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Plastic Pollution and Trophic Niche Differentiation in Herbivorous Reef Fish from an Urbanised Reef in Maceió, Brazil.

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Abstract

Marine coral reefs are experiencing rapid degradation, particularly in urbanised coastal areas where anthropogenic pressures such as pollution and habitat disturbance are most intense. Plastic pollution has been damaging for ocean wildlife by physical entanglement and ingestion, leading to the entry and accumulation of plastic in the trophic food chain. This study aims to assess the extent of plastic pollution on the Ponta Verde reef and nearby beach in Maceió, Brazil. In addition, I investigate the niche differentiation of two herbivorous reef fish species, the ocean surgeon (*Acanthurus bahianus*) and the grey parrotfish (*Sparisoma axillare*), and their potential ingestion of plastics. Macro (> 5mm) litter was collected using line transects on the beach and snorkelling transects on the reef once per month from November 2023 to August 2024. Sand samples were collected from each transect once per month from November 2023 to August 2024 and analysed for micro (<5mm) litter particles. Muscle tissue from under the dorsal fins of the two species were used to perform stable isotope analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) to assess ecological niche differentiation and trophic positioning. Observational data of the species' preferred biting material was gathered from reef surveys, and gut content was analysed to evaluate plastic ingestion. There was significantly more macro litter on the beach compared to the reef. However, plastic was the dominant form of litter in both environments, accounting for over 80% of macro litter. Similarly, plastic accounted for over 70% of micro litter in both environments. Stable isotope analysis revealed a clear separation of isotopic niche between the two species. Observational data showed both species preferred to bite the turf material within the EAM (epilithic algal matrix). Neither one of the species showed signs of plastic ingestion. The significant dominance of plastics among macro and micro litter across reef and beach environments underscores the persistent nature of plastic pollution and its tendency to fragment into smaller particles over time. *S. axillare* displayed a broader isotopic niche, suggesting a more varied diet, while *A. bahianus* occupied a higher trophic position, suggesting a more nitrogen rich diet. Given that both species preferred turf as biting material, the differences in isotopic niche suggest that they target different components within the EAM. Despite the presence of plastic pollution in the reef environment, no plastic was detected in the gut contents of either species. This suggests that, under current conditions, plastic ingestion does not pose a significant threat to these herbivorous fish species.

Table of Contents

Acknowledgements	I
Abstract	II
1. Introduction	1
2. Materials and methods.....	4
2.1 Study site	4
2.2 Study species	5
2.3 Transect surveys	7
2.4 Microplastics in sand and algae samples.....	8
2.5 Observational surveys	8
2.6 Stable isotopes.....	9
2.7 Stable isotope analysis.....	9
2.8 Microplastic ingestion	10
2.9 Data analysis.....	10
3. Results	12
3.1 Plastic pollution in the environments	12
3.2 Niche overlap and trophic position.....	14
3.3 Feeding behaviour	15
3.4 Microplastic in fish and algae.....	16
4. Discussion	16
4.1 Plastic pollution in the environment.....	16
4.2 Isotopic niche & feeding behaviour	18
4.3 Plastic consumption.....	19
4.4 Conclusion.....	19
5. References	21

1. Introduction

Marine coral reefs are known to be one of the most biodiverse ecosystems on Earth (Knowlton et al., 2010). They occupy less than 1% of the world's ocean area, yet they provide a home for at least 25% of all marine species (Mulhall, 2008). In addition to being the home to a vast variety of marine wildlife, they also provide goods and services for human populations, such as fisheries, tourism, protection from waves, aesthetic and cultural values, all of which coastal communities and cities close to the reefs depend upon (Bellwood et al., 2004). Still, coral reefs all over the world are extremely susceptible to anthropogenic activities and global warming. As a result, coral reefs globally are seeing a decrease in coral reef health, which has a detrimental cascading effect on the marine ecosystem (Hughes et al., 2018).

Coral reefs worldwide have experienced substantial declines in both coral cover and species diversity (Bellwood et al., 2004; Bruno, 2007), driven largely by the combined impacts of pollution (Dubinsky & Stambler, 1996), overfishing (Jackson, 1997), disease (Aronso & Precht, 2001) and climate change (Hughes et al., 2003). Rising sea temperatures due to global warming have caused thermal stress, triggering mass bleaching of corals (Hughes et al., 2017). Coral bleaching is the whitening of corals due to the loss of their dinoflagellate symbionts, commonly known as zooxanthellae (Brown, 1996; Muller et al., 2015). Bleaching is frequently described as a stress response, that often leads to high mortality, reduced growth rates and lower fecundity among corals (Hughes et al., 2003; Baird & Marshall, 2002). Notably, mass coral bleaching events have become more frequent over the last 50 years (De Moura et al., 2001).

The resilience of a complex system such as a coral reef lies in its capacity to absorb and adapt, or recover from disturbances, while maintaining its functions and services without shifting to an alternate state (Hughes et al., 2010). There have been instances of coral reefs being able to completely recover from disturbances, especially when a fast-acting episode such as a cyclone, bleaching event or predation occurs (Pisapia et al., 2016). These events quickly push the system away from its equilibrium and are a normal part of the dynamics of functional coral reefs (Hughes et al., 2010). Human stressors, in particular fishing pressure, nutrient enrichment and coastal pollution are slow drivers that are more persistent and accumulate over time (Nyström et al., 2000). This accumulation of pressure can have a detrimental effect on the resilience of coral reefs, decreasing their ability to recover from disturbances (Hughes et al., 2003).

Coral reefs near urbanised areas are increasingly exposed to anthropogenic stressors threatening their ecological integrity. They are directly impacted by nutrient runoff, overfishing and anthropogenic pollution, resulting in the decrease of reef resilience (Hughes et al., 2003). A prominent decrease of coral reef resilience will promote the growth of fast spreading organisms like algae or sponges to dominate the sea floor. This will prevent coral recruitment and decrease coral growth, which in return can shift the reef ecosystem from a coral-dominated to an alternate stable state (Scheffer et al., 2001). This is a phenomenon referred to as a phase shift (McManus & Polsenberg, 2004; Knowlton & Jackson, 2008). These phase shifts are concerning for reef health and integrity, biodiversity, and social, cultural and economic values (Hughes et al., 2003). Previous research indicates that macroalgal dominance is correlated to areas close to urbanised coastlines, suggesting that urban-related stressors contribute to these shifts (Cruz et al., 2018).

Herbivorous fish play a key role in maintaining the health and resilience of coral reefs (Bonaldo et al., 2014), as they regulate algal cover on benthic communities and can consume over 90% of daily algae production in shallow coral reefs (Burkepile & Hay, 2006). This works by grazing on turf algae, macroalgae and components of the epilithic algal matrix (EAM), which is a complex layer of turf forming filamentous algae (< 1 cm high), detritus, sediment and associated fauna (Wilson et al., 2003; Bonaldo et al., 2014). Therefore, with herbivores present, corals and coralline algae are able to survive and grow (Ogden & Lobel, 1978). Consequently, a decline in herbivores on the reef will promote the growth of macroalgae and ultimately lead to a coral-algal phase shift (Hughes et al., 2007).

A phase shift may alter the trophic structure of the ecosystem (Cruz et al., 2018). The different trophic levels include primary producers, detritivores, primary consumers and secondary consumers (Dodds & Whiles, 2010), the structure is complex and shaped by the interactions between organisms and their environment. A shift in the environment will alter resource availability, potentially forcing species to exploit new niches or compete with other species they previously did not overlap with (Schoener, 1974). Additionally, the introduction of foreign material such as plastic presents an additional level of complexity. As plastic litter is entering the environment, the potential consumption of plastics increase (Dantas et al., 2024). As of 2021, it has been reported that 1288 marine species ingest plastic (Santos et al., 2021). Therefore, understanding the quantity of plastics entering the environment and its uptake by species at the bottom of the food chain is key to evaluating its ecological consequences. One important mechanism by which pollution, specifically plastic, can affect ecosystems is through

its entry and accumulation in food webs. Plastics have been detected in herbivorous and planktivorous fish, which occupy the base of many tropical reef food chains (Santos et al., 2021). This can result in the accumulation of plastic in the trophic chain and results in the accumulation of plastic in individuals further up the food chain. This raises concerns about the potential for plastic to not only harm individual organisms, but also disrupt energy flow, trophic interactions, and nutrient cycling.

Urbanised coral reefs are increasingly threatened by plastic pollution (Ilechukwu, et al., 2024), threatening reefs by spreading throughout food webs, increasing disease transmission and causing structural damage to reef organisms (Aronson & Precht, 2001; Lamb et al., 2018; Pinheiro et al., 2023). An estimated 4.8-12.7 million metric tons of plastic waste enters the ocean every year (Jambeck et al., 2015). Reefs near large population centres are expected to accumulate more consumer-derived litter than reefs located further from direct human influence (Pinheiro et al., 2023). As plastic litter enters the environment, it can degrade over time resulting in the accumulation of microplastics (Jambeck et al., 2015). As coral reefs near urbanised areas are already subject to several other local stressors, plastic pollution represents a compounding threat that may decrease resilience and accelerate ecological degradation (Pinheiro et al., 2023).

To further understand how pollution affects trophic dynamics, it is essential to investigate how species use available resources. One way to do this is through stable isotope analysis, which can provide insight into an organism's trophic position and potential resource use over time. By examining the ratios of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$), we can understand differences in feeding ecology, including isotopic niche width, which is an indicator of dietary breadth or ecological specialization (Layman et al., 2007). Despite the growing concern around plastic ingestion in marine systems, few studies have combined isotopic niche analysis with plastic pollution data to examine how these may interact, particularly on urbanised reefs.

In this study, my focus is on an urbanised reef on the north-east coast of Brazil. I investigate trophic position and niche differentiation of two herbivorous tropical reef fish species using stable isotope analysis. In addition, I quantify plastic pollution in both the reef environment and adjacent beach and assess plastic ingestion in the two fish species (ocean surgeon, *Acanthurus bahianus* and gray parrotfish, *Sparisoma axillare*). More specifically, I ask the following questions: 1) How prominent is macro- and micro-plastic pollution in the reef environment and on the nearby beach? 2) Do *A. bahianus* and *S. axillare* differ significantly in their trophic position and niche portrayed by isotope analysis? 3) Do they exhibit similar feeding behaviour

and are they targeting the same food resource? 4) Do their intestines contain microplastics and, if so, at differing quantities? Results are discussed in light of existing literature.

2. Materials and methods

2.1 Study site

This study was conducted on the coral reef and beach at Ponta Verde (9839' 56"S– 35841' 30"W), Maceió, which is in the central part of Alagoas state in north-eastern Brazil. This coast extends approximately 600 km from Natal to the mouth of São Francisco River (Castro & Pires, 2001). Maceió is home to several types of reef ecosystems, including coral and sandstone reefs. The Ponta Verde reef is categorised as a fringing reef attached to the shoreline, where many of the reef's platform surfaces emerge during low tide. This creates a path that is possible to walk on and is popular for both tourists and fishermen. The reef has deteriorated over the last decade, and it has been estimated that approximately 90% of the corals in Maceió are dead (G1 Alagoas, 2024).



Figure 1: Map of study area in north-east Brazil outside the city of Maceió (inset). Aerial photograph shows the Ponta Verde beach and reef with the beach transects (black dots), marine transects (white dots), and fish observation zone (red polygon) indicated.

2.2 Study species

This study focuses on two herbivorous reef fish species found in the coastal waters of Maceió, Brazil: *Acanthurus bahianus*, commonly known as the ocean surgeon (Figure 2) and *Sparisoma axillare*, known as the gray parrotfish (Figure 3). These species were selected due to their ecological importance as primary consumers in reef ecosystems. Herbivorous reef fish can be

divided into four functional groups based on their feeding strategy and role on the reef. These groups are scrapers/small excavators, large excavators/bioeroders, grazers/detritivores and browsers (Green & Bellwood, 2009).

A. bahianus is one of the most widely distributed reef fish in the west and central Atlantic (Roberston et al., 2005). They are easily recognised by their oval-shaped bodies with colours varying between light yellow to blue-grey or dark brown (Figure 2). They are diurnal feeders, falling under the functional group of grazers. Grazers are recognised by their ability to limit establishment and growth of macroalgae by grazing on the algal turf within the EAM (Green & Bellwood, 2009). They have established a feeding strategy that combines herbivory and detritivore characteristics, feeding on fleshy and filamentous algae, and consuming large amounts of detritus and inorganic material that resides in the EAM (Hogan, 2011).

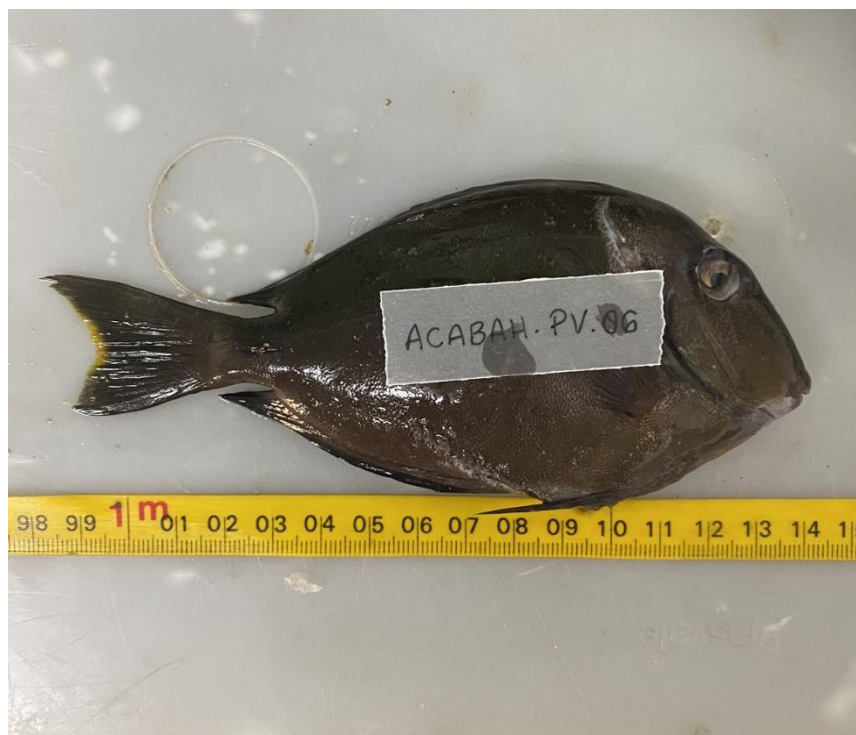


Figure 2: Photo of *Acanthurus bahianus* (ocean surgeon).

S. axillare is a species endemic to Brazil and is restricted to the southwestern Atlantic. They are easily recognised by their grey colouring and their large smooth cycloid scales (Figure 3). They have fused teeth forming a parrot-like beak used for scraping algae and detritus from substrate, common for all parrotfish (Bellwood, 1985). Functionally, *S. axillare* belongs to the

scrapers/small excavator group, known for grazing on algal turf with the EAM and for scraping the substrate surfaces they feed on. This behaviour helps with limiting the growth of macroalgae and creates clean substrate, promoting coral recruitment (Green & Bellwood, 2009). In addition to its beak, *S. axillare* has a second pair of jaws, the pharyngeal jaw apparatus, located in the throat (pharynx), which is equipped with specialised grinding plates that allow the species to crush and process tough algal material and substrate (Bonaldo et al., 2014). Due to intense exploitation by local fisheries, *S. axillare* has been classified as threatened (de Queiroz-Véras et al., 2023).



Figure 3: Photo of *Sparisoma axillare* (gray parrotfish).

2.3 Transect surveys

To assess the abundance of plastic pollution along the Ponta Verde beach, transect surveys were performed for which all visible litter was collected within a 2m wide transect, with transect length ranging from 5 – 50 m depending on the tide line at the time of the survey. There were 10 transects in total on Ponta Verde beach (Figure 1), which were all completed on the same day to ensure consistency in environmental conditions. Litter items were categorised into macro (> 5 mm) and micro (< 5 mm) litter. The collected litter was transported to the Laboratory of Ecology and Conservation in the Anthropocene (ECO lab), where each item was measured

and categorized by material, colour, usage and origin. In the current study, I utilise data collected by the ECOA lab at Universidade Federal de Alagoas (UFAL) between November 2023 – August 2024.

Similarly, plastic pollution on the Ponta Verde reef was assessed by analysing litter composition and abundance collected from transect surveys completed on the reef. A total of 15 transects (each 2 x 20m) were surveyed one day each month from November 2023 to August 2024 (Figure 1). Within each transect, all visible litter was collected by snorkelling along a pre-laid rope, ensuring standardized coverage of the survey area. All collected litter was subsequently measured and categorized following the classification method described previously.

2.4 Microplastics in sand and algae samples

To quantify microplastic pollution in the environment, sand was collected from each of the beach and reef transects. The samples were dried in an oven at 60°C for at least 48 hours until completely dry. Subsequently, the samples were weighed. The sand was then sieved through three mesh sizes: 1 cm, 1 mm, and 500 µm. Sifted material was transferred into petri dishes for further examination. Microplastics were identified as any particles smaller than 5 mm. If present, plastic particles were subsequently counted and categorized following the classification proposed by GESAMP (2019), based on their size, shape, and colour. Once the particles were classified, they were tested to ensure that the particles were plastic. This was done by heating up a needle point and using this to touch the particles, depending on how they reacted to the heat, they could be classified as plastic e.g. if the particle melted.

To further investigate the microplastic pollution, three large macroalgae (*Bryopsis sp.*, Turf forming algae *Gelidium sp.* and *D. deliculata*) samples were collected. These macroalgae were chosen due to their abundance on the reef, as well as being the preferred bite material for the focal fish species during observational surveys. These samples were put through sieves of the same size as above, and if microplastics were present, they were counted and categorised following the same guidelines from GESAMP (2019). To ensure the particles were from plastic, the test described previously was used.

2.5 Observational surveys

To assess the feeding behaviour of *A. bahianus* and *S. axillare*, observational surveys were conducted on the Ponta Verde reef (Figure 1). The surveys were conducted by snorkelling along the reef during low tide. Snorkelling during low tide provides greater visibility for underwater

observations. Eleven individuals each of *A. Bahianus* and *S. axillare* were observed. When an individual was identified, it was subsequently followed at 1-2 m distance to ensure that the natural behaviour of the fish remained unaltered. For each fish, the first ten bites observed were recorded and the substrate or material targeted was noted. Bites recorded as “turf” included various components of the EAM, the complex benthic community of turf forming algae, detritus, sediment and associated fauna (Wilson et al., 2003).

2.6 Stable isotopes

In this study, stable isotopes were used to compare and visualise the trophic niches of *A. bahianus* and *S. axillare*. The ratio of stable isotopes of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) is a powerful tool for estimating trophic links between a consumer and its diet. This is done by estimating the trophic position of, and carbon flow to, consumers in food webs (Post, 2002). When estimating the trophic positions of a consumer, the $\delta^{15}\text{N}$ value is typically enriched by 3–4‰ relative to its diet (Deniro & Epstein, 1981; Post, 2002). This means that when the $\delta^{15}\text{N}$ value of an individual increases, the trophic position of this individual becomes higher. The $\delta^{13}\text{C}$ values reflect where consumers accumulate most of their carbon/energy. This is due to the $\delta^{13}\text{C}$ values of consumers closely mirror those of their dietary sources (Peterson & Fry, 1987). By comparing $\delta^{13}\text{C}$ values of consumers and their potential prey, we can estimate what the consumers are eating and help explain the carbon flow in ecosystems (Layman et al., 2012).

2.7 Stable isotope analysis

To quantify the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *A. bahianus* and *S. axillare*, local fishermen provided ECOA lab with 12 individuals of *A. bahianus* and 16 individuals of *S. axillare*. White muscle tissue from each fish was extracted from under the dorsal fin. These samples were dried in an oven at 60 °C for 24 hours. Earlier research has shown that utilizing an oven temperature of 60°C does not change the isotopic compositions of the samples (Bessey & Vanderklift, 2014).

To determine feeding behaviour, and where the fish accumulate most of their carbon in their diet, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of several algal species were quantified. These algal samples were collected during the observational survey, including at least one brown, red and green algae. In addition, samples of algae the fish appeared to prefer were collected. In total, 9 algal samples were collected (Table 1). The algal samples were similarly dried in an oven at 60°C, but the time varied between the different types of algae, due to their different morphology and ability to hold water.

After the samples were dried, they were ground into a homogenous powder using a mortar and pestle. Between 650 - 750µg of the fish powder was transferred into a tin capsule that needed to be pressed into a spherical shape without contamination. The same procedure was done for the algae, except that the amount of powder increased to 350 – 450mg. After all samples were placed into a microplate, they were sent to the Integrated Analysis Centre of Federal University of Rio Grande for isotope analysis. The permit for the work presented herein was provided by Comissão de Ética no Uso de Animais (CEUA) of UFAL (protocol number 25-2022).

Table 1: Algal species collected for stable isotope analysis, categorised functional group and colour.

Genus/Species	Functional group	Category
<i>Bryopsis sp.</i>	Filamentous macroalgae	Green
<i>Caulerpa lentillifera</i>	Corked macroalgae	Green
<i>Dictyopens delicata</i>	Leafy macroalgae	Brown
<i>Dictyota ciliolata</i>	Leafy macroalgae	Brown
<i>Gelidium sp.</i>	Turf forming algae	Red
<i>Halimeda sp.</i>	Calcareous macroalgae	Green
<i>Sargssum sanganese</i>	Leathery Macroalgae	Brown
<i>Spermothamnion repens</i>	Filamentous algae	Red
<i>Wrangelia decumbens</i>	Filamentous algae	Green

2.8 Microplastic ingestion

To assess microplastic ingestion by each fish, the collected fish were dissected, and the gastrointestinal tract removed. The gut content from each individual was extracted, transferred to a petri dish and analysed for microplastics utilizing a stereo microscope. All particles were collected and quantified by counting, anything resembling plastic was classified using the guidelines from GESAMP (2019). The same test as described previously was applied to ensure that the particles were of plastic material.

2.9 Data analysis

To compare total macro litter abundance per transect between the environments (beach vs reef), the mean item density per m² was firstly tested for normality using a Shapiro-Wilk test. This test confirmed that the macro litter data were not normally distributed. Therefore, a Wilcoxon rank-sum test was applied to evaluate whether the mean density of litter items per m² differed

between the two environments. Litter samples of unknown origin (NA) were excluded from the analyses. The items, sorted by category and density, were visualised using bar plots. The dominance of plastic was then tested in each environment using an exact binomial test to evaluate whether the proportion of plastic items significantly exceeded 50% of the total macro litter.

Similar analyses were conducted for the micro litter data. The Shapiro-Wilk test was utilized to test for normality. The Wilcoxon rank-sum test was applied to evaluate a significant difference in density of litter items per 100 g of sand. Visualization of the mean litter density per 100g of sand by category was completed with bar plots. An exact binomial test was performed to evaluate whether the proportion of microplastic particles significantly exceeded 50% of the total micro litter in both environments.

To visualise and compare the isotopic niches of *A. bahianus* and *S. axillare*, standard ellipse areas (SEA) were calculated for each species, with corrections for small sample size applied (SEAc), following Jackson et al. (2011). SEA and SEAc values were similar, but SEAc is used in this thesis for consistency and to reduce bias. These ellipses represent 40% of the most central data points to eliminate the effects of extreme outliers. A PERMANOVA test was used to further detect any significant differences in the isotopic niches.

Additionally, convex hull areas (TA) were calculated to represent the total isotopic space occupied by each species. The calculations were based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, with trophic position (TP) estimated using the equation in Post (2002). The TA provides a measure of niche breadth by encompassing all isotopic values for each species, thus reflecting the full range of trophic diversity. It is worth noting that the TA metric is further influenced by outliers than SEAc.

A Pearson's Chi-square test was utilized to see if *A. bahianus* and *S. axillare* differed significantly in the overall distribution of bites on the different materials. Additionally, a one-way ANOVA was applied to test if the percentages of turf bites were significantly different between the two species.

All statistical analyses and visualizations were performed in R studios (version 4.4.3)

Portions of the data analysis guidance, code structuring and language support were assisted by OpenAI's ChatGPT (version 4, 2025 release). Final interpretations, critical assessments, and all academic decisions were made independently.

3. Results

3.1 Plastic pollution in the environments

A Shapiro-Wilk test indicated that the macro litter data were not normally distributed ($W = 0.91$, $p < 0.001$). However, a Wilcoxon rank-sum test ($W = 899$, $p < 0.001$) showed that macro litter density was significantly higher on the beach (mean = 2.82 items/m²) compared to the reef (mean = 0.54 items/m²; Figure 4b).

An exact binomial test showed that litter containing plastic comprised a significantly greater proportion than 50% of all macro litter collected on the beach ($p < 0.001$). Plastic items accounted for 83.3% of the total litter (Figure 4a), with a 95% confidence interval ranging from 82.5% to 100%. Similarly, the macro litter found on the reef had a significantly greater proportion of plastic material ($p < 0.001$) compared to any other material found. Plastics found on the reef accounted for 81.4% of all items recorded (Figure 4a), with a 95% confidence interval ranging from 77.8% to 100%.

A Shapiro-Wilk test indicated that the micro litter was not normally distributed ($W = 0.72$, $p < 0.001$). A Wilcoxon rank-sum test showed no significant difference in micro litter density (items per 100g of sand) between the beach and reef environments ($W = 131.5$, $p = 0.352$; Figure 5b).

An exact binomial test showed that plastic items made up a significantly greater proportion than other materials. Plastic accounted for 84.5% of micro litter (Figure 5a), with a 95% confidence interval of 80% to 100% on the beach with a highly significant result ($p < 0.001$). On the reef, plastics made up 72.9% of micro litter ($n = 48$), with a confidence interval of 60.4-100% ($p = 0.001$).

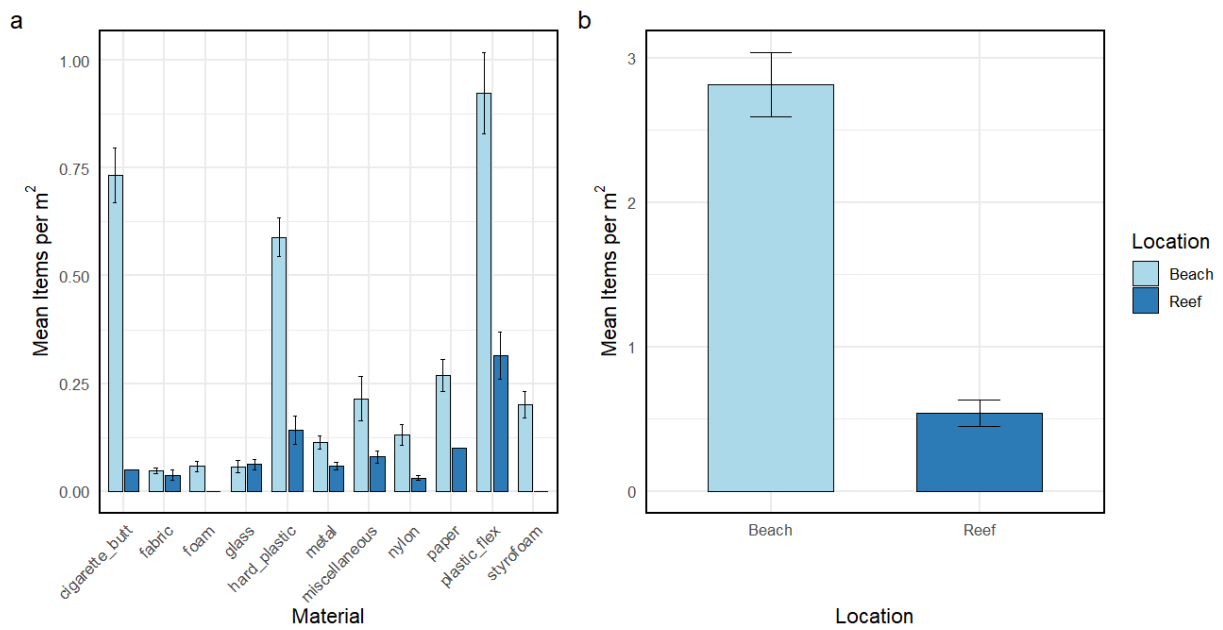


Figure 4: The mean number of items per m² of each litter material (a) and mean number of items per m² combined (b) collected from the Ponta Verde beach and reef environment.

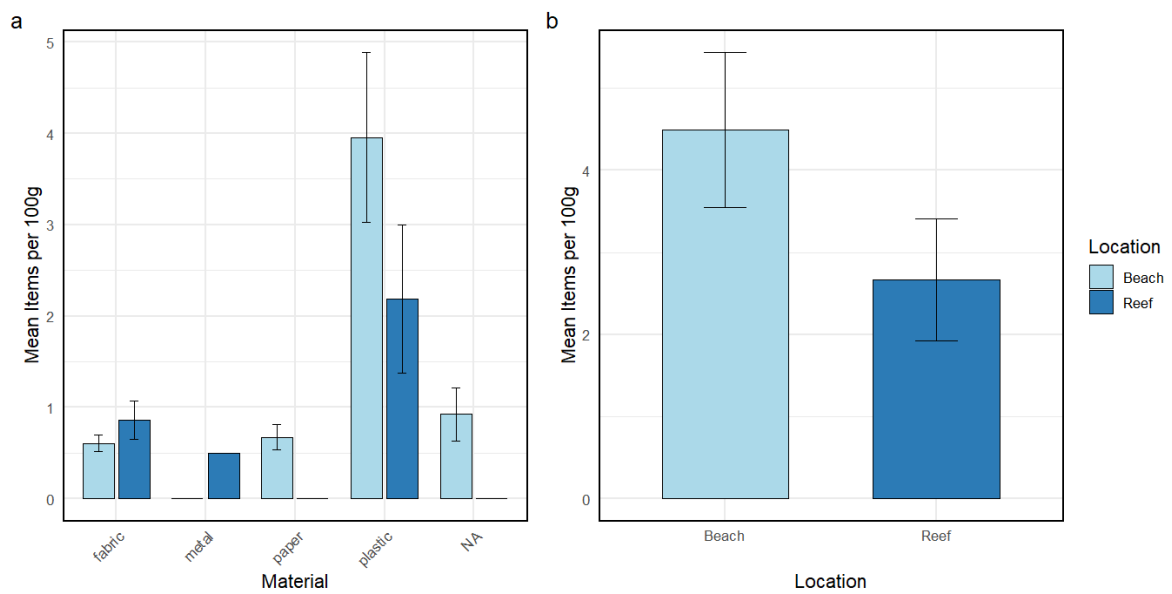


Figure 5: The mean number of items for each micro litter per 100g of sand (a) and mean number of items per 100g of sand combined (b) collected from the Ponta Verde beach and reef sand samples.

3.2 Niche overlap and trophic position

The isotopic niches of *A. bahianus* and *S. axillare* were significantly different (PERMANOVA $R^2=0.629$, $F=44.09$, $p=0.001$; Figure 6). *S. axillare* exhibited a broader isotopic niche ($SEAc = 0.967\text{‰}^2$) than *A. bahianus* ($SEAc = 0.471\text{‰}^2$). Additionally, the total area (TA) occupied by *S. axillare* (2.99‰^2) was larger than that of *A. bahianus* (1.00‰^2) (Table 2). *S. axillare* had closer $\delta^{13}\text{C}$ values to the potential algal prey samples compared to *A. bahianus* (Figure 7).

Table 2: Corrected standardised ellipse areas (SEAc), convex hull areas (TA), mean values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and trophic position ($\pm SD$) of *A. bahianus* and *S. axillare*.

Species	SEAc (‰^2)	TA (‰^2)	Mean (‰)	$\delta^{13}\text{C}$ (‰)	Mean (‰)	$\delta^{15}\text{N}$ (‰)	Trophic Position \pm SD
<i>A. bahianus</i>	0.471	1.00	-12.1		11.5		2.15 ± 0.06
<i>S. axillare</i>	0.966	2.99	-14.0		10.6		1.91 ± 0.107

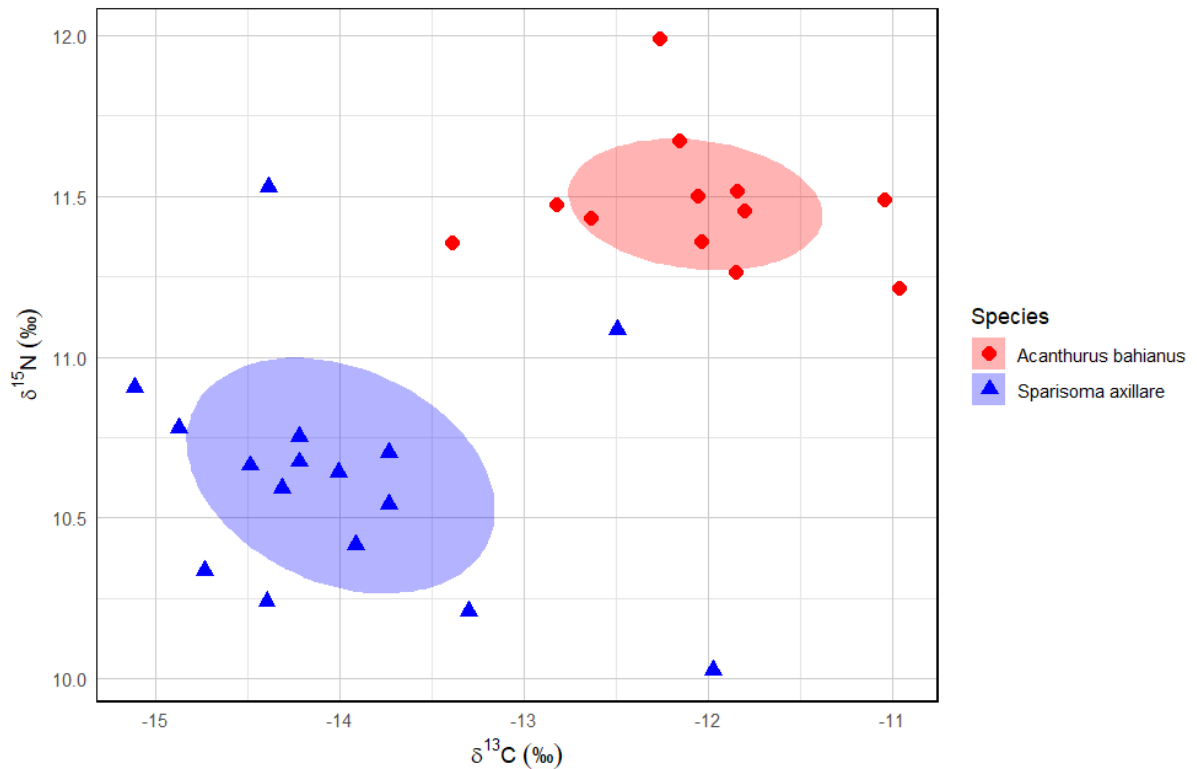


Figure 6: Isotopic niches of *Acanthurus bahianus* and *Sparisoma axillare* based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Shaded ellipses represent corrected standard ellipse areas (SEAc), encompassing 40% of the data based on maximum likelihood estimates.

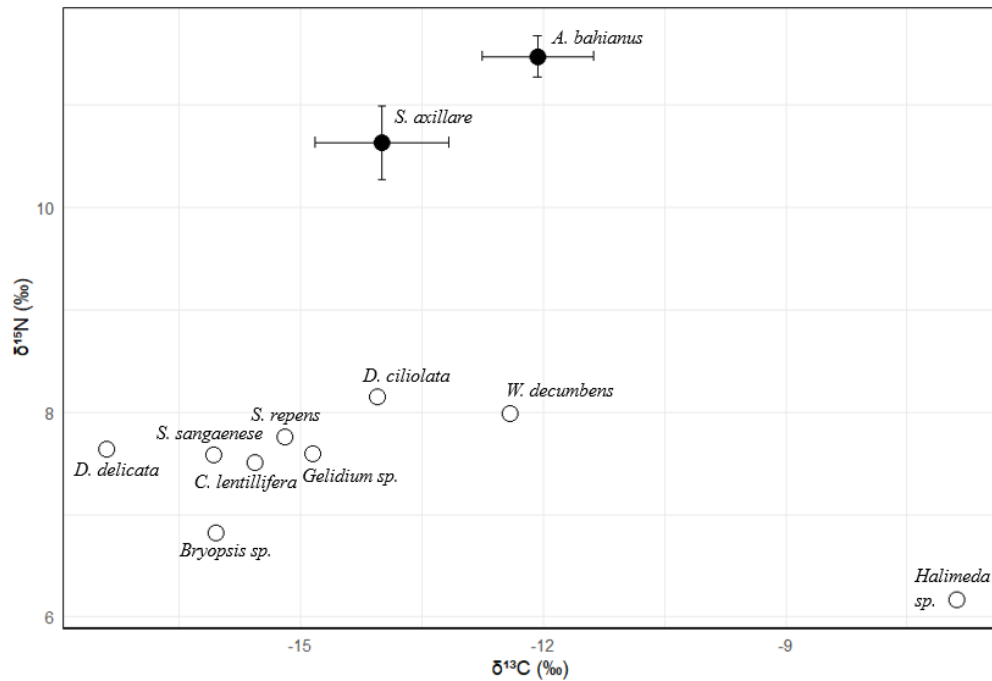


Figure 7: Isotopic niches for *A. bahianus* and *S. axillare* based on mean values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, as well as the niches for their potential algal prey. Error bars represent standard error (SE) for both $\delta^{13}\text{C}$ (horizontal) and $\delta^{15}\text{N}$ (vertical) values.

3.3 Feeding behaviour

The feeding behaviour of *A. bahianus* and *S. axillare* were significantly different as a Pearson's Chi-squared test revealed a significant difference in the overall distribution of bites performed on different substrates between species ($X^2 = 10.13$, $df = 4$, $p = 0.03816$). However, turf dominated the bites for both species (Figure 8) and there was no significant difference in the percentage of turf bites between the species (ANOVA: $F=3.23$, $p = 0.088$).

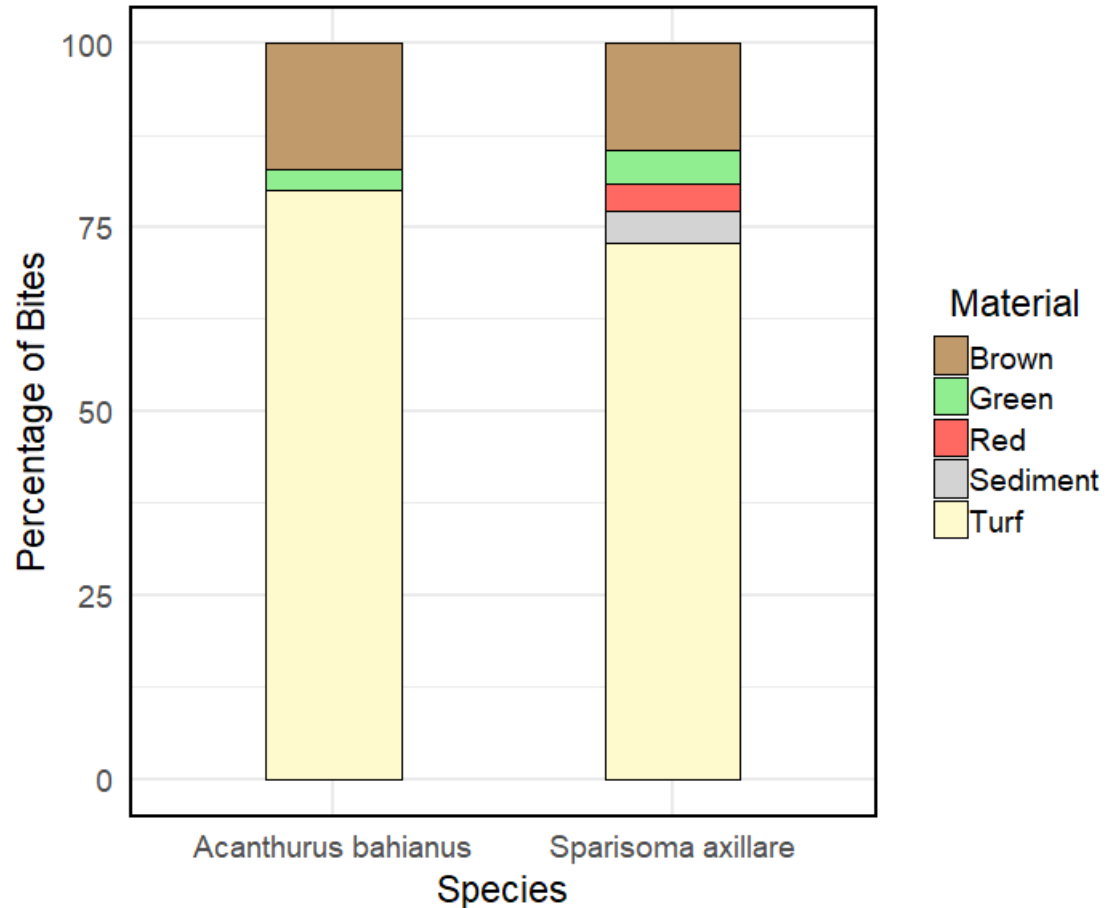


Figure 8: Percentage of bites performed on different substrates by *A. bahianus* and *S. axillare*.

3.4 Microplastic in fish and algae

No evidence of microplastics were found in any of the algae samples analysed. Similarly, there were no microplastics found in the gut content of either fish species.

4. Discussion

4.1 Plastic pollution in the environment

The exact binomial test showed that plastic materials comprised the majority of all macro litter collected on the Ponta Verde beach, accounting for 83.3% of the total litter. A similar pattern has been observed on other Brazilian beaches, where plastics dominate the coastal litter (Andrades et al., 2020). Such findings are consistent with anthropogenic pressures commonly observed in urbanised coastal zones, where improper waste management and maritime activities contribute substantially to the accumulation of pollutants in nearshore environments (Correia, 2014). Similar findings have been recorded globally, with studies conducted in Southeast Asia

and coastlines located in northeastern Atlantic reporting a clear dominance of plastic litter (Browne et al., 2010; Fruergaards et al., 2023). These global parallels underscore the widespread nature of coastal plastic pollution and highlight the urgency for integrated local and international mitigation efforts.

The density of macro litter was significantly higher on the beach than on the reef (Figure 4b). Nevertheless, plastics remained the dominant form of litter on the reef, representing 81.4% of the macro litter collected (Figure 4a). This pattern suggests a strong link between coastal pollution and reef litter, potentially reflecting the transport of land-derived litter into marine habitats. Comparable findings were reported on the coral reefs of Darvel Bay, Malaysia, where plastics contributed to 91% of marine litter, primarily from single-use items associated with human activities (Santodomingo et al., 2021). These results highlight the critical need for effective management of beach litter, as well as decreasing human reliance of single use plastic items to mitigate the distribution of litter and the potential downstream impacts on adjacent coral reef ecosystems.

Beyond visible macro litter, an abundance of microplastics was also detected. There was no statistically significant difference in microplastic density between the beach and reef environments (Figure 5b). However, both environments exhibited high dominance of micro plastic particles, with significantly greater proportions of microplastic compared to other materials (Figure 5a), mirroring the composition pattern observed for macro litter. The dominance of plastic litter across both litter sizes and environments suggests a strong connection between macro litter inputs and the microplastic particles resulting from their degradation. This supports the growing concerns that plastic pollution is not only persistent but fragmenting into smaller particles that remain in the environment (Zhang et al., 2021). In fact, the results from this thesis are consistent with findings that reefs may accumulate microplastics in sediment and even within the coral assemblages, potentially affecting coral health, reproduction and resilience (Hall et al., 2015; Reichert et al., 2018). Particularly reefs close to urban centres, may act as sinks for microplastics due to terrestrial runoff, sewage discharge and degraded shoreline litter (Browne et al., 2011). In addition to gathering sand samples, it would be interesting for future studies to collect water samples to investigate potential microplastic particles suspended in the water column.

4.2 Isotopic niche & feeding behaviour

The isotopic niche analysis based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values revealed a clear separation between *A. bahianus* and *S. axillare*, with no overlap in isotopic niche space (Figure 6). *A. bahianus* occupied a higher position along the $\delta^{15}\text{N}$ axis compared to *S. axillare*, indicating a higher trophic level, suggesting that *A. bahianus* has a diet with higher nitrogen values. These isotopic differences can potentially reflect the distinct feeding modes of the two species, with *S. axillare* functioning more as a true herbivore and *A. bahianus* having more detritus in their diet. Wilson et al. (2007) explains that detrital material is often enriched in nitrogen compared to fresh algal tissue due to microbial processing and decomposition, which can lead to elevated $\delta^{15}\text{N}$ values in detritivorous species.

Sparisoma axillare exhibited a broader isotopic niche ($\text{SEAc} = 0.967 \text{ ‰}^2$) than *Acanthurus bahianus* ($\text{SEAc} = 0.471 \text{ ‰}^2$), suggesting that *S. axillare* exploits a wider range of food resources. This was also evident in the total area (TA) occupied by each species, with *S. axillare* covering a larger isotopic space ($\text{TA} = 2.99 \text{ ‰}^2$) than *A. bahianus* ($\text{TA} = 1.00 \text{ ‰}^2$; Table 2). The absence of overlap and differences in niche breadth point to clear resource partitioning, likely reducing competition and supporting their coexistence on the Ponta Verde reef. These findings align with observations by Francini-Filho et al. (2010), who documented differing foraging strategies between these species on eastern Brazilian reefs.

To further explore these foraging differences, direct observations of feeding behaviour were performed. These observations revealed that there was a significant difference in bite distributions between the two species, where *S. axillare* fed on a greater diversity of material compared to *A. bahianus* (Figure 8). This suggests that *S. axillare* displays greater flexibility in its feeding behaviour, targeting a wider range of materials, and is consistent with this studies' finding that this species occupies a broader ecological niche. However, both species appeared to prefer feeding on the turf material within the EAM, the complex matrix of short filamentous algae, detritus, sediment and microbial life (Wilson et al., 2003). This suggests that while both species rely on the EAM, they may target different components within it.

S. axillare has a $\delta^{13}\text{C}$ value closer to the cluster of algal prey present on the reef compared to *A. bahianus* (Figure 7), while *A. bahianus* exhibited higher $\delta^{15}\text{N}$ values, potentially reflecting a greater consumption of nitrogen-enriched detritus within the matrix. These trophic differences resemble behavioural patterns observed on other reefs, where herbivorous fishes partition resources through microhabitat selection and feeding mode (Brandl & Bellwood, 2015). These

findings support this ecological model by demonstrating how trophic separation enables coexistence even in an urbanised reef impacted by anthropogenic pollution. An interesting avenue for future research would be to conduct a similar study on a more pristine reef system to compare isotopic niche structure, trophic position and feeding behaviour under lower anthropogenic pressure. Such a comparison would help to determine whether urban associated stressors influence resource use and ecological roles of herbivorous reef fish like *A. bahianus* and *S. axillare*.

4.3 Plastic consumption

Despite the clear abundance of plastic pollution observed in the reef environment and on the nearby beach at Ponta Verde, the two fish species analysed in this study showed no evidence of plastic ingestion. This suggests that the presence of plastic in their habitat may not pose an immediate threat to these species. Supporting this observation, a broader study on plastic ingestion by marine fish found that even in heavily polluted environments, not all species ingest plastic (Markic et al., 2020). These findings suggest that some fish either actively avoid plastic or are less susceptible to ingesting it, potentially due to differences in feeding behaviour or habitat use.

4.4 Conclusion

This study provides valuable insight into the extent of anthropogenic pollution on the Ponta Verde reef and nearby beach, revealing plastic as the most dominant litter form of at both macro and micro litter level. Positively, microplastics were not detected in the digestive systems of the two herbivorous fish species studied (*Acanthurus bahianus* and *Sparisoma axillare*), suggesting that these species may not be directly impacted by plastic ingestion through their current feeding strategies. However, this result contrasts significantly with findings for many other marine taxa. Numerous studies have documented plastic ingestion in marine birds (Azzarello & Van Vleet, 1987), turtles (Tomás et al., 2002), cetaceans (Baulch & Perry, 2014), and several carnivorous fish species (Mirana & de Calvalho-Souza, 2016), often with serious physiological consequences. Therefore, the absence of plastic ingestion in *A. bahianus* and *S. axillare* should not be taken as evidence that plastic pollution poses no risk to herbivores in reef ecosystems. On the contrary, it highlights the need to expand plastic ingestion studies to include a broader range of species, especially those feeding at different trophic levels or with different foraging behaviours.

The findings in this study contribute to a growing understanding of how urbanised coastal environments are affected by human activities and offer a small but positive result regarding herbivorous reef fish. The broader ecological threat of plastic pollution remains urgent, and its persistence in marine ecosystems continues to affect wildlife across taxa (Thushari & Senevirathna, 2020). This study therefore supports the continued global push to reduce human reliance on plastic, plastic production, improve waste management, and implement conservation strategies to protect vulnerable marine habitats.

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