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# IMPACT OF MICROPLASTICS IN JELLYFISH

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Trabajo de fin de máster para la obtención del título de Máster Interuniversitario en Oceanografía

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# ABSTRACT

For some years now, plastic has become a concern issue among population, so much so that "microplastic" was named word of the year 2018 for the Fundéu BBVA. Currently they can be found in almost all ecosystems and environments around the world, from the top of Everest to the deep sea. This ubiquity makes them have a severe impact on ecosystems, affecting all the food chain. Although plastics and their effects are increasingly studied, especially on key species for humans, there is still much to know about how they affect other organism such as medusa and siphonophores. The above motivates this study to determine if plastic ingestion occurs in medusa and siphonophores and, on the other hand, if factors such as microplastic concentration or the presence of prey affect ingestion and retention time.

**Keywords:** microplastic, microspheres, medusa, siphonophore, plastic pollution.

Los desechos plásticos se están convirtiendo desde hace unos años en un tema de preocupación entre la población, tanto es así que “microplástico” fue nombrada palabra del año 2018 para la Fundéu BBVA. Actualmente se pueden encontrar en prácticamente todos los ecosistemas y ambientes alrededor del mundo, desde la cima del Everest hasta las profundidades marinas. Esta ubicuidad hace que tengan un severo impacto en los ecosistemas, afectando a toda la cadena trófica. A pesar de que cada vez se estudia más acerca de los plásticos y sus efectos, especialmente en las especies clave para los seres humanos, aún queda mucho por saber acerca de cómo afectan a otros organismos como medusas y sifonóforos. Lo anteriormente expuesto motiva la realización de este estudio, con el objetivo de determinar si ocurre ingestión de plástico en medusas y sifonóforos y, por otra parte, si factores como la concentración de microplásticos o la presencia de presas afectan a la ingestión y el tiempo de retención.

**Palabras clave:** microplásticos, microesferas, medusa, sifonóforo, contaminación, plásticos.

# INDEX

# 1. INTRODUCTION

The large number of types of plastics and its properties, such as resistance to chemical elements, different temperature ranges, durability and due to its low cost and easy handling, makes it an essential material for most industries in our society (Andrady & Neal, 2009). In the year 2017, 348 million tons of plastic were produced worldwide, coming mainly from Asia (50.1%), followed by Europe with 18.5% of production (PlasticsEurope, 2018). In a study conducted in 2010 by Jambeck et al. (2015) it was estimated that, of the plastics used up to a distance of 50 km from the coast, 8 million tons ended up in the sea.

The same properties that make plastic popular make it a material so harmful for the environment. Due to its durability and resistance it can take hundreds or thousands of years to degrade, especially in the marine environment, where it can spend long periods of time before any degradation begins. (Rutkowska et al., 2002). Despite this, in the case of compostable plastics, such as bags, the degradation time is considerably reduced, especially in the marine environment if compared with conventional plastic bags (Napper & Thompson, 2019).

When conventional plastic is degraded, at least in the short term, it does not form simpler chemical structures, but suffers a physical fragmentation that, as it reduces the size of its pieces, it forms what is called microplastics. The size from which it is considered microplastic has varied over time and depends on the author or research group. For Graham & Thompson (2009) the size considered was <10 mm, Derraik (2002) used the range of 2 – 6 mm, authors like Ryan et al. (2009) used <2 mm sizes and even sizes <1 mm in the case of Browne et al. (2007), Browne et al. (2010) and Claessens et al. (2011). In this case, <5 mm is the recognised size to consider a fragment as microplastic, similar to studies like Barnes et al. (2009), Betts (2008) and Hartmann et al. (2019).

Microplastics can be divided into primary or secondary (Cole et al., 2011). The primary microplastics are manufactured with that size initially, for example, the pellets, the raw material for the fabrication of plastic objects, that usually have a size of 2 – 5 mm in diameter or the microspheres used as exfoliants in cosmetic and hygiene products. On the other hand, secondary microplastics are those that originally had a larger size and, due to physical, chemical and / or biological degradation, are fragmented into particles smaller than 5 mm.

These plastics, whether macro or micro are not limited only to urban beaches or coastal areas of zones populated by humans, but can be found widely distributed by the oceans (Eriksen et al., 2014) and in many of the most remote and deepest places on the planet

(Barnes et al., 2009) due to the transport they suffer from wind, currents and even organisms (Choy et al., 2019).

The fact that plastic is such a widely distributed waste has, as one of its multiple consequences, an impact on a multitude of organisms such as fish (Baalkhuyur et al., 2018; Zhu et al., 2019), corals (Chapron et al., 2018; Connors, 2017; Hall et al., 2015; Hankins et al., 2018), turtles (da Silva Mendes et al., 2015; Mrosovsky et al., 2009), bivalves (Naidu, 2019; Sussarellu et al., 2016) or medusa (Macali et al., 2018), affecting their feeding, growth, reproduction and even their behaviour, it can also cause death. The way plastics impact on organisms depends fundamentally on their feeding behaviour, for example filtering organisms mistake plastic as their prey due to its colour and form (Constantino & Salmon, 2003; Q. A. Schuyler et al., 2014; Q. Schuyler et al., 2012). The consumed plastic can be transferred along the food chain (Nelms et al., 2018) and can even affect humans (Herrera et al., 2019; Van Cauwenberghe & Janssen, 2014).

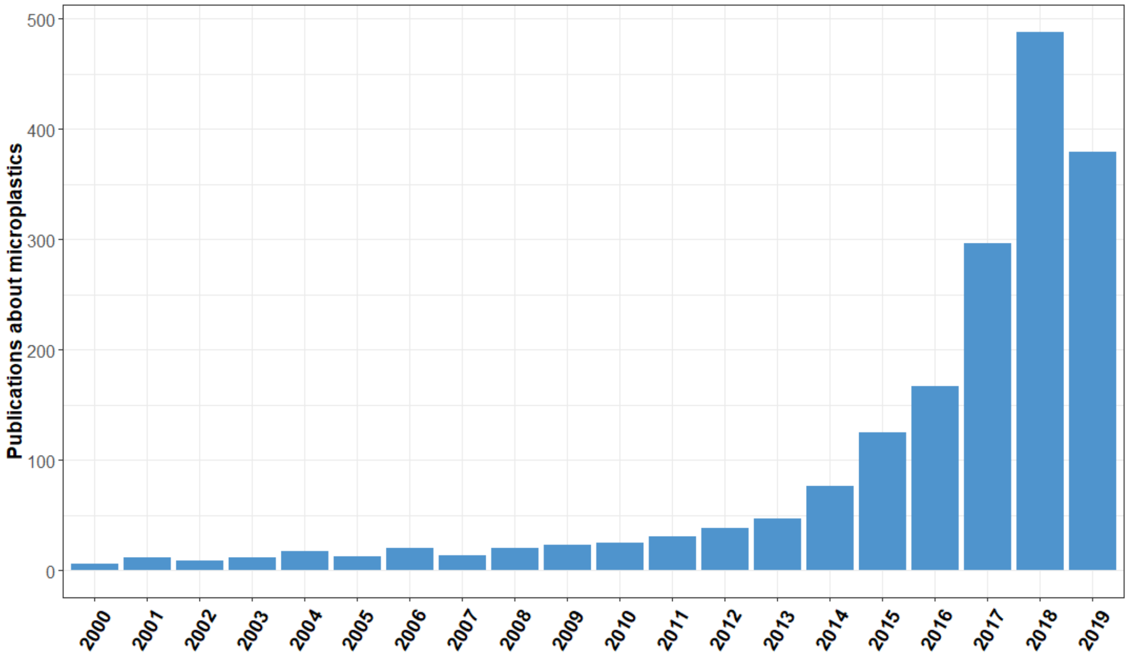



*Fig. 1. Publications per year on microplastics according to the Web of Science database in July 2019.*

Even though microplastics and their effects are increasingly studied (Fig. 1), there is still much to investigate, especially its relationship with organisms such as medusa and siphonophores. These animals belong to the phylum Cnidaria, are characterized by the presence of urticating cells called cnidocytes, used mainly to capture prey. In the present study we analyzed the presence of plastics in *Physalia physalis*, *Pelagia noctiluca* and we studied ingestion of microplastics in *Aurelia aurita* in different conditions.

*P. physalis* is usually misidentified as a medusa, belonging to the class Hydrozoa, order Siphonophora, is actually a floating colony of highly specialized organisms. Its sting can be very dangerous, especially for allergic people and can even cause death in more extreme cases. It is widely distributed in warm and temperate waters around the world (Tibballs, 2006). *P. noctiluca*, belonging the class Scyphozoa, is the most dangerous medusa, indigenous to the Mediterranean, although it can also be found in the Atlantic Ocean and more rarely in Australia, California or Hawaii. It commonly forms large blooms that cause great damage, especially to fisheries. *A. aurita*, also belonging Scyphozoos, is a cosmopolitan medusa of temperate and cold waters that, although is usually considered harmless to humans, can be problematic for bathers when there is a great number of them. (Mariottini & Pane, 2010).

The order Siphonophora is a poorly understood group. Due to the fragility of these animals, most are damaged or destroyed when collected by nets. Each suborder has a characteristic diet, being fish larvae and copepods the main food of suborder Cystonectae, which includes *P. physalis*. Siphonophores can have many small gastrozooids per colony, which are the polyps responsible for the digestion, usually consuming their prey outside gastrozooids, or can have few large gastrozooids which have great percentage of containing prey. Siphonophores with many small gastrozooids have close-spaced tentacles and branches that are spread in a three-dimensional net. Siphonophores with large gastrozooids, like *P. physalis*, often have fewer and more widely spaced tentacles, more suited to the capture of large, less abundant prey types (Bardi & Marques, 2008; Purcell, 1981). The organisms belonging to class Scyphozoa have the ability to swim, unlike most of siphonophores. This allows them to actively hunt their prey stinging them using their tentacles located on the medusa's umbrella margin. Using their four oral arms move the prey to the mouth and into the gastric pouch, where it would be digested (Gasca & Loman-Ramos, 2014; Sandrini & Avian, 1989; Vazquez Archdale & Anraku, 2005)

Due to the lack of information about how microplastics affect these animals, the present study aims to investigate if plastic can be found adhered to or inside them in their natural habitat and to study for the first time the ingestion of plastic microspheres in cultures of *A. aurita*, and the effect of the concentration and presence of prey on ingestion. In order to determinate the possible origin of the plastics found in jellyfish, the abundance and types of microplastics found on the beaches on the same day that the organisms were collected were studied.

# 2. MATERIALS AND METHODS

## 2.1 Study of microplastic on the beach

On the same day that the *P. physalis* were obtained, sand samples were also taken at points 1 (28.147320° N, 15.431171° W), 2 (28.143625° N, 15.433297° W) and 3 (28.141586 ° N, 15.435111° W) of Las Canteras beach (Fig. 2).


*Fig. 2. Sampling points of Las Canteras beach.*

In each point, three squares of 50 x 50 cm were placed following the tide line, the first 2 cm of sand was collected in order to quantify and identify the plastics present. First, the sand was separated from the other elements by passing the samples through a 1 mm sieve. Mainly what remained after the sieving were plastics and organic matter, then the samples were introduced in 96% ethanol in order to separate by density plastic and organic matter. Most plastic except foams sink to the bottom, while the organic matter float. Then the foams and plastic were separated and, subsequently, with a 5 mm sieve, the plastics were separated by size in two fractions, macro and mesoplastics ( >5 mm) and microplastics (1 - 5 mm).

After, each size fraction of each sample was weighed, counted and classified according to the type of plastic and colour.

## 2.2 Study of microplastics in *P. physalis and P. noctiluca*

On April 5, 2019, a notice was received that specimens of *P. physalis* (Fig. 3A) were arriving at Las Canteras beach, in the northeast of Gran Canaria island (Spain), dragged by the currents, so they were collected as they were stranded on the shore. Later, on June 19 of the same year, some medusa of the species *P. noctiluca* (Fig. 3B) could be obtained from the water in Tenerife island (Spain).

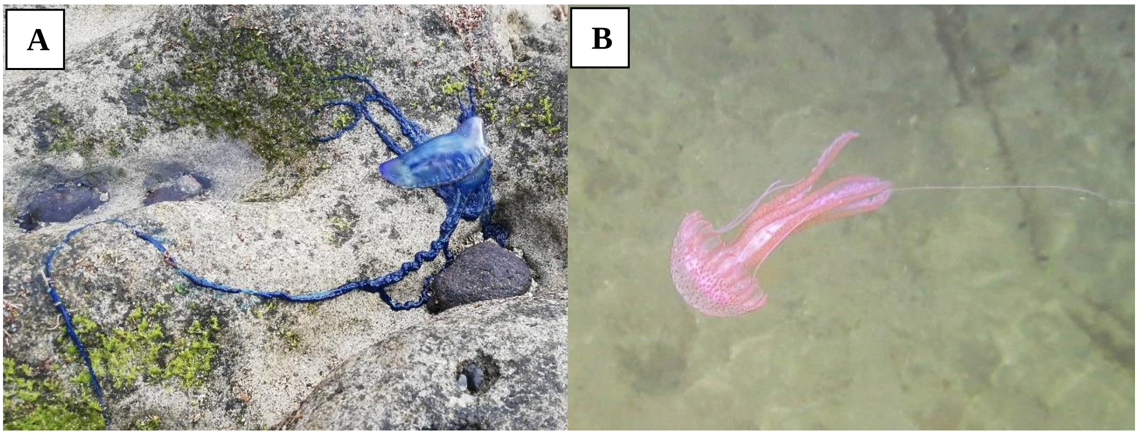



*Fig. 3. Specimens of P. physalis (A) and P. noctiluca (B) collected.*

Once the specimens were obtained, they were taken to the laboratory, where fifteen of the *P. physalis* sampled that day and fifteen *P. physalis* collected previously in February 2017 were observed under the microscope, in order to check the presence of plastic and differences between the two different samplings. This was also done for six *P. noctiluca*. Because this last one is rarely found in coastal waters of the Canarian archipelago, it was not possible to obtain more organisms or do a statically comparison with individuals previously obtained.

In the laboratory each individual was introduced separately in 10% KOH solutions and left in an oven at 60 °C for 24 h in order to dissolve the organic matter and to identify the plastic particles. After 24 h the samples of *P. physalis* were correctly dissolved, but it was necessary to leave *P. noctiluca* 72 h more to digest most of the organic matter.

When the samples were properly digested, they were filtered and observed again under the microscope to separate and identify plastic particles.

## 2.3 Microplastic ingestion experiments

Faced with the inability to breed, obtain and maintain *P. noctiluca* for this experiment, it was decided to use the medusa *A. aurita*, since in our facilities we have cultures and we have developed the techniques for their maintenance.

Four 1.7 L plankton kreisel tanks (Fig. 4) specially designed for this experiment were used, one *A. aurita* individual was placed in three of them, leaving the fourth empty as control. These plankton kreisel generate a circular water flow thanks to an air pump that produce a bubbling on the side of the tank. So the medusa were kept in suspension in the water column, because in the absence of this circulation they remained at the bottom of the tank.

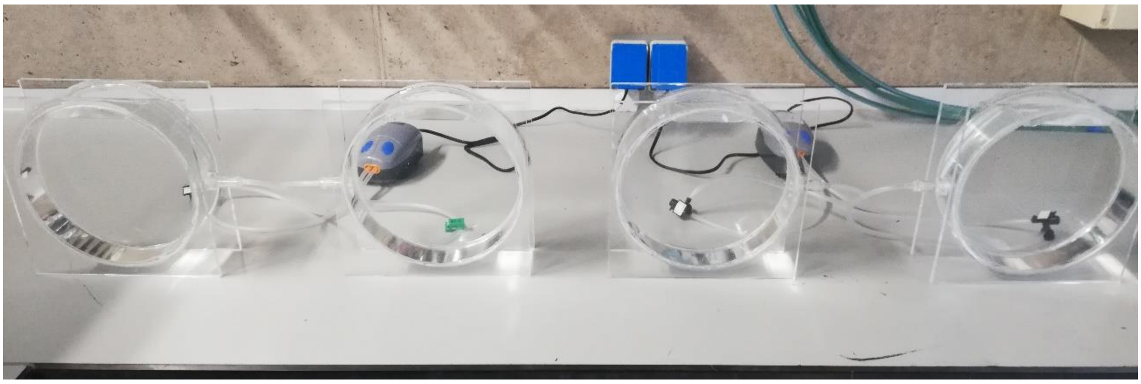


*Fig. 4. Plankton kreisel used in microplastic ingestion experiments.*

The first experiment was designed to determine the ingestion of microplastic at different concentrations, and if the presence or absence of prey affect the ingestion of microplastics in medusa. Fluorescent plastic microspheres (Cospheric fluorescent green polyethylene microspheres 1.025 g / cc, 75 - 90 μm) were added to the plankton kreisel tanks so that the resulting concentration was 5000 ± 326 microspheres / L in the four tanks. Every 10 min and during 8 h, with the help of ultraviolet light it was checked if the medusa had ingested plastic or not inside their gastric cavity (Time of presence). In the following days the experiment was repeated, but with concentrations of 10000 ± 652 and 20,000 ± 1034 microspheres / L (ANNEX Table 2). To see if the presence of prey is relevant in the consumption of microplastic the experiment was repeated with the three previous concentrations and, 500 48h-artemia nauplii added at each tank (ANNEX Table 2).

We performed another experiment to determine the retention time of the microspheres in the gastric cavity and whether this time varies with concentration, as well as the variability of the number of ingested microspheres. To do this, *A. aurita* was feeding with 48h-artemia nauplii together with the three previous concentrations of microspheres and, after 30 min to let medusa enough time to eat, three medusa were placed separately into three tanks filled exclusively with seawater, without more nauplii or microspheres than they could already take inside, so that, unlike the previous experiment, we could find out if the microspheres that could have inside were always the same or if they were ingesting and expelling different microspheres. With ultraviolet light, it was checked every 10 min if they had microspheres inside, in the manubrium or in the gastric cavity (Retention time) until they had already expelled or digested them all (ANNEX Table 3).

## 2.4 Statistical analysis

The normality of the data was verified by the Shapiro-Wilk test, and the homogeneity of variances was analyzed with the Bartlett test. Since both normality and homogeneity of variances were fulfilled, no transformation was necessary and ANOVA test was applied to determine statistical differences between treatments. Both the statistical analyses and the graphs were made with the statistical software R (version 3.5.3) and its integrated development environment Rstudio (version 1.1.463).

# 3 RESULTS

## 3.1 Study of microplastic on the beach

All the plastics that were found on the beach could be divided into three types of plastic: fragments, foams and lines. Among them, most were identified as fragments (Fig. 5) while only 1% of the plastics found were lines. Considering the colour, the white and the degraded white due to the sun, represent 65% of items found (Fig. 6), followed by transparent and semitransparent plastics that reached 11%. The colours that were less frequent in the sand were red, orange, gray and brown.

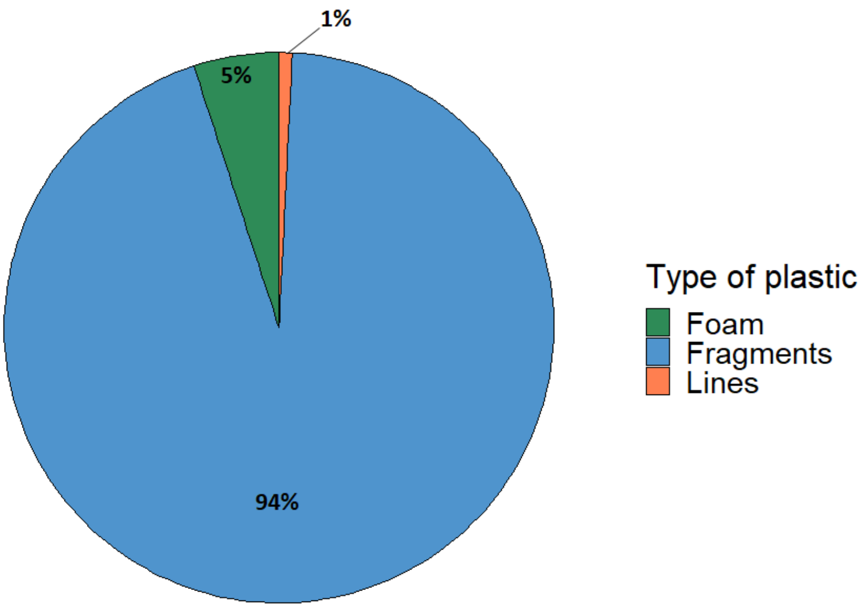



*Fig. 5. Types of plastic found on Las Canteras beach.*

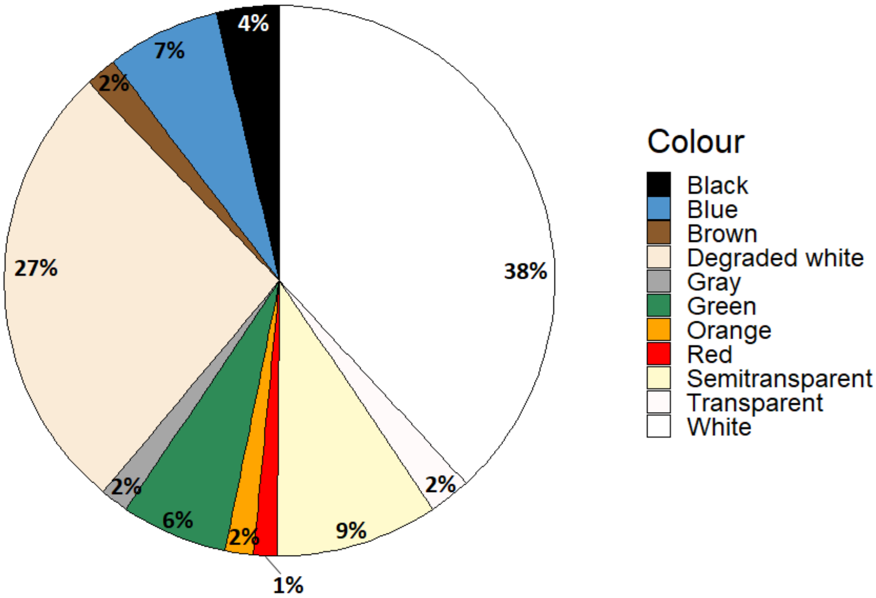



*Fig. 6. Colour of the plastics found on Las Canteras beach.*

The amount of microplastics found in the different sampling points along the beach (Fig. 7A) was compared. In zone 1 there is a smaller amount of microplastics, and apparently in zones 2 and 3 there is a similar amount. To certify this, an ANOVA was performed to see if there were differences in the number of microplastics between the different zones. The results were that zones 1 - 3 were different (p-value = 0.035) and nevertheless between zones 1 - 2 and 2 - 3 no significant differences were found (p-value = 0.065 and p-value = 0.870 respectively).

No differences were found in the weight of plastic (Fig. 7B) between the two size fractions (p-value = 0.726), ergo, there were similar weights regardless of whether the size was >5 mm or 1 - 5 mm. When comparing the weight found in the three zones differences were found. Specifically, zone 3 is significantly different to zones 1 and 2 (p-value = 0.0002 and p-value = 0.018 respectively) and between zones 1 - 2 there were no significant differences in the weight of plastic found (p-value = 0.140).

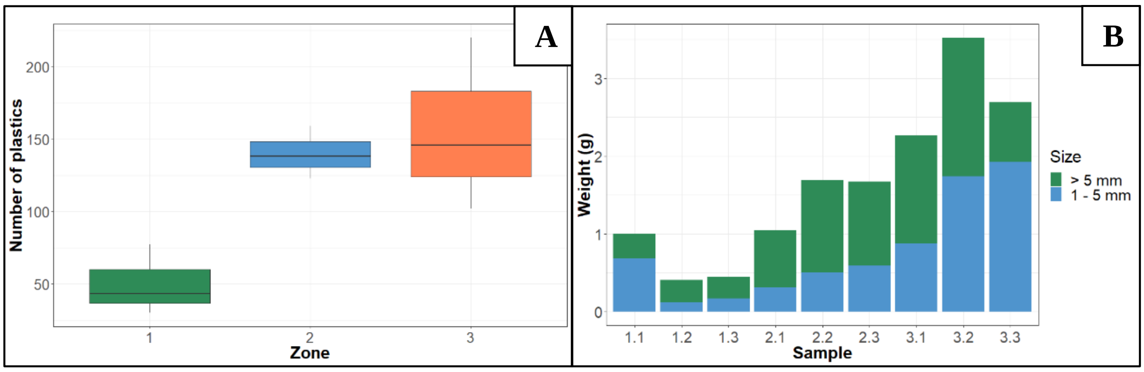
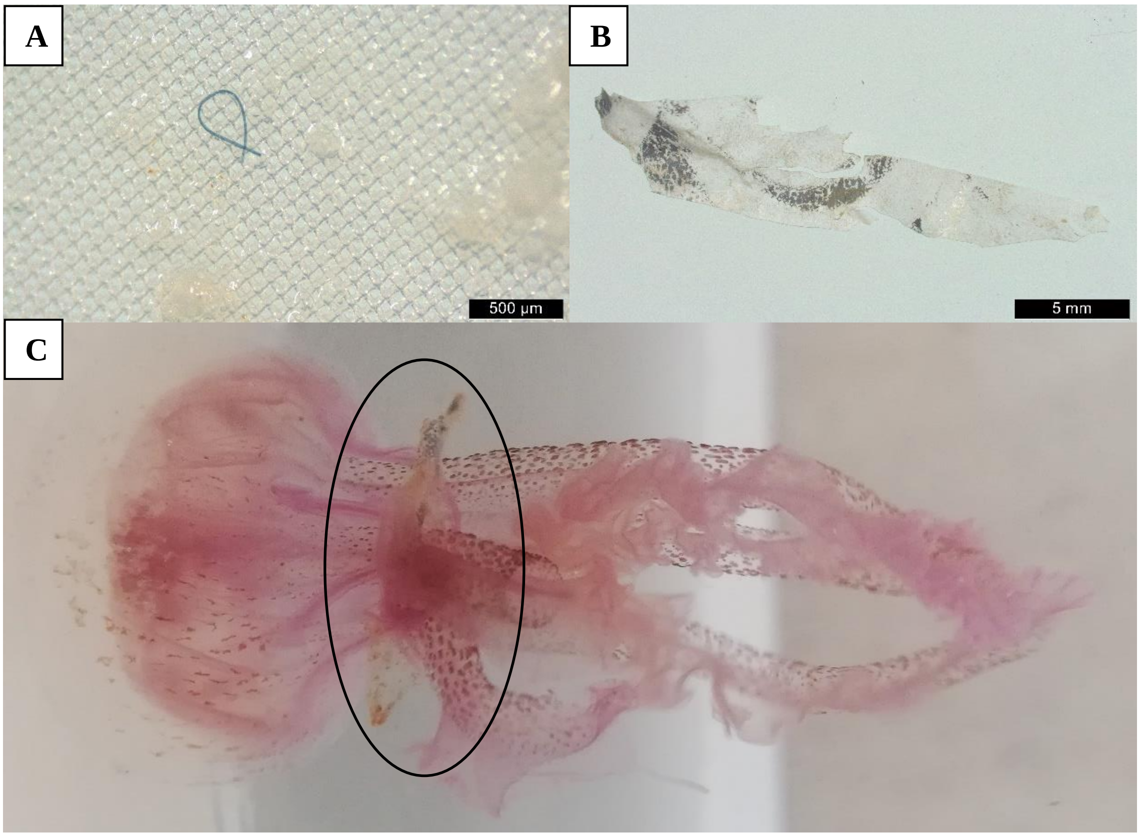



*Fig. 7. Number of plastics in each zone (A) and weight in grams for each sample depending on size of the particle.*

### 3.2 Study of microplastics in *P. physalis and P. noctiluca*

After digestion and examination of *P. physalis* samples, the only plastics found were textile microfibers (Fig. 8A) in all samples. These microfibers were also found in *P. noctiluca*, however in both cases they were not considered for the results, due to a possible contamination in the beach or during transport to laboratory by synthetic microfibers in the atmosphere (Dris et al., 2016).

In case of *P. noctiluca*, in three of the six specimens, other plastics were found in addition to synthetic microfibers. In the first one, a transparent plastic fragment was found (Fig. 8B) that could be seen with the naked eye prior to the digestion of the medusa and that was entangled between its tentacles (Fig. 8C). The second contained a black line (Fig. 8D), probably from a fishing net or similar. In the third one (Fig. 8E), another line was found, although in this case it was transparent and longer, about 21 mm instead 1.5 mm. In the three remaining individuals of *P. noctiluca* the items found were only microfibers.



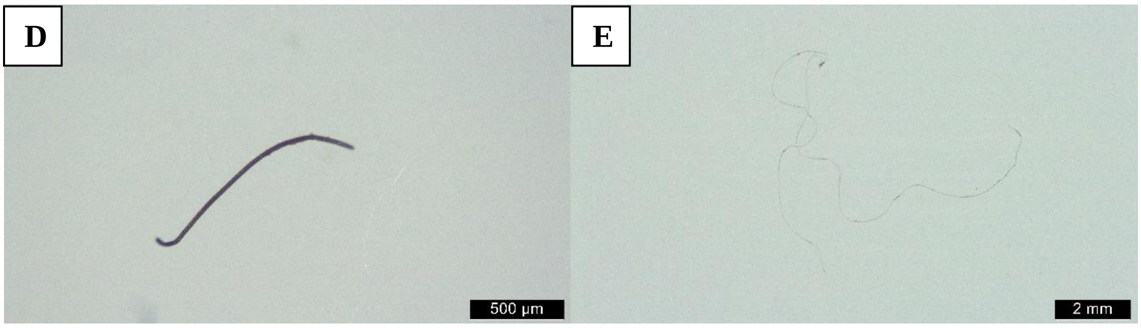



*Fig. 8. Plastics found in the samples of P. physalis (A) and P. noctiluca (B, C, D, E).*

### 3.3 Microplastic ingestion experiments

Table 1 shows the results of the two experiments carried out in cultures of *A. aurita*. An important finding is that ingestion of microspheres only occurs in the presence of prey, independently of the concentration. Otherwise, it seems that the tendency of the time of presence of microspheres in the gastric cavity (Fig. 9A) increases with the concentration of microspheres (Fig. 10). However, no significant differences were found between the time of presence at the three different concentrations (p-value = 0.307). In the retention time experiment (Fig. 11) no significant differences were found between the different concentrations of microspheres (p-value = 0.441) nor is there an appreciable upward trend as in the previous case, so it seems that the retention time is independent of the beads present in the environment.

| Treatment | Ingestion | Average time of presence (min) | Retention time (min) | Average microspheres |
|---|---|---|---|---|
| 5000 / L | ✗ | - | - | - |
| 5000 / L + nauplii | ✓ | 103.33 ± 136.67 | 150 ± 60 | 1 ± 1 |
| 10000 / L | ✗ | - | - | - |
| 10000 / L + nauplii | ✓ | 176.67 ± 16.67 | 166.67 ± 33.33 | 2 |
| 20000 / L | ✗ | - | - | - |
| 20000 / L + nauplii | ✓ | 226.67 ± 96.67 | 123.33 ± 33.33 | 3 ± 2 |

*Table 1. Results obtained from the ingestion experiments.*

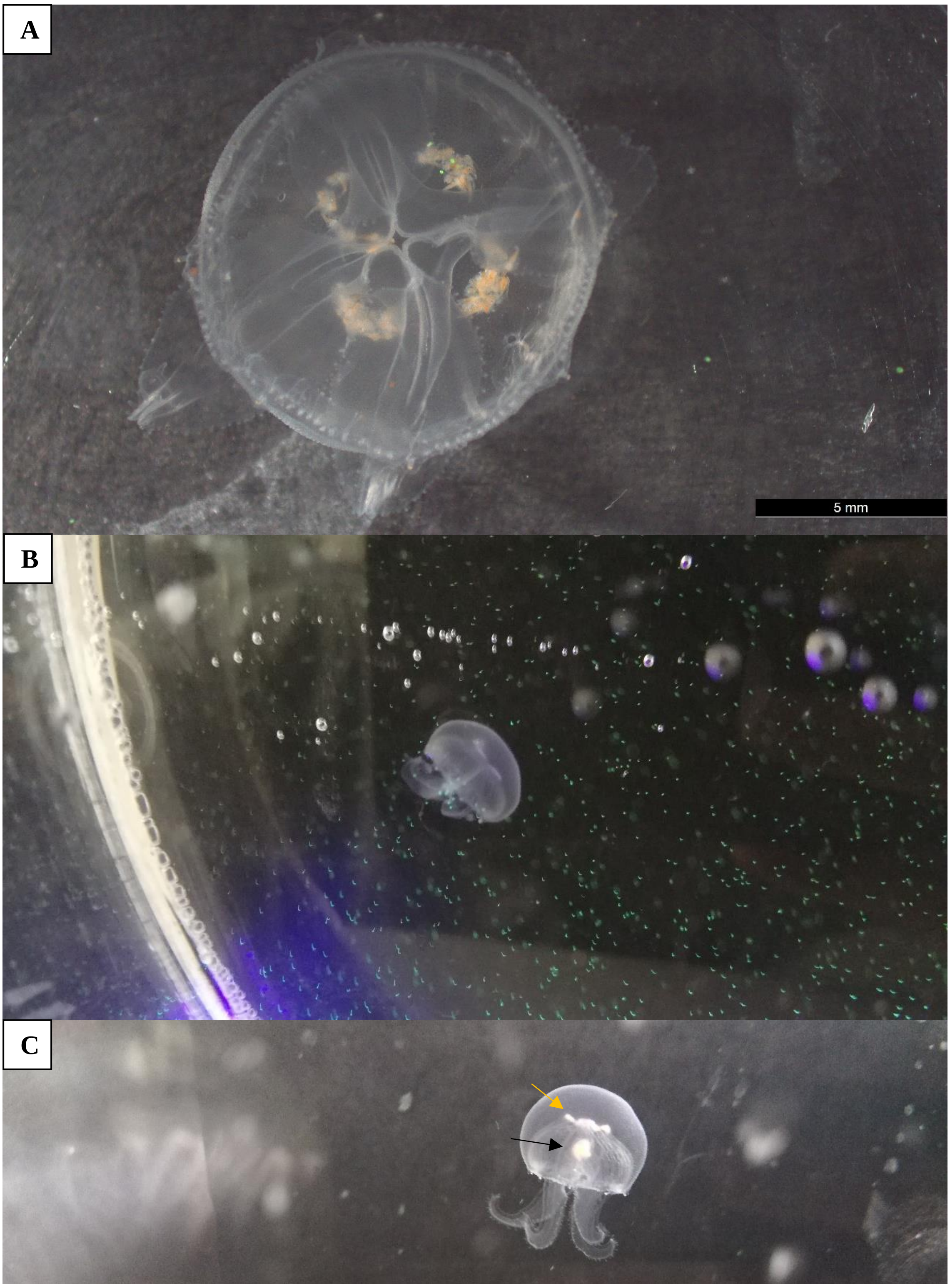



*Fig. 9. Ingested microspheres next to the nauplii in the gastric cavities (A). Adhered microspheres at 20000 microspheres / L to A. aurita (B). Ingested food in the manubrium marked with a black arrow and the aliment in the gastric cavities pointed with a yellow arrow (C).*

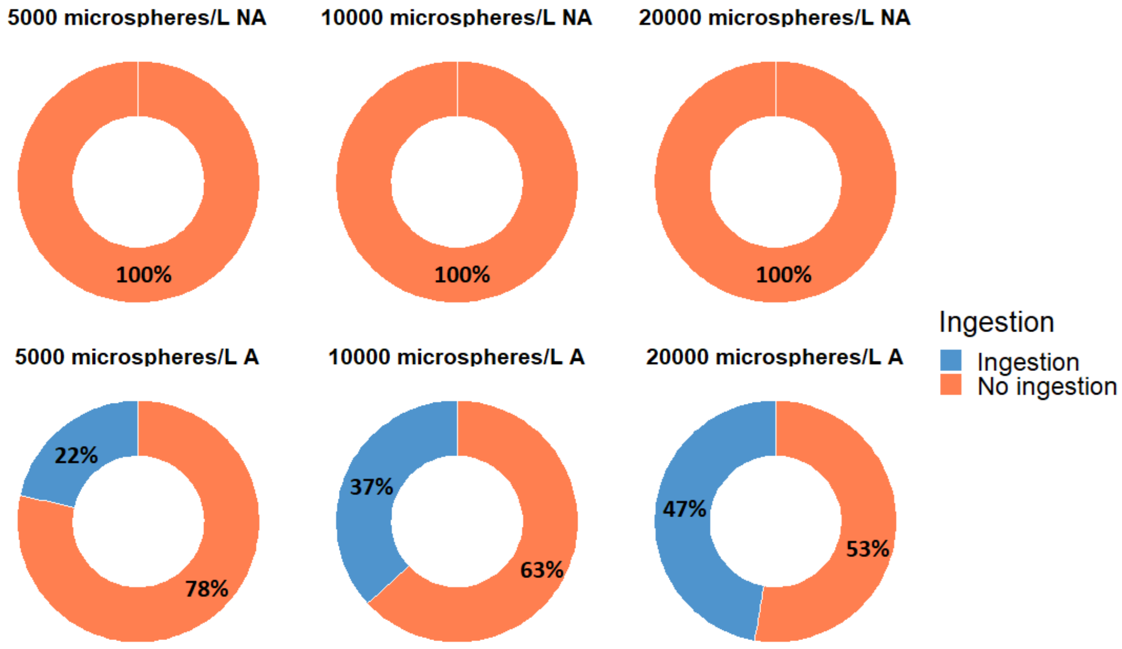



*Fig. 10. Ingestion of microsphere and percentage of the time with presence of microspheres in the gastric cavity.*

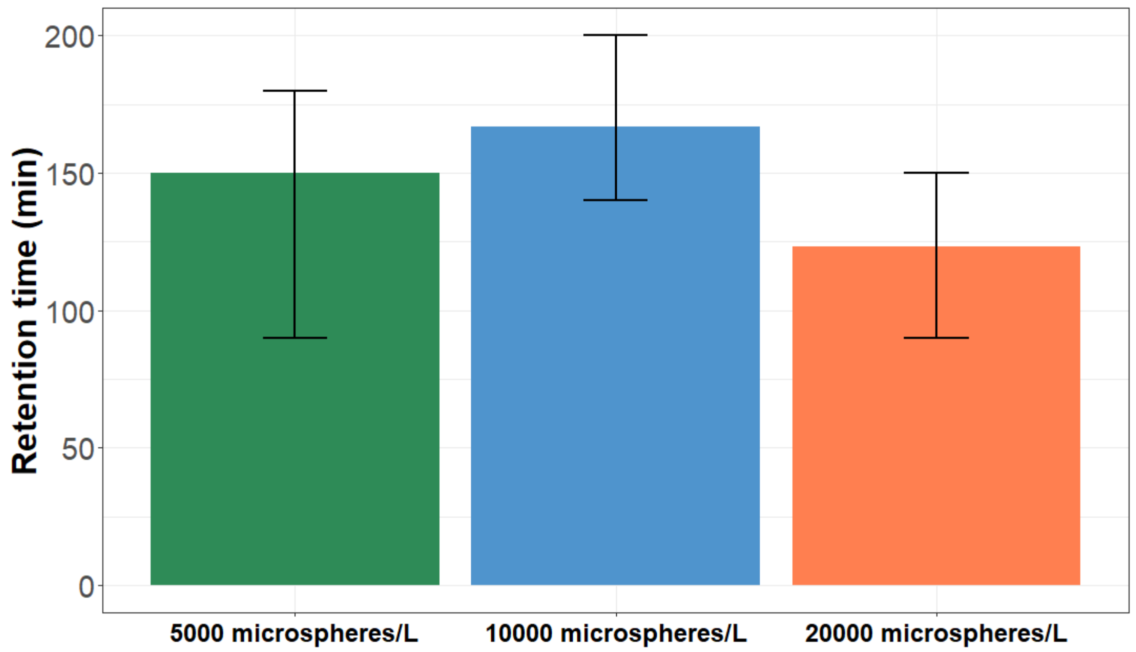



*Fig. 11. Retention time of microspheres for each concentration.*

# 4 DISCUSSION

In view of the above results, it can be concluded that plastic affects differently medusas and siphonophores, or at least, *P. physalis* distinctly from *P. noctiluca* and *A. aurita,* since in none of the siphonophores was found nothing more than synthetic microfibers. This may be due to the different feeding behaviour that both groups have. *A. aurita* and *P. noctiluca* are active hunters. It means that they look for and capture their prey, then carry out an internal digestion taking them to the mouth and to the gastric cavities in which they are digested. In contrast, *P. physalis* does not have the capacity to search and capture the prey, but they extend the tentacles, once the prey is captured, it is brought closer to polyps that secrete digestive enzymes and an external digestion occurs. Precisely the fact that *P. noctiluca* is an active hunter makes it more vulnerable to the impact of plastic in them, because in this case with the entangled plastic, it could not move correctly, making it difficult to hunt their prey and causing be more susceptible to being eaten by one of its predators.

Perhaps there is a difference in the number of synthetic microfibers present in medusa and siphonophores, but to prove this it is necessary to carry out a sampling and have a protocol in the laboratory that takes into account and eliminates as much as possible the atmospheric contamination, such as manipulating the samples in the laboratory inside an extractor hood and collect and process the samples with special clothes. In addition, it would also be convenient to collect the specimens while they are still in the ocean, since when they reach the beach, they are also exposed to the plastics present there.

These plastics and microplastics existing on the beaches, and more specifically in this case of Las Canteras beach, are not evenly distributed, but may depend on several factors such as currents, waves and human activity. The main current that reaches Las Canteras beach comes from the north (ANNEX Fig. 12), so the breakwater that is north of the beach (Fig. 2) protects that area from the current and the waves. In addition, there is also a submerged barrier in the northern half that also helps protecting that area. As we move south of the beach, the activity of the waves and the current is increased when the effect of the breakwater and the submerged barrier disappears. This may explain why in zone 1, further north, it is the one with the least amount of plastics, and the other two more to the south have a greater quantity, especially in zone 3 which statistically has been found differences with zone 1 and with zones 1 and 2 in weight.

The most common plastics found on Las Canteras beach are white or transparent fragments, followed by blues and greens. This coincides with different authors found in different parts of the world (Ivar Do Sul et al., 2014; Lavers et al., 2016; Shaw & Day, 1994). Some studies like Shaw & Day (1994) suggest that this may be due to darker colours receive more solar radiation than light colours, accelerating their decomposition, which is why light colours are the most frequent. In the sea, however, the conditions are the same for all colours and the radiation is minor, so this differentiation in the amount of plastics of each colour would be less pronounced. As plastics were not found in *P. physalis* it would be necessary a study in situ in order to minimize the synthetic microfiber contamination and be able to compare the microfiber present in the organisms in the environment. In *P. noctiluca* transparent was the most common colour, coinciding with the beach. Regarding the type of plastic, lines were the majority in *P. noctiluca*, in contrast with fragments, which were the most common on the beach.

From the experiments carried out with *A. aurita* it was clearly seen that in the absence of prey that motivated the medusa to eat, they did not ingest the microspheres, although they did stick to the body (Fig. 9B), especially when increasing the concentration. A distinction must be made between the microspheres that were in the manubrium and those that were in the gastric cavities (Heeger & Möller, 1987). In these experiments, only those into the gastric cavities were counted as ingested microspheres (Fig. 9C) since many did not reach the cavities, but they were expelled directly from the manubrium. Some of those that reached the gastric cavities after a few minutes returned to the manubrium, and from there they could either return to the cavities or be expelled. This may be because the organisms recognize that the microspheres are not food and expel them, as consequence they do not eat the microspheres without the presence of prey. In addition, the few ingested probably were by mistake, since having so many adhered, when they take to the mouth the nauplii some of these microspheres are also dragged.

The time that *A. aurita* present microspheres in the cavities seems to be independent to the concentration of these in the environment. Although the time of presence of microspheres in the gastric cavities tends to increase with concentration, the variability found among the different individuals was very large, so for future experiments it would be convenient to have more replicates and concentrations to try to improve this, as well as considering other factors that may affect.

Finally, for the retention time, it does not seem to follow any trend and is not influenced by the concentration of microspheres. However, the number of microspheres ingested seems to tend to increase with concentration.

# 5 CONCLUSIONS

1. There are differences between the plastic presence in medusa and siphonophores, ranging from 0% in *P. physalis* to 50% in *P. noctiluca*, probably due to the different feeding behaviour.

2. Plastic found in *P. noctiluca* were a transparent line, a transparent fragment and a black line.
3. The most common plastics found in Las Canteras beach were fragments of whitish and transparent colours, which correspond to the main types found in medusa.
4. *A. aurita* ingestion of microspheres only occurs when there is also presence of prey in the environment.
5. Neither the average time of presence nor the retention time are affected by the concentration of microspheres in the environment, however the number of microspheres ingested increased at higher concentrations.
6. Further studies are needed to understand the impact of microplastics on medusa and their physiological state.

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# 7 ANNEX

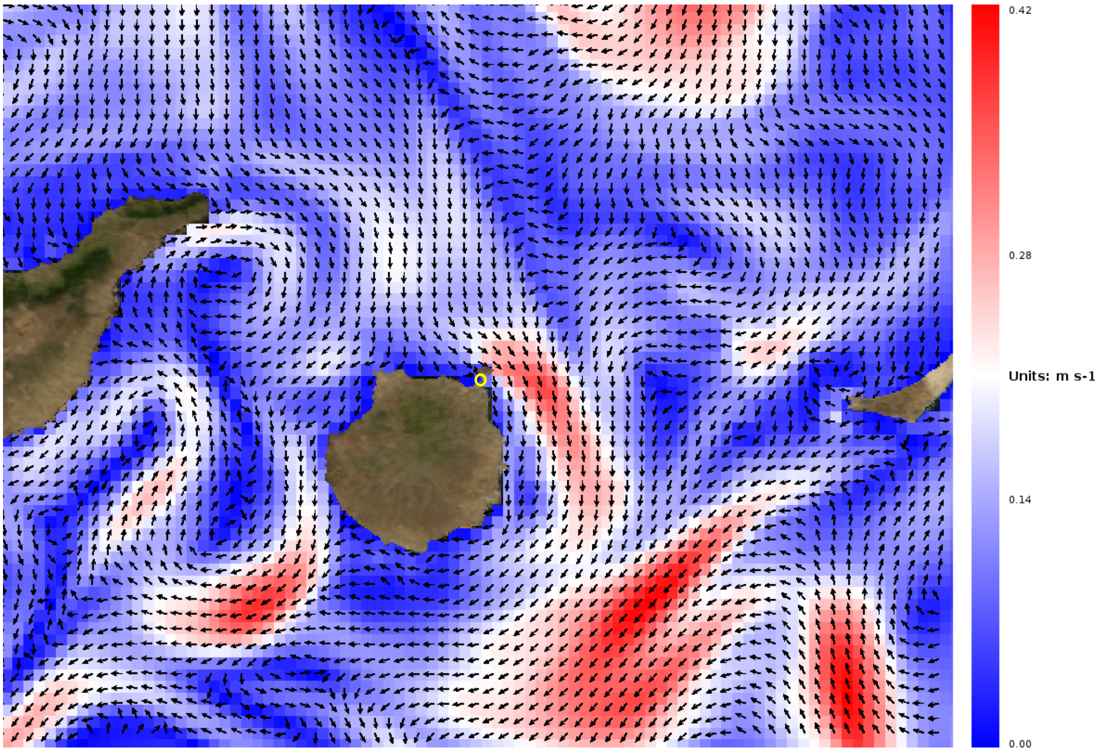


*Fig. 12. Superficial current speed of April 5 obtained from CMEMS, (http://marine.copernicus.eu) dataset IBI_ANALYSIS_FORECAST_PHYS_005_001. Las Canteras beach marked with a yellow circle.*

| | 5000 µesferas/L sin artemia | | | 5000 µesferas/L con artemia | | | 10000 µesferas/L sin artemia | | | 10000 µesferas/L con artemia | | | 20000 µesferas/L sin artemia | | | 20000 µesferas/L con artemia | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tiempo (min) | Individuo 1 | Individuo 2 | Individuo 3 | Individuo 1 | Individuo 2 | Individuo 3 | Individuo 1 | Individuo 2 | Individuo 3 | Individuo 1 | Individuo 2 | Individuo 3 | Individuo 1 | Individuo 2 | Individuo 3 | Individuo 1 | Individuo 2 | Individuo 3 |
| 10 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ |
| 20 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ |
| 30 | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ |
| 40 | ✗ | ✗ | ✗ | ✗ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ |
| 50 | ✗ | ✗ | ✗ | ✗ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ |
| 60 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ |
| 70 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ |
| 80 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ |
| 90 | ✗ | ✗ | ✗ | ✗ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ |
| 100 | ✗ | ✗ | ✗ | ✗ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ |
| 110 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ |
| 120 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ |
| 130 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ |
| 140 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ |
| 150 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ |
| 160 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ |
| 170 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✗ | ✓ |
| 180 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✓ | ✗ | ✓ | ✗ | ✗ | ✗ | ✓ | ✗ | ✓ |
| 190 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✓ | ✗ | ✓ | ✗ | ✗ | ✗ | ✓ | ✗ | ✓ |
| 200 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✓ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✗ | ✓ |
| 210 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✓ |
| 220 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✓ | ✗ |
| 230 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ |
| 240 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ |

| | 5000 µesferas/L sin artemia | | | 5000 µesferas/L con artemia | | | 10000 µesferas/L sin artemia | | | 10000 µesferas/L con artemia | | | 20000 µesferas/L sin artemia | | | 20000 µesferas/L con artemia | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tiempo (min) | Individuo 1 | Individuo 2 | Individuo 3 | Individuo 1 | Individuo 2 | Individuo 3 | Individuo 1 | Individuo 2 | Individuo 3 | Individuo 1 | Individuo 2 | Individuo 3 | Individuo 1 | Individuo 2 | Individuo 3 | Individuo 1 | Individuo 2 | Individuo 3 |
| 250 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✓ |
| 260 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ |
| 270 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ |
| 280 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ |
| 290 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ |
| 300 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ |
| 310 | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ |
| 320 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ |
| 330 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ |
| 340 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ |
| 350 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ |
| 360 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ |
| 370 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ |
| 380 | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ |
| 390 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ |
| 400 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ |
| 410 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ |
| 420 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ |
| 430 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ |
| 440 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ |
| 450 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ |
| 460 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ |
| 470 | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ |
| 480 | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ |

*Table 2. Results of time of presence experiment.*

| | 5000 µesferas/L | | | 10000 µesferas/L | | | 20000 µesferas/L | | |
|---|---|---|---|---|---|---|---|---|---|
| Tiempo (min) | Individuo 1 | Individuo 2 | Individuo 3 | Individuo 1 | Individuo 2 | Individuo 3 | Individuo 1 | Individuo 2 | Individuo 3 |
| 0 | 1 | 1 | 2 | 1 | 1 | 1 | 3 | 1 | 4 |
| 10 | 0 | 1 | 2 | 0 | 0 | 1 | 2 | 0 | 4 |
| 20 | 0 | 1 | 2 | 2 | 0 | 1 | 3 | 1 | 3 |
| 30 | 1 | 1 | 0 | 2 | 2 | 1 | 2 | 0 | 3 |
| 40 | 1 | 1 | 0 | 0 | 0 | 1 | 3 | 0 | 3 |
| 50 | 1 | 1 | 1 | 0 | 0 | 1 | 3 | 0 | 3 |
| 60 | 1 | 0 | 0 | 0 | 1 | 0 | 3 | 0 | 3 |
| 70 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 0 | 3 |
| 80 | 0 | 0 | 1 | 0 | 0 | 1 | 3 | 0 | 3 |
| 90 | 0 | 0 | 2 | 0 | 0 | 1 | 3 | 0 | 1 |
| 100 | 0 | | 2 | 0 | 0 | 2 | 3 | | 1 |
| 110 | 0 | | 1 | 0 | 0 | 2 | 3 | | 1 |
| 120 | 0 | | 1 | 0 | 0 | 2 | 2 | | 0 |
| 130 | 0 | | 1 | 0 | 0 | 1 | 1 | | 0 |
| 140 | 0 | | 1 | 0 | 0 | 1 | 0 | | |
| 150 | 0 | | 1 | | 0 | 1 | 0 | | |
| 160 | 0 | | 0 | | 0 | 0 | | | |
| 170 | 0 | | 0 | | 0 | | | | |
| 180 | 0 | | 0 | | 0 | | | | |
| 190 | | | | | 0 | | | | |
| 200 | | | | | 0 | | | | |

*Table 3. Results of retention time experiment.*