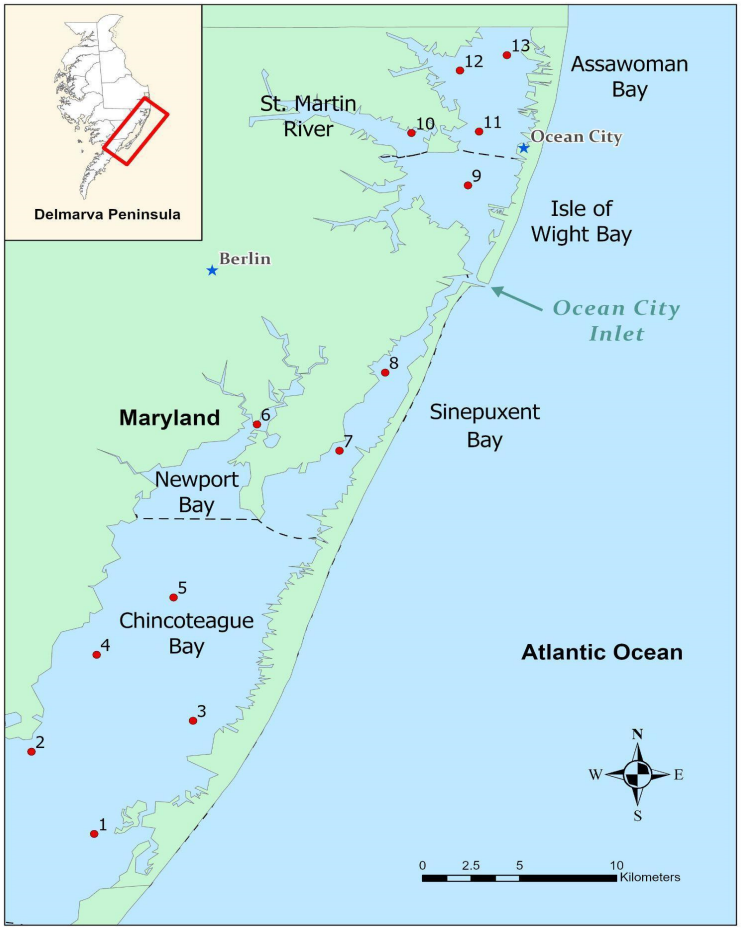


Figure 1. Map of Maryland's coastal bays showing sampling sites 1–13.

2.2. Sample Collection

Samples of *P. dentatus*, *A. mitchilli*, *L. xanthurus*, and *B. chrysoura* were collected by a beam trawl at 13 sites in MCBs (Figure 1) from April to October in 2017 and 2018. The fish specimens were kept on ice until arrival in the laboratory, where they were stored in a −20 °C freezer until analyzed. The length (cm) and weight (g) measurements for each individual fish were obtained before muscle tissues were removed from the side of the fish between the dorsal and ventral fin. All the fish sampled were young-of-year (YoY) fish except *A. mitchilli*, which consisted of YoY and age-2 fish. The fish were classified as YoY by the following: *P. dentatus*, 1.3–16 cm TL [28]; *L. xanthurus*, <17.0 cm TL [29]; and *B. chrysoura* <9.6 cm TL [30]. *A. mitchilli* YoY were <6.5 cm, and age-2 were 6.5–7.9 cm TL [31].

2.3. Stable Isotope Analyses

Freeze-dried and homogenized muscle tissues (1.2–1.3 mg) were placed in tin capsules and sent to the Stable Isotope Facility, University of California Davis, for analysis of carbon and nitrogen isotopes. Samples were analyzed with a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The isotopic composition (^{13}C and ^{15}N) was expressed in parts per thousand deviations from an internationally accepted standard:

$$\delta(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

where R is the ratio of heavy-to-light isotope, R_{sample} is the ratio in the sample, and $R_{standard}$ is the ratio in the standard. A positive delta (δ) signifies that the sample has more of the

heavy isotope than the standard, while a negative δ signifies the sample has less of the heavy isotope compared to the standard [32].

2.4. Data Analyses

The $\delta^{13}C$ and $\delta^{15}N$ values were reported as averages for each year, season, and sampling location. The $\delta^{15}N$ values for each fish species were used to calculate trophic levels (TL) according to the following formula [3]:

$$TL = \lambda + \left(\delta^{15}N_{consumer} - \delta^{15}N_{base}\right)/\Delta n$$

where λ is the trophic position of the organism used to estimate $\delta^{15}N_{base}$ (1 was used as the trophic position of suspended particulate organic matter (SPOM), the base organism in this study), $\delta^{15}N_{consumer}$ is the mean of $\delta^{15}N$ values for each fish species, and $\delta^{15}N_{base}$ is the mean of $\delta^{15}N$ values (~6.0‰) for SPOM collected in summer and fall 2016 from the bays; no significant differences were observed in the mean values from the northern and southern bays [33]. Finally, Δn is the trophic fractionation per trophic level, and 3.4‰ is the typical average fractionation per trophic level [3,34,35]. No lipid correction was applied to the samples, as the mean values of the C:N ratios from the results of the mass spectrometry generally did not exceed 3.5 [36].

The trophic niche sizes of the four fish species based on their $\delta^{13}C$ and $\delta^{15}N$ values were determined using SIBER within the stable isotope package analysis in R (SIAR; v. 4.2). The standard ellipse area (SEA) and corrected standard ellipse area (SEAc) for small sample sizes were used for comparison of niche size. The percentage of ellipse overlap was also calculated by the ratio between the area of overlap between paired ellipses of species and each individual ellipse area for a species [37]. When the overlap percentage between two species was >60%, it was considered significant [38,39].

Multivariate statistical analyses were conducted using Sigma Plot. Kruskal–Wallis analysis was used to determine if there were intraspecific and interspecific differences in stable isotope values, and Dunn's pairwise test was used to determine which groups were different. The Mann–Whitney *U* test was used to determine if there were differences between years and between the northern and southern MCBs in stable isotope values, as well as to compare fish lengths between years and between northern and southern MCBs.

3. Results

A summary of data on the fish used in the diet study is presented in Table 1 for each species, including the mean total length, minimum and maximum length, and number of fish analyzed each season and year. *A. mitchilli* and *L. xanthurus* lengths (cm) were significantly different between 2017 and 2018 (Mann–Whitney, $p < 0.05$). *A. mitchilli* were larger in 2017 (7.3 ± 0.3SE) than in 2018 (6.2 ± 0.2SE), while *L. xanthurus* were larger in 2018 (13.7 ± 0.5SE) than in 2017 (11.4 ± 0.4SE). No significant differences were observed between 2017 and 2018 in the mean lengths of *P. dentatus* and *B. chrysoura* ($p > 0.05$). There was also a significant difference between the northern and southern bays in the mean length of *L. xanthurus* collected in 2017 (Mann–Whitney, $p < 0.001$), with fish collected in the northern MCBs being larger, but no differences were observed in 2018. For the other species, no significant differences were observed between the northern and southern bays in the mean length of the fish ($p > 0.05$).

Table 1. A summary of the number (*n*) and total length (mean, minimum, maximum) of each fish species used in the stable isotope analyses of diet of fishes in Maryland coastal bays (a dash indicates there were either no length data or not enough data for that year and/or location).

	2017 (April–October)	**2018 (June–October)**
	Mean ± SE (*n*); Range (cm)	**Mean ± SE (*n*); Range (cm)**
P. dentatus	11.9 ± 0.5 (141); 5.5–39.5	11.7 ± 0.4 (90); 7.2–33.0
A. mitchilli	7.3 ± 0.3 (16); 6.1–12.1	6.2 ± 0.2 (44); 4.0–9.9
L. xanthurus	11.4 ± 0.4 (41); 7.1–16.5	13.7 ± 0.5 (21); 8.5–18.3
B. chrysoura	8.4 ± 0.2 (53); 5.5–11.2	8.7 ± 0.2 (50); 4.5–15.0
	2017 (North MCBs)	**2017 (South MCBs)**
	Mean ± SE (*n*); Range (cm)	**Mean ± SE (*n*); Range (cm)**
P. dentatus	12.0 ± 0.8 (51); 7.7–39.5	11.9 ± 0.6 (90); 5.5–35.0
A. mitchilli	-	7.3 ± 0.3 (16); 6.1–12.1
L. xanthurus	13.6 ± 0.4 (13); 12.0–16.5	10.4 ± 0.4 (28); 7.1–16.3
B. chrysoura	8.5 ± 0.3 (24); 5.9–11.2	8.3 ± 0.2 (29); 5.5–10.5
	2018 (North MCBs)	**2018 (South MCBs)**
	Mean ± SE (*n*); Range (cm)	**Mean ± SE (*n*); Range (cm)**
P. dentatus	12.0 ± 1.2 (21); 7.6–33.0	11.5 ± 0.4 (69); 7.2–32.4
A. mitchilli	6.5 ± 0.2 (20); 4.0–8.3	5.9 ± 0.3 (24); 4.0–9.9
L. xanthurus	13.4 ± 0.7 (11); 11.5–18.3	13.9 ± 0.7 (10); 8.5–15.7
B. chrysoura	8.6 ± 0.3 (41); 4.5–15.0	9.3 ± 0.8 (9); 6.0–13.9

3.1. Interspecific Differences in Isotopic Compositions of the Four Fish Species in MCBs

Mean values of $\delta^{13}C$ and $\delta^{15}N$ in the years 2017 and 2018 for *P. dentatus*, *A. mitchilli*, *L. xanthurus*, and *B. chrysoura* are shown in Table 2. There were significant differences among fish species in $\delta^{13}C$ values in 2017 (Kruskal–Wallis, $p < 0.001$). Pairwise comparisons revealed that *L. xanthurus* had higher $\delta^{13}C$ values than *A. mitchilli*, *B. chrysoura*, and *P. dentatus* (Dunn's, $p < 0.050$). In 2018, there were also significant differences (Kruskal–Wallis, $p = 0.013$) between fish $\delta^{13}C$ values; pairwise comparison (Dunn's, $p = 0.026$) showed that *P. dentatus* and *B. chrysoura* were different. The $\delta^{15}N$ values were also significantly different among species (Kruskal–Wallis, $p < 0.001$) in both years. In 2017, $\delta^{15}N$ values for *A. mitchilli* were significantly higher than for *L. xanthurus*, *P. dentatus*, and *B. chrysoura*; *B. chrysoura* were significantly higher than for *P. dentatus* and *L. xanthurus*; and *P. dentatus* and *L. xanthurus* were significantly different. In 2018, the pairwise test (Dunn's, $p < 0.001$) revealed that $\delta^{15}N$ values in *L. xanthurus*, *A. mitchilli*, and *B. chrysoura* were not significantly different but were higher than in *P. dentatus*.

Table 2. Mean values ± standard error of $\delta^{13}C$ and $\delta^{15}N$ values in fish species from MCBs collected in 2017 and 2018. $\delta^{13}C$ and $\delta^{15}N$ isotopic values of fish species with similar letters within each year are not significantly different ($p > 0.05$).

	2017	**2017**	**2018**	**2018**
	$\delta^{13}C$	**$\delta^{15}N$**	**$\delta^{13}C$**	**$\delta^{15}N$**
P. dentatus	−18.7 ± 0.1 a	10.9 ± 0.1 c	−19.1 ± 0.1 a	10.7 ± 0.1 b
A. mitchilli	−18.9 ± 0.3 a	13.0 ± 0.2 a	−19.5 ± 0.2 c	12.5 ± 0.2 a
L. xanthurus	−17.2 ± 0.3 b	10.2 ± 0.2 d	−18.8 ± 0.5 c	12.4 ± 0.2 a
B. chrysoura	−18.0 ± 0.2 a	11.5 ± 0.1 b	−19.4 ± 0.2 b	12.0 ± 0.1 a
p-value	<0.001	<0.001	0.013	<0.001

3.2. Interannual Differences in Isotopic Composition of Each Fish Species in MCBs

The $\delta^{13}C$ values were significantly higher in 2017 than in 2018 for all four fish species (Mann–Whitney, $p < 0.05$) (Table 3). *A. mitchilli*, *L. xanthurus*, and *B. chrysoura* also showed annual intraspecific differences in $\delta^{15}N$ values between 2017 and 2018 (Mann–Whitney, $p < 0.05$), but no difference was observed for *P. dentatus* ($p > 0.05$).

Table 3. Mean values ± standard error of $\delta^{13}C$ and $\delta^{15}N$ in fish species from MCBs collected in 2017 and 2018. $\delta^{13}C$ and $\delta^{15}N$ isotopic values of fish species with similar letters between years are not significantly different ($p > 0.05$) as in Table 2.

	2017	2018		2017	2018	
	$\delta^{13}C$	$\delta^{13}C$	*p*-Value	$\delta^{15}N$	$\delta^{15}N$	*p*-Value
P. dentatus	−18.7 ± 0.1 a	−19.1 ± 0.1 b	<0.05	10.9 ± 0.1 a	10.7 ± 0.1 a	>0.05
A. mitchilli	−18.9 ± 0.3 a	−19.5 ± 0.2 b	<0.05	13.0 ± 0.2 a	12.5 ± 0.2 b	<0.05
L. xanthurus	−17.2 ± 0.3 a	−18.8 ± 0.5 b	<0.05	10.2 ± 0.2 a	12.4 ± 0.2 b	<0.05
B. chrysoura	−18.0 ± 0.2 a	−19.5 ± 0.2 b	<0.05	11.5 ± 0.1 a	12.0 ± 0.1 b	<0.05

Isotope biplots (Figure 2a,b) indicated that in 2017, *A. mitchilli* was the most enriched in $\delta^{15}N$ (13.0‰) and most depleted in $\delta^{13}C$ (−18.9‰) compared to the other fish species. In contrast, *L. xanthurus* was the most depleted in $\delta^{15}N$ (10.2‰) but most enriched in $\delta^{13}C$ (−17.2‰) compared to the other fish species. *P. dentatus* and *B. chrysoura* had intermediate levels of $\delta^{15}N$ and $\delta^{13}C$ relative to the other species. The biplots in 2018 revealed that *A. mitchilli* (12.5‰), *B. chrysoura* (12.0‰), and *L. xanthurus* (12.4‰) had enriched $\delta^{15}N$ values and relatively depleted $\delta^{13}C$ values (−19.5 to −18.8‰), whereas *P. dentatus* had depleted $\delta^{15}N$ (10.7‰) but an intermediate $\delta^{13}C$ value (−19.1‰).

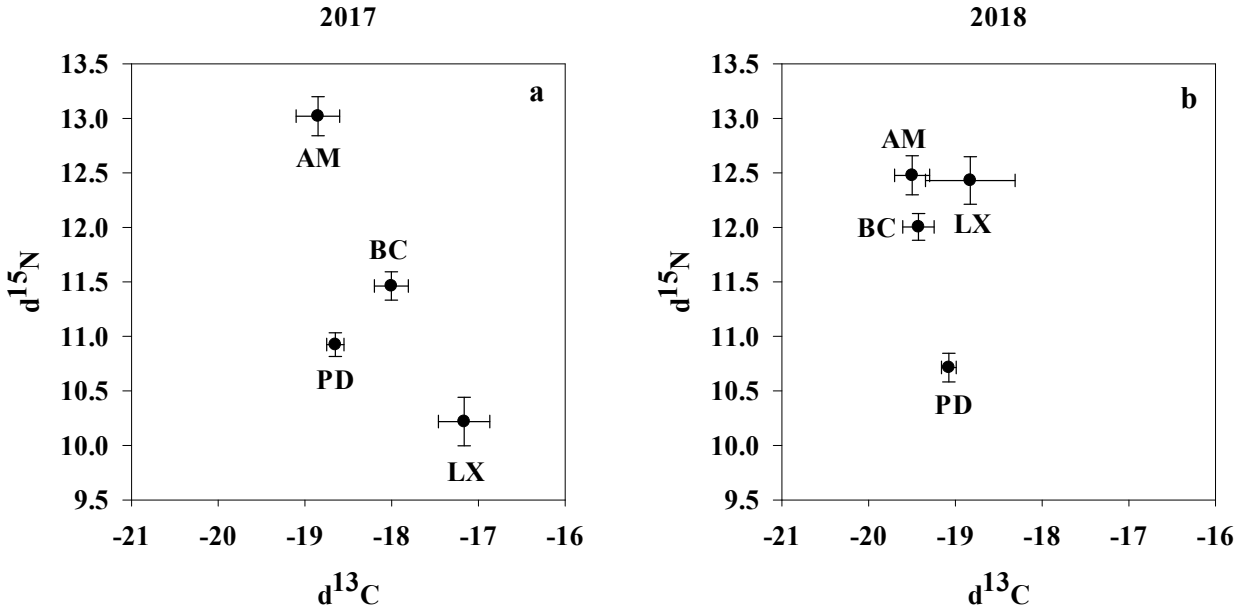


Figure 2. Annual biplots of $\delta^{13}C$ and $\delta^{15}N$ of fish species in 2017 (**a**) and 2018 (**b**). PD—*P. dentatus* (2017, $n = 140$; 2018, $n = 90$), AM—*A. mitchilli* (2017, $n = 16$; 2018, $n = 44$), LX—*L. xanthurus* (2017, $n = 41$; 2018, $n = 21$), and BC—*B. chrysoura* (2017, $n = 53$; 2018, $n = 50$).

3.3. Seasonal Differences in Isotopic Composition of Each Fish Species in MCBs

Seasonal comparisons were made only for *P. dentatus* and *B. chrysoura* in 2017 and for all species in 2018 because of insufficient sample sizes in spring and summer months in 2017. In 2017, *P. dentatus* showed no significant difference between spring (−18.4 ± 0.2), summer (−18.6 ± 0.1), and fall (−19.0 ± 0.2) for $\delta^{13}C$, while significant differences (Kruskal–Wallis, $p = 0.001$) were noted for $\delta^{15}N$ in spring (10.8 ± 0.4), summer (11.2 ± 0.1), and fall

(10.4 ± 0.2). Pairwise testing revealed that $\delta^{15}N$ values of *P. dentatus* caught in summer and fall were significantly different (Dunn's method, $p = 0.003$). In 2017, *B. chrysoura* showed significant differences (Kruskal–Wallis, $p = 0.001$) between summer (−17.8 ± 0.2) and fall (−20.0 ± 0.1) for $\delta^{13}C$, as well as for $\delta^{15}N$ in summer (11.4 ± 0.1) and fall (12.7 ± 0.3).

In 2018, no significant differences were observed between summer and fall in $\delta^{13}C$ content of the fish species examined, except for *B. chrysoura*, which showed significant differences for $\delta^{15}N$ (Kruskal–Wallis, $p = 0.001$).

Seasonal biplots (Figure 3a–c) indicated that in summer 2017, *B. chrysoura* was enriched in $\delta^{15}N$ and had intermediate levels in $\delta^{13}C$ compared to the other fish species. *P. dentatus* was enriched in both isotopes, and *L. xanthurus* was depleted in both isotopes compared to the other fish. In summer 2018, *L. xanthurus* was enriched in both $\delta^{15}N$ and $\delta^{13}C$ compared to the other fish species, while *A. mitchilli* and *B. chrysoura* were enriched in $\delta^{15}N$ and depleted in $\delta^{13}C$. *P. dentatus* was fairly depleted in both isotopes compared to the other species. Comparing the fish species in fall 2018, *A. mitchilli* was enriched in $\delta^{15}N$ and had intermediate levels of $\delta^{13}C$, *L. xanthurus* was enriched in $\delta^{15}N$ and depleted in $\delta^{13}C$, *B. chrysoura* had intermediate levels of both isotopes, and *P. dentatus* had depleted levels of both isotopes.

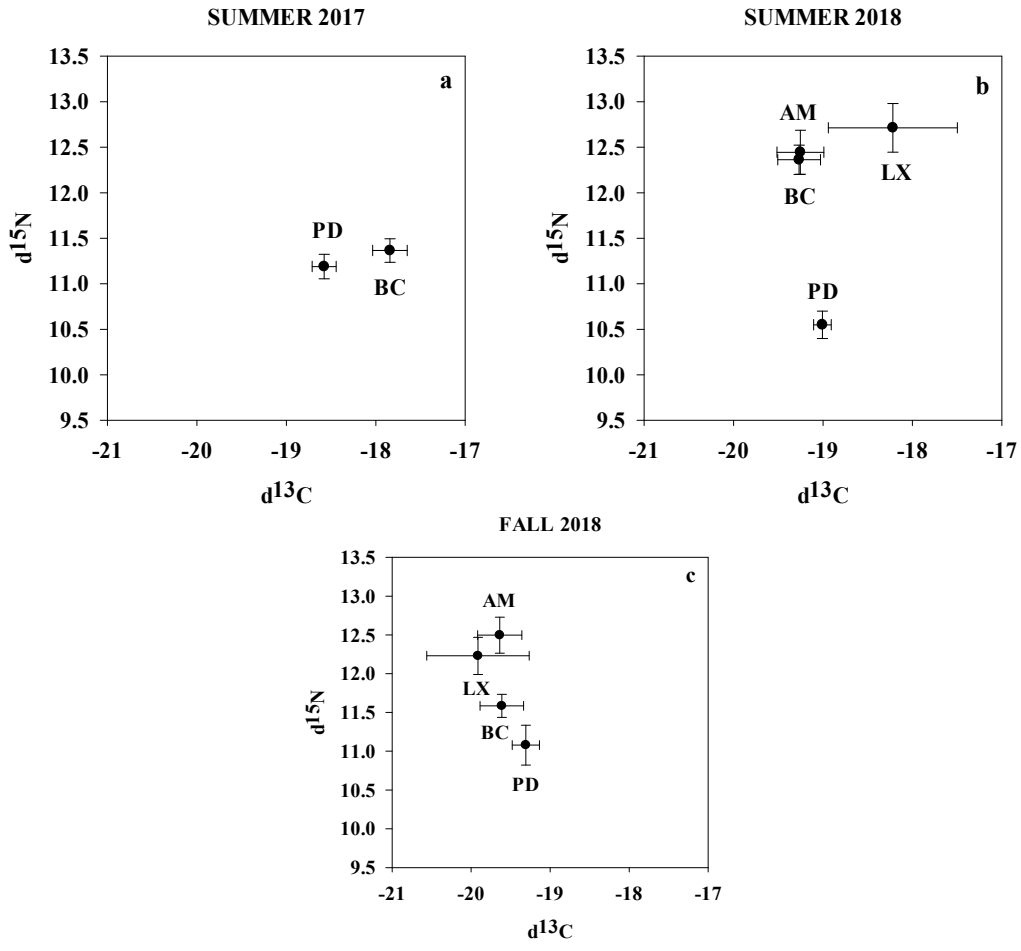


Figure 3. Seasonal biplots of $\delta^{13}C$ and $\delta^{15}N$ for the fish in 2017 (**a**) and 2018 (**b**,**c**). PD—*P. dentatus* (summer 2017, $n = 83$; summer 2018, $n = 67$; fall 2018 $n = 21$), AM—*A. mitchilli* (summer 2018, $n = 16$; fall 2018, $n = 28$), LX—*L. xanthurus* (summer 2018, $n = 13$; fall 2018, $n = 7$), and BC—*B. chrysoura* (summer 2017, $n = 49$; summer 2018, $n = 27$; fall 2018, $n = 23$).

3.4. Spatial Variation in Stable Isotopes of the Four MCB Fish Species

Mean values of $\delta^{13}C$ and/or $\delta^{15}N$ in 2017 and 2018 for *P. dentatus*, *A. mitchilli*, *L. xanthurus*, and *B. chrysoura* differed between the northern and southern bays (Table 4). In 2017, the $\delta^{13}C$ contents of *P. dentatus* and *B. chrysoura* were higher in the southern than

northern parts of MCBs, but no spatial differences were observed for *L. xanthurus*. For *P. dentatus*, *B. chrysoura*, and *L. xanthurus*, the $\delta^{15}N$ contents were higher in the northern bays than southern bays (Mann–Whitney, $p < 0.05$). In 2018, *P. dentatus* and *B. chrysoura* $\delta^{13}C$ contents were higher in fish collected from the southern than northern MCBs, while $\delta^{15}N$ contents for *P. dentatus* were significantly higher in the northern than southern bays (Mann–Whitney, $p < 0.001$), but no differences were observed between areas for the other species (Table 4).

Table 4. Mean ± standard error of $\delta^{13}C$ and $\delta^{15}N$ values of fish species collected in the northern and southern MCBs in 2017 and 2018 (a dash indicates there were either no isotopic data or not enough data for that location). For each species and year, values in the north and south bays with similar letters were not significantly different.

	2017			**2018**		
	$\delta^{13}C$			**$\delta^{13}C$**		
	North	**South**	***p*-Value**	**North**	**South**	***p*-Value**
P. dentatus	−19.1 ± 0.1 a	−18.4 ± 0.1 b	<0.05	−19.6 ± 0.1 a	−18.9 ± 0.1 b	<0.001
A. mitchilli	-	−18.9 ± 0.3	-	−19.4 ± 0.4 a	−19.6 ± 0.2 a	>0.05
L. xanthurus	−17.4 ± 0.6 a	−17.0 ± 0.3 a	>0.05	−19.3 ± 0.8 a	−18.3 ± 0.7 a	>0.05
B. chrysoura	−18.5 ± 0.3 a	−17.6 ± 0.2 b	<0.05	−19.8 ± 0.2 a	−17.8 ± 0.3 b	<0.001
	$\delta^{15}N$			**$\delta^{15}N$**		
	North	**South**	***p*-value**	**North**	**South**	***p*-value**
P. dentatus	11.4 ± 0.2 a	10.6 ± 0.1 b	<0.05	11.9 ± 0.2 a	10.4 ± 0.1 b	<0.05
A. mitchilli	-	13.0 ± 0.2		12.7 ± 0.3 a	12.3 ± 0.2 a	>0.05
L. xanthurus	11.3 ± 0.2 a	9.7 ± 0.3 b	<0.05	12.9 ± 0.2 a	11.9 ± 0.3 a	>0.05
B. chrysoura	11.8 ± 0.1 a	11.2 ± 0.2 b	<0.05	12.1 ± 0.1 a	11.7 ± 0.1 a	>0.05

Isotopic biplots (Figure 4a–d) indicated that in 2017, the fish had similar enrichment/depletion trends for both the northern and southern MCBs. *A. mitchilli* was enriched in $\delta^{15}N$ and most depleted in $\delta^{13}C$. *B. chrysoura* had intermediate $\delta^{13}C$ but the most enriched $\delta^{15}N$ levels. *P. dentatus* had an intermediate $\delta^{15}N$ level and the most depleted $\delta^{13}C$, and *L. xanthurus* was the least depleted in $\delta^{15}N$ but most enriched in $\delta^{13}C$. In 2018, *L. xanthurus* and *A. mitchilli* from the northern MCBs were enriched in both $\delta^{15}N$ and $\delta^{13}C$ compared to the other species, while *B. chrysoura* and *P. dentatus* were relatively depleted in both $\delta^{15}N$ and $\delta^{13}C$. In the southern MCBs, *L. xanthurus* and *B. chrysoura* were enriched in both isotopes, *P. dentatus* was depleted in both isotopes, and *A. mitchilli* was enriched in $\delta^{15}N$ but depleted in $\delta^{13}C$.

The trophic levels of *A. mitchilli*, *B. chrysoura*, *L. xanthurus*, and *P. dentatus* were determined to be 3.04, 2.77, 2.55, and 2.51, respectively, based on their $\delta^{15}N$ values. The standard ellipse areas estimated for the species are presented in Table 5 and Figure 5. The SEA and SEAc values for the species are similar. *L. xanthurus* had the largest SEA/SEAc values compared to the other fish species, while *A. mitchilli* had the smallest SEA/SEAc values.

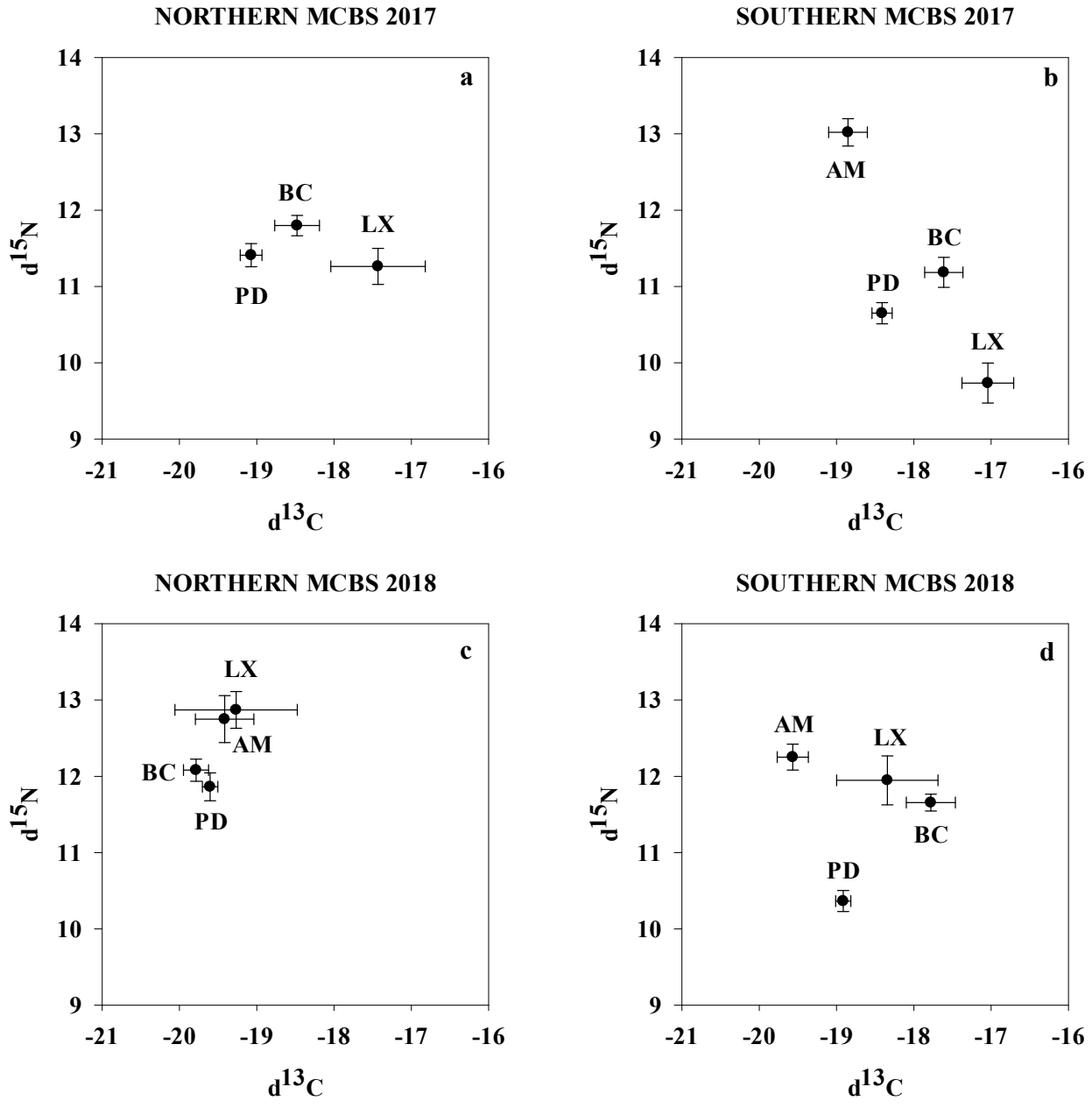


Figure 4. Spatial biplots of $\delta^{13}C$ and $\delta^{15}N$ for the fish in 2017 (**a**,**b**) and 2018 (**c**,**d**). PD—*P. dentatus* (2017 north, *n* = 51; 2017 south, *n* = 89; 2018 north, *n* = 21; 2018 south, *n* = 67), AM—*A. mitchilli* (2018 north, *n* = 20; 2018 south, *n* = 24), LX—*L. xanthurus* (2017 north, *n* = 13; 2017 south, *n* = 28; 2018 north, *n* = 11; 2018 south, *n* = 9), and BC—*B. chrysoura* (2017 north, *n* = 20; 2017 south, *n* = 29; 2018 north, *n* = 41; 2018 south, *n* = 9). Trophic levels and isotopic niches of the four MCB fish species.

Table 5. Standard ellipse area (SEA) and standard ellipse corrected area (SEAc) for the four MCB fish species.

Species	SEA (δ^2)	SEAc (δ^2)
P. dentatus	4.2	4.3
A. mitchilli	3.8	3.8
L. xanthurus	10.6	10.8
B. chrysoura	4	4

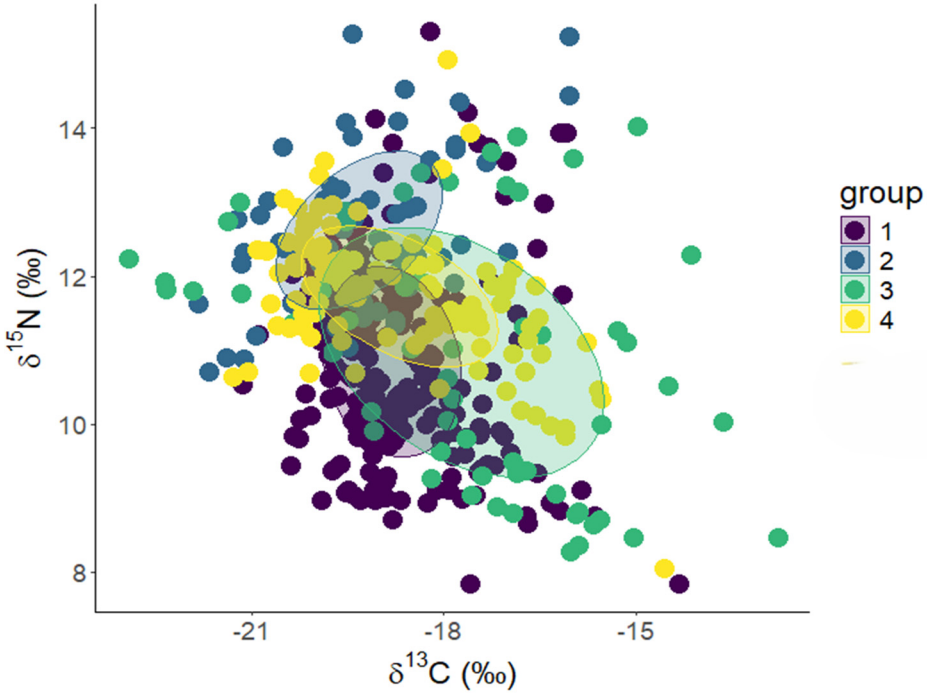


Figure 5. Isotopic niche width based on carbon ($\delta^{13}C$) and ($\delta^{15}N$) values of the four MCB fish species (Group 1—*P. dentatus*, Group 2—*A. mitchilli*, Group 3—*L. xanthurus*, and Group 4—*B. chrysoura*).

The trophic niche overlap between species based on $\delta^{13}C$ and nitrogen $\delta^{15}N$ ranged from 20.1 to 57.2% (*P. dentatus*), 22.2 to 76.4% (*A. mitchilli*), 22.7 to 33.6% (*L. xanthurus*), and 46.1 to 89.1% (*B. chrysoura*) (Table 6). The percentage of the *B. chrysoura* ellipse that overlapped *L. xanthurus* was the highest (89.1%), while the ellipse of *P. dentatus* that overlapped *A. mitchilli* was the lowest percentage (20.1%).

Table 6. Percentage area overlap between the SEA of the four MCB fish species. (The table should be read horizontally. For example, the ellipse for *P. dentatus* overlaps the ellipse for *A. mitchilli* by 20.1%, whereas the ellipse of *A. mitchilli* overlaps the ellipse of *P. dentatus* by 22.2%.)

	P. dentatus	***A. mitchilli***	***L. xanthurus***	***B. chrysoura***
P. dentatus		20.1	57.2	49.8
A. mitchilli	22.2		76.4	48.5
L. xanthurus	22.7	27.4		33.6
B. chrysoura	52.3	46.1	89.1	

4. Discussion

4.1. Interspecific Differences in Stable Isotope Composition of the Four MCB Fish Species

There were significant differences between species in the $\delta^{13}C$ and $\delta^{15}N$ mean values of the muscle tissue. In 2017, the fish were most enriched to most depleted in $\delta^{13}C$ as follows: *L. xanthurus*, *B. chrysoura*, *P. dentatus*, and then *A. mitchilli*, which was similar to the pattern observed in 2018. The higher enrichment of $\delta^{13}C$ in *L. xanthurus* than in the pelagic species such as *A. mitchilli* is probably related to a more benthic feeding that might have caused the addition of a benthic microalgal carbon source, which has $\delta^{13}C$ values around −17.0‰ [40,41]. Nevertheless, the $\delta^{13}C$ values overlapped between fish species and indicate that the fish species were dependent on a similar organic matter source [42]. The mean ± SE value of $\delta^{13}C$ ranged from −18.9 ± 0.5‰ to −17.2 ± 0.3‰ in 2017 and −19.5 ± 0.2‰ to −18.8 ± 0.5‰ in 2018 and suggest that the carbon source for the fish were mainly of marine phytoplankton, which ranges from −24‰ to −18‰ [5,40].

In 2017, the fish were most enriched to most depleted in $\delta^{15}N$ as follows: *A. mitchilli*, *B. chrysoura*, *P. dentatus*, and then *L. xanthurus*, which is slightly different from what was

observed in 2018, when the most enriched to most depleted $\delta^{15}N$ values were *A. mitchilli*, *L. xanthurus*, *B. chrysoura*, and then *P. dentatus*. *A. mitchilli* had the highest $\delta^{15}N$ values ($13.0 \pm 0.2‰$ in 2017 and $12.5 \pm 0.2‰$ in 2018) in both years compared to the other species. In another study, Winemiller et al. [8], found that *A. mitchilli* had the lowest $\delta^{15}N$ value ($12.8 \pm 0.8‰$) compared to *L. xanthurus* ($13.3 \pm 2.0‰$) and *B. chrysoura* ($14.7 \pm 2.0‰$), though the $\delta^{15}N$ value they observed in *A mitchilli* was comparable to the value we observed in our study in the MCBs. The *A. mitchilli* used for stable isotope analysis were approximately age-2 fish based on the length values, while the other fish species were age-0 [28–31]. It is known that $\delta^{15}N$ increases with the size and age of a species [5,42,43].

It was found that in 2017, *A. mitchilli* was the most enriched in $\delta^{15}N$ and most depleted in $\delta^{13}C$ compared to the other fish species; *L. xanthurus* was the most depleted in $\delta^{15}N$ and the most enriched in $\delta^{13}C$, while *P. dentatus* and *B. chrysoura* had intermediate levels of $\delta^{15}N$ and $\delta^{13}C$ compared to the other two fish species. In 2018, *A. mitchilli*, *B. chrysoura*, and *L. xanthurus* had enriched $\delta^{15}N$ values and depleted $\delta^{13}C$ values, and *P. dentatus* had depleted $\delta^{15}N$ and an intermediate $\delta^{13}C$ level. When fish have relatively enriched $\delta^{13}C$ values and depleted $\delta^{15}N$ values, this indicates that they were feeding on a more benthic-based prey; depleted $\delta^{13}C$ values and enriched $\delta^{15}N$ values indicate that the fish were feeding on more of a planktonic- or pelagic-based prey [6,41]. This suggests that *L. xanthurus* in 2017 and *P. dentatus* in 2018 had a more benthic influence in their diet, while *A. mitchilli* in 2017 and 2018 and *B. chrysoura* in 2018 had a more planktonic influence on their diet, which is consistent with what is known about their feeding habits [10].

4.2. Temporal Variations in Stable Isotope Composition of the Four MCB Fish Species

The $\delta^{13}C$ values of the fish were more enriched in 2017 than in 2018. In 2017, *L. xanthurus* mean $\delta^{13}C$ value was slightly more enriched and might have had a benthic microalgal carbon source, which has $\delta^{13}C$ values around $-17.0‰$ [40,41]. MacKenzie et al. [44] attributed annual variations in $\delta^{13}C$ to the feeding areas of salmon (*Salmo salar*) being subjected to interannual variability in primary production. It has also been shown that $\delta^{13}C$ can be more depleted in wet years compared to dry years because more terrestrially derived carbon is carried by rivers into an estuary in the wet years [45]. There was more precipitation in the year 2018 compared to 2017 in Maryland. The average annual rainfall values in 2017 and 2018 were 104.7cm and 164.1cm, respectively [22]. Since 2018 had more depleted $\delta^{13}C$, especially in the northern bays, it is possible that the higher rainfall increased freshwater inputs into the MCBs and affected the carbon signatures of organisms at the base of the food web [33,46] which, in turn, resulted in variations in the fish species' $\delta^{13}C$ [32,33,47–50].

4.3. Spatial Variations in Stable Isotopes of the Four MCB Fish Species

The $\delta^{13}C$ values in *P. dentatus* and *B. chrysoura* collected from the southern bays in both 2017 and 2018 were enriched compared to the individuals collected from the northern bays. In contrast, $\delta^{15}N$ values in *P. dentatus*, *B. chrysoura*, and *L. xanthurus* collected from the northern bays in 2017 were more enriched compared to the individuals collected from the southern bays, whereas only *P. dentatus* had significantly higher $\delta^{15}N$ in the northern than southern bays in 2018. Thus, the fish that displayed significant differences were more depleted in $\delta^{13}C$ and enriched in $\delta^{15}N$ when collected from the northern MCBs but enriched in $\delta^{13}C$ and depleted in $\delta^{15}N$ when collected from the southern MCBs. This spatial difference reflects the differences in the amount of freshwater inflow from MCB tributaries in the northern and southern bays and the extent of anthropogenic impact. St. Martin River, the main tributary that transports water directly into the MCBs, is located in the northern bays and thus carries organic materials from land and river into the bays, thereby influencing the $\delta^{13}C$ values in the fish. It has been determined that increased anthropogenic nutrients influences productivity, causing increases in isotopic values [51]. The northern MCBs have more anthropogenic eutrophication from residential and urban development compared to the southern MCBs [13,52]. It is also plausible that the observed difference

between the northern and southern MCBs was due to different prey assemblages that influenced the diet of the species in the areas. Estrada et al. [53] found isotopic variations in bluefin tuna (*Thunnus thynnus*), suggesting that the diets of individual bluefin tuna differed and explained by the fact that the bluefin tuna came from areas with different prey assemblages.

In a study on stable isotopes of juvenile fish in MCBs, O'Brien [54] found that *A. mitchilli* collected in the northern MCBs had $\delta^{13}C$ values around −20‰, indicating the presence of a more pelagic carbon source. The $\delta^{13}C$ values (−19.8 ± 0.2‰ to −17.4 ± 0.6‰) for the fish collected from the northern and southern bays in this study are similar to the values reported by O'Brien [54].

4.4. Trophic Levels and Niche Overlap of the Four MCB Fish Species

The trophic levels estimated for *A. mitchilli*, *B. chrysoura*, *L. xanthurus*, and *P. dentatus* were 3.04, 2.77, 2.55, and 2.51, respectively. The higher trophic level of *A. mitchilli* relative to the other species may be because they were older and likely included more fish in their diets. Fish size/age has a positive relationship with trophic level [55,56].

The isotopic niche breadth differed between the species, with *L. xanthurus* having a higher SEA/SEAc than the other fish species, indicating a larger niche, and *A. mitchilli* having the lowest SEA/SEAc, indicating a smaller niche. The broader niche width of *L. xanthurus* reveals a wider use of MCBs' habitat and/or prey sources [57]. The variation in niche widths could be related to species with smaller niche widths having a trophic behavior that is more flexible compared to species with larger niche widths, which include individuals with specialized trophic behavior [58]. Generalist species are believed to have a narrower isotopic niche width than specialist species due to generalists having similar isotopic signatures because they are sampling the same prey items in similar proportions and thus integrate their diet [59,60]. The niche width data from this study suggest a higher variability in the diets among *L. xanthurus* individuals, resulting in specialized diets [61], compared to individuals belonging to other fish species that were thus, relatively, generalists.

The percentage of the *B. chrysoura* ellipse that overlapped *L. xanthurus*, as well as the *A. mitchilli* ellipse that overlapped *L. xanthurus*, was >60%, meaning they had significant niche overlaps [38,39]. Greater niche overlaps suggest that the species are feeding on similar prey resources [62,63]. This high dietary overlap between the species can increase resource competition [64].

5. Conclusions

The $\delta^{13}C$ values of the fish species in both years indicate marine phytoplankton as the dominant source of carbon and that interannual variations in $\delta^{13}C$ in some species were likely associated with interannual variability in the amount of precipitation and terrestrially derived organic carbon transported via freshwater discharge into the bays. *A. mitchilli* had the highest $\delta^{15}N$ in both years, probably because the individuals used in the study were ages-1 to 2 fish, while the other fish species were juveniles. Overall, the species were more $\delta^{15}N$ enriched when collected from the northern MCBs than when collected from the southern MCBs, especially in 2017. This was probably due to a higher anthropogenic influence in the northern MCBs and different prey assemblages in the two locations. *L. xanthurus* had a broader niche breadth than the other fish species, whereas *B. chrysoura* and *A. mitchilli* had the highest niche overlap with *L. xanthurus*; thus, there is a potential for competition between the two species and *L. xanthurus*. Our results are useful for interpreting food web dynamics in estuaries and underscore the need to take into account spatial and temporal variability of $\delta^{13}C$ and $\delta^{15}N$ when examining trophic relationships of fishes.

Author Contributions: Conceptualization, C.R., P.C. and A.I.; methodology, C.R.; software, C.R.; validation, C.R., P.C. and A.I.; formal analysis, C.R.; investigation, C.R.; resources, P.C. and A.I.; data curation, C.R.; writing—original draft preparation, C.R.; writing—review and editing, P.C. and A.I.; visualization, C.R., P.C. and A.I.; supervision, P.C. and A.I.; project administration, P.C. and

A.I.; funding acquisition, P.C. and A.I. All authors have read and agreed to the published version of the manuscript.

Funding: This project was funded by the US National Science Foundation award (grant number: 1547821), in part by the NOAA Educational Partnership Program (grant number: NA16SEC4810007) to the University of Maryland Eastern Shore, and by Title III HGBI.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data available upon request from authors.

Conflicts of Interest: The authors declare no conflicts of interest.

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