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Derian Javier Del Ángel-Guzmán , [Claudia Araceli Dávila-Camacho](#) * , [Fabiola Lango-Reynoso](#) , [María del Refugio Castañeda-Chávez](#) , Rocío de Guadalupe Bernal-Ramírez , [Jesús Montoya-Mendoza](#) , [Leonardo Martínez-Cárdenas](#)

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Article

Microplastics in the Digestive Tract and Gills of *Dormitator maculatus* (Perciformes, Eleotridae) from Alvarado Lagoon

Derian Javier Del Ángel-Guzmán ¹, Claudia Araceli Dávila-Camacho ², Fabiola-Lango-Reynoso ¹, María del Refugio Castañeda-Chávez ¹, Rocío de Guadalupe Bernal-Ramírez ¹, Jesús Montoya-Mendoza ¹ and Leonardo Martínez-Cárdenas ^{2,*}

¹ Laboratorio de Investigación de Recursos Acuáticos (LIRA). Tecnológico Nacional de México/Instituto Tecnológico de Boca del Río (ITBOCA). Km 12 Carretera Veracruz-Córdoba, C.P. 94290, Boca del Río, Veracruz, México; e-mail@e-mail.com

² Unidad Académica de Agricultura. Universidad Autónoma de Nayarit (UAN), Boulevard Tepic-Xalisco 325 C.P. 63155, Ciudad de la Cultura Amado Nervo, Tepic Nayarit Méx.; e-mail@e-mail.com

* Correspondence: e-mail@e-mail.com; Tel.: (optional; include country code; if there are multiple corresponding authors, add author initials)

Abstract: 127 “naca” fish, *Dormitator maculatus* from the Alvarado lagoon, were analyzed, of which 1134 microplastics (MP) were obtained: 561 found in the digestive tract and 573 in gills. The predominant MP found in *D. maculatus* were fibers with 97.53%, the most abundant color was blue (62%), followed by transparent (12%) and of these the MP in the form of fibers predominated. Regarding the biological parameters, there was only a positive correlation with the weight of the intestine and the weight of the gills; However, this ratio is very low. The biological parameters analyzed: weight, height, condition index and intestinal fullness, lack a relationship between the amount of MP present in the intestines and gills. The type of PM obtained indicates that they come from urban areas, a product of daily activities such as washing clothes and fishing

Keywords: microplastics; *dormitator maculatus*; alvarado lagoon; gastrointestinal tract contents; gills

1. Introduction

Plastics are used in large quantities due to their versatility; weight, function and resistance, are present in almost all articles of daily use, and are used in the food sector to package [1,2,3]. Plastics are considered emerging pollutants, since there is still a lack of regulatory standards and much of their impact on the environment is unknown [4,5]. The classification of plastic waste according to its size is: macroplastics (> 25 mm), mesoplastics (5 mm to 25 mm), microplastics (MP) (1 µm to 5 mm) and nanoplastics (1 nm to 1 µm) [6].

Microplastics (MP) are pollutants present in the environment, they have emerged due to the great demand for articles made of plastic [7], and due to improper handling and being in the open air, they are fragmented by UV radiation, wind and rain, generating MPs which are transported to aquatic ecosystems [1]. Excessive pollution by marine litter is a global environmental problem, due to the damage it causes to ecosystems, preventing physiological functions, growth and survival of the biota that resides in it. MPs present in aquatic ecosystems, sediment and biota, generate great problems, due to their persistence and their function as a vector of diseases and toxic and persistent substances, since they are ingested by marine fauna such as polychaetes, worms and fish [8,9,10,11,12].

MPs are ingested and accumulate over time, which can cause a reduction in the quality of life in the biota, a decrease in reproductive performance, malnutrition and starvation of animals that consume them. In addition to this, it is known that MPs adsorb contaminants that will be transported by these species that live in the aquatic environment, most of which are of economic interest for

human consumption [1]. There is great concern due to the presence of low molecular weight chemicals that are adsorbed by PM, representing a toxic danger to the food chain [13,14].

The Alvarado Lagoon (LA) has high ecological, social and economic value belongs to the Alvarado Lagoon System (SLA), this system was named a RAMSAR Site in 2004 by the Commission of Protected Natural Areas (CONAP), it is located in the central coastal plain of the State of Veracruz, southwest of the Gulf of Mexico. *Dormitator maculatus* (Bloch, 1792) is a species native to the SLA, it is a commercially important fishing resource in the area, and also has great ecological value due to its position in the food chain [15]. It is a euryhaline and demersal species; It lives in fresh and brackish water, inhabits coastal lagoons, estuaries, mangroves, river mouths, streams and swampy areas [16,17]. *D. maculatus* carries out massive migrations to the sea for spawning during its peak reproduction period between the months of September and October [18,19], It is omnivorous and feeds on detritus (algae and diatoms), protozoans, invertebrates, shrimp and small fish [20,21,22].

The intestine functions as the main digestive organ of aquatic animals; contributes to nutrient absorption and metabolism [23,24], It is worth mentioning that the intestine plays an important role in the immune system, due to its large content of immune cells [25], a quality that could be affected by the presence of MP. PM retention in some fish species can lead to obstruction of the digestive tract, resulting in reduced energy reserves, malnutrition, hormonal alterations, delayed sexual maturity and growth inhibition. [26,27], early inflammatory responses, vacuolization, infiltration and necrosis have been observed in the hepatocytes of zebrafish (*Danio rerio*) treated with MP, they also showed an alteration of the metabolomic profiles in the liver of the fish, 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, inducing bioaccumulation that increases with age [28], in addition to biomagnification that alters the trophic chain [29]. The objective of this study was to determine the presence, quantity and variety of MP present in gills and intestines and their relationship with the biological parameters of *Dormitator maculatus* from the Alvarado lagoon

2. Materials and Methods

In September 2021, during the fishing season, samples of *Dormitator maculatus* captured and landed in LA were sampled using a gillnet with a 1.5-inch mesh size (Figure 1) [30,31]. The specimens were transported in a plastic container with ice to the Aquatic Resources Research Laboratory (LIRA) of the Technological Institute of Boca del Río (ITBOCA).

2.1. Biometrics and extraction of gills and intestines

For each specimen, was recorded the sex, weight (g with a Core Adam brand electronic digital scale Mod. CQT202 with a capacity of 200g x 0.001g), and total length (cm with a 40 cm ichthyometer brand Biotechnology Aquatic® Model IK2). Subsequently, dissections were performed to extract the complete intestines, the length was recorded with the help of a digital vernier with precision 0.001mm, General® brand, Mod. H-7352, Furthermore, each of them was weighed [32] and they were visually classified, according to the technique proposed by [28]. The gills were also separated and weighed [33].

For the extraction of MP from intestines and gills, the samples obtained were placed in properly labeled bottles for degradation by means of alkaline digestion, using a 10% potassium hydroxide (KOH) solution, accelerating the process in an oven, at a temperature 60 °C for 4 hours, The content was filtered with the help of a vacuum pump and fine-pore filter paper with a retention capacity of 1 µm, a methodology used by [34,35]. For the observation of microplastics, a Quasar Qm1580x stereoscopic microscope was used, the MPs were identified and classified by their shape, color and size. For classification by shape, they were differentiated into: sheets, pearls, fibers, and fragments. For colors in: red, green, blue, yellow, white and transparent. Finally, for size, plastic particles ranging from 0.5 mm to 5.0 mm were identified, and the MP found were photographed [28]

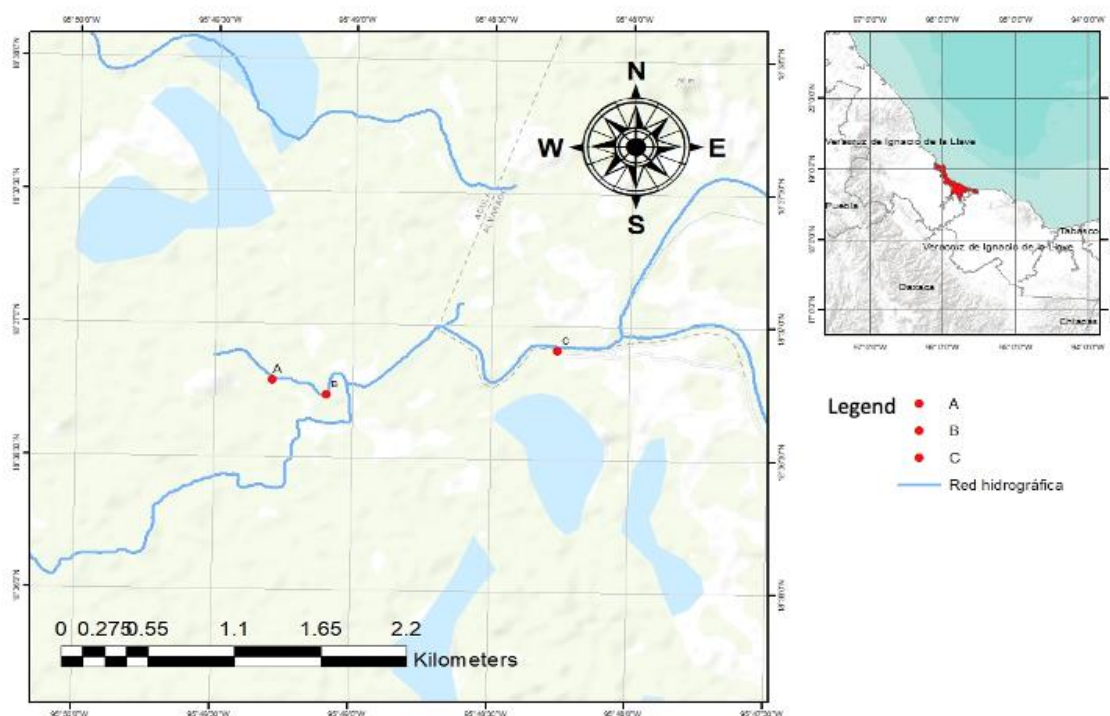


Figure 1. *Dormitator maculatus* sampling site, in Laguna Alvarado, Veracruz.

2.2. Length-weight ratio

The length-weight analysis was performed using linear regression, calculating the values of a and b from the following equation, where a is the intercept, and b is the slope between length-weight. For isometric growth ($b = 3$), negative allometric ($b < 3$), positive allometric ($b > 3$) [36,37,38].

$$W = aL^b \quad (1)$$

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

Statistical analysis was carried out for the total length-weight relationship. This result allows us to compare weight and height between the amount of MP present in *D. maculatus*.

2.3. Fulton Condition Index (K)

To calculate the Fulton condition index and establish the degree of well-being, the equation proposed by Fulton (1904) cited by [37] and modified by [39] was used. The Fulton condition index allows establishing the degree of health and well-being that an animal presents, the value of $0.0 < 1.0$ thin; $1.1 < 1.2$ well-nourished and > 1.21 fat.

$$K = \frac{W_t}{L^3} \times 100 \quad (2)$$

where: K = Fulton condition factor, W_t = total weight of the organism (g), L = total length of the fish (cm).

2.4. Plenitud intestinal

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 [28].

Mann-Whitney statistical tests were carried out to identify if there is a relationship between the amount of MP obtained in the digestive tract and gills of males and females, as well as the difference in the means between the MP in gills present in males and females.

3. Results

127 fish were analyzed, 51 males and 76 females; Table 1 shows the biometric measurements. In general, an average total height and average total weight of 13.79 cm and 44.51 g respectively were obtained and maximum and minimum size of 17.60 cm and 10.50 cm respectively, as well as the maximum weight of 102 g and minimum of 18.30 g. *Dormitator maculatus* has a tubular digestive system (intestine or gastrointestinal tract). The gastrointestinal tract has an average length of 54.87 cm, a maximum of 87 cm and a minimum 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 the average weight = 0.17 g (max= 2.02 g and min= 0.0005 g) (Table 1).

Table 1. Biometrics of the 127 *D. maculatus* fished in the Alvarado Lagoon. .

N:127	LT	PT	LI	PI	PB
Mean	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

Total length = LT (cm), Total Weight = PT (g), Intestinal Length = LI (cm), Intestinal Weight = PI (g) and Gill Weight = PB (g).

MPs were identified in 100% of the fish with a total of 1134 MPs, of which 561 MPs were present in intestines and 573 in gills (Table 2).

The total MP forms present were 1106 fibers, 10 pellets, 13 films and 5 fragments. In the digestive tract, 545 fibers, 9 pellets, 5 films and 2 fragments were found. In gills there were 561 fibers, 1 pellet, 8 films and 3 fragments (Figures 2 and 3).

Table 2. Type of PM located in the digestive tract and gills of *Dormitator maculatus* according to their relative abundance and percentage.

Forms	digestiv tract	gills	total	%
Fibra	545	561	1106	97.53
Pellet	9	1	10	00.88
Film	5	8	13	01.15
Fragment	2	3	5	00.44
Irregular	0	0	0	00.00
Total	561	573	1134	100.0



Figure 2. MP found in the digestive tract, blue and red fibers.



Figure 3. MP extracted from gills, colored fibers: blue and red, and white and blue film.

Regarding the classification by color and total abundance of MP, 704 blue, 200 black, 53 red, 9 green, 141 transparent, 17 yellow and 10 white were found. In intestines: 353 blue, 111 black, 28 red, 3 green, 51 transparent, 9 yellow and 6 white. In gills: 351 blue, 89 black, 25 red, 6 green, 90 transparent, 8 yellow and 4 white, as shown in table 3.

Table 3. Abundance of MPs classified by color present in the digestive tract and gills of *Dormitator maculatus*.

Color	Intestine	Gills	Total	%
Blue	353	351	704	62.08
Black	111	89	200	17.64
Red	28	25	53	04.67
Green	3	6	9	00.79
Transparent	51	90	141	12.43
Yellow	9	8	17	01.50
White	6	4	10	00.88
Total	561	573	1134	100.0

According to the analysis of the total length-weight relationship, a value of $\alpha = 0.022$ and a value of $b = 2.8895$, $r^2 = 0.08236$ was obtained, which means that these fish present negative allometric growth ($b < 3$), which means that the increase in weight is proportionally less than the increase in length, specifically in the adult stage, after reaching a height of fifteen centimeters

3.1. Variation in the amount of MP between males and females

In the Mann-Whitney analysis to identify the relationship between the amount of MP obtained in intestines and gills, of males and females, the values of $U = 1620.500$ and $P = 0.117$ were obtained, which means that no statistically significant difference was found. between the amount of MP present between males and females. Also, this same test was carried out to compare whether there is a significant difference between the number of MPs present in the digestive tract of males and females, from which $U = 1748,000$ and $P = 0.347$ were obtained, therefore, it does not have a statistically significant difference. For gills in males and females, $U = 1583.500$ and $P = 0.078$, the difference in means between gill MPs present in males and females is not large enough to exclude the possibility that the difference is due to sampling variability. random; therefore, there is no statistically significant difference.

3.2. Variation between the amount of MP in gills and intestines

A Mann-Whitney test was performed comparing the total number of MPs in intestines and gills, and the values of $U = 7809.500$ and $P = 0.661$ were obtained. These results indicate that the average difference between the MPs present in intestines and gills is not large enough to exclude the

possibility that the difference is due to random sampling variability; therefore it does not present a statistically significant difference.

3.3. Fulton Condition Index (*k*)

The K index showed that 100% of the fish are under the condition of “ready to spawn” or “fat”, with a value = 1.66, which suggests that the amount of MP ingested has not affected their appetite. since no degree of malnutrition was observed.

3.4. Intestinal fullness

41 individuals were obtained with the empty category, 55 with the half-full category and 31 with the full category, to which a Kruskal-Wallis test was performed to identify the differences between the mean values of MP present in intestines according to the classification of intestinal fullness. The test results show that there are no statistically significant differences between the treatment groups ($H = 1.038$, $df = 2$, $P = 0.595$), which are not large enough to exclude the possibility that the difference is due to the variability of random sampling.

3.5. Pearson correlation

Various analyzes were carried out to identify if there is a relationship between the biometric measurements, the organs analyzed and the number of MPs found. The Pearson correlation (r) was carried out, with a confidence level of 0.95 and ($\alpha < 0.05$) to establish the relationship between the amount of total PM, in the digestive tract and gills, with the biometrics performed. The correlation analysis between the amount of MP in gills with total length, total weight, and condition index, presented a non-significant correlation. The graphs did not present any apparent linear correlation and the value of r is close to zero.

4. Discussion

Because 100% of the *D. maculatus* organisms presented microplastics (MP), plastic contamination is confirmed and reflected in the SLA. This effect has been documented in other studies of aquatic ecosystems, in different parts of the world, from intercontinental waters to the North Pole [40].

In this study, it was found that MP fibers are the most abundant with 97.53%, while the abundance of films was low with 1.15%, these results coincide with the forms of MP presented by the authors [41,42,43,28] because in their studies, apart from the predominance, there is also a dominance due to the amount of plastic fibers presented in fish. Synthetic fibers are a form of plastic frequently found in the intestines of marine animals, and mainly in fish [45].

[46, 47,48,49] affirm that fibers are particles that come from different sources, such as the wear of synthetic clothing with the use of washing machines, which release large quantities of fibers generated by mechanical action, these are subsequently deposited in rivers and consequently dragged to the oceans, both by runoff and atmospheric deposition, Furthermore [46] mentions that a garment can shed more than 1900 fibers per washing machine, demonstrating that the use of washing machines can, indirectly, add considerable amounts of microplastic fibers to marine habitats. 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 [46]. The previous investigations agree with the result of the MP analysis of this investigation, obtaining the predominance of fibers with 97.53%.

In this research, it was obtained that the colors blue and black were the predominant ones with 62.08% and 17.64% respectively, results that differ from those reported by [50,51] who reported a higher percentage of white microfibers, the color is associated with the fishing nets, lines and ropes [52], black with tires, abrasives and plastic bags [1].

In studies of MPs in wild fish, it has been reported that more MPs are found in the gastrointestinal tract, gills and skin, and with lower abundance in other organs such as muscles and liver [53]. [54] propose that the presence of MP in intestines is caused by intentional or accidental

ingestion due to the availability of these contaminants in water or sediments, or also by predation on prey contaminated with MP. Furthermore [55] mention that MPs 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 species of fish studied. In this research it is inferred that the presence of blue MPs is related to their feeding habits associated with the sediment, given that *D. maculatus* is a detritivorous fish that feeds passively. It should be noted that [56] state that microfibers are more toxic than microspheres for *Hyaletta azteca* due to the longer residence time of the fibers in the intestine of the fish.

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 [57,58,51] who 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 it [51], 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 its high presence in gills, due to water filtration, and in intestines due to incidental consumption.

The present work, in addition to considering the biological parameters such as sex, size, weight, condition index and intestinal fullness, considers the length and weight of the intestine, as well as the weight of the gills as variables among which there was no difference between the amount, shape or color of MP observed between males and females.

The results of the length-weight relationship of *D. maculatus* obtained in this study showed a negative allometric growth, different from what was found by Dávila-Camacho and Galaviz-Villa, who observed that *D. maculatus* presents a positive allometric growth ($b = 3.1718$); The increase in weight is proportionally greater than the increase in length, with this difference in values it can be inferred that there is a negative influence due to the presence of MP for the optimal development of the species, since it is observed that its weight does not increase with the increase. height in adulthood.

The value of the Fulton Condition index of males and females obtained in this study was high, but the sample evaluated only corresponds to a sampling of the month of reproduction, when their gonads are well developed, this value was higher compared to the value obtained during an annual cycle evaluated by [59].

The null relationship between MP and the condition index obtained in this work coincides with what was reported by [44] who found that there is no relationship between the amount of MP and the condition index. Also, in the study carried out by [60] 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 [61,62] they did find a significant relationship between intestinal fullness and MPs, they coincide with those intestines that were classified as "full" which contained a greater number of MPs. Furthermore, they suggest that the presence of MP in mesopelagic fish species, the trend is that the increase in MP abundance occurs in smaller size categories [63].

In the present study, the relationship of the total amount of MP between intestines or gills, with variables such as weight, height, condition factor, classifications of intestinal fullness, the weight of the intestine and gills with 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 is affecting the health of the fish [64,28]. The relationship between the weight of the intestine and the MP may be due to the biomass, although in this case no negative effect is shown 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 [64] and thus influence some biological parameter; However, the intestine has been documented to

be an internal organ sensitive to MP damage [65] and often the entry point for pathogens to reach the interior of the body [66].

Finally, we must consider an issue that has been little studied, but contributes directly to pollution, the COVID-19 pandemic may be one of the causes of the high amount of MP found in this study, a consequence of the high demand of plastic items during this period, according to [67] the pandemic has intensified the demand for plastic for the production of personal protective equipment, medical supplies and packaging.

According to [68] and [46] it is necessary that clothing and washing machine designers should consider the need to reduce the release of fibers into wastewater. More research is required in this regard to develop methods to eliminate or reduce the release of MP into the environment through wastewater; for example, ultrafiltration of wastewater that has a lower amount of plastic particles.

5. Conclusions

The presence of microplastics (MP) is confirmed in 100% of *D. maculatus*. There is no significant difference between the abundance of MPs found in gills and intestines of *D. maculatus*. The biological parameters analyzed: weight, height, condition factor and intestinal fullness, lack a relationship between the amount of MP present in the intestines and gills. The weight of the intestine and gills present a very weak relationship (non-significant) with the amount of plastic MP in these organs. In the *D. maculatus* samples, fibers predominated, which were mostly blue in color. *D. maculatus* is proposed as a bioindicator of MP contamination, due to its wide distribution and its position in the food chain. The present study serves as a reference to establish a contamination index based on the amount of MP present in the intestines and gills of the species.

The color and shape of the MP indicate that they come from urban areas as a result of various daily activities such as washing clothes and fishing, in addition to poor wastewater management. The interpretation and comparison of the results may be used to determine the degree of contamination and thus contribute to the knowledge and development of environmental management proposals for the benefit of the environment.

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