Effects of food availability on the sexual reproduction and biochemical composition of the Mediterranean gorgonian *Paramuricea clavata*

Andrea Gori [a,*], Cristina Linares [b], Nuria Viladrich [c], Ana Clavero [a], Covadonga Orejas [d], Ida Fiorillo [a], Stefano Ambroso [a], Josep-Maria Gili [a], Sergio Rossi [c]

[a] Institut de Ciències del Mar, Consejo Superior de Investigaciónes Cientificas, Passeig Maritim de la Barceloneta 37–49, 08003 Barcelona, Spain
[b] Departament d'Ecologia, Universitat de Barcelona, Avda. Diagonal 645, 08028 Barcelona, Spain
[c] Institut de Ciència i Tecnologia Ambientals, Universitat Autònoma de Barcelona, UAB Campus Cn s/n, 08193 Cerdanyola del Vallès, Barcelona, Spain
[d] Instituto Español de Oceanografía, Centro Oceanográfico de Baleares, Moll de Ponent s/n, 07015 Palma de Mallorca, Spain

ABSTRACT

Food availability strongly affects the ecology and physiology of coral and gorgonian species, as well as their resistance to stress factors. Since the resources available to these species must be allocated among the energy-costly processes of maintenance, regeneration, growth and reproduction, a reduction in food availability is expected to negatively influence these processes. Here, we show evidence that starvation induces a significant decrease in the gonadal volume produced by the Mediterranean asymbiotic gorgonian *Paramuricea clavata*, with a differential effect on male and female colonies. Fed and starved female colonies produced the same number of oocytes per polyp, but the oocytes were significantly smaller in the starved colonies. Conversely, the spermaries showed the same size in fed and starved male colonies, but fewer spermaries per polyp were produced in the starved colonies. The percentage of organic matter in the coenenchyme of the fed colonies did not change during the experiment, and its composition showed an increase in the relative amount of lipids. In contrast, the starved colonies showed a decrease in the percentage of organic matter, and a constant proportion of lipid. These results confirm a direct link between food availability and both reproductive output and biochemical composition in this organism, and underscore the potentially important role of food availability in explaining the spatial variability in the reproduction and energy storage of suspension feeders.

1. Introduction

Environmental conditions strongly influence the ecology and physiology of marine organisms, determining seasonal patterns in feeding, growth, reproduction and abundance. For this reason, studies of the response of marine species to variability in the main environmental factors are crucial for understanding the potential adaptability of species to local conditions, as well as to future changes in environmental features (Gardner, 2000; Wildish and Kristmanson, 1997). Biotic and abiotic factors that influence food availability have a special influence on the life cycles of sessile benthic suspension feeders that rely primarily or entirely on the capture of seston (Gili and Coma, 1998; Wildish and Kristmanson, 1997). The resources available to these species are often limited in time and space and must be allocated among the energy-costly processes of maintenance, regeneration, growth and reproduction (Richmond, 1987; Rinkevich, 1989; Ward, 1995).

Food availability has been shown to affect the principal physiological processes in coral species, whose starved colonies show a significant decrease in calcification, respiration and excretion rates (Houlbrèque and Ferrier-Pagès, 2009; Naumann et al., 2011; Szmant-Froelieh and Pilson, 1984). During thermal stress, food availability significantly promoted survival and recovery in tropical coral species (Connolly et al., 2012; Ferrier-Pagès et al., 2010). Nutrients also directly mitigate the effects of elevated pCO_2 on coral calcification (Holcomb et al., 2010), and act in concert with other abiotic factors to maintain the calcification rates (Edmunds, 2011). In temperate gorgonians, supplemental food availability delayed the appearance of partial mortality (Coma et al., 2009). The nutritional history of an organism will affect not only its physiology and metabolism but also its biochemical composition (Szmant-Froelich and Pilson, 1980). Coral and gorgonian species show depletion of energy reserve materials, such as lipids and carbohydrates (Grottoli et al., 2004), during periods of starvation (Harland et al., 1992; Szmant-Froelich and Pilson, 1980) or under ambient food and/or light constraints (Ben-David-Zaslow and Benayahu, 1999; Rossi et al., 2006). Resources available to benthic suspension feeders are often diluted, patchily distributed, and stochastically variable in time and space (Gili and Ballesteros, 1991; Ribes et al., 1999; Rossi and Gili, 2005, 2007). In this context, the high lipid content of coral and gorgonian species

(Ben-David-Zaslow and Benayahu, 1999; Grottoli et al., 2004; Stimson, 1987; Ward, 1995) can constitute an energy reserve that may be employed to outlast periods of food shortages (Harland et al., 1992; Rossi et al., 2006).

In a temperate sea, such as the Mediterranean, the seasonal cycles of primary production strongly control the food available to benthic suspension feeders (Coma and Ribes, 2003; Rossi and Gili, 2005). Among these organisms, gorgonians represent a structural component and contribute significantly to the biomass, structural complexity, and energy flow of many benthic communities (Ballesteros, 2006; Gili and Coma, 1998; Gili and Ros, 1985). Shallow-water Mediterranean gorgonian species show a marked seasonality in biochemical composition, with an increase in lipid content during the spring. This increase coincides with the principal bloom in Mediterranean primary and secondary production. These gorgonians also show lipid depletion during the summer period of water stratification as well as in late autumn, when the quality of food decreases due to storms and rainfall (Gori et al., 2007; Rossi et al., 2006).

Recent studies have reported substantial interpopulation variability in the reproductive output and biochemical composition of Mediterranean gorgonians (Gori et al., 2007, 2012; Rossi and Tsounis, 2007). However, energy storage and reproductive output do not always appear to be directly related (Gori et al., 2007; Rossi and Tsounis, 2007; Rossi et al., 2006). Understanding the influence of food availability on reproduction and energy storage has major implications for understanding gorgonian population dynamics. Further research on this topic may highlight the potential nature and importance of food availability in the response and recovery of these organisms following environmental stress caused by human and climate-induced disturbances, which are increasingly affecting Mediterranean benthic communities dominated by gorgonians and sponges (Garrabou et al., 2009; Lejeusne et al., 2010). A decrease in food availability could impact the reproductive output and energy storage capability of these organisms. In this case, a future scenario characterized by lower food availability as a consequence of stronger water stratification (Doney, 2006; Smetacek and Cloern, 2008) may have important consequences for the long-term viability of populations of benthic suspension feeders.

For these reasons, the primary goal of this study was to experimentally investigate the effect of food availability on (1) the gonad development and (2) the biochemical composition of the Mediterranean gorgonian *Paramuricea clavata*. The final aim of the study is to investigate how changes in energy availability may potentially affect the reproduction of asymbiotic temperate gorgonian species and, consequently, the potential recovery and survivorship of these species under a scenario of change.

2. Materials and methods

2.1. Study species

P. clavata (Risso, 1826) is an emblematic asymbiotic gorgonian species characteristic of the western Mediterranean and the Adriatic Sea (Carpine and Grasshoff, 1975; Weinberg, 1976), where it displays a strongly patchy distribution associated with vertical rocky walls and bottoms exposed to strong currents (Gori et al., 2011; Linares et al., 2008a; Weinberg, 1978). This species is characterized by a low growth rate, high mortality rates of recruits and juvenile colonies (Coma et al., 2001; Mistri and Ceccherelli, 1994), and low natural mortality of adults (Coma et al., 2004; Linares et al., 2007). Its populations are maintained by sexual reproduction, and the effect of asexual reproduction is negligible. Synchronous spawning occurs annually in mid-June near the times of the new and full moons (Coma et al., 1995). *P. clavata* regularly feeds on pico-, nano- and micro-zooplankton. It feeds less frequently on mesozooplankton, such as calanoid copepods (Coma et al., 1994; Ribes et al., 1999).

2.2. Sample collection

In late February, 60 *P. clavata* colonies were sampled by SCUBA diving at a depth of 18–22 m in the Medes Islands (NW Mediterranean Sea, 43° 2′ 30″ N, 3° 13′ 30″ E). All sampled colonies, haphazardly chosen, were higher than 30 cm (sexually mature colonies according to Coma et al., 1995). From each of the 60 colonies, 6 primary branches (approximately 5 cm in length) were collected. Branches from each colony were preserved separately in a labeled plastic bag filled with seawater (one bag for each colony). In the laboratory, one branch from each colony was preserved for analysis (see Sections 2.3 and 2.4), whereas the other five branches from each colony were attached to a concrete base with a two-component resin. The bases were identified with a colony identification code and placed into two circular tanks (30 colonies per tank) 55 cm in diameter, 20 cm high, and 50 l in volume. Two submersible pumps (Marea 3200, Croci Trading Company, Gazzada, Italy) in each tank provided a continuous circular current with a flow rate of 3200 l h^{-1}. Water renewal was provided by a continuous flow of Mediterranean seawater pumped from a depth of 15 m at a rate of 50 l h^{-1} and filtered with a 4 μm pore size. To avoid interference by any factor other than food availability, the water was maintained at a constant temperature of 12 $\pm$ 1.0 °C, corresponding to the in situ temperature at the moment of sampling. The gorgonians were maintained in the dark. Only one of the tanks was fed 5 times a week with frozen *Cyclops* and a liquid mixture of particles from approximately 10 to 450 μm in size (Bentos Nutrition, Marine Active Supplement, Maim, Vic, Spain), a suitable range of food sizes for *P. clavata* (Coma et al., 1994; Ribes et al., 1999). During feeding, water renewal in both experimental tanks was interrupted for approximately 3 h to allow the gorgonians to feed. During the experiment, a branch from each colony was collected and preserved for analyses after 28 days, 67 days, 95 days, 123 days, and 138 days. At each of these sampling times, every branch was divided into three portions: the first was preserved in 10% formalin to study gonad development, the second portion was analyzed to assess the quantity of organic matter in the gorgonian coenenchyme, and the third portion was frozen at −20 °C and lyophilized for 16 h at −110 °C and at 100 mbar pressure (Lyo Alfa 6, Telstar, Terrassa, Spain) to assess carbohydrate, protein, and lipid content.

2.3. Gonad development

To study gonad development and reproductive output during the experiment, 10 female and 10 male colonies were analyzed at each sampling time for each treatment. Polyps were dissected under a stereo microscope (Wild, Heerbrugg, Switzerland), and the diameters of all oocytes and spermaries present in 5 polyps in each sample were measured with an eyepiece micrometer (± 10 μm), avoiding apical ends where reproductive output may be affected by annual growth (Coma et al., 1995). If the gonads were elliptical, both minor (a) and major (b) diameters were measured, and the diameter of a sphere with an equivalent volume was calculated (d = 2 ((a / 2)^2 · b / 2)$^{1/3}$). To estimate the gonadal volume per polyp, diameters (d) were transformed into volume (V = 4 / 3π (d / 2)^3).

2.4. Biochemical analyses

Gorgonian organic matter in the coenenchyme and its composition in carbohydrates, proteins and lipids were assessed at each sampling time in 10 colonies from each treatment. Approximately 30 mg (± 0.01 mg) of coenenchyme dry weight from each sample was reduced to ash for 4 h at 500 °C in a muffle furnace (Raypa HM-9, R. Espinar, Terrassa, Spain), and the weight of organic matter (OM hereafter) was calculated as the difference between the coenenchyme dry weight and ash weight (Slattery and McClintock, 1995). To determine carbohydrate content, approximately 10 mg (± 0.01 mg) of coenenchyme dry weight from each sample was homogenized in 3 ml of double-distilled water, and

carbohydrates were quantified colorimetrically (Dubois et al., 1956) with glucose as a standard. To quantify protein content, approximately 10 mg (±0.01 mg) of coenenchyme dry weight from each sample was homogenized in 2 ml 1 N NaOH, and proteins were quantified colorimetrically (Lowry et al., 1951) with albumin as a standard. Finally, to determine lipid content, approximately 15 mg (±0.01 mg) of coenenchyme dry weight from each sample was homogenized in 3 ml of chloroform:methanol (2:1), and total lipids were quantified colorimetrically (Barnes and Blackstock, 1973) with cholesterol as a standard. All the colorimetric quantifications were performed with a spectrophotometer (UV mini-1240, Shimadzu, Kyoto, Japan), and the results are presented in μg carbohydrate–protein–lipid mg^{-1} of OM.

2.5. Statistical analyses

Two-way factor PERMANOVA with treatment and time as fixed factors was used to test for significant differences over time and between fed and starved gorgonians for each of the seven variable parameters analyzed in the study (gonadal volume, number of gonads per polyp, diameter of the gonads, organic matter in coenenchyme, carbohydrate, protein, and lipid content). Pairwise comparisons were performed for the factor of interest (here, feeding treatment) separately for each sampling time. Although the variables were univariate, a PERMANOVA was employed because the null distribution of the test statistic in PERMANOVA is produced by permutation (in this case we used 9999 random permutations), thus avoiding the usual normality and sphericity assumptions of ANOVA (Anderson, 2001). The analyses were performed with the Primer v6 statistical package (Clarke and Gorley, 2006) in conjunction with the Windows PERMANOVA + module (Anderson et al., 2008).

3. Results

3.1. Gonad development

Oocyte volume and diameter increased with time in both treatments but presented significantly lower values in the starved than in the fed colonies at the end of the experiment (Fig. 1A, B, Table 1). At the beginnings of the experiment, the mean number of oocytes per polyp showed significant differences between treatments. However, after a clear decrease with time in both treatments, no significant differences were observed between the fed and starved colonies at the end of the experiment (Fig. 1C, Table 1). The volume of the spermaries increased significantly with time in both treatments, showing significantly lower values in the starved than in the fed colonies at the end of the experiment (Fig. 2A, Table 1). The diameter of the spermaries increased with time, with no differences between treatments (Fig. 2B). The mean number of spermaries per polyp decreased in the starved colonies and increased in the fed ones, with significantly lower values in the starved than in the fed colonies at the end of the experiment (Fig. 2C, Table 1).

3.2. Biochemical analyses

The percentage of organic matter in the coenenchyme decreased significantly in the starved colonies (Fig. 3, Table 1). In contrast, the biochemical composition of the organic matter showed a decrease in carbohydrate content in both treatments (Fig. 4A, Table 1), a significant increase in protein content in the starved colonies (Fig. 4B), and a significant increase in lipid content in the fed colonies (Fig. 4C, Table 1).

4. Discussion

The results of this study demonstrate two important effects of differential food availability on gorgonian reproduction: (1) a significant effect was observed on the gonadal volume produced by *P. clavata*,
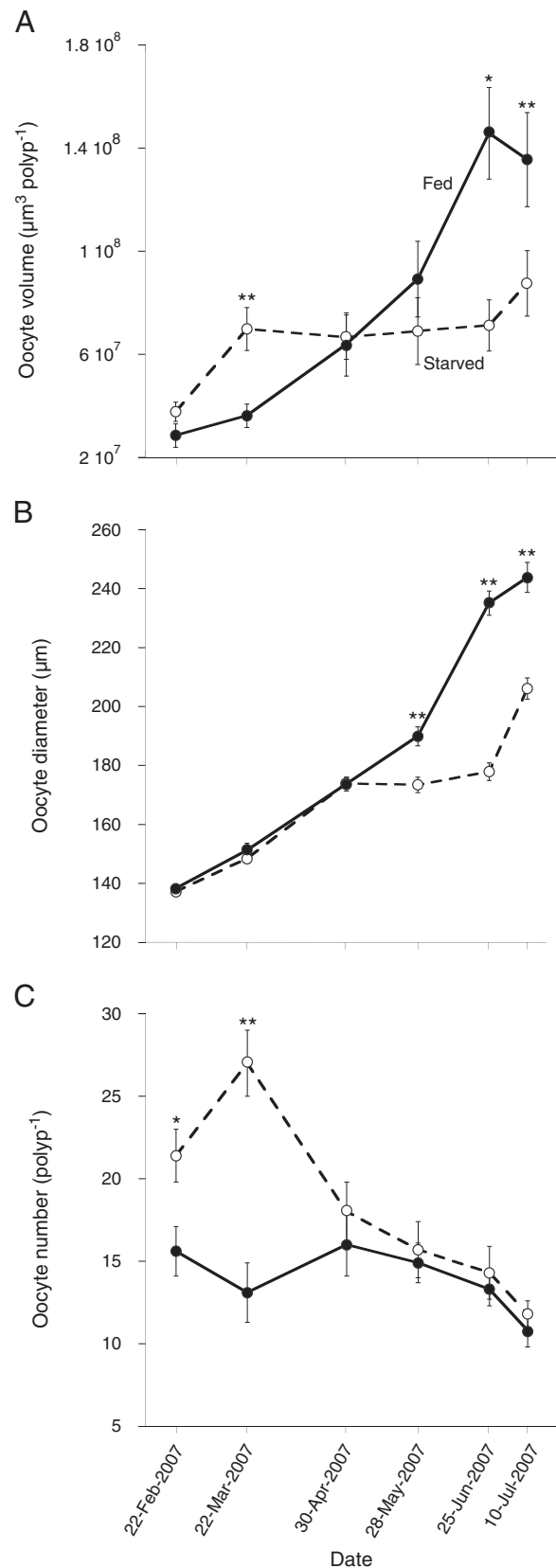

Fig. 1. Temporal changes in (A) mean polyp volume of oocytes, (B) oocyte diameter, and (C) oocyte number per polyp in fed (filled circle) and starved (open circle) *Paramuricea clavata* female colonies (mean ± SE); significant differences between the two treatments shown by the pairwise test are indicated by one (p-value < 0.05), two (p-value < 0.01), or three *asterisks* (p-value < 0.001).

Table 1

Two-way PERMANOVA comparing the mean gonadal volume per polyp, gonad diameter, gonad number, organic matter, content of carbohydrates, proteins, and lipids, during time and between feed and starved gorgonians; significant p-values are indicated by one (p-value < 0.05), two (p-value < 0.01), or three asterisks (p-value < 0.001).

	df	SS	MS	Pseudo-F	p Value	
Female gonadal volume						
Treatment	1	459,561	45,961	7.2	0.008	**
Time	5	498,652	99,729	15.6	<0.001	***
Treatment * Time	5	194,171	38,834	6.1	0.002	**
Residual	595	3,795,411	6378			
Female gonad diameter						
Treatment	1	19,403	19,403	19.8	<0.001	***
Time	5	292,323	58,461	595.4	<0.001	***
Treatment * Time	5	44,295	8859	9.0	<0.001	***
Residual	1088	577,321	981			
Female gonad number						
Treatment	1	2041	2041	17.9	<0.001	***
Time	5	5257	1051	9.2	<0.001	***
Treatment * Time	5	2482	496	4.4	0.002	**
Residual	585	66,528	114			
Male gonadal volume						
Treatment	1	984,611	984,611	55.0	<0.001	***
Time	5	4,874,121	974,822	59.0	<0.001	***
Treatment * Time	5	1,264,911	252,987	15.3	<0.001	***
Residual	555	91,687,699	16,521			
Male gonad diameter						
Treatment	1	59,736	59,736	3.2	0.075	
Time	5	4,388,221	877,651	46.7	<0.001	***
Treatment * Time	5	140,721	28,143	1.5	0.192	
Residual	1168	2,195,277	18,795			
Male gonad number						
Treatment	1	1767	1767	21.3	0.005	**
Time	5	2254	451	5.4	<0.001	***
Treatment * Time	5	7308	1461	17.6	<0.001	***
Residual	556	46,029	83			
Organic matter						
Treatment	1	514	514	23.7	<0.001	***
Time	5	1057	211	9.7	<0.001	***
Treatment * Time	5	833	167	7.7	<0.001	***
Residual	344	7467	22			
Carbohydrate content						
Treatment	1	436	436	2.7	0.112	
Time	5	15,545	3109	19.3	<0.001	***
Treatment * Time	5	4821	964	6.0	<0.001	***
Residual	108	17,387	161			
Protein content						
Treatment	1	80,130	80,130	11.9	<0.001	***
Time	5	353,681	70,736	10.5	<0.001	***
Treatment * Time	5	149,422	29,884	4.5	<0.001	***
Residual	108	710,464	6702			
Lipid content						
Treatment	1	14,266	14,266	31.3	<0.001	***
Time	5	12,991	25,983	5.7	0.002	**
Treatment * Time	5	64,803	12,961	2.8	0.014	*
Residual	108	49,240	4559			

Fig. 2. Temporal changes in (A) mean polyp volume of spermaries, (B) spermary diameter, and (C) spermary number per polyp in fed (filled circle) and starved (open circle) *Paramuricea clavata* male colonies (mean ± SE); significant differences between the two treatments shown by the pairwise test are indicated by one (p-value < 0.05), two (p-value < 0.01), or three *asterisks* (p-value < 0.001).

with a considerable decrease in starved colonies, and (2) a differential effect on the reproductive cycle of male and female colonies, presumably related to the moment of gametogenesis at which the food constraint occurred. Fed and starved female colonies produced the same number of oocytes per polyp, but the oocytes were significantly smaller in the starved colonies. Conversely, the spermaries showed the same size in fed and starved colonies, but fewer spermaries per polyp were produced in the starved colonies. These differences are most likely related to the longer duration of oogenesis, compared with spermatogenesis, in gorgonians from temperate environments
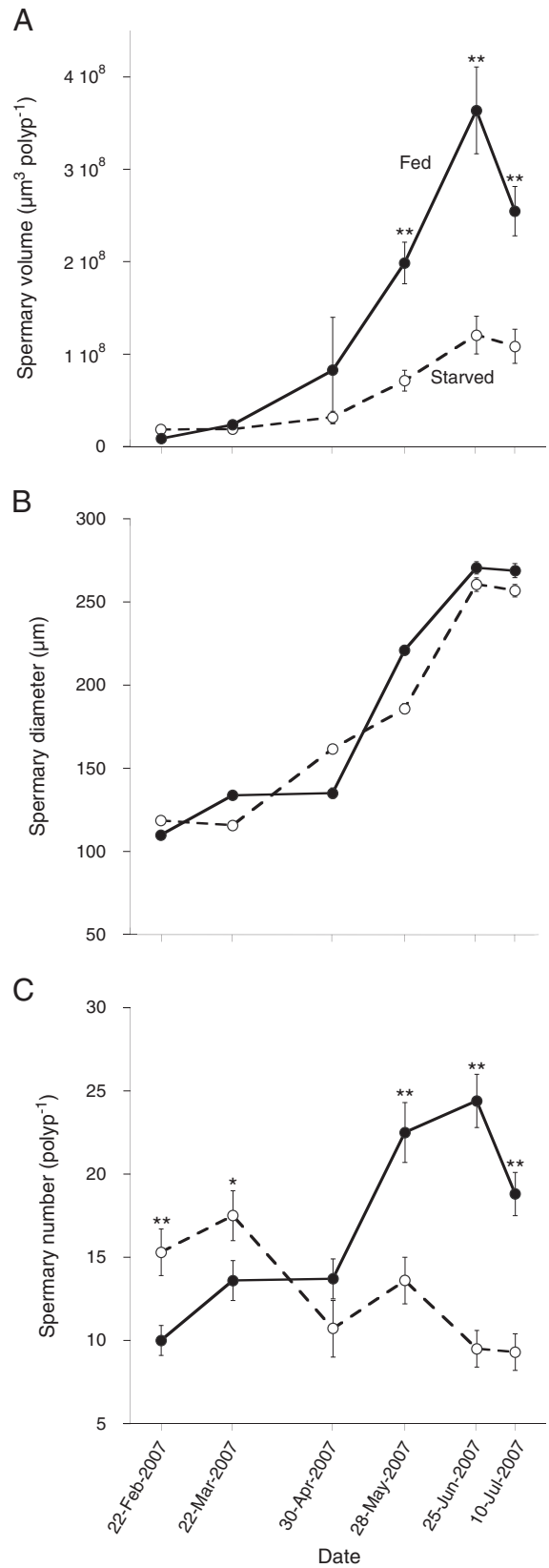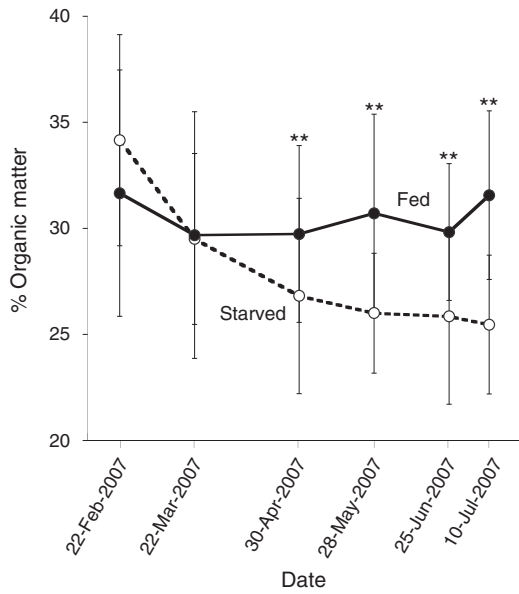
Fig. 3. Temporal changes in the organic matter in the coenenchyme of fed (filled circle) and starved (open circle) *Paramuricea clavata* colonies (mean ± SD); significant differences between the two treatments shown by the pairwise test are indicated by one (p-value < 0.05), two (p-value < 0.01), or three *asterisks* (p-value < 0.001).

(Grigg, 1977; Ribes et al., 2007; Tsounis et al., 2006a). In *P. clavata*, oocyte development begins between February and July and culminates in spawning during the summer (June and July) of the following year (Coma et al., 1995). Thus, at the beginning of this experiment, the number of maturing oocytes was already established, and the difference in energy availability between the fed and starved colonies only affected the size of the oocytes. Conversely, the development of the spermary is considerably shorter, beginning in January (almost coinciding with the beginning of this experiment) and culminating in spawning during the summer of the same year (Coma et al., 1995). Consequently, a smaller number of spermaries developed and matured in the unfed colonies. We have no explanation for the higher number of oocytes and spermaries observed in the starved colonies at the beginning of the experiment. However, since gonads are extremely small at this stage of the gametogenesis, these differences only slightly affected the oocyte volume, and did not affect the spermaries volume (Figs. 1A and 2A). The small dimensions of gonads may have also resulted in a lower precision in their quantification at the beginnings of the experiment. Previous studies focusing on the effects of climate-induced mass mortality events on *P. clavata* (Cupido et al., 2012; Garrabou et al., 2009; Linares et al., 2005) showed a significant decrease in the population reproductive output. A reduction in fecundity was observed in both male and female colonies affected by partial mortality. However, the sizes of the oocytes and spermaries were not affected (Linares et al., 2008b). This result may suggest that if the stress driver operates before the beginning of gametogenesis, both male and female colonies respond by reducing fecundity but maintaining gonad sizes. However, if the stress factor starts to operate after the beginning of gametogenesis (as occurs in this experiment), the gonad sizes could also be affected. Colonies of *P. clavata* colonized by epibionts following injuries were not able to invest as much in reproduction as unaffected colonies and showed a resulting decrease in both male and female fecundity. These results indicate a compensatory response in which energy resources that would normally be used for reproduction were invested in the recovery of injured tissue (Tsounis et al., 2012). Similarly, in tropical corals, stress factors (such as temperature increase, contamination, bleaching and injuries) have been observed to affect reproduction by reducing gonad size (Guzman and Holst, 1993) or fecundity (Kojis
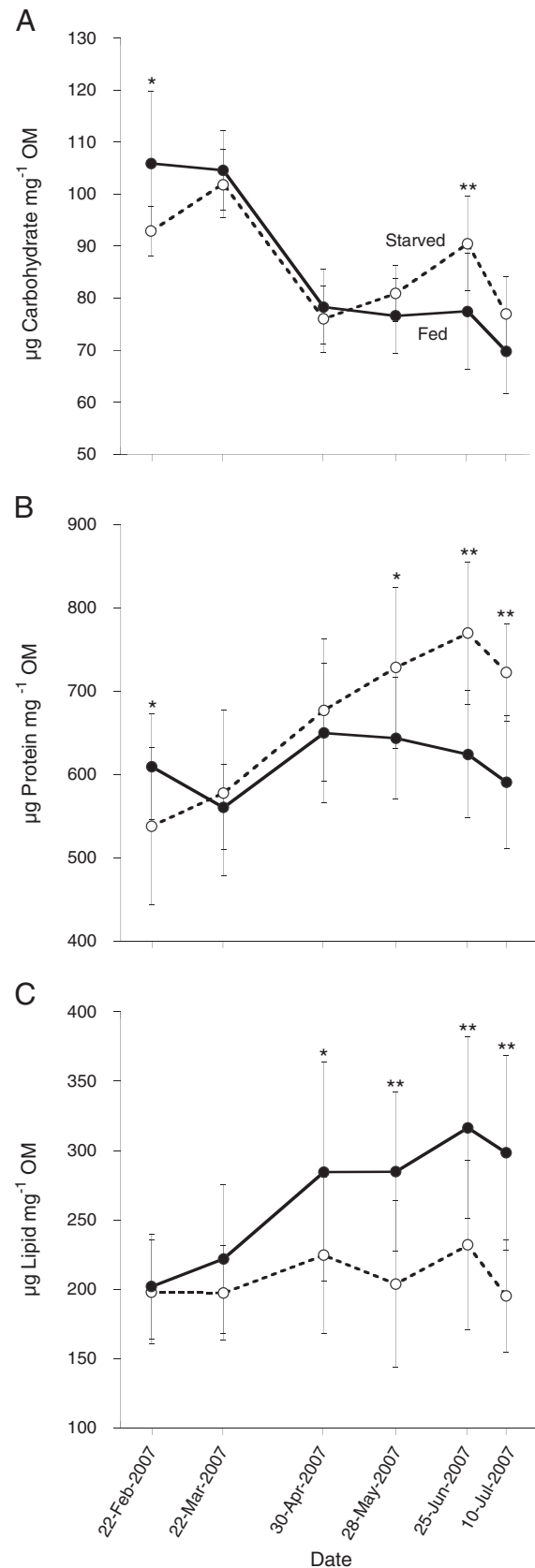

Fig. 4. Temporal changes in the (A) carbohydrate, (B) protein, and (C) lipid content in tissue of fed (filled circle) and starved (open circle) *Paramuricea clavata* colonies (mean ± SD); significant differences between the two treatments shown by the pairwise test are indicated by one (p-value < 0.05), two (p-value < 0.01), or three *asterisks* (p-value < 0.001).

and Quinn, 1984; Michalek-Wagner and Willis, 2001; Szmant and Gassman, 1990; Van Veghel and Bak, 1994). Elevated nitrogen reduced the reproductive output of acroporid corals, while elevated levels of phosphorus increased the number of produced gametes (Ward and Harrison, 2000), and heterotrophic feeding induced the production of bigger oocytes, but overall reduced the fecundity and reproductive effort of the symbiotic coral *Pocillopora verrucosa* (Séré et al., 2010).

Coral and gorgonian ecology and physiology depend strongly on the quantity and quality of available food (Ferrier-Pagès et al., 2003; Gili and Coma, 1998; Naumann et al., 2011; Szmant-Froelich and Pilson, 1984). For this reason, time periods (or sites) with low food availability may show a significant decrease in coral and gorgonian reproductive output (Ben-David-Zaslow et al., 1999; Dahan and Benayahu, 1997). A reduction in the production of spermary volume may affect the probability of fertilization (Coma and Lasker, 1997; Oliver and Babcock, 1992) and then, ultimately, reduce the number of larvae produced. Conversely, in time periods (or sites) of high food availability, more resources can be allocated to reproduction (Ben-David-Zaslow et al., 1999), which could produce high reproductive success and recruitment.

Spatial variability in reproductive output has been previously reported in *P. clavata* and in the other Mediterranean gorgonian *Eunicella singularis* (Gori et al., 2007, 2012; Ribes et al., 2007), as well as in tropical coral species, being attributed to differences in the allocation of resources to gamete production (Fan and Dai, 1995; Kruger et al., 1998; Sier and Olive, 1994). A negative impact on the oocyte size may have important consequences for the long-term viability of populations because smaller oocytes may produce larvae with a reduced energetic content, with an ultimate result of low recruitment success. Tropical soft coral larvae produced during periods of high food availability were larger, and almost all of these larvae were competent to metamorphose, whereas significantly lower rates of metamorphosis were observed in planulae produced during periods of lower food availability (Ben-David-Zaslow and Benayahu, 1996). Moreover, smaller planulae with lower energetic content could reduce their pelagic larval duration, consequently affecting connectivity among populations.

The absence of spawning in both fed and starved colonies (maintained at the constant winter seawater temperature of 12 °C) highlights the importance of temperature as a determinant of spawning in shallow coral and gorgonian populations (Benayahu and Loya, 1983, 1986; Coma et al., 1995; Gori et al., 2007; Grigg, 1977; Weinberg and Weinberg, 1979). However, the observed development of oocytes and spermaries despite the constant seawater temperature (differently from the increase in seawater temperature that occurs in early spring in the Mediterranean Sea) also suggests that temperature interacts with additional factors or with endogenous timing in gonadal development. In any case, the environmental drivers that determine spawning in temperate gorgonian species deserve further research. Both the oocytes and the spermaries of the fed colonies were smaller than those previously observed in the field (Coma et al., 1995; Gori et al., 2007; Linares et al., 2008b), whereas their number per polyp was of the same order of magnitude. These differences could not only be due to a delay in the gametogenesis as a consequence of the low temperature, but also to the aquarium conditions relative to those of the field, as suggested by the rapid reduction in carbohydrates observed after a few weeks in both experimental treatments. Carbohydrates have been shown to have a relatively direct relationship to trophic stress in anthozoan species, showing a rapid response to food depletion (Fitt and Pardy, 1981; Rossi et al., 2006; Willmer et al., 2000). This result might suggest that the quantity or quality of the food used in this experiment resulted in a much poorer diet than the food that *P. clavata* generally uses in the field (Coma et al., 1994; Ribes et al., 1999). In a similar way, *Acropora danae* colonies in the field showed much higher carbohydrate content than colonies maintained in aquaria, possibly as a consequence of variation in feeding frequencies and/or in the nutritional value of the food (Szmant-Froelich and Pilson, 1984).

The percentage of organic matter in the coenenchyme of the fed colonies did not change during the experiment, and its composition showed a significant increase in the relative amount of lipids. In contrast, the percentage of organic matter decreased in the starved colonies, and lipid content remained at the low values (~ 200 µg mg^{-1} OM) previously observed in *P. clavata* during winter (Gori et al., 2007; Rossi et al., 2006), resulting in an overall decrease in the total amount of lipids. This result confirms a direct link between food availability and biochemical composition in these organisms (Harland et al., 1992; Rossi et al., 2006; Szmant-Froelich and Pilson, 1984). Lipids have been reported to represent the principal storage molecules used by corals, and the large amount of lipid content in coral organic matter constitutes an energy reserve that can be partitioned between growth and reproduction (Ben-David-Zaslow and Benayahu, 1999; Rossi et al., 2006). During periods of starvation, as well as in bleached corals, lipids and carbohydrates are depleted relative to proteins (Grottoli et al., 2004; Szmant-Froelich and Pilson, 1984), as observed in this experimental study. Conversely, lipids are stored during times of abundant food and/or light availability (Ben-David-Zaslow and Benayahu, 1999; Glynn et al., 1985; Harland et al., 1992; Rossi et al., 2006; Stimson, 1987).

Due to the temporal and spatial variability of littoral seston quantity and quality (Rossi and Gili, 2005, 2007; Rossi et al., 2003) and the observed high variability in prey capture rates at the colony (Coma et al., 1994) and population level (Tsounis et al., 2006b), the results of this study underscore the potentially important role of food availability in explaining the spatial variability in the reproductive output and energy storage of suspension feeders. To the extent that the responses of *P. clavata* in this experiment are representative of the response of this species to food availability in nature, our results show that a lower food availability due to stronger water stratification in a future scenario for shallow coastal waters (Doney, 2006; Smetacek and Cloern, 2008) will have a direct impact on the capability of gorgonian populations to withstand environmental changes. The present results may help to better understand the sublethal responses expected after environmental stresses and to identify those populations in which food availability may produce greater resistance and resilience to human- and climate-induced disturbances.

Acknowledgments

References

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46.
Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA + for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth.
Ballesteros, E., 2006. Mediterranean coralligenous assemblages: a synthesis of present knowledge. Oceanogr. Mar. Biol. 44, 123–195.
Barnes, H., Blackstock, J., 1973. Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanillin method for "total" lipids. J. Exp. Mar. Biol. Ecol. 12, 103–118.
Benayahu, Y., Loya, Y., 1983. Surface brooding in the Red Sea soft coral *Parerythropodium fulvum fulvum* (Forskål 1775). Biol. Bull. 165, 353–369.
Benayahu, Y., Loya, Y., 1986. Sexual reproduction of a soft coral: synchronous and brief annual spawning of *Sarcophyton glaucum* (Quoy & Gaimard, 1833). Biol. Bull. 170, 32–42.
Ben-David-Zaslow, R., Benayahu, Y., 1996. Longevity, competence and energetic content in planulae of the soft coral *Heteroxenia fuscescens*. J. Exp. Mar. Biol. Ecol. 206, 55–68.
Ben-David-Zaslow, R., Benayahu, Y., 1999. Temporal variation in lipid, protein and carbohydrate content in the Red Sea soft coral *Heteroxenia fuscescens*. J. Mar. Biol. Assoc. UK 79, 1001–1006.

Ben-David-Zaslow, R., Henning, G., Hofmann, D.K., Benayahu, Y., 1999. Reproduction in the Red Sea soft coral *Heteroxenia fuscescens*: seasonality and long-term record (1991 to 1997). Mar. Biol. 133, 553–559.

Carpine, C., Grasshoff, M., 1975. Les gorgonaires de la Méditerranée. Bull. Inst. Océanogr. Monaco 71, 1–140.

Clarke, K.R., Gorley, R.N., 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth.

Coma, R., Lasker, H.R., 1997. Small-scale heterogeneity of fertilization success in a broadcast spawning octocoral. J. Exp. Mar. Biol. Ecol. 214, 107–120.

Coma, R., Ribes, M., 2003. Seasonal energetic constraints in Mediterranean benthic suspension feeders: effects at different levels of ecological organization. Oikos 101, 205–215.

Coma, R., Gili, J.M., Zabala, M., Riera, T., 1994. Feeding and prey capture cycles in the aposymbiontic gorgonian *Paramuricea clavata*. Mar. Ecol. Prog. Ser. 115, 257–270.

Coma, R., Ribes, M., Zabala, M., Gili, J.M., 1995. Reproduction and cycle of gonadal development in the Mediterranean gorgonian *Paramuricea clavata*. Mar. Ecol. Prog. Ser. 117, 173–183.

Coma, R., Ribes, M., Gili, J.M., Hughes, R.N., 2001. The ultimate opportunists: consumers of seston. Mar. Ecol. Prog. Ser. 219, 305–308.

Coma, R., Pola, E., Ribes, M., Zabala, M., 2004. Long-term assessment of temperate octocoral mortality patterns, protected vs. unprotected areas. Ecol. Appl. 14, 1466–1478.

Coma, R., Ribes, M., Serrano, E., Jiménez, E., Salat, J., Pascual, J., 2009. Global warming-enhanced stratification and mass mortality events in the Mediterranean. PNAS 106, 6176–6181.

Connolly, S.R., Lopez-Yglesias, M.A., Anthony, K.R.N., 2012. Food availability promotes rapid recovery from thermal stress in a scleractinian coral. Coral Reefs 31, 951–960.

Cupido, R., Cocito, S., Manno, V., Ferrando, S., Peirano, A., Iannelli, M., Bramanti, L., Santangelo, G., 2012. Sexual structure of a highly reproductive, recovering gorgonian population: quantifying reproductive output. Mar. Ecol. Prog. Ser. 469, 25–36.

Dahan, M., Benayahu, Y., 1997. Reproduction of *Dendronephthya hemprichi* (Cnidaria: Octocorallia): year-round spawning in an azooxanthellate soft coral. Mar. Biol. 129, 573–579.

Doney, S.C., 2006. Plankton in a warmer world. Nature 444, 695–696.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for the determination of sugars and related substances. Anal. Chem. 28, 350–356.

Edmunds, P., 2011. Zooplanktivory ameliorates the effects of ocean acidification on the reef coral *Porites* spp. Limnol. Oceanogr. 56, 2402–2410.

Fan, T.Y., Dai, C.F., 1995. Reproductive ecology of scleractinian coral *Echinopora lamellosa* in northern and southern Taiwan. Mar. Biol. 123, 565–572.

Ferrier-Pagès, C., Witting, J., Tambutte, E., Sebens, K.P., 2003. Effect of natural zooplankton feeding on the tissue and skeletal growth of the scleractinian coral *Stylophora pistillata*. Coral Reefs 22, 229–240.

Ferrier-Pagès, C., Rottier, C., Beraud, E., Levy, O., 2010. Experimental assessment of the feeding effort of three scleractinian coral species during a thermal stress: effect on the rates of photosynthesis. J. Exp. Mar. Biol. Ecol. 390, 118–124.

Fitt, W.K., Pardy, R.L., 1981. Effects of starvation, and light and dark on the energy metabolism of symbiotic and aposymbiotic sea anemones, *Anthopleura elegantissima*. Mar. Biol. 61, 199–205.

Gardner, J.P.A., 2000. Where are the mussels on Cook Strait (New Zealand) shores? Low seston quality as a possible factor limiting multi-species distributions. Mar. Ecol. Prog. Ser. 194, 123–132.

Garrabou, J., Coma, R., Bensoussan, N., Bally, M., Chevaldonné, P., Cigliano, M., Diaz, D., Harmelin, J.G., Gambi, M.C., Kersting, D.K., Ledoux, J.B., Lejeusne, C., Linares, C., Marschal, C., Pérez, T., Ribes, M., Romano, J.C., Serrano, E., Teixido, N., Torrents, O., Zabala, M., Zuberer, F., Cerrano, C., 2009. Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. Global Change Biol. 15, 1090–1103.

Gili, J.M., Ballesteros, E., 1991. Structure of cnidarian populations in Mediterranean sublittoral communities as a result of adaptation to different environmental conditions. Oecol. Aquat. 10, 243–254.

Gili, J.M., Coma, R., 1998. Benthic suspension feeders: their paramount role in littoral marine food webs. Trends Ecol. Evol. 13, 316–321.

Gili, J.M., Ros, J., 1985. Study and cartography of the benthic communities of Medes Islands (NE Spain). PSZNI Mar. Ecol. 6, 219–238.

Glynn, P.W., Perez, M., Gilchrist, S.L., 1985. Lipid decline in stressed corals and their crustacean symbionts. Biol. Bull. 168, 276–284.

Gori, A., Linares, C., Rossi, S., Coma, R., Gili, J.M., 2007. Spatial variability in reproductive cycle of the gorgonians *Paramuricea clavata* and *Eunicella singularis* (Anthozoa, Octocorallia) in the Western Mediterranean Sea. Mar. Biol. 151, 1571–1584.

Gori, A., Rossi, S., Berganzo-González, E., Pretus, J.L., Dale, M.R.T., Gili, J.M., 2011. Spatial distribution, abundance and relationship with environmental variables of the gorgonians *Eunicella singularis*, *Paramuricea clavata* and *Leptogorgia sarmentosa* (Cape of Creus, Northwestern Mediterranean Sea). Mar. Biol. 158, 143–158.

Gori, A., Viladrich, N., Gili, J.M., Kotta, M., Cucio, C., Magni, L., Bramanti, L., Rossi, S., 2012. Reproductive cycle and trophic ecology in deep versus shallow populations of the Mediterranean gorgonian *Eunicella singularis* (Cap de Creus, northwestern Mediterranean Sea). Coral Reefs 31, 823–837.

Grigg, R.W., 1977. Population dynamics of two gorgonian corals. Ecology 58, 278–290.

Grottoli, A.G., Rodrigues, L.J., Juarez, C., 2004. Lipids and stable carbon isotopes in two species of Hawaiian corals, *Porites compressa* and *Montipora verrucosa*, following a bleaching event. Mar. Biol. 145, 621–631.

Guzman, H.M., Holst, I., 1993. Effects of chronic oil-sediment pollution on the eproduction of the Caribbean reef coral *Siderastrea siderea*. Mar. Pollut. Bull. 26, 276–282.

Harland, A.D., Davies, P.S., Fixter, L.M., 1992. Lipid content of some Caribbean corals in relation to depth and light. Mar. Biol. 113, 357–361.

Holcomb, M., McCorkle, D.C., Cohen, A.L., 2010. Long-term effects of nutrients and CO_2 enrichment on the temperate coral *Astrangia poculata* (Ellis and Solander, 1786). J. Exp. Mar. Biol. Ecol. 386, 27–33.

Houlbrèque, F., Ferrier-Pagès, C., 2009. Heterotrophy scleractinian corals. Biol. Rev. 84, 1–17.

Kojis, B.L., Quinn, N.J., 1984. Seasonal and depth variation in fecundity of *Acropora palifera* at two reefs in Papua New Guinea. Coral Reefs 3, 165–172.

Kruger, A., Schleyer, M.H., Banayahu, Y., 1998. Reproduction in *Anthelia glauca* (Octocorallia: Xeniidae). I. Gametogenesis and larval brooding. Mar. Biol. 131, 423–432.

Lejeusne, C., Chevaldonné, P., Pergent-Martini, C., Boudouresque, C.F., Pérez, T., 2010. Climate change effects on a miniature ocean: the highly diverse, highly impacted Mediterranean Sea. Trends Ecol. Evol. 25, 250–260.

Linares, C., Coma, R., Díaz, D., Zabala, M., Hereu, B., Dantart, L., 2005. Immediate and delayed effects of a mass mortality event on gorgonian population dynamics and benthic community structure in the NW Mediterranean Sea. Mar. Ecol. Prog. Ser. 305, 127–137.

Linares, C., Doak, D.F., Coma, R., Díaz, D., Zabala, M., 2007. Life history and viability of a long-lived marine invertebrate: the octocoral *Paramuricea clavata*. Ecology 88, 918–928.

Linares, C., Coma, R., Garrabou, J., Díaz, D., Zabala, M., 2008a. Size distribution, density and disturbance in two Mediterranean gorgonians: *Paramuricea clavata* and *Eunicella singularis*. J. Appl. Ecol. 45, 688–699.

Linares, C., Coma, R., Zabala, M., 2008b. Effects of a mass mortality event on gorgonian reproduction. Coral Reefs 27, 27–34.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Michalek-Wagner, K., Willis, B.L., 2001. Impacts of bleaching on the soft coral *Lobophytum compactum* I. Fecundity, fertilization and offspring viability. Coral Reefs 19, 231–239.

Mistri, M., Ceccherelli, V.U., 1994. Growth and secondary production of the Mediterranean gorgonian *Paramuricea clavata*. Mar. Ecol. Prog. Ser. 103, 291–296.

Naumann, M.S., Orejas, C., Wild, C., Ferrier-Pagès, C., 2011. First evidence for zooplankton feeding sustaining key physiological processes in a scleractinian cold-water coral. J. Exp. Biol. 214, 3570–3576.

Oliver, J., Babcock, R., 1992. Aspects of the fertilization ecology of broadcast spawning corals: sperm dilution effects and in situ measurements of fertilization. Biol. Bull. 183, 409–417.

Ribes, M., Coma, R., Gili, J.M., 1999. Heterogeneous feeding in benthic suspension feeders: the natural diet and grazing rate of the temperate gorgonian *Paramuricea clavata*. Mar. Ecol. Prog. Ser. 183, 125–137.

Ribes, M., Coma, R., Rossi, S., Micheli, M., 2007. Cycle of gonadal development in *Eunicella singularis* (Cnidaria: Octocorallia): trends in sexual reproduction in gorgonians. Invertebr. Biol. 126, 307–317.

Richmond, R.H., 1987. Energetic relationships and biogeographical differences among fecundity, growth and reproduction in the reef coral *Pocillopora damicornis*. Bull. Mar. Sci. 41, 594–604.

Rinkevich, B., 1989. The contribution of photosynthetic products to coral reproduction. Mar. Biol. 101, 259–263.

Rossi, S., Gili, J.M., 2005. Composition and temporal variation of the near-bottom seston in a Mediterranean coastal area. Estuar. Coast. Shelf Sci. 65, 385–395.

Rossi, S., Gili, J.M., 2007. Short-time-scale variability of near-bottom seston composition during spring in a warm temperate sea. Hydrobiologia 557, 373–388.

Rossi, S., Tsounis, G., 2007. Temporal and spatial variation in protein, carbohydrate, and lipid levels in *Corallium rubrum* (Anthozoa, Octocorallia). Mar. Biol. 152, 429–439.

Rossi, S., Grémare, A., Gili, J.M., Amouroux, J.M., Jordana, E., Vétion, G., 2003. Biochemical characteristic of settling particulate organic matter at two north-western Mediterranean sites: a seasonal comparison. Estuar. Coast. Shelf Sci. 58, 423–434.

Rossi, S., Gili, J.M., Coma, R., Linares, C., Gori, A., Vert, N., 2006. Temporal variation in protein, carbohydrate, and lipid concentrations in *Paramuricea clavata* (Anthozoa, Octocorallia): evidence for summer–autumn feeding constraints. Mar. Biol. 149, 643–651.

Séré, M.G., Massé, L.M., Perissinotto, R., Schleyer, M.H., 2010. Influence of heterotrophic feeding on the sexual reproduction of *Pocillopora verrucosa* in aquaria. J. Exp. Mar. Biol. Ecol. 395, 63–71.

Sier, C.J.S., Olive, P.J.W., 1994. Reproduction and reproductive variability in the coral *Pocillopora verrucosa* from the Republic of Maldives. Mar. Biol. 118, 713–722.

Slattery, M., McClintock, J.B., 1995. Population structure and feeding deterrence in three shallow-water Antarctic soft corals. Mar. Biol. 122, 461–470.

Smetacek, V., Cloern, J.E., 2008. On phytoplankton trends. Science 319, 1346–1348.

Stimson, J.S., 1987. Location, quantity and rate of change in quantity of lipids in tissue of Hawaiian hermatypic corals. Bull. Mar. Sci. 41, 889–904.

Szmant, A.M., Gassman, N.J., 1990. The effects of prolonged "bleaching" on the tissue biomass and reproduction of the reef coral *Montastrea annularis*. Coral Reefs 8, 217–224.

Szmant-Froelich, A., Pilson, M.E.Q., 1980. The effects of feeding frequency and symbiosis with zooxanthellae on the biochemical composition of *Astrangia danae* Milne Edwards and Haime 1849. J. Exp. Mar. Biol. Ecol. 48, 85–97.

Szmant-Froelich, A., Pilson, M.E.Q., 1984. Effects of feeding frequency and symbiosis with zooxanthellae on nitrogen metabolism and respiration of the coral *Astrangia danae*. Mar. Biol. 81, 153–162.

Tsounis, G., Rossi, S., Araguren, M., Gili, J.M., Arntz, W., 2006a. Effects of spatial variability and colony size on the reproductive output and gonadal development cycle of the Mediterranean red coral (*Corallium rubrum* L.). Mar. Biol. 143, 513–527.

Tsounis, G., Rossi, S., Laudien, J., Bramanti, L., Fernández, N., Gili, J.M., Arntz, W., 2006b. Diet and seasonal prey capture rate in the Mediterranean red coral (*Corallium rubrum* L.). Mar. Biol. 149, 313–325.

Tsounis, G., Martinez, L., Bramanti, L., Viladrich, N., Gili, J.M., Martinez, A., Rossi, S., 2012. Anthropogenic effects on reproductive effort and allocation of energy reserves in the Mediterranean octocoral *Paramuricea clavata*. Mar. Ecol. Prog. Ser. 449, 161–172.

Van Veghel, M.L.J., Bak, R.P.M., 1994. Reproductive characteristics of the polymorphic Caribbean reef building coral *Montastrea annularis* III. Reproduction in damaged and regenerating colonies. Mar. Ecol. Prog. Ser. 109, 229–233.

Ward, S., 1995. Two patterns of energy allocation for growth, reproduction and lipid storage in the scleractinian coral *Pocillopora damicornis*. Coral Reefs 14, 87–90.

Ward, S., Harrison, P., 2000. Changes in gametogenesis and fecundity of acroporid corals that were exposed to elevated nitrogen and phosphorus during the ENCORE experiment. J. Exp. Mar. Biol. Ecol. 246, 179–221.

Weinberg, S., 1976. Revision of the common Octocorallia of the Mediterranean circalittoral. I. Gorgonacea. Beaufortia 24, 63–104.

Weinberg, S., 1978. Mediterranean octocorallian communities and the abiotic environment. Mar. Biol. 49, 41–57.

Weinberg, S., Weinberg, F., 1979. The life cycle of a gorgonian: *Eunicella singularis* (Esper 1794). Bijdr. Dierkd. 48, 127–140.

Wildish, D., Kristmanson, D., 1997. Benthic Suspension Feeders and Flow. Cambridge University Press, Cambridge.

Willmer, P., Stone, G., Johnston, I., 2000. Environmental Physiology of Animals. Blackwell, Oxford.