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Presence of Microplastics in the Food Web and Macroplastics in Seabirds

– WASTEREDUCE Project –

(Scientific Study)

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Presence of Microplastics in the Food Web and Macroplastics in Seabirds
– WASTEREDUCE Project –

FINAL REPORT

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In accordance with the Service Agreement "Preparation of a Study on the Presence of Microplastics in the Food Web and Macroplastics in Seabirds – WASTEREDUCE Project", **CLASS 112-07/25-01/15**, **REG. NO. 01/2025**, signed on 1 August 2025 by the Director of Natura Histrica, Silvia Buttignoni, and the owner of Blue Mark Consulting, Ana Markić, within the framework of the Interreg WASTEREDUCE project, a scientific study was conducted with the aim of determining the presence of micro- and macroplastics in the digestive systems of four groups of marine organisms: bivalves, crustaceans, fish and seabirds. The research was carried out within Natura 2000 sites in Istria County, including coastal ecosystems particularly vulnerable to anthropogenic pollution. The collected data will contribute to a better understanding of plastic uptake within the food web across multiple trophic levels. In accordance with the project assignment, this final report contains detailed information on the study, including the introduction, methodology, results, discussion, conclusion and references.

Abstract

Marine plastic pollution affecting marine organisms currently represents one of the most widespread and ecologically significant anthropogenic pressures on marine ecosystems. Within the framework of the study on the presence of microplastics in the food web and macroplastics in seabirds, a total of 183 specimens of bivalves, crustaceans and fish, as well as 10 seabird pellets, collected in Istria County were analysed. The aim of the study was to assess the presence of plastics across different trophic levels and to identify potential bioindicator groups of organisms.

The results showed that bivalves from urban and anthropogenically impacted areas accumulated significantly higher concentrations of microplastics compared to individuals from more remote and less impacted locations, confirming the importance of local pollution sources and the high biomonitoring potential of filter feeders. In contrast, crustaceans contained relatively low quantities of plastic and proved methodologically challenging to analyse, making them less suitable as bioindicators of microplastic pollution. Among fish, pronounced interspecific differences were observed; mullets contained substantially higher quantities of microplastics than bluefish, highlighting the importance of feeding ecology and feeding strategy in plastic ingestion. These results suggest that filterfeeding bivalves and mullets may represent suitable bioindicators of microplastic pollution in coastal environments.

The analysis of seabird pellets revealed an extremely high occurrence of plastic, with all 10 thoroughly analysed pellets containing plastic debris. A total of 274 plastic items were isolated, predominantly plastic films and fibres. Plastic was confirmed at all investigated trophic levels of the food web; however, trophic transfer and biomagnification could not be unequivocally confirmed.

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Abbreviations

PP – polypropylene
PE – polyethylene
PS – polystyrene
PET – polyethylene terephthalate
PVC – polyvinyl chloride
PA – polyamide

1. INTRODUCTION

1.1. MARINE PLASTIC POLLUTION

The problem of plastic pollution began in the mid-20th century with the rapid growth of industrial plastic production following the Second World War, when plastics started to be widely used across nearly all sectors due to their low cost, durability and versatility (Geyer et al., 2017). A major turning point occurred with the proliferation of single-use plastics, as products such as bags, packaging, bottles and disposable utensils became standard in everyday life (UNEP, 2018). Although the first signs of the problem in marine ecosystems were recorded as early as the 1960s and 1970s, when researchers began to observe plastic debris in the oceans and marine organisms (Carpenter & Smith, 1972; Rothstein 1973), these early scientific warnings remained largely within academic circles and did not achieve broader societal attention. Over the following decades, the amount of plastic in the environment continued to increase, driven by globalised consumption patterns and inadequate waste management systems (Jambeck et al., 2015). Only in the past decade has plastic pollution entered global public awareness, largely due to microplastic research, activist media campaigns and documentaries that visually demonstrated the scale of ocean contamination. Today, it is clear that this is a long-term and cumulative problem affecting all levels of marine ecosystems, from surface waters to the deep sea, and that it is the consequence of decades of uncontrolled production and overconsumption of single-use plastics.

Plastic pollution, including microplastics (MP) (<5 mm) and nanoplastics (NP) (<1 µm), has become a major environmental and food safety concern due to its accumulation in aquatic and terrestrial ecosystems and its integration into food webs (GESAMP, 2015; WHO, 2019). These particles move through trophic levels, from phytoplankton and zooplankton to fish and bivalves, and can ultimately enter the human diet (Wright & Kelly, 2017). Microplastics can also act as vectors for additives incorporated during manufacturing as well as for sorbed hydrophobic environmental contaminants, with their contribution to organism exposure being influenced by environmental conditions, particle properties and retention time in the digestive tract (Koelmans et al., 2016).

Primary sources of microplastics include industrial pellets and cosmetic products containing microbeads (Fendall & Sewell, 2009; Andrady, 2011). Secondary microplastics arise from the fragmentation of larger plastic items under the influence of UV radiation, oxidation and mechanical degradation (Barnes et al., 2009; Andrady, 2011).

1.2. EFFECTS OF PLASTIC ON ORGANISMS

1.2.1. Effects of plastic on marine organisms

Experimental studies consistently show that microplastics and nanoplastics can exert a broad range of adverse effects on marine organisms, with mechanisms and outcomes varying substantially depending on species, particle size, concentration and exposure duration (Galloway et al., 2017; Bucci et al., 2020). At the physiological level, reported effects include reduced feeding activity, energy deficits, oxidative stress and inflammatory responses, particularly in filter feeders such as bivalves and zooplankton, where mechanical irritation and obstruction of the digestive tract may occur (Cole et al., 2013; Avio et al., 2015). Reproductive effects include reduced fertility, altered gamete and larval development and decreased offspring survival, effects that are especially pronounced in invertebrates and fish during early life stages (Sussarellu et al., 2016). At the endocrine level, experimental studies indicate disruptions in hormonal regulation, including changes in the expression of genes associated with reproduction and stress responses, suggesting a potential role of microplastics as endocrine disruptors, either directly or indirectly through associated chemicals (Rochman et al., 2013). In addition, nanoplastics demonstrate the ability to cross epithelial barriers and distribute into internal tissues and organs, with experimental and review studies indicating their presence beyond the digestive system and their potential for systemic, cellular-level effects (Mattsson et al., 2017; Ma et al., 2021).

Particle size and shape are key determinants of bioavailability and translocation (Koelmans et al., 2016). Microplastics can adsorb persistent organic pollutants (POPs) and other contaminants, acting as vectors of chemical exposure (Teuten et al., 2009; Rochman et al., 2013).

1.2.2. Trophic transfer and biomagnification

In marine ecosystems, microplastics and nanoplastics enter food webs primarily through filter feeders, including zooplankton, bivalves and small pelagic fish, which act as initial vectors of trophic transfer. Experimental and field studies show that microplastic ingestion can affect feeding efficiency, behaviour and survival of organisms at lower trophic levels, with consequent effects on energy availability for predators (Cole et al., 2013; Botterell et al., 2020). Trophic transfer of microplastics through prey–predator interactions has been confirmed in multiple systems, including planktonic, benthic and nektonic organisms within marine food webs (Farrell & Nelson, 2013; Setälä et al., 2014; Santana et al., 2017; Markic et al., 2018).

Although classical biomagnification of microplastics (i.e. increasing concentration or accumulation with trophic level) is variable and often limited (Koelmans et al., 2016; Wright & Kelly, 2017), plastic particles can act as carriers of chemical contaminants. Adsorption of hydrophobic organic compounds and other pollutants to plastic surfaces contributes to complex “cocktail” effects and may alter their distribution and bioavailability within food webs (Teuten et al., 2009; Koelmans et al., 2016). Therefore, although biomagnification of the particles themselves is not consistently demonstrated, trophic transfer of associated contaminants represents a key ecological risk mechanism, with potential consequences for higher trophic levels, including commercially important species and humans (Diepens & Koelmans, 2018; Mercogliano et al., 2020).

1.2.3. Effects of plastic pollution on humans

Microplastics and nanoplastics have been detected in various human biological matrices, including blood, lungs, stool, placenta and arterial plaques, confirming their widespread exposure and ability to be internalised within the human body (Ragusa et al., 2021; Jenner et al., 2022; Leslie et al., 2022). Of particular concern is the ability of nanoplastics to translocate across epithelial barriers and potentially cross the blood–brain barrier, with experimental and *in vivo* models suggesting possible accumulation in the brain and induction of oxidative stress and neuroinflammation (Prüst et al., 2020; Danopoulos et al., 2022).

Human exposure to microplastics occurs via ingestion and inhalation, including trophic transfer through the consumption of contaminated marine and terrestrial organisms, with seafood recognised as a relevant dietary source of intake (Cox et al., 2019; Mercogliano et al., 2020). A significant exposure pathway is also the indoor environment, including synthetic textiles, furniture and carpets, from which microfibres and plastic particles are continuously released, as well as atmospheric exposure (Dris et al., 2016; Vianello et al., 2019).

Given the presence of microplastics across multiple organ systems and evidence of *in vitro* cytotoxicity, including oxidative stress, inflammatory responses and potential genotoxic effects, research focus is increasingly directed towards the long-term consequences of chronic exposure and tissue accumulation (Danopoulos et al., 2022).

1.3. STATE OF PLASTIC POLLUTION IN THE ADRIATIC AND THE MEDITERRANEAN

In the Adriatic Sea and the wider Mediterranean, microplastics are widely distributed in sediments, the water column and biota, with the Mediterranean recognised as one of the global “hotspot” regions exhibiting elevated microplastic concentrations compared with open ocean systems (UNEP/MAP, 2020; Terzi et al., 2024). In the Adriatic, microplastics have been detected, among others, in bivalves such as *Mytilus galloprovincialis* (e.g. Gomiero et al., 2019; Kovačić et al., 2024) and in crustaceans (e.g. Aliko et al., 2022). They have also been documented in commercial fish species, where microplastics are found in the digestive tracts of a significant proportion of analysed individuals, confirming their integration into local food webs (Savoca et al., 2021; Mistri et al., 2022; Iveša et al., 2024).

1.4. MONITORING AND BIOINDICATORS

Biomonitoring of microplastics in marine ecosystems is based on the selection of bioindicator organisms that integrate spatial and temporal environmental exposure. According to the review by Tang et al. (2026), the most effective bioindicators are organisms that combine wide availability, a well-defined ecological role and the ability to accumulate microplastics at levels that can be reliably quantified. Filter feeders such as bivalves (e.g. *Mytilus* spp.) are particularly prominent, along with other trophically relevant organisms that enable the assessment of microplastic transfer through food webs, thereby allowing evaluation not only of presence but also of particle movement within the ecosystem.

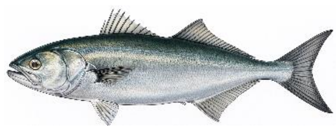
Tang et al. (2026) emphasise that the selection of bioindicators must be aligned with monitoring objectives, as different organism groups provide complementary information on exposure, bioaccumulation and trophic transfer of microplastics. They further highlight the need for standardisation in species selection to ensure comparability of data across regions and temporal series, which is essential for the development of reliable monitoring programmes and ecological risk assessment.

2. METHODOLOGY

2.1. FIELD SAMPLE COLLECTION

During the period from **1 September 2025** to **8 March 2026**, sample collection was conducted over **17 field campaigns** and **5 purchases** of fish samples at the Pula fish market, during which samples of bivalves, crustaceans, fish and seabird pellets were collected. Table 1 provides detailed information on the field sampling of bivalves, crustaceans and fish, including species names of the sampled organisms, locations and geographic coordinates, date and time, and technical details of sampling. Samples were collected opportunistically, resulting in a total of **240 individuals**, comprising **80 individuals of Pacific oyster**, **100 individuals of blue crab**, **60 fish individuals** (30 boxlip mullet and 30 bluefish), and **45 pellets of seabirds** including European shag, great cormorant and sea gull.

Table 1. Field sampling of bivalves, crustaceans and fish during the period from 01.09.2025 to 17.01.2026.

Collected organisms	Location	Date and time	Number of individuals	Brief description of field activity
Magallana Gigas (80) 	Pula Harbour (44°52'23.2"N 13°50'49.0"E)	**30.11.2025.** 12:00 – 13:00	40	Pacific oysters were collected at two sites in Pula Harbour. They were detached using a flat screwdriver, placed in an ice cooler and frozen at −20 °C until analysis.
		17.01.2026. 10:00 – 12:00	40	Pacific oysters were collected in Brijuni National Park from boat piers on Veli Brijun and Mali Brijun.
Callinectes sapidus (100) 	Palud (45°01'32.8"N 13°42'04.6"E)	**01.09.2025.** 17:00 – 19:00	4	Blue crabs were collected in the Palud ornithological reserve in the afternoon. Sampling was conducted by one or two experts using hand nets, with several individuals captured using a set gill net. Crabs were then stored in portable cooler boxes with ice, after which they were transported the same day to the Juraj Dobrila University of Pula, Faculty of Natural Sciences laboratory and stored in a freezer at −20 °C until further processing.
		02.09.2025. 16:00 – 18:00	16	
		06.09.2025. 14:00 – 16:00	18	
		07.09.2025. 13:00 – 16:00	14	
	Šćuza (44°49'11.7"N 13°53'01.6"E)	**07.09.2025.** 21:00 – 22:00	10	Blue crabs were collected in Šćuza Bay, in the inner part of Pomer Bay. Sampling was conducted by one or two experts using hand nets. Collection was carried out in the evening under night conditions using headlamps. After collection, all samples were transported to the laboratory of the Faculty of Natural Sciences, Juraj Dobrila University of Pula, where they were stored in a freezer at −20 °C until laboratory processing.
		09.09.2025. 20:00 – 21:30	17	
		14.09.2025. 19:30 – 21:00	8	
		21.09.2025. 19:30 – 21:00	13	
Pomatomus saltatrix 	Western coast of Istria and	**21.09.2025.**	11	Bluefish specimens were obtained from a spearfisher along the western coast of Istria using a speargun, ensuring an intact digestive tract. Fish were cooled in ice-filled portable boxes and frozen at −20 °C until analysis.
		15.11.2025.	3	Four bluefish specimens were purchased at the Pula fish market. The fish had been caught using set gill nets along the western coast of Istria. Specimens were frozen at −20 °C until further analysis.
		29.11.2025.	1	
		18.11.2025.	1	Two bluefish specimens, caught using set gill nets in the Medulin Bay, were purchased at the Pula fish market.
		29.11.2025.	1	
		13.01.2026	13	An additional 13 specimens were purchased at the Pula fish market.
Oedalechilus labeo (30) 	Medulin Bay, (44°47'34.5"N 13°54'50.7"E)	**01.10.2025.** 15:00 – 17:00	15	Boxlip mullet specimens were collected in Medulin Bay (Lokva and Portić coves) by freediving, using a set gill net (18 mm mesh size). Specimens were stored in portable cooler boxes with ice, transported the same day to the University of Juraj Dobrila Pula, Faculty of Natural Sciences and frozen at −20 °C until processing.
		12.10.2025. 15:00 – 17:00	11	
	Medulin Bay, (44°46'42.4"N 13°55'05.0"E)	**03.10.2025.** 15:00 – 17:00	4	
Ukupno			**240**	

2.1.1. Pacific oyster (*Magallana gigas*)

Pacific oyster (*Magallana gigas*) was selected as the target organism for microplastic analysis in bivalve digestive tracts. A total of 80 individuals were collected from two sites: **40 from Pula Harbour** (Figures 1–2) and **40 from Brijuni National Park**. In Pula Harbour, sampling was conducted on **30 November 2025** around midday during low tide at two accessible stair locations (Figure 3), where oysters occurred in dense intertidal aggregations (Figure 4). In Brijuni National Park, samples were collected on **17 January 2026** from two locations (Veli Brijun and Mali Brijun) at ferry piers (Figure 5). Oysters were detached using a flat screwdriver, avoiding shell damage to prevent contamination. Samples were photographed, stored in ice-filled cooler boxes, transported to the laboratory and frozen at −20 °C until analysis.

Figure 1. Pacific oyster samples from Pula Harbour.

Figure 2. Two sampling locations of Pacific oysters in Pula Harbour (yellow stars) (Source: Google Earth).

Figure 3. Steps providing easier access to Pacific oysters.

Figure 4. Dense aggregations of Pacific oysters.

Figure 5. Two sampling locations of Pacific oysters in Brijuni National Park (yellow stars), Veli Brijun (left) and Mali Brijun (right) (Source: Google Earth).

2.1.2. Blue crab (*Callinectes sapidus*)

The invasive non-native **blue crab** (*Callinectes sapidus*) was selected as the representative crustacean species due to its high abundance at the **Palud** and **Šćuza** sites. Palud, a special ornithological reserve south of Rovinj, is a shallow, highly muddy lagoon habitat with rich marsh vegetation, where blue crabs have been consistently abundant and established in recent years. Šćuza, in contrast, is a less muddy, artificially enclosed inlet within the Medulin Bay and represents one of the oldest preserved traditional fishing systems in Istria. Palud is a hypersaline marsh, whereas Šćuza is a brackish inlet. At Palud, sampling was conducted in the channel connecting the lagoon to the sea and its immediate surroundings (Figure 6, left; Figure 7). At Šćuza, sampling was carried out in the western part of the bay, where field access was easier (Figure 6, right; Figure 8).

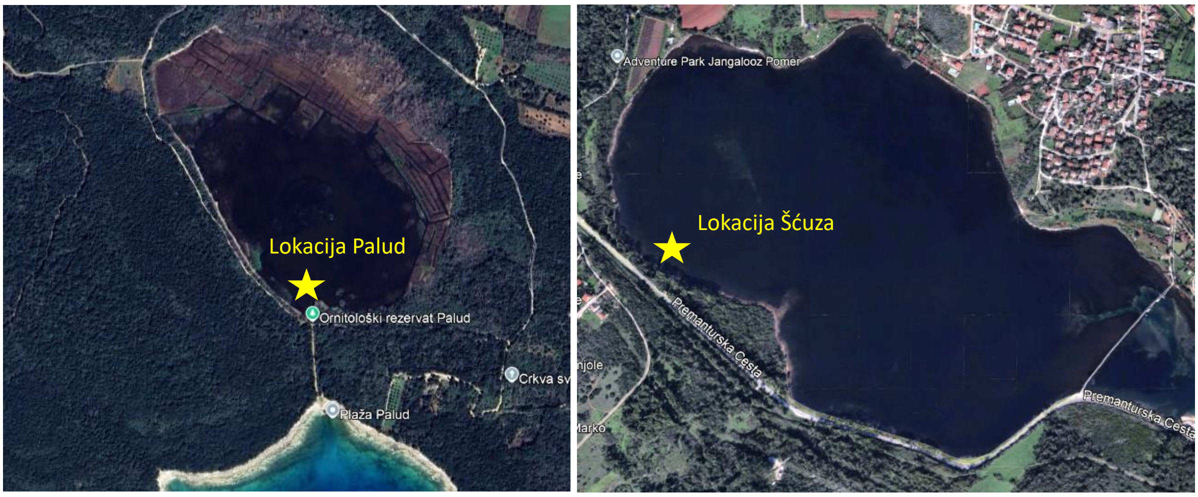


Figure 6. Sampling locations of blue crab – Palud (left) and Šćuza (right).
Yellow star indicates the sampling area (Source: Google Earth).

Figure 7. Blue crab sampling location at Palud (02 September 2025).

Across eight field sampling events, a total of 100 blue crab individuals were collected. On four occasions, 52 individuals were sampled at Palud and 48 at Šćuza. Sampling at the muddy Palud site (Figure 9a) took longer due to reduced visibility and difficulty in locating crabs within the sediment, whereas Šćuza has more rocky substrate and less mud (Figure 9b). To minimise potential plastic contamination, no plastic net traps were used; instead, crabs were collected with a hand net to reduce exposure to plastic materials during sampling (Figure 9c). However, several individuals were collected using a set gillnet intended for a different sampling purposes. Specimens were then placed in ice-filled cooler boxes in metal containers (Figure 9d,e) and transported to the Faculty of Natural Sciences, University of Juraj Dobrila Pula (Figure 9f), where they were frozen at −20 °C until laboratory processing.

Figure 8. Blue crab sampling location at Šćuza (14 September 2025).

Figure 9. Blue crab sampling: (a) muddy habitat at Palud, (b) rocky–muddy habitat at Šćuza, (c) cooler box and sampling bucket, (d) hand net used for crab collection, (e) blue crabs in a metal bowl cooling in an ice-filled cooler box and (f) blue crabs from Šćuza.

2.1.3. Fish: bluefish (*Pomatomus saltatrix*) i boxlip mullet (*Oedalechilus labeo*)

Bluefish (*Pomatomus saltatrix*) and **boxlip mullet** (*Oedalechilus labeo*) were selected as target species for assessing microplastic presence in fish digestive tracts. Bluefish represents a top predator, while boxlip mullet is a benthopelagic species feeding non-selectively on small particles from sediment, rock surfaces and the water surface.

A total of **30 bluefish** individuals were collected using two approaches: direct provision from a spearfisher and purchase from fishmongers at the fish market. The spearfisher collected 11 individuals in a Natura 2000 area (western Istria) using a speargun (Figure 10, left), ensuring intact digestive tracts, and the specimens were promptly frozen at −20 °C. The remaining 19 bluefish were purchased at the fish market and had been caught using set gill nets (Figure 10, right) along the western coast of Istria and in the Medulin Bay.

Figure 10. Bluefish caught by speargun (left) and set gill net with 80 mm mesh size (right).

Across three field campaigns, **30 boxlip mullet** individuals were collected from two locations in Medulin Bay, Lokva Cove and Portić Cove (Figure 11, yellow stars). Fish were captured by freediving and driving them into a set gill net with 18 mm mesh size (Figure 12, upper and middle images), after which they were transported as quickly as possible to the Faculty of Natural Sciences, University of Juraj Dobrila Pula and frozen at −20 °C in the laboratory (Figure 12, lower image).

Figure 11. Lokacije uzorkovanja cipla plutaša (žute zvjezdice) u Medulinskom zaljevu (Izvor: Google Earth).

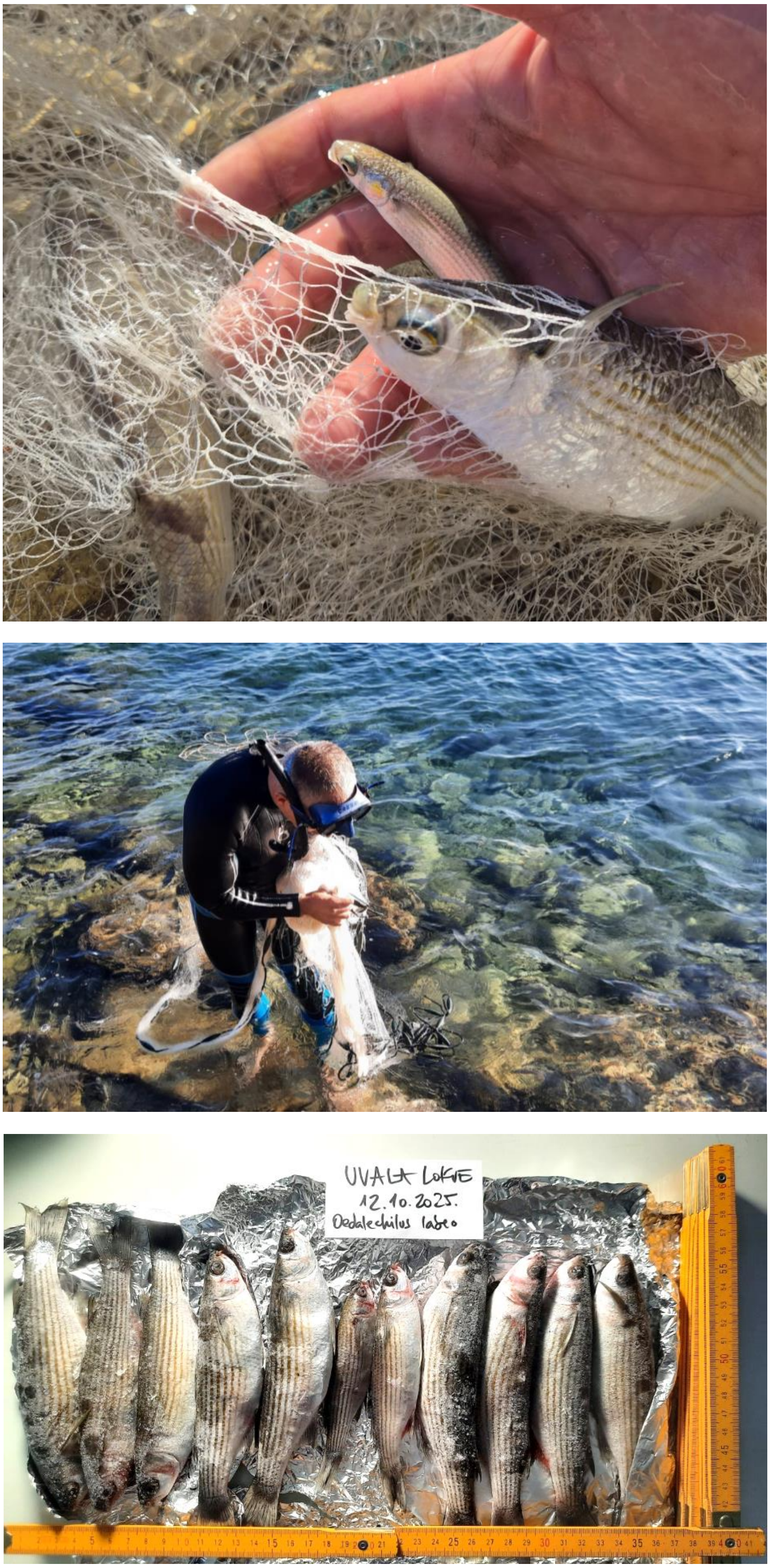


Figure 12. Boxlip mullet sampling using a set gill net with 18 mm mesh size (upper and middle images). Boxlip mullet samples collected at Lokva Cove, Premantura, on 12 October 2025 (lower image).

2.1.4. Seabirds

Systematic field surveys for the collection of dead seabirds or regurgitated pellets were conducted on three occasions: **15** and **16 February 2026** in **Brijuni National Park** (Kozada and Madonna islets) with a BIOM field team and staff of the park authority (Figure 13, top), and on **8 March 2026** on **Levanić islet** in Medulin Bay (Figure 13, bottom).

Figure 13. Seabird sampling locations (yellow stars) in Brijuni National Park on Madonna and Kozada islets (top) and in Medulin Bay on Levanić islet (Source: Google Earth).

Repeated opportunistic searches for dead birds resulted in the recovery of only two individuals: one **juvenile yellow-legged gull** (*Larus michahellis*) found dead on **21 February 2026** on the Marlera Peninsula and one **European shag** (*Gulosus aristotelis*) found on **21 March 2026** in Brijuni National Park. The sea gull died due to ingestion of a fishing plastic lure and entanglement in fishing line, while the cause of death of the shag could not be determined. Following the reported increase in avian influenza cases in wild birds announced by the Ministry of Agriculture, Forestry and Fisheries in late October, the digestive tracts of these specimens were not analysed (Ministry of Agriculture of the Republic of Croatia, 2025).

Field surveys in Brijuni National Park were conducted as part of the **2026 European shag census** across the Brijuni archipelago, in collaboration with park employees and the BIOM association. On Kozada and Madonna islets, where European shags nest in low, dense vegetation, a high number of nests was recorded. Many nests contained plastic debris incorporated into their structure (Figure 14), most commonly white plastic mesh used in mussel aquaculture.

Figure 14. Plastični materijal kao građevni materijal u gnijezdima morskih vranaca.
(Autorice fotografija: gore Ana Markić, dolje Ana Klarin).

At the second site, Levanić islet in Medulin Bay, seabirds do not breed but use the area for roosting and foraging. Recorded species included **juvenile European shags** that are not yet breeding, **great cormorants** (*Phalacrocorax carbo*) and **yellow-legged gulls** (Figure 15).

Figure 15. European shags and great cormorants (top) and yellow-legged gulls (bottom) on Levanić islet (Photo: Filip Prošić).

A large number of pellets (Figure 16) and faeces of shags, cormorants and sea gulls were found on the rocky shoreline of Levanić islet. A total of 45 pellets were of sufficient quality for laboratory analysis. Pellets were carefully collected and individually wrapped in aluminium foil with corresponding labels.

Figure 16. Seabird pellets collected on Levanić islet.

2.2. LABORATORY ANALYSIS

Laboratory analysis of samples began on **24 October 2025** at the **Faculty of Natural Sciences, University of Juraj Dobrila Pula**, following procurement of the necessary chemicals and equipment for microplastic isolation and identification and establishment of the analytical protocol. In brief, the digestive tract analysis consisted of the following steps:

- specimen thawing and recording of biometric data
- removal of the digestive tract and placement in a beaker with a chemical digestion solution
- chemical digestion at room temperature or on a magnetic stirrer at elevated temperature (up to 40 °C)
- vacuum filtration of the digested solution
- stereomicroscopic examination of filters (up to 40× magnification)
- storage of suspected microplastic particles for further chemical analysis
- spectrometric analysis for polymer identification

2.2.1. Quality control and contamination prevention

To **minimise contamination of samples with environmental microplastics**, the entire laboratory was thoroughly cleaned before each working day using wet wiping procedures, with particular attention to floors to reduce airborne particle deposition. In addition, all work surfaces used during analysis were regularly cleaned with 100% cellulose sponges (e.g. Vileda).

After cleaning, a **blank control** ("procedural blank") was processed to assess potential contamination, following the same analytical steps as real samples through to microscopic inspection. Tap water, pre-screened for microplastic presence, was used as the blank matrix. Fibres were occasionally detected in initial blanks; in response, the laboratory was re-cleaned and an additional contamination check was performed, after which no fibres were observed on filters. During blank controls, occasional transparent fibres originating from laboratory coats were also observed and were excluded from the dataset.

2.2.2. Isolation of microplastics and macroplastics

Chemicals required for digestive tract chemical digestion and dissolution of calcium carbonate (calcareous material) included **potassium hydroxide** (10% KOH), **hydrogen peroxide** (15% H_2O_2) and **acetic acid** (9% CH_3COOH). Solutions were prepared, mixed and filtered through a stainless-steel filter (5 µm pore size) and stored in glass bottles until further use (Figure 17).

Figure 17. Filtered chemicals.

After thawing, biometric measurements were taken (Figures 18a and 18b). Depending on the organism group, dimensions (length and width), mass and sex were recorded. For fish, standard length (from snout to the base of the caudal fin), total length (from snout to the end of the caudal fin), total mass and sex were measured. After measurements, the digestive tract was carefully removed (Figures 18c and 18d) and promptly transferred to a glass jar or beaker for chemical digestion to minimise contamination risk. If the digestive tract exceeded a few grams, it was homogenised (chopped and mixed) to ensure more efficient digestion in 10% KOH or 15% H_2O_2 (Figure 19).

The homogenised digestive tract is left in 10% KOH or 15% H_2O_2 until complete digestion of the organic material, which may take from several hours to several days depending on sample composition. To accelerate digestion, samples are kept warm and ideally placed on a magnetic stirrer with heating (up to 40 °C) for at least a short period before filtration (Figure 20).

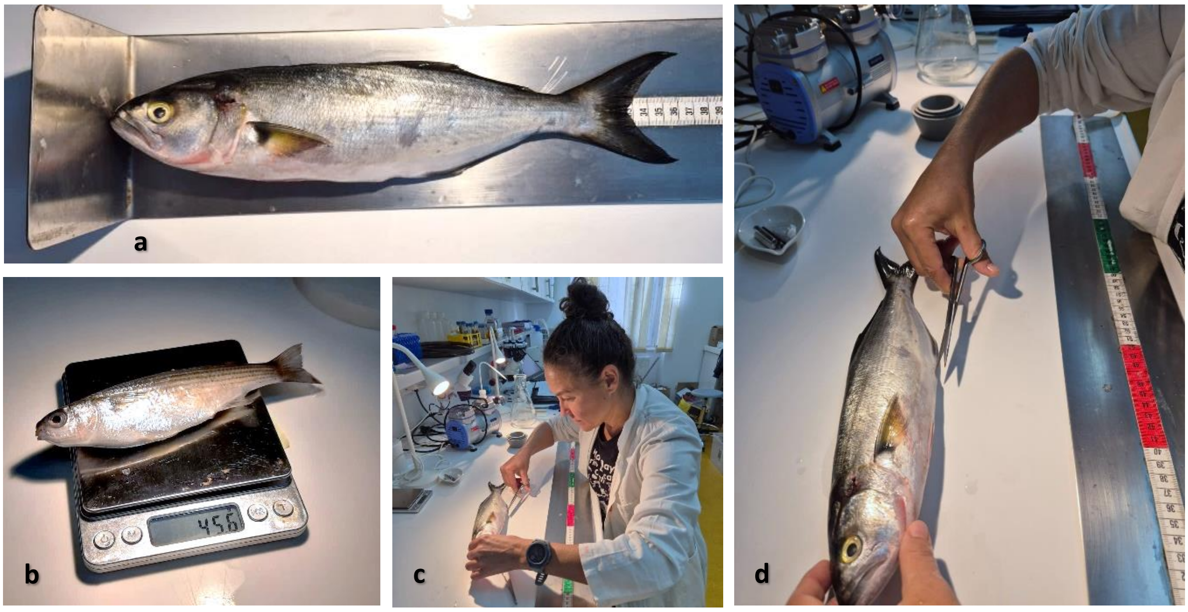


Figure 18. Recording biometric data for (a) bluefish and (b) boxlip mullet and dissection of the bluefish digestive tract (c and d).

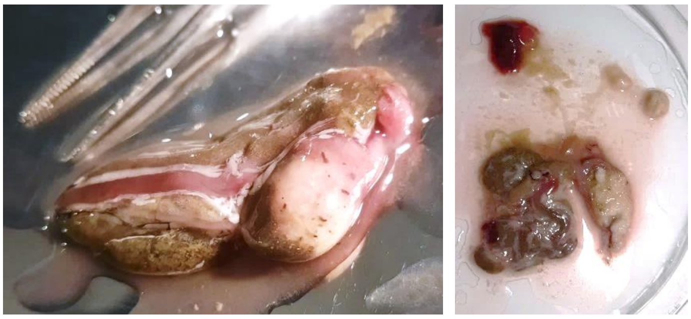


Figure 19. Excised boxlip mullet digestive tract (left), subsequently homogenised and prepared for digestion (right).

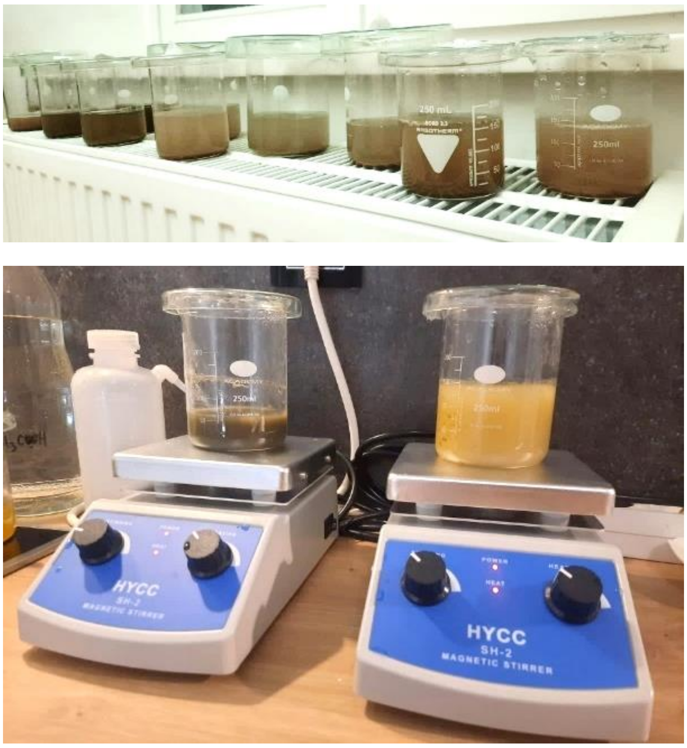


Figure 20. Heating of samples on a radiator (top) and on a magnetic stirrer (bottom).

After chemical digestion, samples are vacuum-filtered through one or two stainless-steel filters depending on particle size distribution in the digestive tract. If the sample is homogeneous and contains fine particles, a single 50 µm pore-size filter is used. When larger particles are present, a two-stage filtration is applied to facilitate microscopic analysis, using a lower 50 µm filter and an upper 250 µm filter. During vacuum filtration, the beaker or container is rinsed with distilled water using a syringe to prevent loss of microplastics adhering to the walls. If the sample contains a high amount of calcium carbonate ($CaCO_3$), including shell fragments from other organisms, it may be additionally treated with acetic acid to enhance dissolution.

Microscopic analysis was performed using a stereomicroscope with up to 40× magnification (Figure 21). A total of **183 digestive tracts** from target organisms were analysed (80 oysters, 43 blue crabs, 30 boxlip mullet and 30 bluefish).

Figure 21. Microscopic analysis of filtered samples.

Seabird pellet analysis for macroplastics included weighing the pellet prior to analysis, extracting plastic items and weighing individual pellet components, as well as counting, measuring, identifying plastic type and colour, and photographing. When necessary, 10% KOH or water was used to soften harder pellet material to facilitate plastic extraction (Figure 22). The slurry was then filtered through a gauze-covered sieve, after which the material was dried and further analysed.

Figure 22. Dissolution of pellet content in 10% KOH (left and centre) and filtration through gauze and a sieve.

2.2.3. Spectrometric analysis of isolated microplastic particles

Raman spectroscopic analysis of particles isolated from digestive tracts was conducted at the **Ruđer Bošković Institute** in Zagreb, Division of Materials Physics, using a **WITec alpha300 Raman spectrometer** (Oxford Instruments, Ulm, Germany) equipped with a 532 nm laser. At the start of the measurements, the spectrometer was calibrated using a standard silicon reference sample, measured at at 520 cm^{-1}. Raman spectra were recorded at room temperature using a laser power of 5 mW and a 50× objective lens was used for signal acquisition.

Material identification was performed by comparing the obtained Raman spectra with reference spectra available in internal and commercial **libraries** within the Raman software package. In addition, the **Open Specy** online platform was used as a complementary tool for microplastic identification, enabling comparison of the obtained spectra with a large database of reference materials (Cowger et al., 2025). Identification was based on the position of characteristic vibrational bands, their intensity and the overall spectral shape, with particular attention given to diagnostic bands specific to individual polymers, pigments or mineral phases. Based on this, the material type was determined, including polymers or additives such as pigments.

Spectrometric analysis was performed on 100% of isolated particles. A conservative approach was applied in result interpretation. In cases where particles were lost during transfer from the filter to the microscope slide and there was no high confidence that the material was a synthetic polymer, such particles were excluded from the analysis. Likewise, particles with a low signal-to-noise ratio, where reliable discrimination between polymeric material and potential natural tissues or mineral particles was not possible, were also excluded. Overall, strict identification criteria for microplastics were applied to ensure high reliability of the final material classification.

2.3. STATISTICAL ANALYSIS

Statistical analyses were performed using the **jamovi** software package (version 2.6.44.0). **One-way analysis of variance (ANOVA)** was used to test differences in the frequency and concentration of microplastics between groups (location and species). When ANOVA results indicated statistically significant differences, post-hoc tests were conducted to determine pairwise differences between individual groups. Normality of distribution and homogeneity of variances were assessed using appropriate diagnostic tests prior to analysis. Results were considered statistically significant at a significance level of $p < 0.05$.

2.4. PERMITS FOR FIELD SAMPLE COLLECTION

Permits for field sample collection were requested and obtained as follows:

- Permit from the Ministry of Environmental Protection and Green Transition
  Class: UP/I-352-02/25-15/75
  Reg. No.: 517-06-2-1-25-2

- Permit from Istria County
  Class: UP/I-352-01/25-01/24
  Reg. No.: 2163-08-02/5-25-3

3. RESULTS AND DISCUSSION

3.1. MICROPLASTICS IN BIVALVES

Bivalve samples were collected from two locations: **Pula Harbour**, which is situated near a traffic road and other potential sources of plastic pollution such as stormwater drainage systems, and **Brijuni National Par**k, which is distant from common pollution sources associated with urbanised areas. From each location, **40 individuals** were collected (N = 40), as shown in Table 2. The average soft tissue mass of oysters sampled in Pula and Brijuni National Park was approximately similar, at 6.0 g and 6.4 g, respectively.

Table 2. Oyster samples and their mean mass (SE – standard error).

	Number of analysed individuals (N)	Average mass (g) ± SE
Pula Harbour 1	20	5.0 ± 0.8
Pula Harbour 2	20	7.0 ± 1.2
Pula Harbour (Total)	**40**	**6.0 ± 1.0**
Mali Brijun	20	8.1 ± 0.8
Veli Brijun	20	4.6 ± 0.5
NP Brijuni (Total)	**40**	**6.4** ± 0.8
Total	**80**	**6.2 ± 0.9**

Microplastic analysis in oysters was performed by digesting the entire soft tissue of bivalves, rather than only the digestive tract. For chemical digestion, 10% KOH was used, which is highly effective for the breakdown of this type of tissue; in some cases, a single sample was fully digested in less than one hour. As digestion was complete, the residual solution was easily filtered through a 50 µm pore-size filter. Overall, oysters showed very little residue on the filters and microscopic analysis was quick and straightforward.

In total, 48 microplastic particles were found in 27 individuals (33.8%), corresponding to an average of almost two particles per oyster. In oysters from Pula Harbour, microplastics were present in 60% of individuals, whereas in oysters from Brijuni National Park, microplastics were detected in only 7.5% of individuals. Further details on microplastic abundance per sub-location are presented in Figure 23 and Table 3.

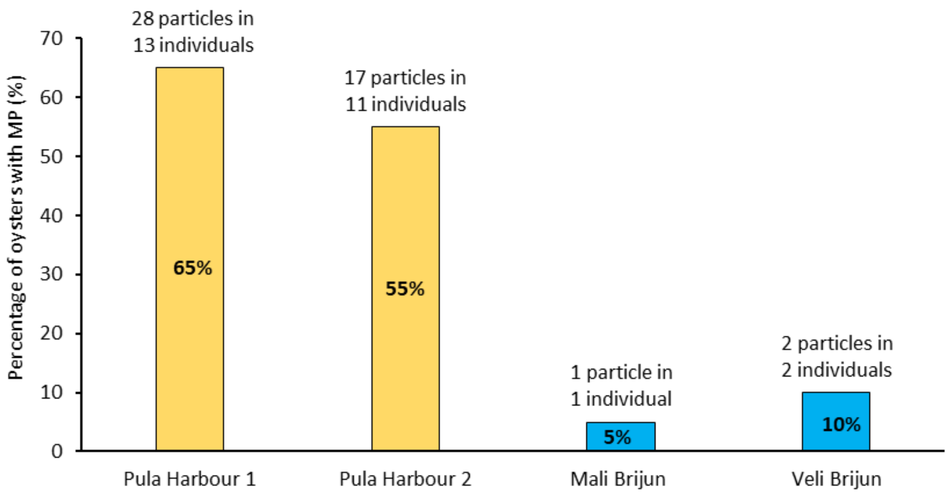


Figure 23. Presence of microplastic particles in analysed oysters.

Table 3. Microplastic abundance in oysters from different locations.

	Number of individuals with microplastics (#)	Percentage of individuals with microplastics (%)	Number of microplastic particles (#)	Number of particles per individual (#MP/individual)
Pula Harbour 1	13	65	28	2.2
Pula Harbour 2	11	55	17	1.5
Pula Harbour	**24**	**60**	**45**	**1.9**
Mali Brijun	1	5	1	1.0
Veli Brijun	2	10	2	1.0
NP Brijuni	**3**	**7.5**	**3**	**1.0**
Total	**27**	**56.3**	**48**	**1.8**

To compare microplastic abundance (MP count) among the four locations (Pula Harbour 1, Pula Harbour 2, Mali Brijun and Veli Brijun), a one-way analysis of variance (**One-way ANOVA**) was performed using the jamovi software package. The results showed statistically significant differences between locations. **Post hoc Tukey tests** indicated that Pula Harbour 1 differed significantly from both Mali Brijun and Veli Brijun ($p < 0.05$), while the difference between Pula Harbour 2 and Veli Brijun was marginally significant ($p = 0.05$). No statistically significant differences were observed between Mali Brijun and Veli Brijun ($p = 0.99$) or between Pula Harbour 1 and Pula Harbour 2 ($p = 0.23$) (Figure 24). Comparable results were reported in a study from England, where populations of the European flat oyster (Ostrea edulis) from urban areas contained significantly higher concentrations of microplastics compared to those from rural locations (Zapata-Restrepo et al., 2025).

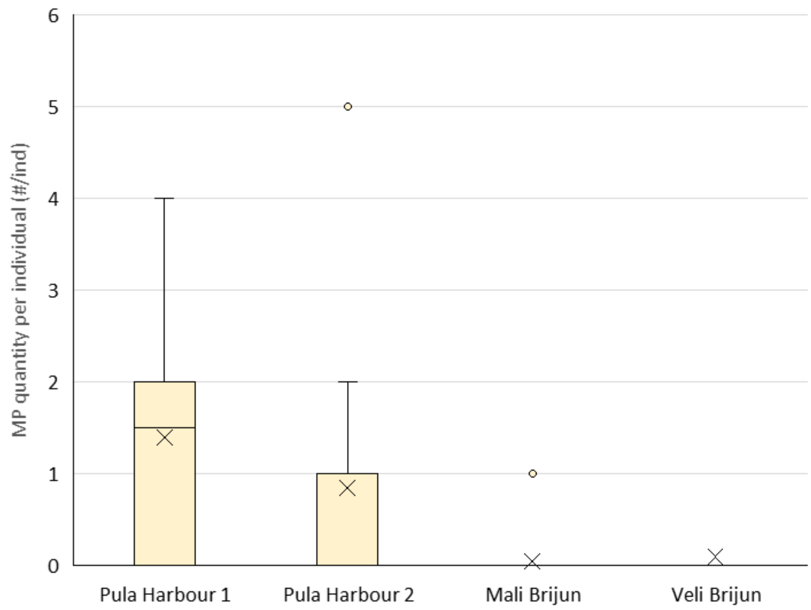
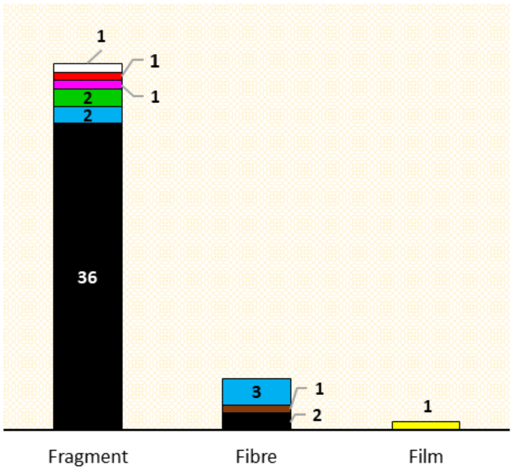


Figure 24. Microplastic abundance per oyster.

Of the 48 isolated microplastic particles, most were **fragments** (41) (Figure 25). In addition to fragments, seven fibres and one film were identified. **Black** was the dominant colour, most commonly occurring in fragment form, followed by blue particles (Figure 25). Mean particle sizes were **below 1 mm**. Fragments averaged approximately 200 µm, fibres around 800 µm and the film approximately 300 µm in size. Under the stereomicroscope, black fragments showed an amorphous and clumped appearance (Figure 26), and probing with a laboratory needle suggested a rubber-like consistency.



MP Type	Quantity	Av. length (µm)
Fragments	41	208
Fibres	6	792
Film	1	300

Figure 25. Types and colours of microplastic particles isolated from oysters. Mean particle sizes are shown in the table on the right. The longest axis was measured and expressed in micrometres (µm).

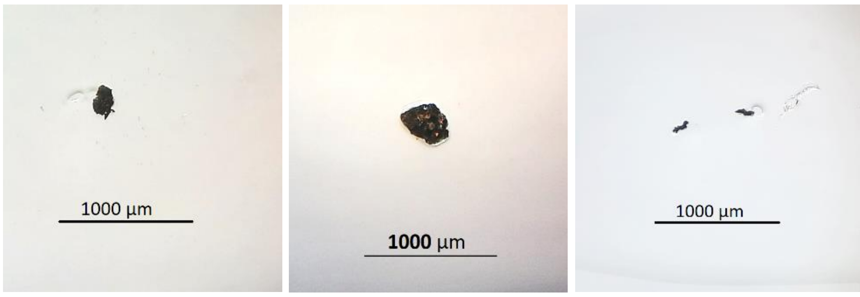


Figure 26. Black particles isolated from oysters from Pula Harbour (samples MAG 41, 10 and 5).

Spectrometric confirmation and identification of microplastic particle composition in oysters proved challenging. The particles visually dominating most samples were small black rubber-like fragments. For a large proportion of these particles, the acquired Raman spectra showed a low signal-to-noise ratio, with pronounced background noise and without clearly defined characteristic bands, which hindered reliable interpretation. In many cases, the spectra also did not match reference spectra available in spectral libraries.

For a subset of particles that produced spectra of sufficient quality, rubber was identified as the most probable material (Figure 27). Since several of the black rubber-like particles yielded reliable spectra, other morphologically identical particles (based on stereomicroscopic observation) were also classified as rubber, although high-quality spectral confirmation could not be obtained for all of them.

Identification of particles in oyster samples therefore relied on a combination of spectral analysis and morphological comparison with particles for which reliable spectra were obtained.

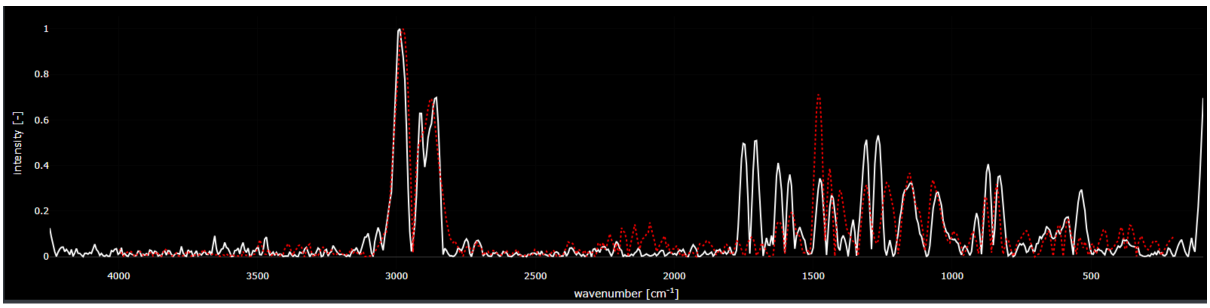


Figure 27. Spectrum of a black particle from an oyster at Pula Harbour 2, for which the Open Specy software indicates a 75% match with Tragacanth rubber (Cowger et al., 2025).

Identification of microplastics by Raman spectroscopy is often challenging for dark and highly pigmented particles, particularly in samples of biological origin such as bivalves. One of the main limitations is fluorescence caused by pigments, additives and residual organic material, which can be several times more intense than the Raman signal itself and may mask characteristic spectral bands (Nava et al., 2021; Aloia et al., 2025). It has also been emphasised that pigments and additives in plastics can substantially reduce identification accuracy by obscuring polymer-specific spectral features (Azari et al., 2024). This issue is especially pronounced in black particles, which are known to frequently produce indistinct or unusable spectra. Black particles commonly contain carbon black, a pigment widely used in consumer products to colour plastics black, protect them from UV degradation and improve material properties (Konings et al., 2024). In addition, environmental weathering, biofilm formation and organic material adhering to particles may increase background signal and spectral noise, resulting in low signal-to-noise ratios and making comparison with spectral libraries more difficult (Umurhan et al., 2025). Due to these limitations, spectroscopic analysis is often recommended to be combined with complementary methods such as Fourier Transform Infrared (FTIR) spectroscopy in order to improve the reliability of particle classification.

Although rubber is not usually the dominant fraction of microplastics, it has been identified as a relevant component of microplastic contamination in marine organisms, including bivalves, and is often associated with sources such as tyre wear and maritime activities (Wang et al., 2025). Kargar et al. (2025) reported that fibres and fragments generally dominate in bivalves, with fibres typically representing the most abundant fraction across different species and environments. A review on microplastics in oysters highlighted that particle composition can vary substantially depending on local pollution sources and analytical methods used for identification (Liu et al., 2023). Since only stormwater, but not sanitary sewage, is discharged into Pula Harbour, and sanitary wastewater is otherwise considered a major source of synthetic microfibres in marine environments (Mishra et al., 2019), fragments rather than fibres dominated oyster samples from this location. Given that a traffic road runs along the entire harbour area, we hypothesise that stormwater runoff and surface washout transport substantial quantities of tyre-wear particles into the sea, where they subsequently accumulate in oysters. Our findings therefore support the interpretation that the presence of rubber-like fragments in samples may reflect specific local pollution sources, in this case traffic-related contamination.

Bivalves are considered among the most suitable bioindicators of microplastic pollution due to their biology and feeding strategy. As filter feeders, they process large volumes of water and non-selectively retain suspended particles, including microplastics, thereby effectively integrating contamination signals from the surrounding environment (Pizzuro et al., 2025). For this reason, they are widely used in monitoring programmes such as the Mussel Watch programme as indicators of environmental quality. An additional advantage is the relatively simple collection and processing of samples, including efficient digestion of soft tissue (e.g. using KOH digestion), which enables standardised microplastic analysis. Furthermore, bivalves respond rapidly to environmental changes and accumulate particles at concentrations reflecting local conditions, making them particularly useful for spatial comparisons of pollution levels (Zapata-Restrepo et al., 2025). Invasive non-native species may provide additional benefits in monitoring programmes because their use avoids sampling native species, while they are often widespread, tolerant of varying environmental conditions and available in high densities, enabling consistent sampling and long-term environmental monitoring.

Although oysters are an invasive species and their contamination with microplastics has limited direct economic relevance, their presence may serve as an indicator of broader environmental pollution, with the assumption that similar contamination patterns also occur in other filter-feeding organisms such as the native oyster *Ostrea edulis* and mussels (*Mytilus* spp.), which have high ecological and economic importance. Tyre-wear particles and associated chemical compounds may negatively affect the physiology, reproduction and early developmental stages of bivalves and other marine organisms, including reduced filtration activity, altered reproductive success and toxic effects of both leachates and the particles themselves (Bernardini et al., 2024; Thomsen et al., 2024; Foscari et al., 2025; Savino et al., 2025; Michelangeli et al., 2026). Similar effects have also been reported in other benthic organisms, including sea urchins, where leachates from rubber particles showed toxicity to early developmental stages (Rist et al., 2026), indicating broader ecotoxicological implications of these particles in coastal ecosystems.

Considering the potential accumulation of microplastics in filter-feeding organisms, the consumption of shellfish from areas with high anthropogenic impact should be avoided and depuration procedures prior to consumption are recommended. Depuration may reduce contaminant loads in shellfish tissues, including microplastics, thereby decreasing the potential intake of particles through food consumption (Liu et al., 2023; Pizzuro et al., 2025).

3.2. MICROPLASTICS IN CRUSTACEANS

Microplastic presence in **blue crabs** (*Callinectes sapidus*) was assessed exclusively through stomach analysis, as the intestines are difficult to separate from surrounding tissues without damage, while crab tissue itself is highly resistant to chemical digestion. For this reason, it was necessary to minimise the amount of material subjected to digestion. Stomachs frequently contained non-digestible or poorly digestible material, such as cellulose and shell fragments. For the first ten stomach samples, a 10% KOH solution was used, but digestion proved slow and limited. Therefore, the remaining samples were treated with 15% H_2O_2, which is commonly applied for efficient digestion of organic matter in biological samples while preserving microplastic particles (Wootton et al., 2025).

A total of 100 blue crab samples were collected, but after analysis of the first 20 individuals it was determined that 40 specimens were sufficient to obtain statistically reliable results due to the consistency of findings. From each location, 20 individuals were randomly selected (N = 20), with an equal sex ratio between males and females. Individuals had an average carapace width of approximately 10 cm, carapace length of around 5 cm and body mass of approximately 75 g (Table 4). Since three individuals were contaminated with fragments of fishing net used during capture (Figure 28), they were excluded from further analysis and replaced with three additional individuals. Consequently, the final analysis was conducted on a total of 43 individuals. The fishing net fragments themselves also represent a valuable and noteworthy finding, confirming that blue crabs are capable of fragmenting larger plastic items and thereby contributing to microplastic generation.

Table 4. Number of analysed individuals and biometric data (SE – standard error).

	Number of analysed individuals (N)	**Mean carapace width, CW ± SE (cm)**	**Mean carapace length, CL ± SE (cm)**	**Mass ± SE (g)**	**Sex (F, M)**
Palud	20	9.8 ± 0.2	4.7 ± 0.1	72.1 ± 4.6	10 F, 10 M
Šćuza	20	10.2 ± 0.3	4.9 ± 0.1	78.9 ± 5.8	10 F, 10 M
Total	40	10.0 ± 0.3	4.8 ± 0.1	75.5 ± 5.2	

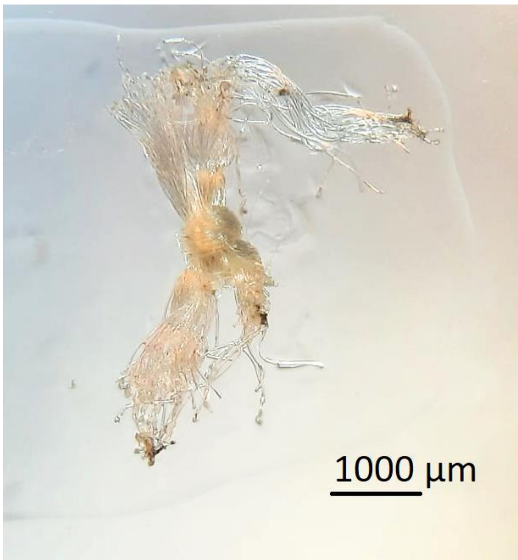


Figure 28. A fragment of fishing net extracted from the digestive tract of a blue crab.

Analysis of stomach contents for the presence of microplastics revealed a relatively low level of contamination. Of the 40 analysed crabs, eight individuals contained microplastics in the digestive tract, representing 20% of the total sample. A total of 15 microplastic particles were isolated from these eight stomachs, corresponding to an average of approximately two particles per individual (Table 5).

Crabs from Šćuza contained higher amounts of microplastics compared to those from Palud (Figure 29). Palud is an area distant from direct anthropogenic influence and protected as a nature reserve, and the lower contamination levels may therefore reflect limited microplastic input into the system. In Palud, microplastics can enter only through the channel connecting the lagoon with the sea, predominantly as floating material. Since crabs inhabit muddy substrates, the likelihood of ingesting floating plastics in such an environment is reduced.

In contrast, the Šćuza site is located near a main road and is potentially influenced by urban pollution sources, including surface runoff and possible stormwater or sanitary sewage discharges into the bay, which may explain the higher exposure to microplastics observed at this location.

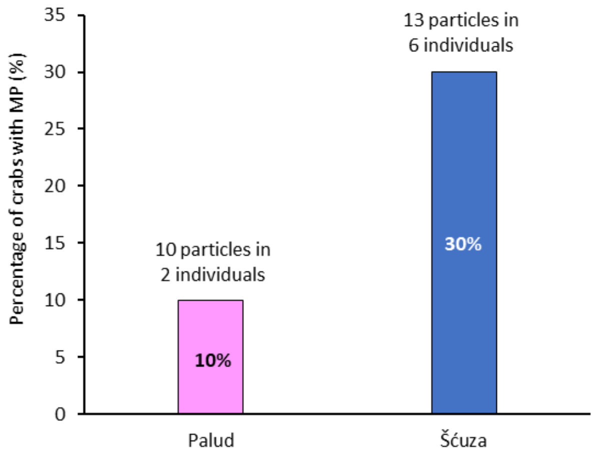


Figure 29. Presence of microplastics in blue crabs.

Table 5. Microplastic abundance in blue crabs from different locations.

	Number of individuals with microplastics (#)	Percentage of individuals with microplastics (%)	Number of microplastic particles (#)	Number of particles per individual (#MP/individual)
Palud	2	10	2	1.0
Šćuza	6	30	13	2.2
Total	**8**	**20**	**15**	**1.9**

A one-way ANOVA was performed in the jamovi software package to examine differences in the number of contaminated individuals and the quantity of microplastics between the two locations. As the assumption of homogeneity of variances was violated, as confirmed by Levene's test, Welch's ANOVA was applied as a robust alternative to the classical ANOVA. The analysis showed that the difference between locations **was not statistically significant** ($p = 0.072$) (Figure 30).

Microplastics isolated from blue crabs were predominantly small fragments and fibres, as shown in Figure 31. As observed in oysters, black was also the dominant colour in crabs. Only two black fibres were found in crabs from Palud, whereas crabs from Šćuza contained eight fragments, four fibres and one film.

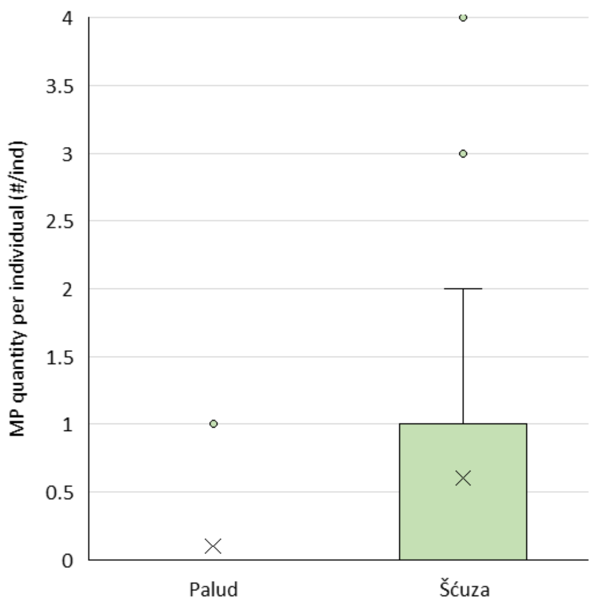
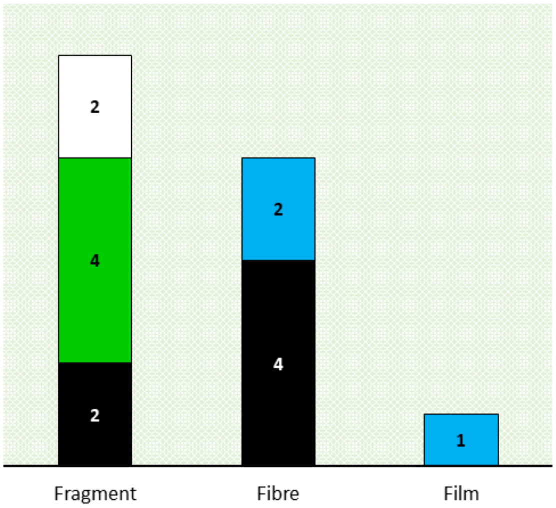


Figure 30. Microplastic abundance per individual.



MP Type	Quantity	Av. length (µm)
Fragments	8	294
Fibres	6	1127
Film	1	1000

Figure 31. Types and colours of microplastic particles isolated from blue crabs. Mean particle sizes are shown in the table on the right. The longest axis was measured and expressed in micrometres (µm).

Spectrometric analysis enabled identification of the synthetic polymer type for six microplastic particles, while the remaining nine produced spectra of poor quality with a low signal-to-noise ratio. Nevertheless, visual examination under the stereomicroscope confirmed that these particles were of anthropogenic origin (Figure 32). Three particles were identified as polyethylene terephthalate (PET) (two fibres and one fragment), two as polypropylene (PP) (one fragment and one film) and one as polystyrene (PS) (fragment).

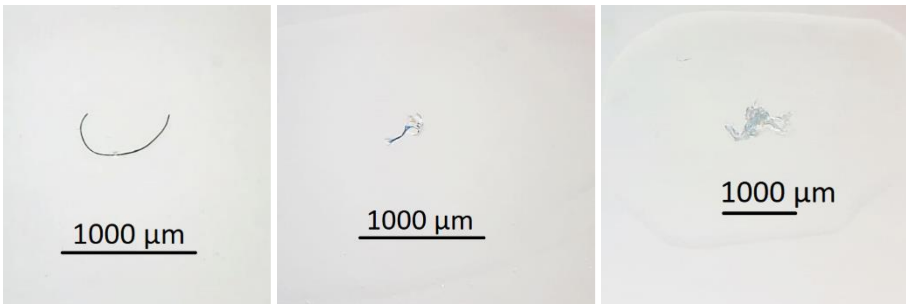


Figure 32. Microplastics from blue crabs under a stereomicroscope (samples CAL 5, CAL 14 and CAL 19).

Several recent studies have confirmed the presence of microplastics in blue crabs, with contamination levels varying depending on location and degree of environmental pressure. For example, a study from the Balearic Islands reported microplastics in 65.8% of individuals, with an average of approximately 1.4 particles per crab, where fibres dominated over fragments (Compa et al., 2023). In blue crabs from the Mar Menor Lagoon in Spain, Villoria-Calvo et al. (2026) reported substantially higher mean abundance (17.28 ± 2.90 particles per individual), with a predominance of fragments (87.7%) and polymers such as PP, PE and PET, whereas Renzi et al. (2020) in the Lesina Lagoon in Italy reported lower values (approximately 2.5 particles per individual) with both fibres and fragments present and a spatial distribution of particles among different tissues. In comparison with these studies, our results show a lower prevalence of contamination (20% of individuals) but a comparable number of particles per contaminated individual (~2 particles), with fragments also dominating over fibres. This pattern suggests a locally limited but present input of microplastics, likely strongly influenced by specific pollution sources and habitat characteristics, consistent with trends observed in other invasive blue crab populations.

The blue crab is an epibenthic, opportunistic and generalist predator feeding on a wide range of benthic organisms, including bivalves, other crustaceans, worms, small fish, detritus and plant material, with its diet strongly dependent on prey availability and environmental conditions (Gencer & Aguilar Vitorino, 2023). Due to this dietary flexibility, it plays an important role in benthic food webs, functioning both as predator and scavenger, while also serving as prey for larger fish and higher trophic levels, particularly in juvenile stages. In invaded areas, it can significantly affect prey community structure, including commercially important bivalves, leading to changes in local trophic relationships and competition with native species (Öndes et al., 2025). Given its position in the food web, microplastics accumulated in benthic and planktonic compartments may be transferred to higher trophic levels through feeding, including human consumption, as the blue crab is increasingly becoming a commercial and food species in the Mediterranean.

Blue crabs inhabiting areas distant from direct anthropogenic influences, such as roads and wastewater discharges, showed low levels of microplastic contamination in this study. Given their role as a seafood species, this suggests that the risk of microplastic intake through consumption is likely reduced in such areas. However, due to variability in contamination depending on location, caution remains warranted, especially for individuals collected from urban and potentially polluted environments.

Although blue crabs are often proposed as potential bioindicators of marine pollution due to their wide distribution and benthic lifestyle, their applicability in routine biomonitoring has certain limitations. Sample collection is logistically demanding, which can hinder standardised and repeatable sampling over time and space. In addition, the complexity of tissues and the presence of hard-to-digest components in the digestive system complicate laboratory processing. Due to these constraints, while they can provide useful information on local contamination, blue crabs are not an optimal bioindicator of microplastic pollution compared to filter-feeding organisms such as bivalves, which allow simpler, more standardised and more comparable monitoring approaches

3.3. MICROPLASTICS IN FISH

Two fish species were selected to assess the presence of microplastics in digestive tracts: **bluefish** and **boxlip mullet**. Bluefish is highly invasive in Istria and was selected to provide insight into microplastic occurrence in pelagic top predators without the need to remove native species from the environment, despite being relatively difficult to sample. In contrast, boxlip mullet is a native, non-invasive species commonly found in shallow coastal areas and feeds in a benthopelagic manner. The trophic level of bluefish is 4.5, whereas that of boxlip mullet is 2.8, indicating that bluefish occupies a significantly higher position in the food web and that boxlip mullet may represent a potential prey item.

A total of **30 digestive tracts per species** were analysed (N = 30), resulting in 60 individuals in total. Bluefish had an average standard length of approximately 32 cm, while boxlip mullet averaged around 13 cm (Table 6). Female individuals predominated in both species.

Table 6. Number of analysed individuals and biometric data (SE – standard error).

	Number of analysed individuals (N)	Mean standard length, SL ± SE (cm)	Mean total length, TL ± SE (cm)	Mass ± SE (g)	Sex (F, M, J)
Bluefish	30	32.3 ± 0.6	41.4 ± 0.8	72.1 ± 4.6	20 F, 10 M
Boxlip mullet	30	12.9 ± 0.2	16.1 ± 0.3	78.9 ± 5.8	17 F, 12 M, 1 J
Total	**60**				

For microplastic isolation via chemical digestion, a 10% KOH solution was used, and when necessary, 9% acetic acid was applied in cases where gastric contents contained calcite particles or shell fragments. In bluefish, digestion was often complete with very little undissolved residue, and in such cases the solution was filtered through a 50 µm pore-size filter. However, boxlip mullet stomachs frequently contained highly heterogeneous material (e.g. rock fragments, fish bones and invertebrate shells) that was not always fully digested. Therefore, a second 250 µm filter was placed above the 50 µm filter. This allowed larger and smaller particles to be separated onto two filters, which significantly facilitated microscopic examination.

In total, **123 microplastic particles** were isolated from **32 individuals of both species**. Bluefish contained considerably less microplastic than boxlip mullet (Figure 33). Of the 30 bluefish individuals, eight contained microplastics (**27%**), whereas 24 of 30 boxlip mullet individuals contained microplastics in their digestive tracts (**80%**) (Table 7). On average, boxlip mullet contained almost five microplastic particles per positive individual, while bluefish contained just over one particle per positive individual.

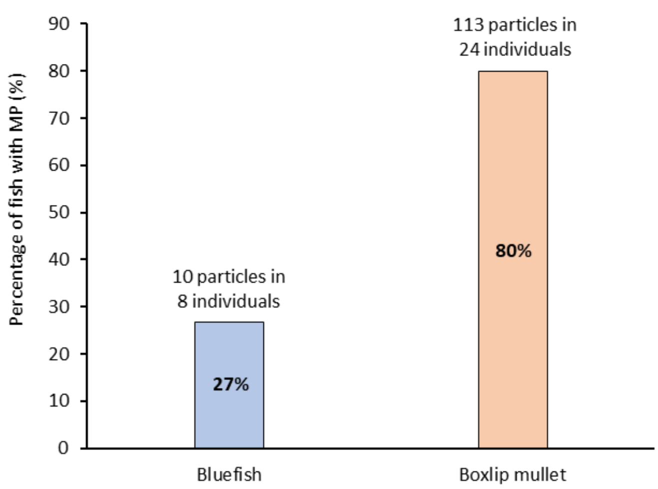


Figure 33. Presence of microplastic particles in analysed fish.

Table 7. Microplastic abundance isolated from the digestive tracts of bluefish and boxlip mullet.

	Number of individuals with microplastics (#)	Percentage of individuals with microplastics (%)	Number of microplastic particles (#)	Number of particles per individual (#MP/individual)
Bluefish	8	27	10	1.3
Boxlip mullet	24	80	113	4.7
Total	**32**	**53**	**123**	**3.8**

Statistical analysis was performed using the jamovi software package, applying a one-way ANOVA test to compare microplastic abundance between bluefish and boxlip mullet. The results indicated a **statistically significant difference between groups** ($p < 0.05$) (Figure 34).

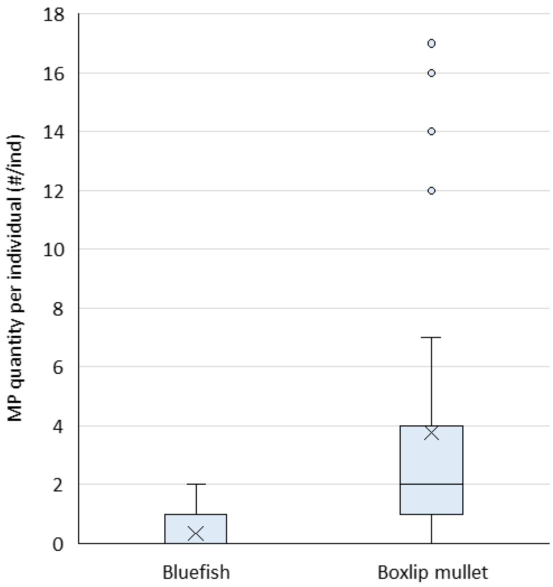


Figure 34. Microplastic abundance per individual.

Of the 123 isolated microplastic particles, more than half were **fragments** (Figure 35 and Table 8). In bluefish, **fibres** were predominant, whereas boxlip mullet digestive tracts mainly contained **fragments and films**, with fibres representing the smallest fraction (Figures 35 and 36). The dominant colours were **blue and white**.

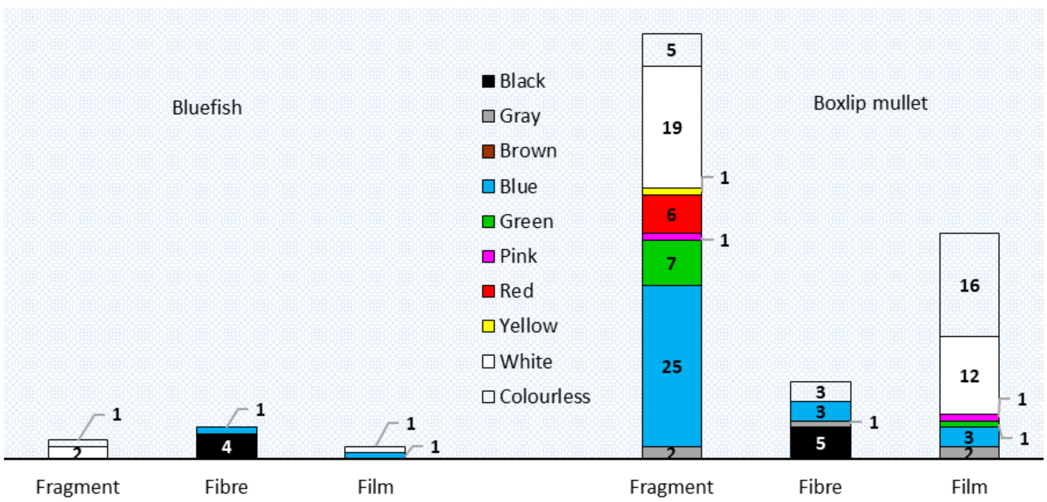


Figure 35. Types and colours of microplastic particles isolated from fish.

Table 8. Particle abundance and mean sizes. The longest axis was measured, expressed in micrometres (µm).

	Bluefish		Boxlip mullet	
MP Type	**Quantity**	**Av. length (µm)**	**Quantity**	**Av. length (µm)**
Fragments	3	435	66	381
Fibres	5	1200	12	1250
Film	2	1075	35	999

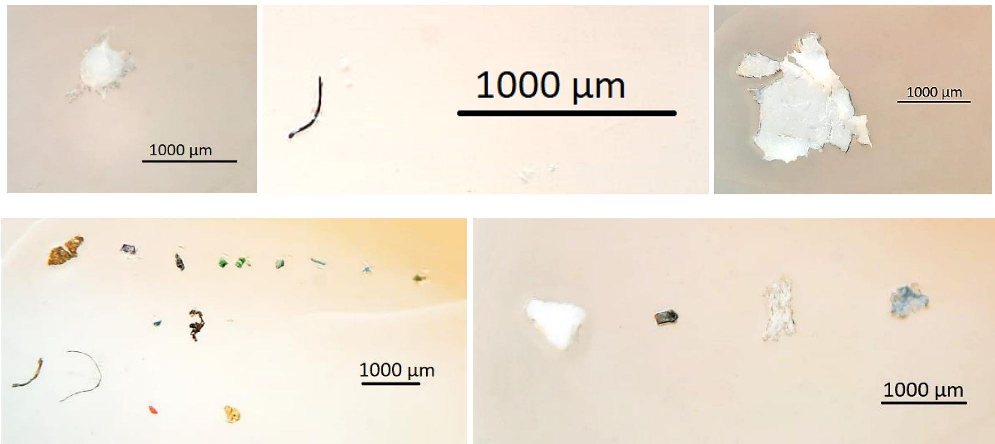


Figure 36. Fragments, fibres and films of microplastics found in the digestive tracts of bluefish (top) and boxlip mullet (bottom).

Raman analysis of isolated particles from fish was considerably more successful than in oysters and blue crabs. It was unsuccessful for three particles extracted from fish digestive tracts (one from bluefish and two from boxlip mullet). Since the obtained spectra were of low quality and visual inspection under a stereomicroscope did not allow unambiguous confirmation that the particles were plastic, these were excluded from further analysis. An additional three particles were identified as minerals calcite (two) and rutile (one) and were also excluded.

Of the remaining 10 particles from bluefish and 113 from boxlip mullet, the polymer type was determined for a total of 52 particles (three from bluefish and 49 from boxlip mullet). For the remaining particles, the spectra showed a low signal-to-noise ratio; however, visual inspection confirmed that they were anthropogenic synthetic material, and these were therefore classified as “synthetic polymer”. In nine cases, pigments rather than the polymer itself were identified, which is consistent with Azari et al. (2024), who noted that dyes and additives can obscure spectral polymer features and hinder identification. In addition, eight particles were lost during transfer from the filter to the microscope slide, although they had previously been confirmed as polymers under microscopic observation. Overall, a conservative approach was applied and only particles with a high level of certainty were classified as microplastics.

For particles in which the synthetic polymer type was determined by comparison with reference spectra from spectral libraries, PE and PP were found to be dominant, followed by PVC and PET (Figures 37 and 38). PE and PP are among the most frequently identified polymers in the environment, which is attributed to their widespread use in packaging, single-use products, textiles and various industrial applications, from which they enter the environment through improper waste disposal, surface runoff and the degradation of larger plastic items.

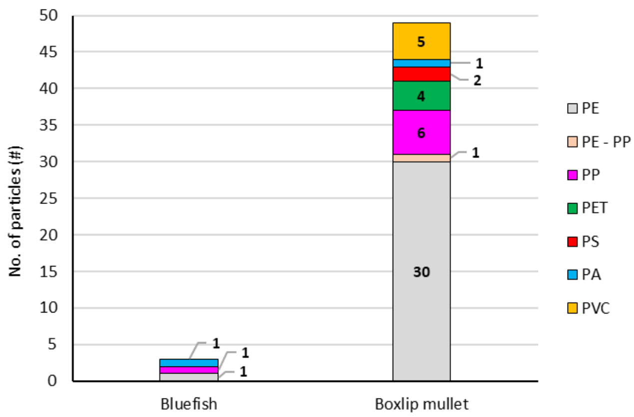


Figure 37. Synthetic polymers detected in the analysed fish.

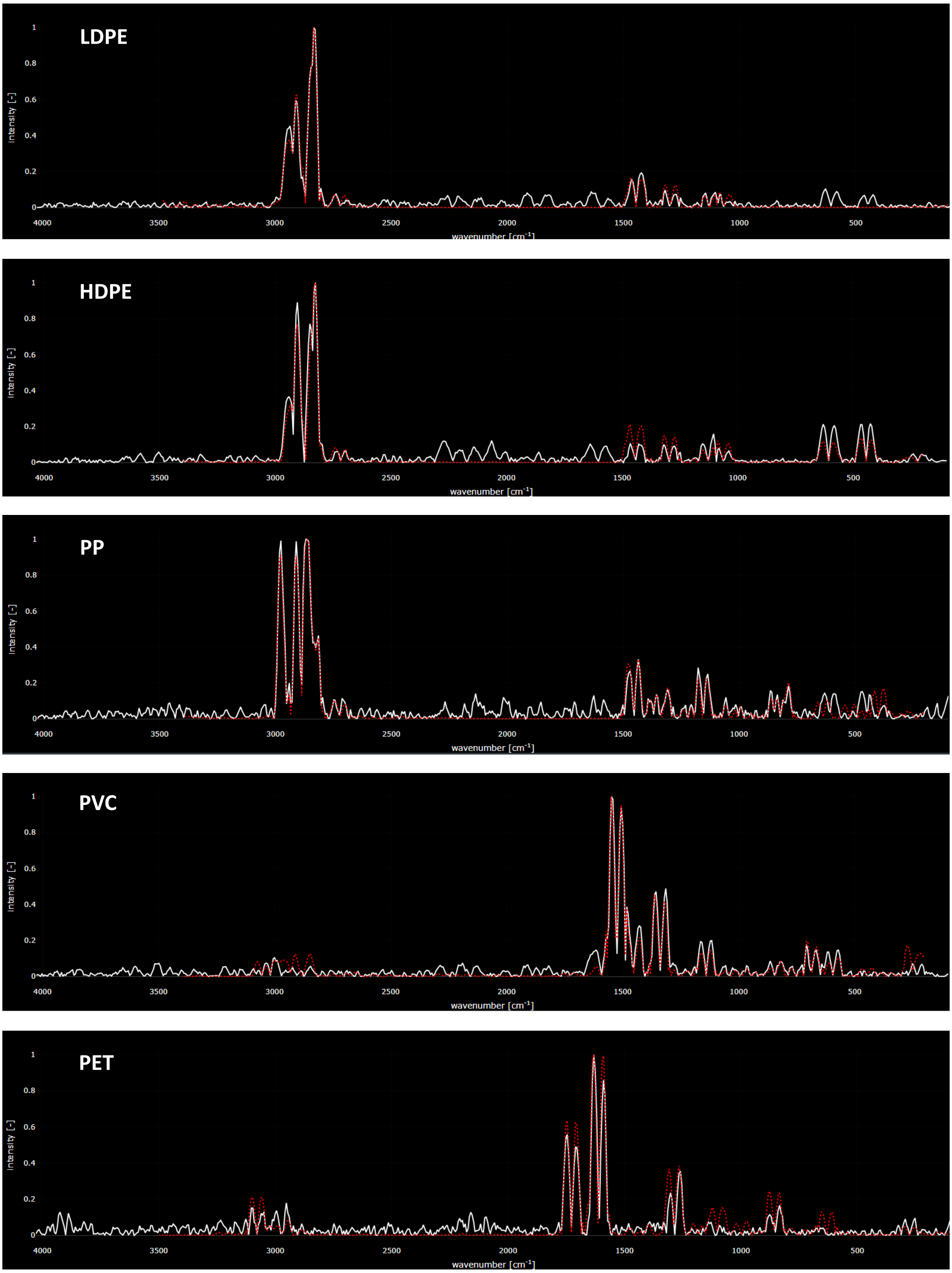


Figure 38. Raman spectra of particles isolated from bluefish and boxlip mullet with >90% match to the spectral library, from top to bottom: LDPE, HDPE, PP, PVC and PET. The white line represents the measured spectrum and the red line represents the reference library spectrum.

Currently, no studies are available on the presence of microplastics in boxlip mullet, making it impossible to directly compare our results with existing literature or to assess whether the observed high ingestion rate (80%) is typical for this species. In contrast, several studies exist for bluefish that allow contextualisation of the results. For example, Aytan et al. (2021) reported microplastics in two out of 17 examined bluefish individuals (12%), which is lower than the proportion observed in our study (27%). In Fıçıcılar et al. (2021), bluefish showed one of the higher ingestion rates, with 67% of individuals containing microplastics in their digestive tracts. In both studies, as well as in the present work, microfibres were the dominant particle type. These differences in microplastic occurrence among studies can be attributed to local environmental conditions and varying levels of anthropogenic pressure in the studied areas, while similarities in particle composition suggest shared sources of pollution in marine ecosystems.

The difference in microplastic prevalence between bluefish and boxlip mullet is most likely driven by differences in feeding strategy. Mullets feed by non-selectively grazing and suction-feeding from the seabed and water surface, thereby coming into direct contact with accumulated microplastics and ingesting higher amounts of environmental particles. In contrast, bluefish are active, selective predators that feed on mobile prey in the water column and are therefore less directly exposed to environmental microplastics. Although boxlip mullet may represent a potential prey item for bluefish, which introduces the possibility of secondary ingestion through the food web, the substantially higher microplastic prevalence in mullet compared to bluefish suggests that the dominant pathway of uptake is not trophic transfer but direct environmental exposure. These differences in feeding behaviour and microhabitat use likely explain the lower microplastic levels observed in bluefish despite their higher trophic position and also suggest that boxlip mullet were probably not represented as prey in the analysed bluefish individuals in this study.

Given the relatively low frequency and concentration of microplastics observed in bluefish in this study, this species can be considered an acceptable species for human consumption. Additionally, bluefish is an invasive species in many regions, including the study area, and its removal from the environment may have a positive ecological effect by reducing pressure on native communities. Therefore, consumption of bluefish may represent a potentially beneficial approach that simultaneously reduces human exposure to microplastics and contributes to the control of an invasive population in marine ecosystems.

For the purposes of microplastic biomonitoring, boxlip mullet appears to be a more suitable bioindicator organism than bluefish, despite the greater technical difficulty associated with processing its digestive tracts. Although sample preparation for mullet is more complex due to the diverse and often highly resistant stomach content, this species exhibits a higher and more stable occurrence of microplastics, thereby enabling more sensitive detection of local pollution levels. In contrast, bluefish are more difficult to sample and showed low levels of contamination in this study, which limits their informative value as a bioindicator. This approach is consistent with the recommendations of Valente et al. (2025), who emphasise that the selection of bioindicator species should be adapted to the local context. In this sense, boxlip mullet represents a better indicator of the actual availability of microplastics in the study area and is more suitable for future monitoring programmes compared to bluefish.

3.4. MACROPLASTICS IN SEABIRDS

Pellet analysis is one of the most widely used and well-established methods for studying the diet of shags, cormorants and sea gulls, as it enables non-invasive determination of prey composition and other ingested materials in seabirds (Barrett et al., 2007) (Figure 39). During a field survey of Levanić islet, where shags, cormorants and sea gulls are present, 45 pellets were collected, of which 10 were randomly selected for detailed analysis. Since plastic was found in all analysed pellets (100% occurrence), and its presence was also observed during field inspection of most remaining samples, it was concluded that the analysed material showed a high level of consistency. Therefore, **N = 10** was selected as a practical and sufficiently informative subsample for further detailed analysis.

Figure 39. Pellets of shags, cormorants and gulls in 6 × 6 cm dishes.

The contents of the pellets indicate that some samples likely belong to shags and cormorants, while others originate from sea gulls. Certain pellets contained highly diverse material such as olive stones, rock fragments, bones of terrestrial animals, glass, paper and plant material, whereas others consisted exclusively of fish remains, including bones and scales. Given that sea gulls have an opportunistic and highly generalist diet often associated with anthropogenic food sources, while cormorants feed predominantly on fish, we assumed that pellets containing exclusively fish remains belonged to cormorants, whereas those with more diverse contents were attributed to sea gulls (Barrett et al., 2007; Vieira et al., 2026; Cosolo et al., 2011).

The total mass of the analysed pellets was 46.65 g, while the mass of plastic was 1.91 g, corresponding to an average of 4.1% of the total pellet mass (Table 9). In some pellets, the mass fraction of plastic exceeded 30%. Regardless of whether the pellets originated from shags, cormorants or sea gulls, all contained plastic, with a minimum of one plastic item (shag or cormorant pellet) and a maximum of 59 (sea gull pellet). In total, 274 plastic items were extracted from 10 pellets, corresponding to an average of 27 items per pellet.

Table 9. Data on pellets and extracted plastic.

Sample	Pellet mass (g)	Plastic mass (g)	Percentage of plastic (%)	Number of plastic pieces (#)
LVNC 3 (sea gull)	2.9	0.09	3.1	9
LVNC 6 (shag/cormorant)	3.48	0.01	0.3	1
LVNC 13 (sea gull)	1.44	0.67	46.5	41
LVNC 17 (sea gull)	9.94	0.27	2.7	56
LVNC 24 (shag/cormorant)	3.61	0.12	3.3	16
LVNC 29 (sea gull)	14.3	0.17	1.2	32
LVNC 30 (sea gull)	0.67	0.23	34.3	15
LVNC 36 (sea gull)	1.75	0.06	3.4	19
LVNC 39 (sea gull)	1.28	0.08	6.3	26
LVNC 45 (sea gull)	7.28	0.21	2.9	59
Total (N = 10)	**46.65**	**1.91**	**4.1**	**274**

The most abundant type of plastic in the pellets was plastic film (Table 10). Of the total 274 plastic items, 204 were classified as film, most commonly in the form of fragments of thin plastic bags of various sizes. Fibres were also present, often occurring as entangled aggregates, while fragments, pieces of expanded plastics (such as polystyrene foam and sponge-like material) and plastic pellets were less frequent. The average size of all plastic items was 1.23 cm, with a range from 2 mm to 7 cm. The most common colours were white, black, green, blue and red and transparent plastic was also observed (Figure 40).

Table 10. Types of plastic isolated from seabird pellets.

Fragments	Film	Fibres	Plastic pellets	Foamed plastic	Total
21	204	37	1	11	**274**

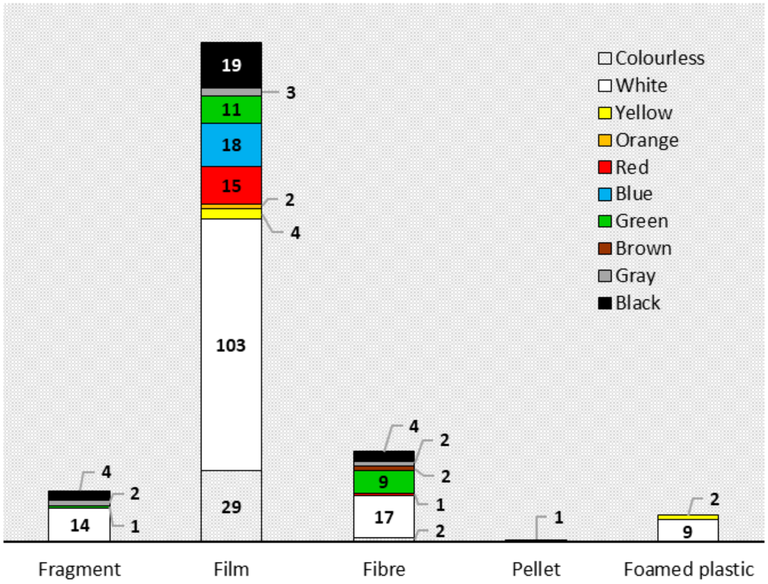


Figure 40. Type and colour of plastic from pellets.

Although the mass fraction of plastic in pellets is relatively low, its numerical abundance (Figures 41 and 42) indicates a high frequency of ingestion. Each individual plastic item represents non-digestible and non-caloric material that occupies space in the digestive system and may displace nutritionally valuable food, which under conditions of high ingestion frequency may potentially lead to energy deficiency and reduced body condition in individuals.

In shags, cormorants and sea gulls, the frequency of pellet formation and regurgitation varies and depends on prey size and type. Russell et al. (1995) found that marine cormorants regurgitate pellets at intervals ranging from 1 to 7 days, with an average of 3.7 days, corresponding to approximately 89 hours. For sea gulls, literature data are available on food retention time in the digestive system rather than pellet formation. Hilton et al. (1998) report that food retention time ranges between 4 and 7 hours, depending on meal size. In addition, prey such as fish and squid is digested significantly faster than crustaceans and other organisms with hard exoskeletons or shells. For illustrative purposes, if an average of eight plastic items per pellet is considered and shags/cormorants produce pellets approximately every 89 hours, this corresponds to an average ingestion rate of one plastic item every 11 hours. In sea gulls, ingestion is likely considerably more frequent. Assuming an average of 32 plastic items over a food retention period of 4 to 7 hours, this corresponds to approximately 5 to 8 plastic items ingested per hour. Such rates suggest continuous and chronic exposure to plastic rather than isolated ingestion events.

As plastics and other pollutants accumulate in the environment and natural food resources become increasingly limited, wild populations will more frequently rely on anthropogenic food sources (Nono Almeida et al., 2023).

Seabirds were among the first marine organisms in which plastic was documented in the late 1960s (Rothstein, 1973) and since then a large body of literature has reported this phenomenon on a global scale.

High rates of plastic ingestion have been recorded in both sea gulls and shags/cormorants. For example, 84% of pellets from the Australasian Pacific gull (Stewart et al., 2020) and 84% of sea gull pellets from France (Nono Almeida et al., 2023) contained plastic. Nono Almeida et al. (2023) extracted 284 plastic items from 143 pellets, predominantly film, which is comparable to our findings, albeit based on a substantially larger sample size.

Figure 41. Pellet content: top row – a sea gull pellet containing 32 plastic bag fragments and 9 fibres and fibrous plastic pieces. The lower row shows natural material consisting of paper and plant material.
Bottom – a sea gull pellet containing 43 plastic bag fragments, 9 fibres and fibrous plastic pieces, 3 fragments and 1 polystyrene bead. The lower row contains olive stones, a terrestrial animal bone and paper material.

Figure 42. Content of a shag or cormorant pellet containing 16 plastic bag fragments and fish remains.

Maaseide et al. (2024) analysed faeces of European shags in Norway and detected microplastics in 69% of samples, with most particles smaller than 300 µm. Álvarez et al. (2018) reported a high proportion of microfibres in 63% of shag pellets. In a more recent study, Santín & Álvarez (2026) also confirmed a high prevalence of microfibres, with a total of 521 fibres in 49 analysed pellets. However, studies such as Álvarez et al. (2018) and Maaseide et al. (2024) carry an increased risk of sample contamination by airborne fibres during collection and laboratory processing, while Santín & Álvarez (2026) also report that procedural blanks were not performed.

In several sea gull pellets from Levanić, expanded polystyrene (Styrofoam) was also found. Bearzi et al. (2022) describe how sea gulls in the western Adriatic occasionally peck at polystyrene buoys used by fishers, causing mechanical damage. This behaviour is mostly recorded in winter, varies between years, and the economic damage to fisheries is relatively small (approximately 3–4 euros per buoy); however, it contributes to the generation of additional microplastics entering the marine environment. The cause of this behaviour remains unclear and the authors highlight the need for further monitoring due to potential impacts on birds and the marine environment.

While shags and cormorants feed exclusively at sea and may accidentally ingest plastic as part of their prey, in our study sea gulls were identified as vectors transferring plastic from land to the marine environment. Their pellets contained numerous terrestrial items such as glass, fabric, rock fragments and olive stones. This suggests that sea gulls frequently ingest anthropogenic materials on land and subsequently introduce plastic and other waste into the marine environment through pellet egestion.

In addition to direct environmental ingestion, plastic can enter seabirds via trophic transfer through consumption of contaminated prey, as experimentally demonstrated in several predator–prey systems (Farrell & Nelson, 2013) and field studies (Markić et al., 2018). However, our analyses could not confirm such transfer, i.e. biomagnification, with certainty. For example, one shag/cormorant pellet contained 16 fragments of plastic bag material, but this is more likely the result of direct ingestion rather than secondary uptake through prey.

Analysis of individual carcasses alone may not always detect plastic, as shags, cormorants and sea gulls regularly regurgitate pellets. For instance, Benjaminsen et al. (2024) found no plastic in the digestive tracts of great cormorants and European shags in Norway obtained as fishery bycatch. In our study, we did not analyse live individuals and we therefore suggest that pellet analysis alone is sufficient for routine monitoring of plastic contamination in seabirds that produce pellets. This approach is considerably less time-consuming, more cost-effective and more practical for long-term monitoring programmes.

4. CONCLUSION

The results of the study "*Presence of Microplastics in the Food Web and Macroplastics in Seabirds – WASTEREDUCE Project*" demonstrate that micro- and macroplastic pollution is widely integrated into marine food webs, with pronounced differences among trophic groups but without a clear linear relationship between trophic level and the amount of ingested plastic. The highest level of microplastic contamination was recorded in fish, while the lowest was observed in crabs.

Pacific oysters confirm their role as sensitive bioindicators, despite not representing the group with the highest frequency of microplastic ingestion in this study (34%). Their value as organisms suitable for monitoring plastic pollution lies in their stable filtration-based exposure and their ability to integrate local pollution signals, making them reliable indicators of spatial differences in contamination, particularly between urban and protected areas.

Blue crabs showed a moderate and locally variable presence of microplastics (20% of individuals), with a clear influence of local anthropogenic pressure. Crabs function as benthic opportunists and their exposure to microplastics arises from contact with sediments and feeding on detritus and benthic organisms. Since blue crab sampling and laboratory processing are technically demanding, we do not consider them an ideal organism for monitoring environmental conditions with respect to plastic pollution.

The boxlip mullet showed an exceptionally high frequency of ingestion (80% of individuals), whereas the frequency in bluefish was considerably lower (27%). This difference clearly indicates the dominant role of feeding strategy in determining exposure to microplastics. Mullets, as benthopelagic and non-selective feeders, continuously ingest particles from sediments and the sea surface layer where microplastics accumulate. Bluefish, as active pelagic predators occupying a higher trophic level, showed lower frequency and abundance of microplastics, suggesting that an increase in trophic level does not necessarily result in greater particle accumulation or biomagnification. Due to its non-selective feeding behaviour, we consider the boxlip mullet to be a more suitable indicator of plastic pollution in a given area.

Seabirds exhibited the most extreme pattern of plastic accumulation at the macroplastic level. Pellets contained high quantities of plastic particles, predominantly films, indicating intensive and repeated ingestion of macroplastics. In this case, the dominant mechanism of intake is not trophic transfer but direct consumption of anthropogenic waste, with birds additionally acting as vectors transporting waste between terrestrial and marine systems.

In the context of trophic transfer and biomagnification, our results do not support classical biomagnification of microplastics through food webs. Instead, the observed distribution pattern suggests that exposure is primarily determined by ecological niche and feeding strategy, while trophic level has a secondary or even limited influence. This confirms that microplastics do not follow typical biomagnification patterns characteristic of persistent organic pollutants, although the transfer of associated chemicals and additives through food webs remains possible.

For biomonitoring purposes, the results clearly indicate that filter-feeding bivalves and some fish species, particularly benthopelagic species such as the boxlip mullet, are informative indicators of microplastic availability in coastal ecosystems. In contrast, blue crabs have limited value as bioindicators due to difficulties associated with sampling and laboratory processing. Seabirds, through pellet analysis, represent a sensitive indicator of macroplastic pollution.

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Note:
Illustrations of *Callinectes sapidus*, *Magallana gigas* and *Pomatomus saltatrix* were generated using ChatGPT, while the illustration of *Oedalechilus labeo* was obtained from Colapisci.it
https://www.colapisci.it/pescitalia/pisces/Mugiliformes/Mugilidi/CefaloLabbrone.htm
Tide data were obtained from the following website: https://www.adip.hr/servisi.html
Satellite images of the sampling locations were obtained from Google Earth.