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A Study of *Arca noae* (Linnaeus, 1758) in Elounda Bay, Crete, Eastern Mediterranean

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Abstract: There is growing interest about marine bivalve aquaculture globally, not only for the market value of the goods produced, but also for the socio-economic and environmental services that this activity can provide. *Arca noae* is an endemic Mediterranean bivalve of commercial value, whose previously undescribed population in Elounda Bay we studied in terms of its structure and reproduction, while constructing a timeseries of the basic environmental parameters of the bay, thus, gaining fundamental knowledge for the potential future exploitation of the species in the area. We found a variable spatial distribution of arks in the study area, with local high peaks in the population density, consisting of smaller size individuals, in comparison to other areas. Because of protandry of the species, human pressure on this population could have a strong negative effect, by targeting the limited numbers of large females in the study area. The reproduction pattern was similar to the reports from other Mediterranean locations. The abiotic conditions in Elounda Bay differed from those in the adjacent coastal zone, confirming that the Bay is a unique semi-enclosed marine area in the island of Crete.

Keywords: *Arca noae*; Elounda Bay; Crete; population structure; reproduction; abiotic timeseries

1. Introduction

Marine bivalve production is a very important part of global aquaculture. In the period between 2010–2015, more than 15 million tonnes/year of bivalves were produced, 89% from aquaculture and 11% from wild fisheries, which accounts for roughly 14% of the global marine aquaculture [1]. FAO states that there are 79 species of marine bivalves currently cultured, and 93 species currently harvested from the wild. Clams, oysters, mussels and cockles are the main species groups of the global bivalve production, with most of that production (85%) in Asia [1]. Furthermore, bivalve aquaculture production offers environmental services, as it can improve sea water quality by removing particulates and absorbing nitrogen and phosphorus, thus, counteracting anthropogenic eutrophication [2]. It can also have an important ecological role by creating substrates for other species on which to settle or take refuge. In this way, it can enhance biodiversity by boosting the species number and biomass of invertebrates and finfish, in comparison with similar areas without bivalve beds [2]. Bivalves and their shells in particular are a source of $CaCO_3$ with possible agricultural, industrial and environmental engineering applications. It is also worth noting that bivalves absorb CO_2 during shell growth; thus, they can potentially counteract the environmental impact of anthropogenic CO_2 [3].

Arca noae (Figure 1) is a commercial, edible bivalve endemic to the Mediterranean Sea, the Black Sea and the Eastern Atlantic Ocean. It attaches by byssal threads to hard substrate and can be found either in clumps or as solitary specimens in depths of over 100

m. Its biology has been studied in several locations in the Mediterranean Sea (e.g., Adriatic Sea, Tunisia) and it is reported that it is a slow growing bivalve, usually reaching a maximum size of 70 to 90 mm and an age of up to 16 years [4–6]. However, exceptional sizes of up to 120 mm and ages of up to 25 years have been reported from a marine protected area (MPA) in Croatia [7]. Up to the present, there exists only limited research about the reproductive biology of the species, conducted in a few locations in the Mediterranean Sea. However, it has already been established that *A. noae* is a protandric hermaphrodite species with males dominating small shell lengths, while females become predominant as size increases [8]. The studies from the Adriatic Sea report a long spawning season, starting in spring and ending in autumn. However, depending on the location, there are reports of single or double spawning peaks within the aforementioned period [9,10]. A similar study from the Bizerte lagoon in Tunisia indicated that *A. noae* is a multiple spawner, exhibiting two spawning peaks each season [11].

Figure 1. Posterior view of an *Arca noae* feeding in Elounda Bay. (Photograph curtesy of Francisco Sedano).

Although there have been a few attempts at cultivating this clam in the Adriatic coasts of Croatia so far, they have not been successful. Specimens were collected and placed in nets designed for mussel culture and were either suspended in the water column or placed directly on the bottom in order to study the survival and growth rates. The survival rates on both occasions were very low, forcing the investigators to characterize the species as unsuitable for aquaculture, since it is highly sensitive to handling [12,13]. Another aspect that can be problematic for the introduction of the species in aquaculture is its rather slow growth rate. It is worth noting that, in order to reach a commercially exploitable size of at least 50 mm, it can take three to seven years, depending on the location of the population. It also appears that other key elements concerning the biology of the species have not been studied adequately in a variety of locations in order to successfully culture *A. noae*, such as distribution and size of stocks and in situ growth rates of the species. In addition, no study about the production of seeds exists so far, leaving seed collection as the only option for acquiring the stock necessary for the culture of the species.

However, natural seed collection has to be synchronized with the spawning period of *A. noae*, which may differ between localities, while the effect of different seed collectors' design and materials have not been studied yet [12].

A unique population, for the island of Crete, of *Arca noae* is present in Elounda Bay. Elounda Bay is a shallow, semi-enclosed bay in the north-eastern part of Crete. Its geomorphology encloses an area of roughly 5 km^2 that is protected from the waves and currents of the adjacent Mirambelo Bay [14,15]. The bottom of the bay below the 2 m depth contour is covered superficially by fine sediments (silt and clay) but most of its coastline is rocky, offering a narrow band of hard substrate in the periphery of the bay to a variety of invertebrates, including arks.

The aim of the present study was to obtain a better insight into the ecology and biology of the population of *Arca noae* in Elounda Bay. For this purpose, the structure of the population was investigated by recording its densities and the morphometry (shell lengths) of individuals in a variety of locations within the bay. Furthermore, aspects of the reproductive biology of the species in this Eastern Mediterranean location were investigated. In particular, the sex ratio in relation to size and the spawning cycle of the population was investigated and compared to the data available for other Mediterranean locations. The study also monitored, for the first time, important environmental parameters of Elounda Bay over a two-year period, such as temperature, salinity and dissolved oxygen. The geomorphology of the area led to the conclusion that these specific parameters may differ in comparison to adjacent coastal waters, granting the bay some unique abiotic characteristics that favor the almost exclusive establishment of *A. noae* locally.

2. Materials and Methods

2.1. Population Structure

The population density of *Arca noae* was measured in 25 sampling sites within and adjacent to Elounda Bay (June–November 2017), using quadrats sized 50 cm × 50 cm (Figure 2). These were placed randomly on hard substrate along a 50 m transect and the individual arks within the quadrats were counted. In each site, we used 30 quadrats and the number of arks measured was converted to individuals/m^2. The densities did not have a normal distribution (Kolmogorov–Smirnov test $p < 0.001$) and were analyzed using non-parametric tests (Kruskal–Wallis ANOVA on ranks and Dunn's test for the multiple comparisons of sites).

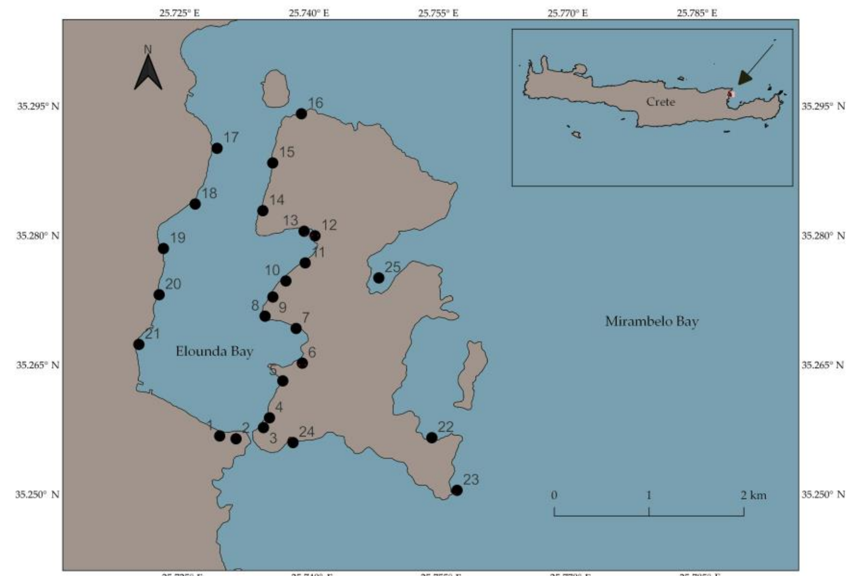

Figure 2. Map of Elounda Bay depicting the sites used for density measurements of *Arca noae*.

The shell lengths of *Arca noae* were measured in 10 of the 25 sampling sites previously mentioned, all located within Elounda Bay (June and July 2019). In order to avoid destructive sampling methods, the measurements were taken in situ, utilizing scuba diving and Vernier calipers. A total of 60 randomly selected individuals were measured in a 50 m transect in each site and the shell length was measured to the nearest mm. The frequencies of the recorded lengths were tested for normal distribution (normal QQ plot and Kolmogorov–Smirnov test) and plotted (histogram).

2.2. Sex Ratio and Reproductive Biology

For the determination of the sex ratio and the reproductive period of *Arca noae* in Elounda Bay, 50 individuals were collected, evenly distributed (10 samples / date) between the summer of 2019 and late spring of 2020 on 5 sampling dates (June, August, September, November of 2019 and May of 2020), with emphasis given to the summer and autumn months, since studies from other Mediterranean locations indicated that the species spawning period is limited within these two seasons [8,10,11]. The individual sizes of all the specimens were measured to the nearest mm and a histological analysis was performed to determine sex and the maturity stage of the gonads, according to the published criteria (Table 1) for the species [10]. In addition, the maturity stage of the female arks was confirmed, when possible, by measuring the diameter of 10 oocytes/female and comparing their mean values to the data from other areas [8]. For histological evaluations, the samples were fixed in 4% formaldehyde: 1% glutaraldehyde [16], dehydrated in a 70–95% ethanol series and embedded in glycol methacrylate resin (Technovit 7100, Heraeus Kulzer, Hanau, Germany). Serial sections were obtained at a thickness of 3–5 μm on a microtome (Biocut 2035, Reichert Jung, Munich, Germany) using disposable blades. After drying, the slides were stained with methylene blue/azure II/basic fuchsin [17] and examined under a light microscope (Nikon Eclipse 50i, Tokyo, Japan).

Table 1. Criteria for the determination of the different maturity stages of *Arca noae*.

	Female	Male
Early & late developing	Initially dense oogonia present in acini, followed by previtellogenic oocytes smaller than 60 μm.	Acini initially filled with germinative cells and spermatogonia, followed by the development of small spermatozoa.
Ripe	Acini filled with large mature oocytes (>60 μm).	Acini filled with spermatozoa. Central part of acini intensively pinkish.
Partially spawned	Acini almost empty with a few mature oocytes remaining.	No spermatogonia present and spermatozoa mainly evacuated.
Spent / Inactive	Empty acini with occasional scarce degenerative oocytes.	Empty acini with occasional small numbers of remaining spermatozoa.
Adapted from Peharda et al. (2006) [10]		

2.3. Environmental Parameters

The measurements of temperature, salinity and dissolved oxygen were made on a monthly basis in five sites in Elounda Bay and two sites in the adjacent open coastal Mirambelo Bay for a two-year period, from January 2019 to December 2020. In addition, pH measurements were also taken using the same protocol, but due to unfortunate events, the pH timeseries covers only a time period of 16 months (January 2019–April 2020). A portable analyzer (Hach, HQ40d) with the appropriate electrodes (CDC 40103, LDO 10101 and PHC 10103) was used for measuring the environmental parameters in surface water. Each month, three consecutive measurements were taken on the same day and a mean value was calculated for each site and parameter. The mean values for the five sites of Elounda and the two sites of Mirambelo were calculated and plotted for each month of the year, for all the parameters.

3. Results

3.1. Population Structure

3.1.1. Population Densities

The mean values of the densities ranged between 0.4 ind m^{-2} and 39.5 ind m^{-2} and varied significantly across the Elounda Bay sites, grouped into the following three characteristic categories: low density, medium density and high density (Figure 3). Sites 8 to 11 exhibited a high population density (>20 ind m^{-2}), site 4 a medium population density (≈10 ind m^{-2}) and all the other sites in the bay had a low population density (<5 ind m^{-2}). Sites 8–11 located in the northwest coast of Elounda Bay were the only ones that offered vertical hard substrate, which seems to favor the highest population densities in the bay. Sites 16, 22, 23, 24 and 25 were located outside Elounda Bay and no arks were found in the 50 m transects.

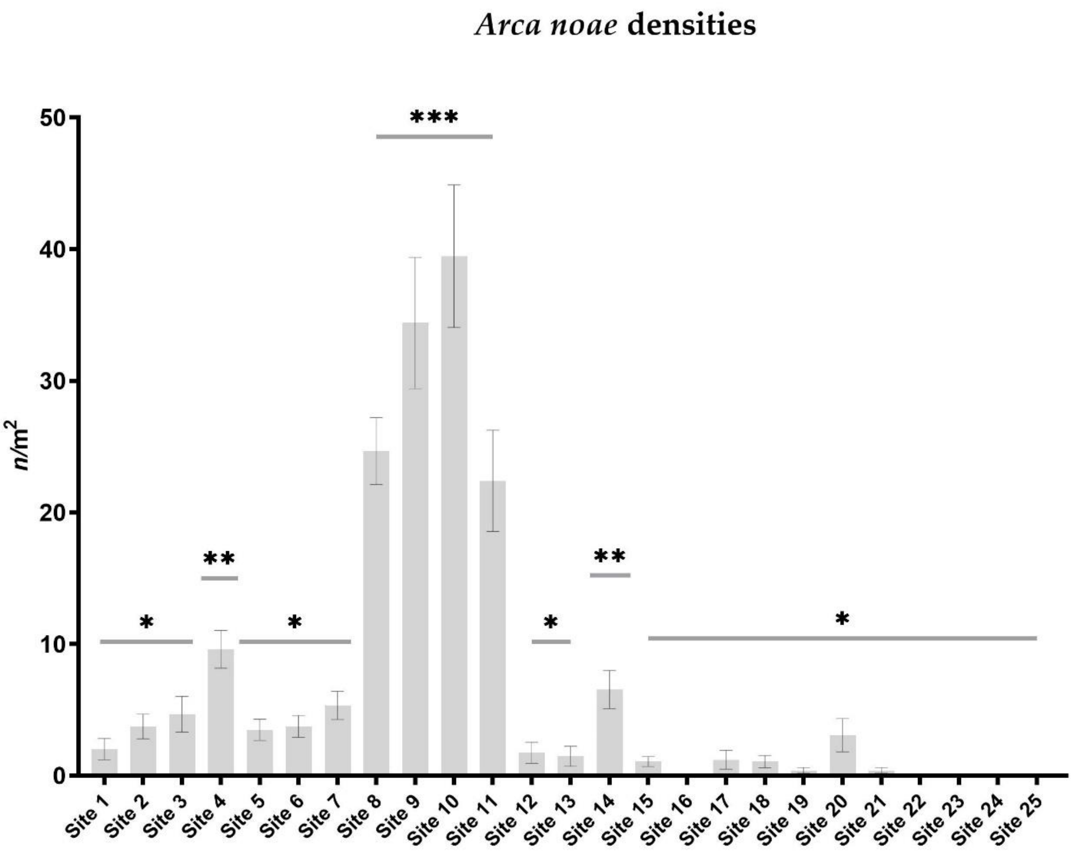

Figure 3. Average densities of *Arca noae* with SEM in all sites of Elounda Bay. Symbols *, ** and *** denote significant differences (p-value < 0.05).

3.1.2. Shell Lengths

The lengths of *Arca noae* measured in Elounda Bay ranged from 13 mm to 61 mm (mean value ± SEM = 36.33 ± 8.58, n = 600). The vast majority of *Arca noae* (93.7%) were below the minimal legal landing size of 50 mm for the species in Greek waters. The length frequencies (Figure 4) did not have a normal distribution (Figure 5), as length values at both extremities of the size range were overrepresented and a few smaller lengths (20–25 mm) were underrepresented. The most frequent lengths (30–50 mm) were found to follow the pattern of a normal distribution.

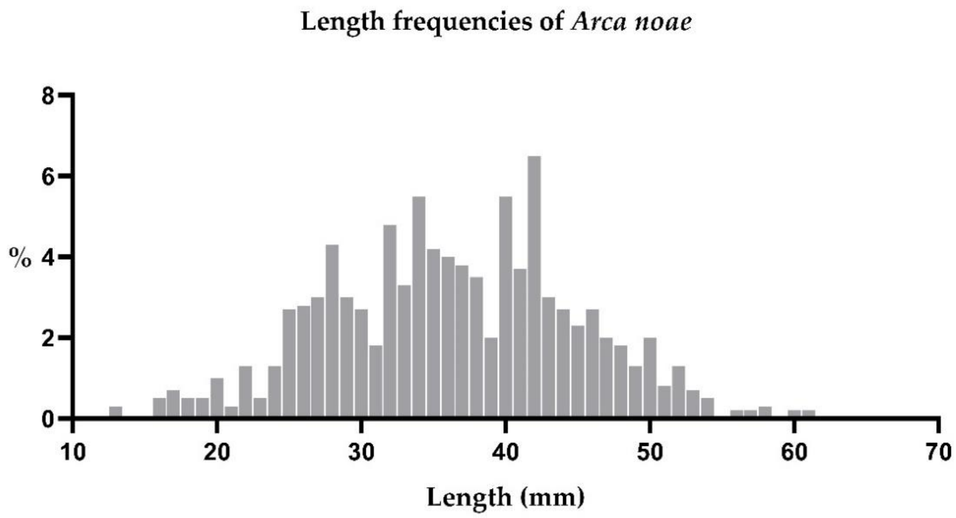

Figure 4. Length frequencies of *Arca noae* in Elounda Bay (n = 600).

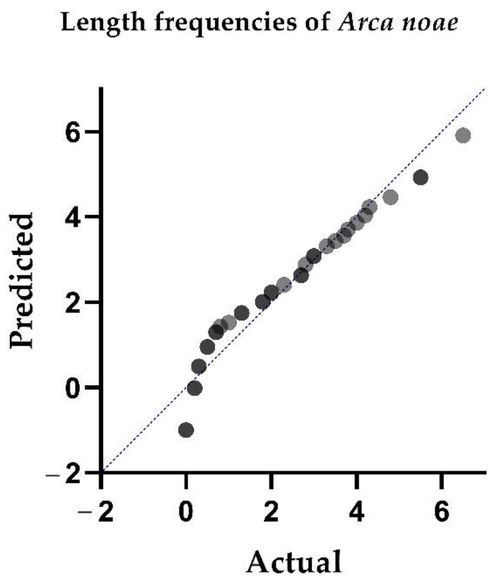

Figure 5. Normal QQ plot of length frequencies of *Arca noae* in Elounda Bay.

3.2. Reproduction

3.2.1. Sex Ratio

Of the 50 individuals of *Arca noae* collected for sex determination and histological analysis, 46 had their sex determined successfully, 2 were possible hermaphrodites and another 2 of unidentifiable sex. Out of 46 arks, 26 individuals (56.5 %) were identified as males, 20 individuals (43.5 %) as females and their distribution per size class is presented in Figure 6. Males were more frequent in the smaller size classes (<30 mm), females were more frequent in the larger size classes (≥50 mm), while their ratio was about 1:1 at intermediate sizes between 30 and 49 mm.

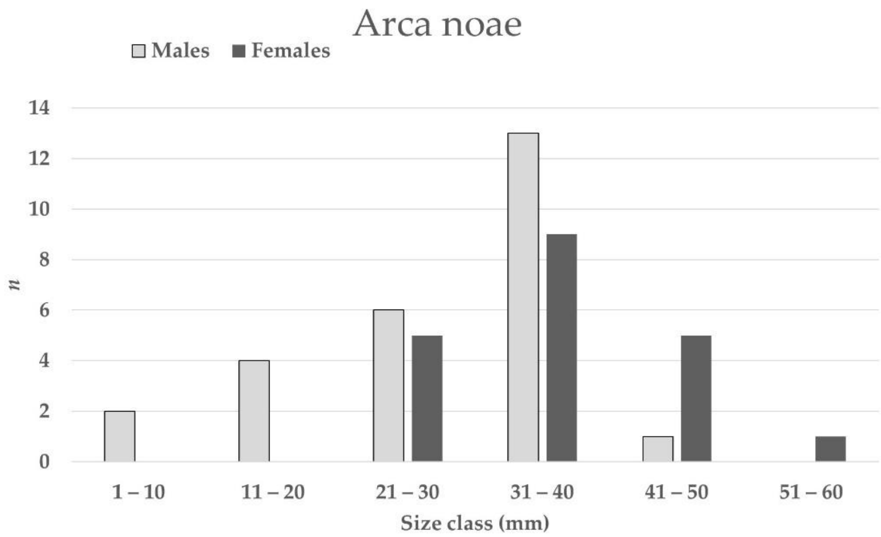

Figure 6. Number of male and female *Arca noae* in size classes found in Elounda bay (n = 46).

3.2.2. Reproductive Cycle

From the histological analysis of the gonad samples, the stage of maturation of each of the *Arca noae* (n = 46) collected in different seasons, as shown in Table 2 and Figure 7, was established. For both sexes, all the individuals in late spring (May) were either at the stage of developing new gametes or possessed fully ripe gonads. However, it was during the early summer (June) that individuals with spent gonads made an appearance. A second period of gonadal development appeared to be taking place in late summer (August and September), as a majority of the arks at this time were found to be developing new gametes. In November, at the approach of winter, most *Arca noae* seemed to have spent gonads and only a few were at the developing stage.

Table 2. Temporal distribution of *Arca noae* (n = 46) at different gonadal maturation stages.

Stage	Early and Late Developing	Ripe	Partially Spawned	Spent/ Inactive
Month				
June	2	1	1	6
August	8	0	0	0
September	5	0	1	3
November	5	0	0	5
May	5	3	1	0

3.3. Environmental Paramaters

3.3.1. Temperature

The mean monthly temperatures of Elounda Bay ranged between 13.78 °C (Jan 2019) and 29.12 °C (Aug 2020), while those of Mirambelo Bay ranged between 16.15 °C (Feb 2019) and 28.65 (Aug 2020) and are shown in Figure 8. It is worth noting the sharp increase in temperature in spring (Apr-May) and the sharp drop of temperature in autumn (Oct-Nov). In both years, there were lower winter temperatures of about 2 °C and higher summer temperatures by more than 1 °C in Elounda Bay, when compared to Mirambelo Bay (Figure 9).

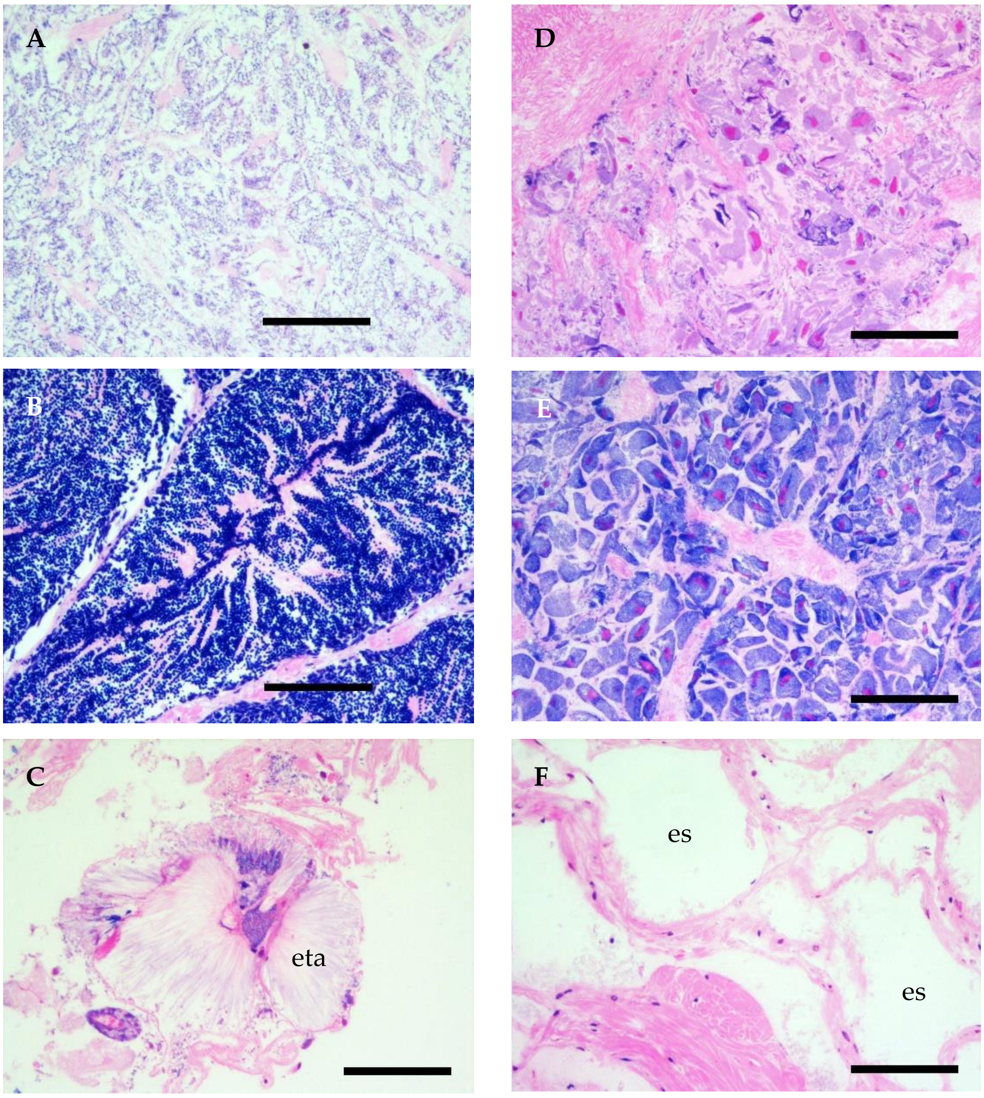

Figure 7. Light photomicrographs of the gonadal stages of *Arca noae*: (**A**) developing male, (**B**) ripe male showing large acini filled with spermatozoa, (**C**) spent male showing empty acini, (**D**) developing female, (**E**) ripe female showing mature oocytes and (**F**) spent female. eta, empty testicular acini; es, empty space. Scale bars: (**A**–**C**,**F**) =100 µm; (**D**,**E**) =200 µm.

3.3.2. Salinity

The mean monthly salinities of Elounda Bay ranged between 38.3 in winter and 41.8 in summer, while those of Mirambelo Bay ranged between 38.9 in winter and 40.6 in summer (Figure 8). In both years, we observed lower winter salinity and higher summer salinity in Elounda bay, when compared to Mirambelo bay (Figure 9).

3.3.3. Dissolved oxygen

The mean monthly dissolved oxygen concentrations of Elounda Bay ranged between 8.98 ppm in winter and 6.38 ppm in summer, while those of Mirambelo bay ranged between 8.74 ppm in spring and 6.49 ppm in summer (Figure 8). Similarly, the mean oxygen saturation values ranged between 99.2% and 121.6% in Elounda Bay and between 99.2% and 122.9% in Mirambelo Bay. In both sites, the dissolved oxygen values and oxygen saturations were within their expected range for the entire duration of the monitoring effort.

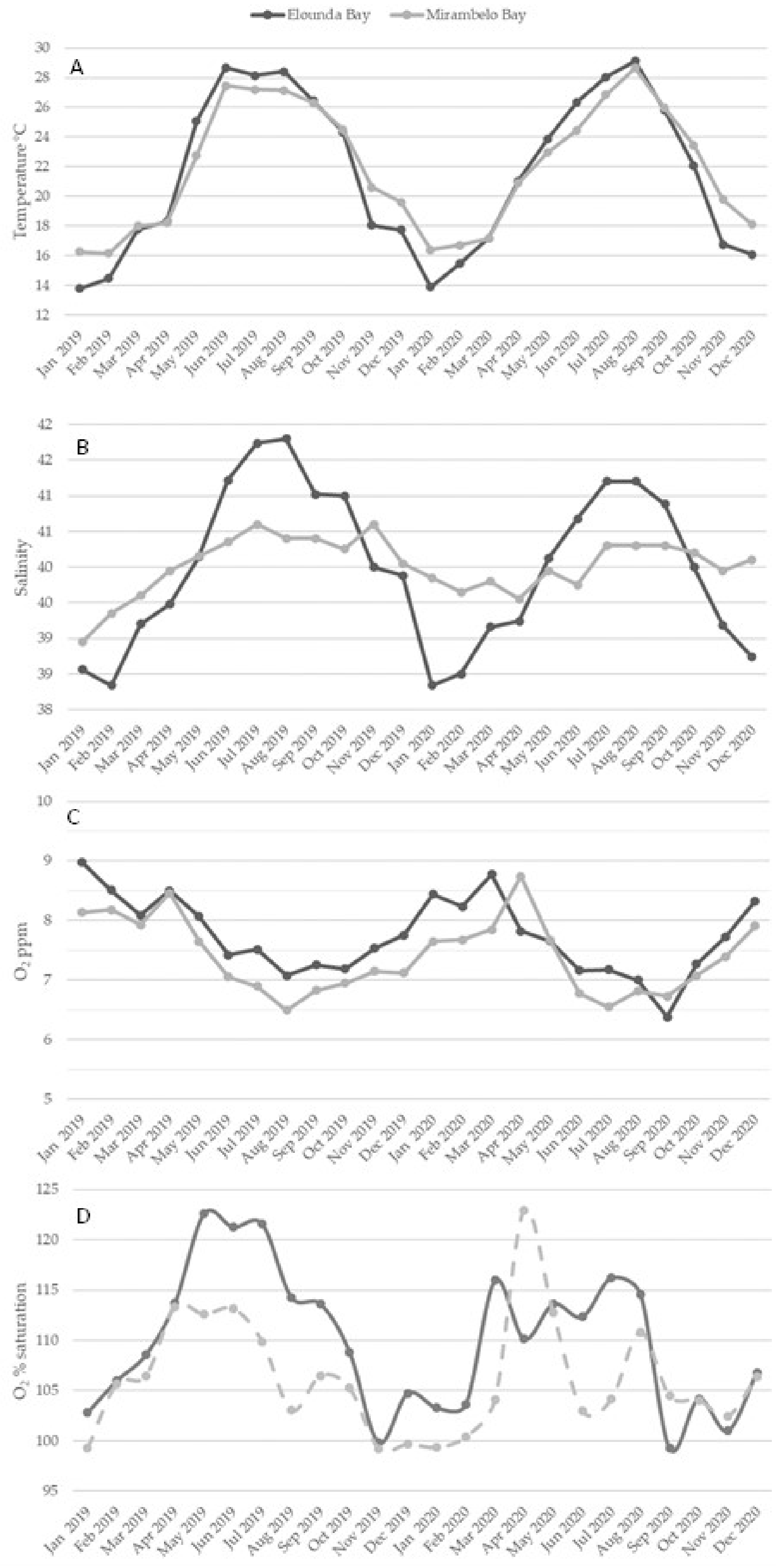

Figure 8. Mean monthly temperature (**A**), salinity (**B**), O_2 concentrations (**C**) and O_2 saturations (**D**) of Elounda Bay and adjacent Mirambelo Bay recorded for a two-year period (2019–2020).

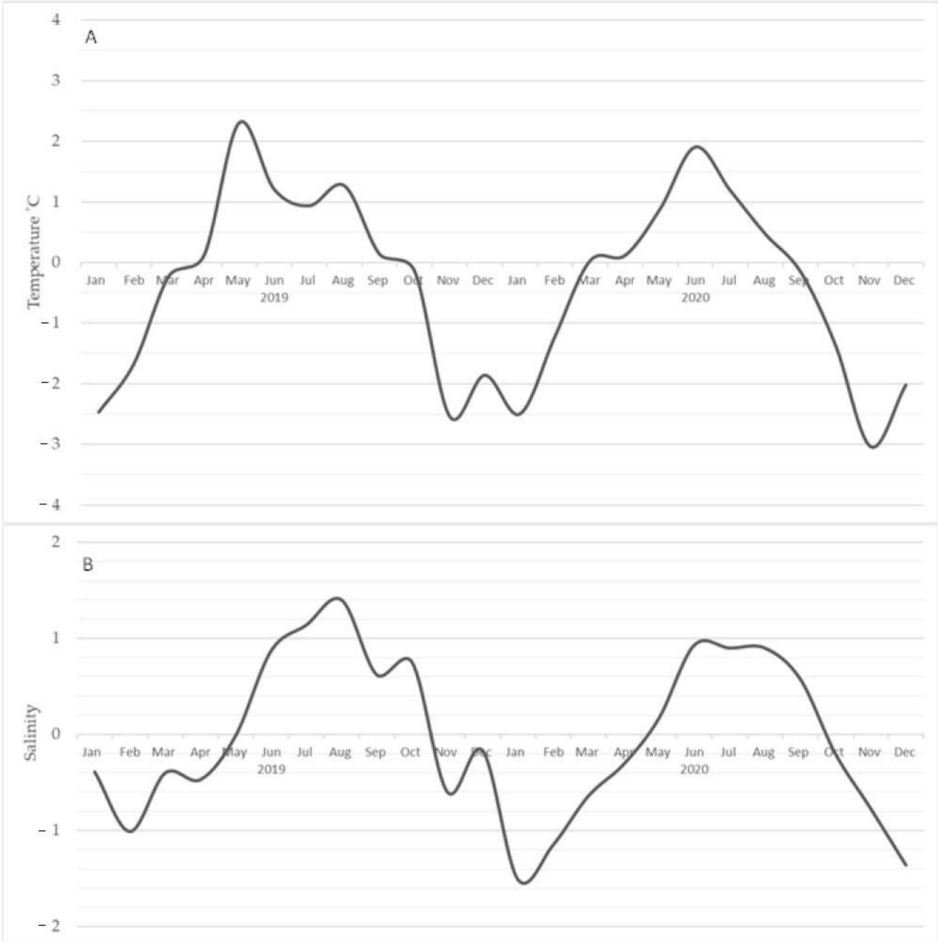

Figure 9. Mean monthly temperature °C (**A**) and mean monthly salinity (**B**) difference between Elounda minus Mirambelo Bay recorded over a two-year period (2019–2020).

3.3.4. pH

The mean monthly pH values of Elounda Bay ranged between 8.10 in winter and 8.40 in summer, while those of Mirambelo Bay ranged between 8.12 in winter and 8.45 in summer and in both sites, the pH values were within their expected range.

4. Discussion

The population of *Arca noae* in Elounda Bay exhibited a large spatial variability, ranging from low to very high mean densities (ind m^{-2}). On horizontal bedrock, which is present in the majority of the sampling sites around the bay, arks were encountered either as solitary specimens or in small clumps, consisting of a few individuals. Their mean density was low, ≤10 ind m^{-2}, comparable to the densities reported for the Adriatic coast of Croatia [4,18]. In those few sampling sites that offered vertical bedrock for establishment, arks were encountered in large clumps and mean densities of ≥20 ind m^{-2} and values as high as 39.5 ind m^{-2} were recorded. It appears that the conditions on vertical substrate were more favorable for arks, since they could sustain significantly larger aggregations. In fact, it has been documented that the orientation of hard surfaces, natural or artificial, does have a significant effect on their epibiota and especially on the assemblages of sessile organisms, as horizontal surfaces are more prone to sedimentation [19–22]. Throughout the bay, *Arca noae* was associated with the bivalve *Modiolus barbatus,* forming common clumps as reported from the other studies in the Mediterranean Sea [4,23]. However, the potentially anti-predatory association of *Arca noae* with sponges [24,25], especially *Crambe crambe*, was never recorded in any of the sampling sites of Elounda Bay. In the five sites outside but adjacent to Elounda Bay, no arks were recorded in quadrats at all.

The size range of *Arca noae* encountered in Elounda showed some deviation from the expected length frequencies recorded in other Mediterranean locations. We believe that an underestimation of the number of the smaller sizes (<15 mm) was to be expected, since most were found between clumps of adults or hidden in crevices, which made them difficult to spot and accurately measure their length without employing destructive sampling techniques, a problem also encountered by other researchers [18]. In addition, the maximum recorded length (61 mm) and the total number of individuals over the 50 mm limit measured in Elounda was lower than expected. In other similar studies conducted in the Croatian Adriatic and in Tunisia [7,11,19,23,26], the reported maximum lengths were over 70 mm and lengths over 50 mm attributed to 30–40% of the whole population. In the present study, a much smaller portion of the population (6.3%) was attributed to lengths over 50 mm. Nevertheless, it should be noted that these locations are more eutrophic [11,26] than Elounda Bay [27] and elevated food availability could explain the higher abundance of large individuals reported in these areas. Unfortunately, information on population densities and length composition from other oligotrophic Eastern Mediterranean locations, which would allow direct comparison with the population of Elounda Bay, is entirely lacking. In conclusion, this population of *Arca noae* locally exhibited some impressive densities that were always associated with the presence of near vertical substrate, but at the same time, they consisted of much smaller individuals than the populations from the Western Mediterranean and the Adriatic Sea [4,7,11,19,23].

The conducted histological analysis gave an important insight into the reproductive cycle of the local population in Elounda Bay. The sex ratios were found as expected from similar studies [9,18], with the males dominating the smaller sizes and females dominating the larger ones, since the species is a known obligate protandric hermaphrodite. However, the low number of large breeding females in the area could be negatively affecting the reproduction dynamics of the population by limiting the number of offspring produced each year. The single gonadal maturation peak observed in late spring (May) and the subsequent spawning that was observed in June in Elounda Bay is comparable to the reported spawning seasonality in other locations of the Mediterranean Sea [8,10,11]. In the following summer months, there was evidence of some gonadal regeneration in only a few arks, while at the same time, most appeared to have spent gonads. This is an indication of a prolonged asynchronous series of smaller spawning events, which lack the intensity of the initial spawning peak and by late autumn, most individuals were found with spent gonads [8,10,11].

The two-year timeseries of the monitoring of environmental parameters sheds light on the abiotic conditions prevailing in the semi-enclosed bay of Elounda, in comparison with the corresponding ones in the open sea (Mirambelo Bay). These records indicate that Elounda Bay behaved as an independent body of water, whose temperature, salinity and pH were more sensitive to change by atmospheric influence, such as heat waves and heavy precipitations.

Furthermore, previous studies in the area have already indicated that this shallow bay is characterized by specific biotic features. A dense canopy of *Caulerpa prolifera* dominated the sediments of the bay, which was home to diverse and abundant benthic macrofaunal assemblages [14]. These, in turn, supported a rich diversity of juvenile fish and cephalopod species in the summer months, indicating the importance of the area as a nursery ground for commercial species [15,16,27].

A possible exploitation of *Arca noae* in Elounda Bay could further boost the economic growth of the area. However, future complementary research is necessary for a successful attempt in the extensive culture of the species in the area. The seasonality of the recruitment, as well as the artificial substrate that is suitable for the settlement and growth of juvenile arks, are to be investigated. In addition, in situ measurement of the growth rates of arks in Elounda Bay will determine the management regime needed for sustainable exploitation. More focused monitoring of water column primary productivity will allow us to investigate the carrying capacity of the bay. The latter will, in turn, dictate the extent of artificial substrates that can be used for the desired population size growth. The predator–competitor interactions of arks in this area are unknown and any impact from illegal fishing should be identified in order to protect a unique and valuable natural resource for the area.

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