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Dietary Habits of Hardhead (*Ariopsis felis*) and Gafftopsail (*Bagre marinus*) Catfish Revealed through DNA Barcoding of Stomach Contents

Ana R. Osowski [1,2,*], Matthew B. Jargowsky [1], Pearce T. Cooper [3], Sean P. Powers [3] and J. Marcus Drymon [1,2]

[1] Coastal Research and Extension Center, Mississippi State University, 1815 Popps Ferry Rd, Biloxi, MS 39532, USA; matthew.jargowsky@msstate.edu (M.B.J.); marcus.drymon@msstate.edu (J.M.D.)
[2] Mississippi-Alabama Sea Grant Consortium, 703 E Beach Dr, Ocean Springs, MS 39564, USA
[3] Stokes School of Marine and Environmental Sciences, University of South Alabama, 600 Clinic Dr, Mobile, AL 36688, USA; pcooper@disl.org (P.T.C.); spowers@southalabama.edu (S.P.P.)
* Correspondence: ana.osowski@msstate.edu

Abstract: A better understanding of trophic interactions between hardhead catfish (*Ariopsis felis*) and gafftopsail catfish (*Bagre marinus*) is crucial for developing multi-species management strategies for the northern Gulf of Mexico (GOM). These two species are often aggregated in food web models; however, limited data are available to substantiate this approach. Therefore, the present study aimed to describe the dietary habits of hardhead catfish and gafftopsail catfish using analysis of stomach contents aided by DNA barcoding. Hardhead (n = 693) and gafftopsail (n = 655) catfish were sampled in the northern GOM from 2015–2019 using both fisheries-dependent and -independent techniques. The average percent number (%N), average percent mass (%M), prey specific number (%PN), prey specific mass (%PM), and prey-specific index of relative importance (%PSIRI) were computed to quantify prey species. The stomach content analysis identified distinct differences in diet between hardhead and gafftopsail catfish. Crustaceans were the most important prey for hardhead catfish, while gafftopsail catfish showed a significantly broader dietary breadth and were primarily piscivorous. Multivariate analyses indicated that the location of capture explained the greatest amount of diet variability for both species. These findings address fundamental knowledge gaps regarding the dietary habits of hardhead and gafftopsail catfish in northern GOM ecosystems.

Keywords: Gulf of Mexico; Ariidae; hardhead catfish; gafftopsail catfish; diet; food webs

Key Contribution: Diet analysis aided by DNA barcoding identified distinct differences between hardhead and gafftopsail catfish in the northern Gulf of Mexico.

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1. Introduction

The family Ariidae includes the hardhead catfish, *Ariopsis felis* (Linnaeus 1766), and the gafftopsail catfish, *Bagre marinus* (Mitchill 1815), both of which are marine catfishes common in tropical and temperate estuaries, coastal bays, and lagoons [1]. Both species have overlapping distributions in the U.S. southeastern Atlantic and the Gulf of Mexico (GOM). While hardhead catfish and gafftopsail catfish frequent coastal waters from Cape Cod to Mexico, gafftopsail catfish also range as far south as Brazil [2–4]. The coastal zone characteristics throughout the GOM favor high abundances of these catfishes [3]. Accordingly, gafftopsail catfish populations support substantial fisheries across the southern GOM, particularly off Tabasco, Mexico, where the species contributes as much as 44% to the state's total fisheries production [5,6]. Although the two species are relatively similar in size, gafftopsail catfish grow larger and more rapidly compared to hardhead catfish [7].

Traditional analysis of stomach contents suggests that hardhead catfish and gafftopsail catfish share similar diets. Both are considered opportunistic feeders and omnivorous scavengers, with a diet that includes algae, sea grasses, sea anemones, gastropods, polychaetes,

crustaceans, and fishes [3,8]. For example, Yanez-Arancibia and Lara-Dominguez [1] noted ontogenetic shifts in diet for hardhead catfish along the Bay of Campeche, where juveniles feed on small shrimp and crabs, mollusks, and annelids, while adults feed primarily on unidentified organic matter, fishes, and crustaceans. Rudershausen and Locascio [9] observed that gafftopsail catfish collected off Florida consume numerous prey items, ranging from benthic infauna to pelagic species, with pink shrimp (*Farfantepenaeus duorarum*), amphipods, and fishes as the most important food items. In Tabasco, Mexico, Mendoza-Carranza [5] found that crabs, fishes, stomatopods, and penaeid shrimp were common prey items for gafftopsail catfish.

Methodological advances can reveal dietary nuances often masked during visual identification of stomach contents. Along the west coast of Florida, nitrogen stable isotope analysis showed that hardhead catfish occupy a lower trophic position relative to gafftopsail catfish from the same region [10], contrary to earlier studies suggesting dietary similarity [3,8]. Stable isotope analysis also indicated that hardhead catfish in Florida [11] and Louisiana [12] show ontogenetic stability, contrary to the ontogenetic shifts reported for hardhead catfish in Campeche [1]. Determining whether these conflicting results reflect temporal or spatial heterogeneity in diets, or simply methodological differences, requires additional investigation.

The expansive distribution and high abundance of hardhead catfish and gafftopsail catfish throughout the coastal GOM suggest that these species fill important ecological roles. Food web models are a tool for understanding the roles of these species in coastal estuarine ecosystems. However, given a lack of detailed dietary data, even modern, comprehensive food web models (e.g., [13]) aggregate hardhead catfish and gafftopsail catfish as "sea catfishes". Given the shortcomings of traditional dietary analysis (e.g., the prevalence of unidentified prey items, a bias against soft-bodied prey, etc.), the objectives of this study were to: (1) investigate the dietary habits of hardhead and gafftopsail catfish by combining morphological examination and DNA barcoding of stomach contents; (2) describe the spatial and temporal variation in diet for hardhead and gafftopsail catfish. These findings will address fundamental gaps in our understanding of the roles these species fill within northern GOM ecosystems.

2. Materials and Methods

2.1. Fish Sampling

Hardhead catfish and gafftopsail catfish were collected from May 2015–September 2019 using both fisheries-dependent and -independent techniques. Catfishes were sampled on Dauphin Island, Alabama, during the annual Roy Martin Young Anglers Tournaments and Alabama Deep Sea Fishing Rodeos in July from 2017–2019. These fishes were captured using hook-and-line in Mississippi or Alabama waters. While exact catch locations were undocumented, general catch locations were obtained when possible. The general catch locations included West Mississippi Sound, East Mississippi Sound, West Mobile Bay, East Mobile Bay, North Mobile Bay, South Mobile Bay, West Offshore, and East Offshore (Figure 1). Anglers were asked if they used non-artificial bait or chum and, if applicable, what species were used. Additional catfishes were also collected with fishery-independent gillnets, trawls, bottom longlines, and seines in coastal Alabama and Mississippi waters throughout the year.

2.2. Biometrics

For each fish, its total length (TL) was measured to the nearest millimeter and its mass was measured in grams. When possible, the sex was recorded based on macroscopic examination of male/female gonads. Two-sample Kolmogorov–Smirnov tests were conducted to examine differences in the length and mass distributions between sexes ($\alpha = 0.05$).

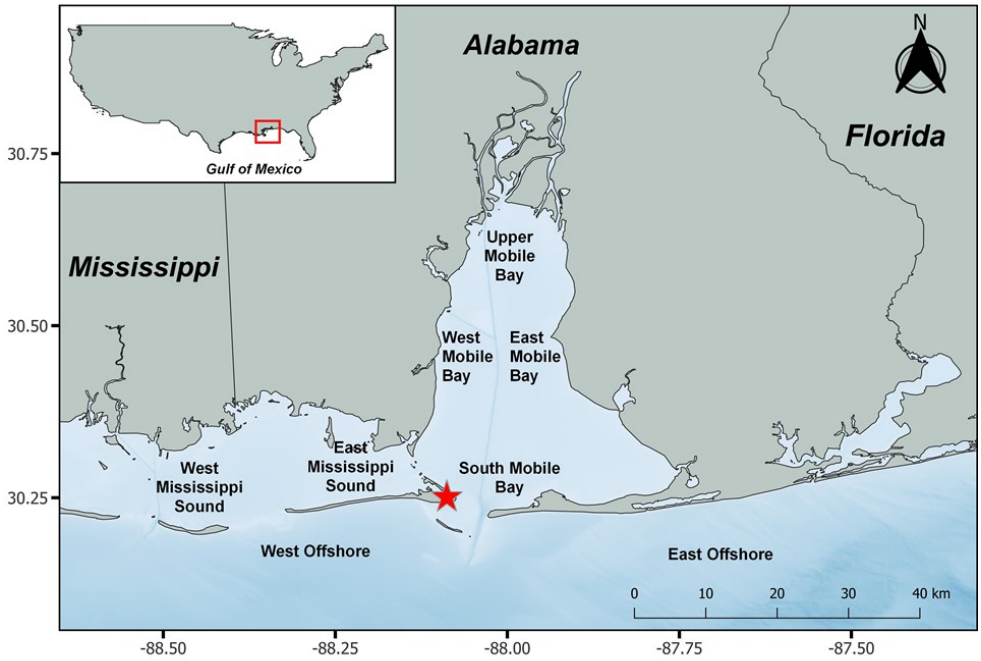

Figure 1. Catch locations of hardhead catfish (*Ariopsis felis*) and gafftopsail catfish (*Bagre marinus*) sampled in the northern Gulf of Mexico from May 2015–September 2019 for dietary analysis. Dauphin Island is noted with a star.

2.3. Morphological Examination of Prey

Stomachs were excised and stored in 200 proof ethanol or frozen at −29 °C until they could be examined. All stomach contents were examined using instruments that were sterilized in a 10% bleach solution. For fisheries-dependent samples, stomach contents that matched the description of the bait or chum used to catch the fish or that showed any evidence that they could have been used as bait were excluded from further analysis. Furthermore, any stomachs that appeared to be artificially stuffed (i.e., the fishermen filled the stomach to increase the mass of the fish) were also excluded from further analysis. All other prey items were separated to the lowest possible taxonomic level (e.g., species), counted, and wet weighed to the nearest 0.01 g. Taxonomic resolution obtained from morphological examination of prey ranged from class to species level. Prey items that could not be visually identified to the species level were stored in 200 proof ethanol until they could be examined genetically.

2.4. Molecular Identification of Prey

2.4.1. Blocking Primer Design

To reduce the amplification of the predator species and increase the amplification of the prey species, blocking primers were designed for gafftopsail catfish (GFT_blk_COIF 5′–CTACCCCCCTCTTGCTGGAAATCTCGCCC/3SpC3/) and hardhead catfish (HH_blk_COIF 5′–CCCTCCTCTTGCTGGTAACCTCGCTCACG/3SpC3/) that competitively annealed to the 5′ end of the forward cytochrome-c oxidase subunit I (COI) priming site and prevented elongation via the presence of the C3 spacer on the 3′ end of the primer. A competitive advantage was granted to the blocking primers by adjusting their molarity to 10× greater (10 µM) than the amplification primers (1 µM).

To assure primer specificity, we designed the gafftopsail catfish blocking primer by aligning the forward universal degenerate primer sequence used in our reactions (mlCOIintF 5′–GGWACWGGWTGAACWGTWTAYCCYCC–3′; [14]) to 34 COI haplotypes, obtained from the National Center for Biotechnology Information (NCBI) GenBank database, including four additional common species of fish, five hardhead catfish, and ten gafftopsail catfish. The hardhead blocking primer was designed in a similar manner using 33 COI

haplotypes, including four additional common species of fish, five gafftopsail catfish, and nine hardhead catfish. Blocking primers were not used on prey items identified as catfish. The additional species included Atlantic croaker, *Micropogonias undulatus*, crevalle jack, *Caranx hippos*, sand seatrout, *Cynoscion arenarius*, and striped anchovy, *Anchoa hepsetus*. To ensure that the blocking primers would block the amplification primer while also being specific, the 5′ end of the gafftopsail and hardhead catfish blocking primers began at the ninth and sixth nucleotide from the 3′ end of the amplification primer.

2.4.2. Library Preparation

DNA extraction, polymerase chain reaction (PCR) amplification, and library preparation were performed by the Texas A&M University–Corpus Christi Genomics Core Lab. The DNA extraction was performed using an Omega E-Z 96 tissue kit (RNase treatment step included) in 200 µL of elution buffer. Polymerase chain reaction was performed to amplify a 313 bp fragment of COI from all metazoans in the sample. The PCR mastermix consisted of 3.8 µL nuclease-free water, 7.5 µL 2X DreamTaq Green Mastermix (ThermoFisher Scientific, Waltham, MA, USA), 0.9 µL 10 µM blocking primer (either GFT_blk_COIF for gafftopsail catfish prey or HH_blk_COIF for hardhead catfish prey unless prey was identified as a catfish species), 0.9 µL 1 µM mlCOIintF primer (5′–/barcode/GGWACWGGWTGAACWGTWTAYCCYCC–3′; [14]), 0.9 µL 1 µM jgHCO2198 primer (5′–/barcode/TAIACYTCIGGRTGICCRAARAAYCA–3′; [15]), and 1 µL DNA template. Both amplification primers had unique five bp barcodes on the 5′ end to identify each sample after pooling. The samples were amplified using a touchdown protocol that included an initial 3 min denaturation step at 95 °C, followed by 13 cycles of denaturation for 10 s at 95 °C, annealing for 30 s at 62 °C (–1 °C per cycle) and elongation for 30 s at 72 °C, followed by 27 cycles at an annealing temperature of 48 °C, and a final 5 min elongation at 72 °C [14]. After PCR, all reactions were subjected to electrophoresis on a 1% agarose gel using an Axygen 100 bp ladder, and the resulting gel image was scored based on the presence of the target band (~313 bp), and three undesirable results indicated improper amplification: DNA smearing from high to low molecular mass, primer dimer, and nontarget amplification. Samples that did not amplify properly the first time either had a DNA smear or primer dimer and were reamplified using a modified PCR protocol. If the DNA was smeared, then the DNA template was reduced to 0.5 µL and the water was increased by 0.5 µL. Alternatively, if there was excessive primer dimer, the template DNA was increased to 2 µL and the water was reduced by 1 µL. Samples that successfully amplified were moved forward to sequencing library preparation. Products were purified using AMPure XP beads (Beckman-Coulter, Brea, CA, USA) in a 0.8X reaction, and the concentration of DNA was quantified in duplicate using AccuBlue High Sensitivity dsDNA Quantitation Solution (Biotium, Fremont, CA, USA) on a SpectraMax M3 plate reader (Molecular Devices, San Jose, CA, USA). Next, 10 ng of DNA from each sample was pooled into one library and concentrated to a volume of 16.67 µL through lyophilization using a refrigerated centrivap (Labconco, Kansas City, MO, USA) and rehydration in nuclease-free water. Sequencing library preparation was completed using the TruSeq DNA PCR-Free Kit (Illumina, San Diego, CA, USA), starting with the blunting step and using 0.33X reactions. Prior to sequencing, the library was adjusted to 2 nM using the Kapa Biosystems Library Quantification Kit on an ABI StepOnePlus real-time thermal cycler (Applied Biosystems, Waltham, MA, USA) and checked for the desired fragment length distribution using an Advanced Analytical Fragment Analyzer and the High Sensitivity NGS kit. The completed library was sequenced on an Illumina MiSeq at New York University's Genome Technology Center using paired-end 250 bp sequencing with an estimated output of 18 million reads.

2.4.3. Bioinformatics and Operational Taxonomic Unit Assignment

Initial processing, read clustering, and operational taxonomic unit (OTU) assignment for the MiSeq library was conducted using the charybdis metabarcoding pipeline (https://github.com/cbirdlab/charybdis, accessed on 21 July 2021) on the Genomics

Core Lab's high-performance computing cluster. The charybdis pipeline uses OBITOOLS version 1.2.9 [16]) with the addition of CROP version 1.33 [17], VSEARCH version 2.3.4 [18], BLAST version 2.6.0 [19], and GENOMETOOLS version 1.5.9 [20] together to cluster putative OTUs and assign them to taxa. Prior to this, for parallel processing, the raw read 1 and read 2 FASTQ files were divided into several smaller files using FASTQ SPLITTER version 0.1.2 (https://kirill-kryukov.com/study/tools/fastq-splitter/, accessed on 21 July 2021). The read pairs were aligned and converted to FASTA format, using the functions illuminapairedend and obiconvert, respectively. The FASTA files were filtered using the function obigrep, removing read pairs with an alignment score lower than 40 or with less than 20 bp of overlapping sequence. Aligned read pairs were demultiplexed and assigned to samples according to the unique barcodes attached during PCR amplification using the function ngsfilter. All the sequences corresponding to each sample were sorted into unique FASTA files for further processing. Duplicate read pairs were quantified and removed using the function obiuniq, leaving only the unique read pairs (variants) and their frequency. Singletons and variants that were likely to result from PCR errors were identified and removed using the obiclean function. Errors in PCR were defined as sequence variants that were, at most, half as frequent as a more abundant variant with one mismatch. Variants that differed in length from the expected 313 bp of COI by more than 15 nucleotides were filtered. Chimeric variants were identified and removed using the uchime_denovo function of VSEARCH. Variants were assigned to OTU using CROP with the block size set to 432 and the number of Markov chain–Monte Carlo iterations set to 10X the block size (4320), as recommended in the CROP manual. Each OTU was assigned to a taxon in a local database of COI sequences from NCBI's GenBank using the top hits of the BLAST alignment algorithm. A local database was created by downloading relevant sequences from the nucleotide database provided by NCBI (https://ftp.ncbi.nlm.nih.gov/blast/db/, accessed on 1 December 2017), with an NCBI ENTREZ query targeting the following search terms: mitochondria, cytochrome, coi, co1, cox1, coxi, mitochondrial genome, and mitochondria genome. The database was additionally filtered to remove entries of uncertain origin with the following search terms: environmental samples[organism], metagenomes[orgn]. The filtered database had 7,692,226 entries. In the pipeline, when an OTU sequence was queried for top alignments using BLAST, if the top hit had an identity of over 97% and a query coverage of 100%, we allowed the OTU to be discriminated at the species level [14]. All OTUs with identity scores less than 97% were described at the family level or a more general taxonomic group. In addition, we manually queried NCBI's complete nucleotide database using the Web-based BLASTn for those 10 OTU sequences with the 10 highest read counts. All had the same taxonomic match.

2.4.4. Analysis of Read Counts and Prey Assignment

The output of the pipeline was a comma delimited table of read pair counts, where each row is an OTU and the columns include a sample ID, putative taxonomic assignment (the traditional full Linnaean hierarchy: KPCOFGS), OTU nucleotide sequence, BLAST coverage, and identity score. The identity of the prey was determined to be the OTU with the highest read count when the predator OTU, known contamination, and OTUs that are not possible prey candidates were removed. Any OTU with less than five reads was also excluded from analysis. In other words, based on the data for each prey item, we chose from all potential species whose genetic material was sequenced and assumed that the potential prey item with the highest read count was the most likely prey item for that particular sample.

2.5. Diet Quantitative Variables

Prey groups were quantified using single and compound indices, including frequency of occurrence (%FO), average percent number (%N), average percent mass (%M), prey-specific number (%PN), and prey-specific mass (%PM) [21–23]. To further characterize hardhead catfish and gafftopsail catfish diets, the prey-specific index of relative importance

(%PSIRI) was used [21]. The equations for %N, %M, %PN, %PM, %FO, and %PSIRI are as follows:

$$\%A_i = \left(\sum_{j=1}^{n} \%A_{ij}\right)(n)^{-1} \quad (1)$$

$$\%PA_i = \left(\sum_{j=1}^{n} \%A_{ij}\right)(n_i)^{-1} \quad (2)$$

$$\%FO_i = (n_i)(n)^{-1} \quad (3)$$

$$\%PSIRI = (FO_i\ (\%PN_i + \%PM_i))(0.5) \quad (4)$$

where $\%A_{ij}$ is the percent abundance (by number or mass) of prey category i in stomach sample j, n_i is the number of stomachs containing prey i, and n is the total number of stomachs containing prey [21]. An index of vacuity was calculated by dividing the total number of stomachs without prey by the total number of stomachs sampled [22].

2.6. Sample Size Sufficiency

Sample size sufficiency was assessed using cumulative prey curves to determine if enough stomach samples had been analyzed to adequately describe the diet of both catfishes. Prey-specific number was used for conducting cumulative prey curves, and sample size was considered sufficient once a prey curve reached an asymptote, which was defined as the slope of a linear regression (b) being <0.05 when fit to the final five randomly sampled stomachs [21,24]. If a prey curve failed to approach an asymptote at the taxon level (e.g., species), new prey curves were generated at higher taxa levels until this criterion was met. If the prey curve failed to reach an asymptote at the species, family, and order levels, diet analysis was performed at the order level.

2.7. Statistical Analysis

The Bray-Curtis index was used to create a dissimilarity matrix for the dependent variables %N and %M, with each sampled stomach treated as an individual sampling event and prey species treated as response variables [25]. Permutational multivariate analysis of variance (PERMANOVA) was then performed on the dissimilarity matrix to measure differences in diet among response variables, which included TL, mass, location, season, and year. Seasons were defined as follows: spring = March–May, summer = June–August, fall = September–November, and winter = December–February. All size ranges of hardhead and gafftopsail catfish were considered in the present study. For statistical analysis, total length was binned into two categories based on available age and reproductive information for these catfishes in the northern GOM. For hardhead catfish, length bins were determined based on a length at 50% maturity of ~250 mm TL [26]. For gafftopsail catfish, fork length at 50% maturity was obtained from Miguez [27] and converted to TL using the length–mass regression provided by Courtney et al. [28]. This value, 359 mm TL, was rounded to 400 mm TL for a conservative estimate. Location, year, length, and season were treated as factors and mass was treated as a covariate. Variables were tested independently, and final models were created using forward, stepwise model selection to determine which combination of explanatory variables best explained diet variability. Permutation tests for heterogeneity of multivariate group dispersions were run for all explanatory variables to test for sample dispersion [29]. All PERMANOVAs were permutated 9,999 times and differences were considered significant at the $p < 0.05$ level.

Canonical correspondence analysis (CCA) was also conducted to complement the final PERMANOVA models. Biplots were constructed to help visualize associations between prey groups and explanatory variables [30]. Prey items that were considered rare (observed in fewer than five stomachs) were removed prior to analysis, as removing rare items can help to maximize the explanatory power of the CCA model [31]. Additional permutational tests were conducted on the CCA to determine the significance of the final CCA models,

constraining axes, and explanatory variables. If an explanatory variable was significant in the PERMANOVA analysis but insignificant in the CCA, it was still included in the CCA model for maximum interpretability as long as $p < 0.1$. All analyses were conducted in R version 4.1.0 [32] using the Vegan Community Ecology Package [33].

3. Results

3.1. Morphometrics

Overall, 693 hardhead catfish and 655 gafftopsail catfish were sampled in the present study (Table 1). The majority (68%, $n = 472$) of sampled hardhead catfish were not assigned a sex; of those that were, 139 were female and 81 were male. In contrast, most (74%, $n = 483$) gafftopsail catfish were assigned a sex; in total, 209 females and 274 males were sampled (Table 2). For hardhead catfish, total length ranged from 64.05–561 mm TL and mass ranged from 1.708–1500 g (n = 686). For gafftopsail catfish, total length ranged from 119–709 mm TL and mass ranged from 13–4000 g ($n = 623$, Figures 2 and 3). Kolmogorov–Smirnov tests revealed significant differences in TL ($D = 0.380$, $p < 0.005$, $D = 0.375$, $p < 0.005$) and mass ($D = 0.367$, $p < 0.005$, $D = 0.390$, $p < 0.005$) between sexes of both species, with females being both heavier and longer than males (Figure 4).

Table 1. Summary of sample sizes by location, season, and year for hardhead catfish (*Ariopsis felis*) and gafftopsail catfish (*Bagre marinus*) sampled in the northern Gulf of Mexico from May 2015–September 2019 for dietary analysis. Food = sampled stomach contained prey items, Empty = sampled stomach contained no prey items, Total = total number of stomachs sampled.

Variable		Hardhead Catfish			Gafftopsail Catfish		
		Stomach Content			Stomach Content		
		Food	Empty	Total	Food	Empty	Total
Location	West Mississippi Sound	25	101	126	20	20	40
	East Mississippi Sound	17	31	48	36	46	82
	West Offshore	20	9	29	21	15	36
	East Offshore	31	54	85	13	9	22
	North Mobile Bay	16	39	55	22	19	41
	South Mobile Bay	15	149	164	34	65	99
	West Mobile Bay	24	27	51	56	99	155
	East Mobile Bay	25	38	63	33	40	73
Season	Spring	75	171	246	38	28	66
	Summer	93	118	211	205	306	511
	Fall	26	210	236	36	42	78
Year	2015	7	49	56	1	15	16
	2016	15	102	117	5	0	5
	2017	95	249	344	158	156	314
	2018	32	42	74	58	114	172
	2019	45	57	102	57	91	148

Table 2. Summary of total length (mm) and mass (g) of hardhead catfish (*Ariopsis felis*) and gafftopsail catfish (*Bagre marinus*) sampled in the northern Gulf of Mexico from May 2015–September 2019 for dietary analysis. Min = minimum, Max = maximum, SD = standard deviation, and n = sample size.

		Total Length (mm)					Mass (g)				
Species		**Min**	**Max**	**Mean**	**SD**	***n***	**Min**	**Max**	**Mean**	**SD**	***n***
Hardhead Catfish	All	64.05	561	248.9	118.03	686	1.708	1500	251	248.3	686
	Females	230	461	363	51.6	135	60	1150	500	213	135
	Males	200	436	318	50	81	50	750	336	151	81
Gafftopsail Catfish	All	119	709	515.6	132.5	623	13	4000	1459.2	745.3	623
	Females	376	709	588	57.9	208	500	4000	1961	660	208
	Males	380	654	543	53.6	273	100	2950	1404	474	273

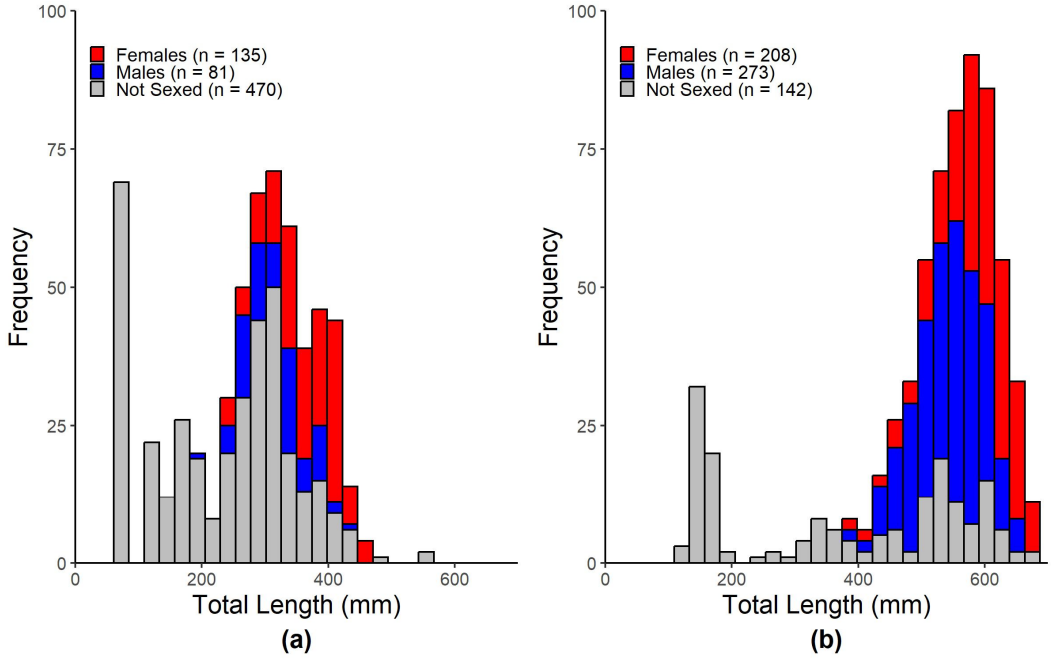

Figure 2. Length–frequency distribution of (**a**) hardhead catfish (*Ariopsis felis*) and (**b**) gafftopsail catfish (*Bagre marinus*) sampled in the northern Gulf of Mexico from May 2015–September 2019 for dietary analysis.

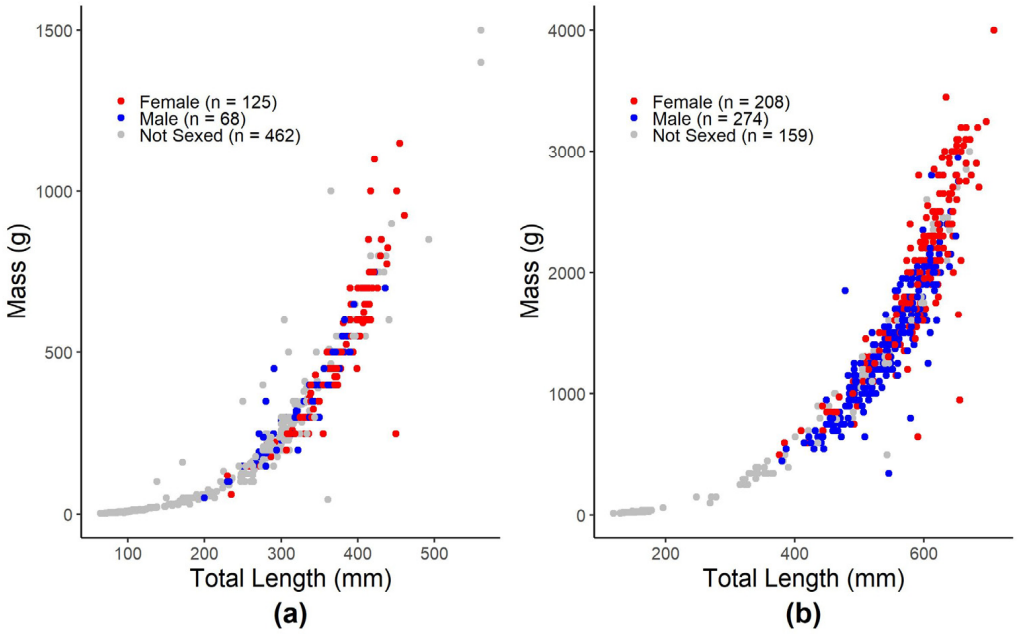

Figure 3. Length–mass relationships for (**a**) hardhead catfish (*Ariopsis felis*) and (**b**) gafftopsail catfish (*Bagre marinus*) sampled in the northern Gulf of Mexico from May 2015–September 2019 for dietary analysis.

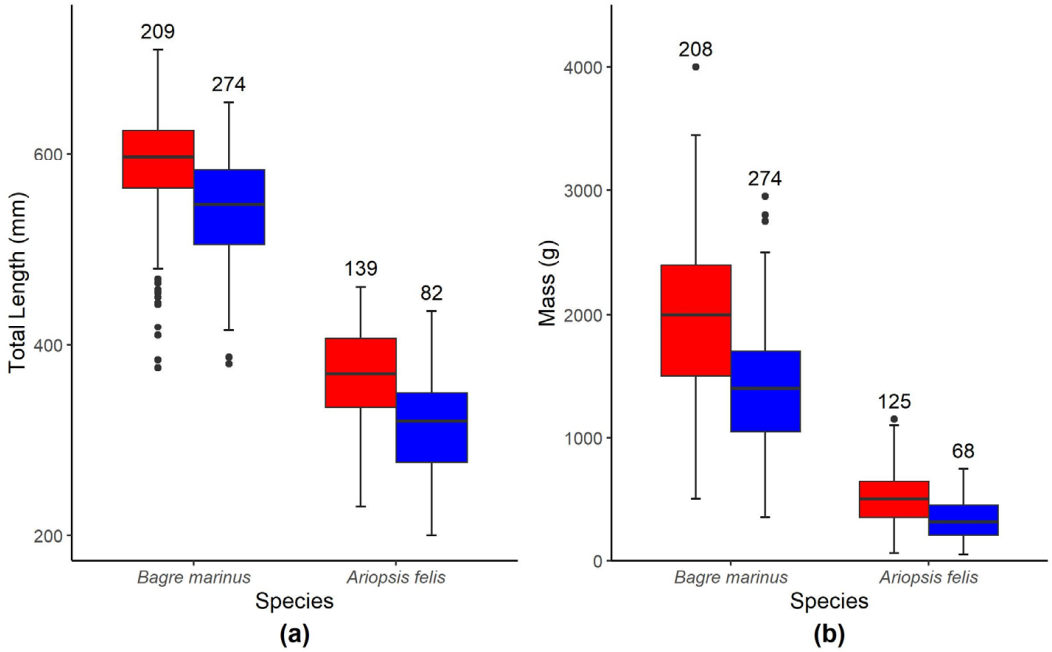

Figure 4. Boxplots showing (**a**) total length (mm) and (**b**) mass (g) for female and male catfishes sampled in the northern Gulf of Mexico from May 2015–September 2019 for dietary analysis. Sample sizes are given above each boxplot, and grouped boxplots are separated by species. Red fill represents females and blue fill represents males.

3.2. Hardhead Catfish Diet

3.2.1. Diet Characterization

Of the 693 hardhead catfish stomachs examined, 194 contained prey, resulting in an index of vacuity of 72%. Small, immature individuals (<250 mm TL) had a higher observed frequency of empty stomachs (n = 262, 92.3%) compared to large, mature individuals (>250 mm; n = 237, 58.4%). Almost half of sampled hardhead catfish stomachs were collected in 2017 (n = 344, 49.6%), and sampling was highest in April (n = 192, 27.7%) and July (n = 173, 25.0%). Total lengths of hardhead catfish with stomachs containing prey ranged from 273–425 mm (mean = 369 mm, n = 51) for females and 200–390 mm (mean = 311 mm, n = 31) for males. From the 194 stomachs containing prey, 364 prey items weighing 714.41 g were identified macroscopically. Of these, 119 prey items were analyzed genetically, and 41 (34.5%) were assigned a final species-level OTU.

Thirty-four prey items were identified, twenty of which were crustaceans (Class Malacostraca; Table 3). Twenty-one prey items were identified to the species level; of these, four (19%) were identified using genetics. Invertebrate prey (decapods and gastropods) were more important than fish prey, with a combined PSIRI of 80.63%. Unidentified decapods constituted the most important prey group (19.23% PSIRI), followed by estuarine ghost shrimp (*Lepidophthalmus louisianensis*, 16.3% PSIRI) and mud crabs (*Panopeidae* spp., 7.25% PSIRI). In contrast, bony fishes (class Actinopterygii) had a PSIRI of 15.52%. Cumulative prey curves indicated that sample sizes for hardhead catfish were inadequate to describe the diet at the species level (b = 0.057) but were acceptable at the family level (b = 0.036, Figure A1). Thus, all multivariate analysis was performed at the family level for hardhead catfish.

Table 3. Diet composition of hardhead catfish (*Ariopsis felis*) sampled in the northern Gulf of Mexico from May 2015–September 2019 for dietary analysis. %FO = frequency of occurrence, %N = average percent number, %M = average percent mass, %PN = prey-specific number, %PM = prey-specific mass, and %PSIRI = prey-specific index of relative importance. Class-level results are in bold.

Class	Order	Family	Species	%FO	%N	%PN	%M	%PM	%PSIRI
Vegetation (Misc.)			**Unidentified**	**0.52**	**0.52**	**100.00**	**0.52**	**100.00**	**0.52**
Unknown			**Unidentified worm**	**1.55**	**1.29**	**83.33**	**1.16**	**75.24**	**1.23**
Malacostraca				**82.47**	**81.14**	**92.94**	**80.23**	**91.96**	**76.25**
	Decapoda			76.29	61.94	93.05	60.96	91.80	70.51
		Alpheidae	*Alpheus heterochaelis*	1.55	1.55	100.00	1.55	100.00	1.55
		Callianassidae	*Lepidophthalmus louisianensis*	18.04	16.32	90.48	16.28	90.24	16.30
		Cambaridae	Unidentified	0.52	0.52	100.00	0.52	100.00	0.52
		Ctenochelidae	*Dawsonius latispina*	0.52	0.52	100.00	0.52	100.00	0.52
		Diogenidae	*Clibanarius vittatus*	3.61	2.15	59.52	2.21	61.29	2.18
		Hippidae	*Emerita benedicti*	3.09	2.15	69.44	2.22	71.79	2.18
		Leucosiidae	*Persephona punctata*	0.52	0.26	50.00	0.25	48.59	0.25
		Palaemonidae	*Palaemonetes* spp.	1.03	1.03	100.00	1.03	100.00	1.03
		Panopeidae	Unidentified	9.79	7.71	78.70	6.79	69.34	7.25
		Penaeidae		3.10	2.10	67.86	2.30	74.35	2.20
			Farfantepenaeus aztecus	2.58	1.76	68.10	2.02	78.48	1.89
			Litopenaeus setiferus	0.52	0.34	66.67	0.28	53.70	0.31
		Pinnotheridae	*Austinixa behreae* *	0.52	0.52	100.00	0.52	100.00	0.52
		Portunidae		10.83	7.51	72.80	7.42	72.02	7.46
			Callinectes sapidus	6.70	5.07	75.60	5.21	77.80	5.14
			Callinectes similis	3.61	2.18	60.37	2.07	57.33	2.12
			Portunus gibbesii *	0.52	0.26	50.00	0.14	27.56	0.20
		Sergestidae	Unidentified	1.03	0.97	94.05	0.39	38.24	0.68
		Unidentified	*Brachyura* spp.	7.73	6.70	86.70	6.90	89.20	6.80
		Unidentified	*Dendrobranchiata* spp.	2.58	2.32	90.00	2.34	90.87	2.33
		Unidentified	Unidentified invertebrate	21.13	19.20	90.84	19.27	91.19	19.23
	Stomatopoda	Squillidae	*Squilla* spp.	5.67	5.15	90.91	5.34	94.12	5.25
Gastropoda	**Neotaenioglossa**	**Naticidae**	***Sinum perspectivum***	**6.70**	**4.59**	**68.54**	**4.18**	**62.30**	**4.38**
Actinopterygii				**18.56**	**32.43**	**79.76**	**34.81**	**87.56**	**15.52**
	Anguilliformes	Ophichthidae	*Myrophis punctatus* *	0.52	0.52	100.00	0.52	100.00	0.52
	Clupeiformes			4.64	5.84	79.53	5.79	79.74	3.61
		Clupeidae	*Brevoortia patronus*	2.06	1.20	58.33	1.61	77.92	1.40
		Engraulidae		2.58	2.32	90.00	2.09	81.20	2.21
			Anchoa mitchilli	2.06	2.06	100.00	2.06	100.00	2.06
			Anchoa hepsetus	0.52	0.26	50.00	0.03	5.99	0.14
	Gobiiformes	Gobiidae	*Gobiosoma bosc*	0.52	0.26	50.00	0.34	66.67	0.30
	Perciformes	Serranidae	*Diplectrum bivittatum* *	1.03	1.03	100.00	1.03	100.00	1.03
	Sciaeniformes	Sciaenidae		3.09	2.06	66.67	2.63	84.75	2.34
			Micropogonias undulatus	2.58	1.80	70.00	2.26	87.53	2.03
			Menticirrhus americanus	0.52	0.26	50.00	0.37	70.88	0.31
	Unidentified			9.28	7.41	79.89	8.04	86.63	7.73
			Unidentified (fish)	7.22	5.95	82.48	6.31	87.37	6.13
			Unidentified (bone)	2.06	1.46	70.83	1.73	84.02	1.60
Unknown			**Unidentified**	**3.61**	**2.15**	**59.52**	**2.05**	**56.93**	**2.10**

* Prey species identified using genetics.

3.2.2. Diet Variability

The PERMANOVA analysis indicated that location and year were significant explanatory variables for %N and %M for hardhead catfish, with location explaining the greatest amount of diet variability (Table 4). The interactions between total length and season, year and location, year and mass, season and location, and season and mass were significant for both %N and %M. Only mass showed evidence of significant heterogeneity in dispersion. The final PERMANOVA models for both %N and %M included the variables location, year, and mass, as well as the interaction between location and year, and explained 27.3% and 26.8% of the diet variability for %N and %M, respectively.

The CCA model for %N for hardhead catfish indicated that season and mass were not significant. However, the CCA model for %M showed that season was significant. Additionally, because $p < 0.1$ for %M, mass was included in this analysis to improve the interpretation of the model and biplots (Figure 5). The final CCA models for both %N and %M were significant, with significant axes (CCA1 and CCA2). The final model for %N included the variables location and year, while the final model for %M included four variables (location, year, season, and mass). The CCA model for %M explained more of the diet variability for hardhead catfish than the CCA model for %N (15.2% vs. 11.3%, respectively).

Table 4. Summary of explanatory variables used in the PERMANOVA models to explain diet composition of hardhead catfish *(Ariopsis felis*) sampled in the northern Gulf of Mexico from May 2015–September 2019 for dietary analysis. %N = average percent number, %M = average percent mass, df = degrees of freedom, F = F-statistic, R^2 = amount of explained variability, and *p* = *p*-value. Significant variables and their interactions are in bold.

			%N			%M		
Model	**Variable(s)**	**df**	**F**	**R^2**	***p***	**F**	**R^2**	***p***
Single Variables	Total Length (binned)	1	0.868	0.005	0.552	0.837	0.004	0.600
	Location	7	2.984	0.114	**<0.001**	2.831	0.108	**<0.001**
	Season	2	1.114	0.012	0.301	1.130	0.012	0.282
	Mass	1	0.948	0.005	0.602	0.939	0.005	0.624
	Year	4	1.658	0.034	**0.007**	1.657	0.034	**0.007**
Interactions	Total Length (binned) × Location	5	0.924	0.025	0.657	0.940	0.026	0.623
	Total Length (binned) × Season	2	1.782	0.018	**0.015**	1.753	0.018	**0.016**
	Total Length (binned) × Mass	1	1.226	0.007	0.219	1.220	0.007	0.225
	Total Length (binned) × Year	2	1.178	0.012	0.229	1.185	0.012	0.219
	Year × Location	13	1.501	0.102	**<0.001**	1.480	0.101	**0.004**
	Year × Season	2	0.968	0.010	0.503	0.971	0.010	0.503
	Year × Mass	4	1.613	0.034	**0.005**	1.547	0.032	**0.011**
	Season × Location	11	1.338	0.079	**0.009**	1.362	0.080	**0.006**
	Season × Mass	2	1.932	0.021	**0.007**	1.900	0.020	**0.008**
	Location × Mass	7	1.025	0.040	0.417	1.031	0.041	0.397
Final Model	**Location**	7	3.051	0.113	**<0.001**	2.894	0.108	**<0.001**
	Season	2	1.754	0.019	**0.027**	1.710	0.018	**0.026**
	Mass	1	1.694	0.009	**<0.001**	1.667	0.009	**<0.001**
	Year	4	1.914	0.041	**0.001**	1.935	0.041	**<0.001**
	Year × Location	12	1.445	0.092	**<0.001**	1.424	0.091	**<0.001**
	Residuals	137		0.273			0.268	

Prey in the family Penaeidae were correlated with the year 2018 and the West Mobile Bay region, while prey items from the families Panopeidae and Portunidae were correlated with the year 2019. Prey items from the family Callianassidae were correlated with the East Mobile Bay region, while prey items from the family Naticidae, as well as unidentified shrimp and invertebrates, were correlated with the South Mobile Bay and East Offshore regions. Unidentified crabs were correlated with the West Mississippi Sound region, and prey items from the family Squillidae were correlated with the West Offshore region (Figure 5).

3.3. Gafftopsail Catfish Diet

3.3.1. Diet Characterization

Of the 655 gafftopsail catfish stomachs examined, 279 contained prey, resulting in an index of vacuity of 57.4%. There was a higher frequency of empty stomachs observed among large, mature individuals (>400 mm TL; *n* = 331, 58.3%) compared to small, immature individuals (<400 mm TL; n = 45, 51.7%). Almost half of sampled gafftopsail catfish stomachs (48%, *n* = 314) were collected in 2017, and sampling was highest in July (*n* = 508, 77.6%) during fishing rodeos. Total lengths of gafftopsail catfish with stomachs containing prey ranged from 419–666 mm (mean = 574 mm, *n* = 75) for females and 422–626 mm (mean = 546 mm, *n* = 112) for males. From the 279 stomachs containing prey, 525 prey items weighing a cumulative 2,141.4 g were identified macroscopically. Of these, 236 prey items were analyzed genetically, and 142 (60.2%) were assigned a final species-level OTU.

Sixty-three prey items were identified, thirty-five of which were fishes (class Actinopterygii; Table 5). Forty-six prey items were identified to the species level; of these, twenty-two (48%) were identified using genetics. Bony fishes (Class Actinopterygii) were more important than invertebrate prey, with a 67.26% PSIRI. Unidentified fishes constituted the most important

prey species (12.63% PSIRI), followed by Gulf menhaden (*Brevoortia patronus*, 12.38% PSIRI), Atlantic croaker (7.84% PSIRI), and brown shrimp (*Farfantepenaeus aztecus*, 7.50% PSIRI). Decapods and allies (Class Malacostraca) had a PSIRI of 27.9%, while Gastropods (Class Gastropoda) had a PSIRI of 0.64%. Cumulative prey curves indicated that sample sizes for gafftopsail catfish were inadequate to describe diet at the species level (b = 0.080) and at the family level (b = 0.065); therefore, multivariate analysis was performed at the order level (Figure A2).

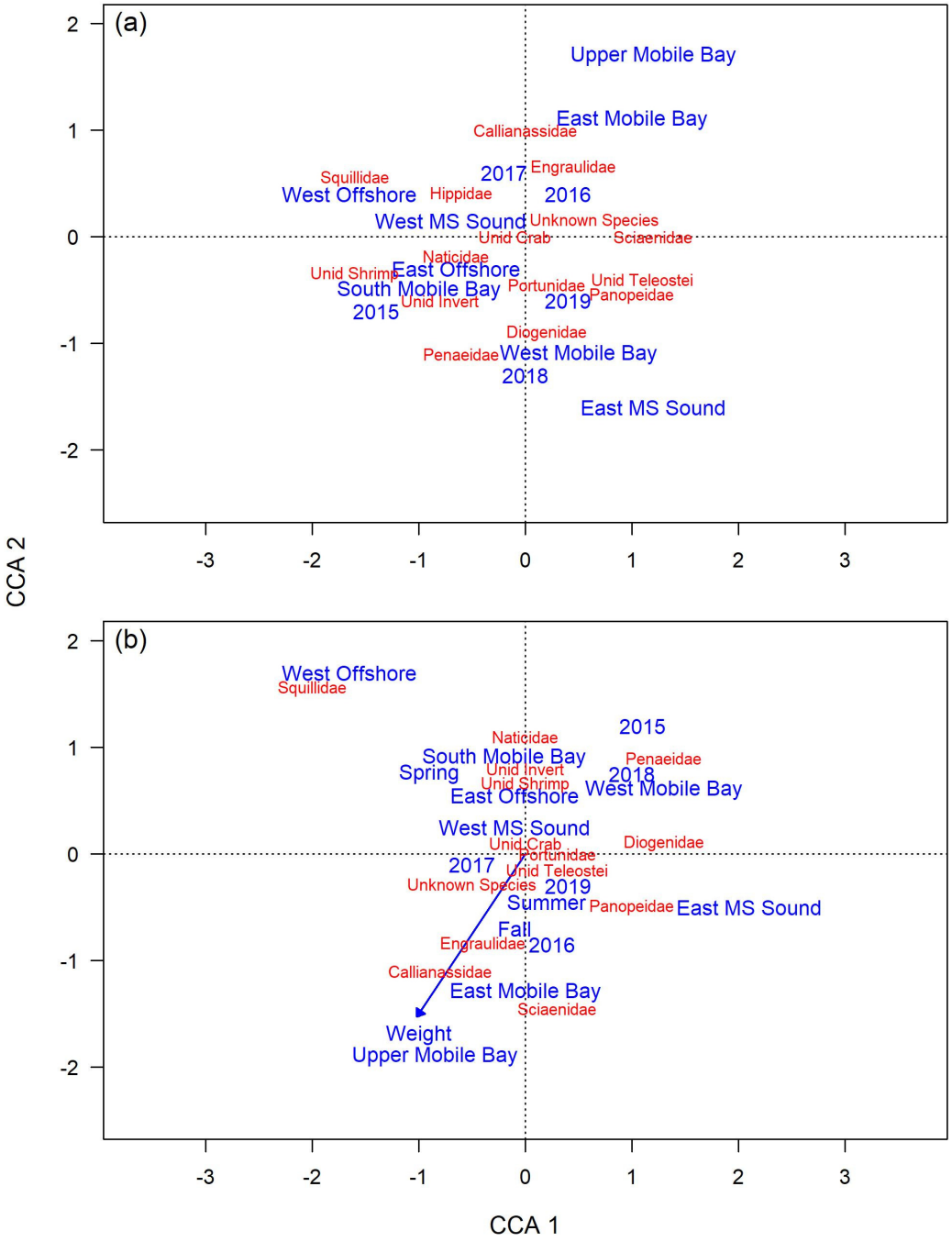

Figure 5. Canonical correspondence analysis plots for (**a**) percent number of prey (%N) and (**b**) percent mass of prey (%M) for hardhead catfish (*Ariopsis felis*) sampled in the northern Gulf of Mexico from May 2015–September 2019 for dietary analysis. Explanatory variables from the final PERMANOVA model (location and year for %N, location, year, season, and mass for %M) are shown in blue and prey families are shown in red.

Table 5. Diet composition of gafftopsail catfish (*Bagre marinus*) sampled in the northern Gulf of Mexico from May 2015–September 2019 for dietary analysis. %FO = frequency of occurrence, %N = average percent number, %M = average percent mass, %PN = prey-specific number, %PM = prey-specific mass, %PSIRI = prey-specific index of relative importance. Class-level results are in bold.

Class	Order	Family	Species	%FO	%N	%PN	%M	%PM	%PSIRI
Vegetation (Misc.)			**Unidentified**	**1.80**	**0.93**	**51.67**	**0.85**	**47.33**	**0.89**
Unknown			**Unidentified worm**	**0.36**	**0.18**	**50.00**	**0.21**	**57.09**	**0.19**
Hydrozoa			**Hydrozoan sp.**	**0.36**	**0.18**	**50.00**	**0.18**	**50.00**	**0.18**
Sipunculidea	**Golfingiida**	**Sipunculidae**	***Xenosiphon* sp.**	**0.36**	**0.21**	**57.14**	**0.33**	**92.49**	**0.27**
Polychaeta				**0.72**	**0.90**	**100.00**	**0.90**	**100.00**	**0.72**
	Leptothecata		*Hydrozoan* sp.	0.36	0.18	50.00	0.18	50.00	0.18
	Phyllodocida	Nereididae	*Alitta succinea* *	0.36	0.36	100.00	0.36	100.00	0.36
	Spionida	Paraprionospio	*Paraprionospio pinnata* *	0.36	0.36	100.00	0.36	100.00	0.36
Insecta	**Coleoptera**		**Unidentified beetle**	**0.36**	**0.36**	**100.00**	**0.36**	**100.00**	**0.36**
Gastropoda				**1.44**	**9.72**	**56.25**	**2.45**	**32.43**	**0.64**
	Littorinimorpha	Littorinidae	*Littoraria irrorata*	0.72	0.54	75.00	0.43	59.17	0.48
	Neotaenioglossa	Naticidae	*Sinum perspectivum*	0.36	0.18	50.00	2.00	4.65	0.10
	Unidentified		Unidentified gastrodpod	0.36	9.00	25.00	0.02	6.71	0.06
Malacostraca				**36.69**	**29.27**	**79.74**	**28.35**	**77.29**	**28.81**
	Decapoda			35.61	28.31	79.48	27.50	77.23	27.90
		Alpheidae	*Alpheus heterochaelis*	2.52	2.22	88.07	2.10	83.34	2.16
		Callianassidae	*Lepidophthalmus louisianensis*	1.80	1.80	100.00	1.80	100.00	1.80
		Diogenidae	*Clibanarius vittatus*	1.08	0.58	54.17	0.76	70.53	0.67
		Hippidae	*Emerita benedicti*	0.36	0.18	50.00	0.04	11.32	0.11
		Penaeidae		16.19	13.02	80.40	12.78	78.96	12.90
			Farfantepenaeus aztecus	9.35	7.62	81.46	7.37	78.83	7.50
			Farfantepenaeus duorarum *	0.72	0.54	75.00	0.54	75.00	0.54
			Litopenaeus setiferus	6.12	4.86	79.41	4.87	79.63	4.86
		Portunidae		8.99	6.21	69.04	5.83	64.86	6.02
			Callinectes sapidus	5.76	4.87	84.58	4.61	80.01	4.74
			Callinectes similis	2.88	1.16	40.33	1.00	34.86	1.08
			Portunus gibbesii	0.36	0.18	50.00	0.22	62.50	0.20
		Sergestidae	Unidentified	0.36	0.36	100.00	0.36	100.00	0.36
		Unidentified	*Brachyura* spp.	3.24	2.19	67.59	2.34	72.34	2.27
			Unidentified invertebrate	2.25	1.45	57.65	1.42	56.38	1.44
		Unidentified shrimp	*Dendrobranchiata* spp.	0.72	0.30	41.67	0.07	9.54	0.18
	Isopoda		Unidentified isopod	0.72	0.54	75.00	0.40	55.60	0.47
	Stomatopoda	Squillidae	*Squilla* spp.	1.08	0.42	38.49	0.45	42.14	0.44
Cephalopoda	**Myopsina**	**Loliginidae**	***Lolliguncula brevis***	**0.72**	**0.27**	**37.50**	**0.19**	**26.56**	**0.23**
Actinopterygii				**74.10**	**90.82**	**89.93**	**92.74**	**91.61**	**67.26**
	Anguilliformes	Ophichthidae		3.60	2.59	71.90	2.19	60.98	2.39
			Bascanichthys scuticaris *	1.80	1.21	67.14	1.07	59.68	1.14
			Myrophis punctatus *	1.80	1.38	76.67	1.12	62.27	1.25
	Atheriniformes	Atherinopsidae	*Membras martinica*	0.72	0.25	35.00	0.30	41.02	0.27
	Aulopiformes	Synodontidae	*Synodus foetens* *	0.36	0.10	28.57	0.04	10.84	0.07
	Beloniformes	Hemiramphidae	*Hyporhamphus unifasciatus* *	0.36	0.18	50.00	0.34	93.35	0.26
	Blenniiformes	Gobiesocidae	*Gobiesox strumosus* *	0.36	0.12	33.33	0.16	45.26	0.14
	Carangiformes	Carangidae		2.16	1.98	91.67	1.82	84.19	1.90
			Chloroscombrus chrysurus *	1.80	1.62	90.00	1.46	81.03	1.54
			Hemicaranx amblyrhynchus *	0.36	0.36	100.00	0.36	100.00	0.36
	Clupeiformes			26.62	45.56	85.56	47.46	89.15	23.25
		Clupeidae		18.71	15.54	83.08	16.56	88.56	16.05
			Brevoortia patronus	14.39	12.00	83.42	12.76	88.71	12.38
			Dorosoma petenense *	0.72	0.72	100.00	0.72	100.00	0.72
			Harengula jaguana *	3.60	2.82	78.33	3.08	85.69	2.95
		Engraulidae		8.27	7.24	87.46	7.17	86.62	7.20
			Anchoa hepsetus	1.80	1.38	76.67	1.37	75.79	1.37
			Anchoa lyolepis *	0.72	0.72	100.00	0.72	100.00	0.72
			Anchoa mitchilli	5.76	5.14	89.27	5.08	88.28	5.11
	Gobiiformes			2.16	3.12	80.56	2.64	69.33	1.62
		Eleotridae	*Erotelis smaragdus* *	0.36	0.36	100.00	0.36	100.00	0.36
		Gobiidae		1.80	1.38	76.67	1.14	63.20	1.26
			Gobionellus oceanicus *	0.36	0.36	100.00	0.36	100.00	0.36
			Microdesmus longipinnis *	1.44	1.02	70.83	0.78	54.00	0.90
	Mugiliformes	Mugilidae	*Mugil cephalus*	0.72	0.48	66.67	0.43	60.22	0.46
	Sciaeniformes	Sciaenidae		16.55	13.03	78.72	14.22	85.97	13.62
			Bairdiella chrysoura	1.08	1.08	100.00	1.08	100.00	1.08
			Cynoscion arenarius	1.08	1.08	100.00	1.08	100.00	1.08
			Larimus fasciatus	1.44	0.81	56.25	0.83	57.96	0.82
			Leiostomus xanthurus	3.24	2.31	71.30	2.59	80.05	2.45
			Micropogonias undulatus	9.35	7.39	79.01	8.28	88.54	7.84
			Stellifer lanceolatus *	0.36	0.36	100.00	0.36	100.00	0.36
	Perciformes	Serranidae	*Centropristis philadelphica* *	0.36	0.09	25.00	0.24	65.34	0.16
	Pleuronectiformes			1.80	1.29	71.67	1.28	70.87	1.28
		Achiridae	*Trinectes maculatus* *	0.72	0.48	66.67	0.42	58.15	0.45
		Paralichthyidae	*Etropus crossotus* *	1.08	0.81	75.00	0.86	79.35	0.83
	Scombriformes	Trichiuridae	*Trichiurus lepturus*	3.60	2.82	78.33	2.61	72.69	2.72
	Scorpaeniformes	Triglidae	*Prionotus scitulus* *	0.36	0.05	14.29	0.00	1.14	0.03
	Siluriformes	Ariidae		6.83	6.18	90.35	6.49	94.99	6.33
			Ariopsis felis	3.60	3.18	88.33	3.27	90.88	3.22
			Bagre marinus	1.08	0.84	77.78	1.06	98.68	0.95

Table 5. *Cont.*

Class	Order	Family	Species	%FO	%N	%PN	%M	%PM	%PSIRI
			Unidentified catfish	2.16	2.16	100.00	2.16	100.00	2.16
	Spariformes	Sparidae	*Lagodon rhomboides*	0.36	0.03	7.14	0.01	3.97	0.02
	Unidentified			16.91	12.95	76.62	12.51	74.00	12.63
			Unidentified (fish)	16.55	12.77	77.20	12.48	75.45	12.63
			Unidentified (scales)	0.36	0.18	50.00	0.03	7.35	0.10
Unknown			**Unidentified**	**0.72**	**0.45**	**62.50**	**0.45**	**63.05**	**0.45**

* Prey species identified using genetics.

3.3.2. Diet Variability

The PERMANOVA analysis indicated that length, location, and mass were significant explanatory variables for %N and %M for gafftopsail catfish, with location explaining the greatest amount of diet variability (Table 6). For both %N and %M, the interactions between total length and location and total length and mass were significant. Only year showed evidence of significant heterogeneity in dispersion. The final PERMANOVA models for both %N and %M included the variables total length, location, and their interaction, and explained 9.7% of the diet variability for both %N and %M. Season was not evaluated for gafftopsail catfish due to small sample sizes.

Table 6. Summary of explanatory variables used in the PERMANOVA models to explain diet composition of gafftopsail catfish (*Bagre marinus*) sampled in the northern Gulf of Mexico from May 2015–September 2019 for dietary analysis. %N = average percent number, %M = average percent mass, df = degrees of freedom, F = F-statistic, R^2 = amount of explained variability, p = p-value. Significant variables and their interactions are in bold.

			%N			%M		
Model	**Variable(s)**	**df**	**F**	**R^2**	***p***	**F**	**R^2**	***p***
Single Variables	**Total Length (binned)**	1	3.446	0.012	**0.006**	3.540	0.013	**0.004**
	Location	7	1.839	0.056	**0.003**	1.769	0.054	**0.006**
	Mass	1	3.082	0.011	**0.007**	3.227	0.012	**0.008**
	Year	4	1.195	0.017	0.218	1.309	0.019	0.126
Interactions	**Total Length (binned) × Location**	4	2.099	0.036	**0.002**	2.206	0.038	**0.002**
	Total Length (binned) × Mass	1	3.023	0.011	**0.012**	3.531	0.013	**0.004**
	Total Length (binned) × Year	2	0.915	0.007	0.574	0.900	0.006	0.583
	Location × Mass	7	1.357	0.041	0.076	1.400	0.042	0.064
	Location × Year	12	1.015	0.053	0.443	0.994	0.052	0.489
	Mass × Year	3	0.770	0.008	0.733	0.734	0.008	0.776
Final Model	**Total Length (binned)**	1	3.671	0.016	**0.004**	3.786	0.016	**0.003**
	Location	7	1.539	0.046	**0.026**	1.464	0.044	**0.042**
	Total Length (binned) × Location	4	2.100	0.036	**0.001**	2.206	0.038	**0.001**
	Residuals	212		0.097			0.097	

The final CCA models for both %N and %M were significant, with one significant axis (CCA1). Total length and location were included in the models. The CCA models for %N and %M explained 6.4% of the diet variability for gafftopsail catfish. Prey items in the orders Siluriformes and Pleuronectiformes were correlated with the South Mobile Bay region and small, immature gafftopsail catfish. Prey items in the orders Carangiformes, Scombriformes, and Clupeiformes were correlated with large, mature gafftopsail catfish and the East Mobile Bay region for %N, and the West Offshore region for %M (Figure 6).

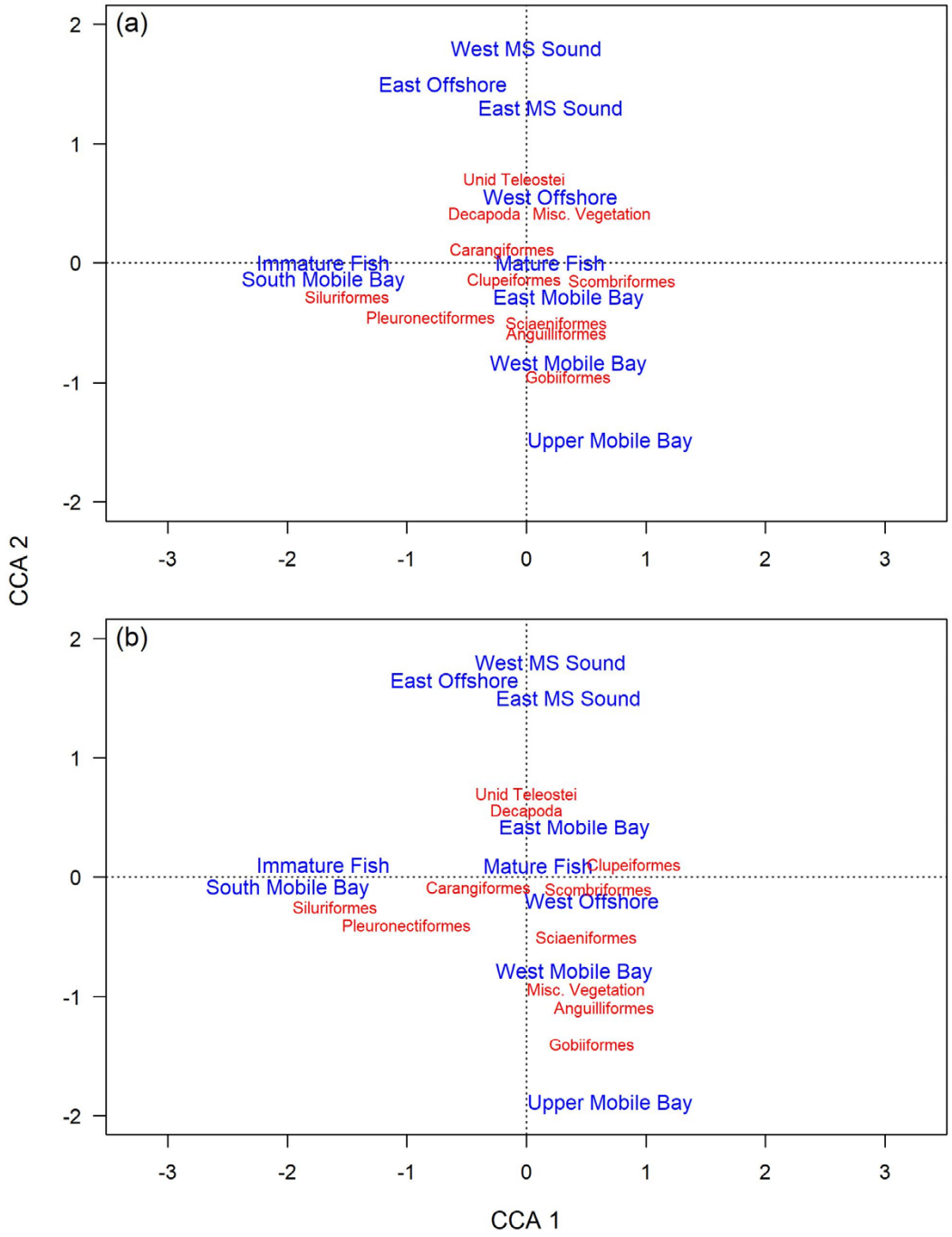

Figure 6. Canonical correspondence analysis plots for (**a**) percent number of prey (%N) and (**b**) percent mass of prey (%M) for gafftopsail catfish (*Bagre marinus*) sampled in the northern Gulf of Mexico from May 2015–September 2019 for dietary analysis. Explanatory variables from the final PERMANOVA model (TL/maturity and location) are shown in blue and prey orders are shown in red.

4. Discussion

Diet analysis aided by DNA barcoding identified distinct differences between hardhead and gafftopsail catfish in the northern GOM. These findings do not align with previous research that suggests both species share similar diets as opportunistic feeders and omnivorous scavengers. Stomach contents of hardhead catfish were dominated by crustaceans, while gafftopsail catfish showed a significantly broader dietary breadth and were primarily piscivorous. Gafftopsail catfish also consumed prey that varied widely in size and habitat, with food items ranging from benthic infauna and epifauna (such as *Lepidophthalmus louisianensis* and *Callinectes sapidus*, respectively) to pelagic species such as *Anchoa mitchilli*. Previous studies reported that crustaceans were a primary prey item for both hardhead catfish and gafftopsail catfish [5,8,9]. In the present study, hardhead catfish CCA biplots show a high and consistent presence of crustaceans, aligning with these earlier studies. These findings support earlier studies that suggest this species is primarily dependent on meio- and macro-benthic invertebrates and epifaunal decapods. However, past results are at odds with the observed diet of gafftopsail catfish in the present study, which largely prey on fishes.

Biological differences could explain the dietary disparities between hardhead catfish and gafftopsail catfish in the present study. The mean length of gafftopsail catfish sampled in this study was more than double that of hardhead catfish, and the mean mass of gafftopsail catfish was more than five-times that of hardhead catfish. Generally, as fish size increases, so do the quantity and size range of its prey items [34]. Thus, a larger size would explain the wider dietary breadth for gafftopsail catfish compared to hardhead catfish occupying the same habitats. Alternatively, the observed differences in diet between gafftopsail catfish and hardhead catfish in this study could be confounded by the taxonomic resolution of the diet analyses. Specifically, the inability to resolve family-level dietary habits for gafftopsail catfish may have weakened the comparison between the two catfishes. However, given the magnitude of the observed differences in prey items, it is likely that these findings reflect true differences in diet between the two species, rather than biological artifacts.

Findings from this study agree with previous hardhead catfish diet studies from the southern [1] and eastern [9] GOM. The significant interaction between location and year in the present study is not surprising, as the extent of the spatial-temporal overlap between hardhead catfish and their prey is reflected in their diet. For example, both estuarine ghost shrimp (*Lepidophthalmus louisianensis*) and crabs of the *Callinectes* genus were important to hardhead catfish diet; estuarine ghost shrimp exhibit high abundances in the northern GOM, specifically off Louisiana and Alabama [35], and *Callinectes* crabs are among the most dominant benthic macroinvertebrates along the Atlantic coast of both North and South America [36]. These prey items exhibit broad distributions, which may contribute to the consistency in hardhead catfish diets between this study and past diet studies in other regions.

In contrast, gafftopsail catfish diet showed significant spatial differences compared to previous studies from the southern GOM. For example, Yanez-Arancibia and Lara-Domingues [1] reported unidentified organic matter as the dominant dietary item for gafftopsail catfish, but only sampled juveniles. Similarly, Mendoza-Carranza [5] found that fish prey were significantly less frequent than brachyuran prey, which were observed in high and consistent abundances throughout the year. However, brachyurans contributed little to gafftopsail catfish diet in the present study. Gafftopsail catfish prey items reported in the present study more closely align with observations off the west Florida coast, where fishes, amphipods, and shrimp were the most dominant prey species [9].

A lack of complete dietary information for ariid populations in the GOM could also explain some of the diet differences between past studies and the present study. Previous research evaluating stomach contents of hardhead and gafftopsail catfish often reported large quantities of unidentifiable material or failed to identify prey items to the species level, making it difficult to elucidate differences in the diets between the two catfish species [1,9].

Those that did not report these difficulties were either conducted outside of the present study's geographic range [5] or lacked the advanced methods used herein to effectively capture diet preferences [3,8]. The use of DNA barcoding in the present study enabled a more comprehensive diet characterization for both catfishes by reporting higher sample sizes and more precise prey identification compared to past diet studies for either species to date in the GOM [1,5,9], thus improving our understanding of the trophic importance of these catfishes in this region.

Final OTU assignment was more successful for vertebrate prey than invertebrate prey, allowing more prey items (i.e., fishes) to be identified for gafftopsail catfish than hardhead catfish. However, our ability to explain gafftopsail catfish diet variability was limited compared to hardhead catfish, which is likely due to the extensive dietary breadth observed for gafftopsail catfish in this study. Despite employing advanced techniques, some prey material still could not be identified for both hardhead and gafftopsail catfish. The substantial proportion of empty stomachs and unidentifiable (i.e., highly digested) material observed in this study for both catfishes and in past diet studies may indicate a rapid digestion rate for these species [9]. Difficulty in identifying prey items could also be due to methodological differences; freezing and thawing samples or preserving them for later analysis [5], rather than processing them shortly after capture [9], may limit accurate prey identification. The freezing and thawing of some stomachs in this study likely explains why the smaller hardhead catfish sampled in this study had such a high index of vacuity. Further investigation is needed to determine the digestion rates and feeding frequency of both hardhead and gafftopsail catfish. Variation in the digestion rates could result in incorrect interpretations of dietary importance, particularly for prey items that have a high %FO but are more easily digestible and are likely under-represented in stomach content analysis [22].

The present study provides some evidence for ontogenetic trophic shifts for gafftopsail catfish, but none for hardhead catfish, in the northern GOM. Curiously, while prey from the family Ariidae were the most important prey for immature gafftopsail catfish, they were less important for mature gafftopsail catfish. However, much of this difference can be explained by a one-time feeding event, as all the immature gafftopsail catfish that consumed other catfish were sampled from trawls performed on the same day. Rudershausen and Locascio [9] provided evidence of ontogenetic changes in the dietary preferences among gafftopsail catfish off the west coast of Florida, although prey items for immature gafftopsail catfish were different from those observed in the present study. Pink shrimp and amphipods were most important for small gafftopsail catfish, while unidentified fishes and crabs were most important for large gafftopsail catfish. No diet shifts with ontogeny were found for hardhead catfish in the present study, which seemed to rely heavily on decapods as primary prey items regardless of catfish size. In contrast, Yanez-Arancibia and Lara-Dominguez [1] reported that hardhead catfish diet changed with ontogeny; however, hardhead catfish were sampled outside of the geographic range of this study, the study suffered from a low (i.e., <100 individuals) sample size, and the most important food group was "unidentified organic matter" for both large (>200 mm TL) and small (<200 mm TL) individuals. Pensinger et al. [12] concluded that these catfish showed consistent trophic niche stability off Louisiana that likely does not change with maturity, aligning with the conclusions of the present study. The high versatility and wide breadth of both hardhead and gafftopsail catfish diets suggested here–along with prey resource partitioning between these two species–likely reduces inter- and intra-species competition, contributing to the high abundances and overlapping distributions of these fishes.

A better understanding of interspecific interactions between hardhead and gafftopsail catfish and their prey–which may be recreationally or commercially valuable–is critical for developing holistic management strategies for the northern GOM [37]. High abundances of these ariids, combined with their extensive use of estuarine and marine habitats and wide dietary breadth, make both species important contributors to ecosystem connectivity [1,3,12]. Results from this study indicate that hardhead and gafftopsail catfish are closely

interconnected as predator and prey, yet show distinct trophic preferences in the northern GOM. Food web models can enhance fisheries management in the northern GOM by improving our understanding of complex ecosystem processes and incorporating multispecies considerations into policy decisions. For example, management efforts aimed at reducing bycatch in the shrimp trawl fishery were unexpectedly predicted to result in decreased productivity of several commercially and recreationally valued species (e.g., *Brevoortia patronus*, *Sciaenops ocellatus*, and *Lutjanus campechanus*) based on an Ecopath/Ecosim model for the GOM [37]. Rather than increasing abundances of these valued species, bycatch reduction would allow the recovery of hardhead and gafftopsail catfish and effectively increase the predation on juveniles of valued species [37]. This counterintuitive prediction highlights the importance of evaluating potential effects of policy changes for interactions that are not yet fully understood. While model projections should be evaluated with caution, ecosystem modeling reinforces the need to establish diet composition and prey preferences for abundant predators such as hardhead and gafftopsail catfish, especially considering that minor shifts in the diets of such predators may significantly increase mortality rates of valuable juvenile species [37].

5. Conclusions

Current food web models aggregate hardhead and gafftopsail catfish into one category (e.g., "sea catfishes" [13]; "demersal coastal invertebrate feeders" [38]). Depending on the questions being addressed and the spatial scale of the model, the results presented herein indicate that this may be inappropriate. The use of DNA barcoding in the present study has not only identified distinct prey preferences for both hardhead and gafftopsail catfish, but also suggests that gafftopsail catfish exhibit ontogenetic dietary shifts. Effective fisheries management is influenced by a species' life history strategy. Both hardhead and gafftopsail catfish appear to be equilibrium strategists [39], showing longevity (lifespan greater than 10 years) combined with low fecundity and high parental investment. However, equilibrium strategists also tend to mature at older ages and exhibit low abundances, yet these marine catfishes do not possess these traits [2,7,26]. A thorough understanding of the ecology of these catfishes is needed to understand how harvested and non-harvested species may respond to changing ecosystems, climate, and fishing pressure. It is unclear how hardhead and gafftopsail catfish population declines may affect GOM ecosystems. Hardhead catfish declined off South Carolina in the 1990s and have not recovered, highlighting the vulnerability of these populations and the challenges to realizing population recovery [26]. Failure to adequately understand and capture these interactions will likely result in significant impediments to effective fisheries management of depleted or declining fish species in the northern GOM. This study represents an important step in evaluating the dietary habits of both hardhead and gafftopsail catfish in northern GOM waters and will enhance our understanding of ecosystem-level interactions for improving the future management of these species.

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

Appendix A

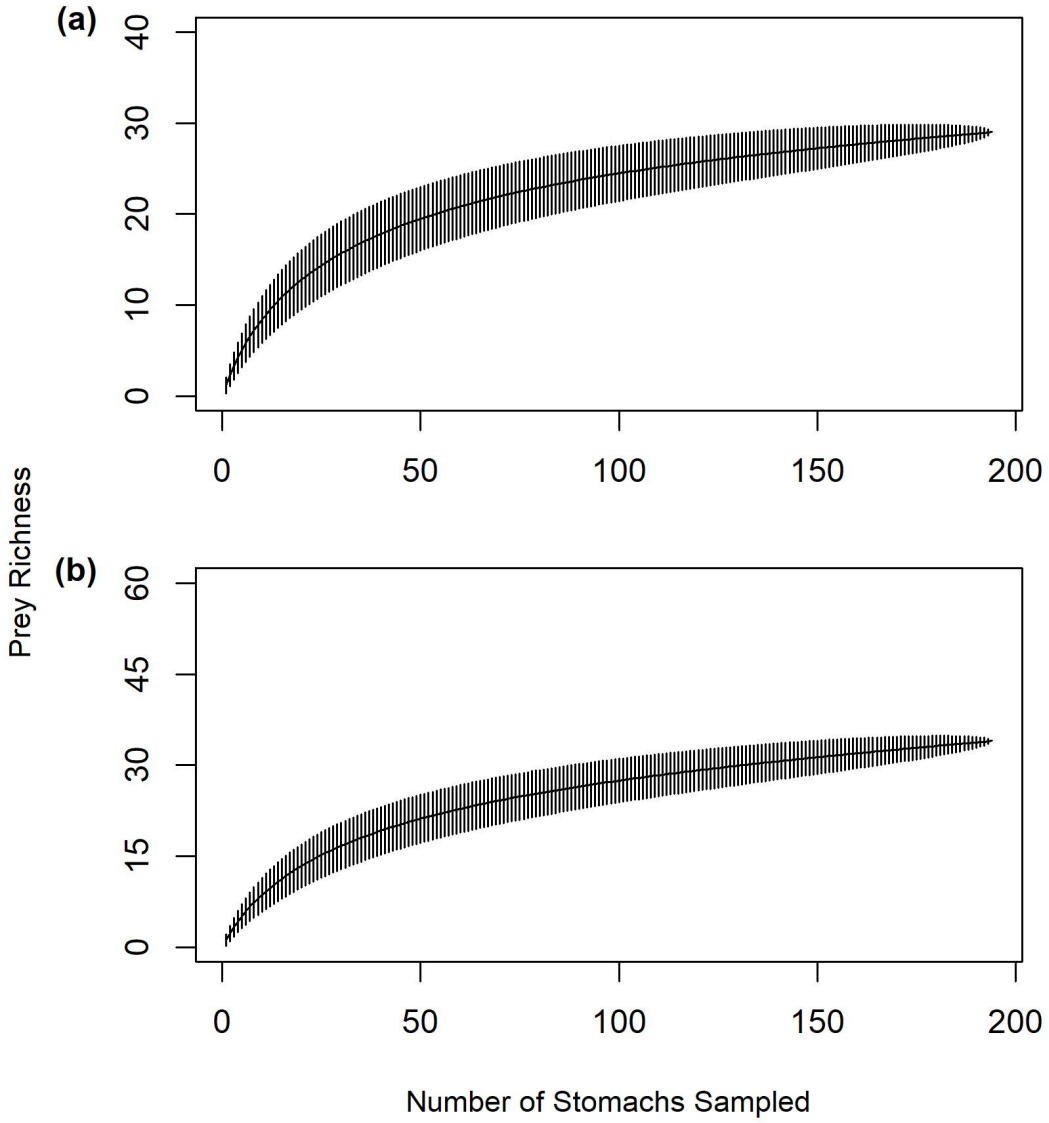

Figure A1. Cumulative prey curves for hardhead catfish (*Ariopsis felis*) at the (**a**) species and (**b**) family level. The species prey curve failed to reach an asymptote (b = 0.057), while the family prey curve showed sufficient sample size for diet characterization (b = 0.036).

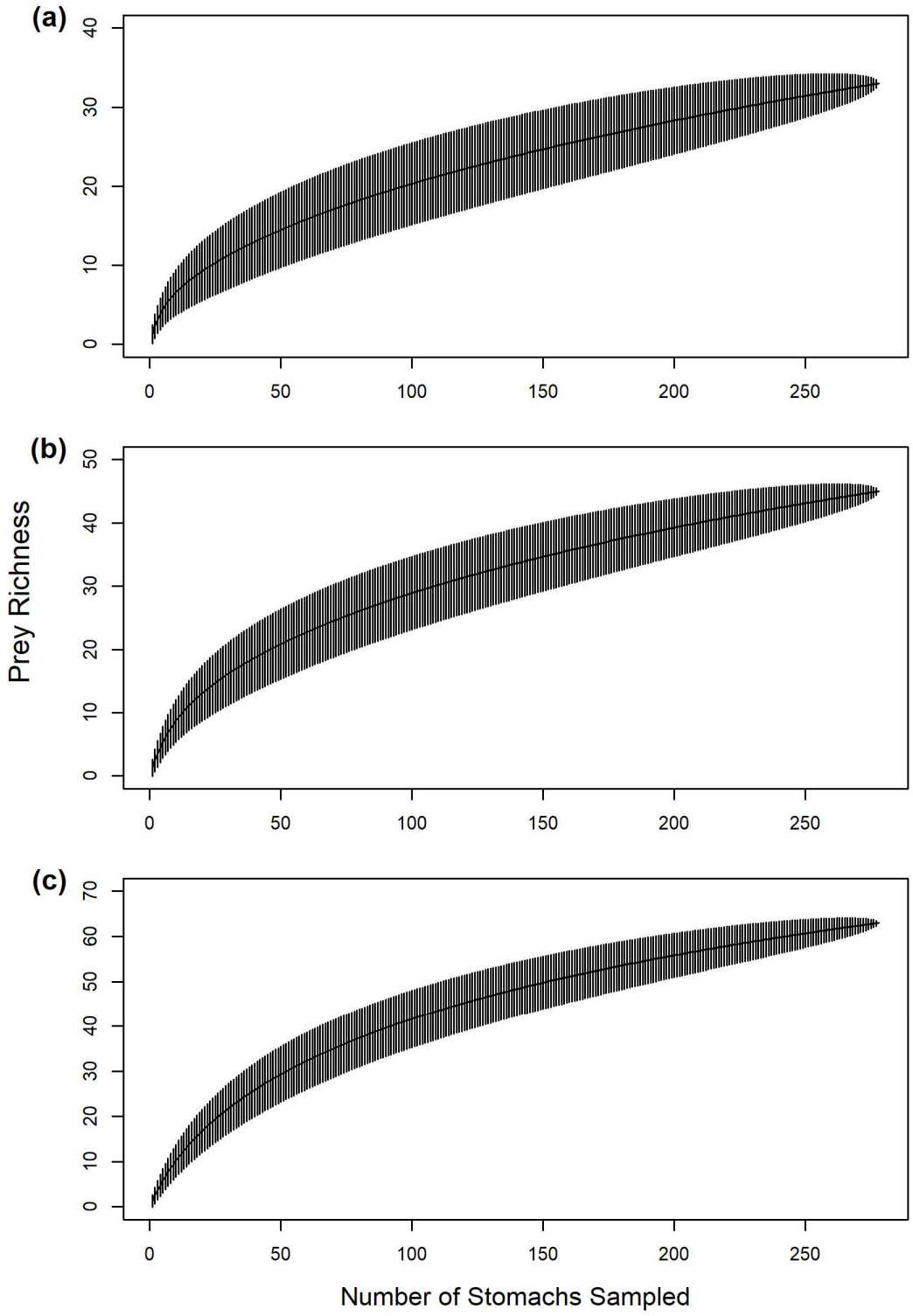

Figure A2. Cumulative prey curves for gafftopsail catfish (*Bagre marinus*) at the (**a**) species, (**b**) family, and (**c**) order level. Prey curves at the species and family levels failed to reach an asymptote ($b > 0.05$), so diet variability was analyzed at the order level.

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