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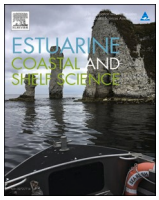

Investigating the reproductive biology of *Anemonia viridis* (Forsskål, 1775) (Cnidaria, Hexacorallia) in the Central-Western Mediterranean Sea, a step towards domestication

Cristina Porcu [a,*,1], Viviana Pasquini [a,1], Marco Secci [b], Martina Francesca Marongiu [a], Colin Hannon [c], Maria Cristina Follesa [a], Pierantonio Addis [a]

[a] Department of Life and Environmental Sciences, University of Cagliari, via T. Fiorelli 1, Cagliari, 09126, Italy
[b] Agricultural Research Agency of Sardinia, Ichthyic Products Research Service, Viale Trieste 111, Cagliari, 09123, Italy
[c] Marine & Freshwater Research Centre, Atlantic Technological University, Galway, Ireland

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ABSTRACT

Knowledge of the reproductive biology of the snakelock anemone (*Anemonia viridis*) is necessary to ensure a science-based management and to develop the biotechnologies of aquaculture of cnidarians. This study investigates the reproductive biology of *A. viridis* collected in the shallow rocky-bottom substrates of the South-Eastern Sardinian seas (Central-Western Mediterranean Sea) at depths between 1 and 5 m. A total of 200 samples with a pedal disc ranging from 6.1 mm to 40.7 mm were histologically examined and sectioned to assess the reproductive activity individually. Female anemones were significantly more abundant than males and exhibited asynchronous oocyte development both within and among individuals. Oocyte diameter data suggested an extended reproductive cycle from February to June, with peaks in March and April. The smallest mature female and male had a pedal disc of 9.7 mm and 13.2 mm, respectively. Our results indicated that temperature was not the main driver of gametogenesis; instead, oocyte maturation was principally linked to the increased primary production in the water, thus correlating with potential higher food availability. Due to the high variability in the spawning period across different geographic areas of the studied species, regional (local) fishing regulations for *A. viridis* should be considered, with a proposed closed season between March and April when spawning peaks occur in the Sardinian populations.

1. Introduction

Although marine invertebrate fisheries in Europe and North America are commonly associated with decapod crustaceans and bivalve molluscs, there are also diverse marine invertebrate groups that are commercially important, including sea anemones (Anderson et al., 2008), primitive animals belonging to the Cnidaria Phylum (Anthozoa: Hexacorallia), the oldest group of eumetazoans. This group has a key ecological role as both predators and preys in marine ecosystems and provides habitat for some marine organisms including fish (Boero et al., 2005).

Many sea anemones, in particular tropical species, are harvested as marine ornamentals for aquaria, and for this reason can be subjected to overexploitation (Shuman et al., 2005; Madduppa et al., 2014). As benthic organisms, sea anemones are also considered good bioindicators for potential toxic elements such as metals bioaccumulation (Mitchelmore et al., 2003; Horwitz et al., 2014,Horwitz et al., 2015; Corrias et al., 2020; Cabeza et al., 2021). In addition, they represent promising putative pharmacological agents for translational research and biomedical applications, having strategies for survival, which includes an articulated and finely defined toxin arsenal (e.g. Nicosia et al., 2018; Thangaraj et al., 2018). Several species of sea anemones are valued as food in various countries including Japan, Samoa, France, Spain, Portugal, and Italy (Thorpe et al., 2000) and their harvesting and consumption diversify greatly at the local regional level, according to specific culinary traditions. Despite the commercial importance of sea anemones, little information about their population status and exploitation on geographical scale are available (Thorpe et al., 2000).

* Corresponding author.
E-mail address: cporcu@unica.it (C. Porcu).
[1] These authors have contributed equally to this work.

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Fig. 1. Map of the study area with indication of sampling sites (Capo Ferrato, Torre delle Stelle and Cala Pira). In purple, the point grid of environmental parameters gathered from Copernicus. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Summary of number of collected samples (N), size (PD, pedal disc) and weight (TW, total weight) ranges of specimens were reported monthly. SD, Standard Deviation.

Month	N	Size range (PD, mm)	Size average (PD, mm ±SD)	Weight range (TW, g)	Weight average (TW, g ± SD)
January	-	-	-	-	-
February	25	6.9-19.9	11.95 ± 3.65	1.75-25.82	7.50 ± 5.60
March	18	11.9-40.7	19.03 ± 9.63	2.77-92.00	21.06 ± 33.23
April	17	6.5-28.5	11.29 ± 5.49	1.48-31.55	6.89 ± 7.38
May	25	9.2-18.9	12.94 ± 1,84	2.6-10.3	4.93 ± 1.71
June	20	7.1-26.2	14.86 ± 4.84	1.8-32.5	10.36 ± 6.35
July	25	7.3-36.2	12.81 ± 5.52	1.3-56.9	5.86 ± 10.79
August	21	6.1-14.3	10.05 ± 2.49	1.38-8.9	4.90 ± 2.16
September	25	7.1-17.6	11.25 ± 2.59	1.4-21.3	7.14 ± 5.73
October	-	-	-	-	-
November	25	6.1-17.2	11.83 ± 2.78	0.94-11.44	4.52 ± 2.57
December	-	-	-	-	-
Total	**200**	**6.1–40.7**	**12.78 ± 5.01**	**0.94–92.00**	**7.75 ± 12.13**

Among the Mediterranean species, *Anemonia viridis* (Forsskål, 1775) is considered one of the most common sea anemones and it is among the largest sea anemones in this region (Porro et al., 2020). Also called snakelock anemone, this species is recognised by its long, green-brownish wavy tentacles and it is often found in rocky coastal areas. It occurs in the intertidal and shallow sublittoral zones of the Mediterranean and Atlantic coast of Europe, from Gibraltar to Scotland, commonly down to 20 m depth and observed on rocky outcrops (Ocaña and den Hartog, 2002; Hofrichter, 2005; Ocaña et al., 2015; Utrilla et al., 2019). Numerous studies reported the presence of another species belonging to the same genus, *A. sulcata* (Pennant, 1777), which shows overlapping distribution ranges in the Northeastern Atlantic Ocean and the Mediterranean Sea. On the other hand, more recent molecular analyses confirmed that these two species can be considered synonymous (Arossa et al., 2021). Hence from here on, *A. viridis* will be used in reference to information from both bibliographic sources.

In recent years, *A. viridis* has attracted interest as possible source of marine natural products and biochemical active compounds, including several secondary metabolites and toxins to capture prey and deterrents from predation (e.g. Rocha et al., 2011; Pejin et al., 2014; Silva et al., 2017; Cabeza et al., 2021). *Anemonia viridis* is considered a delicacy seafood in Sardinia Island (Italy, Central-Western Mediterranean) where it is called "orziadas", in Spain where it is known as "ortiguilla" (Utrilla et al., 2019; Coll et al., 2025) and to a minor extent in France (Thorpe et al., 2000). *Anemonia viridis* is also commercially targeted in Spain, in particular from the coast of Cadiz in the South to Galicia in the North as part of a growing gourmet trade for local harvesters.

Over the past few years, in the Western Mediterranean, the increasing demand for this species, coupled with the absence of regulations and/or illegal fishing and trade, had led to the decline of local populations (Utrilla et al., 2019). For example, in the southern Spain (Andalusia), the habitat loss caused by the expansion of the invasive algae *Rugulopterix okamurae*, is affecting numerous benthic species populations, including those of *A. viridis*, further threatening the population abundances (Coll et al., 2025). On a broader geographical scale, only a few information is available about its conservation status or fishery pressure, with only one conference paper exploring its fishery in the Granadian coast reporting an overall good status of the population (Norman et al., 2008). To allow a scientific-based management of *A. viridis* populations and guarantee a sustainable harvesting, it is necessary gather knowledge about their life history traits, including the reproductive biology and the spawning period. These life history traits can vary at local geographical scale, depending on biotic and abiotic factors, affecting the extension and the reproductive peaks of the species along their range of distribution (Orton, 1920; Tirado et al., 2011; Tirado and Salas, 1998). Nevertheless, investigations on the sexual reproductive cycle of the dioic *A. viridis* are mainly limited on spatial scale to the western Mediterranean Sea (Alboran Sea: South of Spain, Utrilla et al., 2019; Morocco, Daoudi et al., 2022), Tyrrhenian Sea (Gulf of Naples, Schäfer, 1984) and Adriatic Sea (Di Camillo et al., 2021).

To the best of the authors knowledge, no attempts to sexually reproduce sea anemones can be found in literature, except for the starlet sea anemone *Nematostella vectensis* where a method for laboratory spawning and reproduction is available (Genikhovich and Technau,

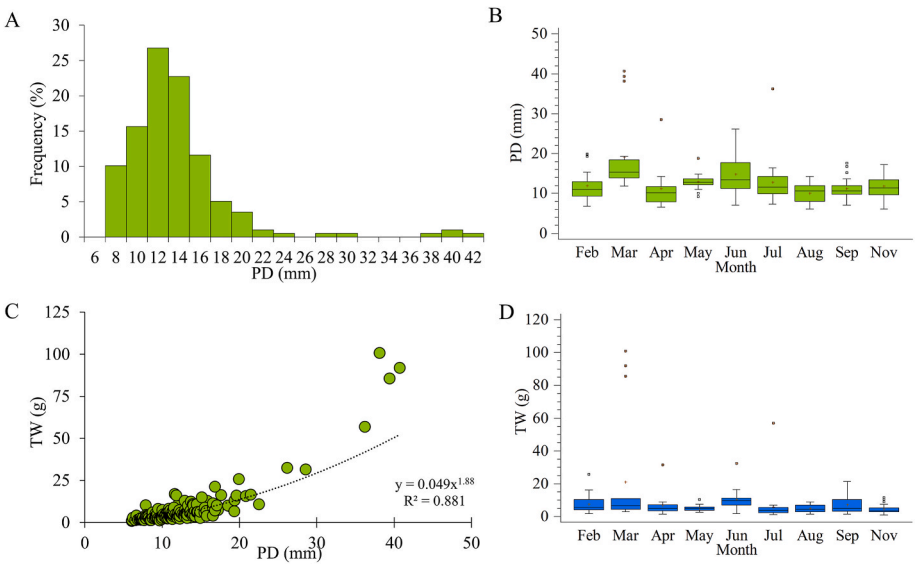

Fig. 2. (A) Size class distribution of *Anemonia viridis* specimens collected during the sampling period. (B) Box-plots showing the pedal disc distribution by month of sampling. (C) Relationship between the pedal disc (PD) and the weight of *A. viridis* collected during the sampling period. (D) Box-plots showing the total weight (TW) distribution by month of sampling. Boxes show the interquartile range, with the median value indicated by the horizontal line and the '+' sign indicating the mean; whiskers show the range. Individual symbols show outliers.

2009; Stefanik et al., 2013).

This species has most recently been assessed for the *IUCN Red List of Threatened Species* in 2014 and listed as Least Concern (Antoniadou et al., 2015). To promote the conservation of snakelock anemone and to encourage its culture for conservative purposes, the aim of this study is to investigate the species biology, by (1) providing a detailed description of the histological features of the gonads in relation to the reproductive cycle and (2) exploring the different maturity stages over seasons and in relationship with the environmental parameters. This study will be useful to define the basic life history traits related to the reproductive biology, including the onset and duration of spawning season, and the role of the environmental factors on them. Indeed, surface temperature and primary production could influence the seasonal timing of reproduction, crucial element for the fishery management and for its potential selection as a new species for aquaculture.

2. Materials and methods

2.1. Sample and data collection

The specimens of *A. viridis* were collected monthly by SCUBA diving, between February and November 2016 from a shallow-rocky bottom substrates in South-Eastern Sardinian seas (Italy, Mediterranean Sea). The study area is characterized by granitic rocky substrates with crevices and small reef formations exposed to moderate hydrodynamic conditions, representing a typical habitat for *A. viridis* in the Mediterranean infralittoral zone. Linear transect of two hundred meters parallel to the coastline were carried out for samplings in three locations namely Torre delle Stelle, Cala Pira and Capo Ferrato, few kilometres apart at depths between 1 and 5m (Fig. 1).

Survey and sampling were not feasible in January, October and December due to unfavourable weather conditions throughout these months. Along the transects, to minimise the possibility of sampling clonemates (Bucklin, 1985; Gomes et al., 2003), care was taken to avoid sampling anemones closer than 2m from one another (Scott and Harrison, 2009).

The specimens were collected by gently detaching them from the rocky substrate using a modified fork, and then transferred alive to the laboratory in a refrigerated plastic container with10L of filtered seawater. A total of 200 sea anemones were acclimatized in aquaria for 2 h with a 7% solution of magnesium chloride ($MgCl_2$) (Messenger et al., 1985) to facilitate the measurement of the pedal disc diameter (PD, mm), made with a vernier calliper to the nearest 0.1 mm. Then they were weighted (TW, g) with a weighing scale to the nearest 0.01 g.

2.2. Environmental data

The relationships between gametes development and sea surface temperature (SST °C) and primary production were gathered using daily SST data and net primary production (nppv) of biomass expressed as carbon per unit volume in sea water (mg C m^{-3} day^{-1}) during the sampling period. Environmental data were retrieved from open access databases provided by Copernicus Marine Service (Copernicus Marine Environment Monitoring Service, Available online at http://marine.copernicus.eu/, Buongiorno Nardelli et al., 2013; Teruzzi et al., 2021a,b). Data were extracted from the nearest geographical grid point to the sampling area (Figs. 1 and 39.09504° N, 9.48806° E), at a depth of 1 m.

2.3. Population structure

All specimens were ordinated according to different size classes of PD (2 mm each) and the length-frequency distribution was performed. To analyse relative changes in *A. viridis* development, the PD-TW relationship was assessed using the allometric equation established by Keys (1928):

$$TW = aPD^b$$

where a is the intercept of the regression and b is the regression and allometric coefficient, estimated from least-squares fitting method of the log10 -transformed variables:

$$\log Y = \log a + b \log X$$

Since *A. viridis* lacks in sexual dimorphism, specimens were sexed after histological preparations.

2.4. Histological procedures

Subsamples of 148 animals from different sizes were first fixed in 5%

Table 2
Results of the univariate PERMANOVA testing differences in the PD and TW in different months (A). Results of the pairwise comparison testing for difference in pedal disc (PD) and total weight (TW) (B). DF = degrees of freedom; MS = mean square; Pseudo-F = F statistic; P(MC) = probability level after Monte Carlo simulations. Significant variations (P < 0.05) are reported in bold.

A

Variable	Source	df	MS	Pseudo-F	P(MC)
PD	Month	8	5.197	6.320	**0.001**
	Res	189	0.822		
TW	Month	8	3.454	3.855	**0.001**
	Res	189	0.896		

B

	PD		TW	
Groups	t	P(MC)	t	P(MC)
Mar, Apr	2.680	**0.014**	1.560	0.137
Mar, Jun	1.713	0.112	1.414	0.174
Mar, Jul	2.681	**0.013**	2.143	**0.038**
Mar, Aug	4.123	**0.002**	2.228	**0.024**
Mar, Sep	3.864	**0.001**	2.061	**0.046**
Mar, Nov	3.553	**0.001**	2.490	**0.018**
Mar, Feb	3.365	**0.005**	2.010	**0.045**
Mar, May	3.097	**0.006**	2.434	**0.018**
Apr, Jun	1.999	0.050	1.462	0.141
Apr, Jul	0.824	0.422	0.317	0.749
Apr, Aug	0.912	0.356	1.173	0.228
Apr, Sep	0.032	0.971	0.116	0.910
Apr, Nov	0.406	0.707	1.469	0.163
Apr, Feb	0.450	0.640	0.287	0.787
Apr, May	1.381	0.180	1.280	0.203
Jun, Jul	1.304	0.184	1.645	0.117
Jun, Aug	4.024	**0.001**	3.717	**0.002**
Jun, Sep	3.198	**0.004**	1.780	0.097
Jun, Nov	2.634	**0.017**	4.191	**0.001**
Jun, Feb	2.293	**0.030**	1.601	0.141
Jun, May	1.824	0.078	4.094	**0.001**
Jul, Aug	2.112	**0.047**	0.403	0.679
Jul, Sep	1.275	0.206	0.522	0.607
Jul, Nov	0.792	0.439	0.607	0.533
Jul, Feb	0.646	0.540	0.672	0.511
Jul, May	0.113	0.913	0.427	0.705
Aug, Sep	1.596	0.097	1.690	0.110
Aug, Nov	2.265	**0.029**	0.536	0.559
Aug, Feb	2.022	0.057	2.000	**0.044**
Aug, May	4.524	**0.001**	0.059	0.968
Sep, Nov	0.758	0.436	2.084	**0.047**
Sep, Feb	0.782	0.434	0.222	0.825
Sep, May	2.660	**0.013**	1.843	0.082
Nov, Feb	0.135	0.902	2.414	**0.023**
Nov, May	1.669	0.106	0.669	0.513
Feb, May	1.208	0.248	2.187	**0.035**

buffered formaldehyde (0.1 mol l^{-1}, pH 7.4) for a maximum period of 48-h. They were dissected into quadrants along the oral–aboral axis by cutting through the centre. A longitudinal quadrant of each specimen was taken for histological process. Tissues were embedded in a synthetic resin (GMA, Technovit 7100, Bio-Optica) following routine protocols and sectioned at 3.5 μm with a rotating microtome (ARM3750, Histo-Line Laboratories). Slides were stained with Gill hematoxylin and Eosin (H&E) for standard histology, following the histological staining protocol proposed by Cerri and Sasso-Cerri (2003). Subsequently, sections were quickly dehydrated in graded ethanol (96–100%), cleared in Histolemon (Carlo Erba Reagents) and mounted in resin (Eukitt, Bio-Optica). Selected sections were observed and photographed using a Nexcope NE600 optical microscope equipped with a digital camera (MD6iS) at different magnifications (40×, 100x and 400x) and edited with Adobe Photoshop CS6 (http://www.adobe.com/products/photoshop.html).

Specimens processed at least two times with negative results will be considered with "non-reproductive."

Size of gametes was measurable only for oocytes, obtained by taking the minimum and maximum diameter, recorded when they were sectioned through the nucleus (image analysis program tpsDig2 (Rohlf, 2009). In addition, the nucleus' size was recorded to estimate the nucleus-plasmatic ratio .This data allowed us to determine frequency of size classes (%) and to define a size range.

2.5. Statistical analysis

The significance of deviation of sex-ratio from the 1:1 null hypothesis was tested for the entire population using the Chi-Square test (χ^2) (Zar, 1999), whereas to assess differences among PD-frequency distributions, the Kolmogorov-Smirnov test was used. In order to verify whether there was a correlation between oocyte size and environmental parameter such SST and nppv, the Spearman rank correlation was performed. A univariate permutational analysis of variance (PERMANOVA) was performed on previously normalised data to test differences on the morphological characteristics of *A. viridis* (pedal disc and weight) and on the oocytes' diameters using the factor all sampled Months as source of variation. PERMANOVA test was based on Euclidean distances, using 999 random permutations of the appropriate units. When significant differences were observed, pairwise tests were also performed. P values in the PERMANOVA and pairwise tests were obtained from Monte Carlo asymptotic distributions (Anderson and Robinson, 2003) using the routines included in the PRIMER 7 software (Clarke and Gorley, 2015).

Relationships between the oocyte size and monthly environmental parameters (SST and nppv) were assessed by a Spearman rank correlation analysis. Correlations were carried out with STATGRAPHICS PLUS 5.1 professional edition (Statistical Graphics 1174 Corp., Rockville, MD, USA) and the graphical output was visualised using PAST statistical software (Hammer et al., 2001).

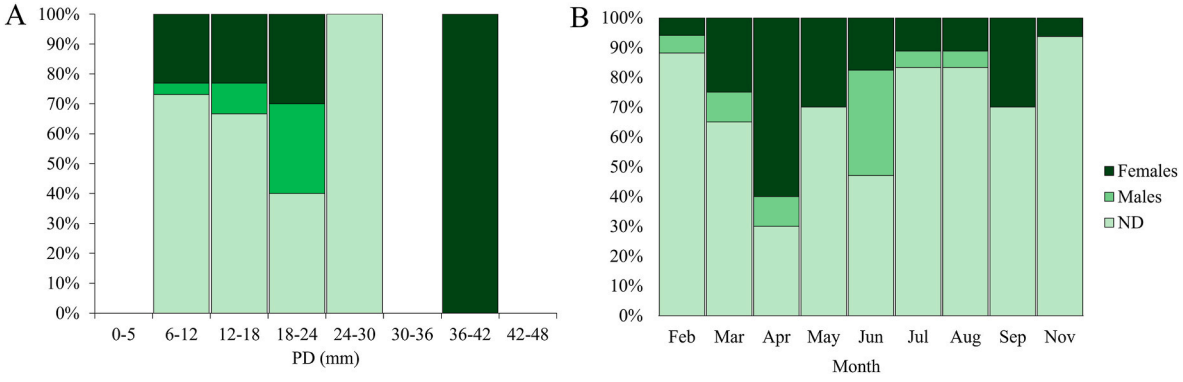

Fig. 3. (A) Size frequency distribution of *Anemonia viridis* specimens; (B) monthly variation of males, females and undifferentiated individuals (ND).

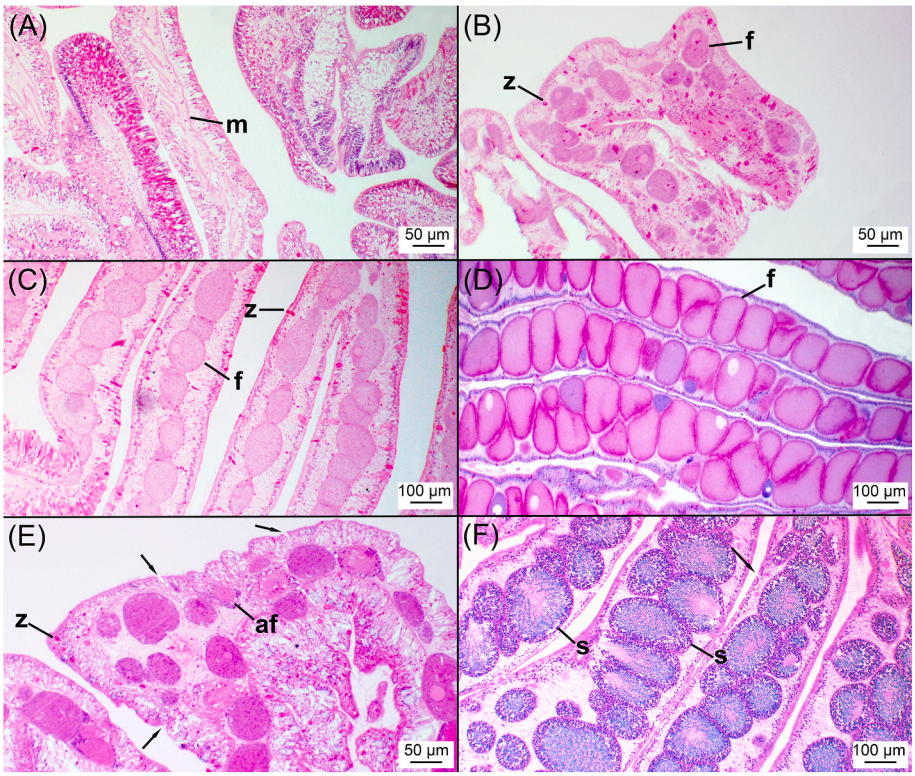

Fig. 4. Histological slides of *Anemonia viridis* at different maturity phases. (A) undifferentiated stage in which neither oocytes nor spermatozoids are observed inside the mesenteries; (B-C) female in the developing stage in which developing oocytes at different stages are visible. At a more advanced phase, oocytes begin to line up in the mesenteries; (D) female in the active stage in which mesenteries are almost fully replenished with active oocytes aligned along the mesenteries. Some preactive basophilic oocytes are present; (E) female in the spawning stage in which mesenteries are disrupted due to the release of the oocytes. Sometimes, atretic oocytes are present; (F) male in the active stage in which spermatozoids are aligned in the mesenteries. Sometimes mesenteric disruption is observed. Abbreviations: ao, active oocytes; ato, atretic oocytes; do, developing oocytes; m, mesenteries; pao, preactive oocytes; s, spermatozoids. Arrows indicate the mesenteries' disruption.

3. Results

3.1. Population structure

The collected specimens of *A. viridis* showed a PD ranging from 6.1 mm to 40.7 mm (12.8 ± 5.01 mm, mean ± sd), and TW ranged from 0.94 g to 100.86 g (7.5 ± 12.13 g, mean ± sd). In Table 1 was reported a summary of the number of collected samples, size and weight ranges on a monthly basis.

The length frequency distribution showed how the 50% of the sampled specimens ranged between 12 and 14 mm PD, while the highest PD classes (38-42 mm PD) were scarcely represented (3%) (Fig. 2A). Furthermore, significant differences were observed at different month (Fig. 2B–D; Table 2A, B), in March and June larger and heavier specimens were collected with the presence of some specimens with larger size, while a similar specimens' size was observed in the other sampling period.

The results of the equation (y = axˆb) showed that there was a relationship ($TW = 0.05*PD^{1.88}$, $R^2 = 0.88$) between the diameter of pedal disc and the weight of *A. viridis* specimens (Fig. 2C). The relationships between these variables show a *b* value lower than three, highlighting a negative allometric growth, with significant power curves (Fig. 2C).

The specimens' sex was assessed exclusively after the histological observation. Among 148 individuals analysed, 44 were females and 16 were males, the others were considered undifferentiated individuals (ND). Therefore, the χ^2 test with Yates' correction indicated that the sex-ratio was significantly different from 1:1 (SR: 2.75:1; $\chi^2 = 12.6$; DF = 1; p = 0.0004) with females outnumbered in respect to males. No simultaneous hermaphrodites were detected in the studied population. The individuals with PD < 9 mm did not show recognisable germ cells and they were indicated as undifferentiated. Females were present in higher numbers than males in the most size classes analysed, dominating in the bigger individuals (36-42 mm PD) (Fig. 3A). Female individuals occurred in all analysed months with a peak in April; then, their number decreased till August, reaching the minimum value in February. Males were detected from February to August with a peak in June (Fig. 3B).

3.2. Gametogenesis

Most individuals analysed appeared undifferentiated, not showing germ cells inside the mesenteries (Fig. 3A). For female specimens, a total of three maturity phases were detected on the basis of oocyte development (Fig. 4).

1) Developing or pre-active stage: developing and pre-active rounded oocytes fully enveloped by a layer of mesoglea and thick endodermal tissue (Fig. 4B and C).
2) Active stage: mature oocytes occupying the majority of the mesenteries. Their cytoplasm was filled with uniformly distributed darkly staining yolk granules. With continued growth, the mesenteries often became tightly packed with oocytes, causing some of the previously round oocytes to become less regular in shape and the edge of some of the mesenteries to become distorted. Some basophilic developing oocytes are present (Fig. 4D).
3) Spawning stage: the mesenteries appear torn and fragmented, to allow the gametes to be released through the gastrovascular cavity to the seawater environment. Large, irregularly shaped empty pockets remained in the endodermal tissue where the mature oocytes previously resided and atretic oocytes are visible (Fig. 4E).

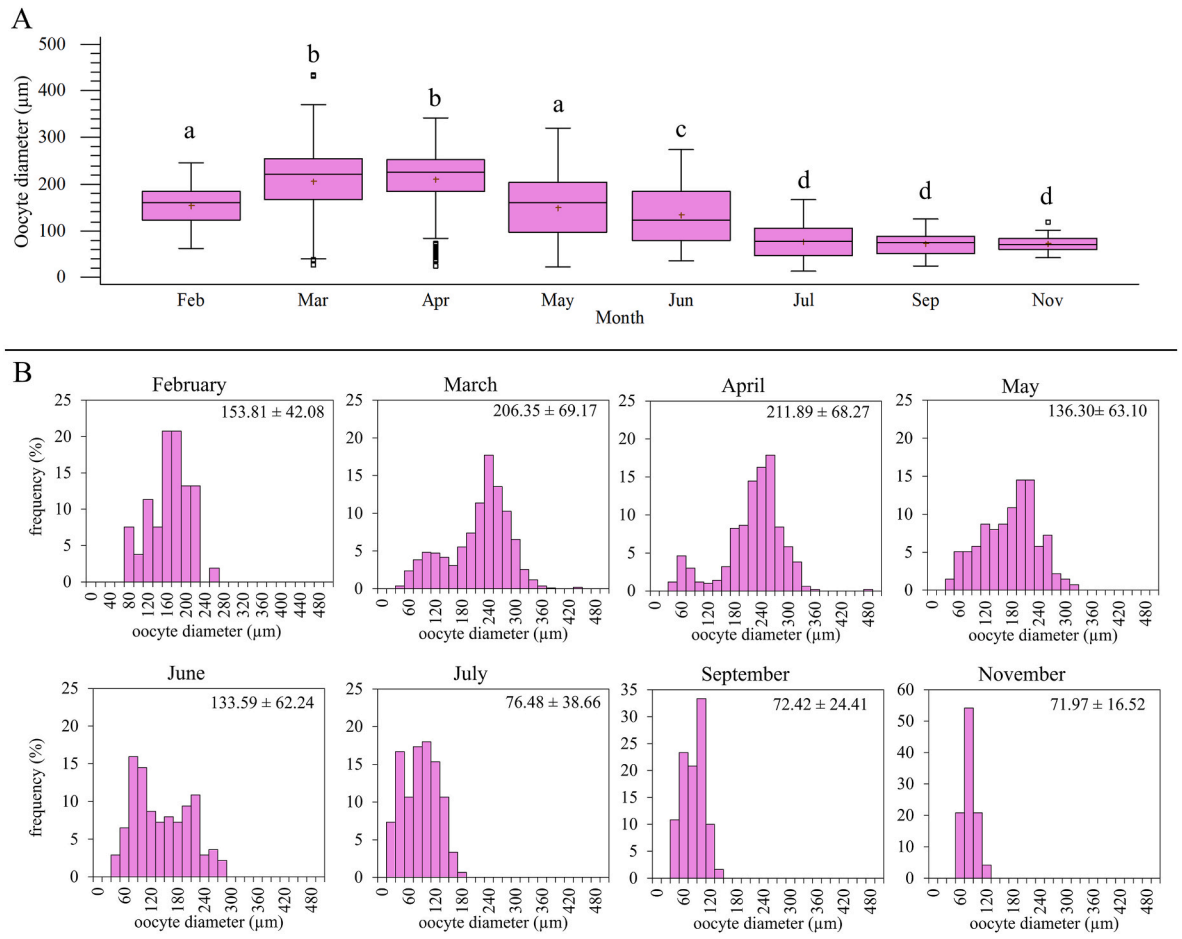

Fig. 5. (A) Variation of the oocyte diameter during the sampled months. Boxes show the interquartile range, with the median value indicated by the horizontal line and the '+' sign indicating the mean; whiskers show the range. Individual symbols show outliers. Lowercase letters indicate the results of the pairwise for the size of the oocytes in different months. (B) Size frequency distribution of oocyte diameter of *A. viridis* at each sampled month. In each graph the mean size ±SD of oocytes is reported.

As oocyte development progressed, the nucleus and cytoplasm grew in size. The rate of cytoplasmic growth exceeded that of the nucleus, resulting in a gradual reduction in the nucleus to cytoplasm ratio (i.e. active oocytes showed small nuclei).

Only mature males were shown, with spermaries made up almost entirely of large numbers of tightly packed spermatozoa with small, condensed heads (Fig. 4F). Spermatogenesis was generally synchronous within each anemone.

3.3. Reproductive period and maturity

The studied population showed the presence of female individuals with developing gonads nearly all year round, while conditioned females were found between February and June. Furthermore, conditioned males were found from March to July.

The maturity stage of the sexual products at the different size classes showed that the smallest mature female *A. viridis* had 9.7 mm of PD, while the smallest mature male 13.2 mm.

Concerning the size of oocytes, a total of 2073 oocytes were measured throughout the sampled period. The oocytes were significantly smaller from July to November (mean range 71.97-76.48 µm, Fig. 5A), while starting from February they began to increase their size reaching the maximum mean values in March and April, considered as peaks of spawning (Fig. 5A and B).

The snakelock anemone showed an asynchrony of germ cell in active females with the simultaneous presence of a small percentage of developing oocytes together with active ones (Fig. 5). This was particularly evident in samples collected in March and April (Fig. 5B).

3.4. Environmental parameters

The considered environmental parameters of SST and nppv showed an opposite trend in the considered period (Fig. 6A). The Spearman rank correlation showed significant relationship between oocyte size and the environmental parameters considered (Fig. 6B). A moderate negative correlation was observed between oocyte diameter and SST ($r = -0.460$, $P < 0.05$), whereas a weak but significant positive correlation was observed oocyte diameter and nppv ($r = 0.315$, $P < 0.05$). Moreover, the results highlighted a strong, significant negative correlation between SST and nppv ($r = -0.851$, $P < 0.05$).

4. Discussion

The snakelock anemone *A. viridis* (syn. *A. sulcata*) is a species of commercial interest in parts of the Mediterranean, where it is consumed as a traditional food, particularly in Southern Spain and Southern Italy. This local demand has contributed to historical overexploitation and reported population declines (Norman et al., 2008). However, information on the current conservation and health status of its populations remains limited.

This study provides new insights into its reproductive biology and ecological traits, with relevant implications for its conservation and sustainable management. Understanding *A. viridis* reproductive strategies is essential to assess population resilience under both natural and

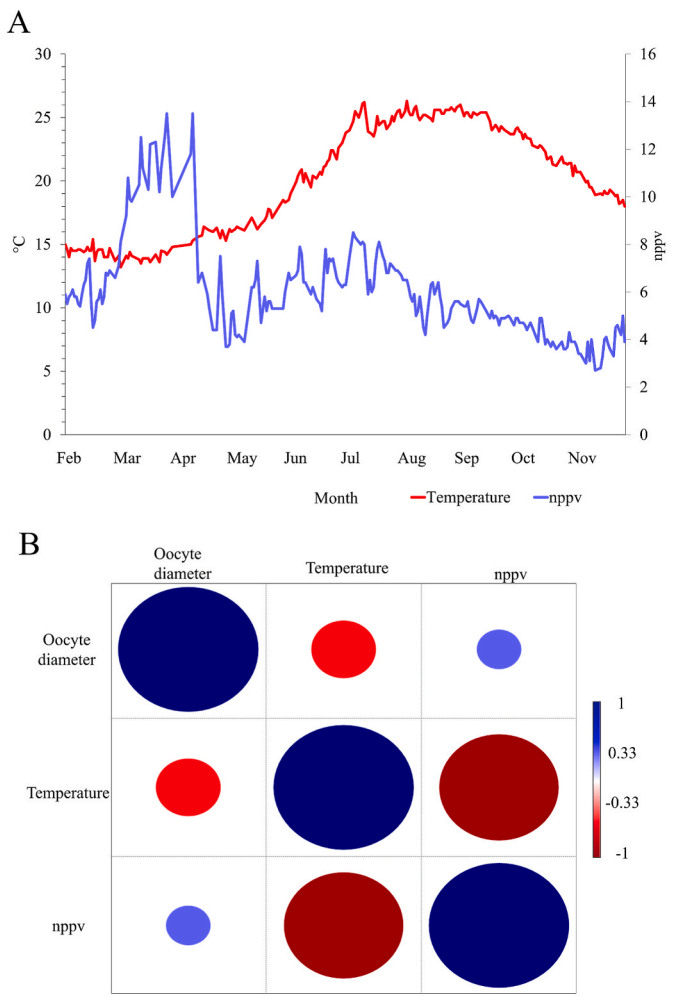

Fig. 6. (A) Temperature variation (°C) and net primary production in sea water (nppv, mg C m^{-3} day^{-1}) along the sampling period. (B) Correlation between oocyte diameter, SST and nppv.

anthropogenic pressures. These findings may also support the development of future breeding protocols and contribute to predicting how this species could respond to environmental changes, including those driven by human activities.

The study of the population structure of *A. viridis* showed that female anemones were significantly more abundant than males in Sardinia Island, confirming the same pattern of the species analysed in the Gulf of Naples (Schäfer, 1984), North Adriatic Sea (Di Camillo et al., 2021), South of Spain (Utrilla et al., 2019) and Morocco (Daoudi et al., 2022) (Table 3). A greater number of females is common among other sea anemone species populations (Dunn, 1982), such as *Anthopleura elegantissima* (Ford, 1964), *Actinia tenebrosa* (Ottaway, 1979), *Urticina lofotensis* (Wedi and Dunn, 1983) and *Entacmaea quadricolor* (Scott and Harrison, 2009). The sex ratio in a population with random matings is usually 1:1 (Maynard-Smith, 1978) and the skewed sex ratio of *A. viridis* populations and spatial distribution of sexes at Sardinia Island is most likely the result of clonal propagation (asexual reproduction), as the species is capable of asexual reproduction via longitudinal fission (Bocharova, 2016).

Overall, individuals of *A. viridis* sampled along the Sardinian coasts were found to be smaller (max PD 40.7 mm) compared to those observed in South of Spain (45-50 mm PD, Utrilla et al., 2019) and Morocco (66 mm PD, Daoudi et al., 2022). Moreover, we observed a significant variation of the size and weight of individuals, with significant larger and heavier specimens in March and June. Both the differences observed between geographical areas and sampling periods can be attributed to several environmental factors including phytoplankton richness, water salinity, temperature, currents and predation pressures (Daoudi et al., 2022). Other elements that can potentially affect the specimens' size can be ascribed to anthropogenic stressors, which seem to negatively affect the "microbiome gardening" of the individuals leading to harmful dysbiotic imbalances (Palladino et al., 2022). Furthermore, high water temperature (25 - 30 °C) and acidification conditions can negatively affect the growth and the diameter of the pedal disc of *Anemonia* species (Chomsky et al., 2004; Horwitz et al., 2015). Indeed, these stressors can modify the fitness of the symbionts that can be massively expelled, a phenomenon also known as bleaching that leads to the death of the sea anemone (Richier et al., 2006). Indeed, high sea temperatures can reduce the photosynthetic rate decreasing the efficiency of photosystem II in the zooxanthellae (Warner et al., 1999) and/or damaging the Calvin cycle (Jones et al., 1998; Leggat et al., 2004), while also causing host cell degeneration and gastrodermal detachment due to heat stress (Richier et al., 2006 and references therein) and promoted cloning propagation (Bingham et al., 2014). These conditions could explain the lower size and weight of the specimens collected in summer (July and August), when higher temperature was recorded and ranged between 24 and 26 °C.

The species shows both asexual and sexual reproduction, and the latter guarantees its long-term ecological success (Ball et al., 2004; Miller and Ayre, 2004; Reuven et al., 2021). Moreover, as all members of the Class Anthozoa, they have no medusoid generation (Bocharova and Kozevich, 2011). Commonly, it is considered broadcast spawner and therefore, the fertilization of oocytes with sperms occurs outside the gastrovascular cavity. Nevertheless, a recent study by Utrilla et al. (2019) reported that an embryo in gastrula stage and an early planula

Table 3
Summary table for *Anemonia viridis* estimates of first reproductive size and reproductive period in different geographical regions. FRS, first reproductive size; L_{50}, size at maturity; N, number of samples; nppv, net primary production expressed in mg C m^{-3} day^{-}; SST, sea surface temperature (° C).

Geographical area	N	nppv	SST	Sex	F:M	FRS*	L_{50}*	Months J F M A M J J A S O N D	References
Sardinia (C-W Med)	200	2.4-14.0	9.8-25.0	F M	2.75:1	9.7 13.2	- -		Present study
North Adriatic Sea	220	-	10.2-26.2	F M	6:1	- -	- -		Di Camillo et al. (2021)
Spain (W Med)	123	-	-	C	1.7:1	10	21.5		Utrilla et al. (2019)
Morocco (W Med)	330		15.0-22.0	F M	2.3:1	- -	- -		Daoudi et al. (2022)
Gulf of Naples (C Med)	-	-	-	F	-	-	-		Schafer (1984)

Reproductive period Peak of spawning

* Pedal di

* Pedal di

was observed attached to the exterior of the mesenteries pointing out that internal fertilisation and suggesting that larval brooding is a possibility for the reproduction of *A. viridis*.

Microscopic analysis of gonad samples confirmed that also in Sardinian waters *A. viridis* specimens were dioecious having separate sexes with no hermaphrodite specimens found. Spermary development was synchronous while oocytes developed in an asynchronous way both within and among individuals, and similarly to what Di Camillo et al. (2021) reported for the Adriatic Sea, the gamete production was observed along the entire sampling period characterized by the presence of small oocyte in the developing stage in the mesenteries. Asynchronous development in females can be caused by sluggish development of certain oocytes that could have been spawned a season later than the bulk or by oocytes continuing to produce at a low rate for a large portion of the year as hypothesised by Scott and Harrison (2009) for *Entacmaea quadricolor*. Annual field reproductive period occurred between February to June with a peak in March and April similarly to previous observations (Table 3) in the Mediterranean Sea. In detail, it was identified from May to June in the Gulf of Naples (Schäfer, 1984) and from March to May in the Alboran Sea, although sexually active individuals were identified almost during the whole year (Utrilla et al., 2019). Similarly to our results, mature individuals were encountered from February to June from Cape Mazari in the Moroccan Mediterranean coast (Daoudi et al., 2022). Oocyte size was higher in March and April when temperature ranged between 14 and 15 °C and the primary production was higher, whereas was minimum from July to November when temperature ranged between 19 and 24 °C. As extensively reported in previous literature, environmental parameters, such as temperature and food availability (van Woesik, 2009; Pasquini et al., 2021, 2022; Ennas et al., 2023; Abyaba et al., 2025), can also significantly influence the reproductive cycle of broadcast marine spawners (Orton, 1920; Guest et al., 2002; Mendes and Woodley, 2002; van Woesik et al., 2006). Our results show that for *A. viridis,* temperature is not primarily driving the gametogenesis. Indeed, oocyte maturation was not led by increasing temperature that was negatively correlated with oocyte size, but it was rather linked to the increasing primary production in water, hence by the increased potential food availability. Previous studies on *A. viridis* showed that this species can obtain the energy from its symbionts, although some feeding on zooplankton is likely to be important for the anemone's nutrients requirements (Bythell, 1988; Davy et al., 1996). Overall, Bell et al. (2006) confirmed this duality showing that the species is a non-selective suspension feeder during daylight, which also relies on the nutrition from its algal symbionts that supply their nutrients requirements through recycling the cnidarian waste products (Furla et al., 1998; Grover et al., 2002; Davy et al., 2012). For example, an alternative way for lipid acquisition in sea anemone is through their symbionts that can synthesise essential fatty acids (Whitehead and Douglas, 2003; Davy et al., 2012; Revel et al., 2016). This interaction is particularly important for specimens from poor waters where autotrophic symbionts can provide a significant competitive advantage by providing nutrient. Overall, these behaviours also known for *A. viridis* (Revel et al., 2016), can help the sea anemone to gather all the energetic requirements (particularly proteins and lipids) necessary for the vitellogenesis, the accumulation of egg yolk, that needs high nutrient influx into oocytes (Revel et al., 2016; Lebouvier et al., 2022).

The only information on first reproductive size and/or size at maturity of *A. viridis* was reported by Utrilla et al. (2019) in Spain (Table 3). However, our data allowed us to detect only the first reproductive size (PD 9.7 mm), in line with the Spanish observations. Further studies should be addressed to complete this gap.

Sea anemones have ben cultured under pilot scale conditions as part of the commercial operations in the South of Spain by the iMARE Natural company for their inclusion in land based IMTA system with the overall objective of commercial production to aid restoration.

Finally, regarding management measures for the fishery of this species, given the high variability in the spawning period along natural geographic areas of the studied species, a regional (local) regulation on *A. viridis* fishing should be considered by proposing a closed period between March and April when there is the peak of spawning in the Sardinian population. In addition, the significant results of the relationship between weight and the pedal disc indicate that they can be both used as reliable proxy for the monitoring of the population structure of *A. viridis* and as management tools.

5. Conclusions

The study of the reproductive cycle and population dynamics of high valuable snakelock anemone *Anemonia viridis,* as well as the definition of its life history traits, are essential steps to pave the way for its culture and developing effective conservation and management strategies, even in changing conditions and anthropogenic pressures. The study conducted on the Sardinian populations revealed significant variability in the size and weight of *A. viridis* individuals and a seasonal variation in size, with larger and heavier specimens during cooler months. Moreover, Sardinian specimens were overall smaller compared to those in other Mediterranean areas, possibly related to environmental factors such as temperature, and food availability. Reproductive timing was found to peak between February and June, with oocyte development linked to primary production rather than to temperature, possibly driven by the dual nutritional strategy, where specimens relay both on autotrophic symbionts and predatory behavior for their nutritional requirements.

CRediT authorship contribution statement

Cristina Porcu: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Viviana Pasquini:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Marco Secci:** Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Martina Francesca Marongiu:** Investigation, Methodology, Writing – review & editing. **Colin Hannon:** Visualization, Writing – review & editing. **Maria Cristina Follesa:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Pierantonio Addis:** Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

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