I/UCRC Executive Summary **Date**: 2/17/25

Title: Metabarcoding the gut contents of predatory fish in coastal Louisiana

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Statement of Problem: Most models quantifying the trophic value of Gulf Menhaden (*Brevoortia patronus*) incorporate traditional diet studies, involving visual identification of stomach and gut contents. These methods have the potential for misidentification of partially digested prey, but also 20% of contents may be unidentified. Molecular techniques provide additional insight through barcoding of tissue and digested remains. Sequencing DNA from the tissue of partially digested and homogenized digested remains, allows more complete characterization of diet. Further, next generation sequencing provides identification of a variety of taxa simultaneously (i.e. metabarcoding) providing input about vertebrate and invertebrate prey.

Deliverables:

Dissect fish (n=400+) collected over the spring and fall sampling season. Metabarcode fish and invertebrates.

Status relative to deliverables:

Sequencing of Spotted Seatrout collected from Spring 2023 has been completed.

Summary of results relative to deliverables:

We have dissected over 352 fish in 2023 and 360 fish in 2024, including target species (Table 1). We are reporting on the most complete portions of the project which are the results from the Spotted Seatrout.

Table 1: Total fish dissected for target predatory fish collected in 2023 and 2024.

Species	N (2023)	N (2024)
Spotted Seatrout	240	171
Red Drum	45	31
Spanish Mackerel	27	15
Atlantic Croaker	16	137

Table 2: Frequency of occurrence (FOO) of prey identified in the gut contents of each target species. Unidentified prey is labeled as Unid. Each column lists the FOO for 2023 and 2024 separated by the pipe symbol (|). No data was collected on visual identification of prey items from Atlantic Croaker in 2023 and is therefore represented with a --.

Species	Empty	Unid	Fish	Crabs	Shrimp
Spotted Seatrout	6% | 45%	15% | 8%	25% | 20%	2% | 6%	20% | 24%
Red Drum	49% | 33%	0% | 7%	3% | 20%	40% | 67%	12% | 40%
SpanishMackerel	54% | 0%	17% | 20%	29% | 100%	0% | 0%	0% | 0%
Atlantic Croaker	-- | 38%	-- | 7%	-- | 4%	-- | 20%	-- | 14%

Metabarcoding: Sequencing of metabarcoding assays for fish prey items using the 12S MiFish markers has been performed for Spotted Seatrout (n=143, Table 2). Sequencing of invertebrate prey using the 18s marker has also been completed for Spotted Seatrout (n=143, Table 3).

12S Assay: A total of 42 fish species were detected (Table 2) in the gut contents of Spotted Seatrout (n=143) using the 12S metabarcoding assay. These data suggest that Spotted Seatrout are opportunistic feeders and eat a variety fish. The most common prey items were *Anchoa mitchelli, Micropogonius undulatus, and Brevoortia sp.* When examined by Coastal Study Area, similar patterns were observed, although sample sizes are unbalanced and small for some CSAs. Only 4% of the fish dissected had mixed gut contents (shrimp and fish) based on visual inspection.

The average number of prey species identified from a single fish was 2.97 and ranged from 1 to 9. An analysis of co-occurrence was performed using the R package, *cooccur*. There is not strong support for co-occurrence of prey items in Spotted Seatrout gut contents. *Fundulus pulvereus* (species #6) appears to occur most frequently with *Atractosteus spatula* (species #16), but all other species either have a negative relationship or no relationship of co-occurrence. *Atractosteus spatula* was only identified as prey items in CSA 3 (Barataria basin) and CSA 5 (Terrebonne basin). This may suggest that Spotted Seatrout tend to feed opportunistically on species that are abundant at the time, such as the most frequently occurring prey, *Brevoortia patronus, Micropogonias undulatus,* and *Anchoa mitchilli.* Alternatively, this may suggest that *Atractosteus spatula* commonly feed on *Fundulus pulvereus* and it is the secondary detection as opposed to co-occurring prey items.

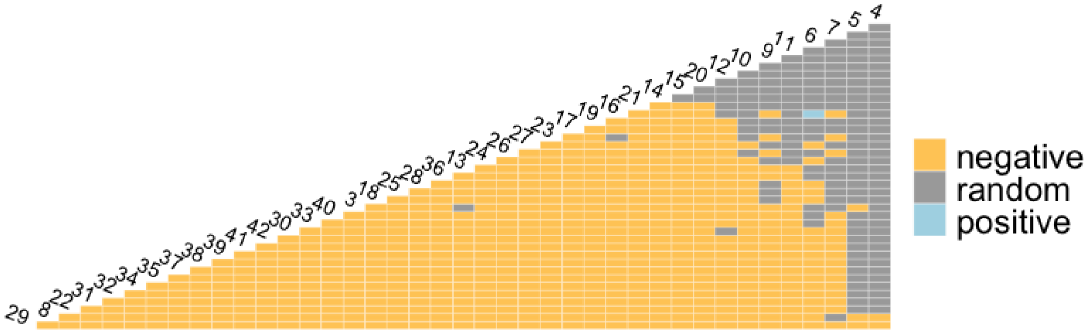

Figure 1: Species co-occurrence matrix of fish detections in the gut contents of Spotted Seatrout (n=143). Species are in the following order: 4) *Archosargus prbatocephalus*, 5) *Ariopsis felis*, 7) *Bagre marinus*, 6) *Atractosteus spatula*, 11) *Ctenogobius boleosoma*, 9) *Citharichthys spilopterus*, 10) *Clupea harengus*, 12) *Ctenogobius shufeldtii*, 20) *Harengula jaguana*, 15) *Eleotris amblyopsis*, 14) *Dorosoma petenense*, 21) *Lagodon rhomboides*, 16) *Fundulus pulverus*, 19) *Gobiosoma bosc*, 17) *Gobiesox strumosus*, 23) *Lepisosteus oculatus*, 27) *Menidia beryline*, 26) *Menidia beryline*, 25) *Membras martinica*, 18) *Gobionellus oceanicus*, 3) *Anchoa mitchilli*, 40) *Sygnathus scovelli*, 33) *Ophidion marginatum*, 30) *Micropterus dolomieu*, 42) *Urophycis tenuis*, 41) *Trichiurus lepturus*, 39) *Smphurus plagiusa*, 38) *Stellifer lanceolatus*, 37) *Scomberomorus maculatus*, 35) *Percina ouachitae*, 34) *Paralichthyse lethostigma*, 32) *Mugil cephalus*, 22) *Leiostomus xanthurus*, 8) *Brevoortia patronus*, 29) *Micropogonias undulatus.*

Red Drum (n=10) gut contents contained *Brevoortia sp.* (50%), *Ctenogobius boleosoma* (50%), *Cynoscion nebulosus* (50%), *Lepisosteus oculatus* (50%), *Atracosteus spatula* (60%), *and Ammodytes dubius* (70%). However, this detection of *Ammodytes dubius* needs to be confirmed because *Ammodytes dubius* does not occur in the Gulf of Mexico (GoM) and no other species of *Ammodytes* has been reported in the GoM.

Spanish Mackerel (n=8) gut contents contained 10 species of fish. The most common was the *Anchoa mitchilli* (88%), *Brevoortia sp.* (63%), *Cynoscion arenarius* (63%), *Trichiurus lepturus* (50%), *Ariopsis felis* (25%), *Bagre marinus* (25%), *Lagodon rhomboides* (13%), *Micropterus dolomieu* (13%), *Harengula jaguana* (13%), *Ammodytes dubius* (13%).

18S Assay: Spotted Seatrout (n-143) diet contents were sequenced, and 48 invertebrates were identified, with a total of 3,396 detections. Of those detections, 47% were bivalves, 41% were crustaceans, 6% were gastropods, and 3% were horseshoe crab. The remainder were intestinal parasites (Trematoda and Rhabdocoela), which are not likely to be prey items. Mollusk detections included scallops, oysters, limpets and clams. Crustaceans included decapods, copepods, and branchiopods. It is also of note that horseshoe crabs were found in 78% of the fish. It is difficult to distinguish between species that are intentionally eaten, those that are accidentally eaten, and those that are detected due to secondary predation. However, by focusing on the prey species that are most common, we can filter out those that are were not intentionally eaten.

The co-occurrence of invertebrates shows non-random associations of 39.8% of the taxa, most of which are positive associations. These associations are likely due to habitat types, but that data is not available in our dataset. *Barbatia sp.* and *Tucetona sp.*, both bivalves, are positively associated and may suggest that they target bivalves during specific feeding bouts.

It is also possible that some of these co-occurrences are due to secondary predation or accidental ingestion, both of which are difficult to identify in metabarcoding studies. A study on detection of secondary predation in a berry-fly-beetle system showed that detection of secondary predation was only possible when flies fed on berries immediately before the flies were ingested by the beetles (Guenay et al. 2019). A similar result was found in a study of omnivorous arthropods (Neidel et al. 2023). Although not directly transferrable, these published studies suggest that detection due to secondary predation may not be as prevalent as suspected in metabarcoding studies. It is very reasonable to assume that some of the invertebrates detected in the gut contents are accidental prey items that are ingested as Spotted Seatrout intentionally prey upon other species, such as fish, bivalves and crustaceans. For instance, it is likely that when Spotted Seatrout feed on benthic organisms, such as bivalves, that they accidentally ingest other benthic organisms. Common crustaceans, such as *Artemia salina* are small and not likely intentionally preyed upon, and may also be detected due to accidental ingestion, which would explain why they are often identified in the gut contents with 21 other prey items. However, a study investigating the impact of secondary predation and accidental ingestion with fish would be beneficial.

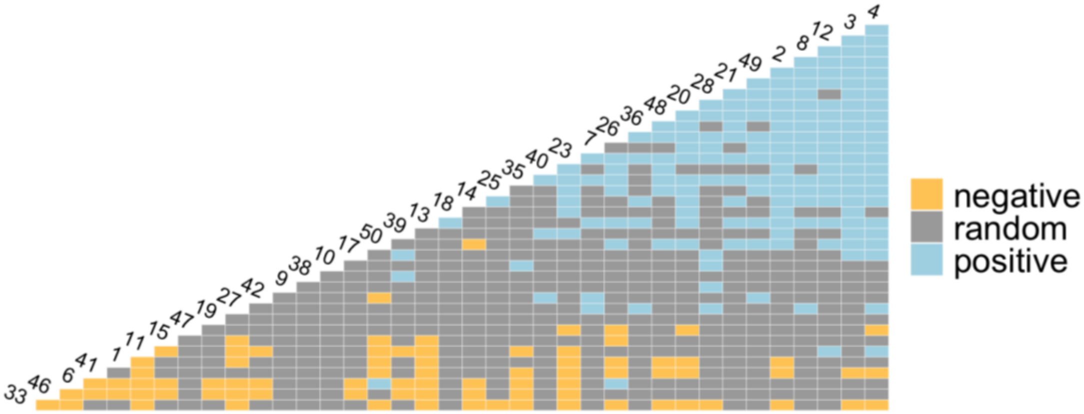

Figure 2. Species co-occurrence matrix of invertebrate detections. Species are in the following order: 4) *Atrina irradians*, 3) *Artemia salina*, 12) *Crassdoma sp.*, 8) *Calanus glacialis*, 2) *Argopecten irradians*, 49) *Temnocephala sp.*, 21) *Heterocypris sp.*, 28) *Macroschisma dilatatum*, 20) *Hematodinium sp.*, 48) *Symbiodinium sp.*, 36) *Palaemon pugio*, 26) *Limulus polyphemus*, 7) *Bosmina longirostris*, 23) *Haliotis discus* , 40) *Ostrea chilensis*, 35) *Microphallidae spp*, 25) *Heterocypris sp.*, 14) *Crassadoma spp*, 18) *Diastylis sp.*, 13) *Chlamys sp.*, 39) *Penaeus duorarum*, 50) *Tucetona pectunculus*, 17) *Derocheilocaris typica*, 10) *Cephaloidophora sp.*, 38) *Pecten maximus*, 9) *Cancrincola plumipes*, 42) *Prosorhynchoides borealis*, 27) *Lopha cristagalli*, 19) *Heliospora caprellae*, 47) *Squilla mantis*, 15) *Geukensia demissa*, 11) *Chlamys sp.*, 1) *Anaspides sp.*, 41) *Placopecten magellanicus*, 6) *Barbatia virescens*, 46) *Squilla empusa*, 33) *Nuculana pernula.*

Red Drum and Spanish Mackerel gut contents have not been sequenced at the 18s marker yet.

Total Diet:

Total diet (12s and 18s combined) with read counts is reported in the supplemental excel file included in this report (Supplemental Table 1). A total of 83 unique sequences were detected and identified to the lowest possible taxonomic level using the Blastn database. Fish were identified to the species level in most cases but invertebrates were not always reliably identified to the species level and in some cases only the genus is reported. Parasites were manually removed from the invertebrate detections. Based on the frequency of occurrence, *Barbatia virescens* was the most common prey item (99%). In fact, the presence of bivalves was very common, including *Barbatia virescens* (99%), *Tucetona sp.* (89%), *Chlamys sp.* (84%), *Atrina pectinata* (57%), *Ostrea sp.* (45%). *Squilla* species also appeared to be important prey items (*Squilla empusa*, 97% and *Squilla mantis*, 3%). *Limulus polyphemus* was identified in 78% of fish. Crustaceans (*Diastylis sp.* (66%) and *Artemia salina* (49%)) were also common in gut contents. *Urophycis tenuis*, *Brevoortia patronus*, *Micropogonias undulatus*, *Trichiurus lepturus*, and *Anchoa mitchilli* were all important fish detected in the gut contents. *Penaeus duorarum* (31%) was the most common shrimp identified but *Penaeus setiferus* (10%) was also identified.

Table 3. Species detected in the gut contents of Spotted Seatrout (n=107) using metabarcoding. Frequency of occurrence (FOO) is the number of fish with the prey species detected divided by the total number of fish.

Organism	FOO
Barbatia virescens	99%
Squilla empusa	97%
Tucetona sp.	89%
Chlamys sp.	84%
Limulus polyphemus	78%
Diastylis sp.	66%
Argopecten irradians	60%
Atrina pectinata	57%
Artemia salina	49%
Ostrea sp.	45%
Urophycis tenuis	44%
Brevoortia patronus	44%
Micropogonias undulatus	42%
Trichiurus lepturus	42%
Anchoa mitchilli	41%
Heterocypris sp.	38%
Lopha cristagalli	37%
Penaeus duorarum	31%
Macroschisma dilatatum	30%
Limnadia lenticularis	28%
Eucyclops serrulatus	27%
Crassadoma sp.	26%
Cynoscion arenarius	26%
Temnocephala sp	24%
Geukensia demissa	22%
Calanus sp.	21%
Fundulus pulvereus	17%

Palaemon pugio	14%
Sciaenops ocellatus	12%
Leiostomus xanthurus	11%
Scomberomorus maculatus	11%
Haliotis sp.	10%
Penaeus setiferus	10%
Atractosteus spatula	8%
Bosmina longirostris	6%
Helicana sp.	6%
Lepisosteus oculatus	6%
Menidia beryllina	6%
Gobiesox strumosus	5%
Nuculana pernula	5%
Bagre marinus	4%
Ctenogobius shufeldti	4%
Gobiosoma bosc	4%
Cancrincola plumipes	3%
Clupea harengus	3%
Ctenogobius boleosoma	3%
Paralichthys lethostigma	3%
Pecten maximus	3%
Squilla mantis	3%
Syngnathus scovelli	3%
Lucania parva	3%
Gobionellus oceanicus	2%
Lagodon rhomboides	2%
Citharichthys spilopterus	2%
Nautilus sp.	2%
Eleotris amblyopsis	1%
Anaspides sp.	1%
Azumapecten farreri	1%
Dorosoma petenense	1%
Goniobranchus tinctorius	1%
Harengula jaguana	1%
Hiatula diphos	1%
Membras martinica	1%
Menticirrhus americanus	1%
Micropterus dolomieu	1%
Micropterus salmoides	1%

Modiolus capax	1%
Mugil cephalus	1%
Oedignathus inermis	1%
Ophidion marginatum	1%
Parvamussium undisonum	1%
Percina ouachitae	1%
Placopecten magellanicus	1%
Pluvialis sp	1%
Pteria macroptera	1%
Spisula subtruncata	1%
Stellifer lanceolatus	1%
Symphurus plagiusa	1%
Turbo sp	1%
Anchoa hepsetus	1%
Ariopsis felis	1%
Ammodytes dubius	1%
Archosargus probatocephalus	1%

The Fisher's Exact test was used to determine if any prey items were found more or less often in CSA 3 (n=25), 5 (n=33) and 6 (n=35). CSA 1 (n=14) was excluded from the analysis due to low sample size. *Micropogonias undulatus*, which was found in 42% of the Spotted Seatrout was found significantly less (p=0.0002) in fish caught in CSA 3 than in CSA 5, but there was no difference between CSA 3 and CSA 6 or CSA 5 and CSA 6. *Fundulus pulvereus* was found in 17% of Spotted Seatrout and was found significantly more (p=0.002) in fish caught in CSA 5 than in CSA 3 or CSA 6. Lastly, *Sciaenops ocellatus* was found in 12% of Spotted Seatrout and was found significantly less in fish caught in CSA 3 than in CSA 5, but there was no difference between CSA 3 and 6 or CSA 5 and CSA 6. These differences occurring in CSA 3 are likely due to the differences in salinity. CSA 3 collects fish from the Barataria Basin, which receives fresh water from the Mississippi River and the lower salinity in the area leads differences in species assemblages.

Species detections from both markers were combined and co-occurrence was done as described above. Most co-occurrences were negative, although it should be noted that this relies on no detection of a species and a negative detection using eDNA could be due to the lack of PCR amplification, biases during sequencing or the lack of adequate DNA in the gut contents. The efficiency can also vary between the two assays (12s for fish and 18s for invertebrates) and may create a bias in the analysis.

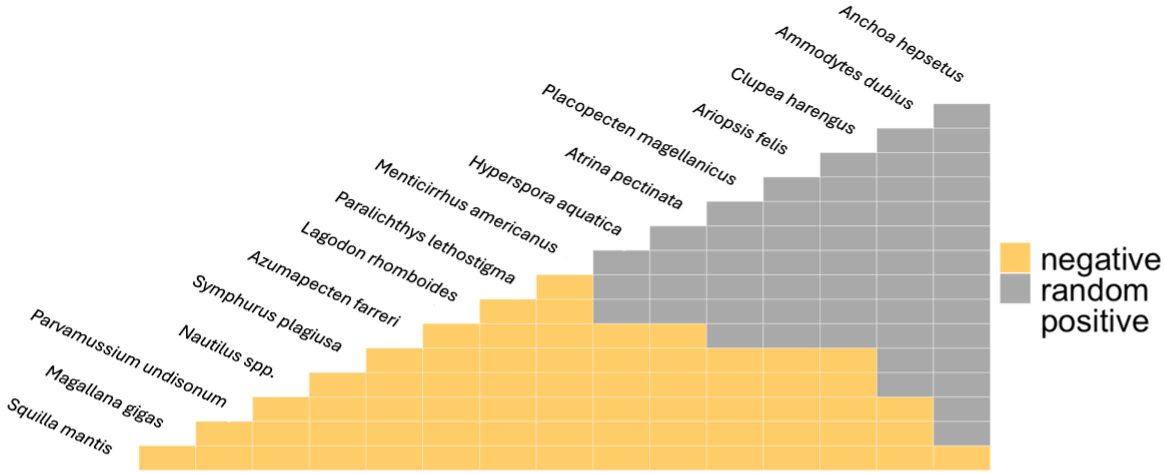

Figure 3. Species co-occurrence matrix of total diet. No positive co-occurrences were detected.

Future work:

This project was funded for 1 year (2023, two seasons of sampling) to collect 400 fish from Louisiana Coastal Study Areas, targeting Spotted Seatrout and Red Drum. This report covers the results of that year of sampling; however additional funding has been secured for future work. An MS student has started at University of Maryland Eastern Shore on a fellowship with the Living Marine Resources Cooperative Science Center (LMRCSC) to continue a second year this study. DNA extractions are currently underway for 2024 Spotted Seatrout gut contents. He will finish 2024 sequencing of the Spotted Seatrout samples and will sequence the gut contents of Red Drum and Atlantic Croaker. We expect to prepare a publication on Spotted Seatrout in 2026 with funding from the LMRCSC.