Reproductive characteristics and gametogenic cycle of the scleractinian coral *Dendrophyllia ramea*

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The present study investigates, for the first time, the reproductive cycle of the scleractinian coral *Dendrophyllia ramea*. This is one of the first reproduction studies conducted in the Mediterranean Sea for a colonial azooxanthellate coral. Coral samples were collected in 2017 (May and October) and 2018 (February and July) in the Alborán Sea (SW Mediterranean), one of the few locations where this species is known to occur at depths shallower than 40 m. These samples were used to study the sexual patterns, fertilization mechanism and gametogenic cycle by means of histological techniques. Additionally, Sea Surface Temperature (SST) and Chlorophyll-a (Chl-a) data from open access databases have been considered to explore the potential influence of these environmental factors as triggers for gamete development and spawning time. The results reveal *D. ramea* as a gonochoric species, since no hermaphroditic specimens have been detected amongst the analysed samples. In the same way, the lack of larvae and embryos in any of the analysed polyps, suggest this species is fertilised externally. Two oocyte cohorts have been detected simultaneously, hinting a yearly reproductive cycle, characterised by a prolonged oocyte maturation and a seasonal spawning, taking place from August to October. Nonetheless, *D. ramea* displays a low fecundity compared to other scleractinians inhabiting deep waters. Lastly, the early stages of gametogenesis seem to be coupled with the highest Chl-a values (i.e. March and December), whereas spawning takes place throughout the warmest period of the year (August to October).

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Abstract

The present study investigates, for the first time, the reproductive cycle of the scleractinian coral *Dendrophyllia ramea*. This is one of the first reproduction studies conducted in the Mediterranean Sea for a colonial azooxanthellate coral. Coral samples were collected in 2017 (May and October) and 2018 (February and July) in the Alborán Sea (SW Mediterranean), one

of the few locations where this species is known to occur at depths shallower than 40 m. These samples were used to study the sexual patterns, fertilization mechanism and gametogenic cycle by means of histological techniques. Additionally, Sea Surface Temperature (SST) and Chlorophyll-a (Chl-a) data from open access databases have been considered to explore the potential influence of these environmental factors as triggers for gamete development and spawning time. The results reveal *D. ramea* as a gonochoric species, since no hermaphroditic specimens have been detected amongst the analysed samples. In the same way, the lack of larvae and embryos in any of the analysed polyps, suggest this species is fertilised externally. Two oocyte cohorts have been detected simultaneously, hinting a yearly reproductive cycle, characterised by a prolonged oocyte maturation and a seasonal spawning, taking place from August to October. Nonetheless, *D. ramea* displays a low fecundity compared to other scleractinians inhabiting deep waters. Lastly, the early stages of gametogenesis seem to be coupled with the highest Chl-a values (i.e. March and December), whereas spawning takes place throughout the warmest period of the year (August to October).

Introduction

The knowledge on the reproductive cycle and characteristics of azooxanthellate scleractinians, and more specifically deep and cold-water scleractinian corals, has increased over the last decade (e.g. Airi et al. 2016; Brooke and Jarnegreen 2013; Feehan et al. 2019; Pires et al. 2014; Prasetia et al. 2017; Shlesinger and Loya 2016; Waller and Feehan 2013). However, although more than 3,000 Cold-Water Coral (CWC) species have been described so far, reproductive information is only known for less than 60 species (see Brooke and Stone 2007; Feehan and Waller 2015; Rakka et al. 2017; Rossin et al. 2017); a disparity that is especially apparent for Mediterranean CWCs (Airi et al. 2016; Orejas and Jiménez 2019). Harrison (2011), summarized the reproductive characteristics of scleractinian corals (encompassing shallow,

mesophotic and CWCs), showing that 71% of scleractinian corals are hermaphrodites compared to a 26% of gonochoric species and only a 3% showing mixing patterns. Nevertheless, despite shallow-water scleractinian corals are mostly hermaphrodites (Fadlallah 1983a; Harrison and Wallace 1990; Richmond and Hunter 1990), most ~~of the~~ CWC scleractinians studied ~~up~~ to date have been reported to be gonochoric (Feehan et al. 2019; Waller et al. 2005; Waller and Feehan 2013). The large number of hermaphroditic species found in shallow waters (which have been more investigated, mainly due to a better accessibility) originally suggested that hermaphroditism was the most ancestral reproductive condition in scleractinian corals (Szmant 1986). However, subsequent studies pointed to gonochorism as the most primitive form of reproduction (Goffredo et al. 2002; Harrison 1990). On the other hand, external mating is the most common fertilisation strategy in scleractinians (Harrison 2011), even for those thriving in deep waters (Waller 2005).

Most of the investigations on scleractinian reproduction have been conducted in tropical species (Fadlallah 1983a; Harrison and Wallace 1990), nevertheless reproduction studies on mesophotic (Shlesinger and Loya 2019) and CWCs have substantially increased in the last decades (i.e. Brooke and Järnegreen 2013; Feehan et al. 2019; Larsson et al. 2014; Strömberg and Larsson 2017; Waller et al. 2002, 2005, 2008; Waller and Feehan 2013; Waller and Tyler 2005, 2011). However, our knowledge on the reproductive biology of scleractinians from temperate and cold waters is still scarce, especially in the Mediterranean (Goffredo et al. 2006). Indeed, reproductive data of Mediterranean corals mostly originate from observations of the species *Caryophyllia smithii*, *Balanophyllia regia*, *Leptosammia pruvoti*, *Astroides calycularis* and *Cladopsammia rolandi* by Lacaze-Duthiers (1873), as well as from more recent studies conducted with the species *Balanophyllia europea* (Goffredo et al. 2002), *L. pruvoti* (Goffredo et al. 2005, 2006), *A. calycularis* (Goffredo et al. 2010, 2011, Terrón-Sigler 2016), *Cladocora caespitosa* (Kružić et al. 2008) and *Caryophyllia inornata* (Caroselli et al. 2017; Goffredo et al. 2012; Marchini et al. 2020). From these species, only *C. smithii, C. inornata* and *L. pruvoti* cover

a wide bathymetric range, from shallow waters to more than 150 m depth (Altuna and Poliseno 2019).

Dendrophyllia ramea is a colonial azooxanthellate scleractinian coral with a Mediterranean-Atlantic distribution and a depth range from 40 to 240 m (Angiolillo et al. 2022; Brito and Ocaña 2004; Dias et al. 2020; Kružić et al. 2002; Orejas et al. 2019a,b; Salomidi et al. 2010; Salvati et al. 2021; Zibrowius 1980). Although some records are documented in Pacific waters in the Ocean Biodiversity Information System (OBIS) data base (https://mapper.obis.org/?taxonid=135187), these might be the consequence of miss-identifications. The species presents a remarkable morphological plasticity, with colonies displaying different shapes (Orejas et al. 2017, 2019a,b). The corallite's diameter ranges from 5 to 12 mm and they are regularly distributed along the branches in two opposite rows (Kružić et al. 2002). Ever since their discovery in the Mediterranean Sea it was thought that this species thrived mostly on hard substrata and covering a depth range from ~40 to ~60 m (Zibrowius 1980), which contrasts with the populations in the Canary Islands, where *D. ramea* tends to live in greater depths (i.e. 80-150 m) and generally on biogenic hard substrata (Brito and Ocaña 2004). However, recent discoveries in the Mediterranean reveal the occurrence of this species in soft substrata at 125–170 m depth in Cypriot waters (Orejas et al. 2019a,b), with the deepest population of *D. ramea* occurring in the Menorca channel at 240 m depth (Jiménez et al. 2016). Furthermore, several populations of this species have been located in the Alborán Sea as shallow as 16 m depth (Terrón-Singler 2016). Therefore, although most occurrences have been documented for mesophotic and deep-water environments, *D. ramea* displays a large bathymetric range. To be consistent with the previous publications on *D. ramea* from the Mediterranean (Orejas et al. 2019a,b), and considering that the species frequently [illegible] under temperatures above 10-12ºC, the term deep-sea coral (DSC) has been chosen in this work to relate to this species instead the term CWC.

The main aim of this work is to describe for the first time the sexual characteristics of *Dendrophyllia ramea* in the Mediterranean Sea, specifically in the coast of Granada (SW Mediterranean, Alborán Sea), as well as to quantify and describe its gametogenic cycle, and reproductive timing. Results are discussed on the light of the current knowledge on reproduction of scleractinian corals, including CWCs.

Materials & Methods

Sampling and Study Site

Samples of *Dendrophyllia ramea* (Fig. 1) were collected between 30 and 37 m depth, in a location inhabited by a dense population of the species (Terrón-Singler 2016). The latter is found off the coast of Granada, in Punta de La Mona (36°43′25″ N 3°43′56″ W, northern Alborán Sea, western Mediterranean Basin) (Fig. 2a,b), within the Marine Protected Area (MPA) of *Acantilados y Fondos Marinos de la Punta de la Mona*. This MPA was established in 2015 as a Special Area of Conservation (SAC) declared by the Autonomous Andalusian government (Junta de Andalucía) (369/2015/BOJA). The MPA is characterised by the upwelling of cold and nutrient-rich water, resulting from the anticyclonic gyres promoted by the incoming Atlantic waters in the Mediterranean Sea (La Violette 1984). Upwelling is more evident in summer, when thermal contrast is stronger. Although the MPA extends below 40–50 m depth, the species tends to occur in relatively shallow waters in the area (Cebrián and Ballesteros 2004). The relatively shallow occurrence of the species in this location allowed to perform 4 sampling events covering all seasons. Sampling was carried out by scuba divers in May (spring) and October (autumn) 2017, as well as in February (winter) and July (summer) 2018 (Table 1, Suppl Mat 1). Three to five polyps were collected from 38 different colonies. Sampling was conducted under the permit (001997/A04D) of the Autonomous Andalusian government (Junta de Andalucía) (Suppl Mat 1). After sampling, polyps were preserved in sea water with buffered

formaldehyd with a final concentration of 4 % and later transferred to the Laboratorio de Investigaciones Marinas y Acuicultura (LIMIA–IRFAP, Mallorca) for histological analyses.

Biometry and histological processing

Biometric and histological analysis were performed for all (74) polyps before the decalcification process was conducted (see Table 1). For the biometric analyses, polyp calyx diameter (D, major axis of the oral disk) and polyp height (H, oral-aboral distance) were measured. The total number of mesenteries per polyp was counted in a total of five haphazardly selected polyps from different months. Polyp measurements (calyx diameter and polyp height) were performed to explore potential relationships between reproductive output and polyp size. Total polyp fecundity was then extrapolated from the number of gametes found during histological analyses (conducted in three mesenteries). Polyp mesenteries were processed for histological analyses in order to: 1) determine the sex of each polyp 2) quantify gametes and describe their developmental stages, and 3) describe the gametogenic cycle. Polyps were decalcified in a 10% formic acid solution for 24-48 hours. Once the polyps were fully decalcified, each polyp was dissected and 3 mesenteries per polyp were extracted to perform gamete counting, analyse and describe their developmental stages and describe the gametogenic cycle. Extracted mesenteries were included in histological cassettes and subsequently dehydrated in a graded ethanol series (70-100%), cleared in Microclearing X0026® and embedded in paraffin wax. Three embedded mesenteries of 3 haphazardly selected polyps from 3 randomly selected colonies were sectioned and analysed, for both males and females in all sampled months (with the exception of October for male colonies, as only 2 colonies were available, see Table 1). For male polyps, mesenteries were sectioned in serial slides (4 µm in thickness), whereas mesenteries of female polyps were serially sectioned (4 µm in thickness) every 50 to 80 µm, depending on the monthly average diameter of oocyte nucleus. Sections were performed with a HM 330 Microm rotary microtome and each slide was examined using an Olympus (BX51)

compound microscope. To avoid counting and measuring the same oocyte twice, only those oocytes sectioned through the nucleus were counted in each slide (a total of 361 for February, 386 for May, 302 for July and 207 for October), following the procedure used by Waller et al. (2014). Maximum and minimum diameter (μm) were measured for each oocyte. Measurements were performed with the imaging software Cell^D (Olympus Europe), connected to an Olympus DP 20 camera.

Reproductive cycle and reproductive output

Gametogenic stages of development were established using a four stage scale of gamete maturation, adapted by the authors to the analysed species (Table 2) and following the criteria used in previous work (Feehan et al. 2019; Mercier and Hamel 2010; Waller et al. 2002; Waller and Tyler 2005). For male polyps, 100 spermatic cysts randomly selected from each of the three randomly selected mesenteries were analysed and classified following the maturation stages described in Table 2. For female polyps, the total number of oocytes in each of the three haphazardly selected mesenteries were counted and classified in maturation stages following the classification of Table 2. Furthermore, in order to describe the gametogenic cycle (developmental stage), the minimum and maximum diameter (as described in the paragraph above) of 100 oocytes haphazardly selected were measured.

Fecundity

The fecundity of *D. ramea* was calculated following the methodology of Mercier and Hamel (2011), where the authors distinguished between potential relative fecundity (PRF), defined as the total number of oocytes per polyp irrespective of their maturity stage, and effective relative fecundity (ERF), defined as the number of mature entities (Stage IV, see Table 2) per polyp. To determine PRF and ERF the total number of oocytes was quantified in three haphazardly selected mesenteries per polyp and colony, which were then averaged. The number of oocytes

per mesentery was multiplied by the mean number of pairs of mesenteries per polyp to obtain the fecundity per polyp.

Environmental factors

In order to explore the potential triggers of *D. ramea* reproductive timing and gamete or larval release, data of sea surface temperature (SST) and primary production (Chl-a) was obtained from open access oceanographic datasets. Monthly Sea surface temperature (SST) data for the years 2017 and 2018 corresponding to the Málaga buoy (the closest buoy to the SAC, 32 nautical miles from the sampling location (36º 41´ N 004º 24´ W)) were obtained from the Ministerio de Fomento de España (www.puertosdelestado.es). The monthly SST data was downloaded from the Historical Data Section and the measuring station (Málaga buoy), whereas monthly Chl-a data from the Alborán Sea were obtained from the Andalusian Environmental Information Network (www.juntadeandalucia.es/medioambiente/site/rediam), specifically from the historical series 2000-2018 available on the Rediam portal. The values of both SST and Chl-a per month correspond to the average among the two years.

Data analysis

A chi-square test was performed to determine whether the sex ratio was significantly different from 1:1. Due to the lack of normality and variance homogeneity in the distribution of the oocyte measurements, the non-parametric Kruskal-Wallis test with a Dunn post hoc test were applied to assess potential differences in average oocyte-size among months and year seasons. Potential differences in oocyte numbers (PRF) were assessed with a one-way ANOVA, after verifying the normality and the variance homogeneity, while the Kruskal-Wallis test with a Dunn post hoc test were used to compare the monthly ERF. A linear regression was used to determine the potential correlation between polyp height (H) and diameter (D) with fecundity (PRF and ERF). All results are presented as mean $\pm$ standard deviation (SD).

208

209 Results

210

211 Morphology

212 Polyp diameters ranged from 6 to 14 mm, whereas polyp heights ranged from 6 to 37 mm
213 (Suppl Mat 1). The dissection of mesenteries of 5 haphazardly selected polyps reveal that *D.*
214 *ramea* polyps hold an average of 20 ± 4 pairs of mesenteries per polyp. No external
215 morphological differences were detected between males and females.

216

217 Sexual pattern and reproductive mode

218 *Dendrophyllia ramea* is a gonochoric species. No hermaphroditic specimens were found in this
219 study. In all the sampled months, colonies with gametes were observed. Out of 38 colonies
220 examined, 17 (45%) were males, 16 (42%) were females, and the remaining 5 (13%) did not
221 contain gametes, therefore it was not possible to determine their sex. Hence, *D. ramea* displays
222 a sex ratio of approximately 1:1 ($X^2 = 0.03$, P= 0.861). Spermatocysts and oocytes are
223 embedded in the mesenteries, surrounded by the mesenterial filament. The fertilization in this
224 species is probably external, as no larva and/or embryos have been detected in any of the
225 analysed histological slides.

226

227 Gametogenesis and reproductive periodicity

228 As the sampling took place across two consecutive years (2017 and 2018) in order to present
229 the gametogenic cycle following the natural sequence of the months of the year, results have
230 been arranged as follows: February 2018, May 2017, October 2017, July 2018.

231

232 Spermatogenesis

233 According to our classification (Table 2), Stage I (SI) spermatocysts (spermaries) were
234 observed in February (Fig. 3a) and in May (Fig. 3b) with 100 % of male colonies displaying this
235 developmental stage (Fig. 4a). In July (Fig. 3 c, d, e), spermatocysts in SII and SIII were first
236 observed, with SIII being the most abundant developmental stage (37 %), followed by SI (26 %),
237 SII (23 %) and SIV (14%) (Fig. 4a). In October (Fig. 3f) most of the spermatocysts were in SIV
238 (78 %), with some in SIII (19 %) and few in SI (3 %) (Fig. 4a).
239
240 Oogenesis
241 According to our classification (Table 2) mature oocytes (Fig. 5e) are observed in all sampled
242 months. Previtellogenic oocytes (SI; Fig 5a, g) were only visible in February and October,
243 representing 7 % and 1 % of the observed oocytes, respectively (Fig. 4b). In May (Fig. 5 c, d),
244 SIII and SIV were the most abundant stages (45 % and 33 %, respectively, Fig. 4b), with the
245 presence of SII in a lower proportion (22%) and absence of SI. In July (Fig. 5 e, f), late
246 vitellogenic oocytes SIII (Fig. 5 d, h) dominated (42%), with high presence of mature oocytes
247 SIV (40 %) and some early vitellogenic oocytes SII (Fig. 5b) (18%) and none in SI. Mature
248 oocytes SIV (Fig. 5e) decreased in October (3%) where oocytes in SIII and II dominate (51%
249 and 44% respectively), with almost no oocytes in SI (2%).
250 The maturity stages of oocytes are also related to different oocyte diameters, with the latter
251 displaying significant differences between months (H= 277.69, P = 2.20 e-16; Fig. 6). Oocyte
252 diameter ranged from a minimum size of 14.41 µm detected in February to a maximum size of
253 642.71 µm detected in July. In February mean oocyte diameter was 154.0 µm ± 99.9, which
254 increased in May (277.0 µm ± 127.0) and reached the largest size in July, with an oocyte mean
255 diameter of 302.4 µm ± 141.1. A significant decrease in oocyte diameter was observed in
256 October (184.8 µm ± 89.5, P < 0.001). Although there were no significant differences between
257 the oocyte average diameter in May and July (Fig. 6, Table 3, P= 0.0742), the oocyte size-
258 frequency distributions (Fig. 7) show two different modes for oocyte size in February, May and

July, while only one oocyte cohort was observed in October, corresponding to small oocytes SI. In all sampled months *D. ramea* polyps presented a standing stock of small oocytes (SI or SII) in their mesenteries, indicating overlapping gametogenic cycles or a continuous production punctuated by periods of rapid maturity, which points to this species as iteroparous.

Fecundity

The highest average PRF was detected in May (574.7 ± 245.0 oocytes per polyp (opp)) (Fig. 8a,b) and the lowest in October (308.2 ± 134.2 opp), decreasing by 46.4 % from May to October (Fig. 8a,b). However, no statistically significant differences in average PRF among the four months have been detected (F= 2.67, P= 0.064). Statistically significant differences were detected among months for the average effective relative fecundity (ERF, oocytes > 350 µm) (K= 17.60, P = 0.00053). As for PRF, the highest average ERF was in May (187.6 ± 81.9 opp) and the lowest in October (10.4 ±5.2 opp), with a significant decrease of 94.4 5% (Fig. 8a,c). The average in February (20.8 ± 25.4 opp) was significantly lower compared to May and July (187.6 ± 81.9 opp and 181.6 ± 90.3 opp) (Fig. 7a,c). There was no statistically significant correlation between polyp height or polyp calyx diameter and the fecundity, either PRF or ERF ($R^2 < 0.1$ and $P > 0.05$ for all combinations; PRF-D: $R^2 = - 0.026$ and $P = 0.741$; PRF-H : $R^2 = 0.045$ and $P = 0.113$; ERF-D: $R^2 = - 0.002$ and $P = 0.338$; ERF-H : $R^2 = -0.018$ and $P = 0.543$; D = polyp calyx diameter and H= polyp height)).

Environmental factors

The decrease in the number of mature oocytes in October 2017 (see Fig. 7 and 8) coincides with lower values of chlorophyll concentration and relatively higher temperatures (Fig. 9). Concentration of Chl-a increased from October to April, with a maximum between March and April, whereas from March to August SST increases, with the highest values occurring in

August. Therefore, the months where ERF is higher (May, July) correspond to the periods with lower Chl-a values and increasing temperatures.

Discussion

The results acquired suggest that *Dendrophyllia ramea* is a gonochoric species, since all colonies examined displayed a single sex and no hermaphrodites were observed. Although according to Harrison (2011) gonochorism is not dominant among scleractinians (26% out of the 416 species), most species considered in that study come from shallow waters, where there is a predominance of hermaphroditism. Instead, gonochorism seems to dominate amongst CWC scleractinian species, as 80% of all studied species have been observed to present this reproductive mode (Table 4). To date, only three solitary CWC belonging to the genus *Caryophyllia* have been documented as hermaphrodites (Waller et al. 2005), although Pires et al. (2014) found some specimens of the colonial corals *M. oculata* and *L. pertusa* with hermaphroditism patterns. However, the overall sexual pattern in scleractinian corals is highly stable within taxonomic groups, from family to species level (Baird et al. 2009; Harrison and Wallace 1990; Kerr et al. 2011). This is also the case of the family Dendrophyllidae, in which 80% of the investigated species, including *D. ramea*, are gonochoric (Table 5). It is important to keep in mind that, although *D. ramea* inhabits areas deeper than 150 meters in many of its documented occurrences, the results from this study have been obtained from colonies collected at ~40 meters. Reproductive features of corals can change across bathymetric ranges as well as geographically (Baird et al. 2009), and differences in reproductive patterns have been found for instance for *Lophelia pertusa* from different areas (Brooke and Jarnegreen 2013). Regarding the reproductive mode of *D. ramea*, the absence of larvae and/or embryos in the analysed colonies suggests that this species is a broadcast spawner (i.e. releases the gametes to the water column). This seems to be the most frequent reproductive mode in CWCs; only 3 of the CWCs species investigated to date are brooders, all of them belonging to the genus

310 *Flabellum*: *F. impensum*, *F. curvatum* and *F. thouarsii* (Waller et al. 2008) (Table 4). However,
311 the reproductive strategy is more variable within the same scleractinian taxon (i.e. family, genus,
312 species) than the sexual pattern (Harrison and Wallace 1990, Kerr et al. 2011). An example
313 would be the genus *Porites* form tropical shallow waters , which includes 10 brooder and 10
314 broadcaster species, while it only presents two gonochoric species in the Atlantic and the Indo-
315 Pacific(Baird et al. 2009). Nevertheless, most of the species of the family Dendrophyllidae are
316 brooders, with 8 broadcast spawner species belonging to the genus *Heteropsammia* and
317 *Turbinaria* (Table 5).
318 The two oocyte cohorts detected simultaneously in *D. ramea*, and the fact that mature oocytes -
319 with migrated nucleus- are present in July (see Fig. 5, f), suggest a yearlong gametogenic cycle,
320 characterised by a prolonged oocyte maturation, and a seasonal spawning occurring from
321 August to October. One of the most striking observations in this study was the lack of initial
322 developmental stages in females (oogonia) and males (spermatogonia). The most plausible
323 explanation for this is the fact that our observations have been focused on the mesenterial
324 mesoglea. Several authors have documented for shallow water corals the migration of gametes
325 in early developmental stages (Stage 0) from the gastrodermis to the mesenterial mesoglea,
326 where differentiation and subsequent maturation takes place (Fadlallah 1983a; Goffredo et al.
327 2002, 2004, 2012; Szmant-Froelich et al. 1980). The lack of occurrence of early oocyte stages
328 in the mesenteries has been also documented in the CWCs *Fungiacyathus marenzelleri* (Waller
329 et al. 2002), *Lophelia pertusa* and *Madrepora oculata* (Waller and Tyler 2005), *Caryophyllia sp*.
330 (Waller et al. 2005) and *Flabellum sp*. (Waller and Tyler 2011). Therefore, we suggest that the
331 migration from gastrodermis to mesenterial mesoglea at the beginning of the gametogenesis
332 also occurs in *D. ramea*. However, further investigations should add specific analyses of the
333 gastrodermis in order to be able to potentially detect oogonia and spermatogonia.
334 Regarding the developmental stages for oocytes, there is a wide range of maturation scales,
335 proposed by different authors. For instance, Waller and Feehan (2013) consider only

previtellogenic and vitellogenic oocytes to describe the gametogenesis of *F. marenzelleri*, whereas in previous works (Waller et al. 2002; Flint et al. 2007), a further stage was considered: late-vitellogenic. In our study we consider 4 stages (without considering oogonia as they have not been observed), since clear differences have been observed amongst them (see Fig. 5). We also described 4 developmental stages for spermatogenesis, following the criteria applied for the CWC species *F. mazzerelli* (Waller et al. 2002), *L. pertusa* (Brooke and Järnegren 2013) and *Caryophyllia sp.* (Waller et al. 2005). The spermatogenesis stages of *D. ramea* are also similar to other Mediterranean corals, although the latter include a further stage: spermatogonia, presenting therefore 5 stages, this is the case for *L. pruvoti*, *A. calycularis* and *C. inornata* (Goffredo et al. 2002, 2010, 2012).

Within scleractinian CWCs and DSC there are some species that reproduce seasonally, whereas others present continuous or quasi continuous reproduction (Waller 2005). *Dendrophyllia ramea* seems to have a seasonal reproductive pattern with gamete release occurring between August and October (Fig. 5, 7). The absence of the larger oocyte cohort in October, together with the high percentage of spermatocysts in SIV (spent stage), suggest that gamete release most probably occurs in that period, from the end of summer and to the start of autumn. Furthermore, the finding of late vitellogenic oocytes of ~200-300µm, may suggest (following Strathman 1987) a pelagic or a demersal development of larvae.

Our results for *D. ramea* showing different oocyte developmental stages simultaneously, suggest two possible reproductive strategies: 1) a quasi-continuous gametogenesis or, 2) a gametogenesis extended over time with a periodic gamete release. The latter option seems to be the most probable considering that: 1) our results show significant differences in average oocyte size within each month compared to other species with quasi-continuous reproduction, which do not show significant differences among oocyte sizes (Flint et al. 2007; Waller et al. 2002), and 2) due to the low number of previtellogenic oocytes (see Fig. 4), as quasi-continuous reproduction implies a larger number of previtellogenic oocytes. A periodic gamete release over

362 time has been previously suggested for other CWC species (Waller and Feehan 2013); these
363 authors also suggested the two potential strategies discussed earlier (i.e. quasi-continuous
364 gametogenesis and long term gametogenesis with periodic gamete release) for the Antarctic
365 solitary coral *F. marenzelleri*. Burguess and Babcock (2005) suggested that the simultaneous
366 presence of oocytes SIII and SIV in *Enallopsammia rostrata* in the Pacific Ocean was due to a
367 delay in oocyte development, which was associated to nutritional resource availability. Periodic
368 gamete releases have been observed for *Oculina varicosa* in the Atlantic, with up to 31 days of
369 difference between spawning events, which reflect that gamete release occurs over long time
370 periods (Brooke and Young 2003). In the present study, the two oocyte cohorts found in *D.*
371 *ramea*, suggest that oocyte maturation may take longer than 12 months. However, as previously
372 mentioned, the low number of previtellogenic oocytes does not allow to determine the initiation
373 of gametogenesis. Analysis of samples from additional time periods may help to solve this.
374 The developmental stages of the spermatocysts reveal an annual cycle, shorter than the
375 oogenesis; which is common for anthozoans (Goffredo et al. 2002; Guest et al. 2005; Harrison
376 and Wallace 1990; Richmond and Hunter 1990; Schleyer et al. 2004). Our data suggest a start
377 of the spermatogenesis in winter time (SI), with increased activity in summer and release of
378 mature sperm in autumn (Fig. 4). Similar patterns of spermatogenesis have been documented
379 for other CWC and DSC species: *L. pertusa* (Brooke and Järnegren 2013; Pires et al. 2014), *M.*
380 *oculata* (Waller and Tyler 2005) and *F. marenzelleri* (Flint et al. 2007; Waller et al. 2002).
381 Although they inhabit shallower waters, the temperate Mediterranean species *B. europaea*, *L.*
382 *pruvoti* and *A. calycularis* (brooding species from the family Dendrophyllidae) also presented a
383 longer oogenesis than spermatogenesis (Goffredo et al. 2002, 2005, 2011).
384 The period of gamete release occurs, in the analysed location where *D. ramea* thrive, when
385 temperature is higher and Chl-a values are low. The investigated *D. ramea* population occurs in
386 a depth range from 16 to 50 meters, in the Alborán Sea, which is characterised by deep water
387 upwellings that play an important role in the temperature as well as in the productivity regime of

388 the area (Sarhan et al. 2000). It is known for several coral species, from both shallow and deep
389 waters, that temperature, photoperiod, lunar cycles and food supply are environmental factors
390 influencing reproduction timing (Goffredo et al. 2006, 2011; Glynn et al. 2000; Harrison 2011;
391 Richmond 1997; Waller and Tyler 2005). Indeed, the reproductive cycle of the temperate
392 broadcast spawner corals *O. patagonica* and *C. caespitosa,* revealed a strong correlation with
393 temperature in the western Mediterranean, with a maximum peak in summer and gamete
394 release occurring at the end of the summer, when the temperature decreases (Fine et al. 2001;
395 Kersting et al. 2013). In the present study the maximum temperature peak occurred in August
396 and the gamete release seems to take place between August-September, when temperatures
397 rapidly decrease and the Chl-a values are displaying minimum values. In October, Chl-a
398 concentrations rapidly increase, which could be beneficial for the larvae (if they were to be
399 planktotrophic) that could take advantage of better nutritional conditions in the surrounding
400 waters. This increase in Chl-a concentration, which extends until April, would be beneficial for
401 the start of the gametogenesis in the early months of the year, allowing to accumulate reserves
402 for further gamete development. In corals (and other organisms) it appears that food availability
403 is the most important trigger influencing the timing of gametogenesis, while other factors, such
404 as temperature, cue spawning (Feehan et al. 2019 and references therein).
405 *Dendrophyllia ramea* presents relatively low PRF values (PRF máx.: 925 opp) compared to
406 other CWCs, and DSC whose values are one order of magnitude higher (Table 4). Nevertheles,
407 similar fecundities have been documented in the NE Atlantic for *C. seguenzae* (in the higher
408 part of the range) and *F. angulare* (Table 4, Waller et al. 2005; Mercier et al. 2011 respectively).
409 Some authors suggest that depth can be a factor constraining fecundity (Flint et al. 2007; Waller
410 et al. 2002, 2008). However, when comparing different species this is not always the case. For
411 instance, *C. seguenzae* and *F. angulare* were sampled at much larger depths (1240-1409 m,
412 925-1430 m respectively, see Table 4) than *D. ramea* (33-37 m) in this study, yet they present
413 similar fecundity values. On the contrary, *D. ramea* and *D. dianthus* sampled at very similar

depths (33-37 m in this study and 18-27 m in Feehan et al. 2019 respectively) revealed very different fecundity values, with a much higher gamete production in *D. ramea* than in *D. dianthus* (925 opp *vs*. 2448-172, 328 opp, see Table 4 and Feehan et al. 2019). However, it is worth mentioning that the comparison of fecundity among species is not always possible, as the applied methodologies are frequently different. For instance, Goffredo et al. (2002, 2006, 2011) calculated fecundity for Mediterranean temperate corals using a formula that considers the length of the “ovary” (which is the mesentery where the oocytes develop) and the frequency and diameter of mature oocytes; other authors calculate the fecundity at colony level (Brooke and Young 2003). In studies conducted with *F. marenzelleri* (Flint et al. 2007; Waller et al. 2002) and *M. oculata* (Waller and Tyler 2005), more than one oocyte cohort have been detected; yet, fecundity was calculated considering the total number of opp (PRF) (Flint et al. 2007; Waller et al. 2002; Waller and Tyler 2005). There is only one study regarding scleractinian CWCs (*F. angulare*, NW Atlantic), by Mercier et al. (2011), in which fecundity was measured by taking into consideration only the mature oocytes (ERF). The study revealed very high values, with a maximum of 10,000 opp, two orders of magnitude higher than the results obtained in our study for *D. ramea* (ERF max.: 536 opp). Up to date , maximum oocyte size diameter for scleractinian CWCs ranges from 100 µm (Brooke and Young 2003) to 5,200 µm (Waller et al. 2008). The maximum oocyte diameter measured for *D. ramea* (617 µm) is in the middle part of the range for CWc and DSC, as from the 17 species investigated (in some cases the same species was analysed for different areas), 12 species have smaller oocytes, whereas the other 12 have larger oocytes than *D. ramea.* The largest diameter (4,800 - 5,167 µm) corresponds to the Antarctic populations of the genus *Caryophyllia*. This might be related to the large lipid deposits included in the oocytes, a typical adaptation for cold-water organisms (Waller et al. 2008).
No relationship has been detected between fecundity and polyp size, considering both polyp calyx diameter and height. In general, the reproductive output is related to body size in most marine invertebrates (Gage and Tyler 1991; Hall and Hughes 1996). However, this does not

seem to apply to CWC and DSC species, as no correlation has been detected for *D. dianthus*, *L. pertusa*, *M. oculata* and *C. ambrosia* (Feehan et al. 2019; Waller et al. 2005; Waller and Tyler 2005), although there are exceptions. For instance, a positive correlation has been found in some solitary CWC species: *F. marenzelli* (Waller et al. 2002), *F. angulare* (Mercier et al. 2011; Waller and Tyler 2011) and *F. alabastrum* (Waller and Tyler 2011), which suggests that this aspect of CWC reproduction needs to be further investigated.

Conclusions

This is one of the first investigations dedicated to describe the reproductive characteristics and gametogenic cycle of a colonial azooxanthellate mesophotic and DSC species from the Mediterranean: *Dendrophyllia ramea.* This coral is gonochoric and the absence of larva suggests a broadcasting reproductive strategy. *Dendrophyllia ramea* presents two oocyte cohorts in winter, spring and early summer months, whereas in October (autumn) a single oocyte cohort is detected in the polyps, suggesting a seasonal reproduction, with spawning taking place in late summer/early autumn. Although this could not be proved with the currently available data, the beginning of the oogenesis and spawning season seems to be related to the higher Chl-a values, which might be beneficial for the coral colonies, as it implies a higher availability of food supply. This is indeed, a factor of paramount importance to promote the energetically costly process of the gametogenesis and larvae production. Knowledge on the reproduction of CWCs is still scarce but absolutely necessary to better understand the functionality of these organisms and population dynamics, as well as to design any potential protection and restoration measures.

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Table 1(on next page)

Dendrophyllia ramea sampling in Punta de La Mona

All samples have been collected in the same sampling location (36º43´25´´N; 003º 43´56´´W). Table includes depth (m), date (day/month/year) and the number of colonies per each sex. F: female colonies, M: male colonies, NI: sex non-identified, T: total number (colony/polyp), C: colony number, P: polyp number.

Table 1: *Dendrophyllia ramea* samples collected in Punta de La Mona.

All samples have been collected in the same sampling location (36º43´25´´N; 003º 43´56´´W). Table includes depth (m), date (day/month/year) and the number of colonies of each sex analysed. F: female colonies, M: male colonies, NI: sex non-identified, T: total number, C: Number of colonies, P: Number of polyps.

	Depth (m)	Date	F		M		NI		T	
			C	P	C	P	C	P	C	P
	36-37	27/05/17	3	9	3	3	0	0	6	12
	34-36	02/10/17	3	9	2	2	3	6	5	11
	36-37	03/02/18	3	9	3	3	1	1	6	12
	33-34	29/07/18	3	9	3	3	1	1	6	12
Total C/P			12	36	11	11	5	8	23	47

Table 2(on next page)

Gamete maturation stages for females and males of *Dendrophyllia ramea*

Maturation stages display in the table have been modified and adapted from previous studies by Feehan et al. (2019); Mercier and Hamel (2011); Waller et al. (2008); Waller and Tyler (2005).

Table 2: Gamete maturation stages for female and male colonies of *Dendrophyllia ramea*.

Maturation stages display in the table have been modified and adapted from previous studies by Feehan et al. (2019); Mercier and Hamel (2011); Waller et al. (2008); Waller and Tyler (2005).

Gamete	Maturation stage	Characteristics of the different maturation stages
Oocyte	Stage I	Previtellogenic oocytes- small oocytes (<42 μm) with basophilic cytoplasm.
	Stage II	Early vitellogenic oocytes (42-160 μm) - small yolk granules visible in the cytoplasm. Cortical granular layer surrounding oocyte absent or incompletely formed.
	Stage III	Late vitellogenic oocytes (160–350 μm)- characterized by displacement of the nucleus from its central position. Abundant yolk granules in the cytoplasm cortical granular layer appear fully defined.
	Stage IV	Mature oocytes- >350 μm in mean diameter. The nucleus is totally displaced towards the animal pole.
Spermatocytes	Stage I	Early- only loosely packed spermatocytes could be seen.
	Stage II	Maturing- spermatocytes and spermatids are observed in a centripetal maturation gradient. Only some spermatozoa tails are visible in the lumen.
	Stage III	Mature- lumen filled with densely packed spermatozoa, with the presence of spermatids in the periphery of the spermatocyst.
	Stage IV	Spent - only relict spermatozoa inside the spermatocyst.

Table 3(on next page)

Results of the Kruskal-Wallis and post hoc Dunn tests, for the average oocyte diameter per month

Table 3: Results of the Kruskal-Wallis and post hoc Dunn tests, for the average oocyte diameter per month.

Kruskal- Wallis			
H	P		
277.69	2.2e-16		
Dunn post hoc			
	October	July	May
February	0.0512	2.2e-16	2.2e-16
May	2.2e-14	0.0742	
July	2.2e-16		

Table 4(on next page)

Reproductive strategies of the species belonging to the Dendrophylliidae family (modified from Goffredo et al. 2010)

h= hermaphrodite; g= gonochoric; ? = unknown; b= brooder; bs= broadcast spawner.

Table 4: Reproductive characteristics of Cold-water corals.

The table is arranged after solitary (S) and colonial (C) species and following the alphabetical order in each category. h= hermaphrodite, g= gonochoric, bs= broadcaster spawner, b= brooder, opp= oocytes per polyp, oo cm^2= oocytes per cm^2. ERF= Effective Relative Fecundity.

*PRF (Potential Relative Fecundity)

Species	Area	Sampling depth (m)	Species depth range (m)	Sex. pattern	Rep.mode	Max oocyte diameter (µm)	Fecundity (ERF) (opp or oo cm^2)	Rep. strategy	Time spawning	Refs.
Caryophyllia cornuformis S	NE Atlantic (Porcupine Seabight)	1,650-2,017	435-2,000	h	bs	350	?	Quasi-continuous	?	Waller et al. 2005
Caryophyllia ambrosia S	NE Atlantic (Porcupine Seabight)	2,315-2,713	1,100-3,000	h	bs	700	200-2,750 opp	Quasi-continuous	?	“
Caryophyllia seguenzae S	NE Atlantic (Porcupine Seabight)	1,240-1,404	960-1,900	h	bs	450	52-940 opp	Quasi-continuous	?	“
Flabellum alabastrum S	NE Atlantic (Rockall Trough)	170-2,190	357-2,000	g	bs	925	2,800 opp (monthly average fecundity)	Quasi-continuous	?	Waller and Tyler 2011
Flabellum angulare S	NW Atlantic (Canada)	925–1,430	900-3186	g	bs	1,200	1,800–10,000 opp	Seasonal	Apr-Jun (spring-summer)	Mercier et al. 2011

“	NE Atlantic (Porcupine Seabight)	2,412–2,467	900-3,186	g	bs	1,015	550 opp (max average fecundity March)	Seasonal / periodic	Aug-Sep (late summer)	Waller and Tyler 2011
Flabellum curvatum S	Antarctica (western Antarctic Peninsula)	500–700	115-1,137	g	b	5,120	1,618±1,071 opp	?	?	Waller et al. 2008
Flabellum impensum S	Antarctica (western Antarctic Peninsula)	270–300	46-2,270	g	b	5,200	1,270±884 opp	?	?	“
Flabellum thouarsii S	Antarctica (western Antarctic Peninsula)	270–650	71-600	g	b	4,800	2,412±1,554 opp	?	?	“
Fungiacyathus marenzelleri S	NE Atlantic (Rockall Trough)	2,200	300–6,238	g	bs	750	2,892±44.4 opp	Quasi-continuous	Jun-Jul (summer)	Waller at al. 2002
“	NE Pacific (California)	4,100	300–6,238	g	bs	750	1,290±407 opp	Quasi-continuous	?	Flint et al. 2007
“	Antarctica (western Antarctic Peninsula)	520-800	300–6,238	g	bs	1,400	2,837±121 opp	Quasi-continuous	?	Waller and Feehan 2013

Dendrophyllia ramea C	W Mediterranean (Alborán Sea)	33-37	40-150 m	g	bs	450	187.6± 81.9 opp	Seasonal	August-Sep (late summer)	This study
Desmophyllum dianthus C	SE Pacific (Patagonian fjords)	18-27	8-2,500	g	bs	380	2,448-172,328 opp*	Seasonal	Aug-Sep (late winter)	Feehan et al. 2019
Enallopsammia rostrata C	SW Pacific(New Zealand)	890-1,130	110-2,165	g	bs	400	>144 opp	Continuous	Apr-May (autumn)	Burgess and Babcock 2005
"	SWAtlantic (Brazil)	565-639	110-2,165	g	bs	1,095	?	Continuous	?	Pires et al. 2014
Goniocorella dumosa S	SW Pacific(New Zealand)	890-1,130	88-1,488	g	bs	135	>480 opp	Seasonal	Apr-May (autumn)	Burgess and Babcock 2005
Lophelia pertusa C	NE Atlantic (Porcupine Seabight)	785-980	100-2,000	g	bs	140	3,146-3,327 oo cm^2	Seasonal	Jan–Feb (winter)	Waller and Tyler 2005
"	NE Atlantic (Norway, Trondheim fjord)	40-500	100-2,000	g	bs	180	?	Seasonal	Jan-Mar (winter-spring)	Brooke and Järnegren 2013
"	SW Atlantic (Brazil)	565-639	100-2,000	g	bs	242	?	Seasonal /periodic	May–Jul (autumn-winter)	Pires et al. 2014

Madrepora oculata C	NE Atlantic (Porcupine Seabight)	870-925	50-3,600	g	bs	350	10-68 opp / 256 oo cm^2	Periodic	?	Waller and Tyler 2005
"	SW Pacific (New Zealand)	890-1,130	50-3,600	g	bs	?	?	?	?	Burgess and Babcock 2005
"	SW Atlantic (Brazil)	565-639	50-3,600	g	bs	650	?	Continuous	?	Pires et al. 2014
Oculina varicosa C	NW Atlantic (Florida)	80-100	3-100	g	bs	100	2,115-4,693 oo cm2	Periodic	Aug-Sep (late summer)	Brooke and Young 2003
Solenosmilia variabilis C	SW Pacific(New Zealand)	890-1,130	220-2,165	g	bs	165	>290 opp	Seasonal	Apr-May (autumn)	Burgess and Babcock 2005
"	SW Atlantic (Brazil)	565-639	220-2,165	g	bs	337	?	Continuous	?	Pires et al. 2014

6

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Table 5(on next page)

Reproductive characteristics of the up to date investigated deep-sea corals

opp= oocytes per polyp, oo cm2=oocytes per square cm. The table is arranged after solitary (S) and colonial (C) species and following the alphabetical order in each category. h= hermaphrodite, g= gonochoric, bs= broadcaster spawner, b= brooder, opp= oocytes per polyp, oo cm^2= oocytes per cm^2. ERF= Effective Relative Fecundity. *PRF (Potential Relative Fecundity)

Table 5: Reproductive strategies of the species belonging to the Dendrophylliidae family (modified from Goffredo et al. 2010).

h= hermaphrodite; g= gonochoric; ? = unknown; b= brooder; bs= broadcast spawner.

Species	Sexual pattern	Reproductive mode	References
Astroides calycularis	g	b	Goffredo et al. 2010, 2011; Terrón-Sigler 2016
Astroides calycularis	h	b	Lacaze-Duthiers 1873
Balanophyllia elegans	g	b	Beauchamp 1993; Fadlallah 1981, 1983b; Fadlallah and Pearse 1982a
Balanophyllia europaea	h	b	Goffredo et al. 2000, 2002; Goffredo and Telò 1998
Balanophyllia regia	?	b	Fadlallah 1983a; Kinchington 1981; Lacaze-Duthiers 1897; Lyons 1973; Yonge 1932,
Balanophyllia sp.	?	b	Abe 1937; Fadlallah 1983a; Richmond and Hunter 1990
Cladopsammia rolandi	h	b	Fadlallah 1983a; Lacaze-Duthiers 1897
Cladopsammia gracilis	?	b	Hizi-Degany et al. 2007
Dendrophyllia manni	?	b	Edmondson 1929, 1946; Fadlallah 1983a; Richmond and Hunter 1990

Dendrophyllia ramea	g	bs	This study
Dendrophyllia sp.	g	b	Babcock et al. 1986; Richmond and Hunter 1990
Heteropsammia aequicostatus	g	bs	Harriott 1983; Richmond and Hunter 1990
Heteropsammia cochlea	g	bs	Harriott 1983; Richmond and Hunter 1990
Leptopsammia pruvoti	g	b	Goffredo et al. 2005; Lacaze-Duthiers 1897
Rhizopsammia minuta	?	b	Abe 1939; Fadlallah 1983a
Stephanophyllia formosissima	?	b	Fadlallah 1983a; Moseley 1881
Tubastrea aurea	?	b	Edmondson 1929, 1946; Fadlallah 1983a; Fan et al. 2006
Tubastrea coccinea	h	b	Creed and De Paula 2007; Edmondson 1929, 1946; Glynn et al. 2008; Hunter 1990; Jokiel et al. 1985, Richmond and Petersen et al. 2007
Tubastrea faulkneri	g	b	Babcock et al. 1986; Richmond and Hunter 1990
Tubastrea tagusensis	?	b	Creed and De Paula 2007
Turbinaria bifrons	?	bs	Babcock et al. 1994
Turbinaria frondens	g	bs	Babcock et al. 1994; Richmond and Hunter

			1990; Willis et al. 1985; Wilson and Harrison 2003
Turbinaria mesenterina	?	bs	Babcock et al. 1994
Turbinaria peltata	?	bs	Babcock et al. 1994
Turbinaria radicalis	?	bs	Babcock et al. 1994; Wilson and Harrison 2003
Turbinaria reniformis	g	bs	Babcock et al. 1994; Petersen et al. 2007; Richmond and Hunter 1990; Willis et al. 1985

5

6

Figure 1

Underwater image of a *Dendrophyllia ramea* colony from study area (scale bar = 5 cm).

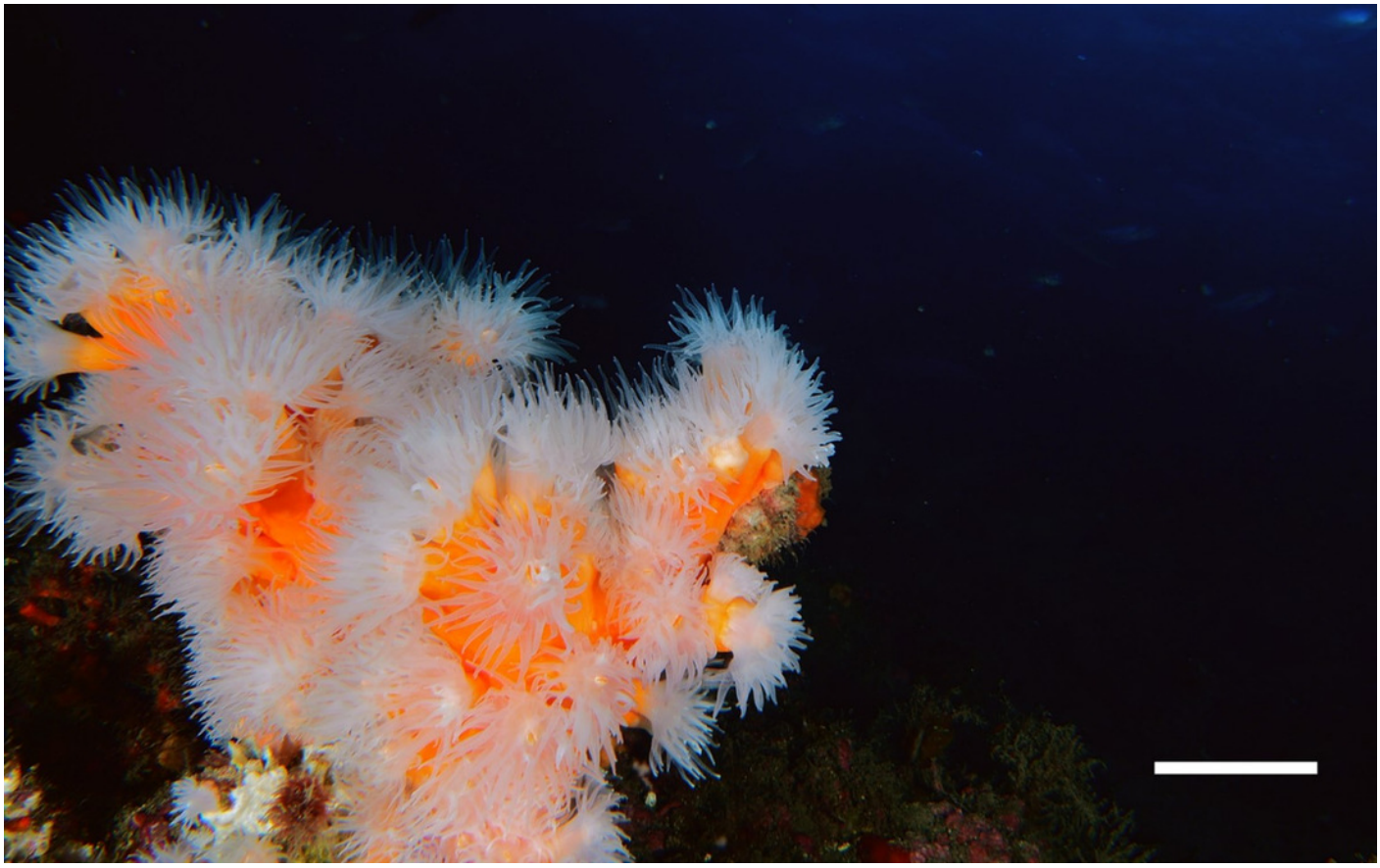


Figure 2

Study area.

(a) Location of the study area, (b) shows the specific site where the sampling took place close to the coast of Granada Punta de la Mona (36º43´25´´N; 003º 43´56´´W) (Figure has been created by A. Terrón-Sigler).

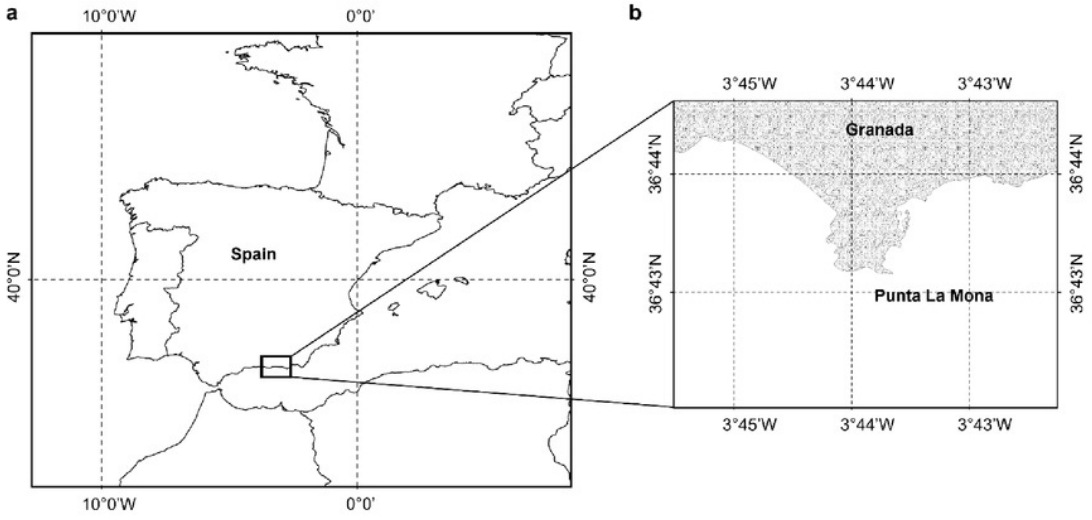

a
10°0'W
0°0'
Spain
40°0'N
b
3°45'W
3°44'W
3°43'W
Granada
36°44'N
36°43'N
Punta La Mona

Figure 3

Histological sections of *Dendrophyllia ramea* reproductive tissues of a male colony.

(a) February 2018, SI= Stage I; (b) May 2017, SI= Stage I; (c-e) July 2018, SI, II, III and IV= Stages I, II, III and IV; (f) October 2017, SIV= Stage IV. *st*= spermatocyte, *sd*= spermatid, *sz*= spermatozoa. Scale bars: a, b, d and f= 50µm; c and e= 100 µm. Note the results have been arranged by month, although the sampling year was different.

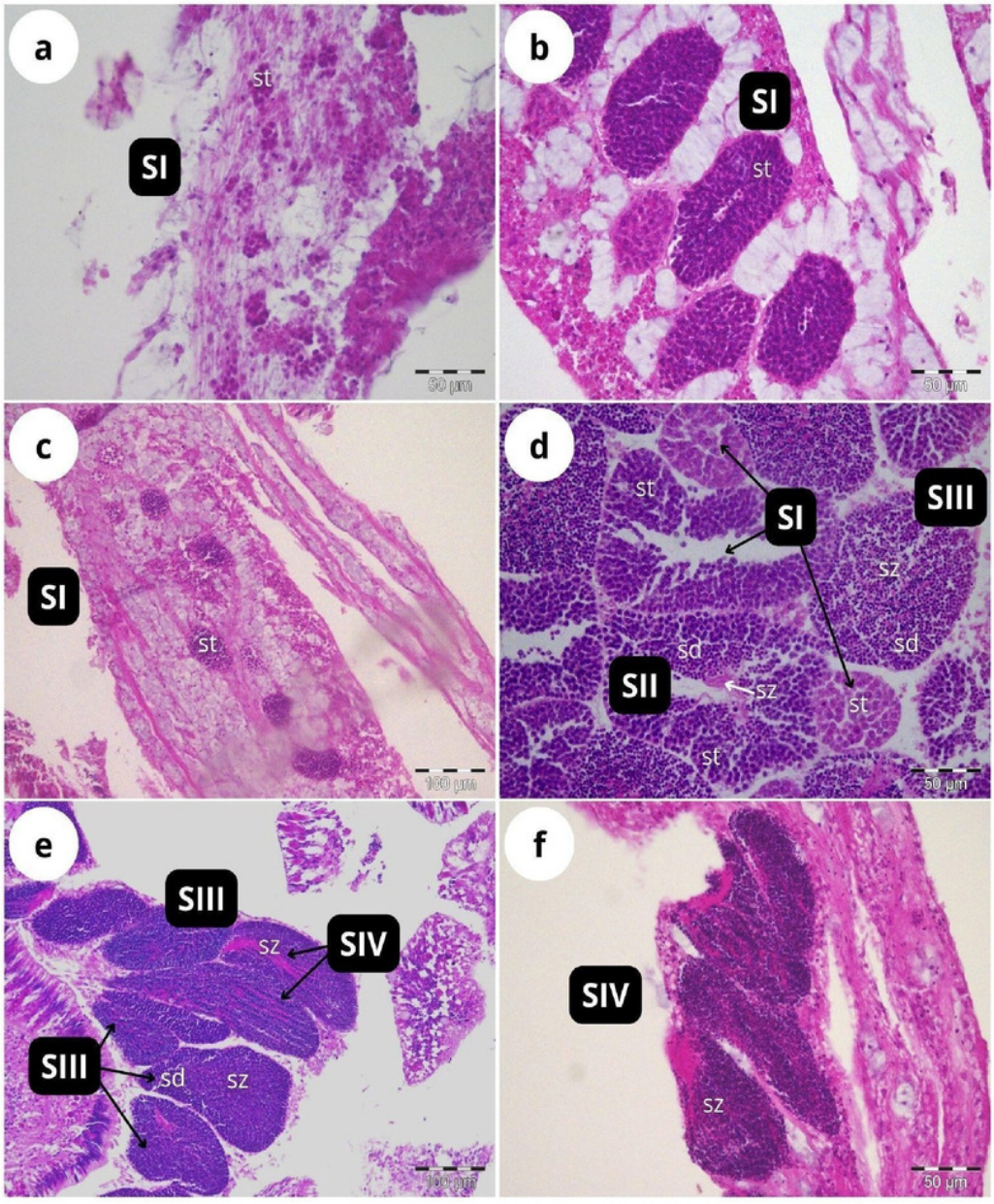

a
st
SI
50 µm
b
SI
st
50 µm
c
SI
st
100 µm
d
st
SI
SIII
sz
sd
sd
SII
sz
st
st
50 µm
e
SIII
sz
SIV
SIII
sd
sz
100 µm
f
SIV
sz
50 µm

Figure 4

Proportion of the different stages of development of spermatocysts and oocytes of *Dendrophyllia ramea*

(a) spermatocysts (100 spermatocysts) and (b) oocytes for each sampled month (361 oocytes in February 2018, 386 in May 2017, 302 in July 2018 and 207 in October 2017). Spermatocysts and oocytes were analysed from three colonies for each sampled month and each sex, with the exception of October where only two male colonies were available. Note the results have been arranged by month, although the sampling year was different.

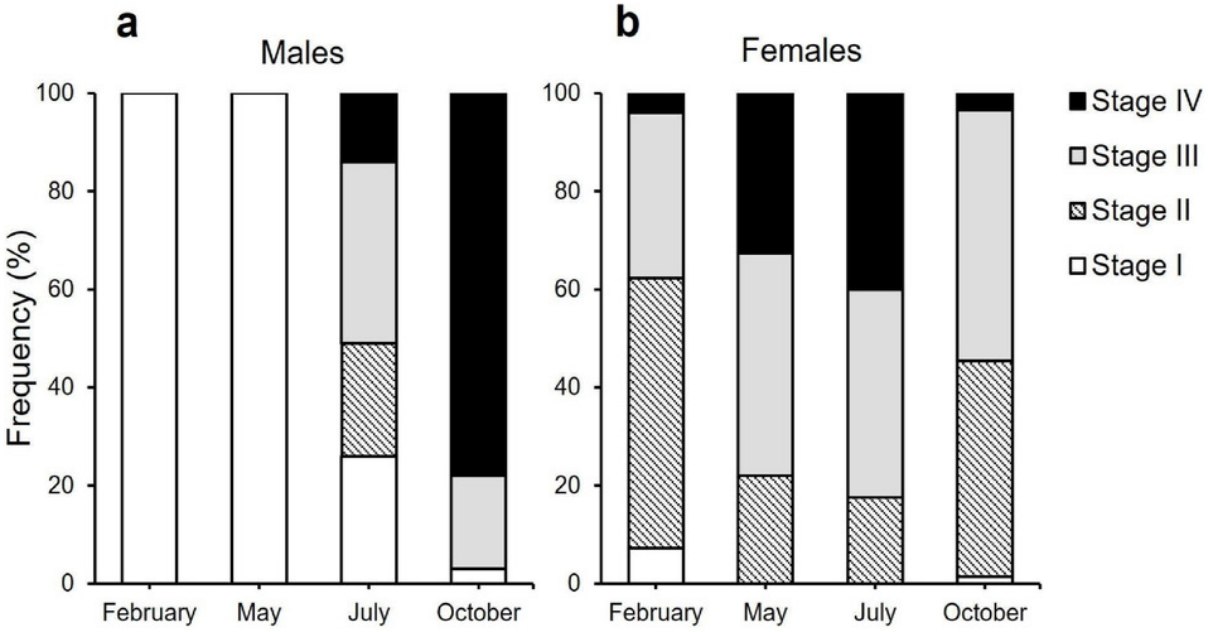

a
Males
b
Females
Frequency (%)
100
80
60
40
20
0
February
May
July
October
Stage IV
Stage III
Stage II
Stage I

Figure 5

Histological sections of *Dendrophyllia ramea* reproductive tissues of a female colony

(a-b) February 2018, SI and S II; c-d: May 2017, S II, S III, SIV; e-f: July 2018, S III and SIV; (g-h) October 2017, SI and SIII. pv= previtellogenic oocyte, evo= early vitellogenic oocyte, lvo= late vitellogenic oocyte, mo= mature oocyte, fc= follicular cells, co= chorion, y= vitellum, N= nucleus, n= nucleolus. Scale: c,e= 200µm, d,h= 100µm, a,b= 50µm, f,g= 20µm. Note the migrated nucleolus (pointed out with an arrow) in panel. Note the results have been arranged by month, although the sampling year was different.

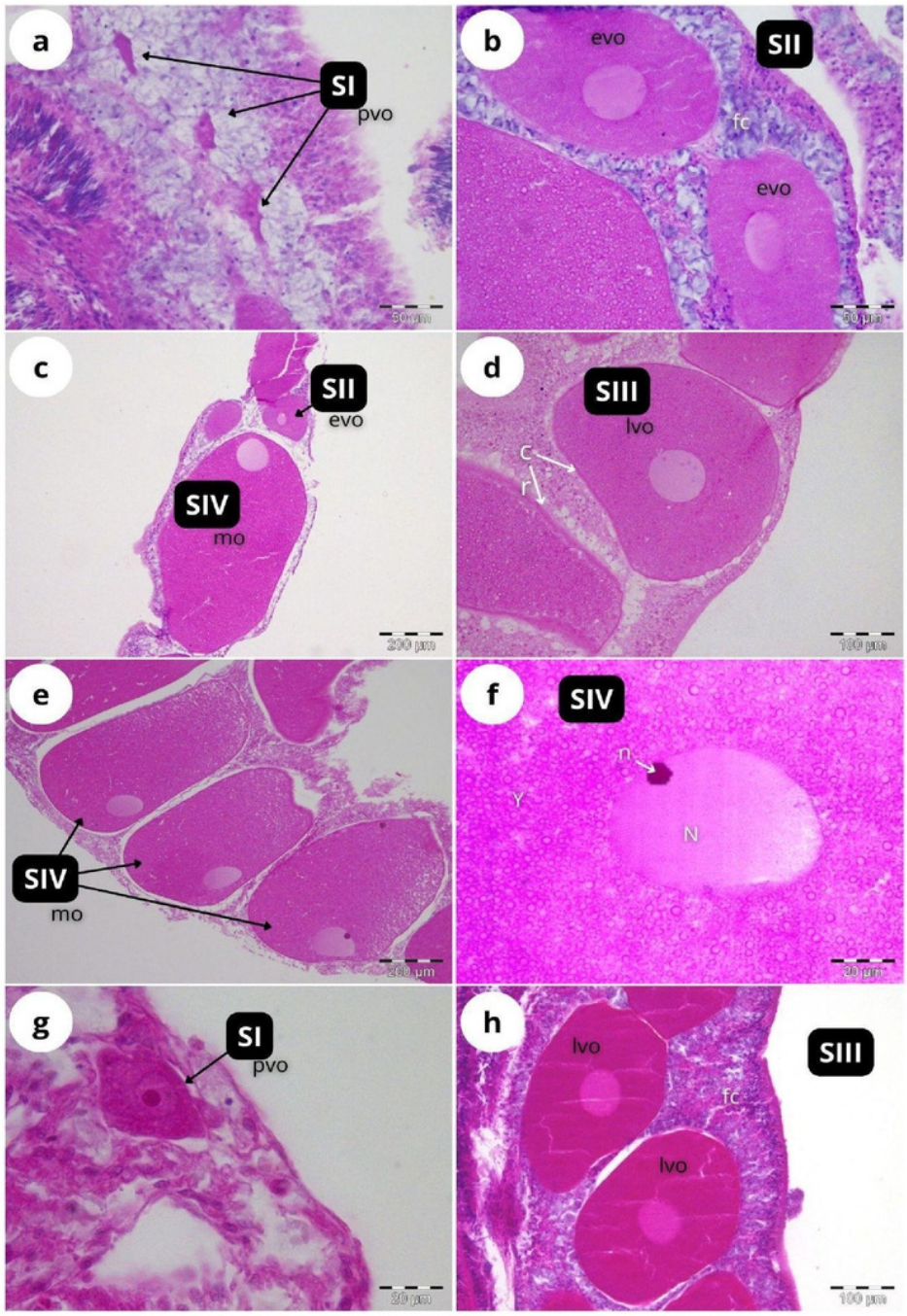

a
SI
pvo
b
evo
SII
fc
evo
c
SII
evo
SIV
mo
d
SIII
lvo
c
r
e
SIV
mo
f
SIV
n
Y
N
g
SI
pvo
h
lvo
SIII
fc
lvo

Figure 6

Oocyte size (mean ± SD) for the three female colonies analyzed per sampling month.

Number of measured oocytes was 361 oocytes for February 2018, 386 for May 2017, 302 for July 2018 and 207 for October 2017.

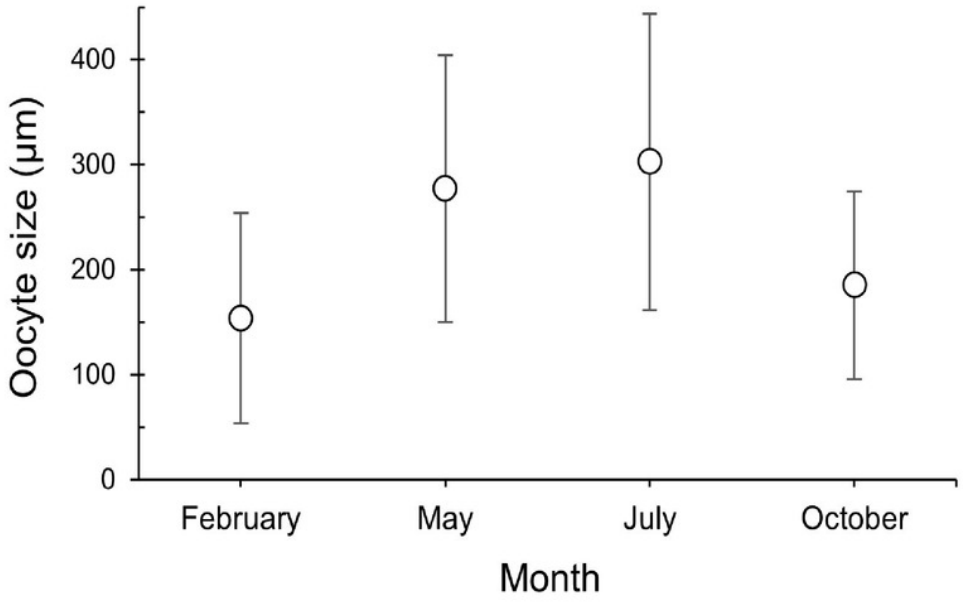

Oocyte size (µm)
400
300
200
100
0
February
May
July
October
Month

Figure 7

Oocyte mean diameter (±SD) size-frequency distributions for *D. ramea* in all the sampled months

N= colony number, *n*= oocyte number. Dashed lines indicate the two cohorts 1 (black) and 2 (grey). Note that May and October samples were collected in 2017 and February and July samples were collected in 2018.

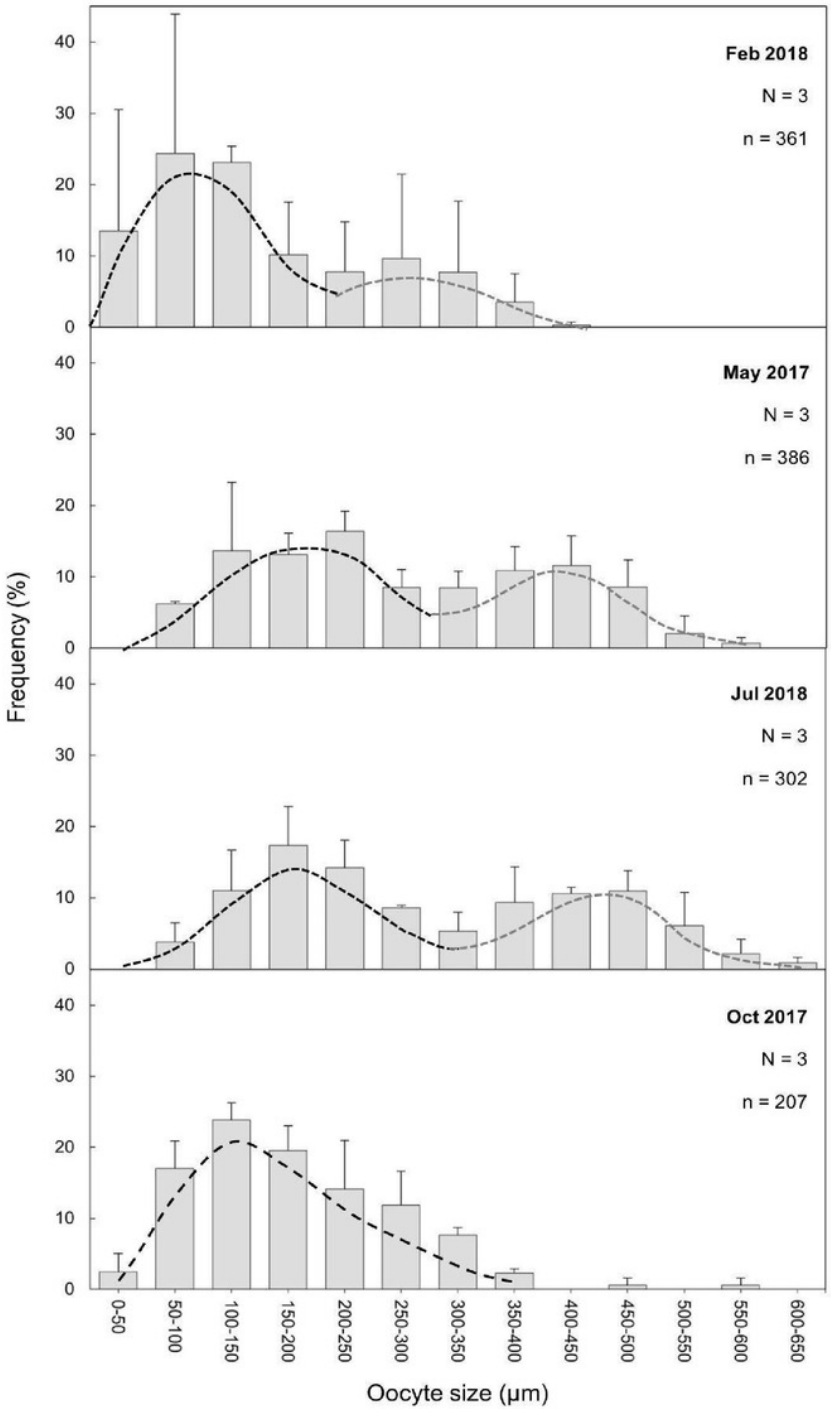

Feb 2018
N = 3
n = 361
May 2017
N = 3
n = 386
Jul 2018
N = 3
n = 302
Oct 2017
N = 3
n = 207
Frequency (%)
0
10
20
30
40
0-50
50-100
100-150
150-200
200-250
250-300
300-350
350-400
400-450
450-500
500-550
550-600
600-650
Oocyte size (µm)

Figure 8

Mean (±SD) Potential Relative fecundity (PRF, grey line) and Mean (±SD) Effective Relative fecundity (ERF, black line) for *D. ramea* for each sampling month.

(a) Mean (±SD) Potential Relative fecundity (PRF, grey line) and Mean (±SD) Effective Relative fecundity (ERF, black line) for *D. ramea* for each sampling month, b) PRF for each analyzed colony (each analysed colony is indicated in the X axis), c) ERF for each analyzed colony. Note that May and October samples were collected in 2017 and February and July samples were collected in 2018.

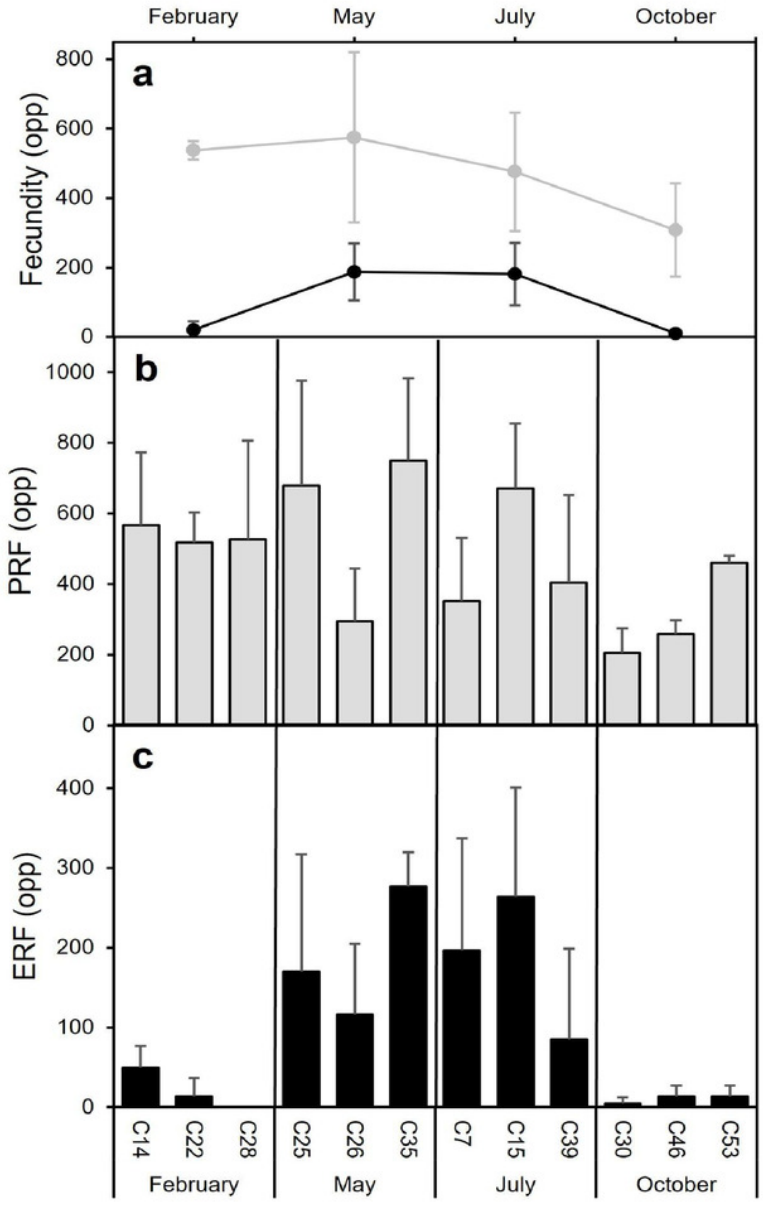
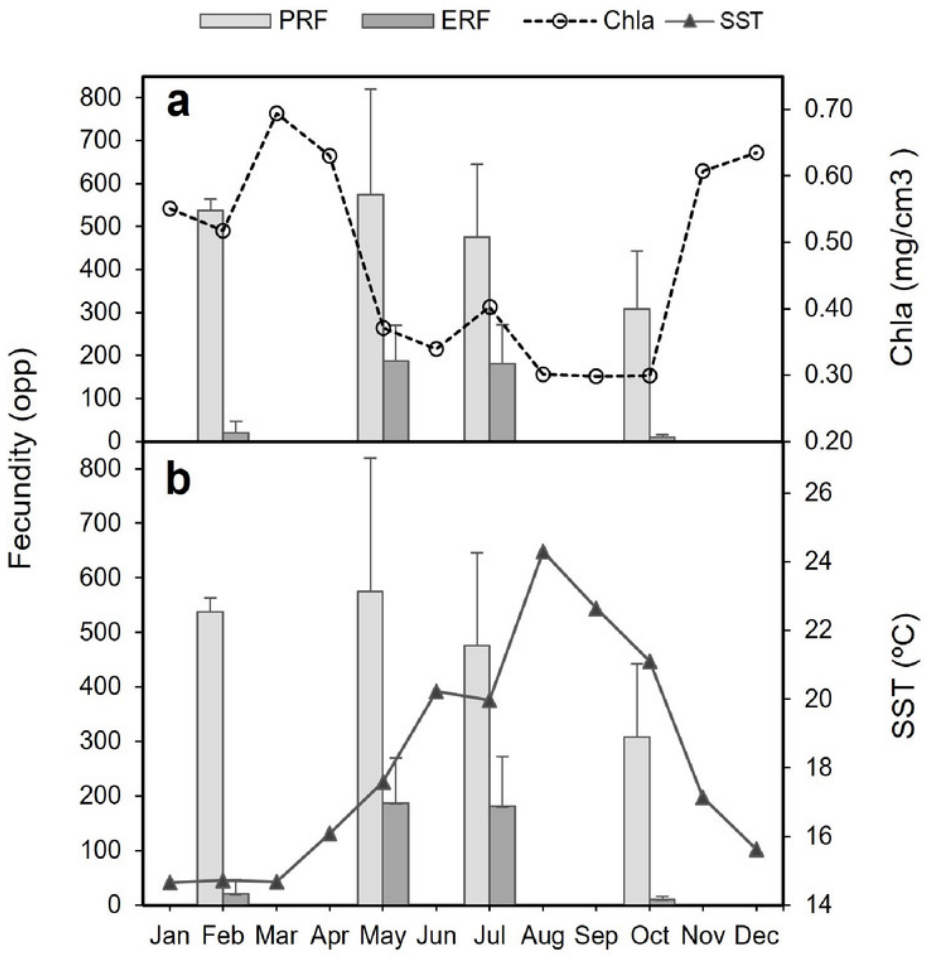

February
May
July
October
a
Fecundity (opp)
800
600
400
200
0
b
PRF (opp)
1000
800
600
400
200
0
c
ERF (opp)
400
300
200
100
0
C14
C22
C28
C25
C26
C35
C7
C15
C39
C30
C46
C53
February
May
July
October

Figure 9

Potential relative fecundity (PRF) and Effective relative fecundity (ERF) of *D. ramea* for the samples months, against Chl-a and Sea Surface Temperature values.

a) Potential relative fecundity (PRF) and Effective relative fecundity (ERF) for the samples months and Chl-a, b) Potential relative fecundity (PRF) and Effective relative fecundity (ERF) for the samples months and SST. Note that May and October samples were collected in 2017 and February and July samples were collected in 2018.


PRF
ERF
Chla
SST
a
b
Fecundity (opp)
Chla (mg/cm3)
SST (°C)
0
100
200
300
400
500
600
700
800
0.20
0.30
0.40
0.50
0.60
0.70
14
16
18
20
22
24
26
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec