ASSESSMENT OF MICROPLASTIC CONTAMINATION IN THE GUTS OF COMMONLY TRADED FISH SPECIES AT A LANDING STATION IN VALENCIA CITY, BUKIDNON

ANG, TRYXZHIA GEM G.
PATRICIO, JO MARIAN ALEXA A.
SALUPADO, JOERY BENEDICT L.
SEVILLENO, KRISTINE CLAIRE P.

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CURRICULUM VITAE

Name	:	TRYXZHIA GEM G. ANG
Grade	:	Grade 11
Research Title	:	ASSESSMENT OF MICROPLASTIC CONTAMINATION IN THE GUTS OF COMMONLY TRADED FISH SPECIES AT A LANDING STATION IN VALENCIA, BUKIDNON
Personal Data	:	Born on JANUARY 03, 2008, in Valencia City, Bukidnon, Middle child of Mr. and Mrs. Michael C. Ang. Presently residing at P-2A, Lumbo, Valencia City, Bukidnon.
Education		
Secondary	:	Central Mindanao University Laboratory High
School	:	University Musuan, Bukidnon
		Academic Year: 2020-Present
Primary	:	His Greatness Christian Academy, Lumbo, Valencia City, Bukidnon
		Academic Year: 2012-2020
Curricular and Extracurricular Achievements	:	1st Place Watercolor Painting Junior Student Convention (ISC) S. Y 2018-2019 and S.Y 2019-2020 at Davao City
	:	2nd & 3rd Place 400meter Track and Field Junior Student Convention (JSC) S. Y 2018-2019 and S. Y 2019-2020 at Davao City
	:	1st Place Science Fair Essay Writing S. Y 2023-2024
		etc.
Seminars Attended	:	2023 Gender Sensitivity Seminar at Central Mindanao University
	:	2024 Career Guidance Seminar at Central Mindanao University Laboratory High School
		etc.

CURRICULUM VITAE

Name	:	JO MARIAN ALEXA A. PATRICIO
Grade	:	Grade 11
Research Title	:	ASSESSMENT OF MICROPLASTIC CONTAMINATION IN THE GUTS OF COMMONLY TRADED FISH SPECIES AT A LANDING STATION IN VALENCIA, BUKIDNON
Personal Data	:	Born on FEBRUARY 01, 2008, in Valencia City, Bukidnon, the first among the two children of Mr. & Mrs. Jose Hermis P. Patricio. Presently residing at P-13, CMU, Musuan, Maramag,Bukidnon.
Education		
Secondary	:	Central Mindanao University Laboratory High
School	:	University Musuan, Bukidnon
		Academic Year: 2020-Present
Primary	:	Musuan Integrated School, Musuan, Bukidnon Academic Year: 2014-2020
Curricular and Extracurricular	:	Class Valedictorian, SY 2019-2020
Achievements	:	3rd Best paper in the Physical Science Category in the Science Congress 2024
	:	1st place high school poster presentation, BIOTA Philippines Annual Convention & International Conference
		etc.
Seminars Attended	:	2023 Gender Sensitivity Seminar at Central Mindanao University
	:	2024 Career Guidance Seminar at Central Mindanao University Laboratory High School
		etc.

CURRICULUM VITAE

Name	:	JOERY BENEDICT L. SALUPADO
Grade	:	Grade 11
Research Title	:	ASSESSMENT OF MICROPLASTIC CONTAMINATION IN THE GUTS OF COMMONLY TRADED FISH SPECIES AT A LANDING STATION IN VALENCIA, BUKIDNON
Personal Data	:	Born on JULY 15, 2007, in Maramag City, Bukidnon, the only child of Mr. and Mrs. Gerry G. Salupado. Presently residing at P-2, Poblacion, Kadingilan, Bukidnon.
Education		
Secondary	:	Central Mindanao University Laboratory High
School	:	University Musuan, Bukidnon
		Academic Year: 2020-Present
Primary	:	Kadingilan Central Elementary School, P-3 Poblacion, Kadingilan, Bukidnon
		Academic Year: 2014-2020
Curricular and Extracurricular	:	Class Valedictorian, SY 2019-2020
Achievements	:	3rd Best paper in the Physical Science Category in the Science Congress 2024
	:	1st place high school poster presentation, BIOTA Philippines Annual Convention & International Conference
		etc.
Seminars Attended	:	2023 Gender Sensitivity Seminar at Central Mindanao University
	:	2024 Career Guidance Seminar at Central Mindanao University Laboratory High School
		etc.

CURRICULUM VITAE

Name	:	KRISTINE CLAIRE P. SEVILLENO
Grade	:	Grade 11
Research Title	:	ASSESSMENT OF MICROPLASTIC CONTAMINATION IN THE GUTS OF COMMONLY TRADED FISH SPECIES AT A LANDING STATION IN VALENCIA, BUKIDNON
Personal Data	:	Born on DECEMBER 31, 2007, in Valencia City, Bukidnon, the only child of Mr. and Mrs. Jose Regner M. Sevilleno. Presently residing at, Grand Meadows, Valencia City, Bukidnon.
Education		
Secondary	:	Central Mindanao University Laboratory High
School	:	University Musuan, Bukidnon
		Academic Year: 2020-Present
Primary	:	Valencia Baptist Christian Academy, Sayre Highway, Valencia City, Bukidnon
		Academic Year: 2011-2020
Curricular and Extracurricular	:	Class Salutatorian, SY 2013-2014
Achievements	:	Most Industrious SY 2019-2020
	:	Class Salutatorian SY 2019-2020
		etc.
Seminars Attended	:	2023 Gender Sensitivity Seminar at Central Mindanao University
	:	2024 Career Guidance Seminar at Central Mindanao University Laboratory High School
		etc.

DEDICATION

This research is dedicated to

Our powerful God;

Our beloved family;

Our inspiring friends;

And to our motivating instructors.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF APPENDIX FIGURES

ABSTRACT

ASSESSMENT OF MICROPLASTIC CONTAMINATION IN THE GUTS OF COMMONLY TRADED FISH SPECIES AT A LANDING STATION IN VALENCIA CITY, BUKIDNON, by Tryxzhia Gem G. Ang, Grade 11 Einstein, Jo Marian Alexa A. Patricio, Grade 11 Newton, Joey Benedict L. Salupado, and Kristine Claire P. Sevilleno, Grade 11 Einstein, Central Mindanao University Laboratory High School, Musuan, Bukidnon, May 2025

Research Adviser: Rica Joy V. Lamparas

Microplastic contamination in marine ecosystems poses significant risks to aquatic life and human health. This study assessed the presence and characteristics of microplastics in the gastrointestinal tracts of commonly traded fish species, *Chanos chanos* (Milkfish) and *Epinephelus sp.* (Highfin grouper), sourced from the Valencia City Fish Landing Terminal in Bukidnon, Philippines. A total of 12 fish samples, six per species were grouped into three pooled samples, each containing two individuals and were analyzed using potassium hydroxide (KOH) digestion, microscopic inspection, and Fourier-Transform Infrared Spectroscopy (FTIR). Results revealed a 100% prevalence of microplastics in both species, with *C. chanos* containing 133 particles and *E. sp.* with 107 particles. Black microplastics were dominant (39.85% in *C. chanos*, 84.11% in *E. sp.*), likely originating from degraded consumer plastics. *C. chanos* exhibited more angular fragments (64.66%), while *E. sp.* had higher proportions of elongated and thin filaments (51.4%). Polymer analysis identified polyethylene terephthalate (PET) or ethylene-vinyl acetate (EVA) and spandex or polyurethane (PU) in *C. chanos*, and predominantly spandex or polyurethane (PU) with traces of polyethylene (PE) in *E. sp.*, suggesting varied pollution sources. Statistical analysis showed no significant correlation between microplastic counts and morphometric parameters (length, weight, GIT weight), indicating prevalent contamination regardless of fish size. The study highlights pervasive microplastic pollution in local fish populations, underscoring the need for improved waste management and further research on ecological and health impacts. These findings contribute to a global understanding of microplastic pollution in aquatic food chains and emphasize the urgency of mitigating plastic waste in marine environments.

***Keywords**: microplastics (MPs), fish contamination, Epinephelus sp., Chanos chanos, FTIR spectroscopy*

Chapter 1

INTRODUCTION

Background of the Study

In the age of convenience, plastic had become a staple of modern life, used in everything from packaging to everyday items. However, once discarded, plastics often break down into microplastics—tiny particles less than 5 mm in size—that infiltrate marine ecosystems (Masura, 2015). Though nearly invisible, microplastics posted significant risks to marine life, as fish and other aquatic organisms mistook them for food. This led to ingestion, potential health issues for the animals, and the possibility of microplastics entering the human food chain through seafood consumption (Wright, 2013).

The scale of plastic waste pollution staggered, particularly in developing nations like the Philippines. According to World Bank Group (2023), the World Bank estimated that the Philippines consumed approximately 163 million plastic sachets daily. This extensive consumption contributed to the generation of 2.7 million tons of plastic waste annually, with only 28% of major plastic resins being recycled. The remaining 72% of improperly disposed plastic waste accumulated in landfills, waterways, and increasingly, in marine environments. The country's status as one of the world's largest contributors to ocean plastic pollution brought the issue closer to home, particularly for coastal communities like General Santos City in South Cotabato and Zamboanga City, where the seas provided not only livelihood but sustenance.

General Santos and Zamboanga are coastal fishing cities where marine resources are vital to the local economy and culture. However, plastic pollution posed a growing threat, with microplastics potentially contaminating commonly consumed fish species like Highfin grouper (*Epinephelus sp.*) and Milkfish (*Chanos chanos*). These fish not only sustained the local population but are also supplied to the wet markets as far as Valencia City in Bukidnon, expanding the impact of any contamination. By studying microplastic contamination in fish, this research aimed to shed light on the risks posed to marine life, public health, and broader communities relying on these coastal resources (Amelia, 2021).

Objectives of the Study

Generally, this study aimed to assess the presence and extent of microplastic contamination in the guts of commonly traded fish species at a landing station in Valencia City, Bukidnon. Specifically, this study aimed to:

1. identify and quantify microplastics found in the guts of selected commonly traded fish species at the fish landing station in Valencia City in terms of:

    a) color

    b) shape

    c) erosion

    d) type

    e) source;

2. describe the adjusted prevalence of microplastic contamination in fish species;

3. evaluate correlation between microplastic contamination levels and morphometric parameters in terms of:

a) standard body length

b) whole body weight

c) GIT weight;

4. compare the microplastic count between microplastic types; and

5. describe the microplastic count in each fish species

Hypotheses of the Study

H_0: There is no significant correlation between the morphometric parameters and the level of microplastic contamination.

H_1: There is a significant correlation between the morphometric parameters and the level of microplastic contamination.

H_0: There is no significant difference in microplastic counts among the microplastic types in each fish species

H_1: There is a significant difference in microplastic counts among the microplastic types in each fish species

Significance of the Study

This study provided important data on the extent of microplastic contamination in fish traded in Valencia City. By examining commonly consumed species, the research aimed to offer insights into the level of plastic pollution affecting the local marine environment. These findings helped raise public awareness about the environmental impacts of plastic waste and its potential entry into the food chain. The results would aid local policymakers in creating informed strategies to address plastic waste and food safety concerns supporting both environmental sustainability and community health.

Scopes and Delimitations of the Study

This study focused only on the analysis of microplastics in the guts of selected fish species traded at the landing station in Valencia City. It did not include water sampling due to budget and logistical constraints. The analysis also did not extend beyond the gut of the fish; as detailed evaluations of other fish tissues are prohibitively expensive. These limitations underscore the need for further research in broader contexts to gain a comprehensive understanding of microplastic pollution.

Operational Definition of Terms

Microplastics. Plastic particles less than five millimeters in size, resulting from the breakdown of larger plastics or manufactured for specific uses.

Prevalence. Refers to the occurrence or proportion of fish with microplastic contamination within a given sample or population of fish species commonly traded.

Plastic polymers. Large molecules made of repeated subunits that comprise various plastics, varying in color, shape, erosion, type, and source.

Fish landing station. A fish landing site is where fishers bring their catch for sorting, trading, and distribution to markets or processors.

Erosion. Microplastics are broken into smaller particles through physical forces like abrasion and waves, contributing to their spread and environmental contamination.

Chapter 2

REVIEW OF RELATED LITERATURE

Microplastic

Microplastic particles are ubiquitous in the environment, from the air we breathe to the food we eat. The majority of plastics are consumed and then disposed on land. However, by 2030, it is expected that the amount of microplastics in some oceanic compartments would have doubled. Plastic debris and microplastics are produced via tire wear, paint failure, washing textiles, at-sea losses, and land disposal. Releases are facilitated by storm water, atmospheric and riverine movement, and natural calamities. Plastics and microplastics, weather, biofoul, agglomerate, sink, are consumed by creatures, and get redistributed by currents in surface waters. The end point is probably ocean sediments. Polymers are known to leak additives, concentrate environmental pollutants, and act as a substrate for pathogenic and exotic biofilms. As the size of the fragments reduces, the amount of microplastics that can be consumed by organisms also increases (Hale et al., 2020). Worldwide, there is an abundance of microplastic contamination in the air, water, and land. Although these habitats are frequently seen as distinct, they are actually closely related (Horton & Dixon, 2018).

Humans have increasingly treated oceans as dumping grounds, with microplastics becoming a significant pollutant affecting both shorelines and freshwater ecosystems worldwide. Microplastics, defined as plastic particles smaller than 5 mm, are commonly found in products like cosmetics, hand cleansers, and airblasting materials. Their pervasive presence across marine environments is due to the durability of plastics, which resists degradation and allows these particles to enter aquatic ecosystems through improper disposal (Thompson et al., 2024).

The small size of microplastics makes them easily ingested by a wide range of aquatic organisms, leading to their accumulation in tissues and subsequent transfer through the food web. In marine life, the buildup of microplastics causes chronic biological effects, while in humans, indirect ingestion of these particles has been linked to potential health risks.

Given the rising threat of microplastics to marine ecosystems and human health, addressing this issue requires reducing plastic additive use, enacting regulations to manage plastic waste, and promoting plastic recycling and awareness programs. Such efforts are essential for mitigating pollution and safeguarding both the environment and public health (Sharma & Chatterjee, 2017).

Microplastic pollution

The accumulation of plastic and microplastic debris in the environment has become a growing concern and a key focus for researchers. Plastics are persistent pollutants that resist environmental degradation, readily attract hydrophobic organic contaminants, and are associated with harm and mortality in many aquatic species. The widespread presence of microplastics reflects the rapid increase in synthetic plastic production and the improper management of plastic waste (Karbalaei et al., 2018).

As an emerging persistent pollutant, microplastic pollution has garnered significant attention. Microplastics are widely distributed across freshwater systems, oceans, and seas, reaching even deep-sea sediments. However, there remains a lack of comprehensive reviews on practical approaches to assessing microplastic pollution, including its distribution, toxicity, sampling methods, and identification in aquatic environments (Yu et al., 2018).

Primary Microplastics

Primary microplastics are directly manufactured small plastic particles used in various commercial products. These microplastics are introduced into the environment during production, consumption, or through industrial processes. They often enter marine ecosystems through wastewater and other forms of pollution, where they persist and are consumed by aquatic organisms (Rogers, 2024). They can start breaking down within 12 hours when subjected to mechanical forces like abrasion from sand or wind (Klein et al., 2019). Laboratory experiments have demonstrated that materials such as high-density polyethylene (HDPE) can undergo substantial degradation after six months of exposure to conditions mimicking the marine environment, especially when ultraviolet (UV) light is present (Vianello et al., 2017). However, the process of fully fragmenting microplastics into smaller particles can take anywhere from several months to years, depending on factors such as temperature, humidity, and the type of plastic involved (Enfrin et al., 2022).

Secondary Microplastics

Secondary microplastics are created when larger plastic items break down due to environmental factors like UV radiation, weathering, and physical damage. This process leads to the fragmentation of plastics into smaller particles, which are easier for marine species to ingest. The breakdown rate of these particles depends on the type of plastic and environmental conditions, such as wave action and temperature. Studies have found that mechanical wear in marine environments can cause fragmentation within a day, but full degradation into tiny microplastics may take several months or even years (Chubarenko et al., 2018; Andrady, 2011; Duis and Coors, 2016). Additionally, over extended periods, these microplastics can break down further into water-soluble organic substances, which makes it more difficult to track how quickly fragmentation occurs (Enfrin et al., 2022). The accumulation of these particles in marine ecosystems contributes to pollution and harms organisms across the food chain, as smaller microplastics are more likely to be consumed by marine life (Rogers, 2024).

Table 1. shows the formation of MPs by the natural degradation/weathering of large plastic debris (Khalid et al., 2021).

Table 1. Microplastics formed in the environment.

Types of MPs	Polymer Types	Products	Shapes of MPs
PRIMARY Produced as it is in industrial products SECONDARY Produced after aging of bigger plastic pieces	PS PE PP PVC PET LDPE HDPE PU PC PA PV PSV SAN PDM	Beverage bottles Containers Cutlery Carry bags Electronics Furniture Food packages Pipes Medical Textile Pillows Forms Tiles Cosmetics Toys	Fragments Films Spheres Foams Pellets

The different polymers of plastics that are found in the aquatic environments and the land are PE (polyethylene), PP (polypropylene, LDPE (low density polyethylene), HDPE (high density polyethylene), PS (polystyrene), PVC (polyvinyl chloride), PET (polyethylene tetra phthalate), POM (polyoxymethylene), PBAT (polybutylene adipate terephthalate) and PLA (poly lactic acid) (Khalid et al., 2020a). Different plastic polymers are made up of a variety of chemical kinds. Examples include tetra Bromo bisphenol A (TBBPA) in vinyl esters and polycarbonate resins, di-2-ethylhexyl phthalate (DEHP) in PVC, hexabromocyclododecane (HBCDD) in polystyrene foam, and polybrominated diphenyl ethers (PBDEs) in plastics, textiles, and polyurethane foams (Services, 2011).

Effects of microplastics on human health

The ingestion of microplastics by marine organisms not only affects marine ecosystems but also poses significant risks to human health. According to Blackburn (2022), studies involving individuals exposed to high concentrations of microplastics, as well as experiments on model animals and cell cultures, have demonstrated potential adverse health effects. These effects include immune system disruption, stress responses, and toxicity to the reproductive and developmental systems. The ability of microplastics to carry harmful chemicals and pollutants, combined with their persistent nature, suggests that long-term exposure through consumption of contaminated seafood could have serious health implicat21ions (Smith et al., 2018). Microplastics may act as vectors for toxic substances like heavy metals, pesticides, and persistent organic pollutants, which are known to cause a variety of health issues, including endocrine disruption, carcinogenesis, and metabolic disorders (Ašmonaitė et al., 2020).

Microplastics on coastal waters

As indicated by Issac & Kandasubramanian (2021), 97% of the water on Earth is contained in the seas, which cover around 71% of the planet's surface. The remaining 3% is found in the atmosphere as water vapor as well as in ponds, streams, glaciers, and ice caps. Microplastics have the ability to both absorb toxic contaminants from the environment and act as vectors for their transportation while also releasing compounds (additives). In the maritime environment, plastics are subjected to mechanical and photochemical reactions that are accelerated by waves and sunlight, respectively, resulting in the formation of micro/nanoparticles.

Microplastic Pollution in Sarangani Bay

Since 2018, large areas of waste, including single-use plastics, have been accumulating in various parts of Sarangani Bay. This bay is a well-known tourist destination in the region, featuring stretches of white sandy beaches and world-class diving spots. The presence of floating plastic waste is harmful to the marine life in the bay, as fish may confuse the debris with food. Despite ongoing clean-up efforts, the trash continues to resurface, largely due to improper waste disposal by nearby residents. A study by Deocaris et al. (2023) revealed that microplastics, largely from plastic waste, have been detected in several areas of the bay. Items like single-use plastics, fishing ropes, and polystyrene break down into microplastic particles. The study reported an average of 1,084 microplastic particles per cubic meter in the surface sediments of the bay's coastal and beach areas.

Plastic Pollution in Zamboanga City

The growing problem of plastic pollution in Zamboanga City, Philippines, has raised serious environmental concerns, particularly affecting marine life and local ecosystems. While several measures and policies have been introduced to address this issue, managing plastic waste effectively remains a persistent challenge. Recent research has revealed that microplastics are widespread in fish sold in local markets, with one study confirming that every fish examined (100%) contained microplastics in their stomachs (Mejos et al., 2023). Additionally, the city struggles with solid waste disposal, as 58% of the waste collected is non-biodegradable. This poor waste management contributes to the increasing presence of microplastics in coastal areas and seabed sediments (Zamboanga City Government, 2022).

Fishes

Epinephelus sp.

Highfin grouper (*Epinephelus sp.*), locally known as lapu-lapu in the Philippines, are highly valued for their excellent texture and flavor, making them a premium choice in aquaculture. Their high market demand, particularly in countries like Hong Kong, Japan, Taiwan, Korea, and Singapore, has made them a lucrative species compared to other farmed fish. However, this growing demand has led to overfishing and significant pressure on wild populations (Southeast Asian Fisheries Development Center, n.d.; Glamuzina & Rimmer, 2022). Belonging to the family Serranidae and subfamily Epinephelinae, they are distributed across tropical and subtropical waters, including the Atlantic Ocean, Mediterranean Sea, and Southeast Asia. This group includes approximately 159 species under 15 genera, many of which are known for their protogynous hermaphroditism (transitioning from female to male during their lifecycle) and their ability to adapt to aquaculture practices. However, some species can accumulate toxins, such as those causing ciguatera poisoning, from their environment, posing risks to consumers (Harikrishnan, Balasundaram, & Heo, 2010; Encyclopaedia Britannica, 2024). To meet increasing demand and counter declining wild stocks, their aquaculture have been practiced globally for over 40 years. Farming involves three phases: hatchery rearing (larvae until 60 days), nursery rearing (juveniles for 1–2 months) and grow-out (fish raised for 6–8 months to reach market size). Proper management practices, including size grading, water quality monitoring, and routine feeding, are essential to optimize production. Fish typically reach market size (300–350 grams) within 5–7 months, with live Highfin groupers. fetching higher prices in both local and international markets (Southeast Asian Fisheries Development Center, n.d.). Despite its economic potential, Highfin groupers aquaculture faces challenges such as habitat destruction, overfishing, limited local supply, and competition in the live fish trade. Addressing these issues is essential to sustain wild populations and ensure the long-term viability of this high-value aquaculture species.

Chanos chanos

The Philippines is an archipelago comprising over 7,000 islands and is home to a wide variety of aquatic species. Fish play a crucial role in the diet of Filipinos, contributing significantly to their protein intake. Consequently, fisheries are vital for the livelihoods of more than 1 million Filipinos residing in coastal areas (Palomares et al., 2014). Aquaculture is a thriving sector in the country, contributing not only to food production but also to job employment and foreign exchange earnings (Hecht, 2006; Magcanta et al., 2021). The annual global production of milkfish reached >1.1M tons in 2015, with the Philippines, Indonesia, and Taiwan as main producers (de Jesus-Ayson et al., 2010) & (FAO, 2017). Milkfish are highly adaptable to different environmental conditions and can thrive in different environments both saline and freshwater environments. They are generally found at depths ranging from 1 to 30 meters and frequently inhabit coastal lagoons, estuaries, and areas near coral reefs (Gaitan et al., 2014). Milkfish undergoes several developmental stages: larval, juvenile, and adult. In the larval stage, milkfish primarily consume zooplankton found in their aquatic environments. As they transition into juveniles, their diet changes to include benthic organisms linked to micro and meiofauna. Common food sources during this stage consist of cyanobacteria, diatoms, detritus, green algae, and small invertebrates like crustaceans and worms. In the adult stage, milkfish continue to have a varied diet that includes benthic invertebrates, different types of algae, and zooplankton (Gaitan et al., 2014; FAO, 2024).

Assessing Microplastic Contamination in Fish

Microplastic contamination in fish is assessed through a structured approach that includes planning, field sampling, laboratory analysis, and reporting. The process begins with careful planning to define the study's objectives, including the selection of target fish species and habitats. Field sampling involves collecting fish from specified environments, often with the assistance of local fishers to ensure representative samples. During collection, key variables such as species, body length, and the condition of the digestive tract are recorded. Following this, laboratory analysis is conducted, where the digestive tracts are treated with chemical solutions like (KOH) & (H_2O_2) to dissolve organic matter, allowing for the isolation of microplastics. Fourier-Transform Infrared Spectroscopy (FTIR) are then employed to characterize the microplastics based on their chemical components and microplastic source. The final stage involves interpreting the data and reporting the findings, which includes assessing contamination levels and discussing potential ecological and health impacts. Throughout this process, quality assurance measures are implemented to mitigate contamination risks during both sampling and analysis (Protocol for Microplastic Monitoring in Fish, 2024). Recent studies indicate that up to 94% of fish samples may contain microplastics, predominantly in fiber form, highlighting the significance of these methodologies in understanding the ecological and health implications of microplastic pollution in aquatic systems (Sulistyorini et al., 2024). Additionally, a comprehensive report by the Food and Agriculture Organization (FAO) discusses how microplastics enter aquatic food chains and their potential impacts on seafood safety, emphasizing the need for further research to address existing knowledge gaps (Lusher et al., 2017).

Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) is an essential analytical technique for identifying and characterizing microplastics, which are plastic particles less than 5 mm in size that pose significant environmental threats. FTIR operates by measuring the absorption of infrared radiation by molecular bonds within a sample, producing a unique spectrum that serves as a "fingerprint" for different polymers. This capability is particularly valuable in microplastics research, as it allows for the differentiation of various types of plastics based on their chemical structures (Osman et al., 2023).

The use of FTIR in evaluating microplastics involves several key applications. It enables the identification of common polymers such as polyethylene (PE), polypropylene (PP), and polystyrene (PS) found in environmental samples like sediments, water, and biota.

The Operational Principles of FTIR

A technique to measure every infrared frequency at once, rather than one by one, was required. A fix was created, which used an interferometer, a fairly basic optical instrument, also known as the Michelson type interferometer. The device is made up of a half-silvered mirror that splits a light beam into two equal pieces. The fixed mirror receives one of the light beam's reflections, while the moveable mirror receives the other (Britannica, 2024). An exclusive kind of signal was generated by the interferometer, containing all of the IR frequencies that are "encoded" in it. Upon measuring the signal in seconds. Most interferometers have a beam splitter, which takes the incoming infrared beam and splits it into two optical beams. This resulted in a reduction of the time element per sample. A fixed flat mirror receives reflections from one beam. The other beam bounces off a flat mirror that can move just a very little distance, usually a few mm away from the beam splitter thanks to a mechanism. The message that emerges from the interferometer is the outcome of these two beams "interfering" with the other, since one beam's path is always the same while its mirror shifts, and 1 beam's path is fixed. The signal that is produced is known as an interferogram (Dutta, 2017).

Conceptual Framework

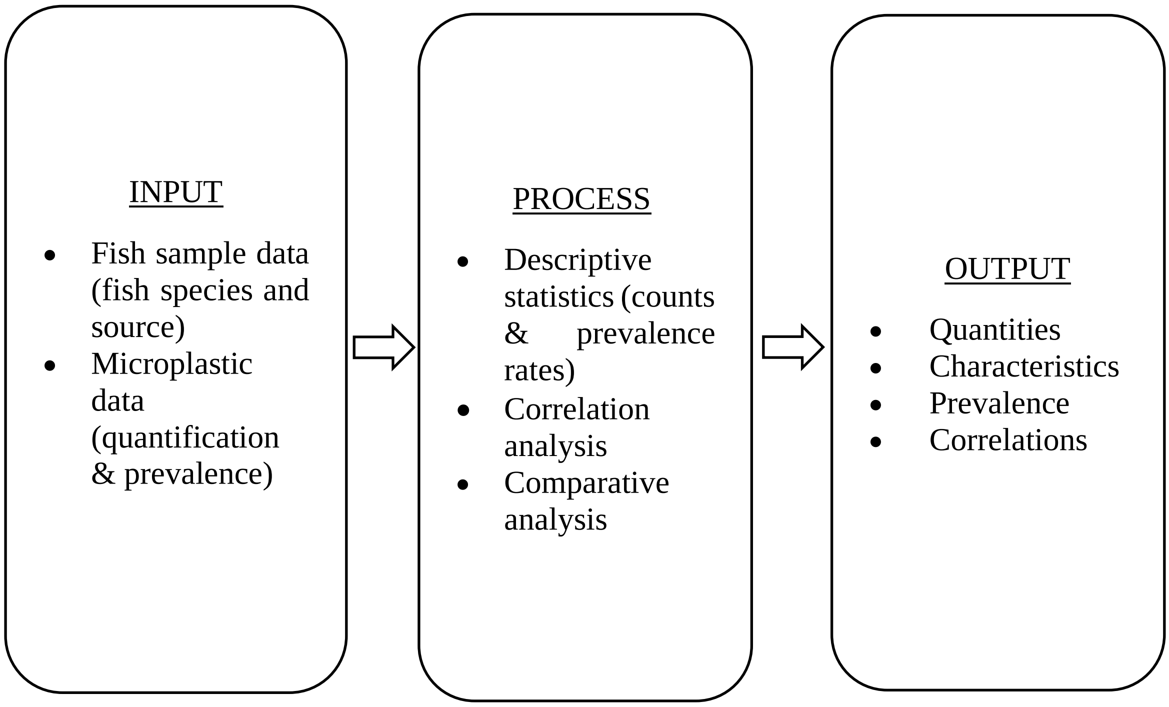

Figure 1. Relationship among inputs, processes, and outputs of the study

Chapter 3

METHODOLOGY

Research Design

This study is an applied research that aimed to evaluate microplastic contamination of guts in fish commonly traded at a landing station in Valencia City. It identified the commonly traded fish species and employed a quantitative, descriptive-correlational design to examine the presence of microplastics in their guts. Additionally, it explored the relationship between microplastic contamination and morphometric parameters such as fish length, weight, and GIT weight.

Location and Duration of the Study

This study was conducted from December 2024 to March 2025. After a series of informal interviews with fish traders in the City Fish Landing Terminal, Bagontaas, Valencia City, Bukidnon, two fish species (*C. chanos* and *E. sp.*) were eventually selected and bought from this site. Fish guts were then removed from these fish samples and subjected for laboratory and microscope analyses at Natural Production Research Development (NPDRC) in Central Mindanao University, Musuan, Maramag, Bukidnon. The duration also encompassed data processing and statistical analysis.

Experimental Procedure

Pre-Fieldwork Preparations

To ensure the legal and ethical implementation of the study, permits were secured prior to the data collection and laboratory procedures. A letter was submitted to the City Economic Enterprise Manager requesting consent to conduct sampling of fish species at the local fish landing site. A separate permit was also obtained from the Natural Production Research Development Center (NPRDC) to authorize the laboratory analysis of the collected samples.

Specimen Identification

For the identification of the Milkfish (*Chanos chanos*) and Highfin grouper (*Epinephelus sp.*), photographs of the collected fish species were submitted to the Central Mindanao University (CMU) Museum. The museum staff assisted in the verification and confirmation of the species based on the provided images.

Sample Collection

Fish samples were collected from the study site, particularly focusing on two commonly traded species: Highfin grouper (*E. sp.*) and Milkfish (*C. chanos*). The Milkfish was sourced from General Santos City, whereas the Highfin Grouper was sourced from Zamboanga City, with two samples per three pooled samples in each species, totaling 12 samples.

Samples were bought from fish vendors in the study site early in the morning (around 4:00 AM) to ensure freshness. These were then stored in an icebox container filled with ice to maintain coolness and prevent spoilage during transport to a designated temporary processing site, where the samples were carefully dissected under controlled conditions (Figure 2).

Figure 2. Selection of fish samples: *C. chanos* and *E. sp.*

Sampling was conducted in December only, due to logistical constraints and tight deadlines that prevented a longer, multi-month approach. Although this limited the assessment of seasonal variations in microplastic ingestion, focusing on a single month streamlined the research process and enabled the study to meet its timeline while still yielding valuable insights into microplastic levels in fish.

Quality Control

To minimize contamination, blanks were processed alongside each batch. These blanks underwent the same procedures but without digestive tracts. Meanwhile, all instruments were thoroughly rinsed with distilled water and dried prior to use. Researchers wore cotton lab coats and polymer-free gloves to maintain cleanliness during the process. Dissection and sample handling were conducted in a laminar flow hood to minimize contamination.

Sample Preparation

Species identification, weight, and length of each fish was recorded using standard methods, and the digestive tracts (from the esophagus to the anus) were dissected after the purchasing of fish samples. Fish samples were measured for standard total length using a measuring tape (Wu et al., 2020). The abdomen was carefully dissected using scalpels, and the alimentary tract, including the stomach and intestines, were removed from the body cavity. Subsequently, the fish was re-weighed after dissection, and the dissected tissue samples were weighed in a pre-weighed beaker (Inocente et al., 2023).

Digestion Procedures

Negative control of samples underwent the same procedure. Distilled water was used, matching the volume of the original sample. Figure 3 shows the digestive tracts being transferred into 250 mL pre-combusted beakers. Each sample was treated with 10% potassium hydroxide (KOH) in a ratio of 1:3, covered with glass lids, and incubated in an oven at 60°C for 48 hours to digest organic matter (Figure 4). During incubation, samples was stirred every four hours. After digestion, the samples were carefully decanted and the top solution filtered using a Whatman GF/C glass microfiber filter (Inocente et al., 2023). These were then stored in a dry room until it reached the desire level of dryness (Figure 5).

Figure 3. Pooled samples treated with KOH

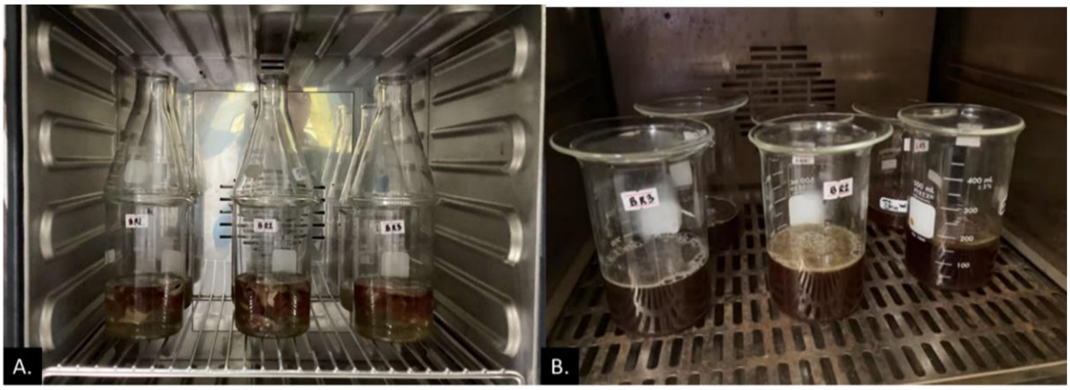

Figure 4. (A) Pooled samples incubated in an oven (B) After 48 hours of incubation

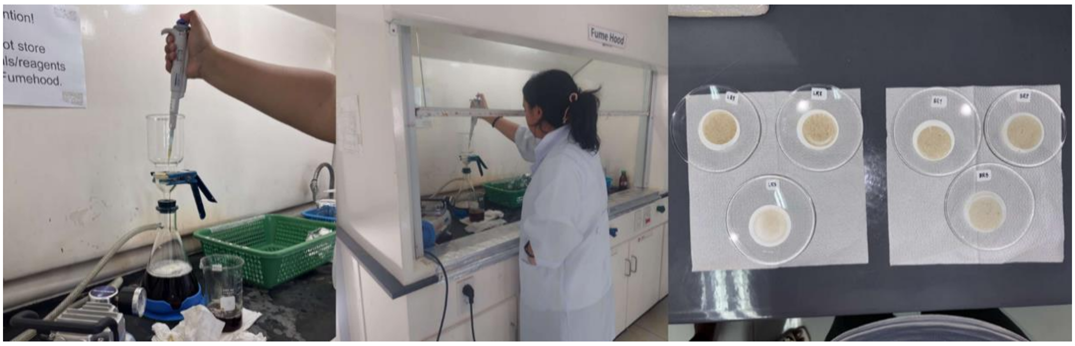

Figure 5. Pooled samples filtered through Whatman. (LR1, 2, & 3-*E. sp.*) (BR1, 2, & 3-*C. chanos*)

Microscopic Inspection and Identification

The filtered replicates were examined under a biological compound microscope at the Natural Products Research and Development, CMU. Figure 6 shows the samples being initially observed under biological compound microscopes at 40x magnification with a microscope digital eyepiece connected to ScopePhoto software for easier identification of particles. The suspected microplastics were then visually identified based on their color, shape, erosion, type, and source, and their abundance was calculated based on the method outlined by Boerger et al. (2010).

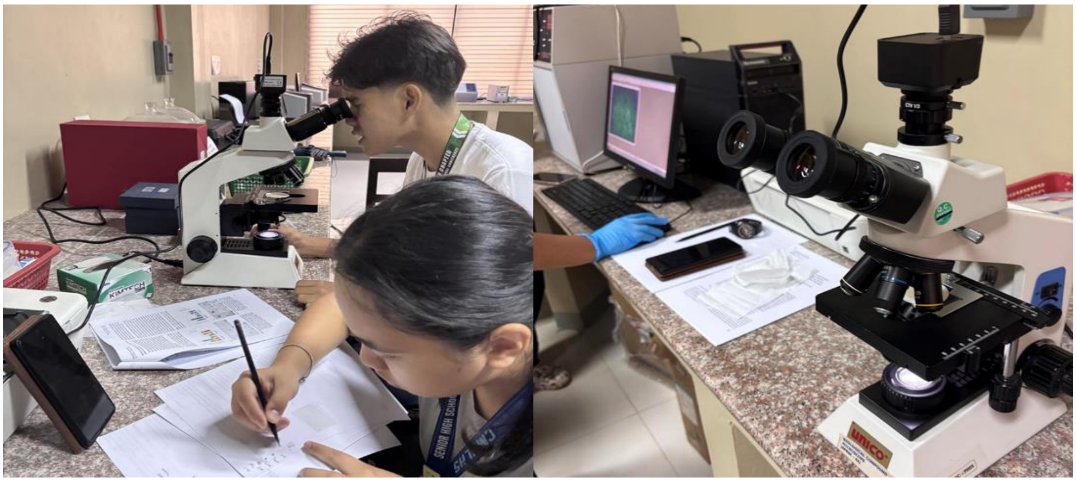

Figure 6. Microplastic inspection and identification

FTIR Analysis

The types of microplastic polymers in the fish samples were analyzed using Fourier Transform Infrared (FTIR) spectroscopy with the potassium bromide (KBr) pellet method, as described by Nor and Obbard (2014). The polymeric wavelength spectra produced by FTIR was analyzed using specialized software and compared against a standard plastic polymer database. This methodology was adapted from the procedures outlined in the study by Wu et al. (2020) and was applied in this study to ensure consistent and reliable results for microplastic identification and analysis.

As shown in Figure 7, Euclidean Distance method was employed to identify the polymer types within the samples (Lusher et al., 2013).

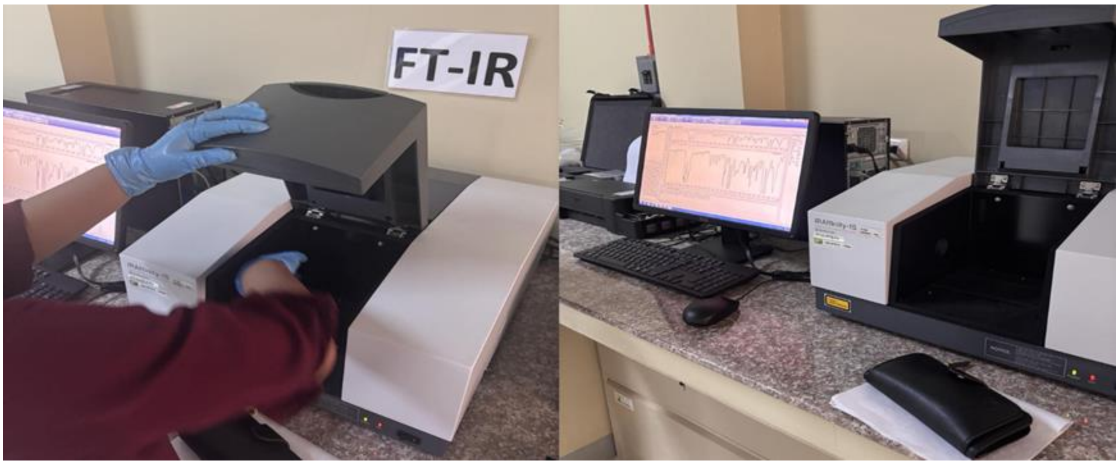

Figure 7. Microplastics were collected and tested through FTIR analysis

Data Gathering Procedure

Fish and Microplastic Characterization

The assessment of fish species and gastrointestinal tracts (GIT) involved measuring morphometric properties, focusing on length, weight, and GIT weight. Microplastics were evaluated based on their physical properties including color, shape, erosion, type and source using FTIR spectra analysis. The fish specimens and microplastics were examined and measured following the procedures outlined below:

Physical Properties

Color, Shape, Erosion, Type, and Source

Color identification in microplastic analysis was done through the combined use of microscopy and Fourier Transform Infrared (FTIR) spectroscopy.

Microscopy enabled the visual sorting and imaging of microplastic particles based on their color and physical appearance (Hidalgo-Ruz et al., 2012). This combination ensured that even visually ambiguous or pigmented particles were accurately identified (Löder & Gerdts, 2015). On the other hand, shape analysis was conducted by visually inspecting isolated particles using a biological compound microscope and noting their physical characteristics, since it can influence how microplastics interact with fish tissues and organs. The level of erosion in microplastics were identified primarily through detailed physical characterization using FTIR spectra. Each microplastic particle type was visually sorted and counted according to these categories, providing insight into the diversity and possible sources of plastic pollution ingested by fish. The source was identified by an FTIR analysis conducted on samples through physical separation of particles from the Whatman filter, transferring them to potassium bromide (KBr) pellets using fine-tipped forceps. The KBr pellets containing the microplastic samples were then placed in FTIR Spectroscopy for analysis (Espiritu et al., 2019).

Microplastic Identification and Quantification

Table 2 presents how microplastics in fish guts were described accordingly based on color, shape, erosion, type and source.

Table 2. Categories used to describe microplastic

CATEGORIES	DESCRIPTION
COLOR	Transparent, crystalline, white, clear-white-cream, red, orange, blue, opaque, black, grey, brown, green, pink, tan, yellow and pigmentation.
SHAPE	For fragments: rounded, sub rounded, sub angular, angular General: irregular, elongated, degraded, rough and broken edges.
EROSION	Fresh, unweather, incipient alteration, and level crazing (conchoidal fractures), weathered, grooves, irregular surface, jagged fragments, linear fractures, sub parallel ridges and very degraded.
TYPE	Fresh, unweather, incipient alteration, and level crazing (conchoidal fractures), weathered, grooves, irregular surface, jagged fragments, linear fractures, sub parallel ridges and very degraded.
SOURCE	Consumer product fragments (e.g. fishing nets) and raw industrial pellets. According to chemical composition (e.g. PE, CS, PU, PP, PVC, PET)

This systematic procedure, based on methodologies outlined by Espiritu (2023), Osman et al. (2023), and Karlsson et al. (2019), ensured the thorough preparation and analysis of gut content from the selected fish species.

Morphometric Properties

Length

Due to the unavailability of standard measuring tools for accuracy and repeatability, the length of *C. chanos* and *E. sp.* was measured using both a ruler and measuring tape at the right angles to the tip of the measurement (Figure 8). The length was first measured at the fish station landing using a measuring tape, and ruler after the fish species samples were transported back to a temporary designated processing site. According to, Man et al. (2016), a ruler is utilized for measuring its standard length, by placing the fish against a flat surface and gently bend the tail upwards and forward and measured from the tip of the snout to the base of the tail fin.

Figure 8. Image of fish species (A) *C. chanos* (B) *E. sp.*

Weight

The weight of *C. chanos*, *E. sp.*, and GIT was measured using a TX2202L Shimadzu digital weighing scale at NPRDC. Each fish samples and GIT were labeled by their corresponding number to familiarize their associated samples and GIT samples and placed into containers to prevent spillage and maintain cleanliness to prevent contaminations (Figure 9). According to Fu et al. (2020), this highlights the use of digital measurement systems for efficiency and accuracy in biological measurements, supporting the use of digital devices for precise and consistent readings. Digital scales provide readable displays, tare features to deduct the weight of trays or containers (handy for weighing dissected organs like the gastrointestinal tract), and fast, consistent readings that improve efficiency.

Figure 9. Weighing of *C. chanos*, *E. sp.*, and their GIT

Statistical Analysis

Descriptive statistics, including frequency and mean, were used to analyze the physical properties of microplastics, specifically color, shape, erosion level, and type. Column graphs and pie charts were utilized to visually represent the distribution, types, and physical characteristics of microplastics, as well as to identify dominant traits and compare prevalence across fish species. To determine the prevalence rate of microplastic contamination across different fish species, the percentage of fish with detectable levels of microplastics was calculated. To address potential bias from small sample sizes, Haldane's correction was applied. For correlation analysis, the study investigated potential relationships between fish species and microplastic contamination levels. Depending on the distribution of data, either Pearson's correlation coefficient (for normally distributed data) or Spearman's rank correlation coefficient (for non-normally distributed data) was used. In comparing the microplastic count and different microplastic types, a One-Way ANOVA test was used to assess whether the microplastic count significantly varied by type in each fish species. A normality test was conducted prior to choosing the appropriate correlation method. All statistical tests, including descriptive analysis, were carried out using Jamovi software. This allowed for clear interpretation of the data and supported the study's objectives by revealing possible associations and trends in microplastic contamination among fish species.

Chapter 4

RESULTS AND DISCUSSION

The presence and extent of microplastic contamination in *C. chanos* and *E. sp.* were determined through microscopic examination. Microplastics were identified and categorized based on their color, shape, type, erosion, and source. Differences in the frequency and distribution of these characteristics between the two species provide insight into potential variations in microplastic exposure and ingestion patterns.

Identification and Quantification of Microplastics

Microplastics found in the guts of commonly traded fish species were classified based on their color, shape, erosion, type, source. These characteristics were used to describe the physical appearance and possible origin of the microplastic particles recovered from the samples. The following findings detail the characteristics assessed:

A. *Color*

Figure 10 shows the color frequency distribution of microplastic (MP) isolated from *C. chanos* and *E. sp*.

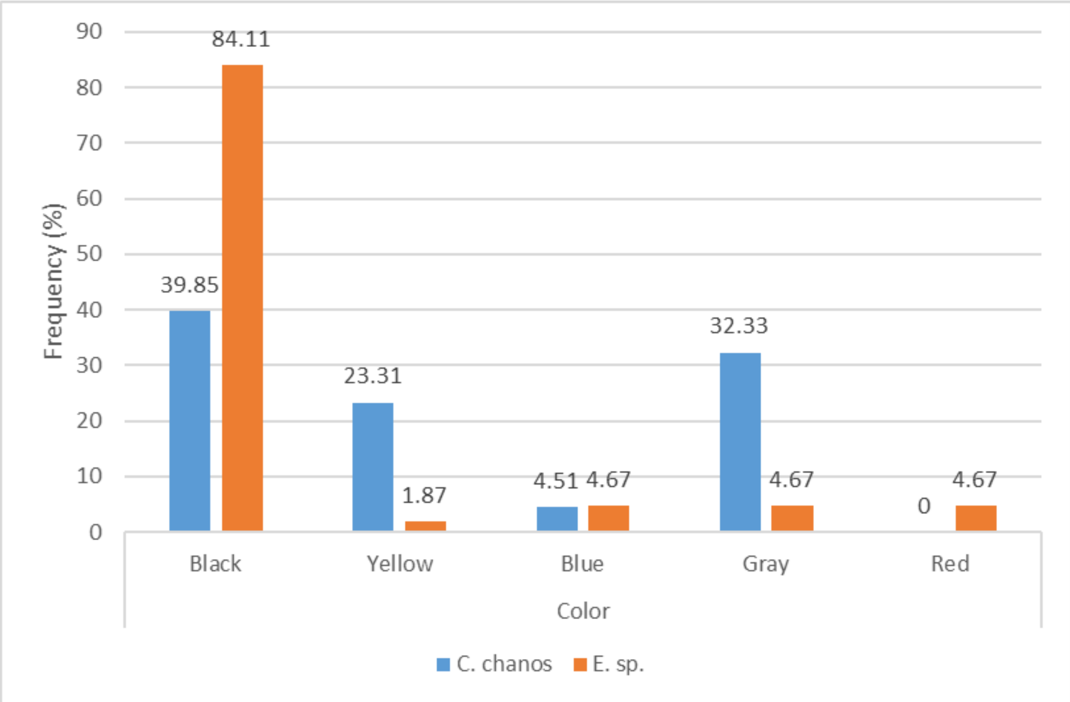

Figure 10. Frequency distribution of microplastic color in *C. chanos* and *E. sp*.

Figure 10 show the results of both fish species, black-colored MPs are the most prevalent, *C. chanos* 39.85% and *E. sp.* 84.11%. According to Huang & Xu (2022), black plastics are some of the least recycled materials, because of their low recycling rates, these are likely to enter the environment, where they break down into microplastics and are attributed to the widespread. The black plastics are consumer products such as industrial sources and diverse plastic pellets, possibly originated from food plastic packaging and their subsequent degradation in aquatic environments (Turner, 2018). The presence of yellow, blue, red, and grayish particles, has less frequent, indicates a diversity of microplastic sources. This suggests that fish are actively consuming microplastics after mistaking them for prey, particularly because brightly colored fragments and fibers resemble natural food sources more than clear plastics, which is known to increase the chances of ingestion (Conowall et al., 2024).

Both fish species has the same mean color percentage (20%), but their distribution also show a significant difference between the color types. *C. chanos* show a relatively balanced distribution disregarding the red color (0%), while the *E. sp.* is heavily predominated by the color black with a low distribution of the other given colors.

According to a study by Seno et al. (2024), it was shown that aquatic environments near urban or industrial areas tend to have diverse microplastic colors due to plastic pollution sources and plastic types present in coastal waters. The detection of various digestive systems of reef and pelagic fish indicates a diverse range of plastic debris in their environment and possible mixing sources.

According to a study by Zhao et al. (2022), because some creatures, like gobies, are visual predators and are likely to consume microplastics that have colors similar to their food, the color of microplastics may have an impact on their consumption by aquatic species. Therefore, while evaluating the ecological risk and toxicity of plastics in the environment, color should also be taken into account.

B. *Shape*

Figure 11 shows the frequency distribution of microplastic shapes in *C. chanos* and *E. sp*.

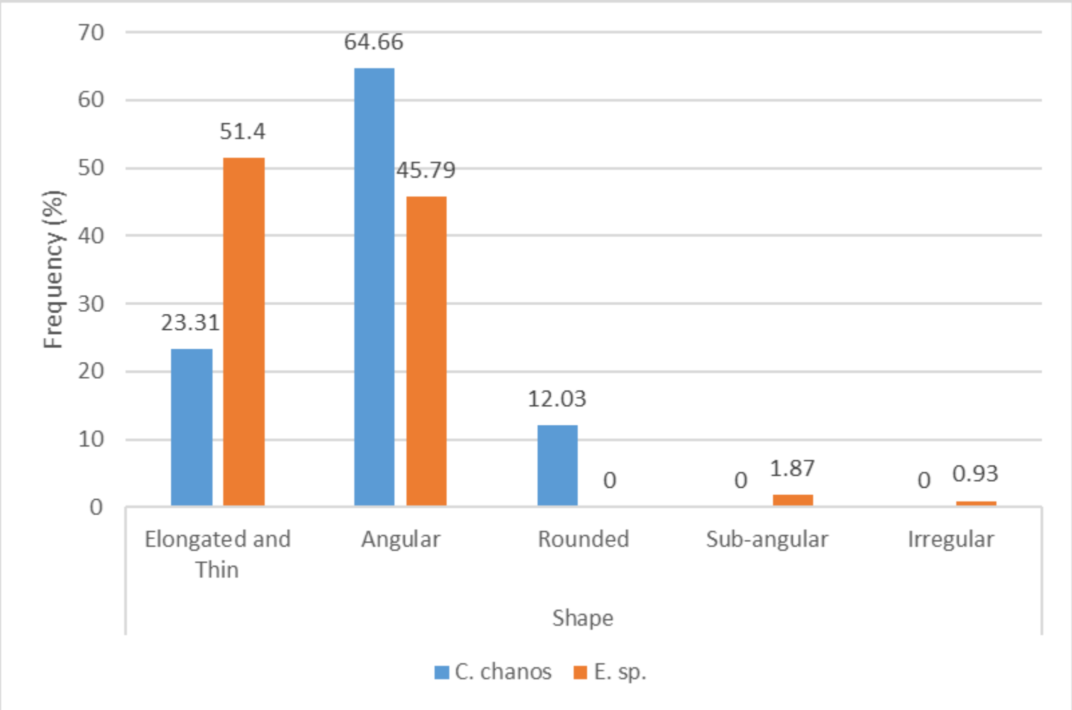

Figure 11. Frequency distribution of microplastic shape in *C. chanos* and *E. sp.*

Based on the results shown in Figure 11, shapes of fragments are predominantly angular, possibly from packaging films such as plastic bags and food containers, filaments are also predominantly elongated and thin, possibly produced from polyester materials and fishing paraphernalia such as fishing nets and lines.

C. chanos contained a higher concentration of angular shaped fragments (64.66%), showing no presence of irregular or subangular shapes, and had other shapes in fragments such as elongated and thin shape (23.31%) and rounded shape (12.03%). *E. sp.* has a more diverse combination with a dominant amount of elongated and thin filaments (51.4%), also containing 1 irregular fragment (0.93%) and 2 subangular filament (1.87%) of each other shape, except for angular shape in fragments (45.79%). Based on these results, *C. chanos* has a stronger concentration in one specific shape (angular), while *E. sp.* is more varied. According to study by Seno et al. (2024), they found that microplastics were mainly categorized into filaments (elongated and thin), fragments (including angular, rounded, and subangular shapes), and films (typically irregular or degraded in appearance). The classification of these shapes, such as angular and rounded fragments, and elongated and thin filaments, was carried out through microscopic analysis, with the presence or absence of subangular and irregular shapes varying based on the sample and type of microplastic observed (Liu et al., 2023).

According to Khan et al. (2023), shape in microplastic such as fragments and fibers can affect a huge impact towards fish, whereas this can translocate to tissues such as gills, muscles, and liver, causing nutritional and growth disorders. A study by Li et al. (2023) states that microplastic shapes affects their capacity to move between tissues after leaving the digestive system. These are likely to cause potential risk factors such as neurotoxicity and organ damage.

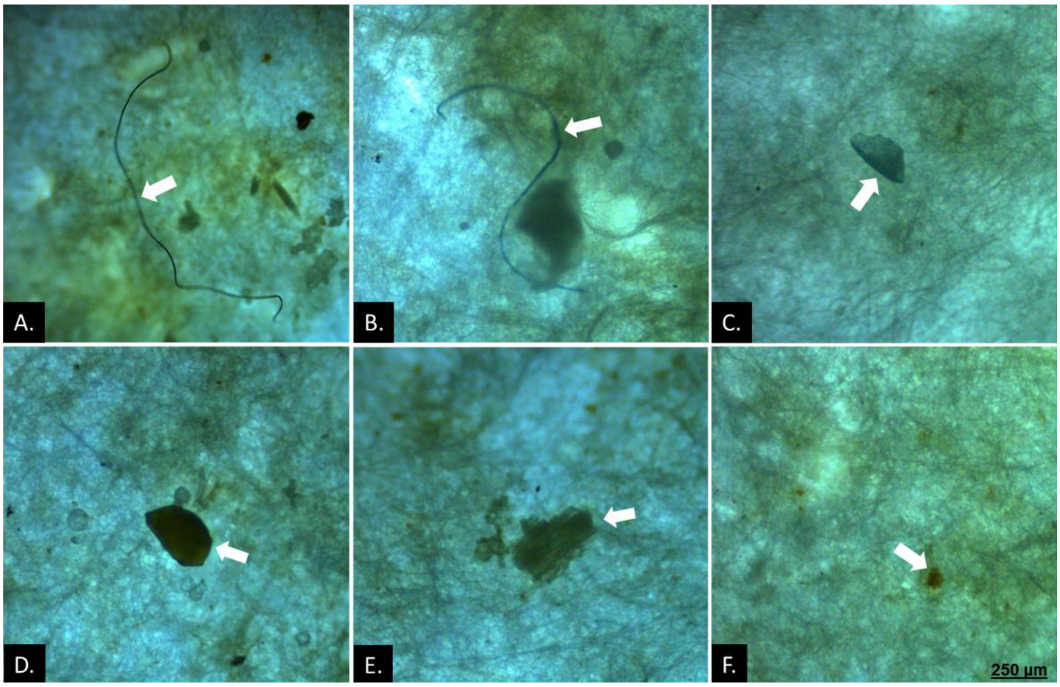

Plate 1. 40x magnification of MP from: *C. chanos* (A) black elongated and thin filament (B) blue elongated and thin filament (C) blue angular fragment (D) yellow angular fragment (E) gray angular film (F) yellow rounded fragment

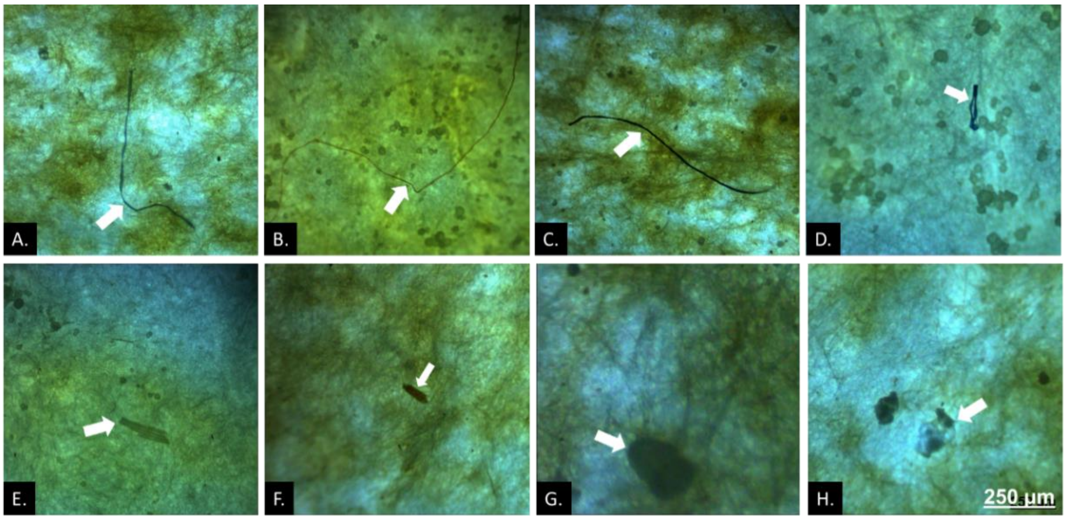

Plate 2. 40x magnification of MP from: *E. sp*. (A) blue elongated and thin filament (B) red elongated and thin filament (C) black elongated and thin filament (D) blue subangular filament (E) gray angular film (F) yellow angular fragment (G) gray angular fragment (H) blue irregular fragment

C. *Erosion*

Figure 12 presents the erosion frequency distribution of microplastics from *C. chanos* and *E. sp*.

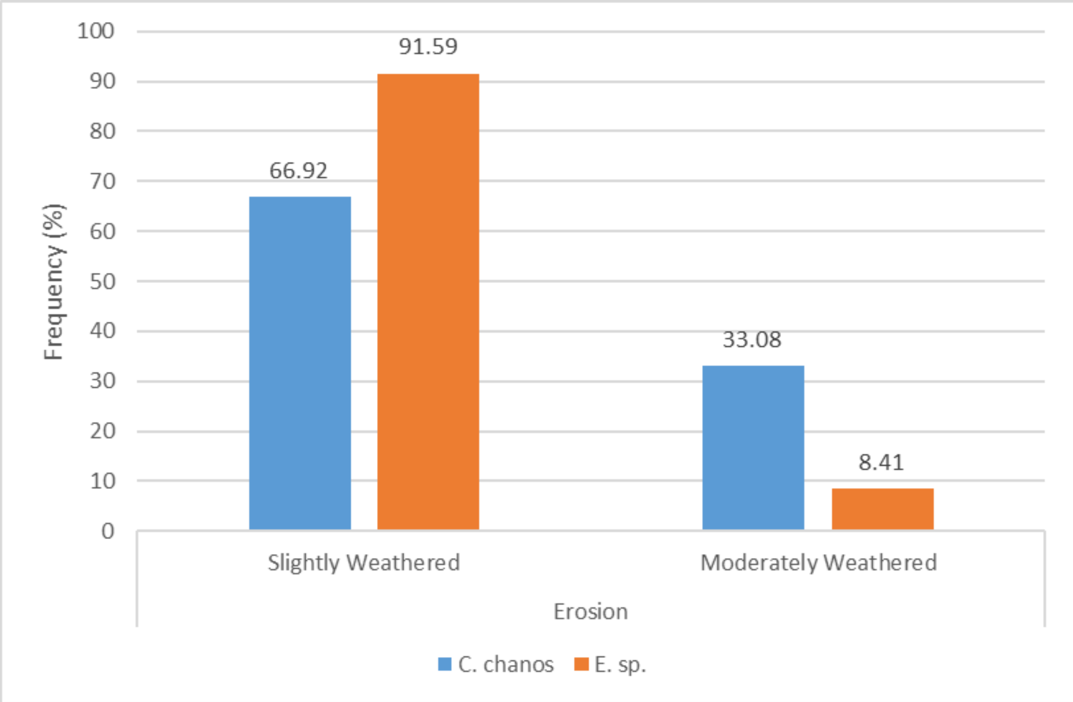

Figure 12. Frequency distribution of microplastic erosion in *C. chanos* and *E. sp*.

Figure 12 shows that *C. chanos* exhibits a more balanced distribution of microplastic erosion levels, with 66.92% classified as slightly weathered and 33.08% as moderately weathered. In contrast, *E. sp.* displays a high concentration of slightly weathered microplastics (91.59%) and a lower concentration of moderately weathered ones (8.41%), suggesting possible recent exposure or limited environmental degradation.

Studies by Similatan et al. (2023) have confirmed the widespread presence of microplastics in commercially important fish species, particularly *C. chanos*, raising concerns about both aquatic ecosystem health and food safety. In the Philippines, research led by the Department of Science and Technology has consistently reported high levels of microplastic contamination in *C. chanos*, with one study finding that 97% of sampled individuals had microplastics in their gastrointestinal tracts (Pustadan, 2024). Although specific data on *E. sp.* remain limited, it is plausible that this species faces similar risks due to its ecological similarities with milkfish and the presence of microplastics in aquaculture environments. The differing proportions of slightly and moderately weathered microplastics between the two species may reflect variations in their habitats, feeding behaviors, and exposure to local pollution sources.

A study by Subaramaniyam et al. (2023) states that erosion levels in microplastics can strengthens their capacity to absorb and move harmful substances including heavy metals and persistent organic pollutants. This can disrupt the gut flora, with absorption of nutrients, causing behavioral changes in habits towards fish. This can also lead to inflammation, oxidative stress, and disruption of gut microbiota, which are linked to a range of health issues such as metabolic disorders and immune dysfunction when consumed by humans (Yuan et al., (2022).

D. *Type*

Figure 13 shows the frequency distribution of microplastic types found in *C. chanos* and *E. sp*.

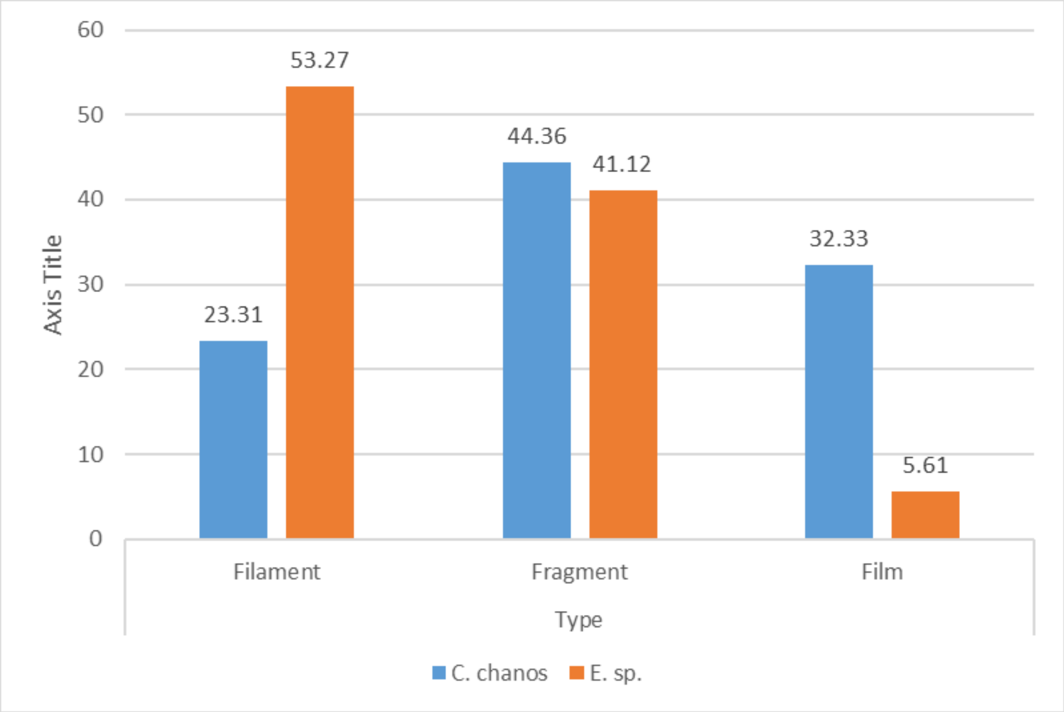

Figure 13. Frequency distribution of microplastic type in *C. chanos* and *E. sp*.

As shown in Figure 13, fragments, films, and filaments are the predominant microplastic types found in the gastrointestinal tracts of the studied fish species. *C. chanos* exhibited a higher proportion of fragment-type microplastics (44.36%), while *E. sp*. had a higher percentage of filaments (53.27%). Both species showed lower percentages of other types: *C. chanos* contained 23.32% filaments and 32.33% films, whereas *E. sp*. had 41.12% fragments and a significantly lower proportion of films at 5.61%, suggesting limited exposure to this type.

These results indicate that *C. chanos* may ingest more fragmented plastic debris such as broken-down food containers whereas *E. sp.* appears more exposed to filament-type microplastics, likely originating from fishing nets or other textile-based materials. This trend aligns with findings from previous studies, which reported similar patterns in fish microplastic contamination (Wang et al., 2019; Jovanović, 2017). Agao-Agao et al. (2024) also found that fragments are often the most abundant microplastic type, with characteristics such as color (e.g., blue) and polymer composition providing insight into their sources, including fishing gear and packaging materials.

Fish often eat these particles by mistake, which can cause internal injuries, block their digestive systems, and harm their gills. These particles also contain toxic chemicals that can build up in fish, affecting their growth, ability to reproduce, and chances of survival (Rochman et al., 2013). When these particles can get into the human body, it may cause inflammation, disrupt hormones, and lead to long-term health problems (Smith et al., 2018).

E. *Source*

Figure 14 presents the frequency of polymer types of microplastics (MPs) isolated from *C. chanos* and *E. sp.*

It shows that the most commonly found MPs in the marine environment include high-density polyethylene (HDPE), low-density polyethylene (LDPE), polyethylene (PE), spandex or polyurethane (PU), and polyethylene terephthalate (PET) or ethyl-vinyl acetate (EVA). This observation aligns with the FTIR spectral data of MPs extracted from the guts of *C. chanos* and *E. sp.*

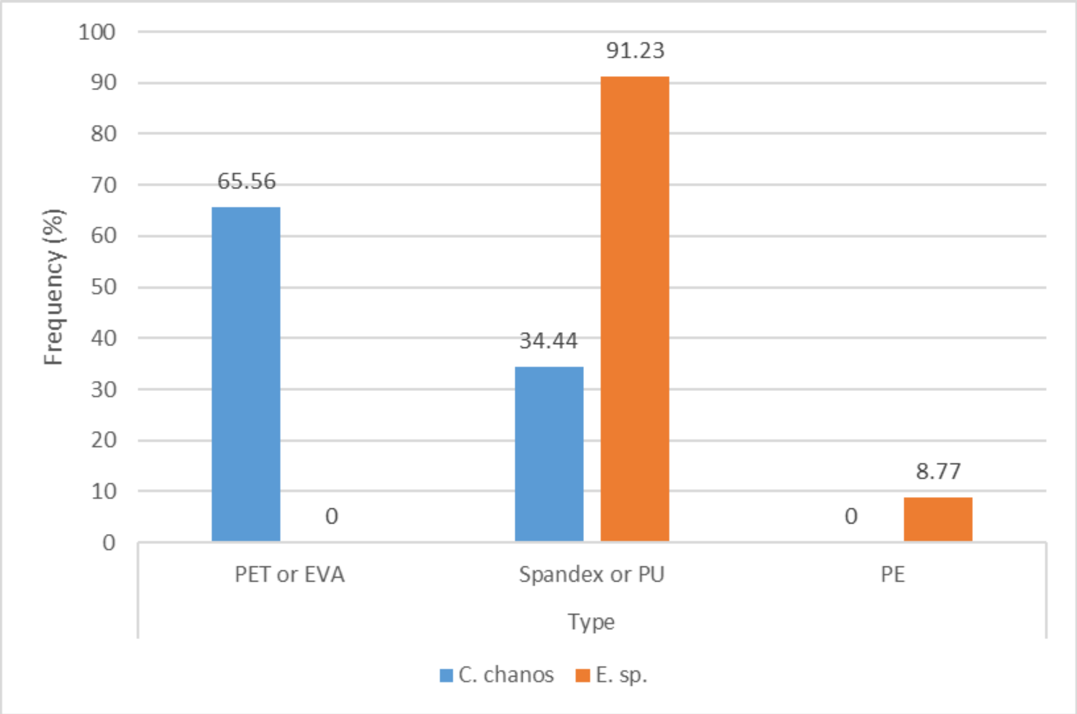

Figure 14. Frequency distribution of polymer in *C. chanos* and *E. sp.*

Results suggest that the predominant types of MPs ingested by *C. chanos* and *E. sp.* are polyethylene (PE) and polyurethane (PU)-based. Specifically, the MPs isolated from *C. chanos* were consistent with compositions of ethylene-vinyl acetate (EVA) or polyethylene terephthalate (PET) and spandex or polyurethane (PU), while those from *E. sp.* appeared to be primarily composed of PE and spandex or PU. This is not unexpected, considering the widespread use of PE-based plastic bags, which are among the most common pollutants in marine environments (Yurtsever & Yurtsever, 2018). Additionally, spandex is extensively utilized in the textile industry, particularly in swimwear, due to its high elasticity and durability (Alam et al., 2020).

Results indicate that *C. chanos* primarily contains PET or EVA polymers, comprising approximately 65.56% of the total microplastics detected, along with spandex or PU at about 34.44%. No PE polymers were identified in this species. In contrast, *E. sp*. exhibited a markedly different polymer profile, with spandex or PU dominating at 91.23%, a small proportion of PE (8.77%), and no detectable PET or EVA. These findings suggest that *C. chanos* is more likely to ingest microplastics originating from food packaging, padding, and containers, whereas *E. sp.* appears to be exposed primarily to sources such as fishing nets and textiles, with limited exposure to materials like detergent bottles or plastic bags.

Due to the hardness and brittleness of certain fragments in *E. sp.* and the film particles found in both species, some samples could not be transferred onto potassium bromide (KBr) pellets for FTIR spectral analysis. As a result, their specific polymer compositions could not be chemically verified. Nonetheless, these particles were still quantified based on their visual identification under a microscope, using morphological characteristics as reference (Espiritu et al., 2019).

Prevalence and Comparative Analysis of Microplastic Contamination by Species

The prevalence of microplastic contamination in *C. chanos* and *E. sp.* was determined by analyzing pooled samples from each species (Table 3). All samples were visually assessed for the presence of microplastics, and the prevalence rate was calculated using Haldane's correction. The results are presented in tabular form to show the extent of contamination across the two fish species.

Table 3. Microplastic Prevalence by Species

Species	**Pooled Samples Tested**	**Pooled Samples Positive**	**Prevalence Rate (%)**
C. chanos	3	3	100%
E. sp.	3	3	100%

Using the adjusted prevalence formula for pooled sampling, the results indicated an estimated 100% prevalence of microplastic contamination in both *C.*

chanos and *E. sp*. All pooled samples tested positive, reflecting widespread contamination across the sampled populations. This uniform result suggests that both species are equally exposed to microplastics in their environment. Consequently, no significant difference in contamination rates was observed between the species. Based on these findings, we fail to reject the null hypothesis (H_0), which states that the prevalence of microplastic contamination is consistent across selected fish species traded at the landing station.

This finding aligns with previous studies conducted in General Santos and Zamboanga. In Sarangani Bay, General Santos, research revealed the presence of microplastic particles in the digestive tracts of commercially important fish species such as yellowfin tuna, skipjack tuna, and spotted rabbitfish, with concentrations reaching up to 2.39 particles per 10 grams in some cases (Earth Journalism Network, 2021). Similarly, a study by Mejos et al. (2023) in Zamboanga City found that all sampled rabbitfish (*Siganus guttatus*) and parrotfish (*Scarus ghobban*) from the Magay public market had ingested microplastics, with rabbitfish containing an average of 51.97 particles and parrotfish 19.29 particles per sample.

Analysis of microplastic ingestion across fish species revealed variations in the number and characteristics of microplastics (MPs) found in different species. For example, certain fish species were more likely to ingest larger microplastic debris, while others consumed smaller particles, suggesting species-specific feeding behaviors and exposure risks (Agao-Agao et al., 2024). While a direct comparison between *C. chanos* and *E. sp.* was not provided in the retrieved articles, similar research by Bakir et al. (2020) on South African fish species demonstrated that sardines ingested the most microplastics (1.58 per fish), followed by herring (1.38) and anchovies (1.13). These findings highlight how feeding habits, habitat choices, and positions in the food chain contribute to species-specific differences in microplastic exposure and ingestion. The study underscores that microplastic contamination varies among fish species, emphasizing the need for focused research to assess ecological risks and guide conservation efforts (Zhang et al., 2020).

In addition, the relationship between biological parameters, specifically fish length, weight, GIT weight, species, and the level of microplastic contamination in fish samples traded at the Valencia City Landing Station, was analyzed. Pearson's correlation coefficient was used for continuous variables, and statistical significance was evaluated at the 0.05 level to determine whether any of the parameters significantly influenced microplastic ingestion. The results offer insights into how body size, feeding condition, and species-specific traits may affect microplastic accumulation in the sampled fish.

Correlation between Microplastic Contamination Levels and Morphometric Parameters

The correlation between morphometric parameters and the total microplastic count in *C. chanos* and *E. sp.* was examined to assess potential associations between body size and microplastic ingestion. Morphometric measurements, including standard total length, whole body weight, and GIT weight, were obtained from individual samples, and statistically analyzed in relation to their respective microplastic counts.

A. *Length*

Table 4 shows the Pearson's correlation analysis between the average length of *C. chanos* and the level of microplastic contamination found in their guts.

Table 4. Correlation between average length and microplastic count in *C. chanos*

	Mean	df	r	p-value
Length	29.3	1	-0.478	0.683
Microplastic Count	44.3			

The correlation coefficient (r) value of -0.478 indicates a very weak negative relationship between the average length of *C. chanos* and the number of microplastics present in their guts. The p-value is 0.683, which is much greater than the standard significance level of 0.05. This means that the observed correlation is not statistically significant, and there is no meaningful linear association between fish length and microplastic contamination in this sample.

The results suggest that the length of *C. chanos* does not significantly influence the amount of microplastic contamination. This implies that microplastic ingestion occurs across all size groups, regardless of whether the fish are small or large. This pattern may be due to similar feeding habits or environmental exposure among *C. chanos* of different sizes, or it could indicate that microplastics are so prevalent in the environment that all individuals are equally at risk. The lack of a significant correlation means that, in this study, fish size cannot be used as a predictor for microplastic contamination levels.

The findings are consistent with Seno et al. (2024), investigated the relationship between fish length and microplastic ingestion in pelagic fish species from Mindanao, Philippines. Their statistical analysis showed no significant correlation between fish length and the abundance of microplastics in the gastrointestinal tract (r = -0.235, p = 0.418). This suggests that fish length is not a determining factor for microplastic contamination levels in pelagic fish, indicating that microplastic ingestion occurs independently of fish size in these species.

The findings of this study are further reinforced by Koongolla et al. (2022), The analysis revealed no significant correlation between fish length and microplastic abundance (p = 0.287), indicating that fish size does not significantly influence microplastic contamination levels. This evidence from a region adjacent to the Philippines strengthens the conclusion that microplastic ingestion in pelagic fish occurs independently of fish length.

Table 5 shows the Pearson's correlation analysis between the average length of *E. sp.* and the level of microplastic contamination found in their guts.

Table 5. Correlation between average length and microplastic count in *E. sp*.

	Mean	df	r	p-value
Length	24.7	1	0.405	0.735
Microplastic Count	35.7			

The correlation coefficient (r = 0.405) indicates a weak positive correlation between fish length and MP count, but the high p-value (p = 0.735) confirms that this is not statistically significant. The mean MP count (35.7) exceeds the mean fish length (24.7 cm), suggesting that MP contamination occurs across all size classes of demersal fish.

The lack of a significant correlation implies that microplastic ingestion in demersal fish is not size-dependent. This aligns with ecological principles where feeding behavior and habitat sediment contamination, rather than individual body size, drive MP exposure. Smaller and larger fish likely encounter MPs at similar rates due to uniform environmental MP distribution in benthic zones.

These findings are consistent with previous studies, such as Bersaldo et al. (2024), which investigated the relationship between fish length and microplastic count in *Mulloidichthys vanicolensis* (yellow striped goatfish), a demersal species from Malita, Davao Occidental. The study found a weak correlation ($r^2 = 0.13$) between fish total length and microplastic count, which was not statistically significant. This suggests that microplastic ingestion in this species occurs independently of the fish's size. In the study by Koongolla et al. (2022), 271 individuals from 32 marine fish species were collected, concluded that there was no statistically significant correlation between microplastic abundance and body length in demersal fish species ($p = 0.287$).

B. *Weight*

Table 6 presents the Pearson’s correlation analysis between the average weight of *C. chanos* and the number of microplastic particles found in their guts.

Table 6. Correlation between average weight and microplastic count in *C. chanos*

	Mean	df	r	p-value
Weight	481	1	-0.167	0.893
Microplastic Count	44.3			

The average weight of the sampled milkfish was 481 grams, while the mean microplastic count was 44.3 particles per fish. The correlation coefficient (r) between fish weight and microplastic count was -0.167, indicating a weak negative relationship. However, the p-value of 0.893, which is much higher than the standard threshold for statistical significance (0.05), indicates that the observed correlation is not statistically significant. Therefore, there is no evidence of a meaningful association between fish weight and the number of microplastics ingested in this sample.

The negative correlation coefficient suggests that, in this sample, heavier milkfish tended to have slightly fewer microplastic particles in their guts. However, given the extremely weak correlation and non-significant p-value, this trend is unreliable and likely the result of random variation. This implies that microplastic ingestion in milkfish is not influenced by weight or size. Instead, other factors, such as feeding behavior, habitat, or environmental availability of microplastics, may play a more significant role. The relatively consistent presence of microplastics across different fish weights suggests that microplastic pollution is pervasive and affects milkfish regardless of their size.

These findings by Cabansag et al. (2021), demonstrated widespread microplastic ingestion among fish, regardless of size or weight. Statistical analysis revealed no significant correlation between fish weight and the number of ingested microplastics, supporting the idea that contamination occurs across size classes. These findings are consistent with the present study's conclusion that environmental exposure and feeding behavior, rather than fish size, are the primary factors influencing microplastic ingestion in commonly traded fish species in the Philippines. Furthermore, the results are corroborated by Hastuti et al. (2019), reported no statistically significant correlation between microplastic count and fish body parameters, including weight, in commercially important fish species. While the direction of correlation in our study differs slightly, both studies suggest that fish weight is not a strong predictor of microplastic ingestion. This reinforces the interpretation that factors such as feeding behavior and environmental exposure likely exert a greater influence on microplastic uptake than morphological characteristics like body weight.

Table 7 shows the Pearson's correlation analysis between the average weight of *E. sp.* and the level of microplastic contamination found in their guts.

Table 7. Correlation between average weight and microplastic count in *E. sp*.

	Mean	df	r	p-value
Weight	361	1	-0.841	0.364
Microplastic Count	35.7			

As shown, the mean average weight of the fish samples is 361 grams, while the mean microplastic count remained at 35.7 particles. The correlation coefficient is –0.841, indicating a strong negative correlation between fish average weight and microplastic count. The p-value of 0.364 confirms that this relationship is not statistically significant.

The results indicate that there is virtually no linear relationship between the weight of fish and the number of microplastics present in their guts. The correlation is nearly -1 and statistically insignificant, suggesting that fish weight does not strongly influence the degree of microplastic contamination. This aligns with the idea that microplastic ingestion is likely influenced by behavioral and environmental factors rather than by body mass alone.

This finding is supported by the study of Hamdhani et al. (2024), suggests that demersal fish such as jacks, blackspot snapper, and coral grouper may ingest higher quantities of microplastic particles. However, the correlation between the average number of ingested microplastics and the average fish weight per species showed a negative relationship ($R^2 = 0.36$) that was not statistically significant ($p = 0.28$). Similarly, the study by Adika et al. (2020b), reported weak positive correlations between microplastic abundance and both the length and weight of demersal fish species. Nonetheless, the low R^2 values indicated that these relationships were not strong or meaningful.

C. *GIT Weight*

Table 8 presents the Pearson's correlation analysis between the average GIT weight of *C. chanos* and the number of microplastic particles found in their guts.

Table 8. Correlation between average GIT weight and microplastic count in *C. chanos*

	Mean	df	r	p-value
GIT Weight	20.1	1	0.091	0.942
Microplastic Count	44.3			

Results show that the mean gastrointestinal tract (GIT) weight of *C. chanos* was 20.1 grams, while the mean microplastic count was 44.3 particles. The correlation coefficient (r) between GIT weight and microplastic count was 0.091, with a p-value of 0.942. This very low r-value suggests a negligible positive relationship, and the high p-value indicates that the correlation is not statistically significant.

These findings imply that there is no meaningful association between the GIT weight of *C. chanos* and the quantity of microplastics ingested. This suggests that microplastic ingestion in these fish is not influenced by GIT weight. Instead, ingestion appears to occur randomly and opportunistically, likely reflecting the pervasive presence of microplastics in the aquatic environment rather than any physiological capacity related to feeding. This is consistent with biological principles, as microplastic exposure is more closely tied to environmental contamination and feeding behavior than to morphological traits such as GIT weight.

The findings are further supported by Ismail et al. (2018), analysis of various fish species, including herbivores, revealed no significant correlation between GIT weight and microplastic ingestion. Despite the presence of microplastics in the digestive systems, GIT weight did not predict microplastic load, reinforcing the conclusion that it is not a reliable indicator of microplastic contamination, even among fish with similar feeding strategies.

Table 9 presents the Pearson's correlation analysis between average GIT weight of *E. sp.* and the number of microplastic particles found in their guts.

Table 9. Correlation between average GIT weight and microplastic count in *E. sp.*

	Mean	df	r	p-value
GIT Weight	8.87	1	-0.953	0.196
Microplastic Count	35.7			

Based on the table, the average GIT weight is 8.87, while the mean microplastic count remained at 35.7 particles. The Pearson correlation coefficient of -0.953 suggests a very strong negative relationship between GIT weight and microplastic levels. The p-value of 0.196 is above the 0.05 threshold, indicating no statistically significant correlation.

The data implies that GIT weight does not significantly correlate with the amount of microplastic ingested. Although a slightly positive trend exists, it is not strong or statistically significant, indicating that higher GIT weight scores do not reliably predict higher microplastic contamination. This may suggest that microplastics can be ingested regardless of the GIT weight, possibly due to their accidental ingestion along with food or their presence in ambient water environments.

These results correspond with the findings of Gad & Midway (2022), suggesting that even among omnivorous fishes, the amount of microplastic does not certainly increase with the fish species' weight of digestive tracts. The study of (Matluba et al., 2023), concluded that microplastic ingestion is not related to body size, condition factor, or digestive tracts. The amount of plastics consumed did not correlate with GIT weight in demersal fish.

Comparison of Microplastic Count Between Microplastic Types

The occurrence of various microplastic types was compared within each fish species to determine which forms were most frequently ingested. Microplastics were classified into filaments, fragments, and films, and their mean counts were analyzed separately for *C. chanos* and *E. sp*.

A. *C. chanos*

Table 10 presents the statistical results of one-way ANOVA results comparing microplastic counts in *C. chanos* based on microplastic types.

Table 10. One-Way ANOVA profiles among microplastic types in *C. chanos*.

	F	df1	df2	p
Microplastic Count	0.718	2	6	0.526

Based on the results, the analysis yielded an F-value of 0.718 with a p-value of 0.526 for the comparison between microplastic counts across different types identified in *C. chanos*. At the 0.05 level of significance, these results indicate that there are no statistically significant differences in microplastic counts among the different types (fragments, filaments, films) found in *C. chanos*.

While there are slight variations in the number of each microplastic type, the differences are not substantial enough to be considered statistically meaningful. This suggests that the observed variation is likely due to random fluctuations rather than actual disparities in microplastic type distribution. The findings imply that *C. chanos* may be exposed to a relatively balanced mixture of microplastic types in its environment.

This interpretation is consistent with the study of Sembiring et al. (2020), which reported that fragments were the most dominant type of microplastic observed in *C. chanos*. However, that study did not perform statistical comparisons among microplastic types. Thus, while fragments may appear more frequent, the lack of significant statistical difference supports the notion that *C. chanos* is likely ingesting various types of microplastics in similar proportions.

B. *E. sp.*

Table 11 presents the statistical results of one-way ANOVA results comparing microplastic counts in *E. sp.* based on microplastic types.

Table 11. One-Way ANOVA profiles among microplastic types in *E. sp*.

	F	df1	df2	p
Microplastic Count	2.98	2	6	0.126

As shown in Table 11, the analysis yielded an F-value of 2.98 and a p-value of 0.126 for the comparison of microplastic counts across different types identified in *E. sp*. Since the p-value exceeds the 0.05 significance level, the results indicate that there are no statistically significant differences in microplastic counts among the various microplastic types found in this species.

The data suggest that the observed differences may be attributed to random variation. Although filaments were the most frequently encountered type, the analysis did not provide sufficient statistical evidence to confirm that their prevalence is significantly different from other types.

Despite the lack of statistical significance, the moderately high F-value and p-value close to the threshold support the descriptive trend that *E. sp.* tends to ingest predominantly filament-type microplastics. This aligns with findings by Cabansag et al. (2021), which reported that marine species like *E. sp.* are likely to encounter greater concentrations of filament microplastics in coastal waters, largely due to human activities and environmental conditions.

These findings suggest that the classification of microplastic types may not significantly influence the quantity of microplastics ingested by either *C. chanos* or *E. sp*. Rather, the relatively uniform ingestion across types indicates a broad and pervasive exposure to microplastic pollution, underscoring the widespread nature of microplastic contamination in aquatic environments.

<u>Microplastic Count in Each Fish Species</u>

The overall microplastic count in *C. chanos* and *E. sp.* was evaluated to determine the level of microplastic ingestion across the two species. The extracted microplastics were categorized into filaments, fragments, and films. The results are presented in a stacked bar chart, with each bar showing the total microplastic count and the distribution of the different microplastic types.

Figure 15 presents the microplastic types and counts observed in the gastrointestinal tracts of *C. chanos* and *E. sp*. The predominant microplastic types identified are filaments, fragments, and films.

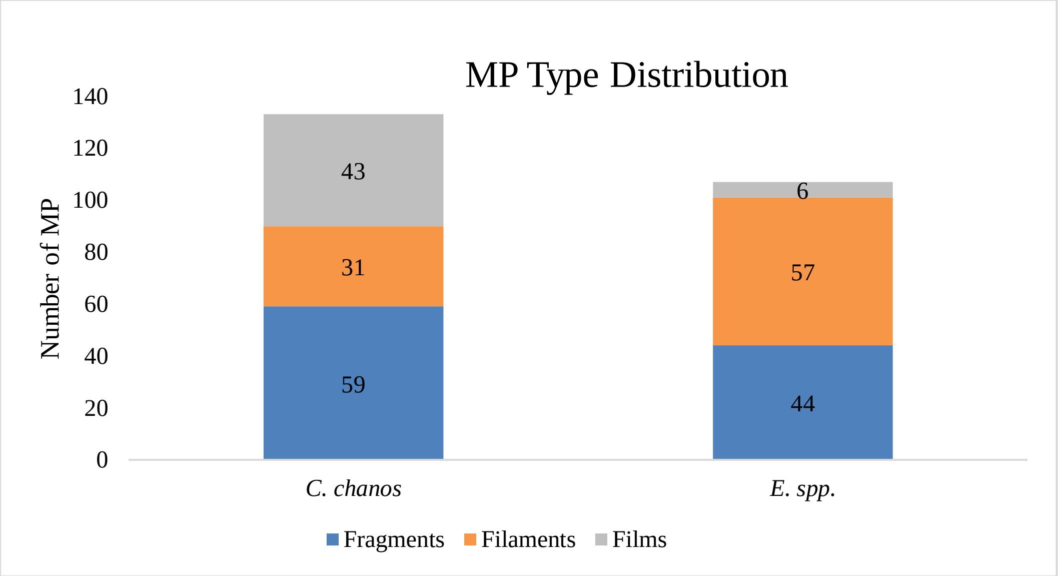

Figure 15. Microplastic types and counts found in the gastrointestinal tracts of *C. chanos* and *E. sp*.

Based on table figure 15, *C. chanos* exhibits a higher microplastic count compared to *E. sp.*, with 133 microplastics versus 107 microplastics, respectively. This suggests that milkfish accumulate more microplastics within their digestive tracts than highfin grouper. When examining the composition of microplastic types, filaments are generally the most abundant in *E. sp.*, followed by fragments and films. In contrast, fragments dominate in *C. chanos*, followed by films and filaments.

The elevated microplastic count observed in *C. chanos* likely reflects greater exposure or ingestion rates, potentially linked to their feeding behavior and habitat preferences. Milkfish are commonly found in coastal and brackish waters, where microplastic pollution can be significant. The predominance of filaments and fragments corresponds with known pollution sources such as textile fibers and degraded plastic debris. Ecologically, this pattern aligns with the fact that many microplastics possess low density and tend to float near the water surface, increasing their availability to pelagic or surface associated fish species like milkfish.

These findings coincide with previous studies reporting similar microplastic contamination patterns in fish species. For example, (Ghosh et al., 2021; Athukorala et al., 2024) found filaments, fragments, and films as the dominant microplastic types in fish GITs. Moreover, a study in Mindanao reported 235 microplastic particles in 30 milkfish samples, confirming the high contamination levels in this species (Vivienne Angeles, 2024). The higher microplastic occurrence in pelagic fish compared to demersal fish is further supported, where pelagic fish species showed a 39.1% occurrence rate versus 10.3% in demersal fish species (Yagi et al., 2021), attributed to the floating nature of many microplastics due to low density, (Hidalgo-Ruz et al., 2012), as well as the ecological niches of these species, since pelagic fish live from the surface to the middle layers of the water column (Susilo, 2010), whereas demersal fish inhabit the bottom of the waters (Wahyuni et al., 2009).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

Conclusion

1. Microplastics were detected in the gastrointestinal tracts of two commonly traded fish species from the Valencia City Fish Landing Port in Bukidnon: *C. chanos* (milkfish) and *E. sp.* (highfin grouper). The microplastics varied in color, shape, degree of weathering, type, and suspected source. Black was the most frequently observed color in both species, with *E. sp.* exhibiting a more concentrated occurrence. Most microplastic particles in *C. chanos* were angular in shape, while *E. sp.* showed greater morphological diversity, predominantly featuring elongated and thin filaments. In terms of surface condition, *C. chanos* displayed a balanced distribution of slightly and moderately weathered particles, whereas *E. sp.* had a higher proportion of slightly weathered microplastics. Fragment-type microplastics were most prevalent in *C. chanos*, while filament types dominated in *E. sp*. Primary polymer types in *C. chanos* included polyethylene terephthalate (PET) or ethylene-vinyl acetate (EVA), and spandex or polyurethane (PU). In contrast, *E. sp.* primarily contained spandex or PU, with some traces of polyethylene (PE). These results suggest that both fish species are exposed to a wide range of microplastic pollutants, likely originating from domestic, industrial, and textile-related sources.

2. Microplastics were detected in 100% of both *C. chanos* and *E. sp.* samples. There are no significant differences in contamination rates between the two species, suggesting that microplastic pollution is consistently present among fish sold at the

landing site. Although the overall prevalence was similar, the types of microplastics varied between species, likely due to differences in habitat, feeding behavior, and levels of environmental exposure. Given that these fish are important dietary protein sources for many Filipinos, the presence of microplastics raises critical concerns regarding potential health risks for consumers.

3. The analysis revealed no significant correlation between morphometric parameters such as length, weight, and gastrointestinal tract (GIT) weight, and microplastic contamination levels in both *C. chanos* and *E. sp*. The correlation coefficients were generally weak, and the p-values were well above the 0.05 significance threshold. These findings suggest that microplastic ingestion is independent of length, weight, or GIT weight. Instead, the results indicate that environmental factors and feeding behaviors likely play a more significant role in microplastic exposure than the individual physical characteristics of the fish.

4. Ingestion rates for different types of microplastics such as fragments, filaments, and films did not show statistically significant differences within each fish species. Although some microplastic types appeared more frequently in descriptive observations, these differences were not significant. This suggests that demersal fish are not selectively ingesting specific types of microplastics. Instead, they are exposed to a relatively uniform distribution of microplastic types, which reflects the widespread presence of these materials in their environment.

5. The analysis of microplastic contamination in the gastrointestinal tracts of *C. chanos* and *E. sp.* reveals that milkfish accumulate more microplastics (133 MPs) than highfin grouper (107 MPs). The dominant microplastic types differ between the

two species: fragments are most prevalent in milkfish, while filaments are most common in highfin grouper. These differences likely reflect variations in feeding behavior and habitat, with milkfish—being pelagic and surface-associated—being exposed to higher levels of floating microplastics, typical of coastal and brackish environments.

Recommendations

1. Consumers should be mindful of the potential microplastic contamination in locally sourced fish and consider moderating consumption from areas with high pollution. Opting for fish from regulated sources can reduce risks.
2. Local authorities should enhance monitoring of microplastic levels in aquatic environments and enforce stricter waste management regulations to reduce plastic pollution in water sources.
3. Future studies should increase the sample size to improve statistical reliability and capture a broader range of microplastic contamination variability across individual fish specimens.
4. Instead of pooled sampling, future research should focus on individual fish analysis to accurately assess the extent of microplastic ingestion in each specimen.
5. To eliminate geographical bias, future studies should source both fish species from the same location for a more controlled and comparable assessment of contamination levels.
6. Researchers should consider analyzing other tissues, such as muscle or liver, to assess microplastic accumulation and its potential risks to human consumers.

7. Incorporating water and sediment samples will provide essential environmental context, helping to identify potential sources of microplastic contamination in aquatic ecosystems.
8. Future research should include seasonal sampling to explore the temporal dynamics of microplastic pollution, offering insights that were not covered in this study due to time constraints.

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APPENDIX

Appendix Tables

Appendix Table 1. Raw mean data of length, weight of GIT, and pooled sample GIT weight in *C. chanos*.

Fish Species	Pooled Sample	Fish Weight, g	Length, cm	GIT Weight, g
C. chanos	BR1	494.285	29.85	16.205
	BR2	487.320	29.30	17.535
	BR3	459.955	28.75	26.700

Appendix Table 2. Raw mean data of length, weight of GIT, and pooled sample GIT weight in *E. sp*.

Fish Species	Pooled Sample	Fish Weight, g	Length, cm	GIT Weight, g
E. sp.	LR1	337.49	26.05	6.630
	LR2	325.93	23.35	9.430
	LR3	420.73	24.80	10.545

Appendix Table 3. Type and number of microplastic in the *C. chanos* per pooled samples.

Fish Species	Types	Microplastic (MP)				
		Pooled Samples and Quantity			Total Types of MPs	Total of Overall MPs
		BR1	BR2	BR3		
C. chanos	Filament	10	18	3	31	133
	Fragment	23	12	24	59	
	Film	0	26	17	43	

Appendix Table 4. Type and number of microplastic in the *E. sp.* per pooled samples.

Fish Species	Types	Microplastic (MP)				
		Pooled Samples and Quantity			Total Types of MPs	Total of Overall MPs
		LR1	LR2	LR3		
E. sp.	Filament	28	13	16	57	107
	Fragment	20	24	0	44	
	Film	5	0	1	6	

Appendix Table 5. Color and number of microplastic in the *C. chanos* per pooled samples.

Fish Species	Colors	Microplastic (MP)			
		Pooled Samples and Quantity			Total Types of MPs
		BR1	BR2	BR3	
C. chanos	Black	12	17	24	53
	Blue	16	12	3	31
	Yellow	5	1	1	6
	Gray	0	26	17	43

Appendix Table 6. Color and number of microplastic in the *E. sp.* per pooled samples.

Fish Species	Colors	Microplastic (MP)			
		Pooled Samples and Quantity			Total Types of MPs
		BR1	BR2	BR3	
E. sp.	Black	45	35	10	90
	Blue	2	0	0	2
	Yellow	2	1	2	5
	Gray	4	0	1	5
	Red	0	1	4	5

Appendix Table 7. Shape and number of microplastic in the *C. chanos* per pooled samples.

Fish Species	Types	Shape	Microplastics (MP)			
			Pooled Samples and Quantity			Total Shapes of MPs
			BR1	BR2	BR3	
C. chanos	Filament	Elongated and Thin	10	18	3	31
	Fragment	Angular	9	12	22	43
		Rounded	14	0	2	16
	Film	Angular	0	26	17	43

Appendix Table 8. Shape and number of microplastic in the *E. sp.* per pooled samples.

Fish Species	Types	Shape	Microplastics (MP)			
			Pooled Samples and Quantity			Total Shapes of MPs
			LR1	LR2	LR3	
E. sp.	Filament	Elongated and Thin	27	13	15	55
		Subangular	1	0	1	2
	Fragment	Angular	19	24	0	43
		Irregular	1	0	0	1
	Film	Angular	5	0	1	6

Appendix Table 9. Erosion and number of microplastic in the *C. chanos* per pooled samples.

Fish Species	Types	Erosion	Microplastics (MP)			
			Pooled Samples and Quantity			Total Shapes of MPs
			BR1	BR2	BR3	
C. chanos	Filament	Slightly weathered	10	18	3	31
	Fragment	Slightly weathered	23	12	23	58
		Moderately weathered	0	0	1	1
	Film	Moderately weathered	0	26	17	43

Appendix Table 10. Erosion and number of microplastic in the *E. sp.* per pooled samples.

Fish Species	Types	Erosion	Microplastics (MP)			
			Pooled Samples and Quantity			Total Shapes of MPs
			LR1	LR2	LR3	
E. sp.	Filament	Slightly weathered	27	12	16	55
		Moderately weathered	1	1	0	2
	Fragment	Slightly weathered	19	24	0	43
		Moderately weathered	1	0	0	1
	Film	Moderately weathered	5	0	1	6

Appendix Table 11. Type of plastic and possible inferred sources of microplastic in the *C. chanos* and *E. sp.* per replicate and per species.

Fish Species	MP	Type of Plastic	Inferred Source
C. chanos	Black Fragment	PET or EVA	Food packaging
	Yellow Fragment	PET or EVA	Padding or food containers
	Blue Filament	Spandex or PU	Fishing nets or clothing
	Black Filament	Spandex or PU	Fishing nets or clothing
E. sp.	Black Filament	Spandex or PU	Fishing net
	Blue Filament	Spandex or PU	Clothing
	Red Filament	PE (HDPE or LDPE)	Detergent bottles or plastic bags

Appendix Table 12. FTIR Spectra of MPs from *C. chanos* and *E. sp.* GIT.

Fish	MP Color and Type	Diagnostic Bands, cm^{-1}	Possible Composition of MP
C. chanos	Black Fragment	2945 (CH stretching), 2031(overtone)1750 (C=O), 611 (aromatic fingerprint)	EVA or PET
	Yellow Fragment	2918 (CH), 2019 (overtone) 1741 (C=O), 615 (aromatic fingerprint)	EVA or PET
	Black Filament	3381 (NH), 2924 (CH), 1734 (C=O), 1537 (C=C aromatic), 613 (aromatic fingerprint)	Spandex or PU
	Blue Filament	3450 (NH), 2920 (CH), 1737 (C=O), 1622 (C=C aromatic), 611 (aromatic fingerprint)	Spandex or PU
E. sp.	Black Filament	3396 (NH), 2916 (CH), 1724 (C=O), 1548 (C=C aromatic), 648 (aromatic fingerprint)	Spandex or PU
	Blue Filament	3630 (NH), 2920 (CH), 1714 (C=O), 1537 (C=C aromatic), 619 (aromatic fingerprint)	Spandex or PU
	Red Filament	2914 (CH),1400 (CH rocking)	PE (degraded LDPE or HDPE)

Appendix Table 13. Jamovi Pearson product-moment correlation coefficient in analyzed relationship of average length and the MP presence *C. chanos*.

Correlation Matrix

		Average Length	Microplastic Count
Average Length	Pearson's r	—	
	df	—	
	p-value	—	
Microplastic Count	Pearson's r	-0.478	—
	df	1	—
	p-value	0.683	—

Descriptive

	Average Length	Microplastic Count
N	3	3
Mean	29.3	44.3
Standard deviation	0.550	11.5

Appendix Table 14. Jamovi Pearson product-moment correlation coefficient in analyzed relationship of average length and the MP presence of *E. sp*.

Correlation Matrix

		Average Length	Microplastic Count
Average Length	Pearson's r	—	
	df	—	
	p-value	—	
Microplastic Count	Pearson's r	0.405	—
	df	1	—
	p-value	0.735	—

Descriptive

	Average Length	Microplastic Count
N	3	3
Mean	24.7	35.7
Standard deviation	1.35	18.0

Appendix Table 15. Jamovi Pearson product-moment correlation coefficient in analyzed relationship of average weight and the MP presence of *C. chanos*.

Correlation Matrix

		Average Weight	Microplastic Count
Average Weight	Pearson's r	—	
	df	—	
	p-value	—	
Microplastic Count	Pearson's r	-0.167	—
	df	1	—
	p-value	0.893	—

Descriptive

	Average Weight	Microplastic Count
N	3	3
Mean	481	44.3
Standard deviation	18.1	11.5

Appendix Table 16. Jamovi Pearson product-moment correlation coefficient in analyzed relationship of average weight and the MP presence of *E. sp*.

Correlation Matrix

		Average Weight	Microplastic Count
Average Weight	Pearson's r	—	
	df	—	
	p-value	—	
Microplastic Count	Pearson's r	-0.841	—
	df	1	—
	p-value	0.364	—

Descriptive

	Average Weight	Microplastic Count
N	3	3
Mean	361	35.7
Standard deviation	51.7	18.0

Appendix Table 17. Jamovi Pearson product-moment correlation coefficient in analyzed relationship of GIT weight and the MP presence of *C. chanos*.

Correlation Matrix

		GIT Weight	Microplastic Count
GIT Weight	Pearson's r	–	
	df	–	
	p-value	–	
Microplastic Count	Pearson's r	0.091	–
	df	1	–
	p-value	0.942	–

Descriptive

	GIT Weight	Microplastic Count
N	3	3
Mean	20.1	44.3
Standard deviation	5.71	11.5

Appendix Table 18. Jamovi Pearson product-moment correlation coefficient in analyzed relationship of GIT weight and the MP presence of *E. sp*.

Correlation Matrix

		GIT Weight	Microplastic Count
GIT Weight	Pearson's r	—	
	df	—	
	p-value	—	
Microplastic Count	Pearson's r	-0.953	—
	df	1	—
	p-value	0.196	—

Descriptive

	GIT Weight	Microplastic Count
N	3	3
Mean	8.87	35.7
Standard deviation	2.02	18.0

Appendix Table 19. One-Way ANOVA test in comparing microplastic counts and microplastic types of *C. chanos.*

One-Way ANOVA (Fisher's)

	F	df1	df2	p
Microplastic Count	0.718	2	6	0.526

Group Descriptive

	Type	N	Mean	SD	SE
Microplastic Count	Filament	3	10.3	7.51	4.33
	Fragment	3	19.7	6.66	3.84
	Film	3	14.3	13.20	7.62

Appendix Table 20. One-Way ANOVA test in comparing microplastic counts and microplastic types of *E. sp.*

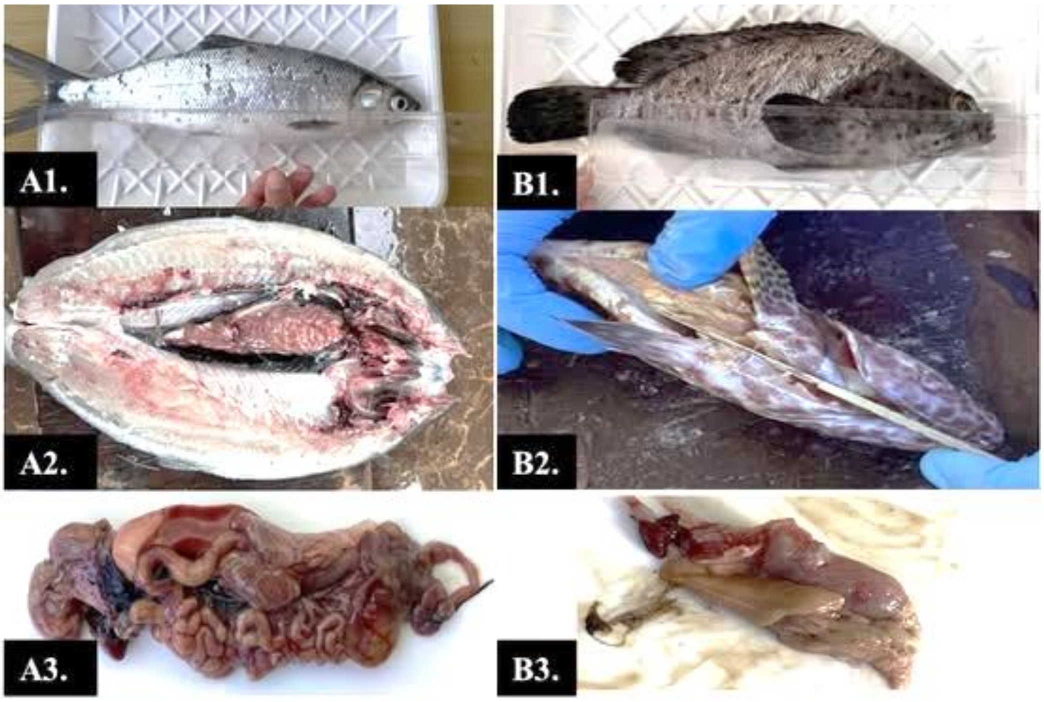
One-Way ANOVA (Welch's)

	F	df1	df2	p
Microplastic Count	2.98	2	6	0.126

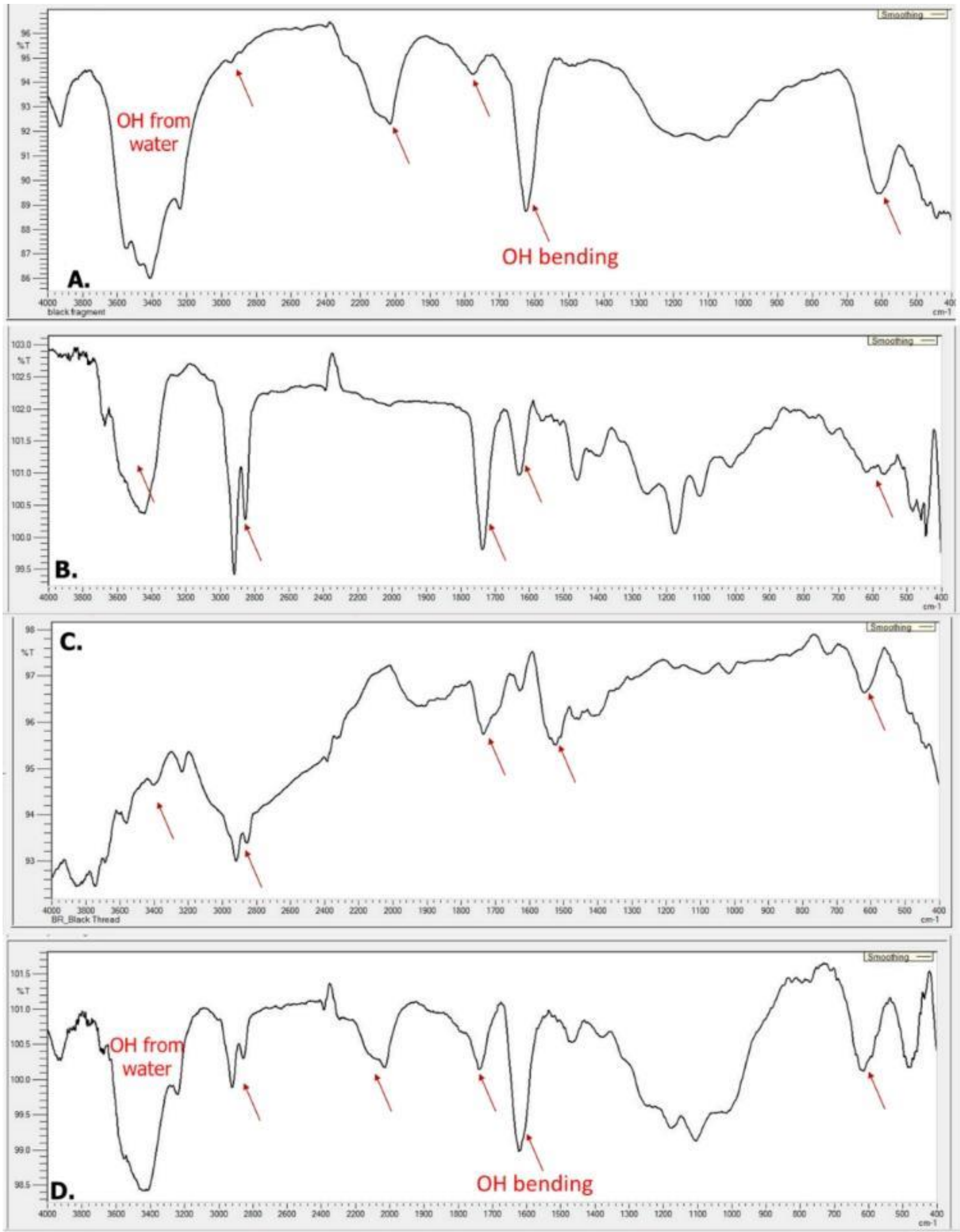
Group Descriptive

	Type	N	Mean	SD	SE
Microplastic Count	Filament	3	19.0	7.94	4.58
	Fragment	3	14.67	12.86	7.42
	Film	3	2.00	2.65	1.53

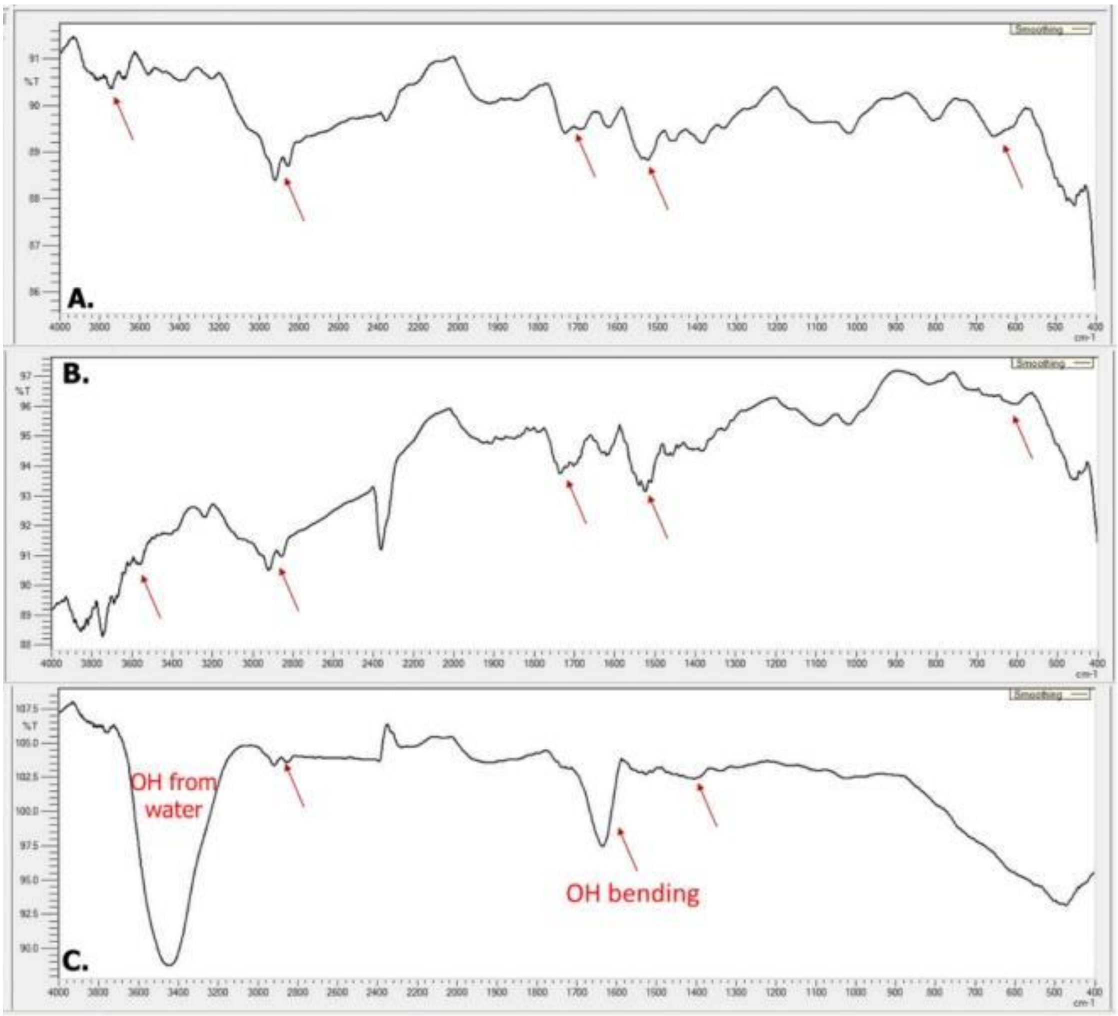
Appendix Figures



Appendix Figure 1. Sample preparation of: A. *C. chanos* and B. *E. sp.* (1–whole fish, 2-dissected, and 3-GIT).



Appendix Figure 2. FTIR spectra and the diagnostic bands (red arrow) of MPs found in the GIT of *C. chanos:* A. black fragment, B. blue filament, C. black filament, and D. yellow fragment.



Appendix Figure 3**.** FTIR spectra and the diagnostic bands (red arrow) of MPs found in the GIT of *E. sp.*: A. black filament, B. blue filament, and C. red filament.

Appendix Figure 4. Biological compound microscope utilized for MP inspection and identification.

TRYXZHIA GEM G. ANG
Grade 11 – Einstein
Central Mindanao University Laboratory High School
University Town, Musuan, Maramag, Bukidnon, Philippines
May 23, 2025

Sir Peter P. Mecisamente
City Economic Enterprise Manager, CEEO
City Hall Compound, Catarata St. Brgy. Poblacion, Valencia City, Bukidnon

Subject: Request for Permit to Conduct Research at the City Fish Landing Station

Dear Sir Mecisamente,

Good day!

We are Grade 11 students from Central Mindanao University Laboratory High School, currently conducting our quantitative research study titled:
"Assessment of Microplastic Contamination in the Guts of Commonly Traded Fish Species at a Landing Station."

We respectfully request your permission to conduct our study at the city's fish landing station, where we plan to purchase fish samples for laboratory analysis. Our study aims to assess the possible presence of microplastics in fish that are commonly traded in the area.

We would like to assure you that our research is strictly for academic purposes, and we do not intend to discourage the public from buying fish from the landing station. In fact, we fully support our local fish vendors and industry, and we will only be buying fish that are already available for sale. We will also follow all rules and guidelines while conducting the study, and we will ensure that there will be no disruption to the daily operations at the station.

We hope for your kind approval of our request. Your support will be a great help in the successful completion of our research.

Thank you very much for your time and consideration.

Respectfully yours,
TRYXZHIA GEM G. ANG
Lead Researcher
0997-326-0675

CITY ECONOMIC ENTERPRISE OFFICE
LGU-Valencia City, Bukidnon
RECEIVED
Date: 05-23-25 Time: 10:12
Received by: John Jerino M. Marinay

JO MARIAN ALEXA A. PATRICIO

JOERY BENEDICT L. SALUPADO

KRISTINE CLAIRE P. SEVILLENO
Student Researchers

Endorsed by:
RICA JOY V. LAMPARAS
Research Adviser
Central Mindanao University Laboratory High School
0918-7355-142

Approved:

PETER P. MECISAMENTE
City Economic Enterprise and Development Officer

Appendix Figure 5. Approved request letter to conduct by the City Economic Enterprise Manager.

Republic of the Philippines
CENTRAL MINDANAO UNIVERSITY
University Town, Musuan, Bukidnon

Center for Biodiversity Research and Extension in Mindanao

UNIVERSITY MUSEUM

May 26, 2025

CERTIFICATION

This is to certify that the animals being studied by Tryxzhia Gem G. Ang, Jo Marian Alexa A. Patricio, Joery Benedict L. Salupado and Kristine Claire P. Sevilleno are identified as:

1. *Chanos chanos*
   Family: Chanidae

2. *Epinephelus* sp.
   Family: Epinephelidae

Identified and Confirmed by:

ALMA B. MOHAGAN, Ph.D.
Zoologist/ Member, Pool of Taxonomist

By: **ROMEO R. PATAÑO Jr. Ph.D.**
OIC, University Museum

Appendix Figure 6. Milkfish (*Chanos chanos*) and Highfin grouper (*Epinephelus sp.*) certification of scientific names.