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Diet and Trophic Ecology of Invasive Blue Catfish *Ictalurus furcatus* in an Eastern Shore Tributary of the Chesapeake Bay

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Abstract

Blue Catfish *Ictalurus furcatus* were first introduced to Virginia tributaries of the Chesapeake Bay in the 1970s and have greatly expanded in population size and distribution, prompting ecological and economic concerns. This study investigates the diet and trophic ecology of invasive Blue Catfish in tidal, low-salinity reaches of the Nanticoke River and Marshyhope Creek, on the Chesapeake Bay's Eastern Shore (Maryland, USA), using complementary stomach content (SCA) and stable isotope (SIA) analyses. Stomach contents from Blue Catfish (n = 363 non-empty stomachs) were collected opportunistically from October 2021 through November 2022. For SIA, 136 individuals across 21 fish species, blue crab *Callinectes sapidus*, and arrow arum *Peltandra virginica* were sampled between October 2021 and October 2023. SCA revealed considerable seasonal and size-based diet variability, suggesting a shift from omnivory to piscivory at 563 mm total length. Primary prey included fish, arrow arum seeds, Asian clams *Corbicula fluminea*, and amphipods, along with seasonally available species of economic and ecological importance (river herring *Alosa* spp., blue crab, White Perch *Morone americana*, and Atlantic Menhaden *Brevoortia tyrannus*). Minimal isotopic niche overlap between large and small Blue Catfish size classes, alongside ontogenetic trends in $\delta^{13}C$ and $\delta^{15}N$, substantiated SCA-detected diet shifts. Trophic level estimates derived from Blue Catfish $\delta^{15}N$ (n = 54 individuals) were higher than those from SCA, particularly for individuals <563 mm. Blue Catfish occupied a relatively high trophic level, and carbon and sulfur SIA indicated marine resource utilization, substantiating SCA results. This research describes localized trophic interactions of invasive Blue Catfish to inform fishery management planning regarding Blue Catfish's potential influence on other species of management interest.

Keywords: predation; estuary; fish ecology; fisheries management; non-native; stable isotope

Key Contribution: Complementary stable isotope and stomach content analyses indicate that invasive Blue Catfish consume marine-derived nutrients and undergo a significant ontogenetic diet shift in tidal, low-salinity reaches of the Nanticoke River watershed—a

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major and previously unassessed Eastern Shore tributary of the Chesapeake Bay. Blue Catfish shift their primary prey from vegetation and invertebrates to fish at around 563 mm, feed at a high trophic level relative to other fishes, and consume seasonally available economic and ecologically important native fauna.

1. Introduction

Humans have broadly introduced fishes outside of their native range for purposes such as aquaculture, aquaria, biocontrol, and establishment of commercial and recreational fisheries [1–4]. Some non-native fishes establish in novel ecosystems with minimal threats to biodiversity and human economic interests [5], and the introduction of sport fish can provide popular angling opportunities and economic benefits to local communities [6–8]. However, non-native fish may cause an array of ecological impacts, scaling from genetic to ecosystem-wide effects [9]. Introduced species that pose significant ecological and economic risks or harm to human health are designated as "invasive" [10]. Competition and predation by invasive fish may negatively impact fisheries, threaten local and regional biodiversity, and cause economic costs [11–17]. Thus, it is important to understand the diet and trophic interactions of invasive fishes in local ecosystems to inform fisheries managers of the implications of species introductions [18,19].

Blue Catfish *Ictalurus furcatus* are native to the Ohio, Mississippi, Missouri, and Rio Grande River basins and have been introduced broadly outside their native range [20,21]. From 1974 through 1985, Blue Catfish were stocked by Virginia fisheries managers into the James, Rappahannock, and Mattaponi Rivers—tributaries to the Chesapeake Bay—to provide new recreational fishing opportunities [22,23]. Blue Catfish have spread to every major tributary of the Chesapeake Bay watershed, likely attributed to the species' relatively high salinity tolerance and fecundity, long spawning season and reproductive life span, parental care of offspring, and ability to reach large sizes [24–29]. The Chesapeake Bay has historically faced threats to conservation, including hydrological modification, habitat loss, overfishing, and water quality degradation, and the introduction of Blue Catfish may represent an additional stressor for native species [30–34]. Tidal freshwater and estuarine habitats in the Chesapeake Bay watershed now support dense and abundant populations of Blue Catfish [35–37], correlating with declines in native White Catfish *Ameiurus catus* [38] and prompting concerns of ecological impacts to native species through competition and predation. As a result of these potential impacts, Blue Catfish were designated as an invasive species in Atlantic Slope waters by the Atlantic States Marine Fisheries Commission in 2011 [39].

Studies of Blue Catfish diet composition in tributaries on the Chesapeake Bay's Western Shore reported a generalist, omnivorous feeding strategy, where common prey items include invasive Asian clam *Corbicula fluminea*, vegetation, macroinvertebrates, insects, estuarine mud crabs (*Rhithropanopeus harrisii* and *Panopeus herbstii*), bivalves, Gizzard Shad *Dorosoma cepedianum*, and White Perch *Morone americana*, amongst others [40]. Documented prey also included species of economic and ecological concern such as blue crab *Callinectes sapidus*, Striped Bass *Morone saxatilis*, American Eel *Anguilla rostrata*, and anadromous alosines [40], with some evidence of possible consumption of early life stage endangered Atlantic Sturgeon *Acipenser oxyrinchus* [41]. Schmitt et al. [40] noted that Blue Catfish undergo an ontogenetic diet shift to piscivory; however, the length at which the shift occurs varies between tributaries (between roughly 550 and 900 mm total length). Diet composition differs among Western Shore tributaries of the Chesapeake Bay and between introduced populations of Blue Catfish in other Atlantic Slope drainages [42,43], suggesting

that trophic interactions depend on the unique physical and ecological characteristics of the receiving aquatic ecosystem.

Comprehensive assessments of diet and trophic interactions are not available for any tributaries on the Chesapeake Bay's Eastern Shore, which are notably different from Western Shore tributaries. Land use on the Western Shore is dominated by urban and industrial development, whereas the Eastern Shore is largely dominated by agriculture for corn, soy, and poultry products [44]. Due to lower human population density and urban development, Eastern Shore tributaries may experience relatively less impact by anthropogenic impairments on fish habitat and anadromous fish passage [44–47]. The topography of the Eastern Shore of the Chesapeake Bay is much flatter than that of the Western Shore; Eastern Shore tributaries are also part of much smaller watersheds [44]. Further, Blue Catfish expanded into Eastern Shore tributaries much later than their introduction to Western Shore tributaries. Therefore, these populations may still be establishing, and population characteristics could differ [48].

Management of Blue Catfish throughout the Chesapeake Bay is complicated, as viewpoints regarding the invasive fish vary across stakeholder groups [23]. The Chesapeake Bay Program Invasive Catfish Management Strategy (2020) advocates for the development of tributary-specific management plans to achieve desired outcomes developed by a diverse group of stakeholders [49]. Assessments of Blue Catfish diet and trophic ecology throughout the Chesapeake Bay watershed are important for informing tributary-specific management. Two common methods for assessing the feeding and trophic ecology of invasive fishes are stomach content analysis (SCA) and stable isotope analysis (SIA) [40,43,50,51]. Stomach contents provide short-term dietary information, as Blue Catfish in the Chesapeake Bay digest prey quickly (maximum gastric evacuation rate = 0.39, or 39% of food digested over a 3 h time interval) [52], likely depending on water temperature, fish size, and prey type. Stomach contents are collected from many individuals in a population over a broad temporal scale to characterize the diet of a consumer [53]. This method can reveal prey at a significant taxonomic resolution, particularly when supplemented with molecular identification [54].

Stable isotope analysis is a powerful tool that can be used to complement traditional dietary analyses of stomach contents [50,55]. Stable isotope analysis may allow researchers to better examine assimilated and long-term diets than is possible using SCA alone, while requiring a much smaller sample size; however, investigating prey items in precise taxonomic detail can be difficult through SIA [50]. Stable isotopic ratios of bulk tissue indicate general trends in diet composition and trophic ecology. For example, carbon stable isotope ratios can indicate basal sources of primary productivity within a consumer's diet [56–58]. Sulfur stable isotopic ratios can indicate assimilation of dietary marine resources in consumers, as primary producers in marine food webs are more enriched in the heavy isotope of sulfur (^{34}S) than those in freshwater ecosystems, and little fractionation of sulfur isotopes occurs during dietary assimilation [59,60]. Typical $\delta^{34}S$ values in riverine and upper-estuarine resident fishes can range from −5‰ to 5‰, while $\delta^{34}S$ in fishes in higher-salinity portions of estuaries can range from 11‰ to 17‰, with marine fishes exhibiting even higher-$\delta^{34}S$ signatures (>17‰) [61,62]. Nitrogen SIA is useful in elucidating trophic interactions, as assimilation of dietary protein discriminates against the light isotope of nitrogen (^{14}N), generally resulting in a $\delta^{15}N$ enrichment of approximately 2–4‰ with each increasing trophic level, varying with factors such as diet source, consumer biology and trophic position, and tissue type [63,64]. Stable isotope analysis can be used to investigate assimilated diet, rather than only what is ingested by a consumer [65]. This is informative when investigating consumers that frequently consume relatively indigestible food items, such as bivalves (calcified shells) and carbohydrate-rich plant matter, as these prey sources can

meet short-term metabolic demands but may be minimally assimilated into muscle tissue, potentially leading to over-representation of their dietary importance through SCA [66,67]. Pairing SIA and SCA can provide greater ecological insights than are possible using either method alone [68].

This study aims to characterize the diet and trophic ecology of invasive Blue Catfish in tidal, low-salinity reaches of the Nanticoke River and Marshyhope Creek, Maryland. Specifically, we investigate seasonal diet trends and examine primary prey resource utilization over ontogeny using complementary SCA and SIA of carbon, nitrogen, and sulfur. We hypothesized that Blue Catfish would shift their diet from vegetation, amphipods, and bivalves to fish over ontogeny and occupy a relatively high trophic level compared to other fishes. Additionally, we expected SCA to indicate ontogenetic increases in trophic position and marine resource utilization, corresponding to positive relationships between Blue Catfish body length and ^{13}C, ^{15}N, and ^{34}S stable isotopic composition. Ontogenetic shifts in primary prey utilization were hypothesized to manifest as significant isotopic niche space segregation. By characterizing seasonal and size-based diet trends and trophic ecology of invasive Blue Catfish in the Nanticoke River, this study expands current knowledge regarding Blue Catfish in the Chesapeake Bay and can inform assessments of ecological impacts on native fauna.

2. Materials and Methods

2.1. Study Area

This study focuses on the largest Eastern Shore tributary of the Chesapeake Bay, the Nanticoke River, where Blue Catfish were first detected in surveys in 2010 (Brett Coakley, Maryland Department of Natural Resources, personal communication, 24 March 2026). From its headwaters north of Seaford, Delaware, the Nanticoke River flows southwest through Maryland's low-elevation Eastern Shore before draining into the Chesapeake Bay, comprising a watershed area of approximately 2994 km^2. Land use throughout the Nanticoke River watershed primarily consists of agricultural areas, forests, and swamps [44,69,70]. The Nanticoke River's relatively intact wetlands and lack of hydrological modification and impediments make it a particularly important tributary for native anadromous fishes, including alosines, Atlantic Sturgeon, and Striped Bass, as well as other important fisheries resources, such as blue crab and Atlantic Menhaden [71,72].

2.2. Collection Methods

Fish collections for SCA and SIA were primarily conducted using low-frequency boat electrofishing from a Smith-Root 9.0 gpp electrofishing boat (pulsed direct current at 15 Hz, 340–680 v, 0.25–5.0 amps) in tidal, low-salinity (fall mean surface salinity: 0.2–1.22 ppt [73]) reaches of the Nanticoke River and major tributary Marshyhope Creek (Figure 1). Monthly boat electrofishing samples were collected during March–November, beginning in October 2021 and ending in November 2022, when water temperatures were suitable for low-frequency electrofishing (18–28 °C) [74]. Low-frequency electrofishing catch rates for Blue Catfish are greatly reduced when water temperatures fall below 18 °C, and variability increases substantially above 28 °C. Thus, high-frequency electrofishing (pulsed direct current, 60–120 Hz, 85 or 170 v, 16 to 75 amps) was utilized, as necessary, to collect fish during the early spring, late fall, and middle of summer. Electrofishing sites were selected opportunistically to maximize catch rates, as Blue Catfish in the Nanticoke River and Marshyhope Creek were often patchily distributed in specific habitat types, and the researchers initially struggled to capture enough fish when testing randomized site selection for this study. During boat electrofishing, a chase boat with two additional netting

personnel was often used in conjunction with the main electrofishing boat to increase catch efficiency and fish storage space [75].

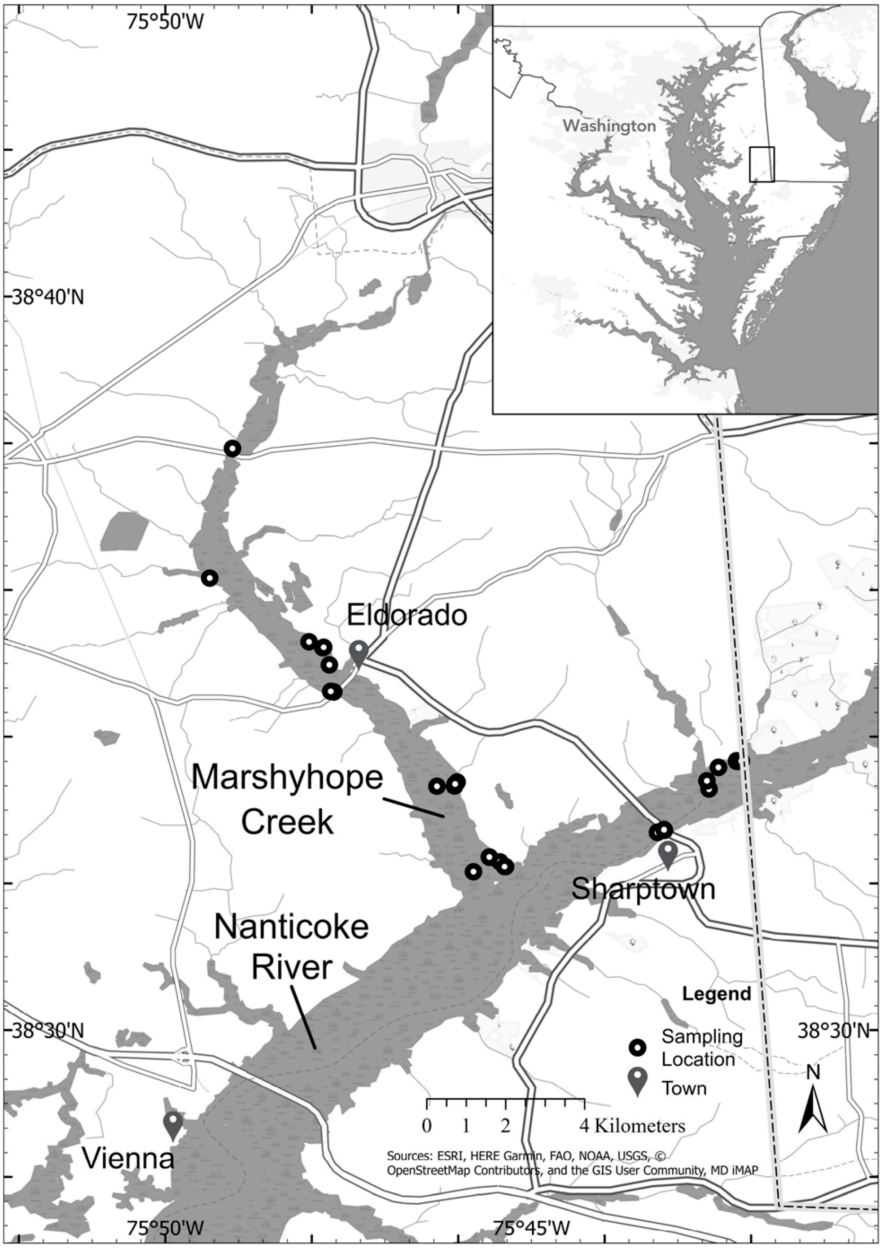


Figure 1. Map depicting the study area and sampling locations within the Nanticoke River and Marshyhope Creek where Blue Catfish were captured for stomach content analysis using boat electrofishing from October 2021 through November 2022. The dashed line indicates the state border betweeen Maryland and Delaware.

A small number of Blue Catfish from tidal, low-salinity reaches of the Nanticoke River and Marshyhope Creek were also collected for SCA through angler donations at fishing tournaments during summer and fall of 2022 (n = 52 fish with n = 13 non-empty stomachs). A total of 696 Blue Catfish ranging from 90 to 1099 mm total length were collected for SCA, with 363 stomachs containing prey (Table 1). During the summer, boat electrofishing was relatively inefficient, and large proportions of stomachs were empty (n = 108 out of 157 individuals), thus resulting in a smaller sample size of non-empty stomachs during the summer.

Table 1. Sample size (*n*) of Blue Catfish containing non-empty stomachs from two size classes (<563 mm and ≥563 mm) collected from tidal, low-salinity reaches of the Nanticoke River and Marshyhope Creek from October 2021 through November 2022 (spring: March–May, summer: June–August; and fall: September–November).

Size Class	Season	*n*
<563 mm	Spring	72
	Summer	36
	Fall	141
	Total	249
≥563 mm	Spring	51
	Summer	13
	Fall	50
	Total	114
All	Total	363

Blue Catfish and other fish and invertebrate species were collected from the Nanticoke River for SIA to assess general isotopic signals across the food web and compare trophic interactions (Table 2). Resident fishes and blue crabs were collected from tidal, low-salinity reaches of the Nanticoke River watershed, aligning with the sampling area for SCA. Migratory prey of Blue Catfish were collected opportunistically throughout the Nanticoke River watershed (including higher-salinity reaches). Specimens for SIA were primarily collected between October 2021 and October 2022 using opportunistic boat electrofishing, dip nets, hook-and-line, trawls, and trot lines. After noting large numbers of arrow arum *Peltandra virginica* seeds in Blue Catfish diets during the fall season, we chose to collect five arrow arum samples from the Nanticoke River (Sharptown, MD) for SIA in a post hoc effort during October 2023. Replicate samples were run for C, N, and S isotopic composition from a proportion of individuals (Table 2).

Table 2. Common name, species name, mean total length (±one standard deviation; * denotes carapace width (mm)), sample size of individuals (*n* indivs), and number of samples (*n* samples) for C/N and S stable isotope analyses for each species collected in the Nanticoke River and Marshyhope Creek from October 2021 through October 2023, listed alphabetically by common name. "-" indicates no data.

Common Name	Species Name	Mean Total Length (mm)	*n* Indivs ($^{13}C/^{15}N$)	*n* Samples ($^{13}C/^{15}N$)	*n* Indivs (^{34}S)	*n* Samples (^{34}S)
Alewife	*Alosa pseudoharengus*	277 ± 17	6	7	6	7
American Eel	*Anguilla rostrata*	367 ± 120	5	6	-	-
Arrow Arum (plant)	*Peltandra virginica*	-	5	5	-	-
Atlantic Menhaden	*Brevoortia tyrannus*	297 ± 5	2	3	2	2
Banded Killifish	*Fundulus diaphanus*	71 ± 11	4	4	4	5
Blue Catfish (<563 mm)	*Ictalurus furcatus*	411 ± 93	21	23	15	16
Blue Catfish (≥563 mm)	*Ictalurus furcatus*	843 ± 143	33	39	25	27
Blue Crab	*Callinectes sapidus*	80 ± 5 *	4	5	-	-
Blueback Herring	*Alosa aestivalis*	277 ± 15	2	3	2	2
Bluegill	*Lepomis macrochirus*	167 ± 6	3	3	1	1
Brown Bullhead	*Ameiurus nebulosus*	251 ± 70	2	2	-	-
Channel Catfish	*Ictalurus punctatus*	402 ± 106	2	2	-	-
Common Carp	*Cyprinus carpio*	452 ± 98	2	2	-	-
Gizzard Shad	*Dorosoma cepedianum*	270 ± 119	6	6	4	4
Hogchoker	*Trinectes maculatus*	111 ± 30	4	4	-	-

Table 2. *Cont.*

Common Name	Species Name	Mean Total Length (mm)	*n* Indivs ($^{13}C/^{15}N$)	*n* Samples ($^{13}C/^{15}N$)	*n* Indivs (^{34}S)	*n* Samples (^{34}S)
Largemouth Bass	*Micropterus nigricans*	314 ± 31	4	5	4	5
Northern Snakehead	*Channa argus*	638 ± 95	6	6	6	7
Satinfin Shiner	*Cyprinella analostana*	82 ± 7	5	5	-	-
Spottail Shiner	*Hudsonius hudsonius*	98 ± 3	3	4	4	4
Striped Bass	*Morone saxatilis*	335 ± 161	6	6	-	-
White Catfish	*Ameiurus catus*	272 ± 61	3	4	-	-
White Perch	*Morone americana*	173 ± 17	5	5	2	2
Yellow Perch	*Perca flavescens*	210 ± 55	3	4	-	-

2.3. Sample Preparation and Processing

Upon collection, Blue Catfish were euthanized, placed in coolers filled with ice, and transported to an offsite laboratory. The total length and weight of each fish were recorded, and stomachs were carefully excised from as close as possible to the connection at the mouth to the pyloric sphincter to ensure all prey items were collected. Stomach samples were stored in individually labeled plastic bags and frozen at −20 °C.

A total of 136 individuals from 21 fish species, blue crab, and one aquatic plant (arrow arum) were analyzed for carbon and nitrogen stable isotopic composition (Table 1). Seventy-five individuals from eight different fish species were analyzed for sulfur stable isotopic composition. A total of 17 and seven individual replicate samples from different species were run for carbon/nitrogen and sulfur analyses, respectively (Table 1). Using a scalpel, small dorsal white muscle tissue samples (1 cm^3) were removed from up to ten individual Blue Catfish from each 100 mm length bin and up to six individuals of other species in the Nanticoke River aquatic food web. Tissue samples were frozen at −20 °C prior to further preparation for stable isotope analysis.

Frozen dorsal white muscle tissue samples from Blue Catfish and competitor or prey species were lyophilized at −84 °C for approximately 24 h using a Labconco FreeZone 6 Console Freeze Dryer (Kansas City, MO, USA) at the University of Maryland Eastern Shore in Princess Anne, Maryland. Lyophilized tissue samples were ground into a fine powder using a mortar and pestle, and no lipid correction was applied. For SIA of ^{13}C and ^{15}N, powdered muscle tissue was weighed to 1.0 mg ± 0.1 mg, packaged into 5 × 3.5 mm or 6 × 4 mm tin cups, and placed into an individually numbered 96-well tray. A note card was placed beneath the lid of each well plate, and trays were sealed tightly using label tape to prevent samples from jumping between wells during shipment. Sample trays were shipped to the University of Maryland Center for Environmental Science Central Appalachians Stable Isotope Facility for carbon and nitrogen stable isotope analysis using a Carlo Erba NC 2500 Elemental Analyzer (Milan, Italy) and ThermoFisher Delta V+ Isotope Ratio Mass Spectrometer (Bremen, Germany). Quality-controlled standards (USGS 40 and USGS 41) are used at CASIF with a long-term analytical precision of 0.1‰ for $\delta^{13}C$ and $\delta^{15}N$.

Up to eight Blue Catfish samples from each 100 mm length bin were analyzed for sulfur isotopic composition. Two to eight tissue samples from six additional species with varying life history characteristics and feeding strategies (Alewife *Alosa pseudoharengus*, Atlantic Menhaden *Brevoortia tyrannus*, Banded Killifish *Fundulus diaphanus*, Blueback Herring *Alosa aestivalis*, Largemouth Bass *Micropterus nigricans*, Northern Snakehead *Channa argus*, and White Perch) were analyzed for sulfur isotopic composition to serve as reference points for sulfur isotopic composition in the Nanticoke River aquatic food web. Samples selected for analysis of ^{34}S were weighed to 4.0 mg ± 0.1 mg, carefully packaged into 5 × 9 mm tin cups, and placed into a 96-well tray. Sample trays were sent to the UC Davis Stable Isotope

Facility for analysis of ^{34}S using an Elementar vario ISOTOPE cube elemental analyzer and Isoprime PrecisION IRMS (Cheadle Hume, Stockport, UK). Reference materials for sulfur SIA included cysteine, bovine gelatin, glutathione, blue-green algae, salmon muscle, and taurine, with a mean standard deviation of ±0.34‰, and a mean absolute accuracy for calibrated reference materials of ±0.40‰.

Frozen stomach samples were thawed to room temperature for analysis, and prey items in the early stages of digestion were visually identified to the lowest taxonomic level possible using morphological characteristics, such as body shape and size, scales, bones, otoliths, and fin rays. Prey items were blotted dry with paper towels, and the wet weight of each diet item was recorded to the nearest tenth of a gram using a digital balance. Any prey items found in donated fish stomachs that matched the bait used were removed from diet analyses. Fish prey items that were too heavily digested to be confidently identified using visual methods were identified using DNA metabarcoding. Tissue samples were removed from visually distinct individual unidentifiable fish prey and placed into individually labeled medical specimen cups filled with 95% ethanol. Bones and other hard parts were excluded from genetic analyses, as these items have a low chance of identification using molecular techniques [76,77].

Tissue specimen cups were sealed and sent to the United States Geological Survey Eastern Ecological Science Center in Kearneysville, West Virginia, where total genomic DNA (gDNA) was extracted according to the manufacturer's instructions for the Zymo quick DNA 96 Kit. Amplicon libraries were prepared in accordance with the Illumina MiSeq 16s Metagenomic Sequencing Guide. Because the workflow involves high-throughput amplification of mixed dietary DNA, this procedure reflects a modified metabarcoding approach rather than conventional single-specimen DNA barcoding. Prior to quality control and sequencing, samples were amplified using primers from Evans et al. (2021) [78], including blocking primers for the Cytochrome C Oxidase Subunit 1 (COI) gene to reduce host DNA bias. Non-template controls (nuclease-free water only) and non-library controls (barcode/index reagents without sample DNA) were included in all PCR plates to detect contamination and tag jump artifacts; taxa detected in these controls were removed from associated samples unless present at more than 10× the control read count. A single positive control consisting of a standardized multispecies mock community was also added to each plate of 96 samples to confirm successful amplification. DNA sequencing was performed in accordance with the methods listed in Clark et al. (2022) [79] with two minor modifications: an Agilent 4150 TapeStation System was utilized instead of an Agilent 2100 Bioanalyzer, and an 8 picomolar (pM) concentration library (rather than 10 pM) with a 6.5% PhiX control spike was added to all pooled amplicon libraries. A custom reference sequence database was created using MetaCurator (v1.0.1) to extract relevant amplicon regions from available NCBI reference sequence data [80]. Sequences were queried against curated databases using VSEARCH (v.2.8.1), and alignments with a threshold of 97% or greater sequence similarity were annotated to genus [81–83]. This similarity threshold was selected because 97% identity is commonly used for COI metabarcoding and balances assignment accuracy with natural interspecific variation [82,83]. Annotations were filtered to remove detection with a likelihood of representing a mistag-associated false detection (using an FDR threshold of 0.01) using standard statistical methods [84]. Taxa that accounted for less than 1% of relative read abundance within samples were excluded from the data to minimize the potential for assigning false-positive occurrences due to sample contamination or the presence of secondary prey. This threshold was chosen because taxa below 1% often fall within levels observed in controls and cannot be reliably distinguished from background noise [83]. Data that passed these filters were assigned as present in the diet and used in percent-frequency-of-occurrence calculations (Section 2.4). Using COI data,

when multiple genera were detected in a sample, the genus that had a read count at least one order of magnitude ($\geq$10$\times$) or higher compared to other genera was assigned the sample weight for purposes of mean-percent-by-weight analysis (Section 2.4). This decision rule was implemented to reduce false-positive weight assignments in mixed-prey samples, particularly given that host DNA (*Ictalurus*) may still appear despite blocking primers. If no genus was identified with one order of magnitude or higher read count compared to other genera, the sample weight was conservatively assigned to unidentified fish. This approach minimized the potential for assigning false-positive prey weight assignments without losing diet breadth data, given that the taxonomic diversity of these multi-genus samples was captured independently for percent-frequency-of-occurrence analyses.

2.4. Analyses

Large numbers of stomachs are often needed to characterize the diet of generalist fishes, and previous research on Blue Catfish diet in the Chesapeake Bay emphasizes the importance of assessing sample size sufficiency in characterizing diets [40,85]. To assess stomach sample size sufficiency across seasons and size classes, we constructed sample-size-based coverage trajectories using the iNEXT package in R (version 4.5.2) [86,86]. Raw prey presence–absence data were compiled into incidence frequency vectors, where individual non-empty stomachs served as sampling units. Coverage trajectories were extrapolated up to double the reference sample size for each size class and season group, and 95% confidence intervals (CIs) were derived from 200 bootstrap replications. Sampling completeness was quantified via Sample Coverage ($\widehat{C}$) [87,88]. To ensure that dominant trophic interactions were captured for each size class and season group, a threshold of $\widehat{C} \geq 0.90$ was established to denote sample size sufficiency [89].

Generalized linear models (GLMs) with logit link functions were used to investigate the relationship between Blue Catfish total length and fish and vegetation presence in stomach contents. Fifty-percent presence probability thresholds (and associated 95% CIs) were estimated from model parameters obtained using maximum likelihood estimation. The fifty-percent probability threshold from the GLM for fish prey, rounded to the nearest millimeter, was used to assign a size class threshold to facilitate further analyses.

Additionally, we employed generalized additive models (GAMs) in the mgcv package in R to model the non-linear relationships between Blue Catfish total length and fish and vegetation prey weight in the diet, using only non-zero observations [90]. For the fish prey weight model, the response was modeled using a gamma distribution with a log link function, and the smooth term for total length was specified using a thin-plate regression spline with the maximum basis dimension set to $k = 10$ to allow flexibility while preventing overfitting. For the vegetation prey weight model, total length was modeled using a Tweedie error distribution family with a log link function, and the smooth term for total length was specified using a thin-plate regression spline (maximum basis dimension set to $k = 10$). Smoothness selection and parameter optimization were performed using Restricted Maximum Likelihood (REML) for both models. Quantile residuals for both GAMs were assessed using the DHARMa package in R to investigate model goodness-of-fit. Using posterior simulation via 10,000 Monte Carlo draws from the respective model parameter covariance matrices across the observed total length ranges, we estimated the total length (and 95% credible interval (CrI)) at which: (1) the effect on fish prey weight exceeded zero and (2) the predicted vegetation prey weight reached its peak.

In investigating diet composition, Blue Catfish were divided into two size classes (<563 mm and $\geq$563 mm) according to the 50% probability threshold for fish prey in the diet from the GLM, and diets were analyzed across seasons, where sample sizes allowed (spring and fall). Incidentally ingested prey items, such as rocks and woody debris, were

removed from analyses. Presence of anthropogenic prey items was noted, and these items were removed from further analyses of the dietary indices presented below. Percent frequency of occurrence, which is an index of how common a particular prey item is in the diet of a consumer, was calculated for each unique prey taxon found in the diet of either size class of Blue Catfish using the following equation:

$$\%FO_j = \frac{N_j}{P} \times 100,$$

where N_j equals the number of stomachs containing prey type j, and P is the total number of stomachs sampled containing prey items [53,91]. Percent frequency of occurrence is useful in assessing diet composition, as this metric may reduce bias associated with differing digestion rates of prey items [92,93]. To assess primary prey composition of Blue Catfish across ontogeny, prey were sorted into groups based on taxonomic similarity, and $\%FO_j$ of prey groups that were found in >2% of stomachs across seasons was calculated for 100 mm total length bins (individuals <200 mm or >800 mm were grouped into single bins due to lower sample sizes of these groups).

Mean percent by weight ($\%MW_j$) was calculated to interpret the relative importance of each prey taxon to the energy budget of Blue Catfish using the following equation adapted from Chipps and Garvey (2007) [92]:

$$\%MW_j = \frac{1}{P}\sum_{i=1}^{P}\left(\frac{W_{ji}}{\sum_{j=1}^{J} W_{ji}}\right) \times 100,$$

where W_{ji} equals the wet weight of prey type j in the diet of predator i, and J equals the number of unique prey types [53,91].

Population-level diet metrics were calculated using pooled stomach content weights across Blue Catfish size classes (k). Individual prey weights were normalized to proportions within each stomach and then aggregated by size class to calculate diet proportions (DC_{jk}) for either size class of Blue Catfish across all seasons. Diet proportions using weight data were used in these calculations, as they may be better approximated by gravimetric diet metrics than occurrence-based metrics [43,92].

Consumer trophic levels ($Troph_k$) were estimated for each size class k using DC_{jk} with the following equation:

$$Troph_k = 1 + \sum_{j=1}^{J}\left(Troph_j \cdot DC_{jk}\right)$$

where $Troph_j$ is the trophic level of the jth prey type sourced from FishBase [94]. Generic values for phylum or family were used when lower taxonomic identification was unavailable, and mean trophic levels from potential genera were utilized when only genus-level identification was available.

To measure variation in prey trophic levels, the omnivory index (OI_k) was calculated following Christensen and Walters (2004) as the variance of prey trophic levels for each size class using the equation [95]

$$OI_k = \sum_{j=1}^{J}(Troph_j - (Troph_k - 1))^2 \cdot DC_{jk}.$$

Omnivory index values approaching or exceeding 1.0 indicate a highly varied diet that spans multiple levels of the food chain. Diet breadth was evaluated for each size class

of Blue Catfish using Levin's standardized index ($B_{A,k}$) according to the following equation from Krebs et al. (1989) [96]:

$$B_{A,k} = \left(\frac{1}{J-1}\right)\left(\left(\frac{1}{\sum_{j=1}^{J} DC_{jk}^2}\right) - 1\right).$$

Levin's standardized index values range from zero to one; values close to zero indicate a limited diet breadth, whereas values closer to one indicate a highly generalist population diet. Statistical uncertainty and 95% confidence intervals for all three indices ($Troph_k$, OI_k, and $B_{A,k}$) were generated via non-parametric bootstrapping with 2000 replications. To account for non-independent prey items within individual stomachs, resampling with replacement was performed at the fish level. For each simulated size class, metrics were re-pooled, and final 95% CIs were extracted using the percentile method in R.

Delta values δ were calculated for all stable isotope data as follows:

$$\delta^X = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000$$

where X is the heavy isotope of each element (^{15}N, ^{13}C, ^{34}S), R_{sample} is the ratio of heavy to light isotopes in an individual sample, and $R_{standard}$ is the ratio of heavy to light isotopes in an internationally recognized standard reference material (Pee Dee Belemite for carbon, air for nitrogen, and Canyon Diablo Troilite for sulfur stable isotope analyses).

To evaluate ontogenetic changes in stable isotope composition while accounting for multiple samples per individual Blue Catfish, we fit separate linear mixed-effects models (LMMs) for δ^{13}C, δ^{15}N, and δ^{34}S with the lme4 package in R [97]. Each model included Blue Catfish total length as a fixed effect and individual as a random intercept.

We evaluated the isotopic niche characteristics of each size class of Blue Catfish (<563 mm and ≥563 mm), as informed by SCA, by modeling bivariate normal distributions over mean individual δ^{13}C and δ^{15}N data with the SIBER package in R [98]. Using maximum likelihood estimators, core isotopic niche widths were first calculated as sample size-corrected Standard Ellipse Areas (SEA_c), which enclose approximately 40% of the data. To incorporate and account for uncertainty in sample data, Bayesian inference was used to estimate the posterior distribution of the Standard Ellipse Areas (SEA_b (Bayesian Standard Ellipse Areas)). Bayesian models were executed using a vague normal prior on the means ($\tau_\mu = 1.0 \times 10^{-3}$) and a vague inverse-Wishart prior on the covariance matrix (k = 2, scaled by a 2 × 2 identity matrix). Markov Chain Monte Carlo simulations were run using three parallel chains for 20,000 iterations per chain (burn-in = 1000, thinning interval = 10, independent posterior samples = 5700), and model convergence was verified for all marginal parameters using the potential scale reduction factor ($\widehat{R}$) (≤1.01) and Effective Sample Size (ESS) (>1000) in the coda package in R [99]. Core resource sharing between size classes was quantified by calculating the Bayesian posterior distribution of the ellipse overlap area across all MCMC draws.

To visualize food web structure and trends in stable isotopic composition in different species, carbon–nitrogen and carbon–sulfur biplots were constructed in R using mean isotopic values and associated standard deviations from individuals.

Trophic level was estimated for species with sample size $n \geq 4$ using Bayesian regression models applied to δ^{15}N data with the trps package in R. The trophic position equation used in the trps package is derived from Post et al. (2002) [100] and Vander Zanden and Vadeboncoeur (2002) [101]. Trophic enrichment factors and associated standard deviations from Stephens et al. (2023) [64] (muscle tissue for omnivorous and carnivorous fishes in mixed-carbon-source systems) were used as informative priors in the models. The mod-

els for carnivorous fish (large Blue Catfish, Largemouth Bass, Northern Snakehead, and White Perch) used a trophic enrichment factor (Δ^{15}N) prior mean of 2.9‰ and a standard deviation of 0.5‰. Models for omnivorous fishes (small Blue Catfish, Banded Killifish, and Satinfin Shiner) used a trophic enrichment factor (Δ^{15}N) prior of 3.3‰ and standard deviation of 0.2‰. All models used the mean δ^{15}N and standard deviation values from Gizzard Shad as a baseline with a trophic position of 2.40 (λ) from FishBase and were run with four chains, 2000 warmups, 4000 iterations, and adapt_delta set to 0.99 [94]. Model convergence was confirmed using $\widehat{R} \leq 1.01$ and ESS > 1000. Median trophic level estimates and 95% credible intervals for each species were extracted from Bayesian posterior distributions from model posterior draws.

3. Results

3.1. Stomach Content Analyses

A total of 363 Blue Catfish stomachs, or 52.2% of fish collected from spring to fall, contained prey items, and the remaining 333 stomachs were empty. Sample Coverage ($\widehat{C}$) was 0.96 from stomach samples pooled from both size classes across all seasons (spring–fall), indicating that this sample size was sufficient to capture 96% of the total prey occurrences in the diet, suggesting sufficiency for inference (Figure 2).

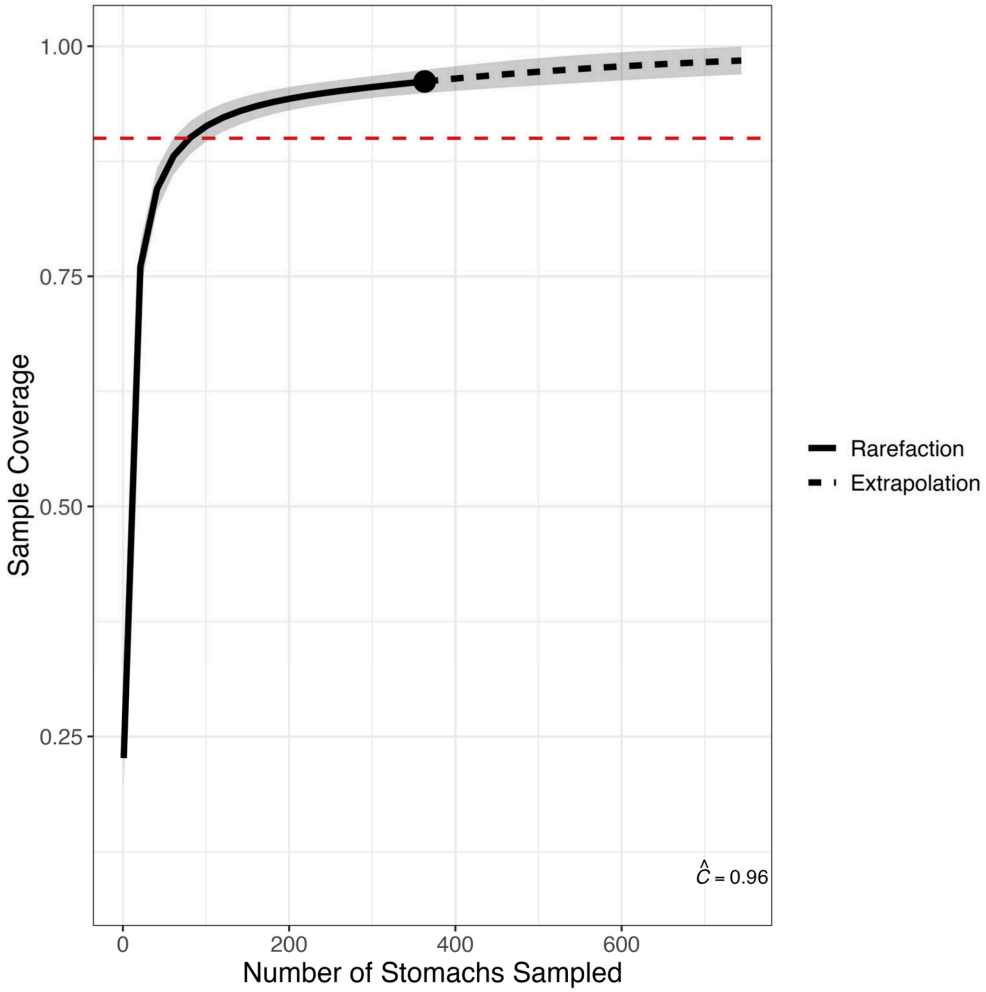


Figure 2. Plot of Sample Coverage ($\widehat{C}$) rarefaction curves from non-empty Blue Catfish stomachs (n = 363) collected from tidal, low-salinity reaches of the Nanticoke River and Marshyhope Creek from October 2021 through November 2022, extrapolated to up to twice the sample size. The horizontal red dashed line indicates the 0.90 $\widehat{C}$ sample size sufficiency threshold.

Fifty-percent probability thresholds from GLMs for fish and vegetation presence in stomach contents were 563 mm (95% CI: 522–605 mm) and 515 mm (95% CI: 465–566 mm), respectively (Figure 3). The Blue Catfish ontogenetic diet shift to piscivory was estimated at 563 mm based on the 50% probability threshold of fish prey in stomach contents, and fish were divided into two size classes to facilitate relevant stomach content and stable isotope analyses: <563 mm (hereafter "small Blue Catfish") and ≥563 mm (hereafter "large Blue Catfish").

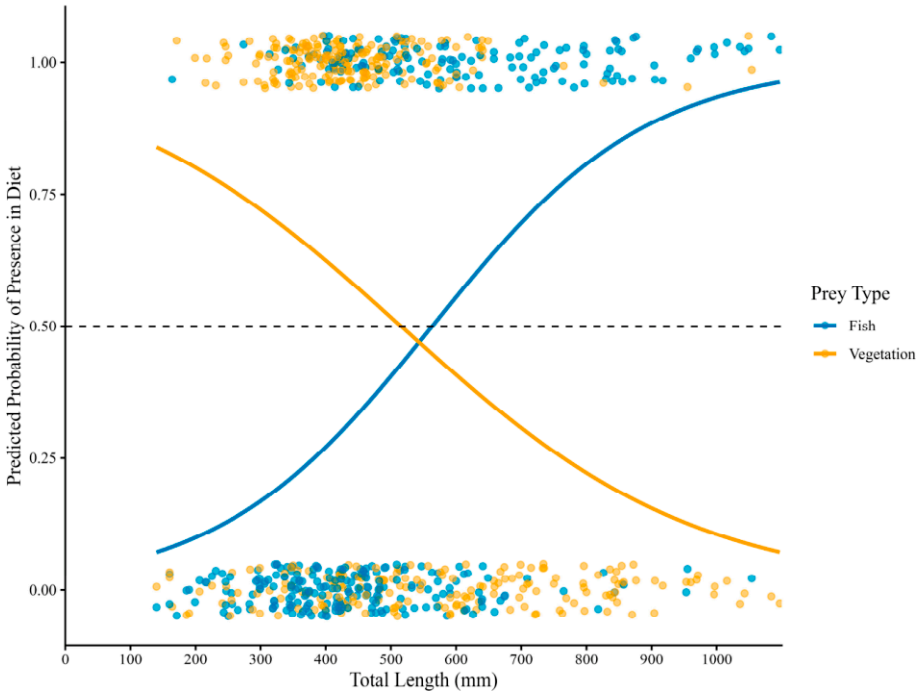


Figure 3. Logistic regression depicting the predicted probability of fish prey and vegetation (1 = present, 0 = absent) in the diet of Blue Catfish (n = 363) in tidal, low-salinity reaches of the Nanticoke River and Marshyhope Creek across ontogeny, with the dashed line representing the 50% probability threshold.

Sample Coverage was insufficient during the summer for both size classes ($\widehat{C}$ = 0.87 and 0.76 for <563 mm ≥563 mm, respectively); therefore, summer diets are not characterized individually using $\%FO_j$ and $\%MW_j$. Sample Coverage ($\widehat{C}$) reached or exceeded the 0.90 threshold for all other size classes and seasons, except for small Blue Catfish during the spring ($\widehat{C}$ = 0.89) (Figure 4). This indicates that while some rare species in the diet of small Blue Catfish during the spring may have been undetected, since $\widehat{C}$ missed our threshold by only 0.01, sample size was considered sufficient to characterize general dietary trends.

The GAM of fish prey weight explained 54% of the total deviance, and Blue Catfish total length had a statistically significant non-linear effect on prey weight (adjusted R^2 = 0.39, n = 152, edf = 3.85, F = 51.87, $p \leq 0.001$). Assessment of quantile residuals from GAMs showed no significant deviations in residual uniformity or heteroscedasticity. The median total length at which Blue Catfish total length began to positively affect fish prey weight from the GAM was estimated at 569 mm (95% CrI: 549–591 mm) (Figure 5). The Tweedie GAM of vegetation weight explained 21.4% of the total deviance (adjusted R^2 = 0.13, n = 187). Total length also showed a strong non-linear relationship with vegetation weight (edf = 3.74, F = 17.76, $p \leq 0.001$), and diagnostic checks confirmed satisfactory residual uniformity and dispersion profiles. The median total length at which vegetation weight in the diet peaked per the GAM was 574 mm (95% CrI: 507–744 mm) (Figure 5).

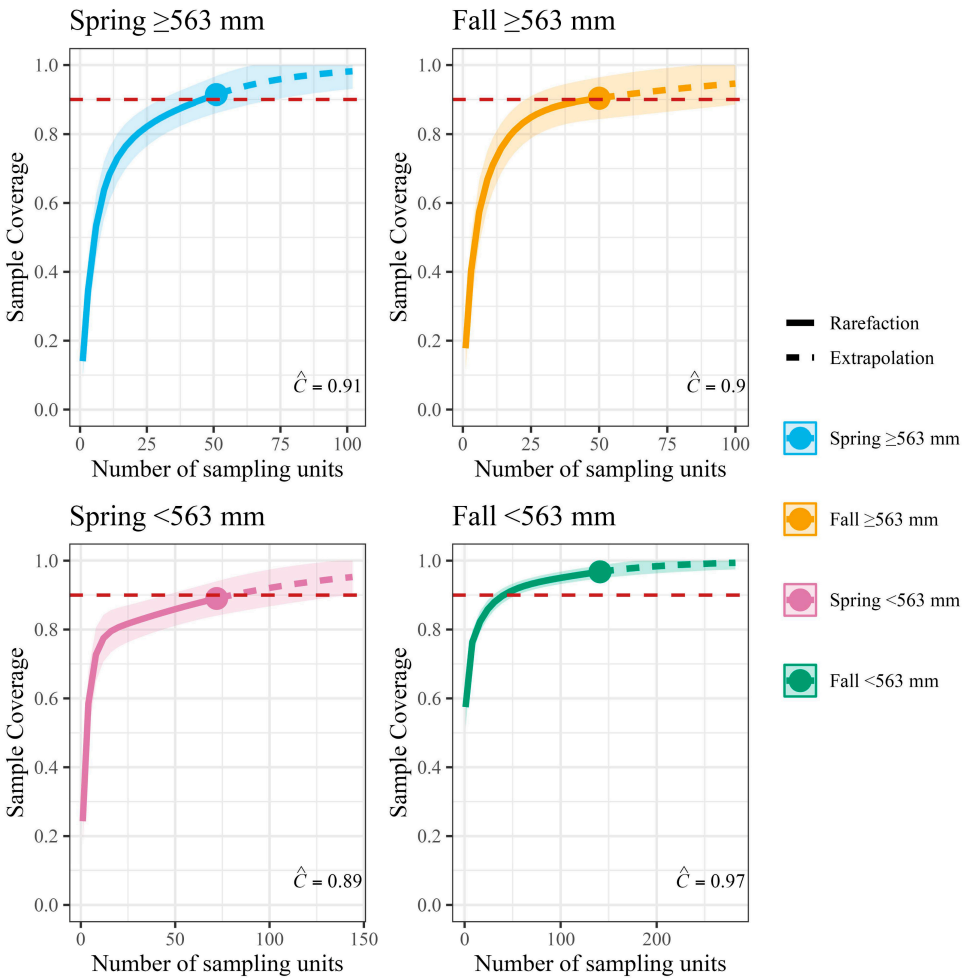


Figure 4. Dietary Sample Coverage rarefaction curves from non-empty Blue Catfish stomachs collected from tidal, low-salinity reaches of the Nanticoke River and Marshyhope Creek from October 2021 through November 2022, split by size class (<563 mm and ≥563 mm) and season, extrapolated to up to twice the sample size. The horizontal red dashed line indicates the 0.90 $\hat{C}$ sample size sufficiency threshold, and $\hat{C}$ values are displayed in the bottom right corner of each panel.

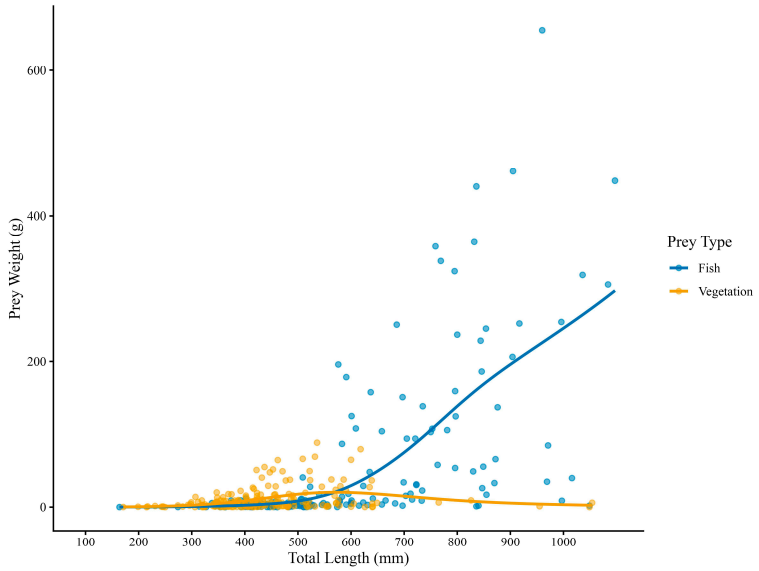


Figure 5. Plot depicting observed fish and vegetation prey weights in the diet of Blue Catfish in tidal, low-salinity reaches of the Nanticoke River and Marshyhope Creek, along with trend lines from generalized additive models, across ontogeny.

Forty-five unique prey types from a wide array of taxa were identified in Blue Catfish stomachs between the spring and fall (n = 363) (Table 3). Diet composition varied between seasons and size classes, reflecting decreased utilization of vegetation, detritus, amphipods, and bivalves and increased predation on fish and blue crab over ontogeny (Figures 6 and 7). The SCA trophic level and diet breadth estimates for small Blue Catfish suggests that small individuals function as mid-level omnivores (2.68, 95% CI: 2.56–2.81), feeding across a narrow suite of primary prey (Table 4). The omnivory index estimate suggests that despite utilizing a narrow taxonomic prey range, small Blue Catfish consume prey across multiple trophic levels. Vegetation was a primary component in the diet of small Blue Catfish, particularly during the fall, when it was found in 85.1% of non-empty stomachs and made up 77.7% MW_j (Table 3; Figure 6). In the fall, vegetation primarily consisted of individual seeds and seed pods of arrow arum, whereas during spring and summer seasons, plant material in stomachs was generally more composed of leaves from riparian vegetation and small amounts of submerged aquatic plant matter. Invasive Asian clams were frequent prey items for small Blue Catfish, especially during the spring season, when they were found in 20.8% of non-empty stomachs and accounted for 8.0% MW_j. Gammarid and corophid amphipods were occasional prey of small Blue Catfish. Unidentified fish were relatively common prey items in small Blue Catfish stomachs across seasons (12.1–22.2% FO_j). Atlantic Menhaden were the most common of the 12 unique fish prey taxa identified in small Blue Catfish stomachs, comprising 5.0% FO_j and 3.0% MW_j during the fall season. Spottail Shiner *Hudsonius hudsonius*, *Ictalurus* spp., and Atlantic Menhaden were amongst the most common fish prey in small Blue Catfish stomachs during the spring. Blue crabs were found in 1.4% and 3.5% of non-empty small Blue Catfish stomachs during the spring and fall, respectively. Other prey items such as estuarine mud crabs, pond snails, other bivalves, grass shrimp *Palaemon vulgaris*, and insects were inconsistently detected in small Blue Catfish stomachs across seasons.

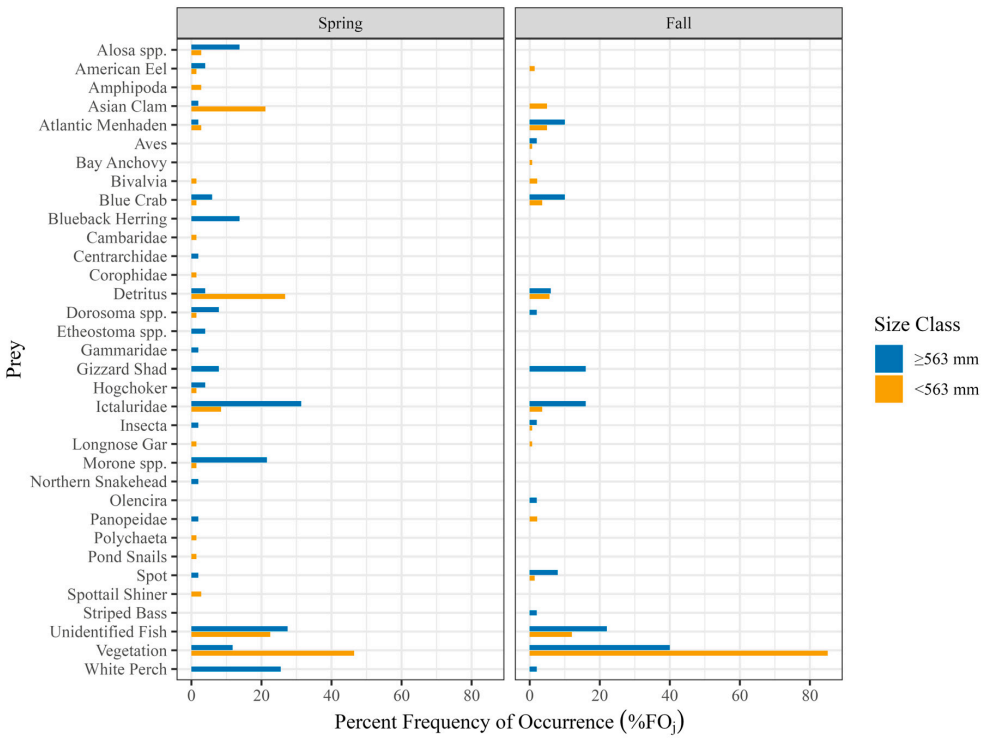


Figure 6. Percent frequency of occurrence ($\%FO_j$) of prey (grouped by species, family, class, order, or phylum (excluding anthropogenic items)) found in the diet of Blue Catfish <563 mm and ≥563 mm total length, listed in alphabetical order from top to bottom, with panels representing seasons (fall: September–November; spring: March–May).

Table 3. Percent frequency of occurrence (%FO_j) and mean percent weight (%MW_j) (rounded to the nearest tenth) of prey items (listed at the lowest taxonomic level possible) found in the diet of Blue Catfish from two size classes (<563 mm and ≥563 mm) during two seasons (spring: March–May; fall: September–November) as well as from all sizes collected from spring to fall (including summer samples; summer diet information is not characterized independently due to insufficient sample size). Samples were collected opportunistically from tidal, low-salinity reaches of the Nanticoke River and Marshyhope Creek in Maryland between October 2021 and November 2022. Prey items are sorted alphabetically by phylum, class, family, and genus and species (* denotes Order). The common name is provided when available. "-" indicates no data, and "*" indicates that the prey item was present but excluded from %FO_j and %MW_j calculations.

		<563 mm				**≥563 mm**				**All Sizes**	
Prey Item	**Common Name**	**Spring**		**Fall**		**Spring**		**Fall**		**All Seasons**	
		%MW_j	**%FO_j**	**%MW_j**	**%FO_j**	**%MW_j**	**%FO_j**	**%MW_j**	**%FO_j**	**%MW_j**	**%FO_j**
Annelida											
Polychaeta		0.1	1.4	-	-	-	-	-	-	0.0	0.3
Arthropoda											
Amphipoda *		1.6	2.8	-	-	-	-	-	-	0.4	1.4
Insecta											
Ephemeridae											
Hexagenia spp.	mayfly	-	-	-	-	0.0	2.0	-	-	0.0	0.3
Formicidae											
Formica spp.		-	-	-	-	-	-	0.0	2.0	0.0	0.3
Malacostraca											
Anthuridae											
Cyathura polita		-	-	-	-	-	-	-	-	0.2	0.3
Cambaridae											
Orconectes limosus	spiny-cheek crayfish	1.4	1.4	-	-	-	-	-	-	0.3	0.3
Corophidae		-	-	-	-	-	-	-	-	0.5	1.1
Apocorophium spp.		0.0	1.4	-	-	-	-	-	-	0.0	0.3
Gammaridae		-	-	-	-	2.0	2.0	-	-	0.4	0.6
Palaemonidae											
Panopeidae	unidentified mud crab	-	-	0.0	0.7	-	-	-	-	0.3	0.6
Rhithropanopeus harrisii	estuarine mud crab	-	-	0.2	0.8	0.7	1.9	0.0	2.0	0.5	1.1
Portunidae											
Callinectes sapidus	blue crab	0.9	1.4	1.6	3.5	4.3	5.9	4.9	10.0	4.0	5.8
Chordata											
Actinopterygii	Unidentified Fish	12.5	22.2	7.0	12.1	18.9	27.5	17.9	22.0	13.5	19.8
Achiridae											
Trinectes maculatus	Hogchoker	1.4	1.4	-	-	1.7	3.9	-	-	0.5	0.8

Table 3. *Cont.*

Prey Item	Common Name	<563 mm				≥563 mm				All Sizes	
		Spring		Fall		Spring		Fall		All Seasons	
		$\%MW_j$	$\%FO_j$	$\%MW_j$	$\%FO_j$	$\%MW_j$	$\%FO_j$	$\%MW_j$	$\%FO_j$	$\%MW_j$	$\%FO_j$
Alosidae											
Alosa aestivalis	Blueback Herring	-	-	-	-	11.9	13.7	-	-	1.7	1.9
Alosa pseudoharengus	Alewife	-	-	-	-	3.1	3.9	-	-	0.4	0.6
Alosa spp.	unidentified *Alosa*	1.4	1.4	-	-	4.0	3.8	-	-	0.9	0.9
Anguillidae											
Anguilla rostrata	American Eel	0.5	1.4	0.8	1.4	0.0	3.9	-	-	0.4	1.4
Atherinopsidae											
Centrarchidae											
Lepomis spp.		-	-	-	-	-	2.0	-	-	0.0	0.3
Channidae											
Channa argus	Northern Snakehead	-	-	-	-	0.0	2.0	-	-	0.0	0.3
Clupeidae											
Brevoortia tyrannus	Atlantic Menhaden	1.4	2.8	3.0	5.0	0.0	2.0	10.0	10.0	5.0	6.9
Dorosomatidae											
Dorosoma cepedianum	Gizzard Shad	-	-	-	-	7.6	7.8	15.0	16.0	3.1	3.3
Dorosoma spp.		1.4	1.4	-	-	0.4	7.8	0.0	2.0	0.3	1.7
Engraulidae											
Ictaluridae		2.1	2.8	1.2	2.1	3.1	3.9	0.6	2.0	1.9	2.8
Ictalurus furcatus	Blue Catfish	1.2	1.4	-	-	3.0	3.9	4.0	4.0	1.2	1.4
Ictalurus spp.		0.5	4.2	0.0	1.4	0.1	23.5	0.1	10.0	0.1	8.3
Lepisosteidae											
Lepisosteus osseus	Longnose Gar	1.4	1.4	0.0	0.7	-	-	-	-	0.3	0.6
Leuciscidae											
Hudosnius hudsonius	Spottail Shiner	1.8	2.8	-	-	-	-	-	-	0.4	0.6
Moronidae											
Morone americana	White Perch	-	-	-	-	20.6	25.5	1.9	2.0	3.2	3.9
Morone saxatilis	Striped Bass	-	-	-	-	-	-	0.1	2.0	0.0	0.3
Morone spp.		0.0	1.4	-	-	5.0	21.6	-	-	1.0	4.4
Percidae											
Etheostoma spp.		-	-	-	-	0.4	3.9	-	-	0.1	0.6
Sciaenidae											
Bairdiella chrysoura	Silver Perch	-	-	-	-	-	-	-	-	0.0	0.3
Cynoscion spp.		-	-	-	-	-	-	-	-	0.3	0.3
Leiostomus xanthurus	Spot	-	-	0.1	1.4	0.0	2.0	6.1	8.0	1.7	3.0

Table 3. *Cont.*

		<563 mm				≥563 mm				All Sizes	
Prey Item	**Common Name**	**Spring**		**Fall**		**Spring**		**Fall**		**All Seasons**	
		%MW$_j$	**%FO$_j$**	**%MW$_j$**	**%FO$_j$**	**%MW$_j$**	**%FO$_j$**	**%MW$_j$**	**%FO$_j$**	**%MW$_j$**	**%FO$_j$**
Aves		-	-	0.0	0.7	-	-	-	-	0.0	0.3
Anatidae											
Aix sponsa	wood duck	-	-	-	-	-	-	2.0	2.0	0.3	0.3
Reptilia											
Kinosternidae		-	-	-	-	-	-	-	-	0.3	0.3
Mollusca											
Bivalvia		0.0	1.4	-	-	-	-	-	-	0.0	0.3
Cyrenidae											
Corbicula fluminea	Asian clam	8.0	20.8	3.1	5.0	0.2	2.0	-	-	2.9	6.9
Tellinidae											
Macoma tenta	elongate macoma	-	-	0.3	0.7	-	-	-	-	0.1	0.3
Dreissenidae											
Mytilopsis leucophaeata	false mussel	-	-	0.7	1.4	-	-	-	-	0.3	0.6
Gastropoda											
Lymnaeidae	pond snail	0.6	1.4	-	-	-	-	-	-	0.1	0.3
Anthropogenic items											
Chicken Neck		*	*	*	*	-	-	-	-	*	*
Plastic Fishing Worm		-	-	-	-	-	-	*	*	*	*
Detritus		21.4	26.4	3.8	5.7	2.8	3.9	3.8	6.0	8.0	10.5
Vegetation		40.4	45.8	77.7	85.1	10.1	11.8	33.7	40.0	45.1	51.5

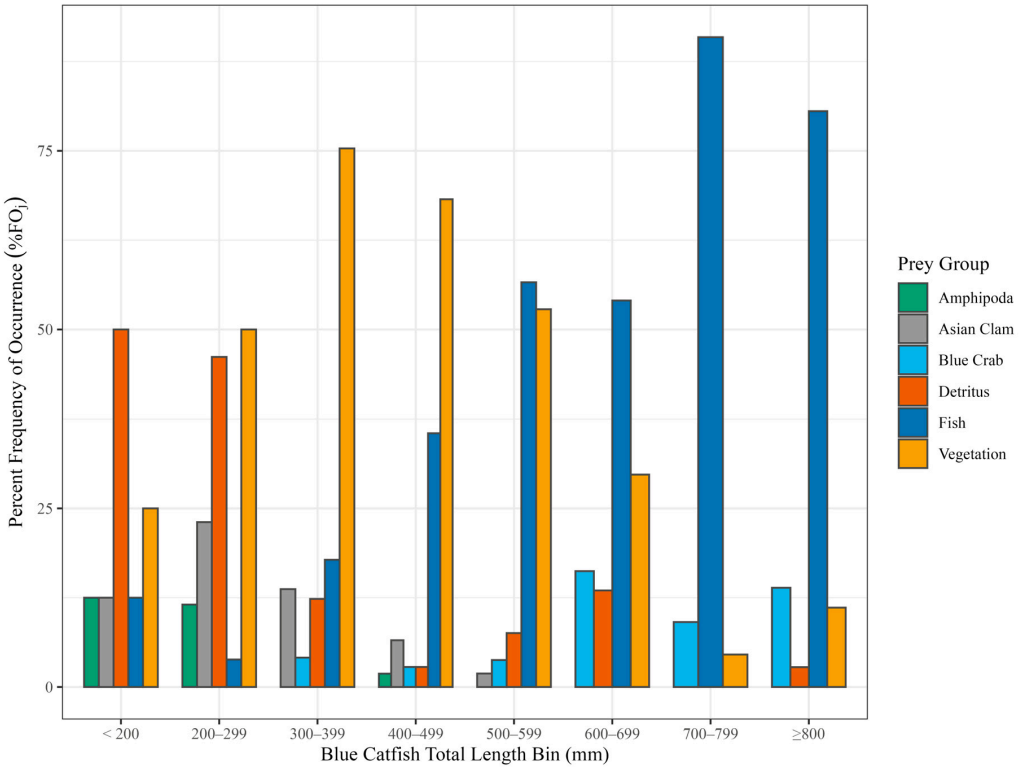


Figure 7. Percent frequency of occurrence of primary prey groups in the diet of Blue Catfish (100 mm total length bins) collected from the Nanticoke River watershed from October 2021 to November 2022.

Table 4. Population-level consumer trophic level (from stomach content analysis), omnivory index (where 0 = limited predation across trophic levels, 1 = considerable predation across trophic levels), and diet breadth (Levin's standardized index, where 0 = limited diet breadth, 1 = considerable diet breadth) estimates rounded to the nearest hundredth and associated 95% confidence intervals for two size classes of Blue Catfish (<563 mm and ≥563) collected throughout tidal, low-salinity reaches of the Nanticoke River and Marshyhope Creek.

Size Class	$Troph_k$ (95% CI)	OI_k (95% CI)	BA_k (95% CI)
<563 mm	2.68 (2.56–2.81)	1.05 (0.90–1.18)	0.05 (0.04–0.06)
≥563 mm	3.69 (3.53–3.86)	0.99 (0.79–1.16)	0.19 (0.14–0.22)

The trophic level estimate for large Blue Catfish using stomach content data was 3.69 (95% CI: 3.53–3.86), indicating that large individuals function as upper-level carnivorous omnivores. Omnivory index and diet breadth values suggest utilization of prey across a broad range of trophic levels and a higher niche width than was observed in small Blue Catfish (Table 4). Fish prey were common in the diet of large Blue Catfish; a total of 22 fish prey taxa were identified, and species composition varied seasonally (Table 3; Figure 6). Unidentified fish were common in large Blue Catfish diet results across seasons due to advanced stages of digestion often obscuring identifying characteristics and preventing identification through genetic analyses.

During the spring, White Perch were the most common prey item of large Blue Catfish and were found in 25.5% of non-empty stomachs, making up 20.6% MW_j. It should be noted that *Morone* spp. were also frequent prey items for large Blue Catfish; this is due to genus-level identification from genetic analyses. We assume the majority of *Morone* spp. were White Perch due to the relative rarity of Striped Bass visually identified in stomach contents. *Ictalurus* spp. were also common prey of large Blue Catfish, which could have been augmented by the presence of host DNA in tissue samples confounding identification

using genetic analyses. Gizzard Shad were common prey in both the spring and fall, accounting for 7.6% MW_j and 7.8% FO_j and 15.0% MW_j and 16.0% FO_j, respectively. River herring and *Alosa* spp. were found relatively frequently in large Blue Catfish stomachs during the spring. Predation on river herring was primarily limited to Blue Catfish greater than 700 mm. River herring (Alewife and Blueback Herring *Alosa aestivalis*) were the only *Alosa* species identified visually within stomach contents. Genetic analyses used in this study provide only genus-level identification; however, it is notable that no American Shad *Alosa sapidissima* or Hickory Shad *Alosa mediocris* were identified visually in catfish stomachs, and no individuals were encountered in boat electrofishing collections during springtime, when they would likely be migrating through the system to spawn. However, both species are documented in the Nanticoke River. Atlantic Menhaden was a common prey item of large Blue Catfish during the fall, making up 10.0% FO_j and 10% MW_j. Spot *Leiostomus xanthurus* and ictalurids were also relatively common fish prey of large Blue Catfish. Other fishes, such as centrarchids, American Eel, Silver Perch *Bairdiella chrysoura*, Hogchoker *Trinectes maculatus*, Northern Snakehead *Channa argus*, and *Etheostoma* spp., were relatively infrequently consumed across seasons.

Blue crabs, present in the diet of large Blue Catfish across all seasons, were frequently encountered prey items during the fall (10.0% FO_j) and made up 4.9% MW_j. Vegetation was also a relatively common prey item for large Blue Catfish, particularly during the fall, when it was found in 40.0% of non-empty stomachs and made up 33.7% MW_j; consumption of vegetation was primarily limited to individuals with total lengths less than 700 mm (Figure 7). Other invertebrates, such as amphipods, bivalves, and estuarine mud crabs, were infrequently detected in large Blue Catfish stomachs. Several unusual prey items were found in the stomachs of Blue Catfish, such as a Wood Duck *Aix sponsa* and a turtle from the mud and musk turtle family, Kinosternidae. Anthropogenic items, including plastic food packaging and chicken necks, were found in stomach samples infrequently.

3.2. Stable Isotope Analyses

Mean $\delta^{15}N$ values for small (n = 21 individuals) and large (n = 33 individuals) Blue Catfish were 16.3‰ and 17.0‰, respectively (Table 5).

The linear mixed-effects model for nitrogen revealed a significant positive relationship between $\delta^{15}N$ and Blue Catfish total length (Figure 8) (β = 0.00123, $t_{51.7}$ = 4.42, $p < 0.001$). Variance partitioning showed that differences between individual Blue Catfish accounted for 66.8% of the random variance, while the remaining 33.2% was due to residual variation between replicate samples. Mean percent nitrogen ranged from 13.7 to 17.5% across species. Mean $\delta^{15}N$ values for mesopredatory fishes, White Perch, Yellow Perch *Perca flavescens*, and American Eel, were 15.0‰, 16.1‰, and 15.6‰, respectively. Bluegill show a relatively enriched mean $\delta^{15}N$ value of 15.9‰ (Figure 9). Native White Catfish have a higher mean $\delta^{15}N$ value (16.0‰) than introduced Channel Catfish *Ictalurus punctatus* (15.0‰). Another invasive fish, Northern Snakehead, exhibited a relatively depleted mean $\delta^{15}N$ value of 13.0‰, though values ranged widely between individuals (standard deviation = 2.4). Planktivorous forage fishes, such as Gizzard Shad, Atlantic Menhaden, and river herring (i.e., Alewife and Blueback Herring), were the least ^{15}N-enriched of all fishes (Table 5). Arrow arum exhibited the lowest mean $\delta^{15}N$ of 7.5‰.

Mean carbon ($\delta^{13}C$) values of all species analyzed range from −27.6‰ to −18.6‰, and migratory species with marine life history components, including river herring and Atlantic Menhaden, were most enriched in ^{13}C (Table 5). Mean percent carbon ranged from 41.3% to 55.3%. Blue Catfish exhibited a significant increasing trend in ^{13}C enrichment across ontogeny (Figure 8) (β = 0.00145, $t_{52.0}$ = 2.68, p = 0.010). Individual fish identity captured nearly all unresolved model variance at 98.4%, leaving 1.6% to variation between replicate

samples. Species that seasonally migrate between fresh and saltwater (diadromous), including Alewife, Menhaden, Blueback Herring, and Striped Bass, had the highest $\delta^{13}C$ values. Hogchoker *Trinectes maculatus* and White Catfish were relatively enriched in ^{13}C (mean $\delta^{13}C$ of −23.0‰ and −23.7‰). White Perch exhibited relatively low ^{13}C enrichment (mean $\delta^{13}C$ = −27.1). Fishes that primarily reside in freshwater habitats, including Common Carp *Cyprinus carpio*, Gizzard Shad, Largemouth Bass, Bluegill, and Brown Bullhead *Ameiurus nebulosus*, had relatively depleted $\delta^{13}C$ values, along with arrow arum.

Table 5. Common names, mean $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values (±one standard deviation), percentages of C, N, and S (±one standard deviation), and median trophic level estimates from Bayesian regression models using $\delta^{15}N$ (including 95% credible intervals (CrIs)), for each species collected in the Nanticoke River watershed from October 2021 through October 2023. Values are sorted alphabetically by common name, and "-" indicates no data.

Common Name	Mean $\delta^{13}C$	Mean $\delta^{15}N$	Mean $\delta^{34}S$	Mean %C	Mean %N	Mean %S	Trophic Level (95% CrI)
Alewife	−18.6 ± 0.2	12.0 ± 0.3	17.7 ± 0.2	49.7 ± 0.9	16.8 ± 0.8	1.1 ± 0.1	-
American Eel	−24.3 ± 2.1	15.6 ± 0.7	-	46.0 ± 2.7	14.2 ± 1.2	-	-
Arrow Arum (plant)	−26.3 ± 1.4	7.5 ± 1.1	-	-	-	-	-
Atlantic Menhaden	−18.9 ± 0.3	12.9 ± 0.2	16.0 ± 0.6	42.0 ± 0.1	14.6 ± 0.4	0.9 ± 0.0	-
Banded Killifish	−26.4 ± 0.6	14.9 ± 0.2	8.2 ± 0.4	44.3 ± 1.4	14.8 ± 0.6	1.1 ± 0.1	3.26 (3.00–3.54)
Blue Catfish (<563 mm)	−24.5 ± 1.1	16.3 ± 0.5	11.2 ± 3.1	45.0 ± 1.7	15.0 ± 1.1	1.5 ± 0.2	3.70 (3.45–3.96)
Blue Catfish (≥563 mm)	−23.8 ± 1.0	17.0 ± 0.5	9.0 ± 2.0	44.8 ± 3.0	14.2 ± 1.1	1.3 ± 0.1	3.92 (3.62–4.21)
Blue Crab	−26.1 ± 0.9	14.0 ± 0.8	-	41.3 ± 3.4	14.0 ± 1.7	-	-
Blueback Herring	−19.6 ± 0.1	12.1 ± 0.1	17.3 ± 0.1	43.8 ± 0.3	15.1 ± 0.3	0.9 ± 0.0	-
Bluegill	−26.0 ± 0.9	15.9 ± 1.2	7.9	45.6 ± 0.4	14.9 ± 0.2	1.1	-
Brown Bullhead	−27.6 ± 1.3	13.2 ± 0.6	-	50.3 ± 0.8	17.5 ± 0.0	-	-
Channel Catfish	−25.1 ± 0.9	15.0 ± 0.1	-	50.2 ± 0.3	17.5 ± 0.1	-	-
Common Carp	−27.4 ± 1.9	13.4 ± 0.1	-	55.3 ± 0.9	10.3 ± 0.0	-	-
Gizzard Shad	−25.1 ± 5.2	12.0 ± 1.7	5.0 ± 2.1	52.2 ± 6.5	10.8 ± 3.0	0.9 ± 0.3	-
Hogchoker	−23.0 ± 1.9	13.9 ± 0.8	-	49.5 ± 1.3	17.3 ± 0.4	-	-
Largemouth Bass	−26.1 ± 0.3	17.0 ± 0.5	4.0 ± 1.0	48.4 ± 2.5	17.0 ± 1.1	1.5 ± 0.1	3.91 (3.43–4.41)
Northern Snakehead	−24.1 ± 1.6	13.0 ± 2.4	8.7 ± 2.4	46.8 ± 2.8	13.7 ± 1.1	1.2 ± 0.1	2.72 (2.10–3.64)
Satinfin Shiner	−25.7 ± 0.7	13.9 ± 0.6	-	45.6 ± 0.6	14.3 ± 0.4	-	2.97 (2.63–3.32)
Spottail Shiner	−26.1 ± 0.9	14.4 ± 0.1	-	49.7 ± 1.3	17.0 ± 0.6	-	-
Striped Bass	−21.8 ± 4.1	15.2 ± 1.2	-	50.5 ± 0.7	17.2 ± 0.2	-	-
White Catfish	−23.7 ± 0.5	16.0 ± 0.2	-	49.3 ± 1.9	17.0 ± 0.8	-	-
White Perch	−27.1 ± 1.8	15.0 ± 1.0	9.2 ± 6.7	46.7 ± 2.9	16.2 ± 1.3	1.4 ± 0.0	3.32 (2.82–3.84)
Yellow Perch	−27.2 ± 1.2	16.1 ± 0.8	-	49.2 ± 1.1	17.1 ± 0.6	-	-

Mean sulfur ($\delta^{34}S$) values ranged from 4.0‰ to 17.7‰ (Table 5). The most enriched ^{34}S tissues were found in the migratory river herring and Atlantic Menhaden, while the most depleted tissues were in Largemouth Bass (mean $\delta^{34}S$ = 4.0‰) and Gizzard Shad (mean $\delta^{34}S$ = 5.0‰) (Table 5; Figure 10). Northern Snakehead were enriched in ^{34}S (mean $\delta^{34}S$ = 8.7‰) relative to another resident predatory freshwater fish, Largemouth Bass. Other freshwater and estuarine species had intermediate values for $\delta^{34}S$, including Bluegill, with a $\delta^{34}S$ of 7.9‰, and Banded Killifish (mean = 8.2‰). White Perch showed a moderately enriched mean $\delta^{34}S$ (9.2‰), though values ranged widely between individuals (standard deviation = 6.7). Blue Catfish were moderately enriched in ^{34}S, with mean $\delta^{34}S$ values of 11.2‰ and 9.0‰ for small and large size classes, respectively. We observed a significant negative relationship between $\delta^{34}S$ and Blue Catfish total length (Figure 8) (β = −0.00410, $t_{45.9}$ = −2.84, p = 0.007). The random intercept for individual accounted for 90.5% of the random error structure, with the remaining 9.5% associated with variance between replicate samples.

Sample size-corrected Standard Ellipse Areas (SEA_c) showed that core isotopic niche breadths were nearly identical between size classes. The SEA_c for large Blue Catfish was = 1.55‰^{2} (95% CrI via SEA_b: 1.09–2.18‰^{2}), and that for small Blue Catfish was 1.56‰^{2} (95% CrI via SEA_b: 1.02–2.36‰^{2}). Despite showing similar isotopic niche breadths, the two size classes exhibited minimal core resource overlap, sharing a maximum likelihood overlap

area of only 0.07‰^{2}, suggesting a shift in core resource use with increasing size (Figure 11). Bayesian overlap analysis confirmed distinct isotopic niche segregation, estimating a median posterior overlap of 0.05‰^{2} (95% CrI: 0.00–0.79‰^{2}, $\widehat{R} \leq 1.002$, ESS ≥ 5580).

All Bayesian regression trophic position models converged ($\widehat{R} = 1.00$), and no divergent transitions were encountered during model fitting. Bulk and Tail effective sample sizes (ESSs) exceeded 6667. Median trophic level estimates range from 2.72 to 3.92 across the species assessed (Table 5). Large Blue Catfish had the highest median trophic level estimate (3.92; 95% CrI: 3.62–4.21), followed by Largemouth Bass (median trophic level = 3.91, with a large 95% CrI of 3.43–4.41). Northern Snakehead notably showed the lowest median trophic level estimate of the species assessed (2.72), though the 95% CrI ranges widely from 2.10 to 3.64. Small Blue Catfish exhibited the third highest median trophic level estimate of the species analyzed at 3.70, with the 95% CrI ranging from 3.45 to 3.96. The median trophic level estimate for White Perch was 3.32, though the 95% CrI ranges widely from 2.82 to 3.84. Notably, SIA median trophic level estimates for Blue Catfish of each size class differed greatly from the trophic level estimates from SCA.

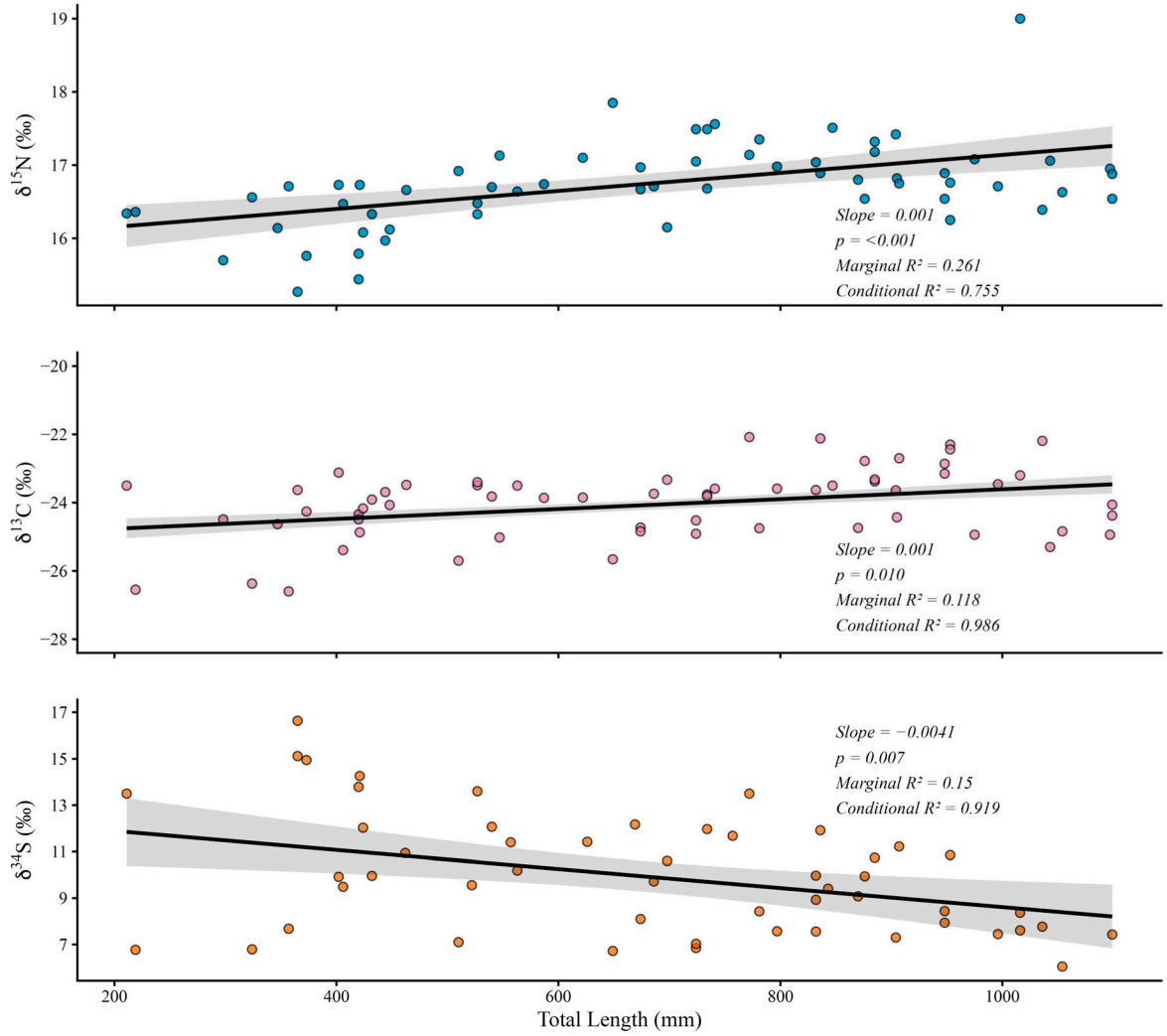


Figure 8. Carbon, nitrogen, and sulfur delta values (δ^{13}C, δ^{15}N, δ^{34}S) plotted against total length of Blue Catfish to evaluate ontogenetic isotopic trends. Regression lines denote fixed-effects trends modeled with a random intercept for individual fish to account for replicate samples. Shaded areas represent 95% confidence intervals, and summary statistics are annotated within each respective panel.

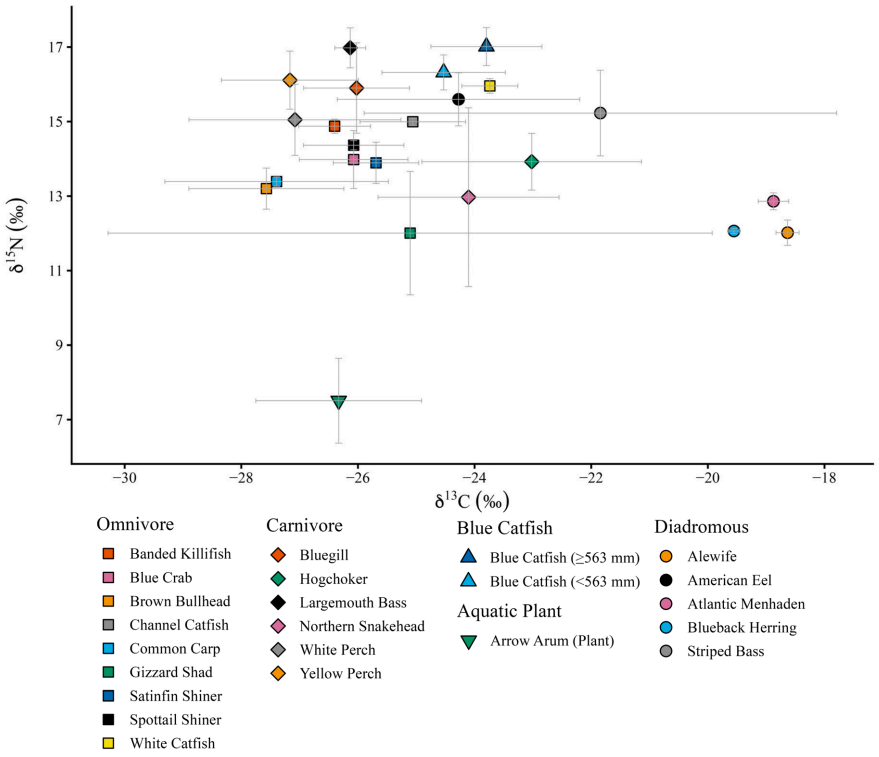


Figure 9. Carbon ($\delta^{13}C$)–nitrogen ($\delta^{15}N$) biplot depicting mean stable isotopic composition of Blue Catfish from two size classes (<563 mm and ≥563 mm) and 21 additional species (shaded shapes) grouped into categories (aquatic plant, diadromous fishes, and resident fishes (split by omnivorous and carnivorous feeding strategies)) to facilitate visual distinction in the plot, with error bars representing ± one standard deviation.

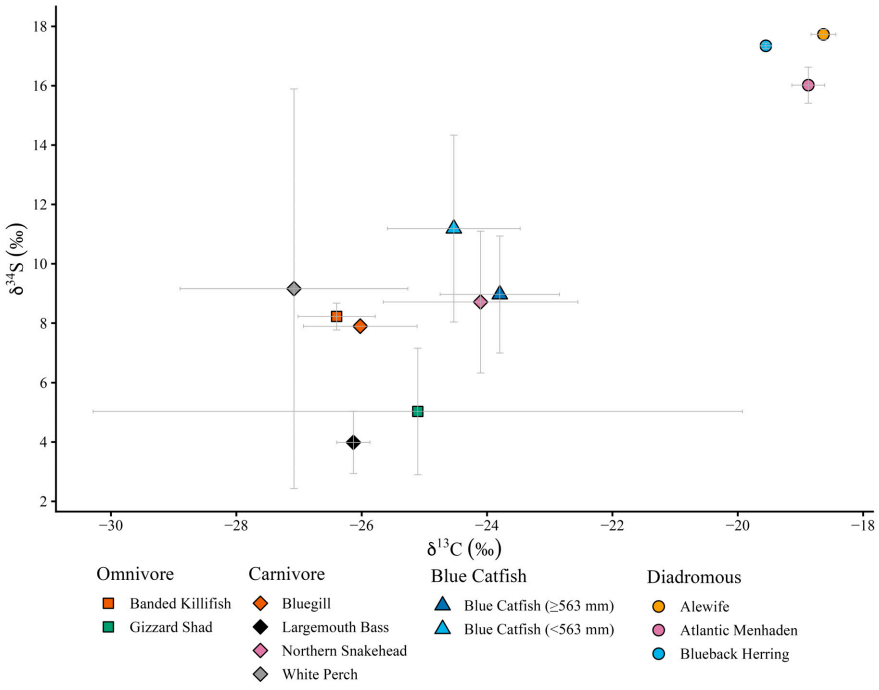


Figure 10. Carbon ($\delta^{13}C$)–sulfur ($\delta^{34}S$) biplot depicting mean stable isotopic composition of Blue Catfish from two size classes (<563 mm and ≥563 mm) and nine additional species (shaded shapes) grouped into categories (aquatic plant, diadromous fishes, and resident fishes (split by omnivorous and carnivorous feeding strategies)) to facilitate visual distinction in the plot, with error bars representing ± one standard deviation.

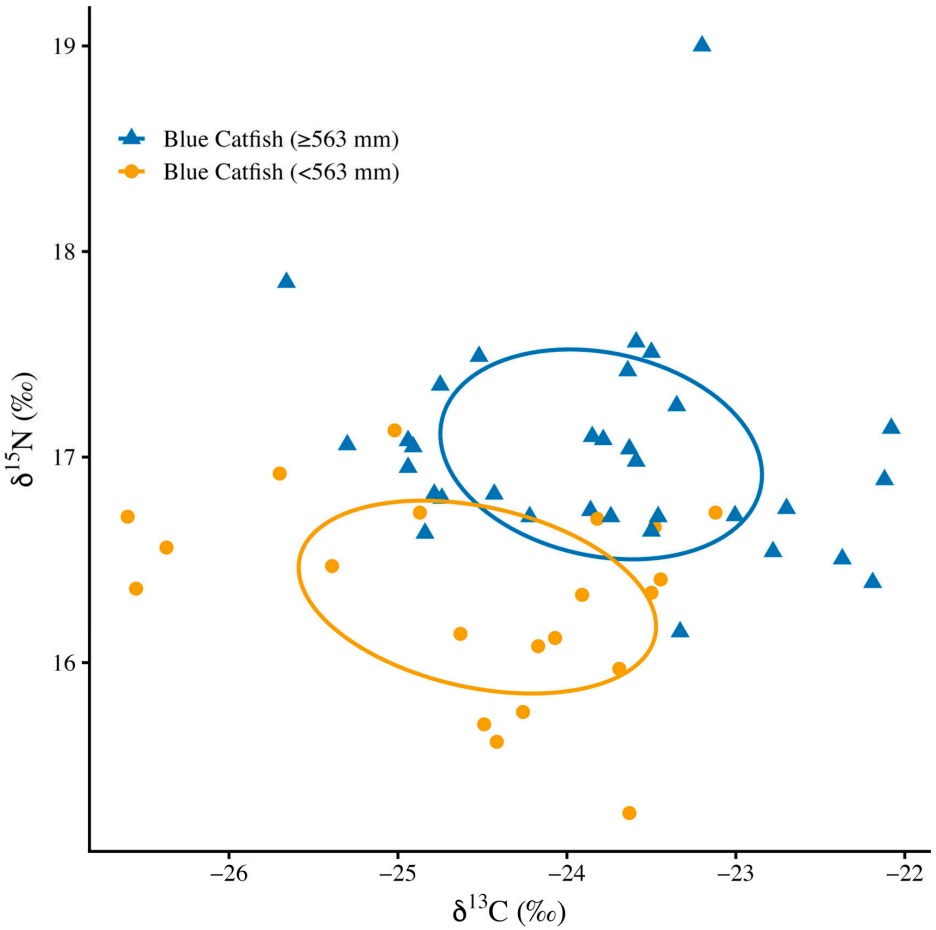


Figure 11. Isotopic niche space for small (<563 mm) and large (≥563 mm) Blue Catfish separated into size bins based on stomach content analysis. Bivariate points (shapes) represent mean individual isotopic values and ellipses illustrate core dietary niches represented by Standard Ellipse Areas (SEA_c).

4. Discussion

The stomach contents collected in this study and the associated omnivory index OI_k values indicate that Blue Catfish in the Nanticoke River watershed prey on a wide array of taxa across a broad range of trophic levels. Results from stomach content analysis (SCA) (logistic regression) estimate an ontogenetic diet shift to piscivory at 563 mm, which was closely supported by the GAM of the relationship between fish prey weight and total length. Diet Breadth (BA_k) estimates suggest that the dietary niche of large Blue Catfish is significantly broader than that of the small size class. The ontogenetic diet shift observed in SCA is strongly supported by analyses of Standard Ellipse Areas from stable isotope analysis (SIA), which suggest minimal overlap in core prey resources between size classes of Blue Catfish. The increasing trends in ^{15}N and ^{13}C enrichment over ontogeny from linear mixed-effect models, along with trophic position estimates of Blue Catfish from SIA, support SCA in suggesting that Blue Catfish increase in trophic level with size. Blue Catfish sulfur ($\delta^{34}S$) and carbon ($\delta^{13}C$) isotope signatures generally suggest utilization of marine and/or estuarine derived nutrients, supporting SCA results, which confirmed consumption of diadromous fishes. Factors such as gape limitation, intra- and interspecific competition for fish prey, and ontogenetic changes in habitat use and metabolic demands may influence the ontogenetic diet shift to piscivory [102–104].

Vegetation, particularly the native aquatic plant arrow arum, is a frequent diet item for small and large Blue Catfish. To our knowledge, this is the first study to note substantial Blue Catfish consumption of arrow arum seeds and seed pods. Interestingly, introduced

Channel Catfish are known to feed on invasive Russian olive *Elaeagnus angustifolia* in the southwestern United States, facilitating olive germination and dispersal. Olives offer a reliable energy source requiring low foraging effort but provide minimal nutritional value, and assimilation to muscle tissue is limited [66,105,106]. Arrow arum is abundant throughout the Nanticoke River and could function similarly to Russian olives' role in subsidizing metabolic demand of Blue Catfish while being minimally assimilated into tissue. While the nutritional value of arrow arum to Blue Catfish is presently unknown, stable isotope data show high ^{15}N enrichment of Blue Catfish across ontogeny, suggesting minimal contribution of arrow arum. The negative relationships observed between Blue Catfish total length and both vegetation occurrence and weight in the diet generally suggest reduced vegetation consumption once Blue Catfish shift to piscivory. These data could suggest that Blue Catfish subsidize their diet with vegetation to meet metabolic demands at small sizes, potentially becoming less reliant on vegetation when they are large enough to reliably consume and compete for fish prey. When combined with the low metabolic demands and high dietary plasticity of Blue Catfish, the ability to meet short-term energy demands by foraging on abundant vegetation (such as arrow arum seeds) could have contributed to the invasion success of Blue Catfish in Chesapeake Bay tributaries [107].

Trophic level estimates from Bayesian regression models using nitrogen SIA data, which reflect assimilated (rather than ingested) diet, suggest that both size classes of Blue Catfish feed at a trophic level similar to that of another fish predator, Largemouth Bass, and higher than that of invasive Northern Snakehead. The median trophic level estimates from SIA for both size classes of Blue Catfish are higher than the estimates from SCA. This is particularly significant for small Blue Catfish, which had an SIA trophic level estimate of 3.70, almost one trophic level higher than the SCA estimate (2.68), with no overlap in uncertainty intervals. Variability in the digestion and assimilation rates of prey and isotopic routing and the single isotopic baseline used in calculations (Gizzard Shad) could contribute to the observed differences between estimates [66,67,108]. The relative indigestibility of forage such as arrow arum seeds and Asian clam could also lead to over-representation in the diet through SCA, correspondingly reducing the trophic level estimate from SCA. Minimal assimilation of vegetation into muscle tissue may also drive lower trophic level estimates from SCA compared to SIA, though further studies on dietary assimilation are needed to support this hypothesis [67,105]. While nitrogen stable isotope composition of aquatic species in the Nanticoke River watershed can be subject to ^{15}N enrichment from sources such as composted animal waste, synthetic fertilizers, and treated wastewater, it is notable that no other producers or consumers exhibited unusually high ^{15}N enrichment or trophic level estimates, including the primary prey of small Blue Catfish, arrow arum [109–118]. These results could suggest that small Blue Catfish primarily rely on higher-nutritional-value prey, such as fish, macroinvertebrates, and crustaceans, for body growth. The discrepancy in trophic level estimates between SCA and SIA underscores the importance of using complementary methods in assessing trophic interactions of fishes.

Several notable similarities and differences exist between Blue Catfish in the Nanticoke River and other invasive populations of this species. Similar to other Chesapeake Bay tributaries, Asian clams were a relatively frequent prey item for small Blue Catfish [40]. Compared to studies from river basins in North Carolina and Georgia, the contribution of Asian clams to the diet of Blue Catfish in the Nanticoke River is notably lower, and no predation on Asian clams was observed by fish greater than 700 mm [42,43], illustrating potential differences in ecological characteristics between systems. Blue Catfish in the Nanticoke River undergo an ontogenetic diet shift to piscivory at a relatively small size (563 mm), similarly to fish in the James River, though much smaller than in the Rappahannock, Mattaponi, and Pamunkey Rivers [40]. Large Blue Catfish in the Nanticoke River feed

at a trophic level higher than that of large individuals in Western Shore Chesapeake Bay tributaries, as indicated by both SCA and SIA. Omnivory index values are similar to those from studies conducted in Western Shore tributaries of the Chesapeake Bay and the Cape Fear River, North Carolina [40,43]. However, diet breadth values for small Blue Catfish are considerably lower than were observed in individuals below the length at which the ontogenetic diet shift to piscivory occurs in other systems. This is likely due to the dominant contribution of vegetation (primarily arrow arum) to small Blue Catfish diets observed here, particularly during the fall. Heterogeneity in trophic position and diet breadth may also reflect general differences in prey availability, fish community composition, and habitat characteristics between systems, as well as the relatively limited geographic range sampled during this study and the smaller stomach-sample size.

Variability in carbon isotopic signatures suggests that Blue Catfish likely derive carbon from both allochthonous and autochthonous sources, and isotopic variability of Blue Catfish of similar sizes may suggest individual diet specialization [119]. Blue Catfish are more enriched in both ^{13}C and ^{34}S relative to other resident fish species in the Chesapeake Bay watershed, suggesting that Blue Catfish consume marine-derived nutrients from enriched ocean-going prey, such as river herring and Atlantic Menhaden, which aligns with SCA results [58,120,121]. The trend in ^{13}C enrichment with increasing total length of Blue Catfish suggests that individuals consume greater amounts of marine-derived nutrients from ocean-going fish prey over ontogeny, also supporting findings revealed by SCA. Our results align with findings from MacAvoy et al. (2000) [120] and MacAvoy et al. (2009) [122], which suggested that higher $\delta^{13}C$ and δ ^{34}S values found in Blue Catfish in the tidal Potomac River were attributed to their consumption of marine-derived nutrients from migratory alosines. However, contradictory to our hypothesis, our SIA results show that ^{34}S enrichment decreases in large Blue Catfish in the Nanticoke River, seemingly suggesting decreased utilization of marine resources over ontogeny, contrary to SCA results. This could be attributed to small Blue Catfish's consumption of juvenile Atlantic Menhaden, which were relatively common prey, as well as potential migratory fish egg predation [123]. Fish eggs, which may be difficult to detect with SCA due to small size and fast digestion, are similar in nitrogen and sulfur isotopic composition to parent muscle tissue [124–126], thus suggesting the potential for ^{15}N and ^{34}S enrichment in predator muscle tissue. Notably, Blue Catfish in Marshyhope Creek have been documented with stomachs full of eggs when collected via trawls in close vicinity of spawning adult Striped Bass (C. Stence, Maryland Department of Natural Resources, 6 April 2023, personal communication), suggesting that further research on anadromous fishes' early life stage predation may be warranted. Future studies may also benefit from analysis of ^{34}S in plant tissues to evaluate for potential isotopic influences of watershed land use practices.

Stomach content analysis indicates that Blue Catfish in the Nanticoke River consume an array of economically and ecologically significant prey taxa. While additional data on consumption, abundance, and mortality are needed to quantify impacts on species in the Nanticoke River watershed, predation indicates that impacts could potentially occur. Seasonal shifts in primary fish prey are evident across size classes and correspond with inter-annual migratory patterns of several fish species. Adult alosines are consumed relatively frequently by large Blue Catfish in the spring season, coinciding with annual spring alosine spawning migrations. Predation of spawning adult river herring by invasive Blue Catfish is an important consideration in fisheries management, as river herring populations are significantly reduced below historic levels, and Blue Catfish are abundant and widely distributed within Chesapeake Bay tributaries [35–37]. Presently, population-level effects of Blue Catfish predation on alosine populations in the Nanticoke River are unknown.

White Perch are another frequently consumed springtime prey item, which coincides with their annual spawning migration into tidal freshwater tributaries. Widely distributed in the Chesapeake Bay, White Perch form a significant part of commercial and recreational fisheries in the state of Maryland, and the species ranks among the top five fish harvested by Maryland anglers [127]. While impacts on White Perch populations are unquantified in this study, due to the recreational and commercial economic benefits provided by White Perch and their large contribution to the diet of Blue Catfish during their spawning season, continued monitoring of population trends could be beneficial for tracking impacts through time.

Atlantic Menhaden were well-represented diet items of Blue Catfish in the Nanticoke River. As important prey for many species along the Atlantic Coast, such as Striped Bass, Weakfish *Cynoscion regalis*, Atlantic Bluefin Tuna *Thunnus thynnus*, osprey *Pandion haliaetus*, and others, Atlantic Menhaden serve a critical ecosystem role [128–130]. Declines in the brood provisioning and reproductive rate of osprey, whose populations in the Chesapeake Bay have faced historic and recent declines, may be attributed to reductions in Atlantic Menhaden abundance [131,132]. Consumption of Atlantic Menhaden by Blue Catfish may signal the potential for predatory and competitive impacts. In and around the Chesapeake Bay, a large-scale reduction fishery annually harvests as much as three quarters of the Atlantic Menhaden catch allocated by the Atlantic States Marine Fisheries Commission to the entire Atlantic Seaboard [133]. In the James River, it is estimated that Blue Catfish annually remove 98.3 metric tons (95% CI: 54.3–169.9 metric tons) of Atlantic Menhaden through predation—only a small fraction of commercial harvest, which annually numbers in the hundreds of thousands of metric tons across Virginia waters [134]. Due to potential competitive and predatory interactions of invasive Blue Catfish, as well as the high economic and ecological value of Atlantic Menhaden, management that considers ecosystem-level interactions could be beneficial for multispecies fishery management.

Striped Bass, which are a current target of conservation efforts in the Chesapeake Bay and have shown reduced recruitment in recent years, are infrequently consumed by Blue Catfish in the Nanticoke River [135]. However, the primary prey of Striped Bass in the Chesapeake Bay includes Atlantic Menhaden, Gizzard Shad, and White Perch, frequent prey items of Blue Catfish in the Nanticoke River, indicating the potential for competitive interactions [128].

In tidal, low-salinity reaches of the Nanticoke River and Marshyhope Creek, large Blue Catfish feed relatively frequently on blue crab, whose populations have declined within the Chesapeake Bay [136,137]. Predation on blue crabs is considerably higher in mesohaline reaches of the James River compared to oligohaline and tidal freshwater reaches [40,138]. Mesohaline areas were not sampled in this study due to the sampling constraints of boat electrofishing; thus, it is possible that blue crab predation is more common closer to the mouth of the Nanticoke River. Fisheries managers may consider the potential additive negative impacts that predation by Blue Catfish could have on Maryland's economic and culturally significant blue crab fishery. Population monitoring data, along with predation estimates, could be useful to quantify predatory impacts of invasive Blue Catfish and inform fisheries management [134,138,139].

Despite seasonally high levels of predation on various important species and the high trophic position of Blue Catfish in the Nanticoke River, it is unlikely that this invasive species is the sole factor impacting populations of commercially and ecologically important species. Instead, invasive Blue Catfish may act as a back-seat driver of ecological change [40,140,141]. Factors such as climatic variability, anthropogenic development, habitat degradation, and impassible hydrological barriers likely have significant negative ecological impacts, particularly on sensitive species that rely on connectivity to upstream

habitats for spawning and offspring development [142–144]. Research to evaluate large-scale drivers impacting populations, while exploring ecosystem-based solutions to lessen the impacts of invasive Blue Catfish on native species of ecological and economic concern, could be beneficial for implementation of ecosystem-based management approaches. Additional research on consumption rates and population sizes of Blue Catfish and prey species throughout Eastern Shore tributaries will generate a greater understanding of interspecies interactions. Given the high abundance and wide geographic distribution of Blue Catfish across Chesapeake Bay tributaries, potential competitive and predatory impacts on native species are important considerations for fisheries and invasive species management in the Chesapeake Bay.

5. Conclusions

This study characterizes the diet composition and trophic ecology of invasive Blue Catfish in tidal, low-salinity reaches of the Nanticoke River and Marshyhope Creek, along the Chesapeake Bay's Eastern Shore (Maryland, USA). Stomach content analysis estimates an ontogenetic diet shift to piscivory at 563 mm and suggests decreased utilization of vegetation over ontogeny. These results are substantiated by ontogenetic trends in carbon and nitrogen stable isotopic composition, alongside minimal isotopic niche overlap between large and small individuals. While sulfur SIA indicates marine resource utilization by Blue Catfish, the negative ontogenetic relationship observed in $\delta^{34}S$ enrichment contrasts with SCA, potentially warranting additional study. Blue Catfish occupy a relatively high trophic position, indicated by both SCA and SIA, and consume species of economic and ecological importance, while exhibiting considerable seasonal and size-based diet variability. By describing these trophic interactions using complementary SCA and SIA methods, this study advances the ecological understanding of invasive Blue Catfish in a Chesapeake Bay Eastern Shore tributary and can inform assessments quantifying predatory and competitive impacts on native species.

Author Contributions: Conceptualization, Z.C., C.B., C.H. and N.R.B.; methodology, Z.C., C.B., C.D.R. and C.H.; software, Z.C., R.R. and R.T.; validation, Z.C., R.T., C.D.R. and R.T.; formal analysis, Z.C. and R.R.; investigation, Z.C. and N.R.B.; resources, C.B., C.D., R.R., R.T., C.D.R., Z.C. and N.R.B.; data curation, Z.C., C.D.R., R.T. and R.R.; writing—original draft preparation, Z.C.; writing—review and editing, Z.C., C.D.R., C.D., C.H., R.T., R.R. and N.R.B.; visualization, Z.C.; supervision, C.B. and N.R.B.; project administration, N.R.B.; funding acquisition, Z.C., N.R.B. and C.D. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal study protocol was approved by the Institutional Animal Care and Use Committee of Salisbury University (Protocol Code: 0065, approved on 1 October 2021).

Informed Consent Statement: Not applicable.

Data Availability Statement: All genetic sequence data generated for this study are openly available through the National Center for Biotechnology Information (NCBI) Sequence Read Archive under BioProject PRJNA1330277, including 18S amplicon BioSample accessions SAMN51647759–SAMN51647838 and COI amplicon BioSample accessions SAMN51493002–SAMN51493081. All

tabular prey-count datasets and stomach content summary data associated with this research are accessible through the U.S. Geological Survey data release [145]. Additional data presented in this study are available on request from the corresponding author.

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Abbreviations

The following abbreviations are used in this manuscript:

CrI	Credible Interval
CI	Confidence Interval
SEA_b	Bayesian Standard Ellipse Area
SEA_c	Sample Size Corrected Standard Ellipse Area
SIA	Stable Isotope Analysis
SCA	Stomach Content Analysis
$\%FO_j$	Percent Frequency of Occurrence
$\%MW_j$	Mean Percent by Weight

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