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Reproductive Biology of Pearl Oyster (*Pinctada radiata*, Leach 1814) Based on Microscopic and Macroscopic Assessment of Both Sexes in the Eastern Mediterranean (South Evia Island)

Dimitris Pafras, Chrysoula Apostologamvrou, Athina Balatsou, Alexandros Theocharis, Alexios Lolas, Marianthi Hatziioannou, Dimitris Vafidis and Dimitris Klaoudatos *

Department of Ichthyology and Aquatic Environment (DIAE), School of Agricultural Sciences, University of Thessaly (UTh), Fytokou Street, 38446 Volos, Greece; dpafras@uth.gr (D.P.); chapostol@uth.gr (C.A.); abalatsou@uth.gr (A.B.); thalexandros@uth.gr (A.T.); allolas@uth.gr (A.L.); mxatzi@uth.gr (M.H.); dvafidis@uth.gr (D.V.)

* Correspondence: dklaoud@uth.gr

Abstract: The Atlantic pearl oyster, *Pinctada radiata* (Leach, 1814), is an Indo-Pacific bivalve of the subtidal zone that has colonized habitats all over the Mediterranean. There is a lack of detailed information on the reproductive activity of *P. radiata* in Hellenic waters, especially following the recent amendment of national legislation aiming to regulate the fishery and prevent illegal fishing and trafficking as a substitute for indigenous oysters. A total of 703 individuals were collected by scuba diving from the southwest part of Evia Island. Gonad microscopic examination indicated that gametogenesis occurs from February to September, with a recovery stage in early autumn. Synchronicity occurs for both sexes, with temperature being the main controlling factor. The annual recruitment pattern indicated two prominent peaks of similar magnitude. Analysis of covariance indicated a significant temperature effect on gonad index above 22 degrees. Onset of sexual maturity (L_{50}) was estimated at 47.2 mm in shell height and 27.09 gr in total weight for the entire population. The sex ratio was in favor of females at 1:1.70, with 7% and 19% of the individuals assessed macroscopically and microscopically identified as hermaphrodites, respectively. Results offer valuable information for the management and conservation of pearl oyster populations.

Keywords: reproduction; pearl oyster; invasive species; stock assessment; histology; ovaries

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1. Introduction

The removal of natural barriers following the opening of the Suez Canal in 1869 allowed species from the Pacific, Indo-Pacific, and Red Sea to flow into the Mediterranean [1]. Recently, the introduction of new species into maritime habitats has increased, driven by climate change and human activities [2,3]. These non-indigenous species alter the plant and animal makeup of invaded ecosystems, directly impacting their sustainability [4,5]. Since 1970, the ease of transporting organisms and inadvertent introductions through aquaculture have made these species more prevalent in maritime environments [6,7]. As a result, many foreign molluscan species have been identified in various regions [8–11].

The first Lessepsian bivalve species to enter the Mediterranean Sea through the Suez Canal was the Atlantic pearl oyster, *Pinctada radiata* (Leach, 1814) [12]. Its introduction to new areas has been facilitated by fouling from commercial shipping lanes, notably in France and Italy (including the Adriatic Sea and the Gulf of Trieste), as well as through intentional introduction for mariculture in Greece [13,14]. This Indo-Pacific bivalve has colonized new habitats all over the Mediterranean, with great success and widely distributed in Greece and Italy [15,16], the Adriatic Sea and Croatian coasts [17–20], as well as the eastern [21–23] and western [24] basins. It is a Lessepsian migrant [19,22], occurring in abundance throughout Tunisia, Sicily (Italy), Malta, Croatia, Portugal, Montenegro, Greece, and Turkey [20,25–30]. It is a species highly adaptable to a variety of climatic conditions, and its ability to spread via ocean currents has enabled it to colonize the northern and western Mediterranean regions [31]. In addition to adjusting to the subtropical climate of the southeast Mediterranean, the species has spread throughout enclosed, contaminated ecosystems due to its durability against chemical pollution [32,33].

The Atlantic pearl oyster is one of the three non-native members of the Pteriidae family found in the Mediterranean, alongside *Pinctada margaritifera* (Linné, 1758) and *Electroma vexillum* (Reeve, 1857) [34]. Oysters belonging to the Pteriidae family are highly valued for their ability to produce pearls, an attribute that offers significant economic potential for the food, ornamental, and pearl industries [33,35–38]. *Pinctada radiata* is a fouling species that attaches to hard substrates by its byssus, making it an epifaunal suspension feeder in the subtidal zone. It typically reaches a length of 50–65 mm, though it can grow up to 100 mm. This protandric hermaphrodite species undergoes sex inversion in shells ranging from 32 to 57 mm in length, with gonad maturity regulated by temperature [25].

Determining the relative size, shape, and color of the gonads or examining developmental stages based on histological characterization are two methods for evaluating gamete development in bivalves [39–41].The most reliable approach for assessing the reproductive cycle progression in oysters involves examining gonadal histology [42]. Histological techniques classify the oysters’ reproductive cycle based on the tissue cell characteristics observed at various stages of gonadal development [43]. Nonetheless, this analysis can be subjective and should be complemented by quantitative methods, such as the condition index (CI), to enhance the evaluation of reproductive aspects [43,44]. Furthermore, several studies have recently employed indices to assess gonad status, including the mean gonad index (MGI) for populations [45,46] the gonad development index (GDI) for individuals, and the mean oocyte diameter [47,48]. Histological methods are continually utilized to confirm reproductive occurrences since they offer a wealth of information regarding gonad development [48].

Research on the reproductive cycle, spawning periods, and larval occurrences of oyster species is crucial for comprehending the population dynamics of wild stocks, which is fundamental for managing natural oyster banks and applying conservation or exploitation strategies for commercial marine and food species [49]. Additionally, a deeper understanding of the reproductive cycle of native oysters and their environmental interactions is necessary for aquaculture. This knowledge will enable the development of more effective and efficient techniques for maintaining oysters and optimizing breeding conditions in laboratory settings, ultimately leading to successful seed production [50,51].

Detailed information on the reproductive activity of *P. radiata* in Hellenic waters is lacking, especially in view of a recent amendment of the national legislation by Presidential Decree (A’14/29.01.2024) enforced to regulate the fishery to prevent illegal fishing and trafficking as a substitute for indigenous oysters. Our aim was to obtain essential information on the reproductive biology of *P. radiata* in Hellenic waters (Aegean Sea) by microscopically and macroscopically assessing its reproductive cycle, sex ratio, the condition, and gonad indices relative to the prevailing environmental conditions.

2. Materials and Methods

2.1. Field Sampling and Maturity Classification

A total of 703 individuals were collected using scuba diving, between February 2023 and June 2024 from the southwest part of Evia Island (Eastern Mediterranean) (Figure 1) at depths between 1.5 and 4 m at a distance between 25 and 40 m from the shore.

Monthly population density and distribution pattern were estimated by a random placement of quadrant plots (50 × 50 cm metal frame) covering each time a sampling area of 0.25 m^2 repeatedly for twenty times and recording *P. radiata* numbers at each quadrant placement.

Figure 1. Map of the study area (black outline) and location of the sampling area (blue outline) in the south-west island of Evia.

Following sample collection, individuals were cleaned, and a range of morphometric parameters from the left shell that included shell height (SH), shell width (SWI), and hinge length (HL) were recorded (0.01 mm accuracy) using a digital caliper (Figure 2). Additionally, total weight (TW), shell weight (SW), and flesh weight (FW) were recorded using a digital scale (0.1 g accuracy).

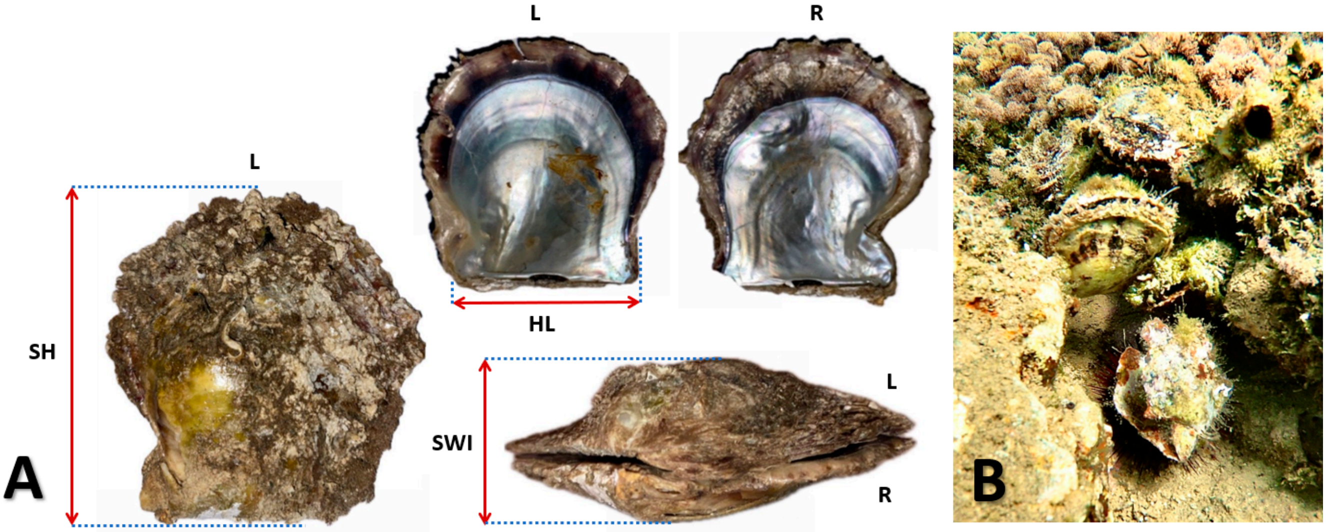


Figure 2. (**A**) Morphometric measurements recorded from sampled *P. radiata* individuals and (**B**) image of *P. radiata* from the study area.

Temperature and salinity were recorded monthly during each sampling period using the D5 Lime dive computer (SUUNTO, Vantaa, Finland) and the portable HI98319 Marine Salinity Tester (Hanna Instruments, Athina, Greece), respectively.

Sex and gonad maturity stage were assessed macroscopically (only for stages III and IV) and microscopically (all stages), and stages adopted were identified as: I: inactive; II: developing; III: mature; IV: spawning; V: spent, modified from [52]

The monthly gonad index (GI) was estimated to assess reproductive activity according to [53] (Equation (1)) as:

$$GI = \frac{[n\ stage\ (I+V)\times 1] + [n\ stage\ (II)\times 2] + [n\ stage\ III + IV \times 3]}{Total\ number\ of\ individuals} \tag{1}$$

2.2. Length at First Maturity and Recruitment

Length at first maturity, representing the length at which 50% of the individuals are sexually mature (L_{50}), was estimated by fitting a binary logistic regression to the data. Mature individuals (stages III, IV, and V) received a score of 1 and immature individuals (stages I and II) received a score of 0.

Recruitment pattern was assessed using a reconstruction of recruitment pulses based on a time series of length-frequency data using the FiSAT II software (FAO, Rome, Italy) (version 1.2.2) [54]. The routine involved the use of time series length-frequency data and growth parameters $L\infty$ and K, allowing for the determination of annual recruitment pulses and their relative strengths [55].

2.3. Microscopic Examination

Microscopic analysis was carried out on both sexes for every stage of maturity. Gonads were instantly fixed in a 10% neutral buffered formalin solution for a minimum of 48 h. The fixed gonads underwent additional processing via histological assessment. The middle sections of each gonadal tissue were placed in a tissue-processing apparatus (Histokinette) (Leica TP 1020, Leica Microsystems GmbH, Nussloch, Germany) for dehydration (immersion in ethanol solutions of increasing concentrations), clearing (immersion in xylene solutions to replace ethanol with an organic dissolvent), and embedding in liquid paraffin wax. Each block of gonad tissue was placed onto a microtome (Leica EG 1150 H Leica Microsystems GmbH, Nussloch, Germany) for sectioning (5 µm sections) after cooling (Slee Mainz Cut 5062, SLEE medical GmbH, Mainz, Germany). Once sections were mounted with Canada balsam mounting media and stained using the hematoxylin-eosin regressive staining technique, they were examined under a light microscope attached to a digital camera (ProgRes Plus 2.1, JENOPTIC Optical Systems GmbH, Jena, Germany). The Progress Capture 2.1 software was utilized to capture images of histological sections at a suitable magnification scale. To determine the oocyte size distribution, only oocytes with a clear nucleus were counted. Due to their irregular shape, the diameter was determined using the Tps.dig v. 2.12 software with the maximal and minimal diameters averaged to reduce variance and prevent artificially increasing the overlap between different groups of oocytes [56].

2.4. Condition Index and Spatial Distribution

Condition index (CI) (the general physiological condition of the mollusk) was calculated according to [57] (Equation (2)) as:

$$CI = \frac{Dry\ mass\ of\ soft\ tissues}{Shell\ weight} \times 100 \tag{2}$$

Spatial distribution was assessed with the use of the Morisita index of aggregation (IM) according to [58,59] (Equation (3)).

$$\mathrm{I}M = \frac{S \times \left(\sum n^2\right) - N}{N \times (N-1)} \tag{3}$$

where n the number of individuals within each sampling quadrant, S the number of quadrants employed, and N the number of individuals within all quadrants employed. To test the null hypothesis of randomness (significance of departure from random distribution), (IM = 1), the chi-square test was used (d.f. = $n - 1$) [60].

2.5. Statistical Analysis

The assumption of normality was assessed with the Shapiro-Wilk test and heteroscedasticity with Bartlett, Levene's, and Variance ratio tests. Independent samples a *t*-test was employed to test the null hypothesis of no significant difference between sexes when data met normality and homoscedasticity assumptions, whereas the Brunner-Munzel Test was employed when data failed both assumptions [61]. One-way ANOVA was employed to test the null hypothesis of non-significant seasonal differences in the GI, IM, and CI. Tukey's pairwise comparisons were used to identify possible differences between sample means.

The temporal (season) and environmental (temperature) effects and their interaction on each estimated index (GI, CI, and IM) were assessed using the general linear model (GLM) analysis of covariance [62] using season as a factor and temperature as a covariate with a least squares regression approach. The main effects plot was employed as a graphical tool to assess monthly and seasonal changes on estimated indices. A Pearson's correlation was employed to assess the correlation between each estimated index, temperature, and salinity [63]. A one sample T-test was employed to assess the significant deference of L_{50} and other published studies. The null hypothesis of equal proportions between males and females and comparison with published literature were assessed with a chi-square goodness-of-fit test [64]. Statistical analysis was performed with Jamovi (ver. 2.5.5) [65] at an α level of 0.05.

3. Results

3.1. Population Structure

Total population characteristics (703 individuals) were 48.44 ± 20.70 gr in total weight, 10.64 ± 3.79 gr flesh weight, 63.41± 13.24 mm shell height, and 38.42 ± 15.11 shell weight.

In total, 119 individuals were sexually identified microscopically and macroscopically: 75 females (63%) and 44 males (37%) at a M:F ratio of 1:1.70.

Shell height and total weight frequency distributions for 119 male and female individuals identified are shown in Figure 3. Females exhibited significantly higher shell height and total weight ($p < 0.001$) compared to males.

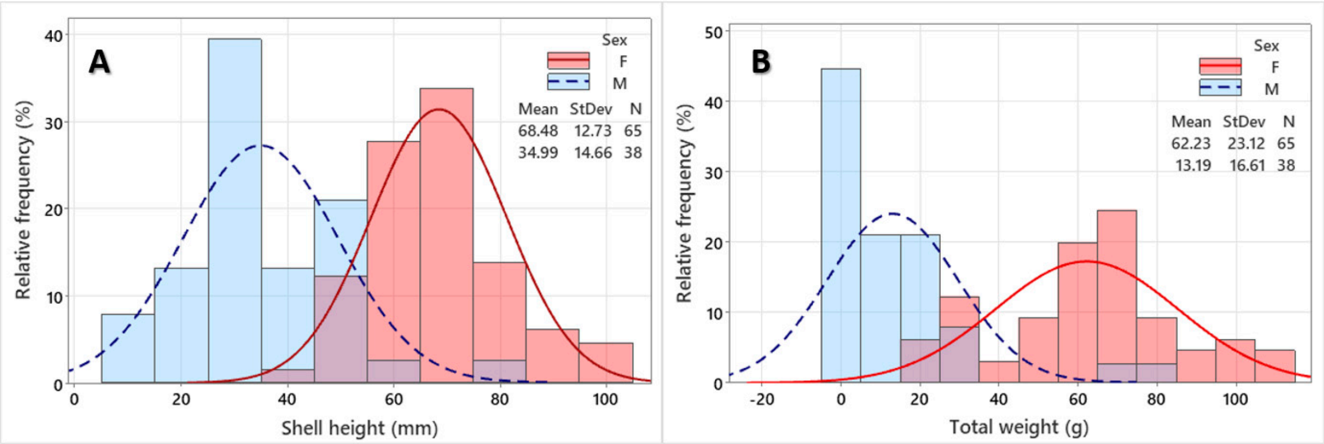


Figure 3. (**A**) Shell height-frequency and (**B**) total weight-frequency distribution of *P. radiata* with overlayed fitted normal distribution for both sexes.

3.2. Reproduction and Dispersal

The gonad index (GI) displayed a gradual increase between January and May and a significant increase in the summer months ($p < 0.05$) lasting through to September before rapidly decreasing in October (Figure 4).

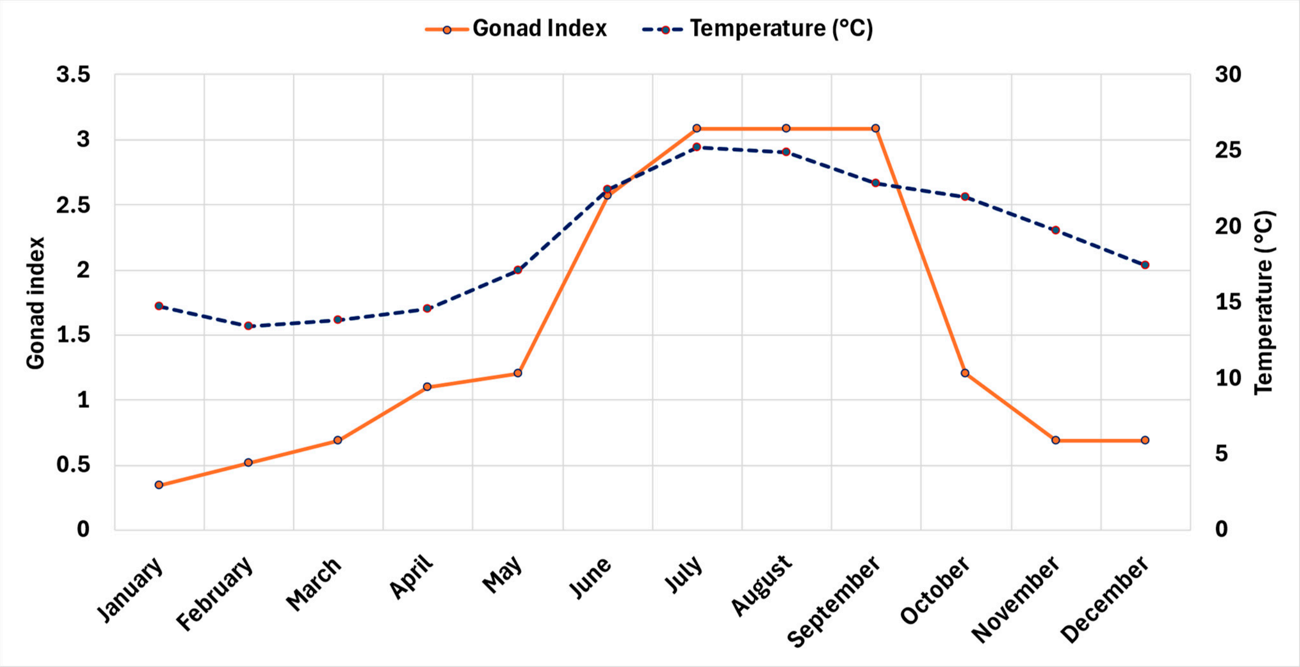


Figure 4. Monthly GI and temperature variation.

The GI exhibited a highly significant positive correlation with water temperature (r = 0.8, $p < 0.01$), indicating the importance of water temperature on the annual regulation of the reproductive cycle for *P. radiata*.

Seasonally, the GI exhibited a significantly higher value during summer (2.91 ± 0.3) (June–August), followed by autumn (1.66 ± 1.26) (September–November), spring (1.43 ± 0.69) (March–May), and winter (0.51 ± 0.17) (December–February).

The condition index (CI) displayed higher values between February and June and lower values between July and November (Figure 5). A significant negative correlation between the CI and water temperature ($r = -0.61$, $p < 0.05$) was indicated.

Seasonally, the CI exhibited a significantly higher value during spring (36.32 ± 0.63), followed by winter (28.10 ± 2.43), summer (27.14 ± 6.71), and autumn (24.88 ± 0.57).

No significant seasonal difference was indicated for I*M*, indicating a uniform dispersal mode throughout the year. I*M* values were above the value of 1 throughout the year (1.52 ± 0.28), indicating a clustered distribution throughout the year, however not significant, with higher values observed between November and January (1.94 ± 0.23). Additionally, no correlation was indicated between I*M* and water temperature.

The annual recruitment pattern of *P. miles* (Figure 6) indicated that recruitment occurred throughout the year with two prominent peaks of similar magnitude. The first from February to May (41.9%) and the second from August to October (42.12%).

Results of GLM (Table 1) indicated that there is a significant temperature effect on GI, a significant seasonal effect and interaction effect on I*M*, and a temperature, seasonal, and interaction effect on the CI.

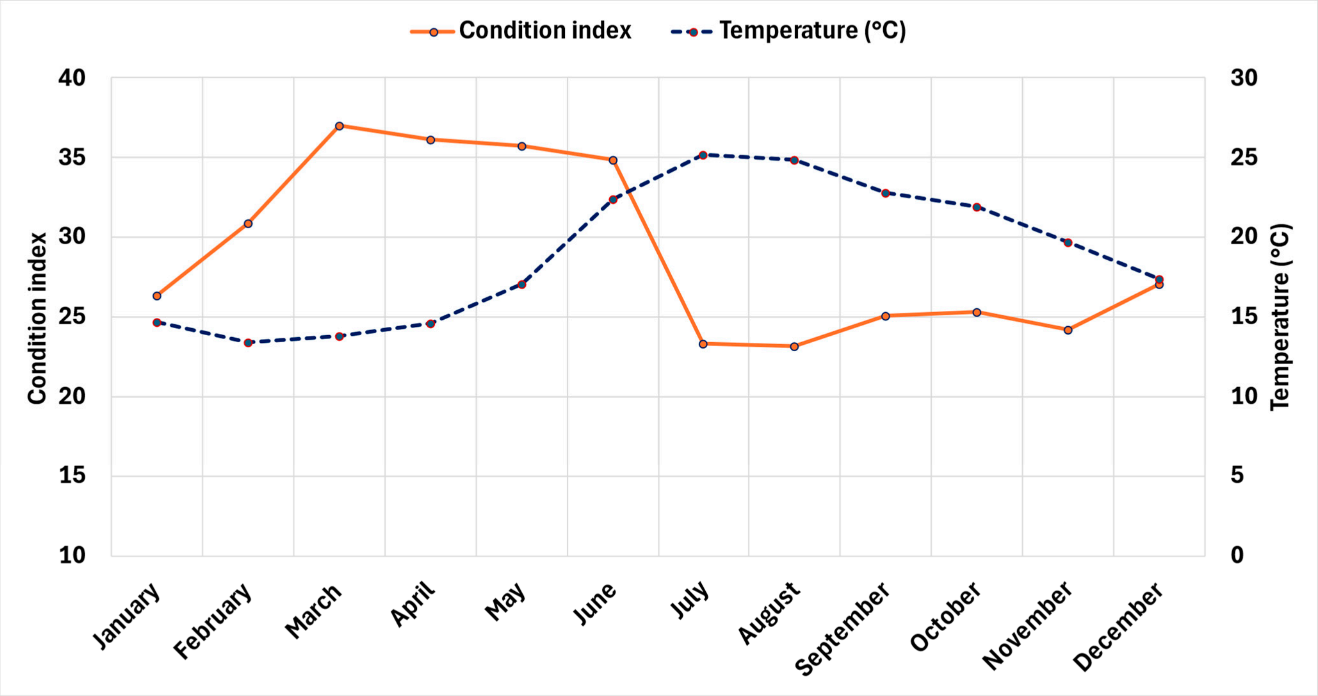


Figure 5. Monthly CI and temperature.

Table 1. ANCOVA results of the effects of season, water temperature, and their interaction, sum of squares (SS), degrees of freedom (DF), test statistic (F), and associated probability (p) on GI, *IM*, and CI of the *P. radiata* population.

GI (R-Squared = 0.92)	SS	df	F	*p*
Model	12.1	7	6.32	*
Season	2.03	3	2.47	ns
Temperature (°C)	2.37	1	8.68	*
Season × Temperature (°C)	1.28	3	1.56	ns
Residuals	1.09	4		
Total	13.2	11		
IM (R-squared = 0.93)				
Model	0.82	7	7.76	*
Season	0.31	3	6.75	*
Temperature (°C)	0.01	1	0.63	ns
Season × Temperature (°C)	0.46	3	10.17	*
Residuals	0.06	4		
Total	0.89	11		
CI (R-squared = 0.97)				
Model	318.99	7	21.66	**
Season	106.05	3	16.8	**
Temperature (°C)	37.42	1	17.79	*
Season × Temperature (°C)	64.18	3	10.17	*
Residuals	8.41	4		
Total	327.4	11		

Significance level: ns not significant, * $p < 0.05$, ** $p < 0.01$.

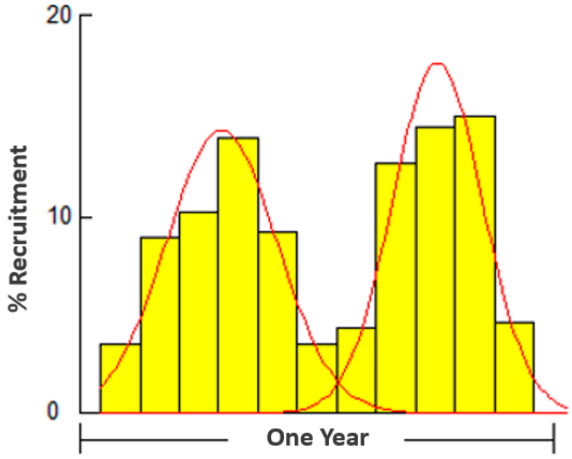


Figure 6. Annual recruitment pattern of *P. radiata* (red line indicates the overall annual pattern of recruitment).

Main effects plots (Figure 7) indicated a higher GI in spring, a higher CI in spring, and a higher *IM* in autumn and winter. Furthermore, the rise in water temperature above 22 degrees corresponded with a spike in the GI.

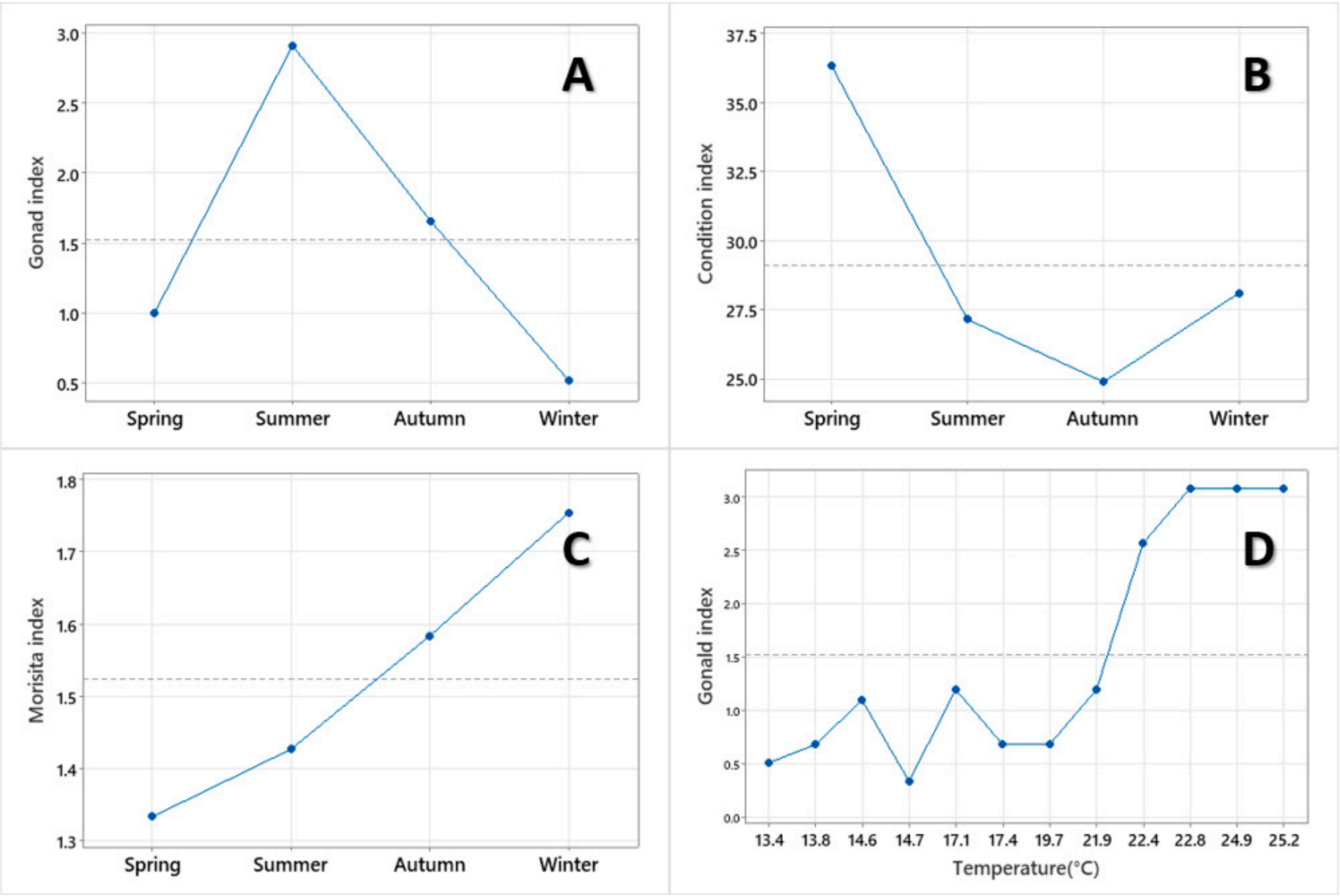


Figure 7. Main effects plots of the seasonal (**A**) GI, (**B**) CI, (**C**) *IM*, and (**D**) effect of water temperature on GI (dotted line indicates mean values).

The L_{50} was estimated at 47.2 mm in shell height at a total weight of 27.09 g for the total population (Figure 8).

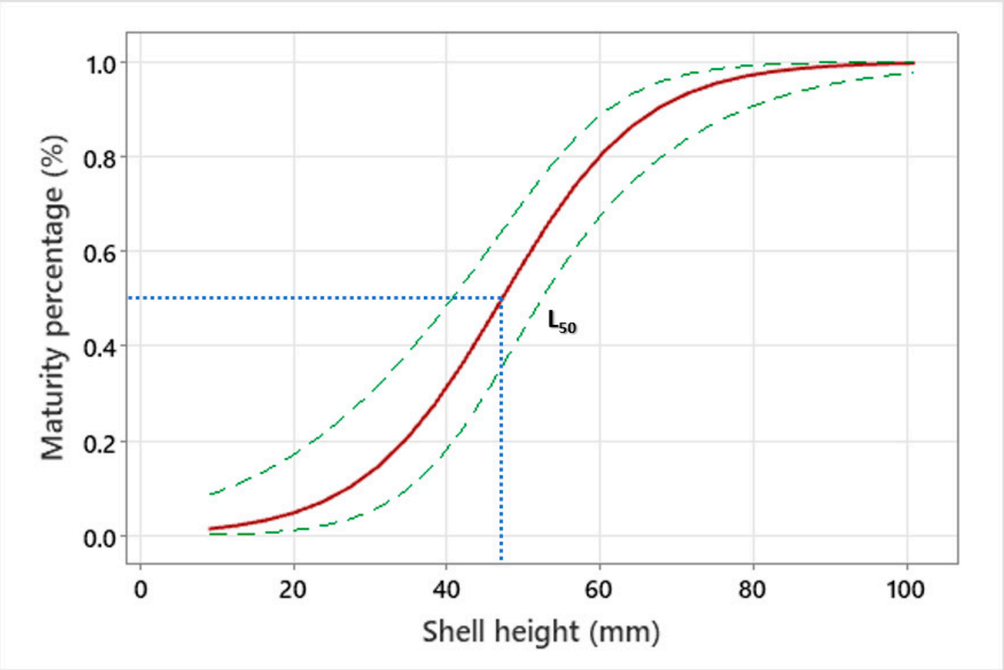


Figure 8. Binary logistic regression of the proportion of mature *P. radiata*, relative to its shell height (red line indicates model fit, green dashed line indicates 95% C.I., blue dashed line indicates L_{50}).

3.3. Maturity Stages

Microscopically, five distinct phases in the female reproductive cycle of *P. radiata* were identified. The position of the oocytes within the gonadal segment, size, and thickness of the ovary wall was used to define the microscopic stages of maturity in females. During the inactive (I) dormant phase, female reproductive cells are not visible. Only connective tissue was observed (Figure 9A). Developing ovules aligned along the germinal epithelium. After transition to the developmental stage (II), the germ layer displayed oocytes in various developmental phases, including developing and pre-vitellogenic; however, the tubule lumen was not filled. The gonadal wall maintained its thickness but gradually decreased over time (Figure 9B). In the next mature stage (III), the thickness of the ovary wall was greatly reduced, with oocytes visible in various developmental stages. With continued vitellogenesis, fully developed ovules were located near the center of the lumen (Figure 9C). Spawning (IV) was distinguished by an abundance of fully matured oocytes with well-defined nuclei, densely accumulating in the lumen, reaching their maximum size (Figure 9D). Following spawning, the final stage of exhausted gonads (V) was characterized by a thicker and wrinkled ovarian wall. The lumen contained phagocytes and no oocytes in the atresia, attributed to phagocytic activity (Figure 9E).

Similarly, to the female reproductive stages, five distinct reproductive stages were recorded for *P. radiata* males. The stages of gonadal maturity in males were assessed by considering several priority factors, namely testicular thickness, size of the genital layers and folds, and the amount of sperm occupying the gonadal lumen. During the inactive (I) dormant male and reproductive cells are not visible, and only connective tissue was observed (Figure 10A). At the transition to developmental stage (II), the wall of the gonads contained spermatocytes arranged in columns along the germinal epithelium, and spermatozoa began to fill the lumen. The walls of the gonads gradually thinned as development progressed, accompanied by a reduction in folds of the germinal epithelium (Figure 10B). The mature stage (III) exhibited a rapid progression of spermatogenesis, with spermatozoa occupying the central lumen. (Figure 10C). Spermiation (IV) showed a thin, single wall with a sperm-rich lumen with some spermatocytes along the germinal epithelium (Figure 10D). The wall of the gonads was thick and wrinkled after spermatogenesis at the expected stage (V), with the lumen containing some undeveloped spermatozoa (Figure 10E).

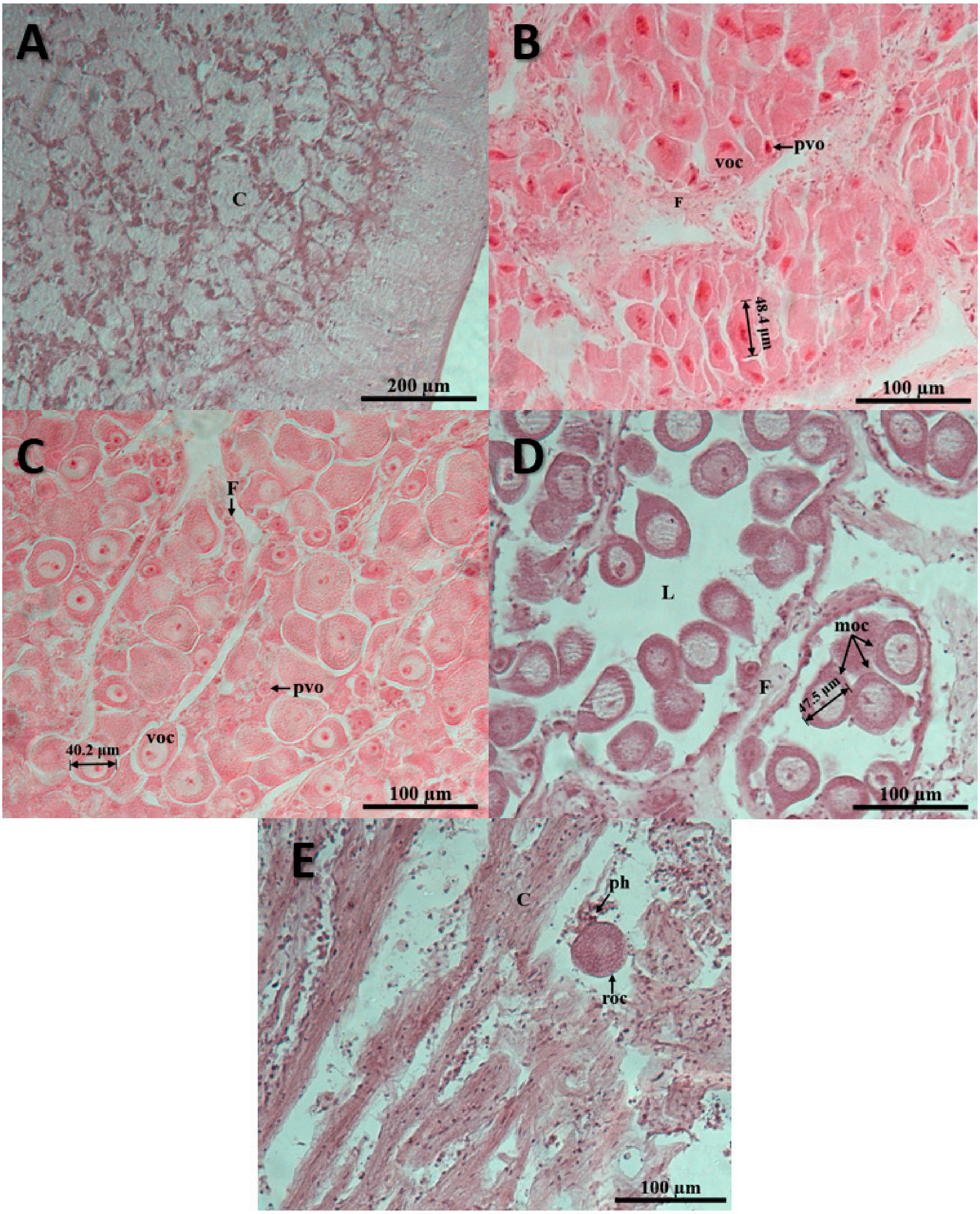


Figure 9. Histological characterization of the maturity stages in *P. radiata* females (hematoxylin and eosin staining). The inactive stage (**A**), development stage (**B**), mature stage (**C**), spawning stage (**D**), and spent stage (**E**) are shown. (C: connective tissue; pvo: previtellogenic oocyte; voc: vitellogenic oocyte; L: lumen; F: follicular cells; roc: relict oocyte ph: phagocytes). The scale bars in (**B**–**D**) are 100 µm.

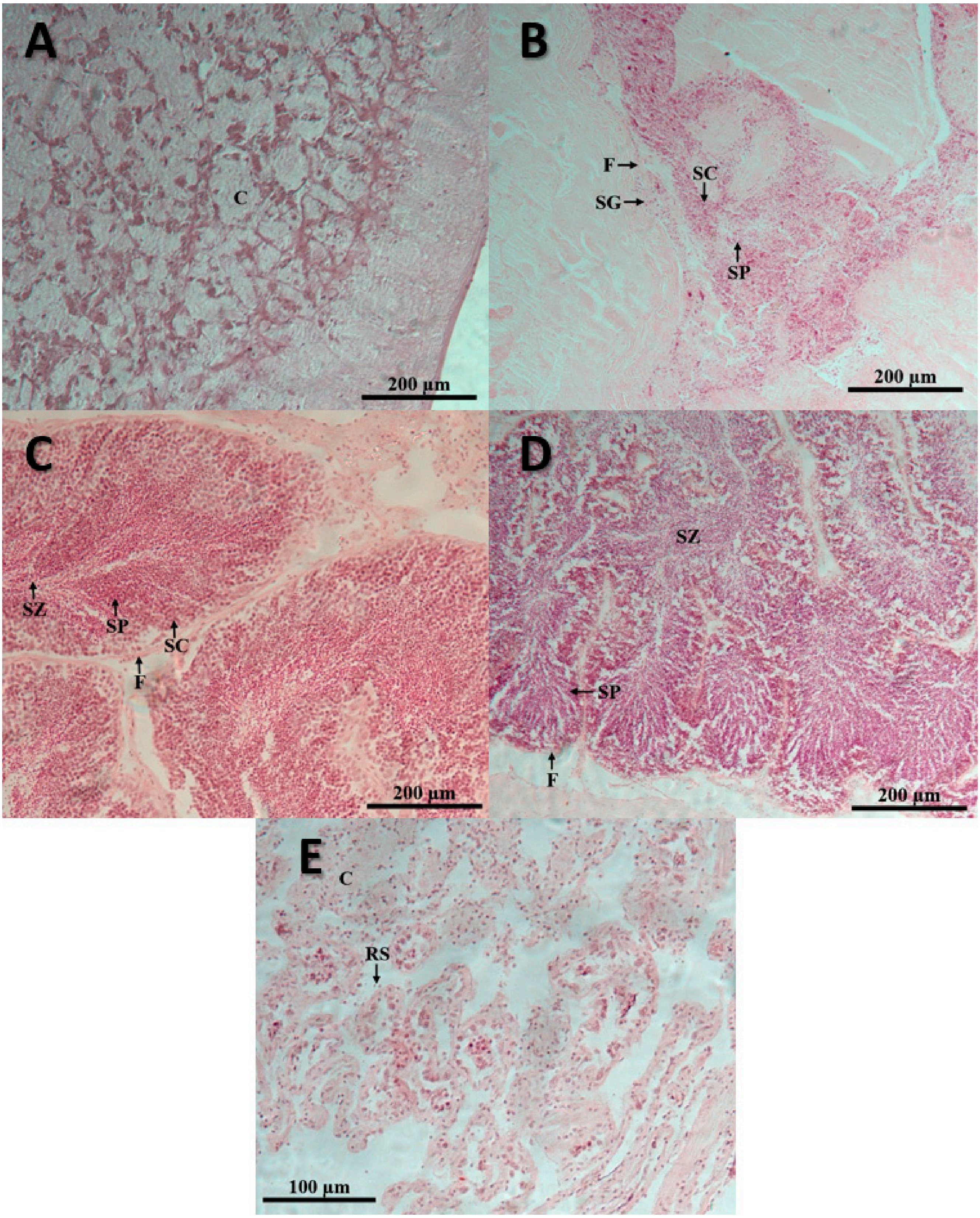


Figure 10. Histological characterization of maturity stages in males in *P. radiata* using hematoxylin and eosin staining. The inactive stage (**A**), development stage (**B**), mature stage (**C**), spawning stage (**D**), and spent stage (**E**) are shown. (C: connective tissue; SG: spermatogonia; SC: spermatocyte; SP: spermatid; F: follicular wall; SZ: spermatozoa; and RS: relict spermatozoa). The scale bars in (**A**–**C**) are 200 µm, while the scale bar in (**D**) is 100 µm.

Microscopic examination of male and female reproductive stages indicated that gametogenesis occurred from February to September, with a recovery stage in early autumn. The gametogenic cycle exhibited synchronization between males and females with a characteristic reproductive pattern reaching the mature stage during warmer months (June-August) and breeding in September. Gonadal development began in spring and reached full maturity in summer.

Hermaphrodites

During microscopic examination, several hermaphrodites were identified during April, May, June, September, and October (Figure 11). Overall, 19% of the individuals assessed microscopically were identified as hermaphrodites. The presence of mature oocytes within the gonadal segment, together with mature spermatozoa, was used to define the hermaphroditic microscopic stages.

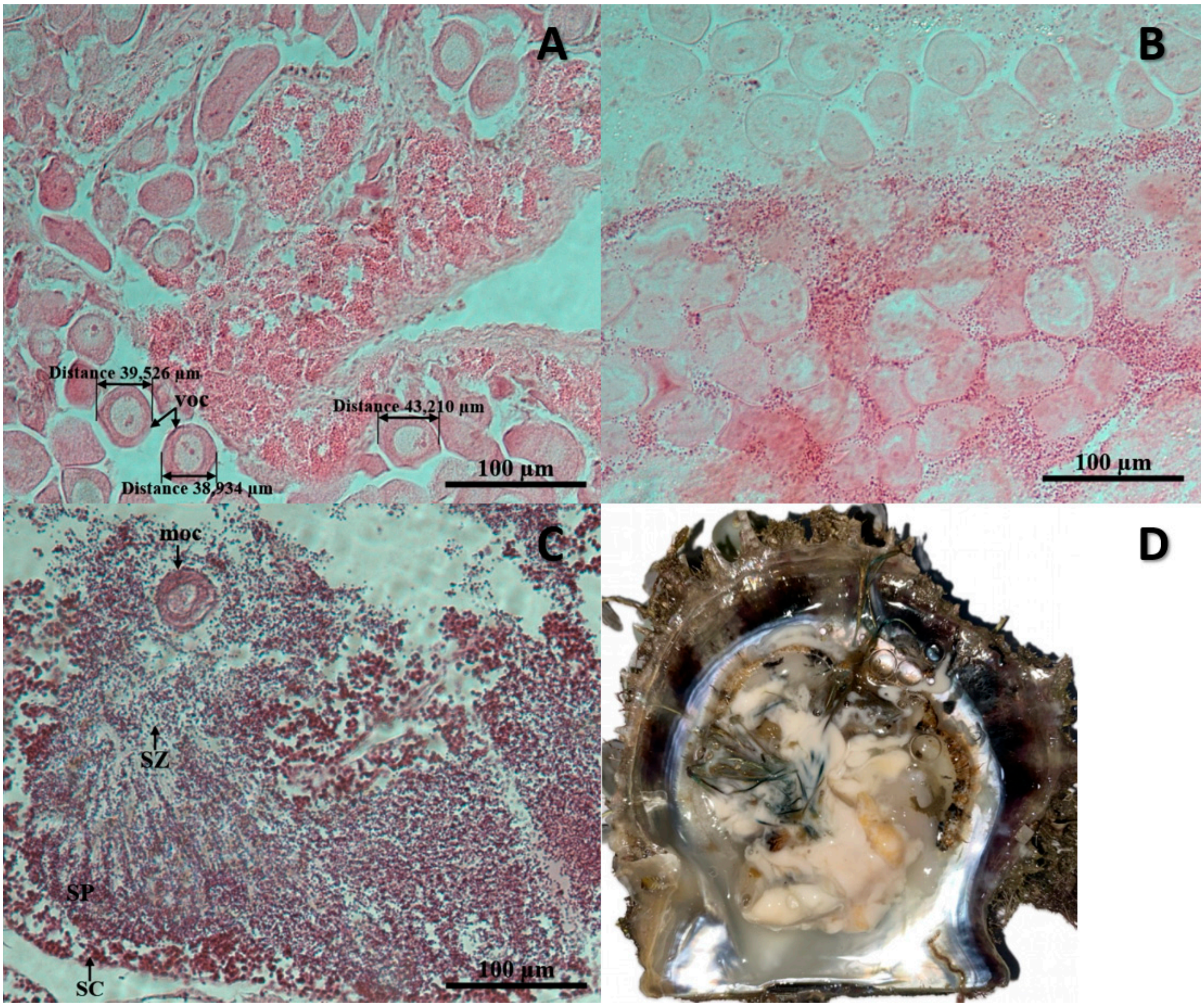
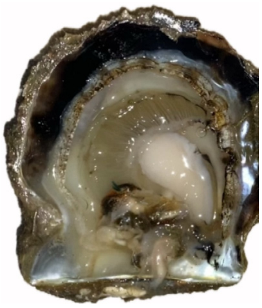
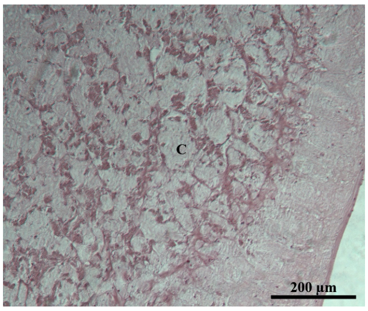
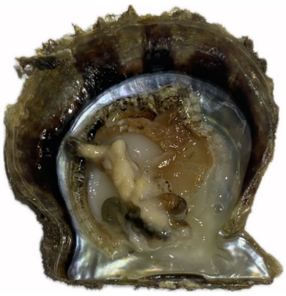
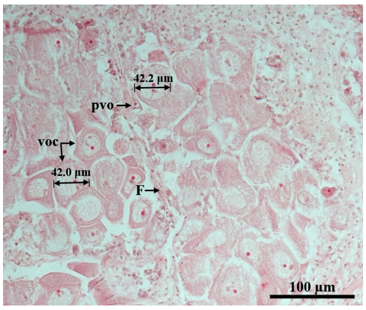
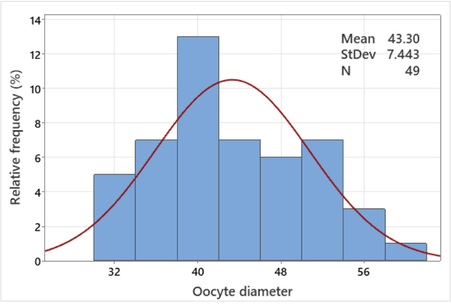
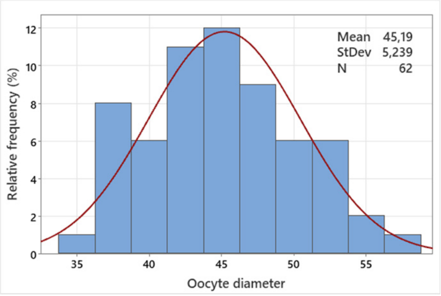
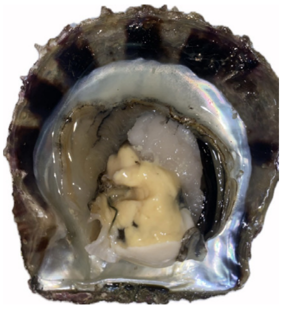
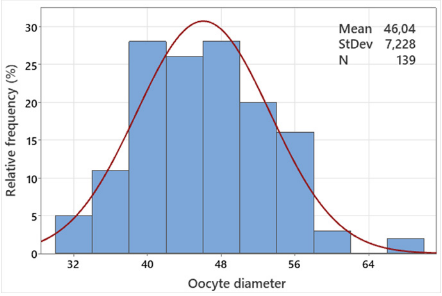
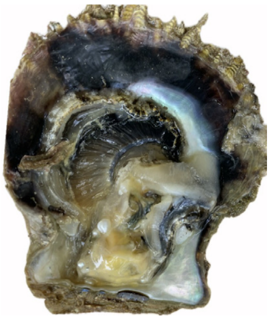
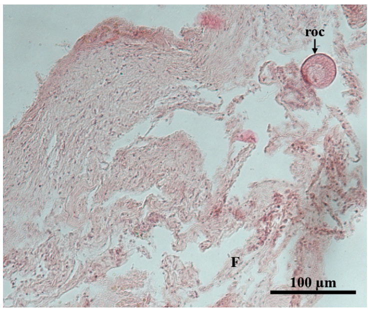


Figure 11. (**A**–**C**); Histological depiction and characterization of *P. radiata* hermaphrodites sampled during April, May, June, September, and October (SC: spermatocyte, SP: spermatid, SZ: spermatozoa, moc: mature oocyte); and (**D**); macroscopic depiction of a hermaphrodite during June.

3.4. Oocyte Size Diameter Frequency Distribution

A macroscopic and microscopic depiction as well as the oocyte size frequency distribution of each female reproductive stage is presented in Table 2.

Table 2. Oocyte diameter frequency distribution for each female reproductive stage (I: inactive; II: developing; III: mature; IV: spawning; V: spent) and associated macroscopic and microscopic depiction.

Stage	Macroscopic Depiction	Microscopic Depiction	Oocyte Diameter (µm)
I			
II			
III			
IV			
V			

Oocyte size frequency for each female reproductive stage between stages II and IV indicated a gradual increase in the mean oocyte size with time.

4. Discussion

The present study provides new and updated information on the life history characteristics of the Atlantic pearl oyster in Hellenic waters (Aegean Sea). Our results confirmed the hermaphroditic status as previously documented [25,66,67]. Many other pearl oysters, such as *P. margaritifera* (Linnaeus, 1758), *P. mazatlanica* (Hanley, 1855), *P. albina* (Lamarck, 1819), *P. imbricata* (Röding, 1798), and *P. fucata* (Gould, 1850), have been found to exhibit protandry [50,68–71], resulting in a metabolic cost due to the energetic and physiological burden of maintaining both male and female reproductive systems [72] leading to increased metabolic demands and potential trade-offs in other physiological functions, such as growth, decreased reproductive success, and immune response [73,74].

Our results indicated that a significant proportion of the adult population processes both reproductive organs. In total, 19% of the microscopically examined individuals were identified as hermaphrodites, with only a fraction of these identified macroscopically (7%), indicating that identification of hermaphrodites should be conducted microscopically; otherwise, their representation in the population could be grossly underestimated. In a study by [66] from 2360 pearl oysters captured from Tunisian waters, only 1% were identified as hermaphrodites. Similarly, from 3360 pearl oysters (*Pinctada margaritifera*) gathered from Takapoto atoll (French Polynesia), only 0.2% were identified as hermaphrodites [75]. Hermaphroditism can be stress-related, natural, and affected by naturally occurring factors (food or temperature) [68] or non-natural (handling or cleaning), causing alterations in both male and female characteristics [76]. According to [75], stress situations will inhibit female growth while favorable conditions will promote it.

Our results suggested that gametogenesis is not continuous during the year and exhibits a higher intensity during the summer months and in synchronicity for males and females throughout the annual reproductive cycle, with seawater temperature being the main controlling factor in agreement with [67,71]. Many studies have demonstrated that temperature is an environmental factor that significantly affects reproduction [40,77–79]. In fact, bivalves exploit reserve material more easily and expedite gametogenesis when temperatures rise [80]. According to [81], variations in temperature, salinity, and food availability may be the cause of reproductive behavior deviations that are seen within a species in the same geographic location.

Gonad index (GI) further indicated that gonad development is not continuous for *P. radiata* in the study area, with spawning occurring as a single pulse during the summer months. *P. radiata* exhibited two main spawning pulses with continuous recruitment [82] in the Gulf of Gabes, Tunisia. It has been suggested that tropical bivalves, which exhibit higher growth rates with shorter lifespans, tend to demonstrate a pattern of continuous recruitment [83–85]. Individuals' reproductive status can be ascertained by the GI [45,46,86], with an increase in the index signaling the gonad development and a reduction suggesting that ovulation is still occurring. Condition index (CI) exhibited an inverse relationship to GI. Numerous bivalves have shown a correlation between seasonal changes in body weight and reproductive activity [50,75]. Gonadal biomass typically indicates gamete production in bivalve seasonal reproduction [87]. As a result, the CI can directly evaluate the activity of gametogenesis and serve as an indicator of food availability [88].

Despite the single spawning event during summer indicated by microscopic examination, the annual recruitment pattern was suggested to occur in two prominent peaks in agreement with [89] in north Evia island. This occurrence could be attributed to several factors influencing the survival and settlement of larvae after the initial spawning event. While a single spawning event typically produces a cohort of larvae, a combination of environmental conditions [90], larval behavior [91], predation [92,93], and hydrodynamics [94] could lead to multiple recruitment pulses from that single event. This could contribute to a more successful recruitment process, enhancing the survival of the species.

The Morisita index of dispersion indicated a clumped distribution in the study area throughout the year, with higher values indicated between November and January corresponding to increasing recruitment following spawning. The Morisita index is a valuable tool for ecologists studying bivalve distribution patterns, offering insights into spatial clustering, habitat preferences, and the effects of environmental factors [95]. Its robustness to sample size and density variations makes it particularly useful in diverse ecological contexts [96].

Sex ratio in the present study was in favor of females with M:F ratio at 1:1.70 in agreement with [97] in Izmir Bay, Turkey, who reported a M:F ratio at 1:1.32 with no significant difference observed among the ratios ($X^2 = 1.82$, $p > 0.05$). In contrast, the M:F ratio was reported in favor of males by [71] in Gazi Bay, Kenya. It has been suggested by [98] that sex ratio could be related to age, with a significant proportion of the oysters maturing initially as males with changing occurring to females in later years (i.e., protandrous hermaphroditism). These sexual changes are potentially related to food availability, with good conditions in favor of females and bad conditions, or stress, favoring males [75]. The L_{50} estimated in the present study was at 47.2 mm in shell height, considerably larger compared to 38 mm reported by [66] in the Gulf of Gabes, Tunisia, possibly attributed to the different environmental conditions. Gonadal development, defined as changes in the gonads during active reproductive phases, occurs as seasonal variations in complex processes when the appropriate biological and physical conditions are fulfilled [99].

In contrast with [67], who reported a biannual reproduction for *P. radiata* in the gulf of Gabes Tunisia. Inactive gonads (stage I) for *P. radiata* in the south Evian region occurred during February, followed by early development (stage II) between February and April and late development (stage III) between April and July. Spawning (stage IV) occurred between August and September, followed by gonad recession (stage V) between October and January. In comparison, *P. radiata* spawning peak exhibits temporal variation; in Bahrain, spawning peak occurs during June-July [100], in Australia, during December-January/March-May [101], and at Kerkennah Island, Tunisia during July/November [66]. Hydrodynamic conditions can directly affect food availability, energy allocation, maintenance, and reproduction [71]. It is also known that bivalves change their reproductive strategies [81] and sex expression [102] according to environmental conditions.

A comparative table of the reproductive status and seawater temperature range between the present study and published literature indicated that in tropical regions spawning frequency tends to be continuous, whereas in more temperate regions spawning occurs in one annual pulse and is more pronounced during the summer months (Table 3).

Table 3. Comparison of reproductive status and temperature range in pearl oysters in different areas.

Spawning Frequency	Spawning Peak	Min Temp	Max Temp	Study Area	Reference
Annual	June–September	13.4	25.2	Evian region, Greece	Present study
Annual	June–September	14.2	27	Izmir Bay, Turkey	[97]
Biannual	June-August, September-December	17	32	Gulf of Gabes, Tunisia	[67]
Biannual	Summer and Autumn	12	30	Gulf of Gabes, Tunisia	[66]
Annual	Summer	-	-	Persian Gulf, Iran	[86]
Biannual	July and October	26	32	Gazi Bay, Kenya	[71]

The histological analysis of *P. radiata* revealed distinct and well-defined reproductive phases for both sexes, contributing valuable insights into their spawning cycles and reproductive strategies. These findings underscore the importance of targeted conservation efforts and sustainable management practices to support the continued health and productivity of pearl oyster populations in the study region.

5. Conclusions

The histological analysis of the reproductive biology of Pearl oyster, *Pinctada radiata* (Leach 1814), in the Eastern Mediterranean (Evian region) provides significant insights into the gonadal development and sexual maturation of this species. The detailed examination of both male and female gonads revealed distinct stages of gametogenesis, highlighting the synchronous development patterns that are influenced by seasonal variations in environmental factors such as temperature and food availability. These findings not only contribute to our understanding of the reproductive strategies of *P. radiata* in the study region but also offer valuable information for the management and conservation of pearl oyster populations. By identifying the reproductive cycles, this study lays the groundwork for future research on the impacts of climate change and anthropogenic activities on the reproductive health and sustainability of this economically and ecologically important species.

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