



# Reproduction of the anthozoan *Anemonia sulcata* (Pennant, 1777) in southern Spain: from asexual reproduction to putative maternal care

Olga Utrilla<sup>1</sup> · Juan Diego Castro-Claros<sup>1</sup> · Javier Urra<sup>2</sup> · Francisco David Navas<sup>3</sup> · Carmen Salas<sup>1</sup>

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## Abstract

*Anemonia sulcata* (Pennant, 1777) is a common shallow water cnidarian from rocky platform and boulder beaches in southern Spain, where it is a popular seafood item with an increasing fishery. To aid in the management of a sustainable fishery, a study on the reproduction of *A. sulcata* in the littoral of Malaga (southern Spain) was performed from November 2014 to September 2015, using histological methods. A total of 123 specimens were examined, with a size range (as diameter of the pedal disc) from 1.1 to 48.2 mm. The sex ratio was significantly biased to females, with 1.7 females: 1 male ( $\chi^2 = 4.45$ ,  $p < 0.01$ ). The spermatozooids and oocytes arise from the endodermal cells. The mature oocytes receive nutritive filaments (trophonema) from the endoderm cells. There were zooxanthellae in the mesenteries, tentacles and also inside the oocytes. A gastrula was observed in one individual, as well as several planula larvae in different degree of development in others. Asexual reproduction by internal budding was observed in some individuals. The studied population showed an extended reproductive cycle with a peak of spawning in April. The size and weight of sexual maturity of the studied population were 21.5 mm and 16.5 g, respectively. A positive significant correlation was observed between size and weight of individuals. We suggest that the diameter of the pedal disc should be used as the legal parameter for the management of this fishery, as this measurement is easier to take by fishermen at sea than the weight, the current legal parameter.

## Introduction

The sea anemone *Anemonia sulcata* (Pennant 1777) (Fig. 1) is a common cnidarian that occurs in the intertidal and shallow sublittoral zones, mainly on rocky platforms or boulder beaches down to 20-m depth (Hofrichter 2005). The species has a broad distribution from the Atlantic coast of Europe to West Sahara, and in the Mediterranean Sea (Ocaña and den Hartog 2002; Ocaña et al. 2015). *A. sulcata* is a popular

seafood item (known locally as “ortiguilla”) in southern Spain, and it is currently being promoted in eastern and northwestern Spain. Because of that, there is an increasing fishery of this species with a strong regression of populations from the littoral of Cadiz (Spain). According to the Spanish environmental surveillance service, the illegal fishing and trade of this anemone have increased in recent years. The fishing grounds of Cadiz province have been exhausted and poaching has moved to Malaga and Granada provinces (Spain).

Sea anemones, as all members of the class Anthozoa, have no medusoid generation; therefore, both sexual and asexual reproduction are developed by polyps. Sexual reproduction can be carried out by fertilization of oocytes and sperm inside or outside the gastrovascular cavity. Parthenogenesis, through the development of new embryos from oocytes without fertilization, is also frequent (Chia 1976; Gashout and Ormond 1979; Fautin 2002). During asexual reproduction, the new individuals are formed from part of the parent body, such as fragments, budding, transverse or longitudinal fission or autotomy of tentacles, among others (Schmidt 1970; Orr et al. 1982; Fautin 2002). The border

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✉ Carmen Salas  
casanova@uma.es

<sup>1</sup> Departamento de Biología Animal, Facultad de Ciencias, Universidad de Málaga, 29071 Málaga, Spain

<sup>2</sup> Centro Oceanográfico de Fuengirola, Instituto Español de Oceanografía, Puerto Pesquero s/n, 29640 Fuengirola, Spain

<sup>3</sup> Servicios Centrales de Investigación, Universidad de Málaga, 29071 Málaga, Spain



**Fig. 1** Specimen of *Anemonia sulcata* in the special area of conservation “Calahonda” (Malaga, Western Mediterranean)

between sexual and asexual reproduction appears to be faint in cnidarians due to the difficulty to distinguish whether a propagule is sexual or asexual in origin and the problematic sex determination in cnidarians (Fautin 2002). This author infers that sexual processes may be less significant to the ecology and evolution of cnidarians than has been thought.

The knowledge of the type of reproduction and the reproductive cycle from different geographical areas and over time is essential for the management of target fishery species, such as *A. sulcata*. Changes in environmental variables, such as the seawater temperature, can modify the reproductive cycle of marine species (Orton 1920). On the other hand, characteristics of habitat and/or the relative proportion of both sexes can influence the type of reproduction in the populations (Stephenson 1929). To regulate the fishery of *A. sulcata*, a study on the reproduction of this species was promoted by the regional authorities of Andalusia (Daza et al. 2002), after which the minimum weight of capture was established at 15 g and January and February as close season of this fishery. However, the study of Daza et al. (2002) was mainly based on changes of biomass; with few scattered histologically processed specimens. Histological techniques are more accurate to define the gametogenesis and the different stages of the sexual cycle (Scott and Harrison 2009).

Most studies dealing with *A. sulcata* have focused on its biotechnological or biomedical applications (Driscoll et al. 1989; Trapani et al. 2014), but few of them have studied morphological (Cazurro 1892–1893) or physiological aspects (Wiedenmann et al. 2000) and only one study was focused on the reproductive cycle through histology (Schäfer 1984, 1985). The latter showed an extended reproductive cycle, with a peak of release in June. The study of Schäfer (1984) was carried out in Naples, an area very far from southern Spain, with different environmental

characteristics. Moreover, it was carried out more than 30 years ago, and during this time, the Mediterranean seawater has been warming (Vargas-Yáñez et al. 2017). The reproduction in marine organisms is related, among other variables, with sea-water temperature, which can modify the extension and the reproductive peaks of the species along their range of distribution (Orton 1920; Tirado and Salas 1998; Tirado et al. 2011). Therefore, the characteristics of the reproductive cycle of marketable species should be analyzed in localities widely apart from fishery management.

Taking into account the current climatic change, with warmer seawater temperatures in the western Mediterranean (Vargas-Yáñez et al. 2008, 2010, 2017), together with the regression of the southern populations, a study of the reproductive cycle by histological methods of *A. sulcata* from the littoral of Malaga was proposed to provide data for a more sustainable fishery and a better management of this resource.

## Materials and methods

The histological study was performed on 123 specimens of *A. sulcata* that were monthly collected in the Special Area of Conservation (SAC) “Calahonda” (36°29.19'N–04°42.4'W), which is also a harvesting ground for invertebrates according to regional authorities. The specimens were collected at a depth between 0 and 1 m from November 2014 to September 2015, except for the month of June (Table 1). To detach the pedal disc from the rock, we carefully used a blunt knife. In the sampled area (ca. 100 m<sup>2</sup>), most of the large specimens had a diameter of pedal disc of around 50 mm; whereas, the diameter of the pedal disc of the small visible specimens was ca. 10 mm. We tried to collect many specimens with a diameter of pedal disc between 20 and 30 mm to better check the size of sexual maturity of the population.

The specimens were relaxed in the laboratory during 4 h with a solution of MgCl<sub>2</sub> (71 g of hexahydrate powder in 1 l of freshwater) to measure the diameter of the relaxed pedal disc with a vernier calliper to the nearest 0.1 mm. They were then weighted with a weighing scale to the nearest 0.1 g to analyze the possible correlation between size and weight for management criteria. We considered that there was an unpredictable bias in the measurement of diameter of the contracted pedal disc, because it was very difficult to know the degree of contraction of the specimens, but all relaxed specimens could be considered to be in similar conditions. Moreover, we consider that the additional size of relaxed pedal discs gives an advantage for the sustainability of this fishery. After this, the specimens were fixed in 10% formaldehyde.

**Table 1** Number of specimens (*N*) of *A. sulcata* by sampling date and size classes analyzed in the present study

Sampling date	Size classes	<i>N</i>
18/11/2014	0–10	2
	10–20	1
09/12/2014	10–20	1
	20–30	5
	30–40	2
08/01/2015	0–10	1
	10–20	5
	20–30	8
07/02/2015	0–10	1
	10–20	4
	20–30	8
08/03/2015	0–10	4
	10–20	7
	20–30	4
17/04/2015	0–10	3
	10–20	7
	20–30	2
	30–40	3
08/05/2015	40–50	1
	0–10	3
	10–20	8
	20–30	3
01/07/2015	0–10	3
	10–20	4
	20–30	5
20/08/2015	0–10	1
	10–20	9
	20–30	2
26/09/2015	10–20	11
	20–30	5

## Histological preparation

Due to their large size, animals were first sliced into quadrants along the oral–aboral axis. A longitudinal quadrant of each specimen was taken for histological process. All pieces were dehydrated through a graded ethanol series, embedded in paraffin, transversally sectioned at 10  $\mu$ m and stained with haematoxylin of Carazzi and Eosin. Two additional specimens were stained with haematoxylin and trichrome (Light Green, Orange G and Acid Fuchsin) (Gutiérrez 1967) for anatomical observations, such as the localization of zooxanthellae. The sex of the specimens of *A. sulcata* could not be distinguished macroscopically by dissection and direct observation, and had to be identified microscopically. Cnidarians have gametogenic tissue and no true gonads; however, the term “gonad” is usually applied to the strips of tissue within the mesenteries where sexual

products accumulate (Fautin and Mariscal 1991; Scott and Harrison 2009; Bocharova and Kozevich 2011) and we follow here this terminology. The stages of gonad development were scored according to the following scale (Fig. 2A–I):

- Cytolized or undifferentiated stage* Neither follicles nor gametes are observed inside the mesenteries (Fig. 2A).
- Preactive or developing stage* Some follicles are present in the mesenteries, with small gametes adhered to their walls (Fig. 2B, C).
- Active or developed stage* The follicles take up the whole of the mesenteries and are fully replenished with gametes (Fig. 2D, G). The oocytes are large and they appeared usually aligned along the mesenteries (Fig. 2D).
- Spawning stage* The walls of follicles are broken and in males, the spermatozooids are located in the center of the follicles. The mesenteries are broken to allow the gametes be released through the gastrovascular cavity to the seawater environment (Fig. 2E, H).
- Postactive or regressing stage* There are wide follicles and/or wide spaces inside the mesenteries (Fig. 2I). Sometimes, residual gametes are present inside them, pointing out a recent spawning event (Fig. 2F). Atresia (degeneration of those male follicles and oocytes which do not are spawned) is frequently observed, particularly in females (Fig. 2F).

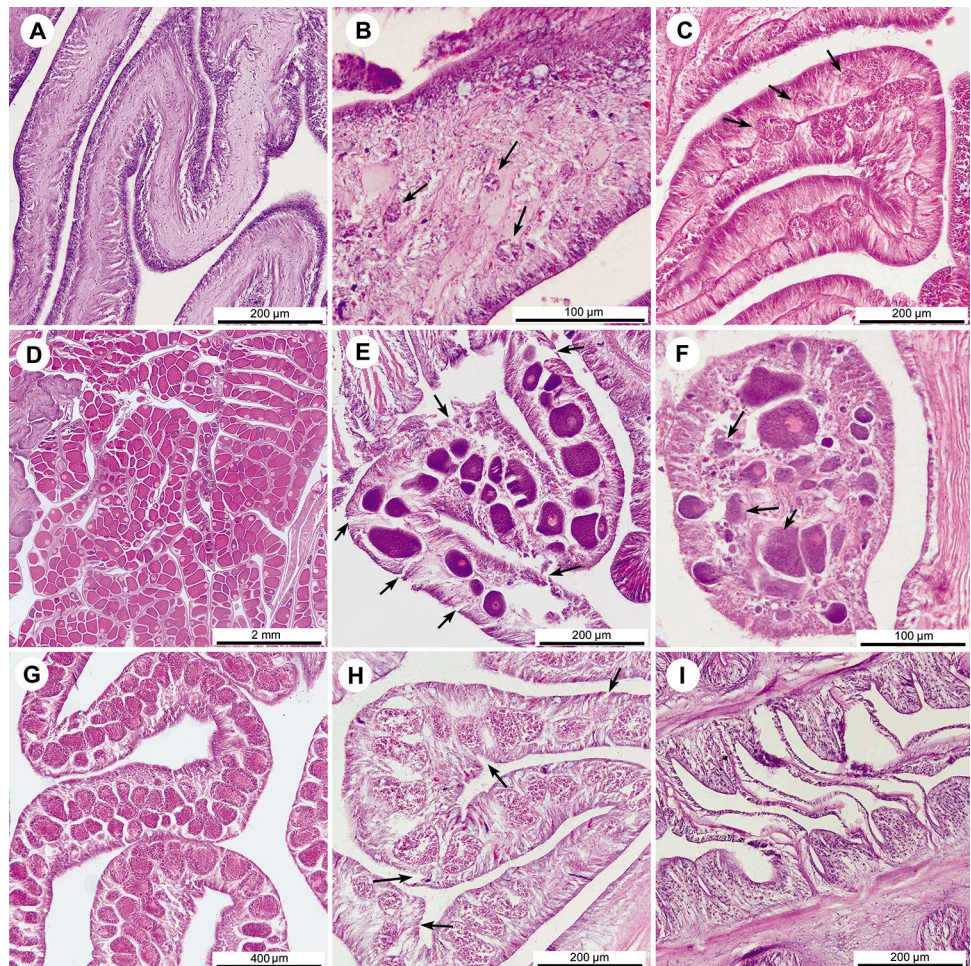
Some specimens partly sectioned for histological analysis were also prepared for Scanning Electron Microscopy (hereafter SEM) observations. The specimens were put in xylene to eliminate the paraffin before dehydration and critical point process. Some larvae obtained from the gastrovascular cavity of one specimen and selected for semithin and Transmission Electron Microscopy (hereafter TEM) observations were postfixed in OsO<sub>4</sub> (2%) for 2 h at 4 °C, and then embedded in epoxy resin Aname Epon 812 (Electronic Microscopy Science, EMS). Semithin sections (1  $\mu$ m) were stained with Epoxy Tissue Stain (EMS Ref. 14950), which had toluidine and acid fuchsin. Ultrathin sections (0.3  $\mu$ m) were stained with uranyl acetate (1%) followed by lead citrate and examined in a TEM (JEOL JEM 1400).

Paraffin and semithin sections were observed using an Olympus VS120 microscope. To localize zooxanthellae in the different body tissues, some paraffin sections were observed with a laser confocal microscope (LEICA SP5 II) with excitation at 405 nm, and maximum emission wavelength at 670 nm.

## Data analyses

The Chi squared test was used to check the sex ratio of the studied population. We also used the cumulative graphic for

**Fig. 2** Optical microscopic views of the stages of gonadal development in *Anemonia sulcata*. **A** Cytolized or undifferentiated. **B** Female in preactive stage. The arrows indicate small follicles. **C** Male in preactive stage. The arrows indicate different follicles in formation. **D** Female in active stage. **E** Female in spawning stage. The arrows show zones where the mesenteries are disrupted due to the release of the oocytes. **F** Females in postactive stage with atresia of the oocytes (arrows). **G** Male in active stage. **H** Male in spawning stage. The arrows show zones where the mesenteries are disrupted due to the release of the spermatozooids. **I** Postactive stage without residual gametes in the wide mesenteries, after the spawning of the gametes



the estimation of the size and the weight of sexual maturity (the size and weight at which the 50% of the individuals have mature gonads, i.e., in active, spawning and postactive stages, (Trippel and Harvey 1991)) to obtain a minimum size and weight for fishery management purposes. The potential relation between size and weight was assessed through Pearson correlation analyses using the software SPSS v.14.

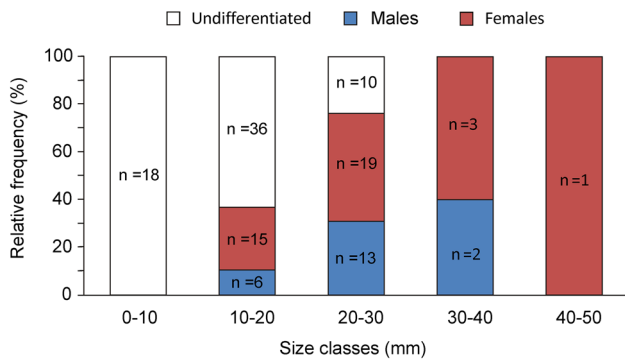
To see if the size of the oocytes was size dependent or if the viable size of oocyte was similar for all the females, the average surface area was calculated. The diameter of the oocytes was determined considering them as spherical. For this purpose, we used image analysis (Image J software) on transversally sectioned oocytes with visible nucleus and nucleolus. Sections of gonadal region of the mesenteries of 31 females were photographed in an Olympus VS120 microscope. A total of 930 oocytes (with visible nucleus and refringent nucleolus in the section) were selected. The area for each sectioned oocyte was registered and from this the diameters were obtained. The relationship between sizes of the females and the average area of their oocytes (for each female) was plotted and a Pearson correlation analysis was carried out using the software SPSS v.14.

## Results

### Sex ratio and the origin of the germ cells

The diameter of the pedal discs of the collected specimens ranged from 1.1 to 48.2 mm, with an average size of 17.9 mm, while the weights ranged from 0.6 to 88.8 g with an average weight of 15.9 g. Among the 123 specimens of *A. sulcata* analyzed, 65 (52.8%) had recognizable germ cells, of which 41 were females and 24 males. Therefore, the sex ratio of the studied population was 1.7 females: 1 male ( $\chi^2 = 4.45$ ,  $p < 0.01$ ), which means a significantly higher proportion of females in this population (Fig. 3). We have not found simultaneous hermaphrodite individuals in the studied population of *A. sulcata*.

The gonads were located in general in the first and second cycles of imperfect mesenteries (Fig. 4A, B). We have not observed gametes inside the first cycle of perfect mesenteries. In males, the spermatozooids arise from the endodermal layer of the mesenteries, being enclosed in small follicles located initially close to the endoderm (Fig. 5A–C). The spermatozooids seem to be originated by the endodermal



**Fig. 3** Relative frequency of sexes by size classes in the studied sample of *Anemonia sulcata*

cells. The presence of flagella in cells close to the periphery (Fig. 5D) frequently hinders the accurate identification of the different phases of the spermatogenesis, which are practically indistinct. Spermatogonia and spermatocytes have been observed in some follicles (Fig. 5E–G). The spermatozooids, with a small rounded tip head of ca. 0.5 μm (Fig. 5E–G) were cumulated in the center of the follicles (Fig. 5D), the diameter of which varies according to the stage of development (Table 2). Some putatively nutritive filaments seemed to enter the follicles located close to the endodermal layer (Fig. 5C).

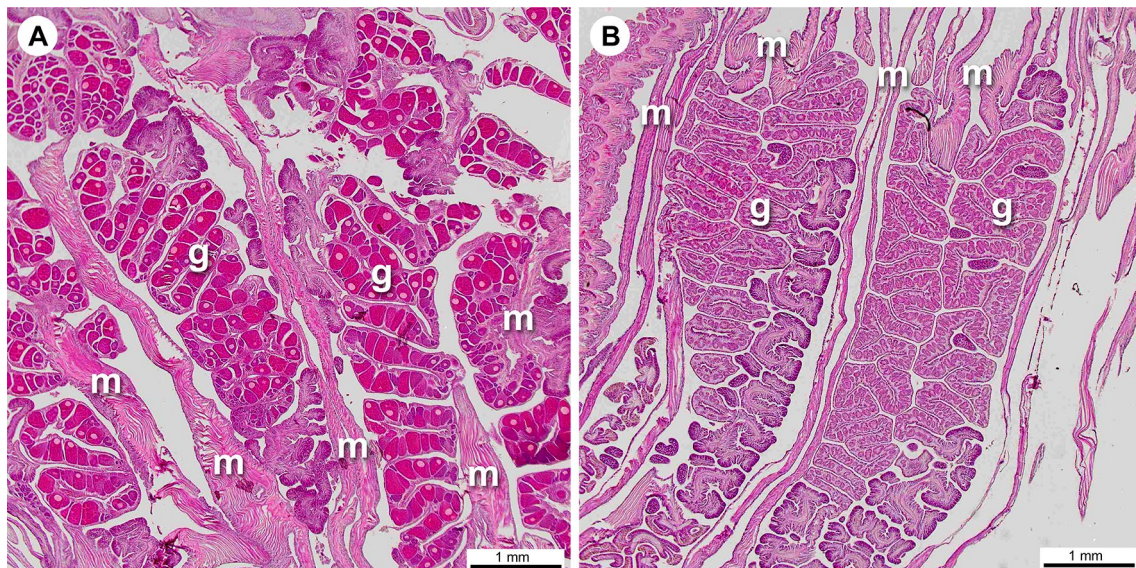
Some small and isolated oocytes were observed in the endoderm layer together with some small follicles in the center of the mesentery at the beginning of preactive stage in females (Fig. 6A). A germ cell asynchrony was frequent, with formation of new oocytes in female in active stage

(Fig. 6B). The oocytes appeared aligned along a folded mesentery, grouped in more or less visible follicles (Fig. 6B). In general, the small follicles appeared in the center of the mesenteries, without apparent connection with the endodermal cells (Fig. 6A). Nevertheless, during the active stage, we observed connection between the oocytes and the endodermal cells, which sent filaments to the mature oocytes (Fig. 6C, D). These filaments seemed to be nutritive and correspond to the so-called “trophonema” in other species. The diameters of the female follicles during the development were measured from the histological sections (Table 2). The average diameter of the mature oocytes from all the females in active stage was  $182.2 \pm 70 \mu\text{m}$ . The oocyte surface was characterized by the presence of numerous bundles of microvilli (Fig. 6E, F).

The studied specimens presented high density of zooxanthellae in the mesenteries, tentacles and female oocytes (Fig. 7A–F). The zooxanthellae were located along the gastric endodermal cell layer and inside the mesenterial filaments (Fig. 7A–C). The use of confocal microscopy at an emission wavelength of 670 nm (the wave length of the chlorophyll *a*) allowed to observe the presence of zooxanthellae in the mesenterial filaments (Fig. 7C), in the wall of follicles and inside the oocytes, where they are frequently located around the nucleus (Fig. 7D–F).

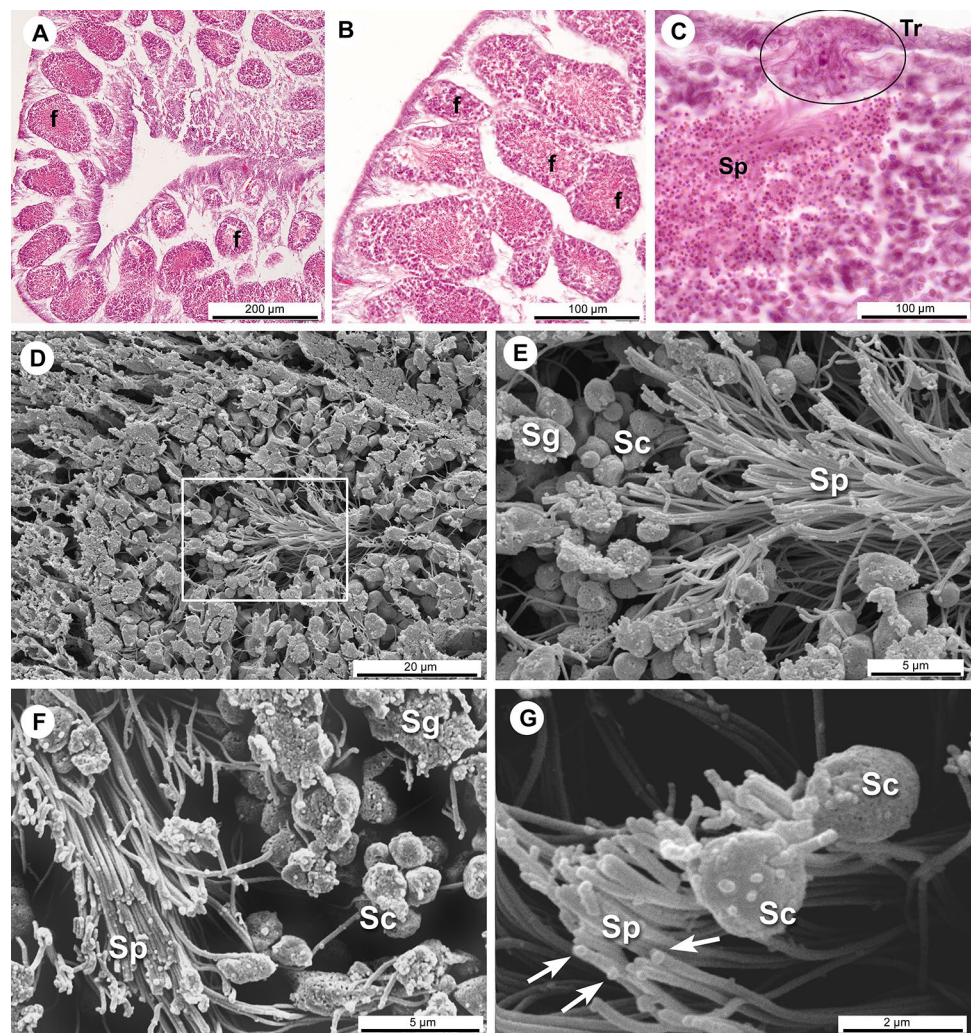
**Reproductive cycle**

The studied population showed an extended reproductive cycle (Fig. 8), with the presence of individuals with developing gonads nearly all year round. During late



**Fig. 4** Optical microscopic view of the development of the gonads (g) in the imperfect cycles of mesenteries (m) of *Anemonia sulcata*. **A** Female in active stage. **B** Male in active stage

**Fig. 5** Development of the male follicles and spermatozooids in *A. sulcata*. Formation of follicles (f) from the endoderm layer: **A** General view of the male follicles. **B** Formation of new follicles (close to the endoderm cells) that push the older ones to the center of the mesentery. **C** Trophonema (Tr) entering a follicle, where there are spermatozooids (sp). **D** SEM view of a male follicle. **E** Detail of the inset of **D**: spermatogonia (sg) spermatocytes (sc) and spermatozooids (sp). **F** SEM view of another male follicle, with spermatozooids (sp) in the left side, spermatocytes (sc) and spermatogonia (sg). **G** Detail of spermatozooids (sp) and spermatocytes (sc) from another follicle. The white arrows show the head of the spermatozooids



**Table 2** Diameters of female and male follicles of *A. sulcata* according to the stage of development

Stage of gonadal. development	Diameter of female follicle (μm)	Diameter of male follicle (μm)
Early preactive	15–25	16–20
Preactive	120–230	42–135
Active	300–835	120–142

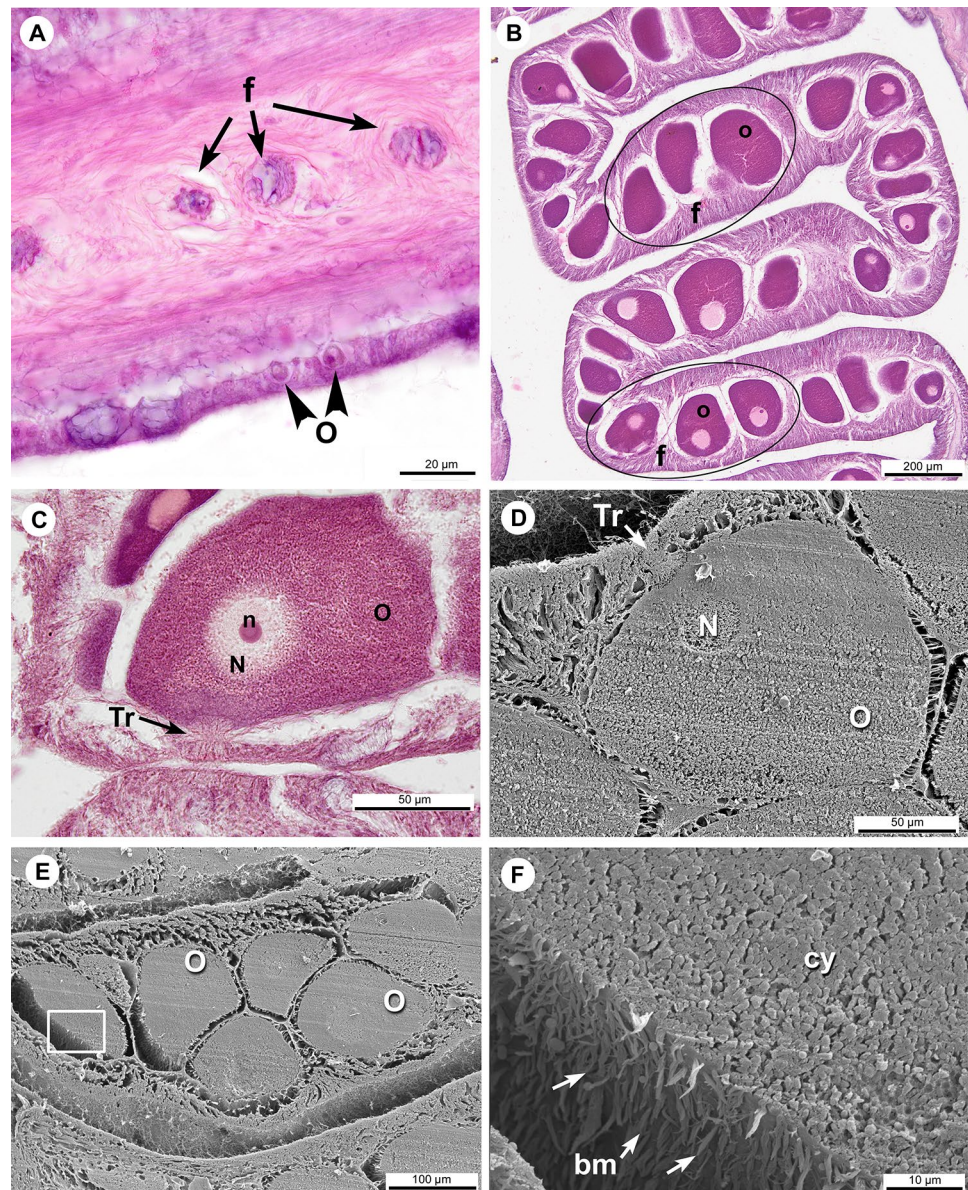
summer (August and September), there were no individuals in active stage (Fig. 8). All the individuals collected in November had undifferentiated gonads; however, a peak of individuals in active stage was found in December, but a posterior peak of spawning in January was not observed. The peak of spawning was in April. During the regressing (postactive) stage, it was frequent to observe atresia of the gametes, particularly visible in the females (Fig. 2F). A positive and significant correlation between the size of the individuals and the size (as surface area) of the oocyte

was obtained ( $R^2 = 0.351$ ,  $p < 0.01$ ) (Fig. 9). The size of the oocytes was greater in larger individuals.

The size of sexual maturity of the studied population from Malaga was 21.5 mm of diameter of pedal disc (Fig. 10A) and the weight at sexual maturity was 16.5 g (Fig. 10B). A positive significant correlation between diameter of pedal disc and weight was obtained ( $R^2 = 0.706$ ,  $p < 0.001$ ) (Fig. 10C).

Although *A. sulcata* is reported as a broadcast spawner species and therefore, the fertilization would be external, an embryo in gastrula stage and an early planula were observed in two sectioned individuals, both of them in postactive stage (Fig. 11A, B). In addition, five early larvae attached to the outside of mesenteries were found in dissected individuals. The larvae had an average diameter of  $266 \pm 21$  μm. The larvae were characterized by the presence of spiral bands of cilia and numerous ciliate protozoan parasites inside (Fig. 11C, D). Juveniles inside the gastrovascular cavity were not found.

**Fig. 6** Development of the follicles (f) and oocytes (O) in females of *A. sulcata*. **A** Female in preactive stage. Oocytes (black arrow) originate from endoderm layer and follicles (f) in the center of the mesentery. **B** Female in active stage. The ellipses show follicles (f). **C** Optical microscopic view of an oocyte (O), showing nuclei (N), nucleolus (n) and trophonema (Tr). **D** SEM view of a partially sectioned oocyte (O) showing the trophonema (Tr) and the nucleus (N). **E** SEM view of a female follicle with oocytes (O) from a partially sectioned gonad. **F** Detail of the oocyte surface from inset of figure E showing part of the cytoplasm (cy) and the bundles of long microvilli (bm) at the surface



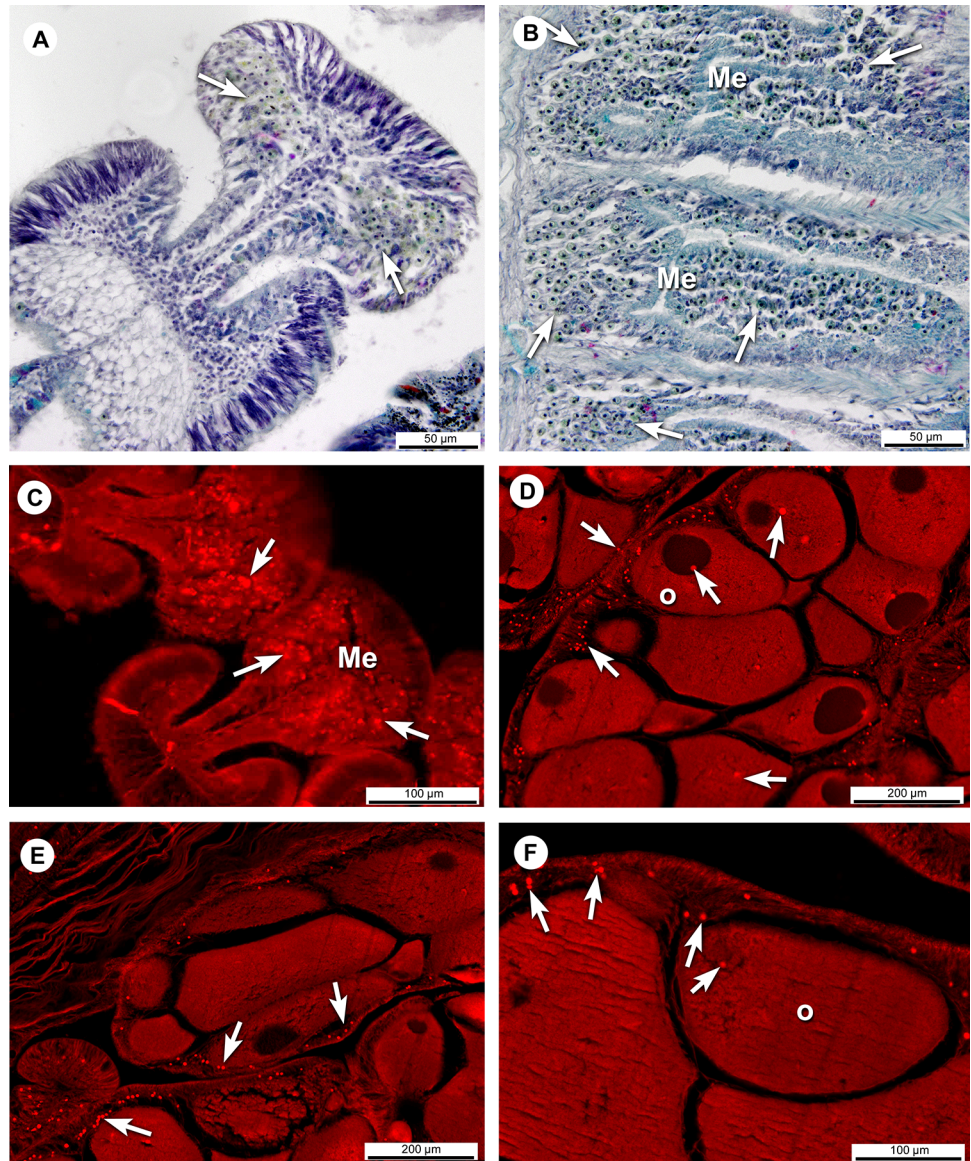
## Asexual reproduction

Some buds were found in three specimens, all of them without developing gonads (Fig. 12A–C). In one individual, several buds appeared still attached to the internal epithelium of the columnar wall (Fig. 12A), but one was apparently free between two mesenteries (Fig. 12C). In other small individual, several buds were observed attached to the mesenteries (Fig. 12B). Nevertheless, the presence of budding as asexual reproduction seems to be much reduced in the studied population compared to the sexual reproduction.

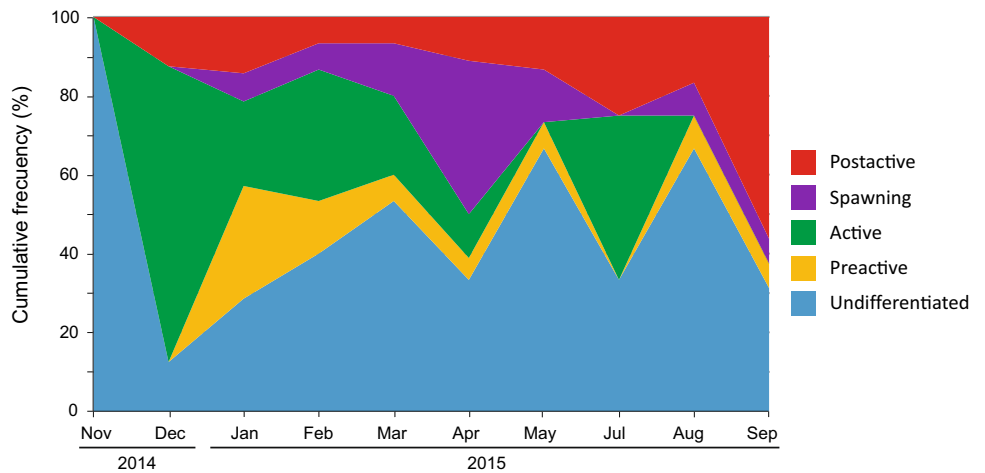
## Discussion

The higher proportion of females found in the population of *A. sulcata* studied within the SAC “Calahonda” seems to be also very frequent in other species (Bocharova and Kozevich 2011). A higher proportion of females in populations of *A. sulcata* from northern Spain was reported by Cazurro (1892–1893) and in Naples by Schäfer (1984). Moreover, in some populations of other species, only females are present (Gashout and Ormond 1979; Chen et al. 2008). Among the reasons suggested by these authors for the female prevalence, the effect of asexual reproduction, also common in

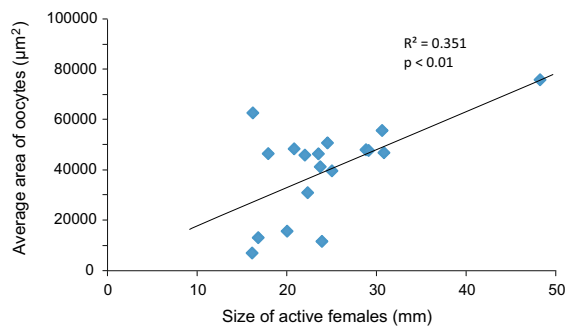
**Fig. 7** Presence of zooxanthellae in *Anemonia sulcata* (white arrows). **A** Optical microscopic view of mesenteric filaments with zooxanthellae. **B** Optical microscopic view of parts of several mesenteries (Me) with zooxanthellae. **C** Confocal image of zooxanthellae (brilliant color points) inside mesenteric filaments. **D–F** Confocal images of the presence of zooxanthellae (brilliant color points) in the walls of the female follicles and inside the oocytes



**Fig. 8** Reproductive cycle of *Anemonia sulcata* in the Special Area of Conservation “Cala-honda” (Malaga)



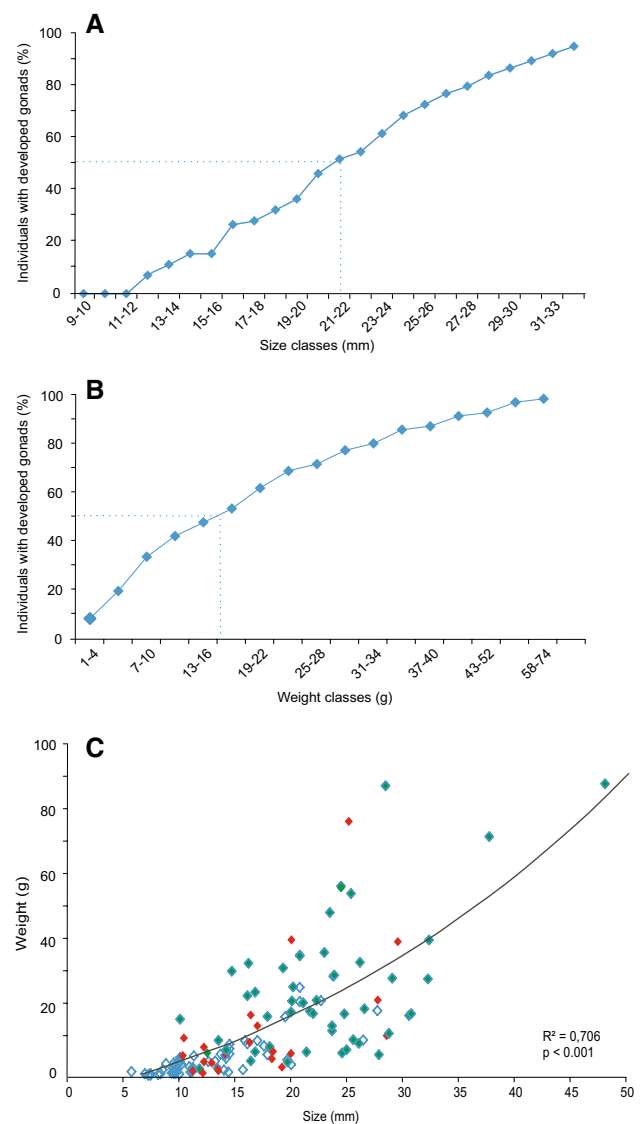




**Fig. 9** Correlation between size of the females in active stage of *Anemonia sulcata* and the average surface area of their oocytes

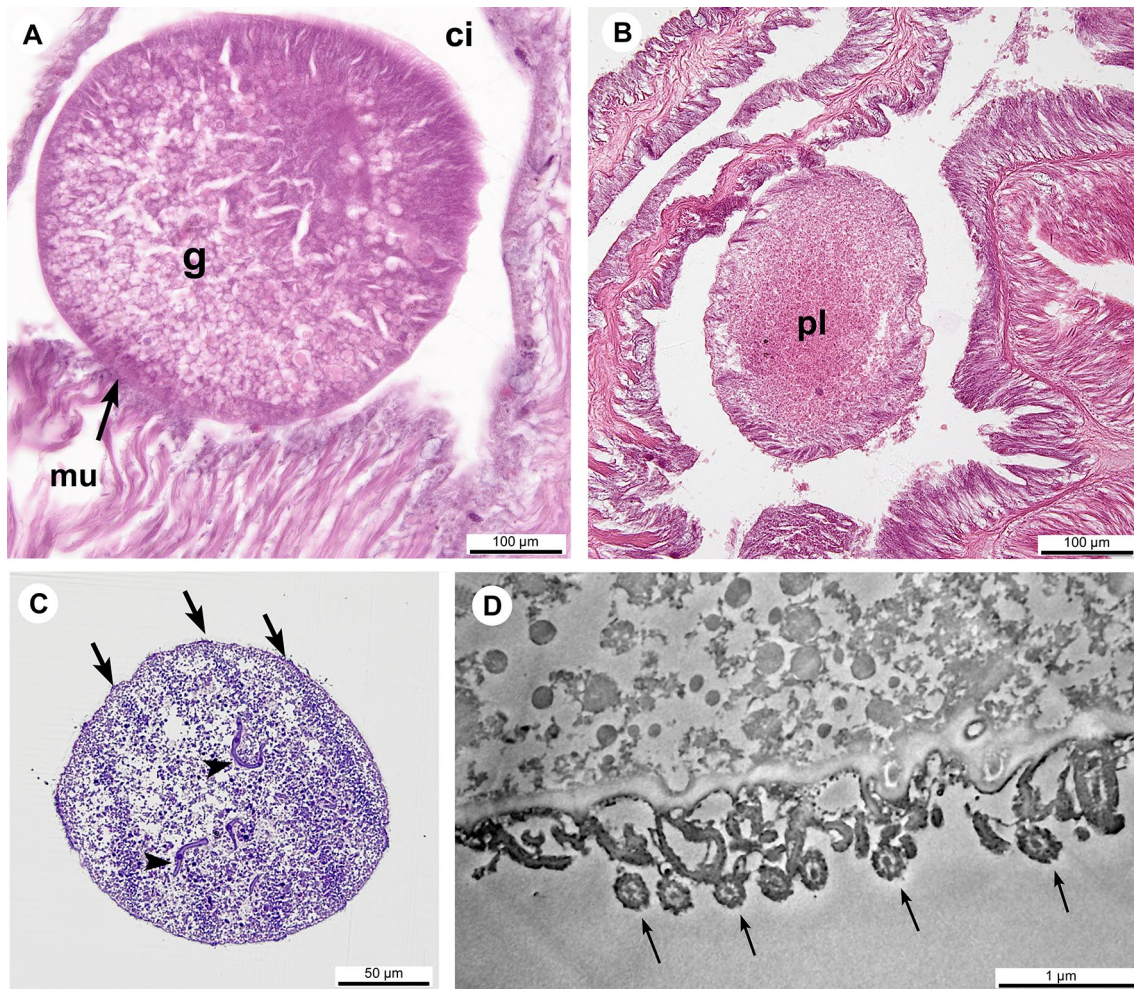
this group, or living in the frontier (geographic or bathymetric) of their distribution, has been highlighted (Shick and Lamb 1977; Sebens 1982; Chen et al. 2008; Bocharova and Kozevich 2011). The studied population of *A. sulcata* from the littoral of Malaga inhabits the lower intertidal zone of a rocky beach that undergoes high temperatures and depletion of oxygen in summer. The latter must cause stress in the population (Smith 1939), which according to some authors (Chen et al. 2008; Bocharova and Kozevich 2011) seems to favor the higher proportion of females by increase of parthenogenesis (Gashout and Ormond 1979). If females are prevalent, the asexual reproduction could favor an increase in the predominance of females.

The origin and development of germ cells in cnidarians are unclear. Some researchers consider that in anthozoans, the germ cells arise from epithelial endodermal layer (Loseva 1971; Dunn 1975); while for others, the origin of germ cells would be in the undifferentiated interstitial cells of the endoderm (Hyman 1940; Dunn 1975). Later on, in both hypotheses, the spermatozooids and oocytes migrate to the mesoglea where their maturation takes place. In *A. sulcata*, the germinal cells originate in the endoderm layer, but we cannot identify the cellular origin with certitude. The sperm seems to originate inside the endodermal cells, as spermatogonia that appear in the periphery of the male follicles. According to Goffredo et al. (2000), in *Anthopleura elegantissima*, it was not possible to distinguish spermatids from spermatocytes. Dewel and Clark (1972) indicated that in *Bunodosoma cavernata*, the peripheral region of the male cysts contain immature gametes; while the central regions contain late spermatids often indistinguishable from spawned sperm. According to Clark and Dewel (1974), the flagellum, destined to be the tail of the mature sperm, is already present in the spermatocyte, thereby obscuring positive identification of these stages. Lyke and Robson (1975) reported also that it is difficult to distinguish between secondary spermatocytes and early spermatids, even at the ultrastructural level. In *A. sulcata*, we found similar problems to identify the successive stages of the



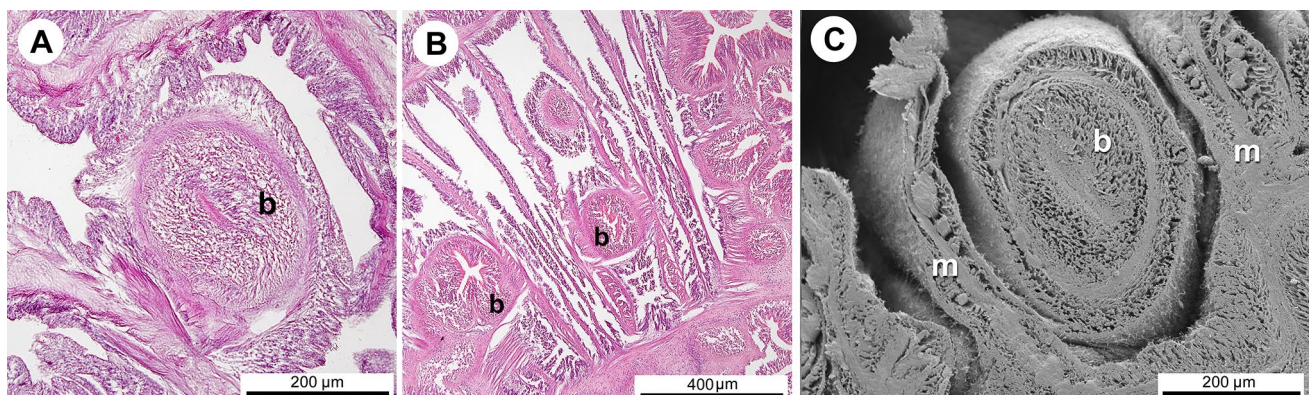
**Fig. 10** Cumulative percentage of individuals with developed gonad by size classes (A) and weight classes (B) in the studied population of *Anemonia sulcata*. C Correlation between size (diameter of the pedal disc) and weight of the studied individuals; open diamonds: undifferentiated individuals; green diamonds: individuals in active and spawning stage; red diamonds: individuals in postactive stage

spermatogenesis. The small sperm cells were enclosed in small follicles that are pushed into the mesoglea by the new forming ones. The oocytes arose as isolated germ cells in the endodermal layer and groups of oocytes inside vesicles were formed in the mesoglea at the center of the mesenteries; therefore, the oocytes have to migrate to the mesoglea (Fig. 6A). The latter was similar to the process described for other sea anemones, such as *Epiactis prolifera* (Dunn 1975) or *Nematostella vectensis* (Eckelbarger et al. 2008; Moiseeva et al. 2017), among others (Bocharova 2016). Moiseeva and co-authors found that the oocytes of *N. vectensis* are



**Fig. 11** Embryo and larvae found in *Anemonia sulcata*. **A** Embryo in gastrula stage (g) connected to a mesentery by mucous filaments (mu) and cilia (ci). **B** Planula (pl) found between the mesenteries. **C** Semi-thin section of an early planula found attached to the mesentery; the

planula shows spiral bands of cilia (black arrows). Inside this larva numerous ciliate protozoans (black arrowhead) have been observed. **D** TEM detail of the spiral band of cilia of the larva from **C**



**Fig. 12** Presence of budding (b) in *Anemonia sulcata*. **A** Optical microscopic view of a bud (b). **B** Optical microscopic view of several buds (b) in an undifferentiated individual. **C** SEM view of a bud (b) between two mesenteries (m)

grouped in the mesenteries (> 10 groups/middle cross section). Schäfer (1984) pointed the presence of spines in the surface of oocytes and of bundles of microvilli in the early embryo (Schäfer 1985); however, we found thousands of bundles of microvilli on the surface of mature oocytes when there are still inside the mesentery (Fig. 6E, F). During the metamorphosis, the bundles of microvilli are transformed in cilia during the gastrula formation (Fig. 11A), and these cilia appeared as spiral bands in the planula larva (Fig. 11C, D).

The presence of filaments connecting the endoderm cells with the oocytes was named by Hertwig (1882) as “filamental apparatus” in several species from Challenger expeditions. This author made a detailed description from that of *Coralliomorphus rigidus*, indicating its presence in other species, such as *Calliactis (Sagartia) parasitica*, and considering its function as nutritive. Nyholm (1943) proposed the term “trophonema” to the filamental apparatus, being the name used in current studies. Larkman and Carter (1982) showed by light microscope autoradiography that oocytes within gonads could take up and incorporate tritiated glucose and leucine from solution. The cells of the trophonema appear more active in precursor incorporation than other gonad epithelial cells (Larkman and Carter 1982). The evidence, therefore, suggests that these cells have a nutritive function during oogenesis. Eckelbarger et al. (2008) made an ultrastructural analysis of the trophonema in *N. vectensis*, showing that the trophonema was constituted by 50–60 modified monociliated gastrodermal cells, that they called trophocytes. The trophonema contacts with < 1% of the oocyte surface and it is restricted to cell junctions connecting peripheral trophocytes with narrow extensions from the oocyte. The trophonema seems to be a structure characteristic of Anthozoa that has been mainly described in sea anemones, although not all the studied anemones have this structure (Wedi and Dunn 1983). Our observations would be the first description of trophonema in oocytes of *A. sulcata*. The presence of a similar structure in males had been reported by Larkman (1984), which indicated that the contact between germ cells and endoderm persisted throughout spermatogenesis. Wedi and Dunn (1983) pointed out that in *Urticina lofotensis*, some sperm packets have a plug-like structure at the mesentery edge that could be homologous with a trophonema. In *A. sulcata*, connections between the sperm follicles and the endoderm were observed (Fig. 5C). The difference between both of them is that in females, each oocyte has one trophonema; whereas in males, the filaments enter the follicle but not the spermatozooids. According to Larkman (1984), the function of the structure observed in *Actinia fragacea* is nutritive, because of that he also called it “trophonema”.

An extended reproductive cycle of *A. sulcata* was also observed by Schäfer (1984) in Naples (Italy), with the peak of spawning in June (late spring); while in the population

from Malaga, the peak of spawning appeared in April (early spring) (Fig. 8). The mismatch of two months in the peak of spawning could be related with the location of our specimens, in the lower intertidal zone, with high irradiance from May to September. Another potential variable that could have influenced this mismatch is the warmer seawater temperatures that have been reported during the last years for the western Mediterranean (Vargas-Yañez et al. 2008, 2010, 2017), as already documented for other marine benthic organisms in the European context (Hiscock et al. 2004). High seawater temperatures can affect *A. sulcata* in two ways: the first one, loss of the zooxanthellae and consequently more difficulty to live in the intertidal zone, where the stress is high (Smith 1939). A second consequence could be a lower rate of sexual reproduction due to the reduction of nutrients in the absence of zooxanthellae and increment of the asexual reproduction. The latter would reduce the size and heterogeneity of the populations. Bingham et al. (2014) found that high irradiance and high summer temperature promoted cloning in individuals of the intertidal sea anemone *A. elegantissima* hosting *Symbiodinium muscatinei* and reduced fitness of aposymbiotic anemones. In this line, further studies regarding the acclimation capacity of reproductive performance of marine benthic organisms, including commercially important species, are essential to understand the impacts of climate change on biological systems, as well as to modify existing marine resources exploiting management plans. The regional authorities have ruled a close season during January and February. However, we consider that the close season should be March and April, when there is the peak of spawning in the population.

Sea anemones are known to have external or external–internal fertilization (Chia 1976; Carter and Thorp 1979; Gashout and Ormond 1979). The external fertilization is usual among gonochoric broadcaster sea anemone species after males and females spawn into the water (Chen et al. 2008). Nevertheless, in these species, some oocytes could remain in the gastrovascular cavity where some spermatozooids can arrive by seawater currents, which could produce internal fertilization. The presence of larvae attached to the exterior of the mesenteries in some dissected individuals, together with the presence of developing embryo and planula larva in two sectioned specimens (Fig. 11), pointed out that internal fertilization and larval brooding is possible for the studied population of *A. sulcata*. Another possibility could be that these larvae were developed by parthenogenesis. The protection of free-swimming offspring is known in other sea anemones, such as *Actinia equina* (Chia and Rostron 1970) or *Urticina felina* (Mercier et al. 2011). Some authors, such as Kahng et al. (2011) consider internal brooding in octocorals when fertilization and embryogenesis occur within the polyp resulting in the release of planula larvae. Others define brooding for actinarians as the retention of

individual offspring by the adult to at least the juvenile stage (Larson 2017). We assume the second view and, therefore, consider that *A. sulcata* is not a true brooding species due to the absence of offspring in juvenile phase in the gastrovascular cavity. Schäfer (1981) considered that the larvae found in males of *A. equina* were originated by parthenogenesis; however, the transport of oocytes by sea currents to gastric cavity where remains of sperm could be present is more likely.

Sea anemones with external–internal fertilization are often viviparous and the offspring develop in the gastrovascular cavity of the female until the end of the metamorphosis, such as in the case of *Cribinopsis fernaldi* (Siebert and Spaulding 1976). The presence of filaments with mucous cells penetrating the gastrula found in the gastrovascular cavity of one individual (Fig. 11A) seemed to indicate some maternal nourishment and care of the embryo by *A. sulcata* when internal fertilization takes place.

The presence of zooxanthellae is common among shallow sea anemones, being known since Brandt (1883a, b). Smith (1939) studied the presence and role of the zooxanthellae in *A. sulcata* and showed that they ensured a supply of oxygen during daylight, which prevented death by asphyxia due to lack of dissolved oxygen in the rock pools of stagnant water where the sea anemone lived. Smith compared the metabolic activity between *A. sulcata* with symbiotic zooxanthellae and *A. equina* without zooxanthellae and pointed that *A. sulcata* had a higher metabolic rate, for which the removal of end products (such as phosphorus) by the algae and the availability of oxygen would be of vital importance. Visram et al. (2006) found that the *Symbiodinium* clade A is restricted to cnidarians from the Mediterranean and Atlantic coast of UK and France. However, Grajales et al. (2016) found that *Aiptasia couchii* from the West Atlantic coast exclusively harbored the cold-tolerant symbiont, *Symbiodinium* sp “Mediterranean or temperate clade A”. The presence of one clade in most of the studied species of cnidarians points out a high specificity that possibly was linked to the high incidence of vertical transmission of this zooxanthella. We have observed the presence of zooxanthellae in the mesenteries of *A. sulcata* (Fig. 7A–C), as well as in the female follicles (Fig. 7D, E) and inside the oocyte (Fig. 7D–F), already reported by Schäfer (1985), most of them around of nucleus, which highlight the importance of the zooxanthella in the life of *A. sulcata*. We have not found zooxanthellae in the male follicles and sperm.

Longitudinal fission is the most widespread type of anemone asexual reproduction; being recorded for *A. sulcata* (Schick 1991; Bocharova 2016). The presence of internal buds in the gastrovascular cavity has been indicated for different species of the genus *Actinia*, among them *Ac. equina* (Orr et al. 1982), but not in *A. sulcata*. Our data on the limited presence of buds in the gastrovascular cavity of *A.*

*sulcata* (Fig. 12) would be the first record of this type of asexual reproduction in this species.

Finally, regarding management measures for the fishery of this species, the minimum weight of capture for *A. sulcata* established by the regional authorities in Andalucía (Spain) is 15 g. According to our data, it should be at least 16.5 g (the weight at which the 50% of the individuals have developed gonads) for the sustainability of this fishery. The positive correlation between the diameter of the pedal disc and the weight of the studied specimens allows the use of both variables as legal parameters for fishery management; however, we consider that the size is a measure easier to take by fishermen at sea than the weight. The measurements of relaxed pedal disc give an advantageous bias for a more sustainable fishery. In addition, this species has frequent asexual reproduction, which form rosette of individuals of similar sizes. Due to this, we propose that the legal requirement should indicate the minimum size of capture instead of the minimum weight of capture, which is the present parameter in the law. Taking this into account, the size of minimum capture should be 21.5 mm of diameter of pedal disc. In relation to the close season, we suggest that for the populations of southern Spain, it must be changed from January and February, to March and April, when the peak of spawning occurs.

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## Compliance with ethical standards

**Conflict of interest** All authors declare that they have no conflict of interest.

**Ethical approval** All applicable international, national and/or institutional guidelines for the care and use of the animals were followed.

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