Suspended culture of *Pinna rudis* enhances survival and allows the development of a seasonal growth model for Mediterranean Pinnids

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Abstract

A two-year growth study of 80 *Pinna rudis* individuals was conducted in offshore cages in the western Mediterranean Sea. A Von Bertalanffy growth model was fitted to monthly measured data of 40 individuals (Group 1), whereas length-dry weight relationship was established with the other 40 individuals (Group 2). Oceanographic data were sampled bimonthly. The individuals showed the fastest growth reported for a bivalve (1.32 mm/day). Temperature acted as the main

factor controlling growth, which showed strong seasonality, but phytoplankton availability acted as a limiting factor during the warmest periods of year. These data will be useful to understand *P. rudis* ecology. Furthermore, the length-dry weight regression is proposed as a tool for captivity diet confection of the critically endangered species *P. nobilis*. Natural mortality was 0% during the study period.

1. Introduction

Pinna rudis is a Mediterranean-Atlantic long living mollusk that can reach 31 years of age (Nebot-Colomer et al., 2016). It ranks among the largest bivalves in the world, with a shell length of 56.5 cm (Schultz and Huber, 2013) only surpassed in the Mediterranean Sea by the endemic species “fan mussel” *Pinna nobilis*, which can reach up to 120 cm (Zavodnik, 1991).

Its populations are threatened due to coastal construction, pollution, fishing and poaching by recreational divers (Barea-Azcón et al., 2008) and the biological information available on the species is scarce (Gvozdenović et al., 2019; Templado et al., 2004). *P. rudis* has been listed as a protected species in Annex II of the Bern Convention and as a threatened or endangered marine species by the Barcelona Convention.

Recently, many young individuals of *P. rudis* have been observed thanks to the strong recruitment event that occurred in summer 2017 (García-March et al., 2020; Kersting et al., 2020b; the present work). This greater availability of *P. rudis* juveniles, has turned the species into the best model to conduct manipulative experiments on Mediterranean pinnids given the mass mortality events associated to a parasitic disease affecting exclusively *P. nobilis* (Catanese, 2020;

Panarese et al., 2019). Using *P. rudis* to assay methodologies prior to their application to *P. nobilis*, a species that is presently endangered with extinction (García-March et al., 2020; Kersting et al., 2020a), could help reducing fan mussel mortality during experimentation. However, it is first necessary to fill up the present knowledge gap on *P. rudis* biology (Gvozdenović et al., 2019; Templado et al., 2004). Furthermore, the scarcity of ecological information about *P. rudis* makes it difficult to render effective conservation programs for the species (Bell et al., 2006; Escudero et al., 2003), especially in the context of climate change and its effect in the survival and distribution of populations (Fitzpatrick and Hargrove, 2009; García-March et al., 2019; Jorda et al., 2012; Márquez et al., 2011; Schwartz et al., 2006). In order to fill this gap, a two-year growth study of *P. rudis* was carried out with juveniles maintained in cages off-shore. Also, dry weight (DW) and morphometric data were obtained. Oceanographic data were simultaneously recorded in order to obtain environmental information that could be correlated with *P. rudis* growth rates. The present study helps improving the knowledge of *P. rudis* ecology with data related to growth, survival, and its association with environmental variables. These data will help both, to use *P. rudis* as a model for the applicability of manipulative experiments to be later conducted on *P. nobilis* and to expand the knowledge relative to *P. rudis*.

2. Material and methods

2.1 Study area and collection of juveniles

Juveniles of *Pinna* spp. of similar sizes (34.9 ± 6.2 mm, N = 39) were found after a massive recruitment on the ropes of an aquaculture installation in Vila Joiosa, Alicante (Spain), on February 19, 2018. Individuals were visually recognized as *P. rudis* or *P. nobilis*, and the identification at the species level was conducted by genetic analyses in a subsample of 5 individuals, which represented the variability of shapes observed in the collected population. The individuals were mostly located in the horizontal ropes of the facility, which featured 60 m length at 4-6 m deep and held three rows of 18 floating net cages. To estimate the number of recruits, 20% of the ropes were surveyed. Four months later, on June 29, 2018, 80 *P. rudis* individuals (shell length ± SD of 48.6 ± 8.8 mm) were collected and transported using an icebox with seawater, to be placed in controlled open-sea cages within a day.

2.2 Design and location of cages for *P. rudis* maintenance in situ

On June 29, 2018, juveniles were divided in two groups; group 1 (n = 40) was used for growth monitoring (length and width) while group 2 (n = 40) was used to obtain the data related to the size-weight relationship. Group 1 was placed in 4 independent cages suspended offshore at 15 m deep in the marine area adjacent to the Natural Park of Penyal d'Ifac in Calp, Alicante (Spain). Each cage included 10 individuals, each fitted within its own box of 35 x 15 x 10 cm (L x W x H) made of 4.5 mm plastic mesh net. Boxes were attached between them and distributed in two rows of 5 boxes each. Cages were anchored to concrete blocks at 20 m depth using a rope and suspended in the water column with four small floats, one in each corner. Juveniles from group 2 were placed in two single cages of 100 x

60 x 10 cm (L x W x H) each at 20 m depth (ca. 1m above the bottom). The location and depth of the cages were selected to avoid self-burial, predation and possible interference of fishermen and recreational divers (Figure 1).

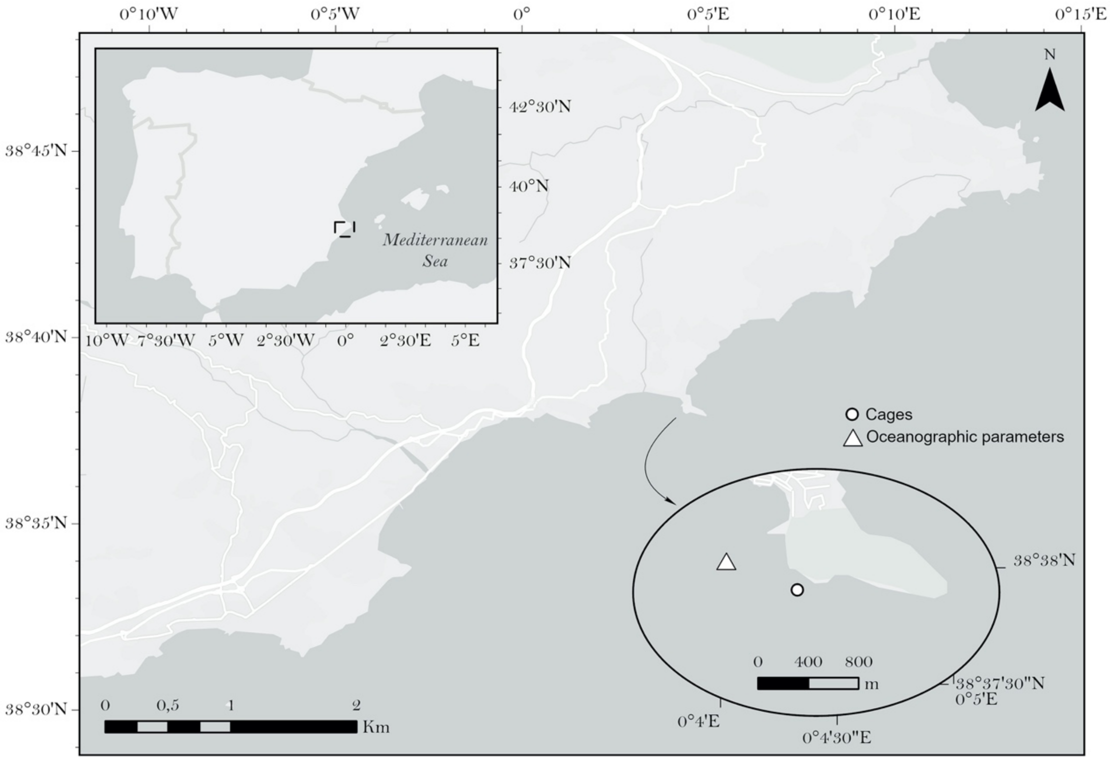

Figure 1: Location of *P. rudis* cages and sampling area of oceanographic data.

2.3 Monitoring

Once per month during 25 months (from June 2018 to July 2020), the maximum antero-posterior length, and dorso-ventral width (measured from the inflexion point to the ventral point with maximum horizontal width (De Gaulejac and Vicente, 1990)), of group 1 individuals were measured to the nearest millimetre in situ by scuba diving. Vernier callipers were used for shell lengths < 15 cm, and tree callipers for shell lengths > 15 cm. The period between measurements varied between 20 to 40 days depending on weather conditions, with the exception of the March-April survey, which was missed due to COVID19 restrictions in Spain. Individuals from group 2 were allowed to grow and then systematically collected during the monitoring period to obtain a range of sizes (25-300 mm of shell length). As in group 1, maximum antero-posterior length and dorso-ventral width were measured to the nearest mm when collected. Individuals were sacrificed, and soft tissues and shells were separated, dried at 105ºC during 48ºC and weighted.

Cages were cleaned monthly from epibionts during the entire monitoring period. Oceanographic parameters (Dissolved oxygen –DO–, Chlorophyll-*a*, turbidity, salinity and temperature) from water column profiles were measured bimonthly in the immediacy of the cages using an oceanographic probe AAQ-RINKO 177 (Figure 1).

2.4 Morphometric relationships and statistical analysis

For the calculations, growth data (group 1) were expressed in mm/day for each monitoring period.

The relationship between length-width (group 1 and group 2) and length-DW (group 2) were tested to fit to the best linear regression equation using the degree of association given by the R^2 coefficient. Length-DW data were previously log-transformed to fit to linear regression.

A growth model was calculated using the non-linear mixed effects model (Vigliola and Meekan, 2009) to fit the size-at-age data to the Von-Bertalanffy growth function. L_∞ was considered random and t_0 and k fixed (García-March et al., 2011).

Growth (mm/day) and oceanographic data were used to calculate Pearson's Correlation Coefficient (Benesty et al., 2009). For this, growth data were detrended to remove the ontogenetic trend using the non-parametric method "Seasonal and Trend decomposition using Loess" (STL) described in Cleveland et al. (1990) using R statistical computing environment.

3. Results

During surveys on fish farm ropes, a total of 635 *Pinna* spp*.* juveniles were located (a mean ± SD of 70 ± 34 individuals in each rope). The projection suggests that around 3175 individuals had recruited in the whole structure. Of those individuals, 94.6% of the individuals were identified as *P. rudis* and 5.4% as *P. nobilis.*

In February 2018, individuals showed a mean length size of 34.9 ± 6.2 mm (N = 39). Four months later (June 2018), the mean size of the individuals collected for the experiment in open-sea cages was 48.6 ± 8.8 mm length and 27.98 ± 5.7 mm width (N = 40, group 1). After the first year in the experimental cages, individuals reached a size of 215.2 ± 17.2 mm in length and 134.0 ± 14.4 mm in width, and at the end of the experiment (758 days) showed a size of 279.0 ± 16.9 mm in length and 163.2 ± 13.1 mm in width (Table 1). Mortality due to natural causes was 0% in both, group 1 and group 2. However, cage 4 disappeared after 15 months together with ropes and concrete blocks, without any hint of the causes.

Maximum growth registered for a single juvenile (individual A1.2) was 34.3 mm on July 7, 2018, over a period of 26 days (1.32 mm d^{-1}). In a year, the maximum growth detected for an individual (A1.1) was 209.0 mm in length (0.57 mm d^{-1}). Mean length growth rate (mm d^{-1}) can be found in Figure 2.

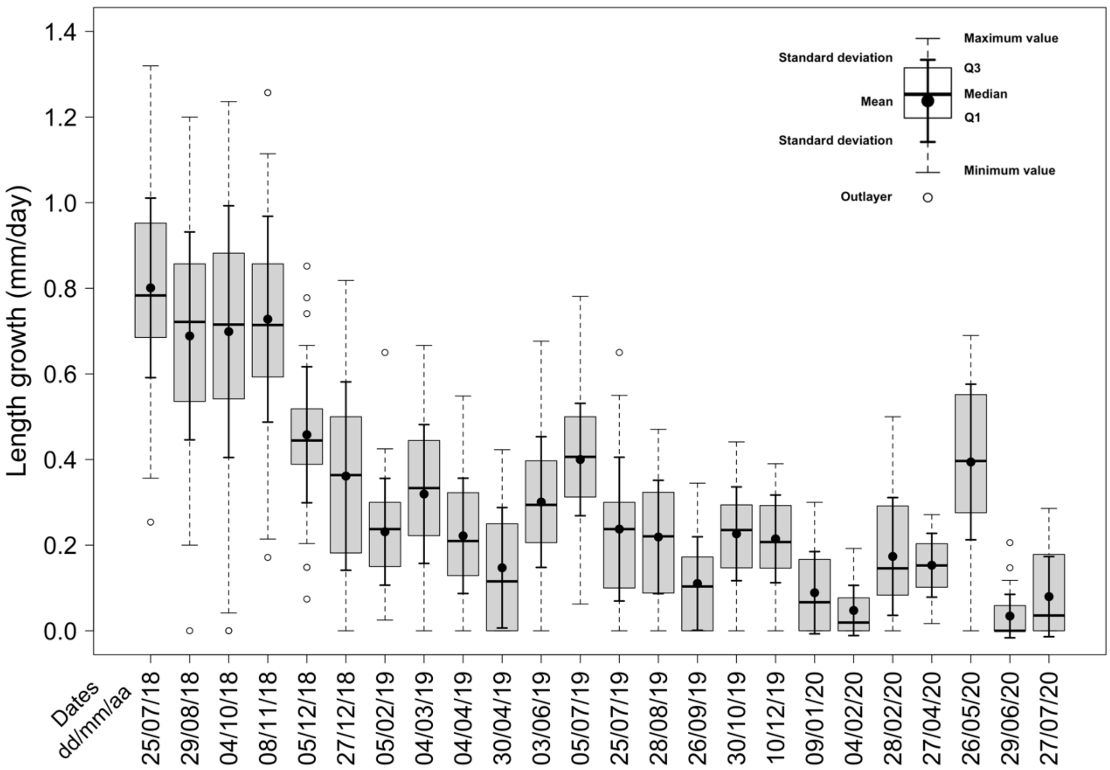

Figure 2: Length growth (mm/day) for each measurement period. Outliers are considered values above Q3 or below Q1 1.5 times the interquartile range.

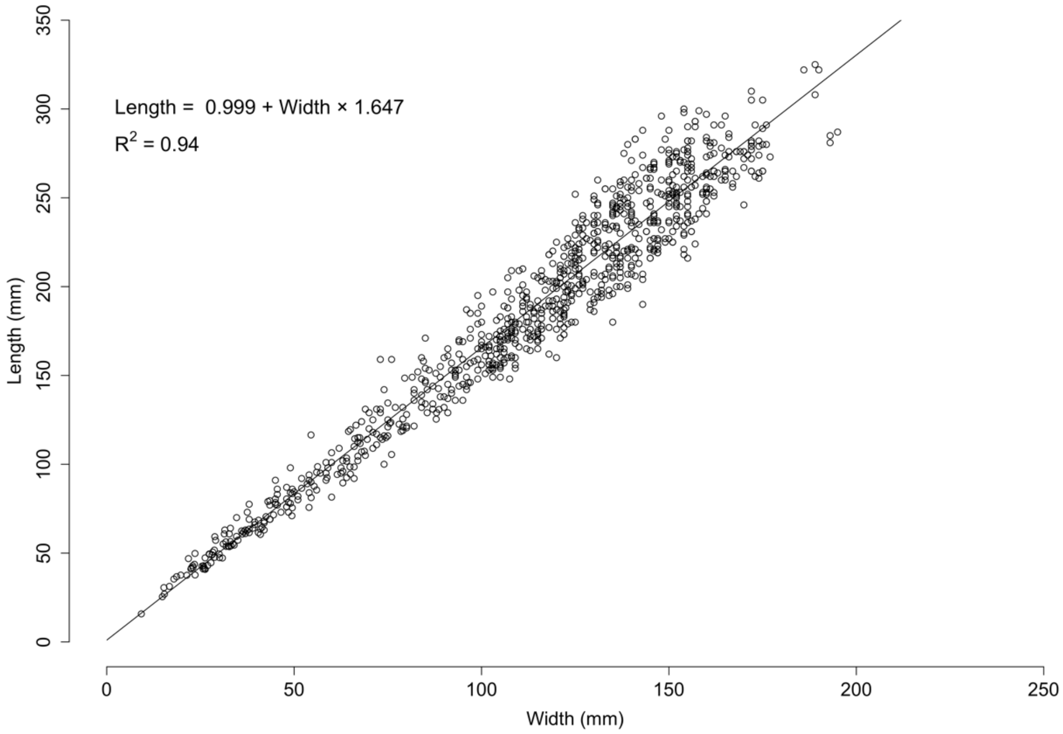

Figure 3: Length-width linear relationship showing equation and R^2.

A positive correlation was found between length-width ($R = 0.94$, p-value < 0.001) and length-DW ($R = 0.97$, p-value < 0.001) using linear regression (Figure 3 and Figure 4).

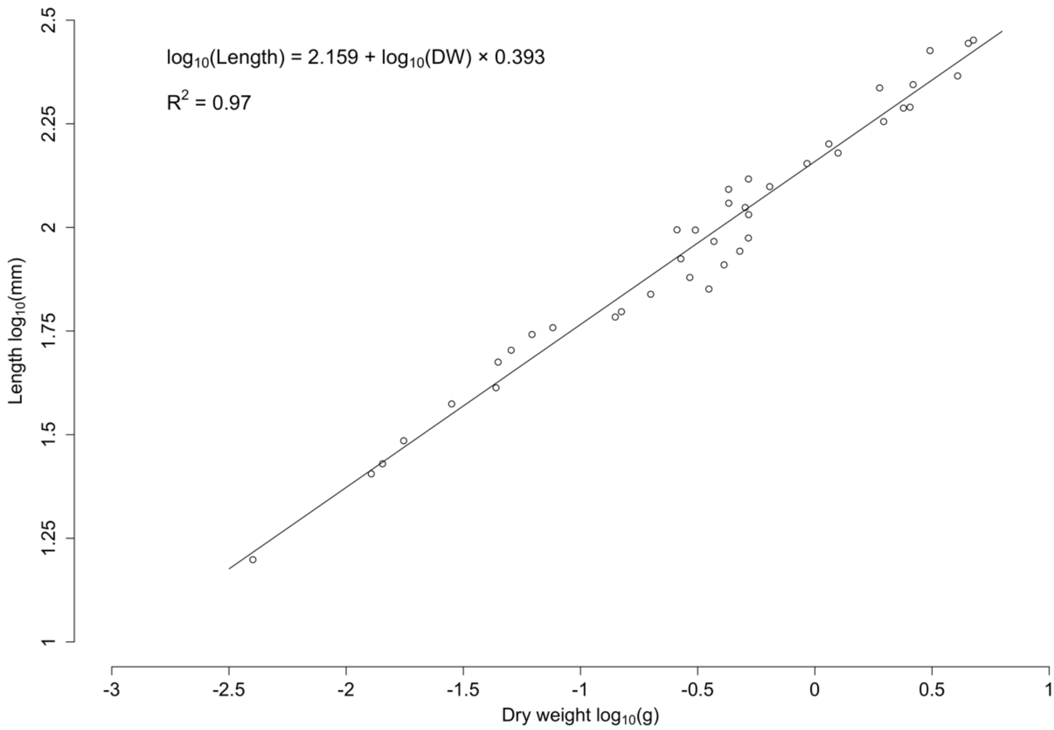

Figure 4: Length-weight (log-transformed) linear relationship, showing equation and R^2.

The growth model gave an L_∞ of 29.06 cm, a K of 1.16 and a t_0 of -0.18. Standardized results showed that 94.56% of the data were within 2 standard deviations of the mean. Detrended growth data (original growth data series, trend of the series and seasonal growth) are presented in Figure 5A. Seasonal growth showed a significant correlation with temperature (0.67, p-value < 0.001), chlorophyll-*a* (-0.56, p-value < 0.01) and DO (-0.58, p-value < 0.01) and no correlation with turbidity and salinity (p-value > 0.05). Seasonal growth, temperature, chlorophyll-*a* and DO data can be found in Figure 5B.

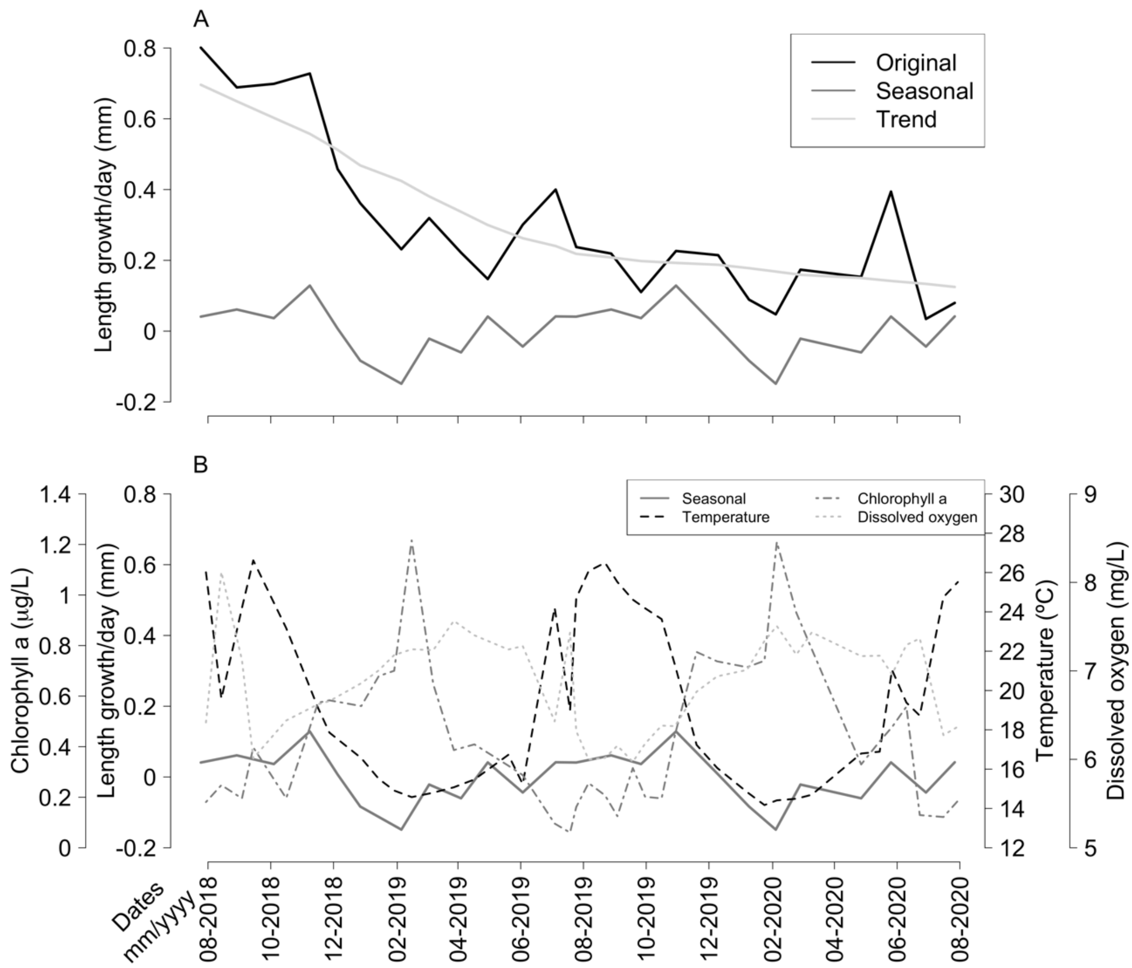

Figure 5: A) Length growth (mm d^{-1}) detrended (original, trend and seasonal). B) Detrended growth mm d^{-1} (seasonal) with temperature, dissolved oxygen and chlorophyll-a.

Table 1: Length and width data for each survey.

Date	N	Mean Length ± SD (mm)	Mean width ± SD (mm)
2018-06-29	40	48.64 ± 8.84	27.88 ± 5.72
2018-07-25	40	69.46 ± 8.46	41.78 ± 6.02
2018-08-29	40	93.53 ± 10.49	57.86 ± 9.40

2018-10-04	40	118.16	±	12.07	72.68	±	9.74
2018-11-08	40	143.64	±	14.72	88.65	±	12.11
2018-12-05	40	156.00	±	13.92	98.43	±	11.71
2018-12-27	40	163.93	±	15.35	106.78	±	11.84
2019-02-05	40	173.18	±	17.10	109.49	±	13.28
2019-03-04	40	181.80	±	17.41	111.71	±	12.79
2019-04-04	40	188.63	±	17.64	118.88	±	13.89
2019-04-30	40	192.20	±	17.44	121.68	±	13.98
2019-06-03	40	202.43	±	17.23	125.45	±	14.73
2019-07-05	40	215.23	±	17.15	134.03	±	14.42
2019-07-25	40	219.98	±	16.78	135.50	±	14.96
2019-08-28	40	227.43	±	17.39	135.65	±	14.79
2019-09-26	30	231.87	±	17.68	137.20	±	15.42
2019-10-30	30	239.57	±	16.80	139.77	±	13.52
2019-12-10	30	248.37	±	16.95	144.67	±	12.31
2020-01-09	30	250.60	±	16.02	145.47	±	12.59
2020-02-04	30	251.40	±	15.40	145.67	±	12.29
2020-02-28	30	255.30	±	15.64	147.93	±	12.07
2020-04-27	30	264.33	±	16.61	156.67	±	14.23
2020-05-26	30	275.77	±	16.75	160.60	±	14.54
2020-06-29	30	276.93	±	16.42	162.03	±	13.34

2020-07-27	30	278.97 ± 16.91	163.17 ± 13.09

4. Discussion

In the present study, suspended culture of *P. rudis* in 4.5 x 4.5 mm mesh net cages kept individuals protected, avoiding predation observed in other studies with *Pinna* juveniles (Arizpe, 1995; Beer and Southgate, 2006; Kozul et al., 2011; Narvaez et al., 2000). Wu and Shin (1998) also observed predation mortality, although the higher losses (above 90%) were observed in individuals transplanted to the bottom without any type of protection. This was also observed by Cendejas et al. (1985), who compared different culture method and obtained 100% survival in those that were fully efficient in keeping predators away. In *Pinna* spp., attachment to the substrate is achieved by byssus threads (Basso et al., 2015). Therefore, recently transplanted individuals need time to attach to the bottom, which makes them more vulnerable to drag forces or predators. Hence, a natural attachment to hold them, anti-predator cages, or an artificial extension of the byssus to fix the individuals to the seabed (Hernandis et al., 2018), appears to be necessary to maximize survival.

The high growth rate recorded in the present study (up to 1.32 mm d^{-1}) suggests that the individuals' feeding capacity was unaffected by the mesh net. Wu and Shin (1998) also reported that feeding capabilities were unaffected by caging in *Pinna bicolor*, although they used a greater mesh opening (65mm), which might have allowed the entrance of predators, and would explain the higher mortality of juveniles reported. In contrast, the 4.5 mm mesh opening used in the present study, together with the monthly cleaning of the cages, avoided the entry of predators, while still allowed for water circulation. Furthermore, it could have provided more constant conditions for *P. rudis* development within the cages, compared to the natural media, where hydrodynamics or other factors often causes shell breakages or erosion, modifying its shape. This could explain the higher allometric correlation of length vs. width observed in the present study ($R^2 = 0.94$, p-value < 0.001), compared to those found by Cosentino and Giacobbe (2006) for *P. rudis* ($R^2 = 0.82$, p-value < 0.001) and *P. nobilis* ($R^2 = 0.57$, p-value < 0.01).

Despite the disappearance of one of the cages with 10 individuals, the 0% of natural mortality detected in either group 1 or 2, supports that the use of a similar methodology would be an effective hatchery method for *Pinna* spp. Individuals obtained through recruitment (Cabanellas-Reboredo et al., 2009; Kersting and García-March, 2017), could be bred in suspended cages and then transplanted when larger sizes were reached, making them less vulnerable to predation. This technique could be a useful tool specially with *P. nobilis*, a critically endangered species with few remaining individuals (García-March et al., 2020). Similarly, the estimation of DW through length in *P. rudis* could be used as an approximation of the DW in *P. nobilis*. DW is a common variable used in bivalves for food ratio determination, which is necessary for the maintenance and maturation of individuals and also to standardize physiological parameters (Albentosa et al., 2012; Bayne and Newell, 1983; Winter, 1978). However, it is usually obtained by sacrificing a random sample of individuals (Helm, 2004), which would involve an invaluable loss for a critically endangered species such as *P. nobilis*.

The maximum growth registered for an individual in the present study (1.32 mm d^{-1}) is the highest monthly rate reported for a Pinnid, although higher growth rates have been reported for longer periods in other species such as *P. rugosa*, 0.65 mm d^{-1} during a hole year (Arizpe, 1995). In fact, rapid growth is a survival feature in Pinnids, because the thin and simple structure of the posterior part of the shell (Schultz and Huber, 2013), enables a quick regeneration and the adaptation of *Pinna* spp. to extreme environments, where shell damage and breakage are common. These environments typically occur in shallow areas exposed to hydrodynamics (García-March et al., 2019) or to other risks such as boating impacts (Prado et al., 2014).

The higher growth rates are usually maintained during the initial period of live. Thenceforth, the growth rate decreases, typically with the arrival of the first winter (Arizpe, 1995; Butler, 1987) at least for non-tropical species (Narvaez et al., 2000). Similarly, *P. rudis* juveniles in the present study showed higher growth rates during the initial four months (Figure 2), from June 29 to November 8 of 2018. The decrease in growth rates coincided with a drop in temperature below 20ºC, a feature already observed for *P. nobilis* by Richardson et al. (1999). Similarly, a positive correlation between temperature and growth was observed by Cendejas et al. (1985) in *P. rugosa*. Other environmental parameters, such as chlorophyll-*a* and DO showed an indirect correlation with growth in the present study. On the contrary, Acarli et al. (2011) found a positive correlation between growth and chlorophyll-*a* and particulate inorganic matter, and no correlation with temperature in *P. nobilis*. The different methods used to measure chlorophyll-*a* in both studies, however, impede a deeper comparison of this variable between them. At first, it seems contradictory that, in the present study, the months with fastest growth of *P. rudis* concur with those with lower presence of chlorophyll-*a*. However, this could be an indicator that in the site where the experiment was located, the food available was enough to fulfill the high growth rates observed. A plausible explanation is that the notable frequency of steady currents in the sampling site (authors. pers. observation) might provide the necessary food by water renovation even when chlorophyll-*a* concertation is relatively low. Wu and Shin (1998) found a higher growth rate in suspended culture individuals compared to those in the bottom, suggesting that higher current flow in the water column may enhance the food availability. In this scenario, indirect correlations observed between chlorophyll-*a* and seasonal growth rates would be due to seasonal of phytoplankton blooms peaking in spring and autumn and decreasing in summer following nutrient trends; these patterns being typical of temperate seas (Cognetti et al., 2001; Miller, 2009) (Figure 5B). Similarly, the indirect correlation between seasonal growth and DO would be the result of the indirect correlation between temperature and DO (Figure 5B). It is worth noting, however, that during the two years, the maximum peaks of seasonal growth have been observed after the thermocline rupture in November, coinciding with increasing chlorophyll-*a* concentration (above 0.4 µg/L) and still moderate water temperatures (above 20 ºC). Furthermore, as soon as temperature drops below 20 ºC, growth rates fall drastically, despite higher chlorophyll-*a* concentrations. This could potentially imply that *P. rudis* might have grown even more than observed during the warmest summer months, if food (chlorophyll-a) had been more abundant during these period, but also that, despite increased food availability, growth drops below a certain water temperature (ca. 20ºC). Food availability has been pointed out as the most important factor modulating growth rates in bivalves (Gosling, 2015), although Killam and Clapham (2018); Saulsbury et al. (2019) found temperature as the best predictor for it, especially for bivalves from temperate seas. As observed in the present study, both factors could actually play an important role modulating growth, sometimes adding or sometimes counteracting their effects, depending on their annual variability and the species tolerance limits to their variation.

The K value of the growth model is much higher than the obtained by Nebot-Colomer et al. (2016) for other *P. rudis* populations and by García-March et al. (2019) and Prado et al. (2020) in different *P. nobilis* populations (maximum value of 0.39 for the population of the Fangar Bay). On the contrary, the L_∞ obtained in the present study is lower than that of other populations of Mediterranean Pinnids (García-March et al., 2019; Nebot-Colomer et al., 2016; Prado et al., 2020), and lower than could be expected for a *P. rudis* growing in, supposedly, good natural habitats conditions. Individuals located in February 2018 probably settled during the recruitment the previous year (Deudero et al., 2017), but they grew slowly while they were attached to the ropes of the aquaculture installation (34.9 $\pm$ 6.2 mm when found in February vs 48.6 $\pm$ 8.8 mm when collected in June 2018). Environmental conditions have been proved to modify the size and growth of individuals in *P. nobilis* (García-March et al., 2019) and it is likely that the extreme conditions of being attached to a rope at shallow depths, constrained growth and forced the small size of individuals. Placing them in the suspended cages in June 2018 could have resulted in enhanced growth rates after months of detrimental conditions compared to what would be expected under normal recruitment conditions, but also had deleterious effects limiting their asymptotic size.

The present study has shown that the size of *P. rudis* can be strongly affected under harsh circumstances limiting their growth. Under normal conditions, growth decreases with temperatures below 20ºC, which means that shorter and warmer winters, as predicted in climate models for the Mediteraean Sea (Molina et al., 2020; Moraitis et al., 2019), will extend the seasonal growth of *P. rudis*. Faster growth is expected to reduce mortality by predation (Kersting and García-March, 2017) and, therefore, increase population density, which could lead, eventually, to an expansion of its ecological niche over a part of that left empty by *P. nobilis*.

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