ACCOBAMS-SC17/2026/Inf05

IMPLEMENTING ACCOBAMS BEST PRACTICES IN POST-MORTEM INVESTIGATIONS ON STRANDED AND BY-CAUGHT CETACEANS FROM ROMANIAN SHORE AND INGESTED MARINE LITTER MONITORING

Note of the Secretariat

At their First Meeting (Monaco, 28 February – 2 March 2002), and as provided by Article IX, paragraph 3, of the Agreement, the Parties to ACCOBAMS established a Supplementary Conservation Grants Fund (SCF) from voluntary contributions of Parties or from any other source in order to increase the funds available for monitoring, research, training and projects relating to the conservation of cetaceans.

Since 2011, projects to be funded under the SCF were selected through calls for proposals launched by the Secretariat, in consultation with the Bureau of the Parties, and following the scientific evaluation made by the Scientific Committee of ACCOBAMS.

The present report is the final report of a project selected from the 2022 call for proposals under the Supplementary Conservation Fund.

Accord sur la Conservation des Cétacés de la Mer Noire, de la Méditerranée et de la zone Atlantique adjacente

ACCOBAMS

Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and contiguous Atlantic Area

FINAL REPORT

Implementing ACCOBAMS best practices in post-mortem investigations on stranded and by-caught cetaceans from Romanian shore and ingested marine litter monitoring (PONTICCET)

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National Institute for Marine Research and Development "Grigore Antipa"

Date
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FINAL REPORT

"Implementing ACCOBAMS best practices in post-mortem investigations on stranded and by-caught cetaceans from Romanian shore and ingested marine litter monitoring" (PONTICCET)

Study funded by:

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Contents

Abbreviations and Acronyms

ACCOBAMS - Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and contiguous Atlantic area;

ASCOBANS - Agreement on the Conservation of Small Cetaceans of the Baltic, North East Atlantic, Irish and North Seas;

NIMRD – National Institute for Marine Research and Development ”Grigore Antipa”;

GFCM - General Fisheries Commission for the Mediterranean;

MOP – Meeting of the Parties;

GIT – gastrointestinal tract;

UAV- Unmanned Aerial Vehicle;

GNS - set gillnets;

DCC – decomposition condition category;

MSFD- Marine Strategy Framework Directive (2008/56/EC);

UASVM- University of Agronomic Sciences and Veterinary Medicine of Bucharest;

D10- Descriptor 10 - Marine Litter;

LiFE DELFI - Dolphin Experience: Lowering Fishing Interactions.

1. Context of the Project

In the Black Sea, three cetacean species—*Delphinus delphis ponticus* (common dolphin), *Tursiops truncatus ponticus* (bottlenose dolphin), and *Phocoena phocoena relicta* (harbour porpoise)—are recognized as endemic with genetic distinctions from Mediterranean populations.

While all three species share overlapping habitats, harbour porpoises and bottlenose dolphins are primarily associated with the circumlittoral area over the continental shelf, while the common dolphin is mainly found in the open sea with circumlittoral areas as a secondary habitat. Their ranges encompass the entire Black Sea. Harbour porpoises can be found in the Marmara Sea, Kerch Strait, and the Azov Sea. Common dolphins are found in the Marmara Sea, though absent in the Azov Sea, and are sporadically sighted in the Kerch Strait. Bottlenose dolphins also inhabit the Marmara Sea, the Kerch Strait, and the waters of the Azov Sea close to the Kerch Strait.

The number of cetaceans significantly declined in the 20th century due to large-scale commercial hunting, capturing dolphins for various purposes. Hunting activities undertaken by the riparian countries of the Black Sea ceased in 1983. Cetaceans were systematically captured for the extraction of raw materials essential to produce various commodities such as oils, paints, adhesives, varnishes, food, medicines, soaps, cosmetics, leather, and fertilizers. Additionally, dolphins were captured for confinement, with dolphinariums operating along the Black Sea coast since 1966.

Treats affecting the Black Sea cetaceans include industrial fishing, poaching, accidental deaths in fishing nets, alien species invasions, epidemics, and sea pollution. One of the major threats affecting marine mammals at the global level, and currently understudied in the Black Sea, is represented by marine litter, especially microplastics. Marine litter is a global pollution problem affecting thousands of marine species, hurting marine wildlife primarily due to ingestion and entanglement. Plastic and other marine debris have been found in the gastrointestinal tracts of cetaceans, likely to cause impairment to digestive processes and even death (Marino et al., 2012).

PONTICCET project tackles cetacean plastic ingestion, wishing to contribute to a better understanding of this threat (i.e., ingested marine litter) and generate new and valuable scientific knowledge through research, taking into consideration that this issue has a high priority for the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and contiguous Atlantic area (ACCOBAMS) Conservation Plan and the Strategic Action Plan for the Rehabilitation and Protection of the Black Sea. The project objectives are strongly related to the negative impacts of marine litter on cetaceans, through monitoring ingested marine litter during necropsies, also a priority in the ACCOBAMS Area (ACCOBAMS Resolution 7.15[1]). Evaluating and addressing threats like marine litter is a key part of the ACCOBAMS objectives and is relevant to the ACCOBAMS Conservation Plan (ACCOBAMS Resolution 7.15[1]).

The overall objective of the PONTICCET project is to establish an operational mechanism for post-mortem investigation of stranded/ by-caught cetaceans at the Romanian shore of the Black Sea following ACCOBAMS/ASCOBANS Best Practices, to assess ingested marine litter and improve knowledge regarding marine litter, one of the major threats for cetaceans.

[1] https://accobams.org/wp-content/uploads/2019/12/Res.7.15_Assessing-Marine-Litter-impacts-on-cetaceans.pdf

The project has six specific objectives:

Objective 1. Setting up a laboratory with the necessary equipment and putting together a trained team for necropsies and gastrointestinal tract (GIT) content analysis.

Objective 2. Performing necropsies following "ACCOBAMS/ASCOBANS Best Practices on cetacean post-mortem investigation and tissue sampling" to all stranded/ by-caught cetaceans and analysing the (GIT) content for marine litter.

Objective 3. Improving knowledge by creating a common database with strandings, by-catches and ingested marine litter (macro-, meso-, micro-litter).

Objective 4. Identifying potential hotspot areas for cetacean ingestion of marine litter.

Objective 5. Raising awareness through the dissemination of the project results.

Objective 6. Updating the National Action Plan for the Conservation of Cetaceans from the Romanian Black Sea waters.

2. Activities carried out during the reporting period

2.1. Strandings monitoring

Strandings monitoring was undertaken along the Black Sea Romanian coastline, between February 2023 and April 2024.

The objectives of the field trips were the collection of biometric data from stranded cetaceans and the collection of the GIT for the analysis of ingested plastics.

In this scope, the National Institute for Marine Research and Development "Grigore Antipa"'s (NIMRD) research team made observations in the field (Figure 1).

Figure 1 Field observations

Furthermore, an unmanned aerial vehicle (UAV) DJI Mavic 2 PRO was used for the aerial surveilling of the hard-to-reach areas of the coastline (Figure 2).

Figure 2 Aerial surveilling of the hard-to-reach areas of the coastline

Examination protocols included external measurements, photographs, and observations of all stranded cetaceans. According to the accessibility and the decomposition condition category (DCC) of the carcasses, the GIT was regularly but not systematically sampled. A Stranded Cetacean Report Form (APPENDIX 1) was filled out for each stranding case.

Each monitoring campaign had a duration of 3 days from south to north (Figure 3, Figure 4) after the following itinerary:

Day 1. Terrestrial observations on the coastal sector between Vama Veche – Port Agigea (Vama Veche, 2 Mai, Mangalia, Saturn, Venus, Jupiter, Neptun, Olimp, Schitu, Costinești, Tuzla, Eforie Sud, Eforie Nord, Port Agigea).

Day 2. Terrestrial observations on the coastal sector between Gura Portiței-Port Midia (Gura Portiței, Grind Chituc, Vadu, Gura Buhaz, Corbu, Port Midia).

Day 3. Terrestrial observations on the coastal sector between Port Tomis – Navodari Beach (Port Tomis, Modern Beach, North Faleza, Reyna Beach, Malibu Beach, Casino Mamaia, Vega Beach, H2O Beach, Năvodari Beach).

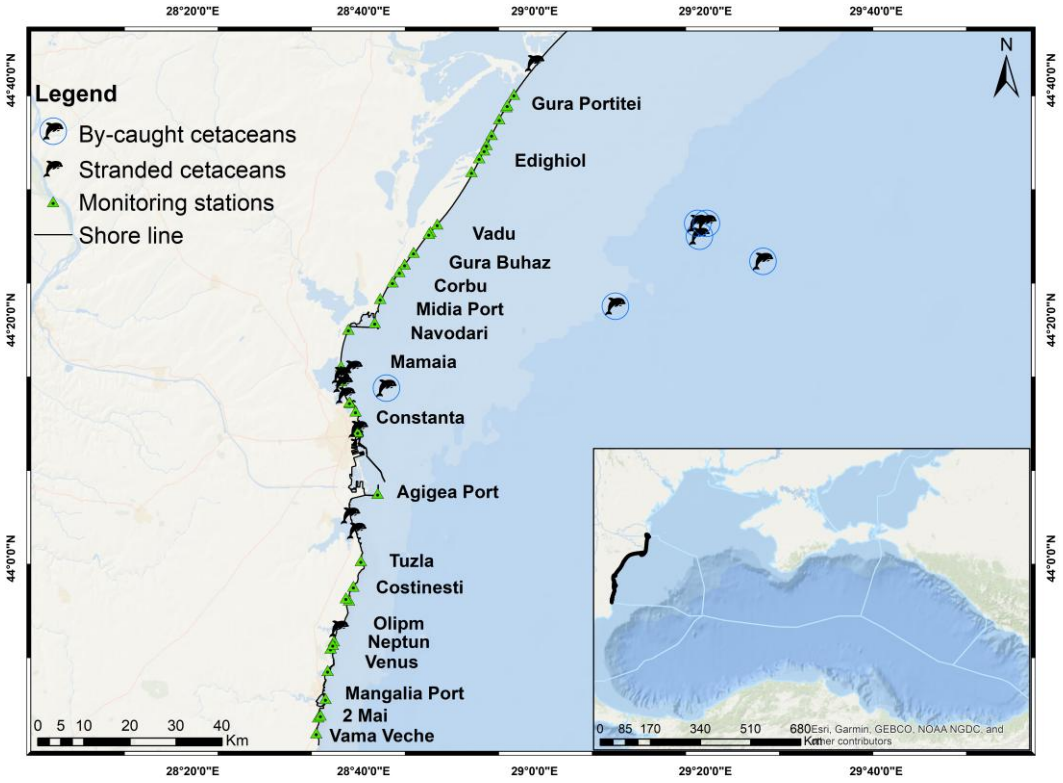

Figure 3 Field observations itinerary

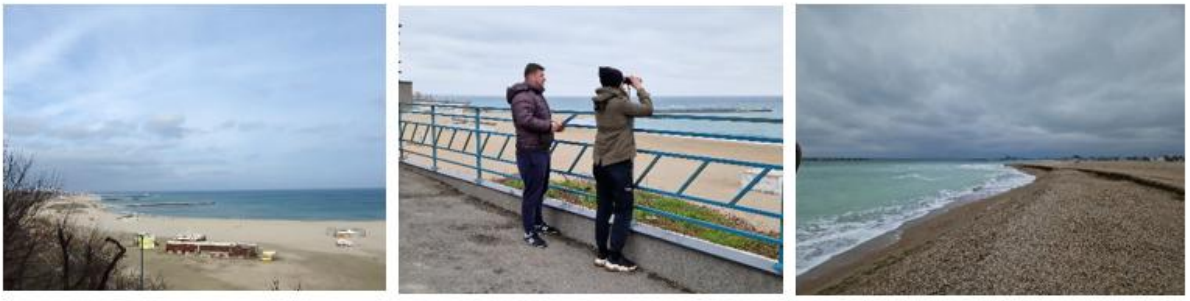

Figure 4 Observations during field monitoring campaigns

In total, 12 monitoring campaigns were made since the beginning of the project, in the following periods:

- ✓ 1st Monitoring campaign - 27-28 February - 1st March 2023;
- ✓ 2nd Monitoring campaign - 29-31 March 2023;
- ✓ 3rd Monitoring campaign - 11-12 April 2023;
- ✓ 4th Monitoring campaign - 27-30 June 2023;
- ✓ 5th Monitoring campaign - 19-20, 25 July 2023;
- ✓ 6th Monitoring campaign - 27-29 September 2023;
- ✓ 7th Monitoring campaign - 19-20, 27 October 2023;
- ✓ 8th Monitoring campaign - 6-7, 11 December 2023;
- ✓ 9th Monitoring campaign - 17, 19, 22 January 2024;
- ✓ 10th Monitoring campaign - 16, 19, 20 February 2024;
- ✓ 11th Monitoring campaign - 27-29 March 2024;
- ✓ 12th Monitoring campaign - 18-19, 22 April 2024.

In addition to the monitoring campaigns, the team responded to reports of the citizens who observed stranded cetaceans on the beach.

Since the beginning of the project, the research team responded to nine reports of cetacean strandings made by the citizens. Of these nine stranding reports, only in two cases, it was possible to collect the GIT, for the other seven cases only the external examination was made and the basic morphometric data were collected due to advanced DCC (Code 5) or the presence of penetrating wounds in the abdomen and the GIT.

The first report of a stranded cetacean was received via social media on the 4th of April 2023 when a citizen reported a stranded bottlenose dolphin (ID: PCETSTR040423#1) on Flamingo Beach, Eforie Sud city. On the 18th of April 2023, another citizen reported a stranded bottlenose dolphin (ID: PCETSTR180423#2) at Pescarie Mamaia, Constanta city. The third stranded cetacean, a bottlenose dolphin (ID: PCETSTR260423#3), was reported on the 26th of April 2023 at Flora Beach, Constanta city. The fourth stranded cetacean, a harbour porpoise (ID: PCETSTR090523#4), was reported on 9th May 2023 on Modern Beach, Constanta city. The fifth stranded cetacean, a bottlenose dolphin (ID: PCETSTR220523#5), reported by a citizen was found stranded on Iaky Beach, Constanta City on the 22nd of May 2023. The sixth stranded cetacean reported by a citizen, a bottlenose dolphin (ID:

PCETSTR270623#6), was found at Cape Tuzla, Olimp resort, Constanta city on 27th June 2023. The seventh stranded cetacean (PCETSTR200723#7), bottlenose dolphin, was found at Gura Portitei on 20th July 2023. The eight cetacean (PCETSTR021023#8), a harbour porpoise, was found at Belona beach in Eforie Nord city on 2nd October 2023. The last stranded cetacean (PCETSTR110324#9), a common dolphin, was found on the Vega beach on 11th March 2024.

ID CODE PCETSTR040423#1: Adult female bottlenose dolphin (*T. t. ponticus*), 194 cm total body length, 81 cm girth, DCC 2, found stranded on the beach on 4th April 2023 (Figure 5). The GIT was collected and stored at -20°C for further content analysis of ingested microplastics.

Figure 5 Bottlenose dolphin PCTESTR040423#1

ID CODE PCETSTR180423#2: Adult male bottlenose dolphin (*T. t. ponticus*), 238 cm total body length, 134 cm girth, DCC 3 (Figure 6), found stranded on the beach on 18th April 2023. The GIT and stored at -20°C for further content analysis of ingested microplastics.

Figure 6 Bottlenose dolphin PCETSTR180423#2

ID CODE PCETSTR260423#3: Adult bottlenose dolphin (*T. t. ponticus*), 238 cm total body length, 134 cm girth, DCC 3, found stranded on the beach on 26th April 2023 (Figure 7). Due to penetrating wounds in the abdomen and infection present in the genital area, sex could not be determined and the GIT was not collected for further analysis of the contents.

Figure 7 Bottlenose dolphin PCETSTR260423#3

ID CODE PCETSTR090523#4: Adult harbour porpoise (*P. p. relicta*), 147 cm total body length, 100 cm girth, DCC 3, found stranded on the beach on 9th May 2023 (Figure 8). Gender could not be determined and the GIT was not collected for further content analysis.

Figure 8 Harbour porpoise PCETSTR090523#4

ID CODE PCETSTR220523#5: Newborn male bottlenose dolphin (*T. t. ponticus*), 117 cm total body length, 40 cm girth, DCC 4 (Figure 9), found stranded on the beach on 22nd May 2023. The GIT was not collected for further content analysis.

Figure 9 Bottlenose dolphin PCETSTR220523#5

ID CODE PCETSTR270623#6: Bottlenose dolphin (*T.t. ponticus*), DCC 5, found stranded on the beach at Cape Tuzla, Olimp resort, on 27th June 2023 (Figure 10).

Figure 10 Bottlenose dolphin PCETSTR270623#6

ID CODE PCETSTR200723#7: Bottlenose dolphin (*T.t. ponticus*), DCC 5, found stranded on the beach at Gura Portiței, on 20th July 2023 (Figure 11).

Figure 11 Bottlenose dolphin PCETSTR200723#7

ID CODE PCETSTR021023#8: Adult harbour porpoise (*P. p. relicta*), 106 cm total body length (without tail fluke), 100 cm girth, DCC 4 (Figure 12), found stranded on the beach on 2nd October 2023. The GIT could not be collected for further analysis of microplastics.

Figure 12 Harbour porpoise PCETSTR021023#8

ID CODE PCETSTR110324#9: Adult common dolphin (*D.d. delphis*), 171 cm total body lenght, 104 cm girth, DCC 3 (Figure 13), found stranded on the Vega beach on 11 March 2024. The GIT could not be collected.

Figure 13 Common dolphin PCETSTR110324#9

2.2. Onboard observations

Two trips for onboard observations on a gill netter were initially planned to be conducted during the project. Subsequently, two more trips were contracted. All observations were made onboard a Romanian fishing vessel of 25.3 m long and 129 gross tonnages. As per the terms outlined in the contract, the identity of both the company and the fishing vessel will remain confidential.

Throughout these trips, meticulous data acquisition was conducted by the NIMRD observers following the stipulations outlined in the document titled "Monitoring the incidental catch of vulnerable species in the Mediterranean and the Black Sea fisheries: Methodology for data collection" (FAO, 2019). A comprehensive On-board Observation Form (APPENDIX *2*) was diligently completed for each expedition.

PCETGN020323

The first trip was performed on 02.03.2023. During the observations, 12,000 m of GNS were recovered after a soak time of 20 days (GNS were deployed on the 11th of January 2023). The work area was in front of Midia Harbour at 53 m water depth. The main capture was the Black Sea turbot (*Scophthalmus maeoticus*) (Figure 14). The bycaught species were spiny dogfish (*Squalus acanthias*) and thornback rays (*Raja clavate*). On the nets were attached various sessile benthic invertebrates (*Mytilus galloprovincialis*, *Spisula subtruncata*, *Ascidiella adspersa*), and very rare living individuals of *Modiolula phaseolina*.

Numerous items of plastic litter, predominantly in the form of plastic bags, were brought onboard during operational activities (Figure 15).

During the first trip, no cetacean was by-caught in the 12 000 m GNS recovered.

Figure 14 Set gillnets for turbot 02.03.2023

Figure 15 Marine litter brought onboard by nets

PCETGN140323

The second trip was performed on 14.03.2023. During the observations, 11,000 m of GNS were recovered after a soak time of 25 days. The work area was in front of Constanta city at 50 m water depth. Recovery of the 110 GNS (100 m each), started from North to South at 08:00 am and ended at 3:00 pm. The main capture was the Black Sea turbot (*Scophthalmus maeoticus*). The bycaught species were spiny dogfish (*Squalus acanthias*) and thornback rays (*Raja clavate*).

Many items of plastic litter were brought on board during operations.

During the trip, one bottlenose dolphin (*T. t. ponticus*) was accidentally captured (ID: PCETGN140223#1) (Figure 16). The cetacean was brought onboard and examined by the NIMRD observer who concluded that the animal was already dead by the time the nets were recovered. For safety reasons, the carcass was measured, photographed and labelled onshore (Figure 17). After all morphometric data were collected it was transported to the NIMRD headquarters and frozen for future analysis (GIT sample).

Figure 16 Bottlenose dolphin (T.t. ponticus) bycaught on 14.03.2023

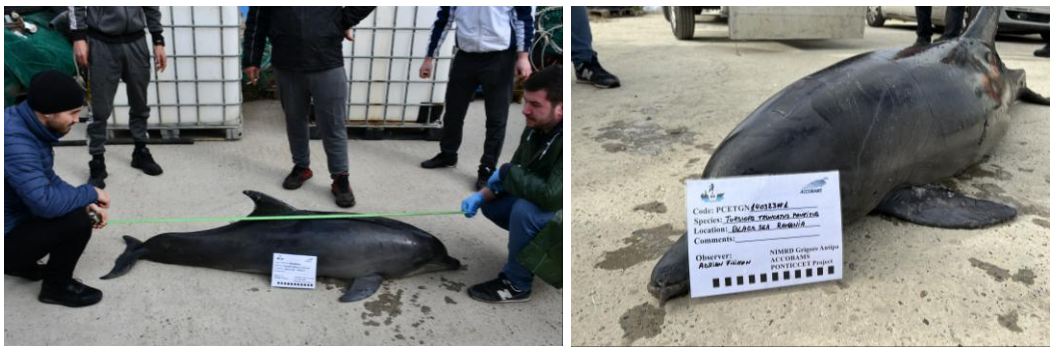

Figure 17 Data collection from bycaught Bottlenose dolphin

PCETGN090423

After the first two trips contracted activities were carried out, and due to good cooperation, the fishing company accepted one NIMRD observer onboard for one more trip. During the observations, 12,000 m of GNS were recovered after a soak time of 25 days. The work area was in front of Corbu at 54 m water depth. The main capture was the Black Sea turbot (*Scophthalmus maeoticus*). The bycaught species were spiny dogfish (*Squalus acanthias*) and thornback rays (*Raja clavate*).

Again, marine litter was observed.

A harbour porpoise (*P. p. relicta*) was accidentally caught in the fishing nets (Figure 18). The cetacean was brought onboard where the NIMRD expert concluded that by the time the nets were recovered the animal was already dead. The data collection was carried out onshore and the carcass was transported to NIMRD laboratory and frozen for subsequent analysis.

Figure 18 Harbour porpoise (P.p. relicta) bycaught on 09.04.2023

On 11th March 2024, the fishing company notified the team members regarding a dead harbour porpoise (*P.p. relicta*), a male juvenile of 93 cm length and 64 cm girth (ID CODE PCETGN110324#3), accidentally caught during their fishing operations (Figure 19). The work area was in front of Midia Harbour. The data collection was carried out onshore and the carcass was transported to the NIMRD laboratory and stored at -20˚C for subsequent analysis.

Figure 19 Harbour porpoise PCETGN110324#3 (P.p. relicta) bycaught on 11.03.2024

PCETGN180324

On 18 March 2024, during onboard observations, an adult female harbour porpoise (*P. p. relicta*) was accidentally caught in the fishing nets (PCETGN180324#4) (Figure 20). The work area was in front of Midia Harbour at 50 m water depth. During the observations, 20,000 m of GNS were recovered. The dead cetacean was brought onboard for investigations. The data collection was carried out onshore and the carcass was transported to NIMRD laboratory and stored at -20˚C for subsequent analysis.

Figure 20 Harbour porpoise PCETGN180324#4 (P.p. relicta) bycaught on 18.03.2024

On 18 March 2024, the NIMRD team was notified by a fishing company that another juvenile male harbour porpoise (*P. p. relicta*) was accidentally caught in the fishing nets (PCETGN180324#5) (Figure 21). The dead cetacean was brought onshore for investigations. The data collection was carried out onshore and the carcass was transported to the NIMRD laboratory and stored at -20˚C for subsequent analysis.

Figure 21 Harbour porpoise PCETGN180324#5 (P.p. relicta) bycaught on 18.03.2024

PCETGN040424

On 4th April 2024, an adult female harbour porpoise (*P. p. relicta*) was accidentally caught in the fishing nets (PCETGN040424#6) (Figure 22). The carcass was brought onshore and transported to the NIMRD laboratory and stored at -20˚C for subsequent analysis.

Figure 22 Harbour porpoise PCETGN040424#6 (P.p. relicta) bycaught on 04.04.2024

2.3. Endowment for necropsy and GIT content analysis

The main equipment for the necropsy laboratory consists of a stainless steel autopsy table and a multi-sieves system for the GIT content analysis. The sieving system was built according to Corazzolla et al. (Corazzola et al., 2021) (Figure 23). The laboratory was also equipped will all necessary tools for necropsy and and GIT sample processing (dissection tools, glass labware, protection equipments).

Figure 23 Necropsy laboratory and GIT content assessment equipment: autopsy table and multi-sieves system

2.4. Performing necropsies and ingested marine litter assessment

In total, fifteen cetaceans were recorded between February 2023 and April 2024, comprising seven bottlenose dolphins (*Tursiops truncatus ponticus*), seven harbour porpoises (*Phocoena phocoena relicta*), and one common dolphin (*Delphinus delphis ponticus*) (APPENDIX 3). Of these, nine were found stranded on the beach (six bottlenose dolphins, two harbour porpoises and one common dolphin) and six (one bottlenose dolphin and five harbour porpoises) were found dead as by-catch during the recovery of turbot gillnets.

Only eight cetaceans were suitable for post-mortem investigations (i.e., DCC 1-4 and absence of penetrative wounds at the GIT level), two stranded and six by-caught in turbot gillnets. Of these eight cetaceans, only four were already necropsied, as four of them were collected between March and May 2024 (see section 2.2. Onboard observation). The four which were not yet investigated (all *P. p. relicta*) are stored at -20°C and will undergo necropsy this year.

All the necropsies were performed according to the methodology described in "Best practice on cetacean post mortem investigation and tissue sampling" by L. IJsseldijk, A. Brownlow and S. Mazzariol (Ijsseldijk et al., 2019).

Fishery interaction was assessed according to "LiFE DELFI: Dolphin Experience: Lowering Fishing Interactions"[2].

The initial approach involved conducting necropsies in the field for stranded cetaceans (Figure 24). However, due to specific national regulations and a lack of established procedures within the

[2] LIFE DELFI Dolphin Experience: Lowering Fishing Interactions LIFE18 NAT/IT/000942 Action A3: Framework for Fishery Interaction
https://accobams.org/wp-content/uploads/2021/07/A3_Framework_Fihery_interaction.pdf

responsible institution for carcass handling, the team opted to conduct the necropsies in a laboratory setting instead.

Both in the field and in the laboratory, the team ensured their safety by donning protective equipment, including protective overalls, cut-resistant gloves, protective gloves, and surgical masks.

Figure 24 Field necropsy performed by NIMRD team

Tier One and Tier Two of the ACCOBAMS necropsy methodology were followed (Ijsseldijk et al., 2019).

Each stranded and bycaught cetacean was labelled and an ID code was assigned. The ID code consists of the first initials of the project PCET followed by STR- for stranded cetaceans or GN- for cetaceans bycaught in gillnets, the date when the cetacean was found in DDMMYY format and an order number #N meaning the order in which the cetaceans were found stranded/bycaught in gillnets (e.g. PCETSTR010123#1 or PCETGN240523#2).

First, the team took photos of the cetacean, basic morphometrics data, assessed the DCC and measured the blubber thickness (mm) (Figure 25) dorsoventrally along the girth line at the level of the cranial insertion of the dorsal fin in three points: dorsal, lateral and ventral. Also, the carcass was examined for any external lesions and any external signs of fishery interaction.

Figure 25 Blubber thickness measurements

The GIT was collected from cetaceans within the decomposition condition category (DCC) 1-4, only if it was intact otherwise the results could be compromised.

The GIT was sealed at both ends before collection to minimize the contamination of the GIT from environmental sources and to avoid the mixing of the content, then it was transported using a portable refrigerator to NIMRD's headquarters where it was frozen at -20˚C until its contents were analyzed.

The ingested marine litter assessment was done according to "*Analysis of the Gastro-Intestinal Tract of Marine Mammals: A Multidisciplinary Approach with a New Multi-Sieves Tool*" (Corazzola et al., 2021) using the multi-sieves tool described in the paper.

2.5. Updating the National Action Plan for the Conservation of Cetaceans

The previous National Action Plan for the Conservation of Cetaceans in the Romanian Waters of the Black Sea was created as part of a project co-financed by the European Union through the Life-Nature Program, titled "Conservation of Cetaceans in the Romanian Waters of the Black Sea." The project aimed to implement the National Action Plan for the Conservation of Cetaceans, which was initiated 20 years ago, in 2004. The need for an update arose due to changes in the legal framework based on European directives concerning the environment and water.

As a result, under the PONTICCET project, funded by ACCOBAMS in 2023, was proposed to revise the National Action Plan for the Conservation of Cetaceans from the Black Sea (Romania).

In this regard, two meetings occurred in 2023. The initial meeting of the working group convened to discuss the National Action Plan, took place in Bucharest, on June 29th, 2023, at the Romanian Ministry of Environment, Waters, and Forests headquarters. The subsequent meeting was held in Constanta, on October 30th, 2023, at the NIMRD headquarters (Figure 26). The decision to relocate the venue for the second meeting was made to facilitate the attendance of fishermen.

Figure 26 The working groups for elaborating the updating of the National Action Plan for Cetaceans Conservation, first and second meeting

Attendance at the meetings for the updating of the National Action Plan for the Conservation of Cetaceans was significant, with active participation from various stakeholders (Table 1).

Table 1 Attendance at the meetings for the elaboration of the National Action Plan for the Conservation of Cetaceans

No.	Name	Institution
1	Basalic Simona	National Sanitary Veterinary and Food Safety Authority Constanța
2	Bîlbă Adrian	National Agency for Fisheries and Aquaculture, Constanța
3	Buhai Dragoș	Black Sea Advisory Council
4	Cioacă Doina	National Agency for Natural Protected Areas
5	Ciucă Andreea-Mădălina	National Institute for Marine Research and Development „Grigore Antipa"
6	Curlișcă Angelica	Natural Sciences Museum Complex Constanța –Dolphinarium
6	Filimon Adrian	National Institute for Marine Research and Development „Grigore Antipa"
8	Giurea Elena	National Environmental Protection Agency
9	Harcotă George	National Institute for Marine Research and Development „Grigore Antipa"
10	Miaută Nela	Romanian Ministry of Environment, Waters, and Forests
11	Mihail Otilia	Romanian Ministry of Environment, Waters, and Forests
11	Mirea Laurențiu	Federation of Fishermen's Organizations from the Black Sea
13	Pacioglu Octavian	National Institute of Research and Development for Biological Sciences,
14	Paiu Marian	NGO Mare Nostrum
15	Pîrlac Georgiana	National Environmental Protection Agency
16	State Lăcrămioara	National Environmental Guard, Constanța
17	Talpeș Vladimir	General Association Of Hunters And Anglers From Romania
18	Tomulescu Caterina	Romanian Ministry of Environment, Waters, and Forests
19	Voicu Mihai	Romanian Ministry of Environment, Waters, and Forests

Following the initial meeting, a Teams group was established, and all members were added to facilitate collaboration. Within the Working Groups, the expert members actively collaborated on refining the draft of the National Action Plan for the Conservation of Cetaceans, which was initially elaborated by the NIMRD team.

Each paragraph was thoroughly discussed during the second Working Group meeting, and necessary changes were introduced and implemented.

Subsequently, the NIMRD team took the feedback and discussions from the meeting to create the final version of the National Action Plan for the Conservation of Cetaceans. The final version of the draft document was then submitted to Dr. Nela Miauta, ACCOBAMS National Focal Point and representative of the Romanian Ministry of Environment, Waters, and Forests, for analysis and filling the draft Plan the required legal steps before approval as a Minister's Order.

2.6. Dissemination of project activities

All PONTICCET activities were disseminated through social media posts on NIMRD's Facebook page[3].

The Newsletter of the Romanian Research, Innovation and Digitalization Ministry published:

[3] https://www.facebook.com/INCDM

"In the PONTICCET project, the team of the Marine Biology and Ecology Department, coordinated by Adrian Filimon, completed the first field expedition. Financed by ACCOBAMS, the project has as the main objective the sampling of GIT and analysis of ingested plastics. Following the first expedition for onboard observations, in the middle of March, the second expedition was organized. Unfortunately, during this, a Bottlenose dolphin (T.t. ponticus) was accidentally caught in turbot fishing nets. The individual was transported to the NIMRD laboratory for GIT sampling and ingested plastics analyses. "Although the loss of marine life is distressing, we hope these data will provide valuable insights and help us improve our efforts to protect cetaceans and minimize the impact of human activity on marine ecosystems," the researchers said".

Based on the preliminary results indicating microplastic contamination in the GIT, the project team authored and published a scientific paper in an ISI journal with an impact factor of 3.[4]

3. Difficulties encountered and measures taken to overcome problems

During the project implementation, the following difficulties have been encountered:

- Delays in the GIT analysis stage due to long delivery times of the necessary equipment (necropsy table and multi-sieving system);
- Non-compliant execution of the ordered equipment. Based on Corazzola et. al. paper (Corazzola et al., 2021), the NIMRD team drew a detailed sketch of the sieving system and contracted a company to build it accordingly. Even if the sketch was very clear the company made some errors and we had to refuse some parts of the equipment. The company had assumed the errors and fixed them according to the plan.

4. Changes introduced in the implementation

There were no major changes in the project implementation.

5. Results on microplastics contamination

The primary goal of the PONTICCET project is to evaluate plastic contamination in the GIT of Black Sea cetaceans through necropsies and utilizing an innovative approach, specifically a multi-sieve system as outlined by Corazzola et al. (Corazzola et al., 2021). The research methodology complies entirely with the requirements of the Marine Strategy Framework Directive (MSFD), and the investigation findings could establish a fundamental comprehension for utilizing these cetacean species in marine litter monitoring endeavours (van Franeker et al., 2018).

The interaction between cetaceans and microplastics is a matter of great concern, whether it occurs through direct ingestion or trophic transfer. Although there are several studies available (Battaglia et al., 2020; Lusher et al., 2015; Philipp et al., 2021; Sá et al., 2023; Yücel et al., 2022; Zhu et al., 2019) for other regions, in the Black Sea the topic is still largely unexplored. Currently, there is only one study conducted in Bulgaria (Mihova et al., 2023). The lack of information on this subject is mostly attributed to limited research endeavours. Collecting samples from cetaceans for microplastic analysis might be difficult because there are only a limited number of stranded or caught individuals available and in proper conditions. Furthermore, thoroughly investigating the ingestion of microplastics by cetaceans requires a substantial commitment of research labour, money, and time, particularly for carefully

[4] https://www.mdpi.com/2076-2615/14/6/886

processing GIT contents and subsequent thorough analysis. The issue of microplastics in the GIT of cetaceans in the Black Sea has received very limited attention.

5.1. Material and methods

2.2. Post-mortem investigation

General data on the stranded and by-caught cetaceans were documented in an Excel database. Concurrently, upon collecting the GIT, the results of plastic contamination analyses were entered into the same database.

Carcasses suitable for post-mortem investigation individuals were transported to the laboratory and stored at a temperature of -20˚C until the post-mortem investigation.

All the post-mortem investigations were performed following the ACCOBAMS and ASCOBANS Best practices necropsy procedure (Ijsseldijk et al., 2019).

All the GIT content processing was performed and further ingested marine litter assessments took place in the NIMRD`s laboratory.

For each cetacean, essential data (e.g., date and location of the sighting, the total length, weight, gender, age, DCC), were recorded (Table 2). The carcass of beached cetaceans was analyzed for any visible external lesions and indications of interaction with fishing activities. The GIT was collected from cetaceans within the DCC 1-4, but only if it was undamaged, otherwise the accuracy of the data could be affected. The GITs were sealed at both ends before extraction to avoid contamination. Subsequently, the GITs were frozen at a temperature of -20˚C until processing. After GITs sampling, the remaining carcasses were delivered to the University of Agronomic Sciences and Veterinary Medicine of Bucharest (UASVM). At the laboratory of the UASVM, additional post-mortem investigations were conducted by veterinarians and the resulting skeletons were prepared for educational purposes. The collaboration between UASVM and the NIMRD was initiated within the framework of this project and was formalized through the signing of a collaborative agreement.

Table 2 General information on investigated specimens

Animal ID	Species	Coordinates	Found	Estimated Age/ Sex	DCC	Organ
PCETSTR040423#1	*T. t. ponticus*	44,039 28.6515	Stranded	Adult female	2	Stomach Intestine
PCETSTR180423#2	*T. t. ponticus*	44.4323 28.6455	Stranded	Adult male	3	Stomach Intestine
PCETGN140223#1	*T. t. ponticus*	44,4153 29,4459	By-catch	Adult male	2	Stomach Intestine
PCETGN090423#2	*P. p. relicta*	44,3527 29,1578	By-catch	Adult male	2	Stomach Intestine

2.3. Gastro-intestinal tract content processing

The processing of the GITs material was conducted following the methodology described by Corazzola et al. (Corazzola et al., 2021).

First of all, the GITs collected and stored at -20°C, were kept in the laboratory at room temperature for thawing for approximately 24 hours.

Before sieving, the GIT (i.e. stomach and intestines) was carefully rinsed with tap water to remove any blood and other particles that could potentially influence the quality of the samples (Figure 27).

Figure 27 GIT rinsing with tap water before the sieving step

The straps used to seal the cranial and caudal parts of the GIT were removed. The stomach was separated from the intestines and each organ was weighed before and after analysis to determine its content weight. Each organ was opened longitudinally, using metal scissors, and its contents were washed through the first 20 mm mesh sieve (Figure 28). Each organ was opened separately, and the contents of the stomach and intestines were collected separately.

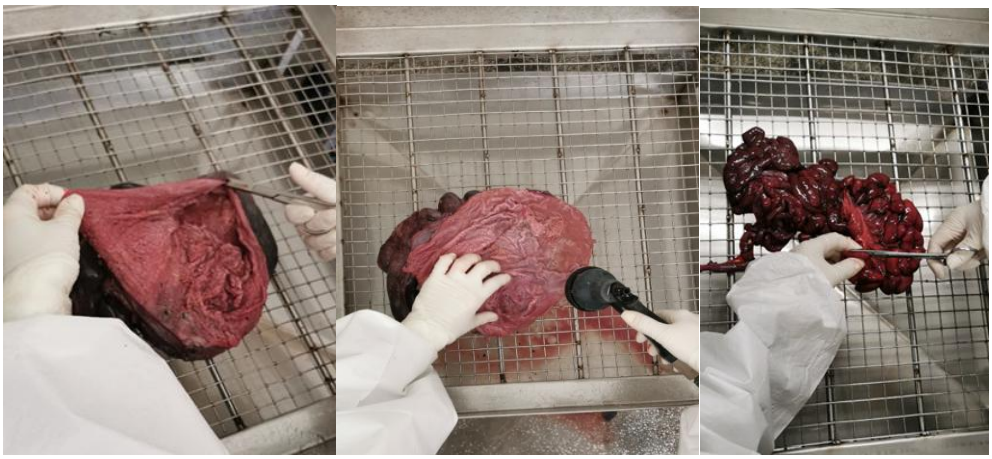

Figure 28 Analysis of GIT contents

After an abundant rinse, the 20 mm, 5 mm and 1000 µm sieves were extracted from the support, and any waste, parasite or food residues were collected in separate containers.

After the collection of the litter items visible with the naked eye from the sieves, the residual material was subsequently collected from the 1000 µm, 500 µm, 250 µm and 100 µm sieves into separate containers by spraying water on the external surface of the sieves fixed above the containers via a funnel (Figure 29).

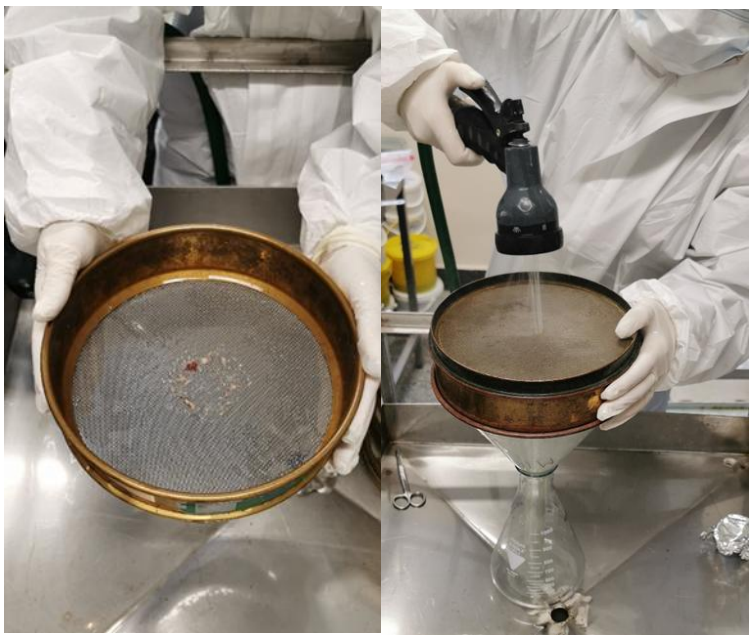

Figure 29 Collection of the GIT residual material from the sieves

2.4. Sample analysis

During sample analysis, a range of foreign objects, including stones, sand shell fragments, plastics, and other types of debris were noticed and recorded. This study especially focuses on microplastics.

The protocol provided by Lusher and Hernandez-Milan (Lusher & Hernandez-Milian, 2018) was followed to process samples for plastics analysis. To decompose the organic (non-plastic) components in the samples, a solution of KOH (10%) was added to the samples in a ratio of 3:1 and the mixture was subjected to incubation at a temperature of 60°C for 24 hours (Figure 30).

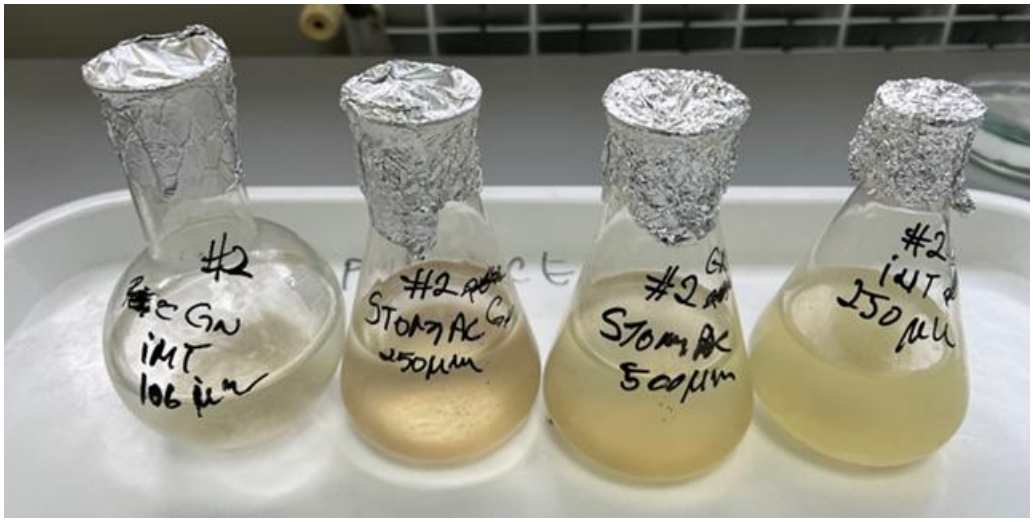

Figure 30 Samples of GIT contents after incubation with KOH solution

Following digestion, the samples underwent vacuum filtration using 1.6 µm glass fibre filters in a fume hood. After that, the filters were left to dry in covered glass Petri dishes (Figure 31).

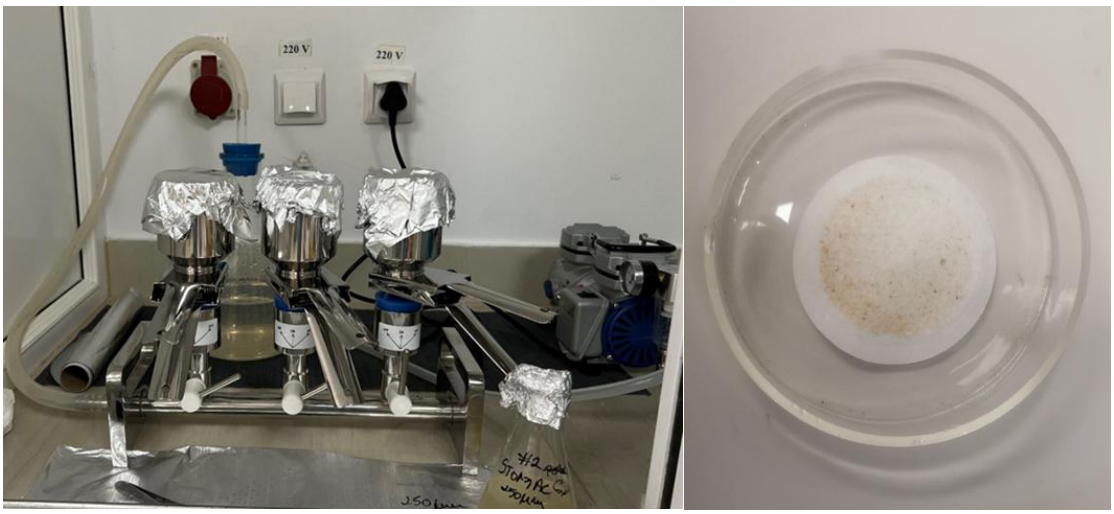

Figure 31 The stage of filtering the samples and drying the filters in Petri dishes

For samples containing sand or other indigestible parts, a prefiltration step was employed to separate plastics. The sorting method involved a saline solution with a density of 1.2 g/cm³ and a separatory funnel. The separatory funnels were well shaken and left to separate for 2-12 h, depending on the amount of material to be separated (Figure 32).

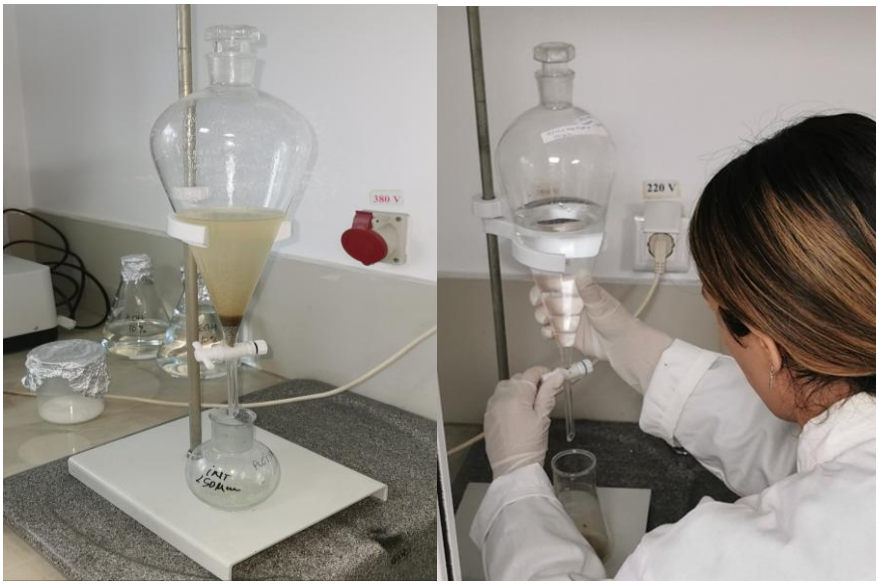

Figure 32 Separation step with NaCl saline solution

The identification of potential plastic items was conducted according to the criteria proposed by Lusher et al. (Lusher et al., 2020) under an Olympus SZX10 microscope foreseen with an SC50 camera (Figure 33). Measurements of plastics were made using cellSens Entry software. All potential microplastics underwent the hot needle test for confirmation.

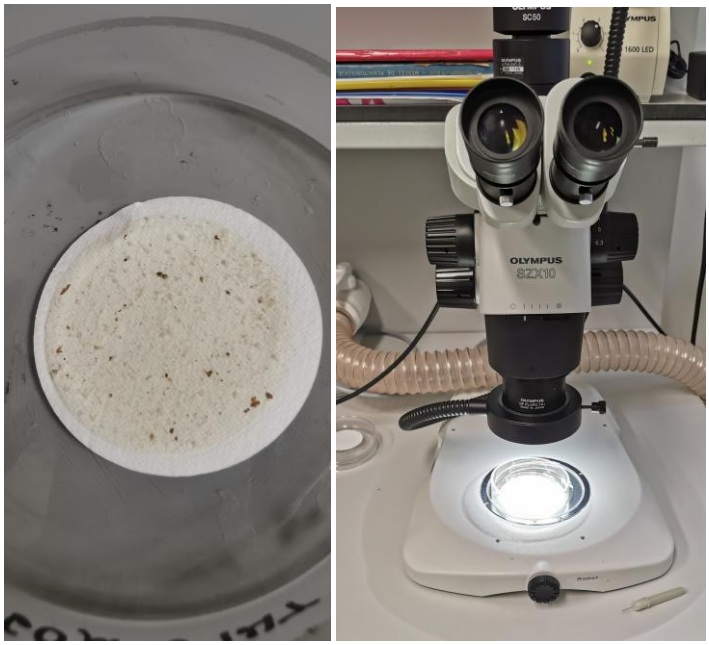

Figure 33 The filter analysis stage under binoculars

2.5. Contamination Control and Procedural Blanks

Strict protocols were enforced during the sample collection and laboratory processing phases to avoid contamination. Before extraction, the GIT was sealed using a white serrated band made of nylon PA66 at the cranial and caudal portions of the stomach and intestine to minimize the contamination of GIT content from environmental sources of microlitter items and to avoid the mixing of the content. In the laboratory, all tools and glassware were thoroughly rinsed with distilled water and ethanol (70%) and stored in aluminium foil. Ethanol (70%) was used to clean the surfaces and equipment in the laboratory. Nitrile gloves and white cotton lab coats were worn during necropsies and laboratory analyses. During GIT sampling and sample analysis, access to the laboratory was restricted. When not in use, samples were always covered with aluminium foil. The materials and tools used were made of glass and stainless steel. To assess contamination, procedural blanks (n = 11) and controls (n = 4) were taken multiple times. For procedural blanks, before GIT content sampling, 500 mL of water was run into the support and into the 500 µm, 250 µm, and 100 µm sieves to capture any microlitter items that may be present in the device. The water was sampled in pre-cleaned glass jars and analysed for microplastics (Corazzola et al., 2021). Controls, represented by ultrapure water blanks and glass microfibre filters, were kept in the working environment during the whole processing (i.e., opening and rinsing the intestines, sample processing, and observation and identification under the stereomicroscope) to collect the microlitter items present in the air. All procedural blanks and controls followed the same treatment as all samples. The microplastic particles found were examined under a stereomicroscope, where they were counted, and details regarding their type, colour, and size were recorded. Subsequently, an equivalent number of particles with matching characteristics were systematically removed from the overall database, maintaining a 1:1 ratio subtraction ratio.

Procedural blank samples were generated by pouring tap water onto the 100, 250, and 500 µm sieves, as well as onto the collector of the multi-sieves tool, before the processing of each sample. These procedural blanks were then collected in glass jars for further analysis. Procedural blanks and control samples followed the same treatment as the samples. Microplastics found in control and procedural blanks were observed under the stereomicroscope and their type, length and colour were recorded. The number of microplastics found in each blank was subtracted from the total number of microplastics with similar characteristics (type, colour, size).

5.2. Results and discussions

Our investigation on microplastics in the GIT content of cetaceans is the first in Romania and the second scientific inquiry within the Black Sea region. Additionally, it introduces a novel approach to the Black Sea, employing a multi-sieve tool for the simultaneous assessment of ingested macro-, meso- and micro-litter across all distinct sections of GIT (Corazzola et al., 2021). The first multi-sieve equipment testing and validation was carried out on five Mediterranean cetaceans in 2021 (Corazzola et al., 2021). Through a programme run by ACCOBAMS in Italy, two of our research team members received training to better understand the methodology and the protocol.

The study examined a total of eight digestive organs, consisting of four stomachs and four intestines, obtained from three *T. t. ponticus* and one *P. p. relicta*. All animals were adults, three males and one female. The analysis revealed the presence of synthetic particles in all of them. All individuals that were analyzed contained plastic litter, resulting in a frequency of occurrence (FO%) of 100%.

A comprehensive investigation indicated a cumulative total of 1055 potential micro-plastics and four mesoplastics, with individual counts ranging from 119 to 388 particles per organism. Out of a total, 91.78% (n=972) were classified as fibres, 8.12% (n=86) were categorized as fragments, and the remaining (0.09%; n=1) was represented by a spherical bead.

The quantity of plastics documented in this study (1059 plastics, including 1055 microplastics) was notably higher than reported in studies conducted in other marine regions. For instance, analyses of the entire gastro-intestinal tracts (i.e., stomachs and intestines) of five cetaceans stranded on the Italian coast using the same methodology for sample processing revealed the presence of only 173 plastic items, including 161 microplastics (Corazzola et al., 2021). Another study involving 38 stranded cetaceans on the Portuguese coast documented 268 plastic items (254 microplastics) (Sá et al., 2023). Similarly, in the digestive tract of 43 striped dolphins stranded on the Mediterranean coast of Spain, a total of 672 plastic items were reported (Novillo et al., 2020). On the British coast, investigations of the stomachs and intestines of 50 marine mammals (43 cetaceans) identified 273 plastic particles (including 261 microplastics) (Nelms et al., 2019). The abovementioned studies reported the prevalence of microplastics ingested by cetaceans, which is similar to our findings. Meso- and macroplastics were either present in low numbers or absent. Comparative data for the Black Sea are limited in availability. The only currently available study that tackles plastic contamination in GIT content, revealed that 84% of the 31 examined individuals had ingested plastic. The analyses carried out revealed a total of 197 plastic particles (Mihova et al., 2023). Because of the large variation in the number of microplastic items identified in the two studies, the comparison needs to be carefully considered. Anyway, in terms of ingested plastic quantity, comparisons between studies are challenging because of differences in the GIT compartments analysed and the methodology followed (Sá et al., 2023). In addition to the already mentioned factors, there could be other variables that can influence the number of ingested microplastics (Nelms et al., 2019).

The most prevalent potential microplastics (27%; *n*=284), were those with sizes ranging from 5000-1001 µm. They were followed by microplastics measuring between 500-251 µm (24%; *n*=256), 1000-501 µm (23%; *n*=243), 250-101 µm (20%; *n*=209), and ≤100 µm (6%; *n*=63) (Figure 34, Figure 35). In each GIT, a single mesoplastic item (i.e., >5000 µm) was found.

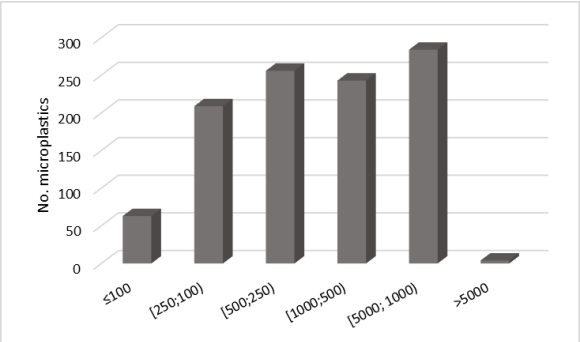

Figure 34 Dominant sizes of potential microplastics

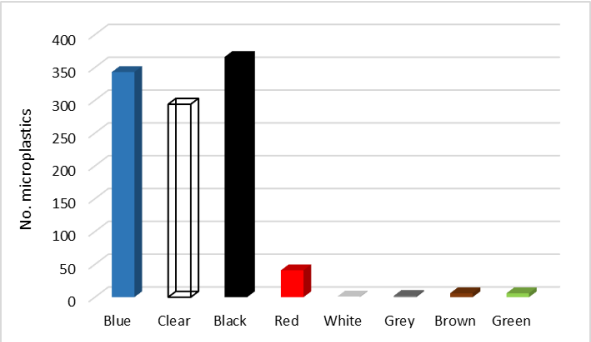

Figure 35 Dominant colours of potential microplastics

The fibres varied in size, ranging from 22.86 µm to 5776 µm, with an average length of 957.20 µm (±920.65 SD). The fragments exhibited a size range of 25.57 x 13.19 µm to 2184.38 x 515.89 µm, with an average dimension of 417.06 (±478.42 SD) x 172.97 (±138.67 SD) µm.

Broadly, our observations align with the outcomes of prior research. In our investigation, we found suspected microplastic particles in all of the analysed samples. In particular, our research showed that fibres were the most common type of microplastic. The fibre prevalence is claimed in the majority of the published studies (Hernandez-Gonzalez et al., 2018; Lusher et al., 2020; Novillo et al., 2020; Zhu et al., 2019, Suaria et al., 2020). Studies conducted in the Black Sea region on various organisms and environmental matrices have also reported the predominance of fibres (Aytan et al., 2021; Cincinelli et al., 2021; Oztekin & Bat, 2017; Şentürk et al., 2020).

Regarding particle colour, a total of eight distinct colours were identified in the samples. The predominant colours were black (34%), blue (32%), and clear (28%). The aggregate of the other encountered colours (red, white, grey, brown, green) constituted a total of 6% (Figure 35, Figure 36). Studies generally indicate a variety of colours of microplastics, ranging from blue to transparent. A comprehensive review of articles focusing on microplastic ingestion in marine biota unveiled that blue (32.95%), white (24.71%), black (18.82%), and transparent (16.47%) are the most prevalent microplastic colours encountered. The most common colours found in marine mammals were blue (50%), transparent (37.5%), and black (12.5%) (Ugwu et al., 2021). The black and blue colours were demonstrated to be prevalent both in the Black Sea environment and in biota (Aydın et al., 2023). Additionally, there is evidence that some species of fish often ingest blue microplastics by mistake, as they resemble their natural prey such as the blue pigmented copepods *Pontella sinica*, *Sapphirina sp*., or *Corycaeus sp*. (Ory et al., 2017). Black and blue were the most common colours in our study, which are comparable to the colours that are the most frequent in cetaceans, as documented by Zantis et al. (Zantis et al., 2021). Certainly, an important source of blue fibres could also be attributed to fishing activities, as the colour blue is commonly used for ropes and nets.

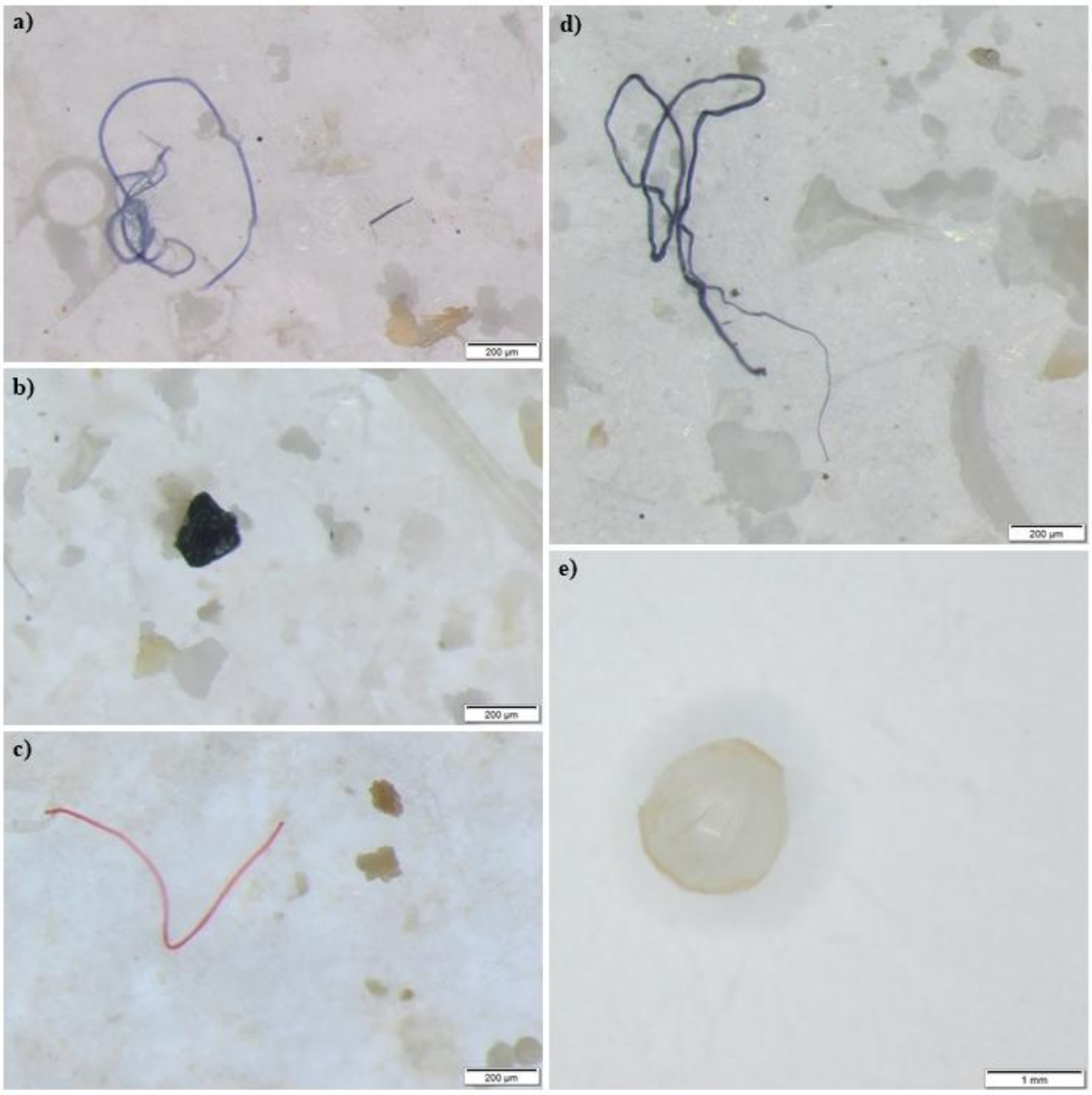

Figure 36 Examples of microplastics found in the GIT of the analyzed cetaceans: a) fibre- blue, b) fragment- black, c) fibre- red, d) fibre- black, e) bead- clear

Among the GIT sections, the number of potential microplastics was higher in stomachs (n=599; mean 149.75±109.9677 SD) compared to intestines (*n*=456; mean 114±89.1291 SD) (Figure 37). The stomachs contained a greater number of both small (1 µm - ≤1000 µm) and large microplastics (1000 - ≤5000 µm) (Bessa et al., 2019), with 419 and 180 particles respectively, compared to the intestine which contained 352 small and 104 large microplastics. Nevertheless, the one-way ANOVA analysis did not show a statistically significant difference in the number of microplastics among the GIT compartments (Pr(>F) = 0.984, p = 0.6298). The results of the present study showed that the stomachs of the cetaceans contained more microplastics than the intestines. The differences between the two sections could be because the stomachs of cetaceans may act as a reservoir for the accumulation of plastic in GIT, as suggested by other scientists (Nelms et al., 2019). Moreover, as prior studies have shown, the existence of microplastics throughout the entire intestine increases the likelihood that they may be excreted (Lusher et al., 2015; Philipp et al., 2021). The finding of microplastics in the scats of various marine mammal species, including *Halichoerus grypus*, *Arctocephalus spp*., and *Callorhinus ursinus*, supports this hypothesis (Desclos-Dukes et al., 2022; Donohue et al., 2019; Eriksson & Burton,

2003). Due to divided opinions among scientists, further research is needed to validate these assertions.

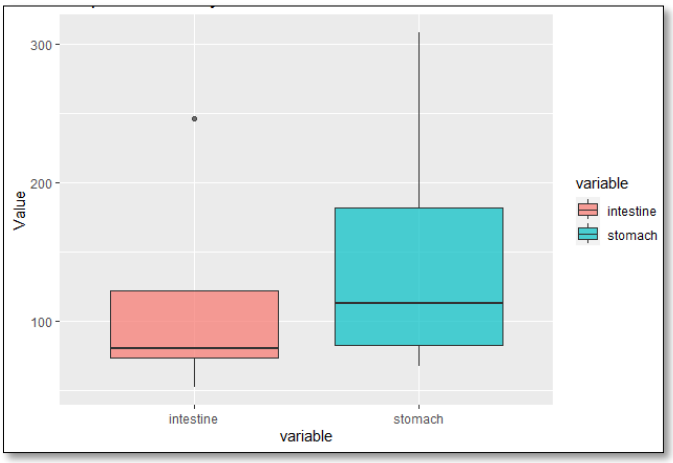

Figure 37 Boxplot showing the median number of suspected plastics number in the intestine and stomach

In terms of environmental contamination, the study adhered to EU guidelines, which specify that background contamination with microplastics should not exceed 10% of the overall average of microplastics found within all analysed samples (Hanke, 2013). In the procedural black and controls, the contamination was 6% (n = 18) of the overall average microplastics found. Of the 18 particles found, 14 items were found in the procedural blank, and 4 items in the controls. Microplastic items with the same characteristics as the items found on the blanks were excluded from the database (Sá et al., 2023).

Our research showed that fibres were the most common type of microplastic ingested. The fibre prevalence was claimed in most of the published studies (Hernandez-Gonzalez et al., 2018; Lusher et al., 2018; Novillo et al., 2020; Suaria et al., 2020; Zhu et al., 2019). While investigating the translocation of microplastics in organs, the dominance of fibres was also reported in the lung tissue, melon, acoustic fat pad, and blubber (Merrill et al., 2023). Studies conducted in the Black Sea region on biota and environmental matrices have also reported the predominance of fibres (Aytan et al., 2021; Cincinelli et al., 2021; Oztekin & Bat, 2017; Şentürk et al., 2020). The Black Sea's microplastics may originate from river and urban runoff, industrial discharges, and the disintegration of larger debris (Karlsson et al., 2018; Kittner et al., 2022; UNEP, 2021; Wang et al., 2022). Fibres could be a result of industrial discharges, whereas fragments are the result of the degradation of bigger plastic products. A study showed that ropes and nets (made of polypropylene, polyethylene, and nylon) used in fishing operations are an important source of fibres (Welden & Cowie, 2017).

There are not many studies on the transfer of microplastics from GIT to blood or other organs. However, microplastics ranging from 24.4 µm to 1387 µm have been detected in the lung tissue, melon, acoustic fat pad, and blubber of twelve different species of marine mammals (Merrill et al., 2023).

Microplastics have been discovered in the digestive tracts of zooplankton, which is a base link in the food chain (Aytan et al., 2020). This suggests that, due to t heir smaller size, microplastics may be able to move up the food chain from lower trophic levels to higher ones, where they may eventually end up in fish, birds, turtles, and marine mammals. Microplastic contamination has been documented in pelagic and benthic fish species (e.g., *Engraulis encrasicolus, Trachurus mediterraneus, Sarda sarda,*

Belone belone, Pomatus saltatrix, Merlangius merlangus, and Mullus barbatus), the primary prey of the cetaceans living in the Black Sea, revealing a high contamination rate (Aytan et al., 2020; Aytan et al., 2021). The high amount of microplastics in all Black Sea compartments exposes the organisms that live there to plastic pollution both through environmental contamination and food ingestion.

There are different ways for cetaceans to ingest microplastics (i.e., direct ingestion from the environment or through trophic transfer). The degree to which microplastics are internalized through direct ingestion from the environment is currently unknown (Lusher et al., 2018). However, we agree with the other statements emphasizing the crucial role of feeding in plastic ingestion (Nelms et al., 2019). Black Sea cetaceans are raptorial feeders that use teeth to catch prey and are more likely to ingest plastic items through trophic transfer (Hocking et al., 2017). As preferred prey, harbour porpoises exhibit a preference for gobies, whereas bottlenose dolphins show a preference for turbots and mullets. Additionally, it is recognized that Black Sea cetaceans undertake mass migrations to the north in spring and to the south in autumn generally associated with the movements of pelagic fish stocks, particularly anchovies. Both harbour porpoise and bottlenose dolphins eagerly eat anchovies, especially when they occur in large and dense schools. The findings of a recent study on microplastic contamination in Black Sea fish species revealed 233 plastic particles (including 157 fibres) in the GIT of 335 anchovies (*Engraulis encrasicolus*), as well as 59 plastic particles (including 38 fibres) in 155 red mullets (*Mullus barbatus*) (Aytan et al., 2021). Considering that the estimated weight of 335 anchovies is approximately 2.5 kg, and a harbour porpoise can consume between 3 and 5 kg of fish per day, while a bottlenose dolphin can consume between 8 and 15 kg of fish per day, there is a potential for significant contamination to occur through trophic transfer.

Given its semi-enclosed basin, high anthropogenic river inputs, and densely populated coasts, the Black Sea is heavily impacted by pollution and litter accumulation (Aydın et al., 2023; González-Fernández et al., 2020; Ioakeimidis et al., 2014; Stanev & Ricker, 2019). The high amount of microplastics in all Black Sea compartments exposes the organisms that live there to plastic pollution. The high plastic contamination in the Black Sea could be highlighted also by the high amount of microplastics found in this study. However, it should be mentioned that the relatively short duration (1 year) of this study, along with the limited number of stranded and by-caught cetaceans, has led to the analysis of a small sample size. Due to these constraints, further studies are required to provide a more comprehensive overview of microplastic pollution in Black Sea cetaceans. Nevertheless, the findings will constitute a significant foundational framework and comparative reference for future investigations in this underexplored domain within the Black Sea region.

Based on these first results, we may state that the monitoring of microplastics in the GIT of Black Sea cetaceans, under the MSFD, could provide valuable insights into this threat. As top predators, the level of microplastic contamination in the GIT of cetaceans provides valuable insights into adjacent trophic levels. Long-term monitoring can bring crucial information for an inaccessible and understudied area (the water-sediment interface), considering that certain cetacean species primarily consume benthic organisms (van Franeker et al., 2018). Furthermore, employing a methodology in line with the MSFD, as utilized in this study, can offer important data for the implementation of this European policy.

Certainly, this approach faces limitations, primarily associated with the collection and analysis of a sufficiently large number of samples to establish thresholds and ultimately assess the ecological status of the marine environment according to Descriptor 10 (D10) - Marine litter criteria. Another crucial factor that could pose challenges is secondary contamination, particularly when handling large samples such as an entire GIT (Philipp et al., 2021). In our study, efforts were made to eliminate all

possible contamination sources; however, in cases where removal was not feasible, such as the white nylon serrated band used for GIT sealing, white safety gloves, and a green hose utilised during GIT washing, their respective colours were noted. This approach allowed us to assess their potential impact on the final results. Subsequent analysis revealed the presence in the samples of only one white particle and six green particles. Green particles were not found in procedural blanks and controls. Even after removing primary contamination sources, procedural blanks and controls remained essential to control secondary contamination during the study. Procedural blanks were taken before washing the GIT, and control samples were maintained throughout the activity. All particles found (n = 18) in procedural blanks and controls were removed from the study's database in a 1:1 ratio to uphold data integrity (Sá et al., 2023).

Nevertheless, with the effective management of research efforts and strengthened collaboration with the fishing sector and with competent authorities, these limitations could be minimized.

General conclusions

Based on these preliminary results, we may state that monitoring of microplastic in the GIT of Black Sea cetaceans, under the MSFD, could provide valuable insights into this threat. Nonetheless, the methodology faces challenges concerning sample accessibility. Because there are few stranded or by-caught cetaceans and more of the carcasses are damaged, obtaining GIT for microplastic analysis can be difficult. Collecting, processing and analysing the samples is time and resources consuming. Usually, the best GITs come from by-caught individuals that are undamaged and usually in DCC 1-2. However, having access on board a fishing vessel necessitates entering into a contractual agreement with the fishing company and covering the associated costs for the service. Assessing Good Environmental Status (GES) under D10 – Marine litter necessitates the establishment of thresholds. Setting these thresholds is crucial and should be based on a representative number of results. The aforementioned restrictions could potentially lead to the impossibility of collecting a representative number of samples.

Cetaceans, positioned at the top of the marine food chain, play a pivotal role in reflecting the nowadays issue of plastic pollution in the Black Sea. Microplastics and mesoplastics were ingested by all analysed individuals in this study. Broadly, our observations on plastic items' colour and form align with the outcomes of prior research. In all samples, microplastics dominated numerically, being much higher than reported in all relevant worldwide studies. This first report of the highest incidence of ingested microplastics in cetaceans could be the consequence of variations in the sample processing methodologies and, more particularly, the level of microplastics in the Black Sea waters, which are considered to be the most plastic-polluted within Europe (UNDP, 2019). Further efforts are required to collect additional data and to harmonize and implement a standardized protocol for the processing of cetacean GIT samples at the regional or even European level.

6. Summary

The PONTICCET project, through its objectives, tackles one of the major threats affecting marine mammals at the global level and currently understudied in the Black Sea, marine litter. During the project period, the project's team made monitoring campaigns for stranded cetaceans and answered citizens' calls that reported stranded cetaceans. The onboard observations task was accomplished as the planned field trips onboard a gillnetter were completed. Moreover, due to our good collaboration, the fishing company has agreed to collaborate with us for the entire year. To address the knowledge gap regarding the interaction of cetaceans and microplastics, a study was conducted using a multi-sieve system to examine plastic pollution in the gastrointestinal tracts of Black Sea cetaceans. The

study, which followed strict protocols, discovered synthetic particles in all analysed organisms, indicating a high frequency of occurrence and emphasising the critical need for monitoring efforts. Despite the study's valuable insights, sample accessibility issues highlight the need for future research with a larger sample size of Black Sea cetaceans to strengthen the findings and improve our understanding of this environmental threat.

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Cetacean report form

1

Date: 05.04.2023

Report written by

Name: Andreea Circa, George Haraka

Address:

Tel. no:

Email:

Notes:

LOCATION OF STRANDING

Name of beach/cove: EFORIE SUD - FLAMINGO

OS map reference: 44°02'20,4"N 28°39'05,5"E

Access to beach: BY FOOT

Nearest town or village and county: EFORIE SUD

INITIAL ASSESSMENT

Number of animals stranded: Total: 1 Alive: 0 Dead: 1 Time assessment made: 13:15

How long stranded: aprox. 8h Estimated / ~~Actual~~

Weather conditions: WINDY CLOUDY

Sea state: 4 BEAUFORT

Tide status: -

Additional notes or useful information:

Number members present at initial assessment:

Note: Stranded Cetacean Report is needed for each individual animal

INITIAL INDIVIDUAL STRANDED CETACEAN REPORT

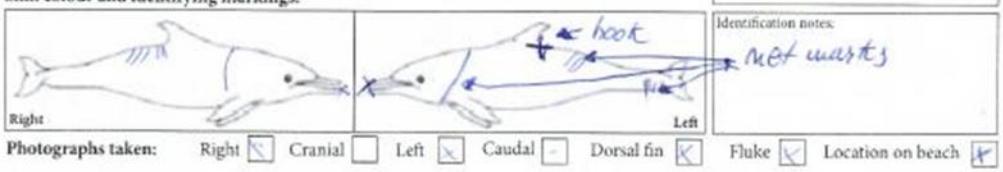
Species: TURSIOPS TRUNCATUS PCET_STR040423#1

Body Length: 194 cm Girth: 40,5 x 2 → 81 cm

Age: ~~Neonate~~/ ~~Juv~~ / Adult Sex: ~~Male~~ / Female / Unknown

If species unknown: Description of beak/snout: Absent ☐ Short ☐ Long ☐

Skin colour and identifying markings:



Identification notes:

Photographs taken: Right ☒ Cranial ☐ Left ☒ Caudal ☐ Dorsal fin ☒ Fluke ☒ Location on beach ☒

POSITION OF CETACEAN WHEN FOUND

Sun: ☐ in direct sunlight ☒ in shade

Sea: ☐ in the surf ☐ above the surf

Beach: ☒ on sand ☐ on shingle ☐ on rocks

TRIAGE

Status: ☐ alive ☒ dead (move on to another animal and record details later)

Decomposition condition criteria (DCC)

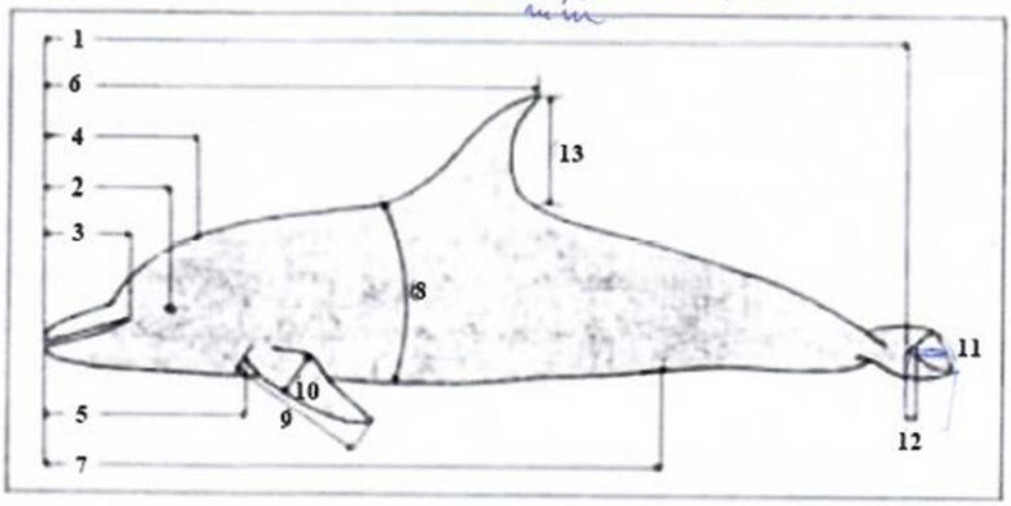
- CODE 1: Extremely fresh carcass, just dead ☐
- CODE 2: Fresh carcass ☒
- CODE 3: Moderate decomposition. ☐
- CODE 4: Advanced decomposition ☐
- CODE 5: Mummified or skeletal remains ☐

Measurements (Codes 1-4)

1. TOTAL LENGHT (tip of upper jaw to deepest part of fluke notch)	194 ?
2. Tip upper jaw- centre of eye	32
3. Lenght of gape (upper jaw to corner mouth)	27
4. Tip upper jaw to blowhole	~~32,5~~ 32
5. Tip upper jaw to front insertion of flipper	48
6. Tip upper jaw to tip dorsal fin	111
7. Tip upper jaw to centre anus	147
8. Max girth half →	40,5
9. Flipper- tip to front insertion	29
10. Flipper- max width	13
11. Tail flukes tip to tip	38,5
12. Depth of fluke notch	4
13. Dorsal fin tip to base	16
Weight	
§Blubber thickness (mm)	V= 16 mm

~~inch~~ 0,7 ~~fixed~~ ~~D= 0,8~~ ~~L=45~~

D=20,32 mm L=17,78 mm



Annex 3.a. On-board observation – vessel characteristics						
Name of data collector(s)	ADRIAN FILIMON					
Data	02 03 2023					
ID. Fishing trip	PCETGN020323					
Country	ROMANIA					
GSA	29					
						Notes
Vessel name*						
Fleet segment						
Total length of the vessel	25,3					
Power (kW)						
Gross tonnage (GT)	129					
Port of departure	MIDIA					
Port of arrival	MIDIA					
	Gear specifications					
	1st gear	2nd gear	3rd gear	4th gear		Notes
Gear type	GNS					
Net length (m)	12.000					
Mesh size (cod-end – mm)						
Number of hooks						
Bait						
Number of lines						
Number of pots/traps						
Soak time (time during which fishing gear is actively in the water)	20 days					
Other						

*if available.

Instructions:
- ID fishing trip: identification code assigned to each fishing trip (unique).
- GSA: insert code of GSA as in Annex 2.
- Fleet segment: insert fleet segment code (i.e. vessel group + length class) as in Annex 10.
- Gear type: insert code of fishing gear, as reported in Annex 11 (e.g. set gillnets [GNS]). If, during a fishing trip, different gear have been used, insert each code separately in the respective columns. Then, based on type of gear, provide the different measures of effort (e.g. mesh size, number of hooks, etc.) in the corresponding column and row.

Annex 3.b. On-board observation – general information by fishing trip			
Date	02.03.2023		
ID. Fishing trip	PCETGN020323		
			Notes
Total number of fishing operations			
Fishing hours	9		
Bycatch of vulnerable species (Y/N)	N		
Number of fishing operations with zero catch of vulnerable species			
General information on catch composition			Notes
Total landing (kg)			
Main commercial species in landing fraction			
Discard (kg and percentage) in catch composition	kg	%	Notes
Main species in discarded fraction			
Marine litter (Y/N)	Y		

Instructions:

- ID fishing trip: identification code assigned to each fishing trip (as in Annex 3.a).
- Total number of fishing operations: insert total number of fishing operations carried out during same fishing trip.
- Fishing hours: insert total number of fishing hours carried out during that fishing trip (i.e. summing the hours of all fishing operations).
- Bycatch of vulnerable species (Y/N): insert 'yes' if during the fishing trip there has been incidental catch of vulnerable species and/or vulnerable marine benthic species (in this case, detailed information, by groups of species, should be reported in Annex 3.c, Annex 4 and Annex 6); otherwise insert 'no'. If, during a fishing operation, the presence of vulnerable species around the vessel has also been recorded, this should be reported in Annex 3.c.
- Fishing operations with zero catches: insert total number of fishing operations carried out during same fishing trip with zero catches of vulnerable species.
- Total landing: insert total landing in kilograms (kg) (or estimate) of commercial species caught during same fishing trip.
- Main commercial species in landing fraction: insert name (preferably scientific name, otherwise the common one) of main commercial species present in landed fraction.
- Discard in catch composition: insert total, cumulative discarded fraction (or estimate) during that fishing trip in kg and percentage (%).
- Main species in discarded fraction: insert name (preferably scientific name, otherwise the common one) of main species discarded.
- Marine litter (Y/N): insert 'yes' if marine litter has been recorded, otherwise insert 'no'. If 'yes', detailed data, by fishing trip, should be reported in the ad hoc template (see Annex 13).

Annex 3.c. On-board observation – general information on vulnerable species		
Date	02.03.2023	
ID. Fishing trip	PCETGN020323-1	
ID. Fishing operation	PCETGN020323-1	
		Notes
Time of starting operation	09:10	
Time of ending operation	16:57	
Latitude (start and end) of fishing operation*	44°24.815N / 44°18.217N	
Longitude (start and end) of fishing operation*	29°26.756E / 29°25.405	
Gear type	GNS	
Some details of gear configuration		
Depth (in metres)	53,5	
Environmental variables*		Notes
Cloud*		
Wind direction*	NE	
Visibility*		
Light condition*		
Sea state*	3-4 BFT	

Vulnerable species caught				
	Species 1	Species 2	Species 3	Notes
Group of vulnerable species				
Family*				
Genus*				
Species				
Photo (Y/N)*				
Total number of individual(s) caught				
Total weight of individual(s) caught (kg)				
Condition at capture*				
Alive				
Dead				
Almost dead				
Not known				
Condition at release*				
Alive				
Dead				
Almost dead				
Not known				
Biological data collected (Y/N)				
Presence of vulnerable benthic species (Y/N)	N			

Presence of specimens around the vessel during fishing operations*			
Species/family/genus	Number*	Behaviour	Notes

* If available.

Annex 4.e. Data on marine mammals

Source						
	On-board observers (Y/N)	Y	ID fishing trip	ACETGN 020323	ID self-sampling operation	
	Self-sampling operation (Y/N)	N	ID fishing operation	ACETGN 020323-1	ID stranding observation	
	Stranding observation (Y/N)	N	Date	02.03.2023		

Species	ID specimen	Total body length (TBL cm)*	Girth in front of the dorsal fin (GFD cm)*	Other body measures (cm)*			Weight (kg)*	Sex*	Photo (yes/no)*	Position of specimen in gear*	Notes

Comments

* If available.

Note: Data should be reported by species. Name of genus or family can be inserted only if detailed information by species is not available.

Instructions:

- Source: indicate source of data and then report code for:
  - ID fishing trip: identification code assigned to each fishing trip (as in Annex 3.a).
  - ID fishing operation: identification code assigned to each fishing observation during a fishing trip (as in Annex 3.c).
  - ID self-sampling operation: identification code assigned to self-sampling operation (as in Annex 8.b).
  - ID stranding observation: identification code assigned to stranding observation (as in Annex 9).
- ID specimen: identification code assigned to each single individual caught.
- Total body length (TBL in cm): insert requested length measure as detailed in Annex 5 (Figure A).
- Girth in front of dorsal fin (GFD in cm): insert requested length measure as detailed in Annex 5 (Figure A1).
- Other body measurements: whenever possible, insert other length measures as detailed in Annex 5 (Figure A2).
- Weight (kg): whenever possible, and for each specimen caught, report total weight, otherwise insert estimate.
- Sex: when available, insert code for sex of individual(s) – M (male), F (female), U (undetermined), ND (not determined).
- Photo (Y/N): insert 'yes' or 'no' to indicate if specimen has been photographed and, if so, assign an identification code to photo. For cetaceans, detail photos of dorsal fin or any remarkable sign would also be useful, facilitating identification of the animal in existing photo-identification catalogue(s) for the area (where available).
- Position of specimen in gear: whenever possible, please specify position of specimen in gear at the moment of capture (e.g. near float or lead lines, in middle of net, etc.).
- Notes: any additional information.

APPENDIX 3 Database

ID Code	Species	SEX	DCC	M1 (cm)	M2 (cm)	M3 (cm)	M4 (cm)	M5 (cm)	M6 (cm)	M7 (cm)	M8 (cm)	M9 (cm)	M10 (cm)	M11 (cm)	M12 (cm)	M13 (cm)	Blubber thickness (mm)			No. of MP
																	D	L	V	
PCETSTR040423#1	*T.t. ponticus*	F	2	194	32	27	32	48	111	147	81	29	15	38,5	4	16	20,32	17,78	16	118
PCETSTR180423#2	*T.t. ponticus*	M	3	238	35	28	35	52	142	170	134	38	16	50	5	18	26	25	30	387
PCETSTR260423#3	*T.t. ponicus*	-*	3	231	31	25	32	52	144	160	174	14	37	45	4	17	-	-	-	-
PCETSTR190523#4	*P.p. relicta*	-*	3	147	15	12	16,5	29	81	110	100	21	9	34	2	9	-	-	-	-
PCETSTR220523#5	*T.t. ponticus*	M	4	117	20	15	20	28	65	78	40	17	6	22	2	9	-	-	-	-
PCETSTR270623#6	*T.t. ponticus*	-*	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PCETSTR200723#7	*T.t. ponticus*	-*	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PCETSTR021023#8	*P.p. relicta*	-*	4	106*	13	10	17	27	74	**	100 **	16	6	**	**	5	-	-	-	-
PCETSTR110324#9	*D.d. delphis*	-*	3	171	30	22	35	60	103	131	104	28	20	36	2.5	13	-	-	-	-
PCETGN140323#1	*T.t. ponticus*	F	2	180	30	24	31	40	104	120	100	29	11.5	41	3	15	25	25	25	166
PCETGN090423#2	*P.p. relicta*	M	2	110	15	10	16	26	63	78	71	18	8	27	2.5	8	19	25	20	384
PCETGN110324#3	*P.p. relicta*	M	2	93	12.5	8.5	14.5	21	44	61	64	15	6.5	22	1.5	5.5	***	***	***	***
PCETGN180324#4	*P.p. relicta*	F	1	137	16.5	11.5	17	29	73	92	94	22	9	33	2.5	9	***	***	***	***

PCETGN 180324#5	*P.p. relicta*	M	2	124	14	95	16	23.5	53.5	68	74	17.5	8	23.5	2	8	***	***	***	***
PCETGN040424#6	*P.p. relicta*	F	2	133	16	11	19	29.5	72	84	39	22	8	36	2.5	8.5	***	***	***	***

* Couldn't be determined.

**Incomplete measurements because the fluke of the cetacean was missing (M1), the body was very bloated (M8, M11, M12) and penetrative wounds in the abdomen made imposible determining de exact position of the genital area (M7).

*** To be completed after the necropsy/ GIT analysis.

M1= TOTAL LENGHT (tip of upper jaw to deepest part of fluke notch);	M10= Flipper- max. width;
M2= Tip upper jaw- centre of eye;	M11= Tail flukes tip to tip;
M3= Lenght of gape (upper jaw to corner mouth;	M12= Depth of fluke notch;
M4= Tip upper jaw to blowhole;	M13= Dorsal fin tip to base;
M5= Tip upper jaw to front insertion of flipper;	Blubber thickness (mm)- D= dorsal, L= lateral, V= ventral;
M6= Tip upper jaw to tip dorsal fin;	
M7= Tip upper jaw to centre anus;	DCC= Decomposition condition category.
M8= Max. girth;	Total number of microplastics found in each individual's GIT.
M9= Flipper- tip to front insertion;