OCCURRENCE AND TYPE OF MICROPLASTICS PRESENT IN ISHMI RIVER WATER AND SEDIMENTS: AN OVERVIEW OF METHODOLOGY FOR SAMPLING AND ANALYSIS

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ABSTRACT

In the past decade, microplastic waste in marine and freshwater ecosystems has received increasing attention. The presence of floating plastic debris, including microplastics (MPs), has been documented since the early 1970s, not only in the North Atlantic, North Pacific, and South Pacific Oceans but also in the Mediterranean Sea. In response to this, the United Nations Environment Program has identified plastic pollution as a critical environmental issue. Alongside climate change, microplastic pollution is emerging as a serious challenge with the potential to significantly affect biodiversity and human health. In Albania, an estimated 198 tons of low-density and 111 tons of high-density plastic waste are produced daily, comprising about 14% of the country's total waste. Due to its durability, plastic can persist in lakes and seas for hundreds of years, eventually breaking down into micro- and nanoplastics, which can infiltrate living organisms and enter the food chain. While most microplastic studies have focused on marine environments, research on riverine systems remains limited. This study addresses this gap by investigating the presence of microplastics in the water and sediments of the Ishmi River, which is considered Albania's most polluted basin by the National Environmental Agency. The Ishmi River, 74 km in length, flows through

Rinas and Fushë Kruja before reaching the Adriatic Sea at Cape Rodoni. The primary sources of pollution in the river are plastic and microplastic waste, stemming from household products, local activities along the river basin, and natural processes. This study presents an analytical methodology for sampling and analyzing microplastic pollution, including the steps of collection, chemical treatment, density separation, and sample filtration. The objective was to adopt protocols that isolate microplastics from large amounts of organic matter in the riverine environment, while preserving their structure. The identification of microplastics was performed using optical microscopy, FTIR, and Raman Spectroscopy.

***Keywords*:** Microplastics, sampling, spectroscopy, freshwater ecosystem

1. INTRODUCTION

A substantial volume of plastic waste has infiltrated aquatic ecosystems due to extensive production and inadequate waste management practices (Filgueiras et al., 2019). Within these environments, plastic waste undergoes fragmentation, producing micro-sized particles such as fragments, fibers, spheroids, granules, pellets, and flakes, ranging in size from 0.1 to 5000 µm, collectively known as microplastics (MPs) (Adomat *et al*., 2021a). Plastics are extensively used in various industries, with packaging accounting for the largest share at 40%. Unfortunately, around 70% of all produced plastic ends up as waste, with only 9% being recycled (Maddela *et al*., 2023). The ecological ramifications of microplastics are profound, affecting degradation processes, environmental interactions, and their potential impact on the food chain and human health (Vethaaka *et al*., 2021; Maddela *et al*., 2023).

Plastic pollution is a significant issue in Albania. According to a recent World Health Organization (WHO) report, Albania is among the most problematic countries, with 73% of its plastic waste left untreated (WWF, 2019). This places Albania among the top four Mediterranean countries with the highest levels of untreated plastic waste, alongside Montenegro, Egypt, and Libya. Moreover, the average amount of total marine litter in Albania's coastal area is estimated to be 0.7 kg at a depth of 241–244 meters, with 0.2 kg of that being plastic (Gjyli *et al*., 2020; Kolitari *et al*., 2020). To date, there are only 2-3 studies that provide data on plastic pollution in the Albanian marine ecosystem, with a similarly limited number of studies on microplastic pollution (Aliko *et al*., 2022).

This study aims to introduce a sample preparation technique designed specifically for the analysis of microplastics in river waters. The correct application of this technique was crucial to ensuring accurate and reliable data, involving the effective selection and isolation of microplastics while minimizing contamination (Löder *et al.*, 2015). The study critically assessed the methodologies used to gauge microplastic pollution in the Ishmi River environment (Marine Debris Program, 2015; Faruk Çullu *et al.*, 2021). The primary focus of this paper is on presenting analytical methods, including a review of non-selective sampling approaches, laboratory processing, and the techniques applied for microplastic analysis.

Using Image-J software, microplastics were classified by size and type. The study also evaluated the presence of various microplastic polymers through Fourier Transform Infrared (FTIR) and Raman spectroscopies, complemented by microscopic examinations

2. MATERIALS AND METHODS

2.1 Sample collection in river water and sediments

Collecting river water samples for microplastic pollution analysis requires meticulous planning to ensure accurate and representative results (AMP *et al.*, 2020). Before selecting the sampling stations, it was essential to gather information on the Ishmi River, including its characteristics and potential sources of microplastics in the area. Three stations were selected along the river based on their potential for microplastic contamination (Diku *et al.*, 2020), representing urban, industrial, and rural zones.

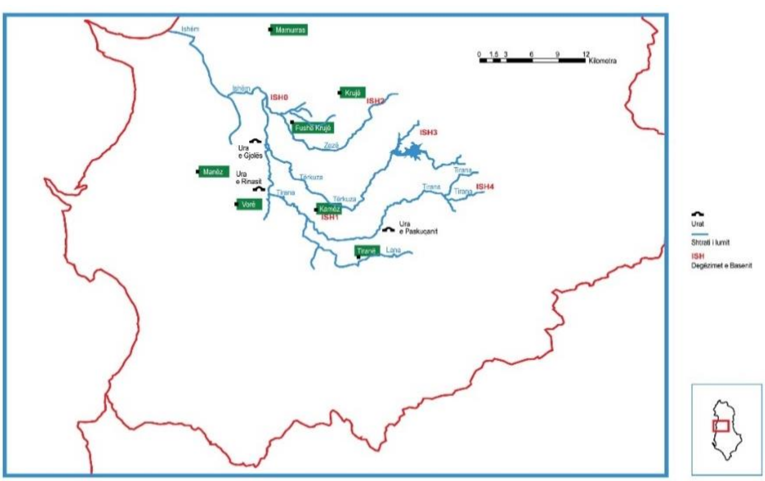


Fig.1. Map of bridges where the samples were taken.

Before sampling, all equipment was sterilized, and cotton clothing and laboratory gloves were worn to prevent contamination. At each station, 2-3 liters of water samples were collected from the coastal areas, with the third station located along the riverbanks and at the river's center. Sterilized glass bottles were used to collect surface water at approximately 10 cm depth (Zheng *et al*., 2021). Sampling took place in June 2023.

For accurate estimation of microplastic concentration in sediment samples, 2-3 kg of sediment was collected from a depth of 1 to 5 cm (Prata *et al*., 2019). All samples were transported to the laboratory and stored at 4°C until further processing.

2.2 Microplastic Separation from samples

The process of microplastic separation is a pivotal stage, crucial for ensuring the precision and reliability of the analysis, thereby aiding in a deeper understanding of microplastic pollution in aquatic environments. Separation of samples (Prata *et al*., 2019) is performed for three main purposes: i) to concentrate microplastics from a larger water volume, facilitating more accurate detection and analysis. ii) to eliminate other organic and inorganic materials present in the sample, and iii) to enhance the concentration of microplastics, allowing for a clearer visual examination under a microscope, aiding in their accurate identification and classification.

The separation of microplastic particles from denser matrices is achieved through flotation, using saturated salt solutions with high density.

2.3 Water samples

To minimize the influence of organic matter in the water sample, 30% H_2O_2 was added (Li *et al*., 2021) to the glass bottle containing the water sample. The bottle was then placed in a constant temperature oscillation box and vibrated at 100 r/min at 70°C for 24 hours. After this treatment, the supernatant was extracted and mixed with NaCl in a ratio of 1 liter to 300 grams and placed on a constant temperature magnetic plate for an additional 24 hours.

To reduce sample volume and improve efficiency, the entire sample was transferred to a separatory funnel and allowed to sit for 24 hours. After decanting the larger particles and separating densities, only the top 1/5 of the water column (surface part) was filtered using a vacuum pump with 2.2 μm filter paper. Prior to analysis, the filter papers were placed in an oven and dried at 30°C for 48 hours.

2.4 Sediment samples

The sediment samples were dried in an oven at 70°C and then sieved through a 500–75 µm mesh for 30 minutes (Claessens *et al.*, 2011; Adomat *et al.*, 2021b). The sieved samples were diluted in a saturated NaCl solution and left on a magnetic plate for 72 hours to ensure thorough homogenization. To minimize the sample volume, the mixture was transferred to a separatory funnel and allowed to settle for 24 hours. After decanting, only the top 1/3 of the water column (surface portion) was treated with 30% H_2O_2 at 60°C on a magnetic plate (Klein *et al.*, 2015). After 48 hours, the solution was transferred to the separatory funnel again and left to settle for another 72 hours. Following this second decantation, the remaining water column was filtered using a vacuum pump through 2.2 µm filter paper. The filtered sample was then placed in a covered Petri dish and dried in an oven at 30°C for 72 hours.

3. RESULTS

3.1 Visual and chemical identification of Microplastics

Over the past decade, FTIR imaging has emerged as one of the most effective techniques for analyzing microplastics. This method allows for the rapid recognition, counting, identification, and classification of particles and fibers based on their spectral information (Janice Brahney, 2021).

Initially, the filter was examined using an optical microscope (Kozo XJPG304, Sony TCC-8.1, version 7.3.1.7, 40x100 zoom) (Fig. 2). Detected microplastic waste was pinpointed with coordinates and subsequently analyzed using a FTIR Spectrometer Nicolet 6700 (Fig. 3). The resulting images provided clear information on the presence of microplastic polymers such as polystyrene and polyethylene.

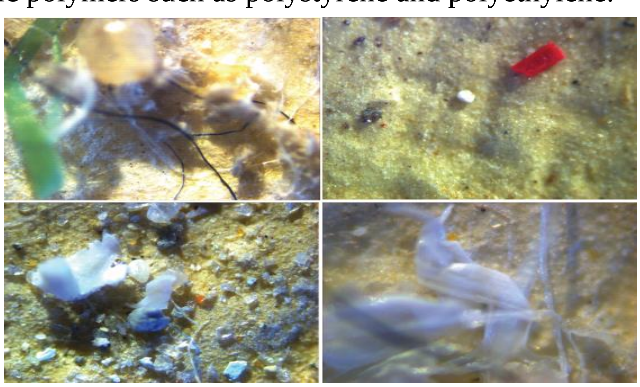


Fig.2. Images of MP waste in samples

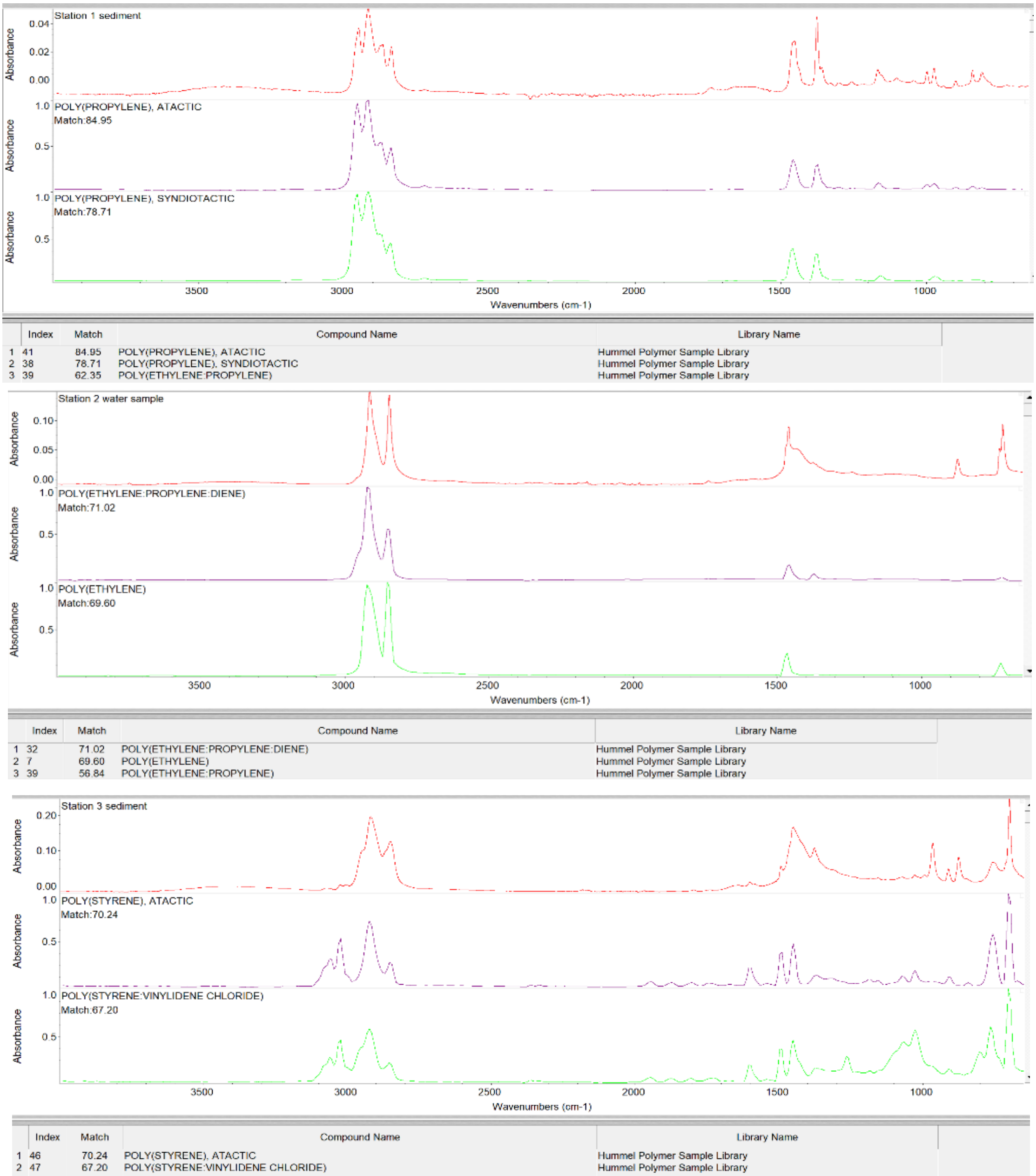
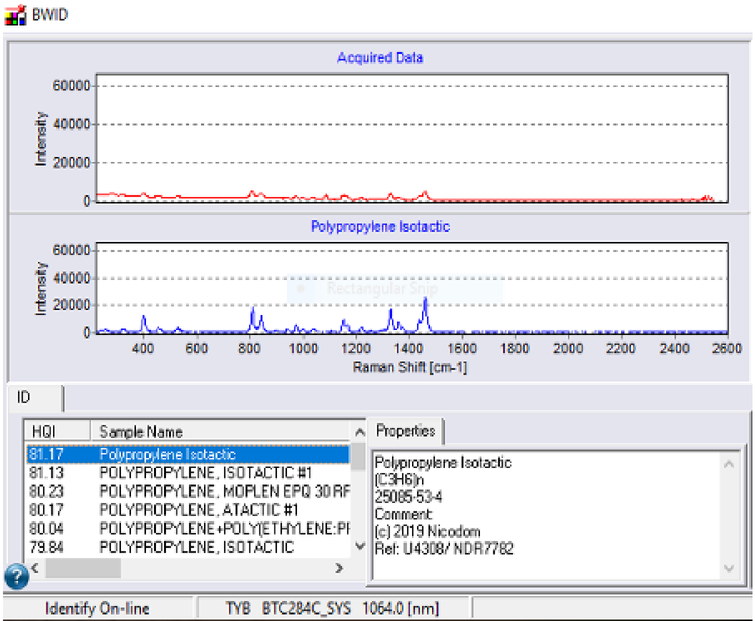


Fig 3: Examples of the FTIR spectra of the three microplastic particles found in the water and sediment samples, which were measured by micro-FTIR spectroscopy: upper panel polypropylene, second panel polyethylene, third panel polystyrene.


BWID
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1600
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81.17
Polypropylene Isotactic
81.13
POLYPROPYLENE, ISOTACTIC #1
80.23
POLYPROPYLENE, MOPLEN EPQ 30 RF
80.17
POLYPROPYLENE, ATACTIC #1
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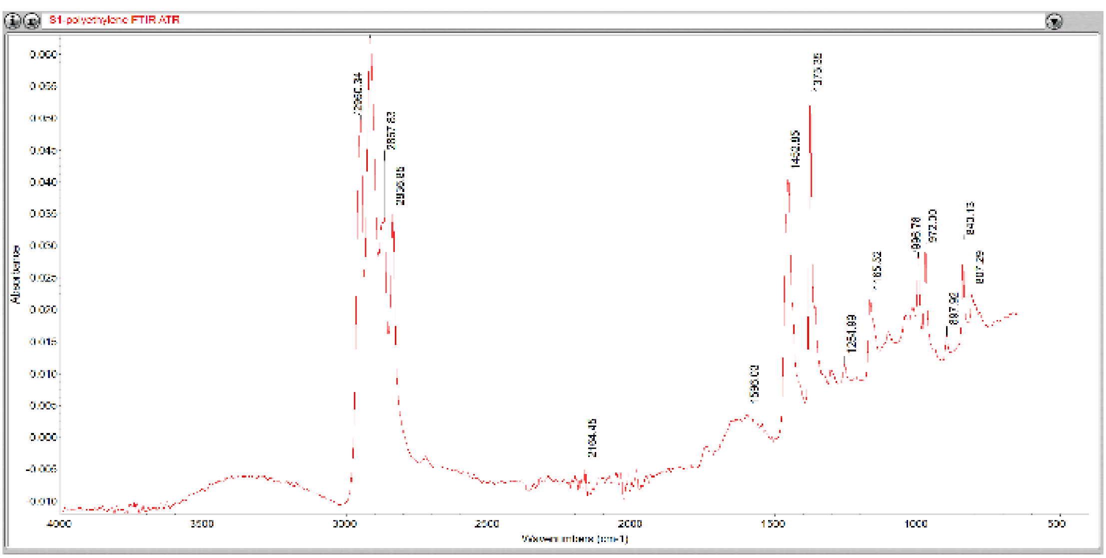
S1 polyethylene FTIR ATR
Absorbance
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Wavenumbers [cm-1]

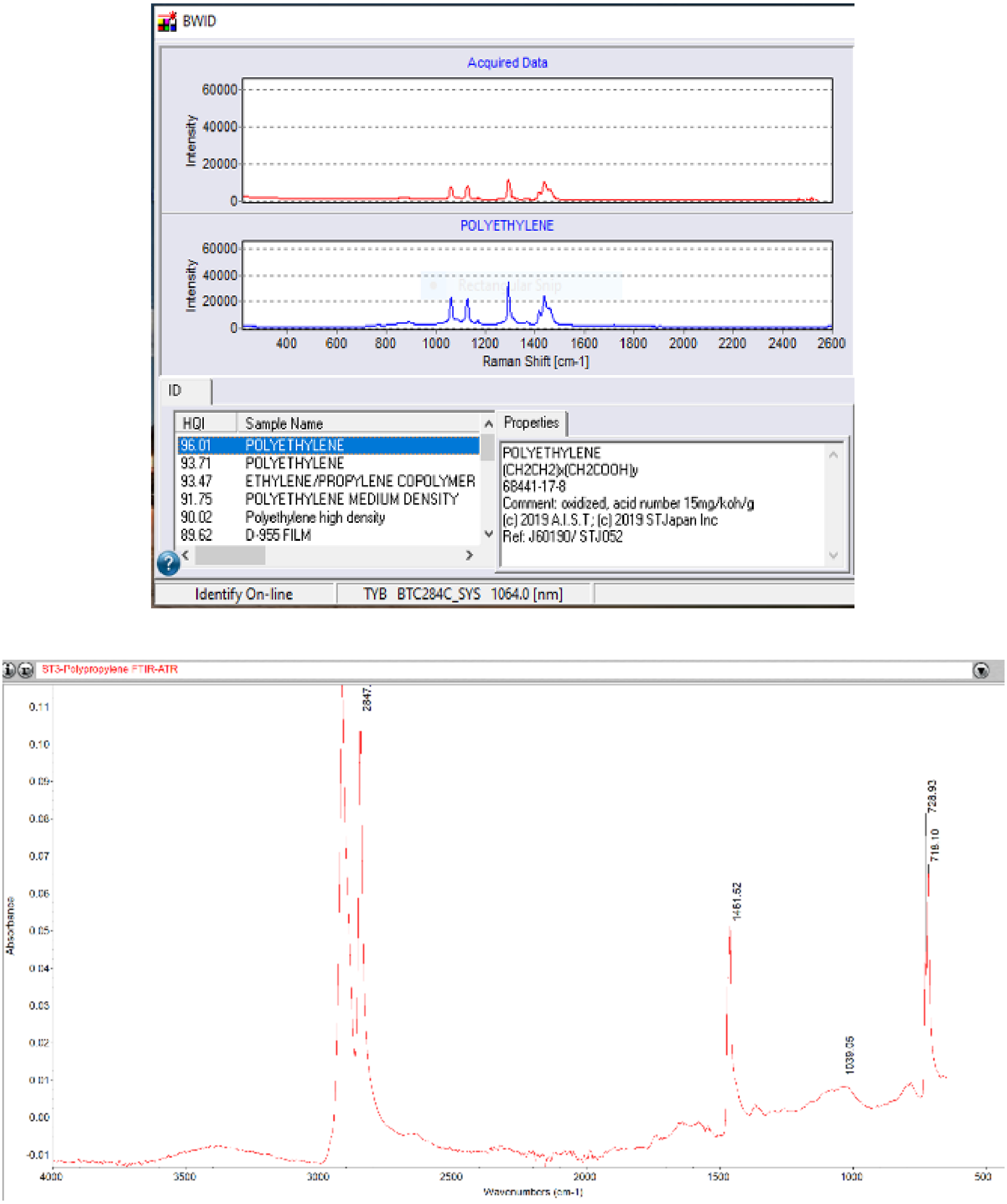

Fig.4. Raman Spectra (left), FTIR Spectra(right) of polypropylene and polyethylene of the same samples.

3.2 FTIR and Raman analysis

To assess the quality of the obtained results, the samples were also analyzed using Raman spectroscopy. The results from both spectroscopic techniques—FTIR and Raman—were consistent, confirming the presence of the same polymers in the samples (Fig. 4). These techniques, being complementary, provided a robust validation of the microplastic polymer identification.

4. Discussion

Due to the techniques used for extracting and purifying the samples, it was expected that they would primarily contain tiny fragments, fibers, particles, and other low-density organic materials. However, plastic fragments were notably scarce, comprising only 20.8% of the particles analyzed. Plastic fibers made up 23%, while particles constituted 22.9%. Our manual screening of bright-field microscopic images for areas with heterogeneous particle appearance revealed a moderate overall abundance of microplastics in the samples.

First, it is noteworthy that a significant portion of the particles were quartz, which was unexpected given that these were supposed to be excluded during density separation. The presence of substantial quantities of sand grains raises questions and suggests a need for further investigation. To address this, it is advisable to use a higher-density salt, such as NaI instead of NaCl, to improve the qualitative differentiation between microplastic waste and the remaining solution.

Second, literature on water sample collection indicates various methodologies, emphasizing the importance of sampling time and river water velocity. Using a Manta net could enhance the capture of a representative sample of microplastic pollution by ensuring a more comprehensive collection of waste abundance (Pasquier *et al*., 2022).

5. CONCLUSION

This case study highlights the essential role of spectroscopic techniques, such as FTIR and Raman spectroscopy, in microplastics analysis. FTIR and Raman spectroscopy have proven to be highly effective for confirming the polymer composition of microplastic particles. The microscopic method used to evaluate the residues was successful, as microplastic particles were clearly visible and identifiable. The analysis revealed that the most common microplastics were fibers ranging from 100 to 200 µm in size, while fragments were predominantly smaller than 100 $µm^2$. The polymers identified in the samples included polypropylene, polyethylene, polystyrene, polyvinyl chloride, and polybutadiene. FTIR spectroscopy offers a broad range of wavenumbers, including fingerprint regions and single bonds, providing comprehensive polymer characterization. Conversely, Raman spectroscopy excels in providing detailed information in the fingerprint region, complementing the data obtained from FTIR.

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ASSESSMENT OF THE POPULATION STATUS OF GLOBALLY THREATENED CRAYFISH *ASTACUS ASTACUS* AND *AUSTROPOTAMOBIUS TORRENTIUM* IN THE LAKES PRESPA, OHRID AND RIVERS OF THE SHEBENIK - JABLLANICË NATIONAL PARK, ALBANIA

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ABSTRACT

Despite being internationally recognized as threatened, there are no comprehensive studies on the crayfish species *Astacus astacus* (Linnaeus, 1758) and *Austropotamobius torrentium* (Schrank, 1803) in Albanian's freshwaters. Existing research, primarily conducted by foreign researchers in the Albanian portions of Lakes Ohrid and Prespa. This study spans one year (2023-2024) and includes 7 monitoring stations within Lake Ohrid, 5 stations in Great Prespa, 2 stations in Small Prespa Lake, and 2 additional stations on the Qarrishta stream within the National Park Shebenik. The methodology used involves baited traps known as "LiNi-Trap". Alongside crayfish monitoring, water parameters such as pH, temperature, conductivity, PPM and dissolved oxygen were also measured. The study found *A. astacus* present only in Great Prespa and Ohrid lakes, while

A. torrentium was not detected in any of surveyed water bodies. In Lake Ohrid, *A. astacus* was observed at four of the seven monitoring stations in the Lin - Piskupat- Udenisht - Memelisht area, and in Great Prepsa Lake it found in three of five stations in the Kallamas - Gollomboc – Pustec area. The measured water parameters were within the optimal range values for species growth, suggesting that factors other than water quality, such as reduced rainfall, are contributing to the displacement of individuals to deeper habitats, potentially affecting their population numbers in monitored areas.

Keywords: Astacus astacus, Austropotamobius torrentium, Ohrid lake, Prespa lake,Shebenik, bait trap, monitoring

1. INTRODUCTION

In Europe, the genera Astacus and Austropotamobius comprise a total of 5 species: *Astacus astacus, Astacus leptodactylus, Astacus pachypus, Austropotamobius torrentium,* and *Austropotamobius pallipes* (Trožić-Borovac, 2011). According to Holdich (2002), while two of the four European crayfish species found in the Balkan Peninsula—*A. astacus* and *A. torrentium*are—are believed to be present in Albania, no studies on their distribution have been published to date, to the best of our knowledge.

Pârvulescu *et al.* (2011), asserts that crayfish taxa are vulnerable to various threats, including overexploitation, habitat modifications, water pollution, increasing pressure from invasive crayfish species, and the crayfish plague. Over the past few decades, stone crayfish declined significantly across their Europea range (Kouba *et al*., 2014), highlighting the need for comprehensive studies, including Albania. As noted by Lindqvist and Lathi (1983), noble crayfish engage in mating during autumn, and the duration of their life cycle is affected by the climate and habitat in which they live. The breeding season begins with a drop in temperature in autumn, while the maturation of testes and ovaries occurs between July and September (Lindqvist and Lathi 1983).

The noble crayfish, Astacus astacus (Linnaeus, 1758), from the genus Astacus, is widely distributed across Europe, both as a native species and through introductions in various regions (Souty-Grosset *et al*., 2006). According to the IUCN, A. astacus is classified as "vulnerable" (Edsman *et al*., 2010). Holdich *et al.*, (2009), and Kouba *et al*., (2014) describe it as the most widespread indigenous crayfish species in Europe, with a range that includes central and northern Europe, encompassing the North Sea, Baltic Sea, Black Sea, Adriatic Sea basins, and the Balkan Peninsula.

The assumption of *A. astacus* presence in Albania has primarily been based on records from transboundary water bodies outside the country (Mrugała 2017). Between 2004 and 2015, ichthyological studies conducted on major rivers across Albania investigated crayfish distribution, identifying *A. astacus* at two locations in Lake Ohrid. In addition to sightings in Lake Ohrid and the upper Devoll River, *A. astacus* populations have also been observed in the Albanian section of Lake Prespa (Đuretanović *et al*., 2017). Earlier studies have consistently reported A. astacus presence in transboundary waters of neighboring countries and in several streams near Albania (Mrugała 2017).

The species *Austropotamobius torrentium* (Schrank, 1803) is classified as "data deficient" by the IUCN (Füreder *et al*., 2010) and is recognized as a "priority species" under the Habitats Directive (European Communities, 1992). It has also been reported in Albania (Subchev 2011), with a specimen collected from the Fani i Madh River in the northern part of the country in 2003 (Subchev, 2011). The presence of *A. torrentium* has also been documented in the Montenegrin part of the Skadar Lake basin (Trontelj *et al*., 2005) and is widespread in northern Greece, where its western distribution extends to the area around the city of Kastoria, near the Albanian border (Koutrakis *et al.*, 2007). Conservation policies for Astacus astacus and Austropotamobius torrentium include protections under the Bern Convention (1982) and the EU Habitats Directive (1992), which classify these species as part of the sensitive freshwater fauna in need of conservation.

In Albania, the Biodiversity Protection Law (No. 9587, 2006) mandates measures to preserve species and their habitats, and the National Biodiversity Strategy (2016-2020) aims to monitor and restore habitats where these species naturally occur.

This limited knowledge about the distribution of these two species underscores the need for further research to clarify their presence in the rivers of Shebenik National Park and the lakes of Ohrid and Prespa. Participation in this one-year study in these aquatic bodies is further encouraged by reports from local fishermen and residents indicating the presence of these species.

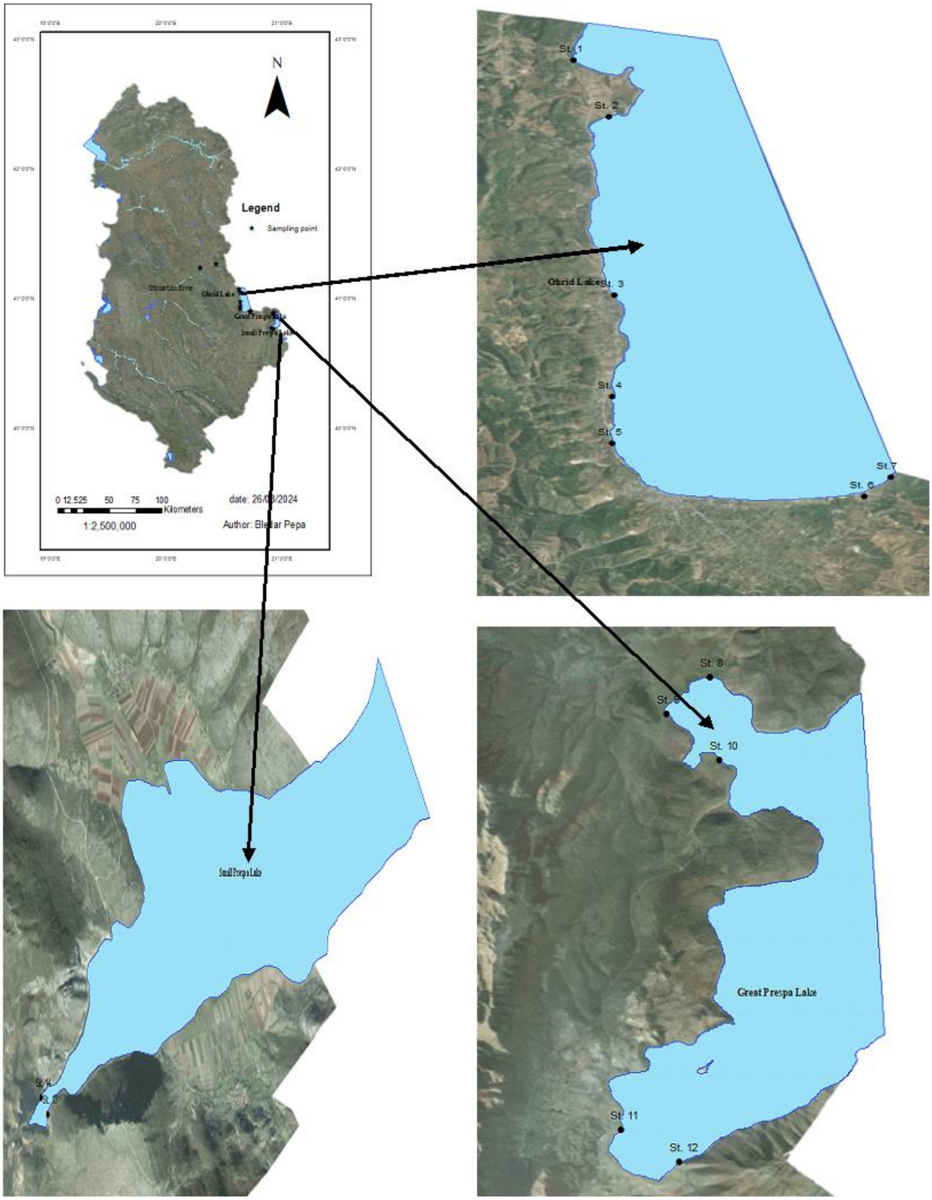
2. MATERIALS AND METHODS

2.1. Study area:

The study area encompasses the Prespa and Ohrid Lakes, along with rivers located within the Shebenik National Park. Seven monitoring stations were established along the shoreline of Lake Ohrid, five stations in Great Prespa Lake, two stations in Small Prespa, and two stations within Shebenik National Park. The sampling stations (Table 1) are positioned according to the coordinates in the table below, as as shown on the accompanying map (Map 1).

Table 1. Sampling stations and respective coordinates

No. Stacion	Name Stacion	Latitude	Longitude
St. 1	Lin	41.07213	20.62835
St. 2	Erlin Park, Lin	41.04986	20.63974
St. 3	Udenisht	40.96393	20.64607
St. 4	Memelisht	40.93349	20.64122
St. 5	Gur iKuq Station	40.92209	20.64151
St. 6	Drilon	40.90189	20.72227
St.7	Tushemisht	40.90116	20.71371
St. 8	Kallamas	40.88387	20.92434
St. 9	Gorica	40.86189	20.94099
St. 10	Gollomboç	40.7895	20.90823
St. 11	Pustec	40.76638	20.91859
St. 12	Zaroshka	40.76831	20.9264
St. 13	Tren Village 1	40.67400	20.99742
St. 14	Tren Village 2	40.68922	21.01108
St. 15	Allaj	41.22809	20.2936
St. 16	Qarrishta	41.26592	20.44122



Map 1. Sampling point in Ohrid Lake, Great Prespa Lake, Small Prespa Lake, and Shebenik National Park.

2.2. Methodology

In our study, we focused on sampling noble crayfish (*Astacus astacus*) and stone crayfish (*Austropotamobius torrentium*) in lakes and rivers. We consulted sampling and monitoring protocols developed in various countries, including Sweden (Edsman and Söderbäck 1999), the United Kingdom (Peay 2003), Poland (Strużyński 2015), and the UW-Madison Center for Limnology (2007). Following the protocols suggested by Larson & Olden (2016) in Field Sampling Techniques for Crayfish and the Protocol for Wisconsin Crayfish Sampling, WAV Version 2007, this study aims to detect the presence or absence of these two crayfish species and to estimate their population status using baited traps in deep water.

Site Selection: Both lakes and rivers were considered as ecological sampling sites. For lakes, the recommended sampling technique involved collecting crayfish along two transects on opposite sides of the lake. During the eight-month survey period, traps were typically placed near stones or tree roots along the shorelines. Traps were set each evening and retrieved the following morning after being left in the water for ten to twelve hours.

Setting of traps

In streams and rivers: Following our methodology, we set 5–10 traps at each sampling site. Traps were placed at least 10 meters apart at water depths ranging from 0.5 to 3.0 meters.

In lakes: The number of traps used depended on habitat characteristics and trap availability, with 10 traps recommended per lake (5 per transect, spaced 10 meters apart). We used LiNi traps (Westman *et al*., 1978), made of nylon netting with a mesh size of 0.5 cm. These traps featured openings and a designated area for securing bait. The dimensions of each trap were 60 cm in length, 20 cm in diameter, and 5 cm for the funnel opening.

Choosing a large number of monitoring stations and a high monitoring frequency is essential for obtaining a comprehensive, accurate, and representative understanding of environmental conditions. This approach is particularly beneficial for monitoring biodiversity, water quality, or ecological health. Key benefits include increased spatial coverage, higher temporal resolution, improved data accuracy and reliability, enhanced detection of emerging issues, and support for policy and conservation efforts.

3. RESULTS AND DISCUSSION

Summary of data on individuals collected in Lake Ohrid

Only the *Astacus astacus* species was found in the monitored habitats of Lake Ohrid. During our recent survey, a total of 92 crayfish individuals were collected. At the first station, 22 individuals were recorded; 8 individuals were recorded at the second station; and 43 individuals were recorded at the third station. No crayfish individuals were recorded at the other four stations. The highest number of individuals was recorded in June, while the lowest number was recorded in December. The male-to-female ratio was 61:3.

In the Lin-Memelisht segment, where the substrate and environmental conditions are more favorable, crayfish populations are more widely distributed. In other areas, where the substrate is primarily sandy, conditions are less suitable for crayfish. The absence of crayfish individuals at some stations may be influenced by factors such as urbanization, particularly in the Drilon area, and the presence of mining-related dams near Gur i Kuq Station. Mining waste can significantly impact water quality, introducing heavy metals and other toxic substances released during mineral extraction and processing. The presence of pollutants has created unsuitable conditions for crayfish, leading to environmental stress and reduced biodiversity in these areas. In June, warmer temperatures and abundant food resources stimulated crayfish activity and reproduction, resulting in the highest number of recorded individuals. In December, however, lower temperatures reduced crayfish activity, leading to a decrease in recorded numbers. The observed male-to-female ratio of 61:3 may reflect a natural population imbalance or seasonal variations in the behavior and availability of each sex (see Map 2, Table 2, and Figures 1 and 2).

Table 2: The number of individuals collected from the Ohrid Lake

Session	Month	Ohrid Lake							M	F	Total Month
		St. 1	St. 2	St. 3	St. 4	St. 5	St. 6	St. 7			
Autumn 2023	October	2	2	4	1	0	0	0	9	0	9
	November	1	2	4	0	0	0	0	6	1	7
Winter 2023	December	0	0	3	0	0	0	0	3	0	3
Spring 2024	March	2	1	4	1	0	0	0	7	1	8
	April	3	2	4	1	0	0	0	9	1	10

	May	3	2	9	1	0	0	0	14	0	15
Summer 2024	June	4	3	7	1	0	0	0	15	0	15
	July	7	6	9	3	0	0	0	26	0	25
	Total St.	22	18	43	9	0	0	0			
	Total lake								**89**	**3**	**92**

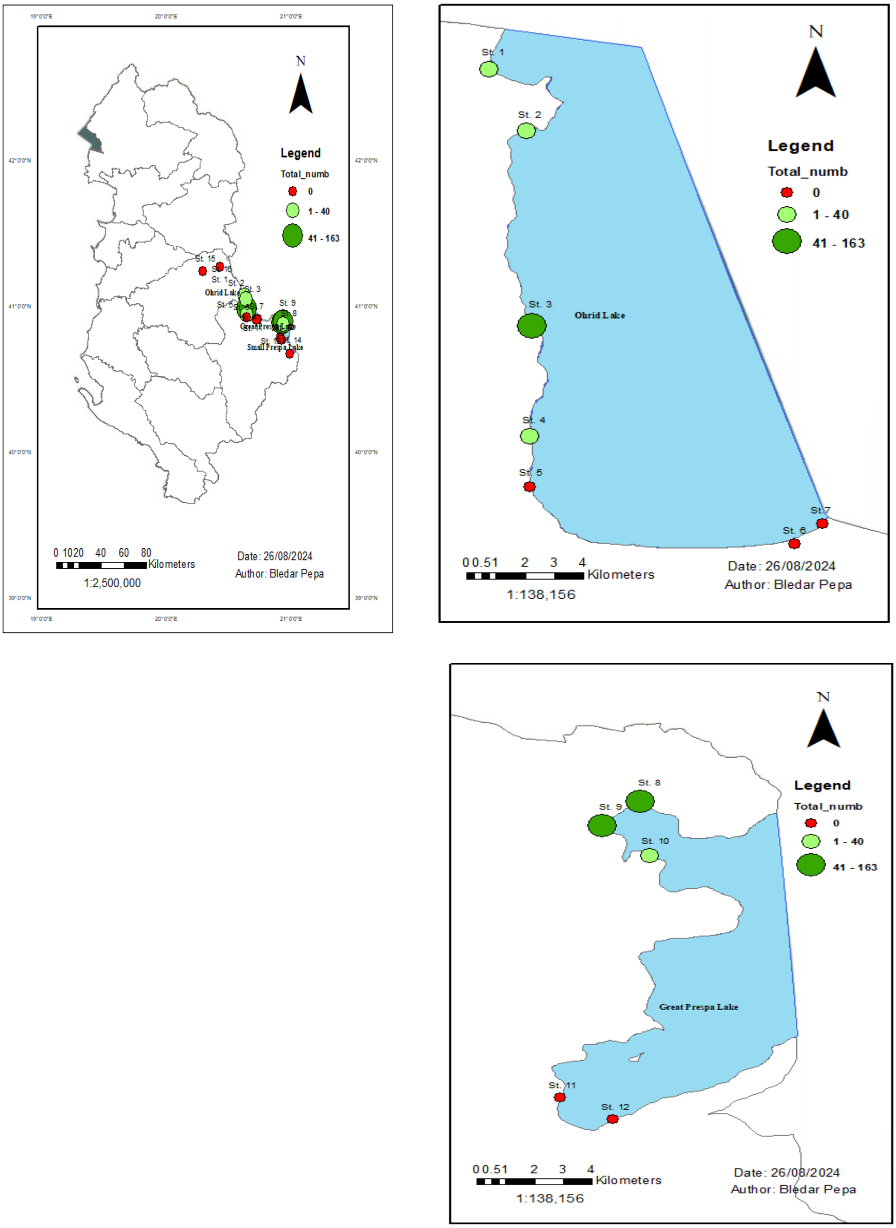
Summary of data on individuals collected in Lake Great Prespa

Only the *Astacus astacus* species was found in the waters of Prespa Lake. During crayfish population monitoring at five stations, a total of 137 individuals were recorded at the first station, 163 at the second, and 40 at the third. No individuals were found at the remaining two stations. The highest number of individuals was recorded in June, and the lowest in December. The male-to-female ratio was 333:7.

The absence of crayfish at stations 11 (Pustec) and 12 (Zaroshka) may be attributed to an unsuitable substrate characterized by psamal and lymal composition. Another likely factor affecting the crayfish population in Prespa Lake is severe water withdrawal, which has degraded the optimal crayfish habitat. Observations from the administration of Prespa National Park and local fishermen indicate a significant drop in lake water levels, possibly linked to reduced winter snowfall. This habitat degradation has created unfavorable conditions for crayfish survival and reproduction, impacting their population in these areas (see Map 2, Table 3, Figures 1 and 2).

Table 3. The number of individuals collected in Prespa Lake

Session	Month	Prespa Lake					M	F	Total Month
		St. 8	St. 9	St. 10	St. 11	St. 12			
Autumn 2023	October	9	16	5	0	0	29	1	30
	November	10	13	3	0	0	26	0	26
Winter 2023	December	6	9	4	0	0	19	0	19
Spring 2024	March	17	22	5	0	0	43	1	44
	April	20	20	3	0	0	40	1	43
	May	24	25	5	0	0	52	2	54
Summer 2024	June	23	26	7	0	0	54	2	56
	July	28	32	8	0	0	67	1	68
	Total St.	137	163	40	0	0			
	Total lake						**333**	**7**	**340**



Map 2. Distribution of the total number of individuals caught according to stations

No crayfish species were found in Small Prespa. The conversion of Small Prespa into a marsh has had negative consequences for animal populations such as crayfish (see Map 2). Habitat alteration is the primary reason for the disappearance of crayfish in this area, as marshes provide unsuitable conditions for their survival, leaving no viable environments for monitoring.

In the Qarrishta and Rapun Rivers, located within Shebenik National Park, no crayfish were found during the 8-month monitoring period. Surveys were conducted along transects covering nearly the entire length of both rivers, yet no evidence of crayfish presence was detected.

Distribution of individuals according to Time Series Plot in both lakes

Following the trend observed in the figure above, the Time Series Plots below show the monitoring data for each month. It is evident that Station 3, which is located in Lake Ohrid, as well as Stations 8 and 9, which belong to Lake Prespa, have the highest number of individuals. The peak in the number of individuals occurs during the summer, specifically in July (see Fig. 1).

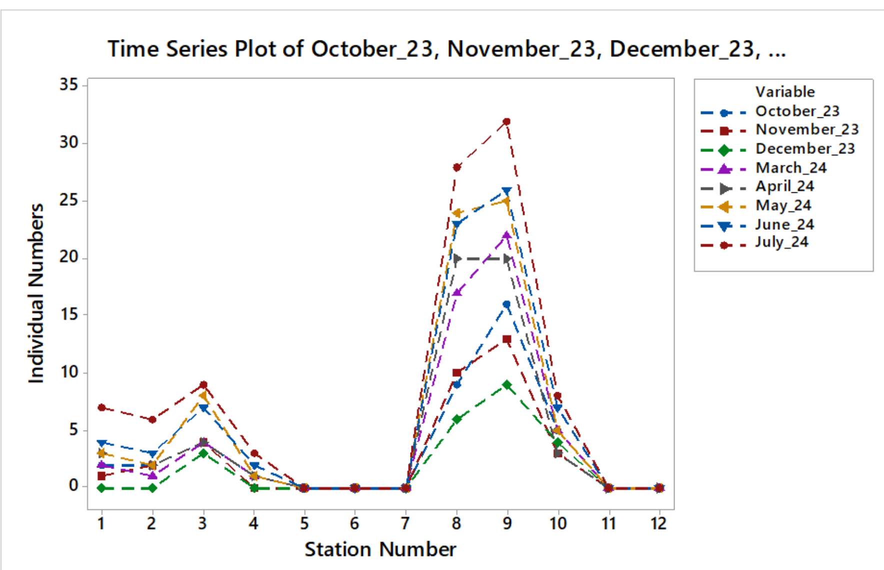


Fig. 1: Monthly distribution of individuals according to Time. Series Plot

Temperature range for crayfish A. astacus

During our monitoring, we have not found temperatures that are outside the critical conditions both in summer and winter (Fig. 2). The optimal temperature for *Astacus astacus* lies between 15°C and 25°C (Holdich, 2002).These temperatures are ideal for growth, reproduction, and overall health.Temperatures above 28°C to 30°C are generally stressful for the species, and long-term exposure to such heat can lead to a decline in growth, impaired immune function, and potential death. On the lower end, temperatures below 4°C to 6°C can cause the crayfish to become lethargic, reducing feeding and growth. If temperatures drop much lower, the crayfish may experience cold-induced mortality, especially if they lack sufficient shelter during the winter.

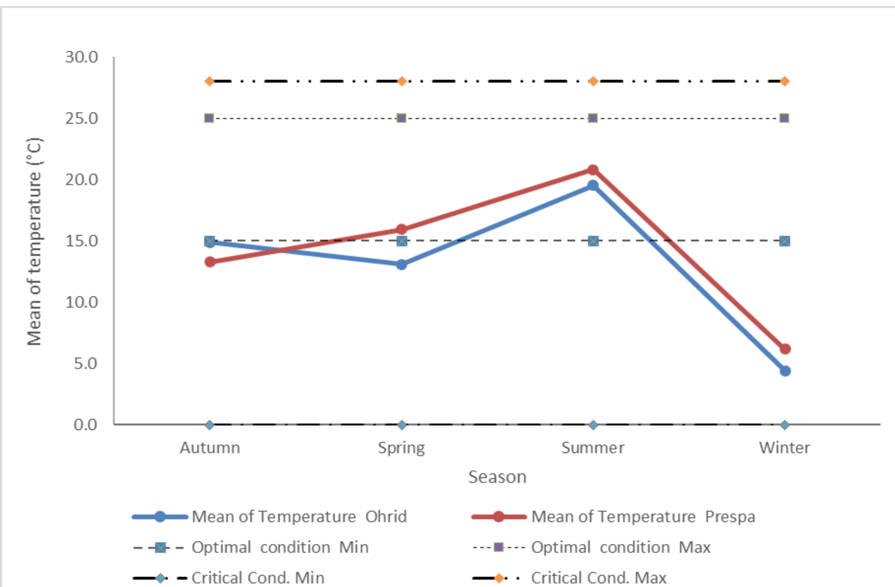


Fig.2: Average Temperature measured in both lakes and optimal condition for species *A. astacus.*

Descriptive statistics of morphological traits of captured and measured individuals in two lakes (Great Prespa and Ohrid)

In the descriptive analysis of the morphometric parameters measured in the crayfish individuals caught, the following average measurements were found for Ohrid and Prespa Lakes: average size—11.4 cm for Lake Ohrid and 10.1 cm for Lake Prespa; body width—2.39 cm for Lake Ohrid and

2.36 cm for Lake Prespa; abdomen length—3.59 cm for Lake Ohrid and 4.34 cm for Lake Prespa; antenna length—5.53 cm for Lake Ohrid and 6.30 cm for Lake Prespa; chelicera length—3.26 cm for Lake Ohrid and 4.30 cm for Lake Prespa; and average rostrum length—approximately 0.90 cm for both lakes. The largest coefficient of variation was observed in the length of the chelicerae, as the species exhibits anisometry in this organ. Crayfish individuals from Lake Prespa were found to be larger, which is reflected in the measurements of the abdomen, antennae, and chelicerae (see Table 4).

Table 4: Descriptive Statistics of Height, Width, Abdomen, Antenna, Chelicera, and Rostrum

Variable	Lake	Total Count	Mean	CV %	Minimum	Median	Maximum
Height	Ohrid	92	11.04	11.98	7.80	11.10	13.60
	Great Prespa	340	10.01	16.31	5.50	9.80	14.90
Width	Ohrid	92	2.39	14.24	1.70	2.30	3.20
	Great Prespa	340	2.36	24.52	1.00	2.30	3.90
Abdomen	Ohrid	92	3.59	7.38	2.90	3.60	4.30
	Great Prespa	340	4.34	21.40	2.00	4.45	6.80
Antenna	Ohrid	92	5.53	5.82	4.80	5.50	6.10
	Great Prespa	340	6.30	24.02	3.00	6.40	9.30
Chelicera	Ohrid	92	3.26	8.06	2.70	3.30	3.90
	Great Prespa	340	4.30	43.05	0.60	4.70	8.60
Rostrum	Ohrid	92	0.90	17.37	0.45	0.90	1.25
	Great Prespa	340	0.90	22.48	0.25	0.88	1.35

4. DISCUSSION

The study on the monitoring of two crustacean species, *Astacus astacus* and *Austropotamobius torrentium*, led to important conclusions regarding the status of these species in the Prespa and Ohrid lake regions, as well as in other areas.

Presence of Species: Only *Astacus astacus* was identified in the region, while *Austropotamobius torrentium* was not found during the study. *A. astacus* is present in Great Prespa Lake and Ohrid Lake but is absent from Small Prespa Lake and the Qarrishta and Rapun rivers, part of Shebenik National Park.

Geographical Extent: In Lake Ohrid, *A. astacus* was found in only 4 out of 7 monitoring stations, while in Great Prespa Lake, it was present in 3 out of 5 stations. The physico-chemical parameters of water, such as temperature and quality, were within the tolerance values for this species in both lakes.

Ratios and Dimensions: The male-to-female sex ratio (M/F) is 96% in both lakes. The largest individuals measured 14.9 cm, and the smallest were 5.50 cm, both of which were found in Lake Prespa.

Changes in Population: The monitoring revealed that *A. astacus* is present throughout the year, with an increase in the number of individuals during the summer months. However, in Lake Prespa, the number of individuals has decreased, likely due to a drop in the water level and the displacement of the habitat to deeper areas. In Lake Ohrid, the habitat of *A. astacus* has moved to depths exceeding 5 meters, making it more difficult to capture them with traps, though they are easier to catch with nets.

Threats and Impacts: *A. astacus* faces several threats, including habitat changes due to climate change, mining, and pollution from urban and agricultural areas. Fishing no longer poses a major threat, as fishermen typically return caught crayfish to the lake without harming the population.

Compared to other Balkan countries, Albania's research efforts are still in the early stages. While research is growing, much of the data on *Astacus astacus* and *Austropotamobius torrentium* are more detailed in countries like Croatia, Serbia, Bosnia (Jelić *et al.*, 2016), Bulgaria, Greece (Marković *et al.*, 2019), and Romania (Varga *et al.*, 2017). This study offers insights into how Albania compares to its Balkan neighbors in terms of the conservation of these species and highlights the ongoing challenges in the region.

Due to the absence of *A. astacus* on the Albanian Fauna Red List and the complete lack of information about the species in the IUCN Red List, it will be proposed that both institutions include *A. astacus* and *Austropotamobius torrentium* on these lists, along with the specific sites where they are found.

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