



Some Observations on the Reproductive Biology of the Mediterranean Pennatulacean *Pteroeides spinosum* (Ellis and Solander, 1786) (Cnidaria: Octocorallia: Pennatulacea)

Francisco J. García-Cárdenas¹ · Pablo J. López-González¹

Received: 19 November 2021 / Revised: 27 October 2022 / Accepted: 5 November 2022 / Published online: 27 December 2022
© The Author(s) 2022, corrected publication 2023

Abstract

Sea pens are some of the most important structural species in soft bottom benthic communities. *Pteroeides spinosum* is a shallow-water pennatulacean (Cnidaria: Octocorallia: Pennatulacea) habiting NE Atlantic-Mediterranean bottoms. In this study, sixteen colonies of *Pteroeides spinosum* collected from NW Mediterranean Sea (Cap de Creus, Spain) at 99–106 m depth in July (2012), were used to acquire information of their reproductive biology, and to enable a comparison with other pennatulaceans. Our colonies of *Pteroeides spinosum* are gonochoric showing a bimodal oocyte frequency distribution and an unimodal spermatocyst diameter distribution. All polyp leaves contained oocytes and spermatocysts in different size classes, with those small and translucent spermatocysts/oocytes (size-class I) often found near the base or lower end of the gastrovascular cavities of polyps. The largest observed diameter was 668 μm for spermatocysts and 687 μm for oocytes. Mean potential relative fecundity (PRF) ranged between 7–17 oocytes per polyp. The effective reproductive effort (ERE) reached a value of ca. 30,000 large oocytes per colony. As was observed for all pennatulaceans previously studied, no sign of hermaphroditism or internal fertilization was detected in our colonies of *Pteroeides spinosum*.

Keywords Reproductive effort · Fecundity · Reproduction · Sea pen · *Pteroeides griseum*

Introduction

Despite all efforts to improve our knowledge of octocoral biology (see Orejas et al. 2002; Page and Lasker 2012; Coelho and Lasker 2014; among others) many groups remain poorly studied from a reproductive point of view. Information on the reproductive biology of the species is essential to understanding population dynamics and potential responses to disturbances (Servetto et al. 2013).

This lack of knowledge limits our current ability to assess the survival potential of species, as well as other biological processes, such as the resilience of the population after a reduction in the number of individuals due to natural or

anthropogenic disturbances. Determining the reproductive features of a species (such as reproductive effort, reproductive cycle, fecundity, sex ratio, spawning events, etc.) not only improves our knowledge of the particular species, but also provide us with information that constitutes a useful tool for designing conservation plans (Connor et al. 2003; Davies et al. 2017), as well as to infer the vulnerability of that species, which in turn will indirectly affect a set of associated species that coexist with it (Roberts et al. 2006).

In this sense, octocorals form essential habitats that host an important reserve of associated fauna (Baillon et al. 2012; Clippele et al. 2015). Banks or gardens of these bioconstructor organisms are often referred to as biodiversity hot spots, similar to tropical coral reefs (Buhl-Mortensen et al. 2010) with a high vulnerability to natural or anthropogenic disturbances (Roberts 2002; Carney 2005).

Among the octocorals, sea pens (Octocorallia: Pennatulacea) seem to be the most specialized group, with significant morphological adaptations, such as a muscular peduncle that serves as an anchoring system on muddy and sandy bottoms (Kükenthal 1915; Williams 2011), where they frequently act as bioconstructors of a benthic

✉ Francisco J. García-Cárdenas
frangarca@us.es

Pablo J. López-González
pjlopez@us.es

¹ Biodiversidad y Ecología Acuática, Departamento de Zoología, Facultad de Biología, Universidad de Sevilla, Reina Mercedes 6, 41012 Seville, Spain

tree-dimensional landscape on which a rich fauna is housed (Baillon et al. 2012; Chimienti et al. 2018). This important ecological role has been internationally recognized by including sea pen beds and their associated megafauna in the OSPAR list of threatened and/or declining species and habitats (Jones et al. 2000; Curd 2010).

Our knowledge about the reproductive characteristics of sea pens has increased over the last 20 years (Eckelbarger et al. 1998; Tremblay et al. 2004; Lopes et al. 2012; Servetto and Sahade 2016; Hamel et al. 2020; Couillard et al. 2021; among others). Thanks to these previous studies we know that pennatulaceans are exclusively gonochoric and broadcast spawning organisms, and to date, no solid evidence of internal larval development or parthenogenesis events have been found (e.g. Baillon et al. 2014; Hamel et al. 2020; Couillard et al. 2021).

Sea pen species with a high fecundity have been described, producing up to 200,000 oocytes per colony in one season (Chia and Crawford 1973). Large oocytes up to 1,200 μm in diameter have been found (Pires et al. 2009), which after the spawning event and external fertilization process, produce lecithotrophic planula larvae that actively swim before settling and metamorphosing when suitable substrate is detected (Chia and Crawford 1973).

Various reproductive temporal patterns have been reported in sea pens. On the one hand, shallow-water pennatulaceans seem mainly to follow an annual cycle in which oocyte development takes at least 12 months to complete and for the oocytes to be released into the water column, in a one single spawn event (Tremblay et al. 2004; Edwards and Moore 2008). As occurred frequently in octocorals (see Simpson 2009), this prolonged cycle of oocyte development commonly involves the presence of a bimodal (or trimodal, see Edwards and Moore 2008, 2009; Couillard et al. 2021) frequency distribution of oocyte sizes, with a constant pool of immature oocytes throughout the year, and another category involving a smaller quantity of larger-sized oocytes developing over time to the maximum sizes at which they will be emitted (Edwards and Moore 2009; Servetto and Sahade 2016). On the other hand, some species have been reported to exhibit a “continuous” or “non-seasonal” reproductive cycle (Rice et al. 1992; Eckelbarger et al. 1998; Pires et al. 2009).

In short, despite these efforts, most of the sea pens species (around 92%) remain unexplored from a reproductive point of view (Baillon et al. 2014; Servetto and Sahade 2016), some of the reproductive traits analyzed vary among pennatulacean species (Baillon et al. 2015) (e.g. continuous or seasonal cycle) and there is a complex methodology (e.g. fecundity indices) difficult to apply to the whole set of diverse morphologies known in sea pens (see Williams 1995). In this early stage of knowledge, any new reproductive information will expand our comprehension about these sometimes inaccessible organisms.

The genus *Pteroeides* Herklots, 1858 presents a wide distribution, from the Eastern Atlantic and Mediterranean Sea to the West Indo-Pacific, and it is known to inhabit a bathymetric range from 15 to 400 m in depth (Williams 1995, 2011; García-Cárdenas and López-González 2022). Colonies of *Pteroeides* are mostly stout and feather-like, with bilateral symmetry in the rachis throughout, the axis is present throughout the colony and polyp leaves are well-developed and rigid due to the presence of one to many supporting rays composed of long needle-like sclerites (Williams 1995).

Pteroeides spinosum (Ellis and Solander, 1786) has been mainly reported from the Mediterranean Sea (Herklots 1858; Gray 1870; Kükenthal 1915; Pax and Müller 1953, 1955, 1959; Rossi 1971; Gili and Pagès 1987; Abdelsalam 2014; Porporato et al. 2014; Topçu and Öztürk 2015), but also in the Northeastern Atlantic Ocean (Madeira island, Ocaña and Wirtz 2007; Tangier bay, Marshall and Fowler 1887), in a bathymetric range from 20 to 295 m in depth.

Moreover, there are no published studies on its reproductive characteristics, perhaps due to the difficult access to these colonies. Only a cool-water, indeterminate population of *Pteroeides* studied by Duncan (1998, unpub. MSc thesis) have been recorded in the literature. In the present contribution, we provide, at the first time, some observations on the reproductive stage of a NW Mediterranean Sea population of the type species *Pt. spinosum*.

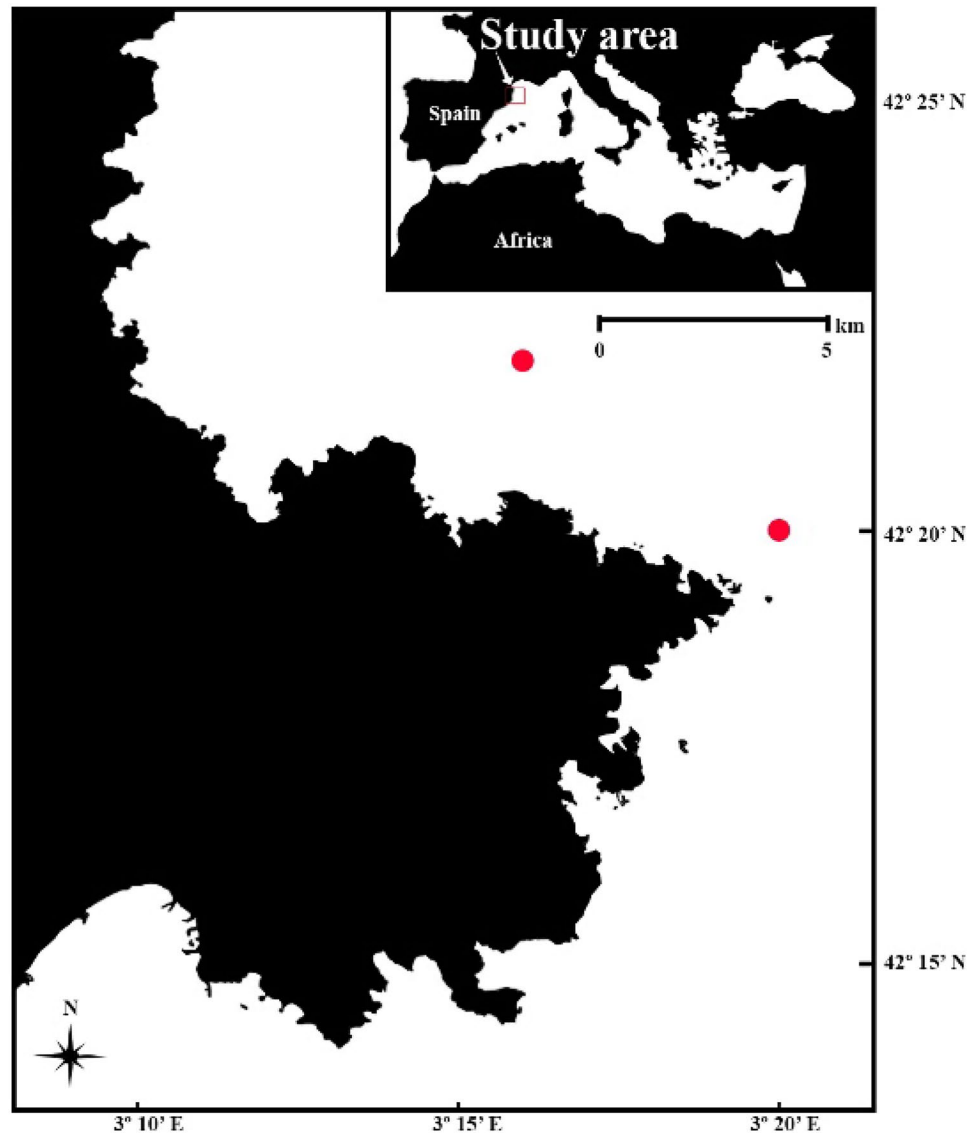
Materials and Methods

Sampling and Sex Determination

The colonies of *Pteroeides spinosum* here studied were collected from NW Mediterranean Sea (Cap de Creus, Spain), using a Rauschert dredge (RD) during the oceanographic campaign INDEMARES VI, carried out between June and July 2012 (Fig. 1). The material analysed in this study was collected at the stations 131 (42° 22,199'N 3° 16,887'E) and 132 (42° 20,011'N 3° 20,085'E), between 99 and 106 m depth, on July 8, 2012. A total of sixteen colonies were collected. The specimens were sorted and labelled on board. The colonies were fixed in hexamethylenetetramine-buffered 4% formalin-seawater. After the fixation period (a week), all colonies were preserved in 70% ethanol. Sixteen colonies were examined and total colony length was measured using ImageJ 1.38 \times software. Colony sizes ranged between 73 to 138.5 mm in total length, and were a priori grouped into two size classes (< 100 mm, and > 100 mm of total length) in order to check possible morphological and reproductive differences.

The terminology used in colony anatomy mainly follows Bayer et al. (1983). The studied material is deposited in the Zoological Museum of Barcelona (Spain) and in the collection of the research group Biodiversidad y Ecología Acuática at the University of Seville (BECA).

Fig. 1 Study area during the oceanographic campaign INDEMARES VI. Red dots indicate stations 131 ($42^{\circ} 22,199'N$; $3^{\circ} 16,887'E$) and 132 ($42^{\circ} 20,011'N$; $3^{\circ} 20,085'E$) (99–106 m depth)



The polyps were dissected and their sexual products extracted for examination under a Motic SMZ-140 stereo microscope. Feret diameter (fD) measures (Flint et al. 2007) of oocytes and spermatocysts were obtained using a calibrated eyepiece. Initial sex determination was done on the observation of gametes (from one or two fragments of different polyp leaves) in lactic acid, and corroborated along the counting phase of the study. Oocytes and spermatocysts were readily distinguishable: oocytes had a relatively large nucleus with nucleolus, and a bright yellow coloration; spermatocysts had a granulate aspect with multiple sperm heads and a translucent appearance. The deviation of a 1:1 sex ratio was assessed using a chi-squared test (Zar 1984).

Intracolony Variation

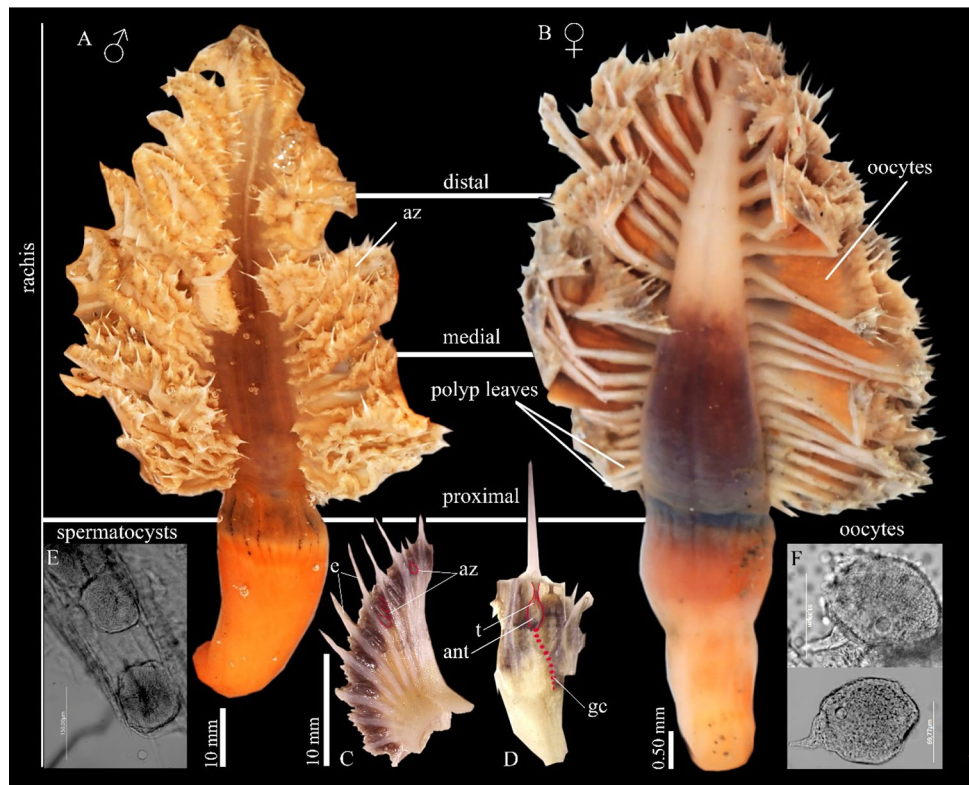
The total length of rachis of male and female colonies was divided into three zones of similar length, namely proximal,

medial and distal (Fig. 2), to evaluate possible differences in reproductive traits among zones, following previous studies on reproduction in octocorals (see Orejas et al. 2002; Soong 2005; Edwards and Moore 2008; among others).

On the one hand, intracolony variation in macro-morphological traits, such as polyp leaf lengths and number of autozooids per leaf, were analysed. On the other hand, the mean value of the oocytes and spermatocysts number and size (fD) from two polyp leaves in each zone was registered.

In summary, the effects and interactions of the factors “size” and “zone” were evaluated in the following variables: (1) mean polyp leaf lengths; (2) mean polyps per leaf; (3) mean gametes per leaf; and (4) mean gametes per polyp, also called potential relative fecundity (PRF, Baillon et al. 2014) [note that “gametes” includes here oocytes and spermatocysts]. Possible variations within and among colonies

Fig. 2 Details of a male (A) and female (B) colony of *Pteroeides spinosum* showing the considered zones in the rachis; C details of a polyp leaf showing the arrangement of autozooids (az) and the prominent groups of spicules (rays) (e); D a fragment of a polyp leaf showing the retracted anthocodia of autozooids (ant), and gastrovascular cavities (gc); E–F, MO images



were tested using homogeneity of variance (Levene) and ANOVA tests.

Gamete Sizes

Different spermatozoa/ oocyte sizes were classified into two size categories, according to the diameter gamete distribution and following previous studies in order to ensure further comparisons (e.g. Mladenov and Brady 1987; McClary and Mladenov 1989; Duncan 1998; Servetto and Sahade 2016): size-class I or small gametes (<300 μm) and size-class II or large gametes (> 300 μm) (see Discussion). Size-class I is a priori considered immature stage; and size-class II is considered close to gamete mature stages, which would be released at the spawning event. The mean number and percentage of spermatozoa/ oocytes in each size class was obtained at zone and at colony level. To determine the maturity stage of a given colony, we identified the leading cohort of gametes (LCO, leading cohort of oocytes; LCS, leading cohort of spermatozoa), using the ten largest oocytes/ spermatozoa in each one, following previous studies (Couillard et al. 2021).

Gamete-diameter Frequency Distribution

Data of spermatozoa and oocyte measurements were used to construct diameter-frequency histograms, taking into account the factors “size” and “zone” of colony per sex. A cluster analysis for K-means to clearly determine the “hidden” groups in

the point cloud was driven (Laverick et al. 2017; Nurdin et al. 2019). A robust one-way Analysis of Variance (ANOVA) to verify statistically differences among groups was applied.

Fecundity

Potential relative fecundity (PRF) defined as the total number of oocytes/ spermatozoa per polyp was estimated irrespective of their size (or maturity stage) by dividing the total number of oocytes/ spermatozoa per polyp leaf by the number polyps on that polyp leaf (Baillon et al. 2014, 2015). Here, this PRF was obtained using the two gamete size classes. As the reproductive cycle of *Pteroeides spinosum* is unknown, this value must be considered with caution to further validation (see Discussion).

$$\text{PRE} = \sum [\text{Mean gametes per polyp leaf in a given zone} \\ \times \text{N polyp leaves in that given zone}] \text{ of the three zones}$$

$$\text{ERE} = \sum [\text{Mean large gametes per polyp leaf in a given zone} \\ \times \text{N polyp leaves in that given zone}] \text{ of the three zones}$$

Statistical Analysis

Colony zones, polyp leaves and spermatozoa/ oocyte dimensions were measured using ImageJ 1.38x software. Normality

was tested using Shapiro–Wilk test, and homogeneity of variances was examined using Levene's test (Servetto and Sahade 2016). When an assumption of normality was not confirmed (e.g. the bimodal distribution of oocytes) and variances were not homogeneous, a cluster analysis for K-means was driven followed by a robust one-way Analysis of Variance (ANOVA, Welch and Brown-Forsythe tests), and then multiple range tests (such as HSD Tukey) were used post hoc (Servetto and Sahade 2016). Statistical data analyses were performed using IBM SPSS Statistics v22 (SPSS INC., Chicago, USA).

Nomenclatural Remark

There is still some confusion about what the appropriate binomen and authorship should be considered currently valid for the species here studied. Many publications used *Pteroeides griseum* (Bohadsch 1761) as the accepted name, while others cited it as *Pteroeides spinosum* (Ellis, 1764). As noted by Williams (1995: 130), the binomen *Penna grisea* Bohadsch, 1761 is not admissible since Bohadsch (1761) was suppressed by ICZN (1944). However, there is also some confusion regarding to who and when the specific epithet *spinosa* appeared in the literature, because Ellis (1764) did not mention the name *spinosa* in his paper. The name *spinosa* as specific epithet for this species appeared for the first time in Ellis and Solander (1786) (see Suppl. Mat. Doc.1 for a detailed review of the nomenclatural vicissitudes on the binomen and taxonomic authorities for this species). In summary, the valid name for the species here studied is considered *Pteroeides spinosum* (Ellis and Solander 1786).

Results

All our *Pteroeides spinosum* colonies were in reproductive stage. In the live state, females with oocytes in advanced state of maturation are clearly differentiated by pale apricot to bright orange colour as their oocytes are visible through the lateral sides of the polyp leaves by transparency (see Fig. 2B), while in male colonies lateral sides of the polyp leaves are cream to yellow (not shown). A part of this gametogenic structure of oocytes and spermatocysts is the typical already observed in other octocoral species (Fig. 2E, F). Specimens examined had no significant deviation from a sex ratio of 1:1 (comprising 7 male and 9 female colonies) ($p = 0.617$; $\chi^2 = 0.25$; $df = 1$).

Differences Among Colony Size Classes

Although two size classes in each sex were differentiated [small and large males (86–99 mm, 106–107 mm, $p < 0.05$; $\chi^2 = 10.32$; $df = 1$); small and large females (73–91 mm, 101–138 mm

$p < 0.05$; $\chi^2 = 19.87$; $df = 1$) (Table S1)], some features analyzed did not appear to differ between both size classes, such as the number of polyp leaves per colony (small: 34–47 polyp leaves; large: 36–60 polyp leaves, Table S1), the mean polyp leaf lengths or the mean polyps per polyp leaf. Only the mean oocytes per polyp leaf (Table S2) showed significant differences between certain small and large colonies ($p < 0.05$; $\chi^2 = 505.74$; $df = 1$). The highest value of spermatocysts/ oocytes per polyp leaf was reached in larger colonies (1,805 spermatocysts per leaf and 3,829 oocytes per leaf) (Table S2).

Statistical analyses supported the idea that two dependent variables (mean polyp leaf length, mean polyps per leaf) showed no significant variation between colony sizes. However, the other variables (mean oocytes per polyp leaf and mean oocytes per polyp) were useful to differentiate among some of the small and large colonies ($p = 0.018$, $df = 8$, Brown-Forsythe; $p = 0.001$, $df = 8$, Brown-Forsythe). These colonies with significant differences were the smaller females ($\text{♀}1$, $\text{♀}4$, and $\text{♀}5$) with the lowest values of oocytes per polyp leaf and per polyp, and two larger females ($\text{♀}7$ and $\text{♀}8$) with the highest mean oocytes per polyp ($p = 0.001$, $df = 8$, Brown-Forsythe and post hoc HSD Tukey test).

Intracolony Variation

Contrary to the variation among size classes, the variation within the colony or comparisons between zones, showed differences strongly supported by the majority of studied features, regardless of the size and sex of the colonies.

The medial zone of the rachis presented the lowest mean number of polyp leaves ($\text{♂} 13 \pm 2$, 9 ± 2 , 16 ± 3 ; $\text{♀} 13 \pm 3$, 12 ± 3 and 16 ± 3 polyp leaves for proximal, medial and distal zones, respectively), the largest polyp leaf lengths ($\text{♂} p = 0.043$; $\chi^2 = 4.06$; $df = 1$; $\text{♀} p = 0.001$; $\chi^2 = 9.98$; $df = 1$), the highest mean polyps per leaf and the highest mean oocytes per leaf ($p < 0.05$; $\chi^2 = 853.43$; $df = 1$) (Table S2). In summary, the mean number of oocytes (and spermatocysts) per polyp leaf was higher in the medial zone and decreased towards the proximal and distal ends of the rachis.

In general, the lowest means were found in polyp leaves from the proximal zone of rachis, although with little variation with respect to the distal zone, the medial zone having the highest means of oocytes per polyp leaf and PRF values (Table S2).

Gamete Sizes

All the polyp leaves observed contained oocytes and spermatocysts in different sizes probably as result of different stages of maturation (as size-classes in this study) (Fig. 2). Those more opaque and large spermatocysts/ oocytes (size-class II) were frequently found at the base and middle part of

the mesenterial filaments of polyps, near the mouth. While those small and translucent spermatocysts/ oocytes (size-class I) were most often found near the lower end of the gastrovascular cavities of polyps (anchored to the lower end of filaments), embedded in the axillary area of the polyp leaves (Fig. 2).

In male colonies, a total of 27,553 spermatocysts were counted (15,499 and 12,054 cysts in small and large colonies, respectively), with a size range between 37.50 and 668.20 μm (Table 1). Regardless of colony size, the highest number of spermatocysts was in size-class I (19,954 cysts) and a few in size-class II (7,599 cysts). The medial-distal zones presented the highest percentage of spermatocysts in size-class II, while the proximal zone showed the highest percentage of spermatocysts in size-class I, the lowest percentage in size-class II, and no spermatocysts were found $> 500 \mu\text{m}$ (Tables 1 and S3).

In female colonies, a total of 57,850 oocytes were counted (21,719 and 36,131 oocytes in small and large colonies, respectively), with a size range between 25 and 687.50 μm (Table 1). The largest number of oocytes was in size-class I (37,875 oocytes), followed by size-class II (19,975 oocytes). As with spermatocysts, the proximal zone had the highest percentage of oocytes in size-class I, and the medial-distal zones had the highest percentage of oocytes in size-class II. In this case a low percentage of large oocytes was found in the proximal zone (Tables 1 and S3).

Diameter Frequency Distribution

Spermatocyst and oocyte diameter-frequency distributions of all *Pteroeides spinosum* colonies are shown combined in Fig. 3 and individually in Figs. 4 and 5. Spermatocyst distributions (Fig. 3, upper row) revealed a unique large cohort of small cysts, ranged between 50 and 300 μm (mainly formed by spermatocysts in size-class I). This distribution was similar among the three zones within the colony (i.e. there was synchrony), but the highest values were reached in the medial zone. This spermatocyst distribution was observed in the seven male individual colonies (Fig. 4).

On the other hand, oocytes showed a well-defined bimodal diameter-frequency distribution (Fig. 3, bottom row), with a more numerous younger oocyte cohort ($< 250 \mu\text{m}$, in size-class I), and another large oocyte cohort ($> 250 \mu\text{m}$, in size class II) that included the highest diameter but with the lowest frequencies. This bimodal distribution is maintained in all zones, showing a clear within-colony synchronization.

In female colonies, the cluster analysis for K-means statistically supported these two cohorts ($K = 2$; K_1 mean of small oocytes with a mean of 117.65 μm and 38,551 units; and K_2 mean of large oocytes with a mean of 454.37 μm and 18,751 units) in the three zones ($p < 0.00$; Welch and

Brown-Forsythe). It can be seen that *Pt. spinosum* females presented two overlapping oocytes cohorts with synchronized harmony within the colony. The second cohort would include oocytes at advanced stages of maturity. Individually analysed female colonies clearly showed this bimodal distribution, with no differences between size-class colonies (Fig. 5). The maturity stage of female colonies of *Pt. spinosum* based on the mean diameter of the largest oocytes (i.e., leading cohort), was similar between the size classes, and ranged between 400–700 μm regardless the total number of oocytes examined (Table 1 and Fig. 6). The relationship between the mean number of spermatocysts and oocytes and the colony height was positive and more pronounced in male than female colonies (R^2 0.6599 and R^2 0.3421, respectively); similarly, the relationship between leading cohort oocyte size (LCO) and the total colony length was also positive but less pronounced (R^2 0.1033) (Fig. 7).

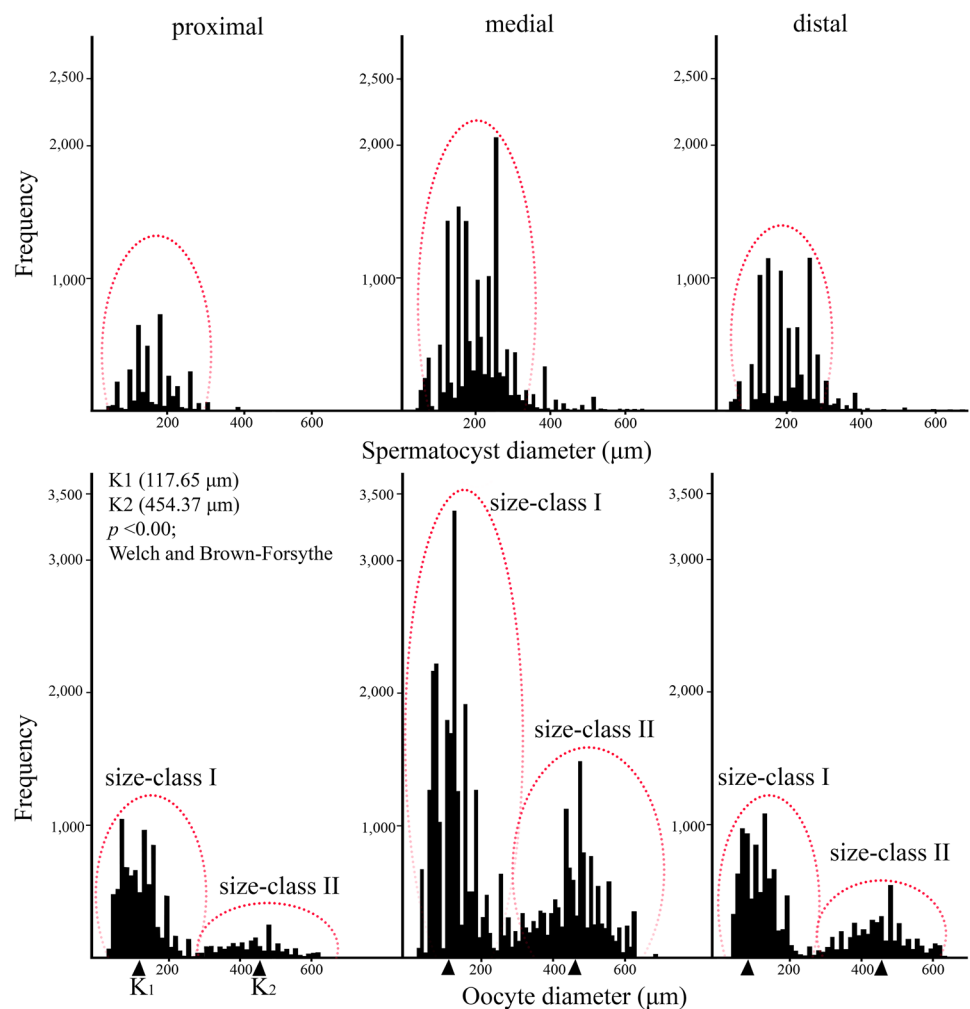
Mean potential relative fecundity (PRF) was estimated to be 7 oocytes per polyp at the proximal and distal zones, and 10 oocytes per polyp at the medial zone in the smaller females; while in larger females, 12–13 oocytes per polyp were found at the proximal and distal zones, and 17 oocytes per polyp at the medial (Table S2). Although the higher values were presented at the medial zone of the rachis, they did not show significant differences with the other zones ($p > 0.05$, HSD Tukey). The relationship between PRF and the total colony length using individual colonies, although positive, did not show a clear lineal increase (Fig. 8).

The potential reproductive effort (PRE) of our colonies increased from small to large colonies, and from lower to upper zones (medial and distal) of the rachis in all the colonies examined (Table 2). In the smaller males, PRE was $19,502 \pm 1,228$ spermatocysts per colony (ranging from 6,611–32,364); and in larger males PRE was $35,646 \pm 1,530$ spermatocysts per colony (ranging from 24,308–46,645) (Table 2). Measures of PRE taken within colony, showed the lowest mean values at the proximal zone (1,572 and 10,294 spermatocysts per zone for small and large males, respectively).

In the smaller females, PRE was $27,379 \pm 2,737$ oocytes per colony (ranging from 8,402–53,140), and in the larger females PRE was $73,114 \pm 7,090$ oocytes per colony (ranging from 26,445–110,283) (Table 2). Within the colony, in both colonial sizes, the highest mean PRE values were at the medial zone (16,621 and 42,802 oocytes per zone, respectively) ($p < 0.05$; $\chi^2 = 93,683.8$; $df = 1$), the lowest PRE values always being found at the proximal zone of the rachis (Tables 2 and S4).

The effective reproductive effort (ERE) increased from the smaller to the larger colonies, and was strongly lower at the proximal zone of the rachis in all colonies examined.

Fig. 3 Diameter frequency distributions of spermatocysts and oocytes in three colony zones of all *Pteroeides spinosum* colonies combined: K_1 , mean diameter of small oocytes (size-class I); K_2 mean diameter of large oocytes (size-class II). The statistical analyses shown correspond to the three zones of female colonies combined



In males, ERE was $5,008 \pm 801$ and $9,946 \pm 1,448$ large spermatocysts per colony for small and large colonies, respectively. Measures of ERE taken within the colony again showed the lowest values at the proximal zone (proximal < medial \approx distal) (Table 2). In females, ERE was $6,828 \pm 1,417$ and $29,876 \pm 6,871$ large oocytes per colony for small and large colonies, respectively. Within the colony, in both colonial sizes the medial zone had significantly the highest ERE values (4,973 and 19,142 oocytes per zone, respectively) ($p < 0.05$; $\chi^2 = 24,031$; $df = 1$) (Tables 2 and S4).

In short, one of our larger *Pt. spinosum* females might contain up to 110,000 oocytes, but maybe only 27% (~30,000) reach a mature stage.

Discussion

Despite an annual sampling programme was not feasible from a logistic point of view for this study, especially difficult when studying deep sea benthic species (as mesophotic

depths), relevant information from a single or limited number of samples of sea pen populations can contribute to improving our current knowledge about the reproductive biology of these species (see Chia and Crawford 1973; Eckelbarger et al. 1998).

Many of the reproductive features observed in our colonies of *Pteroeides spinosum* are shared with the population of *Pteroeides* sp. studied by Duncan (1998) from Fiorland (New Zealand), as well other Mediterranean pennatulaceans (as *Veretillum cynomorium*, see Lopes et al. 2012) and alcyonaceans such as *Eunicella singularis*, *Paramuricea clavata*, *Alcyonium acaule* or *Alcyonium coralloides* (see Theodor 1967; Weinberg and Weinberg 1979; Coma et al. 1995a, b; Fiorillo et al. 2013; Quintanilla et al. 2013). All of them had spermatocysts and oocytes at different sizes corresponding to different stages of maturity, as well as a distinguishable bi- or tri-modal oocyte size distribution and a high reproductive effort at colony level. In our colonies no sign of hermaphroditism or internal fertilization was observed, as is also assumed in the rest of pennatulaceans (e.g. Baillon et al. 2014, 2015).

Fig. 4 Diameter frequency distributions of spermatocysts of individual male colonies of *Pteroeides spinosum* used in this study

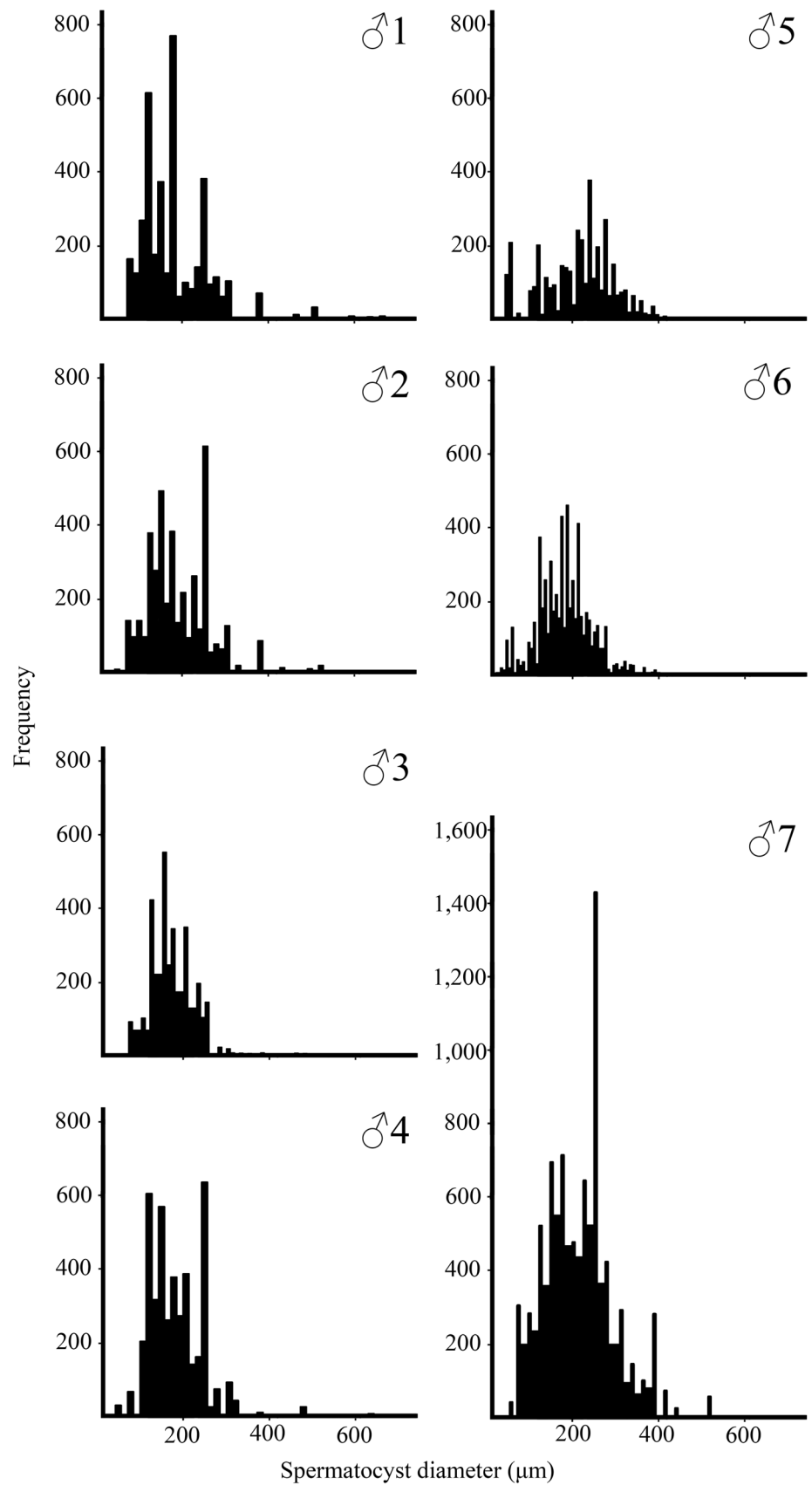
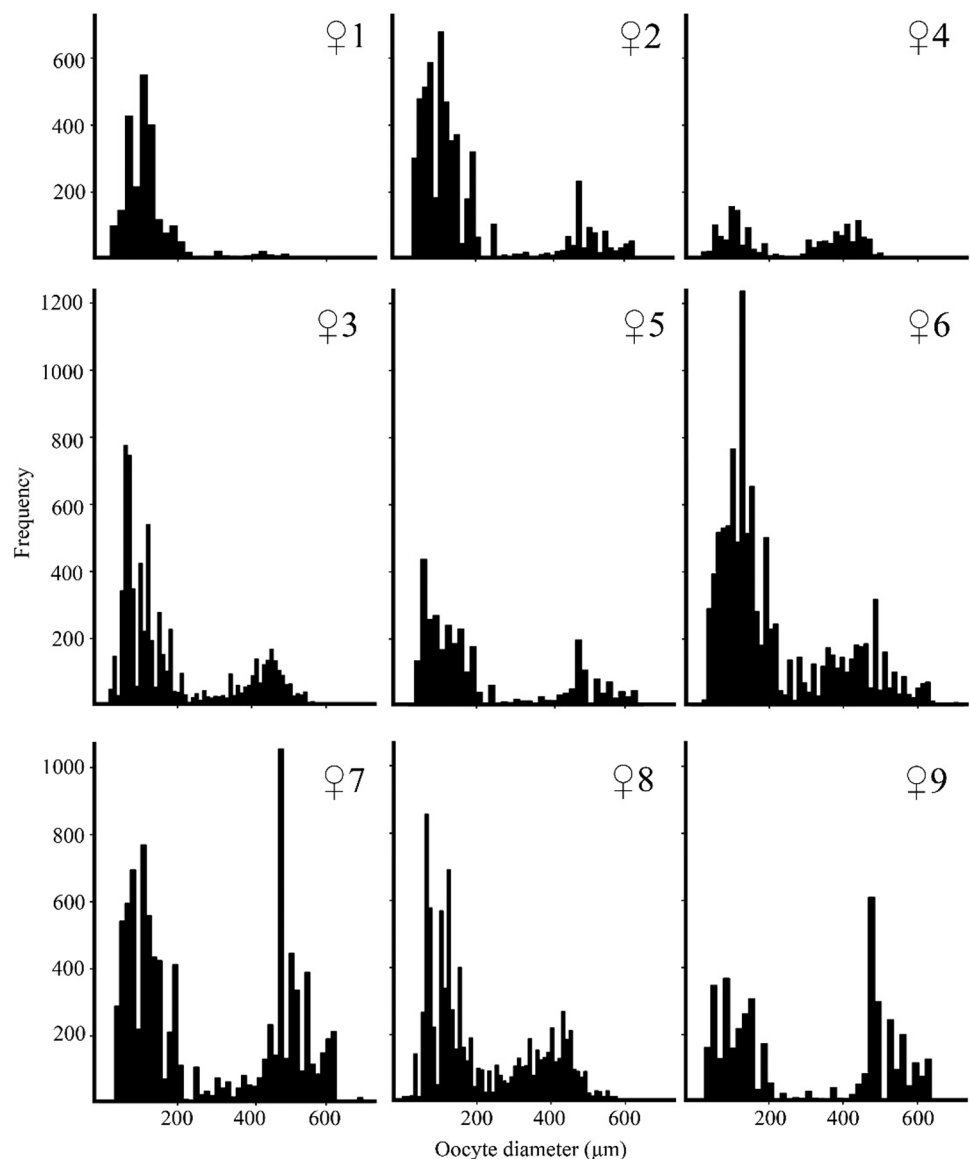


Fig. 5 Diameter frequency distributions of oocytes of individual female colonies of *Pteroeides spinosum* used in this study



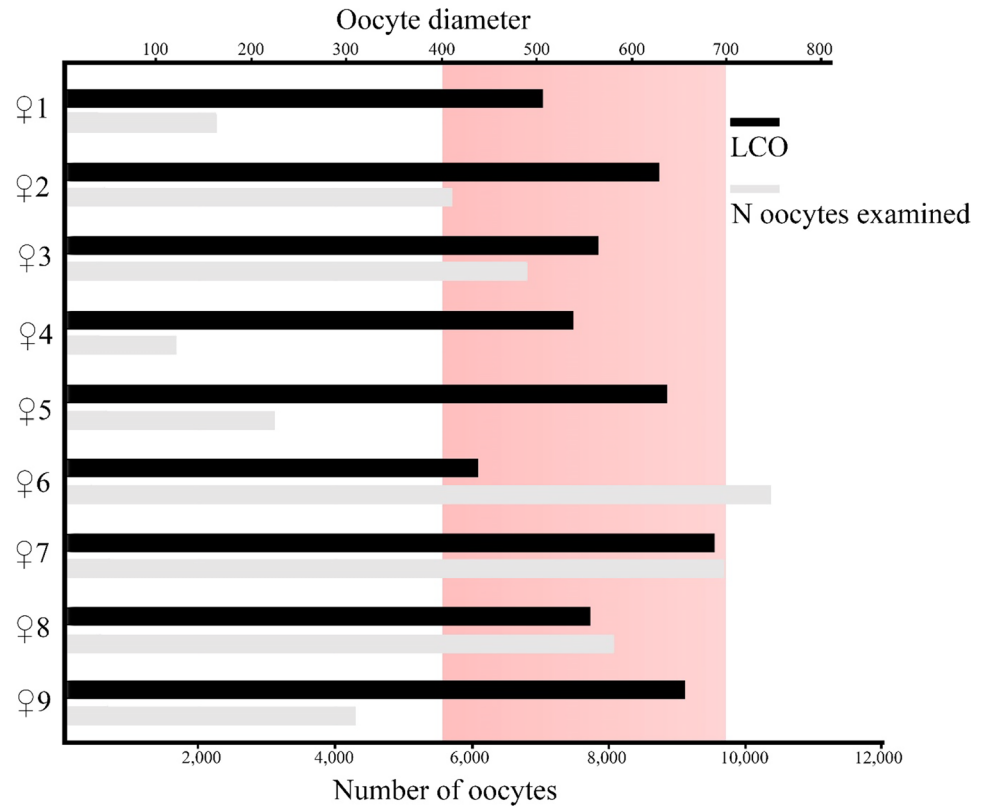
In population studies the importance of spatial distribution and the proportion of male and female colonies is well known (see Simpson 2009). Certain authors have reported that the highest fertilization percentage occurs in a population with both sexes (Benayahu and Loya 1983; Brazeau and Lasker 1992; Coffroth and Lasker 1998) and with a ratio close to 1:1 (Coma et al. 1995a, b; Pires et al. 2009). The colonies examined in the present study displayed gonochorism and equality of sex-ratio, in line with most pennatulacean species studied (Rice et al. 1992; Edwards and Moore 2008; Baillon et al. 2014, 2015; Hamel et al. 2020; Couillard et al. 2021).

Influence of Colony Size

Considering that further statistical analyses are needed (see Hamel et al. 2020; Couillard et al. 2021), and although a priori

different size classes were observed in our *Pteroeides spinosum* specimens collected, most features analyzed here did not support significant differences between the small and large size classes considered (Table S2), all colonies being in reproductive stage (Figs. 6 and 7). However, two features, mean oocytes per polyp leaf and mean oocytes per polyp (fecundity), enabled differentiation between some smaller colonies, with the lowest values. In this sense, the relationship between fecundity (PRF) and the colony height examined using individual colonies data (Fig. 8) was positive although lower pronounced than in other species (see Hamel et al. 2020 for *Umbellula encrinus*). This influence of colony size on the reproductive effort was also reported in other sea pens (see Baillon et al. 2015 for *Halipteris finmarchica*). Perhaps a considerable increase of *Pt. spinosum* colonies would offer a more accuracy relationship between PRF-colony height factors.

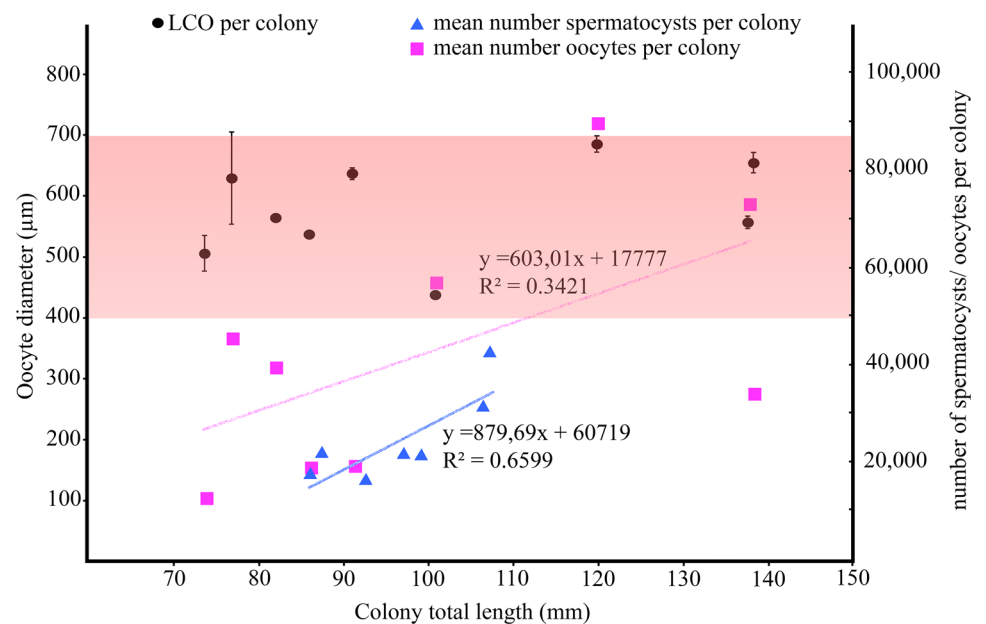
Fig. 6 Diameter of the leading cohort of large oocyte (LCO) of individual female colonies of *Pteroeides spinosum*. In red the LCO range observed in our colonies



In our colonies, it was not possible to determine the number of large oocytes per polyp (known as effective relative fecundity, ERF) because the elongated gastrovascular cavities of autozooids are embedded within large and fleshy polyp leaves (see also Duncan 1998). This implies, in this case, that fecundity can only be estimated at the polyp level as PRF (Soong 2005, see Discussion).

Although spawning time and whether there is a single or more than one spawning events are also unknown, we offer a speculative estimate at colony level using the potential reproductive effort (PRE hereafter) and the effective reproductive effort (ERE) within and among our colonies, in line of previous studies (see Baillon et al. 2014, 2015; Servetto and Sahade 2016). These data must be considered

Fig. 7 Relationship between the colony total length of individual colonies of *Pteroeides spinosum* (blue for males, pink for females) and the leading cohort of oocytes (LCO, black), the mean number of spermatocysts (blue) and oocytes (pink) per colony. In red the LCO range observed in our colonies



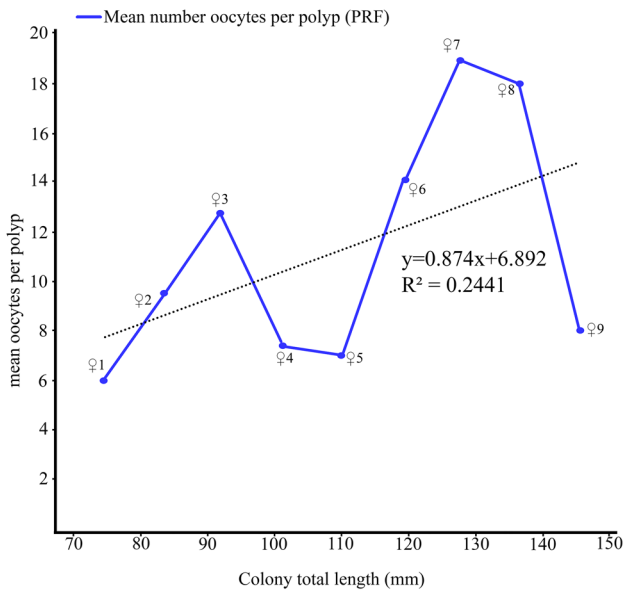


Fig. 8 Relationship between the potential reproductive fecundity (blue) and the colony total length (black) of individual female colonies of *Pteroeides spinosum*

with caution when compared with those of the other species presented in Table 3 with a known reproductive cycle. In general, those morphologies with polyp leaves present the highest values in reproductive efforts.

Oocyte Diameters

As do other octocorals, sea pens show, within the same autozooid, different oocyte (or spermatocyst) diameters that would correspond to different maturity stages (Chia and Crawford 1973; Servetto and Sahade 2016). However, previous studies on the reproductive cycle of pennatulaceans with polyp leaves, such as *Ptilosarcus*, *Pteroeides* (including our present study) and *Pennatula*, reported that it requires a huge and often unsuccessful effort to try to separate the autozooids individually to assess their sexual products (see the autozooid arrangement within polyp leaves, Fig. 2C, D) (Chia and Crawford 1973; Duncan 1998; Edwards and Moore 2008).

Levitan (1993, 1996) argued that large oocytes would be an easier target for sperm, increasing the probability of fecundation. This also appears to be favored by releasing sperm as cysts into the water column (Eckelbarger et al. 1998). Maximum oocyte diameter in sea pens ranged between 300 μm in *Virgularia juncea* and 1,200 μm in *Anthoptilum murrayi* (see Table 3). In our study, and assuming that the largest diameter of spermatocyst and oocyte of our colonies is the largest diameter at the sampling time, the largest oocyte diameter observed was 687 μm, slightly larger than those registered by Duncan (1998) for *Pteroeides* sp. (550 μm). As occurred in previous studies (e.g. Mladenov

Table 1 Spermatocyst and oocyte diameter and size classes in the examined colonies of *Pteroeides spinosum*

size	z(s)	number cysts	diameter (μm)				LCS ± SE	size categories			
			mean	± SE	max	min		size-class I	%	size-class II	%
small	proximal	1,228	167.54	69.06	487.62	37.50	438.61 ± 42.84	1,040	84.7	188	15.3
	medial	8,396	201.79	79.47	660.24	50.00	484.82 ± 79.87	5,892	70.2	2,504	29.9
	distal	5,875	193.71	74.79	668.20	48.41	577.18 ± 144.32	4,468	76.1	1,407	23.9
	total	15,499									
large	proximal	2,745	168.54	56.58	385.50	48.41	385.50 ± 10	2,448	89.2	297	10.8
	medial	6,597	216.39	80.70	514.00	43.30	481.47 ± 61.94	4,387	66.5	2,210	33.5
	distal	2,712	216.15	71.36	514.00	64.25	421.48 ± 25.37	1,719	63.4	993	36.6
	total	12,054									
Total		27,553						19,954		7,599	
size	z(s)	oocytes	mean	± SE	max	min	LCO ± SE	size-class I	%	size-class II	%
small	proximal	4,178	139.09	107.20	621.87	25.00	563.4 ± 37.16	3,729	89.3	449	10.7
	medial	13,638	208.51	163.69	687.50	25.00	598.87 ± 52.48	9,753	71.5	3,885	28.4
	distal	3,903	195.15	159.21	675.00	25.00	526.95 ± 70.53	2,870	73.5	1,033	26.4
	total	21,719									
large	proximal	5,980	199.36	154.21	687.50	25.00	636.87 ± 26.71	4,407	73.7	1,573	26.3
	medial	21,401	269.68	176.71	687.50	25.00	561.45 ± 117.57	11,830	55.3	9,571	44.7
	distal	8,750	245.46	175.95	687.50	25.00	636.23 ± 63.93	5,286	60.4	3,464	39.6
	total	36,131									
Total		57,850						37,875		19,975	

Z(S) zone nested in size, LCS mean (± SE) leading cohort of spermatocysts per zone, LCO mean (± SE) leading cohort of oocytes per zone (see also Table S3 for statistical results)

Table 2 Reproductive effort in the examined colonies of *Pteroeides spinosum*

Male Reproductive Effort							
size	z(s)	PRE				ERE	
		mean	± SE	max	min	mean	± SE
small	proximal	1,572	73.0	2,880	525	241	19
	medial	8,060	789.6	16,666	3,466	2,404	501
	distal	9,870	365.3	12,818	2,621	2,364	281
	Total	19,502	1,228	32,364	6,611	5,008	801
large	proximal	10,294	388.4	13,815	6,720	1,114	149
	medial	14,843	143.2	16,245	13,248	4,973	553
	distal	10,509	998.7	16,585	4,340	3,848	747
	Total	35,646	1,530	46,645	24,308	9,946	1,448
Female Reproductive Effort							
size	z(s)	PRE				ERE	
		mean	± SE	max	min	mean	± SE
small	proximal	4,249	262	9,234	456	512	45
	medial	16,621	1,696	26,317	6,451	4,973	1,166
	distal	6,509	779	17,589	1,495	1,343	207
	Total	27,379	2,737	53,140	8,402	6,828	1,417
large	proximal	9,531	1,468	19,227	4,883	2,507	787
	medial	42,802	4,540	61,264	10,656	19,142	4,786
	distal	20,781	1,082	29,792	10,906	8,227	1,299
	Total	73,114	7,090	110,283	26,445	29,876	6,871

PRE potential reproductive effort (number of spermatocysts/ oocytes per colony), ERE effective reproductive effort (number of large spermatocysts/ oocytes per colony; large spermatocysts and oocytes $\geq 250 \mu\text{m}$), Z(S) zone nested in size

and Brady 1987; McClary and Mladenov 1989), Duncan (1998) shown as different oocyte sizes found within polyps of *Pteroeides* sp. corresponded to different stages of development and were classified into five stages of maturity: stage I (post-spawning, a few remnant large mature oocytes, $< 250 \mu\text{m}$); stage II (early growth, $\sim 100 \mu\text{m}$); stage III (previtellogenic oocytes, $100\text{--}200 \mu\text{m}$; and vitellogenic oocytes, $> 200 \mu\text{m}$); stage IV-V (mature oocytes, 300 to $520 \mu\text{m}$; and ready to spawn, $> 300 \mu\text{m}$) (Duncan 1998: Fig. 5.2). Then, these oocyte stages of development can be summarized in immature or remnant stage ($< 250 \mu\text{m}$), and mature stage ($> 250 \mu\text{m}$) and they are consistent with our oocyte size classes (small oocytes $< 250 \mu\text{m}$, and large oocytes $> 250 \mu\text{m}$). However, to validate this assumption the complete cycle of reproduction in *Pt. spinosum* must be known. This relationship between the size of gametes and their stage of development has also been documented in other sea pens (Servetto and Sahade 2016; Couillard et al. 2021).

Considering that oocyte developmental times of *Pteroeides spinosum* have been still unknown, similar types of large oocyte diameter has been related to long developmental periods (Orejas et al. 2002). Large oocytes also appear to be

related to longer survival of larvae, providing a high nutritional content essential for lecithotrophic larvae (Chia and Crawford 1973). Although it has not been directly observed, oocyte sizes in *Pt. spinosum* are large enough to facilitate lecithotrophic development, as was suggested for *Ptilosarcus guernei* ($687 \mu\text{m}$) and *Kophobelemnion stelliferum* ($800 \mu\text{m}$) (Chia and Crawford 1973; Rice et al. 1992). Larvae were never observed in the gastrovascular cavities of polyps of our *Pt. spinosum* colonies. After determining the diameter of the leading cohort of oocytes (LCO) in all *Pt. spinosum* individual colonies (see Figs. 6 and 7), the size distribution of these LCO did not discriminate between colonies at different stages of maturity, all female colonies had LCO $> 400 \mu\text{m}$, thus not following the LCO:colony height relationship presented in *Pennatula aculeata* colonies (Couillard et al. 2021). Perhaps further captures that considerably increase the colonies to examine may offer additional information in this case.

Intracolony Variation

In octocorals, intracolony variation in reproductive features has been largely documented as a differential contribution

Table 3 Reproductive studies in Pennatulacea. Note that ERE value is corresponding to ECF in other studies (Baillon et al. 2014, 2015)

Species	Geography	Max length colony (mm)	Max. oocyte Diam (µm)	Sampling period	PRE	ERE	Study
<i>Ptilosarcus guernei</i>	NE Pacific (Seattle, USA)	500?	600	March 1972		200,000–210,000	Chia and Crawford (1973)
<i>Kophobelemnion stelliferum</i>	NE Atlantic (Porcupine Seabight)	250	800	Between 1977–1986			Rice et al. (1992)
<i>Umbellula lindahli</i>	NE Atlantic (Porcupine Seabight)	300	800	Between 1977–1986		2,000	Tyler et al. (1995)
<i>Pennatula aculeata</i>	NW Atlantic (USA)	235	880	August 1993			Eckelbarger et al. (1998)
<i>Pteroeides</i> sp.	SW Pacific (New Zealand)	190	550	Several months during 1997–1998		36,334	Duncan (1998)
<i>Renilla koellikeri</i>	NE Pacific (California, USA)		400	May-Aug during 2002–2003	1,400		Tremblay et al. (2004)
<i>Virgularia juncea</i>	NW Pacific (Taiwan)	800	300	Every 1–2 months between Sep.1990 and Nov. 1993	87,000		Soong (2005)
<i>Pennatula phosphaea</i>	NE Atlantic (Scotland, UK)	120	600	Over a 12-month period from Feb 2004 to Jan 2005	53,534	3,000–8,000	Edwards and Moore (2008)
<i>Funiculina quadrangularis</i>	NE Atlantic (Scotland, UK)	2,000	900	Over a 12-month period from Feb 2004 to Jan 2005			Edwards and Moore (2009)
<i>Anthoptilum murayi</i>	SW Atlantic (Brazil)	580	1,200	Feb, Jun, Jul, Aug (2000?)	25,713–35,918		Pires et al. (2009)
<i>Veretillum cynomorium</i>	NE Atlantic (Portugal)	< 400	967	Bimonthly, Apr 2010–Feb 2011			Lopes et al. (2012)
<i>Malacobelemnion daytoni</i>	Antarctica	110	350	Monthly, Jan 2009–Dec 2010		22–745	Servetto et al. (2013), Servetto and Sahade (2016)
<i>Anthoptilum grandiflorum</i>	NW Atlantic (Canada)	700	1,100	Several months during 2006–2007; Apr and May during 2009–2010		998–4,331	Baillon et al. (2014)
<i>Balticina finmarctica</i>	NW Atlantic (Canada)	148	1,000	Several months during 2006–2007		500–6,300	Baillon et al. (2015)
<i>Umbellula encrinus</i>	Arctic Ocean (Canada)	1,240	950	Aug–Nov 2006; Jun–Jul 2008; Oct 2010		6,460	Hamel et al. (2020)
<i>Pennatula aculeata</i>	NW Atlantic (Canada)	169	< 740	Jul 2014; May, Ago, Oct 2015			Couillard et al. (2021)
<i>Pteroeides spinosum</i>	NW Mediterranean Sea	138.5	687	July 2012	110,283	29,876	This study

PRE and ERE data must be considered with caution when species with different sampling periods are compared

PRE potential reproductive effort, ERE effective reproductive effort

by polyps according to their location within the colony (Brazeau and Lasker 1990; Soong and Lang 1992; Coma et al. 1995a, b; Brito et al. 1997; Orejas et al. 2002; among others).

Some studies in pennatulaceans determined a lineal increase in reproductive contribution of the polyps located from proximal to upper zones (medial and distal), the former commonly appearing empty of reproductive products (e.g. *Funiculina quadrangularis* in Edwards and Moore 2009; *Anthoptilum grandiflorum* in Baillon et al. 2014; *Halopteris finmarchica* in Baillon et al. 2015; *Malacobelemnion daytoni* in Servetto et al. 2013, Servetto and Sahade 2016; *Pennatula aculeata* in Couillard et al. 2021). However, the highest estimated mean fecundity in *Virgularia juncea* was found at the medial zone (Edwards and Moore 2009), as occurred with our colonies of *Pteroeides spinosum*, in which features such as polyp leaf lengths, mean polyps per leaf and mean oocytes per leaf, reached the highest values at the medial zone of rachis, decreasing towards to both extremes. Some *Pt. spinosum* male colonies showed slight differences between medial and distal zones, with a similar contribution. In this case, the lower number of spermatocysts per polyp leaf was compensated by a higher number and more congested polyp leaves at the distal portion of the rachis. On the one hand, the higher reproductive contribution in medial-distal zones may be related to the higher number and size of autozooids and polyp leaves in these zones, in comparison to the shorter polyp-leaves often bearing less developed autozooids, as previously suggested for other pennatulacean species. On the other hand, the access to nutritional resources falling from the upper water layers by those autozooids located in the medial-distal zone can provide more matter and energy than that obtained, in quality and amount of particles, by autozooids located in the lower polyp-leaves (Edwards and Moore 2008; Baillon et al. 2014).

Oocyte Diameter Frequency Distribution

It is commonly accepted in octocorals that a frequency-distribution in two clearly defined oocyte size groups represents the overlapping of two cohorts: the first cohort gathering immature oocytes, and kept practically throughout the year; the second cohort, gathering a less numerous oocyte pool at different degrees of maturity, formed in the previous year and that will be released in that season, often over one night (Orejas et al. 2007; Simpson 2009). As an alternative the possible occurrence of a trimodal size distribution of oocytes has been discussed for some pennatulacean species (see Edwards and Moore 2008; Couillard et al. 2021). In the reproductive cycle of *Pteroeides* sp. mature oocytes were only present from February to April (corresponding to summer and autumn), with very few remnant unspawned oocytes observed in May (late autumn) (Duncan 1998).

This means that between late summer and autumn nearly all vitellogenic oocytes became mature and were spawned. In parallel, Duncan observed a cohort of immature oocytes in all captures made over the two years. Although, as seen before, the developmental periods of oocytes in *Pteroeides spinosum* are still unknown, our results based on colonies collected in early summer and with a high percentage of large oocytes, follow the developmental line observed in *Pteroeides* sp. by Duncan (1998) (see below). The oocyte diameter frequency distribution for each individual colony not revealed the potential presence of a third modal ‘peak’ (as occurred for example in *Pennatula aculeata*, see Edwards and Moore 2008, Fig. 2) but rather the bimodal distribution was constant in all colonies examined, as occurred in *Pteroeides* sp. (Duncan 1998).

Fecundity

In most sessile invertebrate marine organisms, fecundity is often determined indirectly from counts of oocytes present, with the assumption that all or most become viable offspring (Brazeau and Lasker 1989; Benayahu and Loya 1983; Langton et al. 1990). In agreement with other authors (e.g. Duncan 1998), we consider here the term reproductive effort to be more appropriate, because it was not possible to assess the actual number of larvae produced. The mean potential relative fecundity (PRF) of our *Pteroeides spinosum* colonies (ranged between 7 and 17 oocytes per polyp) showed a similar range to other pennatulaceans, such as *Renilla koellikeri* (7–8 oocytes per polyp, Tremblay et al. 2004).

Quantifying different oocyte maturity stages within the colony offers more reliable information about the reproductive stage of the colony and its reproductive effort (Servetto and Sahade 2016). However, comparisons with previous reproductive studies must be made with caution because some data are based on potential reproductive effort (PRE) (e.g. Tremblay et al. 2004; Soong 2005; Pires et al. 2009) while others did not include immature oocytes in their fecundity estimates (ERE) (e.g. Chia and Crawford 1973; Tyler et al. 1995; Baillon et al. 2014; Hamel et al. 2020) (see Table 3). Moreover, some of these species have the complete reproductive cycle examined while others are based on scarce samples or punctual samplings (Chia and Crawford 1973; Eckelbarger et al. 1998). Our colonies of *Pteroeides spinosum* showed ERE values similar to the ERE of *Pteroeides* sp. (Duncan 1998). However, without a seasonal sampling program, inferences on colonial maturity stages and spawning events in *Pt. spinosum* must remain speculative. Future samplings of this population in different seasons might offer more information about the maturation cycle and verify spawning events with more accuracy.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s41208-022-00505-6>.

Acknowledgements The authors would like to thank the scientists and crew on board the R/V García del Cid during the "INDEMARES-CSIC VI", cruise to Cap de Creus, as well as many colleagues on board, without whose help it would not have been possible to collect the specimens for this work. Special thanks are addressed to Josep-Maria Gili for his continuous support to benthic research in the NW Mediterranean Sea. This research was funded by LIFE+ INDEMARES project (LIFE07/NAT/E/000732). The authors are also grateful for the criticisms and suggestions provided by three anonymous referees and the editorial board of Thalassas. Mr. Tony Krupa is thanked for reviewing the English version.

Funding Funding for open access publishing: Universidad de Sevilla/CBUA.

Data Availability All data generated or analysed during this study are included in this published article [and its supplementary information files].

Declarations

Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Abdelsalam KM (2014) Faunistic study of benthic Pennatulacea (Cnidaria, Octocorallia) from the Northern coast of Egypt. *Egypt J Aquat Res* 40(3):261–268
- Baillon S, Hamel JF, Wareham VE, Mercier A (2012) Deep cold-water corals as nurseries for fish larvae. *Front Ecol Environ* 10(7):351–356
- Baillon S, Hamel JF, Wareham VE, Mercier A (2014) Seasonality in reproduction of the deep-water pennatulacean coral *Anthoptilum grandiflorum*. *Mar Biol* 161(1):29–43
- Baillon S, Hamel JF, Mercier A (2015) Protracted oogenesis and annual reproductive periodicity in the deep-sea pennatulacean *Halipterus finmarchica* (Anthozoa, Octocorallia). *Mar Ecol* 36(4):1364–1378
- Bayer FM, Grasshoff M, Verseveldt J (1983) Illustrated trilingual glossary of morphological and anatomical terms applied to Octocorallia. *EJ Brill/ Dr. Backhuys, Leiden*, pp 75
- Benayahu Y, Loya Y (1983) Surface brooding in the Red Sea soft coral *Parerythropodium fulvum fulvum* (Forsk., 1775). *Biol Bull* 165(2):353–369
- Bohadsch JB (1761) De quibusdam animalibus marinis, eorumque proprietatibus, orbi litterario vel nondum vel minus notis, liber cum nonnullis tabulis aeri incisus, ab auctore super vivis animalibus delineatis. *Dresdae: apud G.C. Walther*
- Brazeau DA, Lasker HR (1989) The reproductive cycle and spawning in a Caribbean gorgonian. *Biol Bull* 176(1):1–7
- Brazeau DA, Lasker HR (1990) Sexual reproduction and external brooding by the Caribbean gorgonian *Briareum asbestinum*. *Mar Biol* 104(3):465–474
- Brazeau DA, Lasker HR (1992) Reproductive success in the Caribbean octocoral *Briareum asbestinum*. *Mar Biol* 114(1):157–163
- Brito TA, Tyler PA, Clarke A (1997) Reproductive biology of the Antarctic octocoral *Thouarella variabilis* Wright and Studer 1889. In: Hartog JC den (ed) *Proc 6th Int Conf on coelenterate biology*, Leiden, pp 63–69
- Buhl-Mortensen L, Vanreusel A, Gooday AJ, Levin LA, Priede IG et al (2010) Biological structures as a source of habitat heterogeneity and biodiversity on the deep ocean margins. *Mar Ecol* 31:21–50
- Carney RS (2005) In: Gibson RN, *Oceanography and marine biology: An annual review* 43:211–278
- Chia FS, Crawford BJ (1973) Some observations on gametogenesis, larval development and substratum selection of the sea pen *Ptilosarcus guernei*. *Mar Biol* 23:73–82
- Chimienti G, Angeletti L, Mastrototaro F (2018) Withdrawal behaviour of the red sea pen *Pennatula rubra* (Cnidaria: Pennatulacea). *Eur Zool J* 85(1):64–70
- Clippele LH, Buhl-Mortensen P, Buhl-Mortensen L (2015) Fauna associated with cold water gorgonians and sea pens. *Cont Shelf Res* 105:67–78
- Coelho MA, Lasker HR (2014) Reproductive biology of the Caribbean brooding octocoral *Antillologorgia hystrix*. *Invertebr Biol* 133(4):299–313
- Coffroth MA, Lasker HR (1998) Population structure of a clonal gorgonian coral: the interplay between clonal reproduction and disturbance. *Evolution* 52(2):379–393
- Coma R, Ribes M, Zabala M, Gili JM (1995a) Reproduction and cycle of gonadal development in the Mediterranean gorgonian *Paramuricea clavata*. *Mar Ecol Prog Ser* 117:173–183
- Coma R, Ribes M, Zabala M, Gili JM (1995b) Sexual reproductive effort in the Mediterranean gorgonian *Paramuricea clavata*. *Mar Ecol Prog Ser* 117(1):185–192
- Connor DW, Allen JH, Golding NEIL, Lieberknecht LM, Northen KO et al (2003) The national marine habitat classification for Britain and Ireland. *JNCC Peterborough*
- Couillard CM, Sainte-Marie B, Dionne H (2021) Late maturity and evidence for female biennial spawning in the sea pen *Pennatula aculeata* (Anthozoa, Pennatulacea) in eastern Canada. *Invertebr Biol* 140(4):e12351
- Curd A (2010) Background document for sea pen and burrowing megafauna communities. *Publication 481(2010):26*
- Davies JS, Guillaumont B, Tempera F, Vertino A, Beuck L et al (2017) A new classification scheme of European cold-water coral habitats: implications for ecosystem-based management of the deep sea. *Deep Sea Res Part II Top Stud Oceanogr* 145:102–109
- Duncan JC (1998) Biology of the sea pen *Pteroeides* sp. in Fiordland, New Zealand. PhD dissertation. University of Otago, New Zealand, pp 88
- Eckelbarger KJ, Tyler PA, Langton RW (1998) Gonadal morphology and gametogenesis in the sea pen *Pennatula aculeata* (Anthozoa: Pennatulacea) from the Gulf of Maine. *Mar Biol* 132:677–690
- Edwards DCB, Moore CG (2008) Reproduction in the sea pen *Pennatula phosphorea* (Anthozoa: Pennatulacea) from the west coast of Scotland. *Mar Biol* 155:303–314
- Edwards DCB, Moore CG (2009) Reproduction in the sea pen *Funiculina quadrangularis* (Anthozoa: Pennatulacea) from the west coast of Scotland. *Estuar Coast Shelf Sci* 82:161–168
- Ellis J (1764) An account of the sea pen, or *Pennatula phosphorea* of Linnaeus; likewise a description of a new species of sea pen, found on the coast of South-Carolina, with observations on sea-pens in general. In a letter to the honourable Coote Molesworth,

- Esq; M.D. and F.R.S. from John Ellis, esq; F.R.S. and member of the Royal Academy of Upsal. *Philos Trans R Soc Lond B* 53:419–435
- Ellis J, Solander D (1786) The natural history of many curious and uncommon zoophytes, collected from various parts of the globe by the late John Ellis systematically arranged and described by the late Daniel Solander. London, printed for Benjamin White and Son, at Horace's Head, Fleet-Street; and Peter Elmsly, in the Strand. 206 pp
- Fiorillo I, Rossi S, Alvà V, Gili JM, López-González PJ (2013) Seasonal cycle of sexual reproduction of the Mediterranean soft coral *Alcyonium acaule* (Anthozoa, Octocorallia). *Mar Biol* 160(3):719–728
- Flint HC, Waller RG, Tyler PA (2007) Reproductive ecology of *Fungiacyathus marenzelleri* from 4100 m depth in the northeast Pacific Ocean. *Mar Biol* 151(3):843–849
- García-Cárdenas FJ, López-González PJ (2022) Biogeographic patterns in sea pens (Octocorallia: Pennatulacea). *Mar Biodivers*. Accepted MS.
- Gili JM, Pagès F (1987) Pennatuláceos (Cnidaria, Anthozoa) recolectados en la plataforma continental catalana (Mediterráneo occidental). *Misc Zool* 11:25–39
- Gray JE (1870) Catalogue of sea-pens or Pennatulariidae in the collection of the British Museum. British Museum, London, p 40
- Hamel JF, Wareham-Hayes VE, Mercier A (2020) Reproduction of a bathyal pennatulacean coral in the Canadian Arctic. *Deep-Sea Res I* 162:103321
- Herklots JA (1858) Notices pour servir à l'étude des polypiers nageurs ou pennatulidés. *Bijdr Dierkd* 7:1–31
- International Commission on Zoological Nomenclature (ICZN) (1944) Opinion 185. Suppression of Bohadsch (J.B.), De quibusdam Animalibus marinis, 1761, and of the German translation thereof published by Leske (N.G.) in 1776). Opinions and Declarations Rendered by the International Commission on Zoological Nomenclature 3 (Part 4):37–52
- Jones LA, Hiscock K, Connor DW (2000) Marine habitat reviews, a summary of ecological requirements and sensitivity characteristics for the conservation and management of Marine SAC's. UK Marine SAC's Project Report. Peterborough: Joint Nature Conservation Committee, pp 178
- Kükenthal W (1915) Pennatularia. *Das Tierreich*. Verlag von R. Friedländer Und Sohn, Berlin 43:1–132
- Langton RW, Langton EW, Theroux RB, Uzman JR (1990) Distribution, behaviour and abundance of sea pens, *Pennatula aculeata*, in the Gulf of Maine. *Mar Biol* 107:463–469
- Laverick JH, Andradi-Brown DA, Rogers AD (2017) Using light-dependent scleractinia to define the upper boundary of mesophotic coral ecosystems on the reefs of Utila. Honduras *Plos One* 12(8):e0183075
- Levitán DR (1993) The importance of sperm limitation to the evolution of egg size in marine invertebrates. *Am Nat* 141:517–536
- Levitán DR (1996) Predicting optimal and unique egg sizes in free-spawning marine invertebrates. *Am Nat* 148:174–188
- Lopes VM, Baptista M, Pimentel MS, Repolho T, Narciso L et al (2012) Reproduction in Octocorallia: synchronous spawning and asynchronous oogenesis in the pennatulid *Veretillum cynomorium*. *Mar Biol Res* 8(9):893–900
- Marshall AM, Fowler GH (1887) Report on the Pennatulida dredged by H. M. S. 'Porcupine.' *Trans Roy Soc Edinburgh* 33(2):453–464
- McClary DJ, Mladenov PV (1989) Reproductive pattern in the brooding and broadcasting sea star *Pteraster militaris*. *Mar Biol* 103(4):531–540
- Mladenov PV, Brady K (1987) Reproductive cycle of the Caribbean feather star *Nemaster rubiginosa* (Echinodermata: Crinoidea). *Mar Ecol* 8(4):313–325
- Nurdin N, Lanuru M, Akbar AS, Kartika I, Komatsu T (2019) Accuracy of unsupervised classification to determine coral health using SPOT-6 and Sentinel-2A. *Int Arch Photogramm Remote Sens Spat Inf Sci Vol XLII-4/W16 6th Int Conf on Geomatics and Geospatial Technology*
- Ocaña Ó, Wirtz P (2007) New records of Pennatulacea (Cnidaria, Octocorallia) from Madeira island. Arquipélago. *Life Mar Sci* 24:49–51
- Orejas C, López-González PJ, Gili JM, Teixidó N (2002) Distribution and reproductive ecology of the Antarctic octocoral *Aimigmartilon antarcticum* in the Weddell Sea. *Mar Ecol Prog Ser* 231:101–114
- Orejas C, Gili JM, López-González PJ, Hasemann C, Arntz WE (2007) Reproduction patterns of four Antarctic octocorals in the Weddell Sea: an inter-specific, shape, and latitudinal comparison. *Mar Biol* 150:551–563
- Page CA, Lasker HR (2012) Effects of tissue loss, age and size on fecundity in the octocoral *Pseudopterogorgia elisabethae*. *J Exp Mar Biol Ecol* 434–435:47–52
- Pax FA, Müller I (1953) Die Anthozoen fauna von der Bucht von Kastela bei Split. *Acta Adriat* 5(1):14–35
- Pax FA, Müller I (1955) Die Korallentiere der Adria. *Die Aquarien- und Terrarien Zeitschrift*, 8,10–12; 39–40; 67–69
- Pax FA, Müller I (1959) The role of the Wrocław Zoological Institute and Museum in the study of the fauna of Anthozoa in the Adriatic Sea. *Przeglad Zoologiczny* 3(1):44–53
- Pires DO, Castro CB, Silva JC (2009) Reproductive biology of the deep-sea pennatulacean *Anthoptilum murrayi* (Cnidaria, Octocorallia). *Mar Ecol Prog Ser* 397:103–112
- Porporato EMD, Mangano MC, De Domenico F, Giacobbe S, Spanò N (2014) First observation of *Pteroeides spinosum* (Anthozoa: Octocorallia) fields in a Sicilian coastal zone (Central Mediterranean Sea). *Mar Biodivers* 44:589–592
- Quintanilla E, Gili JM, López-González PJ, Tsounis G, Madurell T et al (2013) Sexual reproductive cycle of the epibiotic soft coral *Alcyonium coralloides* (Octocorallia, Alcyonacea). *Aquat Biol* 18(2):113–124
- Rice AL, Tyler PA, Paterson GJL (1992) The pennatulid *Kophobelemnon stelliferum* (Cnidaria: Octocorallia) in the porcupine seabight (north-east Atlantic Ocean). *J Mar Biol Assoc UK* 72:417–434
- Roberts CM (2002) Deep impact: the rising toll of fishing in the deep sea. *Trends Ecol Evol* 17:242–245
- Roberts JM, Wheeler AJ, Freiwald A (2006) Reefs of the deep: the biology and geology of cold-water coral ecosystems. *Science* 312(5773):543–547
- Rossi L (1971) Guida a cnidari e ctenofori della fauna italiana. *Quaderni Sta Idrobiol Milano* 2:1–101
- Servetto N, Sahade R (2016) Reproductive seasonality of the Antarctic sea pen *Malacobelemnon daytoni* (Octocorallia, Pennatulacea, Kophobelemnidae). *PLoS ONE* 11(10):e0163152
- Servetto N, Torre L, Sahade R (2013) Reproductive biology of the Antarctic "sea pen" *Malacobelemnon daytoni* (Octocorallia, Pennatulacea, Kophobelemnidae). *Polar Res* 32(1):20040
- Simpson A (2009) Reproduction in octocorals (Subclass Octocorallia): a review of published literature. <http://www.ucs.louisiana.edu/~scf4101/Bambooweb>
- Soong K (2005) Reproduction and colony integration of the sea pen *Virgularia juncea*. *Mar Biol* 146:1103–1109
- Soong K, Lang JC (1992) Reproductive integration in reef corals. *Biol Bull Rev* 183(3):418–431
- Theodor J (1967) Contribution à l'étude des gorgones VII. Ecologie et comportement de la planula. *Vie Milieu* 18:291–301
- Topçu EN, Öztürk B (2015) Composition and abundance of octocorals in the Sea of Marmara, where the Mediterranean meets the Black Sea. *Sci Mar* 79(1):125–135

- Tremblay ME, Henry J, Anctil M (2004) Spawning and gamete follicle rupture in the cnidarian *Renilla koellikeri*: effects of putative neurohormones. *Gen Comp Endocrinol* 137:9–18
- Tyler PA, Bronsdon SK, Young CM, Rice AL (1995) Ecology and gametogenic biology of the genus *Umbellula* (Pennatulacea) in the North Atlantic Ocean. *Int Rev Hydrobiol* 80(2):187–199
- Weinberg S, Weinberg F (1979) The life cycle of a gorgonian: *Eunicella singularis* (Esper, 1794). *Bijdr Dierkd* 48:127–140
- Williams GC (1995) Living genera of sea pens (Coelenterata: Octocorallia: Pennatulacea): illustrated key and synopses. *Zool J Linn Soc* 113:93–140
- Williams GC (2011) The global diversity of sea pens (Cnidaria: Octocorallia: Pennatulacea). *PLoS ONE* 6(7):e22747
- Zar JH (1984) *Biostatistical analysis*. 2nd. Prentice Hall, USA

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.