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Sexual reproduction and biometry of the non-zooxanthellate papillose cup coral *Paracyathus pulchellus*

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Basic information on the reproductive biology of many scleractinian corals species is limited or entirely lacking, particularly from temperate zones, though it is essential for a better understanding of their ecology. This study describes the morphological aspects and the annual cycle of gametogenesis and biometric parameters of the papillose cup coral *Paracyathus pulchellus* collected at Palinuro (Italy, Southern Tyrrhenian Sea), filling a knowledge gap about the reproductive biology of a widespread Mediterranean and Northern Atlantic coral. Samples of *P. pulchellus* were collected by SCUBA diving between 5- and 10-meters depth during 18 monthly collections from June 2010 to December 2011. Sexually active polyps displayed either oocytes or spermaries, indicating that *P. pulchellus* was gonochoric. The sex ratio of sexually active polyps was 1:1. Gametogenesis began with undifferentiated germ cells arose in the gastrodermis that migrated towards the mesoglea of the mesentery where they completed the development. During spermatogenesis, spermary diameter increased from 25 to 83 μm . Oocyte diameter ranged from 9 to 146 μm and during oogenesis the nucleus/cytoplasm ratio decreased due to the accumulation of yolk. The nucleus migrated to the periphery of the oocyte adhering closely to the cell membrane. No embryo was observed in the coelenteric cavity of the polyps, suggesting an external development of planktotrophic larvae due to the small-sized mature oocytes and a possible broadcast spawning reproductive mode. Gonadal index of both females and males increased significantly from August until November and fertilization took place from November to January. Only sexually inactive individuals were observed from February to April, suggesting a quiescence period in both males and females. Seasonal variations in water temperature and photoperiod may have a key role in regulating gametogenesis. The analysis of the main biometric parameters (polyp width, height, dry skeletal mass,

volume, surface/volume ratio and bulk skeletal density) showed a negative correlation between size and skeletal density, and no sexual dimorphism.

KEYWORDS

sexuality, reproductive mode, gametogenesis, sex ratio, scleractinia, Mediterranean Sea

Introduction

Reproduction has a fundamental role in the population structure, dynamics, and distribution of benthic animals, which are an important component of marine ecosystems. Studying reproductive traits can provide relevant evolutionary information, which may be crucial for understanding the short- and long-term impacts of climate change that are occurring in the Mediterranean Sea (Airi et al., 2014; Airi et al., 2017; Gizzi et al., 2017; Marchini et al., 2020; Marchini et al., 2021). Within Scleractinian corals, the current global knowledge on reproduction is the result of many decades of research in which studies have identified an exceptional diversity in the reproductive patterns (Harrison and Wallace, 1990). Corals are modular organisms and the variety of reproductive processes and modes they employ is testament to their exceptional regeneration ability, developmental plasticity, and adaptability (Baird et al., 2009; Harrison, 2011). Substantially, four combinations of reproductive patterns can be recognized: propagation mode (asexual or sexual), sexuality (hermaphroditic or gonochoric), reproductive mode (broadcasting or brooding) and embryonic development (coeloblastic or stereoblastic; Harrison, 2011).

Most scleractinians are hermaphrodites (Harrison and Wallace, 1990; Richmond and Hunter, 1990; Harrison, 2011), while gonochorism occurs only in 26% of the species studied (Harrison, 2011). Gonochoric coral species include colonies or individual polyps that produce only male or female gametes over their lifetime (this condition is also referred to as dioecy, separate sexes) (Harrison and Wallace, 1990; Richmond, 1997; Harrison, 2011). Oocytes and spermaries originate from interstitial cells, they grow and develop within the mesenteries, enclosed by mesoglea and gastrodermis (Harrison, 1990).

Fertilization can be either external, when corals release gametes in the water column (broadcast spawning), or internal, when gametes encounter within the celenteric cavity of the polyp (brooding) (Harrison and Wallace, 1990). Broadcast spawning is typical for 82.7% of the 428 coral species for which the mode of development has been documented (Harrison, 2011). Most corals studied so far are hermaphroditic and broadcast spawning, while gonochoric brooding is the least frequent combination (Baird et al., 2009; Harrison, 2011).

The reproductive traits have been reported in more than 450 (Harrison and Wallace, 1990; Richmond and Hunter, 1990; Richmond, 1997; Baird et al., 2009; Harrison, 2011) of the more

than 1,500 coral species identified (Harrison, 2011). Most of these studies were carried out on tropical and subtropical scleractinians, while information on the reproductive biology of scleractinians from temperate zones is limited. Although there are 33 species of scleractinians in the Mediterranean Sea (Zibrowius, 1980), data on their reproduction come from old observations (Lacaze-Duthiers, 1873) and from studies on the species *Oculina patagonica* (Fine et al., 2001), *Balanophyllia europaea* (Goffredo and Telò, 1998; Goffredo et al., 2002), *Leptopsammia pruvoti* (Goffredo et al., 2005; Goffredo et al., 2006), *Astroides calycularis* (Goffredo et al., 2010; Goffredo et al., 2011a), *Cladocora caespitosa* (Kružić et al., 2008; Kersting et al., 2013; Hadjioannou, 2019), and *Caryophyllia inornata* (Goffredo et al., 2012; Marchini et al., 2015).

The analysis of biometric parameters has been used in different studies to investigate aspects of coral individual growth patterns (Goffredo and Chadwick-Furman, 2003; Goffredo et al., 2004; Goffredo et al., 2011b) and how these can change in correlation to environmental factors such as solar radiation and seawater temperature (Goffredo et al., 2007; Caroselli et al., 2015) and depth (Özalp et al., 2018). In these studies, polyp width, height, volume, surface/volume ratio and bulk skeletal density are correlated to polyp length. The study of the correlation between width and length can indicate either an isometric growth, in which the coral has a more circular shape of the calyx, or an allometric growth, where length increases more rapidly than width and which leads to a more oval oral surface (Goffredo and Chadwick-Furman, 2003).

Paracyathus pulchellus (Figure 1A) is a caryophylliid coral widespread in the Western and Eastern basins of the Mediterranean Sea (Fenner et al., 2013). Moreover, this species is found in the Eastern Atlantic between Portugal, the Azores, the Gulf of Guinea (Zibrowius, 1980), and in the Western Atlantic from North Carolina to Brazil (Cairns, 1979; Cairns, 2000). *P. pulchellus* colonizes hard substrates such as vertical walls, caves, and wrecks (Zibrowius, 1980; Fenner et al., 2013). Its reported depth range varies from 17 to 1260 m (Casado-Amezua et al., 2015; World Register of Marine Species¹). The species is classified as Data Deficient by the IUCN and investigation on its occurrence is highly recommended (Casado-Amezua et al., 2015). The purpose of this study was thus to provide the first investigation on the reproductive biology of *P. pulchellus*, using specimens from a Mediterranean population (Figure 1).

¹ <https://marinespecies.org>

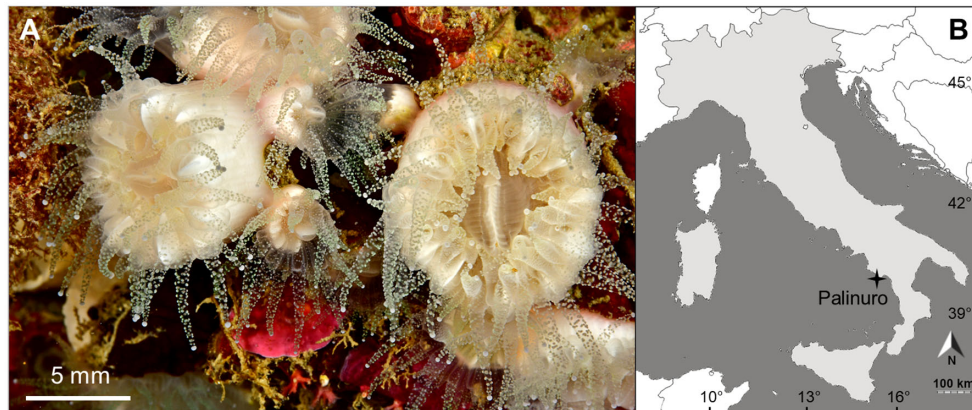


FIGURE 1
(A) Living specimens of *Paracyathus pulchellus* photographed by Francesco Sesso; **(B)** Map of sampling site located at Palinuro (Italy, 40° 02'N and 15° 16'E).

Materials and methods

Sampling

Samples of *Paracyathus pulchellus* were collected in a cave named Grotta Azzurra (Blue Cave) at Palinuro (Italy, Southern Tyrrhenian Sea: 40°02'N and 15°16'E; [Figure 1B](#)), during 18 monthly collections from June 2010 to May 2011. Twenty polyps were haphazardly collected each month by SCUBA diving between 5- and 10-meters depth. Polyps were immediately fixed in saturated formalin solution (90% seawater and 10% formaldehyde) and transferred to the lab for biometric and histological analyses.

Environmental parameters

Photoperiod data were obtained from an online database (<http://www.eurometeo.com>). Depth Thermometer Temperature (DTT; °C) was measured by digital thermometers (I-Button DS1921H, Maxim Integrated Products) placed at the depth and site of collection for the entire sampling period. Sea Surface Temperature data (°C) were recorded from the National Mareographic Network of the Institute for the Environmental Protection and Research (ISPRA). These data were measured by mareographic stations (SM3810 manufactured by the Society for the Environmental and Industrial monitoring; SIAP + MICROS), placed close to the sampling sites. A linear regression was produced between DTT and SST data (Depth Temperature, DT = 1.3818*SST + 7.243) to estimate temperatures during periods in which sensors were lost due to bad weather conditions. In this study we considered the monthly average Depth Temperature (DT; °C) of one year of sampling (n = 12 monthly temperatures).

Biometric analysis

Biometric analyses were performed on 102 polyps of which we measured the length (l, maximum axis of the oral disc; [Figure 2](#)), width (w, minimum axis of the oral disc) and height (h, oral-aboral axis of the polyp) using a caliper. The volume was calculated using the formula $v = h \times (l/2) \times (w/2) \times \pi$ ([Goffredo et al., 2002](#)). Surface/volume (S/V) ratio was calculated by dividing S by V. The surface parameter was obtained as a sum of the surface of the oral disc $\pi \times (l/2) \times (w/2)$ and the lateral surface of the coral calculated with the formula $\pi \times [3 \times (l/2 + w/2) - \sqrt{((3 \times l/2 + w/2) \times (l/2 + 3 \times w/2))}] \times h$ ([Ramanujan, 1914](#); [Caroselli et al., 2015](#)).

The wet mass of all 102 corals was measured using a digital precision balance. The wet mass of 10 additional corals spanning the length range 4.4 - 9.65 mm was measured, then they were immersed in a 10% commercial bleach solution for 3 days to remove the soft tissue and then skeletons were dried for 4 days. Each of these 10 corallites was observed under a binocular microscope to remove fragments of substratum and deposits produced by other organisms, then their dry skeletal mass was measured with a digital precision balance. Using the linear regression between the wet and the dry skeletal mass of the 10 selected corallites the dry skeletal mass of all corals was then estimated. Skeletal bulk density (D) was calculated by dividing the dry skeletal mass (M) by the volume (V; [Caroselli et al., 2015](#)).

Histological analysis

Histological analysis was performed on 57 polyps ([Supporting Information Table S1](#)). After a post fixation in

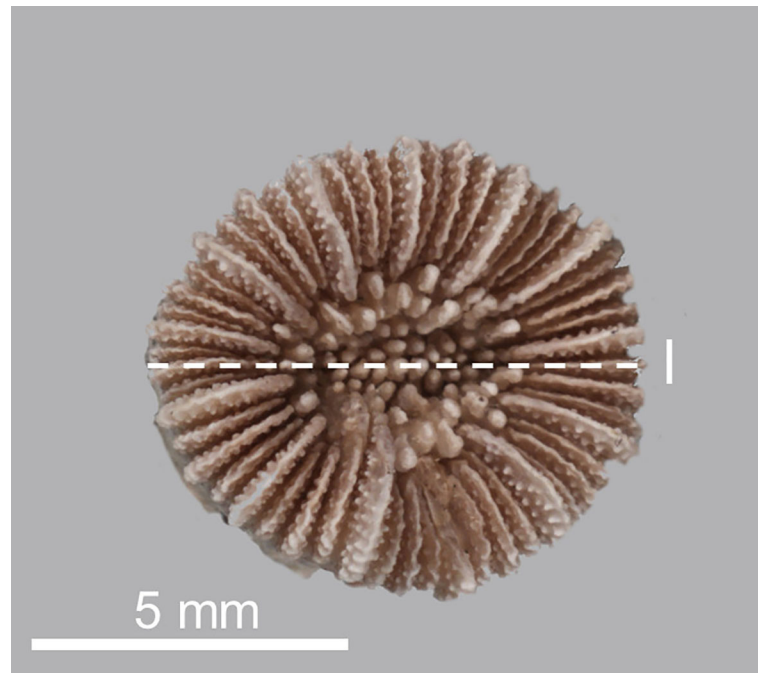


FIGURE 2
Skeleton of *Paracyathus pulchellus*. Dotted line indicates polyp length (l , maximum axis of the oral disc).

Bouin solution to ensure a better fixation and staining of the tissues, polyps were decalcified using EDTA, dehydrated through a graded alcohol series from 80 to 100%, clarified in histolemon and embedded in paraffin. Serial transverse sections were cut at 7 μm intervals along the oral-aboral axis, from the oral to the aboral pole of the entire polyp, using a microtome. Finally, tissues were stained with Mayer's hematoxylin and eosin (Goffredo et al., 2002; Marchini et al., 2015).

Gamete size

Histological observations and cytological readings were performed using the light microscope NIKON Eclipse 80i and the image analysis system NIKON NIS-Elements D 3.1. The maximum and minimum diameter of the spermaries and oocytes in nucleated sections were measured and classified into developmental stages in accordance with other studies on gametogenesis in scleractinians (Goffredo et al., 2005; Goffredo et al., 2010). Gamete size was determined as the mean of the two diameters (Goffredo et al., 2005; Goffredo et al., 2010). A gonadal development index was calculated as the percentage of body volume occupied by the germ cells (Hall and Hughes 1996). The volume of each reproductive element was estimated as described in the subsection "Gonadal index" of Goffredo et al., 2002.

Definitions

The following definitions were used: sexually active polyps, individuals that displayed a gametogenetic activity; male polyps, individuals that displayed spermaries; female polyps, individuals that displayed oocytes; sexually inactive polyps, individuals that did not display a gametogenetic activity.

Statistical analysis

To determine the presence of sexual dimorphism, 12 female and 13 male polyps were used for a comparative analysis of the biometrical parameters (length, width, height, volume, S/V ratio, dry skeletal mass and bulk skeletal density). These comparisons were made by conducting a one-way permutation multivariate analysis of variance (PERMANOVA) using the software Primer v6 – Quest Research Limited (Anderson, 2001). The analysis was conducted with 999 permutations and included the Monte Carlo correction for small sample size. The test was performed using sex as the only fixed effects factor and was based on Euclidean distances. A Student's t -test was used to compare each biometrical parameter between female and male polyps and to compare length between sexually active and sexually inactive polyps.

Results

The population of *P. pulchellus* was found and collected from 5 meters depth, shallowing the bathymetric range reported by IUCN for this species that previously started from 17 meters.

Sexual condition and reproductive mode

Paracyathus pulchellus was found to be gonochoric since all specimens analyzed had either spermaries or oocytes, and none showed both types of germ cells. Sexual dimorphism was not observed, neither at level of overall morphology (PERMANOVA, $df = 24$, $p = 0.834$) nor according to each biometric parameter (Tables 1, 2; Figure 3). The sex ratio of sexually active polyps was 1:1 (chi-square test, $\chi^2 = 0.04$, $df = 1$, $p > 0.05$). In the months in which both males and females showed germ cells (from May to January), 18 sexually inactive polyps were observed (Supporting Information Table S1). The mean size of the sexually inactive polyps was homogeneous to that of the sexually active ones (mean length: sexually inactive = 7.6 mm, 95% CI = 7.1-8.1, $N=18$, sexually active = 7.8 mm, 95% CI = 7.4-8.2, $N=25$, Student's test $p > 0.05$). No embryo was observed in the coelenteric cavity, indicating an external development and a possible broadcast spawning reproductive mode.

Male gametogenesis

Spermaries were found in the mesentery and were made up of groups of germ cells and delineated by mesogleal envelope (Figure 4). A total of 4,531 spermaries were identified and measured (Supporting Information Table S1). Five stages of maturation were identified:

Stage I - undifferentiated germ cells arose in the gastrodermis and then migrated toward the mesoglea of the mesentery where they regrouped forming the spermary. The spermary was made up of an early aggregation of spermatogonia (Figure 4A). Spermaries had a mean diameter of 25 μm , 95% CI = 24-26, $N= 213$.

Stage II - the spermary was made up of a group of spermatocytes undergoing meiosis. The mesogleal layer enveloping the spermary had not yet formed a wall (Figure 4B). Spermary mean diameter was 38 μm , 95% CI = 37-39, $N= 331$.

Stage III - the spermary, still made up of a group of spermatocytes undergoing meiosis, was delineated by a clearly differentiated wall formed by the mesoglea (Figure 4C). Spermary mean diameter was 61 μm , 95% CI = 60-62, $N= 1,683$.

Stage IV - the spermary showed a centripetal maturation gradient: less mature and larger germ cells (spermatocytes) were located at the periphery of the spermary, whereas more mature and smaller ones (spermatids) were located in the center (Figure 4D). Spermary mean diameter was 83 μm , 95% CI = 82-84, $N= 1,663$.

TABLE 1 General PERMANOVA results of biometric parameters distribution between male and female polyps.

Source	df	SS	pseudo-F	p (MC)
Sex	1	382.46	0.230	0.834
Residual	23	38179		
Total	24	38562		

df, degrees of freedom; SS, sum of squares; p (MC), significance after Monte-Carlo correction for small sample size.

TABLE 2 Mean biometric parameters, 95% confidence intervals (CI) and t-test p-value of sexually active polyps (males and females).

Biometric parameters	Male	Female	p-value
Length (mm)	7.6 (7.0-8.2)	8.0 (7.6-8.5)	0.271
Width (mm)	6.8 (6.3-7.3)	6.9 (6.4-7.4)	0.853
Height (mm)	9.4 (8.6-10.2)	8.6 (7.0-10.3)	0.423
Volume (mm^3)	397 (318-477)	385 (284-485)	0.850
S/V ratio (mm^{-1})	0.68 (0.63-0.73)	0.68 (0.64-0.72)	0.997
Dry skeletal mass (g)	0.36 (0.31-0.41)	0.35 (0.28-0.35)	0.834
Bulk skeletal density (mg mm^{-3})	0.96 (0.84-1.07)	0.97 (0.81-1.12)	0.904

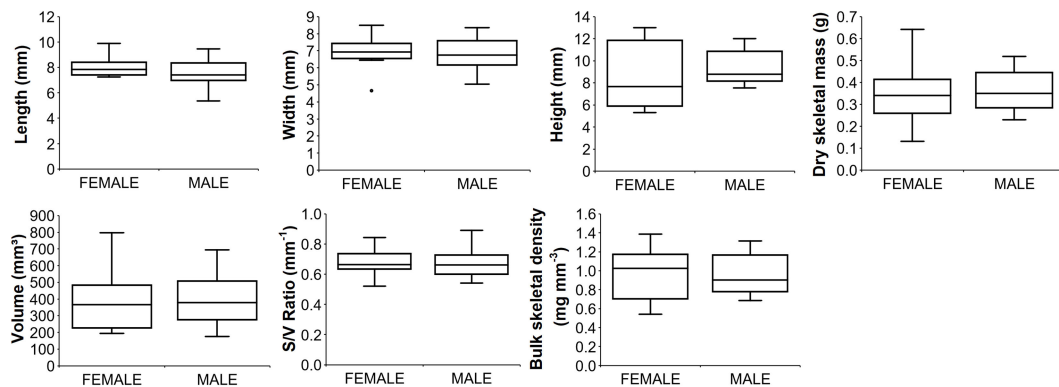


FIGURE 3

Boxplots of biometric parameters for male and female polyps. Median (solid horizontal line), first and third quartiles (box outline), and outliers (circles). Number of polyps analyzed: 12 for females; 13 for males.

Stage V - the spermary was made up of a mass of spermatozoa with their tails all facing in the same direction (an arrangement known as a “bouquet”; Fadlallah and Pearse, 1982a; Figure 4E). Spermary mean diameter was 83 μm , 95% CI = 81–85, N= 641.

Female gametogenesis

The oocytes were oval-shaped and located in the mesenteries (Figure 5). A total of 25,102 oocytes were identified and measured (Supporting Information Table S1). The diameter of the oocytes ranged from 9 to 146 μm and their mean diameter was 46 μm , 95% CI = 45.7–46.3, N= 25,102. The early stages of oogenesis were visible in the mesentery gastrodermal layers. Early oocytes had a centrally located spherical nucleus and a high ratio of nucleus to cytoplasm (Figure 5A). In the intermediate stages the nucleus/cytoplasm ratio decreased due to the accumulation of yolk (Figure 5B). In the more advanced stages, the ratio further reduced since cell size increased in parallel to the storage of yolk in the oocyte cytoplasm (Figures 5C, D). The nucleus had also migrated to the cell periphery and adhered closely to the cell membrane (Figures 5C, D). During oogenesis, the nucleolus was always positioned on the periphery of the nucleus (Figures 5C, D).

Annual reproductive cycle

Gonadal index of both females and males increased significantly from August until November, the months in which both the photoperiod and water temperature decreased after the maximum of the year (Figures 6A, B). During late autumn and winter, late maturation stages of both oocytes (mean diameter ranged from 67.3 to 93.8 μm) and spermaries (IV and V) were observed (Supporting Information Table S2). After this period, both

type of gametes disappeared (Figure 6B and Supporting Information Table S2), suggesting that the fertilization was occurred. Immediately after the fertilization period, we observed the emptying of gametes in both males and females (Figure 6B and Supporting Information Table S2) and the consequent increase in sexually inactive polyps (Figure 6C). In late spring and summer new oocytes (mean diameter ranged from 20.9 to 38.8 μm) and the development of early stages of spermary maturation (I, II, III) were detected (Supporting Information Table S2).

Biometry

Polyp length was selected as the main biometric parameter since it was the best indicator of dry skeletal mass and has been used as the measure of size in biometric, reproductive biology, and population dynamic studies of other solitary corals (Bell and Turner, 2000; Goffredo and Chadwick-Furman, 2003; Goffredo et al., 2010; Caroselli et al., 2015). Polyp width, height, dry skeletal mass and volume all correlated positively with polyp length (Figure 7). Surface/volume ratio and bulk skeletal density correlated negatively with polyp length (Figure 7). Since the regression equation exponent was < 1 (95% confidence interval = 0.798–0.959), the increase in polyp width compared with that of polyp length showed an allometric growth, with polyp length increasing more rapidly than width.

Discussion

Sexual condition and reproductive mode

Each sexually active polyp of *Paracyathus pulchellus* contained a single type of germ cell, indicating gonochorism. As expected for

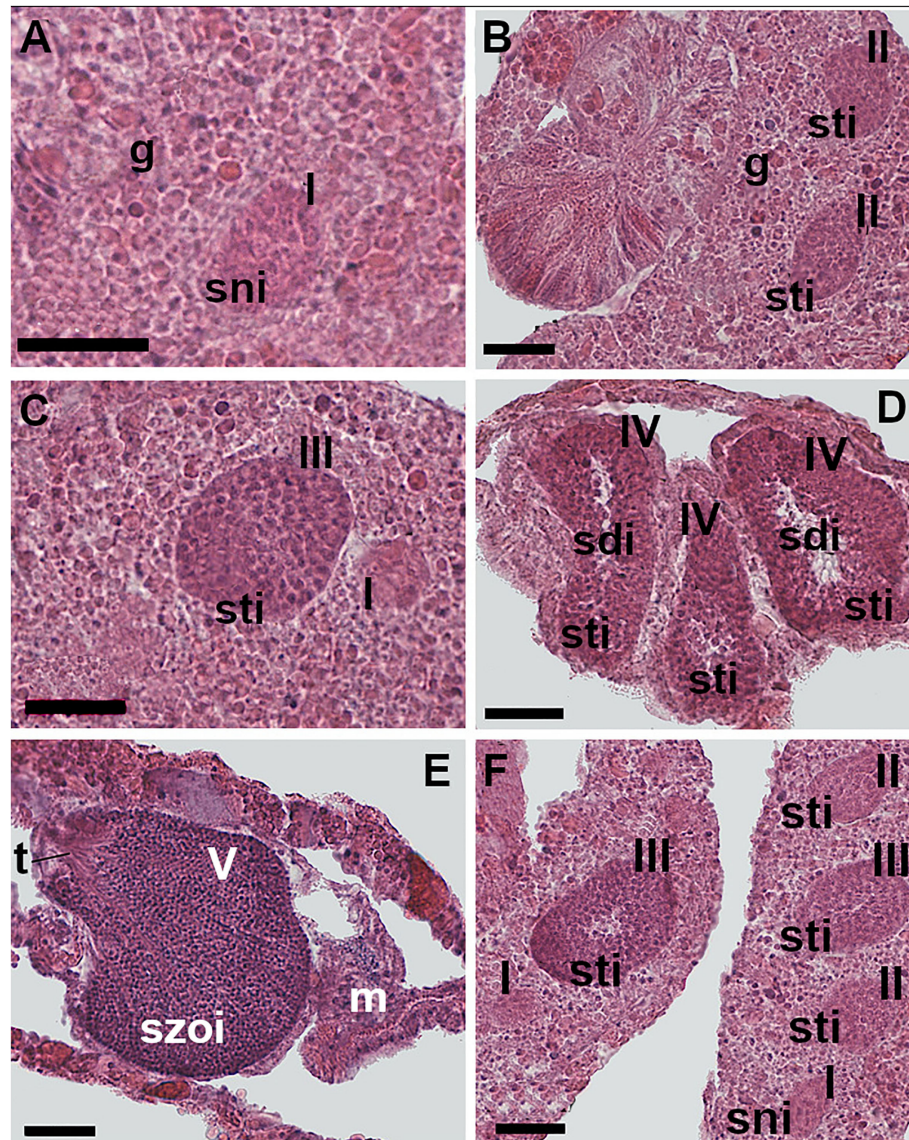


FIGURE 4

Spermatogenesis. (A) Stage I: undifferentiated germ cells disposed in the gastrodermis layers of the mesentery. The spermaries are made up of a group of spermatogonia. (B) Stage II: the spermaries are made up by spermatocytes involved in the process of meiosis. (C) Stage III: the spermary, containing spermatocytes undergoing meiosis, is delineated by a wall that has arisen from the mesoglea. (D) Stage IV: the spermary presents an external layer of spermatocytes and an internal mass of spermatids, recognizable by the presence of a tail. (E) Stage V: the spermary is made up of a mass of spermatozoa. Shortly before leaving the spermary, mature spermatozoa form a bouquet, with their tails all facing in the same direction. (F) Spermaries of stages I, II, and III located in the same mesentery. lg: gastrodermis; m: mesoglea; sni: spermatogonia; sti: spermatocytes; sdi: spermatids; szoi: spermatozoa; t: spermatozoa tails; I, II, III, IV, V: spermary developmental stage). Scale bars are 25 μ m.

gonochoric corals, male and female polyps did not show sexual dimorphism, likely excluding protandry or protogynous hermaphroditism (Harrison, 2011). Within the genus *Paracyathus*, *P. stearnsii* (Fadlallah and Pearse, 1982b) is another case of gonochorism. The sex ratio in a population with random mating is normally 1:1 (Maynard-Smith, 1978), as it has been observed in this population of *P. pulchellus*. Similarly, the sex ratio among individuals of *P. stearnsii*, collected off the coast of

California, did not seem to differ from 1:1 (Fadlallah and Pearse, 1982b). Based on the absence of embryos in all the polyps analyzed, it is highly likely that the embryos of *P. pulchellus* have an external development. Within the Caryophylliidae family, almost all species studied develop the embryos externally, with the exceptions of *Caryophyllia cyathus*, *C. inornata*, *C. huinayensis*, *Tethocyathus endesa*, and some records of brooding in *C. smithii* (Table 3). Sexual condition (gonochorism vs. hermaphroditism)

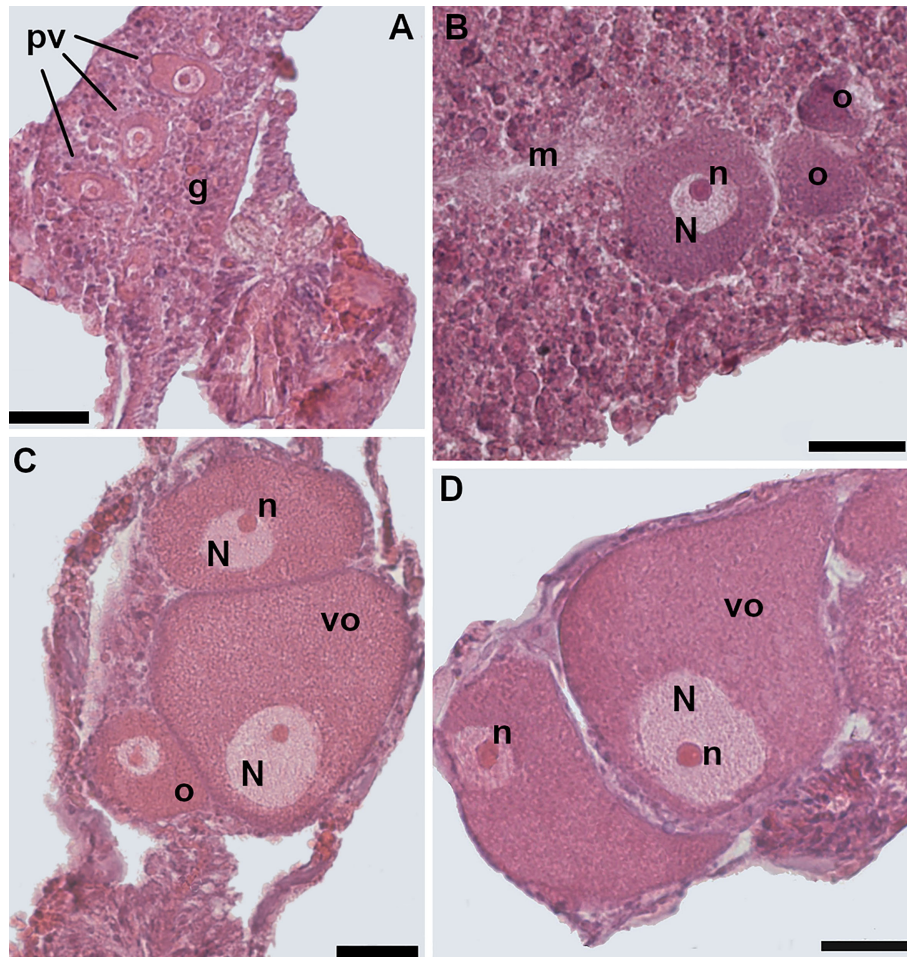


FIGURE 5

Oogenesis. (A) Early stage: three previtellogenic oocytes in the gastrodermis of the mesentery, characterized by a high nucleus/cytoplasm ratio. (B) Intermediate stage: vitellogenic oocyte located in the mesoglea. The spherical-shaped nucleus is still located in the central portion of the cell. The nucleus/cytoplasm ratio is reduced. (C) Late stage: the nucleus of the oocyte has started to migrate toward the cell periphery. Note two smaller vitellogenic oocytes in the same section. (D) Late stage: two mature oocytes, located in the mesentery; the nucleus/cytoplasm ratio is greatly reduced. [o, oocyte; m, mesoglea; N, nucleus; n, nucleolus; pv, previtellogenic oocyte; vo, vitellogenic oocyte; g, gastrodermis]. Scale bars are 25 μ m.

in scleractinians tends to be phylogenetically correlated and therefore a constant feature within each species, genus, and family, while reproductive mode (brooding vs. external development) tends to be a plastic trait and more variable (Harrison, 1985; Harrison and Wallace, 1990). However, in an increased number of families the presence of both gonochoric and hermaphroditic species has been reported (Harrison, 2011). Sexuality can vary not only at a genus and family level but also among populations of the same species. For example, *Astroides calycularis* collected at Palinuro (Southern Tyrrhenian Sea, Italy) and at Punta de la Mona (Alboran Sea, Spain) was described as gonochoric species both at the polyp and colony level (Goffredo et al. 2010; Goffredo et al. 2011a; Casado-Amezua et al. (2013), while Lacaze-Duthiers (1873) described *A. calycularis* from the

Algerian coasts as monoic hermaphroditic, observing hermaphroditic colonies formed by gonochoric polyps with some cases of polyps that exhibited a simultaneous hermaphroditism. Another example is the species *Cladocora caespitosa*, the only shallow water scleractinian coral that can be considered a reef-building in the Mediterranean basin. Colonies from the Western and Eastern Mediterranean Sea were observed to be gonochoric (Kersting et al., 2013; Hadjioannou, 2019), while Kružić et al. (2008) described this species as hermaphroditic in the Adriatic Sea. Given the wide distribution of *P. pulchellus*, which spans the entire Mediterranean and Northern Atlantic, investigations on populations from other sites are needed to check if the reproductive traits observed in this study are maintained across the whole species areal.

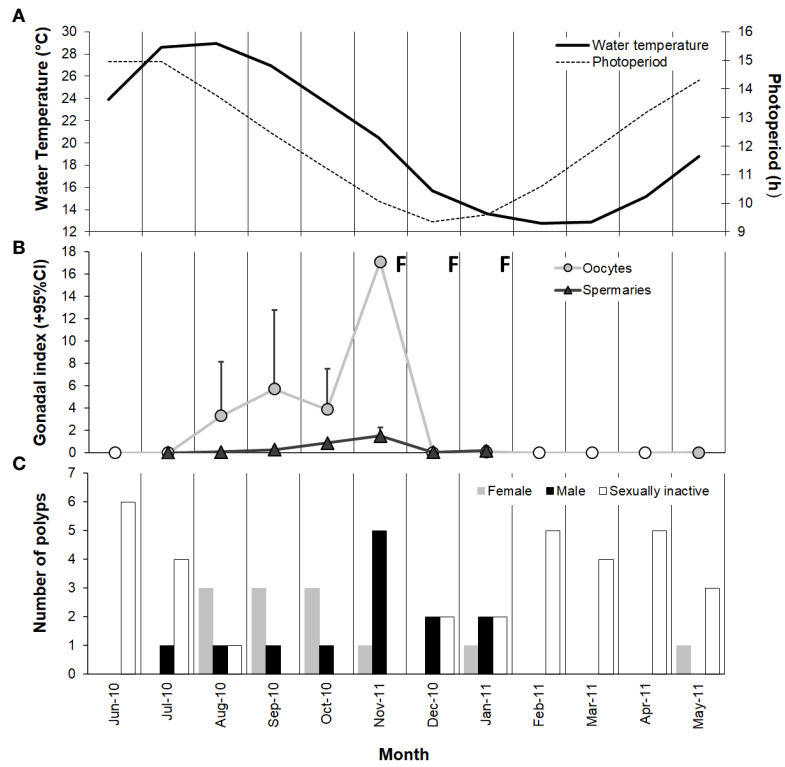


FIGURE 6 Variation in (A) water temperature and photoperiod, (B) gamete development (monthly mean + 95% confidence interval), and (C) number of female, male, and sexually inactive polyps from June 2010 to May 2011 at Palinuro. Note that the value ranges on the ordinate axes are different. O no oocytes detected; Δ no spermaries detected; F, fertilization.

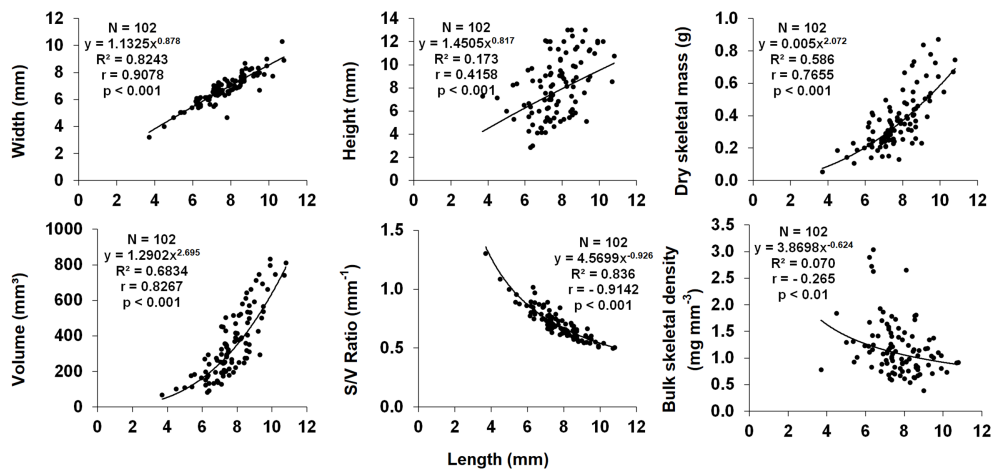


FIGURE 7 Dependence of biometric parameters on polyp length. N, number of individuals; R², coefficient of determination; r, Pearson correlation coefficient.

TABLE 3 Reproductive traits found in the literature on Caryophylliidae family.

Species	Sexual condition	Reproductive mode	Source
<i>Caryophyllia ambrosia</i>	hermaphroditic	broadcast spawner	Waller et al., 2005
<i>Caryophyllia cornuformis</i>	hermaphroditic	broadcast spawner	Waller et al., 2005
<i>Caryophyllia cyathus</i>	–	brooder	von Koch, 1897 in Fadlallah, 1983
<i>Caryophyllia huinayensis</i>	–	brooder	Heran et al., 2018
<i>Caryophyllia inornata</i>	gonochoric	brooder	Goffredo et al., 2012; Marchini et al., 2015
<i>Caryophyllia sequenzae</i>	hermaphroditic	broadcast spawner	Waller et al., 2005
<i>Caryophyllia smithii</i>	gonochoric	broadcast spawner brooder	Tranter et al., 1982 Hiscock and Howlett, 1977
<i>Desmophyllum dianthus</i>	gonochoric	broadcast spawner	Feehan et al., 2019
<i>Goniocorella dumosa</i>	gonochoric*	broadcast spawner	Burgess and Babcock, 2005
<i>Heterocyathus aequicostatus</i>	gonochoric	broadcast spawner	Fisk, 1981 in Harriott, 1983; Harrison and Wallace, 1990; Heltzel and Babcock, 2002
<i>Hoplangia durotrix</i>	hermaphroditic	–	Baird et al., 2009
<i>Desmophyllum pertusum</i> (= <i>Lophelia pertusa</i>)	gonochoric **	broadcast spawner	Waller and Tyler, 2005; Brooke and Järnegren, 2013; Pires et al., 2014
<i>Paracyathus pulchellus</i>	gonochoric	broadcast spawner	present work
<i>Paracyathus stearnsii</i>	gonochoric	broadcast spawner	Fadlallah and Pearse, 1982b
<i>Solenosmilia variabilis</i>	gonochoric	broadcast spawner	Burgess and Babcock, 2005; Pires et al., 2014
<i>Tethocyathus endesa</i>	–	brooder	Heran et al., 2018

(-) unknown; (*) only colonies of one sex have been found; (**) in one colony oocytes and sperms were found in different polyps.

No significant differences were found in the average size between sexually active and inactive polyps during the reproductive period, thus, the inactive polyps could be quiescent. Since most of the inactive polyps have been observed at the beginning of gametogenesis (May-July; Supporting Information Table S1), it is likely that they do not have yet begun to develop gametes. While the inactive polyps observed at the end of gametogenesis (December-January; Supporting Information Table S1) may have already released their gametes for fertilization.

Male gametogenesis

The morphological aspects of male gametogenesis in *Paracyathus pulchellus* correspond to those of other scleractinians of this and other families with the same or different reproductive traits. For example, aspects of spermatogenesis observed in *P. pulchellus* have been described, among Caryophylliidae family, in the gonochoric and broadcasting *P. stearnsii* (Fadlallah and Pearse, 1982b), *Solenosmilia variabilis* (Pires et al., 2014), *Desmophyllum dianthus* (Feehan et al., 2019), and *Desmophyllum pertusum* (Brooke and Järnegren, 2013; Pires et al., 2014), in the gonochoric and brooding *Caryophyllia inornata* (Goffredo et al., 2012), and in the hermaphroditic and broadcasting species *C. ambrosia*, *C. sequenzae*, and *C. cornuformis* (Waller et al., 2005). Spermatogenesis with similar morphological traits was also observed in other families: e.g., in the gonochoric and brooding *Balanophyllia elegans* (Dendrophylliidae; Fadlallah

and Pearse, 1982a), *Leptopsammia pruvoti* (Dendrophylliidae; Goffredo et al., 2005), and *Astroides calycularis* (Dendrophylliidae; Goffredo et al., 2010), in the hermaphroditic and brooding *Balanophyllia europaea* (Dendrophylliidae; Goffredo et al., 2002), *Madracis mirabilis*, *M. senaria*, *M. decactis*, *M. formosa*, *M. carmabi*, and *M. pharensis* (Pocilloporidae; Vermeij et al., 2004), in the gonochoric and broadcasting *Euphyllia ancora* (Euphylliidae; Shikina et al., 2012), *Enallopsammia rostrata* (Dendrophylliidae; Pires et al., 2014) and *Fungiacyathus marenzelleri* (Fungiacyathidae; Waller et al., 2002), in the hermaphroditic and broadcasting *Mussismilia hispida* (Mussidae; Neves and Pires, 2002), *Acropora cervicornis* (Acroporidae; Vargas-Angel et al., 2006).

Female gametogenesis

In corals, oocyte development varies according to the reproductive mode. Generally, brooding species produce a relatively small number of large-sized oocytes rich in reserves (Shlesinger et al., 1998), while broadcasting species produce and release a relatively large number of small oocytes (Fadlallah, 1983; Richmond and Hunter, 1990). *Paracyathus pulchellus* showed many relatively small oocytes that ranged from 9 to 146 μm with a mean diameter of 46 μm , similar to those described for *P. stearnsii* which produces oocytes ranged from 5 to 175 μm in diameter (Fadlallah and Pearse, 1982b). The morphological aspects of oogenesis observed in *P. pulchellus* are also similar to those described for some species of the

Caryophylliidae family. For example, both *Caryophyllia inornata* (that broods its embryos but probably of agamic origin with a possible external fertilization) and *C. smithii* produce oocytes with spherical or oval shape and a diameter respectively ranging from 12 to 382 μm with a mean diameter of 69 μm (Goffredo et al., 2012; Marchini et al., 2015) and from 130–150 μm (Tranter et al., 1982). However, oocyte diameter can vary considerably among individuals of the same species inhabit different environments (Harriott, 1983) or within a genus (Babcock et al., 1986; Richmond and Hunter, 1990). In contrast, the three deep-coral species *C. cornuformis*, *C. sequenzae*, and *C. ambrosia* have oocytes with maximum sizes that are 2–5 times greater ranged from 350 to 700 μm (Waller et al., 2005). It seems that the size of mature oocytes of the genus *Caryophyllia* increases with an increment in depth of the habitat of the species, as an adaptation to oligotrophic environments, since large oocytes and a consequent development of lecithotrophic larvae should not require an exogenous food supply (Shilling and Manahan, 1994; Waller et al., 2005). Species that produce small oocytes, as for *P. pulchellus*, which contain less energy reserves, usually generate small planktotrophic larvae (Jaekle, 1995; Sewell and Manahan, 2001; Prowse et al., 2008) with a long pelagic larval phase, during which they must find food sources in the water (Pechenik, 1990). To enhance growth and larval development, the release of gametes should occur during periods that coincide with the presence of a higher amount of food in the water (Tyler et al., 1982). In *P. stearnsii*, Fadlallah and Pearse (1982b), through artificial fertilization, observed the formation of swimming planulae, but none were able to settle, probably due to lack of food. Similarly, *C. smithii* was observed to have planktotrophic planulae which start to feed 48 hours after fertilization when they are totally formed, and that swim and develop for 8 to 10 weeks before starting to settle (Tranter et al., 1982). In *P. pulchellus*, when the oocytes shifted from immature to mature, their shape started to change from round to elliptical. This pattern was found in other species of the same family but also in corals of different genus and different regions such as *Acropora hyacinthus* in Northwestern Philippines (Jamodiong et al., 2018), *Plesiastrea versipora* in Sidney Harbour (Madsen et al., 2014), and *Diploria labyrinthiformis* in the Colombian Caribbean (Alvarado et al., 2004), and others. As was observed in *P. pulchellus*, and in other species belonging to the same family, such as *Goniocorella dumosa* and *Solenosmilia variabilis* (Burgess and Babcock, 2005), during the final phases of oogenesis in scleractinians and more generally in anthozoans, the nucleus migrates to the cell periphery and adheres to the plasma membrane (Szmant-Froelich et al., 1985). The typical U-shaped phase (in which the nucleus becomes indented assuming a sickle) that is observed in some scleractinians (e.g., Goffredo et al., 2005; Shikina et al., 2012; Park et al., 2020) was not observed in *P. pulchellus*, as in no other species within the Caryophylliidae family, such as *G. dumosa*, *S. variabilis* (Burgess and Babcock, 2005; Pires et al.,

2014), *Desmophyllum pertusum* (Brooke and Järnegren, 2013; Pires et al., 2014), and *C. ambrosia*, *C. sequenzae* and *C. cornuformis* (Waller et al., 2005).

Annual reproductive cycle

Histological observations showed that sexual reproduction of *Paracyathus pulchellus* follows a single seasonal reproductive cycle. The maturation of gametes required about 9 months (from May to January, though mature males and females predominated from July to January; Figures 6B, C) and occurred simultaneously. Synchronous gametogenesis has also been documented for another solitary coral within the *Paracyathus* genus, *P. stearnsii*, in which spermaries and oocytes follow an annual cycle and are closely paralleled (Fadlallah and Pearse, 1982b). The same pattern has been observed for several coral species from Mediterranean Sea such as *Balanophyllia europaea*, *Leptopsammia pruvoti*, *Astroides calycularis*, and *Caryophyllia inornata* (Goffredo et al., 2002; Goffredo et al., 2006; Goffredo et al., 2011a; Marchini et al., 2015).

Both seawater temperature and photoperiod are often mentioned as important factors that controls gametogenetic cycles in many anthozoans (Babcock et al., 1994; Acosta and Zea, 1997; Colley et al., 2000; Glynn et al., 2008). *P. pulchellus* showed an acceleration in gamete maturation during the months in which both photoperiod and water temperature decrease, while fertilization took place when photoperiod reaches the minimum of the year. However, studies on other Mediterranean corals showed an increase in gonadal development rate in the months during which both photoperiod and seawater temperature increase (Goffredo et al., 2002; Goffredo et al., 2006; Marchini et al., 2015). In individuals of *C. inornata* studied at Elba Island, gonadal index increases from March to May, coinciding with an increase of photoperiod and seawater temperature. Fertilization takes place from April until July, when photoperiod reached the maximum of the year (Marchini et al., 2015). The solitary corals *L. pruvoti* and *B. europaea* at Calafuria have a significant increase in gonadal development in the months during which photoperiod becomes longer after its annual minimum, and fertilization takes place in the period when both photoperiod and seawater temperature are increasing (Goffredo et al., 2002; Goffredo et al., 2006). Thus, the seasonal variation of seawater temperature and photoperiod seems to be crucial in the gamete development for these temperate corals, even though they can trigger different processes of the reproductive cycle. The dissimilarity observed in the timing of gonadal development of these Mediterranean corals, could be explained by the different larvae development mode. In fact, for the developing larvae also food availability is an important factor (Lessios, 1981). Since Mediterranean Sea is characterized by the highest Chlorophyll-a values in winter (Barale et al., 2008), a gonadal development in this period could be a strategy for

brooding corals that need energy to produce large oocytes with subsequent development of lecithotrophic larvae whose feeding depends on the yolk present in the large oocytes. On the other hand, planktotrophic larvae that develop in the water column (as in the case of *P. pulchellus*), need a constant supply of food throughout the early stages of their lives.

Biometry

All biometric parameters analyzed in this study correlated significantly with polyp length (Figure 7), consistently with previous studies on other solitary coral species (Goffredo et al., 2007; Caroselli et al., 2015). Bulk skeletal density decreased with increasing polyp size. The relationship between skeletal density and size can be interpreted in terms of relationship between calcification and linear extension (calcification = skeletal density \times linear extension rate; Lough and Barnes, 2000). In *Paracyathus pulchellus*, as known for other solitary corals (Goffredo et al., 2004; Goffredo et al., 2007; Caroselli et al., 2015), the linear extension rate is expected to decrease as size (age) increases. The decrease of skeletal density with increasing size could be explained as a greater decrease in calcification than in linear extension rate with increasing size, as already suggested for *Caryophyllia inornata* (Caroselli et al., 2015) and *Leptopsammia pruvoti* (Goffredo et al., 2007).

The exponent of the w/l relationship for *P. pulchellus* polyps was < 1 , (CI 95% = 0.798–0.959). This indicates an allometric growth of the corallite, in which polyp length increases more rapidly than width, thus producing a more oval-shaped oral disc. This shape of the oral disc has been put in relation to sedimentation stress in some coral species (e.g., *Balanophyllia europaea*, Goffredo et al., 2007, and *C. smithii*, Bell and Turner, 2000), as it can facilitate sediment removal compared to circular oral discs (Foster et al., 1988). The width:length relationship has also been studied for other solitary corals. However, also several solitary species living on walls or under overhangs (e.g., with the oral disc facing downwards) such as *L. pruvoti* (Goffredo et al., 2007) and *C. inornata* (Caroselli et al., 2015) show an allometric growth, leading to a more circular oral disc even if they are less subject to sedimentation, because sediment removal is mainly carried out by gravity (Goffredo et al., 2007). Other factors such as a better zooplankton capture ability may thus be linked to the shape of the oral disc (Caroselli et al., 2015).

Conclusions

The population of *Paracyathus pulchellus* from Palinuro shallows the bathymetric range of this species up to 5 m depth. It was gonochoric with a sex-ratio 1:1. No embryo was found in the coelenteric cavity of the polyps, suggesting an external development of the larvae and a possible broadcast spawning reproductive mode. The study of the gametogenesis morphology

showed a spermary development similar to other scleractinians (even those not belonging to the same family) and mature small-sized oocytes, such as those found in other caryophyllids living in the same habitat conditions, suggesting a possible planktotrophic larval development. Gametogenesis follows an annual cycle and appears to be influenced by temperature and photoperiod variations. Finally, the study of biometry showed a correlation between the main biometric parameters and polyp length and an allometric growth that leads to a more oval-shaped calyx. This is the first investigation on gametogenesis of *P. pulchellus* whose data could be useful for future studies assessing the impact of global climate change on reproduction, and thus the survival of these endangered organisms.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

GF, ZD, and SG conceived the research; CM, EC, and SG performed the fieldwork; CM, VF, MC, and EC performed the analyses; CM, VF, MC, and EC wrote the manuscript; GF and ZD gave conceptual advice; SG contributed to the reagents, materials, and analysis tools. All authors participated to the scientific discussion and gave final approval for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2022.925586/full#supplementary-material>

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