Vol. 573: 117–128, 2017
https://doi.org/10.3354/meps12141

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published June 21

Variation of lipid and free fatty acid contents during larval release in two temperate octocorals according to their trophic strategy

N. Viladrich[1,*], L. Bramanti[2], G. Tsounis[3], A. Martínez-Quintana[4], C. Ferrier-Pagès[5], S. Rossi[1]

[1]Institut de Ciència i Tecnologia Ambientals (Universitat Autònoma de Barcelona), Edifici Z Campus UAB, Cerdanyola del Vallès 08193, Spain

[2]UMR 8222, LECOB, Observatoire Oceanologique, UPMC, 66650 Banyuls-sur-Mer, France

[3]Department of Biology, California State University, 18111 Nordhoff Street, Northridge, CA 91330-8303, USA

[4]Geology Department, 126 Cooke Hall, University at Buffalo - The State University of New York, Buffalo, NY 14260-3050, USA

[5]Equipe Ecophysiologie Corallienne, Centre Scientifique de Monaco, 8 Quai Antoine 1er, MC-98000, Monaco

ABSTRACT: This study investigated the energetic investment in larval release of 2 Mediterranean gorgonians with different trophic strategies: *Eunicella singularis* (mixotroph) and *Corallium rubrum* (heterotroph). Both are internal brooders, releasing larvae within a few weeks in summer. A biochemical approach, based on the analysis of stable isotopes, total lipid and free fatty acid (FFA) content, was applied in combination with the quantification of sexual products. Stable isotopes showed that food source varied seasonally for *E. singularis*, while it remained constant throughout the year for *C. rubrum*, suggesting a higher trophic plasticity of *E. singularis*, likely due to its mixotrophic feeding strategy. Although total lipid and FFA content were higher in *E. singularis* than in *C. rubrum*, both species showed low energetic investment in reproduction, probably linked to their low fecundity and reproductive output, compared to other gorgonians (e.g. *Paramuricea clavata*). The higher FFA content in *E. singularis* compared to *C. rubrum* can be explained by a higher metabolic demand and metabolite exchange between the host and its symbiotic algae. The higher inter-annual variability in total lipid and FFA content in *C. rubrum* suggests that this species is more sensitive to food constraints than *E. singularis*. Indeed, the inter-annual consistency in the trophic ecology (showed by the stable isotopes) means that *C. rubrum* is more affected by changes in food availability. Conversely, the mixotrophy makes *E. singularis* less sensitive to stress conditions caused by starvation or thermal stress. These physiological differences could partly explain the wider distribution of *E. singularis* compared to *C. rubrum*.

KEY WORDS: Energy investment · Gorgonian · Fatty acids · Lipids · Heterotrophic · Autotrophic

INTRODUCTION

Lipid content in animal tissues (Cejas et al. 2004, Rossi et al. 2006, Seemann et al. 2013) and trophic strategy (Grottoli et al. 2006, Viladrich et al. 2016a) are 2 main drivers of the capacity to resist environmental stressors. According to their trophic strategies, species can be grouped in 2 main categories: monophagous or polyphagous (Broom 1981). The diet of monophagous species is focused on a single specific food source and thus their capacity to accumulate lipid reserves depends on the availability of the food source. Conversely, polyphagous species can adapt their diet according to the food availability,

*Corresponding author: viladrich.nuria@gmail.com

and lipids accumulated by these species are rather related to food quantity and quality. Differences in trophic strategies will induce differences in the quantity and quality of lipids accumulated in animal tissue, with consequent impact on the organisms' health, survival, reproduction and growth (Szmant & Gassman 1990, Anthony & Connolly 2004, Grimsditch & Salm 2006). Despite the major role they play in organism health, studies on trophic strategies are not common, probably due to the difficulty in correcting for feeding behavior. Therefore, passive suspension feeders, lacking the ability to pursue prey, are useful models to study trophic strategies and the effect of food availability on metabolism.

Heterotrophic passive suspension feeders acquire nutrients through the capture of zooplankton and suspended particulate organic matter (POM) (Gili & Coma 1998), and in some cases also through the uptake of dissolved organic matter (DOM) (Al-Moghrabi et al. 1993). In suspension feeders living in symbiosis with intracellular dinoflagellates, photosynthates (autotrophic carbon) fixed by the symbionts cover most of the nutritional needs of the host (Muscatine et al. 1981, 1984, Tremblay et al. 2014), which can also acquire heterotrophic nutrients (e.g. Goreau et al. 1971, Ferrier-Pagès et al. 2003, Houlbrèque et al. 2004). The combination of auto- and heterotrophy (mixotrophy) maximizes the nutrient acquisition and ecological success of suspension feeders in environments where light and plankton concentrations are variable and often limiting (Muller-Parker & Davy 2001, Grottoli et al. 2006). Indeed, food availability may occasionally become a constraining factor for non-symbiotic corals (Coma & Ribes 2003, Rossi et al. 2004, Houlbrèque & Ferrier-Pagès 2009), while the trophic plasticity of mixotrophic species allows increased energy acquisition (Goreau et al. 1971, Anthony & Fabricius 2000, Ferrier-Pagès et al. 2011, Gori et al. 2012).

Nutritional condition of parental colonies may also affect the survival of future generations, as it directly affects the number of offspring and their survival (Strathmann 1985, Simpson 2009, Gori et al. 2013). The amount of energy allocated to reproduction can then inform on the survival capacity of parental colonies and offspring, as well as help to understand their distribution patterns (Cocito et al. 2013). Mixotrophic species, which have higher or more constant levels of energy reserves, should show a higher or more constant energetic investment in reproduction than heterotrophic ones.

In corals, reproductive investment is commonly estimated by quantifying the number of oocytes produced (fecundity) and/or their volume (e.g. Hall & Hughes 1996). However, this approach can underestimate the reproductive investment, because it does not take into account the energetic costs of gamete production and tissue repair associated with spawning, nor the energetic cost of the survival of parental colonies and larvae (Calow 1979). A more accurate estimate of the energetic reproductive investment can be obtained by quantifying the changes in lipid content of tissues before and after larval release (Stimson 1978, Ward 1995, Leuzinger et al. 2003).

In corals, the total lipid content includes wax esters, sterol esters, phospholipids, glycolipids and free fatty acids (FFAs), among others (Imbs et al. 2015). Most lipid components are energy reserves that can be oxidized to obtain FFAs (Gurr et al. 2002), which in turn are beta-oxidized to provide a source of immediate and highly efficient energy (high ATP/FA molecule; Sargent et al. 1988). Therefore, FFA content can trace the metabolic demands of corals according to the reproductive period or their trophic strategy (Díaz-Almeyda et al. 2011, Imbs 2013, Viladrich et al. 2016a). Indeed, it was shown that the FFA content can increase in stress situations, probably as a response to higher metabolic demand (Sargent et al. 1999).

In the Mediterranean Sea, *Corallium rubrum* and *Eunicella singularis* are 2 of the most common octocorals in coastal areas (Ballesteros 2006). Whereas *C. rubrum* is heterotrophic, feeding on POM and zooplankton (Tsounis et al. 2006a), *E. singularis* is mixotrophic, due to its symbiosis with the dinoflagellate *Symbiodinium* sp. (Forcioli et al. 2011). Even if the quantity of carbon acquired from symbionts by *E. singularis* is 2 to 3 times lower than in tropical scleractinian corals, photosynthates can sustain the daily respiratory needs of this species in summer, due to highly efficient light utilization (Ferrier-Pagès et al. 2015). On the other hand, heterotrophic feeding is also important for *E. singularis* (Coma et al. 2015), since the wide distribution of the species includes habitats with lower light levels, colder waters and higher nutrient concentrations compared to tropical areas (Gori et al. 2012). Thus, *E. singularis* is able to use heterotrophic and autotrophic energy to sustain its basic metabolism (Ezzat et al. 2013) and optimize its nutrient input (Gori et al. 2012, Ferrier-Pagès et al. 2015). The trophic plasticity of *E. singularis* was the subject of several studies in recent years (Cocito et al. 2013, Ezzat el al. 2013, Coma et al. 2015, Ferrier-Pagès et al. 2015), but its effects on reproduction are still unknown.

E. singularis and *C. rubrum* are long-lived gonochoric species with annual reproduction. Lecithotrophic ciliated larvae (planulae) are internally brooded and released once a year over a period of approximately 2 wk in June to July and July to August, respectively, for *E. singularis* and *C. rubrum* (Santangelo et al. 2004, Bramanti et al. 2005, Tsounis et al. 2006b, Gori et al. 2007, Ribes et al. 2007). Planulae of the 2 species have a pelagic larval duration (PLD) of approximately 1 mo (Theodor 1967, Weinberg & Weinberg 1979, Martínez-Quintana et al. 2015). Therefore, these 2 species represent a good model to understand how passive suspension feeders, with similar reproductive features but different trophic strategies, invest their stored energy in offspring. To achieve this objective, oocyte and sperm sac production (number, size and volume), organic matter (OM), total lipid and FFA content were quantified in *E. singularis* and *C. rubrum* colonies before and after spawning, as a proxy for parental investment. The analyses were carried out during 2 yr (2010 and 2011) in order to take into account temporal variability and its effect on the reproductive output and energetic condition of the population. Stable isotope composition was also seasonally assessed in both species to display their trophic strategies. The results will help in understanding the functioning of passive suspension feeder populations, their distribution patterns, and their capability to survive acute and/or recurrent perturbations.

MATERIALS AND METHODS

Sampling procedure

Samples of *Corallium rubrum* (N = 30) and *Eunicella singularis* (N = 30) were collected by SCUBA diving at Punta s'Oliguera in Cap de Creus (42° 17′ 03″ N; 003° 17′ 95″ E, northwestern Mediterranean Sea; Fig. 1). Populations of both species were located in the same rocky wall at different depths (25 to 30 m for *C. rubrum* and 13 to 16 m for *E. singularis*). Owing to their small size, *C. rubrum* colonies >4–5 cm height (sexually mature according to Tsounis et al. 2006b) were sampled haphazardly. For *E. singularis*, colonies >20 cm height (sexually mature colonies according to Ribes et al. 2007) were tagged and sampled.

For each sampling date (see the following subsections), 1 fragment of primary branch was collected from each colony and divided into 2 portions. The first portion (~1 cm, base) was fixed in 10% formalin for the study of sexual product development and output (Coma et al. 1995, Rossi & Gili 2009). The second portion (1 to 2 cm, top) was immediately frozen in liquid nitrogen stored at –80°C, freeze-dried (–110°C and 100 mbar) for 24 h, and finally stored at –20°C for biochemical analyses.

Stable isotopes

The stable isotope ($\delta^{13}C$ and $\delta^{15}N$) composition of the tissue of *E. singularis* and *C. rubrum* was assessed to identify the trophic position of the 2 species (Gori et al. 2012, Cocito et al. 2013). For this purpose, 3 colonies for each species were sampled seasonally (May, August, November and January). Two portions of 2 ± 0.001 mg (Mettler Toledo model XS3DU) of coenenchyme dry weight (DW) were taken from each sample. One portion was analysed for $\delta^{15}N$ and the other portion was fumed with concentrated HCl during 48 h to eliminate the inorganic fraction, and then analysed for $\delta^{13}C$. $\delta^{13}C$ and $\delta^{15}N$ were determined by using a Thermo Flash EA 1112 analyser and a Thermo Delta V Advantage spectrometer following the same methodology as Gori et al. (2012). Isotope ratios are expressed as parts per thousand (‰) (different from a standard reference material)

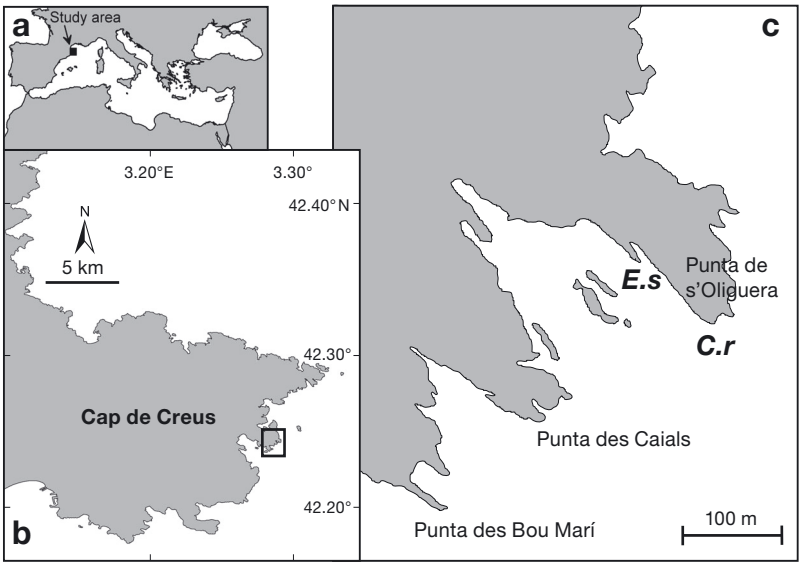

Fig. 1. (a,b) Study area and (c) location of the sampling site. *C.r* and *E.s*: *Corallium rubrum* and *Eunicella singularis* populations, respectively

according to the equation $\delta^X = [(R_{sample}/R_{standard}) - 1] \times 10^3$, where X is ^{13}C or ^{15}N and R is the corresponding ratio C^{13}/C^{12} or N^{15}/N^{14}. $R_{standard}$ values for ^{13}C and ^{15}N are from Pee Dee Belemnite (PDB) and atmospheric N_2, respectively.

Production of oocytes and spermaries

Oocyte and sperm sac production of *C. rubrum* and *E. singularis* was followed from May to August 2010 and from April to August 2011 to describe the sexual product development and to determine the exact moment of larval release in both years (see 'Results: Production of oocytes and spermaries'). For each species, 5 colonies of each sex were identified (Santangelo et al. 2004, Ribes et al. 2007), 6 polyps per colony were dissected under a stereomicroscope (Olympus SZ-STS) and the sexual products found in each polyp were photographed (Moticam 5, 5.0 million pixels). Pictures were then analysed with Macnification 2.01 software (www.orbicule.com/macnification/) to measure the area (A) and circularity of each oocyte or sperm sac. Circularity, defined as the ratio between the area measured and the area of a circle with the same perimeter, determines the extent to which a shape can be approximated to a circle. As circularity was always >90%, sexual products were considered spheres. Their diameter (d) was calculated according to the equation $d = 2(A/\pi)^{-1/2}$ and their volume (V) was calculated according to the equation $V = 4/3\pi(d/2)^3$.

OM content

OM was quantified before and after larval release in 2010 and 2011, using 5 colonies for each sex and species. Approximately 100 ± 0.1 mg of coenenchyme DW from each sample was weighed, combusted for 4 h at 500°C in a muffle furnace (Relp 2H-M9) and weighed again. OM was then calculated as the difference between dry and ash weight (Slattery & McClintock 1995). Results are expressed in percentage with respect to the initial DW.

Lipid content

The total lipid content in the OM was quantified in 5 female and 5 male colonies from each species before and after larval release in 2010 and 2011, following the colorimetric method of Barnes & Blackstock (1973). Approximately 10 ± 0.1 mg of coenenchyme DW from each sample was homogenized in 3 ml of chloroform:methanol (2:1), and total lipids were quantified colorimetrically according to the sulphophosphovanilun methods with absorbance quantified at 520 nm, using cholesterol as a standard. Results are presented in µg lipid mg^{-1} OM.

FFA content

FFA content was assessed in 3 female and 3 male colonies from each species before and after larval release in 2010 and 2011, according to Viladrich et al. (2016b). Approximately 10 ± 0.1 mg of coenenchyme DW from each sample was dissolved in dichloromethane:methanol (3:1). The extraction solvent was eluted through an aminopropyl glass column resulting in 3 fractions (neutral lipids, FFAs and polar lipids). Only the FFA fraction was used in this study, and it was methylated using a solution of 20% methanol/boron trifluoride heated at 90°C for 1 h. The methyl esters of FFA obtained were identified and quantified with gas chromatography (GC) (Agilent Technologies 7820A GC) by comparing their retention times with those of standard FAs (37 fatty acid methyl ester compounds, Supelco Mix C^4–C^{24}) and by integrating areas under peaks in the GC traces (Chromquest 4.1 software). Results are presented in µg FFA mg^{-1} OM.

Statistical treatment

Differences in volume, diameter and number of sexual products between years, sexes and species were tested using a non-parametric Wilcoxon-Mann-Whitney test, (R software, function 'wilcox.test'; R Development Core Team 2008), as the data were not normally distributed. Differences in stable isotopes were tested using a 2-way ANOVA with season (4 levels: spring, summer, autumn and winter) and species (2 levels: *C. rubrum* and *E. singularis*) as independent variables. Differences in OM, lipid and FFA content were tested using a 4-way ANOVA with year (2 levels: 2010 and 2011), species (2 levels: *C. rubrum* and *E. singularis*), release period (2 levels: before and after larval release) and sex (2 levels: male and female) as independent variables. Before performing ANOVAs, normality of data residuals and variance homogeneity were tested with Shapiro-Wilks and Bartlett's tests (R language function 'shapiro.test' and 'bartlett.test'). When variances were not homogeneous, logarithmic transformations were applied.

ANOVA tests were performed with the R language function 'aov' (Chambers & Hastie 1992) followed, when appropriate, by a Tukey post hoc test (R language function tukeyHSD).

RESULTS

Stable isotopes

Stable isotope composition did not change between seasons in *Corallium rubrum* (1-way ANOVA, $p > 0.05$; Fig. 2), whereas *Eunicella singularis* showed significant differences in $\delta^{13}C$ between spring and summer, and in $\delta^{15}N$ between spring, summer and autumn (1-way ANOVA, $p < 0.01$; Fig. 2). While $\delta^{13}C$ showed significant differences between species in all seasons (2-way ANOVA, $p < 0.001$; Fig. 2), $\delta^{15}N$ was significantly different only in spring (2-way ANOVA, $p < 0.01$; Fig. 2).

Production of oocytes and spermaries

Changes in the frequency distribution of sexual products in *C. rubrum* (Fig. 3a,b) and *E. singularis* (Fig. 3c,d) indicated the approximate period of larval release in 2010 and 2011, which corresponded to the disappearance of larvae inside the female polyps. Larvae of *C. rubrum* were released between 19 July and 8 August 2010 (Fig. 3a), and between 29 June and 4 August 2011 (Fig. 3b). On the basis of these observations, samples collected on 9 June 2010 and 5 June 2011 were considered 'pre-release', and samples collected on 8 August 2010 and 4 August 2011 were considered 'post-release'.

E. singularis released larvae between 9 June and 8 August 2010 (Fig. 3c), and between 5 June and 4 August 2011 (Fig. 3d). Thus, samples of 9 June 2010 and 5 June 2011 were considered 'pre-release', and samples collected on 8 August 2010 and 4 August 2011 were categorized as 'post-release'.

In *C. rubrum*, no significant difference in oocyte size was found between years, while spermaries in 2011 were significantly larger in size, lower in number and with a smaller total volume with respect to 2010 (Wilcoxon-Mann-Whitney test, $p < 0.001$; Fig. 4). In *E. singularis*, the diameter of oocytes and spermaries was significantly bigger in 2011 (Wilcoxon-Mann-Whitney test, $p < 0.001$; Fig. 4b). Conversely, the number and volume of sexual products per polyp did not show any significant difference between years and sexes (Wilcoxon-Mann-Whitney test, $p > 0.05$; Fig. 4a,c). The volume and number of oocytes in both years, and spermaries only in 2011, were different between the 2 species (Wilcoxon-Mann-Whitney test, $p < 0.001$; Fig. 4a,c), being higher in *E. singularis* than in *C. rubrum*. The diameter of sexual products was higher in *C. rubrum* than in *E. singularis* (Wilcoxon-Mann-Whitney test, $p < 0.01$; Fig. 4b), except between female colonies in 2011, for which no difference was found (Wilcoxon-Mann-Whitney test, $p > 0.05$; Fig. 4b).

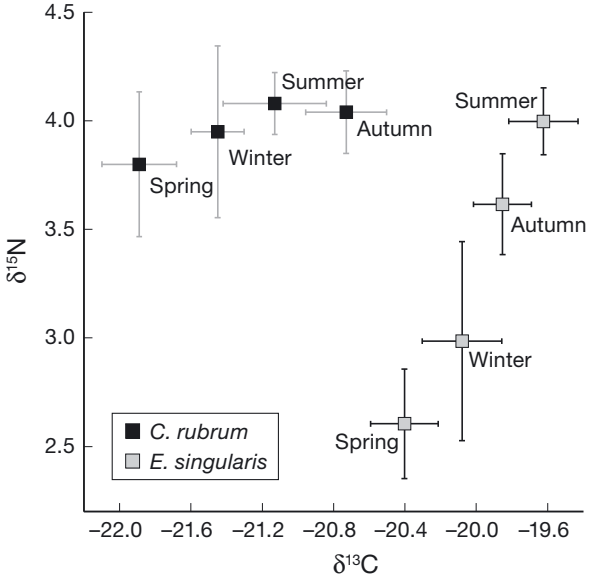

Fig. 2. Stable isotope ($\delta^{15}N$ and $\delta^{13}C$) composition in *Corallium rubrum* and *Eunicella singularis*. n = 3 for each point; mean ± SD

OM content

OM content was 24.1 ± 6.4% (mean ± SD) for *C. rubrum* and 24.4 ± 5.8% for *E. singularis*, with no significant differences either within species, considering the sex and sampling periods (ANOVA 4-way, $p > 0.05$), or between species (ANOVA 4-way, $p > 0.05$).

Lipid content

Lipid content of *C. rubrum* significantly decreased after larval release only in female colonies during 2010 (ANOVA 4-way, $p < 0.001$; Fig. 5a). On the other hand, *E. singularis* did not show any significant difference between sexes or between sampling periods (ANOVA 4-way, $p > 0.05$; Fig. 5b). Lipid content in female colonies was significantly higher in *E. singularis* than in *C. rubrum* in 2010 (ANOVA 4-way,

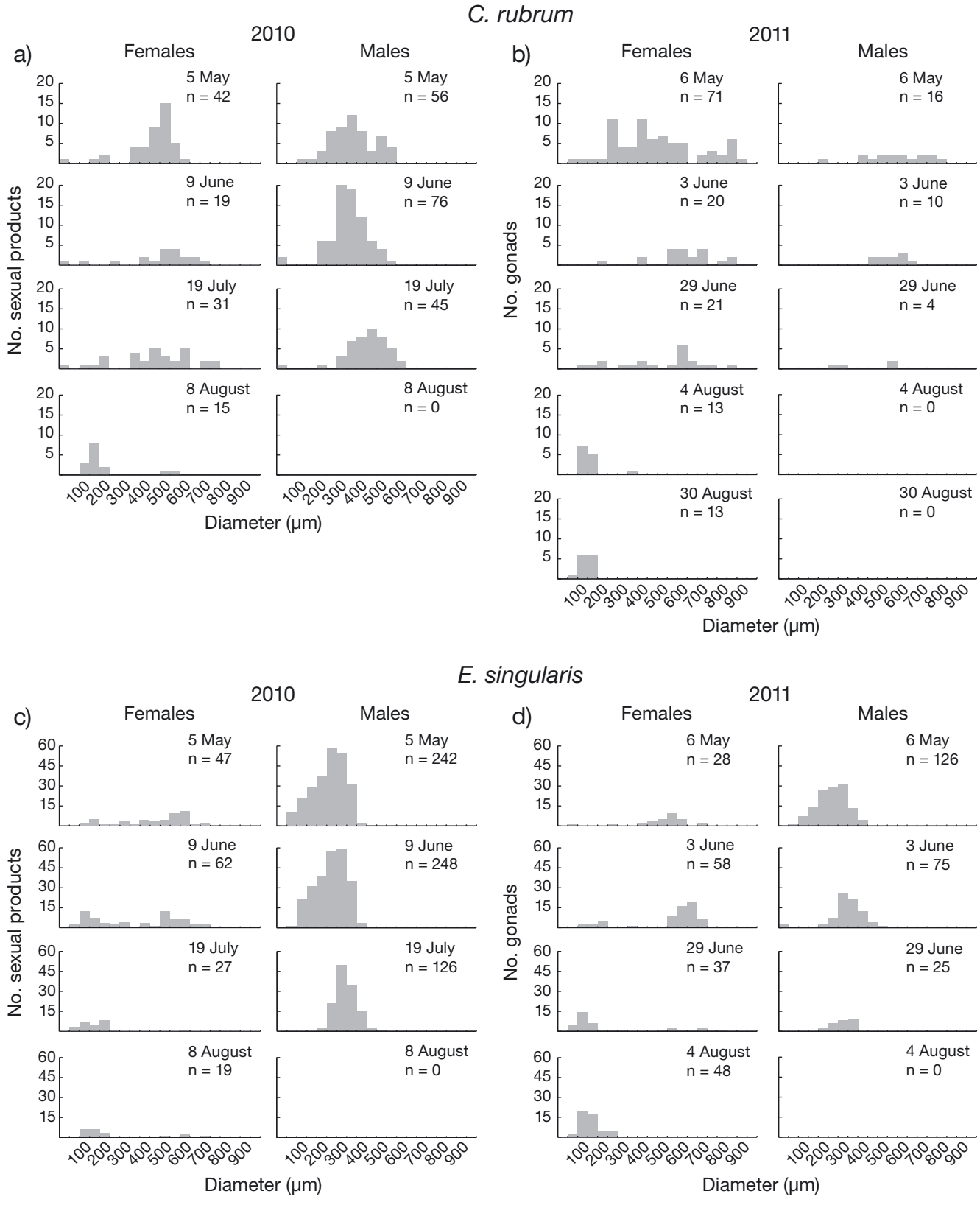

Fig. 3. Distribution of gonadal diameter frequency (µm) in 30 female and male polyps of (a,b) *Corallium rubrum* and (c,d) *Eunicella singularis* in year (a,c) 2010 and (b,d) 2011. n = sexual product number

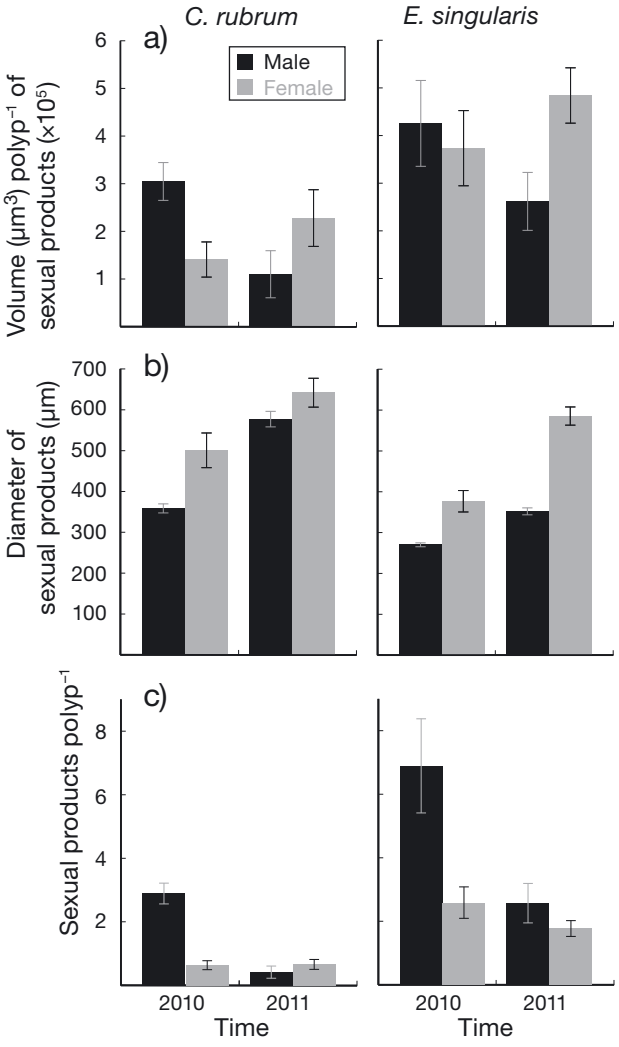

Fig. 4. (a) Volume, (b) diameter and (c) number of sexual products per polyp of 5 male and female colonies (mean ± SE) in 2 yr (2010, 2011) for *Corallium rubrum* and *Eunicella singularis*, respectively

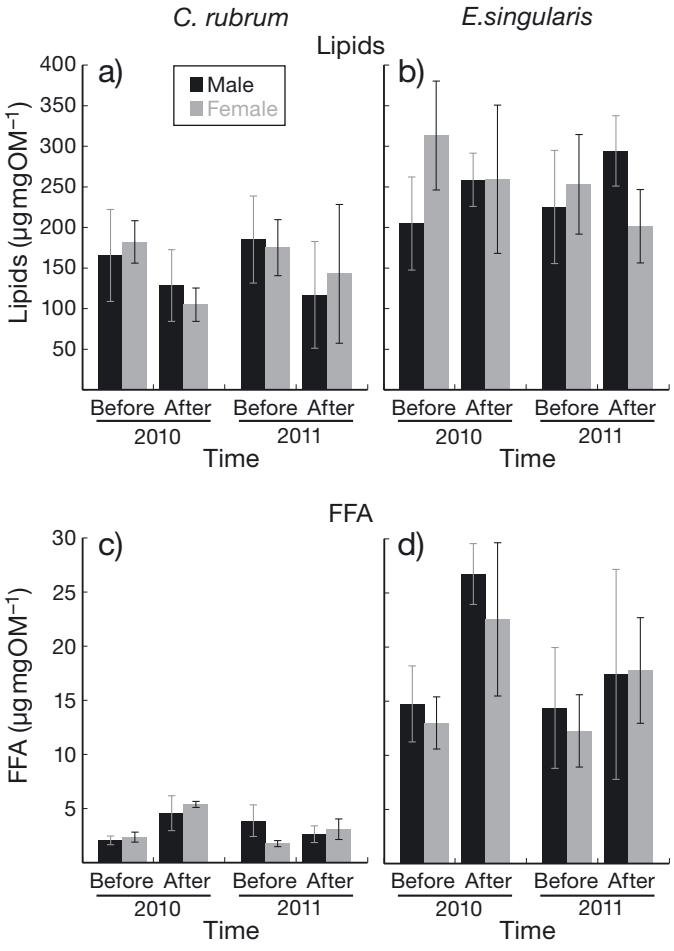

Fig. 5. (a,b) Lipid and (c,d) free fatty acid (FFA) content (µg mg^{-1} OM) (mean ± SD) in tissue of (a,c) *Corallium rubrum* and (b,d) *Eunicella singularis* in male and female colonies (n = 5 for lipids, n = 3 for FFAs) before and after spawning in 2010 and 2011

$p < 0.05$; Fig. 5a,b), but not in 2011 (ANOVA 4-way, $p > 0.05$; Fig. 5a,b). Male colonies presented significantly higher lipid content in *E. singularis* than in *C. rubrum* in both years after larval release (ANOVA 4-way, $p < 0.01$; Fig. 5a,b), but not before larval release (ANOVA 4-way, $p > 0.05$; Fig. 5a,b).

FFA content

FFA content in *C. rubrum* colonies did not show significant differences between sexes or before larval release (ANOVA 4-way, $p > 0.05$; Fig. 5c). However, after larval release, the FFA content significantly increased in 2010 (ANOVA 4-way, $p < 0.05$; Fig. 5c), and it was overall significantly higher than in 2011 (ANOVA 4-way, $p < 0.05$; Fig. 5c). FFA content in colonies of *E. singularis* was similar in all conditions, except in male colonies after the larval release in 2010, where it increased (ANOVA 4-way, $p < 0.05$; Fig. 5d). The comparison between species showed a higher FFA content in *E. singularis* than in *C. rubrum*, considering the sex and sampling period (ANOVA 4-way, $p < 0.001$; Fig. 5c,d). Finally, another difference between both species was the high variability (i.e. SD) of *E. singularis* with respect to *C. rubrum*.

DISCUSSION

This study analysed variations in energetic reserves during larval release in 2 gorgonian species with different trophic strategies: *Corallium rubrum* (hetero-

trophic) and *Eunicella singularis* (mixotrophic). The results showed that the energy allocation during larval release was similar between the 2 species, but *E. singularis* was characterized by a better nutritional condition (higher total lipid content) with a lower inter-annual variability with respect to *C. rubrum*, likely due to the presence of symbionts, which buffered the environmental variability in food availability.

Trophic strategies confirmed by stable isotope analysis

$\delta^{13}C$ and $\delta^{15}N$ signatures of animal tissue serve as a long-term index of carbon and nitrogen assimilation (Muscatine et al. 1989, Bergschneider & Muller-Parker 2008), and are also a useful tool for understanding trophic interactions in animals. While the $\delta^{13}C$ signatures of the consumers and the food sources are similar (DeNiro & Epstein 1978), the enrichment factor in $\delta^{15}N$ between 2 consecutive trophic levels ranges from 2.3 to 3.4‰ (Minagawa & Wada 1984, McCutchan et al. 2003). The $\delta^{13}C$ and $\delta^{15}N$ values obtained in the present study for *C. rubrum* and *E. singularis* were in agreement with the values measured for other temperate suspension feeders (Fig. 2) (Carlier et al. 2007, Cocito et al. 2013). The $\delta^{13}C$ and $\delta^{15}N$ values of *C. rubrum* did not present large seasonal changes, suggesting that this species has a defined diet (monophagous trophic strategy), which does not change throughout the year. In addition, the depleted $\delta^{13}C$ and high $\delta^{15}N$ values found in *C. rubrum* suggest that the diet is mainly based on POM (Darnaude et al. 2004a,b, Carlier et al. 2007), in agreement with the findings of previous feeding experiments (Tsounis et al. 2006a). Indeed, POM is usually characterized by very low $\delta^{13}C$ (ranging from –20 to –30‰) and high $\delta^{15}N$ (ca. 3 to 4‰), characteristic of numerous recycling processes occurring in the water column. In contrast, *E. singularis* displayed a higher seasonal variability in its isotopic signature, suggesting a greater trophic plasticity (polyphagous trophic strategy). This result is in agreement with previous observations (Gori et al. 2012) and may be due to both autotrophic and heterotrophic inputs. However, the small difference in the $\delta^{13}C$ signature of *E. singularis* between summer and winter (less than 1‰) suggests that the input from autotrophy is limited (Gori et al. 2012, Cocito et al. 2013, Ferrier-Pagès et al. 2015), and also points to mixotrophic feeding throughout the year. Indeed, scleractinian temperate species (e.g. *Cladocora caespitosa*), which benefit from a high autotrophic input in summer, present a much larger variation in their $\delta^{13}C$ signature between winter and summer (up to 8‰; Ferrier-Pagès et al. 2011). The constant difference in $\delta^{13}C$ between *C. rubrum* and *E. singularis* (maximum of ca. 1.6‰ in spring) can be explained by the small autotrophic carbon input derived from *E. singularis* symbionts, a different diet, or a different carbon fractionation in these 2 species, although no firm conclusion can be drawn at this point. On the other hand, results of $\delta^{15}N$ in *E. singularis* showed a high seasonal variation. In general, $\delta^{15}N$ signature augments with increasing trophic level (Minagawa & Wada 1984, Post 2002, Carlier et al. 2007). Therefore, the results suggest that the high $\delta^{15}N$ observed in summer and autumn is due to heterotrophic feeding, whereas the low $\delta^{15}N$ observed in winter and spring is due to a very depleted source, probably dissolved inorganic nitrogen (DIN) directly taken up by the symbionts. This would indicate that DIN is a constraining factor for the autotrophy in *E. singularis*. However the increased nitrogen isotopic signature observed in summer could also be due to the use of the host nitrogenous wastes by the symbionts (Reynaud et al. 2009, Ferrier-Pagès et al. 2011, Cocito et al. 2013). In this case, the depleted $\delta^{15}N$ would indicate a high degree of nitrogen recycling in summer (Gori et al. 2012, Cocito et al. 2013, Ezzat et al. 2013).

Energy investment in reproduction according to trophic strategy

Volume and number of oocytes and sperm sacs per polyp were higher in *E. singularis* than in *C. rubrum* (Fig. 4), likely due to the energetic surplus deriving from its mixotrophic feeding. Reproductive output, in fact, can vary according to the quantity and quality of available food (Qian & Chia 1991, 1992, Gori et al. 2013). A recent study by Gori et al. (2012) on *E. singularis* showed that asymbiotic populations of this species (60 m depth) have a lower volume of oocytes and sperm sacs than symbiotic ones (20 m depth).

Apart from differing between the 2 species, volume, size and number of oocytes and sperm sacs showed an inter-annual variability in both species (Fig. 4), probably linked to environmental variability (Brey 1995, Tsounis et al. 2006b, Gori et al. 2007, Torrents & Garrabou 2011, Gori et al. 2013). However, we are unable to link these 2 factors, as we lacked environmental data for spring 2009, when oocyte and sperm sacs obtained in 2010 started to develop (Vighi 1972, Santangelo et al. 2004, Tsounis et al. 2006b, Gori et al. 2007, Ribes et al. 2007, Gori et al. 2013).

Values of lipid content found for both species are in line with previous studies (Fig. 5a,b) (*C. rubrum*: Rossi & Tsounis 2007, Bramanti et al. 2013; *E. singularis*: Gori et al. 2007, Gori et al. 2012). Moreover, the lipid content of *E. singularis* is in agreement with that measured in other symbiotic corals (Harland et al. 1993, Yamashiro et al. 1999, Grottoli et al. 2004, Shirur et al. 2014), while lipid content found in *C. rubrum* is comparable with other non-symbiotic octocorals (Rossi et al. 2006, Hamoutene et al. 2008, Tsounis et al. 2012). Therefore, the higher values found in *E. singularis* with respect to *C. rubrum* are probably due to the acquisition of photosynthates transferred to the host by *Symbiodinium* cells. However, the higher lipid content in adults of *E. singularis* does not result in a higher reproductive investment of this species compared to *C. rubrum*. These findings do not support the common hypothesis that mixotrophy should result in a higher energetic investment in reproduction (Mckillup & Butler 1979, Thompson 1983, George 1994). Hence, at least in *E. singularis*, there is no direct relationship between energetic reserves and parental investment in reproduction.

After spawning, organisms face high energetic costs to repair the processes associated with spawning (Calow 1979). Therefore, animals with a high initial amount of lipids and energetic reserves should recover after spawning faster than those with lower reserves (Grottoli et al. 2006). This is exactly what the present study revealed, as male colonies of *E. singularis* displayed higher lipid content after spawning than *C. rubrum* colonies. This difference cannot be observed in female colonies, due to their higher investment in reproduction (Arai et al. 1993, Raymond et al. 2007). Therefore, these observations suggest that male colonies should recover faster than female colonies.

In 2010, the lipid content in female colonies was lower in *C. rubrum* than *E. singularis* both before and after larval release. Summer 2010 was characterized by lower food availability in the water column compared to 2011 in the studied area (Viladrich et al. 2016b), which could have induced a starvation in *C. rubrum* and *E. singularis* (Tsounis et al. 2006a, Coma et al. 2015). Unlike *C. rubrum*, *E. singularis* could have compensated the decrease in feeding rate with the energy input from *Symbiodinium*, therefore being less affected by food availability (Previati et al. 2010, Cocito et al. 2013). The wider distribution of *E. singularis* with respect to *C. rubrum* (Rossi et al. 2008, Gori et al. 2011, Angiolillo et al. 2016) can also be partly related to its mixotrophic feeding, conferring a higher resistance to environmental stressors (Grottoli et al. 2006).

Energetic demands before and after spawning

Besides the differences observed in lipid content, the 2 species also differed in their FFA content. The FFAs are compounds that can be beta-oxidized to provide a source of immediate and highly efficient energy (high ATP/FA molecule) (Sargent et al. 1988) and, therefore, reflect specific cellular physiological functions and physiological states (Sargent et al. 1990, 1999). The FFA content in *E. singularis* was higher than in *C. rubrum* (Fig. 5c,d), suggesting that *E. singularis* has a higher metabolic demand, which is probably linked to the maintenance costs of the symbiosis (i.e. to cope with high oxidative stress and possible regulation of the symbiotic algal growth; Muller-Parker & D'Elia 1997). On the other hand, fast-growing species such as *E. singularis* (Weinberg & Weinberg 1979, Munari et al. 2013, Viladrich et al. 2016b) also have high respiration rates, and thus high metabolic demands (Gates & Edmunds 1999, Marschal et al. 2004). Finally, the high variability (i.e. SD) in FFA content of *E. singularis* may be partly explained by an uneven distribution of *Symbiodinium* in the tissue (Bachok et al. 2006), which results in differences in photosynthetic rates and in cellular energy demand between different areas of the tissue (Oku et al. 2002).

Furthermore, the FFA content can increase during stress conditions, such as the presence of pathogens or as a consequence of starvation (Sargent et al. 1999). The present results show that the FFA content in *C. rubrum* increased after spawning only in 2010 (Fig. 5c), suggesting that *C. rubrum* suffered physiological stress in that year. In fact, summer 2010 was characterized by lower POM and zooplankton availability in the water column in comparison to 2011 (Viladrich et al. 2016b), and therefore lower food availability, which can affect the nutritional condition of *C. rubrum* (Tsounis et al. 2006a).

In conclusion, the present study reveals that the lipid content of the tissues of octocorals increased with mixotrophic feeding, but this increase did not result in a higher energy investment in the reproductive activity. The results also suggest that mixotrophic species are probably less affected by environmental variability due to their trophic plasticity, which gives an energy surplus with respect to heterotrophic organisms. This conclusion is corroborated by the study of Sbrescia et al. (2008), in which a quick recovery of *E. singularis* after a thermally induced mass mortality event was observed. The implications of this sensitivity to environmental conditions, in light of global climate change affecting shallow water benthic com-

munities (<40 m depth), warrant further studies to evaluate the potential for shifts from heterotrophic to mixotrophic coral communities.

Acknowledgements. The authors thank N. Moraleda for laboratory work, A. Sandu for help with English and 2 anonymous reviewers for their constructive comments on the manuscript. N.V. was funded by an FI AGAUR research grant (FI-2010-03824), and S.R. by a Ramón y Cajal contract (RyC-2007-01327) and a Marie Curie International Outgoing Fellowship (ANIMAL FOREST HEALTH, Grant Agreement Number 327845). The work was supported by the BENTOLARV project (CTM2009-10007) and the authors are grateful for the support of the Generalitat de Catalunya MERS (2014 SGR - 1356) project.

LITERATURE CITED

Al-Moghrabi S, Allemand D, Jaubert J (1993) Valine uptake by the scleractinian coral *Galaxea fascicularis*: characterization and effect of light and nutritional status. J Comp Physiol B 163:355–362

Angiolillo M, Gori A, Canese S, Bo M and others (2016) Distribution and population structure of deep-dwelling red coral in the northwest Mediterranean. Mar Ecol 37: 294–310

Anthony KRN, Connolly SR (2004) Environmental limits to growth: physiological niche boundaries of corals along turbidity–light gradients. Oecologia 141:373–384

Anthony KRN, Fabricius KE (2000) Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity. J Exp Mar Biol Ecol 252:221–253

Arai T, Kato M, Heyward A, Ikeda Y, Iizuka Y, Murayama T (1993) Lipid composition of positively buoyant eggs of reef-building corals. Coral Reefs 12:71–75

Bachok Z, Mfilinge P, Tsuchiya M (2006) Characterization of fatty acid composition in healthy and bleached corals from Okinawa, Japan. Coral Reefs 25:545–554

Ballesteros E (2006) Mediterranean coralligenous assemblages: a synthesis of present knowledge. Oceanogr Mar Biol Annu Rev 44:123–195

Barnes H, Blackstock J (1973) Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanilun method for 'total' lipids. J Exp Mar Biol Ecol 12:103–118

Bergschneider H, Muller-Parker G (2008) Nutritional role of two algal symbionts in the temperate sea anemone *Anthopleura elegantissima* Brandt. Biol Bul 215:73–88

Bramanti L, Magagnini G, De Maio L, Santangelo G (2005) Recruitment, early survival and growth of the Mediterranean red coral *Corallium rubrum* (L. 1758), a 4-year study. J Exp Mar Biol Ecol 314:69–78

Bramanti L, Movilla J, Guron M, Calvo E and others (2013) Detrimental effects of ocean acidification on the economically important Mediterranean red coral (*Corallium rubrum*). Glob Change Biol 19:1897–1908

Brey T (1995) Temperature and reproductive metabolism in macrobenthic populations. Mar Ecol Prog Ser 125:87–93

Broom DM (1981) Biology of behaviour: mechanisms, functions and applications. CUP Archive, Cambridge

Calow P (1979) The cost of reproduction — a physiological approach. Biol Rev Camb Philos Soc 54:23–40

Carlier A, Riera P, Amouroux JM, Bodiou JY, Grémare A (2007) Benthic trophic network in the Bay of Banyuls-sur-Mer (northwest Mediterranean, France): an assessment based on stable carbon and nitrogen isotopes analysis. Estuar Coast Shelf Sci 72:1–15

Cejas JR, Almansa E, Jérez S, Bolaños A, Felipea B, Lorenzo A (2004) Changes in lipid class and fatty acid composition during development in white seabream (*Diplodus sargus*) eggs and larvae. Comp Biochem Physiol B 139: 209–216

Chambers JM, Hastie TJ (1992) Statistical models in S. CRC Press, Boca Raton, FL

Cocito S, Ferrier-Pagès C, Cupido R, Rottier C and others (2013) Nutrient acquisition in four Mediterranean gorgonian species. Mar Ecol Prog Ser 473:179–188

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, Zabala M, Gili JM (1995) Sexual reproductive effort in the Mediterranean gorgonian *Paramuricea clavata*. Mar Ecol Prog Ser 117:185–192

Coma R, Llorente-Llurba E, Serrano E, Gili JM, Ribes M (2015) Natural heterotrophic feeding by a temperate octocoral with symbiotic zooxanthellae: a contribution to understanding the mechanisms of die-off events. Coral Reefs 34:549–560

Darnaude AM, Salen-Picard C, Harmelin-Vivien ML (2004a) Depth variation in terrestrial particulate organic matter exploitation by marine coastal benthic communities off the Rhone River delta (NW Mediterranean). Mar Ecol Prog Ser 275:47–57

Darnaude AM, Salen-Picard C, Polunin NV, Harmelin Vivien ML (2004b) Trophodynamic linkage between river runoff and coastal fishery yield elucidated by stable isotope data in the Gulf of Lions (NW Mediterranean). Oecologia 138:325–332

DeNiro MJ, Epstein S (1978). Carbon isotopic evidence for different feeding patterns in two hyrax species occupying the same habitat. Science 201:906–908

Díaz-Almeyda E, Thomé P, El Hafidi M, Iglesias-Prieto R (2011) Differential stability of photosynthetic membranes and fatty acid composition at elevated temperature in *Symbiodinium*. Coral Reefs 30:217–225

Ezzat L, Merle PL, Furla P, Buttler A, Ferrier-Pagès C (2013) The response of the Mediterranean gorgonian *Eunicella singularis* to thermal stress is independent of its nutritional regime. PLOS ONE 8:e64370

Ferrier-Pagès C, Witting J, Tambutte E, Sebens KP (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, Peirano A, Abbate M, Cocito S and others (2011) Summer autotrophy and winter heterotrophy in the temperate symbiotic coral *Cladocora caespitosa*. Limnol Oceanogr 56:1429–1438

Ferrier-Pagès C, Reynaud S, Béraud E, Rottier C, Menu D, Duong G, Gévaert F (2015) Photophysiology and daily primary production of a temperate symbiotic gorgonian. Photosynth Res 123:95–104

Forcioli D, Merle PL, Caligara C, Ciosi M and others (2011) Symbiont diversity is not involved in depth acclimation in the Mediterranean sea whip *Eunicella singularis*. Mar Ecol Prog Ser 439:57–71

Gates RD, Edmunds PJ (1999) The physiological mecha-

nisms of acclimatization in tropical reef corals. Am Zool 39:30–43
George SB (1994) Population differences in maternal size and offspring quality for *Leptasterias epichlora* (Brandt) (Echinodermata: Asteroidea). J Exp Mar Biol Ecol 175: 121–131
Gili JM, Coma R (1998) Benthic suspension feeders: their paramount role in littoral marine food webs. Trends Ecol Evol 13:316–321
Goreau TF, Goreau NI, Yonge CM (1971) Reef corals: autotrophs or heterotrophs? Biol Bull 141:247–260
Gori A, Linares C, Rossi S, Coma R, Gili JM (2007) Spatial variability in reproductive cycles of the gorgonians *Paramuricea clavata* and *Eunicella singularis* in the western Mediterranean. Mar Biol 151:1571–1584
Gori A, Rossi S, Berganzo E, Pretus LJ, Dale MRT, Gili JM (2011) Spatial distribution patterns 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 JM, Kotta M, Cucio C, Magni 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
Gori A, Linares C, Viladrich N, Clavero A and others (2013) Effects of food availability on the sexual reproduction and biochemical composition of the Mediterranean gorgonian *Paramuricea clavata.* J Exp Mar Biol Ecol 444:38–45
Grimsditch G, Salm R (2006) Coral reef resilience and resistance to bleaching. IUCN, Gland
Grottoli AG, Rodrigues LJ, 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
Grottoli AG, Rodrigues LJ, Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals. Nature 440: 1186–1189
Gurr MI, Harwood JL, Frayn KN (2002) Lipid biochemistry. Blackwell Science, Oxford
Hall VR, Hughes TP (1996) Reproductive strategies of modular organisms: comparative studies of reef-building corals. Ecology 77:950–963
Hamoutene D, Puestow T, Miller-Banoub J, Wareham V (2008) Main lipid classes in some species of deep-sea corals in the Newfoundland and Labrador region (northwest Atlantic Ocean). Coral Reefs 27:237–246
Harland AD, Navarro JC, Spencer Davies P, Fixter LM (1993) Lipids of some Caribbean and Red Sea corals: total lipid, wax esters, triglycerides and fatty acids. Mar Biol 117:113–117
Houlbrèque F, Ferrier-Pagès C (2009) Heterotrophy in tropical scleractinian corals. Biol Rev Camb Philos Soc 84: 1–17
Houlbrèque F, Tambutté E, Allemand D, Ferrier-Pagès C (2004) Interactions between zooplankton feeding, photosynthesis and skeletal growth in the scleractinian coral *Stylophora pistillata*. J Exp Biol 207:1461–1469
Imbs AB (2013) Fatty acids and other lipids of corals: composition, distribution, and biosynthesis. Russ J Mar Biol 39: 153–168
Imbs AB, Dang LP, Rybin VG, Svetashev VI (2015) Fatty acid, lipid class, and phospholipid molecular species composition of the soft coral *Xenia* sp. (Nha Trang Bay, the South China Sea, Vietnam). Lipids 50:575–589
Leuzinger S, Anthony KRN, Willis BL (2003) Reproductive energy investment in corals: scaling with module size. Oecologia 136:524–531
Marschal C, Garrabou J, Harmelin JG, Pichon M (2004) A new method for measuring growth and age in the precious Mediterranean red coral *Corallium rubrum* (L.). Coral Reefs 23:423–432
Martínez-Quintana A, Bramanti L, Viladrich N, Rossi S, Guizien K (2015) Quantification of larval traits driving connectivity: the case of *Corallium rubrum* (L. 1758). Mar Biol 162:309–318
McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378–390
McKillup SC, Butler AJ (1979) Modification of egg production and packaging in response to food availability by *Nassarius pauperatus.* Oecologia 43:221–231
Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochim Cosmochim Acta 48:1135–1140
Muller-Parker G, D'Elia CF (1997) Interactions between corals and their symbiotic algae. In: Birkeland C (ed) Life and death of coral reefs. Chapman & Hall, New York, NY, p 96–113
Muller-Parker G, Davy SK (2001) Temperate and tropical algal–sea anemone symbiosis. Invertebr Biol 120: 104–123
Munari C, Serafin G, Mistri M (2013) Structure, growth and secondary production of two Tyrrhenian populations of the white gorgonian *Eunicella singularis* (Esper 1791). Estuar Coast Shelf Sci 119:162–166
Muscatine L, McCloskey LR, Marian RE (1981) Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. Limnol Oceanogr 26:601–611
Muscatine L, Falkowski PG, Porter JW, Dubinsky Z (1984) Fate of photosynthetic fixed carbon in light and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. Proc R Soc Lond B Biol Sci 222:181–202
Muscatine L, Falkowski PG, Dubinsky Z, Cook PA, McCloskey LR (1989) The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral. Proc R Soc Biol Sci Ser B 236:311–324
Oku H, Yamashiro H, Onaga K, Iwasaki H, Takara K (2002) Lipid distribution in branching coral *Montipora digitata.* Fish Sci 68:517–522
Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83: 703–718
Previati M, Scinto A, Cerrano C, Osinga R (2010) Oxygen consumption in Mediterranean octocorals under different temperatures. J Exp Mar Biol Ecol 390:39–48
Qian PY, Chia FS (1991) Fecundity and egg size were mediated by quality of diet in the marine polychaete worm, *Capitella* sp. J Exp Mar Biol Ecol 148:11–25
Qian PY, Chia FS (1992) Effects of diet type on the demographics of *Capitella* sp. (Annelida: Polychaeta): lecithotrophic development vs. planktotrophic development. J Exp Mar Biol Ecol 157:159–179
R Development Core Team (2008) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
Raymond JF, Himmelman JH, Guderley HE (2007) Biochemical content, energy composition and reproductive effort in the broadcasting sea star *Asterias vulgaris* over the

spawning period. J Exp Mar Biol Ecol 341:32–44
Reynaud S, Martinez P, Houlbrèque F, Billy I, Allemand D, Ferrier-Pagès C (2009) Effect of light and feeding on the nitrogen isotopic composition of a zooxanthellate coral: role of nitrogen recycling. Mar Ecol Prog Ser 392:103–110
Ribes M, Coma R, Rossi S, Michelli M (2007) The cycle of gonadal development of *Eunicella singularis* (Cnidaria: Octocorallia). Invertebr Biol 126:307–317
Rossi S, Gili JM (2009) The cycle of gonadal development of the soft bottom-gravel gorgonian *Leptogorgia sarmentosa* (Esper, 1791) in the NW Mediterranean sea. Invertebr Reprod Dev 53:175–190
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, Ribes M, Coma R, Gili JM (2004) Temporal variability in zooplankton prey capture of the passive suspension feeder *Leptogorgia sarmentosa* (Cnidaria: Octocorallia) a case study. Mar Biol 144:89–99
Rossi S, Gili JM, 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
Rossi S, Tsounis G, Orejas C, Padron T and others (2008) Survey of deep-dwelling red coral (*Corallium rubrum*) populations at Cap de Creus (NW Mediterranean). Mar Biol 154:533–545
Santangelo G, Maggi E, Bramanti L, Bongiorni L (2004) Demography of the over-exploited Mediterranean red coral (*Corallium rubrum* L. 1758). Sci Mar 68:199–204
Sargent JR, Parks RJ, Mueller-Harvey I, Henderson RJ (1988) Lipid biomarkers in marine ecology. In: Sliegh MA (ed) Microbes in the sea. Ellis Horwood Ltd, Chichester, p 119–138
Sargent JR, Bell MV, Hendersen RJ, Tocher DR (1990) Polyunsaturated fatty acids in marine and terrestrial food webs. In: Mellinger J (ed) Animal nutrition and transport processes: nutrition in wild and domestic animals. Karger, Basel, p 11–23
Sargent JR, McEvoy LA, Estevez A, Bell JG, Bell M, Henderson J, Tocher D (1999) Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179:217–229
Sbrescia L, Russo M, Di Stefano F, Russo GF (2008) Analisi delle popolazioni di gorgonie dell'AMP di Punta Campanella dopo eventi di moria di massa. Oceanol Limnol Sin 19:449–457
Seemann J, Sawall Y, Auel H, Richter C (2013) The use of lipids and fatty acids to measure the trophic plasticity of the coral *Stylophora subseriata.* Lipids 48:275–286
Shirur KP, Ramsby BD, Iglesias-Prieto R, Goulet TL (2014) Biochemical composition of Caribbean gorgonians: implications for gorgonian–*Symbiodinium* symbiosis and ecology. J Exp Mar Biol Ecol 461:275–285
Simpson A (2009) Reproduction in octocorals (Subclass Octocorallia): a review of published literature. www.ucs.louisiana.edu/~scf4101/Bambooweb/reviewrepro.pdf
Slattery M, McClintock JB (1995) Population structure and feeding deterrence in three shallow-water Antarctic soft corals. Mar Biol 122:461–470
Strathmann RR (1985) Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. Annu Rev Ecol Syst 16:339–361
Stimson JS (1978) Mode and timing of reproduction in some common hermatypic corals of Hawaii and Enewetak. Mar Biol 48:173–184
Szmant AM, Gassman NJ (1990) The effects of prolonged 'bleaching' on the tissue biomass and reproduction of the reef coral *Montastrea annularis.* Coral Reefs 8:217–224
Theodor J (1967) Contribucion a l'étude des gorgones (VI): la dénudacion des branches de gorgones par des mollusques predateurs. Vie Milieu 18:73–78
Thompson RJ (1983) The relationship between food ration and reproductive effort in the green sea urchin *Strongylocentrotus droebachiensis.* Oecologia 56:50–57
Torrents O, Garrabou J (2011) Fecundity of red coral *Corallium rubrum* (L.) populations inhabiting in contrasting environmental conditions in the NW Mediterranean. Mar Biol 158:1019–1028
Tremblay P, Grover R, Maguer JF, Hoogenboom M, Ferrier-Pagès C (2014) Carbon translocation from symbiont to host depends on irradiance and food availability in the tropical coral *Stylophora pistillata*. Coral Reefs 33:1–13
Tsounis G, Rossi S, Laudien J, Bramanti L, Fernández N, Gili JM, Arntz W (2006a) Diet and seasonal prey capture rate in the Mediterranean red coral (*Corallium rubrum* L.). Mar Biol 149:313–325
Tsounis G, Rossi S, Aranguren M, Gili JM, Arntz WE (2006b) 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 148:513–527
Tsounis G, Martínez L, Viladrich N, Bramanti L, Martínez A, Gili JM, Rossi S (2012) Effects of human impact on the reproductive effort and allocation of energy reserves in the Mediterranean octocoral *Paramuricea clavata.* Mar Ecol Prog Ser 449:161–172
Vighi M (1972) Étude sur la reproduction du *Corallium rubrum* (L.). Vie Milieu Vol XXIII fase 1, sér A, p 21–32
Viladrich N, Rossi S, López-Sanz A, Orejas C (2016a) Nutritional condition of two coastal rocky fishes and the potential role of a marine protected area. Mar Ecol 37: 46–63
Viladrich N, Bramanti L, Tsounis G, Chocarro B and others (2016b) Variations of lipid and free fatty acid contents during spawning in two temperate octocorals with different reproductive strategies: surface versus internal brooder. Coral Reefs 35:1033–1045
Ward S (1995) The effect of damage on the growth, reproduction and storage of lipids in the scleractinian coral *Pocillopora damicornis* (Linnaeus). J Exp Mar Biol Ecol 187:193–206
Weinberg S, Weinberg F (1979) The life cycle of a gorgonian: *Eunicella singularis* (Esper, 1794). Bijdr Dierkd 48: 127–140
Yamashiro H, Oku H, Higa H, Chinen I, Sakai K (1999) Composition of lipids, fatty acids and sterols in Okinawan corals. Comp Biochem Physiol B 122:397–407

Editorial responsibility: Steven Morgan, Bodega Bay, California, USA

Submitted: March 24, 2016; Accepted: April 4, 2017
Proofs received from author(s): May 25, 2017