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Evaluation des concentrations des polluants chez les poissons à intérêt commercial du Golfe de Bejaia.

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Dedication

To my beloved mother, father, sister, brother and future wife your unwavering love and support have been my guiding light.

To the cherished members of my uncle Abdellah's family, your warmth and kindness have enriched my life in countless ways.

To my dear friends in M'sila, Khenchela and Bejaia, your friendship has been a source of joy and laughter.

And to all who have helped me along this journey, your encouragement and assistance have been invaluable.

In the memory of my grandma Ferroudja Manouva
MEHADI

With deepest gratitude and love.



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Abbreviations list

- AD: Anthropogenic Debris
- ALDFG: Abandoned, Lost, or Otherwise Discarded Fishing Gear
- AP: Anthropogenic Particles
- EW: Eviscerated weight
- FO: Frequency of Occurrence
- FTIR: Fourier Transform Infrared
- GESAMP: Group of Experts on the Scientific Aspects of Marine Environmental Protection
- GHG: Greenhouse Gas
- GIEPW: Global Initiative to End Plastic Waste
- GIT: Gastro-Intestinal Tract
- HDPE: High-Density Polyethylene
- HNO: Nitric Acid
- HSD: Honestly Significant Difference
- KOH: Potassium Hydroxide
- LDPE: Low-Density Polyethylene
- MP(s): Microplastic(s)
- MS: Microsoft
- NW: Northwest
- PA: Polyamides
- PC: Polycarbonates
- PD: Plastic debris
- PE: Polyethylene
- PET: Polyethylene Terephthalate
- PP: Polypropylene
- PS: Polystyrene
- PUR: Polyurethane
- PVC: Polyvinyl Chloride
- TL: Total Length
- TW: Total Weight
- TWP: Tropical Western Pacific
- UNDP: United Nations Development Programme
- UNEP: United Nations Environment Programme
- UV: Ultraviolet

I. GENERAL INTRODUCTION

Marine pollution has reached alarming levels in recent decades, with significant impacts on marine ecosystems (Rios et al., 2007, Rochman et al., 2015). The world's marine ecosystems are facing a serious crisis fuelled by a variety of factors and resulting in widespread marine pollution (Siung-Chang, 1997). Pollution has a negative impact on all marine organisms constituting the marine ecosystems. It disrupts biodiversity, leading to species decline and habitat destruction, particularly through oil spills. Chemical pollutants contaminate water, affecting marine life health and reproduction, while plastics cause physical harm through entanglement and ingestion. Pollution also alters marine behavior and contributes to ocean acidification, harming organisms with calcium carbonate structures (Hidalgo-Ruz et al., 2020). Addressing pollution is crucial to preserving marine ecosystems and the diverse life they sustain particularly on fish species suffer from its consequences in many ways, including biochemical, morphological, physiological and behavioural changes. Biochemically, they may suffer from elevated concentrations of heavy metals in their tissues, disrupting essential metabolic processes. Morphologically, exposure to pollutants like endocrine disruptors can result in deformities and abnormalities in fish anatomy. Physiologically, water contamination can lead to reduced oxygen uptake, causing respiratory distress and impairing overall health. Furthermore, pollution can induce behavioral alterations, such as disrupted migration patterns, often caused by noise pollution from human activities, which can interfere with communication and navigation. These multifaceted consequences of pollution underscore the urgent need for comprehensive measures to mitigate its impact on marine ecosystems and safeguard the wellbeing of fish species (Bengtsson, 1979).

I.1 Anthropogenic pollutants

Because not all of the particles collected could be identified as plastic, the term "Anthropogenic Debris" (AD) refers to the total number of particles found, also known as Anthropogenic Particles (AP), are material particles or fragments created by humans or originating from human activity (Berg et al., 2024).

These particles are frequently non-natural in nature and are the product of human activities such as manufacturing processes, urbanization, and consumer activities. Anthropogenic particles in the context of environmental contamination often includes a wide range of materials, with plastic debris being a prominent example. Plastic debris, such as single-use plastics, microplastics and macroplastics, constitutes a significant portion of anthropogenic particles found in terrestrial and aquatic environments (Villafañe et al., 2023).

After Vetturayasudharsanan et al. (2020) beside plastic debris, AP include:

- •Microbeads: These are tiny plastic particles used in personal care products, such as exfoliating scrubs and toothpaste;
- •Tire Particles: Microscopic rubber particles generated from tire wear on roads and highways.
- •Metal Particles: Fragments of metals resulting from industrial activities, automobile emissions, and brake dust;
- •Paint Particles: Small particles released during painting and other coating processes;
- •Cigarette Butt Litter: Cigarette filters made of cellulose acetate, a type of plastic, discarded after smoking;
- •Fly Ash: Fine particles produced from burning coal in power plants;
- •Industrial Dust and Emissions: Particles released from industrial processes and manufacturing activities;
- •Construction and Demolition Debris: Particles generated during construction and demolition work.

I.2 Plastic debris

Plastic debris (PD) are small anthropogenic particles confirmed as plastic polymers, they comes from a variety of sizes, ranging from microplastics to macroplastics, and it poses a huge environmental concern (Manullang, 2020).

Plastics degrade and lose their initial qualities over time, at varying rates depending on the physical, chemical, and biological environments to which they are subjected. Weathering-related deterioration causes a series of changes, including mechanical integrity loss, embrittlement, further degradation, and fragmentation into secondary microplastics (UNEP, 2015).

I.2.1 Plastic debris classifications

Plastic debris are characterized by size and morphotypes (Lam et al., 2020), and it includes a wide variety of particles ranging from nanoplastics, microplastics, mesoplastics to macroplastics (Xu et al., 2020). This classification approach allows for a more in-depth understanding of the numerous plastic shapes (spheres, beads, pellets, foams, fibers, fragments, films, and flakes) present in the environment, Plastic debris can also be from primary or secondary sources, primary sources involve direct discarding of items like bottles and packaging, while secondary sources result from the breakdown of larger plastics into

microplastics due to weathering and Ultra-Violet radiation. Both types threaten marine life, highlighting the need for reducing plastic use and improving waste management (Hidalgo-Ruz et al., 2020; Lusher et al., 2020).

I.2.1.1 Plastic debris classification by size

Plastics can transform into small particles (nanoplastics, microplastics, mesoplastics, etc.) through several processes, including fragmentation and degradation (Dimassi et al., 2021). Over time, large plastic items exposed to sunlight, wave action, and mechanical abrasion, can break down into smaller particles. This debris, ranging from millimeters to micrometers in size, are considered as plastic debris (Cressey, 2016).

Plastic debris varies in size, and there are no universally defined standards or size thresholds that distinctly categorize nanoplastics, microplastics, mesoplastics, and macroplastics. (Table 01), with microplastics often being less than 5 mm in size, this pioneering definition recognizes the synthetic chemical composition and generally modest size but does not provide a lower size boundary. Traditionally, the lower size restriction corresponds to environmental sampling size limitations and analytical detection limits (Burns et al., 2018).

Table 01: Prevalent size-based nomenclature for plastic particles

Nanoplastics	Microplastics	Mesoplastics	Macroplastics	Authors
(NNPs)	(MPs)			
-	<5mm	-	-	Thompson et
				al., 2004
<1µm	1μm-1mm	>5mm		Browne et al.,
				2007
<20µm	20-5000µm	5-25mm	>2.5cm	Wagner et al.,
				2014
<1µm	1-1000µm	1-25mm	2.5-100cm	Andrady et al.,
				2015
-	<5mm	-	>5mm	Cormier, 2020

While microplastics have received a lot of attention, it is critical to keep studying larger plastic debris categories (mesoplastics and macroplastics) to get a whole picture of the plastic pollution problem.

I.2.1.2 Plastic debris classification by form

Plastic debris degrades and transforms into a variety of forms, including thin plastic films, plastic fibers, and shattered particles. The breakdown of larger plastic things results in the formation of various morphotypes (de Souza Machado et al., 2018). As of the present, researchers and authors have not standardized a single term to encompass the diverse forms resulting from plastic debris degradation. This lack of consensus has led to varying nomenclatures being used across scientific literature.

Some authors refer to these different forms as "types" of plastic debris (de Souza Machado et al., 2018; Amin et al., 2020), while others prefer the terms "morphotypes" (Jaubet et al., 2021; Aves et al., 2022) or "shapes" (Zhao et al., 2021; Lozano et al., 2022) to describe the various configurations resulting from degradation. This variability in terminology highlights the need for a cohesive framework in categorizing and describing the different manifestations of degraded plastic debris.

According to Martínez Silva and Nanny (2020), plastic particle shapes are classified as pellets (spherical primary microplastics), fragments (irregular shapes presumably derived from the physical degradation of larger plastic debris), and fibers (from fishing lines, nets, clothing, and non-woven textiles) (Figure 01).

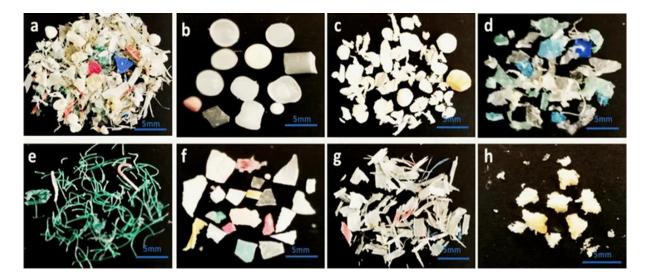


Figure 01: Different morphotypes of partial plastic/microplastic samples collected by Li et al. (2022); (a) mixed MPs, (b) pellets, (c) foams, (d) films, (e) fibers, (f) fragments, (g) flakes, (h) sponges.

Plastic debris morphotypes provide useful insights into potential contamination sources. The different forms and shapes found in degraded plastic particles can provide information about

their origins and the processes that caused them to fragment. Researchers can deduce the likely sources of plastic litter by evaluating the morphological properties of the debris, such as packing materials, fishing gear, or consumer products. Understanding the origins of pollution is critical for designing focused and effective solutions to reduce plastic pollution and encourage sustainable waste management practices (GESAMP, 2015).

I.2.1.3 Plastic debris classification by origin

The origin of plastic debris is a common classifier, particularly for microplastics, which are divided into "primary" and "secondary" microplastics. According to the prevalent viewpoint, primary microplastics are those that are purposely manufactured in that size range, whereas secondary microplastics are those that are formed in the environment by fragmentation or wear and tear of plastic-containing goods, such as tire wear particles (TWP) and fibers released from textiles after usage (GESAMP, 2015).

Marine pollution is a multifaceted matter. The main contributors to marine pollution are as follows:

- (1) Plastic Pollution (Ritchie and Roser, 2018): Plastic debris, such as macroplastics, microplastics and discarded fishing gear. Improper waste management, littering, and inadequate recycling practices lead to large quantities of plastic entering rivers and oceans;
- (2) Oil Spills (Saadoun, 2015): Accidental oil spills during transportation, offshore drilling operations, or tanker accidents release vast amounts of oil into marine environments;
- (3) Chemical Pollution (Elliot and Kyle, 2013): Industrial activities, agricultural runoff, and sewage discharges introduce harmful chemicals and pollutants into the marine ecosystem. Pesticides, heavy metals, pharmaceuticals, and toxic substances can accumulate in the water, sediments, and organisms;
- (4) Marine Debris (UNEP, 2005): Other types of marine debris, including glass, metal, rubber, and wood, contribute to pollution. Abandoned or lost fishing gear, commonly known as "ghost nets";
- (5) Atmospheric Deposition (Duce et al., 2009): Airborne pollutants, such as heavy metals, industrial emissions, and agricultural chemicals, can be moved over long distances and deposited into marine environments through rainfall or atmospheric deposition.

The inappropriate usage of plastic products and inadequate waste management practices have led to a significant buildup of plastic waste in aquatic ecosystems. In recent decades, the

production of plastic has increased to approximately 348 million tons (PlasticsEurope, 2018). The increase in production of plastics is caused by our increasing dependence on plastics in our daily routines (Andrady and Neal, 2009).

Notably, packaging alone accounts for approximately 40% of the total global plastic production (Plastics Europe, 2018). Unfortunately, the majority of plastic items are designed for single-use purposes, leading to a significant accumulation of waste in our aquatic ecosystems.

For example, plastic bags, bottles, synthetic fibers, and thin films are the most common waste in marine ecosystems.

Plastics are compound polymers formed through a chemical process that combines monomer chains. However, plastic polymers are not solely composed of monomers; they may also contain various additives such as plasticizers or flame retardants. These additives are incorporated to enhance specific physiochemical properties like hardness, density, and protection against damage from factors such as heat, UV radiation and oxidation (Andrady, 2011).

Plastics can also undergo degradation, where exposure to environmental conditions like sunlight (UV radiation), heat, and water leads to chemical changes in the polymer structure. This degradation process can cause the plastic to become more brittle and susceptible to breaking into smaller pieces (Andrady, 2022).

It is important to note that microplastics can also be intentionally manufactured and directly released into the environment as small plastic, such as those used in personal care products (microbeads) or as industrial abrasives (van Wezel et al., 2016).

Plastic debris can reach sediments, seas, oceans, soils, and biota through a combination of direct and indirect pathways. Plastic debris can enter water bodies, including seas and oceans, through surface runoff from urban areas, roads, and industrial sites. Rainwater can wash plastic litter and waste from streets and landfills into storm drains and eventually reach waterways (Talvitie et al., 2017).

Small plastic particles also can be transported by rivers and streams, carrying it from inland areas to coastal regions and eventually into the open Sea. Plastic waste from urban centers, agricultural fields, and industrial activities can enter watercourses and be carried by the flow of water (Talukdar et al., 2023).

Plastic debris can be ingested by various organisms inhabiting aquatic and terrestrial environments. Marine animals such as seabirds, turtles, fish, and marine mammals can mistakenly consume plastic, either by mistaking it for prey or accidentally ingesting it along

with their food. Similarly, microplastics can be taken up by plants and terrestrial organisms, potentially entering the food chain (Rebelein et al., 2021).

I.2.1.4 Plastic debris classification by color

In addition to examining morphotypes, the authors consider the colors of plastic debris to be an important component of their research. The varying colors of degraded plastic particles can be indicative of their original composition and the potential additives or dyes used during manufacturing (Hartmann et al., 2019). Researchers get new insights on the sorts of plastics prevalent in the environment and the sources of pollution that contribute to the buildup of different-colored plastic particles by studying the colors of plastic debris. The study of the colors of plastic litter adds to our understanding of pollution causes and aids in the development of comprehensive strategies to prevent plastic pollution and promote sustainable behaviors for a cleaner environment (Angelini et al., 2019; Luna et al., 2022).

I.2.1.5 Plastic debris classification by polymers

Plastics are classified based on their polymeric structure. A polymer is made up of chains formed by the repetition of monomers with the same chemical structure. Distinct monomers cause changes in the physicochemical properties of these polymers, and hence distinct types of plastic. For example, the length of the polymer chain influences the strength of the plastic, so the longer the chain, the more force is required to break it. The presence of polar groups can also boost strength, resulting in hydrogen bonding, which favors attraction between chain polymers. Another distinction between polymer types is their melting point, which can result in distinct properties (Brydson, 1999).

According to Cornier (2020), the main polymers are:

PET: Polyethylene terephthalate (PET) is a linear polyester (polymer comprised of an ester, - COO-) that was originally developed in 1941. PET is the foundation of synthetic polyester fibers and is widely used in clothing, ropes, carpet fibers, and beverage bottles. PET materials can be recycled due to their thermoplastic nature.

PE: Polyethylene is the most basic polymer chain. It is a linear polymer using the monomer ethylene (-CH2- CH2-). It first appeared in use in 1933. It is the most commonly made plastic due to its low cost and multiple properties (electrical insulation, chemical resistance, flexibility, transparency, and so on). PE is classified into two types: high-density polyethylene (HDPE) and low-density polyethylene (LDPE). HDPE is commonly used in bottles, toys, and other

similar applications, but LDPE is mostly utilized in light packaging (e.g., film wraps or plastic bags).

PP: Polypropylene has similar qualities to polyethylene but is harder and more heat resistant. The polymerization of PP began in 1954, with the first commercially manufactured products coming only a few years later. It is the second most prevalent plastic and is frequently used in packaging and labeling. PP is commonly used for medical receptacles and other similar goods since it is resistant to the heat of an autoclave.

PVC: Polyvinyl chloride is a rather unstable polymer that requires additions to stay stable. The polymerization of vinyl chloride exposed to sunshine resulted in the first report of PVC in 1872. It was initially produced in 1926. PVC is now utilized in construction (pipes, doors, and windows) as well as a variety of everyday things (bottles, non-food packaging, and so on).

PS: Polystyrene can be solid or foam and is utilized in a variety of items (CD packaging, containers, lids, construction). PS is a low-cost material with a variety of desired properties, such as electrical insulation, stiffness, and simplicity of molding.

Other: The final category contains a variety of materials, such as polyamides (PA) or polycarbonates (PC), as well as copolymers made up of more than one monomer. This other category includes polyurethane (PUR). In addition to petrochemical polymers, bioplastics and natural plastics are included in this category. These materials are manufactured from carbons obtained from renewable biomass rather than fossil fuels. Bio-based materials are made from rice, corn, potatoes, or sugar cane, however the origin of carbons is not linked to biodegradability, and a bio-based plastic is not necessarily recyclable.

Plastics are made up of more than just polymers. They also contain a variety of additives that are used to increase their properties or to change the chemical affinity between molecules. Plasticizers, flame retardants, antioxidants, light and heat stabilizers, pigments, and other additives are examples of additives. Each additive plays a unique role in the structure and functionality of a plastic product (Hahladakis et al., 2018).

I.3 Plastic pollution and aquatic ecosystems

Plastics are transported via wind activity (from water or coastal areas to beaches), sedimentation (from water to sediments), resuspension (biological and mechanical movements), wash-up (dredging of sediments for beach nourishment), Plastics in freshwater are also transported to oceanic environments by riverine horizontal transport (water and sediments) (Van Cauwenberghe et al., 2015).

I.3.1 Sources of Plastic debris in the aquatic environment

In the marine environment, around 18% of microplastics were introduced into the aquatic system through fishing activities (Derraik, 2002; Andrady, 2011; Cole et al., 2011), while about 80 percent of marine plastic debris originated from terrestrial sources (Jambeck et al., 2015; Mani et al., 2015). Floods and hurricanes, for example, can promote the transfer of MPs from lands to aquatic systems (Barnes et al., 2009). While Waste Water Treatment Plants (WWTPs) do remove some MPs from home wastewater, they are not designed or ideal to remove all plastic particles. As a result, WWTPs are a common source of contamination in the aquatic environment (Wolff et al., 2019). MPs of both sorts are thought to pose a risk to the aquatic ecosystem (Thompson et al., 2009; Cole et al., 2011).

1.3.2 Sea-based sectors generating plastic litter

Maritime activities encompass a diverse array of plastic usage, spanning from disposable items to durable materials. Figure 02 visually delineates the primary sources and pathways through which plastics enter marine environments, while Table 02 provides a detailed breakdown of these materials. While sectors like fisheries or aquaculture may favor certain types or volumes of plastics, it's noteworthy that a cruise ship, hosting thousands of passengers, essentially functions as a medium-sized floating community akin to a small town. This similarity extends to the scale of consumption and waste generation, underscoring the substantial impact such vessels can have on marine ecosystems (Kershaw, 2016).

These floating communities, such as cruise ships, exhibit a complex web of consumption patterns, necessitating a comprehensive approach to managing plastic usage and waste disposal. From single-use plastics like straws and utensils to more enduring materials like deck furnishings, the range of plastics employed reflects the multifaceted needs of onboard operations. Recognizing the scale of these demands is crucial in devising effective strategies for minimizing plastic pollution and promoting sustainable practices within the maritime industry (Kozioł et al., 2022).

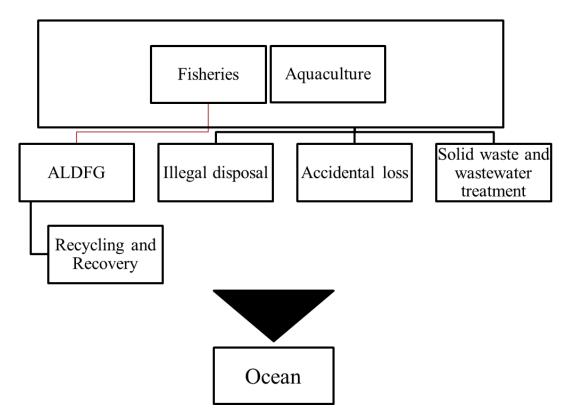


Figure 02: Pathway to the ocean and sea of plastic generated by fisheries and aquaculture (adapted from Kershaw, 2016), ALDFG: Abandoned Lost or otherwise Discarded Fishing Gear.

Table 02: Sources of plastic debris by maritime sector adapted from UNEP (2016)

Sector	Examples	Entry	Importance
Fisheries	Fishing gear Packaging Nets	Coastal Maritime	High
Aquaculture	Nets Lines Packaging	Coastal Maritime	Medium
Shipping	Cargo Packaging	Coastal Maritime	Medium
Ship-based tourism	Packaging Personal goods	Coastal Maritime	Low

A variety of maritime activities result in the direct release of plastic particles into the water, the main fishing gear may be a significant source of synthetic fibers in some regions (Kozioł et al., 2022). Plastic debris from maritime sources eventually makes its way into both sediments and ocean (sea) waters. Once in the ocean, plastic particles can sink and settle in sediments or float on the top, contaminating both marine organisms and coastal surroundings. Plastic debris deposition in sediments and ocean waters creates substantial environmental issues, highlighting the critical need for effective solutions to manage plastic pollution at its source and limit its effects on marine life and ecosystems. Table 03 provides a summary of the materials involved.

Table 03: Sources of primary and secondary plastic debris by maritime sector

Sector	Primary PDs (and MPs)	Secondary PDs (and MPs)	Importance
Fisheries (Spadea et al., 2015)	-	Fragments Fibers	High
Aquaculture (Da Le et al., 2022)	-	Fragments Fibers	Medium
Shipping (Kozioł et al., 2022)	Pellets	-	Medium
Ship-based tourism (Löhr et al., 2017)	PCCPs (personal care and cosmetic products)	-	Low

I.4 Conducted studies on plastic debris

Ingestion of plastic debris by fish species (Atlantic silversides, Menidia) was first reported by Carpenter et al. (1972) in the Sargasso Sea, the majority of studies conducted in recent years have predominantly focused on species found on the Northern Hemisphere (Boerger et al., 2010; Davison and Asch, 2011; Anastasopoulou et al., 2013; Lusher et al., 2013) and the Southern Hemisphere species (Cliff et al., 2002; Di Beneditto and Awabdi, 2014; Naidoo et al., 2016).

Several studies have been conducted to investigate the ingestion of plastic debris by fish in marine ecosystems:

Rummel et al. (2016) studied microplastics in pelagic fish from the North Sea and Baltic Sea. They confirmed that 5.5% of examined fishes were contaminated by plastic particles, with 74% of all particles being microplastics. The authors concluded that both pelagic and benthic fish may ingest microplastics directly or indirectly by consuming them in prey;

Bray et al. (2019) analysed the microplastic ingestion by three deep-water elasmobranch species in the Tyrrhenian Sea. The study found that microplastics can be ingested by a wide range of organisms due to their small size and high abundance

Markic et al., (2020) studied microplastic ingestion by commercial marine fish from the seawater of Northwest Peninsular Malaysia. They found that microplastics are contaminants of emerging concern and may be unknowingly present in high amounts in certain regions. They also found that given their small size, microplastics may be easily ingested by organisms and cause adverse impacts on ecosystem and human health.

Other studies have uncovered evidence of freshwater species ingesting microplastics:

Andrade et al. (2019) mentioned the first evidence of plastic ingestion by freshwater fishes in the Amazon. The study confirmed the presence of plastics in approximately 80% of the species studied, including piranhas and other serrasalmids with diverse feeding habits;

Kasamesiri and Thaimuangphol (2020) on their study about microplastics ingestion by freshwater fish in the Chi River, Thailand found that 72.9% of the collected fish were polluted with microplastics, with a mean abundance of 1.76 ± 0.97 particles per fish. The study investigated eight fish species and found no significant difference in the abundance of microplastics between the species. The research revealed that the percentage occurrence of microplastics was highest in the omnivorous fish *Puntioplites proctozysron* (Bleeker, 1865) (86.7%).

To understand the impact of plastic debris on fish populations and their habitats, it is essential to examine data from both marine and freshwater environments (Silva-Cavalcanti et al., 2017). The Mediterranean Sea has been designated as one of the most contaminated Seas on the planet (Compa et al., 2019). Numerous studies have been conducted in different regions of the Mediterranean Sea. These investigations focused on the presence of plastic debris in various environmental compartments such as fish and sediments. The results of these studies have shown the negative effects and the wide distribution of plastic pollution in the Mediterranean ecosystem.

Many researchers assessed pollution levels by evaluating the prevalence of plastic particles in fish:

Romeo et al. (2015) reported the occurrence of plastic debris in the stomach content of three large pelagic fish species: *Xiphias gladius* (Linnaeus, 1758), *Thunnus thynnus* (Linnaeus, 1758), and *Thunnus alalunga* (Bonnaterre, 1788) caught in the Mediterranean Sea;

Bellas et al. (2016) confirmed the presence of microplastic pollutants in three commercially relevant demersal fish species from the Spanish Atlantic and Mediterranean coasts: the lesser spotted dogfish, the European hake, and the red mullet (respectively: *Scyliorhinus canicular*, *Merluccius merluccius*, *Mullus barbatus*);

Kılıç et al. (2022) reported microplastic abundance in the gastrointestinal tract of three economically important farmed fish species in Turkey: rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792), gilthead seabream (*Sparus aurata* Linnaeus, 1758), and European seabass (*Dicentrarchus labrax* Linnaeus, 1758);

Other researchers have documented the presence of a high concentration of plastic particles in sediments (Vianello et al., 2013, Phuong et al., 2018; Baysal et al., 2020).

Plastic contamination along the Southern Mediterranean coasts is underreported (Abidli et al., 2017), some studies reported the situation of marine litter prevalence on Southern Mediterranean beaches (Bouchentouf, and Tabet, 2013, Mansui et al., 2015; Abidli et al., 2018; Taïbi et al., 2021; Mankou-Haddadi et al., 2021; Setiti et al., 2021; Jaouani et al., 2022; Grini et al., 2022). In addition, Abidli et al. (2022) showed the presence of plastic debris in the gastrointestinal tract of two commercial fish species *Sarpa salpa* (Linnaeus, 1758) and *Liza aurata* (Risso, 1810) from the lagoons of Bizerte and Ghar El Melh, Tunisia.

Investigations on plastic pollutants in fish species from Algerian coasts are still insufficient. Some of them concentrated their research especially on the quantity and distribution of microplastics in sediments (Tata et al., 2020). Recently, Zeghdani et al. (2023a) analysed the presence of plastic debris in two commercial fish species, *Sardinella aurita* (Valenciennes, 1847) and *Lithognathus mormyrus* (Linnaeus, 1758) inhabiting the Gulf of Bejaia (East of Algerian coast). The authors confirmed the presence of various plastic debris in the digestive tracts of examined fish specimens, they also mentioned a positive relationship between ingested plastic debris and measured fish parameters. Otherways, they highlighted the potential threat of plastic debris (microplastic) regarding the high rate (74.30%) of fish specimens ingesting microplastics (Zeghdani et al., 2023b). Investigations on the effects of plastic debris contamination in other marine environmental compartments is highly recommended by the authors.

While the focus has mostly been on sediments, it is very important to investigate the effects of plastic debris contamination on other environmental compartments.

I.5 Effects of plastic debris

The effect of microplastics on marine biota, human health, and ecosystems is a growing concern. Research has shown that microplastic pollution poses a threat to global marine ecosystems and human health (Yu and Sher, 2023). Microplastics can be ingested by a variety of marine organisms, leading to entanglement and ingestion, which can be lethal to marine life (Chatterjee and Sharma, 2019). In terms of human health, microplastics have been found in seafood, raising concerns about the potential health implications of consuming contaminated seafood (Smith et al., 2019). Additionally, microplastics can enter the food chain and negatively impact the health of aquatic organisms, ultimately posing a threat to human health (Gosh et al., 2023). Therefore, the impact of microplastics on marine biota, human health, and ecosystems is a complex and multifaceted issue that requires further research and mitigation efforts.

Microplastics can act as carriers for various contaminants, including heavy metals, bacteria, and viruses, posing risks to marine biota, human health, and ecosystems. Many studies have shown that microplastics can adsorb and transport heavy metals, leading to potential toxic effects on aquatic organisms and human health (Liu et al., 2021). Additionally, microplastics have been found to harbor and enrich antibiotic-resistant bacteria and pathogens, raising concerns about the spread of infectious diseases and antimicrobial resistance (Piergiacomo et al., 2022). Furthermore, microplastics could prolong virus survival and infectivity also these particles have the potential role in the transmission of viruses into the aquatic environments (Lu et al., 2022).

The following sections will explore the specific interactions of plastics with environmental pollutants:

Persistent Organic Pollutants (POPs)

Plastic particles have a great capability for absorbing persistent organic pollutants (POPs) from the ocean. Plastic, because of its hydrophobic nature, quickly adsorbs and absorbs these hazardous compounds from the surrounding seawater. As a result, plastic debris serves as a vector, transferring and delivering POPs to marine species through ingestion. This buildup of POPs in plastic waste raises worries about potential environmental implications and poses major hazards to marine life, especially when plastic particles are swallowed by creatures and enter the food chain. The combination of plastic debris and POPs emphasizes the importance

of coordinated efforts to combat plastic pollution and prevent further contamination of marine ecosystems (Koelmans et al., 2013; Lohmann, 2017; Rodrigues et al., 2019; Tang, 2021).

Hydrophobic Organic Chemicals (HOCs)

Plastic fragments have been shown in studies to be capable of concentrating hydrophobic organic compounds. Plastics absorb and accumulate these substances from their surroundings due to their hydrophobic nature (Koelmans et al., 2016; Velez et al., 2018; Prajapati et al., 2022). As a result, plastic pieces serve as transporters, allowing hydrophobic organic contaminants to be transported and concentrated. This phenomena raises serious worries about the possible effects on marine life, as plastic waste can be mistakenly consumed by creatures, resulting in the transfer of toxic chemicals through the marine food web. The ability of plastic fragments to concentrate hydrophobic organic molecules emphasizes the importance of tackling plastic pollution and its environmental consequences (Brennecke et al., 2016; Lohmann, 2017).

Heavy Metals (HMs)

Many studies (Vieira et al., 2021; Esmaeilbeigi et al., 2023) declare plastic particles as environmental vectors for heavy metals. Microplastics can adsorb and collect heavy metal contaminants in water bodies and sediments due to their small size and huge surface area relative to their bulk. As these microplastics are consumed by aquatic species, the heavy metals accumulated may be transported up the food chain, potentially impacting higher trophic levels, including humans who consume seafood. According to Deng et al. (2020) and Cao et al. (2021), the interaction of plastic debris with heavy metals is complex and the effect of this combination (HMs-PDs) still limited.

I.5.1 Plastics and ecological effects

It is critical to remember that the effects of plastic pollution go beyond microscopic plastic particles. Microplastics, mesoplastics, macroplastics and megaplastics are all examples of plastic pollution. Entanglement incidents have been frequently reported for a wide range of marine mammals, reptiles, birds, and fish (Figure 03). This frequently results in acute and chronic harm or death (Kurtela et al., 2019; Alabi et al., 2019). It is assessed that between 57 000 and 135 000 pinnipeds and baleen whales are entangled globally each year, in addition to the countless fish, seals, birds, and turtles impacted by entanglement in marine plastic ingestion (Butterworth et al., 2012).

I.5.2 Plastic debris ingestion by aquatic organisms

Several researchers concentrated in their investigations on the ingestion of plastic debris by aquatic organisms (Figure 03). All of them reported the occurrence and implications of plastic ingestion in marine life ranging from small organisms to larger fish and marine mammals. The authors hoped to shed insight on the ecological implications and threats posed by plastic pollution in maritime habitats by investigating the occurrence and potential consequences of plastic ingestion in marine biota (Mascarenhas et al., 2004; Romeo et al., 2015; Seif et al., 2018; Im et al., 2020; Nam et al., 2021). Various plastic fragments, including microbeads, filaments from discarded fishing gear and textile fibers have been found in digestive systems of fish (Nie et al., 2019; Harikrishnan et al., 2023). Several researchers attribute the death of some aquatic mammals (Figure 03d), inhabiting Mediterranean (and Algerian) marine waters, to strangulation or ingestion of solid debris especially microplastics (Fossi et al. 2020; Benrekaa-Henda, 2022).

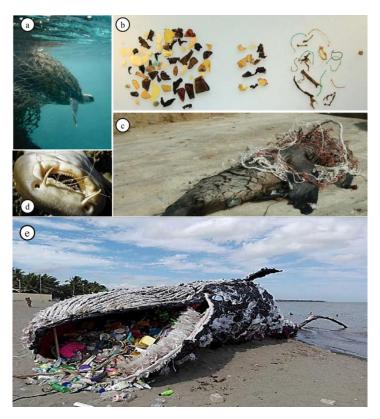


Figure 03: Plastic effects on marine organisms: a) sea turtle entangled in a net (UNEP, 2016), b) plastic debris isolated from gastro-intestinal tract of fish specimens (UNEP, 2016), c) an entangled seal (John Vonderlin flickr ©), d) nurse shark entangled in a filament net (Aaron O'Dea in UNEP, 2016), e) Whale stranded in the Philippines overloaded with plastic debris (Mathieu Doutreligne ©).

I.5.3 Plastic debris and human safety

Potential consequences of plastic-ingestion-mediated chemical influx into consumer products, as well as the accompanying consequences, are speculative and require further investigations (Al Mamun et al., 2023). PDs and MPs are ubiquitous in the aquatic environment, entering aquatic food webs and eventually reaching humans, the primary consumers. Microscopic plastic particles have been found in mollusks (mussels, oysters) (Wang et al., 2019), crabs (Watts et al., 2015), and fish (Savoca et al., 2021). Plastic particles were identified in more than 25% of the seafood commodities on the market (Rochman et al., 2015), according to some estimates. Because marine products are a key dietary component in human diets, this discovery has raised concerns about the possible effects of MPs on human health, food safety, and food quality (Barboza et al., 2018).

I.6 Organizations and international conferences on plastic debris

The Global Initiative to End Plastic Waste (GIEPW), the United Nations Development Programme (UNDP), and the Conference of the Parties (COPs) organization are among the entities working to address the problem of plastic pollution. Plastic pollution is a major environmental issue that poses a threat to marine biota, human health, and ecosystems. The UNDP has collaborated with The Ocean Cleanup and Rare to tackle plastic waste management and behavior change, while the GIEEC and COPs organization have held events and conferences to raise awareness of the issue and promote responsible trade in plastics. The COP27 Side Event discussed the existing governance for trans-boundary movements for plastic waste by the Basel Convention, thus reducing GHG emissions as a climate mitigation strategy. The event displayed the importance of responsible trade in plastics, and the environmentally sound management of plastic waste, which would also tackle climate change, promote healthy oceans and protect biodiversity.

I.7 Problematic, goal and sections of our thesis

Plastic debris are ingested by many aquatic organisms including commercial fish species. Nowadays, it's well known that plastic debris contaminants are transferred up the marine food chain. So, their impacts on ecosystems and human health are important and certain. Unfortunately, plastic debris in marine fish species from Algerian coasts remains unstudied and less documented. In fact, urgent investigation is needed to promote sustainable fisheries management practices in Algeria.

The goal of our thesis is to confirm the presence of plastic debris in the gastrointestinal tracts of examined fish specimens, to characterize and to identify (colors, size and morphotypes) the collected plastic debris ingested by commercial fish from Algerian coast. The aim of our study consists also to analyze the evolution of plastic debris according to environmental (seasonal variation) and fish (habitats and biological parameters).

Our thesis comprises four essential parts, the first part presents a comprehensive overview on the subject, especially plastic particles as a rising environmental threat, their capacity to adsorb or transfer pollutants, their toxicity to aquatic organisms and their impact on the human health. The second part provides methods used to isolate and to characterize collected plastic particles from marine fish. Then, the third part exhibits the obtained results on marine plastic debris ingested by commercial fish from Algerian coast (Gulfs of Bejaia, Skikda and Annaba). The fourth part gives a general discussion relating to plastic debris ingested by commercial marine fish and their risks. In a conclusion, we provide our main findings on microplastic contaminants affecting Algerian marine ecosystems. The future research prospects are defined.

II.1 The study area

Our study area is located in the east of Algerian coast (Figure 4): the Gulf of Bejaia is characterized by a relatively narrow continental shelf (with an average width of 1.5 km) and the coastal area consists of sandy beaches that line the base of the cliffs (Leclaire, 1972).

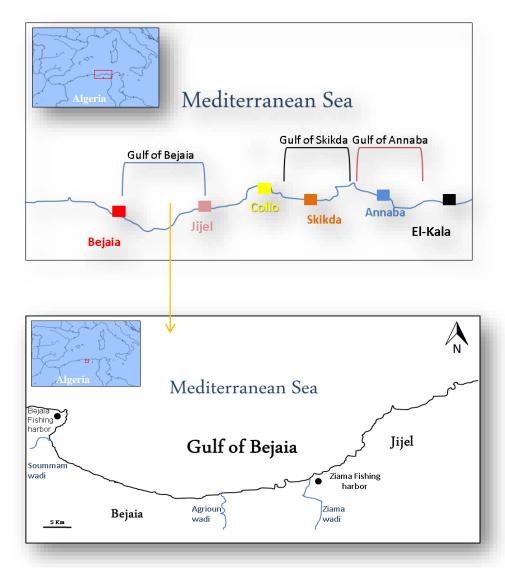


Figure 04: Study area.

In order to have an insight on the distribution of plastic waste, we included two secondary sampling area relatively far away from the Gulf of Bejaia (Gulf of Annaba and the Gulf of Skikda). These two additional sites give us the possibility to study spatial distribution of the plastics contaminants around the Algerian coastline, allowing us to examine potential differences in plastic debris abundance and composition between these different areas.

II.2 Sampling

Commercial fish specimens were obtained from local markets and the port. We included representative samples of commercial fish from the three considered area.

Between January 2018 and April 2023, 606 fish specimens were collected from all stations, 393 of them were sampled from the Gulf of Bejaia (Table 04). These samples were obtained to evaluate the ingestion of plastic debris by selected fish species in the Gulf of Bejaia and the secondary stations (Annaba, Skikda and El Kala) (Table 04). The sampled fish species are: Sardina pilchardus (Walbaum, 1792), Sardinella aurita (Valenciennes, 1847), Boops boops (Linnaeus, 1758), Pagellus acarne (Risso, 1827), Trachurus trachurus (Linnaeus, 1758), Sparus aurata (Linnaeus, 1758) and Lithognathus mormyrus (Linnaeus, 1758). These species were chosen because of their commercial importance. The presence of plastic debris in these fish species maybe offers an insight on the quality of the marine environment (flora and fauna) and consumer health in Algerian coasts.

Table 04: fishes sampled during the study (n = 606)

Species	(n)	Habitat	Mean weight (g) ± SD	Mean length (cm) ± SD	
Gulf of Bej	Gulf of Bejaia (From January 2018 to Mars 2020) GB1				
Sardinella aurita (Valenciennes, 1847)	60	Pelagic	26.95 ± 23.1	16.43 ± 4.1	
Sardina pilchardus (Walbaum, 1792)	60	Pelagic	25.43 ± 11.5	12.49 ± 3.9	
Pagellus acarne (Risso, 1827)	45	Benthopelagic	48.75 ± 18.4	14.61 ± 3.6	
Trachurus trachurus (Linnaeus, 1758)	38	Pelagic	44.08 ± 12.31	16.84 ± 5.1	
Boops boops (Linnaeus, 1758)	40	Demersal	56.91 ± 32.4	17.83 ± 2.7	
Sparus aurata (Linnaeus, 1758)	06	Demersal	304.3 ± 11.2	25.67 ± 2.6	

Gulf of Bejaia (From January 2022 to Mars 2022) GB2				
Sardinella aurita (Valenciennes, 1847)	120	Pelagic	16.11 ± 1.79	36.12 ± 12.29
Lithognathus mormyrus (Linnaeus, 1758)	24	Demersal	22.63 ± 1.65	157.82 ± 37.96
Additional stations (secondary)				
El Kala Coasts				
(From January 2022 to June 2022): (n= 43)				
Gulf of Annaba				
(From January 2022 to June 2022): (n= 54)				
Gulf of Skikda				
(From January 2023 to June 2023) : (n= 57)				
(From January 2023 to June 2023) : (n= 59) BC				

N=number of samples; SD=Standard deviation; BC=Bay of Collo

II.3 Plastic debris extraction Protocol

In this section, we will go over the protocol for collecting and examining the gastrointestinal tracts (GIT) of fish specimens. From the initial fish sampling to the final extraction of plastic debris from the GIT. This comprehensive protocol will explain how to obtain and analyze GIT contents, ensuring accurate and reliable results in assessing the prevalence and impact of plastic debris on the studied fish populations.

Stringent controls are implemented throughout the sampling process to ensure the integrity of our samples and prevent any potential contamination, with special attention paid to avoiding the introduction of plastic particles into the environment. Precautions are being taken to avoid contamination, particularly from plastic particles in the air, which could interfere with our research findings.

II.3.1 Biological parameters measurement

The collected fish specimens were measured for total length (TL), total weight (TW), and eviscerated weight (EW). These measurements are critical for characterizing the physical characteristics of the fish specimens and obtaining pertinent biometric data. Furthermore, these parameters serve as critical reference points for future research and analysis on plastic debris ingestion, trophic interactions, and potential ecological impacts on the studied fish populations.

We can ensure a robust and well-informed evaluation of the interactions between plastic debris and marine organisms in the Gulf of Bejaia and surrounding regions by conducting comprehensive measurements.

For measuring the lengths of the fish specimens, a graduated ruler is utilized (cm), allowing precise and accurate readings. To obtain the weights of the fish, a balance is employed (g), which ensures the determination of the fish's mass with precision.

II.3.2 Dissection and sex determination

Visual observations were used to determine the sexes of fish specimens that were dissected. We were able to identify and differentiate male and female individuals by carefully examining the internal anatomy of the fish. This sexing process is critical for understanding the population structure of the studied fish species as well as conducting additional research into potential gender differences in plastic debris ingestion and its associated impacts. Visual observations were a non-invasive and efficient method of determining the sex of the fish specimens (male, female or indeterminate), resulting in accurate and reliable data for our research.

II.3.3 Age determination

Otolith examinations were used to determine the age of the fish specimens. Otoliths are calcium carbonate structures that grow in distinct rings or layers in the inner ear of fish throughout their lives. Similar to tree rings, these growth rings provide valuable information about the age of the fish.

Otoliths were carefully extracted from the fish specimens during the examination, and cross-sections of the otoliths were prepared for microscopic analysis. We were able to determine the age of each individual fish (GB2) by counting the number of growth rings or annuli present in the otolith.

First annulus and second annulus refer to distinct growth increments found within the structure of fish otoliths, which are calcified structures in the inner ear of fish. These annuli, or rings, are formed periodically as the fish grows and are analogous to the growth rings found in tree trunks. The first annulus typically represents the period of rapid growth during the fish's early life stages, often corresponding to its first year of life. The second annulus, therefore, would represent the subsequent year of growth. By counting these annuli and examining their width and structure, scientists can estimate the age of the fish and gain insights into its growth patterns, life history, and environmental conditions.

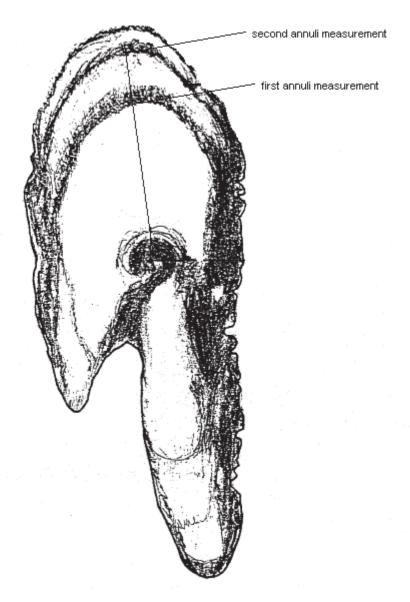


Figure 05: The use of otholits in the age determination (McFarlane et al., 2010)

II.3.4 Gastrointestinal tract extraction

The gastrointestinal tract (GIT) of each fish specimen was carefully removed from the esophagus to the aperture, and its weight was measured. The guts of each individual were then cleaned with distilled water to remove any exterior contamination. After rinsing, the cleaned gut was carefully put into sanitized Petri dishes.

Each specimen was assigned a unique code number to ensure proper identification and organization. This code number served as a reference point throughout the study, connecting each sample to its corresponding data and ensuring accurate record-keeping. The use of code numbers allowed for more efficient data management and analysis, making it easier to assess plastic debris ingestion and its potential effects on the studied fish populations.

II.3.5 Digestion treatments

The digestion treatments used in this study had two primary goals: first, to remove any remaining biological material from the fish specimens' gastrointestinal tract (GIT), and second, to facilitate the isolation of plastic debris (Roch and Brinker, 2017). Any organic matter, such as food remnants and tissue, was effectively broken down and removed by subjecting the GIT to specific digestion procedures. This ensured that the extracted material was mostly made up of non-organic components, making subsequent analysis and identification of plastic debris easier (Karuppasamy et al., 2020).

II.3.5.1 Digestion treatments methods

Different digestion methods are used to examine plastic debris ingestion by marine organisms to isolate plastic particles from the biological material present in the gastrointestinal tract (GIT). Among the most common digestion methods are (Cole et al., 2014):

- Enzymatic Digestion: In studies examining plastic debris ingestion by marine organisms, different digestion methods are used to isolate plastic particles from Enzymatic Digestion. The use of enzymes such as trypsin or pepsin to break down biological matter in the GIT, leaving behind plastic particles, is used in this method.
- Chemical Digestion: Chemical digestion uses strong acids, such as hydrochloric acid or potassium hydroxide, to dissolve organic material while leaving plastic debris intact (Table 5).
- **Physical Digestion**: Mechanical processes, such as agitation or shaking, are used to separate plastic particles from the biological content of the GIT.
- Combination Digestion: To achieve the most effective isolation of plastic debris while minimizing interference from biological material, a combination of different digestion methods is frequently used.

Each digestion method has advantages and disadvantages, and researchers choose the best method based on their research objectives, sample characteristics, and the types of plastic particles they want to isolate and analyze. For accurate and reliable quantification and characterization of plastic debris ingestion by marine organisms, digestion methods must be carefully chosen. Chemical digestion is the most commonly used method among the various digestion methods used in various studies on plastic debris ingestion. Chemical digestion is

frequently used by researchers due to its effectiveness in breaking down biological material in the gastrointestinal tract (GIT) while preserving plastic particles (Mai et al., 2018).

When strong acids or alkaline solutions are used during chemical digestion, organic matter is completely dissolved, leaving behind plastic debris for further analysis. This method has been shown to be effective in isolating microplastics and other plastic fragments from marine organisms' GIT (Piarulli et al., 2019).

Table 05: Examples of some methods used in chemical digestion

Author	Chemical method	Biota
Prata et al. (2019)	30% hydrogen peroxide (H2O2) and iron (Fe(II)) catalyst	animal tissues
Thiele et al. (2020)	10 % KOH	-
Schirinzi et al. (2020)	(KOH/HNO)	dolphinfish
Catarino et al. (2017)	1 M NaOH	mussels
Jin-Feng et al. (2018)	69% HNO3	bivalves

Chemical digestion involves not only the use of acids and alkaline solutions, but also the careful control of temperature and digestion time (Munno et al., 2018).

Two digestion methods were used in our study to isolate plastic debris from the gastrointestinal tracts (GIT) of the fish specimens:

Digestion by sodium hydroxide (NaOH)

In our initial sampling (GB1), the gastrointestinal tract (GIT) samples were subjected to the following protocol adapted by Baalkhuyur et al. (2018):

- GIT samples incubated for one hour at 60°C. This step aimed to remove any excess moisture and thoroughly dry the samples.
- To improve the efficiency of plastic extraction from GIT tissue, we modified a digestion protocol described by Cole et al. (2014). This method employs varying concentrations of sodium hydroxide (NaOH) solutions.
- There were two NaOH solutions used: 1M NaOH solution and a 10M NaOH solution. These solutions have previously been shown to be effective in removing biogenic material from GIT contents.
- 30 ml of a 1 M NaOH solution was added to the samples to reinforce digestion and remove any remaining biological material and non-digestible residues. This procedure was carried out in accordance with the protocols established by Cole et al. (2014) and Catarino et al. (2017).

▶ Digestion by hydrogen peroxide (H₂O₂)

A slightly modified protocol adapted from the method described by Digka et al. (2018) was used in our second sampling (GB2) to ensure comprehensive plastic extraction from the gastrointestinal tracts (GIT) of the fish specimens. The following actions were taken:

- Each fish specimen's gut was carefully transferred into a clean petri dish. Following that, the mixture was baked in a 60°C oven for 24 hours. This extended duration and temperature control were chosen to ensure thorough and efficient removal of plastic debris while also removing biogenic and other biological materials.
- The digestive tracts were then treated with a 30% hydrogen peroxide (H_2O_2) solution (Chem Lab in Germany) after the oven treatment. The use of hydrogen peroxide aids in the breakdown of any remaining biological residues and non-plastic materials in the GIT, thereby improving plastic debris isolation.

II.3.6 Plastic debris inspection and identification

A binocular stereoscope was used to inspect the samples visually. This specialized microscope provided a detailed and magnified view of the extracted contents, facilitating the identification and extraction of plastic debris from the fish specimens' gastrointestinal tracts (Hidalgo-Ruz et al., 2012; Free et al., 2014; Jabeen et al.; 2017; Al Muhdhar et al., 2021).

We were able to distinguish plastic particles from other materials and accurately record the quantity, size, and type of plastic debris present in each sample by using a binocular stereoscope. The stereoscope enabled us to collect precise data and further analyze the ingestion patterns of plastic particles by the studied fish species. Plastic debris were identified visually based on color, brightness, and the absence of cellular structures. This method is commonly used in studies of microplastic pollution and has been validated by previous studies (Herrera et al., 2018 and Bellas et al., 2016).

Ensuring the quality and accuracy of plastics extraction and analysis from the gastrointestinal tracts (GIT) of fish requires meticulous attention to detail and adherence to standardized procedures. The first step is sample collection and handling. During field collection, it is essential to use stainless steel or glass tools to avoid contamination from plastic materials. Samples should be stored in pre-cleaned, non-plastic containers, such as glass jars, to prevent any introduction of extraneous MPs. Once in the laboratory, sample preparation must be carried out in a controlled environment to minimize airborne contamination. This includes using clean, dust-free areas and wearing non-fibrous laboratory coats. All equipment and surfaces should be thoroughly cleaned with filtered water before use. It is advisable to conduct blank controls by processing samples without fish tissues alongside actual samples to detect any potential contamination during the extraction process. During the extraction of MPs from the GIT, enzymatic digestion or chemical digestion should be performed carefully to dissolve organic matter while preserving plastic particles. Filtration should be done using stainless steel or glass filters rather than plastic ones. (Lin et al., 2023)

Sample Handling Equipment Cleaning Plastic Extraction Accurate Plastic Analysis Blank Controls Laboratory Preparation

Ensuring Plastic Extraction Quality

Figure 06: Quality Assessment and Control for PD Extraction.

II.3.6 Plastic particles shapes and length measurements

After visually identifying the plastic particles, we used (ImageJ) 1.4 to count, photograph, and measure them. This specialized software enabled accurate and precise measurements of each plastic particle's size and dimensions.

The color and shape data were critical in characterizing the plastic debris found in the fish specimens' gastrointestinal tracts (GIT).

ImageJ is a well-established method for particle analysis, and it is consistent after Jabeen et al. (2017). We improved the reliability and validity of our research findings by using this software to ensure standardized and consistent quantification and characterization of plastic debris.

II.3.7 Data analysis

SPSS 14.0 and MS Excel 2013 were used for statistical analysis of our data. A Pearson correlation test was used to investigate potential relationships between the occurrence of microplastics (FO% = Frequency of occurrence of microplastics) and fish parameters such as TL (total length), Tw (total weight), and the Fulton's condition factor K (K=100× (TW/TL³)). The data was then analyzed using ANOVA (Analysis of Variance) to determine whether there were any significant differences between the variables. The Tukey test's HSD (Honestly Significant Difference) was used to determine specific group differences with ** = p < 0.01 and * = p < 0.05 values.

The number of affected specimens was subsequently calculated based on the total count of individuals exhibiting plastic debris ingestion or external contamination.

The quantification of plastic debris within the studied population required a precise counting methodology. Each plastic particle found within the gastrointestinal tract or adhered to the external surface of a specimen was carefully enumerated. Additionally, specialized techniques, such as filtration or sedimentation, may have been employed for the collection and quantification of microplastics from environmental samples. The total number of plastic particles was recorded, and meticulous attention was given to documenting the count per individual specimen.

The occurrence (FO %) of plastic particles present in the digestive tracts was calculated using the following formula:

FO (%) = $(Ni/N) \times 100$, where

- FO% = frequency of occurrence of plastic particles;
- Ni = number of GITs that contained plastic particles;
- N = total number of GITs examined.

III. Results

This chapter contains a detailed analysis of data obtained from examined fish specimens (Gastrointestinal tracts of different fish species), in which we identified, quantified, and characterized plastic particles based on their color, shape and size.

From the first sampling (GB1), plastic debris were found in 146 specimens among the 249

III.1 Frequency of occurrence for ingested plastic debris (by fish specimens) (FO%)

examined fish specimens (58.63%). These small pollutants were collected in all fish species (Table 06), indicating that they are present in pelagic, benthopelagic, and demersal fish species. The average number of ingested particles was 8.45 ± 14.69 particles per individual. *Boops boops* has the highest average (21.03 \pm 23.49 particles per individual). The second sampling (GB2) revealed that microplastics were found in 107 individuals among 144 (74.30%) of *Sardinella aurita* and *Lithognathus mormyrus* (Table 06). Additionally, the results from the secondary regions, including Annaba, Skikda and El Kala, are also presented in Table 06. Plastic debris with varying densities were discovered in 462 gastrointestinal tracts (GIT) among 606 examined samples (FO%=76.23%). Our results suggest that plastic ingestion is common among the studied fish populations in the Gulf of Bejaia and also in the secondary stations (Annaba, Skikda and El Kala).

III.2 Morphotypes of plastic debris retrieved

A total of 3,352 plastic derbis were extracted from fish specimens collected in the Gulf of Bejaia. Plastic debris were isolated from the fish's gastrointestinal tracts (GIT) and characterized based on their color, shape, and size. In the first sampling (GB1), our investigation focused on all plastic debris size-types, ranging from microplastics to macroplastics. Notably, the highest number of plastic debris was recorded in this sampling, 2,347 plastic particles and the average number of particles ingested was 8.45 ± 14.69 particles per GIT.

In the second sampling (GB2), we concentrated exclusively on microplastics. A total of 1,005 microplastic particles were discovered in the 144 examined gastrointestinal tracts (GIT) with a density of 9.38 ± 20.11 Plastic debris/individual.

Two plastic particles types were reported. The most observed morphotype is fiber with 85% from the first sampling (GB1) and 71.71% from the second sampling (GB2). In the first sampling (GB1), fragment morphotypes were extracted only from three fish species: *Boops boops*, *Sparus aurata* and *Pagellus acarne* (Figure 07). These fragment morphotypes accounted for 15% of the total number of plastics found in the fish specimens' gastrointestinal tracts (GIT),

the microplastics identified in the gastrointestinal tracts (GIT) of the fish from the second sampling (GB2) were fragments, accounting 28.35% from the total microplastic particles (Figure 08).

Table 06: Occurrence of plastic debris in examined fish species.

Fish species	(n)	number of contaminated samples	FO%
	GB1		
Sardinella aurita	60	35	58.33%
Sardina pilchardus	60	26	43.33%
Pagellus acarne	45	33	73.33%
Trachurus trachurus	38	24	63.16%
Boops boops	40	22	55.00%
Sparus aurata	6	6	100.0%
	GB2		
Sardinella aurita	120	87	72.50%
Lithognathus mormyrus	24	20	83.33%
	Gulf of An	naba	
All species (2022)	54	52	98.11%
	Gulf of Ski	kda	
All species (2023)	57	57	100.0%
All species (2023) BC	59	58	98.30%
	El Kala co	asts	
All species (2022)	43	42	97.67%

III.3 Colors of the collected plastic debris

In the first sampling (GB1), the extracted plastics exhibited a variety of colors (Figure 09), with red, blue, and transparent being the most prominent. Among these colors, blue plastic particles were the most dominant, constituting 56.4% of the total plastics identified.

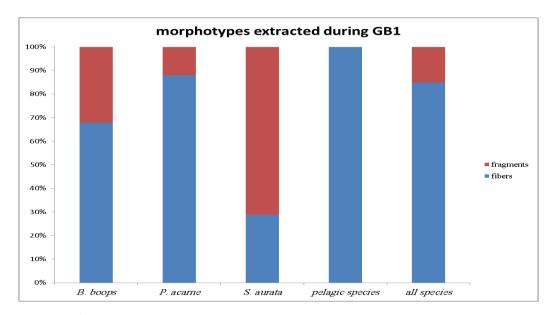


Figure 07: Variability of PDs extracted from samples GIT (GB1)

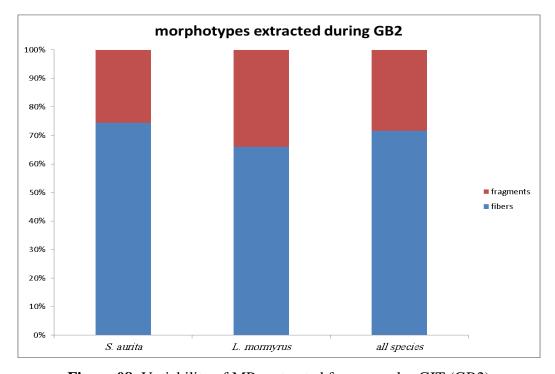


Figure 08: Variability of MPs extracted from samples GIT (GB2)

The red plastic particles followed closely, accounting 41.3% of the extracted plastics. However, transparent and other colors were observed less frequently and prevalent. The microplastic particles retrieved from the fish during the second sampling (GB2) came in a variety of colors, including translucent, black, blue, red, yellow, and white. Blue microplastics were the most common of these colors, accounting 49% of the total microplastics identified in *Sardinella aurita* specimens and 30% in *Lithognathus mormyrus* specimens. Following blue, black, and clear microplastics were also found in lesser quantities (Figure 08). The remaining colors,

including red, yellow, and white, accounted for only a minor portion of the total microplastic particles.

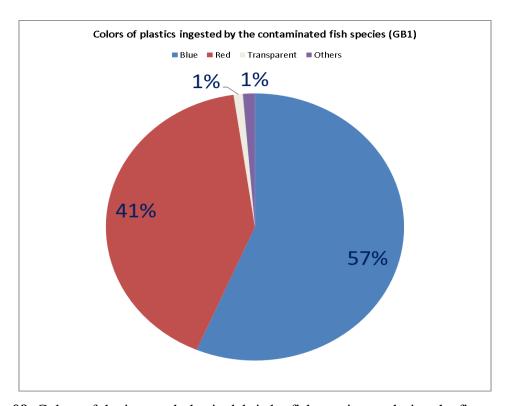


Figure 09: Colors of the ingested plastic debris by fish specimens during the first sampling (GB1).

Our results illustrated in Figure 07 show the observed microplastic color data during the second sampling (GB2). The diagram represents the distribution and prevalence of various microplastic types detected in the gastrointestinal tracts (GIT) of fish specimens from the Gulf of Bejaia. The colors illustrated in the figure 09 are blue, black, translucent, red, yellow, and white, each represented by a percentage. Figure 10 (a) represents the results of microplastic colors observed in *Sardinella aurita* specimens during the second sampling (GB2). The figure visually displays the distribution and prevalence of different microplastic colors found in the gastrointestinal tracts (GIT) of *Sardinella aurita*. Figure 10 (b), on the other hand, represents the results of microplastic colors observed in *Lithognathus mormyrus* specimens during the same sampling zone (GB2). This figure visually presents the distribution and prevalence of different microplastic colors found in the gastrointestinal tracts of *Lithognathus mormyrus* fish from the Gulf of Bejaia.

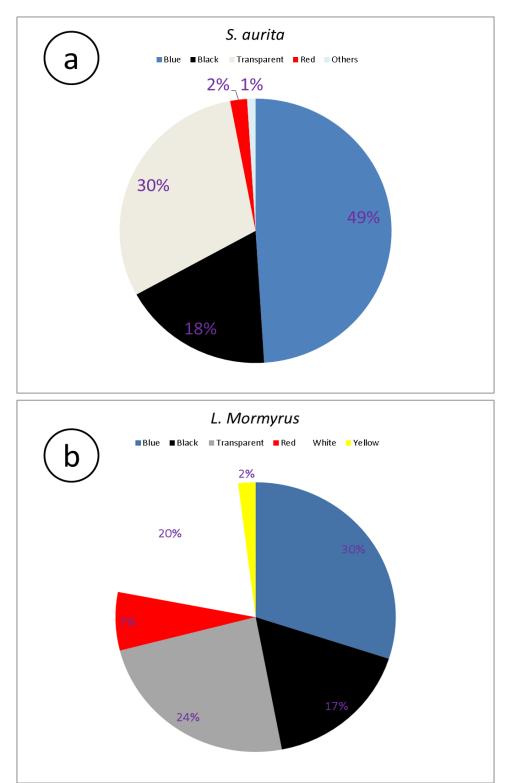


Figure 10: Colors of the ingested microplastics during the second sampling (GB2); (a): by *S. aurita* and (b) by *L. mormyrus*.

III.4 Plastic debris under binocular stereoscope

Shape and color were used to analyze the contents of the gastrointestinal tract (GIT). We were able to reliably identify and characterize plastic trash detected in the GITs of fish species using

this method. The form and color of the plastic particles facilitate to understand their composition and potential origins.

Figures 11 and 12 show a visual summary of some photographed plastic debris (from GB1 and GB2 samples). These photos show a variety of plastic particles with various forms and colors found in the GIT of examined fish species.

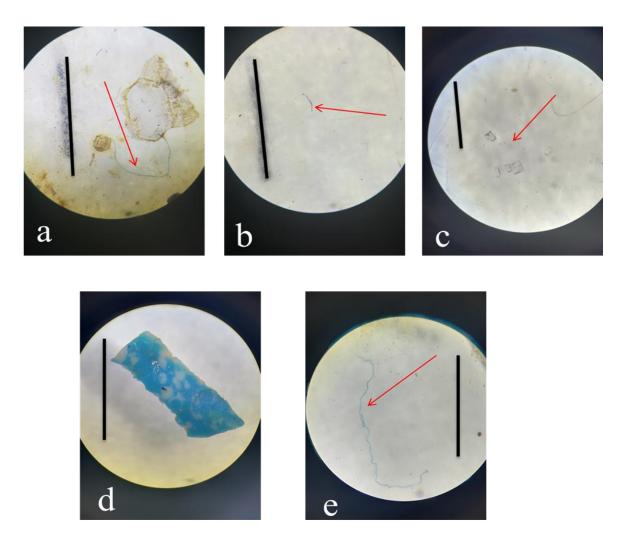


Figure 11: Plastic debris extracted from demersal fish specimens; (a): green fiber founded in the GIT of *B. boops*, (b) blue filament extracted from *B. boops*, (c) transparent fragments exctracted from *L. mormyrus*, (d) blue fragment retrieved from the GIT of *S. aurata*, (d) macroplastic blue fiber. Scale=5mm.

III.5 Plastic debris classification by particle size

Microplastics accumulated in the digestive systems of each fish species tested range in size from 0.01 to 0.5 cm (Figure 13). The number of microplastics discovered in these fish specimens exceeds the number of macroplastics. The previous picture also shows that microplastics identified in the examined species are smaller than 2mm in length for filaments

and diameter for items. The presence of macroplastics is uniformly low in all of the species studied.

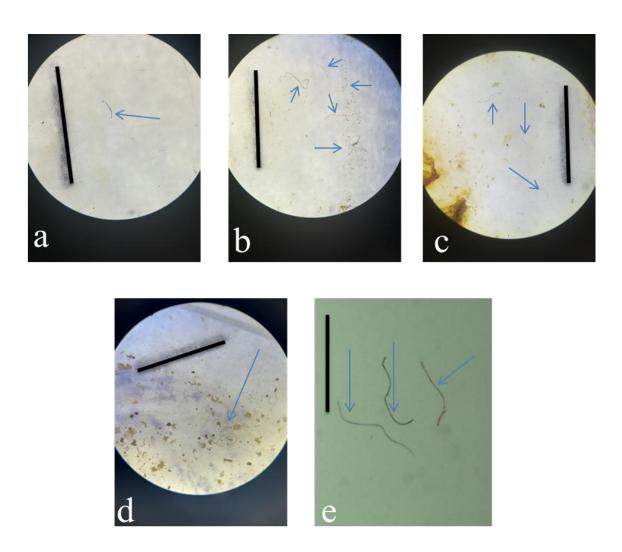


Figure 12: Plastic debris extracted from pelagic fish specimens; (a): blue fiber founded in the GIT of *S. pilchardus*, (b) filaments (blue, red and black) extracted from *S. aurita*, (c) fibers exctracted from *S. pilchardus*, (d) fibers with different colors retrieved from the GIT of *T. trachurus*, (d) microplastic fibers. Scale=5mm.

The percentage of microplastics (MPs) in total plastic varies according to fish species tested (figure 13). Microplastics account 89% of the total plastic waste collected in the gastrointestinal tracts of *Sardina pilchardus*. *Sardinella aurita* represents a greater percentage, with microplastics accounting 92% of the total plastic particles found. *Boops boops*, on the other hand, contains the largest concentration of microplastics, accounting 98% of the total plastic waste. *Pagellus acarne* reveals that microplastics made up 88% of the accumulated plastic detritus. *Sparus aurata* contains the highest percentage of microplastics of any tested species, accounting 100% of the total plastic debris in their gastrointestinal tracts. *Trachurus trachurus*

shows a significantly lower percentage of microplastics, accounting 84% of the total plastic waste. In summary, when all of the fish species evaluated together, microplastics account 92% of the total plastic debris identified in GITs.

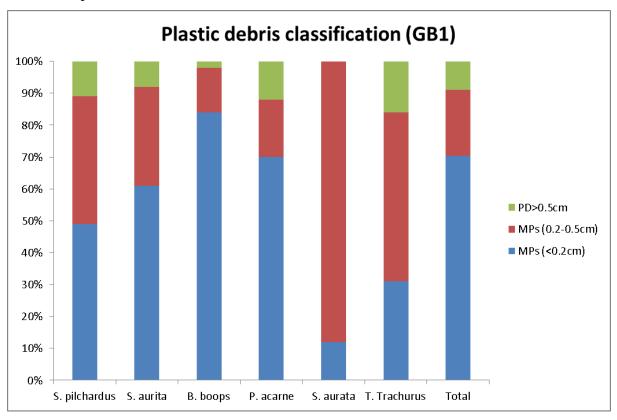


Figure 13: Classification by particle size (MPs and Mesoplastics) for plastic debris extracted during the first sampling (GB1)

III.6 Plastic debris occurrence according to fish sex

The total population sampled in GB1 was 123 females, 96 males, and 30 individuals with undetermined gender. In females specimens, 74.1% of their gastrointestinal tracts is contaminated, whereas males present a low frequency of occurrence (21%). Females ingested somewhat more than males (1055 and 1022 particles, respectively), while undetermined individuals ingested only 270 particles (Figure 14). The ANOVA analysis reveals no significant differences in the mean amount of plastic particles across the three genders, with a Tukey HSD p-value greater than 0.05.

In GB2, there were no significant differences (p > 0.05) in the number of microplastics ingested by females (545 particles), males (302 particles), and indetermined specimens (158 particles) in the two studied species (Figure 15). No significant differences were observed in the secondary study areas; however, females ingested slightly more microplastics in terms of quantity than males.

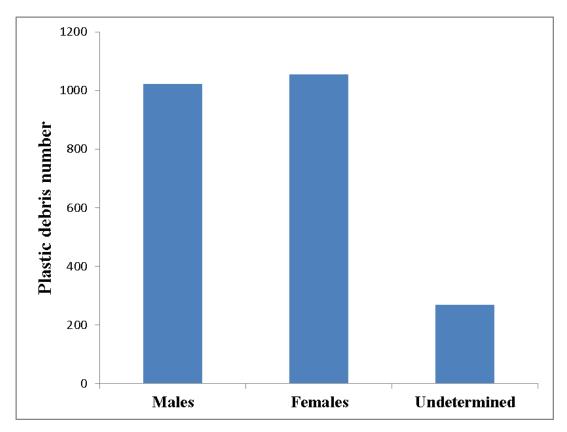


Figure 14: Number of PDs ingested by males, females and undetermined fishes during the first sampling (GB1)

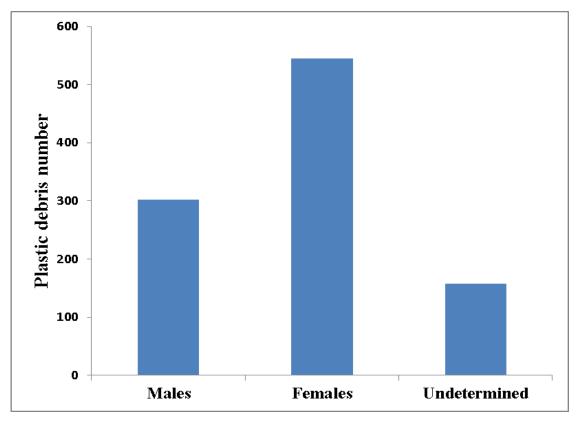


Figure 15: Number of MPs ingested by males, females and undetermined fishes during the first sampling (GB2)

III.7 Plastic debris occurrence and fish's age

One of the main objectives of starting the second sampling (GB2) was to look into the potential relationship between plastic debris ingestion and fish age.

The aim is to verify the influence of age on plastic ingestion patterns among the studied fish species.

The age of *Sardinella aurita* specimens varies from 1 to 6 years. For *Lithognathus mormyrus*, the age varies from 4 to 8 years.

The linear correlation (Figure 16) analysis reveals that there is no significant relationship between the age of fish and microplastic (MPs) ingestion for both *Sardinella aurita* (y = 0.308x+6.717; $R^2 = 0.004$) and *Lithognathus mormyrus* (y = -1.784x+31.56; $R^2 = 0.030$). The low R-squared values (0.004 for *Sardinella aurita* and 0.030 for *Lithognathus mormyrus*) indicate that the age of the fish does not have a strong influence on the extent of microplastic ingestion in either species.

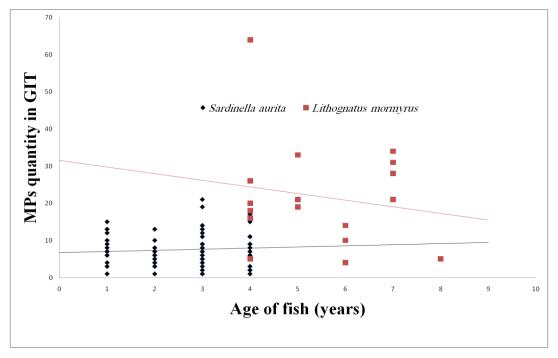


Figure 16: MPs quantity-Age relationship in *Sardinella aurita* and *Lithognathus mormyrus*.

III.8 Plastic debris, total length and total weight relationship

The relationship between total length and total weight was studied in *Sardinella aurita* and *Lithognathus mormyrus*. To better understand the influence of physical characteristics on fish ingestion of microplastics in the Gulf of Bejaia ecosystem, we examined the relationship between total length and total weight of the fish. This analysis aimed to investigate whether variations in these physical attributes correlate with differences in microplastic ingestion rates.

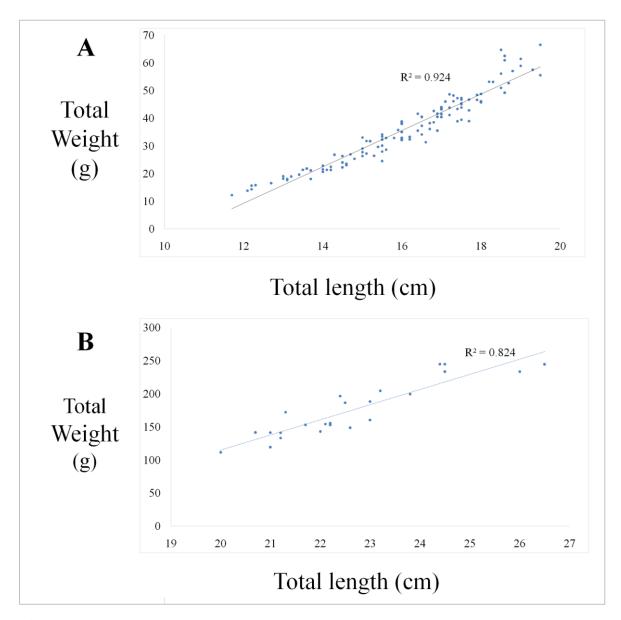


Figure 17: Total length and total weight relationship in (A) *S. aurita* and (B) *L. mormyrus*.

The length-weight relationship (Tl-Tw) was analyzed using a linear regression equation (R2) for both *Sardinella aurita* (R^2 = 0.924) and *Lithognathus mormyrus* (R^2 =0.824). Our results show a positive correlation between total length and total weight for both species, as illustrated in Figure 17. This correlation indicates that as the total length of the fish increases with the total weight.

The Fulton's condition factor, calculated as the ratio of the fish's weight to its length cube, provides useful information about the fish's overall health and well-being. The positive correlation between Tl-Tw in this case indicates that changes in the condition factor of the fish can be attributed to variations in both total length and total weight. Therefore, the Fulton's condition factor serves as a useful metric to assess the condition and physiological state of the

fish specimens in relation to their size metrics and may have implications for their susceptibility to microplastic ingestion and overall health in the Gulf of Bejaia ecosystem (Figure 18).

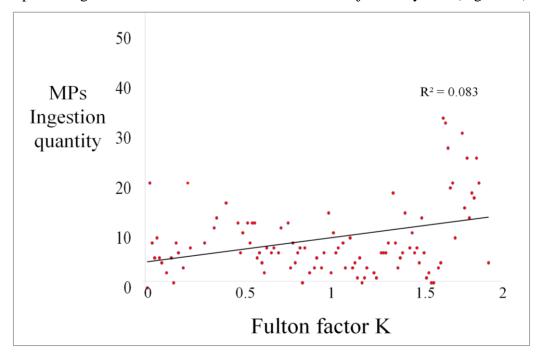


Figure 18: Relationship between Fulton's Condition Factor and Microplastics (MPs) ingestion quantity in fish specimens

The examination of the impact of Fulton's condition factor K on microplastic ingestion was one of the objectives of the second sampling (GB2). The relationship between the fish's physiological condition, and ingested microplastics was investigated. Our results (Figure 18) show no correlation between Fulton's condition factor K (R²=0.083) and ingested plastic particles in *Sardinella aurita* and *Lithognathus mormyrus* Fish condition factor is not influenced by plastic particles ingested by the studied fish species.

III.9 Effect of seasonal variation on plastic debris ingestion

In this section, we wanted to see if there were any patterns or changes in plastic consumption by fish over different seasons. Seasonal conditions and fish behavior can have a substantial impact on the quantity and abundance of plastic particles in the marine ecosystem. We present here the results of the relationship between seasonal variation and the quantity and quality of the ingested plastic debris by the studied fish species.

The fish specimens used in this study were acquired during the period spanning from January 2018 to March 2020. This time frame allowed us to include samples collected during both dry and wet seasons (GB1).

III.9.1 Effect of seasonal variation on plastic debris quantity

Figure 19 and figure 20 depicts the incidence of contaminated fish specimens with plastic particles during the wet season. The rainy season had a mean of 26.71 ± 4.2 individuals containing plastic debris, while the dry season had a smaller mean number of contaminated fish specimens, with 8.71 ± 1.7 individuals.

The collected plastic debris was significantly higher during the wet season compared to the dry season, according to statistical analysis using a one-way ANOVA test (p = 0.05). During the wet season, fish consumed a total of 2092 plastic particles (an average of 418.4 particles per species). The dry season, on the other hand, had a much lower count of 129 plastic particles (25.8 per species).

Figure 19 illustrates the mean number of contaminated fish specimens, showcasing the higher prevalence of plastic ingestion during the wet season compared to the dry season. Figure 20 represents the mean number of ingested plastic particles during both the wet and dry seasons.

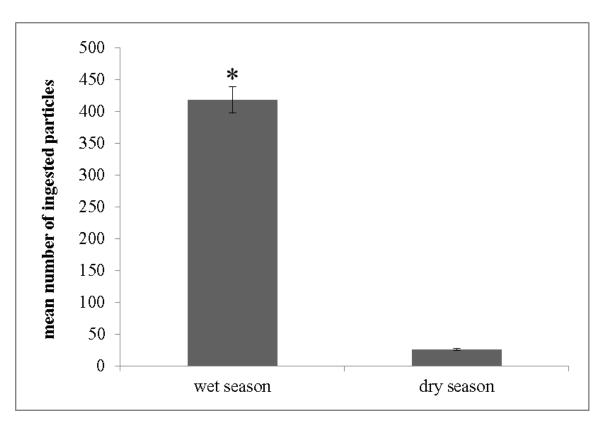


Figure 19: the mean number of plastic debris ingested by fish specimens in wet and dry seasons. **: p<0.01; *: p<0.05

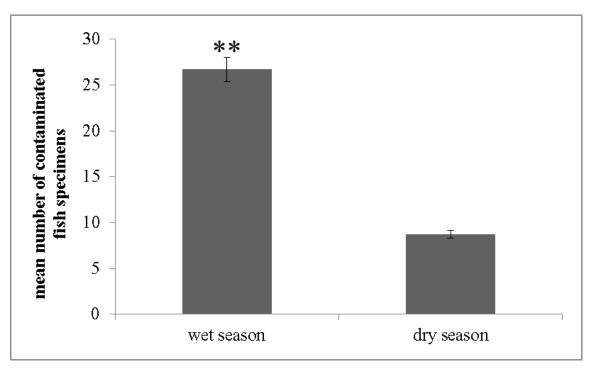


Figure 20: the mean number of contamined fishes by seasonal variation. **: p<0.01; *: p<0.05

III.9.2 Effect of seasonal variation on plastic debris morphotypes variability

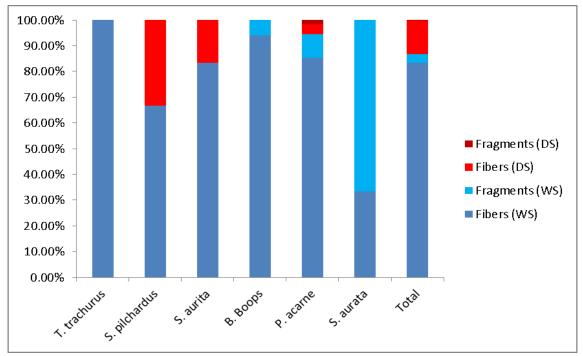


Figure 21: Variability of plastic debris morphotypes according to the season. DS= dry season; WS= wet season.

The amount of plastic debris consumed by fish increased significantly during the wet season. Furthermore, a major fraction of the plastic debris during the wet season consisted of fragments, which were especially prevalent during this season.

In contrast, the dry season shows significantly lower levels of plastic debris ingestion. During the dry season, the majority of the plastic debris fibers, with only four (4) fragments (Figure 21).

III.9.3 Effect of seasonal variation on plastic debris colors

Examined individuals attached to *Sardinella pilchardus*, *Pagellus acarne*, *Sardinella aurita*, and *Trachurus trachurus* during the dry season showed a limited color variety of consumed plastic particles as shown in Figure 22. The spectrum of colors detected among the ingested plastic particles was limited during this season, indicating a lower diversity when compared to other seasons.

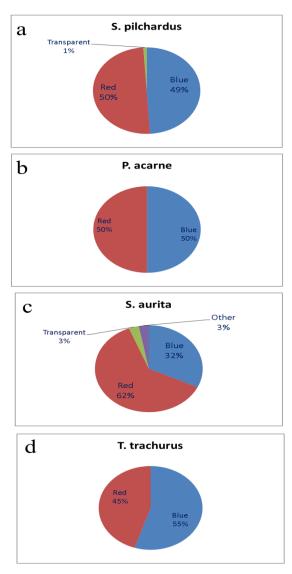


Figure 22: Colors variety in plastic debris ingested by (a) *S. pilchardus*, (b) *P. acarne*, (c) *S. aurita* and (d) *T. trachurus* during the dry season.

During the wet Season, however, *Sardinella pilchardus*, *Pagellus acarne*, *Sardinella aurita*, and *Trachurus trachurus* consume plastic particles with a wider range of colors, (Figure 23). When compared to the dry season, the plastic debris consumed by these fish species revealed a more wide range of coloration. In the wet season, for example, the color green was notably marked in both types of sardines (*Sardinella pilchardus* and *Sardinella aurita*).

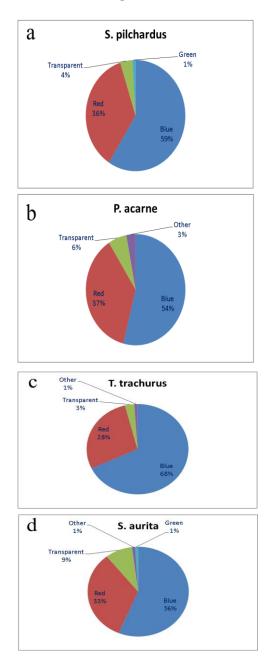


Figure 23: Colors variety in plastic debris ingested by (a) *S. pilchardus*, (b) *P. acarne*, (c) *T. trachurus* and (d) *S. aurita* during the dry season.

III.10 Density of plastic debris ingestion

The density of plastic particle ingestion, expressed as the number of plastic debris per gram of gastrointestinal tract (GIT) weight, varies significantly among different fish species. Our analysis revealed distinct patterns of plastic ingestion across the studied species, indicating that the quantity of plastic debris found within the GIT per gram of tissue weight differs from one species to another. The gray data points in the above graph represent the average density of plastic debris per gram of gastrointestinal tract (GIT) for the various species tested throughout both the dry and rainy seasons. *Boops boops* (21.21 PDs/ (GIT) g) and *Sardina pilchardus* (18.38 PDs/ (GIT) g) have the greatest average microplastic density. Furthermore, as shown in Figure 24, the average microplastic density is higher in species examined during the wet season compared to those sampled during the dry season. This finding indicates that during the wet season, fish species consumed more plastics per gram of GIT, whereas during the dry season, the average microplastic density was lower.

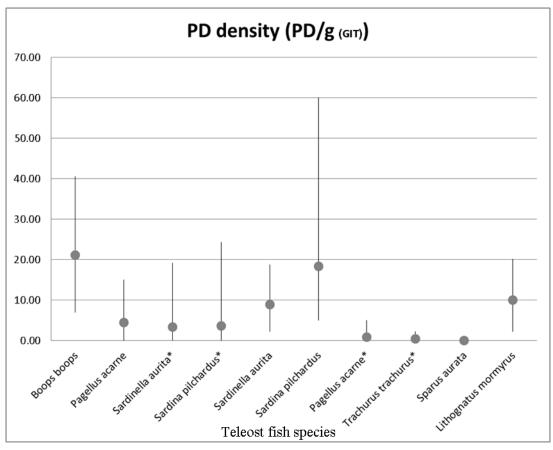


Figure 24: The minimum, average and maximum density of plastics (PD/GIT g) across all studied species. *=Dry season

IV. Discussion

In this study, commercial fish species from three ecological regimes were gathered to assess the prevalence of plastic particles in their gastrointestinal tracts: pelagic, benthopelagic, and demersal. We focused on the changes in plastic ingestion patterns among species with varied eating behaviors and habitats in the considered marine ecosystem.

Our findings will be compared to those of other relevant studies conducted in various regions and ecosystems. In addition, we will include data and insights added from our secondary study sites, which include Annaba, Skikda and El Kala, to present a more comprehensive picture of plastic contamination in the study region.

IV.1 Plastic ingestion levels

Plastic debris were observed in 462 of the 606 fish gastrointestinal tract (GIT) samples analyzed, suggesting a 76.23% frequency of occurrence (FO%). This rate of plastic debris intake is notable and not uncommon on a global scale. Various investigations reported similar results of high plastic intake by many fish species: *Pseudophycis bachus* (Hutton, 1872), Nemadactylus macropterus (Bloch & Schneider, 1801), Chelidonichthys kumu (Lesson, 1828), Helicolenus barathri (Waite, 1916), Latridopsis ciliaris (Cuvier, 1829), Squalus acanthias (Linnaeus, 1758), Thyrsites atun (Euphrasen, 1791), Trachurus declivis (Jenyns, 1841) and Seriolella brama (Richardson, 1846) were among the commercial fish species studied by Clere et al. (2022). According to this study, the frequency of occurrence (FO) of microplastics in these fish species reached 75.2%. This discovery emphasizes the extensive occurrence of microplastics in the maritime environment and their possible influence on the region's commercial fish species. Chen et al. (2021) examined 117 fish samples representing 39 species and 18 families in the coastal waters of Taiwan's Hengchun Peninsula. The study discovered that nearly 95% of the fish tested positive for plastic debris in their gastrointestinal tracts. The fish species studied belonged to the Labridae, Pomacentridae, Serranidae, Pseudochromidae, and Cirrhitidae families. In their study on commercial marine fish from Malaysia, Karbalaei et al. (2021) demonstrated that African catfish and Threefinger threadfin exhibited high ingestion rates of plastic debris. Specifically, African catfish showed a plastic ingestion rate of 90%, while Threefinger threadfin had a 100% ingestion rate. These findings indicate a significant prevalence of plastic debris ingestion in these particular fish species in the marine environment of Malaysia. Ferreira et al. (2018) investigated microplastic contamination in the GIT of Cynoscion acoupa, a commercially significant top predator in the Western Atlantic. The study looked at the spatiotemporal and ontogenetic use of a tropical

estuary. Microplastic contamination was found in more than 50% of the analyzed fish specimens. This discovery emphasizes the widespread presence of microplastic pollution in the gut of *Cynoscion acoupa*, highlighting the potential impact of plastic debris on economically important marine species in the region's estuarine ecosystem. Silva-Cavalcanti et al. (2017) found that 83% of the fish *Hoplosternum littorale*, a common freshwater fish heavily consumed by humans in semi-arid regions of South America, had plastic debris inside their gut. This finding indicates a high prevalence of plastic ingestion in this freshwater fish species, which could have implications for both the fish population and human health in areas where this fish is a major part of the local diet. In their study, Sparks and Immelman (2020) investigated the presence of microplastics in several fish species, including *Trachurus capensis* (Castelnau, 1861), *Merluccius paradoxus* (Franca, 1966), *Etrumeus whiteheadi* (Ege, 1953), *Scomber japonicus* (Houttuyn, 1782), *Chelidonichthys capensis* (Kaup, 1858) and *Argyrozona argyrozona* (Valenciennes, 1830) Their results showed that microplastics were present in 87% of the sampled fish from the Agulhas Bank, South Africa.

Many studies on plastic debris ingestion by fish have consistently found that the frequency of occurrence (FO%) of plastic ingestion exceeds 50% (Nadal et al., 2016; Güven et al., 2017; Sbrana et al., 2020; Pennino et al., 2020). These authors confirmed that plastic pollution is a prevalent and widespread issue affecting fish populations in the Mediterranean region. Studies conducted in the Mediterranean Sea have reported varying levels of plastic debris ingestion by fish, ranging from low to medium and high occurrences. The findings across these studies show that the frequency of plastic ingestion by fish varies significantly among different species and locations within the Mediterranean region (Table 07). While some studies have found relatively low levels of plastic ingestion in specific fish species, others have found medium to high levels of plastic debris in the gastrointestinal tracts of various fish specimens. These variations in ingestion rates may be influenced by factors such as species-specific feeding habits, geographical location, and the abundance of plastic pollution in various areas of the Mediterranean Sea.

Table 07: Summary of studies conducted on plastic debris ingestion by fish from the Mediterranean Sea.

Location	Species	FO%	References
Algerian East coast	Sardinella aurita Sardina pilchardus Pagellus acarne Trachurus trachurus Boops boops Sparus aurata Sardinella aurita Lithognathus mormyrus	76.23%	Current study
Spanish Mediterranean coast	Sardina pilchardus Engraulis encrasicolus	15%	Compa et al. (2018)
Spanish Mediterranean coast	Scyliorhinus canicula Merluccius merluccius Mullus barbatus	17.5%	Bellas et al. (2016)
NW Iberian shelf	Engraulis encrasicolus Sardina pilchardus Callionymus lyra Mullus surmuletus	78%	Filgueiras et al. (2020)
Spain, France, Italy and Greece	Boops boops	46.8%	Tsangaris et al. (2020)
Salento coastal seas (Italy)	Sardina pilchardus Boops boops Mullus barbatus	12-88%	Trani et al. (2023)
Italy and Croatia	Mullus barbatus Merluccius merluccius	23.3%	Giani et al. (2019)
Eastern Aegean Sea (Greece)	Paracentrotus lividus	100%	Hennicke et al. (2021)
Turkey	Oncorhynchus mykiss Sparus aurata Dicentrarchus labrax	50- 63 %	Kılıç (2022)

Lebanon	Engraulis encrasicolus	83.3%	Kazour et al. (2019)
Egypt	Siganus rivulatus Diplodus sargus Sardinella aurita	100%	Shabaka et al. (2020)
Egypt	Siganus rivulatus Diplodus vulgaris Serranus scriba Boops boops Sparus aurata Scomberomorus commerson Nemipterus japonicus Parupeneus macronemus Pomatomus saltatrix	100%	El-Sayed et al. (2022)
Egypt	Siganus rivulatus Diplodus sargus Sardinella aurita	100%	Shabaka et al. (2020)
Libya	Sparus aurata Sphyraena chrysotaenia Mugil cephalus Epinephelus marginatus Seriola fasciata Oblada melanura	100%	Hamid et al. (2022)
Tunisia	Sarpa salpa Liza aurata	100%	Abidli et al. (2021)
Tunisia	Serranus scriba	100%	Zitouni et al. (2020)
Morocco	Diplodus cervinus Auxis thazard	10%	Alshawafi et al. (2018)

The high level of plastic debris (PD) was observed in fish samples from the southern Mediterranean Sea, this result corroborate with our findings is then confirmed.

The presence of plastic particles throughout the Mediterranean Sea's water column. Furthermore, it is likely that some of these plastic particles will form complexes with other

organic or chemical elements, indicating the possibility of interactions and impacts on the region's marine ecosystems.

IV.2 Plastic debris morphotypes variability

The plastic debris found in the fish samples' gastrointestinal tracts (GITs) was entirely made up of fibers and fragments. During our investigation, we found no other types of plastic particles in the GITs. Observations across various studies highlight the prevalence of these specific types of plastic particles as the most common forms of plastic debris ingested by marine organisms, including fish, in different regions and ecosystems:

In their comprehensive study investigating microplastics (MPs) in the gastrointestinal tracts (GITs) of demersal fishes, Chan et al. (2017) focused on several fish species from Hong Kong, namely *Eutrigla cardinalis* (Lacepède, 1802), *Istiblennius japonicus* (Cuvier, 1829), *Rhinogobiops richardsonii* (Bleeker, 1854), *Sillago ovata* (Richardson, 1846), and *Lophius stellatus* (Akazaki, 1983). Their findings provided important insights on the types of microplastics discovered in these fish's GITs. According to Chan et al. (2017), a consistent pattern appeared, with 84% of the microplastics identified in GITs being in the form of fibers and the remaining 16% being fragments. This finding emphasizes the importance of fibers as the principal kind of microplastics consumed by these demersal fish species.

Similarly, Neves et al. (2015) investigated microplastics in fish gastrointestinal tracts (GITs) of many species: Alosa fallax (Lacepède, 1803), Boops boops (Linnaeus, 1758), Brama brama (Bonnaterre, 1788), Dentex macrophthalmus (Bloch & Schneider, 1801), Helicolenus dactylopterus (Delaroche, 1809), Lepidorhombus boscii (Risso, 1810), Lepidorhombus whiffiagonis (Walbaum, 1792), Lophius piscatorius (Linnaeus, 1758), Merluccius merluccius (Linnaeus, 1758), Mullus surmuletus (Linnaeus, 1758), Pagellus acarne (Risso, 1827), Polyprion americanus (Bloch & Schneider, 1801), Raja asterias (Delaroche, 1809), Sardina pilchardus (Walbaum, 1792), Scomber japonicus (Houttuyn, 1782), Scomber scombrus (Linnaeus, 1758), Scyliorhinus canicula (Linnaeus, 1758), Solea solea (Linnaeus, 1758), Torpedo torpedo (Linnaeus, 1758), Trachurus picturatus (Bowdich, 1825), Trachurus trachurus (Linnaeus, 1758), Trichiurus lepturus (Linnaeus, 1758), Trigla lyra (Linnaeus, 1758), Trisopterus luscus (Linnaeus, 1758) and Zeus faber (Linnaeus, 1758) they confirmed that 65.8% of the microplastics discovered in GITs were fibers, with the remaining of 34.2% being fragments.

Previous studies have repeatedly indicated that fibers are the primary type of plastic debris (PD) identified in fish gastrointestinal tracts, in addition to fragments, fibers were found as the

most abundant particles swallowed by fish in research conducted by Ferreira et al. (2018), Sathish et al. (2020), Merga et al. (2020), Hamed et al (2023) and Atamanalp et al. (2022). These data confirme the hypothesis that fibers account for a considerable fraction of plastic waste consumed by marine animals, including fish, in various study areas.

These findings can be linked to the prevalence of fibers in the studied marine environments. Fibers are the most common sort of plastic waste discovered in the environment, as emphasized by Valente et al. (2019). The sources of these fibers may be linked to their ubiquity, with fishing nets and textiles recognized as potential main contributors (Kane and Clare, 2019). The extensive usage and poor disposal of fishing nets and textiles can result in the release of fibers into the marine environment, where marine creatures, especially fish, can consume them. According to Thushari et al. (2017), the deterioration of fishing gear, fish cages, or nylon ropes is a significant source of fiber microplastics. When these things are subjected to environmental conditions, they can degrade into tiny microfibers that eventually infiltrate the marine ecosystem. Furthermore, effluent from laundry has been identified as a substantial source of microfiber pollution (Browne et al., 2011). When synthetic clothes are washed, microscopic threads are released into the wastewater, which eventually makes its way to the ocean. As a result, anthropogenic activities considerably contribute to the prevalence of fiber microplastics in the marine environment. So, their ingestion by marine creatures such as fish and the number of fibers in the environment most certainly leads to their dominance in the gastrointestinal tracts of the fish species studied.

Plastic fragments detected in fish gastrointestinal tracts, according to Tanaka and Takada (2016) may reveal information about their origin or history of deterioration in the environment. However, the particular sources of these fragments were not found during their research. Given the features of the fragments discovered in the fish gastrointestinal tracts, Zhang et al. (2021) proposed that the fragments observed in the fish gastrointestinal tracts could be originated from the breakdown of fishing nets or fishing lines. The exact reasons and mechanisms of fragmentation may differ based on the individual environmental conditions and anthropogenic activity in the researched areas.

Fragments were found primarily in the gastrointestinal tracts of *B. boops*, *S. aurata*, *P. acarne*, and *L. mormyrus*, which are known to exhibit semipelagic to demersal behavior, resulting in interactions with a diverse range of plastic morphotypes as they move through different water layers (Sbrana et al., 2020).

IV.3 Plastic debris colors variability

In both GB1 and GB2 sampling, the dominant color of plastic debris found in the gastrointestinal tracts of fish specimens was blue. Numerous studies have consistently reported that the most abundant color of plastic debris found in fish samples is blue (Bessa et al., 2018; Ferreira et al., 2018; Kasamesiri & Thaimuangphol, 2020; Dantas et al., 2020; Sulistyo et al., 2020; Kasamesiri et al., 2021; Liu et al., 2021; Azizi et al., 2021; Pequeno et al., 2021; Jaafar et al., 2021; Clere et al., 2022; Atamanalp et al., 2022; Kalaiselvan et al., 2022; Pradit et al., 2023;).

Regarding the prevailing color of plastic debris in our study, it is crucial to highlight that interpreting the color can be difficult due to a variety of factors, including bleaching processes in river and marine environments (Stolte et al., 2015). According to Pradit et al. (2023), the preponderance of blue fibers may be region-specific. Blue fibers could also be derived from fishing nets, fishing wires, or other thermoplastic materials that degrade by hydrolysis, photodegradation, or thermo-oxidative degradation, eventually breaking down into smaller pieces and short strands.

It is critical to establish a link between the number of blue particles discovered in fish gastrointestinal tracts and their frequency in marine environments. Understanding the amount of blue particles in marine habitats can provide useful information on the causes and distribution of plastic pollution. We can acquire a more thorough knowledge of the overall impact of plastic pollution on marine ecosystems by evaluating the abundance of blue particles in various maritime areas (Ferreira et al., 2018).

Because PDs resemble natural prey, Barboza et al. (2020) revealed that fish are more likely to mistake them for food. Microplastics are frequently identical in size, shape, and color to plankton and other microscopic creatures that fish consume. Because of their similar appearance, fish may mistake microplastics for food in their native environment, leading to inadvertent consumption.

After blue, black was found to be the second most prevalent color of microplastics in GB2. Similarly, black microplastics were identified in significant concentrations in some GB1 species. Primus and Azman (2022) reported a similar order of abundance for microplastic colors in fish samples in their investigation done in the Melayu River, Johor. Blue microplastics were found to be the most common, followed by black, red, and yellow microplastics.

It is possible that the abundance of black microplastics found in fish samples, as well as in other studies, could be attributed to photodegradation. Sunlight and UV radiation can degrade

the molecular structure of polymers, causing color changes. Photodegradation is a process that can cause plastics to fade or discolor (Nelms et al., 2018).

In the majority of the results obtained from the secondary sampling sites, the dominant color of plastic particles was black. Color identification of plastic particles during visual inspection might be error-prone at times, especially when dealing with microscopic particles. Furthermore, the quality of the binocular microscopes used for investigation can alter color perception. As a result, blue particles may seem dark in some cases, potentially leading to misidentification. Several researchers have chosen to blend these two colors in their investigations. By doing so, they hope to reduce the possibility of color identification errors and provide a more thorough picture of the prevalence of plastic particles in various color groups (Nel & Froneman, 2015; Lots et al., 2017;).

IV.4 Plastic debris classification by size

The retrieved marine debris in the first sampling (GB1) was primarily composed of microplastics, accounting 94.58% of the total collected debris. Mesoplastics, on the other hand, made only a small percentage of the total collected debris. Interestingly, all fish investigated in this study consumed more microplastics than mesoplastics, which is consistent with prior research findings (Romeo et al., 2015; Murphy et al., 2017; Jabeen et al., 2017). The presence of microplastic particles in the gastrointestinal systems of several fish species. Maybe attributed to the ease with which marine species can consume them due to their small size, as emphasized in the study conducted by Neves et al. (2015).

IV.5 Microfibers contaminants

The new topic of "microfibers" research has gotten a lot of attention, and various studies have concentrated on comprehending these anthropogenic particles, which include not only plastic debris but also other elements. Microfibers are distinguished by their small size (usually less than 5mm) and a distinct morphotypes resembling fibers. Because of their possible environmental implications and extensive dispersion in diverse ecosystems, these particles have piqued the curiosity of researchers.

Microfiber sources, distribution, and ecological effects in many environmental situations have been studied by many researchers. They revealed that microfibers can come from a variety of sources, such as synthetic textiles, industrial processes, and domestic items. Because of their small size, they are especially vulnerable to ingestion by marine species, which could result in

bioaccumulation and negative consequences on wildlife and ecosystem health (Volgare et al., 2022; Santonicola et al., 2023; Kim et al., 2023; Angulo-Olmos et al., 2023).

IV.6 Plastic debris occurrence and fish sex

In our investigations, we observed that female fish ingested somewhat more plastic particles than males and those of unknown gender. No significant difference (p>0.05) was observed between these groups. This shows that ingestion of plastic trash is pretty equal across genders in the fish populations studied. Our results corroborate with those reported by Frank et al. (2022).

Interestingly, Sbrana et al. (2020) discovered that male of *B. boops* taken from Italian shores swallowed more plastic particles than females. Such disparities in results could be due to a variety of variables, including differences in environmental circumstances, feeding patterns, and the availability of plastic particles in both pelagic and benthic environments.

It is probable that the prevalence of plastic contaminants in the marine environment is generally homogeneous, giving all genders of fish an equal opportunity to encounter and absorb these particles. Ingestion of plastic waste, regardless of gender, is a cause for concern because it can harm marine organisms and contribute to the wider problem of plastic pollution in aquatic ecosystems. More research is needed to fully understand the elements that influence plastic ingestion patterns in fish populations and to design effective conservation strategies to reduce the effects of plastic pollution.

IV.7 Plastic debris-Length, weight and condition parameters of fish relationship

Numerous investigations on the association between fish length and microplastic buildup in the gastrointestinal tract (GIT) have shown a variety of results. McNeish et al. (2018) found no association between fish length and microplastic consumption in some of these investigations. However, other research has shown a strong positive association between fish length and microplastic deposition in the GIT (Peters et al., 2016; McNeish et al., 2018; Pegado et al., 2021).

Interestingly, Bessa et al. (2019) discovered a substantial negative connection between fish length and microplastic consumption. These disparities could be related to differences in fish species, environmental circumstances, and microplastic sources in the various study regions.

Pazos et al. (2017) evaluated the relation between the quantity of microplastics (MP) and numerous parameters such as fish length, weight, and feeding patterns in their study. Their analyses found that the quantity of MP had no significant link with these variables. In other

words, the amount of microplastics consumed by the fish in the examined population did not appear to be influenced by their size and weight, as well as their feeding patterns.

In our study (GB2), we focused on the condition factor K rather than the fish weight and length correlation with microplastic (MP) ingestion. We observed no significant relationship between the condition factor K and the number of microplastics ingested by both species. This suggests that the condition factor, which represents the fish's overall health and well-being, did not appear to influence microplastic ingestion, Kerubo et al. (2021) concluded that fish size or age have no significant influence on microplastic ingestion. Similarly, Roch et al. (2020) state that the mechanisms underlying how these microplastic particles are ingested by fish are largely unknown. Despite numerous studies investigating the relationship between fish size, age, and microplastic ingestion, the precise reasons and mechanisms underlying this phenomenon remain unknown. More research is needed to gain a thorough understanding of the factors influencing microplastic ingestion in fish species.

IV.8 Effects of seasonal variation

Plastic debris in the gastrointestinal tracts of contaminated fish specimens increased significantly during the wet season compared to the dry season (p<0.01). With 2092 plastic particles, the wet season had a higher contamination level than the dry season, which had only 129 plastic particles. Our study's findings align with those of Dantas et al. (2012), as they reported similar results. This difference in contamination can be attributed to a variety of factors during the wet season, including increased precipitation and strong winds (63.7 mm in our study), both of which promote the movement and degradation of plastic debris, allowing it to enter the marine ecosystem (Cheung et al., 2016).

Rivers are also important sources of plastic contamination, with many microplastics and mesoplastics potentially entering the sea via several rivers related to the Gulf of Bejaia as mentioned by Rowley et al. (2020) and Xu et al. (2020). These rivers' seasonal activity may result in a greater input of marine trash into coastal waters, making plastic particles readily available to fish species during the wet season.

In our study (GB1), it was observed that samples collected during the wet season exhibited a higher diversity of colors and morphotypes compared to samples from the dry season. Several variables could lead to seasonal fluctuations in the composition of plastic waste. For starters, increased runoff and river flow during the wet season can introduce a bigger load of plastic

trash from inland sources, resulting in a wider range of plastic types and colors into the marine environment. Furthermore, during the wet season, storms and high winds may break down big plastic items into smaller fragments, increasing the number of different-sized particles and colors.

IV.9 Density of plastic debris ingestion

Many researchers in the field of plastic pollution and marine biology have opted to calculate the number of plastic particles per unit weight of GIT to analyze the buildup of plastic debris in the gastrointestinal tracts (GIT) of fish. This method enables a more uniform and quantitative measurement of fish plastic ingestion. Notably, Yuan et al. (2021), Mistri et al. (2022), and Rodrigues et al. (2023) used this method to assess microplastic contamination in fish samples. Other researchers in the field of microplastics investigation have chosen to assess plastic debris ingestion in fish by calculating the number of plastic particles per individual fish rather than per unit weight of gastrointestinal tracts (GIT) (Zheng et al., 2019; Abidli et al., 2021; Hamed et al., 2023).

According to our findings, *Boops boops* had a greater density of plastic debris ingestion in the gastrointestinal tract per unit weight, averaging 21.21 plastic particles per gram of GIT. Similarly, *Sardina pilchardus* ingested a significant amount of plastic trash, averaging 18.38 plastic particles per gram of GIT. several findings show that microplastics are abundant in the digestive tracts of several fish species. El-Sayed et al. (2022) reported a higher density of microplastics in the gastrointestinal tracts of fish in their investigation. MP were observed in 91.8% of the fish, with an average density of 11.7 pieces per fish, similar to places with high pollution levels in the southeastern Mediterranean Sea. *Sparus aurata*, in particular, had a substantially higher average concentration of MP, with 38.3 items per fish. These findings highlight the worrying prevalence of microplastic pollution in the marine ecosystem, particularly in specific fish species' digestive systems.

Boops boops, also known as the bogue fish, has been extensively identified and used as a bio-indicator of worldwide plastic pollution. Many scientists have chosen this species to study the presence and impact of plastic litter in maritime habitats (Dizer et al., 2001; Garcia-Garin et al., 2019; Tsangaris et al., 2020; Sbrana et al., 2020; Capó et al., 2022). The highly mobile nature of *B. boops* is one of the reasons it was chosen as a bio-indicator. This species is noted for its broad travels, frequently migrating between oceans and rivers, making it an ideal candidate for studying plastic contamination in various aquatic ecosystems. Furthermore, *B.*

boops feeds both in the water column and near the seafloor, providing insight into the probable sources and routes of plastic ingestion (Garcia-Garin et al., 2020).

IV.10 Plastic debris and human health

While no direct evidence has been found to suggest that eating fish contaminated with plastic debris (PD) directly affects human health, researchers such as Barboza et al. (2018) have highlighted the potential implications of marine microplastic debris on human food security, safety, and health.

To yet, no research has directly shown that eating fish polluted with plastic debris causes negative health impacts in people. It is, nevertheless, critical to recognize that plastic contamination in marine settings can have far-reaching implications.

The Mediterranean is a closed and polluted sea, ranking at the forefront worldwide in terms of microplastic pollution (Ferrari et al., 2020). In coastal areas, demersal species ingest significantly more plastic debris than other species (Murphy et al., 2017). Metallic contaminants can adsorb onto microplastic polymers (Brennecke et al., 2016; Aissioui et al., 2021a, b, c; Aissioui, 2022), which tend to sink into the water column (Lagarde et al., 2016). These polymers can penetrate into the internal organs of organisms (especially benthic ones), carrying with them the contaminants they have absorbed (e.g., Pb and Hg). The effects of metallic contaminants, mainly mercury (Hg), lead (Pb), and cadmium (Cd), on human health have long been well-known (Aissioui, 2022).

Microplastics are known for their deleterious effects on ecosystems. Many researchers report that microplastics present in wastewater can also transport pathogens and even contribute to increasing bacterial resistance to antibiotics (Zhang et al. 2020).

Wastewater treatment plants not only serve as a convergence point for numerous microplastics but also for various chemicals and bacteria and other pathogens (Habib et al., 2020).

Experiments conducted by Pham et al., (2021) have clearly demonstrated that bacteria tend to thrive on microplastics. Indeed, these researchers have confirmed the presence of two emerging human pathogens (respiratory infections related to the presence of *Raoultella ornithinolytica* and *Stenotrophomonas maltophilia*).

V. Conclusion

New data on the presence of plastic particles in fish species are reported in the present study for the first time. No previous scientific studies were conducted in our study area. Our findings confirm the presence of anthropogenic particles in Algerian fish species particularly for those having commercial interest.

Our findings provide vital insights into the degree of microplastics (and other plastic debris) impacts in Algerian marine ecosystems.

High levels of plastic particle ingestion were observed in the Algerian eastern coast confirming a significant issue of marine pollution. These findings highlight the urgent need for better waste management techniques and environmental protection measures.

Plastic debris in marine biota, particularly fish, is a clear indicator of the degree of plastic pollution in these considered areas. Plastics appear to be omnipresent and have formed a fundamental component of the marine ecosystem, posing potential risks to both marine life and human health.

Plastic debris detected in fish gastrointestinal tracts (GIT) came in a variety of sizes, morphotypes, and colors. Our research exposed a diverse range of plastic shards, fibers, and particles with varied diameters, sizes, and colors. Plastic waste ranged in size from minuscule microplastics (less than 5mm) to bigger mesoplastics. These anthropogenic particles contained both macroscopic and microscopic constituents, demonstrating how widespread plastic contamination is in marine environments. Plastic debris came in a variety of morphotypes (fibers and fragments). These different morphologies are most likely the result of marine plastic deterioration, weathering, and mechanical breakdown.

Furthermore, the colors of the plastic particles varied, with blue and black being the most common in our samples. In the GITs of the examined fish species, other types, like red, yellow, and white, were also found among the total variety of plastic debris. The variety of plastic waste sizes and morphotypes demonstrates the complexities of plastic pollution in marine ecosystems.

Our investigations reveal no significant relationship between biological parameters and consumed plastic debris by fishes. The sample size including a larger number of examined

fish specimens maybe bring significant results concerning the relationship between plastic debris and biological parameters of fish specimens.

Our results confirm the major influence of seasonal variation on the type and amount of plastic debris. We observed distinct seasonal variations in the types and quantities of plastic particles collected from fish gastrointestinal systems. During the wet season, we noticed a significant increase in the variety and quantity of plastic waste consumed by fish. This observation is consistent with this season's increased precipitation and stronger winds, which may enhance the mobility and breakdown of plastic particles in the marine ecosystem. Furthermore, during the wet season, the inflow of plastic trash from rivers related to the Gulf of Bejaia may contribute to elevated plastic pollution levels in coastal waterways. In the other hand, the dry season had decreased amounts of plastic trash ingestion by fish. The calmer weather conditions and less river input during this season may lead to a decrease in the availability of plastic particles in the marine environment. Our findings show clearly that seasonal variations influence the abundance and fluctuation of plastic contamination in marine ecosystems.

Developing successful strategies is primordial for minimizing marine pollution and improving waste management practices by monitoring seasonal oscillations in plastic trash.

In our study we assessed the ingestion of plastic debris by pelagic, benthopelagic and demersal fish species.

Our results confirm that the plastic particles were found in the gastrointestinal systems of fish from all of these various habitats, showing the ubiquitous presence of plastic pollution along the column water and benthic system. Among the examined fish species, *Boops boops* is considered as a potential bio-indicator, making it an important target for monitoring worldwide environmental degradation, especially plastic contamination. The presence of considerable plastic ingestion in *B. boops* highlights the severity of plastic contamination in maritime ecosystems.

Our research sheds light on the impact of plastic waste on fish populations from varied habitats, emphasizing the critical need for conservation and management measures to reduce plastic pollution in marine settings. Wastewater treatment plants play a vital role in mitigating plastics pollution by employing various mechanisms to intercept and manage plastic waste within the wastewater stream. These facilities are equipped with sophisticated filtration systems designed to capture solid materials, including plastics, effectively preventing their

discharge into natural water bodies. Through processes like sedimentation and separation, suspended plastic particles are removed from the water, subsequently extracted as sludge to prevent environmental contamination. Advanced treatment technologies, such as membrane filtration and activated carbon adsorption, further enhance the removal of microplastics and smaller plastic particles from wastewater effluents. Additionally, wastewater treatment plants implement measures to prevent the loss of plastic pellets, known as nurdles, during production processes, minimizing their release into aquatic environments. Moreover, these facilities play a crucial role in public education and awareness campaigns, promoting responsible waste management practices to reduce the overall input of plastics into the wastewater system. In sum, wastewater treatment plants serve as frontline defenses against plastics pollution, safeguarding aquatic ecosystems and contributing to global efforts to combat plastic contamination.

Marine pollution control methods and ensure the health and sustainability of marine ecosystems. Plastic debris poses risks to human health through chemical exposure, ingestion of microplastics, bioaccumulation of pollutants, microbial contamination, and respiratory issues from burning. Chemical additives in plastics, along with accumulated pollutants, can cause endocrine disruption, inflammation, and developmental disorders. Ingested microplastics may lead to gastrointestinal and immune system issues, while microbial contamination on plastics increases the risk of infectious diseases. Burning plastic releases toxic fumes, exacerbating respiratory conditions. Addressing plastic pollution is crucial to safeguard human health.

V.1 Perspectives

- Investigate potential ways for minimizing the impact of plastic waste on marine ecosystems. We will also look at the broader ramifications of plastic pollution and its possible effects on human health and food security;
- Promote sustainable practices for a cleaner and healthier marine environment;
- -Additional research should focus on the western Algerian coasts. Investigating the prevalence and abundance of plastic debris in marine species throughout the western coast will offer useful information on the degree of contamination and potential biological impacts. Furthermore, examining the distribution of plastic particles in coastal sediments and water samples may help identify potential origins and pathways of plastic contamination;

- Investigations should prioritize the examination of plastic debris polymers found in marine environments (FTIR, Raman Spectroscopy, Py-GC-MS). Understanding the specific types of plastic polymers present in marine organisms and coastal ecosystems is crucial for assessing their environmental fate, persistence, and potential toxicity;
- To understand the association between biological parameters and plastic debris consumption a large sample size with equal gender representation is required;
- Plastic debris ingestion by riverine fish species and other biota should be prioritized in this region's research. Rivers are important entry points for plastic pollution into marine ecosystems, and determining the degree of plastic ingestion by freshwater animals is critical for understanding the total impact of plastic pollution on aquatic environments;
- -The abundance and consequences of plastic litter on riverine biota can provide important insights into the sources and distribution of microplastics, as well as their potential repercussions on both freshwater and marine ecosystems. Researchers can contribute to a more comprehensive knowledge of plastic pollution and its implications for the entire coastal and marine environment in the region by focusing on riverine habitats;
- Future researches should broaden its scope to encompass all sorts of anthropogenic waste, not simply plastics. While plastic debris is a major environmental concern, other types of human-made garbage, such as metals, glass, rubber, and fibers, can also endanger marine ecosystems. Investigating the prevalence and impacts of diverse anthropogenic debris types on aquatic animals and ecosystems will provide a more comprehensive picture of the region's overall pollution burden. Taking into account the interplay of various types of debris and their combined effects on biota can lead to more successful management tactics and conservation initiatives. By broadening the scope of research to include all anthropogenic debris, decision-makers and stakeholders will be better informed in their efforts to combat marine pollution and safeguard aquatic biodiversity;
- We strongly encourage future investigations to be carried out throughout both the dry and wet seasons. Investigating plastic waste ingestion and its effects on aquatic animals across seasons is critical for understanding the entire scope of marine pollution.;
- -Researchers can get useful insights into how environmental parameters such as precipitation, river discharge, and hydrodynamics influence the availability and ingestion of plastic trash by fish and other biota by studying both dry and wet seasons. Furthermore, studying seasonal patterns of plastic ingestion might help in the development of targeted conservation measures and waste management regulations to reduce the impact of marine pollution on aquatic ecosystems;

- To conclude, exploring the relationship between plastic and heavy metals, as well as plastic and pathogens, offers valuable insights into the potential health risks associated with exposure to these substances. By understanding how plastics can absorb and concentrate heavy metals from the environment, we can better grasp the risks posed to human health when ingested or in contact with food. Similarly, investigating how plastics serve as vectors for the growth and transmission of pathogens highlights the potential for infections and illnesses among consumers. These insights underscore the importance of implementing preventive measures to mitigate these risks and protect public health. Moving forward, continued research and regulatory efforts are essential to address these issues effectively and ensure the safety of consumers.

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APPENDICES

Appendices

${\bf A01.} \ \ Biological\ parameters\ and\ plastic\ debris\ characteristics}$ $(GB1\ and\ GB2\ species)$

				Sard	ina pilchardus		
ind	Tw	Ew	Tl	Sl		FO%	43.33%
Mean	25.43	22.37	12.49	10.45		PDs length	0.2
SD	11.5	9.6	3.9	2.8		SD	0.18
				Sar	dinella aurita		
ind	Tw	Ew	Tl	Sl		FO%	58.33%
Mean	26.95	20.08	16.43	14.45		PDs length	0.3
SD	23.1	21.65	4.1	2.99		SD	0.15
				В	oops boops		
ind	Tw	Ew	Tl	Sl		FO%	55.0%
Mean	56.91	51.08	17.83	14.56		PDs length	0.1
SD	32.4	21.65	2.7	1.99		SD	0.13
				Track	urus trachurus	S	
ind	Tw	Ew	Tl	Sl		FO%	63.3%
Mean	44.08	38.68	16.84	/		PDs length	0.3
SD	12.31	6.75	5,1	/		SD	0.18
				Pa	gelus acarne		
ind	Tw	Ew	Tl	Sl		FO%	73.33%
Mean	48.75	46.8	14.61	/		PDs length	0.4
SD	18.4	8.90	3.6	/		SD	0.34
				Sp	arus aurata		
ind	Tw	Ew	Tl	Sl		FO%	100%
Mean	304.3	280.8	25.67	22.5		PDs length	0.36
SD	11.2	8.90	2.6	1.87		SD	0.13
	•			Sardin	ella aurita GB	32	

Mean 36.12 32.3 16.11 12.45 PDs length 0.21 SD 12 8.8 1.79 1.8 SD 0.18	ind	Tw	Ew	Tl	Sl	FO%	72.5%
SD 12 8.8 1.79 1.8 SD 0.18	Mean	36.12	32.3	16.11	12.45	PDs length	0.21
	SD	12	8.8	1.79	1.8	SD	0.18

Lithognathus mormyrus GB2

ind	Tw	Ew	Tl	Sl
Mean	157.82	100.3	22.63	/
SD	37.96	20.8	1.69	/

FO%	83.3%
PDs length	0.31
SD	0. 2

A02. Biological parameters of samples collected from the secondary areas (Skikda, El Kala and Annaba)

Collo (West of Skikda) 2023

Species	N	Tw	Tl	F:M:U
S. aurita	15	29,86 (± 9,48)	15,47 (± 1,66)	08:05:02
S. pilchardus	14	33,92 (± 5,45)	$16,23 \ (\pm 0,28)$	09:03:02
T. trachrus	15	25,8 (± 6,94)	14,68 (± 1,20)	04:06:05
B. boops	15	24,66 (± 12,77)	13 ,32 (± 1,67)	02:07:06
total	59			23 :21 :15

Center and East (Skikda) 2023

Species	N	Tw	Tl	F:M:U
S. aurita	15	34.86	16.44	11:3:1
S. pilchardus	15	30.92	15.5	7:6:1
T. trachrus	15	43.8	17.25	7:5:3
B. boops	12	79.45	20.25	5:5:2
Total	57			30:19:8

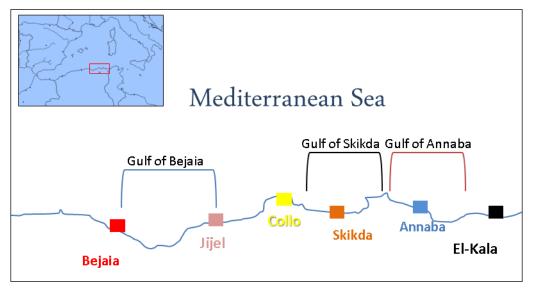
Annaba 2022

Species	N	Tw	Tl	F:M:U
S. aurita	15	34.43	15.66	2:2:11
S. pilchardus	12	35.26	16.05	10:01:01
T. trachrus	15	66.55	19.50	01:11:03
B. boops	12	60.43	17.78	05:07:00
Total	54			18:21:15

El Kala 2022

Species	N	Tw	TI	F:M:U
S. Aurita	15	56.40 ± 13.9	19.58± 1.5	7:4:4
S. Pilchardus	9	35.26 ± 5.7	16.69± 0.6	8:0:1
T. Trachrus	10	66.55 ± 18.5	19.71± 1.7	2:8:0
B. Boops	9	60.43 ± 15.5	19.63 ± 1.3	7:2:0
Total	43			24:14:5

A03. Location of all study area



SCIENTIFIC PRODUCTION

1. Scientific papers

1.1 "A" Class

Zeghdani, Z., Aissa, Z. A., Kherfallah, L., Bouchema, N., Gherbi, R., & Ramdane, Z. (2023). Microplastic ingestion in Sardinella aurita Valenciennes, 1847 and Lithognathus mormyrus (Linnaeus, 1758) along the Gulf of Bejaia, Algeria. Indian J. Fish, 70(2), 95-100.

Belkoum, Noureddine, **Zouhir Zeghdani**, and Horiya Bouali. "CONTRIBUTION TO THE HYDROCHEMICAL STUDY OF GROUNDWATER IN THE PLAIN OF MELLAGOU-BOUHMAMA (NORTHEAST ALGERIA)." CARPATHIAN JOURNAL OF EARTH AND ENVIRONMENTAL SCIENCES 19.1 (2024): 115-123.

1.2 "B" Class

Zeghdani, Z., Mehdioui, S., Mehdioui, Y., Gherbi, R., & Ramdane, Z. (2023). Plastic Particles in the Gastrointestinal Tract of Some Commercial Fish Species Inhabiting in the Gulf of Bejaia, Algeria. Jordan Journal of Biological Sciences, 16(2).

2. Seminars and colloques

ZEGHDANI Z. et RAMDANE Z. (LZA, Univ. Bejaia): First record of microplastics in digestive tract ingested by fishes in the Gulf of Bejaia, Algeria. 5ÈMES JOURNÉES D'ETUDE NATIONALE SUR LA ZOOLOGIE APPLIQUÉE ET L'ECOPHYSIOLOGIE ANIMALE (2018).

ZEGHDANI Z.; RAMDANE Z. & GHERBI R. (L.Z.A.U. Bejaia): Microplastics ingestion by type in three species of fish from the Gulf of Bejaia (Algeria). 6ÈMES JOURNÉES D'ETUDE NATIONALE SUR LA ZOOLOGIE APPLIQUÉE ET L'ECOPHYSIOLOGIE ANIMALE (2019).

ZEGHDANI Z.; BELKOUM N. & YETTOUS S. (L.Z.A.U. Bejaia): La technologie des micro-organismes efficaces, quel avenir ? (exemple de valorisation des déchets oléicoles). Univ. Khenchela (GBFS) (2023).

BELKOUM N.; MELLAL H.; AROUA K.; LAICHE A.; **ZEGHDANI Z.**: ETUDE HYDROGEOCHIMIQUE DES EAUX SOUTERRAINES DES HAUTS PLAINS ALGERIENS (EST DE BATNA) Univ. Khenchela (GBFS) (2023).

Jordan Journal of Biological Sciences

Plastic Particles in the Gastrointestinal Tract of Some Commercial Fish Species Inhabiting in the Gulf of Bejaia, Algeria

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Abstract

In the present study we report for the first time, the presence of plastic particles on the gastrointestinal tracts of the examined commercial fish species: $Sardinella\ aurita\ (n=60)$, $Sardina\ pilchardus\ (n=60)$, $Pagellus\ acanne\ (n=45)$, $Trachurus\ trachurus\ (n=38)$, $Boops\ boops\ (n=40)$, $Sparus\ aurata\ (n=6)$. The sampled fish species (n=249) inhabiting in demersal, benthopelagic and pelagic marine ecosystems (Gulf of Bejaia) were examined to test eventual differences in micro and macro plastic particles. Overall, results showed that 58.63% of gastrointestinal tracts were contained plastic particles. An average 8.45 ± 14.69 items per fish were recorded. The most common morphotype particles were fiber (85%) whereas dominant colors of plastic debris were the blue and the red among three types of particle colors. Our results shows that are no significant differences between females and males specimens in terms of ingested plastic particles. The highest abundance of plastic particles in fish gastrointestinal tracts was recorded in wet season.

Keywords: Plastic particles, Microplastics, Commercial fish species, Gulf of Bejaia.

1. Introduction

Plastics are the most widely used material (Millet *et al.*, 2018), inexpensive, lightweight, strong, durable, corrosion-resistant materials, with high thermal and electrical insulation properties (Thompson *et al.*, 2009), and this is why this material persists in the environment (Teuten *et al.*, 2009).

Sunlight, wind, and waves are the mean natural factors transforming plastics into small particles (Matjasic *et al.*, 2021). When the size of this particles is less than 5 mm they are called microplastics (Hartmann *et al.*, 2019), some researchers use 0.5 or 1 mm as a maximum size for microplastics (Andrady, 2011; Cole *et al.*, 2011).

Microplastic particles can have either been manufactured purposely with that size (primary microplastic) or proceeding from fragmentation by different physical, chemical, and biological degradation (secondary microplastic) (Wright *et al.*, 2013; Rainieri *et al.*, 2018).

Plastics are emergent pollutants and have been found almost in every part of the planet (Mishra *et al.*, 2021) and their risk (macroplastics and microplastics) on the marine environment have been addressed by reporters as an emerging global problem that detriments marine organisms (Derraik, 2002). Regarding their size and form (fibers, fragments, etc.), this particle can be unfortunately ingested by biota (Galgani *et al.*, 2010; Andrady, 2011).

For this reasons many scientific works were conducted on microplastic ingestion by fish species (Takarina et al., 2022; Piyawardhana et al., 2022; Thiele et al., 2021; Yin et al., 2019), especially, in the Mediterranean Sea (Bellas et al., 2016; Guven et al., 2017; Pennino et al., 2020), where researchers reported the occurrence of microplastic particles in gastrointestinal tract of fish (Jabeen et al., 2017; Jaafar et al., 2021; Parvin et al., 2021; Akhter and Panhwar, 2022).

The Mediterranean Sea recently classed as the most impacted regions of the world by plastic debris, demersal species from coastal zones ingest more plastic particles than other species (Murphy *et al.*, 2017), polymers can act as vector and adsorb heavy metals from the water column (Holmes *et al.*, 2014; Boucher *et al.*, 2016) which have a tendency to flow (Lagarde *et al.*, 2016).

Low-density microplastics are found in surface waters (Thompson *et al.*, 2004), they held some heavy metals (Brennecke *et al.*, 2016), then ingested by several species of zooplankton (Cole *et al.*, 2013) than by larvae and adults of fish (Browne *et al.*, 2013; Lusher *et al.*, 2013; Rochman *et al.*, 2013).

Researchers have reported that microplastics can enter the human body through the food chain and human exposure to microplastics could lead to harmful health (Couture, 2017), microplastics enriched bacterial pathogens (Junaid *et al.*, 2022) and can serve as carriers of antibiotic-resistant bacteria (Pham *et al.*, 2021).

The implications of the complex microplastics-heavy metals-bacterial pathogens to the human health are

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significant (Wang et al., 2021). In effect, this complex seems to amplify the health risks for humans and animal.

In Algerian coasts, no studies were conducted on plastic particles containing in the gastrointestinal tracts of commercial fish species. The aim of the present study is to examine some commercial fish species (from Algerian coasts) for the presence of plastic debris (microplastics and macroplastics) in the gastrointestinal tract, to characterize the isolated plastic debris (form, color, size, etc.), and finally to analyze the variation of ingested plastic particles according to fish parameters, fish habitat and seasonal variation. These results maybe give us a clear idea on the presence of these toxic pollutants in the Algerian marine ecosystems, and the health risks that can induce to consumers of fish.

2. Materials and methods

A total of 249 specimens of fish belonging to six species (with high commercial value) were sampled in the port of Bejaia, Algeria (Figure.1) or from fish markets.



Figure 1. Location of the study area (Gulf of Bejaia)

In our sampling, we focused on fish species occupying various habitats (demersal, benthopelagic and pelagic) (Table.1).

In the laboratory, we worked in sterile space, in order to reduce the risk of air contamination using clean materials (disinfect with ethanol 70%), after identification of sampled fish specimens, total length (cm) and total weight (g) were measured for each specimen.

Table 1. Habitat and Biological parameters of examined fish species in the study (n = 249).

Fish species	(n)	Habitat	Mean weight (g) \pm SD	Mean length (cm) \pm SD
Sardinella aurita	60	Pelagic	26.95 ± 23.1	16.43 ± 4.1
Sardina pilchardus	60	Pelagic	25.43 ± 11.5	12.49 ± 3.9
Pagellus acame	45	Benthopelagic	48.75 ± 18.4	14.61 ± 3.6
Trachurus trachurus	38	Pelagic	44.08 ± 12.31	16.84 ± 5.1
Boops boops	40	Demersal	56.91 ± 32.4	17.83 ± 2.7
Sparus aurata	6	Demersal	304.3 ± 11.2	25.67 ± 2.6

The protocol adapted by Baalkhuyur et al. (2018) was applied regarding its simplicity and feasibility. The different stapes devoted to detect the plastic particles in the examined fish individuals are: -Fish specimens were dissected and sexed (visual observations). After that, the gastrointestinal tract (GIT) was removed (from esophagus to the opening) and weighted;-The specimen's gut were rinsed with distilled water, and then carefully moved into cleaned Petri dishes. For each sample a code number was given; -After removal, the samples of GIT were placed in an oven for 1 hour at 60 °C; -To increase the efficacy of the extraction of plastic from the tissue, a digestion protocol was adapted from the procedure given by Cole et al. (2014). NaOH (1 M and 10 M), has been successfully applied to remove biogenic material; -30ml of a 1 M NaOH solution were added to reenforce the digestion (remove the remaining biological material and nondigestible residue) (Cole et al., 2014; Catarino et al., 2017); -Samples were manually shaken intermittently during the incubation period in order to facilitate complete digestion, and after the incubation, samples were inspected under binocular stereoscope visually (Hidalgo-Ruz et al., 2012; Free et al., 2014).

After identification, the plastic particles were counted, photographed and measured using the software "imagej" (ver: 1.4.3; https://imagej.nih.gov/), color and shape were also determined for each plastic particle (Jabeen et al.,

The occurrence of Frequency (FO %) of the collected microplastics in the digestive tracts was calculated using the following formula:

FO (%) = $(Ni/N) \times 100$, where

FO% = frequency of occurrence of plastic particles;

Ni = number of gastrointestinal tracts that contained plastic particles;

N = total number of gastrointestinal tracts examined.

Data were analyzed using SPSS 14.0 software and EXCEL 2010. Independent t-test was performed to determine if there are differences on the abundance of plastic particles between wet season and dry season, and between males and females (95% confidence level). A significant difference in the abundance of plastics among individuals was tested applying one-way ANOVA. The Tukey test's HSD test was set at * = p < 0.05 and ** = p <0.01 values.

3. Results

3.1. Biological parameters of fishes

A total of 249 specimens attached to 6 species were analyzed (Table.1). The body weight of fish specimens varies from 10.30 g to 328.20 g (31.74 g \pm 20.42), and the total length varies from 10.90 cm to 24.20 cm (15.11 cm \pm

3.2. Intensity of plastic consumed by fishes

Evidence of plastic particles was appeared to be in 146 specimens from the total 249 (58.63%) examined in this study. These potential contaminants were found in the 6 species (Table.2), and therefore in pelagic, benthopelagic, and demersal species. The average number of particles

ingested was $(8.45 \pm 14.69 \text{ particles per specimen})$ ranging from 0 to 69 particles per gastrointestinal tract, the highest

average was recorded in *Boops boops* L. (21.03 \pm 23.49 particles per specimen).

Table 2. Frequency of plastic particles ingestion by fish species.

Fish species	n of examined GIT	n of contaminated GIT	Rate (%)
Sardinella aurita	60	35	58.33
Sardina pilchardus	60	26	43.33
Pagellus acarne	45	33	73.32
Trachurus trachurus	38	24	63.16
Boops boops	40	22	55
Sparus aurata	6	6	100

GIT: Gastrointestinal Tract

Collected microplastics and mesoplastics from the examined fish specimens (2347 particles) recovered various morphotypes and colors of plastic particles.

3.3. Morphotype, size and color of the plastic particles retrieved

Two morphotypes of plastic particles were extracted (Figure.2), where the most observed were fibers with 85% (p<0.01). In Sardinella aurita, Sardina pilchardus and Trachurus trachurus fibers reached 100%.

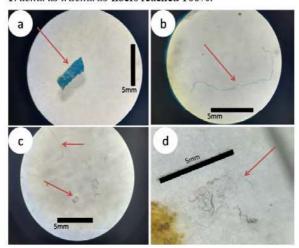


Figure 2. The morphotypes (a) fragment ingested by examined *Sparus aurata*; (b) mesoplastic fiber; (c) transparent fragments and a red fiber observed in the gut of *Boops boops*; (d) various fibers ingested by examined *Sardina pilchardus* (see red arrows).

Fragment morphotypes was exclusively extracted from *Boops boops, Sparus aurata* and *Pagellus acarne* with 15% of the total number of plastics (Figure.3a). The size of plastic particles varied from 0.1 mm to 5 mm for microplastics and the highest size of a mesoplastic was 18 mm, microplastics were the most observed with 94.58% of the total number of particles (p<0.01) (Figure.3b).

Microplastics lower than 2 mm (size <2mm) represent 71% of the number of analyzed plastics.

In the study region, the extracted plastics represent numerous colors (especially red, blue and transparent). The dominant color was the blue plastic particles with 56.4% followed by the red color with 41.3%. Transparent and other colors were not highly observed (Figure.3c).

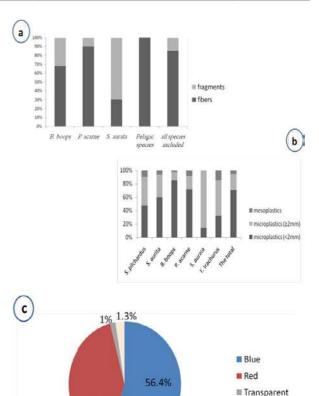


Figure 3. morphotypes (a), size (b) and color (c) of the ingested plastics by the contaminated fish species.

Others

3.4. Occurrence and mean number of collected microplastics according to sex.

From the total of samples, 123 were females, 96 were males and 30 were undetermined individuals. The percentage of females with infected gastrointestinal tract reached 74.1% followed by males (FO reached 21%). When comparing the number of ingested plastics particles we found that females ingest slightly more than males (1055 and 1022 particles respectively) and for undetermined gender, a number of 270 particles was noted. According to sex, the mean number of plastic particles (Figure.4) shows no significant differences between the 3 genders (ANOVA, Tukey HSD p-value> 0.05).

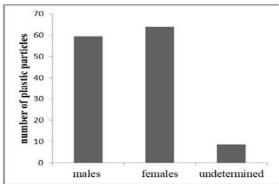


Figure 4. mean number of plastic particles ingested by sex (global examined fish species).

3.5. seasonal variation and plastic ingestion

Contaminated fish specimens by plastic particles were high in the wet season (Figure.5a), with a mean of 26.71 ± 4.2 individuals contain plastic. In the dry season the mean number of contaminated fish specimens was lower (8.71 \pm 1.7). A one-way ANOVA test between the two seasons (wet and dry) confirms that the collected plastic debris was significantly higher in wet season than the dry season (p<0.01).

Our results indicate a significant differences in the mean number of ingested plastics among the two seasons (one-way ANOVA; F=6.99; p<0.05). The total number of plastic particles ingested by fish in wet season was 2092 particles (418.4 per species), in contrast, the dry season counted only 129 (25.8 per species) (Figure.5b).

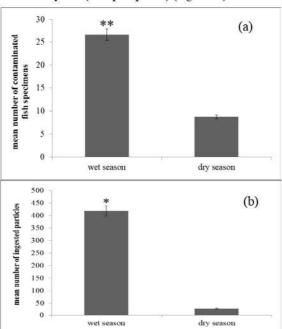


Figure 5 the mean number of contamined fishes (a) and the mean number of ingested plastic particles (b) in the wet and the dry season. **: p<0.01; *: p<0.05

4. Discussion

In the present study, fishes from three regimes e.g. pelagic, benthopleagic and demersal were gathered to investigate the presence of plastic particles in the gastrointestinal tract. Our results revealed higher abundance of these potential contaminants in all examined

fishes especially in demersal fish species. These results confirm that plastic particles are present throughout the water column, and probably, a part of these plastic particles make complex with other organic or chemical (metallic contaminants) enhancing sedimentation and their availability for benthopelagic and demersal fishes in deeper marine water. Lagarde et al. (2016) reported that these plastic particles adsorb heavy metals from the water column, which have a tendency to flow. In this context, a recent study conducted by Akhter and Panhwar (2022) revealed the presence of microplastic materials in crabs and fishes from pelagic and mesopelagic area in Pakistan. The authors stated that microplastics materials are present in pelagic regime than demersal and validate that low weight of plastic particles floats on ocean surfaces.

All examined fish species (Sardinella aurita, Sardina pilchardus, Pagellus acarne, Trachurust rachurus, Boops boops and Sparusaurata) consumed plastic particles. Our results showed that more than a half of the sampled fish specimens contain marine plastics. The recorded frequency of occurrence reaches 58.63%. Ours results corroborate with the previous studies were high value were reported. For example, Ferreira et al. (2016) reported in Cynoscion acoupa from the Goiana Estuary 51%, in the Northwest Atlantic, Wieczorek et al. (2018) assessed the presence of microplastics in the stomachs of nearly three out of every four mesopelagic fish. Karbalaei et al. (2019) highlighted that 9 of 11 examined fish species from Malaysia (Megalaspis cordyla, Epinephelus coioides, Rastrelliger kanagurta, Thunnus tonggol, Eleutheronema tridactylum, Clarias gariepinus, Colossoma macropomum, Nemipterus bipunctatus and Ctenopharyngodon idella) contaminated.

Neves et al. (2015) measured microplastics in stomach contents of 17 fish species from Portuguese coast (comprising B.boops, P.acarne, T.trachurus and S.pilchardus). In this study region, the authors reported relatively low rate (19.8% of 263 specimens) of fish contaminated with plastic particles regarding our results.

In addition, the mean number of plastic items ingested by specimens of *B. boops* was higher (21.03±23.49) than those reported by Nadal *et al.* (2016) in the same fish species around the Balearic Islands (3.75±0.25microplastic items per fish). Many previous studies (Romeo *et al.*, 2015; Anastasopoulou *et al.*, 2013) have related the rate of marine debris or particles ingestion by fish to the variability of feeding habits.

Only two morphotypes of plastics were recorded (fibers with 85% and fragments with 15%) in the sampled specimens of all examined species. Our results corroborate with those of Neves et al. (2015), Ferreira et al. (2018), Sathish et al. (2020) and Merga et al. (2020) who reported clearly, the predominance of fibers as the most common particles ingested by fishes. These results maybe explained by the abundance of fibers in their ecosystems. After Valente et al. (2019) fibers are the most common in the environment. Fishing nets and textiles are possible sources of most fibers (Kane and Clare, 2019). They are similar in shape to the fish feed, which promote the ingestion by specimens (Walkinshaw et al., 2020).

Fragments were extracted only from the gastrointestinal tracts of *B. boops*, *S. aurata* and *P. acarne*. *B. boops* which are semipelagic to demersal fishes and *P. acarne* a

benthopelagic species so they contact various plastic morphotypes than pelagic fishes when they are in movement (Sbrana *et al.*, 2020).

From the total of marine debris extracted, 94.58% were microplastics while mesoplastics represent a low percentage, all specimens contaminated ingest microplastics more than mesoplastics, the same results were reported in previous studies (Romeo *et al.*, 2015; Murphy *et al.*, 2017; Jabeen *et al.*, 2017). The obtained results confirm that the abundance of microplastics was higher than mesoplastics in all investigated fish species. Ingestion of small particles is maybe easier by small size of marine organisms (Neves *et al.*, 2015).

Our findings highlight that the blue particles were by far the most frequent plastics in the gastrointestinal tract contents; this is a result that was reported worldwide (Barboza et al., 2019, Merga et al., 2020). Some authors relate that (abundance blue particles) to their availability in the environment (Ferreira et al., 2018) or because fishes mistake them more as they are like food (Barboza et al., 2019). The red particles came second representing 41.3% of the total plastic debris, the source of red fibers is the fishing industry mainly fishing nets (Cole et al., 2011; Lusher et al., 2013; Nelms et al., 2018).

The results show no significant differences in the ingestion of plastic debris between males, females and undetermined specimens (p> 0.05). Maybe the availability of these pollutants particles in the ecosystems (pelagic and benthic) offers the same chance for all these categories, although they have different feeding habits.

Contaminated fish specimens by plastic debris in gastrointestinal tract was significantly higher in wet season as compared to dry season (p<0.01). The wet season showed a high contamination (2092 plastic particles) compared to dry season (129 plastic particles). Precipitation and strong winds during the wet season (63.7 mm in our study) stimulate the movement and degradation of plastic debris (Cheung et al., 2016). In addition, rivers are important sources of plastic contamination (Rowley et al., 2020; Xu et al., 2020), many microplastics and mesoplastics are maybe introduced to the sea by many rivers connected to the gulf of Bejaia. This high input of marine debris into the coastal waters depends on the seasonal activity making therefore plastic particles available to fish species then to consumers of fish.

5. Conclusion

This study shows high ingestion of plastic particles by commercial fishes from the gulf of Bejaia (Algeria). Extracted plastic debris were analyzed for the first time in the gastrointestinal tract of examined fish species. These pollutants exhibit a variability in colors, morphotypes and size, their abundance is higher in the wet season and in fish species from different ecosystems (pelagic, benthopelagic and demersal), and particularly those having a benthopelagic and demersal behavior. The obtained results maybe give an insight on the state of the marine ecosystem health of Algerian coast (especially in the studied region).

We highly recommend using the digestion process by sex in the future investigations on plastic particles ingested by fish species. We also recommend substantial investigations on the impact of these potential toxic pollutants on the health of consumers, and on depollution techniques reducing this kind of pollutants in the environment.

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Microplastic ingestion in Sardinella aurita Valenciennes, 1847 and Lithognathus mormyrus (Linnaeus, 1758) along the Gulf of Bejaia, Algeria

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Abstract

This study reports for the first time, the relationship between microplastic ingestion and biological parameters in *Sardinella aurita* and *Lithognathus mormyrus* from the Gulf of Bejaia, Algeria. Gastrointestinal tracts content of 144 fishes belonging to two different habitats (pelagic and demersal) were examined for microplastics contamination. Our results showed that 74.30% of individuals ingested microplastics. The most common colour of plastic particles was blue (49% in *S. aurita* and 30% in *L. mormyrus*) and the most of microplastics extracted from gastrointestinal tracts of samples were fibers (71.64%). There was no significant relationship between age and Fulton's factors of both the species and the quantity of microplastics ingested by them.

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Introduction

Pollution by plastic particles is known as a black spot because of its effects on the environment and wildlife (Yan et al., 2015)., The annual global demand for plastics stands at 245 million t (Andrady, 2011), and the plastic waste is expected to reach 12 billion t by 2050 (Geyer et al., 2017). Plastics are lightweight, durable and inexpensive and their advantages have led to the use in several domains (Thompson et al., 2009). The first report on plastic waste in the oceans was in the early 1970s (Khan et al., 2022). In the environment, plastic waste persists and continuously degrades into small particles such as microplastics and nanoplastics (Chen et al., 2022).

There is no recognised standard for determining the maximum particle size of microplastics, which varies with different studies (Cole et al., 2011). However, the term "microplastics" (MPs) is generally used to describe plastic particles less than 5 mm (Padervand

et al., 2020), and most studies refer to microplastics as particles within the size range of 1 to 5 mm (Isobe et al., 2017). Plastic debris in marine environments comes from various sources such as land-based, marine and industrial sources (Zhao et al., 2014; Bullard et al., 2021), agriculture (Tian et al., 2022), and wastewater treatment plants (Magni et al., 2019), and can take various forms such as foams, films, fibers, microbeads, pellets, and fragments (Reznia et al., 2018; Woo et al., 2021). Microplastics can be primary or secondary, depending on their formation process (Huffer et al., 2017; Laskar and Kumar, 2019). Human exposure to microplastics primarily occurs through food ingestion, with studies finding microplastics in commercial aquatic species such as mussels, oysters, crabs, sea cucumbers, and fish (Leslie et al., 2013; De Witte et al., 2014; Van Cauwenberghe and Janssen, 2014).

The objective of our study was to evaluate microplastic (MP) pollution in



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Received: 03.02.2023 Accepted: 07.06.2023 the gulf of Bejaia (Eastern Algeria) in particular, the ingestion of MPs by the pelagic fish *Sardinella aurita* Valenciennes, 1847 and the demersal fish *Lithognathus mormyrus* (Linnaeus, 1758) and to evaluate the relationships, if any, between the biological parameters of fishes and the presence of MPs in their gastrointestinal tracts.

Materials and methods

This study was carried out in the Gulf of Bejaia, located in the southern part of the Mediterranean Sea. For this study, a total of 144 fish samples (Sardinella aurita, n=120 and Lithognathus mormyrus, n=124) landed at the fishing ports (Bejaia and Ziama) (Fig. 1), were collected between March and June 2022

Biological parameters of fishes

Samples were cleaned with deionised water and the following biometric parameters of the samples were estimated (Table 1): (a) Total length (TI): represents a metric parameter used to study fish growth; (b) Total weight (Tw): measured before dissection of each individual using a KERN PCB scale with an accuracy of 0.1; (c) Sex identification (male, female or indeterminate): fish samples were sexed by visual observation of gonads; (d) Age identification: the age of fish was determined by otolith examinations.

Table 1. Biological parameters of fishes sampled during the study (n = 144).

Biological parameters	S. aurita	L. mormyrus
No. of specimens	n = 120	n = 24
Age ± SD (years)	2.8 ± 1.05	5.6 ± 1.43
Total length ± SD (cm)	16.11 ± 1.79	22.63 ± 1.65
Total weight ± SD (g)	36.12 ± 12.29	157.82 ± 37.96
Sex ratio (M:F:U)	65:37:18	18:6:0

The protocol for sampling and analysing microplastics from fish samples was adapted from the method described by Digka et al. (2018) with slight modifications. After dissection, the density separation process was not utilised. The gut of each specimen was transferred into a clean petridish, and the mixture was subjected to a 24 h oven treatment at 60° C to ensure optimal plastic extraction and removal of biogenic and biological materials. The digestive tracts were treated with 30% hydrogen peroxide (H_2O_2 , Chem-Lab, Germany). Each sample in the petridish was assigned a code number. Following complete digestion, samples were visually inspected under a binocular stereomicroscope, and microplastics were counted, photographed, and classified based on their shapes and colours (Hidalgo-Ruz et al., 2012; Free et al., 2014; Jabeen et al., 2017).

Microplastic extraction



Fig. 1. Location of the study area (Gulf of Bejaia, Algeria)

Data analysis

SPSS 14.0 and MS EXCEL 2013 were used for statistical analyses. Pearson correlation test was performed to determine if there were significant relationships between microplastics occurrence (F0% = Frequency of occurrence of microplastics) and TL (total length), Tw (total weight) and the Fulton's condition factor K. Data were analysed using ANOVA. The Tukey test's HSD was set at ** = p < 0.01 and * = p < 0.05 values.

Fulton's condition factor K was calculated using the formula:

k=100

Frequency of occurrence (FO %) of the microplastics in the digestive tracts was calculated using the formula:

FO (%) = $(Ni/N) \times 100$, where, Ni = Number of gastrointestinal tracts that contained plastic particles and N = Total number of gastrointestinal tracts examined.

Results

Microplastics in GIT of fish

The analysis of fish samples in sardines (*S. aurita*) and sand steenbras (*L. mormyrus*) revealed the presence of microplastics in 107 individuals out of 144 (74.30%) as shown in Table 2.

Table 2. Frequency of occurrence (FO) of microplastics (MPs) in the sampled fishes

	Total no. of	No. of individuals that		
Species	samples	contained MPs	FO (%)	
S. aurita	120	87	72.5	
L. mormyrus	24	20	83.3	
Total	144	107	74.3	

Microplastic distributions according to the form

In the gastrointestinal tract of samples examined (n=144), 1005 particles of MPs were identified. The most observed were fibers (71.64%, p < 0.01) and 28.35% were fragments (Fig. 2), the highest density of MPs per individual was observed in $L.\ mormyrus$ with a mean of 16.15 microplastic particles in each GIT versus 7.83 MPs per individual in $S.\ aurita$.

Classification of MPs by colour

The microplastic particles from fish had a variety of colours (transparent, black, blue, red, yellow and white), of which,

blue was highest (49% in *S. aurita* and 30% in *L. mormyrus*), followed by black and transparent microplastics, and the remaining colours represented a small percentage (Fig. 3).

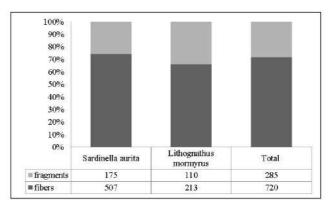


Fig. 2. Variability of microplastic forms ingested by fish samples

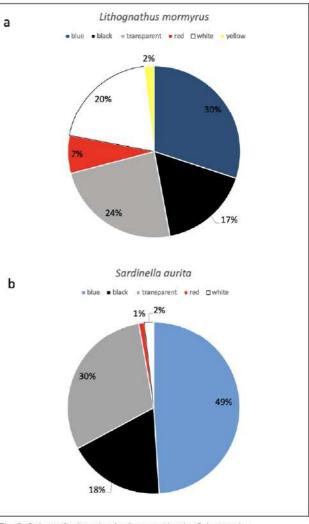


Fig. 3. Colour of microplastics ingested by the fish samples. (a) L. mormyrus and (b) S. aurita

Relationship between biological parameters and MPs ingestion

With no significant difference (p > 0.05), the number of microplastics ingested by females (545 particle) was found slightly more than the number of microplastics ingested by males (302 particles) and indeterminate samples (158) in the two species studied.

The age of *S. aurita* ranged from 1 to 6 years and from 4 to 8 years for *L. mormyrus*. The linear correlation presented in Fig. 4 highlights that there is no relationship between age of fish and MPs ingestion for sardines (y = 0.308x + 6.717; $R^2 = 0.004$) and sand steenbras (y = -1.784x + 31.56; $R^2 = 0.030$).

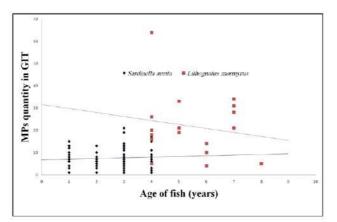


Fig. 4. Age of fish and microplastics (MPs) quantity relationship in *S. aurita* and *L. mormyrus* fish samples

The length-weight relationship (TI-Tw) analysed by linear regression equation (R2), for both species were positively correlated (Fig. 5 a;b). It was observed that no correlation existed between Fulton's condition factor K (representative of total length and total weight) and plastic particles ingestion by sardines and steenbras (Fig. 5c).

Discussion

Ingestion of microplastics by fishes from the Gulf of Bejaia, Algeria was investigated in this study. Of the 144 individuals analysed, 107 (74.30%) had ingested plastic particles, which is comparable to other studies (Boerger et al., 2010; Nadal et al., 2016; Clere et al., 2022) and indicates that microplastics are heavily contaminating marine environments. Microplastics are found in the gastrointestinal tracts of fish, affecting all types of fish. Pelagic species such as *S. aurita* are more likely to ingest low density microfibers and soft fragments from plastic bags floating on the water surface, while demersal species like *L. mormyrus* ingest hard fragments from sand while feeding.

However, a few studies have examined the presence of

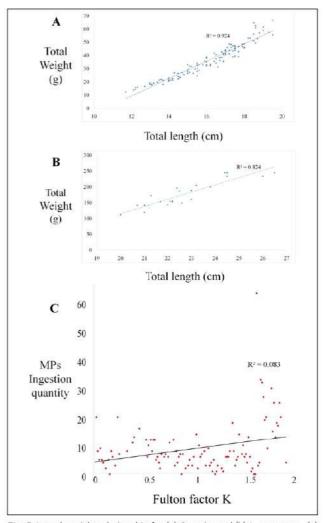


Fig. 5. Length-weight relationship for (a) *S. aurita* and (b) *L. mormyrus*. (c) Relationships between Fulton's factor of all fish samples and microplastics (MPs) ingestion in terms of quantity.

microplastics in *S. aurita* such as that by Adika *et al.* (2020) from the Eastern Central Atlantic Ocean, off the Coast of Ghana and the study of Shabaka *et al.* (2020) in Alexandria, Egypt. Both studies reported a high level of MPs-pollution values. Gundogdu *et al.* (2020) in their study reported the occurrence of microplastics in the gastrointestinal tracts of *L. mormyrus* along the Turkish coast.

In our study, only two forms of MPs were recorded (fibers and fragments). Microplastic fibers are the most abundant forms found, corroborating the results of Neves et al. (2015) and Ferreira et al. (2018). These fibers, originating from persistent and ubiquitous marine environmental pollutants such as fishing ropes and nets, pose a potentially significant threat to the health of aquatic species (Clere et al., 2022). They are similar to fish feed, which further contributes to their ingestion by specimens (Walkinshaw et al., 2020). The blue colour stand out as a predominant colour of the microplastics in the fish

samples studied, which fits with many studies highlighting blue MPs as most frequent in the gastrointestinal tracts of fishes (Kasamesiri and Thaumuangphol, 2020; Ugwu et al., 2021). Authors relate the abundance of blue particles to their availability in the sea environment (Ferreira et al., 2018).

Pooling both species together, our study found that female fish ingest slightly more than males and indeterminate individuals, but there was no significant difference (p>0.05). Results obtained by Sbrana *et al*; (2020) indicated that the males of *B. boops* collected from the Italian coasts ingested more plastic particles than females. Mc Neish *et al.* (2018) reported correlation between length and MPs quantity, while. Kerubo *et al.* (2021) reported that size or age does not influence microplastic ingestion in fish.

Many studies relating fish length and MP accumulation in the GIT reported no correlation (Mc Neish et al., 2018), significant correlation, either positive (Peters et al., 2016; McNeish et al., 2018; Pegado et al., 2021) or negative (Bessa et al., 2019). Pazos et al. (2017) observed no relationship between quantity of MPs and fish length, weight, and also feeding habit. Replaced by the condition factor K, in our study we excluded weight and length correlation with MP ingestion from analysis. Our results showed no significant relation between the condition factor K and the number of microplastics ingested by both species. The underlying mechanisms of how these particles are ingested are largely unclear (Roch et al., 2020).

The contamination of aquatic ecosystems by microplastics is a significant environmental issue. To assess the characteristics of microplastics pollution in gastrointestinal tracts, two fish species were selected from the Gulf of Bejaia, Algeria. Plastic particles were detected in fish's gastrointestinal tracts with a high occurrence frequency and with variability in colours and shapes. The study established that fish size, age and sex did not influence ingestion of microplastics by fish specimens. Further investigations are needed incorporating more variability in species and habitats as well as large number of individuals with different maturity levels, in order to pinpoint the relationship of microplastics occurrence with biological parameters.

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UNIVERSITÉ ABDERRAHMANE MIRA - BÉJAÏA LABORATOIRE DE ZOOLOGIE APPLIQUÉE ET D'ÉCOPHYSIOLOGIE ANIMALE



ATTESTATION

- DE PARTICIPATION

LA ZOOLOGIE APPLIQUÉE ET L'ECOPHYSIOLOGIE ANIMALE **4**EMES JOURNÉES D'ETUDE NATIONALE SUR BEJAIA DU 23 AU 24 OCTOBRE 2018

Journées d'Etude Nationale sur la Zoologie Appliquée et l'Ecophysiologie Animale, atteste que Mr: Je soussignée Dr. KADJI-DJOUDAD Hafsa, présidente du comité d'organisation des 4èmes

ZEGHDANI ZOUHIR

Co-auteurs: RAMDANE Z

A participé avec une communication affichée intitulée: « First record of microplastics in digestive tract ingested by fishes in the Gulf of Bejaia, Algeria»

Présidente du comité d'organisation Dr. KADJI-DJOUDAD Hafsa

Directeur du laboratoire LZA
Pr. Riadh MOULAÏ



UNIVERSITE ABDERRAHMANE MIRA - BEJAÏA
LABORATOIRE DE ZOOLOGIE APPLIQUEE ET D'ECOPHYSIOLOGIE ANIMALE



ATTESTATION

DE PARTICIPATION -

LA ZOOLOGIE APPLIQUÉE ETL'ECOPHYSIOLOGIE ANIMALE LES SÈMES JOURNÉES D'ETUDE NATIONALE SUR JIJEL, LES 22 ET 23 OCTOBRE 2019

Je soussignée, Pr RAMDANE Zouhir, président du comité d'organisationdes 5 èmes Journées d'Etude Nationale sur la Zoologie Appliquée et l'Ecophysiologie Animale atteste que Mr:

ZEGHIDANI ZOUHIR

CO-AUTAURS: RAMDANE Z. ET GHERBI R.

«Microplastics ingestion by type in three species of fish from the Gulf of Bejaia (Algeria) A participé avec une «Communication Affichée» intitulée :

Président du comité d'organisation

Directeur du laboratoire LZA



Laboratory of Biotechnology, Water, Environment and Health Ministry of Higher Education and Scientific Research University ABBES LAGHROUR-KHENCHELA People's Democratic Republic of Algeria Faculty of Natural and Life Sciences



CERTIFICATE OF PARTICIPATION

The president of the first International Seminar on Green Biotechnology and Food Security

held between 16 and 17 November 2022, certifies that:

ZEGHDANI ZOUHIR

Participated with ORAL Communication

VALORISATION DES DECHETS OLEICOLES). Entitled: LA TECHNOLOGIE DES MICRO-ORGANISMES EFFICACE (EM), QUEL AVENIR ? (EXEMPLE DE

Co-Authors: BELKOUM NOUREDDINE, YATTOUS SALAHEDDINE Conference president Laboratory manager



بيوتكنولوجيا ماء محيط وصعم الأستاذة : قاضي كنزة Pr. Kenza KADI

Dean of the Faculty



Abstract:

This study aims to analyze plastic particles ingested by commercially valuable fish species in the Gulf of Bejaia (Algeria). During our study, we targeted several species of Teleostean fish: Sardinella aurita, Sardina pilchardus, Pagellus acarne, Trachurus trachurus, Boops boops, Sparus aurata, Sardinella aurita and Lithognathus mormyrus. A specific protocol was applied in the research laboratory to various fish samples, from which several pieces of information regarding the prevalence and characteristics of plastic ingestion were obtained. Our results reveal that the examined fish exhibit a significantly high proportion of plastic debris in their digestive tracts. The predominant color of the ingested particles was mainly blue or black, while fiber forms were most observed. Despite rigorous analysis, no discernible correlation emerged between biological parameters and the incidence of plastic ingestion. Our results demonstrate a marked disparity in ingestion rates between wet and dry seasons, confirming a probable seasonal dynamic of plastic pollution. Although our research provides crucial information confirming plastic pollution, much remains to be done in this specific field to accurately assess the total extent of plastic pollution and its impacts on marine ecosystems. Solutions are proposed to protect communities and marine ecosystems.

Key-words: Micropastic, digestive tract, fishes, marin ecosystem, Gulf of Bejaia.

Résumé:

Cette étude a pour but l'analyse des particules de plastiques ingérés par les espèces de poissons à valeur commerciale du golfe de Béjaia (Algérie). Durant notre étude, nous avons ciblé plusieurs espèces de poissons Téléostéens: Sardinella aurita, Sardina pilchardus, Pagellus acarne, Trachurus trachurus, Boops boops, Sparus aurata, Sardinella aurita et Lithognathus mormyrus. Un protocole spécifique a été appliqué au laboratoire de recherche sur les différents échantillons de poissons où plusieurs informations sur la prévalence et les caractéristiques de l'ingestion de plastique ont été obtenues. Nos résultats révèlent que les poissons examinés présentent une proportion significativement importante de débris de plastique dans leur tractus digestif. La couleur prédominante des particules ingérées était principalement bleue ou noire, tandis que les formes en fibre étaient les plus couramment observées. Malgré une analyse rigoureuse, aucune corrélation discernable n'est apparue entre les paramètres biologiques et l'incidence de l'ingestion de plastique. Nos résultats montrent une disparité marquée dans les taux d'ingestion entre les saisons humides et sèches, ceci confirme une vraisemblable dynamique saisonnière de la pollution plastique. Bien que nos travaux de recherche fournissent des informations cruciales qui confirment la pollution en plastique, beaucoup reste à faire dans ce domaine bien précis afin d'évaluer avec exactitude l'étendue totale de la pollution plastique et ses impacts sur les écosystèmes marins. Des solutions sont proposées afin de protéger les communautés et les écosystèmes marins.

Mots-clés: Micropastique, tractus digestif, poissons, écosystème marin, golfe de Béjaia.

الملخص:

تمثل هذه الدراسة الرائدة أول تحليل شامل لاستيعاب بلع جزيئات البلاستيك من قبل أنواع الأسماك التي تعيش في المناطق الساحلية المتنوعة في الجزائر. من خلال التركيز على الموانئ الرئيسية في الشرق، بشكل مكثف في بجاية، جمعنا عينات بدقة من أنواع Sardinella aurita, Sardina pilchardus, Pagellus acarne, Trachurus trachurus, الأسماك الأكثر أهمية تجاريًا: Boops boops, Sparus aurata, Sardinella aurita

باستخدام عملية تفكيك المادة العضوية لهذه العينات باستخدام NaOH و H_2O_2 بشكل مستقل، تؤكد نتائجنا مشكلة واسعة الانتشار، مع نسبة كبيرة من الأسماك المفحوصة تظهر علامات بلع للبلاستيك. وبشكل ملحوظ، كان لون الجزيئات المستقبلة هو الأزرق أو الأسود بشكل رئيسي، بينما كانت الأشكال الشبيهة بالألياف هي الأكثر شيوعًا في التشكيل.

على الرغم من التحليل الدقيق، لم نجد علاقة ملحوظة بين المعلمات البيولوجية ودرجة بلع البلاستيك، مما يشير إلى تفاعل معقد للعوامل. وبشكل مثير للاهتمام، كشفت در استناعن فارق واضح في معدلات التسمم بين فصول السنة الرطبة والجافة، مما يؤكد على التباين الموسمي المتغير في ديناميكية التلوث بالبلاستيك.

علاوة على ذلك، تكشف دراستنا عن نمط ثابت للتلوث عبر مناطق الخلجان الشرقية في الجزائر، مما يبرز الحاجة الملحة لاتخاذ إجراءات متوازنة لمعالجة هذا التحدي البيئي المنتشر. وبينما توفر بحوثنا رؤى حاسمة، فإنها تؤكد أيضًا على ضرورة إجراء المزيد من الدراسات لفهم النطاق الكامل للتلوث بالبلاستيك وتأثيراته على النظم البحرية.

في الختام، تشكل نتائجنا نداءً للعمل الفوري للتخفيف من التلوث بالبلاستيك على طول السواحل الجزائرية. في المستقبل، تعد الجهود التعاونية ضرورية لتطوير حلول فعالة تحمى صحة النظم البحرية ومعاش الجماعات الساحلية.

كلمات مفتاحية: جزيئات البلاستيك، السبيل الهضمي، الأسماك، النظام البيئي البحري، خليج بجاية.