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Using DNA metabarcoding to reveal prey diversity in diets of juvenile black sea bass (*Centropristis striata*) in Long Island Sound in the Northwest Atlantic Ocean

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Abstract—The abundance of black sea bass (*Centropristis striata*) has increased in coastal waters of the northeastern United States, and the implications of that change for regional food webs remain poorly understood. We used DNA metabarcoding of 2 gene regions, the mitochondrial cytochrome *c* oxidase subunit I (COI) region and the V9 hypervariable region of 18S rRNA, to classify prey in gut contents of juvenile black sea bass collected from a nearshore habitat in Long Island Sound (LIS). Using sequence numbers for the COI and V9 regions, we found that gut contents were dominated by the crustacean superorder Eucarida, followed by 2 polychaete species, and an invasive amphipod, *Grandidierella japonica*. Bivalves, gastropods, and ostracods were less abundant. Fish prey, including northern pipefish (*Syngnathus fuscus*) and Atlantic silverside (*Menidia menidia*), were present (>10% of sequences) in the stomachs of 2 large black sea bass. High variability in sequence numbers was evident between individuals for both the COI and V9 gene regions. Taxonomic composition of prey items also differed: eucarids contributed >87% of V9 gene sequences in samples from 51 stomachs, amphipods composed >50% of sequences in samples from guts of 21 fish, and polychaetes dominated with >75% sequences in samples from 6 stomachs. We concluded that, during settlement pulses, juvenile black sea bass may compete with other benthic predators for decapod, polychaete, and amphipod prey in LIS nearshore habitats.

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Human intervention, exploitation, and climate change have increasingly led to rapid ecosystem and regime shifts worldwide (Perry et al., 2005; Poloczanska et al., 2013). Long Island Sound (LIS), a tidal estuary of the Northwest Atlantic Ocean located at 41°19′24″N, 72°0′55″W, borders one of the most densely populated metropolitan areas in the world and consequently is part of one of the most anthropogenically affected coastal regions in North America. The waters of LIS have been warming at a rate about 4 times faster than the global average over recent decades (Snyder et al., 2019), causing a rapid, ongoing reorganization of the LIS ecosystem evidenced by a shift from cold-water adapted to warm-water adapted marine communities (Howell and Auster, 2012; Crosby et al., 2018; Snyder et al., 2019). Regime shifts are often associated with rapid increases in the abundance of new or previously rare species, which then assume new, self-reinforcing trophic roles (Alheit et al., 2005). In LIS, this type of response to a regime shift is a likely explanation for the increase in abundance of black sea bass (*Centropristis striata*), an omnivorous mesopredator (a predator that occupies a mid-ranking trophic level in a food web) of the grouper family (Serranidae), in waters on the continental shelf of the Northwest Atlantic Ocean (Bell et al., 2015; McMahan et al., 2020). This species has become more abundant while populations of cold-water species have continued to decline in abundance (Collie et al., 2008; Howell et al., 2016; Frisk et al., 2018). Indeed, abundance of black sea bass in LIS has risen by one order of magnitude since 2010 (Zavell et al., 2024).

The large increase in abundance of black sea bass in LIS could be affecting the food web and productivity in profound ways, but little is currently known about diets of black sea bass in the region (Taylor et al., 2023). Results from previous diet studies of black sea bass captured south of the Hudson Canyon, an extension of the Hudson River Valley located about 160 km southeast of New York City, indicate a wide, generalist diet, including crustacean, mollusk (especially squid), and fish species (Sedberry, 1988; Hood et al., 1994; Steimle et al., 1999). However, the diet of black sea bass north of the Hudson Canyon is less known, particularly for

juveniles, which now arrive during July–September in large numbers to settle in shallow nursery habitats in LIS. It is in these LIS nursery habitats that settling black sea bass could first affect local food webs by potentially outcompeting other benthic predators for prey. No data are currently available on the diets of juvenile black sea bass in LIS, and this knowledge gap limits projections of possible ecosystem effects.

Research on fish diets has traditionally relied on morphological inspection of preserved gut contents, requiring diverse taxonomic expertise and a considerable time commitment of research staff (e.g., Taylor et al., 2023). Moreover, morphological approaches detect large, hard-bodied prey like fish more reliably than soft-bodied, small, and less abundant prey items like gelatinous zooplankton (Amundsen and Sánchez-Hernández, 2019). The use of DNA metabarcoding has provided new insights into marine biodiversity (Bucklin et al., 2016; Govender et al., 2023). Metabarcoding allows identification of taxa from a sample based on analysis of short regions of DNA sequences called *barcodes* (Taberlet et al., 2012). One commonly used barcode is a portion of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene region (Bucklin et al., 2021); additional markers include hypervariable regions in rRNA, including the V9 hypervariable region of 18S rRNA (Amaral-Zettler et al., 2009). Metabarcoding relies on high-quality databases of reference gene sequences to identify species or taxonomic groups in a sample (Coissac et al., 2012; Bucklin et al., 2021; O'Brien et al., 2024).

Metabarcoding holds promise for fish diet studies because DNA samples can be processed in a relatively short amount of time and highly digestible, soft-bodied prey items that may elude morphological analyses can be detected (Schroeder et al., 2020). The diets of yellowfin seabream (*Acanthopagrus latus*) (Su et al., 2018; Pan et al., 2021) and Arctic lamprey (*Lethenteron camtschaticum*) (Shink et al., 2019) have been successfully examined by using metabarcoding. The utility of metabarcoding with the V9 region of 18S rRNA has been previously demonstrated in an examination of diets of 2 zooplanktivorous clupeids: European pilchard (*Sardina pilchardus*) and European sprat (*Sprattus sprattus*) (Albaina et al., 2016).

In this study, we used metabarcoding to examine the diet of juvenile black sea bass collected from a shallow, coastal habitat in LIS. We used the COI and V9 barcodes to characterize the prey spectrum and identify dominant hard- and soft-bodied prey taxa that could be affected by the expansion of the range of black sea bass in coastal regions of the Northwest Atlantic Ocean. We also tested the hypotheses that the use of the 2 gene regions for metabarcoding would yield qualitatively consistent results and that diet composition would vary with fish size and between 3 sampling dates in August 2020.

Materials and methods

Sample collection and DNA extraction

Juvenile black sea bass (≤14 cm in total length [TL]; Miller et al., 2016) were collected by using beach seine nets (30 × 2 m) in Mumford Cove, a small, seagrass-dominated embayment off Connecticut in the eastern area of LIS. On each of 3 sampling dates, 6, 14, and 26 August 2020, collections were composed of up to 35 juveniles of similar lengths, with ranges of 22–51 mm TL, 29–62 mm TL, 31–69 mm TL, respectively, and an overall mean of 38 mm TL (standard deviation 7). Four large juveniles (>135 mm TL) were also collected for comparison of diets with those of smaller juveniles. The fish were removed from the seine net and immediately flash frozen in liquid nitrogen. A total of 107 specimens were analyzed in this study. In the laboratory, fish were partially thawed to dissect stomach contents but kept on ice to ensure stability of DNA. The stomach lining was removed, and gut contents were homogenized in buffer solution of sodium dodecyl sulfate and digested in a 55°C water bath for 6–7 h. DNA was extracted by using phenol-chloroform-isoamyl alcohol (mixture ratio: 25:24:1) and then a DNeasy PowerClean Cleanup Kit[1] (Qiagen, Hilden, Germany).

Amplicon generation and sequencing

Purified DNA was amplified for the target COI and V9 regions through polymerase chain reaction (PCR). MiSeq adapters (Illumina Inc., San Diego, CA) were added to all primers used for amplification. A 313-base-pair (bp) region of the COI gene sequence was amplified with Invitrogen Platinum *Taq* DNA Polymerase (Thermo Fisher Scientific Inc., Waltham, MA) reagents and 20 ng of DNA by using PCR primers, mlCOIintF and jgHCO2198, and following published protocols (Geller et al., 2013; Leray et al., 2013). For the amplification of the V9 gene region, 10 ng of DNA from each individual stomach was mixed with KAPA HiFi PCR Kit (Roche Diagnostics Corp., Indianapolis, IN) and 1 µL of each primer (10 µM), 1510R and 1380F, by using published protocols (Amaral-Zettler et al., 2009). The sample products from amplification of both the V9 and COI regions were examined by using electrophoresis in a 2% gel with a 50-bp marker.

Library preparation entailed adding Nextera XT index primers (Illumina Inc.) for a second PCR cycle. Successful library attachment was verified by using a High Sensitivity D1000 ScreenTape Assay for 4200 TapeStation Systems (Agilent Technologies Inc., Santa Clara, CA). Bidirectional sequencing of libraries was carried out at the University of Connecticut Center for Genomic Innovation by using a MiSeq System sequencer and the MiSeq Reagent Nano Kit v2 (Illumina Inc.) spiked with a minimum of 20% PhiX Sequencing Control v2 (Illumina Inc.) to account for amplicon sequence redundancy.

Bioinformatics

Gene sequences from 35 of 100 samples taken from stomachs of black sea bass were available for COI analysis.

[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

Paired-end reads for COI sequences were processed with a custom mothur script (vers. 1.44.3; Schloss et al., 2009), executed on the Xanadu computing cluster (Xanadu Quantum Technologies, Toronto, Canada) at the University of Connecticut. Sequences were filtered by using length and sequence quality control values. Generated contiguous sequences (>150 bp) were aligned to sequences in the global MetaZooGene Atlas and Database (O'Brien et al., 2024; database available from website). The PCR errors and chimeras were filtered out by using Unoise and Vsearch in mothur (Rognes et al., 2016). Taxonomic classification and species identification were based on sequences and operational taxonomic units for the COI region by using a naive Bayesian classifier algorithm in mothur against the data set for the North Atlantic Ocean in the MetaZooGene Atlas and Database (Bucklin et al., 2021). Species-level taxonomic identification was carried out by using >97% bootstrap values after 100 iterations. Bootstrap values in COI metabarcoding analyses usually range from 95% to 100% to maximize recovered genetic diversity while controlling for sequence errors (Porter and Hajibabaei, 2020).

Sequences from 99 of 100 samples taken from stomachs of black sea bass were available for analysis of the V9 region. The reads of the V9 gene sequences were also analyzed with the tailored mothur pipeline script (Schloss et al., 2009) and executed on the Computational Biology Core at the University of Connecticut. Contiguous sequences were assembled from forward and reverse MiSeq reads. Contiguous sequences containing ambiguous base calls and quality Phred scores <30 were discarded. To mitigate any PCR biases, we screened for and removed chimeras using Vsearch in mothur (Rognes et al., 2016). Remaining sequences were aligned to the sequences in the SILVA reference database, release 132 (Quast et al., 2012). Aligned sequences that did not span the entire V9 region were removed to improve the accuracy of the generated operational taxonomic units. To further address potential PCR bias, we used the Unoise 37 method (Edgar[2]) in mothur to impose a 2-bp difference limit between sequences before clustering of operational taxonomic units. Taxonomic assignment with identification bootstrap values ≥80% after 100 iterations were carried out by using a Bayesian classifier (Wang et al., 2007) and a modified database developed from the data set of the small subunit (18S rRNA) sequences in the SILVA database, release 132 (Blanco-Bercial, 2020). Bootstrap values for the V9 region are lower than those for the COI region because of the slower rate of evolution. This difference in rate of evolution results in conserved regions, facilitating the design of primers and allowing taxonomic assignment to family level at best (Amaral-Zettler et al., 2009).

On the basis of the taxonomy summary files for COI generated in mothur (Wang et al., 2007), we focused on gene sequences for 6 taxonomic prey groups: Eucarida (i.e., Decapoda and Euphausiacea), Polychaeta, Amphipoda, Actinopterygii, Copepoda, and Gastropoda. For the V9 region, we additionally included sequences for Ostracoda, Bivalvia, Hemichordata, and Ascidiacea, while disregarding sequences for Actinopterygii because the V9 region cannot be used to distinguish between the tissues of fish prey and the stomach of the predator. Taxa represented by <100 sequences were removed from further analysis.

We used cumulative COI and V9 sequence numbers across all stomach samples to describe the overall prey composition of juvenile black sea bass. Variation between samples was examined by comparing taxon-specific sequence numbers expressed as proportions of the total sequence number for all samples. Kruskal–Wallis tests were used to compare log-transformed (log10[N+1]) V9 sequence numbers of Polychaeta, Amphipoda, and Copepoda between the main cohort sample (i.e., juveniles 22–69 mm TL) and the 4 individuals >135 mm TL. To determine whether prey composition changed between the 3 sampling dates in August 2020, we used log-transformed V9 sequence numbers of the 9 prey taxa for each individual as input for a principal component analysis. Components with an eigenvalue >1 were extracted, rotated component scores were used to test for significant differences between sampling dates with a multivariate general linear model, and Fisher's least significant difference post-hoc tests were conducted. To test for collinearity between V9 and COI sequence numbers, we restricted the data set to only those individuals for which data for both barcodes were available (sample size [n]=35) and used Pearson correlation and linear regression to compare the log-transformed sequence numbers within each major prey group. These analyses were done in SPSS Statistics, vers. 20.0 (IBM, Armonk, NY).

All metabarcoding sequence data and associated metadata have been deposited in the GenBank Sequence Read Archive (National Center for Biotechnology Information, available from website) under BioProject PRJNA1101022. The GenBank accession numbers for COI gene sequences are SRR28750955–SRR28750989 and for V9 gene sequences are SRR28745280–SRR28745384.

Results

Through metabarcoding of the COI region, we detected a total of 2.79 million sequences across 35 samples of the stomach contents of black sea bass (Fig. 1A). Over half of all sequences belonged to the superorder Eucarida (1.47 million sequences), represented almost completely by 3 decapod crustacean species: the sevenspine bay shrimp (*Crangon septemspinosa*) (59%), zostera shrimp (*Hippolyte zostericola*) (30%), and longwrist hermit (*Pagurus longicarpus*) (6%). The class Polychaeta (0.52 million sequences) was the second-most abundant taxon, consisting largely of sand worms (*Alitta virens*) (43%) and clam worms (*A. succinea*) (37%). Amphipod sequences (0.35 million sequences) were attributable almost exclusively to *Grandidierella japonica* (97%). An additional 20,000 COI

[2] Edgar, R. C. 2016. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. BioRxiv 081257. [Available from website.]

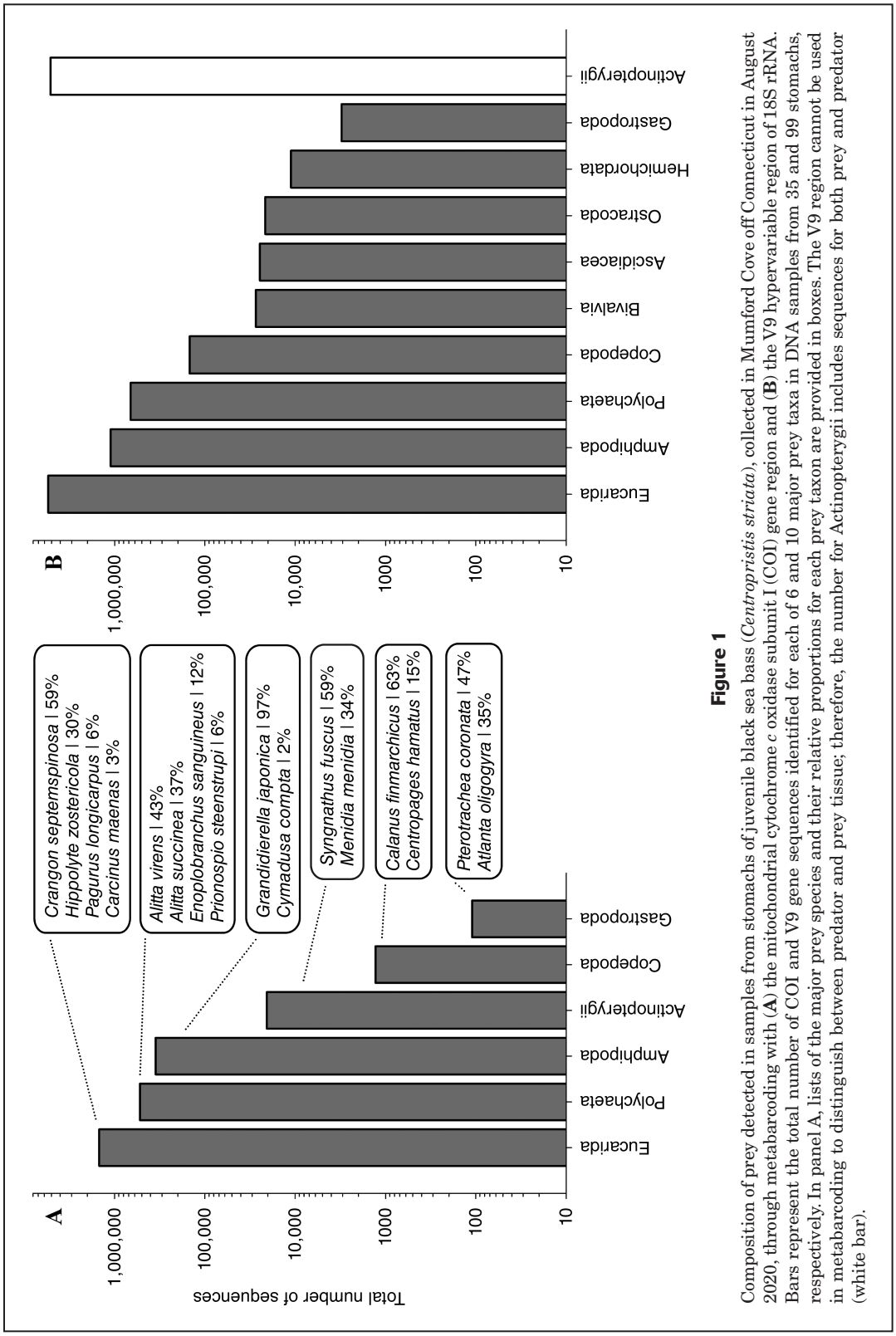

Figure 1

Composition of prey detected in samples from stomachs of juvenile black sea bass (*Centropristis striata*), collected in Mumford Cove off Connecticut in August 2020, through metabarcoding with (**A**) the mitochondrial cytochrome *c* oxidase subunit I (COI) gene region and (**B**) the V9 hypervariable region of 18S rRNA. Bars represent the total number of COI and V9 gene sequences identified for each of 6 and 10 major prey taxa in DNA samples from 35 and 99 stomachs, respectively. In panel A, lists of the major prey species and their relative proportions for each prey taxon are provided in boxes. The V9 region cannot be used in metabarcoding to distinguish between predator and prey tissue; therefore, the number for Actinopterygii includes sequences for both prey and predator (white bar).

sequences of fish (Actinopterygii) were attributable to northern pipefish (*Syngnathus fuscus*) (59%) and Atlantic silverside (*Menidia menidia*) (34%), and COI sequences of about 1000 Copepoda and about 100 Gastropoda were identified across all stomach samples (Fig. 1A).

Metabarcoding of the V9 region returned a total of 12.6 million sequences for 99 stomach samples from juvenile black sea bass (Fig. 1B). This number included 5.1 million sequences for Actinopterygii that were not included in analysis because V9 gene sequences cannot be used to distinguish between fish prey tissue and predator stomach tissue. Consistent with results from COI metabarcoding, most V9 sequences for taxa that were not fish were attributable to Eucarida (5.45 million sequences), followed by Amphipoda (1.11 million sequences), Polychaeta (0.67 million sequences), and Copepoda (0.15 million sequences). Sequences from the V9 region attributed to Bivalvia, Ascidiacea, Ostracoda, and Hemichordata were one order of magnitude rarer (0.01–0.03 million sequences) than sequences attributable to the previously mentioned more common taxa, but they were more frequent than sequences attributed to Gastropoda (0.003 million sequences) (Fig. 1B).

Both the number and taxonomic composition of detected sequences varied widely between juvenile black sea bass (Fig. 2). The total number of COI sequences ranged from 20 to 120,000 (mean: 66,000) per stomach sample, and the total number of V9 sequences ranged from 7000 to 416,000 (mean: 120,000). In analysis with V9 sequence data, Eucarida were detected in all stomach samples, and in half of those samples (n=51) Eucarida accounted for >87% of all V9 sequences. Amphipoda and Polychaeta sequences were also detected in all samples, representing an average of 20% and 10% of sequences, respectively. In addition, results of gut content analysis indicate that 21 samples had >50% of total sequences classified as Amphipoda and that another 6 samples had >75% of total sequences classified as Polychaeta. Low numbers of Actinopterygii sequences were detected by using the COI region in 32 of 35 stomachs from black sea bass, but only the 2 largest individuals (138 and 145 mm TL; Fig. 2) had notable amounts of fish in their stomachs (i.e., >10% of all the sequences detected in each stomach). Shifts in the diet of black sea bass with size are also indicated by the V9 sequence data: comparisons with Kruskal–Wallis tests revealed that the 4 largest specimens had significantly lower average sequence numbers of Polychaeta (P<0.001), Amphipoda (P=0.001), and Copepoda (P=0.002) than smaller specimens (Fig. 3).

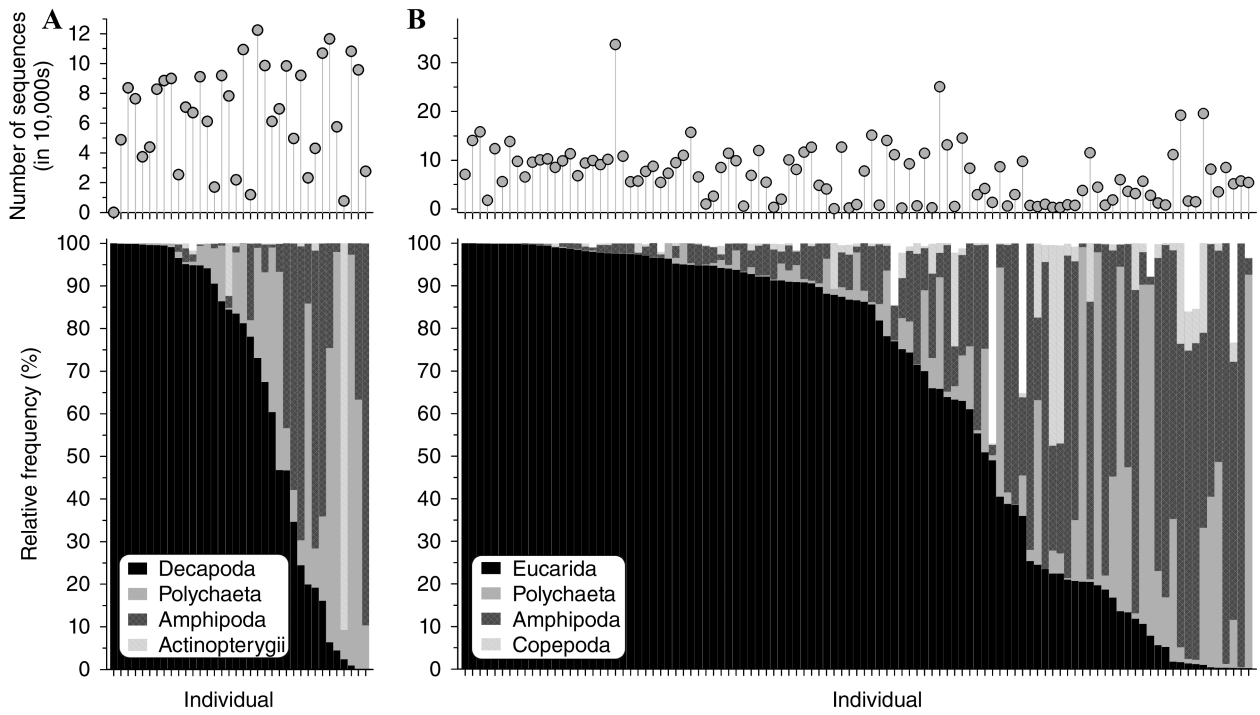

Figure 2

Results from analysis of stomach contents of juvenile black sea bass (*Centropristis striata*) collected in Mumford Cove off Connecticut in August 2020, through metabarcoding with (**A**) the mitochondrial cytochrome *c* oxidase subunit I gene region and (**B**) the V9 hypervariable region of 18S rRNA. In the top panels, data points indicate the variability between individuals in the number of sequences detected in stomach samples. In the bottom panels, bars indicate the relative frequencies of 4 major prey taxa found in stomach samples. Data for Actinopterygii are not reported because the V9 region cannot be used in metabarcoding to distinguish between predator and prey tissue.

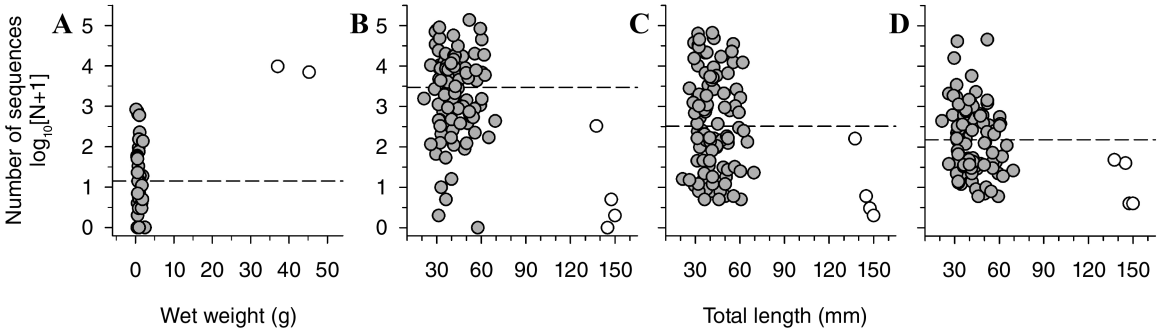

Figure 3

Log-transformed sequence numbers (log10[N+1]) (**A**) for the prey class of Actinopterygii, from metabarcoding stomach samples with the mitochondrial cytochrome *c* oxidase subunit I (COI) gene region, in relation to the wet weight of juvenile black sea bass (*Centropristis striata*) collected in Mumford Cove off Connecticut in August 2020 and for the prey taxa (**B**) Amhipoda, (**C**) Polychaeta, and (**D**) Copepoda, from metabarcoding with the V9 hypervariable region of 18S rRNA, in relation to the total length of the juvenile specimens. White circles depict the number of sequences for the largest juveniles analyzed (2 and 4 largest specimens in weight and length, respectively), and gray circles indicate the numbers for the main sample of small juveniles. The dashed lines depict the means for the main sample.

In the principal component analysis of V9 sequence data for 9 prey taxa, 4 principal components (PCs) were identified that together explain 74% of the overall variability (PC1: 29%; PC2: 19%; PC3: 14%; PC4: 12%; Fig. 4). With the multivariate general linear model, significant differences between sampling dates were detected (Pillai's trace=0.55, df=8, P<0.001), and results from Fisher's least significant difference post-hoc tests indicate that PC1–3 all separated the first 2 collection dates (6 and 14 August) from the third date (26 August) (P<0.035), whereas PC4 separated 6 August from 14 and 26 August (all: P<0.002). Amphipoda and Polychaeta sequences were primarily responsible for distinguishing between samples collected on different dates along PC2. Gastropoda sequences separated sampling dates on PC3, and Eucarida sequences separated sampling dates along PC4 (Fig. 4).

The log-transformed COI and V9 sequence numbers from the same stomach samples were positively correlated for the 3 most important prey groups, Eucarida (coefficient of multiple determination [R^2]=0.35, P<0.001), Polychaeta (R^2=0.57, P<0.001) and Amphipoda (R^2=0.48, P<0.001), but not for the other, less abundant prey groups (Fig. 5).

Discussion

Through DNA metabarcoding of stomach contents, we identified the diets of juvenile black sea bass recently settled in LIS, corroborating and refining the general view of this species as a benthic omnivore that consumes mostly crustaceans, including shrimp, crab, and amphipod species (Richards, 1963; Link, 1980; Sedberry, 1988). The study site is geographically close to Narragansett Bay, in Rhode Island, where Taylor et al. (2023) found mainly amphipod, shrimp, crab, nematode, and fish species in the diets of juvenile black sea bass by using morphological inspection. Comparison of both studies revealed several noteworthy contrasts.

First, Taylor et al. (2023) found the diet of small juvenile black sea bass (<50 mm TL) to be dominated by amphipods (83%) over shrimp species (7%), whereas the results from our study indicate the opposite, a consistent dominance of shrimp species over amphipods and polychaetes. Because both crustacean groups have hard, chitinous carapaces, they likely remain detectable in fish stomachs (Buckland et al., 2017), indicating that juvenile black sea bass in Mumford Cove consumed more shrimp than similarly sized black sea bass in Narragansett Bay. The sevenspine bay shrimp and zostera shrimp are both very common species in coastal waters of the Northwest Atlantic Ocean (Price, 1962; Haefner, 1979; Modlin, 1980). These 2 shrimp species have been found to be particularly abundant in habitats, such as Mumford Cove, with dense subaquatic vegetation like the eelgrass *Zostera marina* (Bologna, 2007; Vaudrey et al., 2010; H. Baumann, personal observ.), a type of habitat not included in the varied habitats and substrates sampled by Taylor et al. (2023). Juvenile black sea bass may prefer shrimp, when abundant, as an energetically denser prey over smaller amphipods. Future work could examine this further by testing whether diets of juveniles of other common fish species in this region, such as the cunner (*Tautogolabrus adspersus*) and tautog (*Tautoga onitis*), have the same spatial variation in prey as the diets of juvenile black sea bass (Taylor et al., 2023).

The occurrence of polychaetes in stomachs of black sea bass is a second notable contrast between the studies, given that Taylor et al. (2023) reported generally low incidence of this class (<2%) but we found substantially greater percentages of polychaete sequences in samples from stomachs of similarly sized juvenile black sea bass (10% on average). The 2 identified polychaete species, sand worm and clam worm, are common benthic organisms in habitats of the

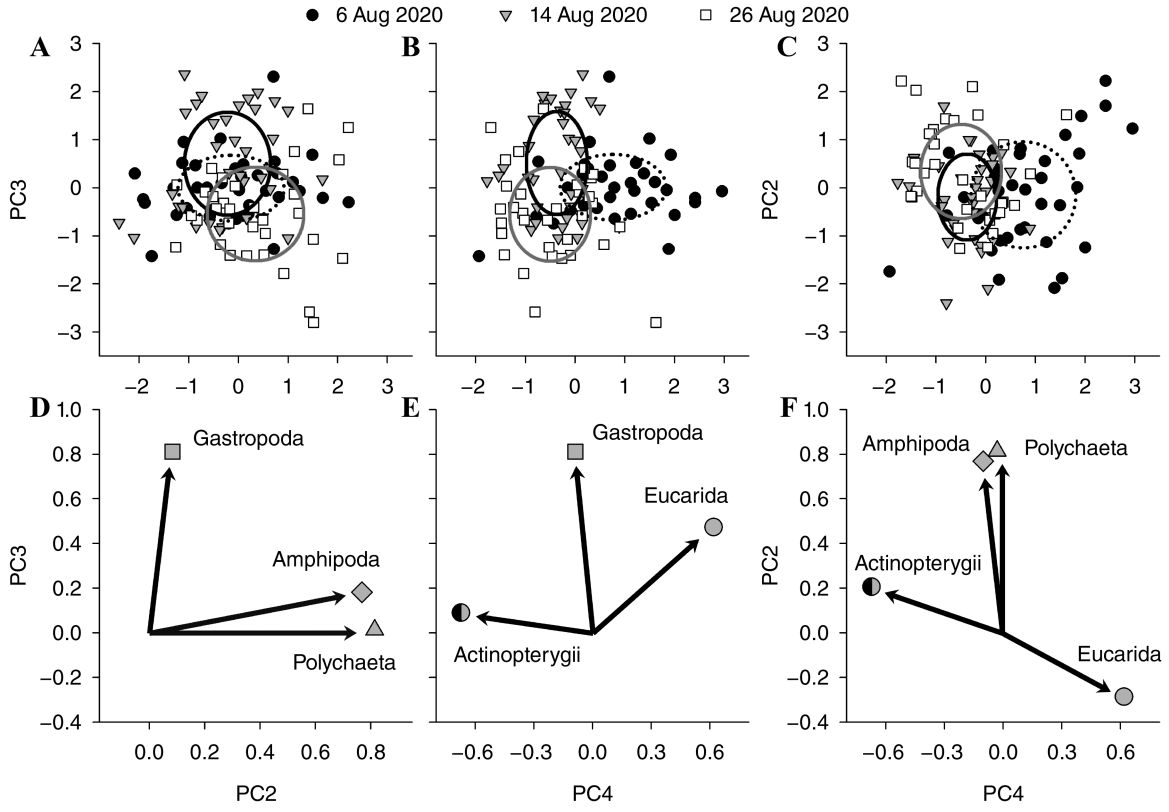

Figure 4

Plots based on principal component (PC) analysis of sequences from metabarcoding with the V9 hypervariable region of 18S rRNA, showing shifts in the taxonomic composition of prey detected in stomachs of juvenile black sea bass (*Centropristis striata*) between 3 dates of sampling in Mumford Cove off Connecticut. (**A**–**C**) In the plots of PC scores for individual black sea bass, the ellipses are centered on the mean of the scores for each date (dotted, black, and gray ellipses for 6, 14, and 26 August 2020, respectively) and their size corresponds to the standard deviation on each PC. (**D**–**F**) The rotated component vectors in these plots indicate the prey groups that influence separation along each PC. The symbols are different for each taxon.

Northwest Atlantic Ocean (Whitlatch, 1982; Lopez et al., 2014), but genuine differences in polychaete abundances could exist between the study sites in Mumford Cove and Narragansett Bay. However, we suggest that polychaetes compose a more substantial fraction of the prey of juvenile black sea bass than previously known. Polychaetes are soft-bodied prey that may disintegrate rapidly in fish stomachs, making them difficult to detect through traditional morphological inspection (Buckland et al., 2017). In contrast, metabarcoding can be used to classify disintegrated tissue; therefore, we argue that this approach is better at detecting polychaetes. Similarly, this difference in detection between methods may also apply to other soft-bodied prey groups that are orders of magnitude rarer. For example, Hemichordata were detected in low sequence numbers through metabarcoding of the V9 region in our study but were absent or undetected in stomachs of juvenile black sea bass captured from Narragansett Bay.

The third important contrast between studies concerns the occurrence of amphipod prey in stomachs of juvenile black sea bass. Taylor et al. (2023) found amphipods, including species from both Gammaridae and Caprellidae, to be the dominant prey (83%) of small juvenile black sea bass, a result that is consistent with analyses by Sedberry (1988) of the stomach contents of small black sea bass (<100 mm in standard length) caught in habitats off South Carolina, Georgia, and northeastern Florida in the North Atlantic Ocean. In contrast, in our study, much smaller average proportions of amphipod sequences were found in samples from stomachs of black sea bass, and these sequences belonged almost exclusively (97%) to a single species of Aoridae, *G. japonica*. This finding is noteworthy because *G. japonica* is native to the western Pacific Ocean (off northern Japan; Stephensen, 1938). Over the past 60 years, it has invaded and established itself first in waters of the Northeast Pacific Ocean off California, then in waters of the Northeast Atlantic Ocean and in regions off Australia, and most recently in the Northwest Atlantic Ocean.

Specifically for Long Island Sound, the first evidence of *G. japonica* was reported in 2013 (Trott et al., 2020), and

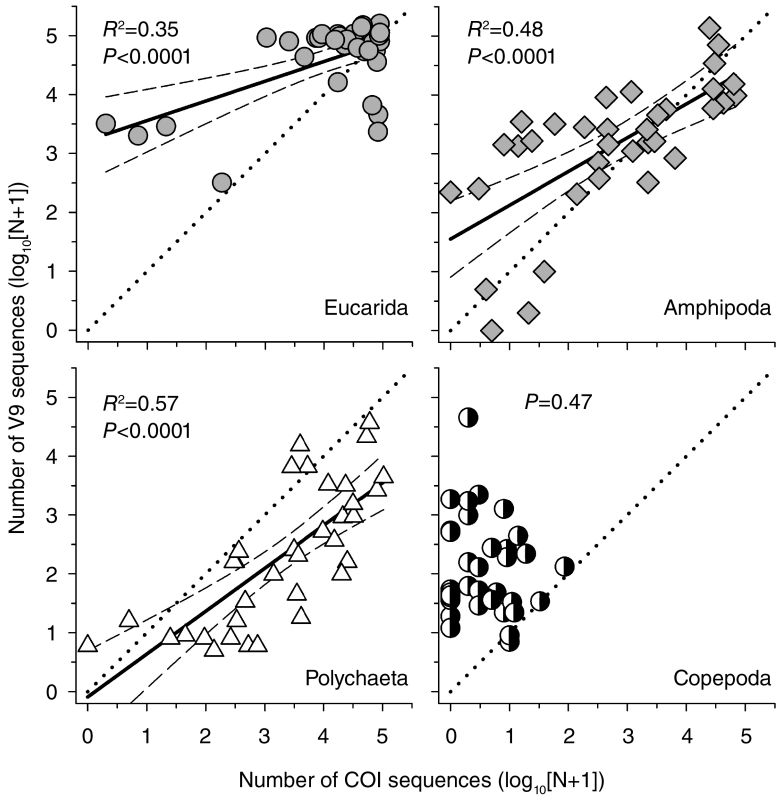

Figure 5

Relationship between log-transformed numbers of sequences (log10[N+1]) from metabarcoding with the mitochondrial cytochrome *c* oxidase subunit I (COI) gene region and those from metabarcoding with the V9 hypervariable region of 18S rRNA for the 4 major prey groups, Eucarida, Amphipoda, Polychaeta, and Copepoda, detected in stomach samples from 35 juvenile black sea bass (*Centropristis striata*) collected in Mumford Cove off Connecticut in August 2020. Symbols are data points for individual juveniles, and the solid and dashed lines depict linear regression lines and their 95% confidence intervals. The dotted line in each graph represents the 1:1 line. The coefficients of multiple determination (R^2) and *P*-values from regression analysis are provided.

yet, at our study site, this invasive amphipod now appears to have become the exclusive prey for small juvenile black sea bass (<70 mm TL). This result is especially significant, given that other amphipods, especially caprellids, remain highly abundant in Mumford Cove (H. Baumann, personal observ.). The reference database used for the metabarcoding analysis confirmed our findings with geolocation and reference sequences for 2 species in the North Atlantic Ocean (out of 10 species of *Grandidierella* worldwide). Both species, *G. bonnieroides* (Gulf of Mexico and Florida) *and G. japonica* (North Atlantic Ocean), have geolocation records. However, more efforts are needed to generate COI reference sequences (or barcodes) of other amphipod prey species.

Taylor et al. (2023) reported a notable proportion (13% occurrence) of nematodes among prey items in the guts of black sea bass and commented that these could include endoparasites or pseudoparasites, which have been documented to live in fish intestines or stomachs (Buchmann, 2012; Abolafia et al., 2015). In contrast, we found negligible numbers of nematode sequences (43 sequences across 8 samples) in samples from stomachs of black sea bass, perhaps, because we focused on the smallest, most recently settled individuals that may not have had sufficient exposure to prey containing nematodes. Similarly, because of our focus on the smallest juveniles, stomach samples were analyzed for very few juvenile black sea bass ≥100 mm TL. Nonetheless, the stomachs of these large individuals contained significantly fewer amphipods and polychaetes (on the basis of metabarcoding of the V9 region) and more fish prey (on the basis of metabarcoding of the COI region) than the stomachs of the smaller fish (20–70 mm TL) in our study, a result that is consistent with Taylor et al. (2023) reporting a steep decline in the importance of amphipods in juvenile black sea bass ≥50 mm TL and a greater occurrence of fish and decapods in their stomachs. Both fish species, the northern pipefish and Atlantic silverside, detected as prey in our study are among the most common species in Mumford Cove during summer (e.g., Pringle and Baumann, 2019).

In aquaculture or laboratory settings, cannibalism is known to occur among juvenile black sea bass (Watanabe et al., 2021; H. Baumann and M. Zavell, personal observ.), but cannibalism has not been reported from previous studies in which stomach contents of black sea bass collected in the field were analyzed (Sedberry, 1988; Taylor et al., 2023). Cannibalism cannot be confirmed through molecular approaches, given the likelihood that stomach homogenates used to extract DNA will also contain traces of the predator's stomach tissue. This limitation is particular to the use of the V9 gene marker, which cannot be used to identify prey items more specifically than the class Actinopterygii and therefore results in the grouping of sequences of potential teleost prey with sequences of the predator. Another limitation is the potential for detecting DNA sequences of taxa consumed by the prey of the organism of interest; it is not possible through metabarcoding to distinguish between sequences for taxa in the guts of the prey and sequences for taxa in the stomach of the predator. In our study, this limitation may be apparent in the large number of detected Copepoda sequences, given that settlement of juvenile black sea bass entails transition from pelagic, planktivorous larvae to benthivorous adults that are unlikely to consume copepods. This issue could be effectively resolved with future laboratory experiments in

which natural plankton would be offered to newly settled juvenile black sea bass.

Although we found that the use of both COI and V9 gene markers produced qualitatively consistent results for the 3 most abundant prey groups (Eucarida, Amphipoda, and Polychaeta), the choice of gene regions for metabarcoding analysis can potentially affect the patterns of biodiversity observed (Djurhuus et al., 2018; Questel et al., 2021). The 313-bp region of the mitochondrial COI gene targeted in our study (Geller et al., 2013; Leray et al., 2013) has been widely used for metabarcoding analysis of marine metazoan diversity (Hirai et al., 2015; Blanco-Bercial, 2020; Govindarajan et al., 2021). Through metabarcoding of the COI gene region, we identified species of some—but not all—groups of organisms and may not have detected some prey that are also overlooked in morphological analyses of gut contents. In contrast, the V9 region can be used to reliably classify sequences at varying taxonomic levels for a broad range of phyla (de Vargas et al., 2015), but it lacks sufficient sequence variation for species identification. In this study, use of the V9 region in metabarcoding resulted in detection of 3 groups (Ascidiacea, Ostracoda, and Hemichordata) that were not detected by using the COI region, indicating the advantage of using multiple gene regions for metabarcoding analysis to fully capture the diversity of prey of juvenile black sea bass.

Accurate classification of prey groups or species through metabarcoding also relies on the geographic coverage and completeness of reference sequence databases. These databases must be accurately curated and contain sequences from specimens identified morphologically by taxonomic experts (Bucklin et al., 2021). In our study, we used a tailored database created, following the method described by Blanco-Bercial (2020), from the data set of small subunit sequences in release 132 of the SILVA database that includes eukaryotic marine sequences from GenBank (available from website) for the V9 region. Similarly, the MetaZooGene Atlas and Database is continuously updated, providing confidence in the use of COI sequences from that database in classification of prey taxa based on results from metabarcoding (Bucklin et al., 2021; O'Brien et al., 2024).

Conclusions

Given the rapid environmental and ecological changes unfolding in LIS and other coastal regions of the Northwest Atlantic Ocean (Howell and Auster, 2012; Crosby et al., 2018; Snyder et al., 2019), monitoring, sampling, and diet studies of species, such as the black sea bass, are critical tools for prediction of changes to local and regional food webs. The combination of rapid warming and altered ecological interactions (i.e., predation and competition) may result in further displacement of cold-water species that may be outcompeted by warm-water species like the black sea bass (Mercaldo-Allen et al., 2020). In this study, we identified prey groups and species that could be affected by large, mid-summer settlement pulses of black sea bass in LIS habitats, with consequences for other fish species with similar diets, including juvenile cunner and tautog (Taylor et al., 2023). Future studies of the effects of black sea bass on food webs in LIS will require efforts to sample black sea bass of a larger size spectrum, from a range of habitats and seasons and with concurrent sampling of associated benthic communities. The results of this study indicate that molecular approaches, such as DNA metabarcoding of stomach contents, can play an important role alongside morphological approaches in investigating fish diets and their effects on marine ecosystems.

Resumen

La abundancia de cabrilla negra (*Centropristis striata*) ha aumentado en las aguas costeras del noreste de Estados Unidos, y las implicaciones de ese cambio para las redes tróficas regionales siguen siendo poco conocidas. Utilizamos la metabarcodificación del ADN de 2 regiones génicas, la región mitocondrial citocromo c oxidasa subunidad I (COI) y la región hipervariable V9 del ARNr 18S, para clasificar las presas en el contenido intestinal de juveniles de cabrilla negra colectados en un hábitat cercano a la costa en Long Island Sound (LIS). Utilizando los números de secuencia de las regiones COI y V9, descubrimos que el contenido intestinal estaba dominado por crustáceos del superorden Eucarida, seguido de 2 especies de poliquetos y un anfípodo invasor, *Grandidierella japonica*. Los bivalvos, gasterópodos y ostrácodos fueron menos abundantes. En los estómagos de 2 cabrillas negras grandes se observaron (>10% de las secuencias) restos del pez pipa del norte (*Syngnathus fuscus*) y el pejerrey del Atlántico (*Menidia menidia*). Se observó una gran variabilidad en el número de secuencias entre individuos, tanto para la región COI como para la región del gen V9. La composición taxonómica de las presas también difirió: los eucaridos contribuyeron con >87% de las secuencias del gen V9 en muestras de 51 estómagos, los anfípodos con >50% de las secuencias en muestras de vísceras de 21 peces, y los poliquetos dominaron con >75% de secuencias en muestras de 6 estómagos. Concluimos que, durante los pulsos de asentamiento, los juveniles de cabrilla negra pueden competir con otros depredadores bentónicos por las presas de decápodos, poliquetos y anfípodos en los hábitats cercanos a la costa de LIS.

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