# INNOVATIVE APPROACH TO SAMPLING OF MICROPLASTICS IN THE MARINE ENVIRONMENT

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**RESEARCH SCIENTIFIC PAPER**

Received: 24 July 2026.
Accepted: 27 August 2026.
Published: 16 November 2026.
DOI: https://doi.org/10.63395/STEDConf15022026158R377

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**Citation:** Radunović, F. et al. (2026). Innovative approach to sampling of microplastics in the marine environment. In Kojić, D. & Sredić. D. (eds.) *Conference proceedings, XV International Conference on Social and Technological Development: STED 2026* (pp. 377-386). Trebinje, B&H: University PIM.

**ABSTRACT**

MARPOL Convention recognises various types of marine pollution, including oil, garbage, sewage, and greenhouse gases pollutions. Garbage-sourced microplastics (MPs), due to their small size and high resistance coupled with their chronic biological effects, pose a serious omnipresent environmental threat. This paper's research was motivated by the lack of methodology innovations in the field of sea environment monitoring and sampling of MPs.

Traditionally, microplastic sampling was conducted using big manta-ray nets, requiring deployment from motor-propelled vessels. Driving the question of possible usage of manta-ray net in combination with unmanned vehicles for a more economical, eco-friendly, flexible and precise approach. The innovative contribution of this research is highlighted through the design of a mini manta-ray net, successfully integrated on Remotely Operated Underwater Vehicles (ROV). Mini manta-ray was constructed in accordance with all relevant technical specifications using a special 300 micron net. First, the prototype was constructed for preliminary testing of structural integrity and stability. Based on experience and the results of preliminary prototype testing, the construction of the final product was used for our first MP sampling using an ROV. Sample of 1.5 liters of seawater obtained from the filtration of 5000 liters of seawater was then sent for laboratory analysis to evaluate system functionality under realistic operational conditions.

Practical testing confirmed stable and functional operational integration of mini manta-ray with ROV. From good water worthiness to positive results from laboratory analysis, indicating reliable sampling performance. The results show realistic functionality and potential for further improvements and optimization of the sampling of microplastics by ROV

**Keywords:** MARPOL Convention**,** Microplastics (MP), Innovative, Mini manta-ray net, Remotely Operated Underwater Vehicles (ROV).

## INTRODUCTION

Oceans and seas are some of the most precious parts of our ecosystem. They are a source of life, food, resources, tourism, climate regulation and transport. However, marine pollution remains one of the major environmental challenges of modern society [1]. Their pollution and preservation are primarily regulated by the International Maritime Organisation (IMO) under the International Convention for the Prevention of Pollution from Ships (MARPOL) [2].

The International Convention for the Prevention of Pollution from Ships (MARPOL) recognizes the following types of pollution: Annex I Regulations for the Prevention of Pollution by Oil (entered into force 2 October 1983), Annex II Regulations for the Control of Pollution by Noxious Liquid Substances in Bulk (entered into force 2 October 1983, provisions took effect from 6 April 1987), Annex III Prevention of Pollution by Harmful Substances Carried by Sea in Packaged Form (entered into force 1 July 1992), Annex IV Prevention of Pollution by Sewage from Ships (entered into force 27 September 2003) and Annex V Prevention of Pollution by Garbage from Ships (entered into force 31 December 1988), Annex VI Prevention of Air Pollution from Ships (entered into force 19 May 2005). For the purposes of this research, the most relevant is Annex V (pollution by garbage) as an emerging marine pollutant [3].

Driven by garbage pollution and the degradation of a wide variety of plastic and synthetic objects, including even sea buoys, microplastic pollution has become a widespread problem. Microplastics (MPs) include all plastic fragments smaller than 5 mm [4, 5]. The most common sources of MPs in today's aquatic ecosystems are wastewater treatment plants, fishing, shipping, landfills, illegal dumping, and industrial pollution [6, 7]. Their small size and high resistance make them a serious challenge for environmental protection and preservation, triggering chronic biological effects such as inflammation and affecting the growth and reproduction of organisms [8]. Microplastics are distributed throughout all water depths, from the surface to the seabed, where they attach to and are ingested by marine flora and fauna. In fish, one of the most commonly consumed seafood products, MPs can be found in the gills and even inside the stomach and intestines. However, the highest human intake of MPs through seafood is considered to occur from the consumption of bivalves, as they are eaten whole [9, 10]. An additional concern is that MPs can act as transport surfaces for other contaminants, such as heavy metals, resulting in coupled toxic effects [11, 12].

Today, there are highly diversified types of MPs that can be found in marine environments. Polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET), and polystyrene (PS) are the dominant types due to their widespread use [13]. However, PE and PP are the most commonly found polymers in surface waters because of their lower density, whereas higher-density polymers, such as PVC, tend to sink and become part of the marine sediment [14].

Even today, there are still gaps in research on microplastics. From their movement and transport in our environment as well as their toxic and harmful effects caused by their accumulation. Even a lack of properly standardized sampling and analysis on a global level. Traditionally, they are sampled with manta-ray nets with 300- 350 μm mesh, with notable differences in results depending on the net's mesh size [15, 16]. Their concentration is calculated with the amount of filtered seawater that goes through the frame of the net based on a flow meter. That is then compared to the number of positive MPs found after laboratory analysis in the given sample, giving us X number of MPs per $m^3$ [17].

Despite the widespread application of conventional microplastic sampling methods, limited methodological innovation and optimization have been introduced in recent years. Traditional microplastic sampling is typically carried out using motor boats towing large manta-ray nets [14]. Although this approach provides reliable sampling results, it also has several limitations. Motor boats contribute to environmental pollution, are unsuitable for sampling in shallow waters, and provide limited manoeuvrability and positioning accuracy during sampling operations. The application of a Remotely Operated Vehicle (ROV) integrated with a compact manta-ray overcomes many of these limitations. Enabling sampling in shallow and difficult-to-access areas

while simplifying and making the sampling process more economically and environmentally sustainable.

Through the development, design and construction of an innovative manta-ray prototype, named *Mini*, surface water sampling was conducted. Sampling was performed using the *Mini* manta-ray integrated with the QY SEA *FIFISH W6* ROV. Sampling was carried out in the Boka Kotorska Bay in Montenegro. Development of the Mini manta-ray, acquisition of the ROV, and laboratory analysis of the collected sample were funded by MARROBO (Interreg IPA CBC Montenegro – Bosnia and Herzegovina, see Acknowledgement).

The sampling of surface water in a micro-location in front of the Maritime Faculty Kotor (PFK) involved filtering seawater through a *Mini* manta-ray that gave a sample of microplastics. Laboratory analysis of the collected sample provided feedback on the concentration of microplastics in surface water levels. Showing us data on area, perimeter, maximum Feret diameter, color distribution of collected microplastic particles, and indicated the level of local microplastic pollution. Results of sampling at depths of up to 10 cm gave us nearly average-level results regarding microplastic concentration in the Mediterranean Sea [18] and operational validation of the *Mini* manta ray prototype.

Grounded in prior research on microplastic particles, their sources, and associated pollutants, this study explores an innovative approach to microplastic particle sampling and the assessment of their concentrations in surface seawater. A laboratory-based analytical methodology was applied to determine the physical composition and characteristics of the collected water samples. The principal objective of this work is to establish a scientific foundation for a more efficient and modern sampling methodology for assessing environmental health based on microplastic concentrations. The study includes the design and construction of a specially optimized manta-ray microplastic sampler for integration with an ROV, followed by its practical validation through the analysis of the collected samples.

The present research has several limitations. A single sample was collected and analysed, representing a preliminary result. Requiring multiple samples for further validation as part of future research, together with a direct comparison with results obtained using traditional sampling methods.

The structure of the paper is organized into four main sections. The second section outlines the research methodology, detailing the data sources and analytical techniques employed. The third section presents the key findings and their interpretation. Finally, the fourth section provides concluding remarks and proposes directions for future research.

## METHODOLOGY SECTION

This study follows a structured methodology for the development and evaluation of an innovative microplastic sampling system based on a new Mini Manta-Ray sampler integrated with a Remotely Operated Vehicle (ROV), can be seen in Figure 1. The research was motivated by the growing concern regarding microplastic pollution in the marine environment and the lack of innovative sampling methodologies capable of providing flexible, precise and environmentally sustainable monitoring solutions compared to traditional methods.

A summary of the research methodology is presented in Figure 2. The methodology included the design, fabrication and testing of two Mini Manta-Ray prototypes. The initial prototype was developed for preliminary assessment of structural integrity, hydrodynamic stability and operational compatibility with the ROV platform. Based on the results obtained during experimental testing, an improved prototype was constructed and integrated with the ROV for field sampling operations. Seawater samples were collected using a specially designed 300 μm sampling net and subsequently prepared for laboratory analysis to evaluate the functionality and effectiveness of the developed sampling system under realistic operational conditions.

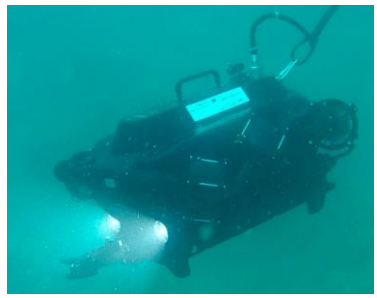


Figure 1. *FIFISH W6* ROV

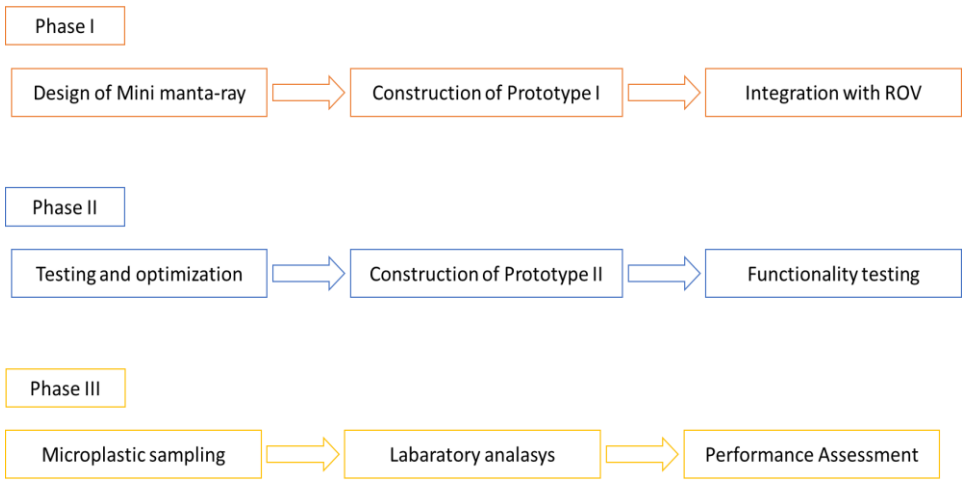



Figure 2. Research methodology summary

Phase I focused on the design and development of the Mini Manta-Ray sampling system. First was the conceptual design stage (Figure 3), followed by the construction of the prototype and its integration with the ROV platform (Figure 4) to assess structural compatibility and operational capability.

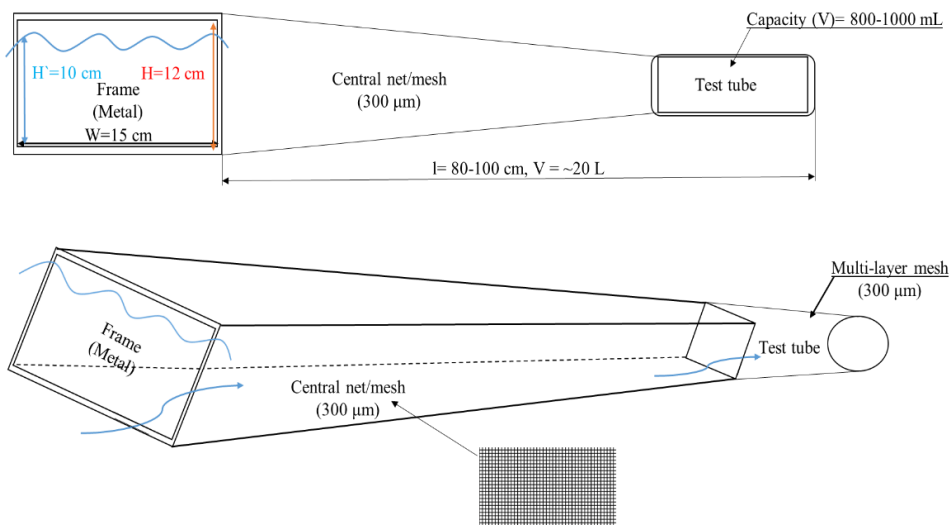



Figure 3. Conceptual design of *Mini* manta-ray



Figure 4. Construction of *Mini* manta-ray prototype I

Phase II involved testing of the initial prototype and optimization of the design based on gathered experience. Followed by the development of an improved Mini Manta-Ray design. With performance assessment of the improved Mini Manta-Ray (Prototype II).

Phase III consisted of the Mini Manta-Ray deployment with ROV *FIFISH W6* for microplastic sampling under real operating conditions (Figure 5). The gathered sample was then taken for laboratory analysis to provide a preliminary validation of this novel methodological approach to microplastic sampling.



Figure 5. Construction and deployment of *Mini Manta-Ray prototype* II for microplastic sampling

**Database**

A total of 5,000 L of seawater was filtered using the final prototype of the Mini Manta-Ray sampling system integrated on an ROV. Sampling was conducted in the waters surrounding the Faculty of Maritime Studies in Kotor, under relatively stable marine conditions and a seawater temperature of approximately 17 °C.

To estimate the volume of filtered seawater, the flow rate of the sampling system was calculated using the basic parameters presented in Figure 3. First, the opening area ($A = W \times H$) was determined using the opening width (W) and height (H). The estimated volumetric flow rate ($Q = A \times v$) was then calculated using the obtained opening area. Determination of the ROV travelling speed ($v$) represented the most challenging part, as a flow meter was not available. This limitation was addressed by measuring the time required for the ROV to travel between two predefined reference lines. The total volume of filtered seawater ($V$filtered) was then calculated by multiplying the volumetric flow rate (Q) by the sampling time (t). Although the calculated ROV speed was not 100% accurate, it provided a sufficiently reliable estimate for the purposes of the analysis.

Upon completion of the sampling procedure, the collection net was carefully retrieved and rinsed with fresh water to ensure the removal of all MPs and other potential particles into a sample bottle. The resulting concentrated sample of approximately 1.5 L was then preserved and sent to the Ruđer Bošković Institute in Croatia for laboratory analysis and identification of microplastic particles.

**Methods**

The collected sample was subjected to laboratory analysis at the Ruđer Bošković Institute. Potential microplastic particles were identified and quantified through visual examination using a stereomicroscope (Nikon SMZ745T, Figure 6). Particle identification was based on visible physical characteristics, including shape, colour, uniformity and the absence of recognizable biological structures.

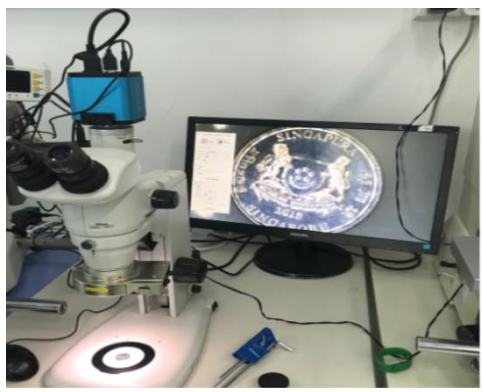


Figure 6. *Nikon SMZ745T*

Image acquisition and particle measurements were performed using *Image J/Fiji* software. Since no spectroscopic confirmation methods, such as FTIR or Raman spectroscopy, were applied, all detected particles were classified as putative microplastics. Therefore, the obtained results represent a preliminary assessment of microplastic presence and should be interpreted as visually identified plastic-like particles (putative microplastic particles) pending further analytical verification.

## RESULTS AND DISCUSSION

From a single sampling site in the Kotor area, 5000 L of seawater was filtered with a mini manta-ray net, mesh size 300 μm. A total of 92 putative microplastic particles were identified by visual inspection from a 1,5 L freshwater sample.

The perimeter histogram for the Kotor sample (Figure 7) depicts a strongly right-skewed, unimodal distribution with the highest frequency in the 1–2 mm bin (61 putative particles, 66.3%). Nearly 97% of the putative microplastics have perimeters of 5 mm or less, while only one particle reaches the 20–50 mm range. This pattern reflects a sample dominated by putative microplastics with small-to-moderate perimeters, with a sparse tail of larger, likely elongated or fibrous outliers contributing to the high skewness, high kurtosis, and elevated mean observed in the descriptive statistics.

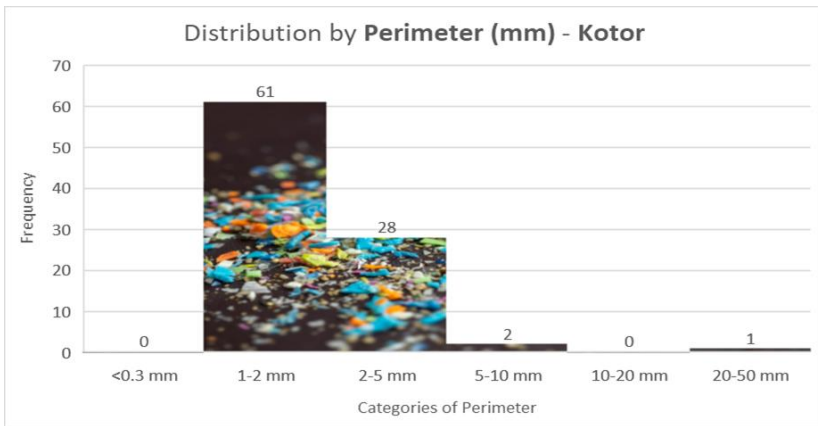



Figure 7. Perimeter histogram

The maximum Feret diameter histogram for the Kotor sample (Figure 8) shows a unimodal, right-skewed distribution with the highest frequency in the 0.5–1 mm bin (36 putative particles, 39.1%). The vast majority (98.9%) of putative microplastics are 2 mm or smaller in their longest dimension, with only one putative particle exceeding 2 mm and none larger than 3 mm. This pattern reflects a sample overwhelmingly composed of small putative microplastics, with moderate positive skew and leptokurtosis driven by a small number of larger outliers, consistent with the descriptive statistics for the Kotor location.

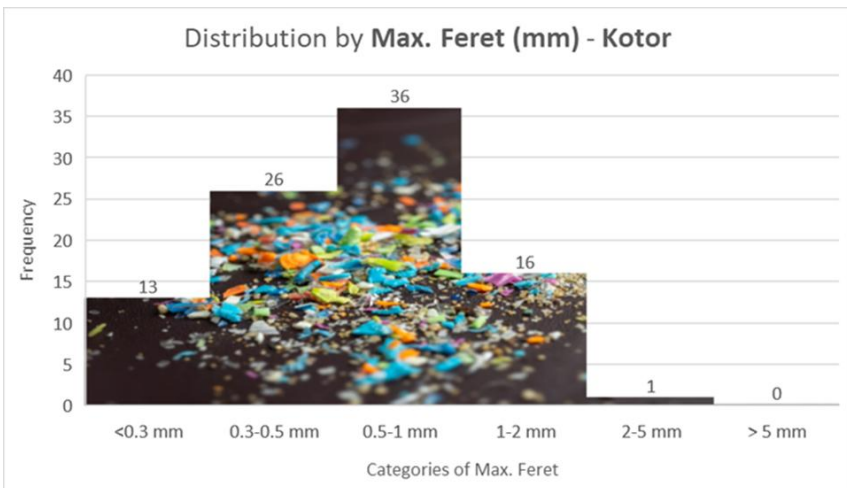


Figure 8. Feret diameter histogram

The area histogram for the Kotor sample (Figure 9) reveals an extremely skewed distribution in which 91 out of 92 putative particles (98.91%) are smaller than 0.5 mm², with only one particle in the 0.5–1 mm² bin and none larger. This pattern demonstrates a sample overwhelmingly dominated by very small putative microplastics, consistent with the low mean (0.04 mm²), median (0.02 mm²), and the strongly right-skewed, extremely leptokurtic distribution described in the summary statistics.

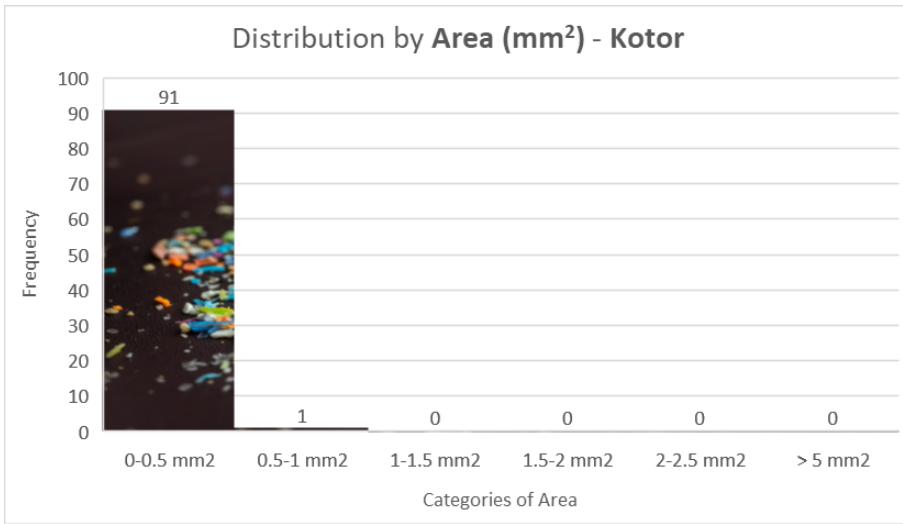



Figure 9. Distribution by area histogram

The color distribution in the Kotor sample (Histogram 10) is highly skewed, with black putative particles dominating overwhelmingly (86 putative particles, 93.5%). Red (4 putative particles, 4.3%) and blue (2 putative particles, 2.2%) appear in trace amounts, while all other colors are absent. This indicates an exceptionally uniform color profile dominated by black putative microplastics at the Kotor site. With a notable mention that 91 out of 92 were fiber-shaped particles, and only one filament.

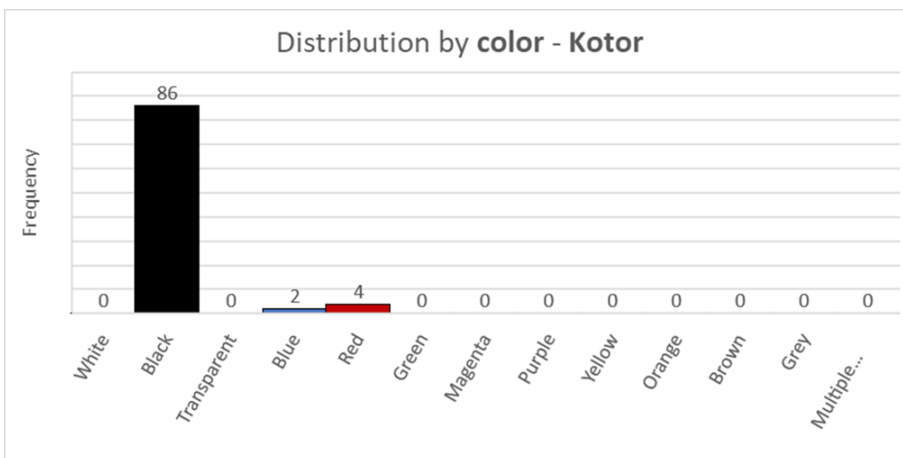



Figure 10. Distribution by color

The analysis of 92 putative microplastic particles collected from 5000 L of seawater in the Kotor area using a 300 µm mesh net reveals a highly uniform, fiber-dominated pollution profile characterized by very small particle sizes. The particles are overwhelmingly tiny, with 98.9% having an area below 0.5 mm² (median 0.02 mm²), a median maximum Feret diameter of 0.55 mm. Almost exclusively black colored and fiber-shaped.

## CONCLUSION

The developed Mini Manta-Ray prototype successfully demonstrated its potential as an innovative microplastic sampling system integrated with an ROV platform and represents a preliminary result. Field testing and laboratory analysis confirmed its operational feasibility, sampling capability and potential as an alternative to conventional sampling methods. Future development of *Mini* manta-ray should focus on the integration of a flowmeter for accurate measurement of filtered water volume, as well as the integration of the sampler on a fully autonomous unmanned platform, enabling more efficient, cost-effective and large-scale environmental monitoring. While, at the research level, further validation should be based on a larger number of samples, supported by additional laboratory analyses and direct comparison with traditional sampling methods.

## ACKNOWLEDGEMENT

This work is supported by the project "Fostering the innovation capacities and skills for marine robotics deployment for monitoring and marine environment protection" (MARROBO), which has been approved for funding under the First Call of the Interreg VI-A IPA Program Croatia – Bosnia and Herzegovina – Montenegro 2021 – 2027, from July 15, 2024, to July 14, 2026. The MARROBO project aims to boost research and innovation in marine robotics for monitoring and protecting the marine environment. It focuses on developing advanced robotics to measure microplastics, metals in seawater, and noise pollution while building new skills. The project seeks to encourage smart investments in robotics that enhance biodiversity protection in the Adriatic Sea, reduce pollution, and promote a sustainable blue economy.

## DECLARATIONS OF INTEREST STATEMENT

The authors affirm that there are no conflicts of interest to declare in relation to the research presented in this paper.

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