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Study on Plastic Ingestion in Carcasses Sea Turtles on Enggano Island, Indonesia

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ABSTRACT

Plastic pollution in the ocean has a harmful impact on marine life and ecosystems including the decline in turtle populations. The decreasing of turtles populations caused by plastic pollution affects the management of regional turtle conservation units. Therefore, the current study was conducted on five green turtles (*Chelonia mydas*) and five hawksbill turtles (*Eretmochelys imbricata*) in the transitional and juvenile size range (29–49 cm) that were found dead on Enggano Island, Bengkulu Province. The aim of this study was to assess their consumption of plastic waste. The results showed that macroplastics and microplastics were 100% detected in the intestines of *C. mydas* and *E. imricata*. In *C. mydas*, a total of 164 macroplastic items were found, with the most common category of USE FRA (35.37%). In *E. imbricata*, 20 macroplastic items were identified, divided into two categories: USE THR (55.56%) and USE SHE (44.44%). Microplastic particles in the digestive tracts averaged from 14 to 18 and from 7 to 12 per 50 grams of sample in *C. mydas* and *E. imbricata*, respectively. Those microplastic types were fibers, films, and fragments. In C. mydas, fragments were the most common type of plastic found (65.67%), while fibers were most prevalent in *E. imbricata* (48.89%). Fourier Transform Infrared (FT-IR) spectroscopy revealed the presence of synthetic polyethylene in both turtle species. In *C. mydas*, larger individuals showed a higher prevalence of film and fragment microplastics, while *E. imbricata* was predominantly characterized by fiber microplastics (no significant influence). Turtles are predicted to digest both macro- and microplastic particles either by eating them directly with their food or indirectly through the food chain in areas or migration routes polluted with plastic waste.

INTRODUCTION

Turtles are found landing and nesting on the coast of Bengkulu including the Enggano District. The turtles commonly found on the coast of Enggano are the green turtle (*Chelonia mydas*), the leatherback turtle (*Dermochelys coriacea*), and the hawksbill turtle (*Eretmochelys imbricata*). Based on the red list **(IUCN, 2010)**, the status of these three turtles falls into the endangered category for the green turtle and critically endangered for the leatherback turtle and hawksbill turtle. In addition, turtles are also protected by the state as outlined in Law No. 5 of 1990 **(Peraturan Pemerintah No. 7, 1999)**. Turtles are migratory animals with a wide range, and during this migration process, turtles can travel less than 100km to several thousand km to return to their nesting sites, known as natal homing **(Jensen *et al.*, 2020; Gaos *et al.*, 2021; Madden Hof *et al.*, 2023; Sani *et al.*, 2024)**. The extensive coverage of turtle migration areas requires a regional management mechanism involving several countries within a single management unit known as Regional Management Units (RMUs), first established in 2010 **(Wallace *et al.*, 2010)** and updated in 2023, making the boundaries for RMUs much more accurate **(Wallace *et al.*, 2023)**. Enggano is included in the RMUs East Indian and Southeast Asia (CM_16_IND_E_SEA) for the green turtles and RMUs Southeast Asia (EI_36_PAC_SEA) for the hawksbill turtles. RMUs allow for better conservation management of turtles whose existence has long been threatened due to human activities and natural phenomena. More specific threats to turtle populations include habitat destruction, trade (meat, eggs, accessories), bycatch **(Kirishnamoorthie *et al.*, 2023)**, and marine pollution by plastic waste.

Plastic waste pollution, both macro and micro, has a detrimental impact on ecosystems and biodiversity and has become a concern in the last decade **(Herawati *et al.*, 2024; Purba *et al.*, 2024)**. Until now, 557 species of marine organisms have been entangled or known to ingest marine debris **(Kühn *et al.*, 2015)**, including 86% of all turtle species **(Asuquo, 2018)**, either entangled or found in their digestive tracts **(Wilcox *et al.*, 2018; Domènech *et al.*, 2019; Matiddi *et al.*, 2019)** since turtles rely on their vision, hence the presence of floating plastic in foraging areas increases the likelihood that they will mistake for food and actively choose to consume it **(Abreo *et al.*, 2016; Taylor *et al.*, 2016; Clukey *et al.*, 2017)**. This can lead to death due to gastrointestinal (GI) blockage or perforation **(Bergmann *et al.*, 2015; Santos *et al.*, 2015; Yaghmour, 2020)**. In addition to direct impacts, plastic can also have indirect ecological effects **(Pham *et al.*, 2014; Nelms *et al.*, 2016)**.

Until now, there has been little research focused on plastic waste contamination in turtles in Indonesia, including in Bengkulu, particularly Enggano. Enggano is not a focus of research for global researchers, even though this area is classified as a kawasan stregis nasional tertentu (KSNT), where disturbances to the turtle population on this island will impact other regions, especially within the same RMU area. For this reason, this research analyzed five green turtles (*C. mydas*) and 5 hawksbill turtles (*E. imbricata*) that were found dead, followed by identifying the categories of macroplastics and types of microplastics, colors, as well as counting the number of plastic fragments found in the digestive tracts, determining whether there are differences between turtle species. This research is anticipated to become an important scientific analysis related to plastics in turtles in Indonesia. In addition, this study would provide sufficient, comprehensive data, which can serve as an accurate reference for better plastic waste management on Enggano Island, with additional information regarding green turtles and hawksbill turtles in Bengkulu for conservation management in the RMU, where Enggano Island is part of it.

MATERIALS AND METHODS

1. Collection and handling of turtle samples

Digestive tract samples were collected from five dead *C. mydas* and five dead *E. imbricata* turtles. The turtles were from Enggano Island, North Bengkulu Regency, Bengkulu Province (Fig. 1.). Before further analysis, data on carapace curved length (CCL) and weight (kg) were collected **(European Commission, 2013)**. Afterward, the live stage was determined based on the CCL size **(Bjorndal, 1977)**. The total length of the digestive tract was measured with a tape measure in cm, and subsequently, the weight of the full and empty tract (after contents were removed) was recorded (g). Each section of the digestive tract was divided according to the natural division of the organs into: esophagus; stomachc, and intestines.

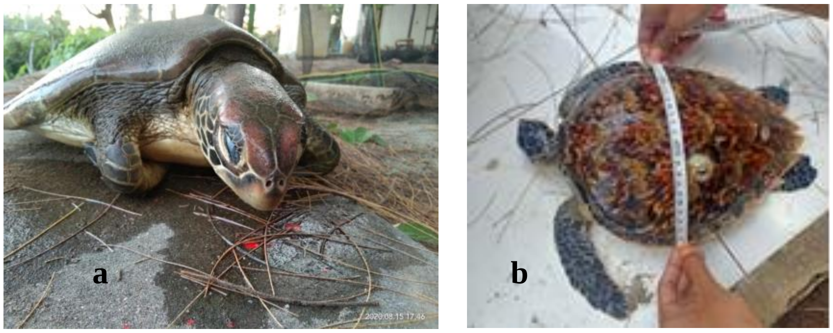


Fig. 1. Sea turtle from Enggano: **a.** *C. mydas*; **b.** *E. imbricata*

During the necropsy, the entire digestive tract, from the beginning of the esophagus to the end of the large intestine, was dissected. All contents of the digestive tract were removed, and the total weight of the intestinal contents was measured. Debris fragments were washed with running water using a 2.8mm sieve. All plastic particles retained on the sieve were dried and categorized as macroplastics, and subsequently identified based on their categories (Table 1). All macroplastics collected on the sieve were stored in jars **(Ryan *et al.*, 2016; Matiddi *et al.*, 2019)**.

Table 1. Categories of macroplastics in the digestive tract of turtles **(European Commission, 2013; Matiddi *et al.*, 2019)**

No	Category	Sub-categori
1.	Industrial Plastic (IND PLA)	plastic pellets and granules, usually in cylindrical and round shapes, but also in oval or cubic shapes
2.	Sheets (USE SHE)	plastic bags, agricultural sheets, or foil plastic. They appear in irregular shapes but are always thin and flexible

3.	Nylon (USE THR)	rope, filament, and materials like other threads such as remnants of fishing gear
4.	Foamed plastics (USE FOA)	Polystyrene foam or soft rubber foam
5.	Hard Plastic (USE FRA)	Fragments of hard plastic items in various different colors, usually rigid, with irregular shapes and sharp, bent edges
6.	Other user plastic (USE POTH)	solid rubber, balloon pieces, and soft airgun pellets
7.	Other than plastic (OTHER)	All non-plastic marine waste such as cigarette butts, newspapers, trash, and hard pollutants

2. Sample handling and microplastic identification

Microplastics in turtles were extracted using the method of **Caron *et al.* (2018)**. Subsamples were taken from each part of the digestive tract, namely the esophagus, stomach, and 5 sections of the intestine, each weighing 50 grams, which were homogenized for analysis to measure microplastic contamination. The homogenized subsamples were taken in amounts of 50 grams, transferred into a beaker, and divided into 4 replicates. All samples were processed in 50mL glass test tubes (~ 6g wet weight (w/w) per test tube). Nitric acid (HNO_3, 69.5%) was added to each sample (3:1, HNO_3 mL: chyme g b/b), left overnight at room temperature (~20 °C), and heated for 2 hours using an oven at 50°C. The samples were then resuspended in 200mL of hypersaline saline solution (NaCl, 1.2 g/cm^3), prepared by saturating RO water with NaCl (solubility in water at 20 °C = 357g/ L). Then, stirring was performed manually for 30 seconds using a glass stirring rod, and the outcome was let to sit for 1 hour.

Microplastic sampling in the water was conducted using a plankton net (mesh size 0.4mm) with a filtered water volume of 25 liters. Water samples were taken from the surface of the intertidal waters at 5 different stations, with 3 repetitions. After all the water samples were filtered, the plankton net was rinsed with water to ensure that no microplastics were left or stuck to the net. Afterward, the water was stored in a cool box for analysis in the laboratory. Identification of microplastic particles in water samples using the NOAA (National Oceanic and Atmospheric Administration) method was carried out in several stages **(Masura *et al.*, 2015)**. The first stage carried out was wet sieving to obtain microplastic samples < 2.8mm using stacked 2.8 and 0.3mm stainless steel mesh sieves. The second stage involved drying the samples in an oven at 90˚C for 24 hours or longer to obtain dry weight. The third stage involved treating the samples with several solutions, such as adding a 0.05 M Fe solution to separate the microplastic samples from the metals. After that, 20ml of H_2O_2 was added to dissolve the organic substances. Then, 6 grams of NaCl per 20ml of sample was added to increase the density. After that, the separation of organic matter and microplastics was carried out using a density separator. The abundance of microplastics was calculated by comparing the number of particles found with the volume of filtered water **(Masura *et al.*, 2015)**.

The amount of microplastics in the sediment was expressed per bulk wet sediment, per volume, or per surface area. Sediment samples were taken using petri dishes with a surface area of 50cm² and a volume of 60mL at five different stations, each station with 3 replicates. Next, the sample analysis procedure followed the method described in **Ikejima (2020)**. The density separation stage was carried out by adding 60ml of Fenton's solution to the sediment and allowing it to sit for 24 hours at room temperature. Next, $ZnCl_2$, at a concentration of 1.5g/ cm³ in OC-T, was added, and the mixture was allowed to sit for another 24 hours.

All samples extracted from the turtle, water, and sediment were then processed by separating the supernatant using a Pasteur pipette and transferring it to a beaker for further filtration. The pipette was rinsed three times with aquades to remove any particles adhering to its inner walls. The collected supernatant was then filtered using a vacuum system with a Millipore HA cellulose filter membrane (pore size 0.45µm).

Each filter was placed in a closed Petri dish and was dried in an oven at 60°C for 4 hours, followed by identification under a microscope. To prevent contamination during the procedure, all laboratory equipments were rinsed with aquades before use, and the filters were kept covered at all times.

FTIR analysis is used to determine the polymer composition through Fourier transform-infrared spectroscopy **(Caron *et al*., 2018; Duncan *et al*., 2019)**. Briefly, FTIR spectra are obtained in transmission mode on a PerkinElmer FTIR Spectrometer 100, using the Attenuated Total Reflectance (ATR) accessory, as described by **Kroon *et al.* (2018)**. Samples with a match below 60% are considered a low match, 60–70% are considered medium, and 70–100% are considered a high match.

All spectra were further examined, and unexplained bands were investigated by reviewing lower-percent matches and consulting relevant literature. This technique was employed during the research to confirm the target polymers extracted from turtles, water, and sediment.

3. Statistical analysis

The relationship between sampling stations (Water and Sediment) and the abundance of microplastics in each category (Fiber, Fragment, and Film) was analyzed using Correspondence Analysis (CA) **(Bengen & Boer, 2023)**. The data matrix consists of stations (in rows), as well as the variable categories of microplastics in water and sediment (in colom). This analysis was conducted using the XLStat program. Subsequently, using the same program, a Principal Component Analysis (PCA) was performed, utilizing the correlation between variables based on the Curve Carapace Length (CCL) and the categories of microplastics (Fiber, Fragment, and Film) found in 10 individuals of the species *C. mydas* (five individuals) and *E. imricata* (five individuals) **(Bengen & Boer, 2023)**.

RESULTS

1. Categories and abundance of ingested plastics

As many as 40% of *C. mydas* and 66.67% of *E. imbricata* found dead had ingested macroplastic particles. The analysis reveals that commonly ingested waste includes fragments, sheets, foam, nylon ropes, and other unidentified materials (Table 2 & Fig. 2).

Table 2. Frequency of marine debris ingestion in *C. mydas* and *E. imricata* in the study

Species	Sample	CCL (cm)	Weight (kg)	Age stage	Abundance and Weight Ingested plastic			Intestinal content weight (g)
					Count (≥ 2.8 mm) per ind.	Weight (g)	Count (20-300 µm) per 50 g	
C. mydas	1	47	13	sub adult	144	3.903	17	341.1
	2	36	6	transitionals	0	0	0	1195.4
	3	41	9	sub adult	0	0	0	1887.7
	4	49	17	sub adult	20	0.706	16.5	3671.9
	5	46	15	sub adult	0	0	0	2613.8
E. embricata	1	29	11	transitionals	0	0	8.5	559.2
	2	35	16	transitionals	7	1.101	8	923.4
	3	41	3	sub adult	2	0.018	7	31
	4	42	7	sub adult	0	0	9.25	1176.7
	5	49	11	sub adult	0	0	12.25	2309.5

(CCL: curved carapace length)

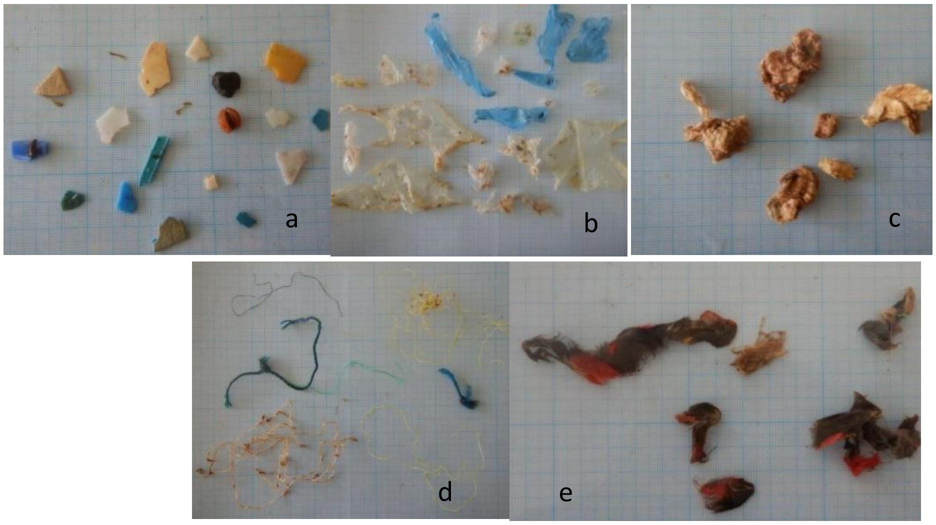


Fig. 2. Examples of ingested plastic objects found in *C. mydas* and *E. imricata*: (a) USE FRA, (b) USE SHE, (c) USE POTH, (d) USE THR, and (e) OTHERS

The total number of macroplastics ingested by *C. mydas* is 164 items, with the highest being in the USE FRA category (35.37%), consisting of fragments of hard plastic items in various colors with irregular shapes and sharp edges. Next, the USE SHE category (35.37%) includes sheets of plastic bags, followed by USE THR (21.95%) consisting of nylon strings/threads from fishing gear remnants. Then, USE POTH and OTHERS each account for 3.66%, consisting of rubber pieces and other waste materials. Next, for the *E. imbricata species*, 2 out of 3 samples were found to have plastic in their intestines, with a total of 20 items, and there were only two categories: USE THR at 55.56% and USE SHE at 44.44% (Figs. 2, 3a).

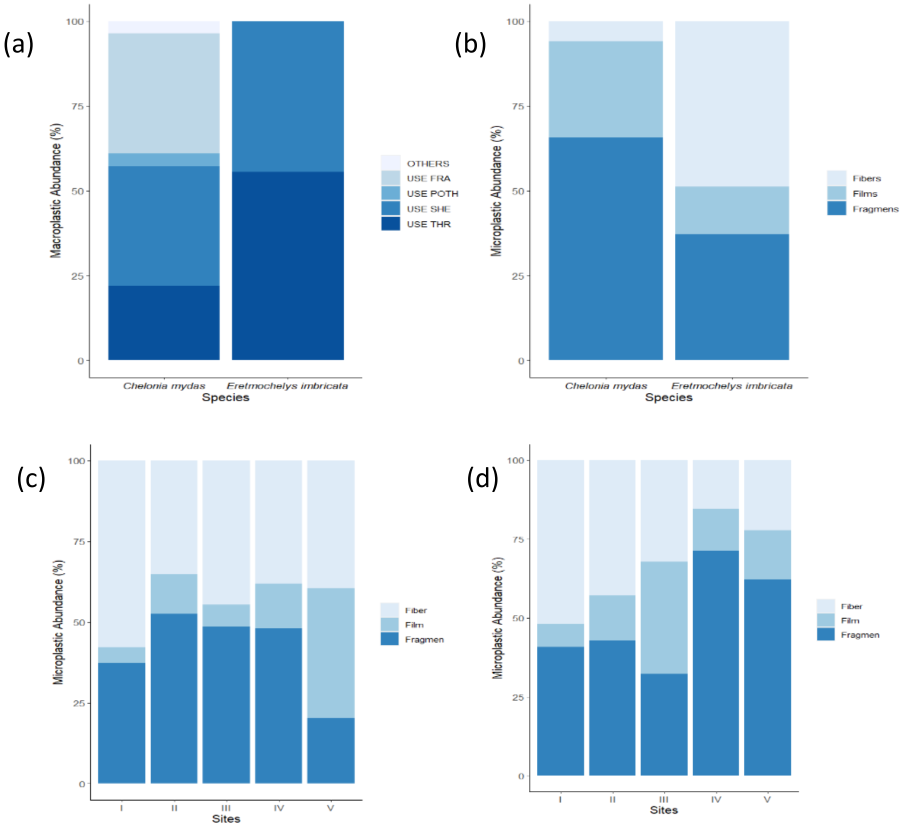
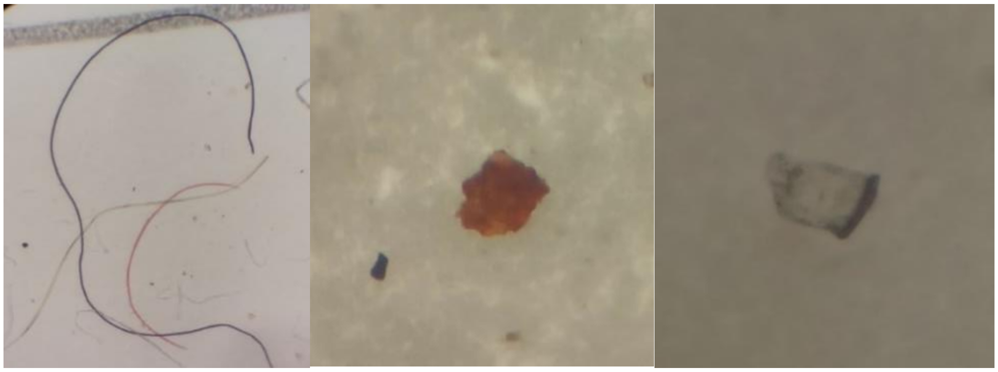


Fig. 3. Percentage distribution of plastic abundance ingested by *C. mydas* and *E. imricata* collected in this study (a) macroplastic abundance in turtles (%); (b) microplastic abundance in turtles (%); (c) microplastic abundance in water (%); (d) microplastic abundance in sediment (%)



(a) (b) (c)

Fig. 4. Types of microplastics found in the digestive tracts of turtles (a) Fiber, (b) Fragment, (c) Film

Microplastics found in the digestive tract of *C. mydas* averaged 14-18 particles per 48g sample, and in *E. imbricata* averaged 7-12 particles per 50g sample, with different types of microplastics, namely fiber, film, and fragment types. Based on the results of this study, the highest microplastic type in *C. mydas* was fragments (65.67%), followed by films (38.36%), and the least was fibers (5.97%). In *E. imbricata*, the highest was fibers (48.89%), followed by fragments (37.22%), and the least was films (13.89%) (Figs. 4, 3b). At each sampling station, microplastics in the water are found to be almost equally abundant in fiber and fragment types, with an average of 1.75 particles/m³ (44.43%) and 1.66 particles/m³ (42.14%), respectively. Film particles are present at 0.53 particles/m³ (13.43%) (Fig. 3c).

2. Plastic color ingested

In this study, differences were observed in the macroplastic waste debris most commonly ingested, based on color. In *C. mydas*, eight color categories were identified, while in *E. imbricata*, five color categories were found (Fig. 5). The highest percentage for *C. mydas* was for transparent debris, accounting for 46.95%, followed by white and blue at 11.59% each. In contrast, for *E. imbricata*, the highest percentage was white at 33.33%, followed by transparent and black at 22.22% each. The prevalence of transparent and white colors includes used plastics, ropes, filaments, and materials such as fishing gear remnants, typically made of nylon (especially woven plastic bags and monofilament ropes), along with various fragments that are abundant in the digestive tract, often irregular in shape with sharp, bent edges. Brown represents the fifth most common color, followed by green, yellow, and red.

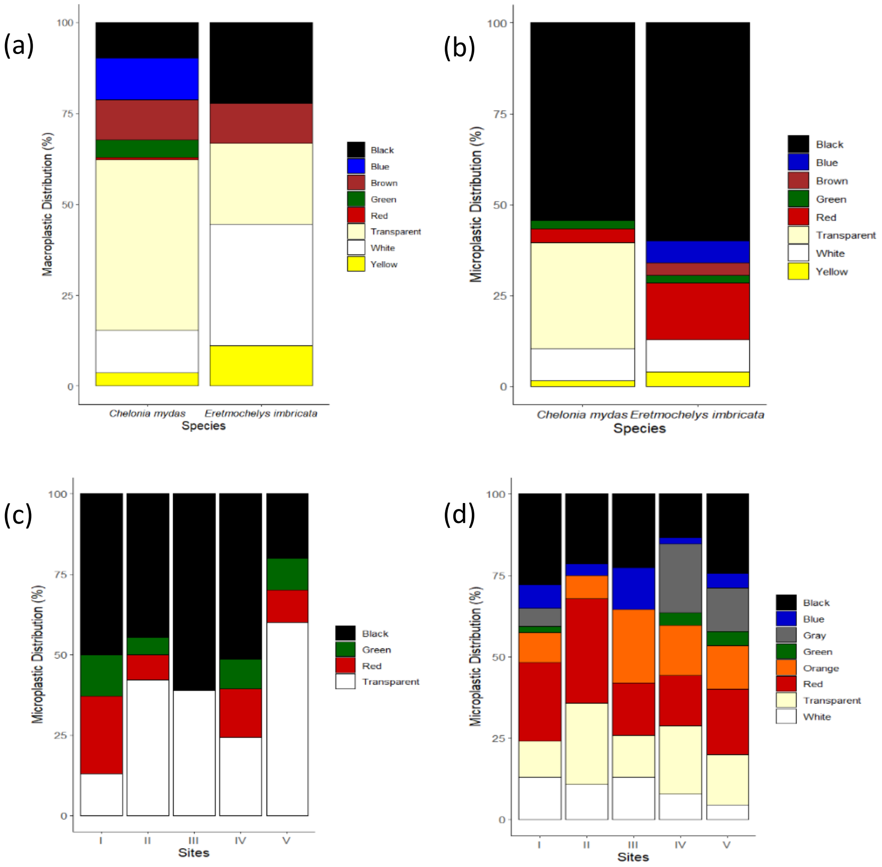


Fig. 5. (a) Macroplastic distribution (%); (b) Microplastic distribution (%) ingested by *C. mydas* and *E. imricata* collected in this study; (c) Water; (d) Sediment

3. Microplastic distribution

The results of the correspondence analysis (CA) revealed three distinct groups of stations and microplastic categories, centered on axis 1 (F1) with a variance of 55.63% and axis 2 (F2) with a variance of 42.84% (Fig. 6). The first group indicates that the sediments at stations A2 and A3 are characterized by a high presence of microplastics in the film category. The second group shows that the sediments at station A4 are dominated by microplastics in the fragment category. The third group reveals that the sediments at station A1 are characterized by a high presence of microplastics in the fiber category.

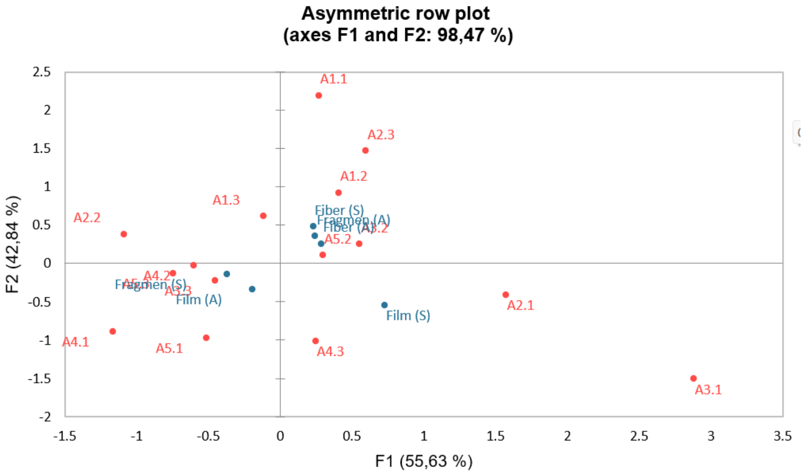


Fig. 6. Results of the correspondence analysis (CA) on the relationship between stations and microplastic categories in water and sediment 1 (F1) and axis 2 (F2)

The distribution of microplastic categories and CCL is centered on axis 1 (F1) with a variance of 55.84% and axis 2 (F2) with a variance of 24.98%. The distribution of CCL is divided into several zones, where each zone has different characteristics of microplastic categories according to the correlation results between variables (Fig. 7).

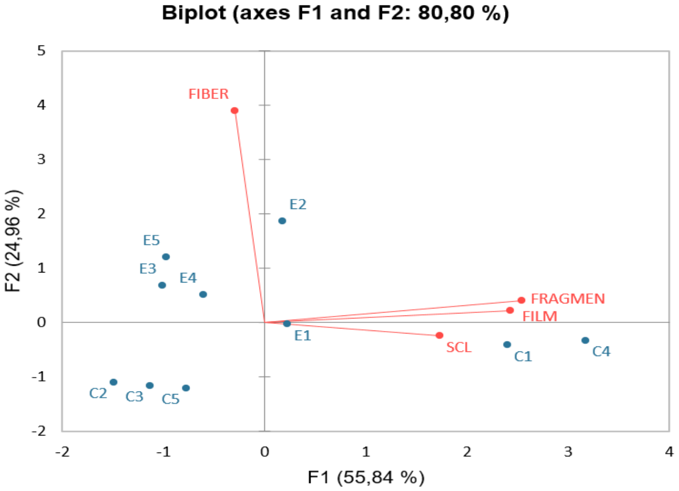


Fig. 7. Results of the principal component analysis (PCA) of the distribution of microplastic categories based on CCL size on axis 1 (F1) and axis 2 (F2)

Based on the division of zones, *C. mydas* with a larger curved carapace length (CCL) is characterized by higher levels of microplastics in the film and fragment categories, while *C. mydas* with a smaller CCL shows lower levels of these microplastics. The *E. imbricata* species, on the other hand, is characterized by high levels of fiber-category microplastics, with no influence of CCL. Overall, high levels of microplastics in the fragment and film categories were found in the sediments of stations A2 and A3 (Fig. 6), particularly in sub-adult *C. mydas* turtles.

DISCUSSION

1. Categories and abundance of ingested plastics

The results of this study are consistent with the research by **Choi *et al.* (2021)**, where the most common categories of plastic are sheets (37.8%) and fragments. Rigid plastic and sheets are the most frequently ingested plastics, and they are generally concentrated along the coastline **(Nunes *et al.*, 2021)**. Based on observations, macroplastics were not found in the esophagus and stomach. A total of 100% of macroplastics, both *C. mydas* and *E. imricata*, were detected in the intestines, and it can also be seen that the esophagus is empty. Only a small amount of food remains in the stomach, which means that the food has already entered the intestines for further digestion. The research by **Choi *et al.* (2021)** found that most plastic waste was found in the intestines (84.1%), followed by studies by **Cheng (2020)** and **Digka *et al.* (2020a)**, which found 81 and 78% of ingested plastic, respectively, in the intestines. These results are consistent with findings in other studies **(Nicolau *et al.*, 2016; Matiddi *et al.*, 2017)**. The presence of a long intestine likely serves as an area for the accumulation of plastic waste ingested by turtles **(Matiddi *et al.*, 2017)**.

Both types of turtles that are the subjects of this research fall into the category of transition from juvenile to sub-adult and sub-adult with a size range of 29 – 49cm (Table 2). According to **Casale *et al.* (2016)**, small stranded turtles are likely to originate from coastal/neritic areas, as indicated by the condition of the turtles which have not yet decomposed, suggesting that they have not been floating for long and are likely not far from the shore before becoming stranded. Juvenile turtles live in pelagic areas with a carnivorous feeding type. Based on the analysis **(Bjorndal, 1977)** of immature *C. mydas*, it is known that they are carnivorous and forage in waters with depths of less than 100m, showing a greater preference for soft-bodied organisms similar to soft plastics. Juvenile to adult *C. mydas*, in addition to consuming various types of seagrass, algae, and animals, particularly jellyfish, salps, mollusks, crustaceans, and sponges throughout their life cycle **(Schuyler *et al.*, 2014)**, are known as the turtle species most frequently reported to interact with anthropogenic debris. *E. imbricata* hatchlings initially live in pelagic areas, but as they mature, they move to benthic areas of coral reef ecosystems, where their diet primarily consists of jellyfish **(Bjorndal, 1977)**. **Camedda *et al.* (2014)** found that small juvenile loggerhead turtles (CCL < 40cm) ingested more plastic than sub-adult or adult loggerhead turtles (CCL > 40cm) in the Sardinian Sea. Research by **Digka *et al.* (2020)** found that the juvenile loggerhead turtles ingested more plastic, at 88%, compared to transitional turtles (66%) and adult turtles (70%).

The variation in ingested macroplastic, both in type and quantity, is likely related to the feeding preferences of each species (Table 3). In general, various research results show that smaller or juvenile turtles ingest more fragment-type plastics compared to larger turtles. According to **Schuyler *et al.* (2012)**, this is possible due to the smaller size of their intestines, where fragment-type plastics occupy less space than sheet-type plastics. This is supported by the research findings of **Choi *et al.* (2021)**, where smaller turtles (SCL < 25cm) were found to ingest more hard plastic or fragments, while larger or juvenile turtles ingested more sheet plastic.

Table 3. Frequency of marine debris ingestion in turtles of various species and age classes

Species	N	Age stage	Location	Occurrence (%)	References
C. mydas	1	Transitionals	Enggano Island	40	This Study
	4	Subadults			
E. imbricata	2	Transitionals		66.7	
	3	Subadults			
C. mydas	59	Pelagics	the coast of Texas, USA	76.3	**Choi *et al.*, (2021)**
	244	Recruit		57.0	
	98	Transitionals		25.5	
	61	Subadults		26.2	
D. coriacea	13	Adult	Canary Islands (Spain)	84.6	**Orós *et al.*, (2021)**
C. mydas	92	Adult	Atlantic Florida beaches	100	**Rice *et al.*, (2021)**
C. caretta	86	Adult		84	
C. mydas	22	Transitionals	southern Brazil	93	**Nunes *et al.*, (2021)**
	18	Subadults			
C. mydas	43	Transitionals & Subadults	Brazilian coast	70.3	**Machovsky-Capuska *et al.*, (2020)**
C. caretta	8	Transitionals	the Greek coastline area East Mediterranean Sea	72	**Digka *et al.*, (2020a)**
	18	Subadults			
	10	Adult			
C. mydas	3	Recruit	Eastern Taiwan	85	**Cheng *et al.*, (2020)**
	145	Transitionals			
	37	Subadults			
	8	Adult			
C. mydas	14	Subadults & Adult	United Arab Emirates	85.7	**Yaghmour *et al.*, (2018)**
C. mydas	96	Transitionals & Subadults	Uruguayan coast	70	**Vélez-Rubio *et al.*, (2018)**
L. kempii	15	Subadults	Pacific longline fisheries (Hawaii &Samoa)	100	**Clukey *et al.*, (2017)**
	22	Adult			
C. mydas	10	Transitionals		90	
C. caretta	2	Subadults		80	
	2	Adult			
	1	Na			

D. coriacea	3	Subadults		0	
C. caretta	24	Pelagics	Atlantik (Pulau Azores/Portugal)	83	**Pham *et al.*, (2017)**
C. caretta	40	Subadults & Adult	South Africa	60	**Ryan *et al.*, (2016)**
C. mydas	1	Adult	Philippine	100	**Abreo *et al.*, (2016)**
C. caretta	95	Subadults & Adult	Portugis	59	**Nicolau *et al.*, (2016)**
C. caretta	24	Subadults	Atlantik (Afrika Selatan)	99	**Ryan *et al.*, (2016)**
C. mydas	265	Transitionals & Subadults	Brazil	70	**Santos *et al.*, (2015)**
C. caretta	74	Subadults & Adult	South-West Indian Ocean	51.4	**Hoarau *et al.*, (2014)**
C. caretta	31	Transitionals & Subadults	Italia	71	**Campani *et al.*, (2013)**
multiple	12	Juvenile	southeast Queensland	33.9	**Schuyler *et al.*, (2012)**
	93	Transitionals			
C. caretta	54	Transitionals & Subadults	Bosnia Hezegovina	35.2	**Lazar and Gračan (2011)**

(N: number of specimens)

Various cases of turtles ingesting plastic waste show that *C. mydas* consumes more plastic compared to other turtle species **(Schuyler *et al.*, 2014; Lynch, 2018; Nunes *et al.*, 2021; Rice *et al.*, 2021)**. Different results were found in this study, where *E. imbricata* ingested more plastic compared to *C. mydas* (Table 3). The abundance of plastic in the oceans, whether with high or low density **(Morét-Ferguson *et al.*, 2010)**, usually accumulates on the ocean surface **(Carman *et al.*, 2014)**. This explains the high incidence of plastic ingestion by turtles, and because turtles rely on visual cues, the presence of floating plastic in foraging areas increases the likelihood that they will mistake it for food and actively choose to consume it **(Abreo *et al.*, 2016; Taylor *et al.*, 2016; Clukey *et al.*, 2017)**. **Schuyler *et al.* (2012)** and **Nelms *et al.* (2016)** stated that turtles in the pelagic life stage are usually less selective in food sorting compared to larger turtles. The occurrence of plastic ingestion is one of the most dramatic threats to turtle populations and can hinder conservation efforts due to the increasing accumulation of plastic waste in the oceans. The research results by **Choi *et al.* (2021)** show that there was an increase in plastic ingestion by turtles, from 32.5% in 1987–1999 to 65.5% in 2019. In both types of turtles, film type was found the least (Table 4). Plastic fragments or microplastics that are ingested are known to be vectors for toxic chemicals; these chemicals can be absorbed internally after ingestion and enter the trophic flow or accumulate through the food chain **(Hamlin *et al.*, 2015; Bejgarn *et al.*, 2015; Gardon *et al.*, 2020; Rendell-Bhatti *et al.*, 2021)**.

Microplastics in the water at each sampling station, on average, the fiber and fragment types are almost equally abundant, with 1.75 particles/m³ (44.43%) and 1.66

particles/m³ (42.14%), respectively, and film 0.53 particles/m³ (13.43%) (Fig. 3c). The research results **(Ayuningtyas, 2019)** showed that the total abundance of microplastics in the waters of Banyuurip, Gresik, East Java, was 57.11 x 10^2 particles/m³, with fragments being the most abundant type of microplastic (50%). The research results **(Syakti *et al.*, 2018)** regarding the waters found 0.45 particles per m³, with fragments being the most abundant type (50.9 ± 4.9%). Another study by **Cordova *et al.* (2019)** reported an average of 0.49 n/L in the northern coast of Surabaya, with the highest abundance being foam (58.44%). **Hiwari *et al.* (2019)** reported an average microplastic concentration of 0.018 ± 0.175, with the highest abundance being fragments. The abundance of microplastics in water is much lower compared to the microplastics that have accumulated in sediments.

Microplastics in the sediment at each sampling station were, on average, the highest in the fragment type, with 242.22 particles/kg (50.9%), followed by the fiber type with 151.11 particles/kg (32.38%), and the film type with 73.33 particles/kg (15.71%) (Fig. 3d). These results are consistent with the study by **Lestari *et al.* (2021)**, which found that microplastics in the sediment of seagrass beds on Panjang Island, Jepara, were dominated by fragments (54.83%), followed by fibers (36.56%) and films (8.6%). In the coral reef habitat sediments of Sekotong, Lombok, Indonesia, the average microplastic concentration was 48.3 ± 13.98 (SD) particles per kg, with the highest percentage being foam (41.20%) and fragments (32.51%) **(Cordova & Prayudha, 2018)**. Additionally, **Sulistiowati *et al*. (2023)** found that in the sediments of Tidung Island, Kepulauan Seribu, Jakarta Bay, microplastics were dominated by fibers (87%) in the first year of study, and fragments (65.71%) in the second year. Similarly, a study by **Ramili and Umasangaji (2022)** found that the average abundance of microplastics in the seagrass bed sediments of the Pulau Mare conservation area, North Maluku, ranged from 18,368 ± 10,625 to 27,090 ± 13,908 particles/kg, with fibers and fragments being the dominant types.

Microplastics can sink and accumulate in sediments through the biofouling process **(Kaiser *et al.*, 2017)**, with aggregation accelerating the sinking rate **(Porter *et al.*, 2018)**. According to **Matiddi *et al*. (2019)**, fragment-type microplastics are characterized by irregular shapes and sharp edges, and are not easily broken using tweezers. **Digka *et al.* (2020)** found that fragments are the largest type of microplastics discovered. Fiber-type microplastics, which have elongated and thin shapes, often originate from fishing lines or nets **(Lie *et al.*, 2018)**. **Azizah *et al.* (2020)** also noted that fiber-type microplastics resemble strands or fishing nets. Finally, **Ayuningtyas (2019)** describes film-type microplastics as thin, transparent pieces resembling paper, typically with a lower density, and often found in irregular shapes and transparent colors.

Table 4. Microplastic research on turtles in several locations

Species	Lokasi	Abundance	Dominant Shape	Size	Dominant Color	Dominant Polymer	References
C. mydas	Indian (Enggano Island/Indonesia)	14-18	Fragments	< 300 µm	Black	polyethylenimine	This Study
E.imricata		7-12	Fiber		Black		
C. mydas	Pasifik (Queensland/ Australia)	11	Fiber	-	Black & Blue	polyethylene (PE), ethylene propylene, polyester	**Duncan *et al.*, (2019)**
C. caretta		6					
N.depressus		6					
E. imricata		5					
L. olivacea		4					
C. mydas	Atlantik (Northern Cyprus/Turki)	10	fiber	-	Black & Blue		
C. caretta		12.5					
C. mydas	Atlantik (North Carolina/Amerika)	5	fiber	-	Black & Blue		
C.caretta		2.5					
L. kempii		3					
D. coriacea		4					
C.mydas	Pasifik (Chairns/Great Barrier Reef/Australia)	3.5	-	0.45 mm–2.51 mm	Transparent	polyethylene	**Caron *et al.*, 2018)**
C. caretta	Atlantik (Azores island/Portugal)	15.83 ± 6.09	Fragments	1–5 mm	Blue	polyethylene and polypropylene	**Pham *et al.*, (2017)**

2. Plastic color ingested

Brown represents the fifth most common debris found, followed by green, yellow, and red. Based on several studies, transparent and white colors are the most common or frequently ingested waste by turtles. This is because transparent and white plastics are the most widely floating and dispersed plastic waste, such as plastic bags **(Camedda *et al.*, 2014; Nelms *et al.*, 2016; Yaghmour *et al.*, 2018; Cheng, 2020)**. An investigation of 14 *C. mydas* by **Yaghmour *et al.* (2018)** showed similar results where white/transparent was the most common color, with white appearing with a frequency of 78.6% followed by transparent with a frequency of 64.3%. Another study conducted by **Nicolau *et al.* (2016)** on 95 loggerhead turtles showed that white was the most common color followed by transparent, blue-black, green, brown, red, orange, yellow, and multicolored. **Digka *et al.* (2020a)** found that in loggerhead turtles, there is a prevalence of white/transparent plastic at 48%. **Schuyler *et al.* (2012)** observed that transparent color (followed by white, black, brown, green, blue, red, yellow, and orange) is the most dominant color ingested by *E. imricata* and *C. mydas*. The dominance of white and transparent objects among the marine debris ingested by various turtle species has often been demonstrated in the existing literature **(Lazar & Gračan, 2011; Hoarau *et al.*, 2014; Nicolau *et al.*, 2016)**. It is hypothesized that the white/transparent suspension patterns on these objects may inadvertently mimic potential prey such as jellyfish **(Hoarau *et al.*, 2014; de Carvalho *et al.*, 2015; Cheng, 2020; Digka *et al.*, 2020a)**.

Turtles in shallow coastal waters have a stronger color preference compared to marine species, as most of the food for coastal species consists of jellyfish or cephalopods **(Narazaki *et al.*, 2013)**. Therefore, it is very likely that turtles mistake white and/or transparent plastic for food and actively ingest it **(Schuyler *et al.*, 2014; Casale *et al.*, 2016; Yaghmour *et al.*, 2018; Domènech *et al.*, 2019)**. Other colors that turtles might also commonly ingest could be related to the mixture of those colors with dominant colors like their natural prey **(Casale *et al.*, 2016)**. This means that the turtles swallowing a mixture of colors may be caused by the mixing of marine debris that is ingested along with their food.

Microplastics ingested by *C. mydas* were found in 6 color categories, while *E. imbricata* had 7 categories, each dominated by the color black (Fig. 5b). This result is consistent with the research by **Duncan *et al.* (2019)** on various types of turtles from different regions, where black is the dominant color of microplastics, followed by blue. The color at all observation stations for water samples was predominantly black (45.47%), followed by transparent (35.64%). In sediments, the highest average colors were black (21.94%), red (21.55%), and transparent (17.14%). Overall, various studies on microplastics in biota, water, and sediments found that the most commonly observed colors with the highest percentages were black, blue, red, and transparent **(Basri *et al.*, 2021)**. **Hiwari *et al.* (2019)** found that the dominant microplastic color in water was black (50%), with blue and transparent each at 8%. For sediments, according to **Firdaus *et al.* (2020)**, the dominant colors were transparent (43%), black (21%), and blue (14%). In contrast, **Yona *et al.* (2023)** found that the microplastics were predominantly blue (49%) and red (26%). All the dominant colors found in turtles were also predominantly found in water and sediments. Since both color and quantity were more abundant in sediments, it is assumed that the microplastics found in the turtle's digestive tract likely originated from the microplastics in the sediments. Several studies found that the microplastic colors in biota matched those in the sediments **(Hennicke *et al.*, 2021; Sawalman *et al.*, 2021; Sulistiowati *et al.*, 2023)**.

The results of polymer testing on samples from the turtle's digestive tract, water, and sediment revealed the presence of microplastic polymers. The type of polymer Polyethylenimine, with a similarity percentage to the functional group of Polyethylene of 61.04%, is the most abundant polymer found. Polyethylenimine (PEI) polymer is a high-performance amorphous polymer with excellent heat resistance, good chemical resistance, inherent flame resistance, and excellent dimensional stability **(Julie, 2021)**.

3. Microplastic distribution

Microplastics can be ingested either directly or indirectly through trophic transfer. Ingestion can occur when particles adhere to the surface of food items, for example, particles adhering to seagrass will be ingested by grazing turtles **(Pantos, 2022)**. At both stations, there are seagrass ecosystems. Research by **Utami *et al.* (2023)** in Kaana (A3) found 3 types of seagrass: *Enhalus acoroides*, *Halophila ovalis*, and *Cymodocea rotundata*, with the highest coverage of 86%. Furthermore, research by Hirotaka **Sejati *et al.* (2024)** in Malakoni (A2) found 2 types of seagrass: *Thalassia hemprichi* and *Halophila ovalis*, with coverage reaching 3.43%. Additionally, in Kahyapu, 2 types of seagrass were found: *Enhalus acoroides* and *Cymodocea rotundata*, with coverage ranging from 80 to 95% **(Purnama, 2013)**. Microplastic particles isolated from species occupying different trophic levels indicate the possibility of several absorption pathways, namely exposure from contaminated seawater and sediment and/or transfer from contaminated prey/food **(Duncan *et al.*, 2018)**. Microplastic contamination in seagrass is a concern given the high dependence of many species on this habitat and the potential for plastic to move up the food chain **(Unsworth *et al.*, 2021)**.

One of the food sources for *C. mydas* and *E. imricata* is jellyfish, which are themselves an important component of the marine ecosystem **(Bjorndal, 1977; Schuyler *et al.*, 2014)**, and they serve as a significant vector for the entry of microplastics into the marine food chain **(Macali *et al.*, 2018)**. Jellyfish have recently been reported as target organisms capable of internalizing a number of anthropogenic fragments, from macro to microplastics, making them a potential new bioindicator for plastic pollution on a global scale **(Macali & Bergami, 2020)**. In addition to ingesting plastic in their habitat and through the food chain, turtles can also ingest plastic on migration routes contaminated with plastic. According to **Hoarau *et al.* (2014)**, ingested plastic waste can remain in the body for more than 40 days before being excreted; within 40 days, turtles can migrate up to 1000km. The extent of this migration range has been proven through various satellite tag experiments, such as those conducted by the Landaa Giraavaru Maldives conservation center (https://marinesavers.com/satellite-tracking-map-2/).

CONCLUSION

This study confirms the presence of both macro- and microplastic waste in the intestines of transitional and sub-adult *C. mydas* and *E. imbricata*. The type and color of plastic varied between the two turtle species, as well as between the water and sediment samples. Approximately 40% of the turtles had ingested plastic waste, and it was found that 100% of the macroplastics in both *C. mydas* and *E. imbricata* were located in their intestines. The most common types of macroplastics found were fragments, sheets, and nylon. For microplastics, fragments were the most prevalent in *C. mydas*, while fibers dominated in *E. imbricata*. Fibers and films were the dominant microplastics found in water, while fragments were the most common in sediment.

Regarding macroplastic color, the most frequently ingested by turtles were transparent and white. Black and transparent macroplastics were most common in water samples, while black and red dominated in sediment samples. Additionally, *C. mydas* with a larger curved carapace length (CCL) showed higher levels of microplastics in the film and fragment categories, while smaller CCLs were associated with lower levels of these microplastics. However, CCL did not influence microplastic levels in *E. imbricata*, which had high levels of fiber-type microplastics. The macroplastic particles were ingested alongside food, whereas microplastics may result from the fragmentation of macroplastics during the digestive process.

We evaluate the possibility that plastic consumption may occur through the food chain along migration routes contaminated by plastic, highlighting the importance of our data for improving conservation management efforts within the regional management units (RMUs), including those on Enggano Island.

ETHICTS STATEMENT

Animal research has been approved by the secretariat of the scientific authority for biodiversity of the National Research and Innovation Agency (BRIN) Indonesia. This research was conducted in accordance with local laws and institutional requirements. The sampling for this research has been approved by the ministry of environment and forestry of the republic of indonesia, with permit number: **SK.85/KSDAE/SET.3/KSA.3/3/2022.**

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