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IMPACT OF MICROPLASTICS IN *Cronius ruber* SPECIES.

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Curso

2021/2022

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Trabajo Fin de Título para la obtención Grado en Ciencias del Mar

TÍTULO

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Proyecto de investigación: Microtrofic

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Acknowledgements

Thanks to the EOMAR group for their support and help during the work, especially to my tutors Alicia Herrera and May Gomez, as well as to Ico Martinez and doctors Raül Triay-Portella and Alejandro Rodriguez Gonzalez for their help and assistance.

I also thank my family and friends for sharing this stage with me.

Abstract

The presence of microplastics in the stomach contents of the invasive species *Cronius ruber* (Lamarck,1818) has been studied on the island of Gran Canaria. For this purpose, four beaches were selected from which individuals were extracted for further analysis. The beaches chosen were Playa de las Nieves beach, El Puertillo beach, La Laja beach and Anfi del Mar beach. The species *Cronius ruber* of the Portunidae family is commonly known as invasive crab and since 2016 its presence in the Canary Islands has been known. Of the individuals studied, 52% were found to be contaminated. Of the microplastics found, a total of 89% corresponded to microplastic fibers. The most frequent colors were blue (52%) and black (19%). This study demonstrates for the first time the contamination by microplastics in the species *Cronius ruber*.

Key words: Microplastics, Canary Islands, *Cronius ruber*, Canary Islands, contamination, fibers, ingestion

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1. Introduction

"Microplastics are any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water". This definition of Frias and Nash (Frias & Nash, 2019), establishes one of the most problematic materials worldwide.

Microplastics have been one of the most prominent marine pollution problems in recent decades. Due to their increasing concentration in both marine waters and sediments and their difficult removal, they are becoming a major problem.

Microplastics can be categorized into primary and secondary. Primary microplastics are manufactured for industrial use as pellets or for other uses such as exfoliants, so they are manufactured with a small size. Secondary microplastics are those plastics that have been fragmented from a larger plastic, this includes microplastic fibers, paint, fragments, lines, or films among others (Laskar & Kumar, 2019).

Microplastics interfere directly with life in the oceans. Marine sediments accumulate most of the microplastics in the water and act as sinks for them. As they accumulate in the sediments, life and microplastics begin to coexist, giving rise to biofouling (Auta et al., 2017). Microplastics can then begin to be incorporated into the food chain, where they bioaccumulate and biomagnifies in the different species (Wesch et al., 2016).

High concentrations of microplastics in organisms eventually lead to unusual behaviors or even death (Wang et al., 2016). In addition, microplastics can have a chemical impact because they are capable of carrying pharmaceuticals or pollutants such as persistent organic pollutants (POPs). Some of them, such as dichlordiphenyltrichloroethane (DDT), which has already been withdrawn in several countries due to its toxicity, are still associated with microplastics due to the resistance of this material (Ugwu et al., 2021).

Marine life ends up being affected by plastics, to a large extent vertebrates such as birds, mammals, and turtles in which there are several studies on their accumulation, although in these organisms it is more difficult to prove if there is damage by possible persistent organic pollutants (POPs). Smaller species such as fish or crabs have suffered visible damage due to the accumulation of toxic substances from the concentration of microplastics, and have even suffered problems in reproduction, embryonic development, and genetic malformations (Ugwu et al., 2021).

The Canary Islands are an archipelago located in the North Atlantic Ocean off the West African coast, between the coordinates (27º 37′ and 29º 25′ north latitude and 13º 20′ and 18º 10′ west latitude). These islands, which have a subtropical climate with temperate waters, have witnessed the increase of pollution in their seas and land.

The archipelago is in the path of the Canary Current, which in turn comes from the Azores Current. The Canary Current brings with it pollutants that accumulate on the coasts of the islands, thus generating a pollution problem (Baztan et al., 2014). In the case of Gran Canaria, there are previous studies that show the contamination already existing on several beaches of this island (Rapp et al., 2020), and therefore the possible contamination and effects on the health of marine biota. Such as the study carried out on chub mackerel (*Scomber colias*), where it was shown that 78% of the individuals fished near the coast of the Canary Islands contained microplastics (Herrera et al., 2019).

Previous studies have shown the presence of microplastics in crab species such as *Carcinus aestuarii* (Piarulli et al., 2019) or *Lithodes santolla* (Andrade & Ovando, 2017). Some of these studies reveal the leaching of microplastics particles into other tissues of the animal such as the gills (Watts et al., 2016), digestive tract (Villagran et al., 2020) or hepatopancreas (Brennecke et al., 2015). Or even how the presence of this type of pollutant affects reproduction and embryonic development of individuals (Horn et al., 2020).

However, there is no evidence of the presence of microplastics in this species *Cronius ruber* (Lamarck,1818), so this study will check for the first time the possible presence of this type of pollutant in the species in wild populations.

The species *Cronius ruber* (Lamarck,1818), is a species belonging to the family Portunidae, known as swimming crabs (Figure 1). This species has been registered in the islands since 2016, being Gran Canaria the first island with record of this animal. It is an invasive species that could have reached the islands through maritime traffic or oil platforms that are in movement in the archipelago (González et al., 2017).

Individuals of this species have a purplish red coloration, with a larger size in females than in males, reaching 85 mm (in females). The carapace is hexagonal and flat. These animals are adapted to pantropical climates. Their distribution is known from the eastern and western Atlantic and eastern Pacific (González et al., 2017).

It is a nocturnal species, with a wide breeding season, established between July and October. This is an important factor when recognizing it as an invasive species since they reproduce for several months. Another interesting fact is that this species is predated mainly by octopuses and cuttlefish. These have decreased their population due to the overcrowding of beaches and fishing (Hernandez et al., 2000). These factors influence the population of *Cronius ruber* growth significantly.

Figure 1. Cronius ruber male individuals (González et al., 2017).

Being a eurythermic and euryhaline species, its habitat is established between rocky bottoms and sandy areas, always in shallow waters, between 1 and 20 meters. In the case of the Canary Islands, it is common to find them in intertidal or shallow waters between 1.5 and 6 meters deep, generally on rocky bottoms (González et al., 2017; Maggio et al., 2021).

In consequence of the preceding, the objectives of this work are to:

- Demonstrate the presence of microplastics in *Cronius ruber* species.
- Establish a new basis for further studies of this species or of microplastics in other crab species.
- Determine whether microplastics have entered the marine food chain.
- To analyze the predominant types and colors of microplastics in order to determine their possible origin.

2. Methodology

2.1. Study area

Samples were collected from four beaches located on the island of Gran Canaria (Figure 2). Playa de las Nieves, (28º 06′ 04,345” N 15º 42′ 40,886” W), in the northwest of the island. La Laja beach (28º 3′ 39,238″ N 15º 25′ 11,95″ W) in the northeast, El Puertillo beach (28º 9′ 9,338″ N 15º 31′ 58,642″ W) in the north and Anfi del Mar beach (27° 46' 22" N 15° 41' 45" W) in the south of the island.

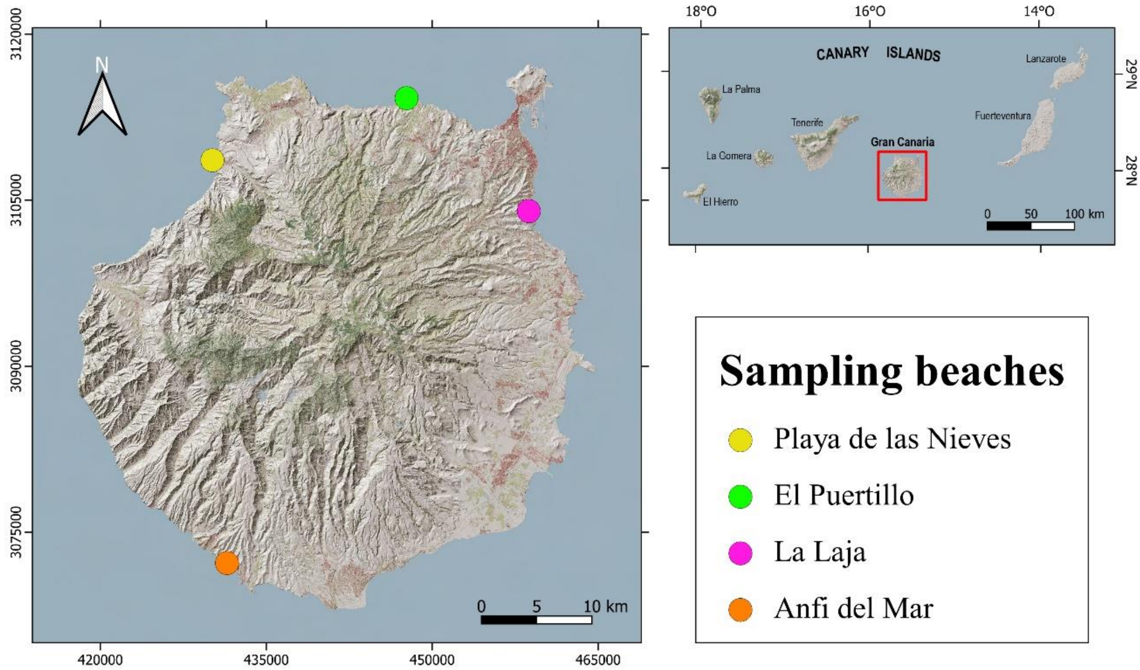

Figure 2. Location map of the sampling beaches. The map of Gran Canaria is in UTM coordinates (zone 28N, EPSG:32628).

2.2.Sampling procedure

Two scientific divers collected the crabs by hand during the night using artificial lights (Figure 3). Samples were collected after sunset and before midnight to find the crabs at their most active time (Triay-Portella et al., 2018). Samples were collected at a depth of between 1 and 7 meters in rocky and sandy bottoms. The sampling was carried out in May, June, July, and October (2021). Samples were immediately frozen to avoid decomposition or digestion within the samples.

Figure 3. Images of the capture of the individuals used in the study (Triay-Portella).

2.3.Laboratory procedures

The samples were thawed at room temperature until ready for manipulation. A dissection was performed in the stomach of each individual was extracted, and the weight of each stomach was measured. For dissection it was necessary to cut the upper part of the cephalothoracic carapace with scissors. After this, a basal membrane covering the internal organs was removed. Finally, the stomach was extracted with forceps. The stomachs were placed in a glass beaker along with a previously prepared solvent of potassium hydroxide (KOH) 10% (Figure 4).

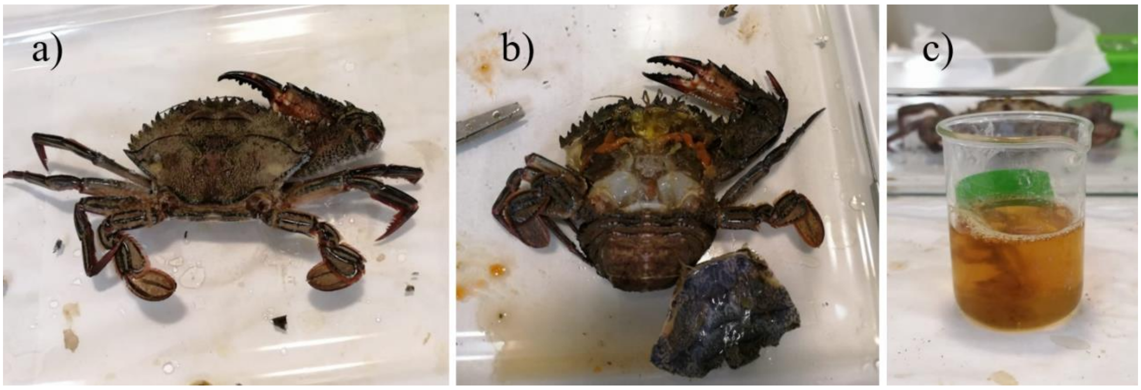

Figure 4. a) Individual of Cronius ruber. b) Dissection of the individual. c) Glass beaker with the stomach extracted into the alkaline solution.

The prepared glasses were placed in an oven, where they remained between 24 and 72 hours (depending on the sample) at 60ºC, producing the alkaline digestion. Because the walls of the crab stomach are extremely hard, the stomachs had to be emptied before placing them in the oven, with the greatest possible precaution to avoid contamination. Once the samples were digested, they were filtered through metal filters (25-micron mesh pore size) with a vacuum pump to remove the excess liquid after digestion. The filters were placed on petri-dishes sealed with flexible tape and labelled until observation.

The samples were visually inspected under binocular stereomicroscope and all particles suspected of being plastic were photographed and measured for further analysis. The microplastics found were classified into fibers, films, fragments, lines or external contamination and in turn by color. In addition, a photo was taken of each microplastic (Figure 5).

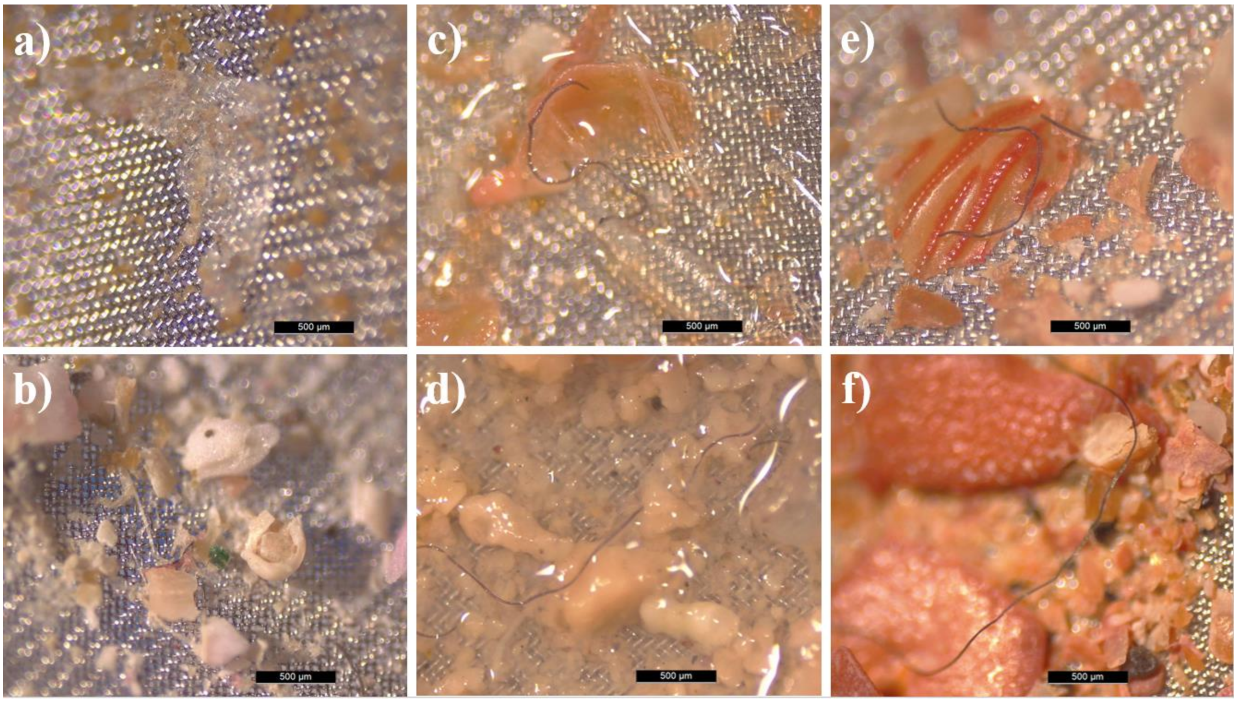

Figure 5. Photos taken of some of the microplastics found. a) Film. b) Fragment. c), d), e) and f) Fibers.

During the whole process of sample analysis, gloves and lab-coat were worn and work was carried out in a fume hood, and all material was washed and examined before use to avoid any contamination during laboratory activities. In addition, a blank was used in all the laboratory activities carried out with the samples.

After the observation and quantification of the number of microplastics found in the samples, the results were processed using Excel to obtain means, standard deviations, frequencies of occurrence and percentages per category.

3. Results

3.1. Frequency of occurrence

For this study, 63 samples were obtained from the beaches of Playa de las Nieves, El Puertillo beach, La Laja beach and Anfi del Mar beach. Of these, 33 were contaminated (52%) with microplastics. Fibers, films, and fragments were found.

Of these 63 samples studied: 22 from Playa de las Nieves, 32 from El Puertillo beach, 6 from La Laja beach and 3 from Anfi del Mar beach. Both Anfi del Mar beach and La Laja beach have the highest mean levels of microplastics per individual.

In total, 57 microplastic particles were found among all samples. The mean number of microplastics per individual was 1.73 ± 1.02 (mean ± SD), as shown Table 1.

Table 1. Summary of data sample by location.

Location	n	Mean MP/ ind	SD	FO%
Playa de las Nieves	22	1.56	0.73	40.9
La Laja beach	6	3.67	2.08	50.0
Anfi del Mar beach	3	3.00	1.41	66.7
El Puertillo beach	32	1.37	0.60	57.6
Total	63	1.73	1.10	52

In case of frequency of occurrence (FO%), plotted in Figure 6, Anfi del Mar beach has the highest percentage of contamination (66.7%), but also the lowest number of samples (n=3). However, in the case of El Puertillo beach, there is also a high percentage (57.6%) and the highest number of samples (n=32). Playa de las Nieves beach had the lowest frequency of occurrence with 40.9% of 22 samples. Only 6 samples were obtained from La Laja beach, with a frequency of occurrence of 50.0%.

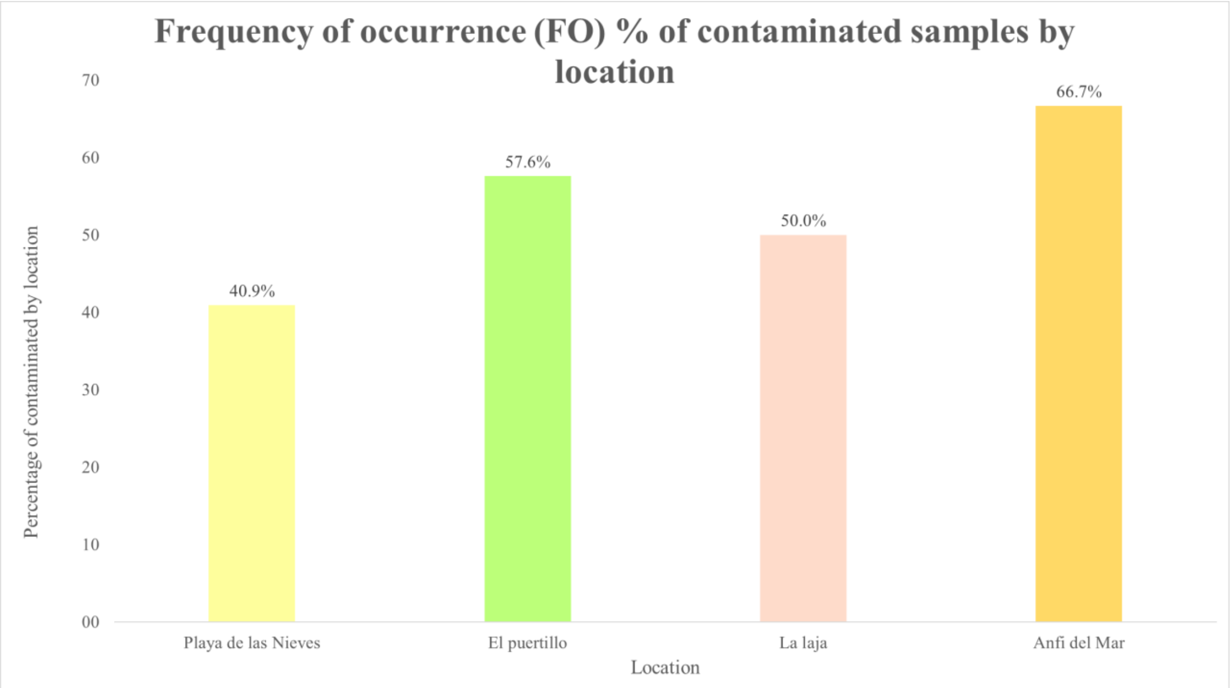

Figure 6. Graph showing the frequency of occurrence (FO%) of contaminated samples by location.

These frequency of occurrence rates can also be viewed on a map to get an overview of the contamination of each beach (Figure 7).

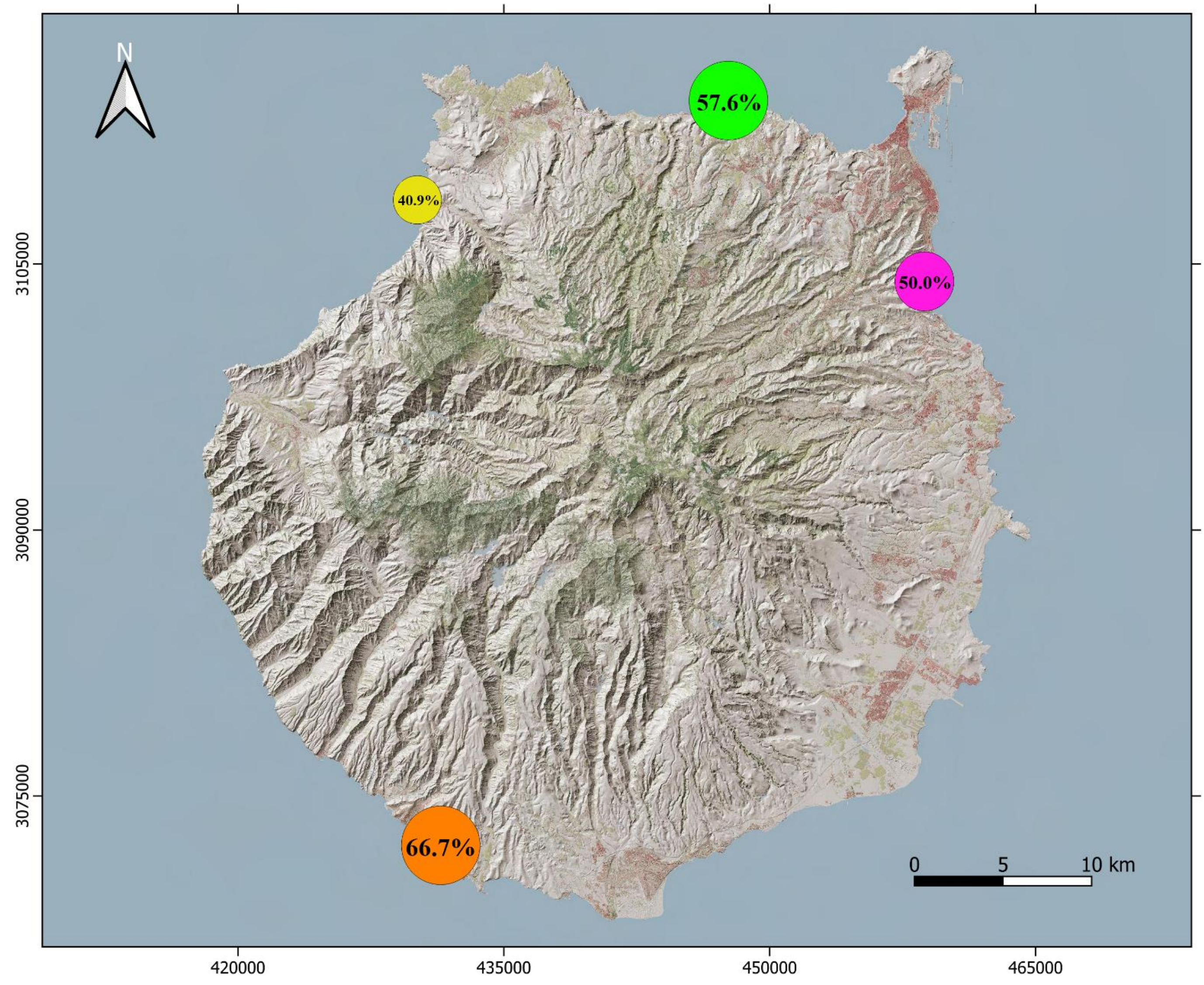

Figure 7. Percentage of contamination at each location.

3.2.Gender distribution

Considering the sex of the individuals, 41% of the samples were from males and 59% from females (Figure 8). Of the total number of males, 53.9% were found to be contaminated by microplastics, while 46.1% were not. In the case of females, 46.0% were found to be contaminated compared to 54.0% without any microplastics. Although the number of females was higher, the males contained a higher percentage of contamination.

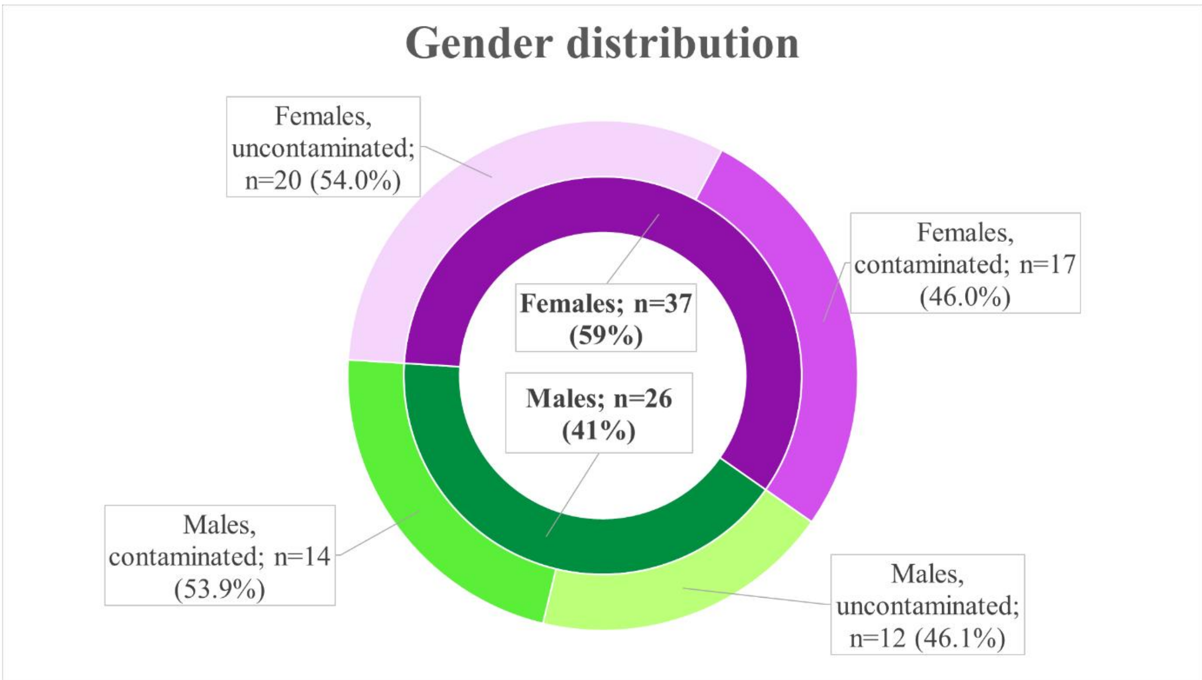

Figure 8. Gender distribution of contaminated and uncontaminated individuals by microplastics.

3.3. Microplastics types

Of the total microplastics found (Figure 9) the vast majority were fibers (89%), although films and fragments were also found in lower concentrations (7% fragments and 4% films).

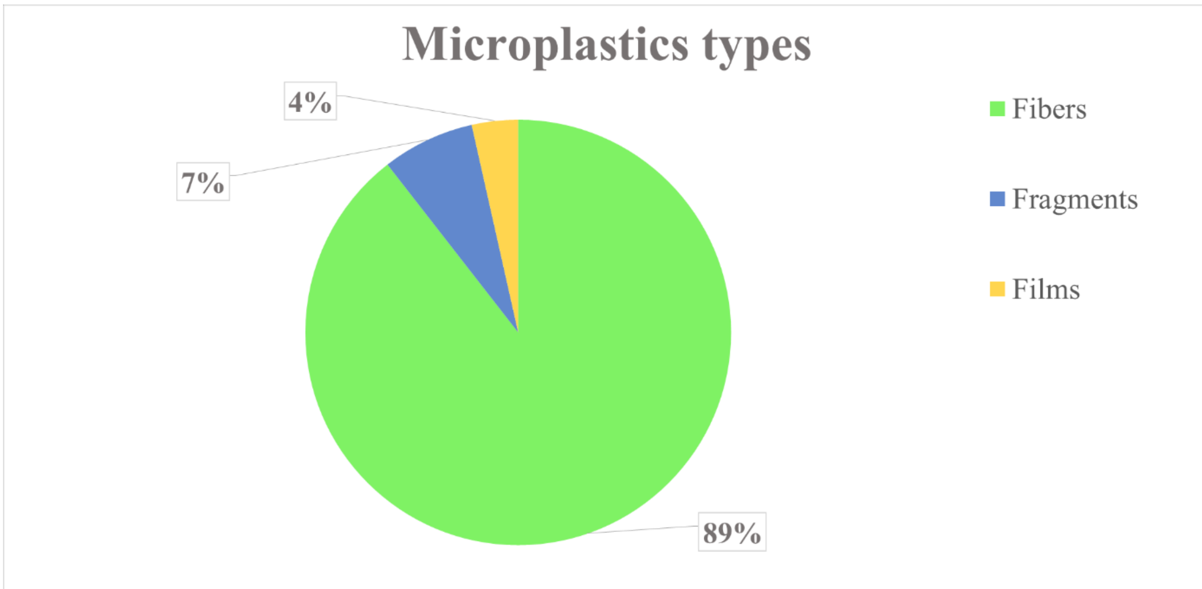

Figure 9. Distribution of microplastics types in percentages.

Different percentages of each type of microplastic were found between localities (Figure 10). Playa de las Nieves was the one with the greatest variety. Among the stomach remains of the samples found on the beaches of La Laja and Anfi del Mar, fibers and fragments were found to a lesser extent. At El Puertillo beach only microplastic fibers were found.

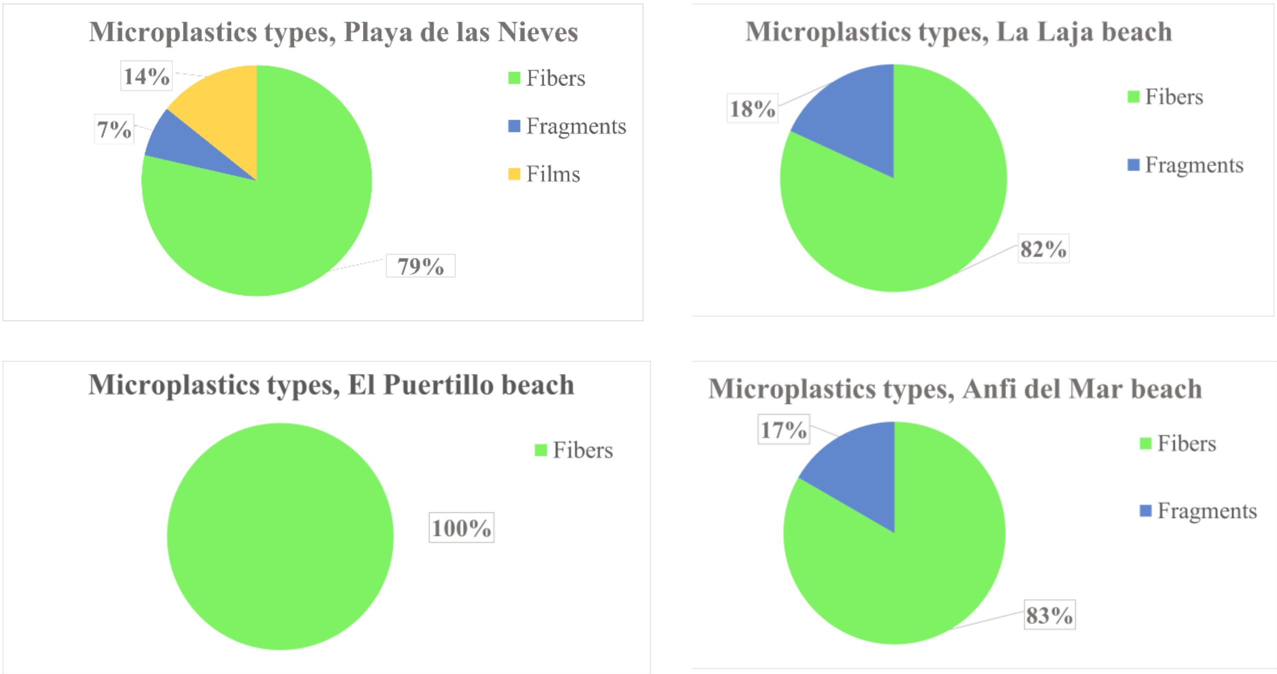

Figure 10. Distribution of microplastics types by locations.

3.4.Color distribution of microplastics

Of the microplastic particles ingested by *Cronius ruber* individuals, it can be seen in Figure 11, that the most prominent color was blue (55%), followed by black (19%) and transparent (12%). In smaller proportions, red (5%), green (7%), and purple (2%) microplastics also appeared.

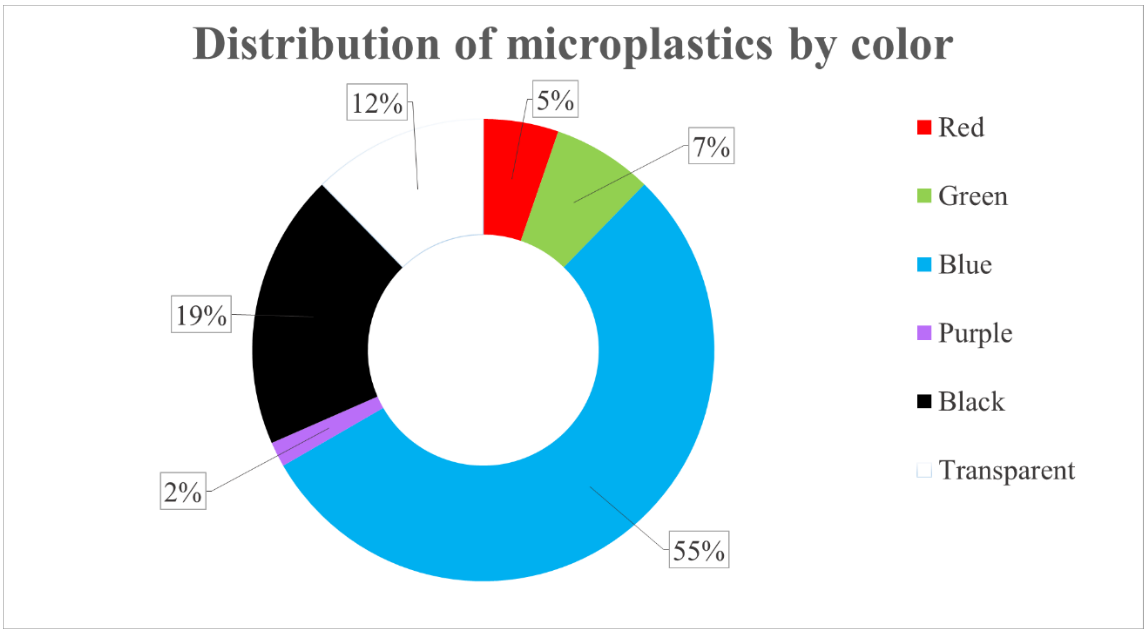

Figure 11. Color distribution of microplastics.

The colors present in all localities were black and blue (Figure 12). Concentrations of red and transparent microplastics were found at Playa de las Nieves and La Laja beach while in the case of El Puertillo beach, some green, purple, and transparent particles were found.

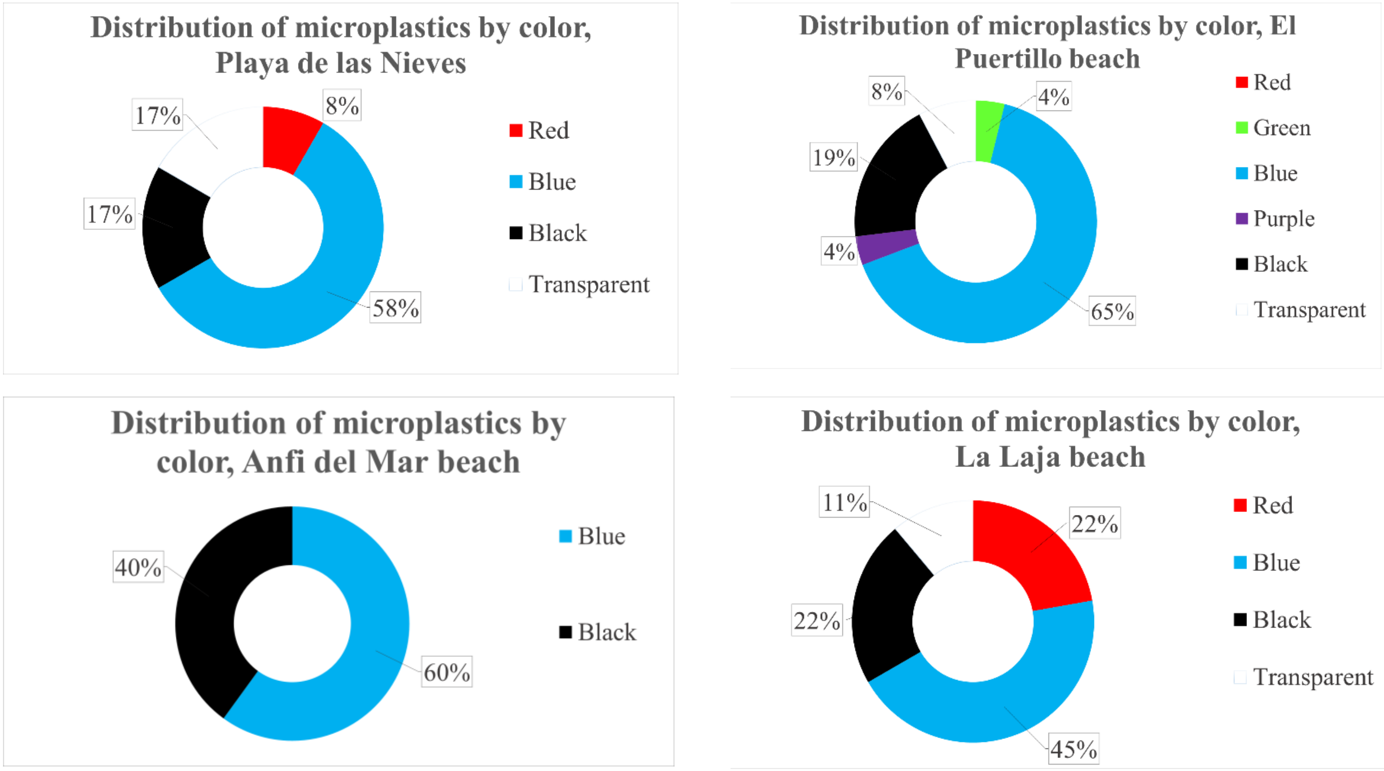

Figure 12. Color distribution of microplastics by locations.

4. Discussion

According to the results, more than half of the samples studied had microplastics in their stomach contents (52%). The presence of contaminants was found in all locations and in none of them below 40% of pollution.

As explained in the introduction, the Canary Current brings with it pollution that is deposited on the islands of the Canary archipelago, which act as a natural barrier to the current. Although this pollution is quite high (Herrera et al., 2018; Rapp et al., 2020), the plastic pollution found on the beaches, and in these four coastal areas, can be understood for the following two reasons.

On the one hand, by the presence of discharges close to them. In the case of Playa de las Nieves, there is an unauthorised discharge close to the beach. At El Puertillo beach, there are four unauthorised dumping points and four that are still being processed. Anfi del Mar beach has 3 unauthorised dumping points nearby. Finally, La Laja beach has one unauthorised discharge point on one side of the beach, while on the other side there are several authorised and pending discharges a little further away (GRAFCAN, https://visor.grafcan.es/visorweb/, Annex 1). All these wastewater discharges could expel microplastics (mainly fibers from the laundry) pollutants that end up on the beach and therefore in the organisms themselves directly through filtration in the case of filter-feeding species, or simple confusion of these anthropogenic debris with the diet of certain generally small species. It can also occur indirectly by trophic transfer in the case of predatory species.

On the other hand, this contamination could also be caused by the presence of anthropogenic pressure. All the beaches sampled are beaches with public access and therefore susceptible to contamination by passers-by. It should be noted that both Playa de las Nieves and Anfi del Mar beach have harbours. In the case of Playa de las Nieves it is a major port where the ferry is located to travel to the island of Tenerife. Hundreds of people pass through the harbour, including vehicles. It also has reactive boats. Anfi del Mar beach, however, has a smaller harbour, but with more recreational boats. This includes both trips in the waters closer to the coast and parties. This, together with the overcrowding of beaches, is a major factor in pollution that can directly affect the study species or the species it preys on.

Cronius ruber is a species with a variable diet, which changes according to age and season. Young adults consume the most food, much more noticeably than old adults, and this difference is greater during the winter. During the summer, both old and young adults increase the amount of food they eat, which coincides with the breeding season (Triay-Portella et al., 2022).

Feed consumption in males is marginally higher than in females. In the case of contaminant ingestion, the percentages are similar in both sexes, being slightly higher in females, as shown in Figure 8. Interestingly, the number of contaminated females (n=17) is higher than the number of contaminated males (n=14) even though they ingest less amount of food. These data would coincide with the study carried out by Triay-Portella (Triay-Portella et al., 2022).

In relation with the diet of this species, the most preyed species are annelids, fish, molluscs and other small crustaceans (Triay-Portella et al., 2022). In the case of molluscs, some of the species consumed are filter feeders. There are already several studies showing how the presence of contaminants in animals such as mussels can affect their predators, in this case crabs (Crooks et al., 2019; Farrell & Nelson, 2013; Wang et al., 2021; Welden et al., 2018). This shows that there is biomagnification and bioaccumulation in individuals.

In addition, females have a greater tendency to consume more annelids, more specifically polychaetes. There is evidence that polychaetes can accumulate microplastics in their bodies due to their diet (Missawi et al., 2022).

As specified in Figure 9, 89% of the microplastic particles found corresponded to fibers. Knowing that fibers are the most abundant type of pollution in the oceans (Browne et al., 2011), it is not surprising that these data appear. Fiber pollution is closely related to wastewater discharges, due to the use of washing machines that are estimated to release more than 700,000 microplastic fibers in a single medium load wash (Napper & Thompson, 2016). From there, it is not difficult that they end up being trapped by filtering organisms such as bivalves, which are already known to filter these fibers and accumulate them inside themselves (Esterhuizen et al., 2022; Jong et al., 2022; Pan et al., 2022).

Therefore, contamination by microplastic fibers in *Cronius ruber* species could come both from the fibers in their environment and from the fibers in the organisms they consume.

Of the colors found, the most abundant is blue with 55% and black with 19% of occurrence according to Figure 11. The color blue also predominates in all locations (Figure 12). Studies in other species show that these two colors tend to predominate in the stomach contents of marine organisms together with white or transparent colors (Boerger et al., 2010; Devriese et al., 2015; Okamoto et al., 2022; Piyawardhana et al., 2022; Rapp et al., 2020). These higher concentrations of blue and black fibers could be explained due to wastewater discharges that carry fibers coming from washing machines, as explained above. These fibers would not have to be exclusively of plastic, they could be of other material but always of anthropogenic origin, being able to transport chemical products such as detergents or laundry conditioners (Napper & Thompson, 2016; Sillanpää & Sainio, 2017). Blue and black fibers are the most common fibers in clothing, and black fibers may also be discolored as they degrade into blue tones. It has also been observed that ingestion of blue and black plastics also occurs in other benthic crab species inhabiting coastal areas, so this possibility could be supported (Ogunola et al., 2022).

As this is the first study that considers the possible existence of microplastics in the species *Cronius ruber*, there is no data with which to corroborate whether the data obtained are the usual ones or not. However, this study confirms the presence of microplastics in this species. Even so, the real impact that these pollutants could have in the long term in the digestive system of the crabs is not yet known.

Studies on other crab species show the damage that microplastics in certain quantities can cause to crabs. In the case of the gills, it is known that microplastics can accumulate, but in small amounts they do not affect respiration, according to a study carried out on the crab *Carcinus maenas* (Watts et al., 2016).

On the other hand, there is evidence that microplastics pass from the stomach to the hepatopancreas of crabs. With the species *Charybdis japonica*, a study was carried out in which it was confirmed that crabs can survive certain levels of microplastics contaminated with plastic paints in their hepatopancreas. The hepatopancreas is the organ where lipids are stored. This organ is able to generate enzymes that combat the stress of microplastics inside the crab. These enzymes manage to prevent cell damage and maintain neuronal activity, as neuronal cells are high in lipids. However, the crab's defense mechanism against pollutants is not perfect, and eventually collapses if the microplastic content is too high (Wang et al., 2021).

Even with these data, it is not yet possible to know how microplastics affect the species *Cronius ruber*, so it would be interesting to see if this species also has this defense mechanism or if it has a greater tolerance to pollutants due to being an invasive species. Undoubtedly much remains to be known about this species and its relationship with microplastics, so it would also be interesting to study this relationship in the different territories where *Cronius ruber* is found.

5. Conclusions

Taking into consideration the results obtained throughout the work, it can be concluded:

- Microplastics pollution are affecting the invasive species *Cronius ruber* on the island of Gran Canaria.
- In this study it has been shown that more than 50% of the individuals have microplastics in their stomachs, which may act in favor of trophic transfer to both predators and humans.
- The contamination of the study species is similar to other crab species of interest, so it would be interesting to investigate the reasons why certain colors and types of plastics are ingested more.
- Future studies are needed to determine the origin of the microplastic contamination that is affecting *Cronius ruber*.

6. Bibliography

Andrade C, & Ovando F. (2017). First record of microplastics in stomach content of the southern king crab *Lithodes santolla (*Anomura: Lithodidadae), Nassau bay, Cape Horn, Chile. Anales Instituto Patagonia (Chile). http://analesdelinstitutodelapatagonia.cl/index.php/analespatagonia/article/view/753/741

Auta, H. S., Emenike, C. U., & Fauziah, S. H. (2017). Distribution and importance of microplastics in the marine environment. A review of the sources, fate, effects, and potential solutions. *Environment International*, *102*, 165–176. https://doi.org/10.1016/J.ENVINT.2017.02.013

Baztan, J., Carrasco, A., Chouinard, O., Cleaud, M., Gabaldon, J. E., Huck, T., Jaffrès, L., Jorgensen, B., Miguelez, A., Paillard, C., & Vanderlinden, J. P. (2014). Protected areas in the Atlantic facing the hazards of micro-plastic pollution: First diagnosis of three islands in the Canary Current. *Marine Pollution Bulletin*, *80*(1–2), 302–311. https://doi.org/10.1016/J.MARPOLBUL.2013.12.052

Boerger, C. M., Lattin, G. L., Moore, S. L., & Moore, C. J. (2010). Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. *Marine Pollution Bulletin*, *60*(12), 2275–2278. https://doi.org/10.1016/J.MARPOLBUL.2010.08.007

Brennecke, D., Ferreira, E. C., Costa, T. M. M., Appel, D., da Gama, B. A. P., & Lenz, M. (2015). Ingested microplastics (>100 μm) are translocated to organs of the tropical fiddler crab *Uca rapax*. *Marine Pollution Bulletin*, *96*(1–2), 491–495. https://doi.org/10.1016/J.MARPOLBUL.2015.05.001

Browne, M. A., Crump, P., Niven, S. J., Teuten, E., Tonkin, A., Galloway, T., & Thompson, R. (2011). Accumulation of microplastic on shorelines woldwide: Sources and sinks. *Environmental Science and Technology*, *45*(21), 9175–9179. https://doi.org/10.1021/ES201811S/ASSET/IMAGES/LARGE/ES-2011-01811S_0002.JPEG

Crooks, N., Parker, H., & Pernetta, A. P. (2019). Brain food? Trophic transfer and tissue retention of microplastics by the velvet swimming crab (*Necora puber*). *Journal of Experimental Marine Biology and Ecology*, *519*, 151187. https://doi.org/10.1016/J.JEMBE.2019.151187

Devriese, L. I., van der Meulen, M. D., Maes, T., Bekaert, K., Paul-Pont, I., Frère, L., Robbens, J., & Vethaak, A. D. (2015). Microplastic contamination in brown shrimp (*Crangon crangon*, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. *Marine Pollution Bulletin*, *98*(1–2), 179–187. https://doi.org/10.1016/J.MARPOLBUL.2015.06.051

Esterhuizen, M., Buchenhorst, L., Kim, Y. J., & Pflugmacher, S. (2022). In vivo oxidative stress responses of the freshwater basket clam *Corbicula javanicus* to microplastic fibres and particles. *Chemosphere*, *296*. https://doi.org/10.1016/J.CHEMOSPHERE.2022.134037

Farrell, P., & Nelson, K. (2013). Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). *Environmental Pollution*, *177*, 1–3. https://doi.org/10.1016/J.ENVPOL.2013.01.046

Frias, J. P. G. L., & Nash, R. (2019). Microplastics: Finding a consensus on the definition. *Marine Pollution Bulletin*, *138*, 145–147. https://doi.org/10.1016/J.MARPOLBUL.2018.11.022

González, J. A., Triay-Portella, R., Escribano, A., & Cuesta, J. A. (2017). Northernmost record of the pantropical portunid crab *Cronius ruber* in the eastern Atlantic (Canary Islands): Natural range extension or human-mediated introduction? *Scientia Marina*, *81*(1), 81–89. https://doi.org/10.3989/scimar.04551.17B

Hernandez, J. (2000). Biología, ecología y pesca del pulpo común *(Octopus vulgaris*, Cuvier 1797*)* en aguas de gran canaria. Thesis.

Herrera, A., Asensio, M., Martínez, I., Santana, A., Packard, T., & Gómez, M. (2018). Microplastic and tar pollution on three Canary Islands beaches: An annual study. *Marine Pollution Bulletin*, *129*(2), 494–502. https://doi.org/10.1016/J.MARPOLBUL.2017.10.020

Herrera, A., Ŝtindlová, A., Martínez, I., Rapp, J., Romero-Kutzner, V., Samper, M. D., Montoto, T., Aguiar-González, B., Packard, T., & Gómez, M. (2019). Microplastic ingestion by Atlantic chub mackerel (*Scomber colias*) in the Canary Islands coast. *Marine Pollution Bulletin*, *139*(July 2018), 127–135. https://doi.org/10.1016/j.marpolbul.2018.12.022

Horn, D. A., Granek, E. F., & Steele, C. L. (2020). Effects of environmentally relevant concentrations of microplastic fibers on Pacific mole crab (*Emerita analoga*) mortality and reproduction. *Limnology and Oceanography Letters*, *5*(1), 74–83. https://doi.org/10.1002/LOL2.10137

Jong, M.-C., Li, J., Noor, H. M., He, Y., & Gin, K. Y.-H. (2022). Impacts of size-fractionation on toxicity of marine microplastics: Enhanced integrated biomarker assessment in the tropical mussels, *Perna viridis*. *Science of The Total Environment*, *835*, 155459. https://doi.org/10.1016/J.SCITOTENV.2022.155459

Laskar, N., & Kumar, U. (2019). Plastics and microplastics: A threat to environment. *Environmental Technology and Innovation*, *14*. https://doi.org/10.1016/J.ETI.2019.100352

Maggio, Y., Núñez, J., Docoito, J. R., Moro, L., Hernández, J. C., & Herrera, R. (2021). *Cronius ruber* (Lamarck, 1818), un nuevo decápodo en los fondos someros de Canarias. *Scientia Insularum. Revista de Ciencias Naturales En Islas*, *4*, 125–142. https://doi.org/10.25145/j.SI.2021.04.07

Missawi, O., Venditti, M., Cappello, T., Zitouni, N., de Marco, G., Boughattas, I., Bousserrhine, N., Belbekhouche, S., Minucci, S., Maisano, M., & Banni, M. (2022).

Autophagic event and metabolomic disorders unveil cellular toxicity of environmental microplastics on marine polychaete *Hediste diversicolor*. *Environmental Pollution*, *302*. https://doi.org/10.1016/J.ENVPOL.2022.119106

Napper, I. E., & Thompson, R. C. (2016). Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions. *Marine Pollution Bulletin*, *112*(1–2), 39–45. https://doi.org/10.1016/J.MARPOLBUL.2016.09.025

Ogunola, S. O., Reis-Santos, P., Wootton, N., & Gillanders, B. M. (2022). Microplastics in decapod crustaceans sourced from Australian seafood markets. *Marine Pollution Bulletin*, *179*, 113706. https://doi.org/10.1016/J.MARPOLBUL.2022.113706

Okamoto, K., Nomura, M., Horie, Y., & Okamura, H. (2022). Color preferences and gastrointestinal-tract retention times of microplastics by freshwater and marine fishes. *Environmental Pollution*, *304*, 119253. https://doi.org/10.1016/J.ENVPOL.2022.119253

Pan, Z., Liu, Q., Xu, J., Li, W., & Lin, H. (2022). Microplastic contamination in seafood from Dongshan Bay in southeastern China and its health risk implication for human consumption. *Environmental Pollution*, *303*, 119163. https://doi.org/10.1016/J.ENVPOL.2022.119163

Piarulli, S., Scapinello, S., Comandini, P., Magnusson, K., Granberg, M., Wong, J. X. W., Sciutto, G., Prati, S., Mazzeo, R., Booth, A. M., & Airoldi, L. (2019). Microplastic in wild populations of the omnivorous crab *Carcinus aestuarii*: A review and a regional-scale test of extraction methods, including microfibres. *Environmental Pollution*, *251*, 117–127. https://doi.org/10.1016/J.ENVPOL.2019.04.092

Piyawardhana, N., Weerathunga, V., Chen, H. sen, Guo, L., Huang, P. J., Ranatunga, R. R. M. K. P., & Hung, C. C. (2022). Occurrence of microplastics in commercial marine dried fish in Asian countries. *Journal of Hazardous Materials*, *423*, 127093. https://doi.org/10.1016/J.JHAZMAT.2021.127093

Rapp, J., Herrera, A., Martinez, I., Raymond, E., Santana, Á., & Gómez, M. (2020). Study of plastic pollution and its potential sources on Gran Canaria Island beaches (Canary Islands, Spain). *Marine Pollution Bulletin*, *153*, 110967. https://doi.org/10.1016/J.MARPOLBUL.2020.110967

Sillanpää, M., & Sainio, P. (2017). Release of polyester and cotton fibers from textiles in machine washings. *Environmental Science and Pollution Research*, *24*(23), 19313–19321.

Triay-Portella, R., Escribano, A., Pajuelo, J. G., & Tuya, F. (2018). Perception of faunal circadian rhythms depends on sampling technique. *Marine Environmental Research*, *134*, 68–75. https://doi.org/10.1016/j.marenvres.2018.01.004

Triay-Portella, R., Martín, J. A., Luque, L., & Pajuelo, J. G. (2022). Relevance of feeding ecology in the management of invasive species: Prey variability in a novel invasive crab. *Estuarine, Coastal and Shelf Science*, 107949.

Ugwu, K., Herrera, A., & Gómez, M. (2021). Microplastics in marine biota: A review. *Marine Pollution Bulletin*, *169*. https://doi.org/10.1016/J.MARPOLBUL.2021.112540

Villagran, D. M., Truchet, D. M., Buzzi, N. S., Forero Lopez, A. D., & Fernández Severini, M. D. (2020). A baseline study of microplastics in the burrowing crab (*Neohelice granulata*) from a temperate southwestern Atlantic estuary. *Marine Pollution Bulletin*, *150*, 110686. https://doi.org/10.1016/J.MARPOLBUL.2019.110686

Wang, J., Tan, Z., Peng, J., Qiu, Q., & Li, M. (2016). The behaviors of microplastics in the marine environment. *Marine Environmental Research*, *113*, 7–17. https://doi.org/10.1016/J.MARENVRES.2015.10.014

Wang, T., Hu, M., Xu, G., Shi, H., Leung, J. Y. S., & Wang, Y. (2021). Microplastic accumulation via trophic transfer: Can a predatory crab counter the adverse effects of microplastics by body defence? *Science of The Total Environment*, *754*, 142099. https://doi.org/10.1016/J.SCITOTENV.2020.142099

Watts, A. J. R., Urbina, M. A., Goodhead, R., Moger, J., Lewis, C., & Galloway, T. S. (2016). Effect of Microplastic on the Gills of the Shore Crab *Carcinus maenas*. *Environmental Science and Technology*, *50*(10), 5364–5369. https://doi.org/10.1021/ACS.EST.6B01187/SUPPL_FILE/ES6B01187_SI_001.PDF

Welden, N. A., Abylkhani, B., & Howarth, L. M. (2018). The effects of trophic transfer and environmental factors on microplastic uptake by plaice, *Pleuronectes plastessa*, and spider crab, *Maja squinado*. *Environmental Pollution*, *239*, 351–358. https://doi.org/10.1016/J.ENVPOL.2018.03.110

Wesch, C., Bredimus, K., Paulus, M., & Klein, R. (2016). Towards the suitable monitoring of ingestion of microplastics by marine biota: A review. *Environmental Pollution*, *218*, 1200–1208. https://doi.org/10.1016/J.ENVPOL.2016.08.076

7. Annexes

7.1.Annex 1

View of all wastewater discharges from the four study locations in an area equal to or less than half a kilometer.

Figure 13. Wastewater discharges near Playa de las Nieves beach.

Figure 14. Wastewater discharges near El Puertillo beach.

Figure 15. Wastewater discharges near La laja beach.

Figure 16. Wastewater discharges near Anfi del Mar beach.