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FECUNDITY STRATEGY OF THE HIGHLY EXPLOITED LIMPET, *PATELLA ORDINARIA*, FROM AN OCEANIC ARCHIPELAGO

AUTORES E INSTITUIÇÕES CORRESPONDENTES:
J. Ferro[1], R. Sousa[2,3], R. Riera[4,5], M. Freitas[2,3] and J. Vasconcelos[3,5,6*]

INTRODUCTION

In the Madeira archipelago (NE Atlantic ocean), limpets have been harvested since the early days of colonisation, dating back to the early 15th century. Due to the continuous long-term exploitation, the reproductive dynamics of *Patella ordinaria* in the Madeira archipelago is thought to have changed. The knowledge of potential fecundity is useful when determining the timing and magnitude of the arrival of recruits on the shore. Unfortunately, there is a lack of fecundity data of the key group of intertidal molluscs, the Patellid limpets.

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 to metacommunities accounting for complex life cycles species along exploitation gradients and fragmented ecosystems to predict future scenarios.

The present study aims to:

(i) characterise the gonads structure at a microscopic level to provide a better understanding of the various maturity stages of *P. ordinaria* along the spawning season.

(ii) identify the fecundity strategy of this species.

MATERIAL AND METHODS

SAMPLING From November 2021 to March 2022, 70 females of the species *P. ordinaria* were randomly collected throughout the mid-to-lower intertidal zone of the rocky shores of Madeira archipelago (Funchal, Porto Moniz, Porto Santo, and São Vicente). Limpets were processed fresh and total length (TL, 0.01 mm) was recorded. All individuals were sexed, gonads dissected out and weighted (GW, 0.01 g), and ovaries preserved in Roti-Histofix ECO PLUS.

HISTOLOGY Small portions of gonad were taken, dehydrated with ethanol at different concentrations (70, 90 and 95%), and embedded in Technovit 7100 resin [1]. Histological sections (5 μm) were made, stained with methylene blue and digitized using a visual image analysis system. Microscopic maturity stages (MS) and oocytes classification into stages of development were assigned according to Prusina et al. [2].

FECUNDITY To investigate the fecundity strategy, the four lines of evidence suggested by Hunter [3], Greer Walker et al. [4], Murua et al. [5] were examined:

(i) presence/absence of an hiatus between previtellogenic (PO) and vitellogenic (VO) oocytes (using digitised images of histological sections from 25 females in ripe and 33 in spawning).
(ii) number of VO over the spawning season (gravimetric method applied to 58 ovaries)
(iii) mean size of VO over the spawning season (gravimetric method applied to 58 ovaries).
(iv) incidence of atresia over the spawning season.

For the 2nd and 3rd lines of evidence, a 100-μm mesh sieve was used, corresponding to the threshold size equivalent to early VO.

RESULTS AND DISCUSSION

A total of 70 females (34.52 - 62.91 mm TL, Table 1) were used in this study for the characterization of gonads structure and fecundity strategy.

Table 1
Number (N) of individuals and the mean total length (TL, mm) and 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 ± SD	Min. – Max.
November	10	46.51 ± 1.96	42.87 – 49.22
January	18	42.96 ± 3.84	34.52 – 49.77
February	16	54.02 ± 4.92	41.61 – 62.91
March	26	51.50 ± 5.73	40.45 – 61.37

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, PO) and other of secondary growth oocytes (vitellogenic oocytes, VO) (Fig.1 and 3). The latter are the oocytes to be spawned during the current spawning season and the primary growth oocytes will be spawned in future spawning seasons.

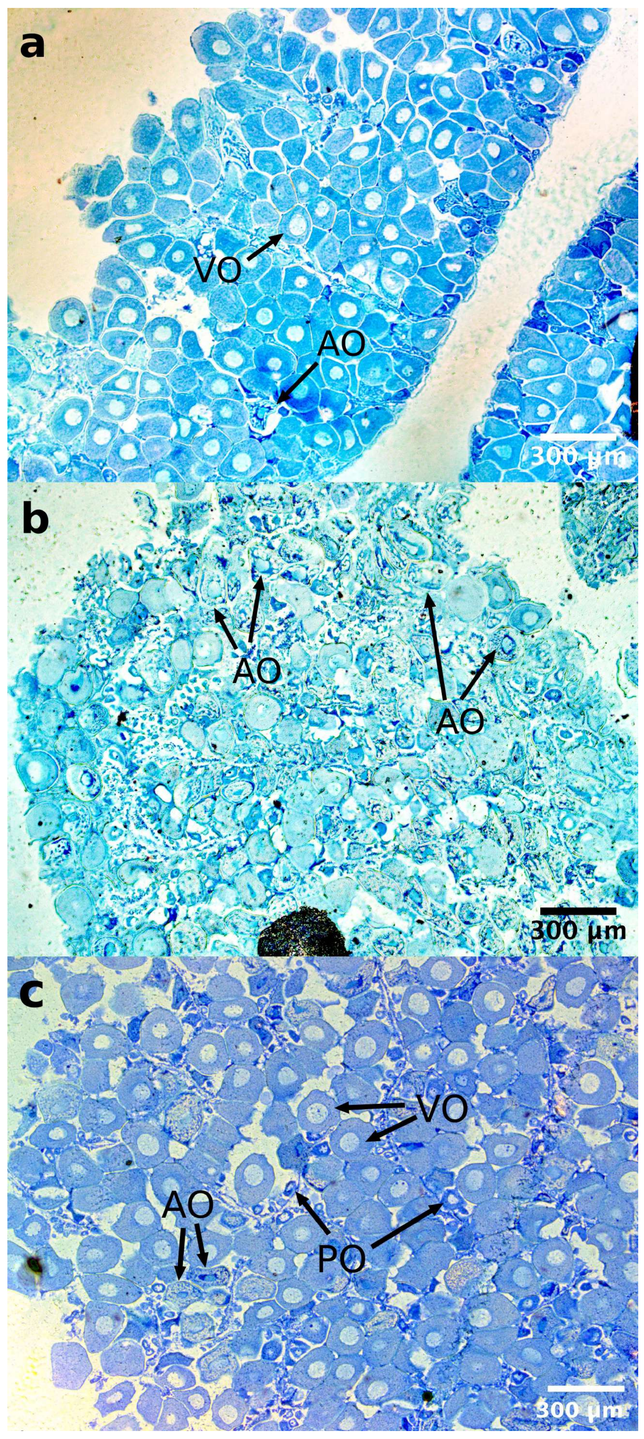

Figure 1 Transverse sections of gonads of the limpet *Patella ordinaria* from the archipelago of Madeira in the maturity stages ripe (a), atresic (b), and spawning (c). **AO** Atretic oocyte, **PO** Previtellogenic oocyte, **VO** Vitellogenic oocyte. Scale bar **300 μm**.

FECUNDITY

The median number of the PO increased from ripe to spawning stages (Kruskal-Wallis: H = 32.448, p = 8.993e-08, Fig.2A). In contrast, the median number of the VO decreased (H = 35.476, p = 1.979e-08, Fig.2B). A decreasing trend in the PO size (H = 18.448, p = 9.863e-05, Fig.2C) and an increasing trend in the VO size (H = 75.086, p < 2.2e-16, Fig.2D) were observed. The post-hoc Wilcoxon Test showed that the MS ripe was responsible for the differences found in the PO size (p < 0.05). For the VO size, all MS were significantly different (p < 0.05).

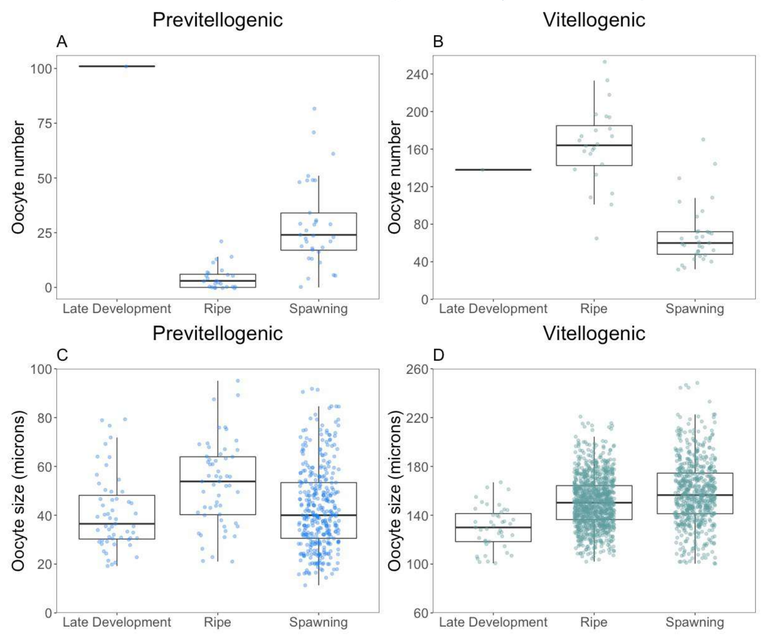

Figure 2 Number and size of previtellogenic (A and C) and vitellogenic oocytes (B and D) in the different maturity stages of *P. ordinaria* from the archipelago of Madeira.

Fecundity strategy was analysed under the four abovementioned criteria. First, the presence of a distinct hiatus in the oocyte size frequency distribution between PO and Vo (Fig.3), indicates a discontinuous oocyte recruitment where the standing stock of oocytes is well defined and fixed before the onset of spawning.

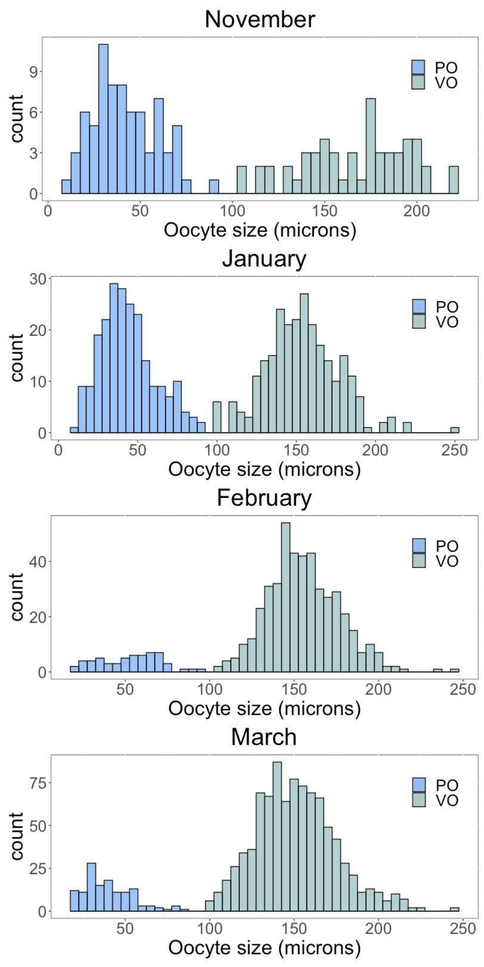

Figure 3 Oocyte size frequency distribution in the limpet *P. ordinaria* sampled across the spawning season (November to March) in the archipelago of Madeira. **PO** Previtellogenic oocytes, **VO** vitellogenic oocytes.

A decreasing trend in the median number of PO across the spawning season was observed (K-W: H=26.249, p=8.46e-06, Fig.4A). The Wilcoxon Test showed that only November and January are not statistically different (p = 0.250). In contrast, an increasing trend was verified in the median number of VO (H = 30.299, p = 1.194e-06, Fig.4B), though with no significant differences between November and January (p = 0.755), and February and March (p = 0.089). A decrease in the number of VO was expected since a hiatus was observed between the two cohorts corroborating the fact that no new VO are recruited to replace those that have been shed.

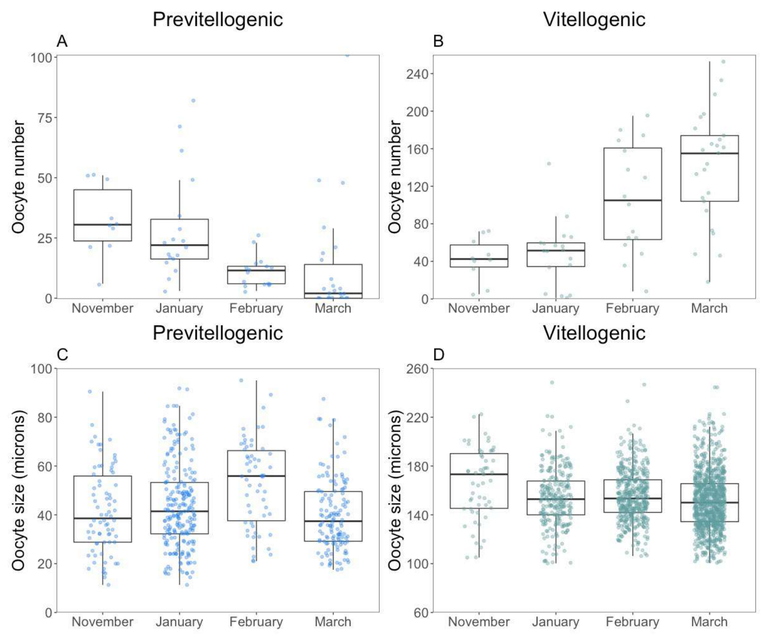

Figure 4 Monthly variation in the number and size of previtellogenic (A and C) and vitellogenic oocytes (B and D) of *P. ordinaria* from the archipelago of Madeira.

Significant differences were found for both PO (H = 22.306, p = 5.633e-05, Fig.4C) and VO (H = 26.981, p = 5.943e-06, Fig.4D) sizes across the spawning season. The Wilcoxon Test showed that February and November were the months responsible for the differences found (p < 0.05). It was expected a significant increase in the mean diameter throughout the spawning season. These unexpected results in second and third lines of evidence, is most certainly due to the higher number of females in the spawning stage observed in November and January and the higher number of gonads in the ripe stage in February and March. In the ripe stage, the mean diameter of the VO is smaller than those observed in the spawning stage. In fact, when the number of oocytes was analysed per maturity stage, a significant decrease was observed in the number of VO. Furthermore, the females observed in November and January in the spawning stage were sampled in the northern part of Madeira island (Porto Moniz and São Vicente). In February and March, females in the ripe stage were sampled in the southern part of Madeira (Funchal) and Porto Santo Island.

Finally, the relative intensity of atresia was observed in low percentages from 7.86% (November) to 20.79% (January) (17.08 ± 14.33%, mean ± SD) with no significant differences between months (K-W: H = 6.722, p = 0.081). Low levels of atresia usually characterise determinate spawners and do not seem to have a greater effect on the potential fecundity [3].

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. 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.

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[1]Faculdade de Ciências Exatas e da Engenharia, Universidade da Madeira, Funchal, Madeira, Portugal
[2]Direção de Serviços de Monitorização, Estudos e Investigação do Mar, Direção Regional do Mar, Funchal, Madeira, Portugal
[3]MARE – Marine and Environmental Sciences Centre / ARNET - Aquatic Research Network, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI), Funchal, Madeira, Portugal
[4]Grupo en Biodiversidad y Conservación, IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria,, Telde, Spain
[5]Departamento de Ecología, Facultad de Ciencias, Universidad Católica de la Santísima Concepción, Concepción, Chile
[6]Faculdade de Ciências da Vida, Universidade da Madeira, Funchal, Madeira, Portugal