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*Research Article*

# Microplastics in the fish *Dormitator maculatus* from the Alvarado Lagoon, Veracruz, Mexico

**Derian Javier Del Ángel-Guzmán[1], Fabiola-Lango-Reynoso[1], Claudia Araceli Dávila-Camacho[1]**
**María del Refugio Castañeda-Chávez[1], Rocío de Guadalupe Bernal-Ramírez[1]**
**Jesús Montoya-Mendoza[1] & Leonardo Martínez-Cárdenas[2]**
[1]Tecnológico Nacional de México, Instituto Tecnológico de Boca del Río
Boca del Río, Veracruz, México
[2]Universidad Autónoma de Nayarit, Tepic, Nayarit, México
Corresponding author: Claudia Araceli Dávila-Camacho (claudia.dc@bdelrio.tecnm.mx)



**ABSTRACT.** This work analyzed the presence of microplastics (MP) in the gills and digestive tract of the fish *Dormitator maculatus* and its relationship with biological parameters in the Alvarado Lagoon (AL). In 127 organisms, 1,134 MP were found: 561 in the digestive tract and 573 in the gills, with an average of 8.9 MP per individual. Central tendency, Mann-Whitney, Kruskal-Wallis, and linear regression tests were performed to examine the relationship between the number of MP and the biological parameters of *D. maculatus*. A slight correlation was found between the number of MP in the gut and gills. The biological parameters analyzed - weight, length, condition index, and gut fullness - lacked a relationship between the amount of MP present in the gut and gills. The MPs were classified by color and type: fibers, pellets, films, and fragments. All fish analyzed presented MP in digestive tracts and gills, which indicates a uniform distribution of contamination and suggests that it originates from nearby populations, resulting from daily activities such as washing synthetic clothes and fishing. Fibers were the most dominant material (97.53%), comprising 62% blue and 12% transparent fibers. The presence of these is a threat to the health of both fauna and humans.

**Keywords:** *Dormitator maculatus*; microplastic in fish; environmental pollution; microplastic in gastrointestinal tract and gills; Alvarado Lagoon

## INTRODUCTION

Plastics are used in large quantities due to their versatility, as they are present in almost all articles of daily use (Andrady 2011, UNEP 2016, Giacovelli et al. 2018). Plastics are considered emerging pollutants, as there is still a lack of regulatory standards and much of their impact on the environment remains unknown (Deblonde et al. 2011, Neves et al. 2015). The classification of plastic waste is based on diameter ranges of: nanoplastics (<0.1 µm), microplastics (0.1 µm-5 mm), mesoplastics (5-25 mm), and macroplastics (>25-100 mm) (Campoy & Beiras 2019).

Microplastics (MP) are pollutants present in the environment, having emerged due to the high demand for plastic-based articles (Ritchie et al. 2023). Due to improper handling and being in the open air, they are fragmented by UV radiation, wind, and rain, generating MP, which are transported to aquatic ecosystems (Andrady 2011). Excessive pollution by marine litter is

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a global environmental problem, as it causes damage to ecosystems, preventing the physiological functions, growth, and survival of the biota that reside in them (Rochman et al. 2013, Ritchie et al. 2023). MP present in aquatic ecosystems, sediment and biota, generate great problems, due to their persistence and their function as a vector of diseases and toxic substances, since they are ingested by marine fauna such as polychaetes, worms and fish (Escobar 2002, Thompson et al. 2004, Rochman et al. 2013, Browne et al. 2015, Li et al. 2021).

MP are ingested and accumulate over time, which can lead to a reduction in the quality of life in the biota, a decrease in reproductive performance, malnutrition, and starvation in animals that consume them. Additionally, it is known that MP adsorb contaminants that are transported by these species living in the aquatic environment, most of which are of economic interest for human consumption (Andrady 2011). There is great concern due to the presence of low-molecular-weight chemicals that are adsorbed by MP, representing a toxic danger to the food chain (Thevenon et al. 2014, Andrady 2017).

Various researchers have stated that despite there being ample literature on MP in marine ecosystems, there is little information on MP in freshwater ecosystems; therefore, they suggest conducting more research on microplastic contamination in freshwater and mangrove ecosystems (Free et al. 2014, Li et al. 2018, Niji 2019).

Some authors, such as Niji (2019), report that the general impact of smaller plastic debris on the marine environment requires further extensive research. They stated that it is not yet possible to fully understand the effects of ingesting plastic waste until it can be determined whether fish can pass MP through the intestine or whether plastic remains in the intestine throughout the fish's life.

The proximity of anthropogenic activity is related to the presence of MP, which comes from various sources, enters rivers, most reach coastal lagoons, and finally the sea (Usman et al. 2020). The study by Pan et al. (2021), off the coast of China's Guangdong province, identified more MP in coastal fish than in fish found in the open sea. Also, carnivorous fish are likely to ingest fewer MPs than herbivorous or omnivorous fish.

The Alvarado Lagoon (AL) is ecologically, socially, and economically important, which is why it was designated a RAMSAR site in 2004 by the Commission for Protected Natural Areas (CONANP, by its Spanish acronym). Located in the central coastal plain of the state of Veracruz, in the southwestern part of the Gulf of Mexico**.** The lagoon's mangroves are home to a wide variety of fauna, and local communities carried out by the populations surrounding the system, especially in Puerto de Alvarado (SISR 2004).

*Dormitator maculatus* (Bloch, 1792) is a species native to the AL, which is a commercially important fishing resource in the area, and it also has great ecological value due to its position in the food chain, as it is a source of food for larger commercially important species (Franco-López et al. 2019). It inhabits the semi-buried bottom in brackish waters from North Carolina, USA to southern Brazil (Schmitter-Soto 1996, Lara-Lara et al. 2008). *D. maculatus* make mass migrations to the sea to spawn during their breeding season between September and October, when they were fished and sampled (Wallace & Van Der Elst 1975, Nordlie 2012).

*D. maculatus* has omnvorous feeding habits (Chávez-López et al. 1994, Winemiller & Ponwith 1998, Froese & Pauly 2023). The intestine serves as the primary digestive organ in aquatic animals, facilitating the absorption and metabolism of nutrients (Yang et al. 2019, Gu et al. 2020). It plays a crucial role in the immune system (Mowat & Agace 2014), a quality that could be affected by the presence of MP. The MP retention in some fish species can lead to obstruction of the digestive tract, resulting in reduced energy reserves, malnutrition, hormonal imbalances, delayed sexual maturity, and growth inhibition (Lu et al. 2016, Xiong et al. 2018).

For example, studies performed with also experiment on zebra fish *Danio rerio* evidenced that MP, showed hepatocytes with early inflammation, vacuolization, infiltration and necrosis, they also demonstrated an alteration of the metabolic profiles in the liver of the fish, as well as an increase in antioxidant enzymes and signs of liver stress (Lu et al. 2016). However, de Vries et al. (2020) studied the presence of MP in the intestines of Atlantic cod (*Gadus morhua*) and (*Pollachius virens*) in Iceland, analyzing their weight, length, gut fullness, and condition index. MP was found in 20.5% of $n$ = 39 *G. morhua* and 17.4% of $n$ = 46 *P. virens*, with no significant correlation between gut fullness, condition index, and the amount of MP, which suggests that in large fish, MP is unlikely to impact these biological parameters. Another problem resulting from the presence of MP is the transport of persistent organic pollutants, which induces bioaccumulation that increases with age (de Vries et al. 2019), in addition to biomagnification that alters the trophic chain (Gallo et al. 2018).

This study is of great importance because it constitutes a reference for understanding the presence of MP and the impact that these emerging pollutants have on the AL. The accumulation in gills and ingestion of MP in *D. maculatus* could affect other commercially important fish in the AL by bioaccumulation. The objective of this study was to determine the presence, quantity, and variety of MP present in gills and digestive tract and their relationship with the biological parameters of *D. maculatus* from the AL.

## MATERIAL AND METHODS

The AL is located in the central zone of the state of Veracruz, southwest of the Gulf of Mexico (Fig. 1). In September 2021, *D. maculatus* samples were caught and landed near the mouth of the AL during the fishing season. When the fish travel in shoals to the sea, they are caught with a gill net, which has a 1.5-inch mesh size and is left passively exposed for 4 or 5 h. The specimens were recovered from the discarded fish caught by fishermen during the breeding season. The specimens obtained were transported in a plastic container with ice (previously reviewed and freed of any plastic particles) to the Aquatic Resources Research Laboratory (LIRA, by its Spanish acronym) of the Technological Institute of Boca del Río (ITBOCA, by its Spanish acronym).

An external review was conducted on each specimen to record its biometric characteristics. The sex and weight (g) were recorded using a Core Adam brand electronic digital scale, Model CQT202, with a capacity of 200×0.001 g. The total length (TL) (cm) was measured using a 40 cm ichthyometer, brand Biotechnology Aquatic Model IK2. Subsequently, dissections were performed to extract the complete digestive tract, and the gut length (0.001 mm, General brand, Model H-7352) was recorded (Ory et al. 2018). The gut was then visually classified according to the technique proposed by De Vries (2020) for intestinal fullness. Regarding the gills, a cut was made from the lower part of the parabranchial chamber to the esophagus to recover the complete gills, including gill arches, gill filaments, and lamellae, which were then separated and weighed (Atamanalp et al. 2022).

The samples obtained were placed in properly labeled bottles for degradation using alkaline digestion with a 10% potassium hydroxide (KOH) solution, and then accelerated in an oven at 60°C for 4 h. The content was filtered using a vacuum pump and fine-pore filter paper with a retention capacity of 1 µm, a methodology employed by Lusher et al. (2013) and Avio et al. (2015).

For the identification of MP, a Quasar Qm1580x stereoscopic microscope was used. Any particle suspected of having organic, cellular, or semi-synthetic structures was discarded. The MP were identified and classified by their shape, color, and size. Then, MP were differentiated by shape, categorized into sheets, pearls, fibers, and fragments, and by color: red, green, blue, yellow, white, and transparent. For size, plastic particles ranging from 0.5 to 5.0 mm were identified, and the microplastics found were photographed. All particles above and below these sizes were excluded from the analysis. The goal was to focus on finding MP because the fish digestive tract is so thin that it is less likely to capture larger particles. Finally, the MP was photographed (De Vries et al. 2020). The particles for which some doubt was generated about their synthetic origin were subjected to the "hot needle" test (De Witte et al. 2014, Filgueiras et al. 2020).

During the analysis of the samples, a previous revision of all the material to be used was carried out, glass petri dishes, aluminum foil to cover the samples from the balance to the filters, in addition to the use of white cotton gowns at all times, natural air currents and the use of air conditioning were avoided at all times, to avoid any contamination by MP from the outside.

The length-weight analysis was performed using linear regression, which calculated the values of $a$ and $b$ from the following equation, where $a$ represents the intercept and $b$ represents the slope between length and weight. For isometric growth ($b$ = 3), negative allometric ($b$ < 3), positive allometric ($b$ > 3) (Teissier 1948, Froese 2006, Froese et al. 2011).

$$W = a L^{b}$$

where: $W$: total weight (g), $L$: length (cm), $a$: shape coefficient (regression constant), and $b$: regression coefficient (allometry coefficient).

Statistical analysis was performed to investigate the total length-weight relationship. This result enables us to compare the weight and height of *D. maculatus* with the amount of MP present.

To calculate the Fulton condition index (K) and assess the degree of well-being, the equation proposed by Fulton (1904), as modified by Ricker (1975) and cited by Froese (2006), was used. This index allows establishing the degree of health and well-being that an animal presents, with values of 0.0 < 1.0 thin, 1.1<1.2 well-nourished, and >1.21 fat.

$$K = \frac{W_t}{L^3} \times 100$$

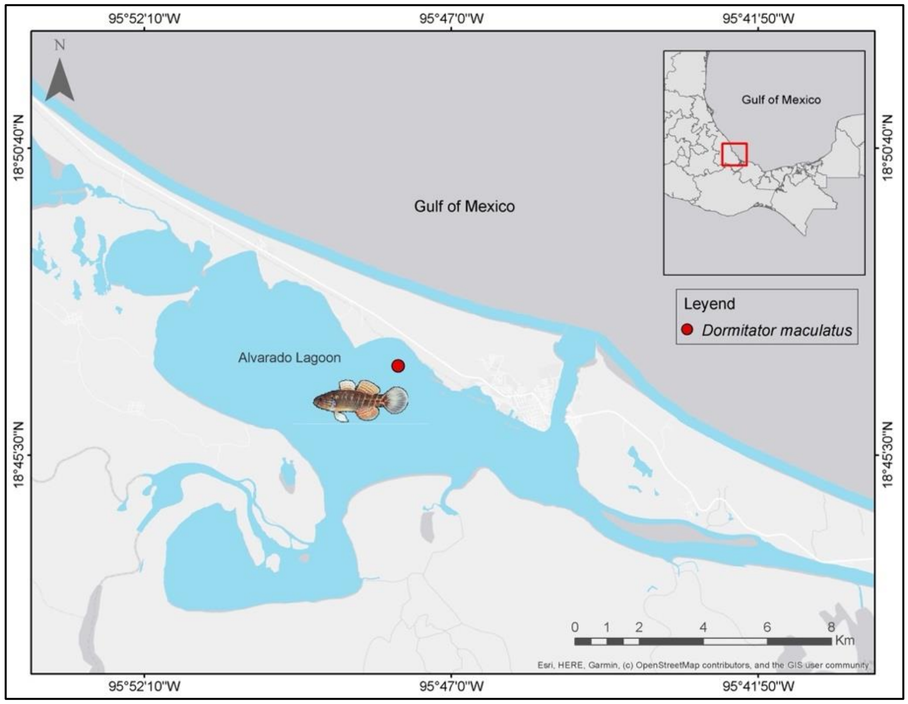


**Figure 1.** Location of the Alvarado Lagoon and location of the *Dormitator maculatus* sampling site, in the Gulf of Mexico, Veracruz.

where: *Wt*: total weight of the organism (g), *L*: total length of the fish (cm).

For the classification of intestinal fullness, the fish were visually classified according to the stomach contents, three categories were identified: "full", "half full", and "empty", to which the values 1, 0.5 and 0.0 were assigned respectively, according to De Vries et al. (2020). Mann-Whitney tests were performed to compare the differences in the number of MP present in the digestive tract between males and females, as well as the differences in MPs between the gut and gills.

The Kruskal-Wallis test was used to identify differences in the median values of MP present in the digestive tract, categorized by intestinal fullness. Finally, different Pearson correlation analyses were carried out between the number of MPs in the intestine and gills with the biological parameters, with a confidence level of 0.95 and ($P$ < 0.05).

## RESULTS

A total of 127 fish were analyzed, comprising 51 males and 76 females; Table 1 presents the biometric measurements. The average TL was 13.79, and the average total weight was 44.51 g respectively were obtained, with a maximum and minimum size of 17.60 and 10.50 cm, with standard deviation (SD) = 1.199, as well as the maximum weight of 102 g and minimum of 18.30 g, with SD =13.365 (Fig. 2).

**Table 1.** Biometrics of 127 *D. maculatus* captured in the Alvarado Lagoon. TL (cm): total length, TW (g): total weight, LDT (cm): length of digestive tract, IW (g): intestinal weight, and GW (g): gill weight.

| N:127 | TL | TW | LI | IW | GW |
|---|---|---|---|---|---|
| Average | 13.79 | 44.51 | 54.87 | 0.19 | 00.17 |
| Minimum | 10.50 | 18.30 | 14.00 | 0.001 | 0.0005 |
| Maximum | 17.60 | 102.00 | 87.00 | 01.87 | 02.02 |

*D. maculatus* has a tubular digestive system. The digestive tract has an average length of 54.87 cm, a maximum length of 87 cm, and a minimum length of 14 cm. The average weight of the gastrointestinal tract was 0.19 g (max = 1.87 g and min = 0.001 g) while the gills presented an average weight = 0.17 g (max = 2.02 g and min = 0.0005 g) (Table 1).

### Microplastics

MP were identified in 100% of the fish analyzed, with a total of 1,134 MP, of which 561 MP were present in the digestive tract and 573 in gills (Fig. 3).

**Figure 2.** Mature specimen of *Dormitator maculatus*. Average length of 13.79 cm.

We identified 1,106 fibers (97.53%), 10 pellets (0.88%), 13 films (1.15%), and 5 fragments (0.44%). In the digestive tract, we found 545 fibers, 9 pellets, 5 films, and 2 fragments, while in the gills, we found 561 fibers, 1 pellet, 8 films, and 3 fragments (Fig. 4).

Of the total MPs identified, 704 were blue, 200 black, 53 red, 141 transparent, 17 yellow, 10 white and 9 green (Fig. 5). In digestive tract: 353 blue, 111 black, 51 transparent, 28 red, 9 yellow, 6 white and 3 green (Fig. 6). In gills: 351 blue, 90 transparent, 89 black, 25 red, 8 yellow, 6 green and 4 white (Fig. 7).

### Length-weight relationship

It was found that there is no relationship between length, weight, and number MP. According to the analysis of the total length-weight relationship, relationship between length to weight and MP a value of Ln $a = 0.022$ and a value of $b = 2.8895$, $r = 0.88147$, was obtained, which means that these fish present negative allometric growth ($b < 3$), which means, the increase in weight is proportionally less than the increase in length, after reaching a size of 15 cm, specifically in the adult stage (Fig. 8).

### Fulton condition index (K)

The *K* index showed that 100% of the fish were in the condition of "ready to spawn" or "fat", with a value of 1.66, suggesting that the amount of MP ingested has not affected their appetite, as no degree of malnutrition was observed.

### Intestinal fullness

Forty-one individuals were assigned to the empty category, 55 to the half-full category, and 31 to the full category. The Kruskal-Wallis test reveals no statistically significant differences between the treatment groups (H = 1.038, df = 2, $P = 0.595$).

### Variation in the amount of MP between males and females

No statistically significant differences were found between the amount of MP present in males and females (U = 1,620.500, $P = 0.117$), nor were there any. In the digestive tract (U = 1748,000, $P = 0.347$) nor gills (U = 1,583.500, $P = 0.078$).

### Variation between the amount of MP in the gills and the digestive tract

The Mann-Whitney test showed no differences between the number of MP in the digestive tract and gills (U = 7809.500, $P = 0.66$).

### Pearson correlation

As shown in Table 2, there were two positive correlations between MP digestive tract and intestine weight ($P = 0.0222$, $r = 0.203$) and between MP in gills and gill weight ($P = 0.00000362$, $r = 0.398$). However, the correlation ratio is very low. The graphs did not exhibit any apparent linear correlation, and the *r*-value indicated a very low relationship.

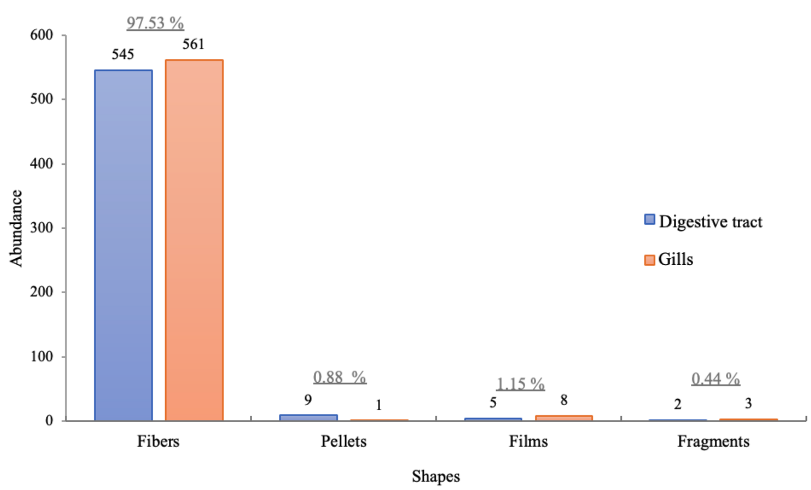


**Figure 3.** Relative abundance of microplastics located in the digestive tract and gills of *Dormitator maculatus* classified by shape.

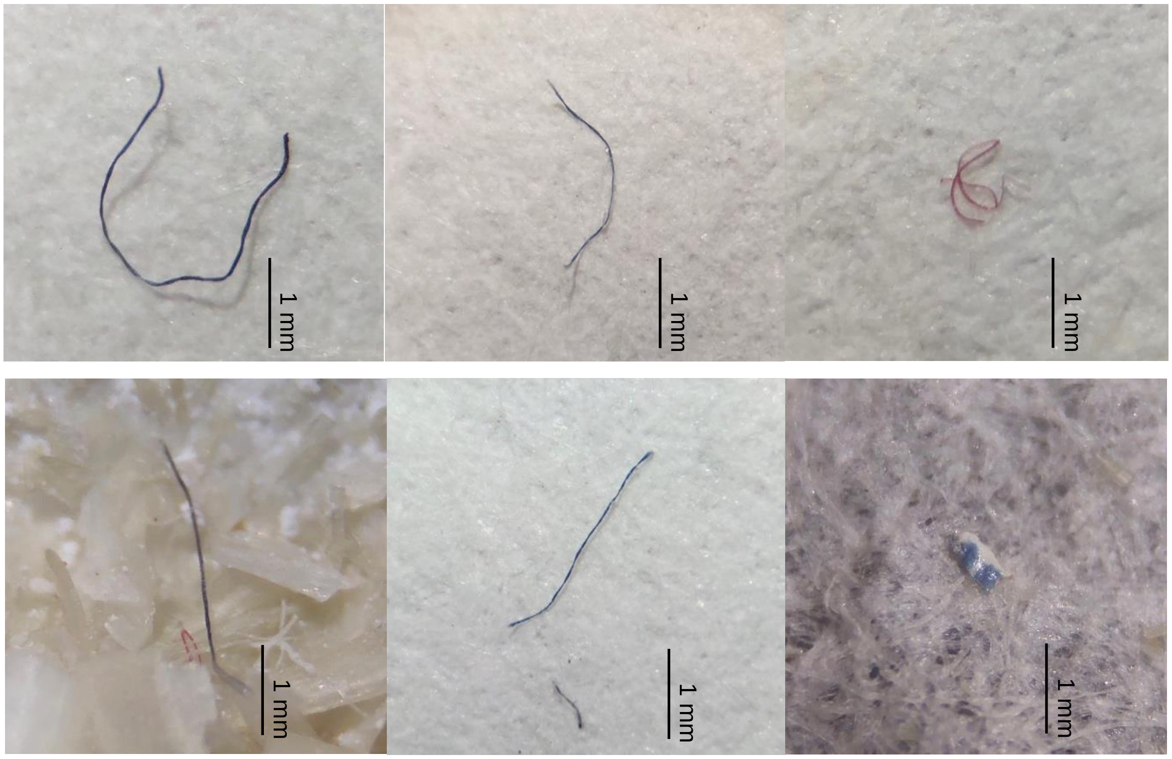


**Figure 4.** Up: microplastics found in the digestive tract, blue and red fibers. Below: microplastics extracted from gills, colored fibers: blue and red, and white and blue film.

## DISCUSSION

Because 100% of the *D. maculatus* organisms presented MP in gills and digestive tract, plastic contamination is confirmed and reflected in the AL. This situation has been documented in other aquatic ecosystems worldwide (Waller et al. 2017). In this study, it was found that MP fibers are the most abundant plastic item. These results align with those of other authors, including Waller et al. (2017), Kumar et al. (2018), Lusher & Hernández-Milián (2018), and Koongolla et al. (2020), as well as De Vries et al. (2020). According to Morgana et al. (2018), synthetic fibers are a form of plastic frequently found in the digestive tracts of marine animals, primarily in fish (Ryan et al. 2016). In this sense, densely populated areas and habitats that receive contributions from wastewater have a greater presence of MP, particularly those that are in the form of fibers (Browne et al. 2011). The previous investigations concur with the result of the MP analysis in this investigation, indicating a predominance of fibers at 97.53%.

In this research, it was found that the MP colors blue and black were the predominant colors, differing from those reported by Phillips & Bonner (2015) and Pan et al. (2021), who reported a higher percentage of white microfibers. Blue is the color associated with fishing activities and clothing (Lusher et al. 2013), while black is associated with tires, abrasives, and plastic bags (Andrady 2011). As stated in Jovanović et al. (2018), the presence of MP in the digestive tract is caused by intentional or accidental ingestion, resulting from the contamination of water or sediments with these substances. Furthermore, Boerger et al. (2010) mention that MP in fiber form can be confused with the food of fish in the ocean, especially blue ones, which could explain the high presence of blue microfibers in some specimens studied. However, the presence of blue MP could be related to the feeding habits of *D. maculatus*, which are associated with the sediment.

In this research, there were no significant differences between the amount of MP present in the gills and digestive tract, unlike the results reported by Koongolla et al. (2020), Park et al. (2020), Pan et al. (2021) found a greater amount of MP in the intestine than in the gills.

The presence of MP in gills is related to its comb-shaped anatomical structure, which allows the MP to be held between its filaments when water passes through

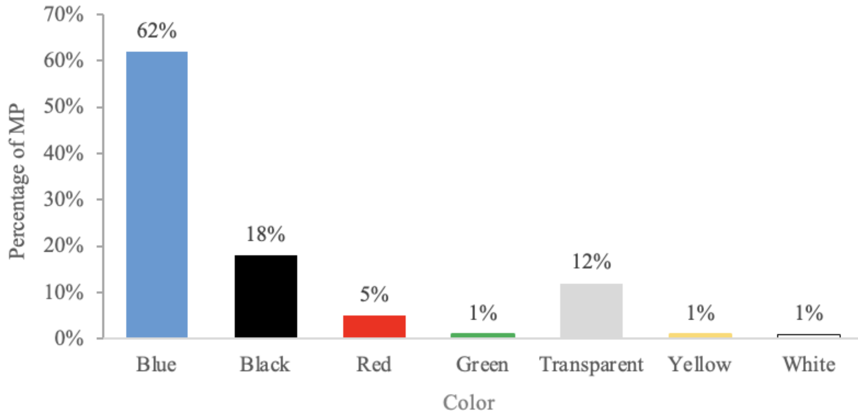


**Figure 5.** Total microplastics (MP) abundance classified by color, present in the digestive tract and grills of *D. maculatus* from Alvarado Lagoon.

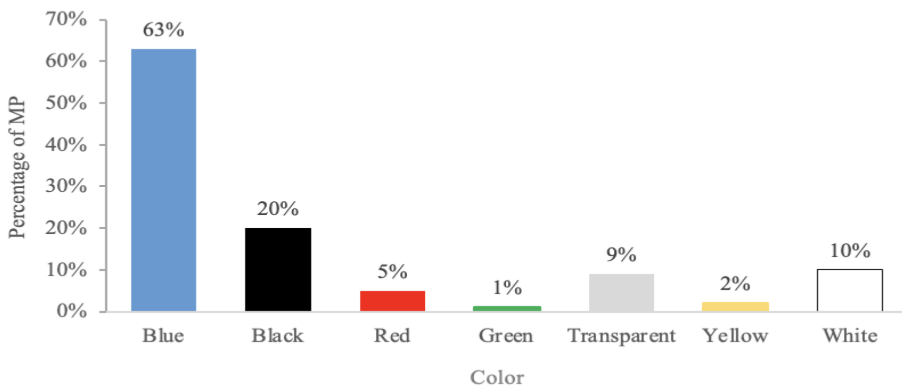


**Figure 6.** Color distribution of microplastics (MP) in the digestive tract in *D. maculatus* from Alvarado Lagoon.

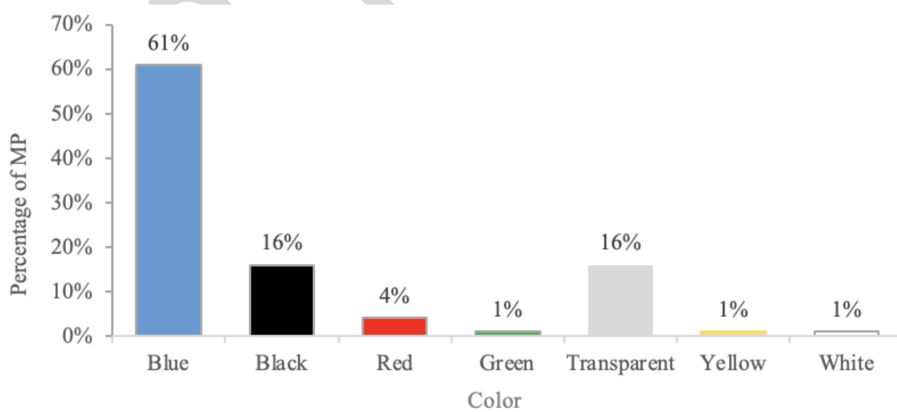


**Figure 7.** Color distribution of microplastics (MP) in gills in *D. maculatus* from Alvarado Lagoon.

it (Pan et al. 2021); in addition, the high availability of this contaminant in the fish habitat contributes to a high frequency of MP contamination in demersal fish such as *D. maculatus*. Therefore, it can be inferred that its high presence is due to water filtration in the gills and incidental consumption in the digestive tract.

The results of the length-weight relationship of *D. maculatus* obtained in this study showed a negative allometric growth, which differs from what was found by Dávila-Camacho & Galaviz Villa (2021), who observed that *D. maculatus* from Alvarado lagoon presents a positive allometric growth ($b$ = 3.1718).

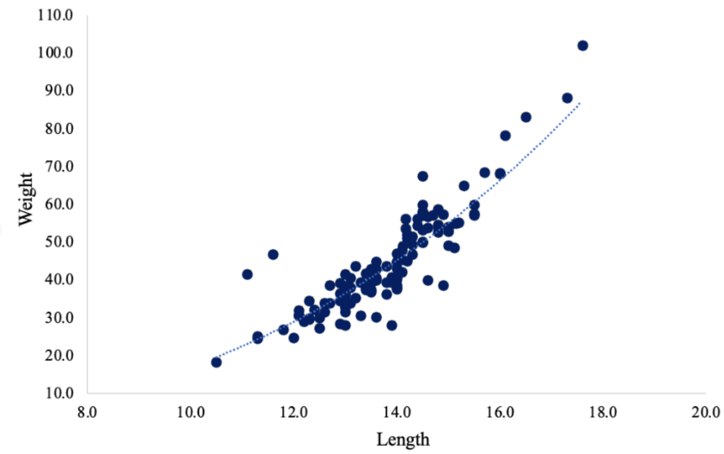


**Figure 8.** *Dormitator maculatus* length-weight relationship.

**Table 2.** Pearson correlation, microplastics (MP) in the digestive tract and gills *vs.* biological parameters.

| Correlation | Biometric | *P*-value | Pearson Correlation (*r*) |
|---|---|---|---|
| MP in the digestive tract. | Total length | 0.954 | 0.0052 |
| | Total weight | 0.623 | 0.0440 |
| | Fulton condition index | 0.046 | 0.0668 |
| MP in grills *vs.* | Total length | 0.929 | 0.008 |
| | Total weight | 0.398 | 0.076 |
| | Fulton condition index | 0.0561 | 0.170 |
| MP in the digestive tract *vs.* | Length of digestive tract | 0.288 | 0.095 |
| | Weight digestive tract | 0.0222 | 0.203 |
| MP in grills *vs.* | Weight of grills | 0.00000362 | 0.398 |

The null relationship between MP and the condition index obtained in this work aligns with the findings reported by Morgana et al. (2018), who also found no relationship between the amount of MP and the condition index. Also, in the study carried out by (Foekema et al. 2013) no relationship was found between the condition factor of the fish and the presence of ingested plastic particles; however, they mention that this result may be due to the sample number they had per species, which was low, the highest number per species was 87 organisms per site and a very low incidence of MP, the authors recommended replicate the research with a larger sample number.

Regarding intestinal fullness (Batel et al. 2016, Liboiron et al. 2016), a significant relationship was found between intestinal fullness and MP. This finding coincides with the digestive tract, which was classified as "full," and contained a greater number of MP. Furthermore, they suggest that the presence of MP in mesopelagic fish species indicates a trend where the increase in MP abundance occurs in smaller size categories (Wieczorek et al. 2018).

In the present study, the relationship between the total amount of MP in the digestive tract or gills and variables such as weight, height, condition factor, classifications of intestinal fullness, and the weight of the intestine and gills regarding the amount of plastics in these organs was not significant. Within this context, it is considered that the presence of particles found per individual reduces the possibility of MP accumulation; therefore, there is a non-visible effect that affects the health of individuals (Foekema et al. 2013, De Vries et al. 2020). The relationship between the weight of the intestine and the MP may be due to the biomass, although in this case no negative effect; given the since the size of the MP seems too small to affect the sensitivity of the fish by causing false sensations of satiety, intestinal blockage or injuries to the digestive tract (Foekema et al. 2013) and thus influence some biological parameter. However, the intestine has been documented to be an internal organ sensitive to MP damage (Hariharan et al. 2021), and it is often the entry point for pathogens to reach the interior of the body (Kinnebrew & Pamer 2012).

The intestine also functions as the main organ of aquatic organisms, contributing to digestion, nutrient absorption and metabolism (Yang et al. 2019, Gu et al. 2020), it is worth mentioning that the intestine plays an important role in the immune system since it contains the largest number of immune cells among the tissues of the body (Mowat & Agace 2014). These qualities could be affected by the presence of MP. The retention of MP in fish can obstruct the digestive tract, leading to other conditions, such as reduced energy reserves, hormonal alterations, delayed sexual maturity, and inhibited growth (Xiong et al. 2018). However, the latter is not fulfilled in its entirety for all species, as is the case in this study. Lu et al. (2016) analyzed early inflammatory responses, vacuolization, infiltration, and necrosis in the hepatocytes of zebrafish *Danio rerio* treated with MP. They also demonstrated alterations in metabolic profiles in the fish liver, as well as an increase in antioxidant enzymes and signs of liver stress. Another problem resulting from the presence of MP is the transport of persistent organic pollutants through these, which induces bioaccumulation that increases with age, in addition to biomagnification that alters the food chain (Lu et al. 2016).

On the other hand, according to Lin et al. (2023), it is necessary to note that various investigations have been conducted, confirming the ingestion of MP by fish. Since it is an emerging contaminant, a standardized methodology is lacking. In the process of extracting and identifying MP, there is a need to develop criteria for its analysis. However, researchers have implemented methodologies with some rigor for the extraction and identification of MP. Despite these implications, the results reflect a sample of the affected fish populations. The presence of MP in different species has been demonstrated, and consistency in the classifications of MP provides information that helps infer their origin. It is worth noting that the results of studies conducted to date demonstrate consistency when compared. Although many institutions in Mexico lack devices for spectrometry or other analytical tools to identify polymers and confirm their origin, their presence has been demonstrated, and the results cannot be ignored. Local actions are required to reduce these pollutants.

## CONCLUSIONS

This work is the first record of MP in the AL, and contamination by MP was evaluated for the first time in the digestive tract and gills of *D. maculatus*. The presence of MP fibers in 100% of the analysed individuals of *D. maculatus*. It is evident that MP are present in this ecosystem, and that *D. maculatus* served as a bioindicator. The results of MP abundance found by sex and by organ lack a significant statistical difference, which is a species of great importance. The results of MP abundance found by sex and by organ lack a significant statistical difference. Regarding the correlations carried out, no significant difference was found between the number of MP present in the gills versus those found in the digestive tract.

The biological parameters analyzed do not show a relationship with the MP found. As for the shapes, blue fibers predominate. The color and shape of the MP indicate that they originate from wastewater in urban areas, likely due to poor wastewater management.

Finally, the results obtained prove the contamination of the coastal lagoon by anthropogenic pollution. *D. maculatus* is proposed as a bioindicator of MP pollution, as 100% of the organisms presented MP, due to its wide distribution and its position in the food chain. In another sense, *D. maculatus* is a commercially important fish and a traditional dish in the region, but it can be toxic for human consumption; thus, contributing to the knowledge and development of environmental management proposals that benefit the environment, the species, and human health.

**Credit author contribution**

D.J. Del Ángel-Guzmán: sampling, execution of methodology and writing-original draft; F. Lango-Reynoso: conception planning, funding acquisition, project administration, methodology; C.A. Dávila-Camacho: supervision, review, data curation and review and editing; M.R. Castañeda-Chávez: design conceptualization, validation, contribution of laboratory equipment, formal analysis; R.G. Bernal-Ramírez: monitoring and advice of the research process, graphic desiggn and mappin; J. Montoya-Mendoza: monitoring and advice of the research process advice of the research, review; L. Martínez-Cárdenas: review and translation. All authors have read and accepted the published version of the manuscript.

**Conflict of interest**

The authors declare no conflict of interest.

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