

## Reproductive patterns in deep versus shallow populations of the precious Mediterranean gorgonian *Corallium rubrum* (Linnaeus, 1758) (Sardinia, Central-Western Mediterranean)

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Handling Editor: Artemis Nicolaidou

Received: 21 July 2016; Accepted: 30 November 2016; Published on line: 13 February 2017

### Abstract

This study quantifies the main reproductive features of the long-lived red coral *Corallium rubrum*, an octocoral endemic to the Mediterranean Sea and neighbouring Atlantic areas and one of the most valuable of all marine species, at different depths (38-40 m versus 96-115 m) in the north-western Sardinian waters (Central-Western Mediterranean Sea). Different population structures were observed with shallow colonies smaller (in basal diameter and height) than deeper ones. Both populations were all gonochoric at polyp and colony level and fertile. The sex-ratio was balanced in the deep red corals while in the shallow ones it was significantly biased towards female. Deep and shallow colonies were found to have a synchronous female sexual products development in the two depth ranges investigated. *C. rubrum* produced large female sexual products in both depth intervals in relationship with its reproductive mode, confirming a long oogenic development. In contrast to reproductive timing, the volume occupied by female sexual products per polyp was different between the populations, being higher in the deep than in the shallow populations. *C. rubrum* exhibited a reproductive strategy characterized by a relative low number of female sexual products per polyp revealing significant differences among the two depth ranges analyzed with a lower mean value of sexual products in the shallow colonies (mean fecundity per polyp: 1.14) than in the deeper ones (mean fecundity per polyp: 2.09). These results indicate differences in reproductive parameters such as sexual products distribution and reproductive output (fecundity) which were observed between shallow and deep populations of *C. rubrum*, in particular within deep dwelling populations.

**Keywords:** Octocoral, red coral, sex-ratio, fecundity, Mediterranean Sea.

### Introduction

Over the past three decades, there has been a substantial increase in research into benthic marine community. Within this topic, knowledge of the reproductive biology of marine invertebrates provides the basis for understanding many aspects of their life-history evolution, population, biology and community structure (Grosberg & Levitan, 1992; Hughes *et al.* 1992) and it is also necessary to supply data for conservation and management plans (Torrents & Garrabou, 2011). Fecundity, defined as the potential reproductive capacity of populations and quantified by the number of sexual products during a reproductive cycle, is one of the most important reproductive traits that usually implies a high energetic investment for living organisms (Torrents & Garrabou, 2011).

Aposymbiotic anthozoan species depend on water circulation and trophic content (e.g. zooplankton, particulate organic matter, etc.) for their energetic inputs (Sebens, 1987). Accordingly, species dwelling in habitats exposed to varying environmental conditions (dependent on environmental features such as water temperature, current speed, light, and the quantity and quality of available food or different depth ranges) showed significant

changes in their life history traits (Sebens, 1987; Gili & Coma, 1998; Gardner, 2000; Gori *et al.*, 2012).

Red coral *Corallium rubrum* (Linnaeus, 1758) is a slow growing, long-lived and habitat-structuring gorgonian, as well as one of the most vulnerable resources in the Mediterranean Sea, because of its use in jewellery worldwide. This precious modular anthozoan (Octocorallia, Alcyonacea) is endemic to the Mediterranean and neighbouring Atlantic rocky shores and can be found at depths ranging from 5 to 820 m (Costantini *et al.*, 2010), but more commonly between 30 and 200 m (Carpine & Grasshoff, 1975; Rossi *et al.*, 2008; Taviani *et al.*, 2010). *C. rubrum* is an iteroparous, dioecious and gonochoric species with few cases of hermaphroditic polyps (Priori *et al.*, 2013). Male colonies release floating sperm cells that find their way to female specimens, where internal fertilization takes place. Red coral is an internal brooder, reproduces annually during spring-early summer (Vighi, 1970, 1972; Santangelo *et al.*, 2003; Tsounis *et al.*, 2006a) and planulae have limited dispersal capabilities (Weinberg, 1979; Bramanti *et al.*, 2005). Moreover, this species is characterized by a higher recruitment rate than most of other octocoral species (Garrabou & Harmelin, 2002; Bramanti *et al.*, 2007) and reaches sexual maturity

at 3–4yr (Santangelo *et al.*, 2003; Gallmetzer *et al.*, 2010; Bramanti *et al.*, 2014).

An operational distinction has been made between shallow and deep red coral populations (Santangelo & Abbiati, 2001; Costantini *et al.*, 2010). In general, in shallow areas, because of the stronger harvesting pressure (Garrabou & Harmelin, 2002; Tsounis *et al.*, 2006a; Rossi *et al.*, 2008; Bavestrello *et al.*, 2009; Montero-Serra *et al.*, 2015), the populations (above a depth of 50 m) show more dense patches with smaller colonies compared to the deep ones (below a depth of 50 m), which are more sparse and large sized. In addition, different genetic structure among populations dwelling at different depths was reported (Cannas *et al.*, 2011; Costantini *et al.*, 2011; Cannas *et al.*, 2016).

Studies on demography (Santangelo & Abbiati, 2001; Garrabou & Harmelin, 2002), reproduction (Vighi 1972; Santangelo *et al.*, 2003; Torrents *et al.*, 2005; Tsounis *et al.*, 2006a; Torrents & Garrabou, 2011; Bramanti *et al.*, 2014), recruitment, settlement and mortality (Garrabou and Harmelin 2002; Bramanti *et al.*, 2003, 2005, 2007), growth (Marschal *et al.*, 2004; Bramanti *et al.*, 2005; Gallmetzer *et al.*, 2010), distribution and space competition (Giannini *et al.*, 2003) and feeding ecology (Tsounis *et al.*, 2006b; Picciano & Ferrier-Pagès 2007) have contributed to a better understanding of the ecology of mainly red coral shallow populations. Because of the wide bathymetric range of this species, which leads to huge sampling limitations for deeper populations (Genin *et al.*, 1992), little attention is given to the deeper populations (50–200 m) (Rossi *et al.*, 2008; Cau *et al.*, 2015b, 2016; Angiolillo *et al.*, 2016; Benedetti *et al.*, 2016).

The island of Sardinia (Italy, central-western Mediterranean Sea) has always been a hotspot for red coral. Recent data on fishery in 20<sup>th</sup> century showed that, in 1991, Sardinia was the most productive site in the western Mediterranean Sea (3 t per year) (Santangelo *et al.*, 1993; Cannas *et al.*, 2010). However, the depletion of shallow-water coral banks led to the exploitation of deeper water banks (Chessa & Cudoni, 1989). The extensive studies undertaken with the ROV (Remote Operated Vehicle) along the Sardinian coasts (Follesa *et al.*, 2013; Cau *et al.*, 2015a) at depths >80 metres, documented a great variability in deep coral colony density within patches between 5.31 col. m<sup>-2</sup>±3.01 SD (2–19 colonies per patch) on the north-western coasts and 54.14 col. m<sup>-2</sup>±32.36 SD (2–97 colonies per patch) on the southeastern margin of the Sardinian continental shelf. This discrepancy was reflected also in coral population structure, showing different sizes patterns characterized by a high occurrence of large colonies, 76% of which had heights >10 cm on the northern coasts and small-medium sized colonies in the southern populations (6% with heights >10 cm). In addition, genetic analyses showed a high genetic diversity between Sardinian deep coral populations and corals from other western Mediterranean localities and even high

differentiation among Sardinian samples, distant from hundreds to less than 1 km (Cannas *et al.*, 2011, 2015, 2016). The highest genetic diversity recorded in Sardinia for all areas and depths with respect to other Mediterranean areas and the substantial demographic stability of the investigated populations (no evidence of bottlenecks) indicate that harvesting has not yet led to a substantial erosion of the genetic pool (Cannas *et al.*, 2015, 2016).

However, information on reproduction of deep colonies sampled over 50 m are very scarce, given only by Priori *et al.* (2013) in Tuscany Archipelago (North Tyrrhenian Sea). In this regard, the present research aims to analyze and compare reproductive features of shallow (<50 m) and deep (>90 m) red coral colonies in an area of north-western Sardinian waters. Specifically, this study focused on the following points: 1) population size structure; 2) sex-ratio; 3) sexual product production; 4) fertility and 5) male and female fecundity.

## Materials and Methods

### Study area and sampling

This study was carried out on rocky bottoms in north-western Sardinian waters (Fig. 1).

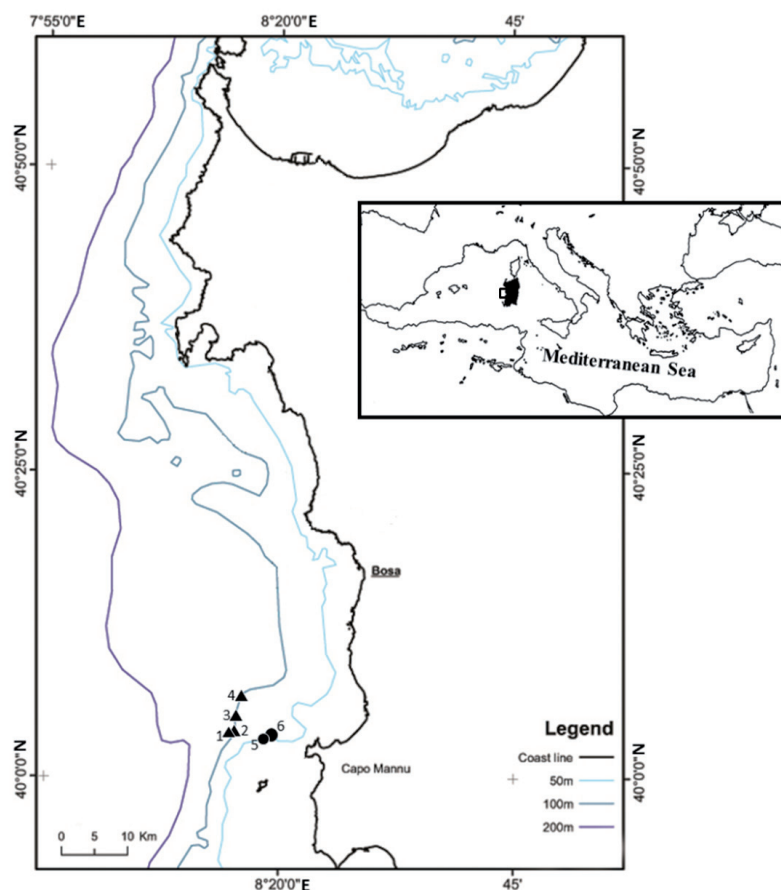
*C. rubrum* colonies were randomly sampled by SCUBA divers from Capo Mannu to Bosa. Two different depth ranges, 38–40 m (shallow) and 96–115 m (deep) were investigated. In order to avoid any potential effects of temporal variability, all colonies were taken between late May and early June 2010–2013 [just before the release season observed by Vighi (1972) and Santangelo *et al.*, (2003)] (Table 1).

### Reproduction features

For each examined colony, basal diameter (BD, in millimetre) and height (H, in centimetre) were registered. During each sampling, a 2–3 cm long apical tip of each colony was extracted and preserved on board in 5% buffered formaldehyde prior to further work in the laboratory. 10–15 polyps for each colony were analyzed for a total of 1486 polyps dissected in this study.

Although the term “gonads” has been widely used in anthozoan publications, we agree with Fautin and Mariscal (1991) and Orejas *et al.* (2007) that the so-called gonads are simply gametogenic areas of the mesenteries. In our opinion, the equivalence of each individual oocyte (or spermatid cyst) to a single gonad, in order to quantify fertility and other reproductive parameters, should be avoided in future contributions. Consequently, in this paper, the term “sexual products” has been used to refer to oocytes or spermatid cysts.

Polyps were randomly selected and the total number of sexual products were counted in each polyp’s gastrovascular cavity. Colonies that did not present gametogenic areas were considered sterile.



**Fig. 1:** Map of the study area, showing investigated locations (black dots: locations < 50 meters; black triangles: locations > 90 meters).

**Table 1.** Summary of the sampling design: sites, coordinates, depth, date and number of *Corallium rubrum* colonies of each sampled population.

Sites	Latitude (N)	Longitude (E)	Depth (m)	Date	N colonies
1(43 NI)	4005280°	814573°	113	11 June 2011	23
2(44 NI)	4005254°	814522°	115	11 June 2011	8
3(ACQP)	4008414°	818734°	100	15 June 2011	40
4 (01T13)	4016550°	819853°	96	05 June 2013	23
Total					<b>94</b>
5(ACQS1)	4003456°	823491°	38	26 May 2010	7
6(ACQS2)	4003457°	823464°	40	17 June 2011	48
Total					<b>55</b>

The dissection was done under the stereo microscope and sexual products were measured using an eyepiece calibrated against a stage micrometer. With the purpose of obtaining the female sexual products volume per polyp, the diameter of sexual products (d) was converted to volume (assuming that they were spherical,  $V = 4/3\pi(d/2)^3$ ), summed within each polyp, and then averaged over 10 polyps in each colony. The effects on the production per polyp among the factor colony size were checked on 28 deep female colonies and 32 shallow females colonies.

Furthermore, to confirm colony sex determination [previously identified from a macroscopic point of view

according to Lacaze-Duthiers (1864) and Vighi (1972)] and sexual product stage, two/three polyps were processed histologically. Each sample was processed as follows: it was dehydrated through a progressively higher series of alcohol concentrations (70-100%), embedded in a synthetic resin (GMA, Technovit 7100, Bio-Optica) and sectioned at 3.5  $\mu\text{m}$  with a rotary microtome (LKB, Histo-range). Subsequently, the sections were stained with Harris hematoxylin and eosin following the histological staining protocol proposed for glycol methacrylate embedded tissue sections by Cerri & Sasso-Cerri (2003).

## Reproductive parameters

Sex ratio (F:M) was calculated for each population and by depth range.

Fertility (number of fertile polyps/total number of polyps, %) and fecundity (number of mature sexual products per polyp) were estimated for the two depth ranges investigated and sex. During data analysis, polyp fecundity values were averaged for each colony. These fecundity values for each colony were considered as replicates within each studied population and were used in the statistical analysis. Relationships between colony size and fecundity were also considered.

## Statistical analyses

The Kolmogorov-Smirnov (KS) two-sampled test was used to test significant differences in the size frequencies by depth ranges. Sex-ratio was tested using a  $\chi^2$  test comparing the observed and expected frequencies in a 1:1 ratio (null-hypothesis). Differences in sexual product diameters and volume per polyp were tested using the non-parametric Wilcoxon–Mann–Whitney test because the data were not normality distributed (Shapiro-Wilk  $p < 0.0001$ ). Differences in fertility and fecundity among depths and sexes were tested using a  $t$ -test (Zar, 1999). Furthermore, a Spearman correlation analysis was carried out to test the relationship between female sexual product volume per polyp/fecundity and colony basal diameter and height.

## Results

### Population size distribution

Shallow colonies (N=55) showed a basal diameter range of 2.6-11.5 mm ( $7.4 \pm 1.5$ ; mean $\pm$ SD) and a height size range of 3.4-9 cm ( $6.3 \pm 1.3$ ; mean $\pm$ SD), while in deep colonies (N=94) the range of BD varied from 2.8 to 22.2 mm ( $10.51 \pm 3.00$ ; mean $\pm$ SD) and H between 5.8 and 38.6 cm ( $16.00 \pm 4.73$ ; mean $\pm$ SD) (Fig. 2). Results of the KS two-sample test indicated statistically significant differences in basal diameter and height frequency distribution (BD: K-S= 3.77,  $p$ -value=0; H: K-S=5.50,  $p$ -value=0) among shallow and deep populations.

### Sex ratio and sexual products distribution

The population sexual structure, from both depths, was completely gonochoric at polyp and colony level: none of the analyzed fertile polyps was hermaphroditic.

Internally, gastrovascular cavities are separated by thin mesoglea walls covered by the gastrodermic epithelium. Mature female sexual products (Fig. 3a) fill all the available space at the basal part of the polyps, showing the same general structure shown in other octocorals. The microstructure of immature and mature female sexual products is similar to those described for other anthozoans. Polyps at both depth ranges showed mainly mature sexual products, finally detached from the mesenteries and (immature) primary sexual products were also seen. No *planula* stages were observed in the gas-

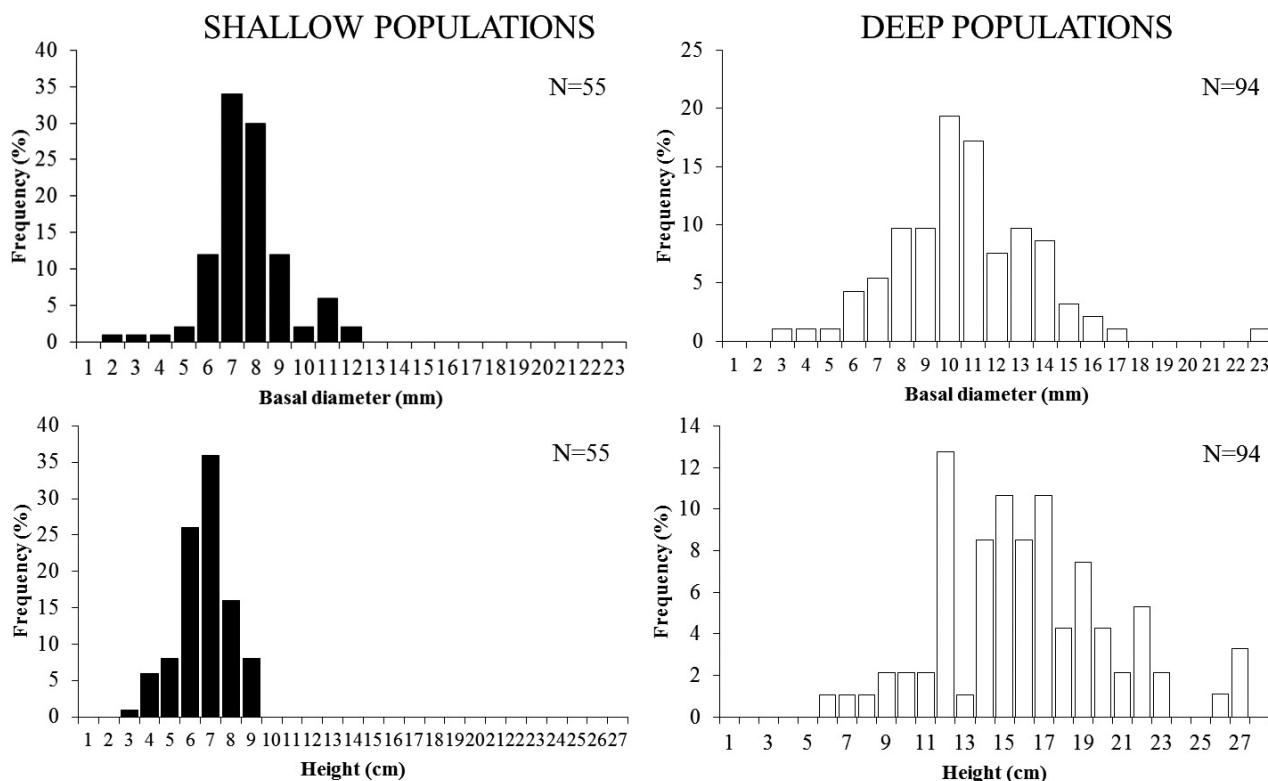
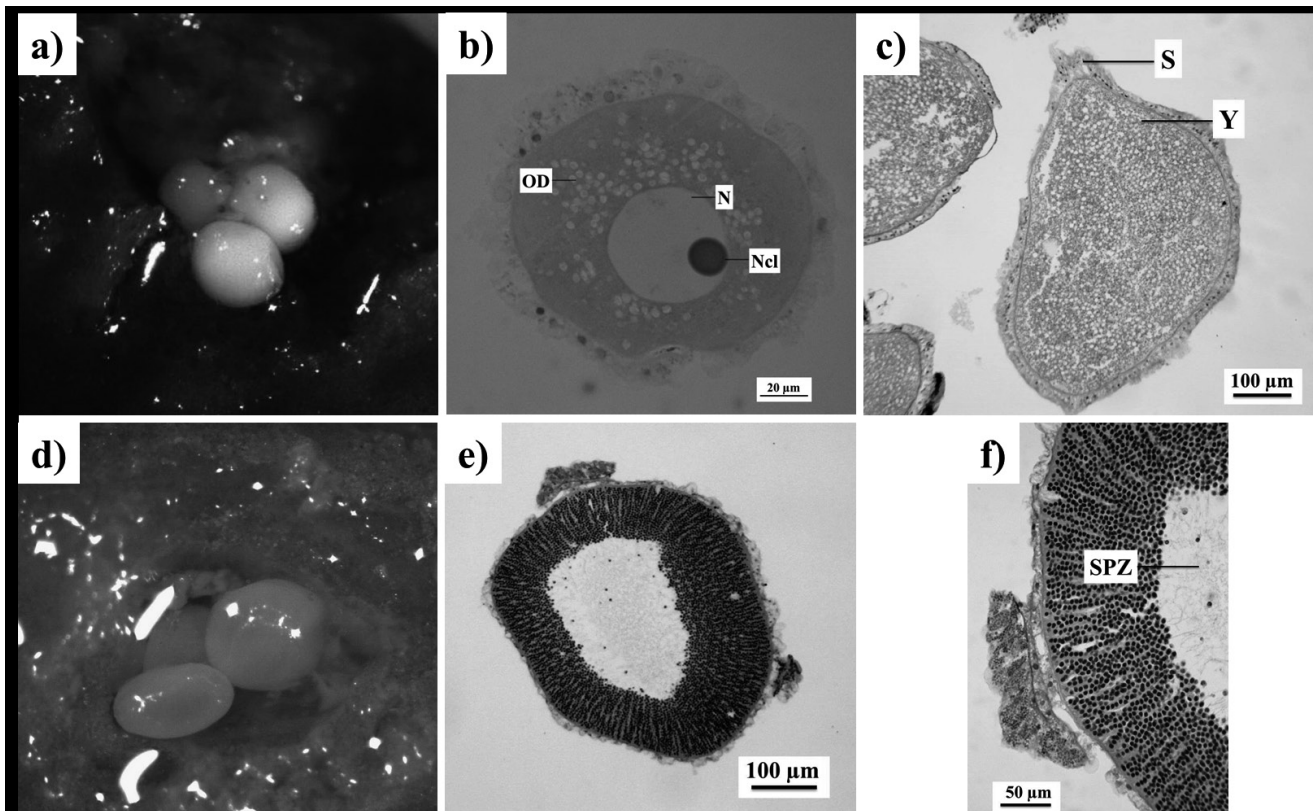


Fig. 2: Size distribution (basal diameter and height) in shallow and deep *Corallium rubrum* populations.



**Fig. 3:** *Corallium rubrum* **a)** female sexual products inside polyp. **b)** primary sexual products located in the gastrodermis with an high nucleus (N) containing a single nucleolus (Ncl) and oil droplets (OD). **c)** mature sexual products with yolk droplets (Y) covering the cytoplasm and a mesogleal stalk (S). **d)** male sexual products inside polyp. **e)** histological section of mature male sexual products. **f)** mature sexual product with spermatozoa (SPZ) visible.

trovascular cavities of the polyps. Female primary sexual products (50-180 µm) were embedded in the mesenteries, surrounded by an endodermal layer. They had little cytoplasm and a large nucleus and a voluminous nucleolus (Fig. 3b), while mature sexual products (198-890 µm), each with its own short mesogleal stalk and surrounded by a thin mesolamella covered by the gastrodermis (very thick), were characterized by a yolk droplets filled cytoplasm and a peripheral nucleus containing a nucleolus (Fig. 3c).

In male colonies, sexual products were surrounded by endodermal cells and the majority of them were ma-

ture (Fig. 3d) and packed with spermatozoa intensely stained with long evident tails inside the lumen (Fig. 3e). The central lumen was usually conspicuous, some sexual products had spermatocytes and spermatozoa tails that could not be distinguished in the preparations (Fig. 3f).

Female and male colonies were found in both populations throughout the entire study period. The sex-ratio showed that female colonies were dominant only in one of the two shallow sites, with a sex ratio of 1.93:1 ( $\chi^2=2.13$ ;  $p<0.05$ ), while in the deep stratum the ratio among sexes did not significantly differ from 1:1 in all investigated populations (Table 2).

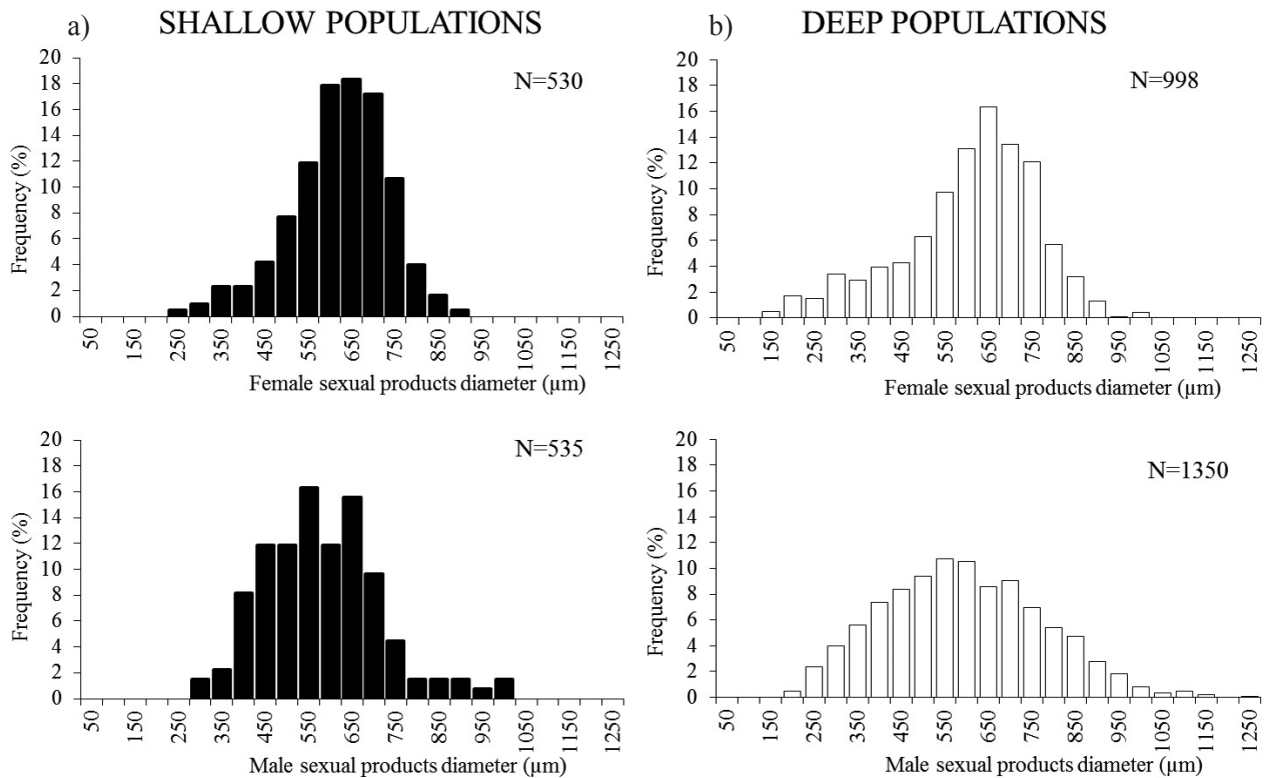
Both shallow and deep *C. rubrum* female colonies exhibited two overlapping sexual product size cohorts. The diameters ranged from 213.57 to 859.30 µm (599.96 ± 124.38 µm, mean ± SD; first cohort 200-400 µm, second cohort 400-860 µm) in shallow colonies and from 101.19 to 985.57 µm (mean ± SD, 561.68 ± 163.92 µm; first cohort 100-400 µm, second cohort 400-990 µm) in deep ones (Fig. 4a, b).

The diameter of male sexual products ranged from 264.72 and 981.79 µm in shallow waters (mean ± SD, 553.63±134.74 µm) and from 164.14 and 1243.34 µm (mean ± SD, 566.40±182.06 µm) in deep ones (Fig. 4a, b).

**Table 2.** *Corallium rubrum* sex-ratio for each site in deep and shallow environment.

Sites	Sex-ratio	$\chi^2$ -test	P-value
1(43 NI)	0.84	1.6	0.68
2(44 NI)	1.66	0.5	0.48
3(ACQP)	0.66	1.6	0.21
4 (01T13)	0.71	0.67	0.41
<b>Total</b>	0.77	1.53	0.22
5(ACQS1)	1.25	0.14	0.7
6(ACQS2)	1.93	2.13	<b>0.04*</b>
<b>Total</b>	1.77	3.92	0.04

\*significant differences



**Fig. 4:** Size distribution of female and male sexual products in **a)** shallow and **b)** deep *Corallium rubrum* populations.

Differences in the female sexual product dimensions among the two investigated depths were not significant (Wilcoxon-Mann-Whitney test,  $p$ -value $>0.05$ ), while diameters of shallow male sexual products were significantly different from deeper ones (Wilcoxon-Mann-Whitney test,  $p$ -value $<0.05$ ).

In shallow female colonies, the volume range of sexual products was comprised between 0.0051 and 0.615 mm<sup>3</sup> with a mean of 0.123 mm<sup>3</sup> ( $\pm 0.07$  SD) (Fig. 5a). In the deepest colonies, the female sexual products volume ranged from 0.000543 mm<sup>3</sup> to 0.501 mm<sup>3</sup> (mean  $\pm$  SD, 0.12  $\pm$  0.08 mm<sup>3</sup>) (Fig. 5a).

The volume per polyp in shallow colonies ranged from 0.01 to 1.03 mm<sup>3</sup> (mean  $\pm$  SD, 0.22  $\pm$  0.14) (Fig. 5b), while the volume per polyp in deep colonies ranged from 0.0021 to 1.627 mm<sup>3</sup> (Fig. 5b) (mean  $\pm$  SD, 0.37  $\pm$  0.12). Similarly to the diameters analysis, differences in the volume of sexual products were not significant among the two depth intervals (Wilcoxon-Mann-Whitney test,  $p$ -value $>0.05$ ), while sexual product volume per polyp was significantly different between shallow and deep populations (Wilcoxon-Mann-Whitney test,  $p$ -value $<0.05$ ).

The analysis of volume of sexual products per polyp for basal diameters and heights (Fig. 6a, b) showed no statistically significant differences in both depth intervals (shallow female colonies  $r$  Pearson = -0.19  $p$ -value=0.24 for BD and  $r$  Pearson = -0.24,  $p$ -value=0.11 for H; deep female colonies  $r$  Pearson = -0.16  $p$ -value=0.24 for BD and  $r$  Pearson = 0.32,  $p$ -value=0.4 for H).

### Fertility and fecundity

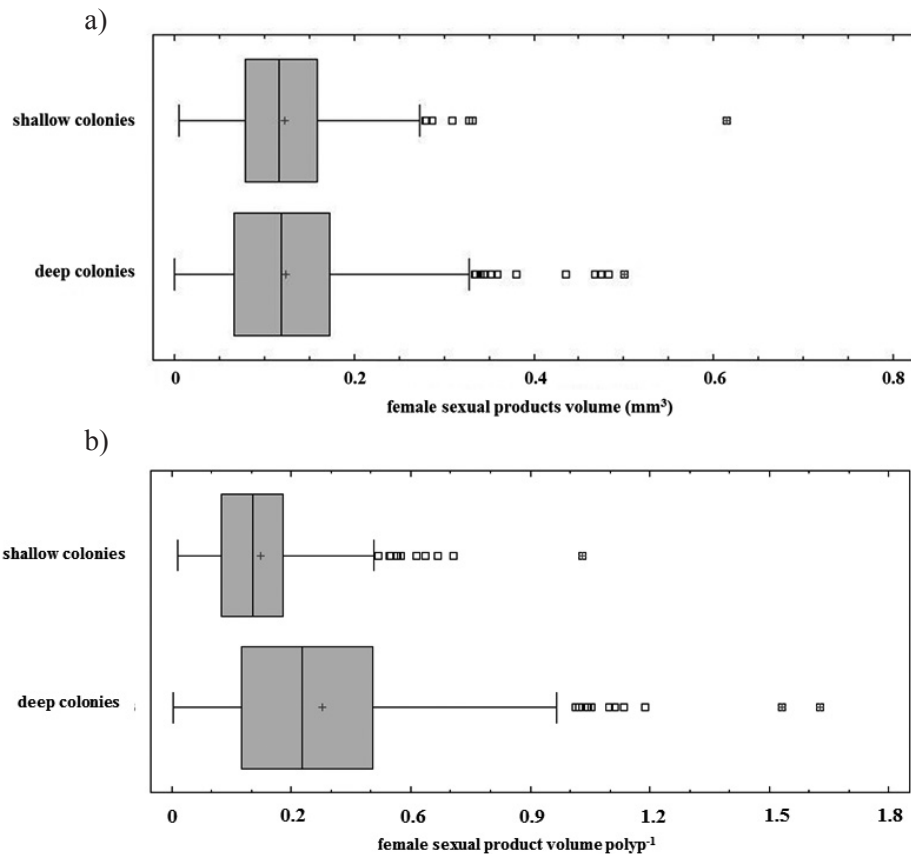
In shallow waters, all sampled colonies were fertile with 55.4% ( $\pm 28.6$  SD) of analyzed polyps capable of reproducing. All investigated deep colonies were fertile as well, with 84% ( $\pm 24.3$  SD) of fertile polyps. No significant differences ( $t$ -test,  $p$ -value $>0.05$ ) were found among male and female polyps fertility for depth intervals (shallow colonies: 50.7% ( $\pm 26.9$ ) of male fertile polyps versus 58 $\pm$ 29.6 % of female fertile polyps; deep colonies: 87 $\pm$ 17.8 % of male fertile polyps versus 81  $\pm$  26.8% of female fertile polyps). However, significant differences ( $t$ -test,  $p$ -value $<0.05$ ) were observed between the two depths in the colony fertility (55.4% versus 84%).

In Table 3 the female and male mean fecundity values for each population are shown.

In shallow waters, each fertile female polyp produced 1 to 7 female sexual products per year. The mean fecundity was 1.14 of female sexual products per polyp ( $\pm 0.61$  SD) and each fertile polyp produced, on mean, 1.81 ( $\pm 0.54$  SD).

No correlation between red coral size (basal diameter and height) and fecundity (Fig. 7a) were observed ( $r$  Pearson=-0.018,  $n=32$ ,  $p$ -value=0.46 for BD and  $r$  Pearson=0.064,  $n=32$ ,  $p$ -value=0.36 for H).

In red coral deep populations, each fertile female polyp produced 1 to 11 sexual products per year. The average fecundity was 2.09 of female sexual products per polyp ( $\pm 0.65$  SD) and each fertile polyp produced, on average, 2.62 ( $\pm 1.35$  SD).



**Fig. 5:** **a)** Boxplot showing the volume ( $\text{mm}^3$ ) of female sexual product in shallow and deep *Corallium rubrum* colonies; **b)** boxplot showing the frequency of female sexual product volume per polyp from shallow and deep populations. Boxplot statistical entries: maximum, third (75%) quartile, median, first (25%) quartile, and minimum, and the open squares represent the outliers.

**Table 3.** *Corallium rubrum*. Female and male mean fecundity for each sampled population. SD, standard deviation; max, maximal number of mature sexual products per polyp found in each population.

Sites	female			male		
	Mean fecundity	SD	max	Mean fecundity	SD	max
1(43 NI)	2.6	1.5	11	5.1	1.5	12
2(44 NI)	3.9	1.9	9	6.5	2.8	12
3(ACQP)	1.6	0.8	6	2.0	0.9	7
4(01T13)	1.4	0.9	6	3.5	1.7	7
<b>Total</b>	2.1	0.8	11	3.4	2.14	12
5(ACQS1)	1.2	0.7	7	1.5	0.8	6
6(ACQS2)	0.8	0.4	3	0.9	0.3	7
<b>Total</b>	1.14	0.61	7	1.3	0.9	7

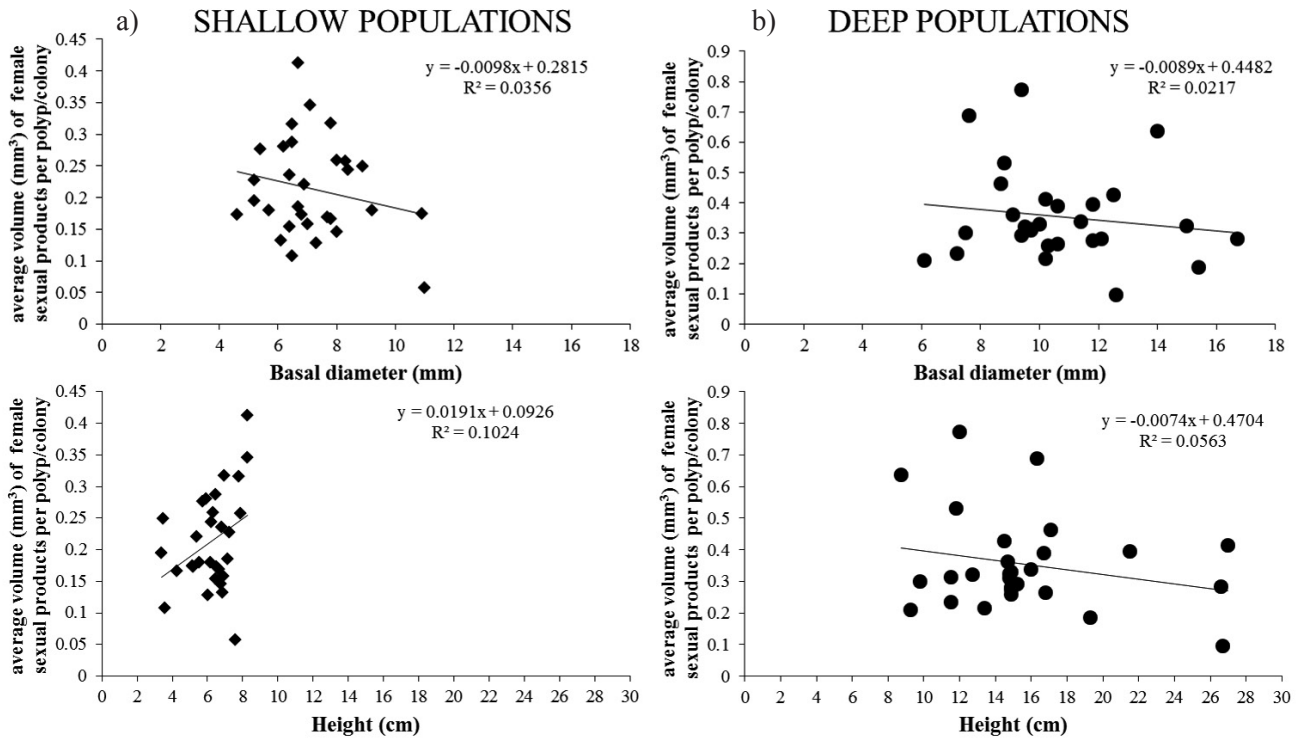
No statistically significant differences in fecundity for basal diameter and height sizes (Fig. 7b) were detected ( $r$  Pearson=-0.12,  $n=41$ ,  $p$ -value=0.23 for BD and  $r$  Pearson=0.03,  $n=41$ ,  $p$ -value=0.43 for H).

The comparison of female fecundity per polyp between shallow and deep populations (Fig. 8) highlighted significant differences among them ( $t$ -test,  $p$ -value<0.05).

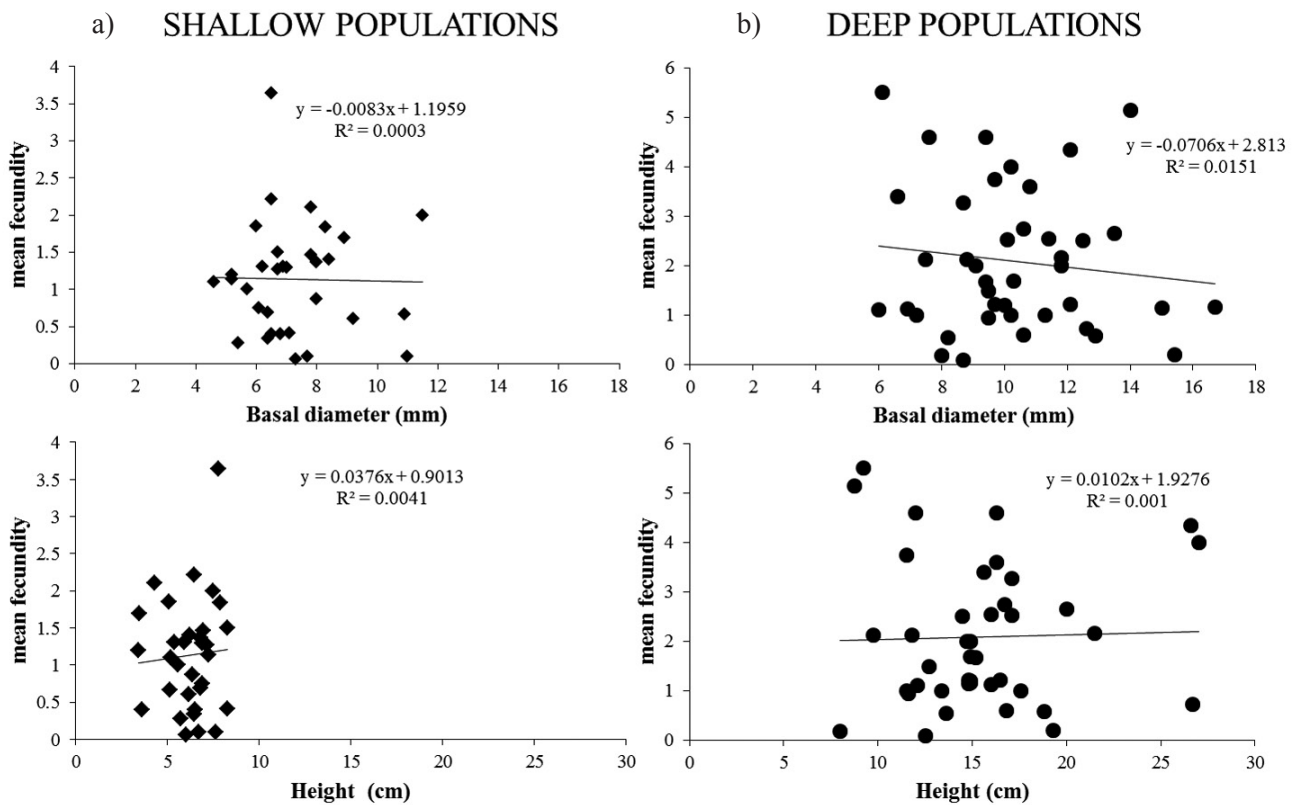
Each shallow waters male fertile polyp produced 1 to 7 sexual products. The average fecundity was 1.34 ( $\pm 0.86$  SD) and each fertile polyp produced on average, 2.62 male products ( $\pm 0.89$  SD) per polyp. In deep popu-

lations, each fertile male polyp produced 1 to 12 sexual products. The average fecundity was 3.38 ( $\pm 2.14$  SD) and each fertile polyp produced on average, 3.70 products ( $\pm 2$  SD) per polyp. The comparison of male fecundity per polyp between the two depths analysed showed significant statistical differences ( $t$ -test,  $p$ -value<0.05).

The comparison between male and female fecundities in shallow waters did not highlight differences statistically significant ( $t$ -test,  $p$ -value>0.05), while fecundity differed significantly between males and female in deep colonies ( $t$ -test,  $p$ -value<0.05).

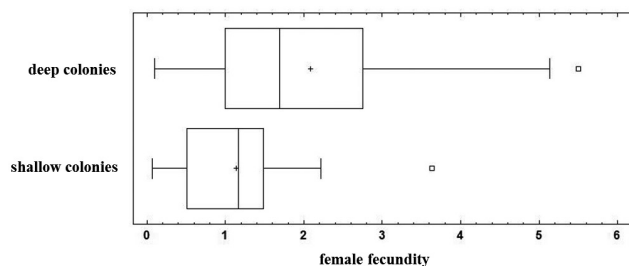


**Fig. 6:** Relationship between average volume of female sexual products per polyp and basal diameter/height in a) shallow and b) deep *Corallium rubrum* populations.

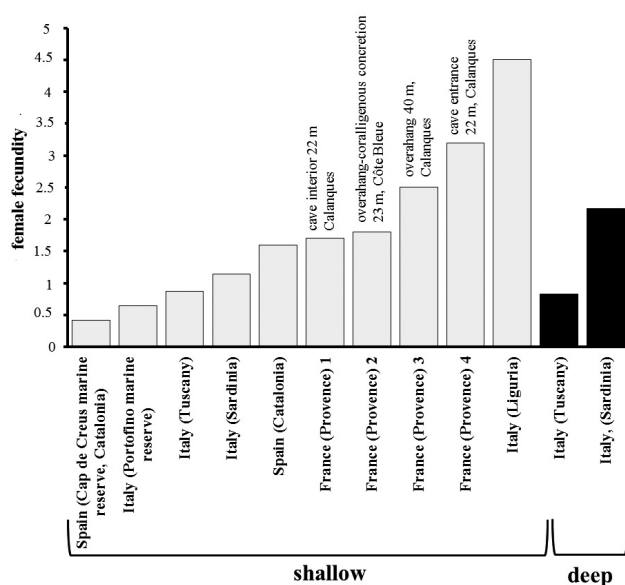


**Fig. 7:** *Corallium rubrum*: mean female fecundity in different sizes of Basal diameter and Height in a) shallow and b) deep populations.





**Fig. 8:** Boxplot showing the female fecundity of *Corallium rubrum* in shallow and deep Sardinian waters. Boxplot statistical entries: maximum, third (75%) quartile, median, first (25%) quartile, and minimum, and the open squares represent the outliers.



**Fig. 9:** Female fecundity of *Corallium rubrum* found in literature: Spain (Cap de Creus marine reserve, Catalonia): Bramanti *et al.*, 2014; Italy (Portofino marine reserve): Bramanti *et al.*, 2014; Italy (Tuscany): Santangelo *et al.*, 2003; Italy (Sardinia): present study; Spain (Catalonia): Tsounis *et al.*, 2006; France (Provence) 1, 2, 3, 4: Torrents and Garrabou, 2011; Italy (Liguria): Vighi, 1970, 1972; Italy (Tuscany): Priori *et al.*, 2013; Italy (Sardinia): present study.

## Discussion

In this paper, the reproductive features of the precious gorgonian *C. rubrum* dwelling at two different depth ranges (shallow and deep) in the north-western Sardinian waters (Central-Western Mediterranean Sea) have been examined.

The analysis of red coral populations at different depths showed a clear different structure in size as observed also in other areas of the Mediterranean Sea (e.g. Cap de Creus, NW Mediterranean, Rossi *et al.*, 2008). Overall, colony diameter and height are higher in deeper than in shallower areas, almost certainly due to the higher harvesting pressure in the shallows (Follesa *et al.*, 2013).

Overall, Sardinian red coral samples were always gonochoric and followed the general pattern found within octocorals (Kahng *et al.*, 2011) where 87% of species studied to date ( $n = 133$ ) are totally dioecious with a very low incidence of hermaphroditism ( $\ll 1\%$ ) (Benayahu & Loya, 1984; McFadden, 1991; Coma *et al.*, 1995; Kruger *et al.*, 1998; McFadden, 2001; Priori *et al.*, 2013).

Histological sections of red coral sexual products, here reported, allowed the definite confirmation of sex and first description of male and female sexual microstructures of this species. Deep and shallow populations were found to have a synchronous female sexual products development in the two depths investigated (no significant differences were found) with two cohorts (the first of immature,  $< 400 \mu\text{m}$  and the second of mature  $< 400 \mu\text{m}$  diameter), confirming the statement of Tsounis *et al.* (2006a) reported for shallow samples in Spain, with deep female products that appeared slightly bigger and larger than the shallow ones. The size of female and male sexual products at the two depths was similar to those observed in other red coral populations sampled just before the release season in shallow waters (Santangelo *et al.*, 2003; Tsounis *et al.*, 2006a). The presence of mature products with a total absence of planulae inside the gastric cavities of all examined specimens at the two depth ranges suggested that the sampled months corresponded to the reproductive period (May-June) of red coral as reported for colonies found in other shallow and deep areas of the Mediterranean Sea (Santangelo *et al.*, 2003; Tsounis *et al.*, 2006a; Torrents & Garrabou, 2011; Priori *et al.*, 2013).

Like other octocorals examined to date, *C. rubrum* produced large female sexual products in both depth intervals (maximum  $986 \mu\text{m}$  in diameter in deep colonies) in relationship with its reproductive mode, confirming a long oogenic development (2-years maturation time: Vighi, 1972; Santangelo *et al.*, 2003). The absence of a clear division in two size classes in male sexual products distribution at both depths confirmed a development cycle shorter than those observed in female ones as well as already demonstrated for the species (e.g. Santangelo *et al.*, 2003) and other gorgonians (e.g. Coma *et al.*, 1995).

In contrast to reproductive timing, the volume occupied by female sexual products per polyp was different between the populations. It was higher in the deep populations than in the shallow populations. Such variability in gonadal output has been previously reported for other coral species being attributed to differences in the allocation of resources to gamete production (e.g. *Eunicella singularis*, Gori *et al.*, 2012).

Given the apparent strong relationship between spatial distribution of male and female colonies within a population and fertilization success, it is reasonable to infer that the sex ratio within a population may have an impact on reproductive success (Simpson, 2009) being an important key to establish a high reproductive output

for marine species (Bramanti *et al.*, 2009, Tsounis *et al.*, 2006c). A balanced sex-ratio represents a common pattern of some octocoral species living in the same red coral habitat: e.g. *Paramuricea clavata* (Coma *et al.*, 1995), *Eunicella singularis* (Weinberg & Weinberg, 1979) and *Malacobelemnion daytoni* (Servetto *et al.*, 2013). Furthermore, skewed sex ratios have been reported in some other octocorals, e.g. *Briareum asbestinum* in favour of male colonies (Brazeau & Lasker, 1990); a male bias of *P. clavata* after a mass mortality episode (Cerrano *et al.*, 2005; Linares *et al.*, 2005), only females among populations in *Plexaura* sp. (Brazeau & Lasker, 1989) or in favour of female colonies as e.g. *Tripalea clavaria* (Excoffon *et al.*, 2004). In Sardinia, a nearly equal sexes distribution has been observed for the deep populations in according to what was reported for deep-dwelling red coral populations of the North Tyrrhenian Sea (Priori *et al.*, 2013). On the contrary, in the shallow populations the sex ratio was significantly biased towards females suggesting that this skewing could probably be a consequence of a differential response of both sexes to perturbations such as mass mortality events more common at shallow levels. In any case, reports on sex ratio rely on data from a single location and are strongly influenced by the local environmental conditions of the habitat. It could be the reason for the different sex-ratio results registered in the several shallow areas of the Mediterranean sea (sex-ratio biased toward female reported by Santangelo *et al.*, (2003) in an over-exploited population in Italy (Tuscany) or sex ratio 1:1 observed by Tsounis *et al.* (2006a) and Bramanti *et al.* (2014) in Spanish marine reserves).

All shallow and deep colonies were found fertile, probably due to the size of samples all above the size at first maturity reported for red coral in the Mediterranean Sea (e.g. Torrents *et al.*, 2005; Gallmetzer *et al.*, 2010). In addition, the highest percentage of deep fertile polyps in deep colonies (84%) was in agreement with those reported for deep-dwelling populations off Tuscany (90.3% Priori *et al.*, 2013).

*C. rubrum* exhibited a reproductive strategy characterized by a relatively low number of female sexual products per polyp (as other internal brooder octocorals, e.g. *Eunicella singularis*, Ribes *et al.*, 2007), revealing significant differences among the two depth ranges analyzed with a lower mean value of sexual products in the shallow colonies (mean fecundity per polyp: 1.14) than in the deeper ones (mean fecundity per polyp: 2.09). Previous studies on shallow populations of red coral reported similar values of fecundity (Fig. 9), even if a high variability in female fecundity was observed within shallow red coral populations inhabiting in different environmental conditions in the North-Western Mediterranean (Provence, France, Torrents & Garrabou, 2011). In general, these latter authors suggested that the fecundity on red coral populations is strictly related to environmental conditions, affirming that it varied significantly for popu-

lations dwelling in particular environments such as cave entrances at a depth of 22 m in which the highest mean fecundity was registered (3.2 mature gonads). Regarding deep dwelling colonies, our results on mean fecundity per polyp differed strongly from the unique data reported in literature for deep populations (Priori *et al.*, 2013) with a higher mean value in Sardinian samples (2.09 mature sexual products per polyp versus 0.83). Our high mean fecundity per polyp was in any case confirmed as well by the high average volume of female sexual products per polyp. Although no correlation was observed between colonies size and mean fecundity per polyps, it seemed that the larger deep colonies (BD > 15 mm) produced a lower number of mature sexual products than the other shallow ones, as confirmed also the analysis of volume of sexual products. This fact could be explained in two ways: i) the largest colonies have already released the planulae; ii) or the oldest have a lower reproductive potential.

Although the observed dissimilarity could be ascribed to the different bathymetrical range investigated, wider in Tyrrhenian samples (50-130 m), other ecological parameters could affect red coral colonies life history traits as showed by Cau *et al.* (2015a) within deep populations dwelling in different environments (i.e. different geological setting and consequent hydrodynamic) with differences in the density and population size structure. To confirm this statement, the application of stock assessments methods on Sardinian deep red coral (Follesa *et al.*, 2013) showed a good condition of the Sardinian colonies in respect to those of the other Mediterranean areas. This result could be related also to the fact that larger Sardinian colonies registered a higher mean fecundity compared to those found in other Mediterranean areas.

In conclusion, differences in reproductive parameters such as sexual products distribution and reproductive output (fecundity) were observed between shallow and deep populations of *C. rubrum*, in particular within deep dwelling populations, when comparing our results with different populations from other locations in the Mediterranean Sea. We stress that further investigations should focus on testing other ecological parameters driving shifts in the populations' demographic traits such as size structure, fecundity and fertility. Scientific efforts focused on increasing knowledge of these parameters will surely lead, in the near future, to the development of more conscious management strategies for this precious resource.

## Acknowledgements

The present study was funded by 1) RAS (Regione Autonoma della Sardegna) L.R. 7 Agosto 2007, and 2) RAS Assessorate of Agriculture - Fisheries Department n.3189/DecA/108 del 19.12.2008.

## Ethical approval

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

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