



Article Dynamics of the Reproductive Cycle of Two Cerastoderma edule Populations (Óbidos and Ria Formosa Lagoons) along with Their Nutrient Storage and Utilization Strategy

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- **Abstract:** The reproductive performance knowledge of economically important bivalves is essential to establish better shellfishery management measures and aquaculture programs. The reproductive cycle, as well as the nutrient storage and utilization of two Portuguese *Cerastoderma edule* populations from Óbidos and Ria Formosa lagoons were characterized by evaluating gonadal development stages, gonadal and condition indexes, as well as glycogen and total lipid content, over several consecutive years. The relationship between reproduction and environmental parameters (sea surface temperature and chlorophyll *a*) was assessed. The reproductive cycle of both populations of *C. edule* followed a clear seasonal pattern. The onset of gametogenesis took place in early autumn for both populations, and its evolution coincided with a temperature decrease which progressed throughout the winter. The mature stage was reached in spring, followed by a spawning period during spring and summer, triggered by a rise in temperature. After spawning, both populations progressed to a prolonged inactive stage. The condition index reflected the accumulation and utilization of glycogen, which was considerably higher in the Óbidos population. However, no relationships were found in the reproductive cycle of the species. Total lipids remained similar not only during the gametogenesis period, but also during the spawning period, which may be related to successive and simultaneous

Keywords: *Cerastoderma edule;* reproductive cycle; condition index; biochemical composition; Portuguese populations; environmental conditions

Key Contribution: The energy allocation strategy of cockles exposes its fragility in self-maintenance and may partially justify the reportedly large inter-annual fluctuations in stock abundance in Ria Formosa.

1. Introduction

gamete production and release.

The common cockle (*Cerastoderma edule*) is a filter-feeding bivalve that is widely distributed along the northeastern Atlantic coast, from Norway to Mauritania, although absent from the Baltic and the Mediterranean Sea [1]. Moreover, it occurs mainly in both intertidal and shallow subtidal areas of temperate climate waters. *C. edule* is commercially and recreationally harvested in several countries, including the United Kingdom, France,



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Netherlands, Spain, and Portugal. With a relatively short lifespan and rapid growth, cockles mature quickly and have a high fecundity [2]. These life features, combined with overfishing, recruitment failure, lethal diseases, and a lack of effective management, have led cockle populations to large seasonal and inter-annual fluctuations in growth, abundance, biomass, and production [3,4].

Knowledge of the species' reproductive cycle and spawning periods is essential to strengthen the sustainability of cockle populations. From a reproductive point of view, *C. edule* is a dioecious species, with a pelagic larval stage of approximately one month after fertilization, afterwards switching to a benthic life after metamorphosis [5]. The reproductive biology of cockles has been extensively studied throughout its distribution area, with different records documenting their reproductive cycle. The interaction between endogenous (genetics) and exogenous factors (e.g., temperature, salinity, photoperiod, food availability) has been proven to be responsible for those differences [6–8].

The reproductive stage and annual cycles of *C. edule* can be determined through classical histological techniques that analyze sections of the gonad or indirectly through the application of condition indexes. These indexes constitute a useful measure of the physiological state of an organism under given environmental conditions, being an indicator of its reproductive output once they provide a quantitative measure of the gametogenic activity [7,9].

Studies on energy allocation in bivalves have shown that the energetic priorities of species may be variable [10–13]. The biochemical composition and cellular structure of bivalves undergo seasonal variations consonant with the reproductive cycle development, depending on the storage and utilization of energy [12–15]. The most common model consists of reserves accumulation before gametogenesis, mainly as glycogen, and then its use in the de novo synthesis of lipids for gametes formation [16,17]. However, this energetic dynamic varies among species, geographical origin, and environmental conditions [18–21]. The timing and rate of energy storage in bivalves are regulated by food and temperature [11,22–24]. Nevertheless, the effect of these variables is complex [13]. The study of the reproductive and energy allocation pattern of individuals in dynamic habitats contributes to a better understanding of the effects of exogenous factors on the physiological processes of organisms.

Although several studies have focused on the reproductive cycle of *C. edule* throughout its geographic range (e.g., [5,7,25–27]), the relative importance of energy allocation in relation to the reproductive cycle remains unknown for this species. Therefore, the current study aimed to characterize and evaluate the dynamics of the reproductive cycle of two cockle populations (Óbidos and Ria Formosa lagoons) along with their strategy for nutrient storage and utilization. This information is essential for implementing better fishery management measures to rebuild stocks and provide aquaculture programs.

2. Materials and Methods

2.1. Study Sites and Samples Collection

Samples were collected in two coastal lagoons, Óbidos and Ria Formosa, 290 km apart (Figure 1). Óbidos Lagoon is a small and shallow coastal system on the west Portuguese coast ($39^{\circ}25' \text{ N } 9^{\circ}13' \text{ W}$). This lagoon has a variable wet surface area of approximately 6.0 km² on average and a maximum length and width of 4.5 km and 1800 m, respectively. This lagoon is permanently connected to the sea through a narrow inlet (around 100 m) [28]. The average depth is small (2–3 m at mean sea level), and the regime of tides is semi-diurnal, with a high amplitude ranging between 0.5 and 4.0 m, depending on location and tidal phase [29]. Ria Formosa lagoon is a large shallow mesotidal lagoon located in the south of Portugal (Algarve coast, $37^{\circ}1' \text{ N } 7^{\circ}49' \text{ W}$), with a wet area of 10,500 ha, in an extension of 55 km, with a maximum width of 6 km and a mean depth of around 3.5 m [30]. Several barrier islands and two peninsulas separate the lagoon from the Atlantic Ocean. The tidal range varies from 1.35 m on neap tides to 3 m on spring tides, with a coefficient of water



renovation of 3.2 m on a spring tide and 1.0 m on a neap tide. Due to its smooth slopes, the intertidal area is air-exposed for several hours over each semi-diurnal tidal period [31].

Figure 1. Location of Óbidos and Ria Formosa lagoons where Cerastoderma edule samples were collected.

Adult specimens of *C. edule* (Óbidos lagoon total length—25.9 \pm 1.3 mm; Ria Formosa Lagoon total length—25.2 \pm 1.2 mm) were collected monthly for a period of twenty months (September 2016–April 2018) in Óbidos Lagoon and 24 months (March 2016–February 2018) in Ria Formosa Lagoon. In Óbidos Lagoon, water samples (4 L) were collected to determine chlorophyll *a* (Chl *a*) concentration, whereas, in Ria Formosa Lagoon, Chl *a* concentration was obtained from an online data system (http://disc.sci.gsfc.nasa.gov/giovanni/overview/index.html, accessed on 1 May 2018). The sea surface temperature (SST) was monitored in situ for both lagoons using a multiparameter probe (HANNA instruments, HI9829). Both bivalves and water samples were transported to the laboratories in cooler boxes for further analysis.

2.2. Water Analysis

Chlorophyll *a* from Óbidos Lagoon was determined according to Lorenzen and Jeffrey [32], following the spectrophotometric method. Briefly, water was filtered through a Whatman GF/C glass paper filter; then, for the extraction of Chl *a*, a glass paper filter was placed in 10 mL of 90% acetone (C3H6O) and kept at 4 °C for 24 h in the dark. Subsequently, samples were centrifuged at 4000 rpm for 10 min. After the first reading of absorbance at 665 nm and 750 nm, samples were acidified with diluted (1:1) hydrochloric acid (HCl) for correction of phaeopigments. Then, the absorbance was read again, with the same wavelengths. The Chl *a* concentration was determined according to the equation of Lorenzen [33]:

Chl
$$a \,(\mathrm{mg}\,\mathrm{m}^{-3}) = \frac{\mathrm{A} \times \mathrm{K} \times [(665_0 - 750_0) - (665_a - 750_a)] \times \mathrm{v}}{\mathrm{V} \times \mathrm{L}}$$

where A is the absorption coefficient of Chl *a* (11), K is the factor used to equate the reduction in absorbance to the initial chlorophyll concentration (2.43), 665_0 and 750_0 are absorbances before acidification at 665 nm and 750 nm, respectively, 665a and 750a are absorbances at 665 nm and 750 nm after acidification, v is the volume of acetone used for extraction (10 mL), V is the volume of water filtered (1 L), and L is the path length of the cuvette (1 cm).

2.3. Bivalves Analyses

Cockles were transported to the laboratory and placed in an aquarium containing aerated filtered seawater at 20 °C for 24 h to purge their stomachs before further analyses (histology, condition index (CI), and biochemistry). Samples for biochemical and CI analyses were prepared and stored at -20 °C until processing.

2.3.1. Histology

Each month, twenty individuals (10 males and 10 females, for the months in which distinction was possible with microscope smear observations) were histologically processed to determine the gametogenic stages. The visceral mass was fixed in a Davidson solution for 24 h and subsequently transferred to 70% ethyl alcohol until further processing. Tissues from these samples were processed and sectioned using standard histological techniques, and sections were stained with haematoxylin-eosin. Slides were examined using a microscope at $40 \times$ magnification, and each was assigned a stage that represented the gonadal state. Cockle reproductive maturity was categorized into six stages [34]: sexual rest—phase 0; beginning of gametogenesis—phase I; advanced gametogenesis—phase II; ripe—phase III; spawning—phase IV; spent—phase V. When more than one developmental stage occurred simultaneously within a single individual, the condition of the majority of the section was used for the assignment of the stage. For each one of those stages, a numerical ranking was assigned as follows: inactive (0); early active (3); late active (4); ripe (5); partially spawned (2); spent (1). A mean gonadal index (GI) was then calculated according to the method proposed by Seed [35]:

$$GI = \frac{\sum ind. each stage \times stage ranking}{total ind. each month}$$

The GI ranged from 0 (all individuals in the sample are resting) to 5 (all individuals are ripe).

2.3.2. Condition Index

Each month, the dry meat and shell weight of ten cockles were determined after oven drying at 80 °C for 24 h. Meat samples were ashed at 450 °C in a muffle furnace for ash weight determination and organic matter weight calculation according to the ash-free dry meat weight (AFDW). The condition index (CI) was calculated according to Walne and Mann [36]:

$$CI = \frac{\text{ash free dry meat weight }(g)}{\text{dry shell weight }(g)} \times 100$$
(1)

2.3.3. Biochemical Analyses

The soft tissue of each individual cockle (n = 10) was homogenized with a tissue homogenizer under an ice bath, and the aliquots of each individual were used for total lipids and glycogen determination. Glycogen content was determined from dried (80 °C for 24 h) homogenate using anthrone as reagent [37], and total lipids were extracted from fresh homogenized material in chloroform/methanol [38] and estimated spectrophotometrically after charring with concentrated sulphuric acid [39]. Duplicate determinations were performed in all analyses, and values are expressed as a percentage of AFDW.

2.4. Statistical Analyses

All statistical analyses were conducted using the Sigma Plot software, version 12.3. Before analyses, percentage data were arcsine transformed to normalize variance, and all data were tested for normality and homogeneity of variance [40]. Condition and gonadal indexes and biochemical composition data were analyzed using a general linear model (ANOVA or *t*-test) followed by a Tukey post hoc test whenever significant differences occurred. Whenever the assumptions (data normality and homogeneity of variances) failed, the non-parametric Kruskal–Wallis test on ranks was performed, followed by Dunn's test in situations where the null hypothesis was rejected. Results were considered significant at p < 0.05. The statistical comparison between the two populations was only carried out when data were available for both sites (between September 2016 and February 2018). A principal component analysis (PCA) was performed to find the association degree of SST, Chl *a*, GI, CI, glycogen, and total lipids. The XLSTAT for Excel software was used to perform this analysis.

3. Results

3.1. Sea Surface Temperature and Chlorophyll a

The evolution of the monthly SST and Chl a during the study period for the Óbidos and Ria Formosa lagoons are shown in Figure 2. Generally, Óbidos Lagoon showed slightly lower temperature values than Ria Formosa Lagoon (except in April and May 2017 and January and February 2018). A typical seasonal cycle in SST was observed in both lagoons, with the lowest temperatures being registered during the winter and the highest between mid-spring and late summer. Monthly mean SST ranged between 11.0 °C in January 2017 and 24.0 °C in May and August 2017 in the Óbidos lagoon and between 13.6 °C in January 2018 and 25.9 °C in June 2017 in the Ria Formosa lagoon. The evolution of Chl a during the studied period also showed seasonal variations in both sites with several phytoplanktonic blooms, especially in the spring, autumn, and winter of 2017, as well as in the spring and summer of 2018 for the Ria Formosa lagoon. Comparatively, Chl a concentration in the Ria Formosa lagoon was much lower than in the Óbidos Lagoon (less than 11.4 ± 5.8 mg m⁻³), with a Chl a average of 11.9 ± 5.4 mg m⁻³ and 0.8 ± 0.4 mg m⁻³ for the Óbidos and Ria Formosa lagoons, respectively.



Figure 2. Monthly sea surface temperature values (SST— $^{\circ}$ C) (**A**) and chlorophyll *a* (Chl *a*—mg m⁻³) (**B**) in Óbidos and Ria Formosa lagoons.

3.2. Gametogenic Cycle

Synchronous gametogenic development and spawning between males and females were observed in both populations throughout the studied period (Óbidos: *t*-test, t = 0.86, d.f. = 38, p = 0.40; Ria Formosa: *t*-test, t = -0.149, d.f = 46, p = 0.88), and no hermaphrodites or sex reversal was encountered. No significant differences were found between populations (*t*-test, t = -1.38, d.f. = 34, p = 0.177) or between years (Ria Formosa: *t*-test, t = 0.36, d.f. = 18, p = 0.72; Óbidos: *t*-test, t = 0.44, d.f. = 6, p = 0.68) (Figure 3). A clear seasonal pattern was apparent across both populations (Figure 4). Gametogenesis generally started

in early autumn in both populations, and its evolution coincided with the decrease in SST. Though there was no statistically significant correlation between Chl *a* and GI, gametogenesis onset of the Óbidos population occurred after phytoplanktonic blooms (October 2016, August 2017). The Ria Formosa population maintained the maximum gonadal maturation for 4 months, from December 2016 to March 2017 (GI highest values: 4.2 and 3.95 in December 2016 and January 2017, respectively), while cockles from Óbidos showed a maximum peak of maturation in February in both sampled years (GI highest value: 4.8 in February 2018). Both populations spawned in spring and early summer (March to July), apparently triggered by the rise in SST. However, in 2017, cockles from the Ria Formosa started spawning one month later but extended the spawning period for another two months (2017 spawning season: April to September). Moreover, the proportion of spawning individuals was higher in 2017 (31%) compared to 2016 (17%).



Figure 3. Monthly variation in gonadal development of *Cerastoderma edule* populations from Óbidos (from September 2016 to April 2018) and Ria Formosa (from March 2016 to February 2018) lagoons, females (**top**) and males (**bottom**).



Figure 4. Monthly variations in gonad index (GI) (mean, n = 20) of *Cerastodema edule* populations from Óbidos (from September 2016 to April 2018) and Ria Formosa (from March 2016 to February 2018) lagoons.

After spawning, cockles progressed to an inactive stage that was prolonged over time (Óbidos: July to January; Ria Formosa: February and May to December, except in July); however, this was more pronounced in the Obidos population than in the Ria Formosa population. In fact, during the resting period, in cockles from Ria Formosa, the co-occurrence and overlapping of other maturation stages (spent, beginning of gametogenesis, and advanced gametogenesis) were more evident.

3.3. Condition Index (CI)

The condition index exhibited statistically significant differences between populations (K-W., H = 38.85, d.f. = 1, p < 0.001), except in March, August, and December 2017 and February 2018 (Figure 5).



Figure 5. Condition index (mean \pm SD; n =10) of *Cerastoderma edule* populations from Óbidos (from September 2016 to April 2018) and Ria Formosa lagoons (from March 2016 to February 2018). * statistically significant differences, p < 0.05 found between populations.

Significant differences were found between years in both populations (Obidos: 2016 vs. 2017—ANOVA, F = 80.39, d.f. = 1, *p* < 0.001, in October, November, and December; 2017 vs. 2018—K-W., H = 4.19, d.f. = 1, p < 0.05, in January and March; Ria Formosa: 2016 vs. 2017—K-W., H = 112,71, d.f. = 1, *p* < 0.001, namely in March, April, May, September, November, and December). In Óbidos, the highest value of CI was found in October 2016 (6.96 \pm 0.59), coinciding with the highest concentration of Chl a in seawater. In the following month, the abrupt drop in Chl a coincided with a decrease in CI; however, this was not reflected in the cockles' GI, which, until February 2017, progressed to the advanced gametogenesis stage. This decreasing pattern of CI generally continued until July 2017 (except in February and April), following the spawning season until the end of the reproductive cycle. The next reproductive cycle followed the same trend, albeit reaching lower CI values, with the lowest value (2.70 ± 0.60) being observed in December 2017 and the highest (6.05 \pm 1.00) in March 2018. In the Ria Formosa cockle's population, differences between years were more pronounced. In May 2016, after the highest peak of Chl a was registered, the species showed the highest value (8.70 \pm 1.62) of CI, coinciding with the start of the spawning season. Afterwards, CI decreased sharply in the following month and remained relatively stable until the end of the year, regardless of gonadal maturity, SST, or food availability (Chl a). In 2017, the maximum condition index value (4.77 \pm 0.53, found in February) was not as sharp as in 2016, reflecting the lower food viability (Chl a) that year. However, this decrease in CI did not reflect the levels of the gonadal maturation of the population that remained relatively high, such as in 2016 and beyond. After that, CI decreased until June, during the spawning season. In August and October, two other smaller peaks occurred after an increase in food availability (Chl a) in August, which may have triggered the next onset of gametogenesis. The lowest value of CI (2.74 ± 0.28) was found in November 2017.

3.4. Biochemical Composition

Significant differences were found between populations in glycogen content (K-W., d.f. = 1, H = 229.96, p < 0.001) except in March 2017 and July 2017 (Figure 6).



Figure 6. Mean values (\pm sd; n = 10) of glycogen and total lipids (μ g mg⁻¹ AFDW) of *Cerastoderma edule* populations from Óbidos (from September 2016 to April 2018) and Ria Formosa lagoons (from March 2016 to February 2018). * statistically significant differences, *p* < 0.05 found between populations.

Scheme 2016. vs. 2017—ANOVA, F = 57.27, d.f. = 1, p < 0.001; 2017 vs. 2018—K-W., H = 12.50, d.f. = 1, p < 0.001; Ria Formosa: 2016 vs. 2017—K-W., H = 82.75, d.f. = 1, p < 0.001). Indeed, these differences were substantially higher in the Ria Formosa population, in which a higher value of glycogen content (130.4 ± 35.0 µg mg⁻¹ AFDW), coinciding with higher CI and Chl *a* values, was obtained in April 2016. However, the differences were also relevant in the Óbidos population when comparing March and April 2017 with 2018. During the first stages of gametogenesis, the Óbidos population exhibited considerably higher glycogen reserves than cockles from Ria Formosa. The highest glycogen content for this population was recorded in December 2017 (139.2 ± 40.7 µg mg⁻¹ AFDW), while the lowest glycogen value was observed in May 2017 (6.1 ± 1.8 µg mg⁻¹ AFDW) and July 2017 (17.4 ± 6.1 µg mg⁻¹ AFDW) for the Ria Formosa and Óbidos populations, respectively.

Generally, cockles from Ria Formosa registered higher values of total lipids than the Óbidos population. Indeed, significant differences were found between populations in total lipid content (K-W., d.f.= 1, H = 72.84, p < 0.05), except in September and November 2016, May and December 2017, and January 2018. In the Ria Formosa population, levels of total lipids were lower during 2016 than in 2017 and 2018. Significant differences were found between years in both populations (Óbidos: 2016 vs. 2017—K-W., H = 8.53, d.f. = 1, p < 0.001 in November; 2017 vs. 2018—K-W., H = 106.44, d.f. = 1, p < 0.001 in January, February, and April; Ria Formosa: 2016 vs. 2017—K-W., H = 125.37, d.f. = 1, p < 0.001) in April, June, July, August, September, October, and November). Indeed, these differences were substantially higher for the Óbidos population in the first three months of 2018, when the total value of lipids peaked (319.7 ± 69.6 µg mg⁻¹ AFDW in February). Although much lower, the highest value of total lipids observed for the Ria Formosa population was recorded in January 2017 (149.1 ± 41.0 µg mg⁻¹ AFDW). The lowest values of total lipids were observed in October 2016 (45.6 ± 11.1 µg mg⁻¹ AFDW) and October 2017 (55.4 ± 11.1 µg mg⁻¹ AFDW) for the Ria Formosa and Óbidos populations, respectively.

3.5. Principal Component Analysis

The PCA analysis resulted in two principal components (F1 and F2) that accounted for 64.79% and 79.51% of the overall data variability for the Óbidos and Ria Formosa populations, respectively (Figure 7). The PCA biplot of both populations illustrates significant inverse correlations between SST and GI. SST was the environmental parameter with more preponderance in explaining the observed reproductive pattern, given the length of the associated vectors. Chl *a* was positively correlated with the CI and glycogen content of only cockles in Ria Formosa Lagoon. Indeed, the positive correlations between glycogen content and CI found for both populations showed that the CI reflected the accumulation and utilization of glycogen. Total lipids were inversely correlated with CI and glycogen in cockles from Ria Formosa Lagoon.



Figure 7. Principal component analysis (PCA) of the parameters used to characterize the reproductive cycle of *Cerastoderma edule* from the Óbidos and Ria Formosa lagoons. Each vector represents one of the parameters analyzed (SST, Chl *a*, GI, CI, glycogen, and TL—total lipids).

4. Discussion

Several authors have reported that differences in the timing and duration of the spawning of bivalves' populations are related to environmental factors, mainly food availability and temperature [6,8,21]. Moreover, the temperature is intrinsically linked to the geographical locations, indirectly affecting food availability; therefore, differences in the reproductive cycle of populations from different latitudes are expected. However, some exceptions to this general pattern of a latitude-direct influence in bivalve reproduction have been found. For example, Guillou et al. [25] observed reproductive pattern variability in cockles along the Atlantic French Coast that were not associated with the latitudinal gradient. Moreover, the same cockle population may even follow differences in the reproductive cycle in consecutive years in response to several environmental factors [41,42]. In this study, despite the similarity of the *C. edule* reproductive cycles, the spawning season showed slight differences between the two studied populations and between years in the Ria Formosa population.

The SST of studied lagoons was characteristic of temperate climates with seasonal variations [14,21]. Generally, Obidos Lagoon had slightly lower temperature values; however, it had substantially more food availability (around 20X more chlorophyll *a* when it peaked) than Ria Formosa Lagoon, as had already been reported by other authors [28,43]. According to Morgan et al. [44], the reproductive cycle alterations of cockles depend not just on temperature, but also on the effect of climate variability on plankton. In our study, the gametogenesis of *C. edule* was much more influenced by temperature. A clear seasonal pattern was apparent across both populations, as previously reported for this and several other bivalve species (e.g., [5,14,45]). The population of Ria Formosa did not reach as high a maturation level as the one of Óbidos; however, the ripe and spawning stages were longer. In the middle of the spawning period (May 2017), the capacity of cockles from Ria Formosa for a slight gonadal regeneration was observed. This reproductive strategy is advantageous for the species since it ensures a supply of gametes for the spawning period extension. On the other hand, the more well-defined spawning period of the Obidos population can provide a higher probability of fertilization. These differences in the reproductive cycle were also observed for *Ruditapes decussatus* in this location or nearby [14,21]. Studies on cockle's reproductive cycle over the years have also provided different records. Spawning can be well defined with one or multiple partial events, as in Southwest UK, Northwest Spain, and the southwest coast of Ireland [7,44,46], or even prolonged throughout the year, without a resting period, as in France [25]. The differences observed between studies have been frequently associated with the influence of geographical location and, consequently, the inherent environmental factors. Nevertheless, these differences were also reported for cockle populations at the same latitude [41]. Maia et al. [26] studied the reproductive cycle of the *C. edule* population from Ria de Aveiro Lagoon for two years (2013 to 2014). This coastal lagoon is distant to the north, approximately 190 km from Óbidos Lagoon. Despite the short distance between these lagoon systems, the reproductive cycle described for the species is significantly different from that found in our study. In Ria de Aveiro Lagoon, gametogenesis occurs in winter, and the spawning peak was reported in summer [26], when the highest temperature was recorded. In contrast, for cockles from the Óbidos Lagoon, gametogenesis started in autumn, when the temperature decreased, after a phytoplankton bloom, and when spawning occurred in spring/early summer. The resting period was also longer in Óbidos Lagoon compared to the population of Ria de Aveiro Lagoon. Given the influence of temperature on the reproductive cycle of cockles found in this study, these differences between various populations may be related to the reproductive adaptation of this species to differences in temperatures, both between latitudes and between years.

Most of the time, the Óbidos population showed a better condition, which could be associated with the more remarkable amount of food availability in Óbidos Lagoon. However, during the studied period, this population never reached the highest CI achieved by the Ria Formosa population in May 2016. Our team has been demonstrating the relationship between the CI and the reproductive cycle in several bivalve species (e.g., Pacific oyster *Crassostrea gigas* [47], white clam *Spisula solida* [20], and European clam *R. decussatus* [14,21]). In this study, cockles' CI reflected the influence of food availability more than the species' reproductive cycle; moreover, despite the fact that the reproductive effort of the species was not substantially affected by the lack of Chl *a* in early 2017 in Ria Formosa, the general condition of cockles was weakened, with an energetic cost.

Several authors have described the energetic costs of the reproductive activity of bivalves [12,48,49]. They found that temperature and food availability control a close relationship between the reproductive cycle and energy storage and expenditure. This energetic balance can be translated into a seasonal pattern of biochemical composition variables according to species and the geographical location of populations [18,21]. Glycogen is considered the main energy reserve in adult bivalves and can be consumed in lipids' biosynthesis during gametogenesis [50]. Likewise, it has long been evident that lipid loss follows spawning [23,51]. In this study, it was found that the CI of cockles reflected the accumulation and utilization of glycogen in both populations; however, no statistically significant relationships were found with the reproductive cycle of the species. The Obidos population exhibited considerably higher glycogen reserves than cockles from Ria Formosa, especially during the first stages of gametogenesis. During this period, and similar to what was shown in the CI, glycogen was accumulated. However, the tendency was inverted during the process in cockles from Obidos Lagoon, and this decrease continued during spawning and the resting period. Significant differences between years were found only in March and April in both populations; nevertheless, those differences were striking for the energetic dynamics, especially in the population from Ria Formosa Lagoon. For these cockles, in 2016, gamete production seemed to depend on the glycogen obtained from the availability of food; however, the lack of Chl a in 2017 substantially decreased the glycogen reserves early in this process. Significant inverse correlations were found between total lipids and CI and glycogen content in the Ria Formosa population; the last one is typical of the de novo synthesis of lipids during gamete formation [52]. The total value of lipids remained similar not only in the gametogenesis period but also during the spawning period, which may be related to successive and simultaneous gamete production and release. According to Iglesias and Navarro [49], the increased costs of reproduction associated with the higher reproductive effort of a *C. edule* population in North Spain did not affect somatic maintenance, since previously stored energetic reserves were primarily used to fuel gametogenesis. However, in the event of less food availability, using energy reserves mainly to support reproduction can favor fertilization to the detriment of energy allocation for growth and self-maintenance, which can weaken the cockles' population

immunity and thus make it more susceptible to pathologies. Matias et al. [21] have argued that the significant energetic effort suffered by *R. decussatus* clams during spawning leads to their weakness, which might be the origin of mass mortality episodes after the reproductive period in the Ria Formosa lagoon.

5. Conclusions

The reproductive cycle characterization of these Portuguese *C. edule* populations (Óbidos and Ria Formosa lagoons) provides valuable knowledge about the biology of this species. For example, the energy allocation strategy of cockles from the Ria Formosa lagoon, exposing its fragility in self-maintenance, may partially justify the reported large inter-annual fluctuations in stock abundance in Ria Formosa [53]. This important finding should be considered in sustainable fishing management, especially in closed seasons' implementation, to ensure that enough broodstock remains to contribute to the future fecundity and sustainability of populations. Moreover, the specific reproductive response of the species in different geographical localization and atypical years in environmental terms, currently so frequent due to climate change, needs to be considered in local fishery management and planning. This study is also important for the future aquacultural development of this species, mainly in terms of providing the optimal time for artificial spawning induction in aquaculture, since a sexually mature wild broodstock could provide an intensive hatchery production of juveniles for restocking actions.

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