



Fecundity strategy of the highly exploited limpet *Patella ordinaria* from an oceanic archipelago

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ABSTRACT

Limpet harvesting dates to the 15th century in Madeira archipelago (NE Atlantic Ocean). This activity is size-selective, removing older and larger individuals with greater commercial value. It can lead to a lower reproductive output as individual fecundity is size-dependent. The decrease of limpet populations has led to the implementation of a series of management measures including a closed season during the reproductive season (November–March). So far, sizes and the reproductive parameters, size and age at first maturity, have been previously used to determine the exploitation status of *Patella ordinaria* in Madeira archipelago, with no study performed on the reproductive strategy of this limpet. This is pivotal to comprehend the population dynamics of any species, being fecundity one of the reproduction parameters that should be analysed. This work aims to provide for the first time data on the fecundity of *P. ordinaria*. An average of 17 females per month were randomly collected over the spawning 2021/2022 season from the subtidal rocky shores of the Madeira archipelago. Four criteria were analysed over the spawning season: (i) the presence of a hiatus between pre- and vitellogenic oocytes; (ii) the number and (iii) mean size of advanced vitellogenic oocytes; and (iv) the incidence of atresia. A hiatus between pre- and vitellogenic oocytes across the spawning season was observed. An increasing trend in the number of vitellogenic oocytes was observed over the spawning season. The size of vitellogenic oocytes showed significant differences among months and the relative intensity of atresia varied from 7.86 to 20.79%. The information on the fecundity strategy leads to a better understanding of the reproductive capacity of this species. It also identifies the best approach for future estimations of relative annual fecundity estimations, with direct implications on the sustainable exploitation of this commercial species.

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1. Introduction

Harvesting affects mollusc assemblages in coastal areas (Ramírez et al., 2009). This extractive activity leads to direct and indirect disturbances of intertidal populations (Kingsford et al., 1991; Lindberg et al., 1998). They disrupt essentially the abundance and size structure, and may induce alterations on the

reproductive output and replenishment of the exploited populations (Sousa et al., 2019b). This is due to the size selective nature of harvesting that removes the largest individuals with greater commercial value (Riera et al., 2016; Sousa et al., 2019b). The decrease of large individuals in accessible coastal areas has a cascading effect that results in changes in their life history, demography, reproductive output, genetics, and changes in ecological interactions and limpet behaviour (Fenberg and Roy, 2008; Espinosa et al., 2009; López et al., 2012), making them highly vulnerable, such as the case of *Patella ferruginea* Gmelin, 1791 on Algerian islands (SW Mediterranean) (Espinosa, 2009). In more extreme cases it can lead to extinction (Guerra-García et al., 2004;

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Núñez et al., 2003). Low connectivity between oceanic islands is equally worrisome as it could also lead to local extinction events (Bird et al., 2007; Goldstien et al., 2009). The risk of complete extinction of the Macaronesian *Patella candei* d'Orbigny complex is considered plausible due to the endemic nature, which shows low genetic diversity and lack of genetic exchange between the archipelagos, and the negative impacts of over-exploitation in coastal communities (Faria et al., 2017) and temperature increase on larval development and survival (Rodríguez et al., 2020).

Harvesting impact has been observed in the archipelago of Madeira for *Patella candei* d'Orbigny 1840 and *Patella aspera* Röding, 1798 (Sousa et al., 2019b), in the Canary Islands for *P. candei* and *P. candei crenata* d'Orbigny 1838 (Ramírez et al., 2009; Núñez et al., 2003), in the Azores for *P. candei* and *P. aspera* (Martins et al., 2008) and in Algeria and Spain for *Patella ferruginea* (Espinosa, 2009; Espinosa et al., 2009). The sharp decrease of limpet populations in Madeira has led to the implementation of various management measures including a minimum harvesting size of 40 mm of total shell length and the establishment of a closed season, from November to March (Portaria n.º 151/2022) to avoid disturbance during the reproductive season (Sousa et al., 2019b). These approaches attempt to preserve the size and age structure, avoiding large changes in sex ratios, switches to early maturation and sperm restriction (Sousa et al., 2019b). Currently, *P. aspera* and *P. ordinaria* are landed in a mixed exploitation that represents ca. 1.5% of the total fisheries in the region with an average of 4C per kg (Sousa et al., 2019b). In 2021, the commercial landings reached an annual catch of 88 tonnes yielding a first value of ca. 0.47M€ (source: Regional Directorate of Fisheries). Despite representing only a small portion of the local fisheries it has a high socio-cultural importance (Sousa et al., 2019b).

The intertidal limpet *Patella candei*, recently denominated *P. ordinaria* (Faria et al., 2017) and *P. tenuis* (Titselaar, 2019), lives on the rocky shores of the archipelago of Madeira from the lower intertidal to 5 m (Côrte-Real et al., 1996). This endemic species breeds during winter with an estimated shell length at maturity of 36.7 mm that corresponds to 1.89 years of age (Henriques et al., 2012). The ripe stage can be observed throughout all year, though in higher proportion in winter (Pinto et al., 2010). Contrastly, spawning maturity stage can only be observed in winter and spring seasons (Pinto et al., 2010). Recruitment occurs throughout the year with a major peak in January (Henriques et al., 2012).

The reproductive dynamics of *P. aspera* and *P. ordinaria* in the Madeira archipelago are thought to have changed due to the continuous long-term exploitation (Sousa et al., 2019b). The knowledge of potential fecundity is useful when determining the timing and magnitude of the arrival of recruits on the shore (Skjærraasen et al., 2006). Unfortunately, fecundity data of this key group of intertidal molluscs, the Patellid limpets is greatly limited to species other than the species under study, e.g., on *Patella vulgata* Linnaeus, 1758 from southwest of England (Ballantine, 1961), *Patella ferruginea* Linnaeus, 1758 from Ceuta (Espinosa et al., 2006) and from the Chafarinas Islands (Gualart et al., 2020), *Scutellastra granularis* (Linnaeus, 1758) from the southeast coast of South Africa (Vat, 2000), *Patella rustica* Linnaeus, 1758 of the Mediterranean (Prusina, 2013; Prusina et al., 2014), *Helcion pectunculus* (Gmelin, 1791) from both the SW and SE coasts of South Africa (Gray and Hodgson, 2003), *Cellana nigrolineata* (Reeve, 1854) from Japan (Catalan and Yamamoto, 1993), *C. sandwicensis* (Pease, 1861) from Hawaii (Mau et al., 2018), *C. denticulata* (Martyn, 1784) (Walters, 1994), *C. flava* (Hutton, 1873) (Walters, 1994), *C. ornata* (Dillwyn, 1817) (Dunmore and Schiel, 2000) and *C. radians* (Gmelin, 1791) (Creese and Ballantine, 1983) of New Zealand, and the Antarctic limpet *Nacella concinna* (Strebel, 1908) (Dopchiz et al., 2018). Up to this point, sizes and the reproductive parameters, body size and age at first maturity

Table 1

Number (N) of individuals and the mean total length (TL, mm) and respective standard deviation (SD) of *Patella ordinaria* from the archipelago of Madeira (NE Atlantic Ocean) sampled between November 2021 and March 2022. Min Minimum, Max Maximum.

Month	N	Mean \pm SD	Min. – Max.
November	10	46.51 \pm 1.96	42.87 – 49.22
January	18	42.96 \pm 3.84	34.52 – 49.77
February	16	54.02 \pm 4.92	41.61 – 62.91
March	26	51.50 \pm 5.73	40.45 – 61.37

have been previously used to establish the exploitation status of *P. ordinaria* in the archipelago of Madeira (Henriques et al., 2012; Sousa et al., 2019a,b, 2020), with only one recent study performed on female fecundity of both patellid limpets, *P. candei* and *P. aspera* (Castejón et al., 2022). This is pivotal to comprehend the population dynamics of any species, being fecundity one of the reproduction parameters that should be analysed. Besides, knowledge of fecundity is critical in fisheries since it determines the method chosen for the estimation of egg production method that should be applied to provide fishery-independent estimates of spawning biomass in fisheries assessment (Jennings et al., 2001; Ganiás, 2013).

This work aims to define the reproductive strategy of the limpet *P. ordinaria* from the archipelago of Madeira, to be included in an ongoing study that extends metacommunity models based on stochastic patch occupancy dynamics (Leibold and Chase, 2017) to metacommunities accounting for complex life cycles species along exploitation gradients and fragmented ecosystems to predict future scenarios. In this sense, the present study aims to (i) characterize the gonads structure at a microscopic level to provide a better understanding of the maturity stages of *P. ordinaria* along the spawning season and to (ii) identify the fecundity strategy of this species.

2. Material and methods

2.1. Sampling

Since in Madeira the population of *P. candei* (now *P. ordinaria*) are winter breeders (with a spawning peak from November to March) (Pinto et al., 2010; Henriques et al., 2012), sampling took place between November 2021 and March 2022 where a total of 70 females (34.52–62.91 mm TL, Table 1) of the species *P. ordinaria* were collected throughout the mid-to-lower intertidal zone of the rocky shores of 3 coastal areas located on the South (Funchal; sampled in February) and North (São Vicente and Porto Moniz; sampled in November and January, respectively) coasts of the island of Madeira and 1 on the South coast of the island of Porto Santo (Porto dos Frades; sampled in March) by an experienced diver.

As winter breeders, we had limitations in the acquisition of mature individuals due to frequent rough sea conditions (Torres and Andrade, 2010; Castejón et al., 2022). Limpets were processed fresh and total shell length (SL, to the nearest 0.01 mm) was recorded. A small incision was made in the mantle using a scalpel and the gonads gently removed from each individual (Cañizares et al., 2021), weighted (GW, to the nearest 0.01 g) and then preserved as a whole in Roti-Histofix ECO PLUS for later histological examination.

2.2. Histology

Small portions of gonad were taken, dehydrated with ethanol at different concentrations (70, 90 and 95%), and embedded in

Technovit 7100 resin (Vasconcelos et al., 2017). The oocyte distribution on the anterior, middle, and posterior regions of the ovary was analysed in histological sections (5 μm). Since no significant differences were found on the oocyte distribution among the 3 regions, sections were made on the middle region of the ovary, stained with methylene blue and digitized using a visual image analysis system (Leica DM2700 P microscope coupled to the Leica DFC 450 C camera and the software Leica Application Suite X – LAS 3.7, Leica Microsystems). Microscopic maturity stages and oocytes classification into different stages of development were assigned according to Prusina et al. (2014) (adopted and modified from McCarthy et al., 2008 and Belkhdja et al., 2011): inactive, early development, late development, ripe, atresic, spawning and spent.

2.3. Fecundity

To investigate female fecundity (number of oocytes per mature gonad) of *P. ordinaria*, we first studied the four lines of evidence suggested by Hunter (1992), Greer Walker et al. (1994), Murua et al. (2003) to determine the type of fecundity: (i) the presence/absence of an hiatus between previtellogenic and vitellogenic oocytes; (ii) the number of vitellogenic oocytes over the spawning season; (iii) mean size of vitellogenic oocytes over the spawning season; and (iv) the incidence of atresia over the spawning season. For the first line of evidence, digitized images of 25 histological sections in the ripe maturity stage and 33 in the spawning (beginning) stage were analysed along the spawning season. Oocyte diameter measurements were performed on 2,169 oocytes with visible nucleus using the ImageJ software v1.53r (<http://imagej.nih.gov/ij/>) (Schneider et al., 2012). For the second and third lines of evidence, the gravimetric method (Hunter et al., 1989) was applied to 58 ovaries of different limpet length classes. From each ovary, three samples of approximately 0.02 g each were extracted, and each sub-sample placed in a tube on a magnetic stirrer to be smoothly stirred and assist the separation of the smaller oocytes. The resulting sample was then passed through a 100- μm mesh sieve (threshold size equivalent to early vitellogenic oocytes) with the help of a wash bottle, to remove the smaller oocytes. The remaining oocytes were placed in a Petri dish and photographed with the Leica S APO Stereo Microscope coupled to the Leica MC170 HD camera and the software Leica application suite (Leica Microsystems). The number and size of advanced vitellogenic oocytes were registered. For the fourth line of evidence, the relative intensity of atresia was calculated as a percentage of atresic vitellogenic oocytes in relation to total number of normal oocytes present in the ovary (Hunter and Macewicz, 1985). Gonads in atresic stage were not considered for this line of evidence. The prevalence of atresia (defined as the proportion of females with oocytes in atresia in the total number of females) was also investigated. The gravimetric method was applied to estimate the absolute batch fecundity. From examining the histological sections, 25 females in the ripe stage were selected and the number of vitellogenic oocytes counted throughout the spawning season. Counts per specimen may be underestimated, due to some oocyte loose or damage during extraction from the gonad sample. These shortcomings have been pointed out by previous researchers (Guallart et al., 2020).

2.4. Statistical analysis

Differences in mean number and mean oocyte diameter throughout the spawning season were tested. Data were analysed for deviations to the parametric assumptions of analysis of variance (ANOVA). Normality of the distribution of the data was verified through the Shapiro–Wilk test (*ggpubr* R package,

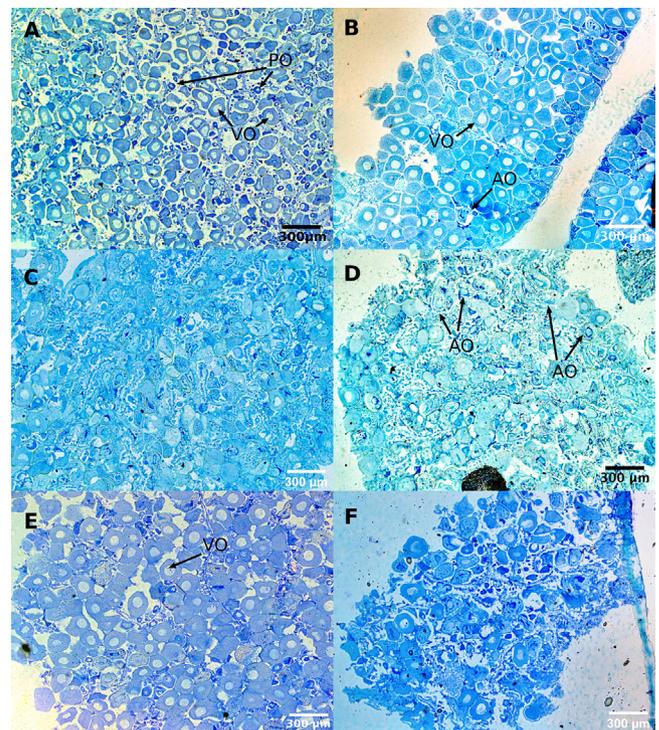


Fig. 1. Transverse sections of gonads of the limpet *Patella ordinaria* from the archipelago of Madeira (NE Atlantic Ocean) in the maturity stages of late development (A), ripe (B), atresic (C, D), spawning (E) and spent (F). AO Atretic oocyte, PO Previtellogenic oocyte, VO Vitellogenic oocyte. Scale bar 300 μm .

Kassambara, 2020; R-Core-Team, 2022). The homogeneity of variance was determined through the Levene's Test (*car* R package, Fox and Weisberg, 2019). As ANOVA assumptions were not met, a non-parametric alternative to one-way ANOVA test was used. The null hypothesis of equal oocyte size medians was tested across the spawning season with the Kruskal–Wallis rank sum test (*stats* R package, R-Core-Team, 2022), without assuming them to follow the normal distribution. The post-hoc Pairwise Wilcoxon Test for non-parametric comparison was applied to calculate pairwise comparisons across the spawning season with corrections for multiple testing (*stats* R package, R-Core-Team, 2022). The method selected for adjusting p values was the “BH” (Benjamini and Hochberg, 1995), a more powerful method than the family-wise error rate. The Kruskal–Wallis rank sum test was used to compare relative intensity of atresia throughout the spawning season. Relationship between absolute batch fecundity and total length was investigated using the Pearson correlation test (*ggpubr* R package, Kassambara, 2020).

3. Results

3.1. Histological analysis

In *P. ordinaria* the oocyte development showed two distinct cohorts of oocytes in the ovaries during the spawning period, one of primary growth (previtellogenic oocytes) and other of secondary growth (vitellogenic oocytes) (Figs. 1 and 3). The last ones are the oocytes to be spawned during the current spawning season and the primary growth oocytes are to be spawned in future spawning seasons. From the histological examination of gonads, it was possible to identify the maturity gonads stages of late development ($n = 1$), ripe ($n = 25$), atresic ($n = 8$), spawning ($n = 33$) and spent ($n = 3$) (Fig. 1). During the whole sampled period, part of the oocytes undergoes lysis and different stages

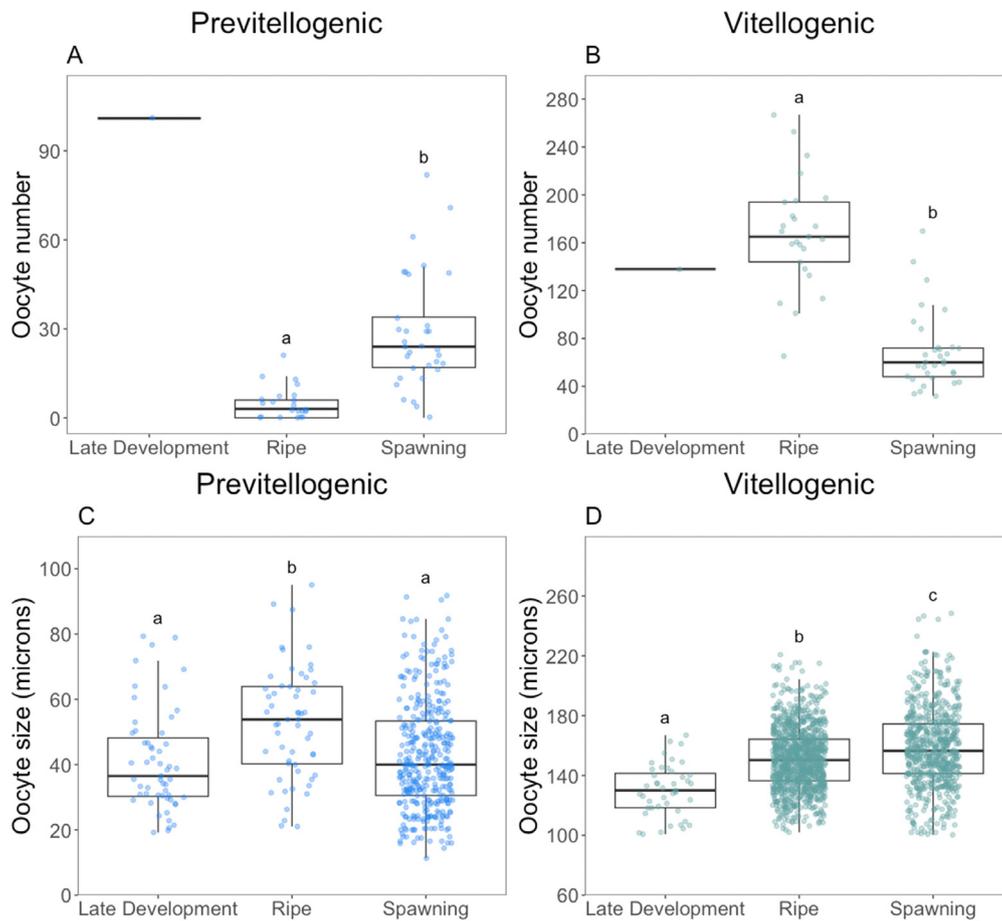


Fig. 2. Number and size of previtellogenic (A and C) and vitellogenic oocytes (B and D) in the maturity stages of *Patella ordinaria* from the archipelago of Madeira (NE Atlantic Ocean). Boxplot showing the minimum and maximum values, the 25th percentile, the median and the 75th percentile.

of atresia, particularly at the beginning of the spawning season. From November to March, females were found in different stages of maturation. In November (70%, n = 7) and January (78%, n = 14), the spawning was the most abundant stage of maturation whilst in February (56%, n = 9) and March (62%, n = 16), the ripe stage was the most abundant.

3.2. Fecundity

Regarding the number of previtellogenic oocytes (Levene's test: $W = 1.0445$, $p > 0.05$) and size ($W = 2.0198$, $p > 0.05$), we can assume the homogeneity of variances across months. Number ($W = 4.8382$, $p < 0.05$) and size ($W = 6.2207$, $p < 0.001$) of the vitellogenic oocytes across months did not exhibit homogeneous variance. The size and number of both previtellogenic and vitellogenic oocytes showed a non-normal distribution (Shapiro-Wilk test: $p < 0.001$).

The number and size of previtellogenic and vitellogenic oocytes was analysed per maturity stage (Fig. 2). The average number of previtellogenic oocytes increased from ripe to spawning stages (Kruskal-Wallis: $H = 32.448$, $df = 2$, $p < 0.001$, Fig. 2A). In contrast, the number of vitellogenic oocytes decreased (K-W: $H = 35.476$, $df = 2$, $p < 0.001$, Fig. 2B). A decreasing trend in the size of previtellogenic (K-W: $H = 18.448$, $df = 2$, $p < 0.001$, Fig. 2C) and an increasing trend in the size of vitellogenic (K-W: $H = 75.086$, $df = 2$, $p < 0.001$, Fig. 2D) oocytes were observed. The post-hoc Pairwise Wilcoxon Test showed that the ripe maturity stage was responsible for the differences found in the size of previtellogenic oocyte ($p < 0.05$). For the size of vitellogenic oocyte, all maturity stages were significantly different ($p < 0.05$). The size of the

vitellogenic oocytes ranged between 80 – 223 μm ($145 \mu\text{m} \pm 21$, mean \pm SD) in the ripe stage, whereas in the spawning stage ranged from 83 – 248 μm ($158 \mu\text{m} \pm 25$, mean \pm SD) (Fig. 2D).

Fecundity strategy was analysed under the four above-mentioned criteria. A hiatus between pre- and vitellogenic oocytes was observed (Fig. 3), indicating a discontinuous oocyte recruitment where the standing stock of oocytes is well defined during the spawning season. The average number of previtellogenic oocytes decreased significantly across the spawning season, from November to March (K-W: $H = 26.25$, $df = 3$, $p < 0.001$, Fig. 4A). During the same period, the average number of vitellogenic oocytes increased significantly (K-W: $H = 30.299$, $df = 3$, $p < 0.001$, Fig. 4B), but with no significant differences between November and January ($p > 0.05$), and February and March ($p > 0.05$). The average size of the previtellogenic (K-W: $H = 22.306$, $df = 3$, $p < 0.001$, Fig. 4C) and vitellogenic oocytes (K-W: $H = 26.981$, $df = 3$, $p < 0.001$, Fig. 4D) varied significantly across the spawning season with February and November, the months responsible for the differences. The prevalence of atresia was 95.71%. The atresia stage (Fig. 5) varied from 7.86% in November to 20.79% in January ($17.08 \pm 14.33\%$, mean \pm SD), with no significant differences across the spawning season (K-W: $H = 6.722$, $p > 0.05$) except in November and January (Pairwise Wilcoxon Test: $p < 0.05$). Total fecundity, estimated based on females in the ripe stage was on average $385,613 \pm 194,902$ oocytes, ranging from 92,098 (45.85 mm SL) to 804,183 oocytes (58.51 mm SL). A significant positive relationship was found between fecundity and total shell length (Pearson's correlation: $R = 0.837$, $p < 0.001$, Fig. 6).

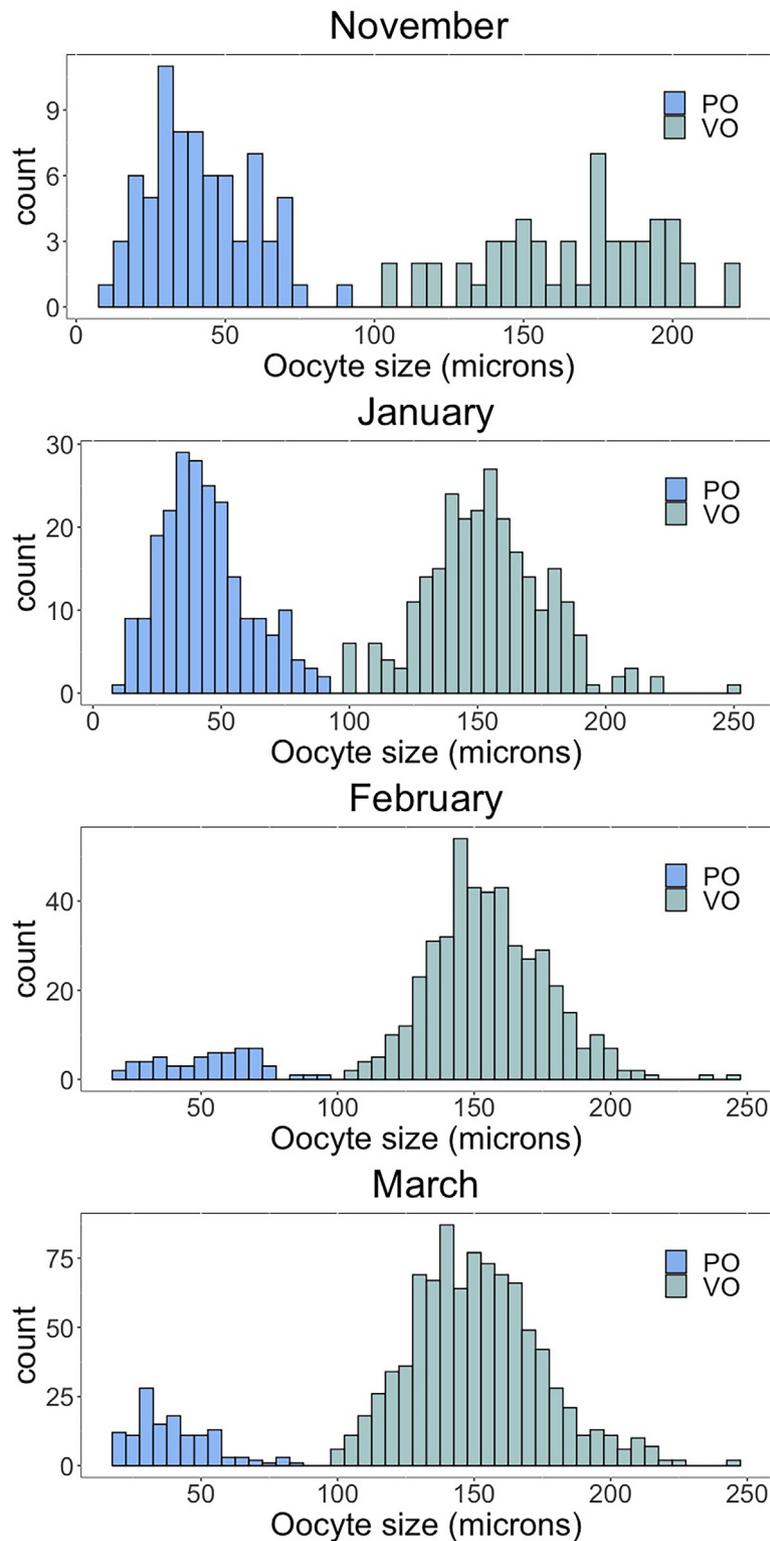


Fig. 3. Oocyte size frequency distribution in the limpet *Patella ordinaria* sampled across the spawning season (November to March) in the archipelago of Madeira (NE Atlantic Ocean). PO Previtellogenic oocytes, VO vitellogenic oocytes.

4. Discussion

In the present study all lines of evidence to assess fecundity strategy were analysed in the limpet *P. ordinaria*. First, the presence of a distinct hiatus in the frequency distribution of oocyte size between previtellogenic and vitellogenic oocytes indicates a disrupted oocyte recruitment, and that fecundity is well fixed

before the onset of spawning (Hunter, 1992). At least two cohorts of oocytes were recognized, a smaller fraction of previtellogenic oocytes and a larger one representing the vitellogenic oocytes. This bimodal distribution of oocyte frequencies was also reported throughout the year for *S. granularis* from the south-east coast of South Africa (Vat, 2000) and *P. rustica* from the south-eastern Adriatic (Prusina et al., 2014). The average size of vitellogenic

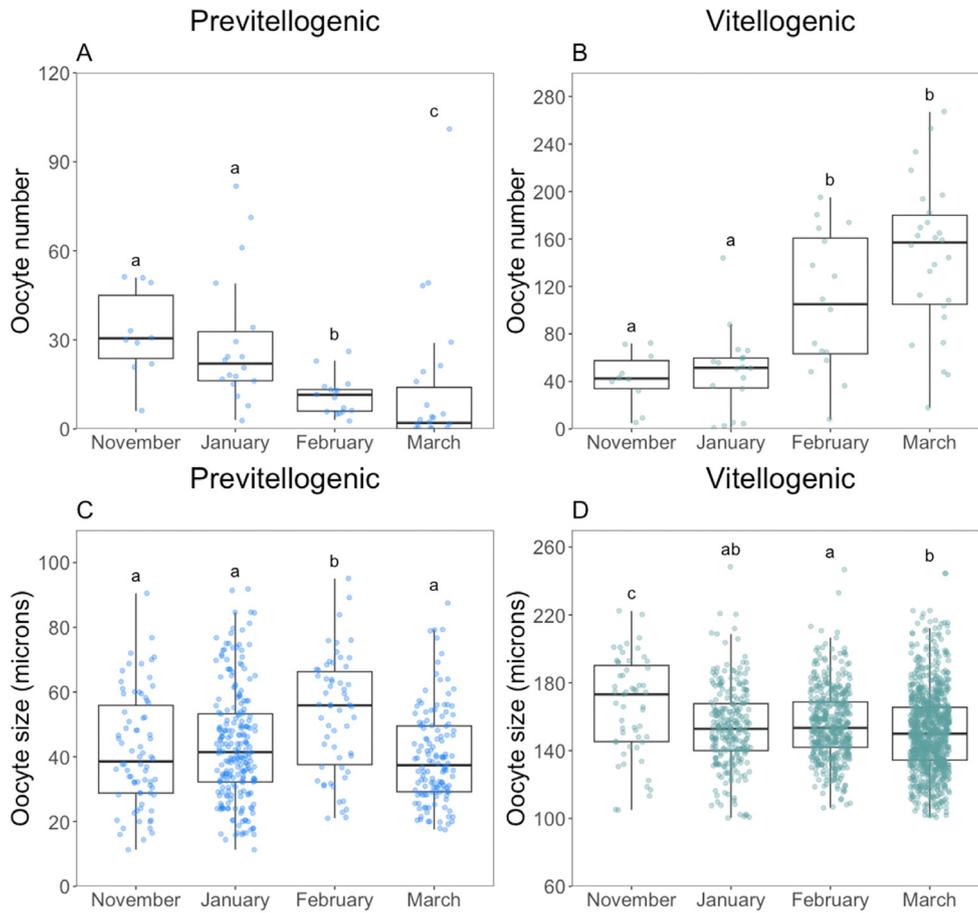


Fig. 4. Monthly variation in the number and size of previtellogenic (A and C) and vitellogenic oocytes (B and D) of *Patella ordinaria* from the archipelago of Madeira (NE Atlantic Ocean). Boxplot showing the minimum and maximum values, the 25th percentile, the median and the 75th percentile.

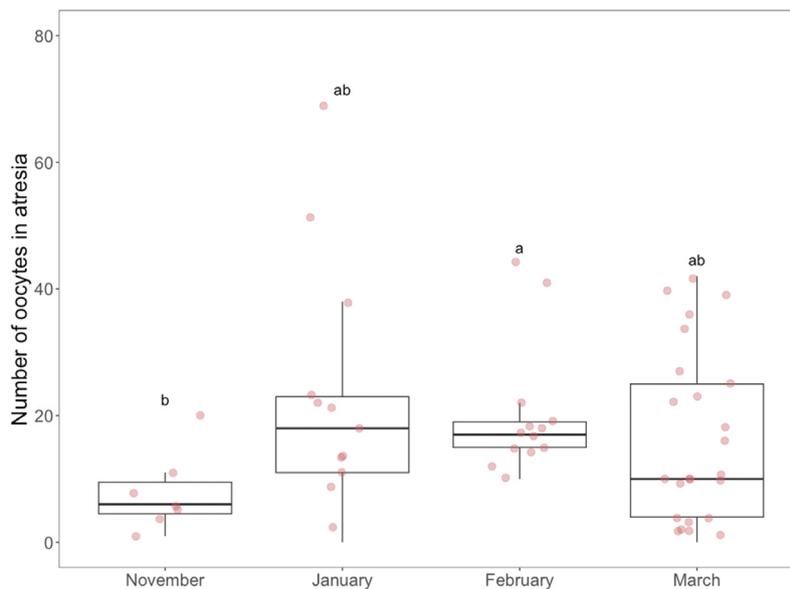


Fig. 5. Relative intensity of atresia estimated for *Patella ordinaria* from the archipelago of Madeira (NE Atlantic Ocean) across the spawning season. Boxplot showing the minimum and maximum values, the 25th percentile, the median and the 75th percentile.

oocytes of *P. ordinaria* ($145 \mu\text{m} \pm 23$; $80 - 248 \mu\text{m}$) was very similar to those documented for other limpet species, such as, *P. ferruginea* from Ceuta, whose mature oocytes had an overall mean

diameter of $149.78 \mu\text{m} \pm 8.32$ (ranging from 142.26 ± 10.05 to $169.9 \mu\text{m} \pm 17.15$) (Espinosa et al., 2006), *H. pectunculus* from both the SW and SE coasts of South Africa with a mean diameter

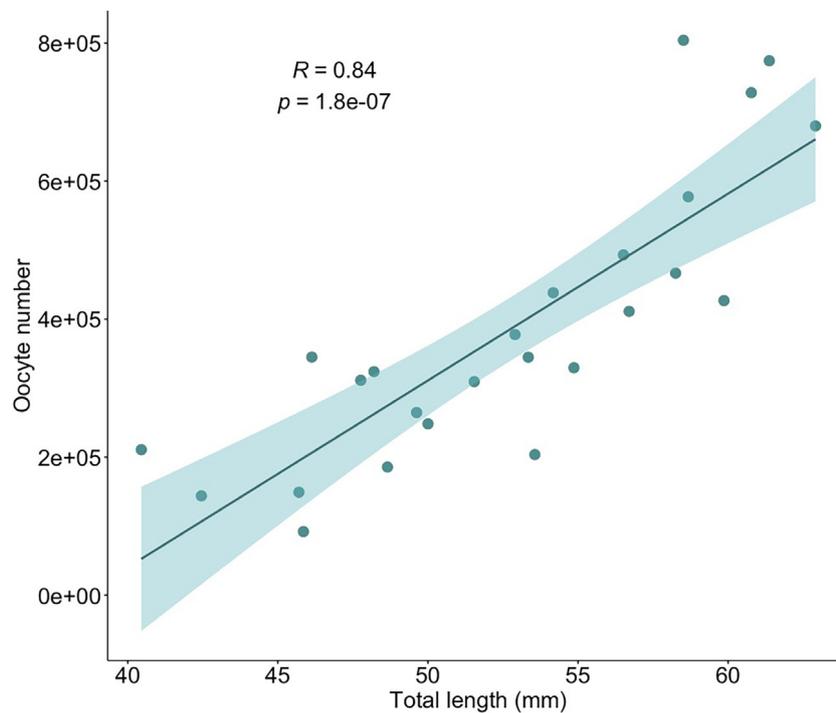


Fig. 6. Linear relation between absolute batch fecundity and total length (TL, mm) for *Patella ordinaria* from the archipelago of Madeira (NE Atlantic Ocean). 95% confidence interval for the model is given by the green area.

of $>120 \mu\text{m}$ (Gray and Hodgson, 2003), *P. vulgata* from the British Isles with an average size of $160 \mu\text{m}$ (Hill, 2000), *S. granularis* from the south-east coast of South Africa with diameter of mature oocytes ranging from 125 to $270 \mu\text{m}$ (Vat, 2000), *Nacella* (*P.*) *deaurata* from Beagle Channel, whose mature oocytes in ripe stage had a diameter that ranged from 120 to $150 \mu\text{m}$ (Morriconi, 1999) and *C. sandwicensis* from Hawaii with spawned eggs with a mean diameter of $136.95 \pm 5.02 \mu\text{m}$ (Mau et al., 2018). *P. rustica* of the Mediterranean ($104.5 \pm 49.6 \mu\text{m}$) (Prusina, 2013; Prusina et al., 2014) and *C. nigrolineata* from Japan (119 – $136 \mu\text{m}$) (Catalan and Yamamoto, 1993) showed lower average oocyte sizes than those reported for the above limpet species. On the contrary, *N. concinna* oocytes showed a much higher average ($189.10 \pm 2.50 \mu\text{m}$; (Dopchiz et al., 2018)) than former species, including the study species.

Secondly, the mean number of previtellogenic oocytes decreased while the vitellogenic oocytes increased over the spawning season. A decrease in the number of vitellogenic oocytes was expected at the end of the spawning season since a hiatus was observed between the two cohorts, corroborating the fact that no new vitellogenic oocytes are recruited to replace those that have been shed. Thirdly, the mean diameter of the vitellogenic oocytes did not increase significantly over the spawning season though a dominant size class of oocytes was observed progressing from November to March. These unexpected results (read Vieira et al., 2016 for determinate fecundity insights) in the second and third lines of evidence are most certainly due to the higher number of females in spawning stage observed in November and January, together with the higher percentage of gonads in ripe stage in February and March. In ripe stage, the mean diameter of vitellogenic oocytes is smaller than those observed in spawning stage. In fact, when the number and size of vitellogenic oocytes were analysed per maturity stage, a significant decrease in the number and an increase in the mean size of the vitellogenic oocytes were observed. Furthermore, the spawning females sampled in November and January were harvested on the northern coast of Madeira Island (Porto Moniz and São Vicente). In February and

March, females in ripe stage were sampled on the southern coasts of Madeira (Funchal) and Porto Santo Island (Porto dos Frades). These differences in the reproductive stage associated with the sampling site may explain the absence of an increasing tendency expected in the average size of vitellogenic oocytes. Finally, the relative intensity of atresia was observed in low percentages (7.86% – 20.79%). Low levels of atresia usually characterize determinate spawners and do not seem to have a greater effect on the potential fecundity (Hunter, 1992). In the present work, fecundity estimates for *P. ordinaria* were on average $385,613$ oocytes ($92,098$ – $804,183$; 46 - to 59 - mm SL), value much higher than the one estimated previously for the same species in the same region, which averaged $186,000$ oocytes ($12,000$ – 1.2×10^6 ; 37 - to 57 -mm SL) (Castejón et al., 2022). For *P. ferruginea* sampled in Ceuta, females larger than 80 mm contributed with a large number of oocytes, 2.3 to 4.5 million eggs (Espinosa et al., 2006) while in Chafarinas Islands, female fecundity varied from $189,200$ oocytes (40.0 mm SL) and $5,019,200$ oocytes (86.4 mm SL) (Guallart et al., 2020). In the southwest of England, fecundity of *P. vulgata* ranged from $27,000$ eggs (28 mm SL) to $500,000$ eggs (52 mm SL) (Ballantine, 1961). The largest females of *Cellana ornata* of the southern New Zealand were estimated to have between $200,000$ and $360,000$ eggs (Dunmore and Schiel, 2000), and over $19,700$ and $26,000$ eggs spawned by *C. denticulata* and *C. flava* respectively, per spawning episode (Walters, 1994). The estimated reproductive output of *C. radicans* ranged from $13,000$ to $80,000$ oocytes (22 to 34 mm SL) (Creese and Ballantine, 1983). The reproductive fitness of *S. granularis* from the south-east coast of South Africa varied according to the substrate, from $366,000$ eggs in limpets inhabiting aeolianite to $119,500$ in quartzite (Vat, 2000). A direct relationship between fecundity and other life-history traits such as female size may be observed (Llodra, 2002), a key feature of reproduction in many taxa related to morphological and physiological restrictions in energy provision and gonad production (Llodra, 2002). *P. ordinaria* exhibits size-dependent fecundity, very common for many broadcast-spawning benthic invertebrates (Llodra, 2002). The results of female fecundity are

in agreement with other species such as *P. ferruginea* (Espinosa et al., 2006; Guallart et al., 2020), *C. ornata* (Dunmore and Schiel, 2000) and *C. sandwicensis* (Mau et al., 2018). Yet, this correlation was not clear for the same species sampled in Madeira, probably masked by differential gonadal development (Castejón et al., 2022). With increasing size, there is a continuing transition in the allotment of energy from growth to reproduction (Langton et al., 1987; Honkoop et al., 1998). Fecundity is intimately related to shell size since the inner shell volume represents the upper limit for reproductive (Nakaoka, 1994; Honkoop and Van der Meer, 1997). However, fecundity shows a wide variation within species, depending on nutrition, population density and adult age and size (Eckelbarger, 1986).

The knowledge of the fecundity strategy leads to a better understanding of the reproductive capacity and allows choosing the best approach to be used in future estimations of the relative annual fecundity (Vasconcelos et al., 2017).

For certain spawners (e.g., determinate spawners), the timing of sampling is critical for estimating potential fecundity. If sampling starts too early, there is a risk that the vitellogenic oocytes will not have developed enough to distinguish them from the pre-vitellogenic oocytes, resulting in inaccurate estimates. If it is carried out too late, spawning may already have taken place, the stock of the advanced oocytes will already have been reduced and the prospective fecundity will be underestimated (Hunter et al., 1989). For future estimates of relative fecundity, this information is essential to avoid underestimates of fecundity. Also, we recommend a more extended sampling period to clearly confirm the type of fecundity strategy of *P. ordinaria* from the Madeira archipelago.

The present results have pivotal implications in the assessment and sustainable exploitation of this resource in the archipelago of Madeira. Currently, the populations of *P. ordinaria* have been managed as a single stock and thus, with no variations in its fecundity features, however, we here observed clear differences in the fecundity of northern and southern populations of this species. This fact indicates that these two populations may have asynchronous spawning seasons. Therefore, both populations may need to be managed separately, in particular the closed season should take into consideration the specificities of these populations for their sustainable management in the archipelago of Madeira.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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