



UNIVERSIDADE DE ÉVORA
ESCOLA DE CIÊNCIAS E TECNOLOGIA
DEPARTAMENTO DE BIOLOGIA



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INSTITUTO SUPERIOR DE AGRONOMIA

Recruitment of the stalked barnacle
Pollicipes pollicipes on an artificial
substratum ("barticle") and transfer to an
extensive system of production: success
techniques and pitfalls

Joana Isabel Rodrigues Nascimento Fernandes

Orientação: Professora Doutora Teresa Cruz
Co-orientação: Doutor David Jacinto
Co-orientação: Mestre Maria Inês Seabra

Mestrado em Gestão e Conservação dos Recursos Naturais

Dissertação

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Recruitment of the stalked barnacle *Pollicipes pollicipes* on an artificial substratum ("barticle") and transfer to an extensive system of production: success techniques and pitfalls

Abstract

Aquaculture potential of the stalked barnacle *Pollicipes pollicipes* was investigated. Artificial collectors (barticles) of larvae and juveniles were deployed at the Cape of Sines and later transferred to a floating platform in the Port of Sines for juvenile growth.

Survival and growth data and/or practical issues, justified the choice of the mid shore as the most suitable barticles's deployment level in the field, and of plates within individual cages as the best option of the analysed growing conditions in the platform. Survival of barnacles transferred to the platform was initially high but decreased to zero over time due to intense biofouling. During winter, growth in the platform was higher than in the Cape of Sines. Barnacles' survival and growth rate during winter in the platform are promising results, although biofouling, namely in spring and summer, is still a major problem to address.

Recrutamento do percebe *Pollicipes pollicipes* em substrato artificial ("barticle") e transferência para um sistema de produção extensivo: sucessos e insucessos.

Resumo

Este estudo analisou o potencial da aquicultura do percebe *Pollicipes pollicipes*. Usaram-se coletores artificiais de larvas ("barticles") colocados no Cabo de Sines (SW Portugal) que foram posteriormente transferidos para uma plataforma flutuante no Porto de Sines para crescimento dos juvenis. Com base nos dados de sobrevivência e crescimento obtidos e/ou aspetos práticos, o nível intertidal médio foi considerado como o mais adequado para a colocação dos "barticles", e as placas em redes individuais como a melhor opção para o crescimento dos percebes na plataforma. A sobrevivência dos percebes transferidos foi alta inicialmente, mas decresceu ao longo do tempo para zero, devido a "biofouling" intenso. Durante o inverno, o crescimento foi superior na plataforma relativamente ao Cabo de Sines. A sobrevivência e a taxa de crescimento dos percebes na plataforma durante o inverno constituem resultados promissores, embora o "biofouling", nomeadamente na primavera/verão, seja ainda um problema que deverá ser mitigado futuramente.

Introduction

Stalked barnacles belonging to the genus *Pollicipes* are crustacean cirripedes that are found mainly on intertidal rocky shores along the eastern Atlantic coast (*P. pollicipes*, from France to Senegal and *P. caboverdensis* in the Atlantic Islands of Cape Verde) and along the eastern Pacific coast (*P. polymerus* and *P. elegans*) (Barnes, 1996; Fernandes *et al.*, 2010).

All *Pollicipes* species inhabit wave-exposed rocky shores and are edible, being exploited as economically important coastal resources (López *et al.*, 2012; Cruz *et al.*, 2015a). Particularly, *P. pollicipes* can be considered the most commercially valuable intertidal resource of the Iberian Peninsula (*e.g.* Molares & Freire, 2003; Cruz *et al.*, 2015b).

P. pollicipes presents several characteristics that qualify it as a marine species with high interest for aquaculture: it is highly appreciated and considered a delicacy in Portugal and Spain (Molares & Freire, 2003; Sousa *et al.*, 2013); it has a high commercial interest and its price can reach up to 200 euros per kilogram in Spanish restaurants; its harvesting is a very dangerous activity and dependent on sea conditions; in Portugal, exploitation has been highly intense, the state of this resource has a negative tendency and its management is considered weak (Cruz *et al.*, 2015b); as a filter-feeder, is a low trophic level species, with consequently low energy requirements; it has potential to be farmed in an environmentally sustainable way, in an extensive system of production.

Besides these characteristics, aquaculture of *P. pollicipes* could diminish harvesting pressure on natural populations and ameliorate the management of this resource. However, there aren't any known cases of success of *P. pollicipes* aquaculture. The only known attempt to produce *P. pollicipes* in the wild was in Santander region (Spain), where pieces of rock with barnacles were transplanted from the exposed intertidal areas and were suspended in the water column for two months (Goldberg, 1984). Although growth rates were high in this system, this procedure would not be sustainable as an aquaculture technique, since it requires the exploitation of barnacles with commercial interest, possibly leading to the destruction of natural populations and its habitats.

P. pollicipes adults are sessile filter-feeding organisms that form dense clusters on rocky shores. They are simultaneous hermaphrodites reproducing through cross-fertilization (Cruz & Hawkins, 1998). Embryos are developed inside the barnacle, in the mantle cavity, until hatching of larvae. This species undergoes a pelagic larval phase comprised of six naupliar stages and one cyprid stage that settles and metamorphoses into a juvenile individual (Barnes, 1996). Although the

cyprids are able to settle directly on the rock or on other organisms, settlement and recruitment of this species is very high on conspecifics (Macho, 2006; Cruz *et al.*, 2010) and in the southwest coast of Portugal it happens mainly during summer and autumn (Cruz *et al.*, 2010). Maturity is reached at a maximum rostro-carinal distance (RC length) superior to 12.5 mm (Cruz & Hawkins, 1998). In its natural habitat, *P. pollicipes* can take one to two years for individuals to grow and reach commercial size (≥ 20 mm RC; Cruz *et al.*, 2010), although growth can be highly variable (Cruz *et al.*, 2010; Jacinto *et al.*, 2015).

The cultivation of *P. pollicipes* in laboratorial conditions has been the subject of several studies regarding larval development (Molares *et al.*, 1994; Kugele & Yule, 1996; Franco, 2014; Franco *et al.*, 2015; Franco *et al.*, 2016a; Franco *et al.*, 2016b), or juvenile and adult growth and survival (Cribeiro, 2007; Franco, 2014; Franco *et al.*, 2015). Several important results were achieved in these studies, such as reproduction and hatching of larvae, larval development from *nauplius I* to cyprid stage or the optimization of larval and adult culture conditions. However, in spite of research efforts, the conditions necessary to promote settlement of cyprids in culture are still poorly understood and this step of the life cycle represents the main bottleneck to the viability of *P. pollicipes* cultivation in captivity. Very low settlement rates of cyprid larvae were found in these studies, even on conspecifics (Kugele & Yule, 1996; Franco, 2014; Franco *et al.*, 2016).

Experimental studies of aquaculture with other cirripede crustaceans regard acorn barnacles of the species *Austromegabalanus psittacus* (“picoroco”) in the Chilean coast (López *et al.*, 2012). This was done on a semi-industrial scale, with larvae collected from the wild with artificial structures and moved to a grow-out system where juveniles within these structures are suspended in the water column (longlines). According to these authors, the productivity of these farming systems was very high in relation to artisanal fishery of this resource, but there was spatial and temporal variability on the production and, consequently, a certain degree of unpredictability of this production. There are also experimental studies to cultivate the Azorean barnacle (*Megabalanus azoricus*) in a similar way, and results revealed a potential of this species for aquaculture (Pham *et al.*, 2011).

Regarding *P. pollicipes*, several authors have tested a wide array of artificial substrates for settlement and recruitment of this species in the field (Coelho, 1991; Cruz, 2000; Franco, 2014; Jacinto, 2016) or in laboratorial conditions (Kugele & Yule, 1996; Franco, 2014). In those studies, there was no success in settlement or low levels of settlement were found (Kugele & Yule, 1996; Jacinto, 2016).

Recently, a new artificial substrate developed by University of Évora (2017, patent pending), named “barticle”, has proved to be efficient for larval attachment, metamorphose and growth of juveniles of *P. pollicipes* in the field (Darras, 2017; Mateus, 2017). The subsequent transfer of

this “barticle” to an extensive cultivation system, is a promising method for the aquaculture of this species.

The Sines region (SW Portugal) presents several features attractive for the implementation of *P. pollicipes* aquaculture: the Cape of Sines is a rocky coastal area of high hydrodynamism where this species is abundant and highly exploited by professional and recreational stalked barnacle harvesters (Sousa *et al.*, 2013); the bay of the Port of Sines is a sheltered area adjacent to the Cape of Sines, and where it is possible to install artificial farming systems under safety conditions.

According to this, the present work is based on the use of “barticles” deployed in the Cape of Sines to collect larvae and juveniles of *P. pollicipes*, and later collected and transferred to support structures placed in a floating platform located in the bay of the Port of Sines, where the barnacles were allowed to grow.

This study had the following objectives:

- 1) To define the most suitable barticle’s deployment level in the field and growing conditions in the floating platform (support structure and anti-predation system);
- 2) To study the temporal variation of survival, size and growth of barnacles transferred to the culture system in relation to different transfer periods.

Additionally the weight of the fouling assemblages associated with “barticles” collected from the platform was quantified.

Methods

Study sites and barticle deployment

This study was carried out in the Cape of Sines (37°57'46"N; 8°53'10"W), a very exposed rocky shore where *P. pollicipes* occurs in abundance along the shallow subtidal and intertidal levels, and on a floating platform located in a sheltered area in the bay of the Port of Sines (37°56'17"N; 8°51'48"W; Figure 1). The floating platform consists of a series of high-density polyethylene (HDPE) pipes assembled together to form two squares of 5x5 m separated by a 1 m wide walkway (total dimensions: 12.5x6 m; Figure 2). The platform was anchored to the jetty nearby and to the bottom at a depth of approximately 25m. In each square, 6 parallel nylon ropes (\approx 70 cm apart), each with 5 superficial buoys (\approx 80 cm apart) were deployed along the surface and fastened to the HDPE pipes for easy access and retrieval of suspended structures. One of the squares had a suspended global cage made with a 1 cm mesh size nylon net (total dimensions: 5x5x2 m) to avoid predators.

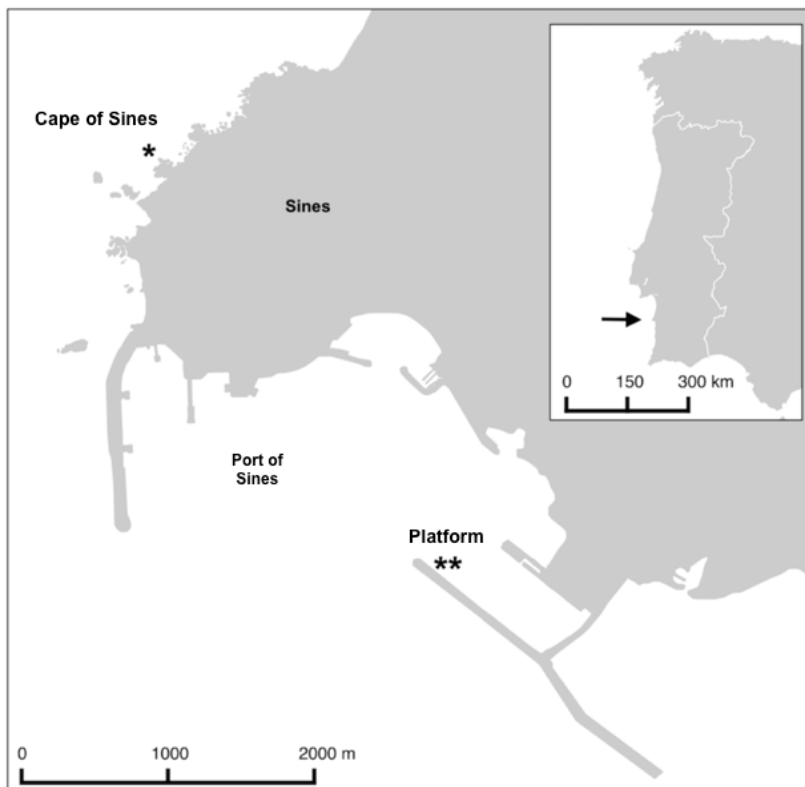


Figure 1 - Location of barticle deployment site at the Cape of Sines (*) and location of the floating platform at the bay of the Port of Sines (**), and its relative position in relation to the coast of mainland Portugal.

The present study involved the deployment of barticles in the Cape of Sines for *P. pollicipes* larval attachment and metamorphosis into juveniles and its subsequent transfer to the platform for barnacle growth.

Barticles are artificial substrates formed by a pvc wall plug (8mm diameter and 38 mm length) with longitudinal grooves and a partially inserted stainless steel screw (4 mm diameter and 40 mm length). The lower end of the plug is inserted and fixed to the rock, while the screw facilitates subsequent barticle removal from the rock (University of Évora, 2017, patent pending).

A total of 832 barticles distributed by 52 plots of 16 barticles each, regularly spaced in a quadrat of approximately 100 cm², were deployed in the intertidal zone of the Cape of Sines with the help of a cordless drill and a hammer. Barticles were deployed on two vertical levels in relation to the intertidal distribution of *P. pollicipes*: mid shore, that corresponds to the middle/upper level of *P. pollicipes* intertidal distribution (~1.5 m to 3 m above MLWS) and low shore that corresponds to the lower intertidal level of *P. pollicipes* distribution (~ MLWS to + 1.5 m) (Sousa *et al.*, 2013). Deployment of barticles was always dependent on sea conditions, but low shore level was only accessible in spring tides and for a shorter period of time compared with mid shore level. Consequently, a higher number of barticles was deployed in the mid shore (n=32 for low shore; n=800 for mid shore).

Barticles were deployed during low tide, in June and July of 2014, before the starting of *P. pollicipes* recruitment season in the southwest coast of Portugal (summer/fall, Cruz *et al.*, 2010).

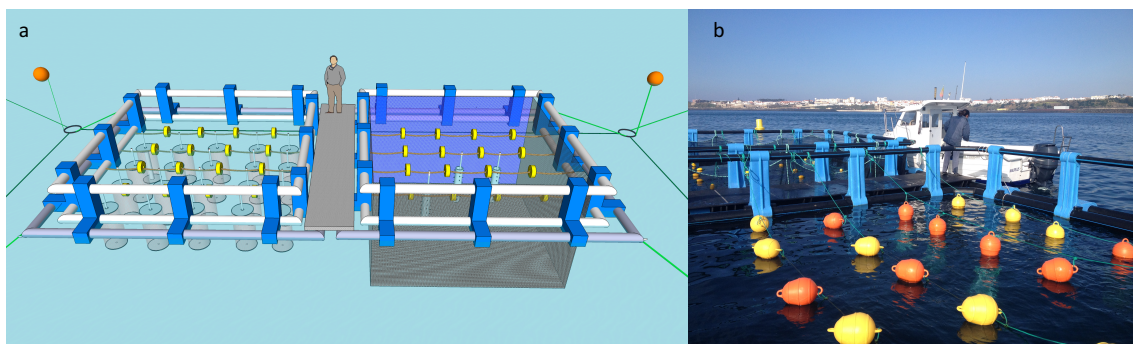


Figure 2 – a) platform scheme with individual cages (left square) and global cage (right square); b) general view of the platform.

Barticle's deployment level and growing conditions

In order to define the most suitable barticle's deployment level in the field, on the 26th, 29th and 30th December 2014, 6th January and 10th, 11th and 12th February 2015, a total of 128 barticles

(32 from low shore and 96 from mid shore) were collected from the Cape of Sines using a hammer claw and taken to the laboratory in a plastic bucket. The initial number of *P. pollicipes* individuals per barticle (Pp_I) was recorded under a dissecting microscope. This procedure was done with a minimum manipulation of the barnacles attached to the barticles, which could have led to an underestimation of this variable. Barticles with no barnacles attached (<10%) were discarded.

Growing conditions in the platform regarded the type of support structures for barticles and an anti-predation system.

To define the most suitable support structure for barticles in the platform, barticles from both vertical levels (mid and low shore) were then fixed to two types of support structures: tubes, consisting in PVC pipes with a diameter of 10 cm, length of 95 cm and thickness of 0.5 cm; plates, consisting in PVC boards with a width of 20 cm, length of 95 cm and thickness of 1 cm. Six replicate tubes and three replicate plates were used in total.

Each structure had 36 insertion points (7 mm holes made with a drill) where barticles were randomly assigned and inserted with the help of a hammer. The support structures (tubes and plates) were then taken to the platform and hung from the superficial buoys in total immersion conditions.

In order to ensure protection against predation of barnacles in the platform, two anti-predation systems were considered for placing the support structures: global cage, as previously described; individual cage, *i.e.* an individualized cage for each plate or tube, placed on the other square of the platform (Figure 2a). The individual cage consisted of an iron structure (20x20x100 cm) wrapped in a nylon net with 1 cm mesh size.

All the procedures, starting from collection in the Cape of Sines until placement in the platform, were made within intervals from 2 to 4 hours after collection of the barticles in the field, in order to minimize the loss or damage of animals and the time required for their transfer.

In order to measure monthly growth of *P. pollicipes* fixed to barticles from different deployment levels and in different growing conditions, a sample of barticles collected from mid (n= 36) and low (n= 13) shore levels in the Cape of Sines was taken to the laboratory and submerged in a calcein solution for barnacle marking (see Jacinto *et al.*, 2015 for method details), randomly assigned to tubes or plates and to the global or individual cages and taken to the platform the following day.

All barnacles were left to grow for three to four months in the platform without further manipulation.

On the 29th and 30th April 2015, all barticles were collected from the platform and were labeled and frozen until further processing in the laboratory.

Following sample unfreezing in the laboratory, *P. pollicipes* barnacles attached to each barticle were detached and individualized. The final number of individuals of *P. pollicipes* per barticle (Pp_F) was recorded and RC length of each individual was measured under a dissecting microscope or with calipers depending on individual size (precision of 0.01 mm and 0.1 mm, respectively).

Barnacles marked with calcein, after having been counted (Pp_F) and measured (RC length), were immersed in commercial bleach, their capitular plates were detached from the rest of the body and the initial (at the time of marking) and final length of scutum plate was measured under an epifluorescent dissecting microscope (Leica M165FC with a UV light source and GFP3 filter) equipped with a digital camera (Leica DF 295), and monthly growth rate (Δ RC30) was estimated according to the method described by Jacinto *et al.* (2015).

The effects of barticle's deployment level (mid and low shore), support structures (tubes and plates) and anti-predation system (global cage and individual cage) on the response variables Pp_I, Pp_F, RC length and Δ RC30, were analyzed by one-way ANOVA to each factor and each response variable. Homogeneity of variance was assessed using Levene's test and data were transformed when appropriate. The software SPSS was used for all analyses.

Survival and size of barnacles transferred to the platform

Transfer of the barticles from the Cape of Sines to the platform was carried out in seven transfer periods (each period comprising one to six dates):

- 1) T1/Dec-Jan (18th, 26th, 27th, 29th, 30th December 2014 and 6th January 2015);
- 2) T2/February (9th-12th February 2015);
- 3) T3/3April (3rd April 2015);
- 4) T4/30April (30th April 2015);
- 5) T5/June (9th Jun 2015);
- 6) T6/August (10th August 2015);
- 7) T7/September (28th-29th September 2015).

On each transfer period, barticles (n= 16 in T7/September to n= 190 in T1/Dec-Jan) were collected in the Cape of Sines and taken to the laboratory.

The initial number of *P. pollicipes* individuals per barticle (Pp_I) was measured as described above.

Additionally, in order to have an independent sample and a more accurate estimation of the number of *P. pollicipes* individuals per barticle and their respective size (RC length) at "time 0"

(t0), a sample of barticles (n= 8 to 25) for each transfer period was frozen until further analysis. RC length data at time 0, for T2/February and T3/3April transfer periods, was missing due to accidental loss of samples.

Barticles were then randomly assigned to twenty support structures and taken to the platform where barnacles were allowed to grow from four to twelve months, without further manipulation until time of sampling.

Five monitoring dates were considered for sampling of barticles in the platform and in the Cape of Sines (control site):

- 1) M1/April (29th and 30th April 2015);
- 2) M2/June (9th and 10th June 2015);
- 3) M3/August (10th and 11th August 2015);
- 4) M4/October (2nd and 8th October 2015);
- 5) M5/January (14th and 15th January 2016).

On each monitoring date, barticles from the different transfer periods were collected from the platform (n= 63 to 152) and an additional sample of barticles (n= 8 to 25) was collected from the Cape of Sines for control purposes. All barticles were labeled and frozen until further processing in the laboratory.

Following sample unfreezing in the laboratory, *P. pollicipes* barnacles attached to each barticle were detached and individualized. The variables Pp_F and RC length of each individual were measured.

The fouling communities (mainly mussels and acorn barnacles) associated with each barticle collected from the platform were weighted, starting on M3/August monitoring date onwards. Differences in Pp_I among monitoring dates, for each transfer period were tested through one-way ANOVA. If no significant differences were found in Pp_I, we considered Pp_F as a proxy of survival.

Differences in Pp_F and in RC length among monitoring dates including at time 0 (t0 – independent sample at time of transfer), were tested through one-way ANOVA for each transfer period. Homogeneity of variance was assessed using Levene's test and data were transformed when appropriate. Post-hoc pairwise comparisons were performed with Student-Newman-Keuls (SNK) tests and the software SPSS was used for all analyses.

Monthly growth of barnacles transferred to the platform

In order to measure monthly growth rates of *P. pollicipes* transferred to the platform and in the Cape of Sines (control site), a total of 67 barticles were collected from the Cape of Sines, then

taken to the laboratory and submerged in a calcein solution for barnacle marking (see Jacinto et al., 2015 for method details), and taken to the platform in two support structures or to the Cape of Sines the following day. Two marking periods were considered: winter, with barnacles marked in December 2014 / January 2015 and collected in March / April 2015 (n= 49), and with a growth period in the field ranging from 82 to 124 days; summer, with barnacles marked in June 2015 and collected in September 2015 (n= 18), and with a growth period in the field ranging from 89 to 90 days.

In the laboratory, *P.pollicipes* barnacles attached to each particle were detached and individualized, and monthly growth rate ($\Delta RC30$) per individual was estimated accordingly to the method previously described.

Variability of $\Delta RC30$ on the two different seasons and on both locations was analysed by permutational multivariate analysis of variance, PERMANOVA (Anderson, 2001) due to unbalanced number of samples in each treatment. Two factors were considered: season (fixed factor with two levels: winter and summer) and location (fixed factor with two levels: Cape of Sines and platform). Homogeneity of univariate dispersion based on Euclidean distance was tested using the PERMDISP routine (Anderson 2006). The software PRIMER 6 and PERMANOVA+ (www.primers-e.com; Anderson et al. 2008) was used to perform these procedures.

Results

Barticle's deployment level and growing conditions

The mean variation of initial (Pp_I) and final (Pp_F) number of *P. pollicipes* individuals per barticle, and of RC length and monthly growth rate (Δ RC30) per individual, regarding different barticle's deployment level (mid and low shore), support structures (tubes and plates) and anti-predation system (global cage and individual cage) is shown in Figure 3.

There was a significant effect of barticle's deployment level on the monthly growth rate (Δ RC30) of *P. pollicipes* ($F_{1, 141} = 14.09$; $p = 0.000$). Δ RC30 in mid shore barnacles (mean \pm SE = 0.9 ± 0.04 mm) was significantly larger than in low shore barnacles (mean \pm SE = 0.7 ± 0.06 mm). There were no significant differences between mid and low shore barticles for the variables Pp_I ($F_{1, 29} = 0.33$; $p = 0.572$), Pp_F ($F_{1, 29} = 0.54$; $p = 0.467$) and RC length ($F_{1, 851} = 3.72$; $p = 0.054$; square root transformed data).

There was a significant effect of the support structures on RC length ($F_{1, 454} = 5.07$; $p = 0.025$) and on Δ RC30 ($F_{1, 383} = 51.93$; $p = 0.000$). RC length in tubes (mean \pm SE = 6.0 ± 0.16 mm) and Δ RC30 in tubes (mean \pm SE = 1.5 ± 0.03 mm) was significantly larger than RC length in plates (mean \pm SE = 5.6 ± 0.18 mm) and Δ RC30 in plates (mean \pm SE = 1.1 ± 0.05 mm). There were no significant differences between tubes and plates for the variables Pp_I ($F_{1, 30} = 0.99$; $p = 0.327$) and Pp_F ($F_{1, 30} = 0.144$; $p = 0.707$).

Regarding the anti-predation system, there were significant differences between individual cages and the global cage for the variables Pp_I ($F_{1, 30} = 13.28$; $p = 0.001$; square root transformed data) and Δ RC30 ($F_{1, 375} = 84.75$; $p = 0.000$). Pp_I in the global cage (mean \pm SE = 25.7 ± 3.31) was significantly bigger than in individual cages (mean \pm SE = 11.4 ± 2.04) while Δ RC30 in the global cage (mean \pm SE = 0.9 ± 0.04 mm) was significantly smaller than in individual cages (mean \pm SE = 1.5 ± 0.03 mm). There were no significant differences between individual and global cages for the variables Pp_F ($F_{1, 30} = 0.38$; $p = 0.545$; $\log_{(x+1)}$ transformed data) and RC length ($F_{1, 616} = 1.94$; $p = 0.164$).

Survival and size of barnacles transferred to the platform

The analyses to the initial number of *P. pollicipes* per barticle (Pp_I) revealed that there were no significant differences for this variable among monitoring dates on most of the transfer periods

(Table 1). The only exception was for barticles transferred in T2/February, although SNK tests failed to identify a defined pattern for this transfer period.

The number of individuals per barticle transferred to the platform varied between 7.8 ± 0.72 (mean \pm SE) in T3/3April and 23.1 ± 5.09 (mean \pm SE) in T6/August (Table 1).

Considering the results of the analyses to Pp_I (no differences among monitoring dates in all transfer periods with the exception of T2), we have considered the final number of *P. pollicipes* per barticle (Pp_F) on each monitoring date and for each transfer period as a proxy of survival.

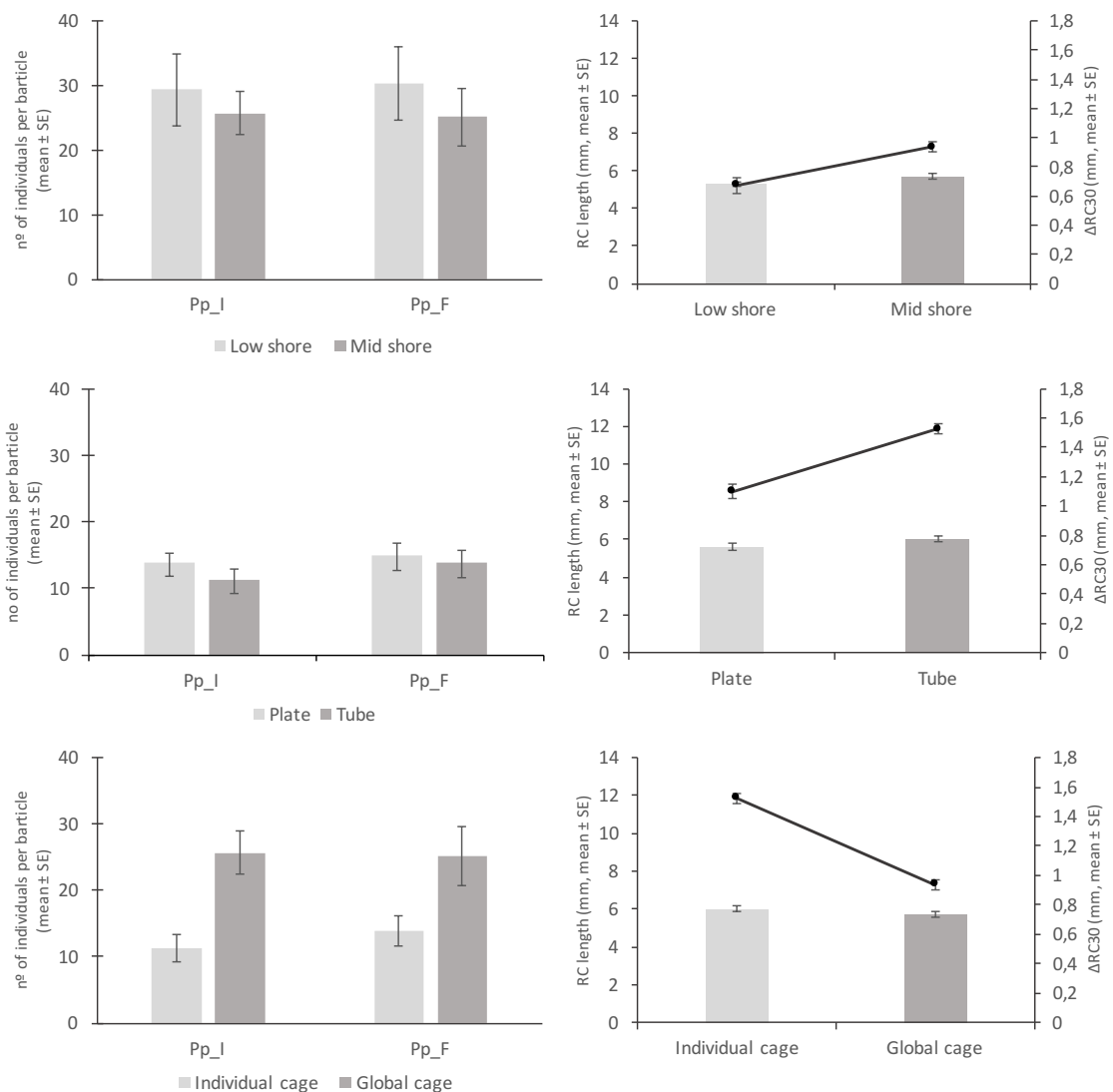


Figure 3 - Initial number (Pp_I; mean \pm SE) and final number (Pp_F; mean \pm SE) of individuals of *P. pollicipes* per barticle for different deployment levels, support structures and anti-predation system (left column graphics). Maximum rostro-carinal distance (RC length, bars; mean \pm SE) and monthly growth rate (Δ RC30, line; mean \pm SE) per individual for different barticle's deployment level, support structures and anti-predation system (right column graphics).

Table 1 –Analyses of variance (ANOVA) on the initial number of *P. pollicipes* per barticle (Pp_I) in relation to factor “monitoring date” for each transfer period and mean values (\pm SE) of pooled data of all monitoring dates for each transfer period. **Bold** – significant p-values ($p \leq 0.05$). Untransformed data.

Transfer period	<i>d.f.</i>	<i>F</i>	<i>p</i>	Mean (\pm SE)
T1/Dec-Jan	4; 175	0.99	0.414	18.1 (\pm 1.15)
T2/February	4; 125	3.57	0.009	11.8 (\pm 0.61)
T3/3April	3; 28	0.57	0.638	7.8 (\pm 0.72)
T4/30April	2; 21	0.30	0.740	17.7 (\pm 2.16)
T5/June	2; 37	1.70	0.196	9.8 (\pm 1.38)
T6/August	1; 14	0,01	0.935	23.1 (\pm 5.09)

The final number of individuals of *P. pollicipes* per barticle (Pp_F) at time 0 (t0) of each transfer period was highly variable and it varied between $8.4 \pm 1,18$ (mean \pm SE) in T3/3April and 58.1 ± 15.54 (mean \pm SE) in T6/August transfer period (Figure 4).

There was a decrease of Pp_F over time and, with the exception of the barticles transferred in T7/September, on the last monitoring date (M5/January 2016), the mean number of individuals per barticle was approximately zero for all the transfer periods (Figure 4).

ANOVA results indicate significant differences in Pp_F among monitoring dates (including t0) for all transfer periods except for T7/September (Table 2). The mean number of individuals per barticle significantly decreased over time from t0 to M5/January. This decrease occurred immediately after t0, as in T4/30April and T5/June transfer periods, or later, as in T1/Dec-Jan transfer period where it occurred only in M4/October monitoring date (Table 2).

The size of *P. pollicipes* individuals at t0 for each transfer period was highly variable and RC length varied between $1,4 \pm 0.08$ mm (mean \pm SE) in T1/Dec-Jan and $4,3 \pm 0.28$ mm (mean \pm SE) on T4/30April transfer periods (Figure 4).

Despite a significant increase in size in the first monitoring date after transfer, for most of the transfer periods (except T2/February in which this increase occurred on M3/August, and T4/30April and T6/August in which there were no significant differences), mean RC length remained similar (T1/Dec-Jan transfer period) or significantly decreased on the last monitoring date (T2/February, T3/3April and T5/June transfer periods) (Table 3).

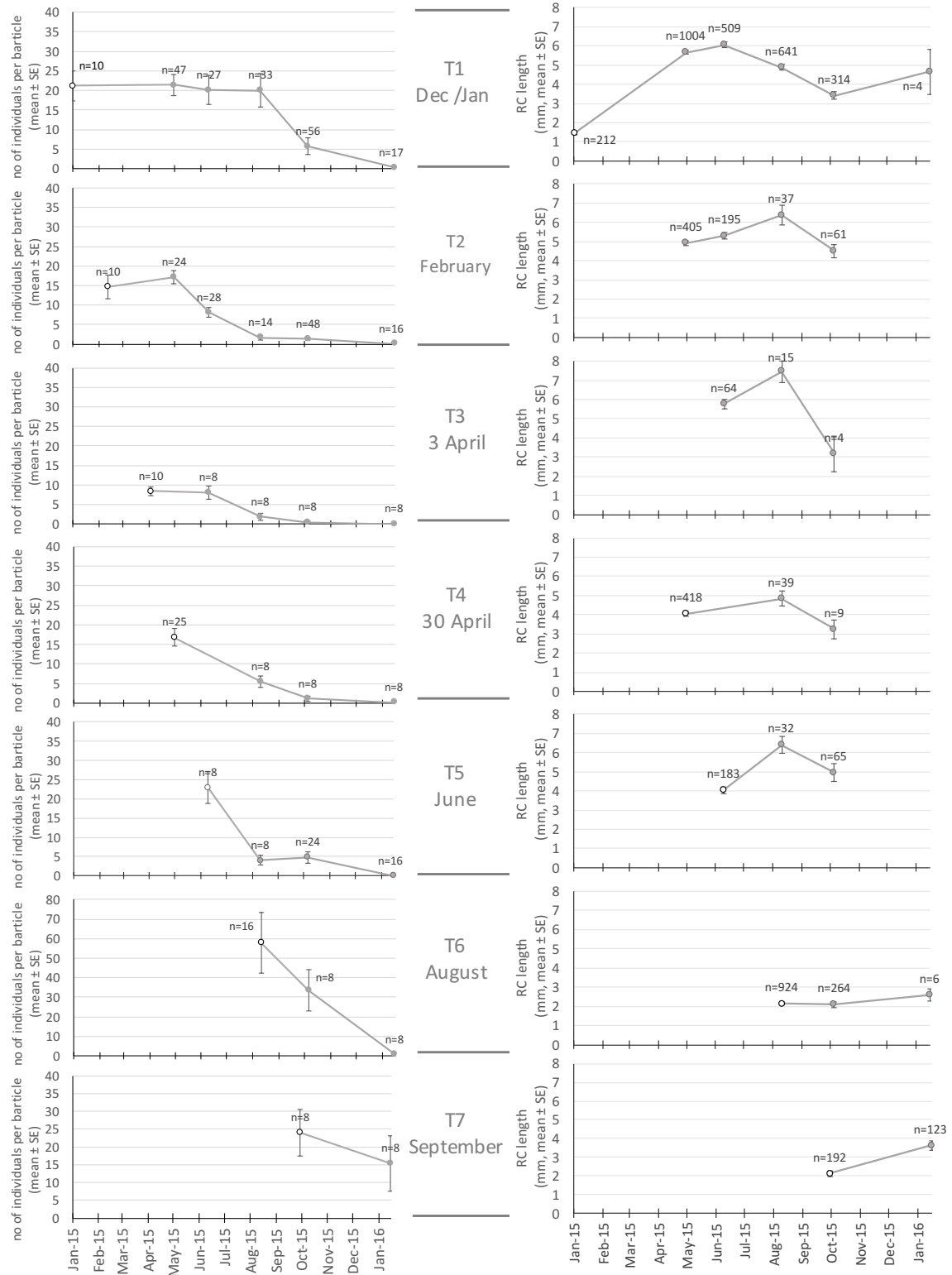


Figure 4 – Number of individuals of *P. pollicipes* per barticle (Pp_F; mean \pm SE) at time 0 (t0; white dot) and at the five monitoring dates (grey dot) (left column graphics) and maximum rostro-carinal distance per individual (RC length; mean \pm SE) at time 0 (t0; white dot) and at the five monitoring dates (grey dot) (right column graphics) for each transfer period of barticles to the platform. RC length data at t0 is missing for T2/February and T3/3April transfer periods. Note the different y-axis scale in T6/August transfer period for the variable Pp_F.

Table 2 –Analyses of variance (ANOVA) on the final number of *P. pollicipes* per barticle (Pp_F) in relation to factor “monitoring date” for each transfer period. **Bold** – significant p-values ($p \leq 0.05$). Monitoring dates: t0– time 0; M1- April 2015; M2- June 2015; M3- August 2015; M4- October 2015; M5- January 2016.

Transfer period	d.f.	F	p	Post-hoc comparisons	Levene statistic	Data transformation
Dec-Jan	5; 184	30.36	0.000	t0 = M1 = M2 = M3 > M4 > M5	4.153	Log10 (x+1)
February	5; 134	55.16	0.000	t0 = M1 > M2 > M3 = M4 > M5	8.537	Log10 (x+1)
3April	4; 37	16.68	0.000	t0 = M2 > M3 = M4 = M5	10.167	no
30April	3; 45	41.02	0.000	t0 > M3 > M4 = M5	3.217	Log10 (x+1)
June	3; 44	40.57	0.000	t0 > M3 = M4 > M5	8.910	Log10 (x+1)
August	2; 29	37.26	0.000	t0 = M4 > M5	1.702	no
September	1; 14	0.72	0.410	t0 = M5	0.227	no

Table 3 –Analyses of variance (ANOVA) on maximum rostrum-carinal distance (RC length) of *P. pollicipes* individuals in relation to factor “monitoring date” for each transfer period. RC length at t0 data not available for T2/February and T3/3April transfer periods. **Bold** – significant p-values ($p \leq 0.05$). Monitoring dates: t0– time 0; M1- April 2015; M2- June 2015; M3- August 2015; M4- October 2015; M5- January 2016. n.d.p – no defined pattern.

Transfer period	d.f.	F	p	Post-hoc comparisons	Levene statistic	Data transformation
Dec/Jan	5; 2678	204.92	0.000	t0 < n.d.p.	155.701	Log10
February	3; 694	5.30	0.001	M1 = M2 = M4 < M3	2.339	no
3April	2; 80	7.60	0.001	M4 < M2 = M3	0.382	no
30April	2; 463	2.36	0.096		1.130	no
June	2; 277	13.91	0.000	t0 = M4 < M3	2.442	no
August	2; 1191	0.17	0.847		1.334	no
September	1; 313	40.99	0.000	t0 < M5	2.630	no

In the Cape of Sines (control site), the number of juveniles of *P. pollicipes* per barticle for the five monitoring dates varied between 13.2 ± 2.16 (mean \pm SE) in M1/April and 58.1 ± 15.54 (mean \pm SE) in M3/August (Figure 5). The RC length for these individuals varied between 2.2 ± 0.14 mm (mean \pm SE) in M3/August and 4.3 ± 0.28 mm (mean \pm SE) in M1/April (Figure 5).

The weight of fouling organisms per barticle in M3/August 2015 monitoring date and for barticles transferred to the platform between the periods T1/Dec-Jan and T5/June varied between 12.6 ± 1.60 g (mean \pm SE) (T5/June) and 89.1 ± 7.23 g (mean \pm SE) (T1/Dec-Jan), while in M5/January 2016, and for the same transfer periods, the fouling weight varied between 177.1 ± 21.27 g (mean \pm SE) (T5/June) and 273.9 ± 36.50 g (mean \pm SE) (T1/Dec-Jan) (Figure 6).

Barticles transferred later to the platform on T6/August and T7/September transfer periods, had a mean fouling weight of 20.6 ± 2.39 (mean \pm SE) and 3.4 ± 1.84 g (mean \pm SE) respectively, on M5/January monitoring date. Acorn barnacles settled often on the capitular plates of *P. pollicipes* and mussels formed dense clumps around the barticles and *P. pollicipes* individuals.

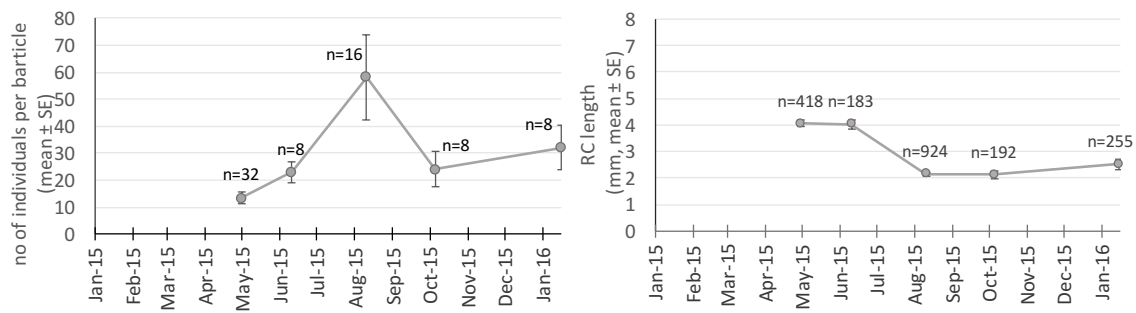


Figure 5 - Number of juveniles of *P. pollicipes* per barticle (mean ±SE) (left side graphic) and maximum rostro-carinal distance (RC length) per individual (mean ±SE) (right side graphic) on the five monitoring dates in the Cape of Sines.

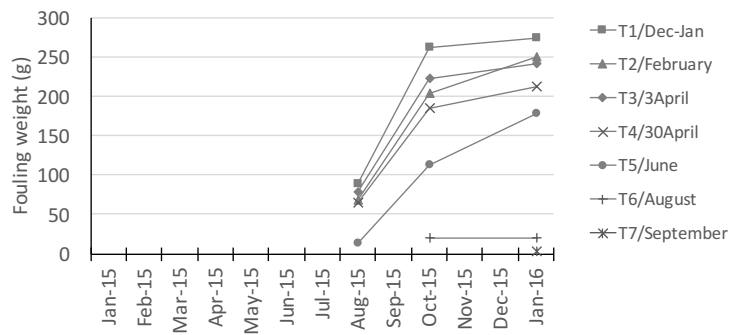


Figure 6 – Mean weight of fouling organisms per barticle on three monitoring dates (M3/August; M4/October; M5/January 2016), for each of the seven transfer periods.

Monthly growth of barnacles transferred to the platform

Monthly growth rate (ΔRC_{30}) of *P. pollicipes* at the platform varied between 0.9 ± 0.04 mm (mean ±SE) in summer and 1.2 ± 0.02 mm (mean ±SE) in winter, while at the Cape of Sines varied between 0.6 ± 0.04 mm (mean ±SE) during winter and 1.0 ± 0.06 mm (mean ±SE) in summer period (Figure 7).

PERMANOVA results revealed a significant interaction between the two main factors (Location*Season) (Table 4a). Pairwise tests to the interaction term indicate that during winter period, monthly growth rate is higher at the platform than in the Cape of Sines while in summer there are no significant differences between the two locations. On the other hand, monthly growth at the platform during winter is significantly higher than during summer, while at the Cape of Sines the opposite pattern is observed, *i.e.* a higher monthly growth during summer than winter (Table 4b).

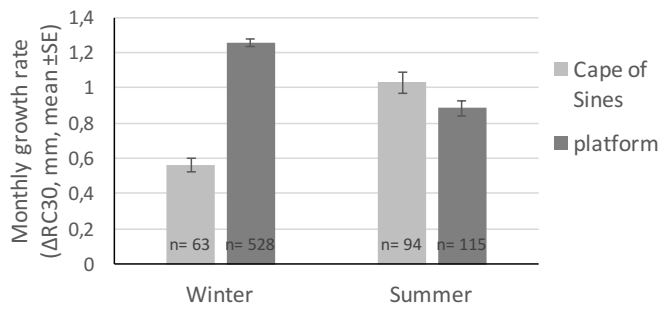


Figure 7 – Comparison of monthly growth rate (mean \pm SE) of *P. pollicipes* during different seasons between the platform and the Cape of Sines (control site).

Table 4 – a) PERMANOVA analysis of factors Season (Se) and Location (Lo) on monthly growth of *P. pollicipes*. All data were square root transformed. PERMDISP test: $F= 2.8182$; $p= 0.057$. **Bold** – significant p-values ($p \leq 0.05$). b) Pair-wise tests to significant interaction term (Lo*Se).

a) PERMANOVA				
Effect	df	MS	Pseudo-F	p-value
Location (Lo)	1	2.5446	32.361	0.001
Season (Se)	1	0.1380	1.7546	0.205
Lo x Se	1	5.0222	63.87	0.001
Res	796	0.0786		
b) Pair-wise tests				
Factor	Level	Pair-Wise tests		
Season:	Summer	platform = Cape		
	Winter	platform > Cape		
Location:	Cape of Sines	Summer > Winter		
	platform	Winter > Summer		

Discussion

This study was based on the use of barticles as artificial substrates for *P. pollicipes* larval attachment and juvenile growth and its subsequent transfer to a grow-out system. The mean initial numbers of *P. pollicipes* obtained in the Cape of Sines and transferred to the platform were substantial, although highly variable and, depending on the transfer period, ranged from 7.8 (Pp_I at T3/3April) to 58.1 (t0 at T6/August) individuals per barticle. Variation is probably due to small scale spatial variability of settlement and recruitment on barticles but also due to time of transfer. In fact, barticles transferred between December 2014 (T1) and June 2015 (T5) collected settlers from the 2014 recruitment season while barticles transferred in August 2015 (T6) and September 2015 (T7) had accumulated settlers from two recruitment seasons (2014 and 2015).

One of the main challenges for the development of aquaculture of barnacles (López *et al.*, 2010) or of many other species around the world (Fuentes & Molares, 1994) is to ensure both a sufficient and reliable quantity of seed for grow-out systems. In the case of *P. pollicipes*, larval settlement in artificial substrates was a main bottleneck for the aquaculture of this species (Cruz, 2000; Franco, 2014). Here we demonstrate that barticles are a relatively easy and low cost method for seed collection and subsequent transfer to grow-out systems.

Regarding barticle's deployment level in the Cape of Sines, results suggest no differences in size between mid and low shore barnacles. However, mid shore barnacles presented a higher monthly growth rate than low shore barnacles in the platform. Jacinto *et al.* (2015) didn't find any effect of shore level on *P. pollicipes* barnacles growth rate on a study at Cape Sardão (~40 Km south of Cape of Sines).

Considering practical issues, mid shore barticles were much easier to access and collect in the intertidal rocky shore. Although collection of the barticles was always dependent on sea conditions, low shore barticles were accessible only in spring tides and for a shorter period of time compared with mid shore barticles. Considering our results and practicality of barticles deployment and collection in the field, mid shore was chosen as the most suitable level for barticle deployment.

The support structures for barticles (tubes and plates) had no effect on survival, but tubes had barnacles with a larger size and a higher monthly growth rate than plates. In a study on acorn barnacle aquaculture, López *et al.* (2012) also found that pvc tubes were more efficient for barnacle growth than plates, which could be associated with the collector surface - water column relationship. However, in our study, the interior part of the tubes ended up serving as

habitat/refuge for several species (ex: sea urchins; polichaetes; sponges; crabs; shrimps) and tended to accumulate more fouling organisms (mussels and acorn barnacles) than plates, making the tubes too heavy and unpractical heavy structures. Considering that there were no differences in barnacle survival, plates were chosen as the most suitable support structures for barticles.

Survival and monthly growth rate were smaller in the global cage than in individual cages. This was probably due to fouling by incrustant organisms, which caused the net aperture occlusion. The global cage in our study is similar to fish cages that are widely used in aquaculture. According to Cardia & Lovatelli (2015) abundant biofouling in fish cages reduces water exchange in the cage and can cause a depletion of dissolved oxygen with deleterious effects on cultivated organisms. Anti-fouling coatings are commonly used in fish cages (Fitridge *et al.*, 2012; Cardia & Lovatelli, 2015) but in our study, considering that *P. pollicipes* is a filter feeding species, we opted for not using any anti-fouling coatings on the cage, in order to prevent any risk of *P. pollicipes* contamination. Fouling was causing a heavy load in the platform and consequently the global cage had to be removed in October 2015. Considering the results obtained and the biofouling problem, individual cages were chosen as the best option to avoid predation.

P. pollicipes barnacles transferred to the platform between December 2014 and September 2015 and monitored during one year had high survival rates on April and June monitoring dates (M1 and M2), particularly barnacles transferred earlier in December-January (T1) and on 3 April (T3). However, the general trend was a decrease in survival of *P. pollicipes* over time and, except for barnacles transferred in September (T7), on the last monitoring date (M5/January 2016), the mean number of individuals per barticle was nearly null for all the transfer periods.

Concurrently, we have observed that fouling organisms started to settle in April and June (initially acorn barnacles and later mussels) and, except for barticles transferred later in August and September (T6 and T7), in August and October monitoring dates (M3 and M4) there was a remarkable increase in the fouling weight per barticle.

Acorn barnacles settled often on the capitular plates of *P. pollicipes*, causing plate deformities and probably interfering with the normal opening of these plates and with feeding rates of the stalked barnacles. Mussels formed dense clumps around each barticle surrounding *P. pollicipes* individuals, with an intricate net of byssus that would probably block any movement of *P. pollicipes*, likely preventing them from feeding and leading to death of these barnacles. Although barticles transferred in August and September (T6 and T7) had low levels of fouling weight, such levels were probably enough to damage the stalked barnacles causing death to almost all individuals (as in the case of T6/August) or a significant decrease in barnacle survival (in the case of T7/September) in the January monitoring date (M5).

Also, the size of the transferred barnacles on the five monitoring dates is probably reflecting the deleterious effect of biofouling in *P. pollicipes* individuals. In fact, RC length in the stalked barnacles either remained similar in all monitoring dates or had an initial increase after transfer, but then remained the same or significantly decreased on the last monitoring date (M5/January 2016).

Biofouling is one of the major management issues in aquaculture causing considerable damage to cultivated organisms and having significant economic impacts (Adams *et al.*, 2011). Several authors have documented the deleterious impacts of biofouling on survival and growth rates of shellfish cultures (ex. Claereboudt *et al.*, 1994; Taylor *et al.*, 1997) such as physical damage, mechanical interference on shell function or competition for food and space (see review by Fitridge *et al.*, 2012).

In contrary, at the Cape of Sines (control site) we did not observe any decrease in survival of *P. pollicipes* neither any fouling problems similar to the ones found at the platform. The mean number of *P. pollicipes* individuals per barticle remained high (>13) at the Cape of Sines across all monitoring dates with a peak in August (M3), when it increased to 58.1 individuals per barticle. The increase in the mean number of individuals per barticle at the Cape of Sines together with a decrease in size at the same monitoring date (M3/August) was probably related with the arrival of new recruits on the barticles, as recruitment is more intense during summer and autumn in this region (Cruz *et al.*, 2010).

Mean growth rate of *P. pollicipes* at the platform reached 1.2 mm RC per month during winter and was higher than growth rate at the Cape of Sines (0.6 mm RC per month) during the same period, while in summer there were no significant differences between the two locations. At the platform, *P. pollicipes* growth rate during summer was smaller (0.9 mm RC length per month) than during winter. The higher barnacle's growth rate found at the platform compared to their natural habitat (Cape of Sines) during winter may be due to the fact that these barnacles are continuously underwater and consequently are not subjected to desiccation during low tide and might receive a steady supply of food. Similar observations were made in two different studies by Hoffman (1988) and Goldberg (1984) where higher growth rates of stalked barnacles (*Pollicipes polymerus* and *P. pollicipes*, respectively) were attained when these individuals were permanently submerged comparatively to barnacles growing in their natural habitat. This environmental difference between locations may have been important during winter, but in summer, the intense settlement of fouling organisms (mussels and acorn barnacles) occurring at the platform, possibly affected the feeding behaviour of stalked barnacles and consequently their growth rates. Recruitment of mussels (*Mytilus galloprovincialis*) on collectors suspended from commercial rafts in Galicia (Spain) occurred from March to September with a more intense

peak in July and August (Fuentes & Molares, 1994). Recruitment of the acorn barnacle *Perforatus perforatus* in panels suspended from a longline on the open coast of SW Portugal occurred mainly in April and May (Fragoso & Icely, 2009). In fact, biofouling problems in the platform started in April 2015 and barnacles transferred in December-January 2015 (T1) and collected on 30 April (M1) were still having a high survival rate during these first months.

Nevertheless, monthly growth rates found in barnacles that were growing at the platform during wintertime are promising results for the development of stalked barnacle aquaculture.

We could expect that considering a growth rate between 0.9 mm and 1.2 mm (RC length) per month and an average initial size of 3 mm (RC length) at time of transfer to the platform, *P. pollicipes* barnacles might reach commercial size (>20 mm RC; Cruz *et al.*, 2010) in 14 to 20 months, if biofouling problems are solved. López *et al.* (2012) have found that the giant acorn barnacle *Austromegabalanus psittacus* cultured in artificial collectors could reach commercial size over a period between 18 and 24 months and the culture of oysters in Canada can take as long as 4 years to be commercially harvestable (Mallet *et al.*, 2009).

The floating platform with its design and present dimensions has proven suitable for our experimental aquaculture studies, although potential future production of *P. pollicipes* at an industrial scale would only be economically viable in a longline system of culture (Ruivo *et al.*, unpublished data).

This study constitutes an important first step towards stalked barnacle aquaculture. It has shown the effectiveness of barnacles as *P. pollicipes* larvae collectors and as substrates for juvenile growth. The high growth rates of *P. pollicipes* found in the platform are promising results and suggest that stalked barnacles can grow to harvestable sizes relatively quickly in culture conditions. However, fouling by incrustant organisms, essentially acorn barnacles and mussels, has caused severe damage to *P. pollicipes* individuals leading to death most of the stalked barnacles transferred to the platform in several periods along the year. Support structures for barnacles and anti-predation cages have also been affected by biofouling and there is still a need to test the application of different anti-fouling techniques in these structures and to improve their design, in order to minimize the available surface area for fouling organisms to settle.

Biofouling is thus a major drawback that needs further investigation. Future studies should evaluate temporal variability in settlement of fouling organisms and test different anti-fouling techniques to avoid or mitigate fouling organisms in this commercially important barnacle species.

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