

Diet composition of the vulnerable fish species *Megalops atlanticus* (Elopiformes: Megalopidae) in a heavily urbanized estuary in Brazil: DNA-based identification of preys

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Submitted January 22, 2025

Accepted July 29, 2025

Epub November 10, 2025

Associate Editor 🟡 Fernando Gibran

Section Editor 🟡 Fernando Pelicice

Editor-in-chief 🟡 Carla Pavanelli

The tarpon (*Megalops atlanticus*) is a species of ecological and economic importance that uses estuaries as nursery grounds during its early life stages. Currently classified as Vulnerable (VU) by the IUCN due to anthropogenic pressures, understanding its trophic ecology is crucial to monitoring environmental changes and fish-habitat interactions. This study examined the diet of juvenile *M. atlanticus* in a highly urbanized estuary, using morphological and molecular (16S rDNA) analyses, comparing diets across different size classes and assessing the consumption of non-native species. The stomach contents of 123 individuals (3.9–64.0 cm standard length) were analyzed. Results show that *M. atlanticus* is a generalist and opportunistic, consuming invertebrates as well as native and non-native fish species, including *Oreochromis niloticus*. The presence of *Tubifex* spp. and evidence of cannibalism indicate dietary plasticity in response to altered environments. The DNA-based approach allowed for more accurate prey identification, reinforcing that the diet of juveniles is shaped by the presence of non-native species, which are common in habitats modified by human activities. These findings highlight the impact of anthropogenic actions on trophic dynamics in estuaries and emphasize the essential role of these habitats as nursery grounds for tarpon.

Keywords: Coastal ecosystem, DNA, Estuarine fish, Feeding habits, Urban ecosystem.

Online version ISSN 1982-0224

Print version ISSN 1679-6225

Neotrop. Ichthyol.
vol. 23, no. 3, Maringá 2025

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O camurupim (*Megalops atlanticus*) é uma espécie de grande importância ecológica e econômica, que utiliza os estuários como berçários durante estágios iniciais de vida. Devido às pressões antrópicas, atualmente está classificado como Vulnerável (VU) pela IUCN. Compreender sua ecologia trófica é fundamental para monitorar mudanças ambientais e interações entre os peixes e o ambiente. Este estudo examinou a dieta de juvenis de *M. atlanticus* em um estuário altamente urbanizado, utilizando análises morfológicas e moleculares (rDNA 16S), comparando as dietas entre diferentes classes de tamanho e avaliando o consumo de espécies não-nativas. O conteúdo estomacal de 123 indivíduos (3,9–64,0 cm de comprimento-padrão) foi analisado. Os resultados mostram que *M. atlanticus* é generalista e oportunista, alimentando-se de invertebrados e de peixes nativos e não-nativos, incluindo *Oreochromis niloticus*. A presença de *Tubifex* spp. e canibalismo indicam plasticidade alimentar em resposta a ambientes alterados. A abordagem com DNA permitiu identificação mais precisa das presas, reforçando que a dieta dos juvenis é influenciada pela presença de espécies não-nativas, comuns em ambientes alterados por atividades humanas. Esses achados ressaltam o impacto das ações antrópicas sobre dinâmicas tróficas nos estuários e destacam o papel essencial desses habitats como berçários para o camurupim.

Palavras-chave: DNA, Ecossistema costeiro, Ecossistema urbano, Hábitos alimentares, Peixe estuarino.

INTRODUCTION

Estuaries serve as nurseries for several fish species, providing essential shelter and food, particularly during their early life stages (Able *et al.*, 2010; Favero *et al.*, 2019). Estuarine fishes are embedded within complex trophic networks, playing a crucial role in the energy transfer between trophic levels and with other ecosystems as they move across different habitats throughout ontogeny (Potter *et al.*, 2011). However, environmental changes and anthropogenic pressures affect the water quality and the biodiversity of these ecosystems (Halpern *et al.*, 2008; Borja *et al.*, 2010; Merigot *et al.*, 2017; Pinto *et al.*, 2025), which influence the dietary characteristics of fish species (Speranza *et al.*, 2020). The increase in pollutants and the introduction of invasive species lead to changes in the diets of fish populations across time and space (Barker *et al.*, 2014; Speranza *et al.*, 2020; Rosa *et al.*, 2021; Griffin *et al.*, 2023). Therefore, studying trophic ecology is crucial for environmental monitoring and ecosystem management in polluted and unpolluted habitats, as it helps to understand the flow of energy and nutrients, ecosystem health, and biodiversity. In polluted habitats, trophic studies become even more critical, as pollution can disrupt these interactions and affect the food web (Costa, Angelini, 2020).

Various techniques are employed to identify the stomach contents of fish. The identification of prey in fish diets, using both morphological and molecular methods, provides crucial data for the development of effective conservation and management strategies (Nielsen *et al.*, 2017; Buckup, 2021; Boza *et al.*, 2022). Traditional methods involve examining stomach contents for items, such as bones, scales, and otoliths (Zavala-

Camin, 1996; Nielsen *et al.*, 2017). However, visual analysis can be challenging due to prey degradation during digestion (Barrett *et al.*, 2007; Teletchea, 2009; Bowen, Iverson, 2012). Recent advances in fish diet studies include DNA analysis, organic macromolecule assessments, and stable isotope evaluations (Nielsen *et al.*, 2017; Boza *et al.*, 2022).

DNA analysis offers high sensitivity and specificity in detecting and identifying consumed prey, providing advantages over the commonly used visual identification methods in predation studies. These advantages include the ability to detect highly digested prey, identify taxa at finer taxonomic levels, standardize methodologies, verify results through sequencing, and analyze large sample sets using high-throughput techniques (Traugott *et al.*, 2021). These features enhance the accuracy of identifying native and non-native species in fish diets, which contributes to understanding their ecological impacts (Baharum, Nurdalila, 2012; Saad, 2019; Herlevi *et al.*, 2023). For instance, studies performed by Brandl *et al.* (2015), Jungbluth *et al.* (2021), and Boza *et al.* (2022) utilized DNA analysis techniques to accurately identify the species consumed in fish diets and demonstrate how this approach can reveal changes in trophic interactions. Furthermore, Sousa *et al.* (2019) illustrate how DNA analysis is an excellent tool for assessing how human-induced changes, such as urbanization, agriculture, and climate change, are affecting the feeding behaviors of various species, both terrestrial and aquatic.

The *Megalops atlanticus* Valenciennes 1847, known as Tarpon, is a diadromous fish with a long-life cycle, slow growth, late sexual maturity, and a leptocephalus larval stage (Silva *et al.*, 2021; Fernandes *et al.*, 2023). This species is classified as globally Vulnerable (VU) by the International Union for Conservation of Nature (IUCN), and also listed as Vulnerable in the Brazil Red List of Threatened Species of Fauna (Adams *et al.*, 2019; Brasil, 2022), due to anthropogenic pressures such as overexploitation, the use of inadequate fishing gear, habitat degradation or loss, and aquatic pollution (Batista *et al.*, 2020).

The Tarpon inhabits tropical, subtropical, and temperate regions of the western Atlantic Ocean, from Canada to northern Argentina (Fricke *et al.*, 2025), limited by its sensitivity to low temperatures (Mace *et al.*, 2020). It is an adaptable species that utilizes a variety of habitats throughout its life cycle. However, the upper zones of estuaries play a crucial role as nurseries for juvenile tarpons (Kurth *et al.*, 2019). In Brazil, Tarpon is mainly captured in the North and Northeast regions, where it is important for both consumption and trade, as well as for traditional communities, who use its scales for handicrafts (Batista *et al.*, 2020). In North America, this species is especially valued for sport fishing (Cianciotto *et al.*, 2019; Batista *et al.*, 2020).

The Tarpon is a generalist, with a diet primarily composed of fish and invertebrates (Jud *et al.*, 2011). It is a highly mobile predator that utilizes different habitats and resources throughout its life cycle, foraging on a wide variety of organisms (Menezes, Menezes, 1968; Jud *et al.*, 2011). The availability of food resources is a significant factor that influences the abundance and distribution of this species in estuarine habitats (Imre *et al.*, 2004). However, limited knowledge exists regarding the feeding and habitat preferences of this species in these ecosystems (Collen *et al.*, 2008; Adams *et al.*, 2014; Wilson *et al.*, 2019), and the patterns of its trophic ecology in tropical estuaries remain poorly understood (Jud *et al.*, 2011; Cianciotto *et al.*, 2019; Kurth *et al.*, 2019). Anthropogenic alterations of recruitment environments also impact the feeding and habitat preferences of this species. Jud *et al.* (2011) demonstrated an increasing dependence of juvenile tarpons on anthropogenically modified estuarine environments, while Rosa *et al.* (2021)

highlighted the significant impact of non-native species on the trophic network. It is suggested that anthropogenically altered estuaries provide favorable conditions for the feeding and growth of juveniles *M. atlanticus*.

Therefore, we aimed to understand how dietary factors may influence the occurrence of juvenile *Megalops atlanticus* in an urbanized and polluted estuary located in the semiarid region of Northeast Brazil. Our study was based on the ecological premise that diet reflects environmental prey availability and that ontogenetic changes in body size can influence feeding patterns. We tested two main hypotheses: (1) the diet of juvenile *M. atlanticus* is primarily composed of non-native fish species, and (2) dietary composition varies with body size, with larger individuals expected to consume a broader diversity of prey or larger prey items. To test these hypotheses, we analyzed the feeding ecology of *M. atlanticus* by identifying stomach contents through morphological characteristics and DNA barcoding techniques, comparing diet across different size classes, and classifying prey items as native or non-native.

MATERIAL AND METHODS

Study area. The Cocó estuary is located within the Parque Estadual do Cocó (PEC) Conservation Unit, in Fortaleza municipality, Ceará, in northeastern Brazil, between the coordinates 03°46'23.7"S 38°26'12.2"W and 03°45'43.3"S 38°30'14.0"W (Fig. 1). The estuary is situated in a densely urbanized area, particularly susceptible to management changes, such as increased discharges of domestic and industrial effluents along the river (Schettini *et al.*, 2017). The climate in this region is characterized by a short and irregular rainy season, followed by a prolonged dry season, with an average annual precipitation of less than 800 mm and an average annual temperature of around 26°C. This affects the seasonal salinity dynamics in the estuaries, leading to hypersalinity in the upper estuarine zones (Barroso *et al.*, 2016; Schettini *et al.*, 2017; Gurgel-Lourenço *et al.*, 2023). However, the Cocó estuary presents a typical saline gradient, with decreasing salinity upstream and freshwater conditions in the middle and upper zones throughout the year (SEMA, 2020).

The Cocó estuary is approximately 13 km long and stands out due to the magnitude and frequency of disturbances (Freires *et al.*, 2013), in addition to hosting the largest population of *M. atlanticus* among regional estuaries (Gurgel-Lourenço *et al.*, 2023). The main sources of pollution are associated with urban development, shoreline occupation, and untreated heavy metals, as well as the introduction of non-native species, such as *Betta splendens* Regan, 1910, *Poecilia reticulata* Peters, 1859, *P. sphenops* Valenciennes, 1846, and *Oreochromis niloticus* (Linnaeus, 1758) (Silva *et al.*, 2004; SEMACE, 2010; Duaví *et al.*, 2015; Gurgel-Lourenço *et al.*, 2023; Pinto *et al.*, 2025). Additionally, the estuary exhibits high concentrations of nitrogen and phosphorus of anthropogenic origin, exceeding natural sources and promoting the proliferation of macrophytes, especially in the middle and upper zones (Barroso *et al.*, 2016).

Biotic and abiotic characterization. The characteristics of the estuarine zones, particularly the physical and chemical parameters of the water, exhibit variations throughout the estuary (Lima *et al.*, 2019), which may be related to habitat selection and prey consumption by the juvenile *M. atlanticus*. To characterize the environment,

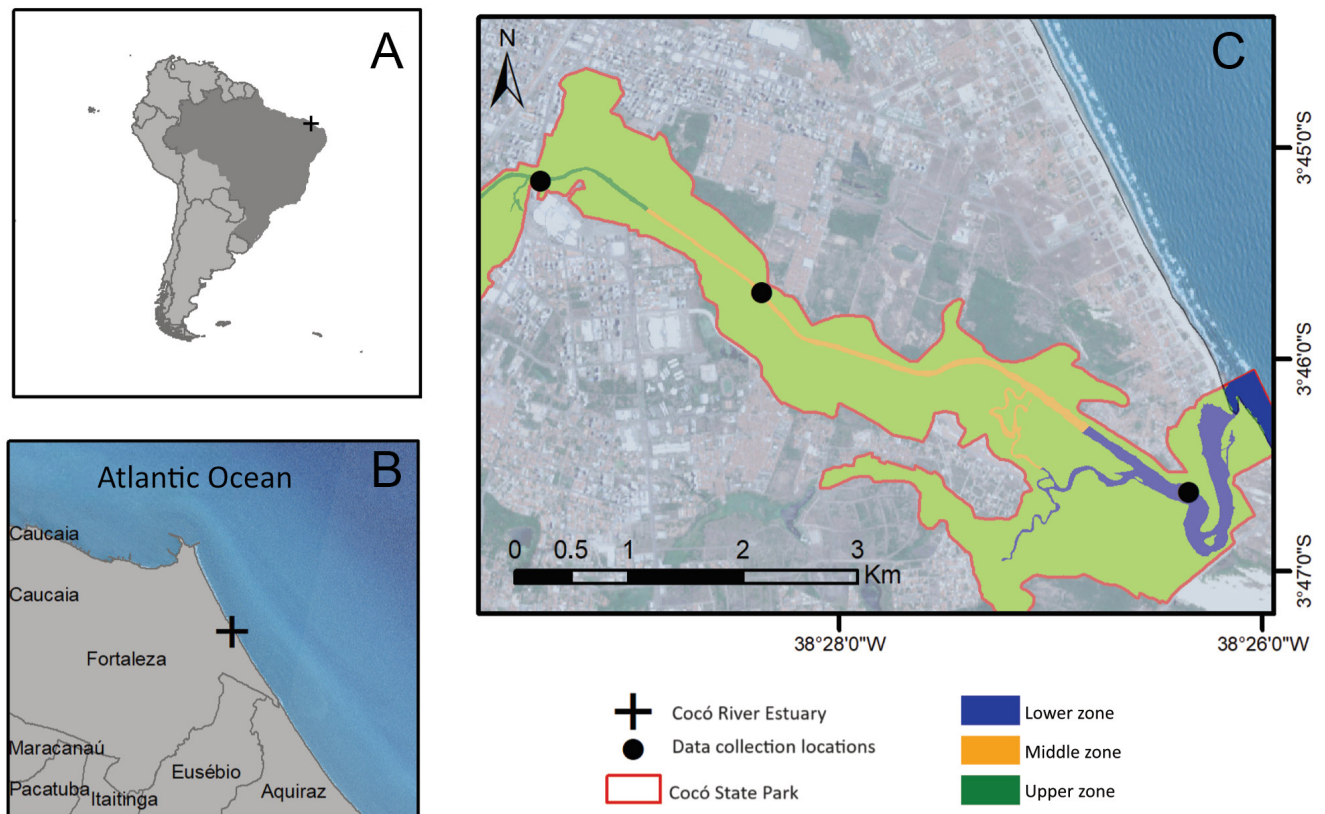


FIGURE 1 | Location of the Cocó estuary in the State of Ceará, northeastern Brazil, showing (A) the position of Brazil in South America, (B) the regional location of the Cocó estuary, and (C) the ichthyofauna sampling points and zones defined in this study.

we recorded the presence and absence of macrophytes in the estuary and measured the river's width and depth using a tape measure and a depth stick (Tab. 1). Furthermore, we assessed temperature ($^{\circ}\text{C}$), salinity, dissolved oxygen (mg/L) and transparency (cm) using, respectively, a thermometer, refractometer, oxygen meter, and Secchi disk.

Sampling. A total of 123 specimens of *M. atlanticus* were collected during the periods of 2017–2018 and 2022–2023. In the first period, 52 individuals were collected, with most captured in May 2017 ($n = 23$), June 2017 ($n = 15$) and November 2017 ($n = 2$), and only six in 2018, primarily in January ($n = 4$), and May ($n = 2$). In the second period, 71 individuals were collected, including 34 in 2022, with the majority captured in June ($n = 24$), and 37 in 2023, mostly in January ($n = 35$).

Smaller individuals were captured by cast nets (6 m^2 and 10 m^2) and a seine net (200 m^2) under license 57780, issued by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio)/Biodiversity Authorization and Information System (SISBio). Larger individuals were captured at the same locations using nine gillnets with areas ranging from 9.2 m^2 to 60 m^2 and mesh sizes of 60, 70, and 100 mm between opposite knots for three hours (9:00 AM to 12:00 PM) under the ICMBio/SISBio license 77988. All the collections were carried out during the ebb tide (2.0 to 0.0) and the flood tide (0.0 to 2.0). The voucher specimen (UFRN 4850) was deposited in the fish collection of the Laboratory of Systematic and Evolutionary Ichthyology, Universidade Federal do Rio Grande do Norte (Natal, RN), Brazil.

TABLE 1 | Characterization of the estuarine zones based on width, depth, and vegetation in the Cocó estuary.

Estuarine zone	Width (m)	Depth (m)	Vegetation
Upper	16.61	1.82	Medium-sized shrubs and trees, and the presence of macrophytes
Middle	30.61	1.91	<i>Avicennia germinans</i> (L.) (Black mangrove) in predominance and the presence of macrophytes
Lower	135.65	1.76	<i>Rhizophora mangle</i> L. (Red mangrove) in predominance and absence of macrophytes

The collected fish were measured for standard length (SL) (cm) using a caliper (0.1 mm) and eviscerated to remove the stomach contents. Additionally, all individuals were classified as immature juveniles based on macroscopic observation of the gonads, following the criteria of Brown-Peterson *et al.* (2011). The stomach contents were preserved in 70% ethanol, stored, and identified through morphological and molecular analysis.

Food items morphological identification. The morphological identification of consumed prey was carried out on Petri dishes with graph paper, under a stereomicroscope using taxonomic keys, species lists, photographs, scientific articles, and fish guides, which allowed the identification of the lowest possible taxonomic level (Menezes, Menezes, 1968; Figueiredo, Menezes, 2000; Pezold, Cage, 2002; Araújo *et al.*, 2004; Marceniuk, 2005; Fischer *et al.*, 2011; Nelson *et al.*, 2016; Buckup, 2021; Sabaj *et al.*, 2022; Botero *et al.*, 2023; Gurgel-Lourenço *et al.*, 2023; Kwun, Kang, 2023). Additionally, specimens were compared with those available in the collection of the Laboratório de Ecologia Aquática e Conservação from the Universidade Federal do Ceará (LEAC-UFC). The analysis included morphological characteristics, such as scale type, body shape, fins, and skeletal structures (Fischer *et al.*, 2011; Buckup, 2021).

Genetic analysis: extraction, amplification, purification, sequencing, and identification of genetic material. For molecular identification of the fish species in the stomach contents, regions with a lower degree of degradation were selected (Aguilar *et al.*, 2017). The next step was to extract a fragment of muscle tissue approximately 25 mm² in size (or smaller, in cases of more degraded samples) using a sanitized graph paper, scalpel, and Petri dish. Two different protocols were used for DNA extraction due to the difficulty of extracting genetic material from the samples.

The first protocol was based on the CTAB 2X method (Warner, 1996) with modifications. In this protocol, the samples were incubated at 60°C in CTAB 2X solution. The second method was adapted from Robles *et al.* (2007) and used samples from the stomach content, which were properly washed with ultrapure water and centrifuged for 1 minute at 3.000 x g, the step was repeated three times. The samples were then resuspended in 600 µL of 1% Sodium Dodecyl Sulfate (SDS) with 10 µL of proteinase K, incubated at 65°C for 3 h. Following this, 200 µL of 7.5 M ammonium acetate was added, and the samples were centrifuged (12,000 x g, 30 min). A total of 700 µL of the supernatant was transferred to 600 µL of isopropanol and incubated at -20°C for 10 min. The samples were then centrifuged at 12,000 x g (4°C), and the pellet was washed with 70% ethanol and evaporated at 37°C.

The mitochondrial 16S region was amplified by Polymerase Chain Reaction (PCR) using primers 16 Sar (5' CGC CTG TTT ATC AAA AAC AT 3') and 16 Sbr (5' CCG GTC TGA ACT CAG ATC ACG 3') (Palumbi, Benzie, 1991). The PCR reactions were performed in a final volume of 35 µL, containing approximately 20 ng of DNA and PCR reagents: 0.14 mM of each dNTP; 0.7 X GoTaq buffer (Promega), 0.7 µL of each primer, 2 mM MgCl₂, 3 mM Bovine Serum Albumin (BSA), and 1 unit of GoTaq polymerase chain enzyme (Promega, USA). The PCRs were carried out in a thermocycler (Eppendorf Mastercycler® Hamburg, Germany) programmed for an initial denaturation step (10' at 94°C), followed by 44 cycles of 1' at 94°C, 45" at 48°C, and 1'45" at 72°C. The final cycle was followed by a final extension step of 10' at 72°C. The amplification products were analyzed by electrophoresis in a 1% (w/v) agarose gel stained with SYBR® safe DNA (Invitrogen, USA). PCR products were purified and precipitated using the potassium acetate and ethanol protocol.

The DNA was sequenced using the Sanger method, employing the primers 16Sar and 16Sbr at the Central de Genômica e Bioinformática (CeGenBio) of the Centro de Pesquisa e Desenvolvimento de Medicamentos (NPDM) at the Universidade Federal do Ceará (UFC). The sequences were edited using Codoncode Aligner v. 6.0.2 (Codoncode Corp, USA). The identification of the fish species in the stomach contents was performed by comparing the sequences with those available in the National Center of Biotechnology Information Database (GenBank), using the Basic Local Alignment Search Tool (BLAST) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

The molecular genus-level identification of these fishes was based on the Expect (E) values resulting from BLAST, where sequences with lower "E" values are those that best match the queried sequence (Zeng *et al.*, 2018). The identity percentage (%) represents how similar the query sequence is to the database sequence. The expectation value (E-value) describes the number of occurrences that may be found by chance, considering the sequence length and the size of the database. For this reason, the smaller the E-value, the higher the likelihood that the result is not due to random chance (Amaral *et al.*, 2007). Finally, the Query cover indicates the proportion of the query sequence that was aligned to the target sequence in the database. Sequences with the highest query coverage are closest to 100%, and lower E-values (closer to zero) indicate maximum alignment with the target sequence (Samal *et al.*, 2021).

Data analysis. We assessed the diet based on genetic sequencing and morphological analysis, using the Food Importance Index (IAi) to determine the relative importance of each food resource. The IAi was calculated by combining the frequency of occurrence (FOi) and volumetric (Vi) methods according to the equation proposed by Kawakami, Vazzoler (1980): $IAi = (FOi \times Vi) \times 100 / \sum (FOi \times Vi)$, where i represents each food item, FOi is the frequency of occurrence (%) of item i, and Vi is the volume (%) of item i. The volume of food items was estimated in cubic millimeters (mm³) by multiplying the area (mm²) occupied by each item on graph paper by its height (mm), measured with a caliper. The IAi values range from zero to one ($0 \leq IAi \leq 1$) and are subsequently converted into percentages, referred to as IAi-% (Teixeira, Gurgel, 2002).

The graphical method was applied to distinguish rare from dominant prey and to synthesize the information from the items found in the stomachs (Amundsen *et al.*, 1996). This method correlates the specific abundance of prey with its frequency of

occurrence through a two-dimensional plot. Mathematically, this is represented as: $(P_i = \sum S_i / \sum S_{ti} \times 100)$, where P_i represents the specific abundance of prey i , S_i is the total volume of prey i , and S_{ti} is the total stomach content for specimens that contain prey i in their stomachs. In this perspective, the $IA_i\%$ is used to represent the specific abundance of prey and FO for the frequency of occurrence of prey in the stomachs. Information about prey relevance and *M. atlanticus* feeding strategy was derived by analyzing the distribution of points along the diagonals and axes of the diagram proposed by Amundsen *et al.* (1996). This diagram allows for the identification of whether the predator consumes dominant or rare prey and evaluates its feeding strategy, classifying it as either a specialist or a generalist.

To assess ontogenetic variation in the diet, length classes were defined based on Sturges guidelines (Vieira, 1980), resulting in four length classes with intervals of 15 cm: (I) lower than 15 cm, (II) 15.1 to 30 cm, (III) 30.1 to 45 cm, and (IV) greater than 45.1 cm, to visualize how consumed items are distributed among the different sizes. Principal Coordinates Analysis (PCoA) from a Bray-Curtis distance matrix was used to evaluate variation patterns in the species' diet. The significance of the size effect on diet composition was tested using the *envfit* function from the vegan package (Oksanen *et al.*, 2019). All analyses and data visualizations were performed using R software (R Development Core Team, 2024).

RESULTS

The highest values for temperature ($29.3 \pm 0.5^\circ\text{C}$) and dissolved oxygen (6.0 ± 1.4 mg/L) were recorded in the lower zone, while the lowest values were observed in the upper zone ($28.2 \pm 0.9^\circ\text{C}$; 2.1 ± 1.2 mg/L). Salinity was also higher in the lower zone (14.8 ± 6.9), as it was the transparency (110.5 ± 15.0 cm). The middle zone exhibited similar values for temperature, dissolved oxygen, and transparency ($28.7 \pm 0.8^\circ\text{C}$, 2.6 ± 1.3 mg/L, and 50 ± 9.0 cm) to those observed in the upper zone, differing primarily in salinity, which was zero in the upper zone and 1.8 ± 4.5 in the middle zone (Fig. 2).

Analysis of variance for water temperature did not reveal significant differences between the zones ($F = 1.91$; $p = 0.19$), suggesting that temperature remained consistent across the upper, middle, and lower zones. In contrast, dissolved oxygen showed significant differences ($F = 9.51$; $p = 0.0049$), with Tukey's post-hoc test indicating that the lower zone had significantly higher dissolved oxygen concentrations compared to the middle ($p = 0.009$) and upper ($p = 0.004$) zones, with no significant difference between the middle and upper zones ($p = 0.79$). Regarding salinity, there were significant differences among the zones (Kruskal-Wallis test: $H = 10.08$; $p = 0.006$), with the lower zone with significantly higher salinity than the middle ($p = 0.02$) and upper ($p = 0.003$) zones, while there was no difference between the middle and upper zones ($p = 0.61$) (Dunn's post-hoc test). Finally, water transparency (Secchi) also showed significant differences among the zones ($F = 45.47$; $p < 0.001$), with the lower zone exhibiting greater water transparency compared to the middle ($p < 0.001$) and upper ($p < 0.001$) zones, and no significant difference between the middle and upper zones ($p = 0.79$).

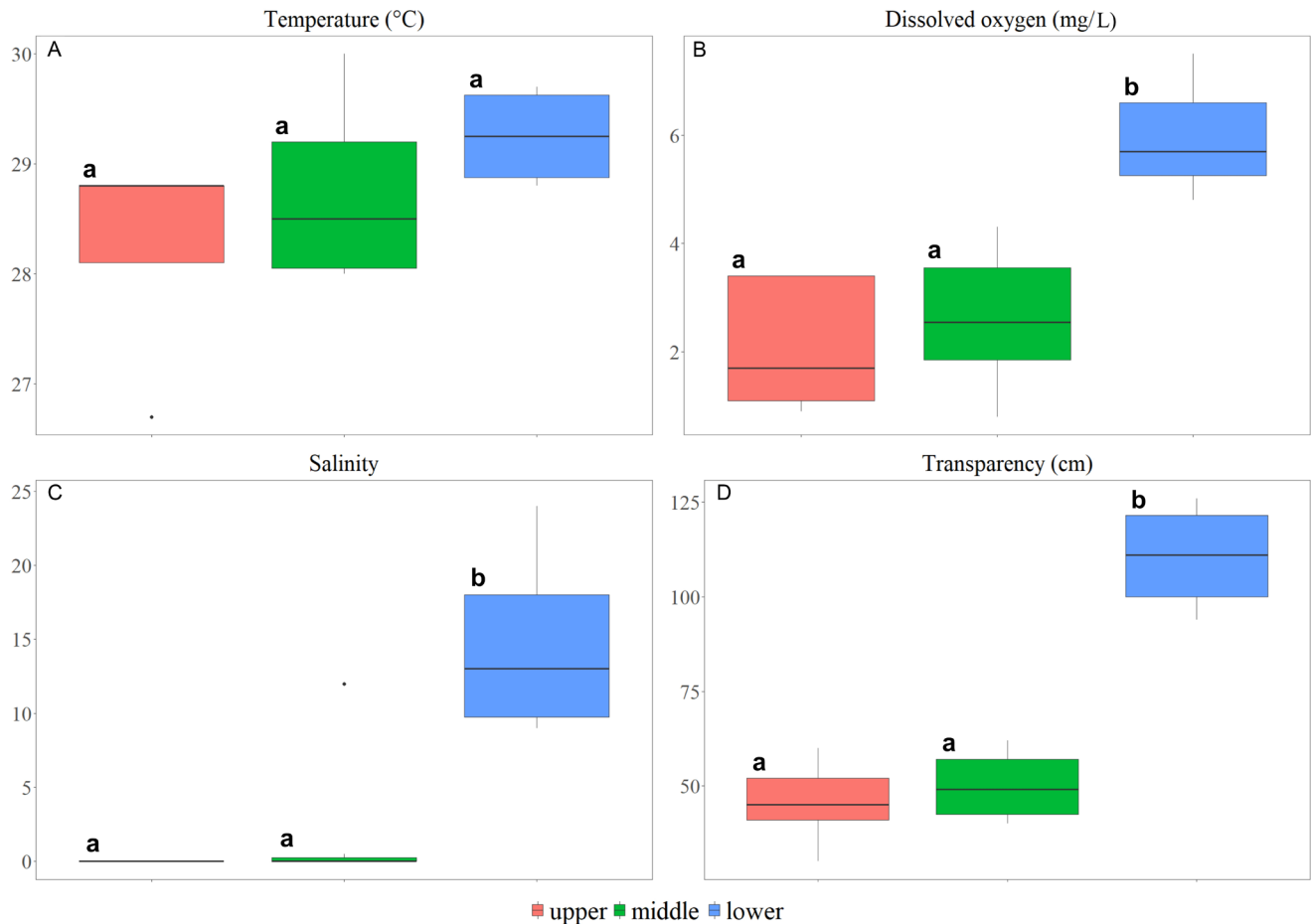


FIGURE 2 | Characterization of temperature (°C), dissolved oxygen (mg/L), salinity, and Secchi transparency (cm) variables in the upper, middle, and lower zones of the Cocó estuary.

Juvenile *M. atlanticus* presented a standard length ranging from 3.9 to 64.0 cm, and the average values in the upper and middle zones were, respectively, 13.3 ± 5.0 cm and 42.0 ± 13.0 cm. All individuals analyzed in this study are considered juveniles. According to Stephens *et al.* (2024), *M. atlanticus* juveniles remain in coastal nursery habitats for several years before migrating to coastal waters as sexually mature adults, which occurs at approximately 10 years of age and 120 cm in fork length. In terms of weight, individuals ranged from 4.6 g to 3.242 g, and the average values in the upper and middle zones were, respectively, 41.5 ± 40 g and $1,127.4 \pm 802$ g. No individuals were captured in the lower zone. Individuals inhabiting the middle zone were considerably larger than those in the upper zone (Wilcoxon rank-sum test $W = 242.5$; $p < 0.001$) (Fig. 3).

A total of 23 consumed items were identified in the diet of *M. atlanticus*, which included insect larvae and eggs, Ephemeroptera nymphs, Belostomatidae, plant material, crustaceans, mollusks, *Tubifex* spp., detritus, and plastic. The most frequent items found were Diptera larvae (FO = 25.33) and *Tubifex* spp. (FO = 24.00) (Fig. 4). Similarly, the relative importance estimate highlighted these same items as the most significant in the diet of juvenile *M. atlanticus* (Diptera larvae IA = 39.02 and *Tubifex* spp. IA = 30.99) (Tab. 2). From the two-dimensional plot, the most important food items in the diet of *M. atlanticus* were Diptera larvae and *Tubifex* spp. worms.

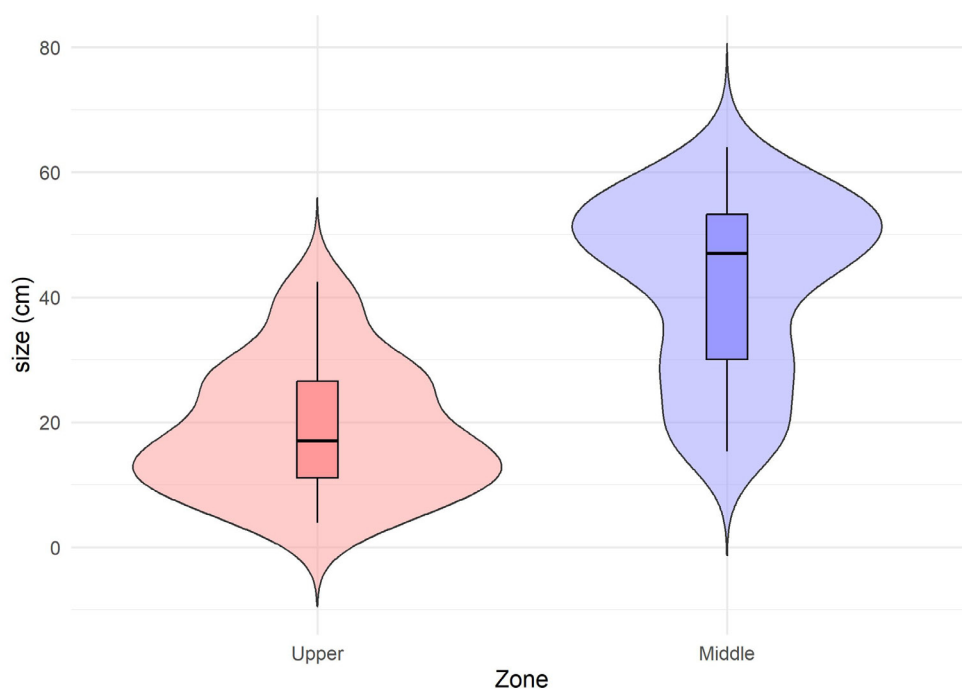


FIGURE 3 | Violin plot of *Megalops atlanticus* size distribution (cm) in the upper and middle estuarine zones.

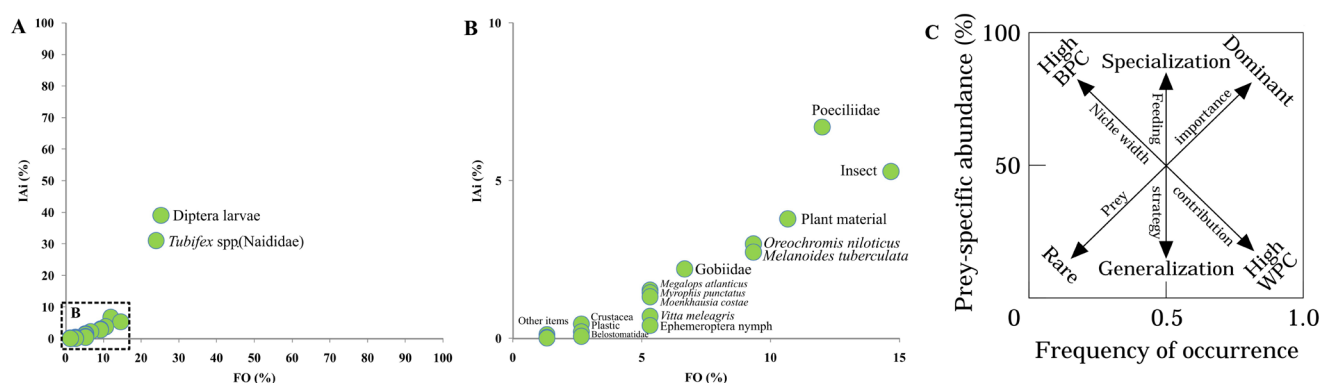


FIGURE 4 | Graphical analysis of the feeding strategy of *Megalops atlanticus* in the Cocó estuary. **A.** Relationship between specific abundance (IAi%) and frequency of occurrence (FO%) of prey. **B.** Enlargement of the lower left region of the plot. Other items: *Eleotris pisonis*, *Bathygobius soporator*, Engraulidae, *Serrasalmus rhombeus*, Syrrhidae larvae, insect eggs, and debris. **C.** Conceptual diagram adapted from Amundsen *et al.* (1996). WPC: Each individual shows variation in its own resource use; BPC: There is variation in resource use among individuals.

TABLE 2 | Frequency of occurrence (FOi%), average volume (Vi%), and dietary importance index (IAi%) of each item consumed by *Megalops atlanticus* in the Cocó estuary. Note: items marked with an asterisk correspond to fish species.

Food item	FOi%	Vi%	IAi%
Diptera larvae	25.33	23.93	39.02
<i>Tubifex</i> spp.	24.00	20.06	30.99
Poeciliidae*	12.00	8.67	6.69
Insect	14.67	5.60	5.29
Plant material	10.67	5.52	3.79
<i>Oreochromis niloticus</i> *	9.33	4.98	2.99
<i>Melanoides tuberculata</i>	9.33	4.54	2.73
Gobiidae*	6.67	5.12	2.20
<i>Megalops atlanticus</i> *	5.33	4.44	1.52
<i>Myrophis punctatus</i> *	5.33	4.21	1.44
<i>Moenkhausia costae</i> *	5.33	3.83	1.31
<i>Vitta meleagris</i>	5.33	2.03	0.70
Crustacea	2.67	2.63	0.45
Ephemeroptera nymph	5.33	1.17	0.40
Plastic	2.67	1.18	0.20
<i>Eleotris pisonis</i> *	1.33	1.33	0.11
Belostomatidae	2.67	0.35	0.06
<i>Bathygobius soporator</i> *	1.33	0.36	0.03
Engraulidae*	1.33	0.24	0.02
<i>Serrasalmus rhombeus</i> *	1.33	0.22	0.02
Syrphidae larvae	1.33	0.16	0.01
Debris	1.33	0.10	0.01
Insect egg	1.33	0.07	0.01

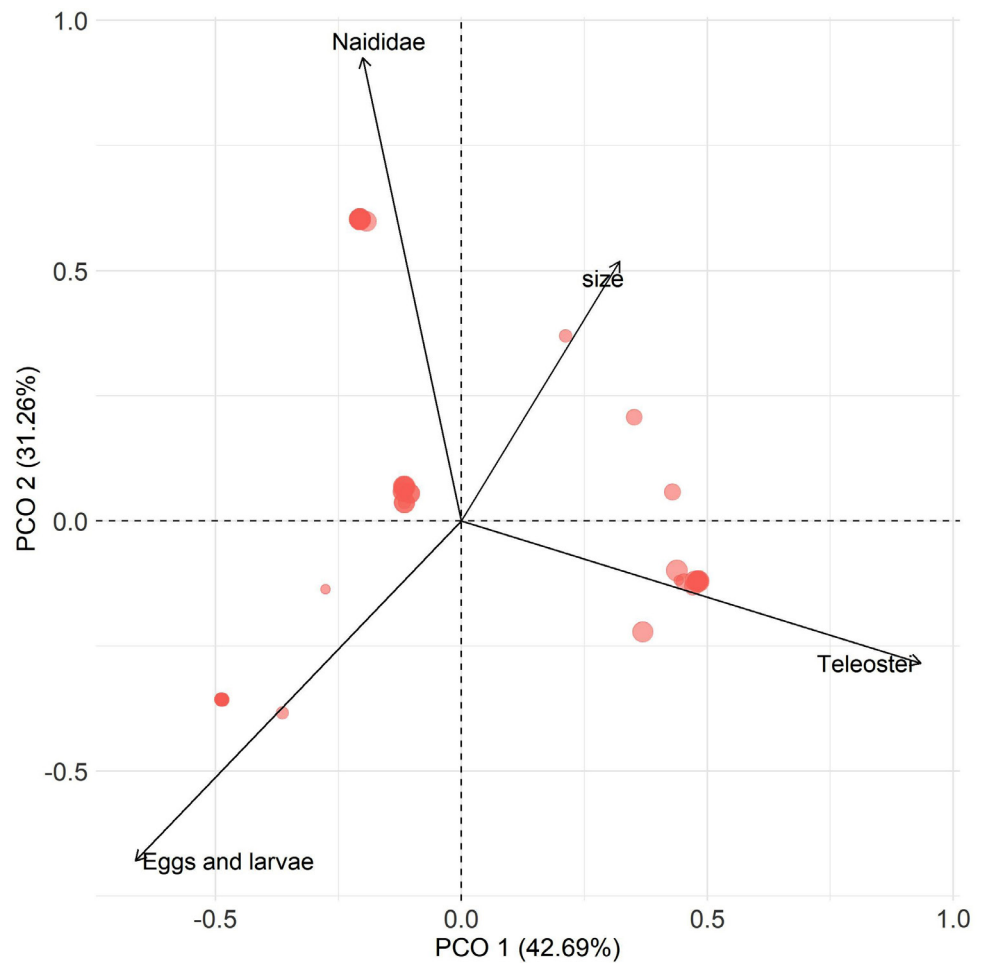
A total of 33 fish samples, either fully or partially digested, found in the stomachs of *M. atlanticus* were genetically evaluated. Eleven of these samples were successfully sequenced, being identified as *Bathygobius soporator* (Valenciennes, 1837), *Megalops atlanticus*, *Moenkhausia costae* (Steindachner, 1907), *Myrophis punctatus* Lütken, 1852, *Oreochromis niloticus* (Linnaeus, 1758), and *Serrasalmus rhombeus* (Linnaeus, 1766) (Tab. 3).

The PCoA revealed three distinct groups based on the predominance of the following items in the diet: eggs and larvae, Naididae (*Tubifex* spp.), and Teleostei. A permutation test using the *envfit* function indicated a significant effect between fish size and diet composition ($p = 0.001$). Smaller individuals (< 15 cm) primarily consumed eggs and larvae, whereas larger individuals preferred Naididae or Teleostei (Fig. 5).

Smaller individuals of Tarpon (< 15 cm) exhibited a less diverse diet, with an emphasis on Diptera larvae, insects, and small fishes (e.g., Poeciliidae). Individuals measuring 15.1 to 30 cm consumed a wider variety of fish, such as *M. costae*, *O. niloticus*, *S. rhombeus*, and Gobiidae, and other invertebrates, signaling a shift toward a more predatory feeding strategy. As *M. atlanticus* grows (30.1 to < 45 cm), its diet becomes more generalist, dominated by fishes and invertebrates, such as Crustacea and Mollusca. The presence of *Tubifex* spp. demonstrated its predominance across different size classes, mainly in larger individuals (Fig. 6).

TABLE 3 | Fish species sequenced in the evaluation of the stomach content of *Megalops atlanticus*.

Code	Largest matches in GenBank	Identities (%)	E-value	Query cover
10A	<i>Oreochromis niloticus</i>	100	0.0	100
12A	<i>Oreochromis niloticus</i>	100	0.0	100
CH19	<i>Oreochromis niloticus</i>	100	0.0	100
PJ05	<i>Oreochromis niloticus</i>	100	0.0	100
20B	<i>Serrasalmus rhombeus</i>	98.94	0.0	100
EVOA	<i>Bathygobius soporator</i>	99.36	0.0	100
EVOB	<i>Bathygobius soporator</i>	99.37	0.0	100
SYMA	<i>Myrophis punctatus</i>	99.04	0.0	100
SYMC	<i>Myrophis punctatus</i>	98.96	0.0	99
20A	<i>Moenkhausia costae</i>	99.66	0.0	99
SI06	<i>Megalops atlanticus</i>	98.54	0.0	99

**FIGURE 5** | Principal Coordinates Analysis (PCoA) ordination of dietary items for different size classes of *Megalops atlanticus* (circle size corresponds to individual size).

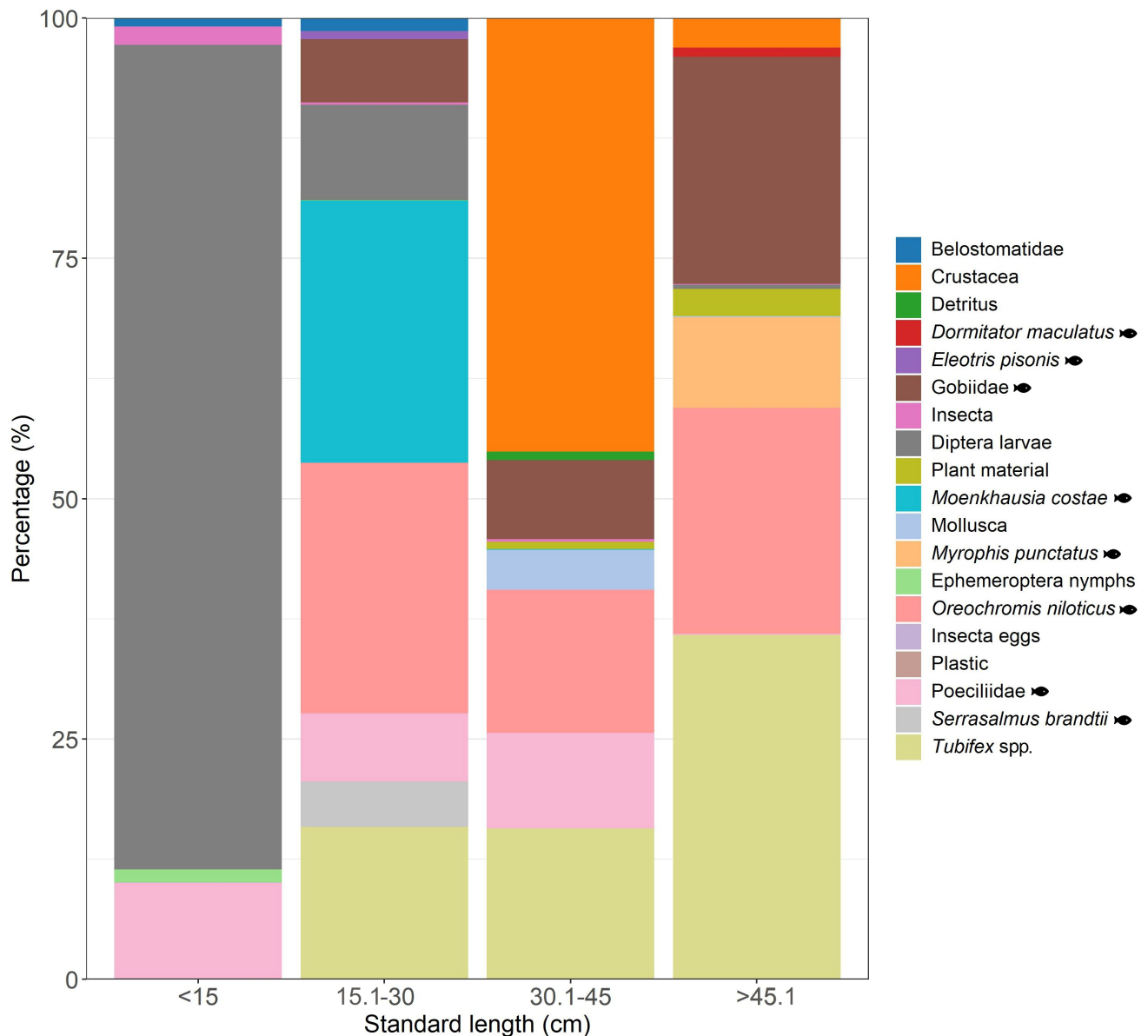


FIGURE 6 | Description of the diet of *Megalops atlanticus* with prey volume (%) and Tarpon size (cm). The fish symbol indicates the items classified as fishes.

DISCUSSION

Megalops atlanticus consumed prey from various trophic levels, showcasing its generalist diet and ability to exploit a wide range of resources, a common characteristic among opportunistic predators (Drenner, Hambright, 2002; Costa, Angelini, 2020). This strategy is particularly advantageous in disturbed environments, which are marked by fluctuations in resource availability (Hall-Scharf *et al.*, 2016). These findings reinforce the species' ecological plasticity and its ability to persist in modified coastal ecosystems. Moreover, ontogenetic changes in the diet of *M. atlanticus* are associated with its growth and shifts in habitat use, transitioning from smaller prey, such as insect larvae, to larger

prey, like fishes (Kurth *et al.*, 2019; Jud *et al.*, 2011). Given the context of an urbanized estuary, the current study evaluated all ingested items, regardless of whether ingestion was intentional, as potential indicators of anthropogenic influence. This approach is especially relevant for a vulnerable species; therefore, even plastic debris was recorded.

The species was recorded exclusively in the intermediate and upper zones of the estuary, which are characterized by low oxygen concentrations, reduced salinity, and lower water transparency compared to the lower zone, conditions found in urban estuaries (Pinto *et al.* 2025). Despite this, *M. atlanticus* survives due to physiological adaptations, such as oxygen storage in the swim bladder and visual mechanisms that facilitate predation in turbid waters (Geiger *et al.*, 2000; Marceniuk, 2005; Schweikert, Grace, 2018). These traits allow the species to exploit abundant prey adapted to the conditions of human-modified habitats, including aquatic invertebrates, such as Diptera larvae and Ephemeroptera nymphs, which are crucial food sources for juvenile fishes (Starks, Long, 2017).

The Cocó estuary faces increasing anthropogenic pressures (Barroso *et al.*, 2016), representing a threat to juvenile survival in these habitats (Wilson *et al.*, 2019). These changes impact not only the habitat quality but also the availability of food resources (Pinto *et al.*, 2025). However, freshwater habitats usually favor species like Cichlidae and Poeciliidae, which often compose the diet of *M. atlanticus*. This dietary pattern was observed in our study and is consistent with the findings from other studies performed in tropical estuaries (Menezes, Menezes, 1968; Jud *et al.*, 2011; Kurth *et al.*, 2019; Navarro-Martinez *et al.*, 2020), favoring the occurrence of Tarpon in the study area despite the increased urbanization. Dietary changes are essential for the occupation of nursery habitats and survival in dynamic habitats (Woodson *et al.*, 2018; Cianciotto *et al.*, 2019; Ríos *et al.*, 2019).

Several biotic and abiotic factors influence these ontogenetic variations, such as prey availability and pollution (Whitfield *et al.*, 2024). In our study, *Tubifex* spp. (Naididae) was identified as the primary food source for the individuals analyzed, while it appeared across a range of size classes, thereby altering the ontogenetic variation. This worm, associated with environments rich in organic matter (Rodrigo, Alves, 2018), reflects the conditions of the estuary, which is characterized by a high organic load. This pattern contrasts with other tropical estuaries, where the diet of *M. atlanticus* is primarily composed of fish and other invertebrates, with clear ontogenetic differentiation (Menezes, Menezes, 1968; Jud *et al.*, 2011). The clear prevalence of *Tubifex* spp. as a food resource emphasizes the impact of anthropogenic alterations on the estuary, as oligochaetes are biological indicators of polluted environments, tolerant of low oxygen levels and organic matter accumulation (Martin *et al.*, 2008; Rodrigues, Alves, 2018).

The presence of the non-native species *Oreochromis niloticus* in the diet of *M. atlanticus* was confirmed, which indicates the consumption of exotic species of freshwater origin. Additionally, three species of Poeciliidae were recorded in the Cocó estuary: *Poecilia reticulata*, *P. sphenops*, and *P. vivipara* Bloch & Schneider, 1801 (Gurgel-Lourenço *et al.*, 2023; Botero *et al.*, 2023; Pinto *et al.*, 2025). However, due to the digestion of the samples, it was not possible to identify each species individually in the stomachs. Given that only *P. vivipara* is native, there is a high likelihood that *M. atlanticus* is consuming other non-native fish species.

The presence of non-native species in the diet of native fishes alters the trophic interactions both directly and indirectly, as well as temporally and spatially, depending on the availability of food resources (Pintor, Byers, 2015; Tran *et al.*, 2015; Basic *et al.*, 2019). The introduction of species can create new feeding interactions, directly impacting the diet of native fishes, which creates a scenario in which exotic predators can compete for resources or directly prey on native species, thereby altering the structure of fish communities (Rosa *et al.*, 2021). The comprehension related to these dynamics is crucial to assessing the ecological impacts of biological invasions and developing effective management and conservation strategies. Nile tilapia (*O. niloticus*), for instance, presents physiological adaptations that, when coupled with frequent records, indicate the species is well-established in degraded urban environments (Cassemiro *et al.*, 2018). Although generalist predators, such as *M. atlanticus* consume non-native species, the presence of these organisms may harm the local biodiversity in the long run (Rosa *et al.*, 2021). In this scenario, these non-native species complement or replace native prey in the diet of fishes, which modifies the energy and matter flows within the ecosystem. However, generalist predators that consume non-native species play a significant role in controlling exotic species, reducing their success (Pintor, Byers, 2015).

When compared to other studies (Menezes, Menezes, 1968; Kurth *et al.*, 2019; Jud *et al.*, 2011; Navarro-Martinez *et al.*, 2020), our results revealed a varied diet with a high frequency of occurrences of prey, with some prey types being more frequent than others. Among the recorded prey, certain species stood out due to their uncommon occurrence in the diet of *M. atlanticus*. In particular, *Myrophis punctatus* had not been previously recorded in the diet of this species. This species typically hides within the substrate (Able *et al.*, 2010), which makes it unavailable to predators with an upper jaw, like *M. atlanticus* (Westneat, 2005). However, daily vertical movements may allow foraging throughout the water column, which could promote the variety of consumed items (Luo, Ault, 2012). It is also possible that secondary predation occurs, which consists of one predator consuming another one that, in turn, has consumed the primary prey (King *et al.*, 2008). This scenario could explain the presence of benthic organisms like *Vitta meleagris* (Lamarck, 1822) and *Melanoides tuberculata* (Müller, 1774).

Cannibalism was also recorded, which may occur in situations of competition where food availability is scarce or in territorial behaviors (Block, Stokes, 2004). This practice had not been previously registered for *M. atlanticus* and can be explained by the limitations in environmental conditions to access alternative prey (Block, Stokes, 2004; Cianciotto *et al.*, 2019). Although molecular analysis can, in some cases, generate false positives due to the amplification of residual DNA fragments in the stomach (Hoogendoorn, Heimpel, 2001; King *et al.*, 2008). Therefore, we highlight the need for further studies, combining behavioral observations and complementary techniques, as well as the continued refinement of molecular protocols to enhance the accuracy of dietary analysis.

All species identified in this estuary have been previously recorded in fauna surveys (Gurgel-Lourenço *et al.*, 2023), which helps validate the morphological identification. In addition to the difficulty of identification due to the high digestion state, the families Characidae and Cichlidae, Eleotridae and Gobiidae, and Ophichthidae and Synbranchidae exhibit significant morphological similarities, which complicates visual analysis. Thus, we demonstrate how molecular data can enhance taxonomic

analysis of fish stomach contents. The combined use of taxonomic methods, molecular analysis, and record history provides realistic estimates of the composition found in the stomach contents of *M. atlanticus*. Furthermore, all fish species identified in the stomach corroborate previous studies on its feeding behavior (Menezes, Menezes, 1968; Jud *et al.*, 2011; Kurth *et al.*, 2019; Navarro-Martinez *et al.*, 2020). Therefore, molecular analysis was crucial to accurately identify the fish species in the *M. atlanticus* diet.

Several procedures can influence the success of DNA amplification from the stomach contents, which include transport, storage, proper use of techniques, and sample quality (Traugott *et al.*, 2020). The use of diet analysis via DNA began expanding in the 2000s and has been continuously refined, offering several benefits, such as greater specificity and sensitivity in detecting and identifying food DNA, as well as validating the identity of detected prey through DNA sequencing.

Despite the success in identifying stomach contents, the limitations encountered included sample degradation and enzymatic activity in the predator's stomach. This made the amplification of some sequences difficult and, consequently, the identification of samples (Piñeros, Calderón-Cortés, 2023). When analyzing the stomach contents of species, it is important to note that the DNA of consumed food is digested and degraded over time. As a result, the genes present in the cells will be partially digested, resulting in fragmented DNA strands (Traugott *et al.*, 2020). This makes the detection of long DNA fragments increasingly difficult as the prey is digested over a longer period (Deagle *et al.*, 2006). This difficulty was also observed by Rosel, Kocher (2002) for Atlantic cod, *Gadus morhua* Linnaeus, 1778, by Boza *et al.* (2022) in the stomach contents of *Trichiurus lepturus* Linnaeus, 1758 (Scombriformes: Trichiuridae), and by Paquin *et al.* (2014) in 12 species of subterranean fish in the North Pacific.

Conservation strategies for *M. atlanticus* should consider the restructuring of altered food webs, which can trigger negative interactions and provide lower-protein trophic resources (Bartley *et al.*, 2019; Costa, Angelini, 2020). This is supported by the identification of non-native species in the diet of *M. atlanticus*, which may offer lower nutritional value compared to native prey (Bartley *et al.*, 2019). To address these challenges, integrated management strategies are essential. In Brazil, there are no specific conservation measures for *M. atlanticus* (Batista *et al.*, 2020). Therefore, habitat restoration and connectivity between breeding, nursery, and migration environments must be prioritized to support the species' persistence in estuaries (Kurth *et al.*, 2019; Luo *et al.*, 2020). Protecting these habitats and understanding the species' habitat, diet, and population dynamics are critical for ensuring successful population recruitment and long-term conservation (Bartley *et al.*, 2019; Kurth *et al.*, 2019).

This study provides new insights into the diet of juvenile *M. atlanticus* in urban estuaries and reveals its foraging strategy and food composition. Anthropogenic activities, along with changes in biotic and abiotic conditions, may influence the fishes' diets (Lyasenga *et al.*, 2021) and the capacity of these environments to function as nursery grounds (Kurth *et al.*, 2019; Toft *et al.*, 2018). The ability of *M. atlanticus* to consume non-native species and adjust its dietary composition according to body size highlights its role as a generalist predator and potential controller of invasive species. These findings emphasize the need for urgent habitat restoration, integrated management, and public awareness efforts to ensure successful population recruitment and preserve the ecological functions of *M. atlanticus* within estuarine ecosystems.

ACKNOWLEDGMENTS

The authors would like to thank the Universidade Federal do Ceará (UFC) for its support. We thank the Conselho Nacional de Desenvolvimento Científico e tecnológico (CNPq) for financial assistance, which supported the fieldwork and provided scholarships for RCGL and LMP. We are grateful for the support of the National Institute of Science & Technology of Materials Transfer at the Continent-Ocean Interface (INCT-TMCOcean), based at UFC. Special thanks are extended to Laboratório de Ecologia Aquática e Conservação (LEAC). We especially thank the managers of the Conservation Unit Parque Estadual do Cocó and Lieutenant Francisco Araújo and his assistant Antônio for their invaluable logistical support during the sampling process. Our sincere appreciation goes to Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (Funcap) for their financial backing.

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ETHICAL STATEMENT

Field data were collected under the authorization of licenses #57780–1 and #77988–1, granted by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/SISBIO).

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

COMPETING INTERESTS

The authors declare no competing interests.

FUNDING

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), MCTI/CNPq Program (Grants 28/2018, 423628/2018–6, and 63/2022, 40354/2022–8). Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (Funcap), Process BMD–0008–01848.01.16/22.

HOW TO CITE THIS ARTICLE

- **Matias GB, Pinto LM, Gurgel-Lourenço RC, Nascimento TCES, Hissa DC, Sánchez-Botero JI.** Diet composition of the vulnerable fish species *Megalops atlanticus* (Elopiformes: Megalopidae) in a heavily urbanized estuary in Brazil: DNA-based identification of preys. Neotrop Ichthyol. 2025; 23(3):e250010. <https://doi.org/10.1590/1982-0224-2025-0010>

Neotropical Ichthyology

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Official Journal of the
Sociedade Brasileira de Ictiologia