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Skeletal growth, respiration rate and fatty acid composition in the cold-water coral *Lophelia pertusa* under varying food conditions

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ABSTRACT: Reefs of the cold-water coral *Lophelia pertusa* form biodiversity-rich habitats in the deep ocean, but physiology, reproduction, feeding and growth in this species remain poorly investigated. Food supply to reef sites varies considerably both spatially and temporarily. In this study we investigated the effects of starvation and zooplankton feeding on respiration and growth of *L. pertusa*. In our first experiment, corals were starved for 6 mo, resulting in a 40% decrease in respiration but no visible effects on coral condition or survival. In a second experiment, corals were fed nauplii of *Artemia salina* for 15 wk at 4 different densities; the organic carbon provided corresponded to between 20 and 300% of the carbon turned over by initial respiration. Respiration rate increased with zooplankton food density, but no effect on skeletal growth could be detected. Skeletal growth remained positive even at low food density. Compared to initial conditions, there was a general decrease in the total concentrations of both structural and storage fatty acids independent of food treatment, but no significant effect among the treatments was discovered. The amount of organic carbon and nitrogen also decreased during the experiment, although significantly less in the highest food density compared to the lowest. The results indicate that *L. pertusa* is highly tolerant to living on minimal resources for periods of several months. Response-times to varying food conditions were slow, but results suggest that tissue content and composition is a better indicator of food conditions in *L. pertusa* compared to calcification rates.

KEY WORDS: Cold-water corals · *Lophelia pertusa* · Coral physiology · Feeding · Growth · Respiration · Fatty acids

INTRODUCTION

Framework-forming scleractinian corals form some of the most spatially complex habitats found on the deep ocean floor (Roberts et al. 2006), and sites with high faunal biomass and carbon cycling on continental margins (Van Oevelen et al. 2009). In the northeast Atlantic, over 1300 species have been found to be associated with reefs of *Lophelia pertusa* (Roberts et al. 2006), which is the most common framework-forming cold-water coral (Roberts et al. 2009). Despite its fundamental importance in building these rich reef communities, little is known about the diet of *L. pertusa* and, in particular, the relationship between food supply and the coral's physiology and growth. Previous analyses of *L. pertusa* indicate that the diet varies from site to site, and that both suspended particulate organic matter and various zooplankton species are ingested. Results from analyses of stable isotope signatures and lipid composition of

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coral tissue indicate the importance of phytoplankton-based resources (Duineveld et al. 2004, 2007, 2012, Kiriakoulakis et al. 2005), and corals in some communities appear to depend mostly on phytoplankton (Duineveld et al. 2007, 2012). Other studies indicate zooplankton is the most important food source (Kiriakoulakis et al. 2005, Carlier et al. 2009, Dodds et al. 2009). Variation in coral fatty acid (FA) composition with depth indicates that shallower corals may be feeding more on herbivorous calanoid copepods, whereas deeper living colonies seem to depend more on carnivorous non-calanoid copepods (Dodds et al. 2009).

Numerical ocean model simulations indicate that *Lophelia pertusa* communities are typically found in areas with high particle encounter rates (Thiem et al. 2006). Accordingly, the most extensive and productive reef structures are often associated with regions of elevated benthic flow e.g. on shelf margins, canyon walls, sills, seamounts or carbonate mounds (Fosså et al. 2002, Dorschel et al. 2005, Huvenne et al. 2007). Hydrodynamic processes such as internal waves, tidally enforced advection of bottom water and downwelling of surface water have been identified as important mechanisms of food supply (White 2007, Davies et al. 2009). The particulate organic matter entering *L. pertusa* reef environments does, however, vary both spatially and temporally; and this has implications for coral nutrition and growth. Temporal variation in physical conditions has been shown to result in large variability in particle supply as indicated by fluorescence recordings (Duineveld et al. 2007); particulate organic matter collected with sediment traps or *in situ* pumps can vary both in quantity and quality with time and reef site (Kiriakoulakis et al. 2007). This periodicity in food supply implies that corals may have to bridge periods of weeks or months at substantially lower food levels, potentially even periods of starvation.

Laboratory studies of *Lophelia pertusa* zooplankton uptake rates at various concentrations and flow conditions have been carried out (Purser et al. 2010, Tsounis et al. 2010), but to date there has been no study of the effect of food conditions on *L. pertusa* growth rates. Coral growth rates are found to decrease with increasing water depth, which may be explained by a decrease of food quantity and quality with water depth (Roberts et al. 2009). Maier et al. (2009) found skeletal growth rates to vary 2-fold between samples from approximately the same depth but from different geographical sites in the NE Atlantic, possibly reflecting a variation in food supply. However, Brooke & Young (2009) did not detect any differences in skeletal growth rates after 1 yr between coral fragments transplanted to different 'high coral' and 'no coral' habitats in the Gulf of Mexico. Recently, Naumann et al. (2011) found a decrease in both respiration and skeletal growth in response to zooplankton exclusion in the solitary cold-water coral *Desmophyllum dianthus*. The respiratory physiology of *L. pertusa* has previously been investigated by Dodds et al. (2007): oxygen consumption rate was found to be sensitive to short-term temperature changes (6.5–11°C), but *L. pertusa* was able to maintain respiratory independence over a broad range of oxygen partial pressures (10–20 kPa). There is, however, no information on how food availability or starvation affects respiration rates of *L. pertusa,* or on which time-scale food availability may influence physiological responses.

The FA content and composition of the tissue reflect the nutritional status of the coral and the coral's diet. Phospholipid derived fatty acids (PLFAs) are an integral part of polar lipids that are the basic matrix of cellular membranes (Dalsgaard et al. 2003). Hence, these PLFAs have a mostly structural role in the organism and can therefore be used to estimate levels of biomass (Boschker & Middelburg 2002). In contrast, neutral lipids act as energy reserves that are destined either for oxidation (to provide energy) or for processing and subsequent incorporation into phospholipids (Dalsgaard et al. 2003). The FA composition of neutral lipids reflects the FA composition of the dietary items because these lipids are taken up and stored with limited modification and can therefore be used to decipher prey contributions to the diets of organisms (Iverson et al. 2004). Dodds et al. (2009) studied FA compositions of *Lophelia pertusa* at various geographical and seasonal scales. Interestingly, these authors did not find a clear seasonal trend in the quantity of storage lipids, despite anticipated large differences in organic matter input to the reefs. Given the above, it is unclear how *L. pertusa* physiologically respond to variations in food supply and what the turnover times of storage lipids are.

This study investigates how variation in food availability influences key physiological processes in the cold-water coral *Lophelia pertusa*. In the laboratory, we measured the response in oxygen consumption rate to long-term starvation and to variation in zooplankton food supply. Simultaneously, we measured skeletal growth rates, and the quantity of phospholipids and neutral lipids as a measure of coral biomass and coral energy reserves respectively, during 3 mo of exposure to various food densities. We hypothesized that the skeletal growth rates, respira-

tion rates and structural lipids would increase with increasing food density. Moreover, the quantity of storage lipids would be positively related to the food density supplied to the corals. The results of this study have implications for understanding of *L. pertusa* metabolism and energy partitioning, together with the time scales involved in physiological responses to food availability.

MATERIALS AND METHODS

Collection and maintenance of corals

Lophelia pertusa coral fragments were collected from the Tisler Reef in the Norwegian part of the Skagerrak (see Table 1). The Tisler reef, most of which is dominated by *L. pertusa*, extends 1200 × 200 m laterally over a depth range of 70 to 155 m, and is situated on top of a northwest–southeast oriented sill forming the connection between the Kosterfjord and the open Skagerrak (Lavaleye et al. 2009). Downwelling and mixing on the downstream side of the sill crest play an important role for the nutrition of the reef (Wagner et al. 2011). Coral fragments were collected from approximately 100 m depth using a Remotely Operated Vehicle (ROV). All coral fragments were of the white morph (there is also a pink/orange morph). Within hours of collection, corals were transported to the laboratory at the University of Gothenburg research station at Tjärnö, Sweden, and divided (when necessary) into manageable pieces 4 to 10 cm long. Corals were maintained in 15 l aquaria with flow-through of sand-filtered seawater with a salinity of 31 to 34 from 45 m depth in the Koster-fjord (sand particle size 1 to 2 mm; water exchange rate of approximately 1 l min^{-1}), at 7 to 8°C, and were fed 5 days per week with *Artemia salina* nauplii until experiments started. Those laboratory conditions are similar to conditions measured *in situ* on the reef with typical salinities of 33 to 35 and with temperatures in September to January of 8 to 9°C, decreasing gradually to 6°C in April (Lavaleye et al. 2009, Wagner et al. 2011, H. Wagner unpubl. data).

Effect of starvation on coral respiration

Coral fragments were mounted in an upright position in 4 separate 15 l aquaria (each containing 8–11 fragments with approximately 90 polyps in total) with a water exchange of approximately 1 l min^{-1} and water temperature of 7 ± 0.2°C. Two treatments were conducted for a period of 28 wk. Fragments in two of the aquaria were maintained in sand-filtered seawater, while fragments in the other two aquaria were maintained in sterile-filtered seawater (Milligard® cartridge filter 0.2 µm, Millipore). No food was added during the 28 wk period, and respiration rates were measured 8 times at ~4 wk intervals. The first respiration measurements were performed before the starvation treatments started. The coral sets were placed into benthic chambers of 'Mississippi type' (Tengberg et al. 2004) with a volume of 8 l. Corals were allowed to acclimatise in the chambers with a flow-through of water for a minimum of 12 h before respiration measurements started. Measurements were performed in sand-filtered seawater; at each measurement, corals were placed in 2 chambers and the background respiration was measured in 2 separate chambers containing seawater only. Chambers were sealed, and the decrease in oxygen concentration was monitored with oxygen optodes (Aanderaa 3830). Chambers were closed for measurements for 8 to 10 h before being aerated for approximately 2 h and then re-sealed for an additional measurement period (resulting in one daytime and one night time measurement). Oxygen concentrations were recorded every minute; the concentration decrease became linear within the first hour after sealing. Oxygen consumption rates were quantified using regression of the linear part of the concentration changes of oxygen versus incubation time. The average background respiration (max. 10% of oxygen consumption in coral chambers) was subtracted from oxygen consumption rates in the coral chambers, and coral respiration rates were quantified using an average value of the 2 measurement periods.

For quantification of total dry weight (DW) of corals in each set, coral fragments were freeze-dried for 48 h immediately after finalisation of experimental measurements and then weighed. The fragments were stored at –80°C before further analysis. For estimation of tissue weight, 5 to 7 fragments from each coral set were ground using a porcelain mortar before being further milled to a fine powder using a ball grinder for 2 to 3 min (care was taken not to overheat the samples). The powder was placed in aluminium cups, dried to constant weight at 60°C and incinerated at 500°C for 4 h. The ash-free DW was subtracted from the total DW resulting in an estimation of tissue DW of the coral fragments. The results were used to calculate the proportion of tissue DW to the total DW of coral fragments, and also to calculate the oxygen consumption rates normalised to tissue DW of corals.

Effect of zooplankton food density on respiration and growth

Experimental set-up

Information on coral collection is summarized in Table 1. Collection of corals for this experiment resulted in a larger sample from a male colony, and due to technical problems with the ROV, only a relatively small sample from a female colony (gender of colonies; A. Larsson unpubl. data). Coral fragments were mounted in an upright position and placed above experimental aquarium floors in specially designed aquaria (Fig. 1A). The 10 l polycarbonate aquaria were in sets of 5 with a common lid and a common horizontal axle in the upper part equipped with 'Mississippi-type' paddle wheels (diameter 90 mm) for internal circulation of water (Fig. 1A,B). Tight seals at the axle between adjacent aquaria and rubber mouldings on the lid ensured a minimal exchange of water and gas. Six coral fragments (one from the female colony and 5 from the male colony) were placed in each of 12 aquaria; one aquarium in each of the 3 sets remained empty for control measurements. The total buoyant weight of corals in each aquarium varied between 36 and 42 g; total DW varied between 63 and 73 g. Aquaria sets were placed in a thermo-regulated room with water- and air-temperature set to 8 ± 0.2°C. The turnover rate of seawater was regulated to approximately 1 l min^{-1} using adjustable house clips.

Four experimental food density treatments were applied to each of 3 aquaria. Corals were provided with *Artemia salina* nauplii in 4 different densities corresponding to a dose of 70, 260, 530 and 1060 µmol C $aquarium^{-1}$ d^{-1}. The organic carbon content of the *A. salina* nauplii (INVE aquaculture) was 0.075 µmol C $ind.^{-1}$ as previously measured by Purser et al. (2010). The 4 food density treatments corresponded to 20, 75, 150 and 300% of the organic carbon turned over by average initial coral respiration. Therefore, we assumed that the 2 lower food densities would be insufficient to meet the coral respiration demands while the 2 higher food densities would represent an over-abundance of food. Coral oxygen consumption was measured by monitoring O_2 decreases in incuba-

Table 1. *Lophelia pertusa* sampling from the Tisler reef, together with experimental start and end dates for the starvation and food density experiments

Experiment	Sampling date	Latitude (°N)	Longitude (°E)	Depth (m)	No. of colonies sampled	Experimental start date	Experimental end date
Starvation	24 Oct 2007	58°59.80	10°58.08	125	1	21 Dec 2007	01 Jul 2008
	30 Nov 2007	59°05.96	10°47.69	100	ca. 3		
Food density	15 Sep 2008	58°59.67	10°58.13	95	1	26 Jan 2009	13 May 2009
	15 Sep 2008	58°59.75	10°58.04	115	1		

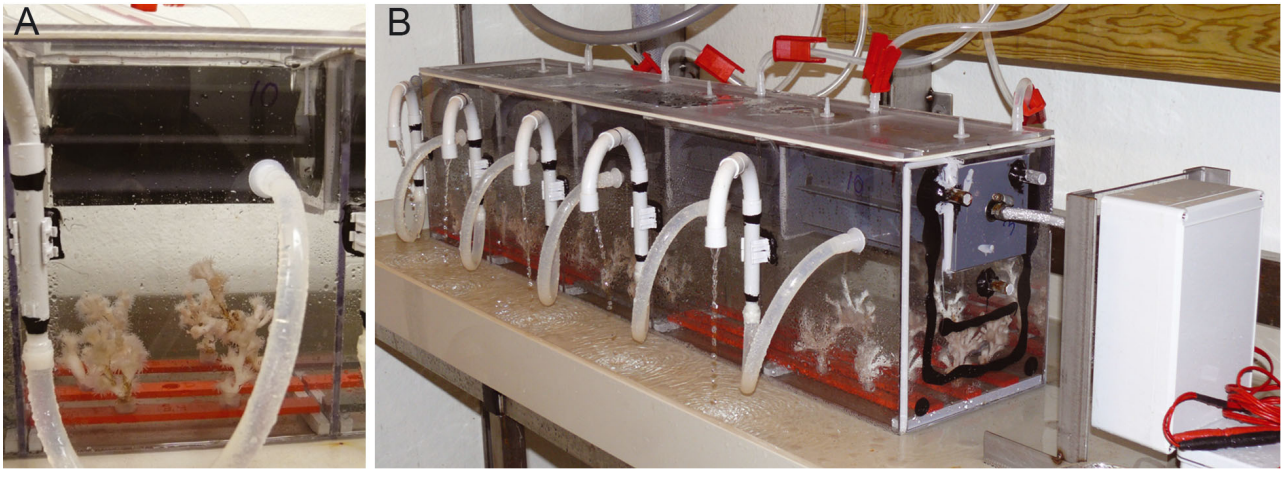

Fig. 1. *Lophelia pertusa* in aquaria used for feeding and for respiration measurements in the zooplankton food density experiment. (A) Mounting of coral fragments in a single aquarium. (B) Set of aquaria with common motor and paddle wheel axis

tion aquaria. Respired O_2 was converted to C units using a respiratory quotient of 1:1 (mol O_2:mol C).

Corals were fed 3 times a week; receiving one third of their weekly dose of food at each feeding. Freshly hatched *Artemia salina* nauplii were used and the food densities of 20, 75, 150 and 300% corresponded to a dose of 21, 80, 160 and 320 nauplii per polyp and feeding occasion. Water flow was shut off and the corals were allowed to feed on the *A. salina* nauplii for 11–15 h before water supply was switched on again. Internal circulation and aeration of water was provided by the paddle wheels at approximately 45 rpm. The corals were fed at the specific food densities for 15 wk. Due to equipment challenges, reliable oxygen consumption measurements were first performed after 2 wk of food treatment. Before this measurement, the given food densities were based on initial oxygen consumption rates from the starvation experiment (adjusted for possible effects of the temperature difference between experiments; Dodds et al. 2007) and were only approximate. Compared to the average coral respiration rate measured after the second experimental week (and on which the *A. salina* doses specified above were based), the amount of *A. salina* given during the first 2 wk was approximately 50% too high.

Monitoring of oxygen consumption rates

Oxygen consumption measurements were performed at 8 ± 0.2°C and in salinities of 31.2 to 33.7 in the same aquaria as the corals were maintained and fed (Fig. 1). The aquaria were thoroughly cleaned and corals were left undisturbed in the aquaria with no food added on the day prior to measurements. The outflow pipe of each aquarium was replaced with a 3 mm tube with a hose clip allowing water samples to be carefully taken. Water flow was cut off, holes in the lid were sealed and the lid was weighed down ensuring tight rubber moulding seals. The aquaria with seawater only were used to monitor background oxygen depletion. During oxygen consumption measurements, the aquaria remained closed for 35 to 47 h. Care was taken so that the oxygen concentration in the aquaria did not fall below the critical oxygen partial pressure of 9 kPa, at which the normal oxygen consumption rate in *Lophelia pertusa* cannot be maintained (Dodds et al. 2007). Respiration measurements were performed at the end of Weeks 2, 6, 9, 13 and 15 of the experiment. The chambers with oxygen optodes used in the starvation experiment were not available for this experiment, and hence oxygen concentrations were measured using Winkler titration. Prior to closure of aquaria, water samples were taken to determine oxygen content at start of the experiment, and new samples were taken after approximately 40 h of respiration (longer measurement times were required when using Winkler titration as opposed to optodes to ensure accurate measurements). The coral respiration rates were calculated by subtracting the average background respiration from the oxygen consumption in coral aquaria. In 2 out of the 3 aquarium sets, there was a small leakage through the wall where the rotating axis (with the paddle wheels) was connected to the motor. As a result, no successful respiration measurements were recorded for one of the 20% and one of the 150% treatment aquaria. Respiration measurements on corals in those aquaria were only carried out at the end of the experiment when corals could be rearranged into other aquaria.

Skeletal growth and budding rate

Coral growth was estimated using the accurate buoyant weighing technique (Jokiel et al. 1978, Davies 1989). To detect an eventual delayed response in skeletal growth to food availability, growth was measured for 2 periods of the experiment. Corals were buoyant weighed in seawater of 8 ± 0.2°C and salinities of 32–33. Buoyant weights of coral pieces were measured 2 wk before the start of experimental treatments, after Week 9, and a few days after Week 15. The initial buoyant weight of individual fragments varied between 4 and 14 g. Following Davies (1989), the contribution of tissue to the total buoyant weight of coral was estimated as 1.9%, which was used to calculate the skeleton buoyant weights. The relative increase in weight, together with data of DW of coral skeleton for each fragment at the end of the experiment, was used to determine absolute skeletal growth. Growth was also measured as the number of new polyp buds that appeared during the experimental period. Number of polyps on each coral piece was noted 2 weeks before the start of food exposure, and again a few days after finalisation of the experiment.

Tissue analyses

Immediately after finalisation of the experimental measurements, the coral fragments were freeze dried for 48 h and weighed. The fragments were

stored at −80°C before further analysis. Four coral fragments from each aquarium were ground to a maximum grain size of 2 to 3 mm using a porcelain mortar; each sample was then divided into 2 parts. The first part was further milled and the amount of tissue was estimated as described for the starvation experiment. The second part was used for analyses of organic carbon (OC), organic nitrogen (ON) and fatty acids (FAs). In addition to the samples from the end of the experiment, 8 coral pieces that had been freeze-dried before the experiment began were analysed to define the initial condition of the corals. OC and ON were analyzed on 50 to 100 mg of ground corals using an automatic CN-analyzer following acidification with 25% HCI in silver sample cups (Nieuwenhuize et al. 1994). This technique has shown long-term precision for OC and ON, irrespective of the calcium carbonate content of the sample. A few grams of ground corals were extracted using a methanol:chloroform mixture by a standard Bligh & Dyer (1959) method to extract the total lipids into chloroform. The chloroform fraction was put into a silicic-acid (Merck Kieselgel 60) column in which it was separated into polarity classes using sequential rinsing with chloroform, acetone and methanol. The methanol elutant contained the polar phospholipid fraction and the chloroform and acetone elutants were combined to form the neutral lipid fraction. The FAs were derivatized to fatty acid methyl esters (FAME) by mild alkaline methanolysis using methanolic NaOH as reagent. Internal FAME standards of both 12:0 and 19:0 were used. FAME concentrations were determined subsequently by gaschromatography-flame ionization detection (GC-FID). In addition, total lipids of 3 samples of freeze-dried *Artemia salina* (no. of ind. 255, 323 and 330, respectively) were extracted as described above into chloroform, and measured on the GC-FID to determine the lipid composition of the *Artemia*. The FAs C18:1ω9c and C18:3ω3 strongly dominated the *Artemia* FAs (average ± SD of 48.3 ± 0.3%), as reported in other studies (Tolosa et al. 2011). Hence, the contribution of these 2 markers to the coral FAs was used to determine whether higher food concentrations indeed resulted in accumulation of FAs from the food source.

Stocks and process rates

The results from the food density experiment were combined to obtain an overview of stock sizes, process rates and food availability in the different treatments. Stocks were estimated from measurements at the end of experimental treatment and were normalized to one average coral fragment per treatment. The amount of carbon from *Artemia* available per coral fragment was summed for the 15 wk of food treatment. Respiration and calcification rates were also summed over the entire experimental period and normalized to 1 coral fragment. Carbon respired was further transformed to energy equivalents under the conversion of 37.56 J mg^{-1} C (Peters 1983). Following Anthony et al. (2002), calcification was converted to energy equivalents using the conversion 0.152 J mg^{-1} DW skeleton. This energy cost for calcification is based on the predicted spending of 1 mol of ATP per 2 mol of Ca^{2+} that are transported through the membrane of calicoblastic cells to the site of calcification, and is considered universal for many different calcifying organisms (McConnaughey & Whelan 1997).

Statistical analyses

Coral respiration and growth data were analyzed using repeated measures ANOVAs with treatment as a between-aquaria factor and time as a within-aquaria factor (Tables 2, 3 & 4). p-values were adjusted for repeated measurements in time using Greenhouse-Geisser epsilon and Hunyh-Feldt epsilon. The dependent variables were rate of oxygen consumption (μmol O_2 g^{-1} DW coral h^{-1}) and skeletal growth (% d^{-1}), respectively. For the food density experiment, all respiration data from Week 2 (when corals were spawning) were excluded from the analysis (see 'Discussion'). Furthermore, due to experimental problems already described, the respiration analysis in the food density experiment was based on 2 replicate measurements for the 20 and 150% treatments and 3 replicates for the 75 and 300% treatments. As a complement, a one-way ANOVA was performed on the respiration rates after 15 wk of feeding, when all 3 replicate chambers could be measured. Prior to the repeated measures analysis of growth, the effect of aquarium was tested in a nested ANOVA with food density as a fixed factor and aquarium as a random factor nested under food treatment. As the p-value for the effect of aquarium was >0.25 ($F_{8,60}$ = 1.05, p = 0.41), data from the aquaria within each treatment could be pooled (Underwood 1997). The effect of food density treatment on budding rate, and the effect of starvation treatment and food density treatment on the ratio of tissue DW to total DW (proportion tissue) was analysed in nested

ANOVAs with treatment as a fixed factor and with experimental aquarium as a random factor nested under treatment. The p-value for the effect of aquarium was > 0.25 ($F_{8,60}$ = 0.47, p = 0.87) in the budding rate analysis, therefore data from the aquaria within each treatment could be pooled. For both analyses of proportion tissue, the p-value for the factor aquarium was <0.25 and data could not be pooled. Analyses of lipid composition, and lipid-, ON- and OC-content were carried out using 1-way unbalanced ANOVAs, since data from samples before the experiment started were included. Prior to these analyses, the potential effect of the factor aquarium on the data collected after finalisation of the experiment was tested in nested ANOVAs. In all cases the p-value for the effect of aquarium was >0.25 allowing data from the aquaria within each food treatment to be pooled. Prior to the ANOVA analyses, data were tested for homogeneity of variances using Cochran's test and no transformations were required. For all analyses a Type 1 error (α) rate of 0.05 was used, and for post hoc analyses of differences between means, Student Newman–Keuls (SNK) multiple comparisons tests were carried out.

RESULTS

Effect of starvation on coral respiration

The coral fragments appeared to remain in good health for the duration of the starvation experiment. No polyp mortality was noted, and the tissue coverage of coral skeletons was high and did not seem affected by the starvation. Polyp tentacles were extended to a high degree during the entire experimental period. The average (±SD) respiration rate at start of the experiment was 0.27 ± 0.05 µmol O_2 g^{-1} DW coral h^{-1}, which corresponds to a respiration rate of 4.4 ± 0.8 µmol O_2 g^{-1} DW tissue h^{-1} when normalised to the average tissue content of corals at finalisation of the experiment. The average respiration rate for the coral sets decreased by 39% during 28 wk of starvation (Fig. 2). In the repeated measures ANOVA, there was a significant effect of time on coral respiration rate (Table 2), however, no difference was detected between the rates of respiration decrease depending on starvation treatment (the interaction Time × Starvation treatment was not significant). The proportion of tissue DW to total DW of coral at the end of the experiment was 6.1 ± 0.4% and 6.1 ± 0.04% (average ± SE) for sand-filter and sterile-filter treated corals, respectively, and did not differ between treatments (nested ANOVA, $F_{1,2}$ = 0.033, p = 0.87).

Effect of zooplankton food density on respiration and growth

Respiration

Changes in respiration rates with duration of feeding and *Artemia salina* densities are presented in Fig. 3. The mean (±SD) respiration rate at the first measurement on Week 2 of the experiment was 0.22 ± 0.05 µmol O_2 g^{-1} DW coral h^{-1}, which corresponds to a respiration rate of 4.2 ± 1.0 µmol O_2 g^{-1}

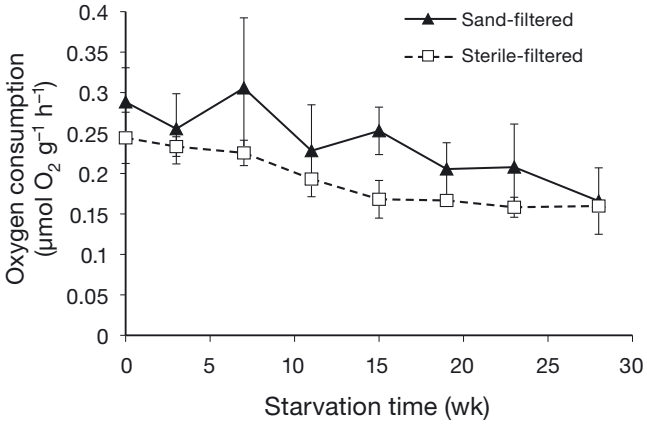

Fig. 2. *Lophelia pertusa*. Effect of 2 starvation treatments on respiration rate. Corals were maintained in sand-filtered or sterile-filtered seawater for 28 wk with no food added. Respiration rates are normalised to total dry weight of coral fragments. Data are presented as mean ± SE, n = 2 replicate aquaria per treatment with 8–11 coral fragments (~90 polyps) in each aquarium. See Table 2 for statistical analysis

Table 2. *Lophelia pertusa*. Repeated measures ANOVA showing the effect of starvation treatment (sand-filtered or sterile-filtered) and starvation time on oxygen consumption rate. G-G and H-F are adjusted p-values according to Greenhouse-Geisser epsilon and Hunyh-Feldt epsilon, respectively. S. tr.: starvation treatment; C. s.: coral set

Source	df	*F*	p	G-G	H-F	Error term
Starvation treatment	1	0.87	0.45			Coral set(S. tr.)
Coral set(S. tr.)	2					
Time	7	9.62	<0.001	0.032	<0.001	Time × C. s.(S. tr.)
Time × Starvation tr.	7	1.13	0.40	0.41	0.40	Time × C. s.(S. tr.)
Time × Coral set(S. tr.)	14					

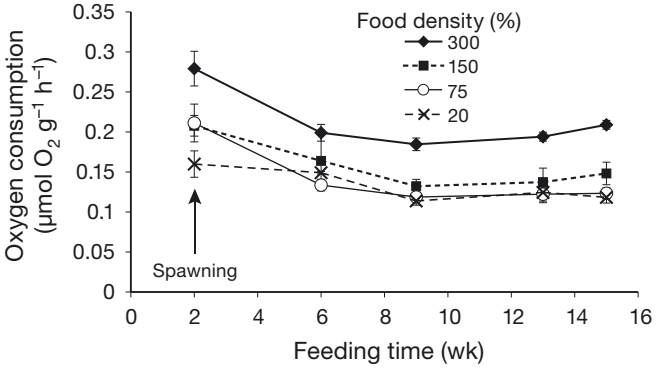

Fig. 3. *Lophelia pertusa*. Effect of zooplankton food density and feeding duration on coral respiration rate. Respiration rates are normalised to total dry weight of corals. A food density level of 100% represents an organic carbon content in the added food equivalent to carbon turned over by average initial coral respiration. Spawning occurred in several of the experimental chambers during Week 2, which may have affected respiration measurements. Data are presented as mean ± SE, n = 2 to 3 replicate aquaria per treatment with 6 coral fragments (~115 polyps) in each aquarium. See Table 3 for statistical analysis

DW tissue h^{-1} when normalised to the average tissue content of corals at finalisation of the experiment. There was a significant effect of both food density and time on coral respiration rate (Table 3). The coral respiration rate in the 300% treatment was higher than respiration rates in the 3 lower food density treatments, which among themselves did not differ significantly (SNK post hoc). The same result was found in the complementary 1-way ANOVA performed on respiration results from only the last measurement occasion when all 3 replicates of each food density treatment could be measured ($F_{3,8}$ = 21.1, p < 0.001). During the first measurement occasion (12 Feb 2009), corals spawned in several of the chambers. In 2 of the 300% treatment chambers and 2 of the 75% treatment chambers both eggs and sperm were released. Results from this measurement occasion were therefore excluded from the analysis, due to the possible confounding effects of spawning on measured oxygen consumption rates (see 'Discussion').

Table 3. *Lophelia pertusa*. Repeated measures ANOVA showing effects of zooplankton food density and feeding time on coral oxygen consumption rate. G-G and H-F are adjusted p-values according to Greenhouse-Geisser epsilon and Hunyh-Feldt epsilon, respectively. Note that data from Week 2 is not included in the analysis, due to spawning activity. F. d.: food density; Aq.: aquarium

Source	df	*F*	p	G-G	H-F	Error term
Food density	3	13.6	0.004			Aquarium(F. d.)
Aquarium(F. d.)	6					
Time	3	12.4	<0.001	0.004	<0.001	Time × Aq.(F. d.)
Time × F. d.	9	1.87	0.12	0.20	0.13	Time × Aq.(F. d.)
Time × Aq.(F. d.)	18					

Skeletal growth and budding rates

No effect of zooplankton food density on coral skeletal growth could be detected even after 15 wk of feeding at 4 different food densities (Table 4). The corals in the 20% treatment grew at the same rate as coral fragments that were fed up to 15 times more (Fig. 4A). However, there was an overall effect of time on skeletal growth (Table 4); coral fragments grew faster during the second part of the experiment (Weeks 10–15) than during the first 9 wk of the experiment (Fig. 4A). Surprisingly, the corals given the lowest amount of food also showed this pattern (Fig. 4A). The corals in the 150% treatment did not grow faster during the second time period, resulting in a significant interaction between food density and time (Table 4). Growth rate varied largely among individual coral fragments, ranging from 0.01% d^{-1} to 0.13% d^{-1} with an average (± SD) growth rate for the entire period of 0.046 ± 0.021% d^{-1}. The average growth rate for the 54 fragments from the male colony was 0.048 ± 0.020% d^{-1}, and for the 12 fragments from the female colony was 0.032 ± 0.017% d^{-1}. The skeletal weight increase was 517 ± 216 mg per coral fragment for the entire period corresponding to a calcification rate of 0.46 ± 0.21 mg $CaCO_3$ g^{-1} skeleton d^{-1}. The budding rate in individual coral fragments also varied largely between 0% (for 42% of coral fragments) and 0.30% new polyps per day and did not differ significantly among zooplankton density treatments (1-way ANOVA, $F_{3,68}$ = 0.42, p = 0.74; Fig 4B). In total, 72 new polyps formed from the 1400 original polyps during the 15 wk experimental period.

Tissue analyses

The proportion of tissue DW of coral fragments after 15 wk averaged 5.3 ± 0.9% (SD) of the total weight, and there were no significant differences among food density treatments (nested ANOVA, $F_{3,8}$ = 0.72, p = 0.55; Fig. 4C). The OC and ON content (% of skeleton) did, however, decrease for all treatments compared to initial

Table 4. *Lophelia pertusa.* Repeated measures ANOVA showing effects of zooplankton food density and feeding time on coral skeletal growth. G-G and H-F are adjusted p-values according to Greenhouse-Geisser epsilon and Hunyh-Feldt epsilon respectively. F. d.: food density; C. f.: coral fragment

Source	df	*F*	p	G-G	H-F	Error term
Food density	3	0.86	0.47			Coral fragment(F. d.)
Coral fragment(F. d.)	68					
Time	1	15.5	<0.001	<0.001	<0.001	Time × C. f.(F. d.)
Time × F. d.	3	3.99	0.011	0.011	0.011	Time × C. f.(F. d.)
Time × C. f.(F. d.)	68					

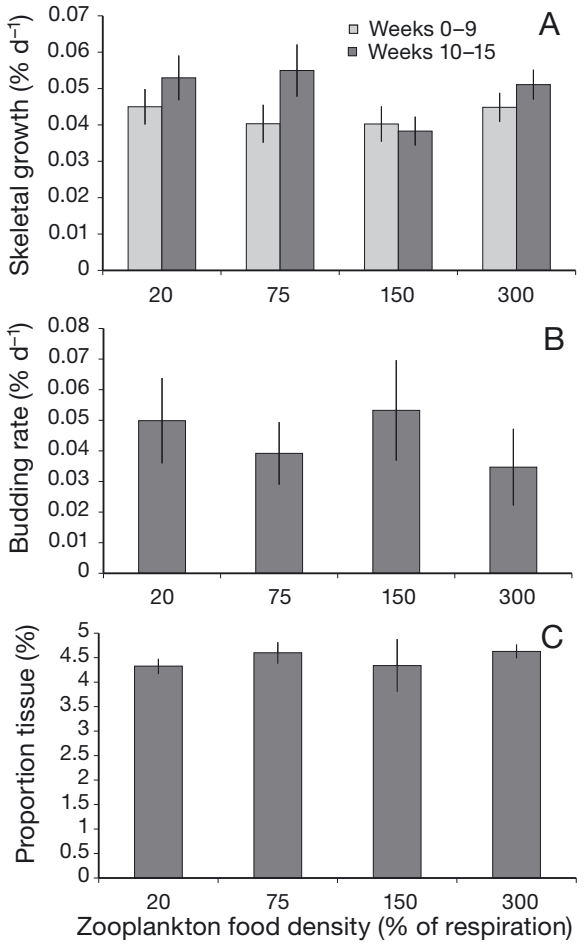

Fig. 4. Effects of zooplankton food density on *Lophelia pertusa* growth and tissue content. Bars = average ± SE. (A) Skeletal growth rates (n = 18 replicate coral fragments per treatment) measured using the buoyant weight technique during 2 time periods of the 15 wk experiment. See Table 4 for statistical analysis. (B) Rates of production of new polyps measured after the feeding period with n = 18 replicate coral fragments per treatment. (C) Proportion tissue (DW, n = 3 replicate aquaria per treatment with 4 coral fragments measured from each aquarium) on coral fragments after finalization of the experiment. The estimations of tissue amount are based on measurements of ash-free dry weight of milled coral fragments

(t_0) conditions (1-way ANOVA; CO: $F_{4,51}$ = 11.0, p < 0.001, SNK Post hoc; ON: $F_{4,51}$ = 22.6, p < 0.001, SNK Post hoc; Fig. 5). The content of both OC and ON increased with food density and was significantly higher in the 300% treatment compared to the 20% treatment (SNK Post-hoc); differences between other treatments were not significant.

The total content of both structural and storage FAs was affected by the experimental treatment (1-way ANOVA; structural: $F_{4,49}$ = 4.31, p = 0.0046; storage: $F_{4,51}$ = 2.60, p = 0.047). The amount of structural and storage FAs was highest in the corals at t_0 with an average (±SD) of 602 ± 255 and 5369 ± 2722 µg FA g^{-1} coral, respectively (Fig. 6A,B). The 20, 75 and 150% treatments were significantly lower than at t_0 for the structural FAs, and the 20 and 75% were significantly lower for the storage FAs (SNK Post hoc tests). There were no significant differences in FA content between the 4 experimental food density treatments. The contribution of the FAs C18:1ω9c and C18:3ω3 (i.e. the FAs that dominated the composition of *Artemia salina*), to the FA compo-

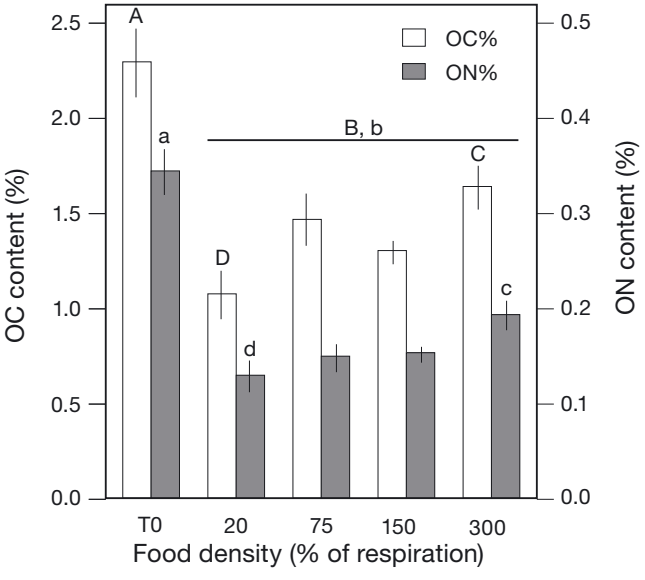

Fig. 5. *Lophelia pertusa.* Percentage of organic carbon (OC) and organic nitrogen (ON) in the coral fragments at the start (t_0) of the experiment and after 15 wk of exposure to different food density conditions (indicated as % of respiration). Data are mean ± SE with n = 8 replicate samples for the t_0 samples and n = 12 replicate samples for each food density treatment. Letters above bars indicate significant differences based on SNK multiple comparisons tests

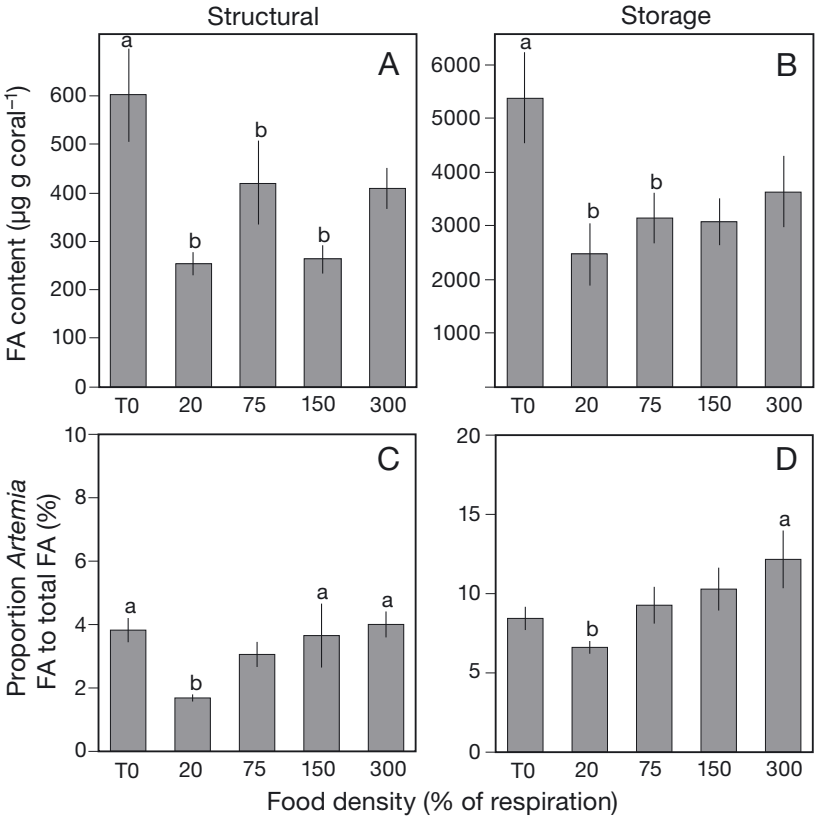

Fig. 6. Summed (A) structural and (B) storage fatty acids (FAs) for the different sets of *Lophelia pertusa* colonies as a function of food density, and normalized to total dry weight of corals. Contribution of *Artemia* FAs to total FAs in (C) structural and (D) storage FAs of corals. The t_0 food density represents the condition before start of the experiment. Bars represent mean ± SE with n = 7–8 replicate samples for the t_0 samples and n = 11–12 replicate samples for each food density treatment. Letters above bars indicate significant differences based on SNK multiple comparisons tests

Table 5. Stocks and process rates during the food density experiment. Stocks are given at the end of the experimental treatment and are normalized to 1 average *Lophelia pertusa* fragment for each treatment. *Artemia salina* expressed in carbon units represents the total amount of carbon available to a coral fragment during the 15 wk of the experiment. Respiration and calcification rates are summed over the entire experimental period and are normalized to 1 coral fragment

	Unit	Treatment (%)			
		20	75	150	300
Stock					
Coral dry weight	g	11.1	10.9	12.1	10.5
Coral tissue dry weight	g	0.57	0.59	0.64	0.59
Organic carbon	mg C	122	155	159	167
Structural fatty acids	mg C	2.5	4.0	2.8	3.8
Storage fatty acids	mg C	24.1	29.8	32.5	33.4
Processes					
A. salina, carbon available	mg C	17	62	125	249
Respiration, carbon	mg C	46.0	48.5	59.1	68.8
Respiration, energy	J	1728.0	1822.3	2219.4	2584.7
Calcification, weight	g	0.48	0.65	0.54	0.52
Calcification, energy	J	72.3	98.6	81.9	78.3

sition of *Lophelia pertusa* was, however, related to the food treatments in both structural and storage FAs (1-way ANOVA; structural: $F_{4,49}$ = 3.06, p = 0.025; storage: $F_{4,51}$ = 2.84, p = 0.033). The percentage *Artemia* FAs of total FAs in the structural FA component was an average (±SD) of 3.8 ± 1.0% at the start of the experiment (as corals were maintained and fed with *Artemia* in the lab prior to experimental start) and increased with food density from 1.7 ± 0.4% (food treatment 20) to 4.0 ± 1.4% (food treatment 300) at the end of the experiment (Fig. 6C). The differences were significant between food treatments 20 and 150% and between 20 and 300% (SNK Post-hoc). The 20% treatment also had significantly lower contribution of *Artemia* FAs in the structural FA compared to the t_0 situation (SNK post hoc). The contribution of *Artemia* FAs to the storage FAs of the corals was an average (±SD) of 8.4 ± 2.1% of total FAs at the start of the experiment and increased with food density from 6.6 ± 1.4% (food treatment 20) to 12 ± 6% (food treatment 300) at the end of the experiment (Fig. 6D). The post hoc test (SNK) revealed that the only significant difference was between the food treatments 20 and 300%.

Stocks and process rates

The overview of stock sizes, process rates and food availability in the different treatments is presented in Table 5. Average carbon respiration rates for the experimental period increased with food density and ranged from 46 to 68.8 mg C per coral fragment. Therefore, although carbon availability was 15 times higher in the highest food treatment compared to the lowest, the carbon that was respired increased by only 1.5 times. The energy investment in skeletal growth is relatively low; energy invested in respiration is about 20–30 times higher than in calcification.

DISCUSSION

Experimental conditions and methodological considerations

For the present study, corals were collected at a water depth of approximately 100 m, whereas the experiments were performed at atmospheric pressure. The resulting pressure difference (1 MPa) has only a minor effect on the partial pressure of dissolved oxygen (1.4%; Enns et al. 1965) and is unlikely to have affected coral respiration, especially since *Lophelia pertusa* can maintain respiratory independence over a broad range of oxygen partial pressures (10 to 20 kPa; Dodds et al. 2007). The pressure difference was also unlikely to have influenced calcification rates, since corals were collected well above the aragonite saturation horizon, which is deeper than 2000 m in the Atlantic Ocean (Davies et al. 2008).

The diet of *Lophelia pertusa* is still relatively unknown, but it appears to feed opportunistically, ingesting whatever is available in the water column (Mortensen 2001, Freiwald 2002, Duineveld et al. 2004, 2007, 2012, Kiriakoulakis et al. 2005, Carlier et al. 2009, Dodds et al. 2009, Van Oevelen et al. 2009). The observed decrease in tissue biomass with time, even in well fed corals, and the lack of response in skeletal growth with *Artemia salina* density, could raise questions about the suitability of *A. salina* as a food source for *L. pertusa* and/or whether the nauplii were really ingested at different rates among the treatments. However, the increased proportion of dominant *Artemia* FAs (C18:1ω9c and C18:3ω3) with increased food density in both structural and storage FAs (Fig. 6C,D), confirms that the various food concentrations indeed resulted in a range of food uptake rates between treatments. A range of food uptake rates is further indicated by the increased oxygen consumption rate with food density (Fig. 3). Although we did not monitor the actual uptake of *A. salina* nauplii in our experiment, visual observations at every feeding occasion indicated that most of the nauplii in all aquaria were ingested. The exception was for the 300% treatment — on a few feeding occasions a significant amount of nauplii was still suspended in the water when feeding was stopped. Measurements of *L. pertusa* uptake rates of *A. salina* nauplii performed by Purser et al. (2010) and Tsounis et al. (2010) show that the amount of *Artemia* given to the corals at each feeding occasion in our experiment was well below the maximum *L. pertusa* uptake capacity of *Artemia* (calculations based on number of polyps per aquaria and initial concentrations of *Artemia*). Although we do not know how suitable *A. salina* is as a food source for *L. pertusa*, we know from previous studies that many corals ingest and assimilate *Artemia,* and that feeding with *Artemia* affects physiological processes such as calcification rates (Houlbrèque et al. 2004a, Hoogenboom et al. 2010, Naumann et al. 2011, Tolosa et al. 2011), tissue growth (Houlbrèque et al. 2004a), respiration rates (Naumann et al. 2011) and lipid content and composition (Al-Moghrabi et al. 1995, Tolosa et al. 2011). In any case, the general decrease in both structural and storage FAs with time indicates conditions in aquaria were suboptimal for the corals. Whether this was a result of the type of food offered or other conditions (e.g. water exchange/movement) is unknown. The spawning likely explains a smaller part of the decrease in biomass (see discussion below).

In the current experiments, corals were maintained in flow-through aquaria of seawater containing possible nutritional sources other than the *Artemia* provided. In addition to zooplankton and detritus, which are documented food sources for *Lophelia pertusa*, heterotrophic feeding by zooxanthellate corals includes nanoflagellates and bacteria (Houlbrèque et al. 2004b); in addition, uptake of dissolved organic matter (DOM) has been reported for some species (Ferrier 1991, Al-Moghrabi et al. 1993, Hoegh-Guldberg & Williamson 1999, Houlbrèque et al. 2004b, Grover et al. 2008, Naumann et al. 2010). Therefore, corals maintained in sand-filtered water may have had access to additional potential food sources such as smaller zooplankton, bacteria, detritus, phytoplankton remains and other smaller particles; for the starved *L. pertusa* corals maintained in sterile-filtered water, the only possible nutritional source was DOM. Although there may have been a small contribution of these additional food sources to coral nutrition in our experiments, it seems that the contribution of particulate sources was minimal given that the decrease in respiration rate was similar between the sterile-filtered and sand-filtered starvation treatments (Fig. 2).

Spawning occurred in some of the aquaria 2 wk into the food density experiment. As a result, biomass was lost, predominantly in the egg-releasing female coral fragments. In zooxanthellate corals, 60 to 85% of the egg dry mass can be lipids (Arai et al. 1993, Harii et al. 2007, Figueiredo et al. 2012). In our experiment, the total loss of lipids and other biomass was likely small, since only 1 of the 6 coral fragments in each aquarium was female. The effect of spawning on coral oxygen consumption measurements in our experiment could be 2-fold. Firstly, the activity of

spawning during the first measurement may have resulted in temporarily increased coral respiration rates. In addition, the presence of spawning products (sperm, eggs and embryos until the 16-cell stage) likely increased oxygen consumption rates in the water (Okubo et al. 2008). Secondly, if a significant amount of the biomass was released as spawning products, the respiration involved in maintenance of tissue biomass should decrease after spawning was complete. We refrain from speculating about the relative contributions of the above mechanisms to the measured oxygen consumption rates.

Effect of food conditions on process rates and resource allocation

We found differences in the respiration rate of *Lophelia pertusa* in response to varying food conditions. The changes in respiration rates were slow but significant, both in terms of a decrease in respiration during prolonged starvation and a difference in respiration among food density treatments. A decrease in respiration with zooplankton exclusion was also found by Naumann et al. (2011) for the solitary Mediterranean cold-water coral *Desmophyllum dianthus*. Interestingly, these authors measured a decrease of 51% within 3 wk, which is rapid compared to the starved *L. pertusa* in our experiment, where the respiration rate decreased by an average of 8% in 3 wk and by 39% after 28 wk. The quicker response of *D. dianthus* may be a result of the higher ambient temperature (12°C) and the associated higher metabolic rate. The respiration rate for *D. dianthus* was 5 times higher at the start of the experiment (Naumann et al. 2011) compared to that of *L. pertusa* in our study. The relatively lower oxygen consumption rate may have allowed starved *L. pertusa* to conserve energy reserves during a longer experimental period. Environmental changes can, however, also cause quick physiological responses in *L. pertusa*. Short-term temperature changes (from ambient of 9°C to 11°C and 6.5°C) have been shown to result in an increase or decrease respectively in the oxygen consumption rate by approximately 50% (Dodds et al. 2007).

Lophelia pertusa skeletal growth rates in this study were comparatively high for long-term laboratory studies. Form & Riebesell (2012) reported average (±SD) calcification rates of 0.009 ± 0.003% d^{-1} under ambient pCO_2 conditions, and Orejas et al. (2011a) reported a growth rate of 0.02 ± 0.01% d^{-1} for Mediterranean *L. pertusa*. Our skeletal growth data compare favourably to freshly collected coral fragments from the Skagerrak (0.033% d^{-1}) and Mingulay reef (0.067% d^{-1}) as measured by Maier et al. (2009), suggesting generally good growth conditions in our laboratory setting. Maier et al. (2009) also found that young polyps grew approximately 10 times faster than older polyps, which likely explains the large variation among coral fragments in the present experiment as both older (proximal) and younger (distal) parts of coral colonies were used. In contrast to our hypothesis, no effect of zooplankton food density on skeletal growth was detected after 15 wk of feeding. This can be compared to Naumann et al. (2011), who found responses in calcification within only 3 wk of starvation of the solitary cold-water coral *D. dianthus*. Surprisingly, skeletal growth rates generally increased during our experiment, although the proportion of OC and ON, as well as both storage and structural FAs decreased during the period. These results raise questions about factors controlling skeletal growth in *L. pertusa*. The increase in skeletal growth could be a result of seasonal variation of nutrient or mineral availability in the ambient water and/or changed resource allocation after spawning. Interestingly, the average growth rate was 50% higher for samples from the male colony than for samples from the female colony, even though the fragments had been equally treated in the laboratory for 8 mo. To what extent the difference in energy allocated to skeletal growth was a result of the nutritional history of the colonies and/or gender is unknown. Our results however, indicate that there is no response in skeletal growth to food conditions on the time scale of months, but food conditions will presumably affect skeletal growth on longer time scales. The coral's nutritional status in terms of stored energy reserves may be one factor controlling calcification rates. In a sediment exposure experiment, Larsson et al. (2013) measured an average (± SD) skeletal growth rate of 0.038 ± 0.024% d^{-1} in a control treatment with *L. pertusa* abundantly fed with *Artemia salina*, although the content of storage and structural FAs at start of the experiment were 7 times and 2 times lower, respectively, than for corals in the present study. Although these corals also lost some FAs during the experiment, a relatively high growth rate compared to previous laboratory studies was maintained, indicating that skeletal growth rate may only be affected when the nutritional status of the coral is quite poor. Calcification in corals requires transport of ions through the coral tissue layers, and the general view for tropical corals is that the extracellular calcifying medium (ECM) between the cali-

coblastic cells and the skeleton is supersaturated with aragonite compared to the surrounding seawater, making precipitation more rapid. (e.g. Cohen & Holcomb 2009, Tambutté et al. 2011). Based on analyses of boron isotopes in coral skeleton, McCulloch et al. (2012) conclude that cold-water corals (including *L. pertusa*) actively pump ions resulting in a strong up-regulation of pH and hence aragonite saturation state in the ECM. This strong up-regulation is energetically costly, and is suggested to be the main step limiting the rate at which azooxanthellate corals calcificate (McCulloch et al. 2012). Interestingly, the calcification rate in our study did not increase despite a 15 times higher *Artemia* availability in the highest food treatment compared to the lowest, indicating there might be other limiting factors to skeletal growth in *L. pertusa* than energy input. Such a limiting factor could be diffusion of ions from seawater if *L. pertusa* has limited capacity to actively regulate the ion concentration in the ECM.

Heterotrophic feeding in zooxanthellate corals can increase both skeletal and tissue growth rates (Anthony & Fabricius 2000, Ferrier-Pagès et al. 2003, Houlbrèque et al. 2003, Houlbrèque et al. 2004a, Orejas et al. 2011b, Tolosa et al. 2011), but tissue growth is more responsive to environmental conditions than skeletal growth (Anthony et al. 2002, Ferrier-Pagès et al. 2003, Houlbrèque et al. 2003, Houlbrèque et al. 2004a). Our results indicate that this is the case also for *Lophelia pertusa*, although it has a dense skeleton with a relatively low tissue to skeleton ratio. Although there were no significant differences in total tissue content after 15 wk at different food densities, other measures of tissue (i.e. content of OC, ON, and structural and storage FAs) generally increased with higher food levels. The amount of storage FAs were in between the reported 55 to 124 mg g^{-1} tissue DW found for 3 locations by Dodds et al. (2009). Interestingly, the latter authors monitored 2 lipid classes in different seasons and at different locations but did not observe seasonal variation in lipid content. They suggested that *L. pertusa* may adjust its metabolism in response to variable food supply, which is also indicated by the results from our respiration measurements. Our estimations of various physiological processes show that *L. pertusa* energy expenditure during the period of constant feeding was dominated by respiration when compared to skeleton growth. Coral tissue decreased during the experiment, which implies that any energy invested in production of organic tissue is offset with a higher consumption of tissue to fuel metabolic processes. It is unclear how much of the energy spent in respiration is used for processes such as tissue maintenance, tissue growth, investment in reproductive tissue, gonad development or calcification, but given the low energy involved in calcification, this is likely a minor energy sink. Also in this respect, it seems skeletal growth in *L. pertusa* is not very sensitive to resource limitations, and that stressful conditions (such as periods of low food availability), would have to occur for a long time before skeletal growth would be affected. It is interesting to note that Brooke & Young (2009), who transplanted Alizarin stained *L. pertusa* nubbins to different 'high coral' and 'no coral' habitats in the Gulf of Mexico, did not detect any differences in branch extension rates after one year. The comparable growth rates resulted in an interpretation that both habitats could potentially support growth of this coral. Given the indication of a very long response time of calcification rate in this case, it may be necessary to first determine the time scale at which an impact of environmental conditions on calcification rates can be expected.

Implications and areas for further study

Our results show that *Lophelia pertusa* is highly tolerant to living on minimal resources for several months. The coral fragments that were maintained in sterile filtered seawater were in a surprisingly good condition after the 6 mo experimental period, and respiration rates had decreased only slowly during this time. Duineveld et al. (2007) monitored fluorescence in the bottom water at a Rockall coral mound community during an 11 mo lander deployment, and found that fluorescence was high from January to July (peaking during the algal bloom period in March and April), but fluorescence was minimal in September to December. Accordingly, cold-water corals may naturally experience periods of several months in which food levels are very low, and may use energy reserves to survive these long periods of starvation. Alternatively, it is possible that *L. pertusa* can use dissolved organic matter (DOM) as nutrition in the absence of particulate organic matter. Flume studies with *L. pertusa* fragments showed that ^{13}C and ^{15}N labelled dissolved amino acids were taken up from the water column and incorporated in the coral tissue within days (C. Mueller unpubl. data). At the same time, *L. pertusa* has been show to release high amounts of DOM into the water column, stimulating oxygen consumption up to meters above the reef (Wild et al. 2008, 2009). The cold-water coral *Desmophyllum dianthus* switched from a net release

of total organic carbon (TOC) to an uptake of TOC after 3 wk of zooplankton exclusion (Naumann et al. 2011). The uptake of TOC (dissolved organic carbon, DOC and smaller particles) after 3 wk of starvation was about 0.25 mg C m^{-2} h^{-1} — approximately 30 times lower than respiration losses at that time. These results indicate that cold-water corals can adjust their feeding with changing environmental conditions. A net release of DOM is probably common under fed or mildly starved conditions, but this may shift to a net uptake of DOM if food limitations persist. The concentration of labile DOM is typically low in the natural marine environment, and the significance of the coral's DOM-uptake ability in terms of the total energy balance can be questioned. However, assuming that DOC concentration in naturally occurring seawater is 100 µM (Vlahos et al. 2002, Neogi et al. 2011) of which 10 % is labile DOC (therefore available for uptake); and assuming a flow rate of 10 cm s^{-1}, the available labile DOC approaching a polyp (1 cm^2) would be 360 µmol h^{-1}. The carbon required to meet the typical respiratory demand in this study was 0.12 µmol C $polyp^{-1}$ h^{-1}; therefore, the delivery of labile DOC to coral polyps in the natural environment is 3 orders of magnitude higher than carbon respired in our experiment. Obviously, other processes are likely limiting the possible DOC uptake in this species, such as means of transportation of DOC into tissue. The mechanisms of DOM uptake by cold-water corals are unknown, but Ferrier-Pagès et al. (1998) demonstrated the role of coral-associated bacteria for substantial uptake of DOM in the zooxanthellate coral *Galaxea fascicularis*. Naumann et al. (2010) measured an average uptake rate of 263 mg DOC m^{-2} coral surface area h^{-1} in zooxanthellate corals of the genus *Pocillopora*, which corresponds to 2.2 µmol cm^{-2} h^{-1} indicating that the estimated uptake of 0.12 µmol DOC cm^{-2} polyp h^{-1} required to meet the *L. pertusa* energy demands is plausible. More research in this area is needed to assess the possible importance of DOM uptake in *L. pertusa*, and the mechanisms involved in such uptake.

In this study, we experimentally manipulated food availability (ranging from starvation to abundant food supply) and monitored the different physiological responses of the cold-water coral *Lophelia pertusa*. Surprisingly, we found no response in calcification rates across the different food treatments. This indicates that there may be important limiting factors to skeletal growth in this species other than energy input. The respiration response to starvation and different food levels was only moderate, although energy investment in respiration was about 20 to 30 times higher than in calcification. Overall, it is clear that the response time of *L. pertusa* to food conditions is long, and acts on a timescale of weeks to months for coral tissue content and respiration, and a timescale of >4 mo for calcification. Similar to Ferrier-Pagès et al. (2003), we suggest that tissue serves as energy storage in *L. pertusa* when food is available, and that skeletal growth may continue at a high rate followed by thinning of the tissue under poor food conditions. This implies that observed skeletal growth rates cannot be used as a short-term indicator of food conditions in the field. This is also true for observed short-term respiration rates, since the respiration responded slowly at a time scale of months. The amount of storage FAs in this study showed an increasing trend with food density, though differences were not significant. The between-sample variability in storage FA levels was comparatively high, which renders translation of these data to food conditions in the field challenging. Out of 3 locations, Dodds et al. (2009) found the highest storage lipid content in samples from the shallow Mingulay reef (~150 m), at which higher food inputs could be expected. Therefore, it appears that storage FA may be a better proxy for food conditions compared to measurements of respiration and calcification rates, given sufficient sampling to deal with the large associated variability.

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