Aquaculture of the stalked barnacle *Pollicipes pollicipes*: improving farming techniques in an extensive system located in Sines (Portugal).

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Master thesis

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Declaraçao da autoria do trabalho:

Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

Resumo :

Pollicipes pollicipes é um crustáceo cirripede que ocorre principalemente em substratos rochosos expostos a forte hidrodinâmica. Os perceves são muitos apreciados na Península Ibérica, mas o declinío nos estoques naturais e sua perigosa captura, faz com que seja uma espécie com alto potencial para a aquacultura.

Neste estudo, várias maneiras de melhorar a aquacultura de *P. pollicipes* foram testadas, com base em trabalhos anteriores da equipe do laboratório CIEMAR. Várias tarefas foram realizadas, nomeadamente para avaliar se o tamanho dos colectores (barticles) (6, 8, 10 e 12 mm) poderia permitir melhorar a intensidade de recrutamento dos juvenis de *P. Pollicipes,* a sua sobrevivência, o seu tamanho e a sua taxa de crescimento, tanto no habitat natural como numa plataforma offshore. Além disso, dois periodos de transferência para a plataforma foram testados, para ver o mais adequado para limitar mortalidade e garantir um tempo de crescimento máximo. Depois, diferentes técnicas de anti-incrustante (limpeza manual, jato de água, revestimento anti-incrustante, em comparação com controlos intocados) foram aplicadas na plataforma em vários grupos de perceves, para melhorar a sobrevivência e crescimento dos perceves transferidos. Um monitoramento das communidades bio-incrustantes foi realizado para estimar os períodos críticos de maior incrustação.

O efeito do tamanho dos barticles sobre a intensidade do recrutamento não obeteve resultados conclusivos. No entanto, os perceves maiores foram coletados com os tamanhos menores de barticles (6 e 8mm), mas essa tendência não foi observada após um período de crescimento nos dois locais de estudo (habitat natural e plataforma offshore). A localização do crescimento dos perceves demonstrou ter um impacto na altura Rostro-Carina (RC) e na sua taxa de crescimento (dRC30). De facto, a jangada offshore permitiu melhorar o crescimento dos perceves e coletar indivíduos maiores. Além disso, os barticles de 6mm mostraram não ser adequados para fins de aquacultura, por serem muito quebráveis, e por não serem convenientes para a sobrevivência. A sobrevivência não dependeu do tamanho dos colectores implantados (8, 10 e 12mm). No entanto, os colectores de 8 e 10 mm devem ser preferidos para uma cultura possível, devido ao seu menor custo.

A data de implementação na plataforma, não revelou diferenças na sobrevivência dos perceves mas revelou diferenças no RC dos indìviduos coletados. De facto, foram coletados perceves maiores quando transferidos anteriormente, isso corroborou as observações anteriores, apresentando a plataforma offshore como uma maneira efetiva de crescer os perceves mais rapidamente.
A sobrevivência de P. pollicipes não foi melhorada pelo tratamento anti-incrustante aplicado à cultura. É possivel que os tratamentos tenham sido aplicados demasiado cedo o que poderá ter induzido a mortalidade nos pequenos perceves. Além disso, mesmo que o impacto dos bio-incrustantes não tenha sido avaliado diretamente neste estudo, eles foram demostrados no passado (ano 2015), como sendo o principal problema para a sobrevivência dos perceves na platforma (competicão por comida e espaço). No entanto, um resultado positivo foi apontado, com crescimento aprimorado nos perceves limpos manualmente ou com jato de água.

O controle das comunidades bio-incrustantes permitiu destacar o período de maior ocorrência dentro de 6 meses e novas sugestões para futuros estudos foram apresentadas de maneira a melhorar a aquacultura de *P. pollicipes.*

Palavras-chave : Aquacultura – Perceves – *Pollicipes pollicipes* – Barticles – sistema extensivo.

Abstract

In this study, several ways to improve *P. pollicipes* aquaculture have been tested. The effect of the size of the barticles on the intensity of recruitment did not give conclusive results. Bigger barnacles were collected however with the smaller sizes of barticles (6 and 8mm), but this trend was not observed after a period of growth in the two study locations. The location of growth was shown as having an impact on the RC of the barnacles and on their growth rate (dRC30). Indeed, the offshore platform allowed to improve the growth of the barnacles and to collect larger individuals. Furthermore, the barticles of 6mm were shown as not suitable for aquaculture purposes, as they were too breakable and they did not favor survival. The survival was not dependent on the size of the three other barticles deployed (8, 10 and 12mm). The barticles of 8 and 10 mm for a possible culture, seemed however preferable, because of their lower cost.

The date of implementation on the platform did not reveal differences on survival, but on the RC of the individuals. Indeed, bigger barnacles were collected, when transferred earlier, this corroborated the previous observations, presenting the offshore platform as an effective way to grow barnacles faster.

The survival of *P. pollicipes* was not improved by the antifouling treatment applied to the culture, probably due to too early applications. However, a positive outcome was pointed out, with enhanced growth on the barnacles cleaned manually or with the waterjet.

The monitoring of the bio-fouling communities allowed to highlight the period of major occurrence within 6 months and new hints for further studies were pointed out, to improve *P. pollicipes* aquaculture.

Key words: Aquaculture - Stalked barnacles -*Pollicipes pollicipes* - Barticles – Extensive system.

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Table of contents

Table of figures :

List of tables

General introduction

Barnacles' ecology and behavior

Naturally, barnacles tend to reproduce from April till September (Cruz and Hawkins, 1998). The life cycle of *Pollicipes pollicipes* consists in a succession of stages, presenting their own specific characteristics.

These hermaphrodite obligate cross fertilizers rely on gregarious settlement (clumps) to ensure their mating behavior (Pavón, 2003). The Fertilized eggs will endure six consecutive naupliar stages, followed by a cyprid stage, before their metamorphosis into juveniles (Cruz *et al.*, 2015).

Nauplii stages are pelagic and therefore evolve in the water column. Nauplii II to VI are planktotrophic stages; Nauplii I and cyprid stages rely on their reserves and do not require to feed during the process. After the transformation into cyprid, the larva adopts a benthic behavior; probes several surfaces and when the adequate one is found, settles and metamorphoses into juvenile (Franco, 2014).

Because of their gregarious behavior, an important number of settlers occurs next to or onto already implanted adults (Cruz *et al.*, 2010; Franco *et al.*, 2016a), these latter tending to offer an additional protection for the early fragile stages. Recruitment on adults' stalk can reach up to 15 larvae/juveniles on average (Cruz *et al.*, 2010). Settlement seems triggered by a larval response to the texture or chemical components of the adults' stalks (Franco *et al.*, 2010).

Stalked barnacles are planktotrophic filter feeders (Girard, 1982). Preys are harvested through a cirral extension. The diet in early stages consists mainly in micro-algae, but the size of prey and variety increases with the barnacle's development. Adults' diet includes small crustaceans and polychaetes (Chan *et al.*, 2008).

In its natural habitat, *Pollicipes pollicipes* can reach market size within 12 months (22 to 25 mm) (Cruz *et al.*, 2010; Franco *et al.*, 2010), but growth is highly variable (Jacinto *et al.*, 2015).

Aquaculture perspectives

Different ways of cultivating aquatic organisms appeared throughout mankind's history and the written records of cultured fish date back 300 B.C, in China (Costa-Pierce, 2002). Several methods of culture were developed afterwards, depending on the type of cultured organisms and on the level of control imposed to the culture itself.

Extensive aquaculture relies on natural nutritional inputs, it requires usually lower economical investments, taking advantage of natural feeding sources and water exchanges (FAO 1988). But this type of aquaculture implies low controls over the culture related factors, predation and disease (Franco, 2014), and the productivity per unit of rearing area is moderately enhanced (Samudra, 1996).

Intensive aquaculture is based on a control of most steps of production to intensify the culture. Water quality, feeds, stock provenance are controlled to improve production efficiency, and monitoring is done throughout the production cycle (FAO, 1988; Samudra, 1996;). This type of culture implies high economical investments. Semi-intensive aquaculture, as extensive methods, depends largely on natural amounts of food but nutritional inputs can be improved through complementary diets (FAO, 1988). It represents a compromise between extensive and intensive cultures but the nuances are still poorly defined (Samudra, 1996).

Conchyliculture, the culture of different species of mollusks, like mussels, oysters, scallops, is widely spread and can be found in the three systems explained before. In 2010, conchyliculture produced more than 13.9 million of tons in the world (FAO, 2012).

Some other types of filter feeders, present commercial interests worldwide. This is the case of a dozen of barnacle species ("acorn" and "goose" or stalked barnacles) (Lopez *et al.*, 2010). A production of acorn barnacles in Chile was done on a semi-industrial scale, in which spats of *Austromegabalanus psittacus* and *Megabalanus azoricus* were collected from the wild and grown in suspended systems (Lopez *et al.*, 2010). In the case of *Pollicipes pollicipes*, previous experimental culture trials were carried out with artificial substrata to collect larvae from the wild (Franco, 2014). Those trials presented few results and to overcome low intensity of settlement (main bottleneck encountered), trials were settled under laboratory conditions (Franco *et al.*, 2010). A 30 to 35% of intensity of settlement was obtained on already implanted adults (Franco *et al.*, 2016a). As explained by Franco (2014), larval development and cyprid settlement in laboratory rely on adequate feed quality and quantity, temperature, photoperiod, salinity, larval density and water quality. Therefore, several steps still need to be improved before adapting *Pollicipes pollicipes* life cycle to an intensive culture. Better controlled parameters represent a very interesting perspective, but further studies are still required to make it possible as well as a consequent higher economical investment.

In case of an improvement in larvae collection, a juvenile grow-out offshore open system (extensive or semi-intensive) would present low costs and is likely to improve growth in comparison with wild specimens. Due to a total submersion, permanent feeding might induce higher growth in the barnacles as it was observed in other studies (Goldberg, 1984; Hoffman, 1988).

Previous studies performed by our group CIEMAR, (Cruz *et al.,* 2016) allowed the development of an artificial substratum "barticle", (AQUAPOLLIS project, patent request submitted in March 2017, see below). Barticles were deployed in the natural habitat (Cape of Sines, Portugal). This type of collector allowed cyprids fixation and metamorphosis into juveniles with success. The barticles can be transplanted to other places and cultivation systems such as floating platforms, as the ones used in the previous studies of the group. Several aspects of this substrate/collector can affect the fixation and growth of barnacles. The size of the barticles could be changed, namely by analyzing how their own diameter could affect the production of barnacles. Indeed, available space (according to different sizes of settlement surface) could have an impact on the number of settlers per collector, and on the survival and growth rates for the individuals collected, as it was already observed for mussels' spats collection on nylon-made ropes (Aghzar *et al.,* 2012).

The age of the transplanted barnacles could also have an impact on their survival. Older transplanted barnacles might have better chances of survival than younger/less developed ones.

<u>Fouling communities and antifouling techniques</u>

Biofouling organisms, composed mainly of periphyton (Biggs *et al.,* 1998) represent an important concern for navigation over centuries (Holm, 2012). The accumulation of biofouling communities, on cages, nets and collectors, is also a major problem and cost factor for aquaculture (Bloecher *et al.,* 2013). Barnacles cultivation is not an exception. These complex communities might compete for space and food with *Pollicipes pollicipes,* and in previous studies made by our group, several fouling issues were encountered, namely by competitive species, such as mussels, algae and other species of barnacles (Cruz *et al.*, 2016)

Therefore, several techniques to reduce biofouling should be applied to allow the survival and growth of *Pollicipes pollicipes.* Antifouling can be achieved by different approaches, such as mechanical, chemical and biological techniques. Mechanical antifouling techniques might imply the use of abrasion, friction or pressurized water/air. Common chemical ways of antifouling rely on the use of different types of coatings, like paints preventing the colonization of surfaces of interest. Most antifouling paints are nowadays getting forbidden because of their toxicity (e.g. former Tributyltin (TBT)) (Callow and Callow, 2002). Non-toxic paints such as Netminder® (LLC Aquaculture coatings), a water based silicone coating, using no organotin compounds (acting as biocides), were already used in scallops culture (Tettelbach *et al.*, 2014) and might represent better environmental-friendly solutions to avoid biofouling species.

Biological antifouling could rely on the use of herbivorous or grazers species to feed on the invasive organisms. For example, limpets or sea urchins, due to their feeding behavior (Cabral, 2006; Lawrence, 2013) are likely to clean the periphyton.

Monitoring the arrival of the different biofouling communities, at a year scale, could help to understand which are the critical periods of high settlement intensity and which groups are the most alarming. Further methods, such as bio-controllers, different immersion depths, could then consequently be administered.

Objectives

The overall aim of this thesis is to improve the methods to cultivate *Pollicipes pollicipes* that have been originally developed in a previous project (AQUAPOLLIS) by our group Cruz *et al.*,2016).

To accomplish this main objective several tasks were performed. First, the effect of the size of the barticles (collectors) was tested on the intensity of settlement, survival, size and growth of the juveniles. This was done by comparing juveniles settled in the barticles that were left in their natural habitat of extraction (Cape of Sines) with transplanted juveniles (originally settled at Cape of Sines) to the floating platform located inside the port of Sines.

In addition, the transfer period to the platform was analyzed by comparing survival rates and size of juveniles settled in the barticles that were transplanted to the platform in two different periods (October 2016 and January 2017).

Finally, several anti-fouling methods were tested and survival rates and size of the juveniles were estimated and compared. Additionally, fouling assemblages were monitored at different temporal scales.

Material and methods

Duration and location of the experiment

The experiments started on the 12th August 2016 (date of deployment of the barticles in the field) and lasted until July 2017. The fieldwork took place in Sines, Portugal (fig. 1). Two study areas were chosen: the wild rocky area (Cabo de Sines) where the barticles were deployed; and the floating platform from CIEMAR, located inside the Port of Sines.

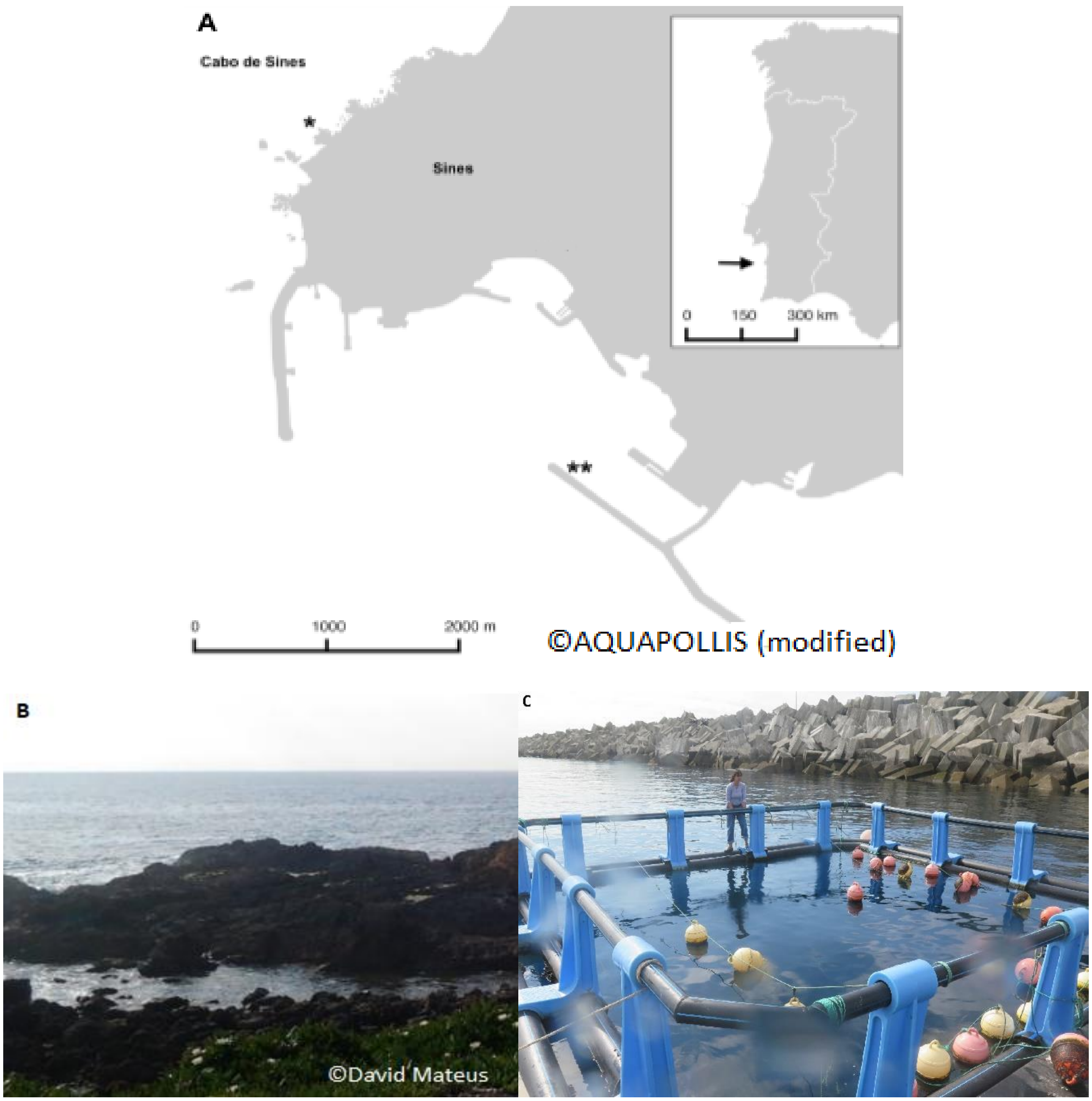

Fig. 1. A) Map of the locality of Sines (Portugal). B) Cabo de Sines (*). C) Platform from CIEMAR (**).

The barticle, an artificial substratum to collect cyprids and juveniles of *P. pollicipes*

The barticles, artificial substrata and the processes to collect larvae and juveniles of *P. pollicipes*, have been developed in project AQUAPOLLIS and were subjected to a patent request submitted in March 2017. They were used for the whole experiment. The concept developed is similar to the systems employed to harvest other filter feeders, such as oysters or mussels. Indeed, those artificial substrata aim to collect cypris and juveniles of *P. pollicipes*. Barticles tend to mimic a stalk of barnacle and to support barnacle cyprids' settlement and growth (fig. 2, 3B). They are composed of a black plastic sleeve of 8 mm of diameter. The sleeve is reinforced by an inox screw of 4mm to maintain rigidity of the whole and to facilitate the retrieval process. Each sleeve includes 6 crenels offering shelter for the fixating larva.

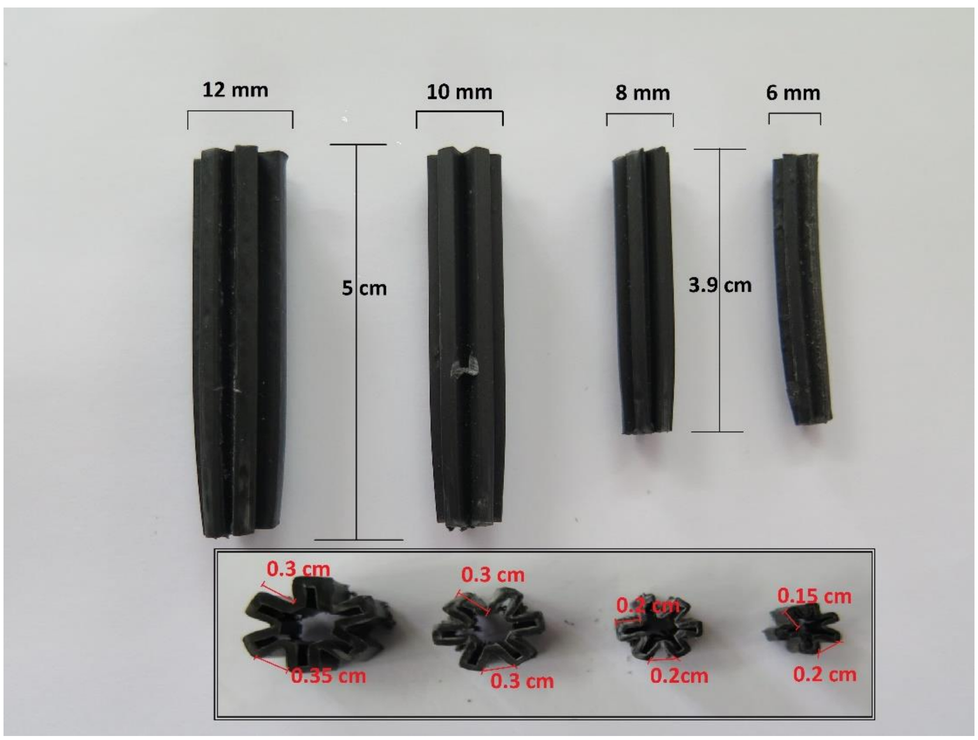

Fig. 2. The different sizes of barticles, horizontal and top view.

The barticles are meant to be deployed close to clumps of naturally implanted barnacles. Barticles' implementation was done in the recruitment season (end of summer and beginning of autumn). Therefore, holes of 7 mm were drilled in the rocks of Cabo de Sines with a drill Bosch® Professional 36V. The barticles were then inserted till mid height (around 2 cm) with a hammer. In several of the following experiments, other sizes of barticles were employed (presenting the same characteristics) with sleeves of 6, 10 and 12 mm of diameter. The drilled holes were then respectively: 5, 9 and 11 mm. A total of 364 barticles of different sizes were deployed in Cabo do Sines on the 12th of August 2016 and were used throughout all experiments. Because of the site exposure to strong waves and currents, the working window during low tides was short and dependent on sea conditions.

Depending on the experiment (see below), some barticles were deployed in the platform (post-withdrawal from the Cape). In this case, barticles were implemented on PVC boards. Each PVC board measured 95 cm x 20 cm and contained 36 slots spaced by 10 cm each (fig. 3B). The PVC boards were inserted in cages equipped with protection nets to diminish predation. The cages were immerged vertically, 50 cm below the surface (fig. 3C).

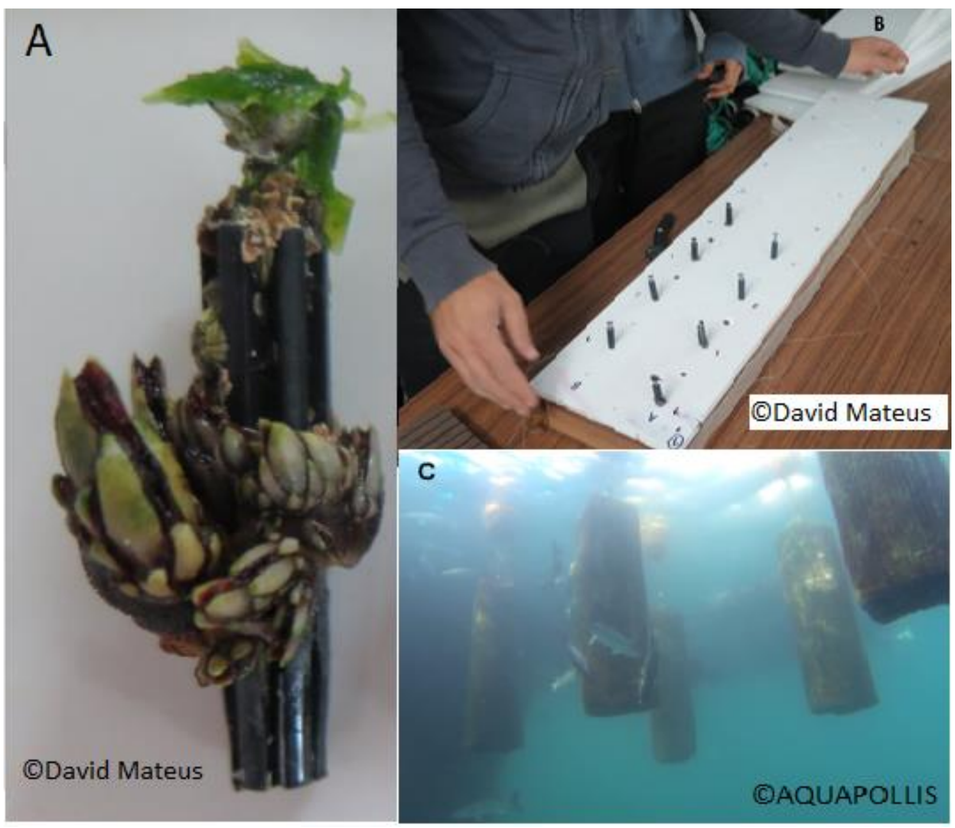

Fig. 3. A) Barticle with barnacles attached; B) PVC board with barticles attached; C) PVC board with barnacles and protective cages + net, submerged at the platform.

Experimental design

I) Effect of the size of the barticle in settlement intensity and size of *P. pollicipes*

The experiment started on the 12th August 2016, when the empty barticles were settled in the Cape. They were withdrawn on the 29th September 2016 (after 48 days). In this experiment two dependent variables (settlement intensity and size of barnacle collected) were tested according to the factor size of the barticle (4 treatments: 4, 8, 10 and 12 mm). A sample of n = 8 barticles for each treatment was analyzed in this experiment.

Settlement intensity was assessed by counting the number of juveniles and cyprids per size of barticle treatment. Juveniles who lost their adherence to the collector were discarded. Size of the barnacles was assessed after their extraction from the barticles. They were measured using their Rostro-Carinal Length (RC) (fig. 4).

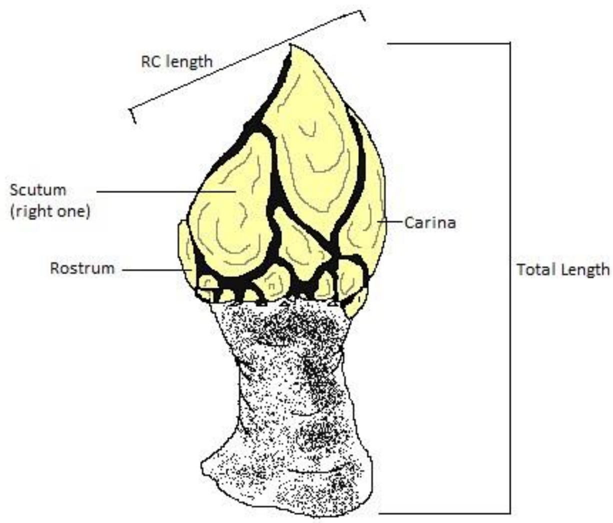

Fig. 4. Scheme of a barnacle, presenting the measures and plates of interest (RC=Rostro-Carinal).

II) Effect of size of the barticle in survival and size of barnacles collected, in the natural habitat and in the platform.

In this experiment, 4 treatments were considered (6, 8, 10 and 12 mm of barticle). The barticles settled in Cabo de Sines on the 12th August 2016 were withdrawn on the 29th of September (48 days later). Pre-counts were performed on the barticles to assess the number of juveniles and cyprids per collector. Two groups were formed and transferred to the locations of interest on the 1st of October (Cabo de Sines and the platform). On the 16th and 17th January 2017 (after 107 and 108 days), both groups were respectively withdrawn. Barnacles were extracted from the barticles, counted and all RC were recorded. The barnacles marked with calcein (see exp III) and the non-marked were separated to process the data.

Survival (%) was calculated as following:

% survival = (Final number of barnacles / Initial number of barnacles) * 100

In this experiment two factors were analyzed, the size of the barticles and their location. The dependent variables recorded were the % survival of marked barnacles per barticle, the % survival of all barnacles withdrawn from the platform in January 2017, the mean RC of the marked barnacles, the mean RC of the individuals that have settled and survived in barticles withdrawn from the platform in January 2017.

III) Effect of the size of the barticle in growth, in the natural habitat and in the platform

In this experiment, 4 treatments were considered (6, 8, 10 and 12 mm of barticle). Barticles were settled in Cabo de Sines on the 12th August 2016. On the 29th September, they were withdrawn, the number of juveniles and cyprids were assessed by barticle. All barnacles (attached to the barticles) were then marked with Calcein.

Calcein Marking procedure

The barticles were immerged in containers (5 L) filled with filtered seawater and 200 mg/L of calcein. A constant aeration has been maintained, through diffusive air stones, during the whole process (around 24 hours). The barnacles were maintained under fasting for the whole process. The marking process, described by Jacinto et al. (2015) has been followed and calcein solution were prepared with 6.25 g/L in distilled water and buffered with *ca*. at pH = 6, adding sodium bicarbonate to improve solubility.

After marking, two groups of collectors were formed. One was implemented on the platform while the other one was settled back in the natural habitat (Cabo de Sines) on the 1st of October 2016. On the 16th and 17th of January 2017, both groups were withdrawn from their respective locations. Chemical marking success was assessed, dividing the number of individuals presenting fluorescent marks by the total of individuals.

Measurement

All barnacles with a maximum Rostro-Carinal length superior to 1mm (RC > 1mm) (fig. 4) were then separated by size, obtained with calipers (±0.1mm). Thus 4 groups were formed (RC<5mm, RC=5-15, RC>15 and adults), and immerged in 3.5% sodium hypochlorite (commercial bleach) for respectively 1, 5, 10 and 30 min for the biggest individuals. Rests of algae and other organisms attached to the plates were cautiously removed thanks to sandpaper and a small electric polisher. This process, time-consuming, required extreme caution, because of the tiny size of the plates. Both pairs of scutum of the capitular plates (fig.) were observed under epifluorescent microscope (Leica M165FC) with UV light source and GFP3 filter + camera. The most visible scutum plate (right or left) was then measured. In the case of successful marking, the original material of the plates presented fluorescence while the new material of growth did not reveal any. Therefore, growth was assessed using the technique developed by Jacinto et *al.* (2015) (fig. 5).

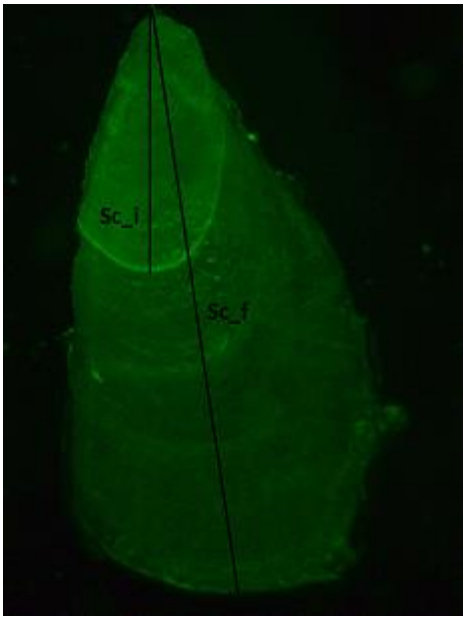

Fig. 5. Marked Scutum plate under epifluorescent microscope (Sc_i: initial scutum size; Sc_f: final scutum size).

In this experiment, the dependent variables considered were the monthly growth rates (dRC30) of the barnacles with a factor size of barticle (4 treatments: 6, 8, 10 and 12 mm) and a factor location (2 treatments: the platform and Cabo de Sines). The sample size used was n = 8.

IV) Effect of the transfer date in the survival and size of the barnacles

In this experiment, two groups of standard barticles (8 mm) were settled in Cabo de Sines on the 12^{th} August 2016. The first group was settled on the platform on the 1^{st} of October. Counts of juveniles fixed and cyprids were previously executed as well as the measurement of the maximum RC per barticle. Barticles were collected on the 21^{st} April 2017. The second group was settled in the platform on the 22^{nd} January (after a pre-count as for the first group) and the collectors were withdrawn on the 21^{st} of April. The dependent variables considered in this experiment, were the % survival (see previous experiment) and the size of the barnacles on the 21^{st} of April, with a factor date of introduction in the platform with two treatments (October and January). Sample size was n = 8.

V) The effect of antifouling treatments on survival and size of the barnacles

In this experiment, standard barticles (8 mm) were settled in Cabo de Sines on the 12^{th} of August 2016. On the 21^{st} of January, barticles were withdrawn from the Cape and attached juveniles and cyprids were counted. The barticles were then implemented on the platform on the 22^{nd} of January. Three treatments were applied monthly from the 21^{st} of February till the 20^{th} of July: manual cleaning, waterjetting and 2 controls.

Manual cleaning

Both sides of each board were scraped with a spatula while paying attention not to harm the barnacles settled at the base of the barticles. With dissection forceps, all barticles were cleaned for a maximum of one minute each, trying to remove as much biofouling organisms as possible.

Waterjet cleaning

The waterjet cleaning was performed using a waterjet Bosch© AQT 5-14x with the maximal spread flow. The flow was applied for 30 s, evenly on each side of the boards about 30 cm above. The water used, was directly extracted from the sea.

Controls

Controls were formed from two batches of barticles, one left in the natural habitat (Cabo de Sines) and one transplanted to the platform. No actions were carried-out during the experiment.

From the 22nd of April onward, a fourth treatment was added: Antifouling paint.

Antifouling paint

An antifouling coating paint was applied for this treatment: Netminder® (white color). The PVC boards were in this case previously painted before receiving the barticles. Two coats of paint were applied with a paintbrush, and the boards were left to dry 3 days before transferring the barticles. No further applications were carried out on the boards till the end of the experiment (20th of July 2017).

The dependent variables considered in this experiment, were the % survival (see previous experiments) and the size of the barnacles in each of the treatment. Each treatment had 3 replicates.

VI) Temporal variation of fouling assemblage

To assess the temporal variation in fouling communities, the average weight of 24 PVC plates (11x13 cm + 2 barticles) was determined (with a scale), they were then settled at the platform in the February 2017. Both sides of each plate were protected by a meshed cage (fig. 6) to reproduce similar conditions encountered by the boards equipped with the barticles. The plates were immerged at a depth ranging from 20 to 50 cm and divided between two ropes. Two plates were withdrawn and replaced with new ones monthly (referred as monthly plates) and two other plates were withdrawn at different month intervals (1, 2, 3, 4, 5 and 6 months, referred as integrated monthly plates) (fig. 7).

Fig. 6. Monthly plate, suspended at the platform.

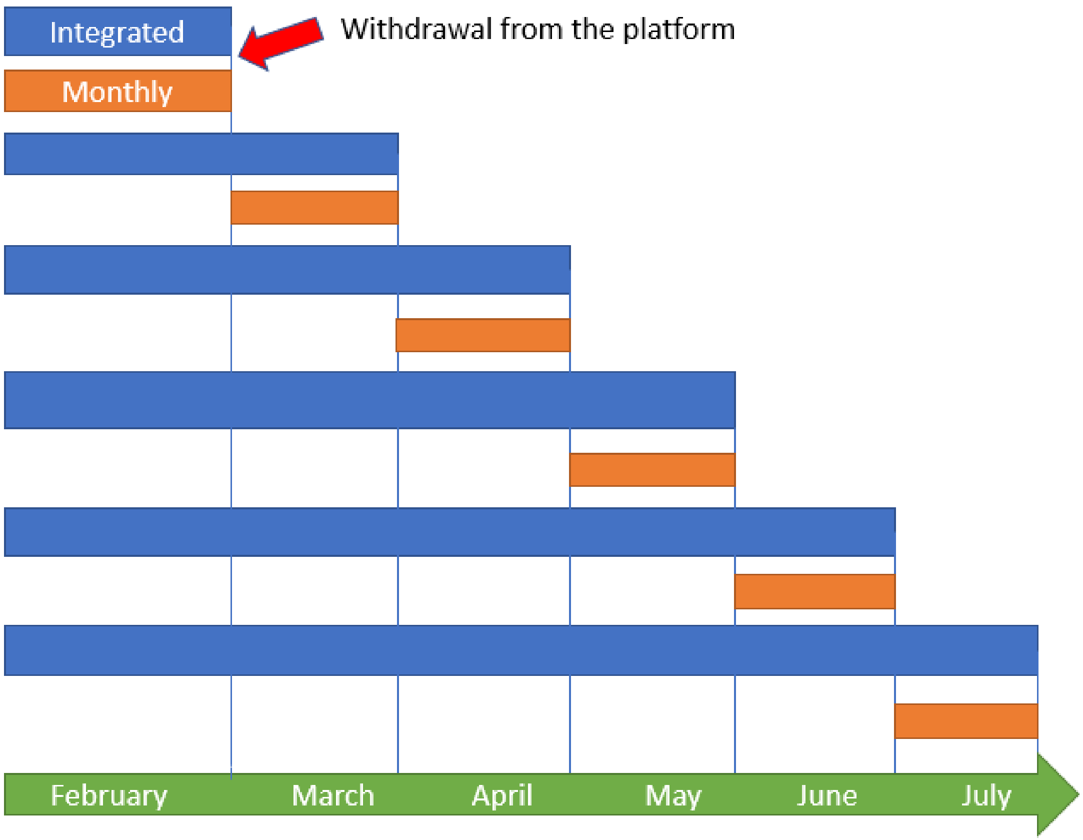

Fig. 7. Planning of insertion/withdrawal of the PVC plates used to assess biofouling assemblages.

The monthly and integrated monthly plates were withdrawn, and placed directly into plastic bags. Once in the laboratory, they were carefully extracted from plastic bags to keep non-sedentary species. The protective net was removed in a tray of water to keep those mobile organisms. The content of the tray sieved in a 0.5 mm mesh, it was directly kept apart in a group "others";

pictures of each protected side of the plates were taken. The other side of the plate was then carefully scraped and cleaned. The side of interest (side with barticles), was turned upside down for 1 minute, to get rid of the excess of water. The fresh weight was then determined (new weight collected during immersion time). A percentage coverage of each group/taxa was assessed with naked eye; using a quadrat (1x1 cm/mesh).

The side with barticles was then scraped and its content collected and sieved in a 0.5mm mesh. The content was then sorted into 3 groups: Mussels, Barnacles and “others”. The group barnacles and mussels were separately weighted to assess the weight of the principal biofouling groups.

All individuals of the group “others” were then sorted per taxa and separated in different flasks with alcohol. They were then gathered per functional groups and the non-sedentary species were countered to estimate their abundance.

In this experiment, the dependent variables considered were the fresh weight, the % weight of the principal taxa, the percent cover of the plates and the abundance of the non-settled taxa, according to a factor time.

Statistical analysis

The statistical analysis were performed, using the statistical program PRIMER 6 + PERMANOVA (Clarke et Gorley, 2006; Anderson *et al.*, 2008). For each analysis, PERMDISP tests (Homogeneity of Multivariate Dispersions) (Anderson, 2006), (with Euclidian or Bray-Curtis distance matrix, depending on the experiment) were done, and in cases of differences, the data was submitted to overall transformations (square roots, fourth roots, Log (x)). PERMANOVA tests (Permutational Multivariate Analysis of Variance) (Anderson, 2001), were conducted with unrestricted permutations of raw data with a sum square of Type III (Anderson et al., 2008). When appropriate, posteriori pair-wise tests (Exp I to V) or Monte-Carlo tests (Exp VI) were conducted to assess differences between factor levels.

The effect of the size of the barticle, on the settlement intensity and the RC of the recruits, was analyzed separately by one-way PERMANOVA test based on a Euclidian distance matrix;

included 1 fixed factor: size of the barticle presenting 4 levels (treatment 6, 8, 10 and 12mm). (Exp I).

The effect of the size of the barticle and the location, on the % survival and RC of the marked barnacles, was analyzed by 2 two-way PERMANOVA test based on a Euclidian distance matrix; including 2 fixed factors: the size of the barticle, (with 3 levels 8, 10 and 12 mm) and the location (with 2 levels Cabo de Sines and platform). The barticles of 6 mm were discarded because no survival was obtained. The barticles of 8mm were discarded for the RC analysis, as the sample size was too small. (Exp II).

The effect of the size of the barticle, on the % survival and the RC of the barnacles collected from the platform in January 2017, was analyzed separately, by a one-way PERMANOVA test based on a Euclidian distance matrix; including 1 fixed factor: the size of barticle (with 3 levels 8, 10 and 12 mm). (Exp II).

The effect of the size of the barticle and the location, on the growth rate (dRC30) of barnacles, was analyzed by a two-way PERMANOVA test, based on a Euclidian distance matrix; including 2 fixed factors: the size of the barticle, (with 3 levels 8, 10 and 12 mm) and the location (with 2 levels Cabo de Sines and platform). (Exp III).

The effect of the date of transfer to the platform, on the % survival and RC of barnacles, was analyzed separately, by a one-way PERMANOVA test based on a Euclidian distance matrix; including 1 fixed factor: the date of implementation on the platform (2 levels: 1st of October or 22nd of January). (Exp IV).

The effect of the antifouling treatments on the % survival and RC of the individuals, was analyzed separately, by a one-way PERMANOVA test based on a Euclidian distance matrix; including 1 fixed factor: treatment, with 4 levels (manual, waterjet, control Cabo and control platform) for the first period and 5 levels for the second (manual, waterjet, antifouling paint, control Cabo and control platform). (Exp V).

The fresh weight, % weight of the 3 principal taxa, % cover and the abundance of the non-settled taxa, of the monthly plates, were analyzed separately; using a one-way PERMANOVA test, based on a distance matrix of Bray-Curtis, with one fixed factor: month, with 6 levels (February, March, April, May, June and July). (Exp VI).

The fresh weight, % weight of the 3 principal taxa, % cover and the abundance of the non-settled taxa, of the integrated monthly plates, were analyzed separately; using a one-way PERMANOVA test, based on a distance matrix of Bray-Curtis, with one fixed factor: integrated months, with 6 levels (1, 2, 3, 4, 5, 6 integrated months). (Exp VI).

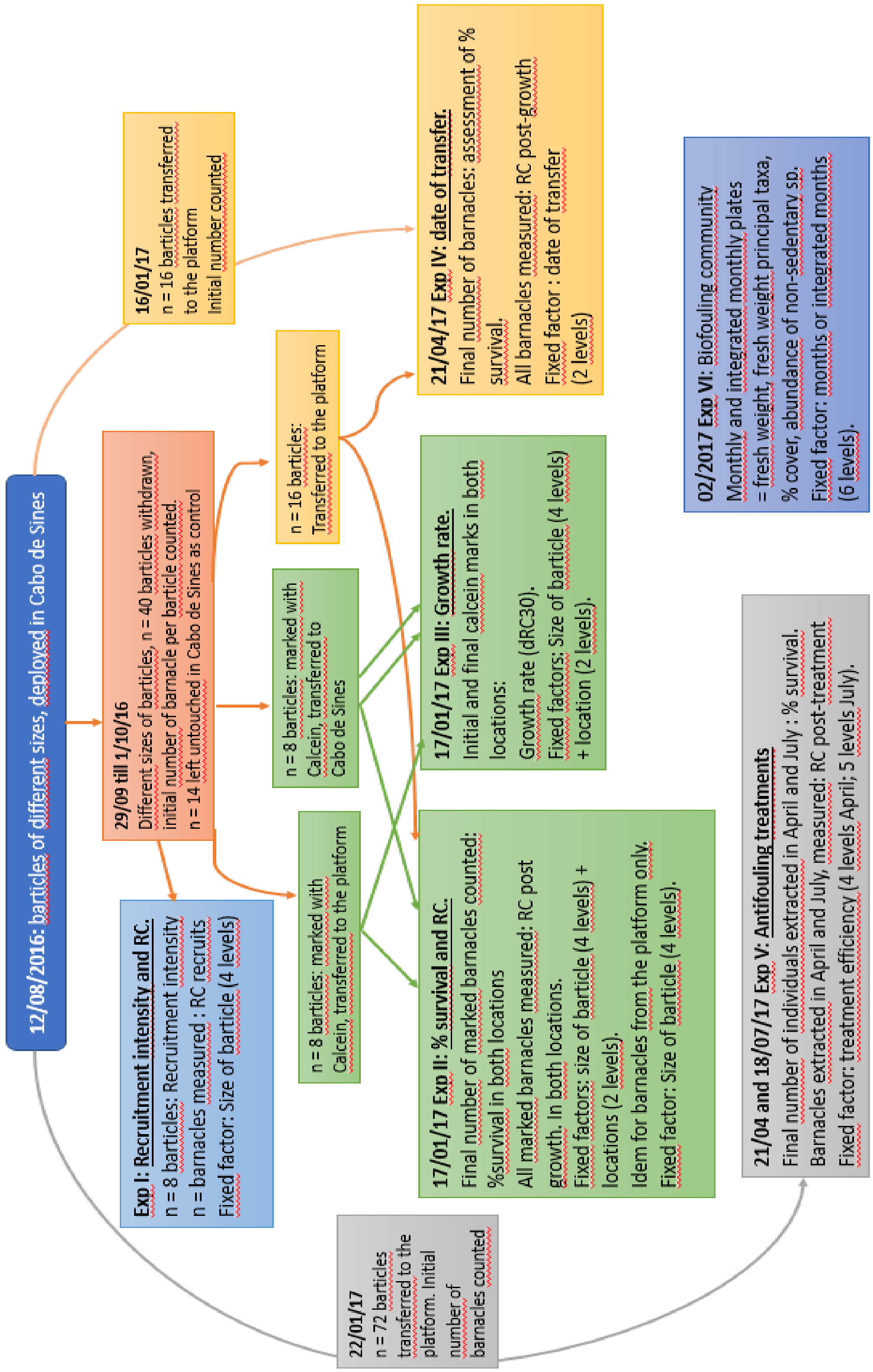

Fig. 8 Scheme of the progress of the experiments, performed during the thesis.

Results

I) Effect of the size of the barticle in recruitment intensity and size of *P. pollicipes*

The impact of the size of barticle on the recruitment is shown in fig. and on the size of barnacles collected in fig 9.

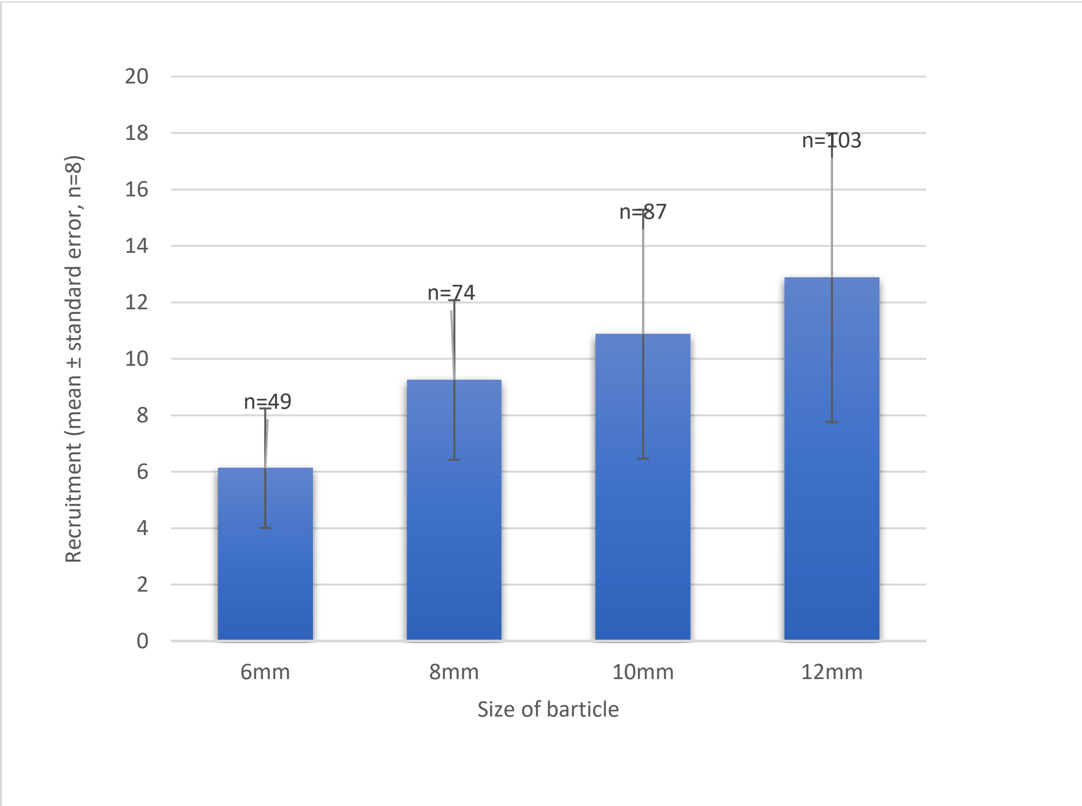

Fig. 9. Mean recruitment according to the different sizes of barticles. N.B: the total number of barnacles collected is mentioned above the standard error bars.

A mean number of recruits of 9.8 barnacles was obtained in all sizes of barticles. Despite an increasing number of recruits with an increasing size of barticles (from a mean number of 6.1 recruits in barticles of 6 mm to 12.8 recruits for barticles of 12 mm), there were no statistical differences ($P>0.05$) (table 1) among the size of barticles treatments.

The impact of the size of the barticle on the mean Rostro-Carinal length of the individuals collected, is shown in fig 10.

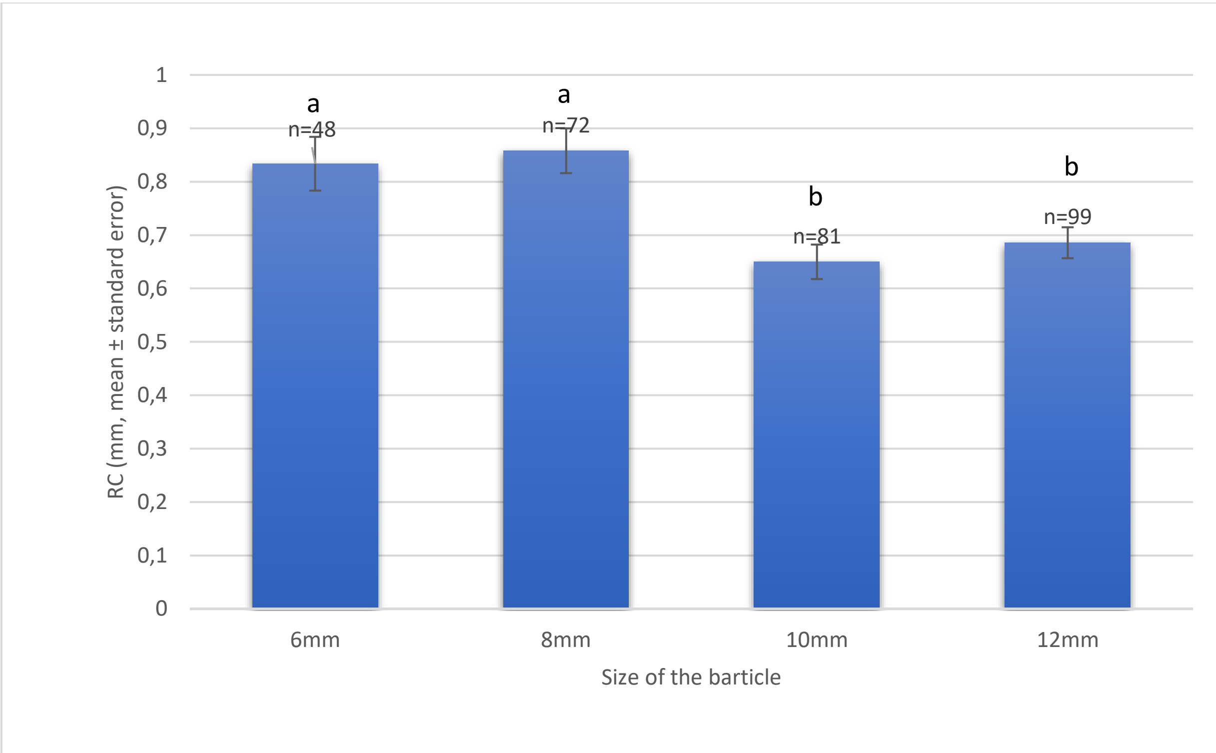

Fig. 10. Mean Rostro Carinal length (RC) in mm, of the barnacles collected, according to the size of barticles. N.B: n = RC used is mentioned above the SE bars. Bars identified with different letters, were statistically different

Two distinctive groups were observed for the mean RC ($P \leq 0.05$) (see table 2) with significantly higher values for the smaller sizes of barticles (6 and 8 mm), 0.845 mm in average and 0.667 mm for the bigger sizes (10 and 12 mm)

II) Effect of size of the barticle in survival and size of barnacles collected, in the natural habitat and in the platform.

The mean % survival of the marked barnacles collected, depending on the size of barticle and location is shown in fig. 11.

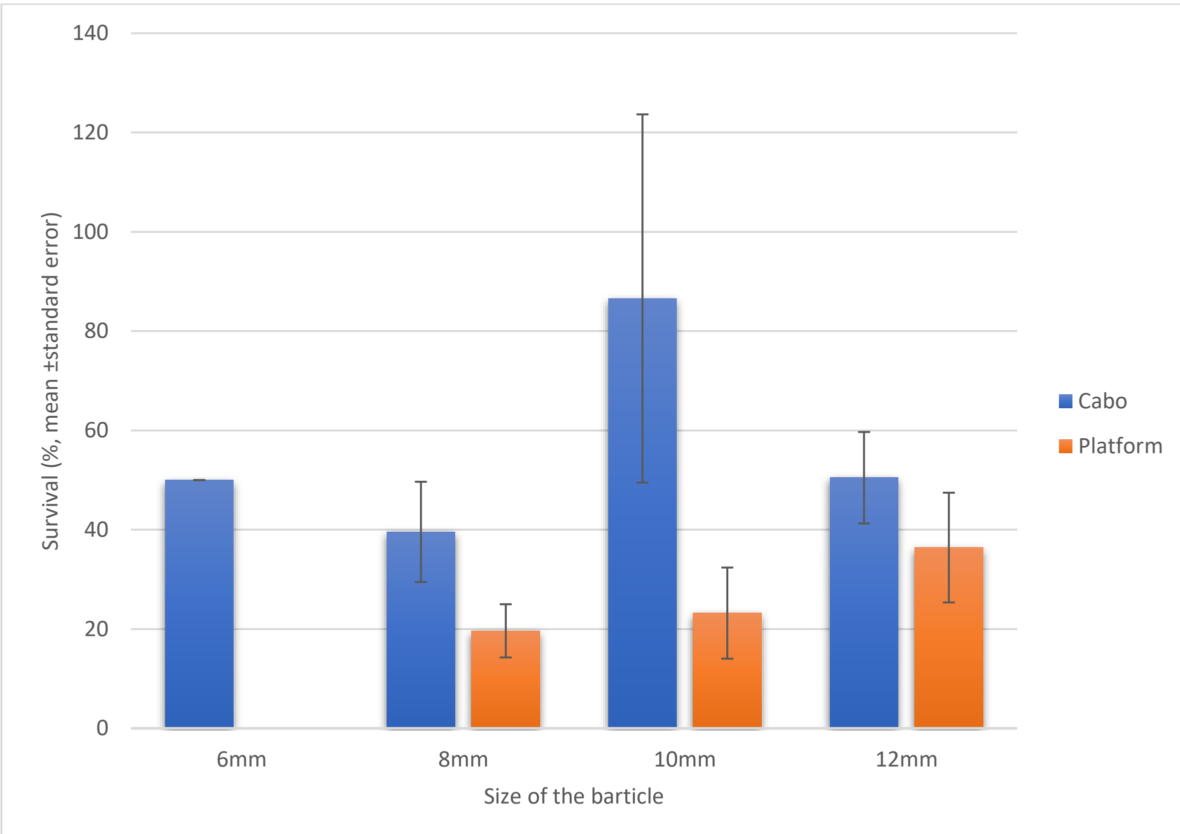

Fig. 11. Mean % survival, depending on the size of the barticle and its location.

No survival was observed on the barticles of 6 mm. Despite higher % survival of marked individuals observed at Cabo de Sines (mean of 56.6% against 26.36% at the platform), no statistical differences were observed between locations (P>0.05) (see table 3). Furthermore, no statistical differences were found among treatments (29.55% for the barticles of 8 mm, 54.9% for the 10mm and 43.5% for the 12 mm) (P>0.05).

The % survival of the barnacles withdrawn in January 2017 from the platform, is shown in fig. 12.

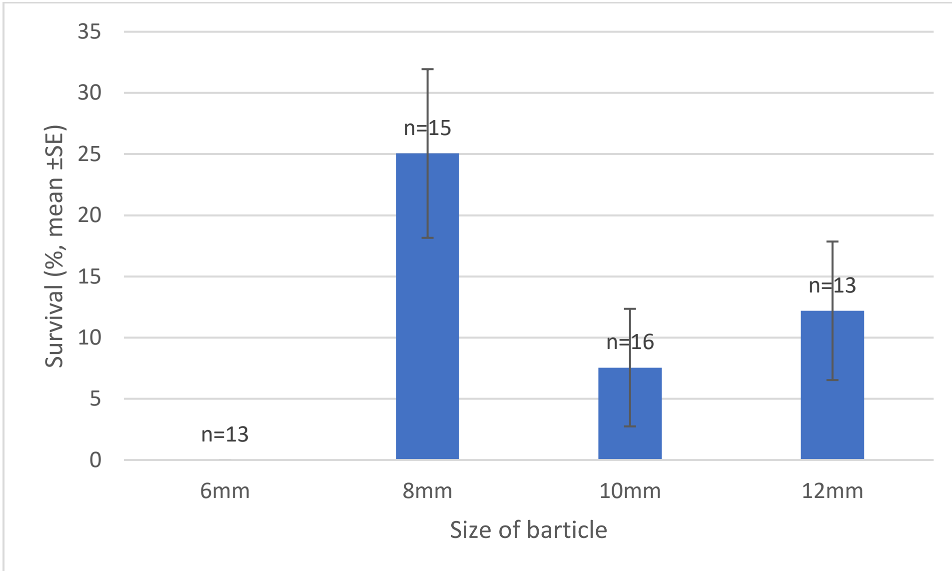

Fig. 12. % survival of the barnacles withdrawn from the platform, per size of barticle. N.B., n=sample size is shown above the SE bars.

No barnacles survived on the barticles of 6 mm in the platform. Despite a doubled %survival observed on the barnacles settled on the barticles of 8 mm, no statistical differences were obtained with the other treatments (P>0.05) (see table 4) and a mean value of 25%, 7.55% and 12.3% for the barticles of 8, 10 and 12 mm respectively.

The mean RC of the marked barnacles collected in the two locations, depending on the size of the barticles deployed, is shown on fig 13.

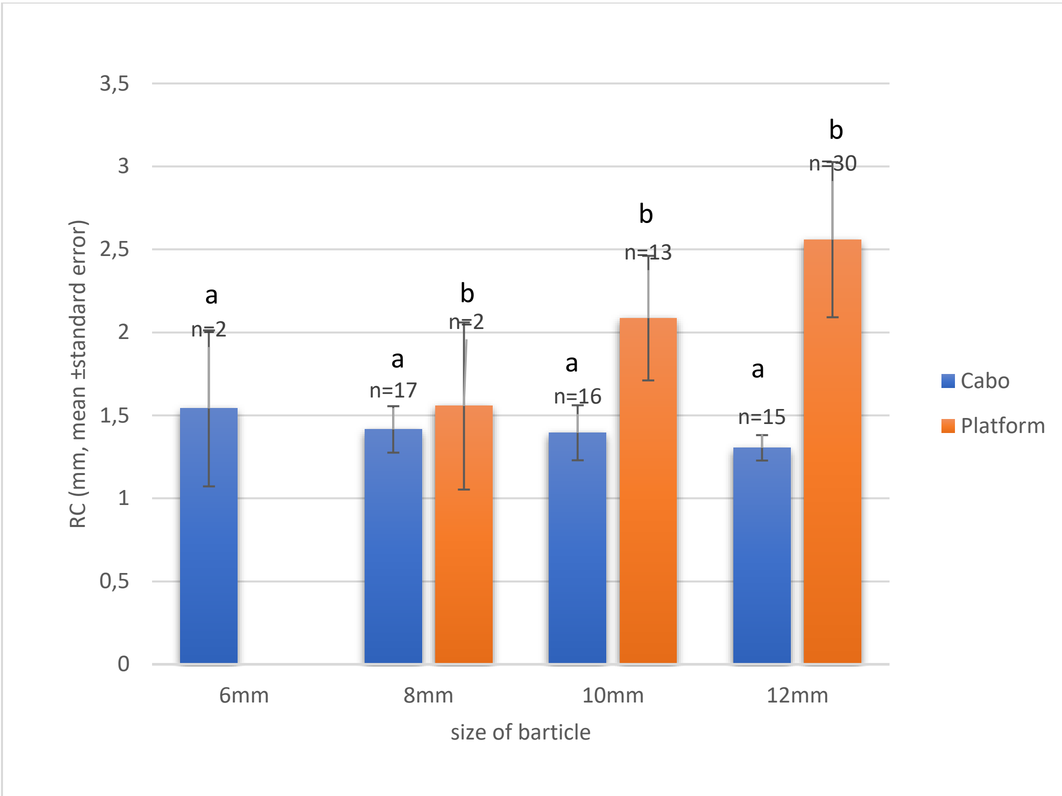

Fig. 13. Mean RC of the marked barnacles from Cabo de Sines and the platform. N.B, n=sample size is shown above the SE bars. Bars identified with different letters, were statistically different.

No RC was obtained for the barnacles settled on the barticles of 6 mm. Indeed, no survival was observed. The mean RC of the individuals collected diverged, according to the location ($P<0.05$) (see table 5). The mean RC of the individuals from the platform was estimated as 51% larger than in Cabo de Sines (average of 2.06mm for all the treatments of the platform and 1.36 mm in Cabo de Sines).

Despite an increase in the RC with the bigger sizes of barticles transferred to the platform, and a slightly inversed tendency in Cabo de Sines, no statistical differences were found among treatments 8, 10 and 12 mm of barticle size ($P>0.05$) (see table 5).

The mean RC of all the barnacles withdrawn from the platform in January 2017, is shown in fig. 14.

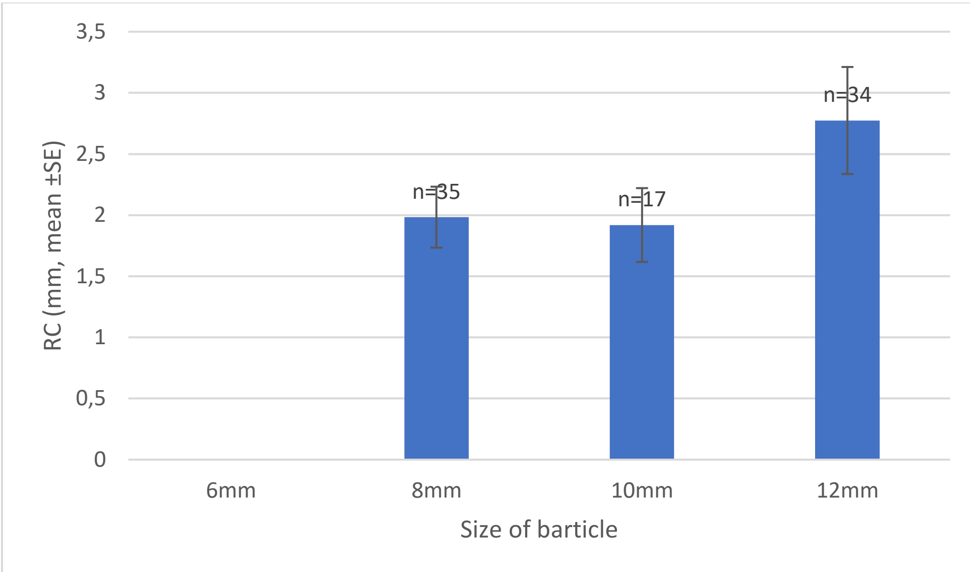

Fig. 14. Mean RC of all individuals withdrawn from the platform in January 2017, per size of barticle. N.B., n=sample size is shown above SE bars.

No RC were obtained for the barticles of 6 mm, as no barnacles survived. Despite larger mean RC observed on the barticles of 12 mm, no statistical differences were found between treatments (P>0.05) (see table 6) withdrawn from the platform and mean values of 1.98, 1.91 and 2.77 mm were observed for the barticles of 8, 10 and 12 mm respectively.

III) Effect of the size of the barticle in growth, in the natural habitat and in the platform.

The relative frequency of marked barnacles at the platform, was almost 3 times more than in Cabo de Sines (respectively 62.69 and 21.14%).

The mean growth over 30 days (dRC30) of the barnacles marked with calcein in the two locations of growth and according of the size of barticle is shown in fig. 15.

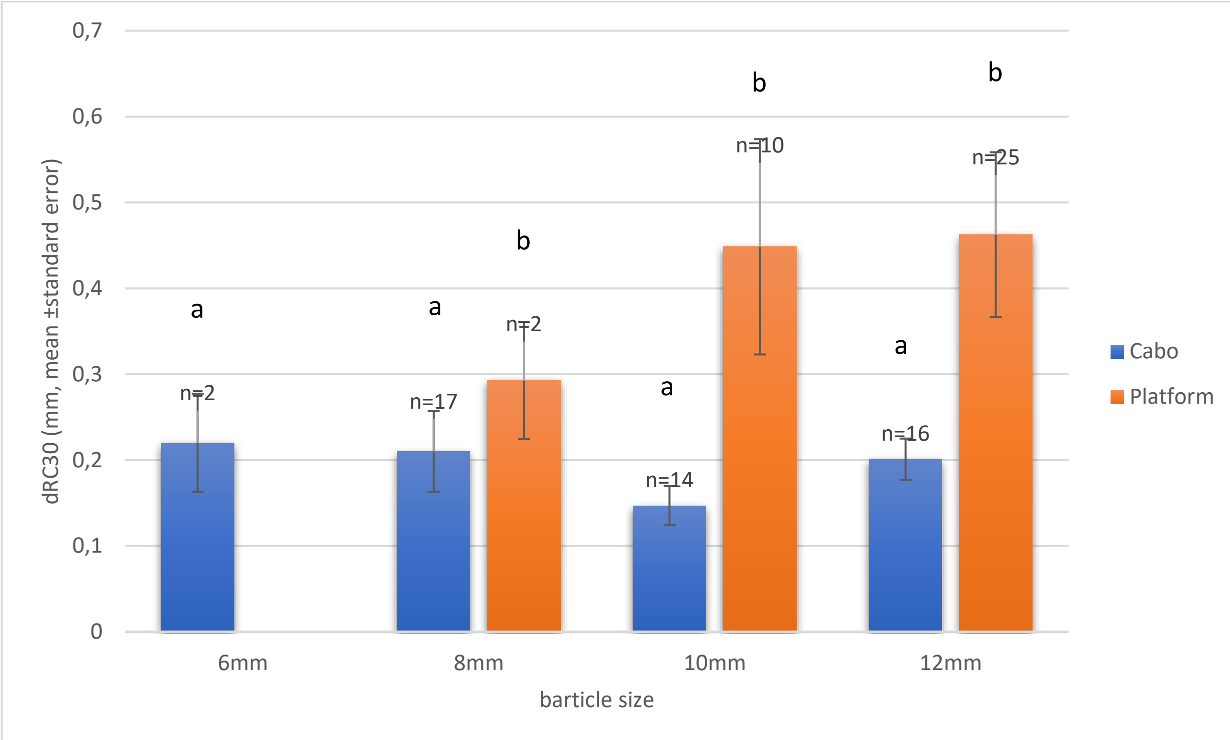

Fig. 15. Monthly mean growth rate according to the location and the size of the barticle. N.B. n = marked barnacles, is shown above the SE bars. Bars identified with different letters, were statistically different.

No mean dRC30 was obtained for the barticles of 6mm from the platform. Indeed, no survival was observed. The mean dRC30 of the marked barnacles among all the treatments combined (8, 10 and 12 mm), reached 0.4 mm for the platform and less than half in Cabo de Sines, with 0.18 mm. The mean dRC30 at the platform was statistically higher than in Cabo de Sines ($P<0.05$) (see table 7). No statistical differences were observed among barticle size treatments ($P>0.05$).

IV) Effect of the transfer date in the survival and the size of the barnacles

The fig. 16, shows the survival of barnacles according to the period of transfer to the platform.

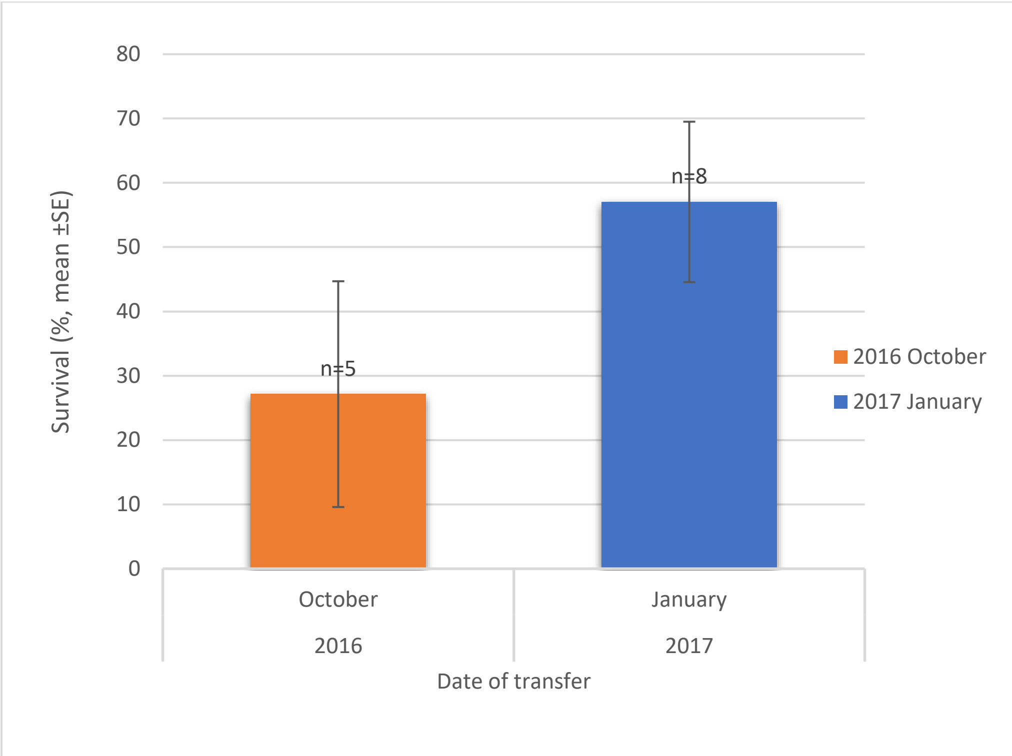

Fig. 16. Effect of the date of transfer to the platform on the %survival. N.B., the sample size is shown above the SE bars.

The barnacles transferred in October 2016 to the platform reached in April 2017 a percent survival of 27.14% ±17.55; whereas, this percentage was more than doubled, with 57.03% ±12.48 for the ones transferred to the platform in January 2017 after 89 days. However, no statistical differences were found between transfer periods (P>0.05) (see table 8) and a mean value of 42.05% survival was observed.

The effect of the transfer date to the platform, on the mean RC of the barnacles is shown in fig. 17.

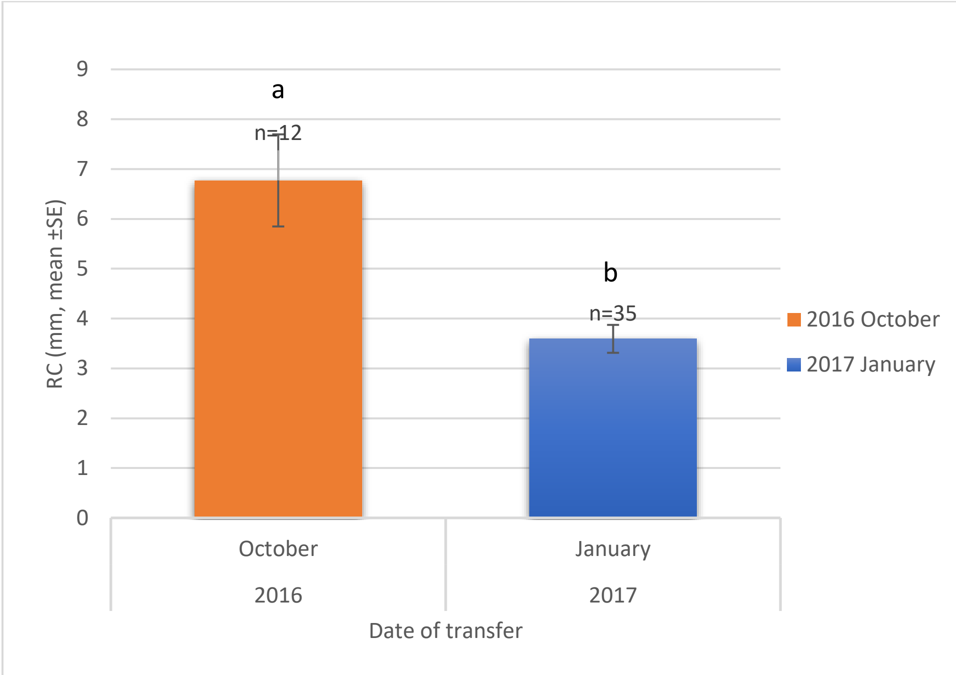

Fig. 17. Effect of the date of transfer to the platform, on the mean RC of the barnacles. N.B., n = sample size is shown above the SE bars. Bars identified with different letters, were statistically different.

The barnacles transferred earlier (October 2016) to the platform, presented a mean RC of 6.77 mm ±0.92, equivalent to almost the double of the ones transferred later (January 2017), with a mean RC of 3.59 mm ±0.28. The barnacles transferred in October had significantly higher RC than the ones transferred later ($P<0.05$) (table 9).

V) The effect of antifouling treatments on survival and size of the barnacles

The effect of the antifouling treatment on the survival of barnacles per period, is shown in fig. 18.

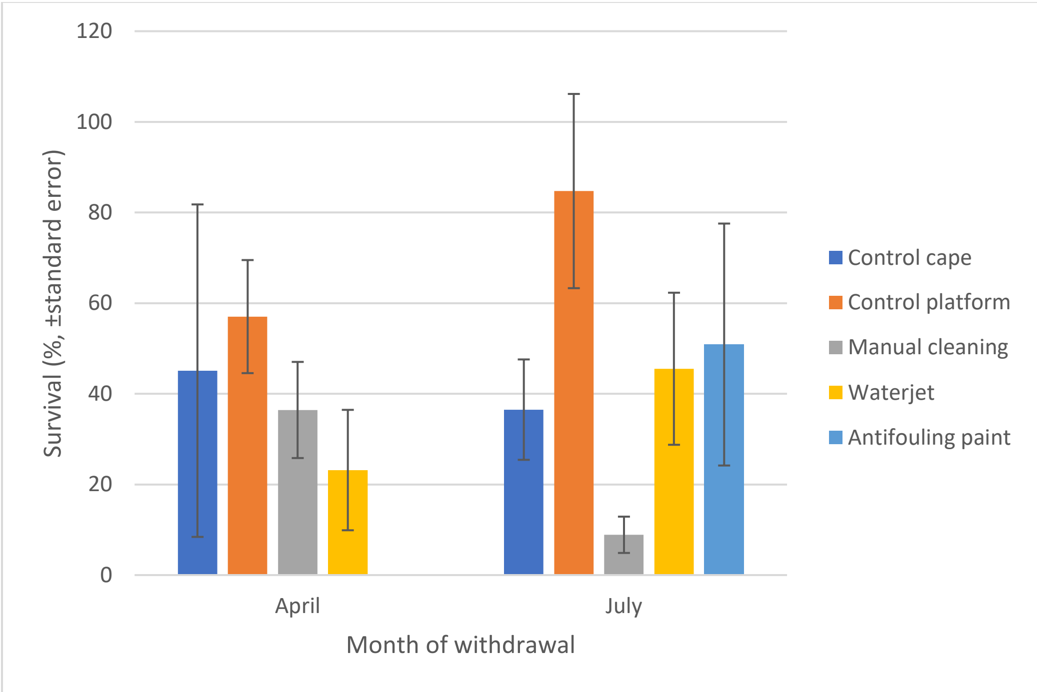

Fig. 18. % survival for each treatment for the two periods of monitoring.

In the first period, no statistical differences among treatments were observed, regarding the % survival of the barnacles (P>0.05) (see table 10), and a mean value of 40.4% of survival was observed. In the second period, no statistical differences were observed in survival, among treatments (P>0.05) (see table 11) and a mean value of 45.3% was observed.

The effect of the antifouling treatment on the mean RC of the barnacles transferred is shown in fig. 19.

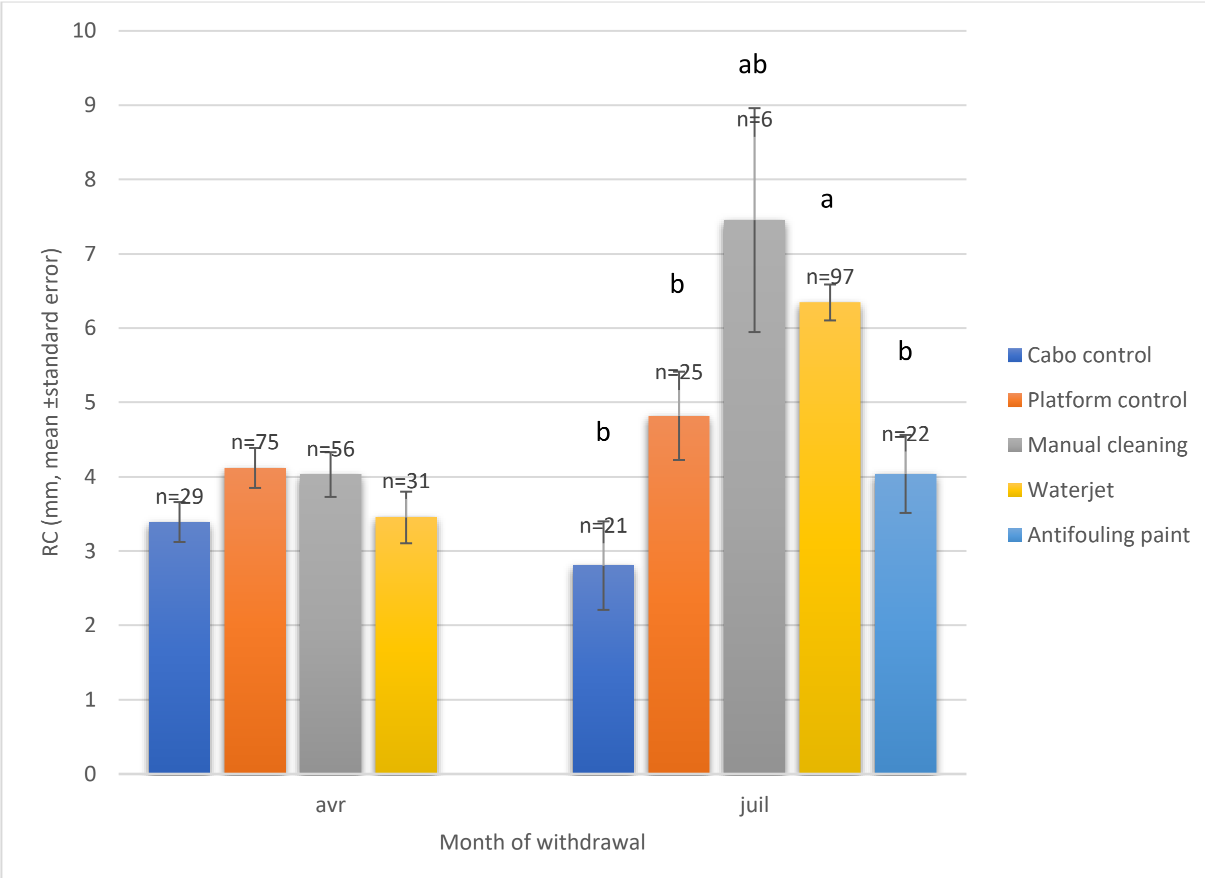

Fig. 19. Mean RC for each antifouling treatment applied. N.B, the sample size is mentioned above the SE bar. Bars identified with different letters, were statistically different.

In the first period, (barticles withdrawn in April), no statistical differences were observed among treatments (P>0.05) (see table 12) and a mean value of 3.73 mm was observed. In the second period, (barticles withdrawn in July) the mean RC of the barnacles who received manual cleaning and waterjet was more than doubled in comparison with the barnacles from the control of Cabo de Sines (respectively 7.45 and 6.34 mm against 2.8 mm). Statistical differences were observed among the control of Cabo de Sines and the other treatments (P<0.05) (see table13) but no clear pattern among treatments in the platform has been found after a pair-wise test.

VI)Temporal variation of fouling assemblages

The mean fresh weight for the monthly plates, at a monthly scale, is shown in fig. 20.

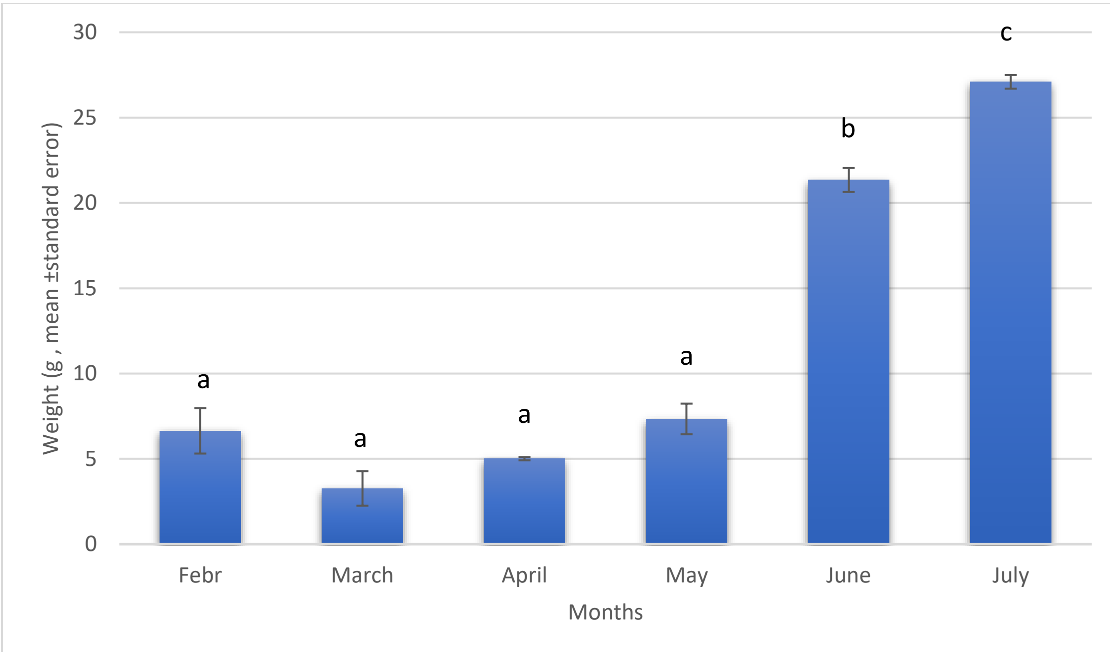

Fig. 20. Mean total fresh weight (g) of the monthly plates, at a temporal scale. Bars identified with different letters, were statistically different.

The plates settled in the platform, presented variations in the mean weights, depending on the month of exposure ($P<0.05$) (see table 14). A clear pattern was obtained, with three statistically distinctive groups. The mean fresh weight of the first group (February, March, April and May) (5.5g) was increased by 4 folds in the second group (June) and more than 5 folds in the third group (July).

The % weight composition of the biofouling assemblage of the monthly plates is shown in fig. 21. for the 3 main biofouling groups (“others”, *Mytilus galloprovincialis.*, *P. perforatus*).

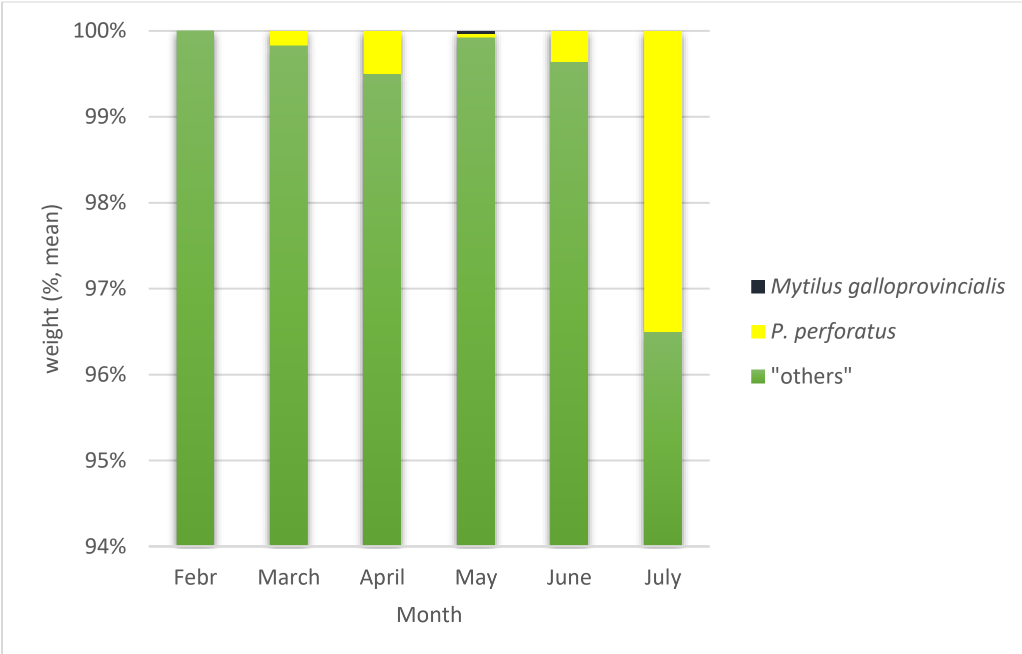

Fig. 21. Mean % weight of the biofouling assemblage at different months in 2017.

The % weight, composed by the *Mytilus galloprovincialis.* was almost not perceptible (<0.1%) and increased for *P. perforatus* between June and July (by eleven folds), reaching almost 3.5% of the total fresh weight.

No statistical differences were obtained among % weights of biofouling groups, within the different months (P>0.05) (see table 15).

The % cover of the different fouling groups of the monthly plates according to the month of the year, is shown in fig. 22.

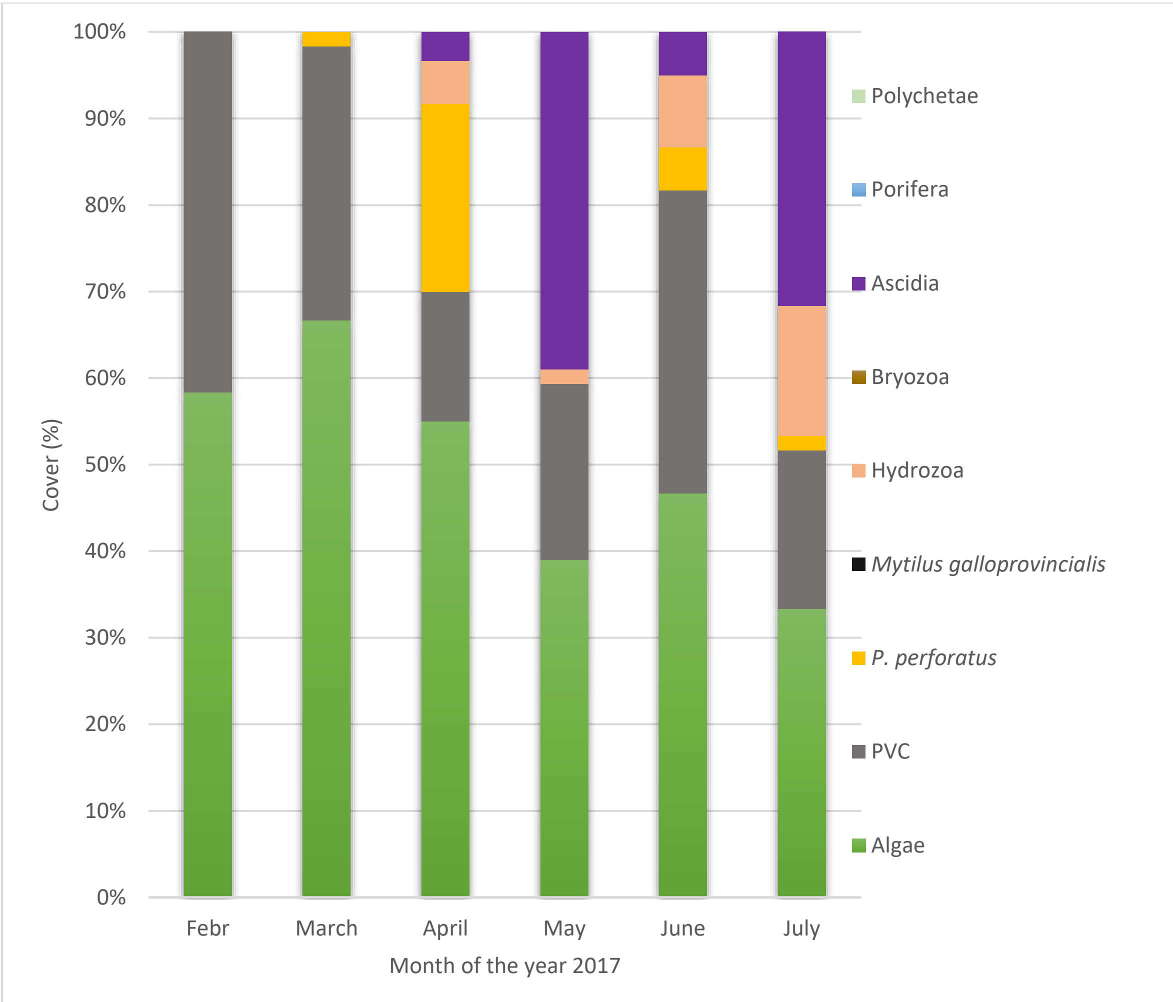

Fig. 22. % cover per fouling groups and PVC in the monthly plates according to the month.

Despite an increased diversification of the biofouling groups and their respective % cover since April, no statistical differences were found between the months (P>0.05) (see table 16).

The mean number of amphipods, on the monthly plates, is shown in fig. 23.

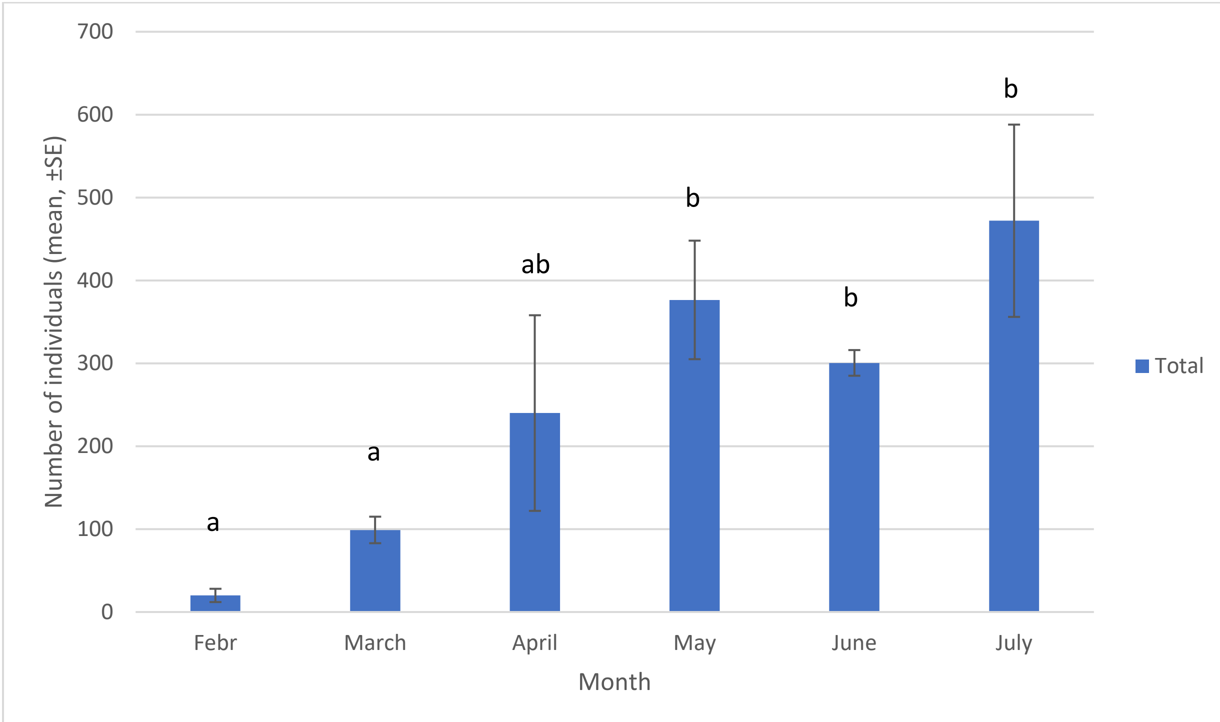

Fig. 23. Mean number of amphipods, on the monthly plates. Bars identified with different letters, were statistically different.

The abundance of amphipods globally increased among the monthly plates. Statistical differences were obtained between months ($P<0.05$) (see table 17), however no clear pattern was observed after a Monte-Carlo test, regarding the abundance of amphipods, per months. A mean number of 251.3 amphipods were observed on the monthly plates.

The mean number of copepods, on the monthly plates, is shown in fig. 24.

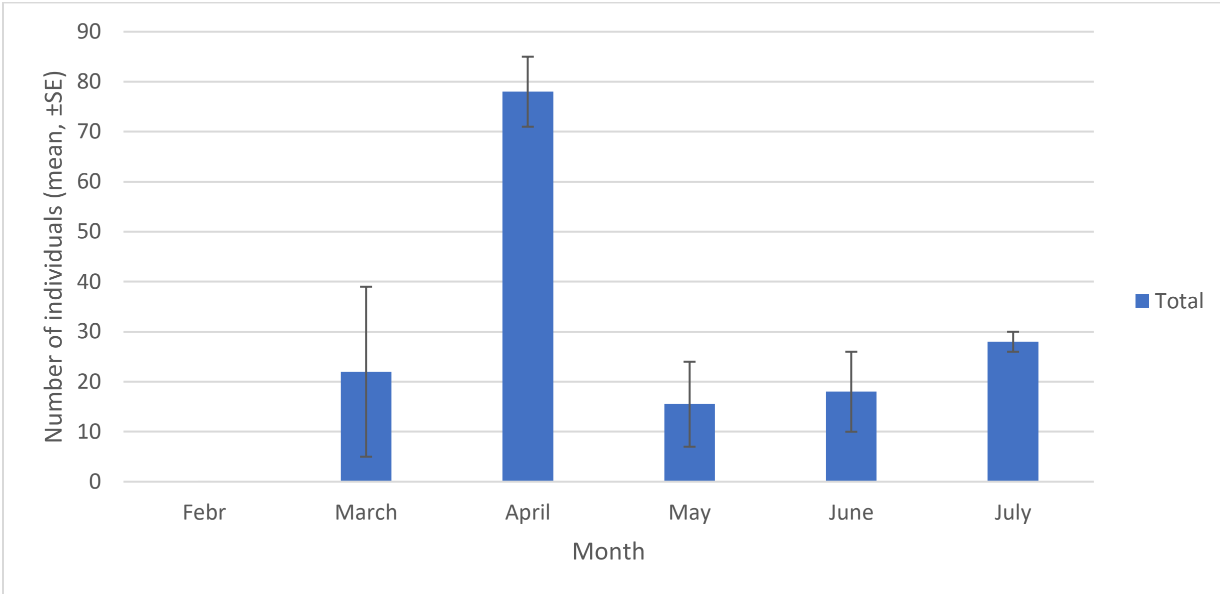

Fig. 24. Mean number of copepods, on the monthly plates.

The abundance of copepods did not give statistical differences among the studied months ($P>0.05$) (see table 18), despite a peak observed in April (almost 4-folds the abundance found in March or May). A mean number of 26.6 copepods were observed for all the monthly plates.

The mean number of polychaetes, on the monthly plates, is shown in fig. 25.

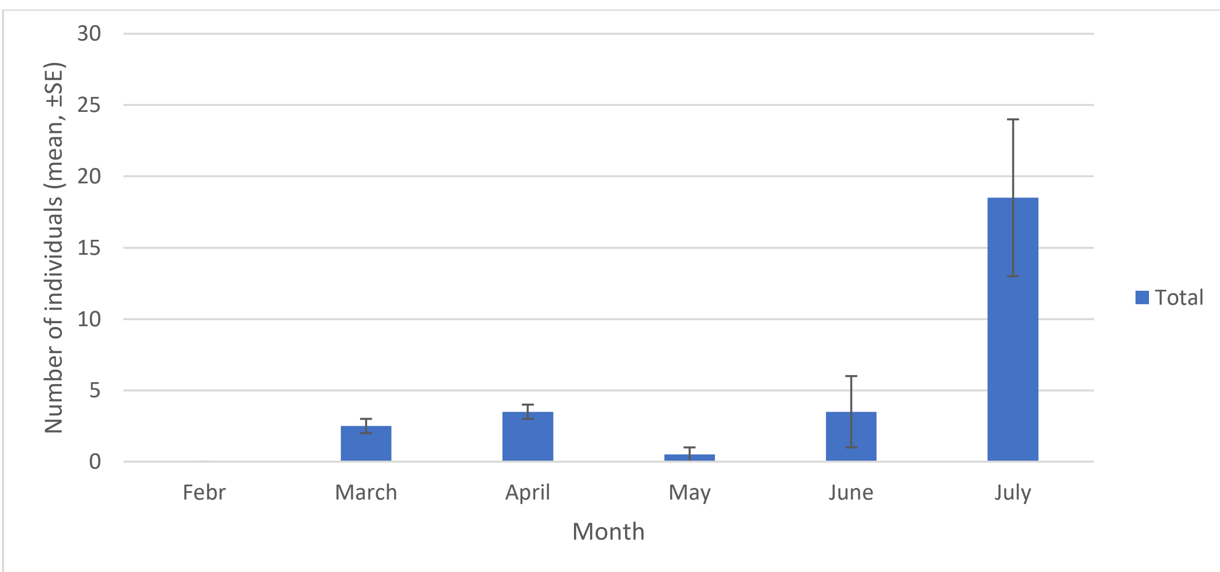

Fig. 25. Mean number of polychaetes, on the monthly plates.

The mean number of polychaetes did not give any statistical differences among the months ($P>0.05$) (see table 19), despite an increased mean number of individuals observed in July (mean value of 18.5 polychaetes against 2.5 for the other months).

Two other functional groups were found on the monthly plates, (echinoderms and gasteropods) but at very low densities (mean values <2), thus they were not taken in consideration.

The mean cumulative fresh weight of the integrated monthly plates, at an integrated monthly scale, is shown in fig. 26.

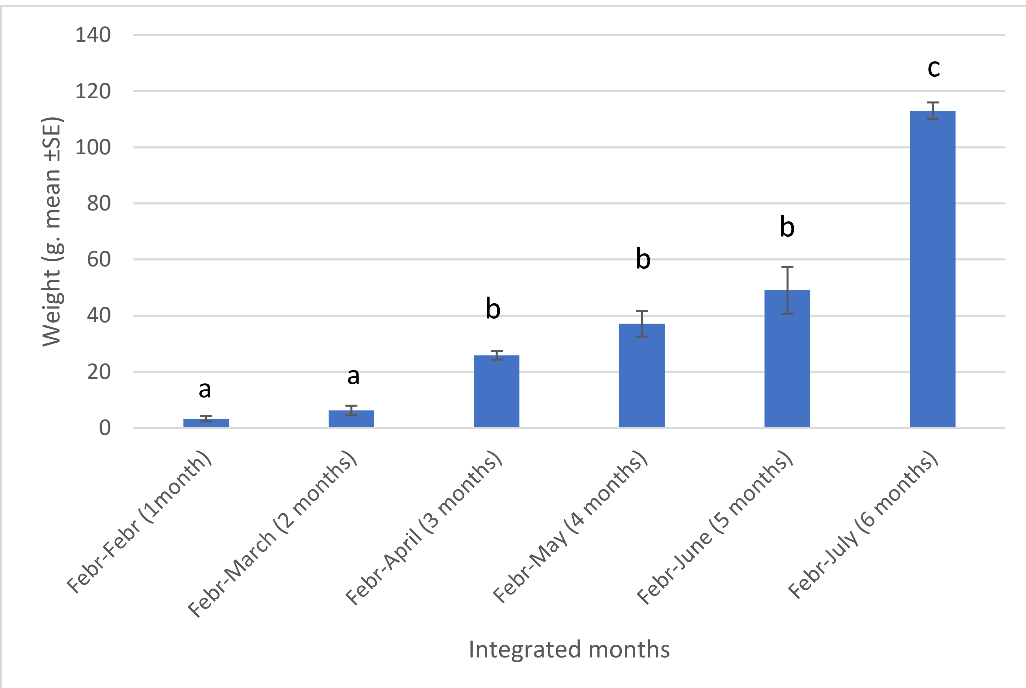

Fig. 26. Mean total fresh weight (g), of the integrated monthly plates. Bars identified with different letters, were statistically different.

The plates settled at the platform, presented a mean cumulative fresh weight of biomass, starting at 3.26 g after 1 month, and increasing until reaching 34 times this value (113g) after 6 months. Statistical differences were found between the fresh weights of each month ($P<0.05$) (see table 20). A clear pattern was obtained with 3 significantly different groups. The mean fresh weight of the first group (Febr-Febr, Febr-March) (4.26g) was significantly smaller than the second group (Febr-April, Febr-May and Febr-June) (37.35g) itself statistically smaller than Febr-July (113g).

The % weight composition of the biofouling assemblage of the integrated monthly plates is shown in fig. 27. for the 3 main biofouling groups.

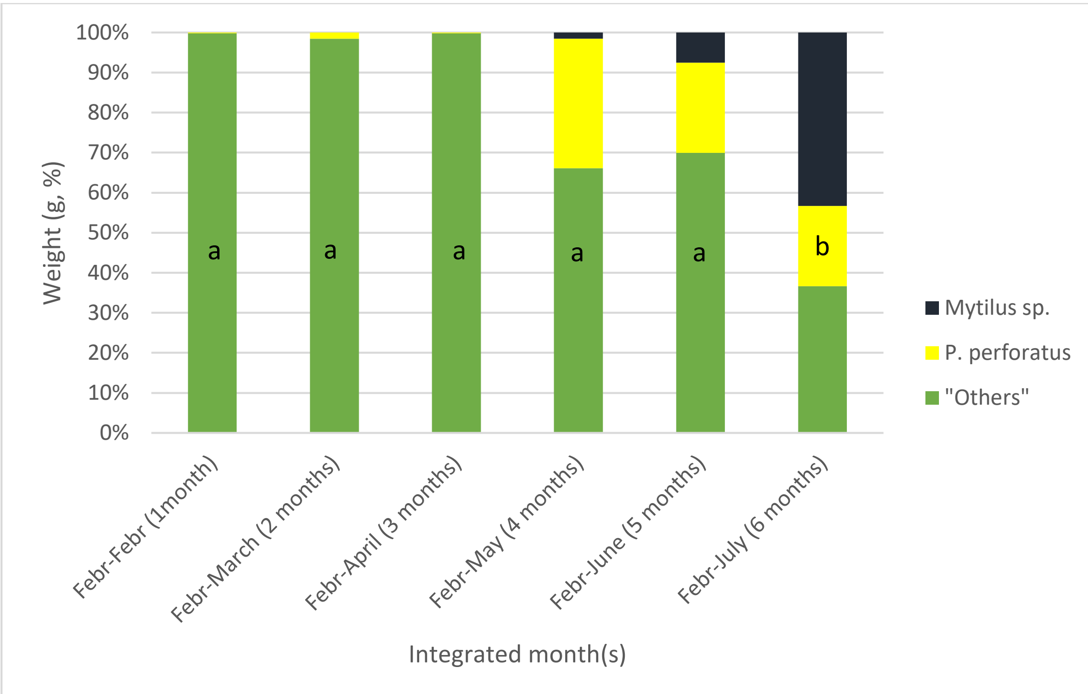

Fig. 27. Mean % weight of the biofouling assemblage at different time intervals in 2017. *s*. Bars identified with different letters, were statistically different.

Statistical differences were found between mean % weights of "others", barnacles and mussels, within the temporal intervals ($P<0.05$) (see table 21). A clear pattern was obtained, with statistical differences between the five first temporal intervals and the last one (Feb-July) in % weight composition, probably due to the larger increase of the mussels relatively to the other groups.

The % weight, composed by the mussels increases for the Feb-May plates till reaching 24 times its value for the Feb-July plates in which, they reached almost half of the fresh weight. The fresh weight of barnacles reached a peak for at the 4 months' interval plates (30 folds in comparison with the 3 months' interval). The % fresh weight composed by the barnacles, then decreased slowly for the 5 and 6 months' interval plates.

The % cover of biofouling groups on the integrated monthly plates, is shown in fig. 28.

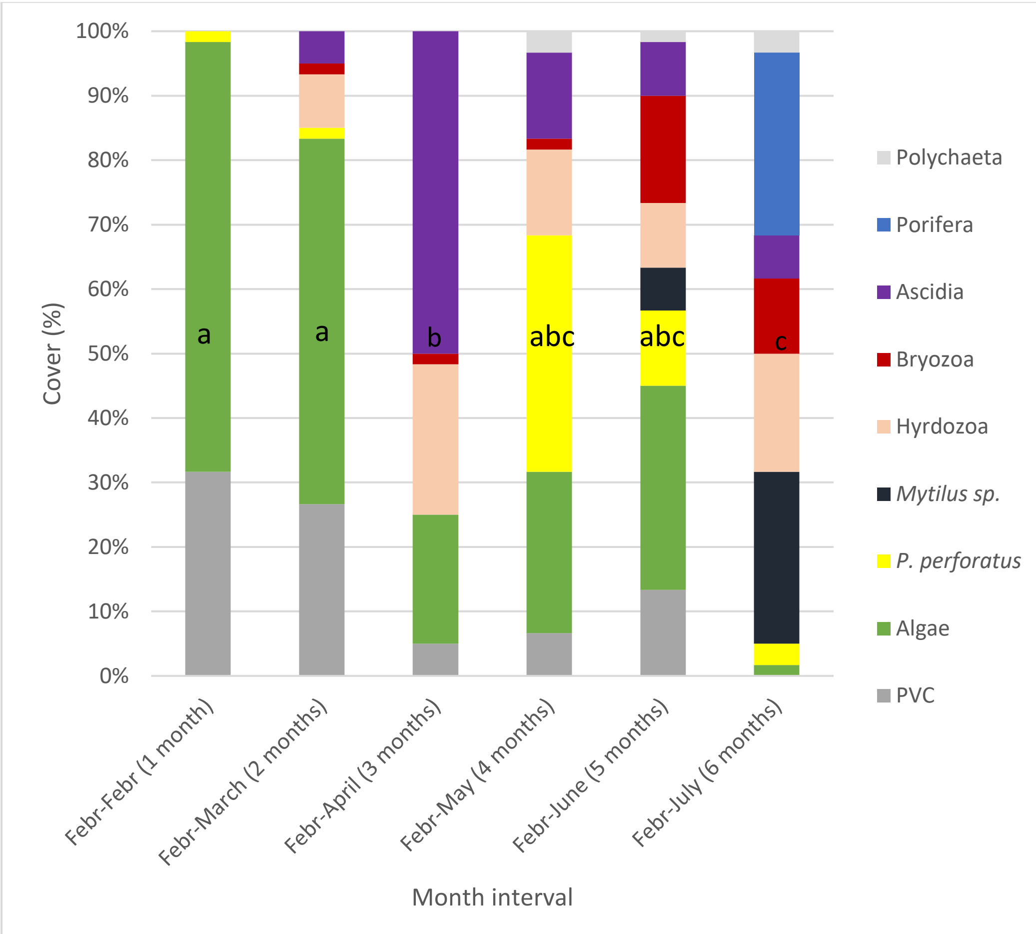

Fig. 28. % cover per taxon and PVC in the integrated monthly plates, according to the integrated monthly plates. Bars identified with different letters, were statistically different.

An increase of the biodiversity on the integrated plates has been observed from the 4months old plates, until reaching a peak (all biofouling groups found) on the 6 months old plates.

This observation was supported by statistical differences in the % cover between integrated plates ($P<0.05$) (see table 22). However, no clear pattern among temporal intervals was obtained by the Monte-Carlo test.

The mean number of amphipods, on the integrated monthly plates, is shown on the fig. 29.

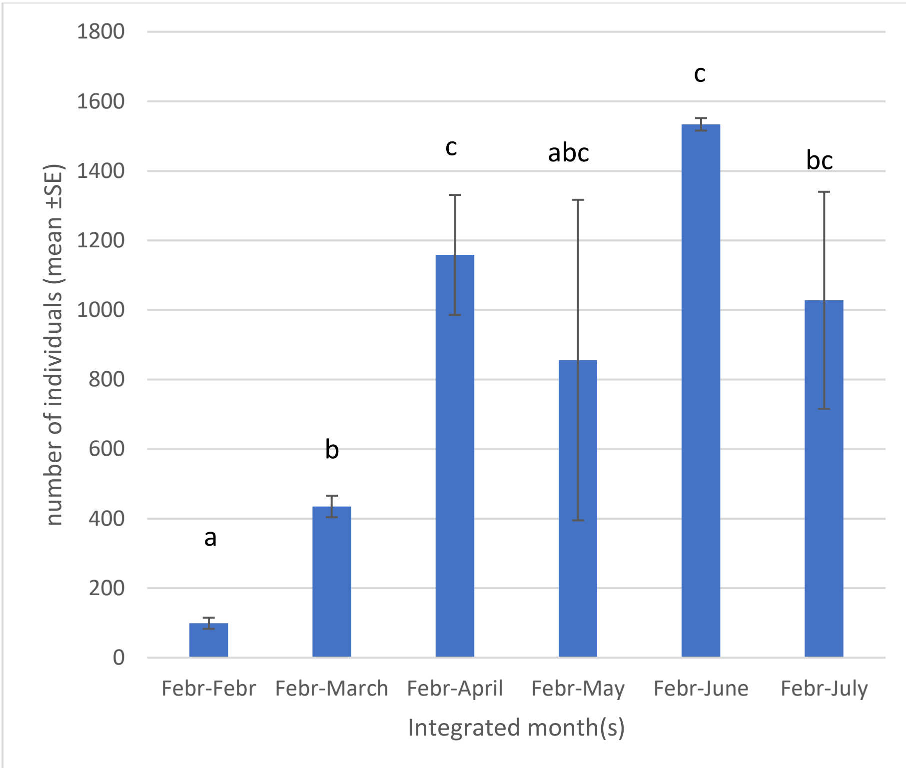

Fig. 29. Mean number of amphipods, on the integrated monthly plates. Bars identified with different letters, were statistically different.

The abundance of amphipods among integrated monthly plates, increased with the length of temporal interval and significant differences ($P<0.05$) (see table 23) were obtained; however, no clear pattern was obtained after a Monte-Carlo test. A mean number of 851.5 amphipods were observed on the integrated monthly plates.

The mean number of copepods, on the integrated monthly plates, is shown on the fig. 30.

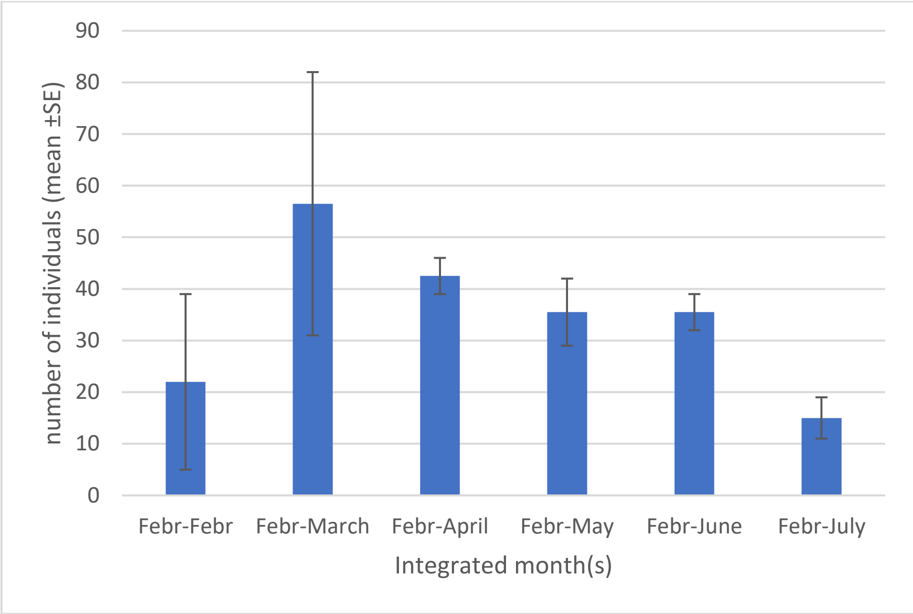

Fig. 30. Mean number of copepods, on the integrated monthly plates.

The abundance of the copepods among the integrated monthly plates did not reveal statistical differences (P>0.05) (see table 24). A mean number of 34.5 copepods were found on the integrated monthly plates.

The mean number of polychaetes, on the integrated monthly plates, is shown on the fig. 31.

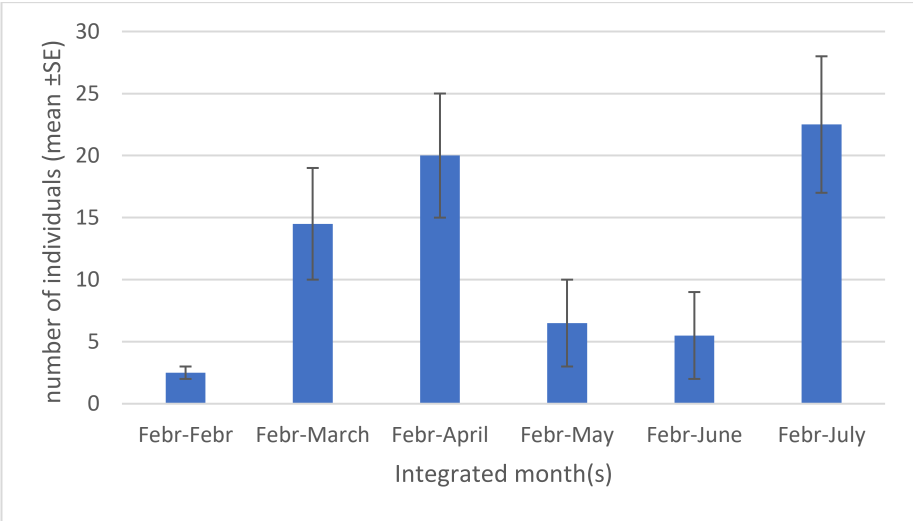

Fig. 31. Mean number of polychaetes, on the integrated monthly plates.

The abundance of polychaetes, was not significantly different ($P<0.05$) (see table 25), despite important variations among integrated monthly plates. A mean number of 11.9 polychaetes were found on the integrated monthly plates.

Two other functional groups were found on the integrated monthly plates (echinoderms and gasteropods) but at very low densities (mean values <2), thus they were not presented in the results.

Discussion

To improve the culture of *P. pollicipes*, several sizes of barticles were used to assess the recruitment intensity, the sizes of barnacles collected, the sizes and growth rates of individuals in the natural habitat or transplanted to the platform and their respective survival. The impact of the transfer date to the platform was tested on the survival and the sizes of the barnacles. To optimize survival and growth, several antifouling technics were applied to the culture system and the biofouling communities were monitored for six months, to assess the peaks of appearance and main taxa encountered.

The mean recruitment intensity for all barticles deployed in the field in August 2016 and sampled in October 2016 was 9.8 barnacles per barticle This value is similar to the ones observed in a previous study (Cruz *et al.,* 2016), in which a mean recruitment intensity of 6 to 12 barnacles per barticle was observed for barticles deployed in the field in summer 2014 and transplanted to the platform in several dates from winter until summer 2015. A higher recruitment intensity was observed in this study (~21 barnacles per barticle) for barticles that were in the field for two consecutive recruitment seasons (Cruz, *et al.,* 2016). The most important recruitment season for *P. pollicipes* is summer and autumn (Cruz *et al.,* 2010).

The absence of differences for the recruitment intensity observed, in the different sizes of barticles, suggests that the number of barnacles collected is not dependent from the size of the barticle deployed. The hypothesis that the diameter of the collector used, could have an impact on the recruitment intensity, was not supported by our data and findings. Contrary results were obtained for spat collection of mussels *Mytilus galloprovincialis* on nylon-made ropes deployed in the Alboran sea (Aghzar *et al.*, 2012), where settlement intensity was dependent on the diameter of the collectors providing more settlement surface. Thicker collectors provided almost seven times more spats.

However, the size of the barticles had an effect on the size of the recruited barnacles. Indeed, larger individuals (RC=0.86mm) were obtained on the smaller sizes of barticles (6 and 8mm) in comparison with the ones recruited in larger barticles (10 and 12mm) that presented a lower mean RC of 0.67mm. A potential higher growth in the smaller barticles might be due to the fact that crenels of the smaller barticles are less prominent (fig. 2) and therefore could provide a better access to waterflow and food for juveniles attached to these smaller barticles.

Survival of barnacles that were transplanted to the platform and that remained in the natural habitat (Cabo de Sines) was studied by marking transplanted animals with calcein according to Jacinto et al. (2015). The relative frequency of marked barnacles at the platform, was almost 3 times more than in Cabo de Sines (respectively 62.69 and 21.14%). The low frequency observed in the Cape location should have been caused by additional recruitment to these barticles that occurred after the marking with calcein. In fact, recruitment is still intense during autumn (Cruz *et al.*, 2010) and the marking date was in October. In relation to the frequency of marked barnacles in the platform, this value is lower than the ones observed in a past study where 100% of marked animals was observed in summer and 90% of marked animals was observed during winter (Cruz *et al.,* 2016). Based on observations made in this study, there is no indication of additional recruitment of barnacles in barticles deployed at the platform, so, as suggested before (Cruz *et al.,* 2016), this low number of marked animals might be due to the small size of the animals at marking time and to a less efficient technique to detect the marks in the plates of very small animals. Indeed, some of the sampled individuals might have lost their scutum plates during the growth period, as not all marked individuals analyzed presented both scutum plates tagged. The low efficiency of this marking technique in the present study might also be caused by a longer marking period (3.5 month) in the present study, in comparison with past studies (e.g., 2.5 months in Jacinto *et al.*, 2015).

Based on the marked animals, the hypothesis of having different %survival according to the size of the barticle, was partially supported. Indeed, no barnacles survived in the barticles of 6mm, but the other sizes of barticles did not give consistent results to identify the most suitable barticle size for survival purposes. No significant differences in % survival between the study locations (Cape and platform) were observed for the marked barnacles, despite higher mortality noted on the platform.

Survival of barnacles in relation to size of the barticle was also studied in the platform for marked and non-marked animals, and the pattern in relation to size of the barticle was similar. Both values of survival in the platform for marked and non-marked animals were lower (20-25% for size of barticle 8mm) that the ones observed in a first phase of previous study (Cruz *et al.,* 2016) where the size of barticle 8mm was the unique size used. In this study, survival in the platform was very high (~100%) for barnacles transplanted to the platform in the beginning of winter and monitored in April. In the present study, barnacles were transplanted to the platform earlier (October) and survival was also monitored earlier (January). This result might indicate that, in terms of survival, a transfer to the platform in the beginning of autumn might increase mortality of small animals (more likely prone to suffer from small predators and competition for space). However, in the present study, the date of transfer to the platform was also studied and no significant differences in survival were found between a situation of transfer to the platform in October versus in January and monitoring in April.

The effect of the size of the barticles was also tested upon the size (RC) of the barnacles and upon the monthly growth rate (dRC30). There was no consistent pattern of differences in the size of the marked and non-marked animals of platform that were present in barticles of sizes 8, 10 and 12mm. The same result was obtained for the monthly growth rate. Size and growth rate of animals from the barticle of size 6mm were not measured, as no animals had survived in this barticle size treatment.

Comparing both locations, in the platform, marked barnacles in January were bigger (mean RC=2.1mm) and had grown faster (mean dRC30=0.4mm) than animals from the Cape, respectively, mean RC of 1.4mm and mean dRC30 of 0.18mm. The barnacles transferred to the platform might have benefited of a full-time immersion in the water and the assumption of getting larger individuals at the platform than in the Cape, was supported. A longer time of immersion seems to favor growth. Indeed, in the natural habitat, low shore barnacles are likely to present a higher RC than the mid-shore individuals (Cruz *et al.,* 2010; Sousa *et al.,* 2013), likely due to a longer period of immersion which would enhance feeding time. In 2015, the mean RC of the barnacles transplanted to the platform was significantly higher in the platform than in the Cape (Cruz *et al.,* 2016). A similar observation was made in a study conducted by Goldberg (1984) and higher growth rates were obtained for individuals of *P. pollicipes,* after 2 months on an offshore system. Some species of acorn barnacles (*Austromegabalanus psittacus* and *Megabalanus azoricus*) showed an increase of biomass up to 15-folds, when placed in offshore suspended systems (Lopez *et al.,* 2010).

The monthly growth rate of the barnacles (0.4mm) in the platform during autumn 2016 is lower than estimates of growth rate from a previous study (Cruz *et al.,* 2016). During winter of 2015, the mean monthly growth rate of barnacles in the platform was almost three times higher (~1.1mm, RC), and during summer 2015, about two times higher (~0.9mm, RC) (Cruz *et al.,* 2016). Also in the Cape, growth rate was higher during winter (~0.6mm, RC) and summer (~0.9mm, RC) of 2015 (Cruz *et al.*, 2016) than the estimate of autumn of 2016 growth from the present study (0.18mm, RC).

Growth of *P. pollicipes* is very variable (Jacinto *et al.,* 2015) and probably dependent on seston availability in the environment as was observed for the giant barnacles (Lopez *et al.*, 2012). Therefore, seasonal variability could be expected in the growth rates of *P. pollicipes*, with possible latency and enhanced growth periods (Cruz, 2000; Cruz *et al.,* 2010; Franco, 2014).

In the present study, the effect of date of transfer of the barnacles to the platform was also tested upon the size of the barnacles monitored in April 2017. A significant effect was detected, as barnacles that were transplanted to the platform in October 2016 had a higher mean size in April (RC=6.8mm) than the ones that were transplanted in January 2017 (RC=3.6mm). As the mean size of the barnacles in the platform in January 2017 was 2.1mm (RC, see above), these results suggest that winter growth might have been higher for barnacles that were already in the platform for a few months. Comparing with a previous study (Cruz *et al.,* 2016), mean size of barnacles in April 2017 that were transplanted to the platform in January of 2017 (3.6mm, RC) was lower that size observed in a similar period in 2015 (mean RC of 6mm in April 2015). These results indicate that inter-annual growth might also be important and that other variables such as mean yearly temperature (Franco *et al.,* 2016b), can contribute to the difference found in similar studies, during the same annual period.

As no significant differences in survival were encountered (despite an important variability) and since larger barnacles (higher RC) were obtained for the barticles transplanted earlier, an early transfer to the platform seems to give better results for aquaculture purposes. The early implementation on the platform of the barticles is likely to give bigger individuals, reaching the market size earlier.

A right implementation period on the grow-out system could maximize the growth rates of *P. pollicipes.* The grow-out offshore system was shown as effective, to improve growth rate of *P. pollicipes* and therefore to obtain larger individuals than in the natural habitat (for the same period of growth). However, the survival was more compromised, and further observations are required to identify the optimal window of recruitment to improve recruitment harvesting and get closer to the maximal carrying capacity of the barticles.

In relation to the change of size of the barticle used in a previous study (8mm, Cruz *et al.*, 2016) to other sizes tested in the present study (6, 10 and 12mm) as a method to improve the cultivation of *P. pollicipes*, results show that barticles of 6mm are not adapted for *P. pollicipes* aquaculture. Indeed, they were considered not stiff enough when placed/withdrawn from the field, and no clear advantage of use could be highlighted (except their lower cost 0.018€/unit). In relation to the other sizes of barticles, results were not consistent in all tested variables (recruitment intensity, survival, size, growth rate) to identify the most suitable barticle size. However, in terms of costs, the barticles of 8 and 10 mm (respectively 0.022 and 0.027€/unit) should be favored in relation to barticles of 12 mm (0.044€/unit for the barticles of 12mm).

The high variability in general that was observed in number of barnacles collected per barticle of a same size, might be due to small scale variations of the rocky shore. Indeed, the complexity of recruitment and populations dynamics relies on several processes, such as larval pools dynamics, larval transport, settlement and post-settlement biotic (e.g. predation, competition) and abiotic processes (Pineda *et al.,* 2009). Those phenomena are likely to influence *P. pollicipes*' settlement and survival on the barticles, and therefore might explain the variability in the number of juveniles collected per barticle.

The hypothesis of improving the survival and getting larger animals at the platform through different antifouling treatments did not meet the expectations. Indeed, no statistical evidences were found in favor of a reduction of mortality of barnacles in antifouling treatments in relation to the control treatment in both periods of experimentation (January-April; January-July).
In fact, there were no significant differences of survival (in April and in July) and of size (in April) of barnacles among antifouling treatments. Contrary to the expectations, the higher value of survival was observed in the control treatment of the platform in July (~82%). The only consistent pattern was a larger size of animals in July in the platform independently of the treatments (RC size from 4 to 7mm, RC) in relation to control barnacles from the Cape (RC=3mm). Again, this result might be due to a higher growth rate in the platform.
In a previous study (Cruz et al. 2016), survival and size of barnacles in the platform were monitored in different dates (from April 2015 to January 2016) and in relation to different transfer dates to

the platform (beginning of winter of 2015, dates during spring, summer and autumn 2015). The most striking result of this study was the steeping decrease in survival of barnacles in the platform from spring/summer forwards, with a total mortality observed in January 2016. This mortality was mainly caused by competition with fouling organisms, namely mussels (Cruz et al., 2016).

The apparent contrary results from the present study and Cruz et al. (2016) might be due to the end date of the experiment (July) being too early to detect the fouling problems that were observed more intensively in the past, during summer and autumn. Monitoring of barnacles from these antifouling treatments and from other periods after July 2017 will be analyzed in the future and this question will be better addressed.

Although there were no differences in antifouling treatments, the fact that survival in the manual cleaning treatment was very low (5%) in July, might be due to a wrong application of this treatment in time. Indeed, manual cleaning when barnacles are smaller, might have been negative for them. Probably, the treatments were applied too early, inducing mortality on the smallest individuals. Delaying the antifouling treatments, until targeted periods of major biofouling blooms (summer), could allow to reduce the mortality induced by their application.

The results obtained for the monitoring of the monthly and integrated monthly PVC plates, revealed low fresh weights and a small biodiversity in winter/spring. During this period of low levels of biofouling, no treatment should be required and leaving the barnacles without manipulation could favor their survival.

Important increase in fresh weight around June/July, and changes in the biofouling communities' structure (%cover and % of weight) were recorded. M. galloprovincialis and P. perforatus populations increased drastically in weight and occupation of space around the end of spring and beginning of summer. Equivalent peaks of recruitment were found in northern Spain for M. galloprovincialis (Cáceres-Martínez *et al.*, 1993; 1994; Molares et Fuentes, 1995). The recruitment of *P. perforatus*, was observed as lasting over 10 months per year (February-November) in Portugal (Cunha et al., 2017), which explains their presence in almost all monthly and integrated monthly paltes. However, the negative impact of the two-latter species, was not directly noticed in this study. Indeed, the mortality of P. pollicipes in the control treatments, did not significantly increased during this period. Their impact on P. pollicipes is likely to increase during the months following the experiment, as it was observed by Cruz (et al., 2016).

Manual cleaning was shown as an effective way to reduce mortality of the barnacles of the platform (Cruz et al., 2016). According to the Collective Research on Aquaculture Biofouling, (CRAB, 2006), manual cleaning was an effective method (only 10% mortality estimated), but is time consuming and expensive to apply (around 30% of the production costs). In the present experiment, the effectivity of manual cleaning has not been assessed (till 90% of mortality in the second period). In addition to the time spent per PVC board (more than 15 minutes), this technique should be revised to be suitable for the culture of P. *pollicipes* and meet the results obtained by the CRAB (2006). Increasing the frequency of treatment application could probably be a good way to limit biofouling development. Waterjet cleaning, was presented as having almost no negative impact on the survival of the cultured stocks (CRAB, 2006). This hypothesis was not supported, as <50% of mean survival was obtained for *P.pollicipes* in both trials. However, waterjet cleaning is presented as well as an effective method of antifouling at an early stage (CRAB, 2006). Hence, waterjet cleaning is shown as very bad after mussel spatfall, the latter ones and hard foulers, being removed with difficulty. In the current experiment, an important part of the very small acorn barnacles and mussels were removed from the PVC boards. This observation suggests that hard foulers can be expelled when at early development. This strengthens the need of increasing the frequency of application of this treatment as it could allow to reduce their settlement at early stages of growth.

The antifouling paint has been applied only in the last trial, and therefore, its efficacy could not really be tested. Indeed, the barticles were transplanted in the same time than in the other treatments and during the first trial, they were left intact. The coating efficiency on survival might have been therefore biased. Therefore, further trials should be realized, from the beginning of the experiment. Water-based silicon coatings, such as the one used, were presented as effective in CRAB (2006), but needed further development to last longer and thus improving the durability, to be economically acceptable at industrial scale. A study using the same antifouling coat (Netminder®), was done for the scallop's culture *Argopecten irradians* (Tettelbach et al., 2014). A higher mortality was observed on the treated nets than on the non-treated (likely due to higher levels of epibionts on the shells). However, an important reduction of biofouling organisms during the grow-out period was highlighted. Furthermore, higher reproductive and overall conditions of the scallops, were observed. This supports the idea that, combined with another treatment realized on the barnacles themselves (manual or waterjet cleaning), the antifouling paint could improve the development conditions on the platform, for P. *pollicipes*.

In CRAB (2006), no species cultured, presented equivalent morphologic characteristics like P. *pollicipes*. The cleaning techniques should be adapted to the organism cultured (Dürr et Watson, 2009) and therefore combining several methods, or increasing the frequency of the treatment (e.g. weekly or twice a month instead of monthly), could improve stalked barnacles' survival. Hence, further studies are required to improve the efficiency of the antifouling techniques employed in this experiment, in survival matters.

No evidences were found that the antifouling treatments had an impact on RC for the first period of study. Statistical differences were obtained in the second one, but no clear pattern was highlighted. However, waterjet cleaning was revealed as having a positive impact with manual cleaning, as the RC of the barnacles transplanted to the platform was observed as being bigger than the controls. The high number of RC measured for the waterjet cleaning, gave more consistency to the results obtained. Waterjet and manual cleaning were shown as having a lower impact on the cultured species (CRAB, 2006) on the survival. The similar outcome on their growth, is to be expected and the results obtained in this experiment confirm that assumption. Furthermore, some fouling organisms such as sponges were shown as having a negative impact on growth for bivalve species (Dürr et Watson, 2009). Indeed, they can affect the shell valves movement and thus reduce the feeding efficiency. The same statement was made while cleaning P. *pollicipes,* whose capitulum plates were frequently entangled with algae or sponges. Waterjet and manual cleaning were the only treatment allowing to clean the barnacles themselves. Thus, those treatments seem to allow a better growth for the individuals than the antifouling paint or no treatment at all. The barnacles whose PVC boards were treated with antifouling coat, did not reveal differences between RC with the individuals from the control. The same observation was realized for scallop culture (Tettelbach et al., 2014) with Netminder®.

The antifouling techniques used during this experiment revealed a certain inefficiency to lower the mortality of P. *pollicipes*. Therefore, new approaches should be tested, to improve culture conditions.

Fouling intensity was shown as decreasing with depth (Dürr et Watson, 2009), and lowering the gear could allow to achieve reduction of levels of fouling. A study carried out in Canada on longlines of *Mytilus* edulis (Bourque et Myrand, 2006) revealed that, lowering the gear before a secondary spatfall, allowed to avoid settlement of new smaller recruits. The latter ones controlled by predators. The growth of P. pollicipes could be improved as well, as in the case of *Austromegabalanus psittacus*, where the collectors were lowered between 4 and 6m of depth (Lopez et al., 2008). According to this study, a drop in energetic costs (induced by lower temperatures and smaller quantity of light hours) could imply a higher energy available for growth. A similar pattern could be observed for *P.pollicipes* when lowering the gear.

Therefore, several depths shall be tested in the case of P. *pollicipes* to assess the efficiency of the method.

Promising results have been obtained, when using bio-control to reduce fouling intensity. The use of Littorina *littorea* showed positive results in oyster cultures, by controlling the algal fouling. An improvement of 30% on the oysters' growth rate has been noticed (CRAB, 2006). Other species, like *Nucella* lapillus were successfully used to decrease the presence of mussels (CRAB, 2006).

Another study on the effects of predators on biofouling communities (Nydam et Stachowicz, 2007) found a positive outcome, when the chiton *Mopalia muscosa* and the limpet *Lottia limatula* were both incorporated to PVC plates. The synergy developed by those two grazers allowed to clean the fouled area four times more efficiently than with the limpets only.

The non-sedentary species were monitored in this experiment and most of the organisms were amphipods. Many species of amphipods are grazers, and they might have a positive impact for the barnacles, preventing the development of periphyton and epiphytes, as it was already observed for *Posidonia sinuosa* (Jernakoff et Nielsen, 1997).

Despite all the promising results from bio-controllers and their environmental-friendly approach, their culture/fishery must be sustainable (Dürr et Watson, 2009) and no large-scale trials have been realized so far.

Prevention is better than cure and so goes for aquaculture. Preventing the heavy spatfalls of biofoulers (Dürr et Watson, 2009) should allow to reduce their impact; namely by lowering the depth of gear or alternating offshore/inland periods. This strategy could represent an effective and low-cost way to optimize P. *pollicipes* aquaculture.

Conclusion

The size of the barticles was shown as not being a determinant variable for recruitment intensity. The recruitment intensity was indeed not dependent of the surface of settlement. Further study might be required to find a most suitable recruitment window, to get closer to the maximal carrying capacity of the barticles. The smaller sizes of barticles allowed to recruit bigger individuals. However, this fact was not observed after a period of growth. The size of the barnacles and their growth rates was not dependent on the size of the barticle. Furthermore, the results obtained for survival on the smaller barticles, coupled with field observations, suggest that the barticles of 6 mm should not be used for aquaculture purposes.

The growth rate of barnacles was enhanced when they were transferred to the offshore platform, in comparison with the ones left *in situ*. This tendency was confirmed when comparing barnacles of same age, transferred at different periods to the offshore system. Bigger barnacles were collected when they were transferred earlier. The grow-out platform, was proven as being an effective system to enhance growth and represents an undeniable advantage for aquaculture perspectives.

Despite improved growth rates, high mortalities were observed for the barnacles transferred to the platform. further studies would be required, to reduce the mortality of the transferred individuals. Indeed, the antifouling treatments used in this study, were shown as not effective enough to improve survival and were probably applied in the wrong timing. The peaks of biofouling competitors, monitored in this study, were recorded in fact at the end of the experiment and a probable treatment-induced mortality could have been avoided, delaying the treatment till the critical period.

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Annexes

Table 1. PERMANOVA results of the effect of the size of the barticle (fixed factor with 4 levels) on the recruitment intensity. PERMDISP test P>0.05.

Source	df	SS	MS	Pseudo-F	P
Size of barticle	3	195.34	65.115	0.56062	**0.655**
RES	28	3252.1	116.15		

Table 2. PERMANOVA results of the effect of the size of the barticle (fixed factor with 4 levels) on the RC of the individuals collected. PERMDISP test P>0.05.

PERMANOVA						**Pair-wise test**
Source	df	SS	MS	Pseudo-F	P	Platform ≠ Cabo de Sines
Size of barticle	3	1,9937	0,66456	6,5931	**0,0002**	
RES	248	24,998	0,1008			

Table 3. PERMANOVA results (two-way) of the mean %survival of the marked barnacles with 2 fixed factors: size of the barticle (3 levels) and location (2 levels). The original data was transformed with a fourth root to homogenize the dispersion. PERMDISP test P>0.05.

Source	df	SS	MS	Pseudo-F	P
Size of barticle	2	0.24074	0.12037	0.51039	**0.6164**
Loction	1	0.99247	0.99247	4.2082	**0.0554**
Size barticle x Location	2	0.22803	0.11402	0.48344	**0.636**
RES	18	4.2451	0.23584		

Table 4. PERMANOVA results of the mean % survival of the barnacles from the platform, with 1 fixed factor, size of barticle (3 levels). PERMDISP test P>0.05.

Source	df	SS	MS	Pseudo-F	P
Size of barticle	2	2504.7	1252.4	2.5023	**0.0851**
RES	41	20520	500.48		

Table 5. PERMANOVA (two-way) results of the RC of the marked barnacles with 2 fixed factors, size of barticle (3 levels) and location (2 levels). PERMDISP test P>0.05.

PERMANOVA						**Pari-wise test**
Source	df	SS	MS	Pseudo-F	P	Platform ≠ Cabo de Sines
Size of barticle	1	0,60932	0,60932	0,19397	**0,6942**	
Location	1	15,803	15,803	5,0305	**0,0172**	
Size barticle x Location	1	1,3258	1,3258	0,42205	**0,5672**	
RES	70	219,89	3,1413			

Table 6. PERMANOVA results of the mean RC of the barnacles from the platform, with 1 fixed factor size of barticle (3 levels). PERMDISP test P>0.05.

Source	Df	SS	MS	Pseudo-F	P
Size of barticle	2	13.589	6.7947	1.797	**0.162**
RES	83	313.83	3.781		

Table 7. PERMANOVA results (two-way) of the mean dRC30 with 2 fixed factors: size of barticle (3 levels) and location (2 levels) Untransformed data, no transformation has homogenized dispersion. PERMDISP test P<0.05.

PERMANOVA						**Pair-wise test**
Source	df	SS	MS	Pseudo-F	P	Platform ≠ Cabo de Sines
Size of barticle	2	1,66E-02	8,31E-03	0,16879	**0,8388**	
Location	1	0,33875	0,33875	6,8839	**0,0149**	
Size barticle x Location	2	3,54E-02	1,77E-02	0,35954	**0,6976**	
RES	78	3,8383	4,92E-02			

Table 8. PERMANOVA results of the mean %survival of the barnacles with 1 fixed factor, date of transfer (2 levels). PERMDISP test P>0.05.

Source	df	SS	MS	Pseudo-F	P
Date of transfer	1	2749.3	2749.3	2.0326	**0.1873**
RES	11	14878	1352.6		

Table 9. PERMANOVA results for the mean RC of the barnacles transferred with 1 fixed factor, date of transfer (2 levels). The original data was transformed with a fourth root (previous PERMDISP value P<0.05) to homogenize the dispersion. PERMDISP test P>0.05.

PERMANOVA						**Pair-wise test**
Source	df	SS	MS	Pseudo-F	P	October ≠ January
Date of transfer	1	0,45429	0,45429	17,68	**0,0003**	
RES	45	1,1563	2,57E-02			

Table 10. PERMANOVA results of the first period for the mean %survival of the barnacles with 1 fixed factor, antifouling treatment (4 levels). PERMDISP test P>0.05.

Source	df	SS	MS	Pseudo-F	P
Antifouling treatment	3	4890.6	1630.2	0.45539	**0.8056**
RES	28	1.00E+05	3579.8		

Table 11. PERMANOVA results of the second period for the mean %survival of the barnacles with 1 fixed factor, antifouling treatment (5 levels). PERMDISP test P>0.05.

Source	df	SS	MS	Pseudo-F	P
Antifouling treatment	4	21121	5280.1	1.9637	**0.115**
RES	33	88731	2688.8		

Table 12. PERMANOVA results of the first period for the mean RC of the barnacles with 1 fixed factor, antifouling treatment (4 levels). PERMDISP test P>0.05).

Source	df	SS	MS	Pseudo-F	P
Antifouling treatment	4	36.861	9.2153	2.022	**0.0965**
RES	186	847.72	4.5576		

Table 13. PERMANOVA results of the second period for the mean RC of the barnacles with 1 fixed factor, antifouling treatment (5 levels). PERMDISP test P>0.05).

PERMANOVA						**Pair-wise test**
Source	df	SS	MS	Pseudo-F	P	Antifouling paint = Control Cabo = Platform control =
Antifouling treatment	4	302,48	75,62	11,395	**0,0001**	Manual cleaning = Waterjet
RES	166	1101,6	6,6361			

Table 14. PERMANOVA results for the fresh weight of the monthly plates (Euclidian distance matrix) with one fixed factor, months (6 levels).

PERMANOVA						**Monte-Carlo test**
Source	df	SS	MS	Pseudo-F	P	Febr = March = April = May ≠ June ≠ July
Month	5	11868	2373,6	18,594	**0,0009**	
RES	6	765,92	127,65			

Table 15. PERMANOVA results for the mean %weight of the 3 main taxa for monthly plates (Bray-Curtis distance matrix) with one fixed factor, months (6 levels).

Source	df	SS	MS	Pseudo-F	P
Months	5	17.826	3.5651	0.8971	**0.6369**
RES	6	23.844	3.974		

Table 16. PERMANOVA results for the %cover per taxon in the monthly plates (Bray-Curtis distance matrix), with 1 fixed factor, months (6 levels).

Source	df	SS	MS	Pseudo-F	P
Months	5	5563.8	1112.8	2.8459	**0.0566**
RES	6	2346	391		

Table 17. PERMANOVA results of the abundance of amphipods (Bray-Curtis distance matrix) on the monthly plates, with 1 fixed factor, months (6 levels).

PERMANOVA						**Monte-Carlo test**
Source	df	SS	MS	Pseudo-F	P	Febr = March = April = May = June = July
Months	5	15294	3058,8	6,9653	**0,0097**	
RES	6	2634,9	439,15			

Table 18. PERMANOVA results of the abundance of copepods on the monthly plates (Bray-Curtis distance matrix) with 1 fixed factor, months (6 levels).

Source	df	SS	MS	Pseudo-F	P
Months	4	6564.2	1641	1.4804	**0.3191**
RES	5	5542.6	1108.5		

Table 19. PERMANOVA results for the abundance of polychaetes on the monthly plates (Bray-Curtis distance matrix) with 1 fixed factor, months (6 levels).

Source	df	SS	MS	Pseudo-F	P
Months	4	12193	3048.4	1.8375	**0.0812**
RES	5	8295	1659		

Table 20. PERMANOVA results for the mean fresh weight of the integrated monthly plates (Euclidian distance matrix) with 1 fixed factor, integrated months (6 levels).

PERMANOVA						**Monte-Carlo test**
Source	df	SS	MS	Pseudo-F	P	Febr-Febr = Febr-March ≠
Integrated months	5	20354	4070,8	22,291	**0,0004**	Febr-Apr = Febr-May = Febr-June ≠
RES	6	1095,7	182,62			Febr-July

Table 21. PERMANOVA results of the %fresh weight composition of the 3 main taxa (Bray-Curtis distance matrix) with 1 fixed factor, integrated months (6 levels).

PERMANOVA						**Monte-Carlo test**
Source	df	SS	MS	Pseudo-F	P	Febr-Febr = Febr-March =
Integrated months	5	6960,9	1392,2	10,181	**0,0126**	Febr-April = Febr-May = Febr-June =
RES	6	820,47	136,74			Febr-July

Table 22. PERMANOVA results of the %cover per taxon (Bray-Curtis distance matrix) with 1 fixed factor, integrated months (6 levels).

PERMANOVA						**Monte-Carlo test**
Source	df	SS	MS	Pseudo-F	P	Febr-Febr = Febr-March =
Integrated months	5	18632	3726,5	6,4188	**0,0015**	Febr-April = Febr-May = Febr-June = Febr-July
RES	6	3483,3	580,56			

Table 23. PERMANOVA results of the abundance of amphipods (Bray-Curtis distance matrix) on the integrated monthly plates, with 1 fixed factor, integrated months (6 levels).

PERMANOVA						**Monte-Carlo test**
Source	df	SS	MS	Pseudo-F	P	Febr-Febr = Febr-March =
Integrated months	5	13005	2601,1	7,1645	**0,0202**	Febr-April = Febr-May =
RES	6	2178,3	363,05			Febr-June = Febr-July

Table 24. PERMANOVA results of the abundance of copepods (Bray-Curtis distance matrix) on the integrated monthly plates, with 1 fixed factor, months (6 levels).

Source	df	SS	MS	Pseudo-F	P
Integrated months	5	5080.1	1016	1.3224	**0.2321**
RES	6	4609.7	768.29		

Table 25. PERMANOVA results for the abundance of polychaetes on the integrated monthly plates (Bray-Curtis distance matrix) with 1 fixed factor, integrated months (6 levels).

Source	df	SS	MS	Pseudo-F	P
Integrated months	5	10964	2192.8	2.7598	**0.0715**
RES	6	4767.3	794.56		