The Journal of Experimental Biology 214, 3351-3357

doi:10.1242/jeb.058354

RESEARCH ARTICLE

Extracoelenteric zooplankton feeding is a key mechanism of nutrient acquisition for the scleractinian coral *Galaxea fascicularis*

Tim Wijgerde*, Rara Diantari, Muhammad Wahyudin Lewaru, Johan A. J. Verreth and Ronald Osinga

Aquaculture and Fisheries Group, Department of Animal Sciences, Wageningen University, Wageningen University and Research centre, 6709 PG Wageningen, The Netherlands

*Author for correspondence (tim.wijgerde@wur.nl)

Accepted 11 July 2011

SUMMARY

Internal and external feeding on zooplankton may provide scleractinian corals with important nutrients. However, the latter process has never been properly quantified. To quantify the dynamics of zooplankton capture, digestion and release for a scleractinian coral, we performed detailed video analyses of *Galaxea fascicularis* feeding on *Artemia* nauplii. A highly dynamic process of prey capture, digestion and release was observed. A single *G. fascicularis* polyp (*N*=3) captured 558±67 and released 383±75 *Artemia* nauplii over a 6 h interval. On average, 98.6% of prey captured was not ingested. Instead, prey items were clustered into aggregates that were digested externally by mesenterial filaments. In addition, we employed carbon, nitrogen and phosphorus analysis of zooplankton before and after digestion by *G. fascicularis* colonies (*N*=6). For total organic carbon, 43.1% (0.298±0.148 μg *Artemia*$^{-1}$) was lost after 6 h of digestion. For total organic nitrogen, total organic phosphorus and orthophosphate (PO_4^{3-}), these values were 51.3% (0.059±0.028 μg *Artemia*$^{-1}$), 50.9% (0.009±0.004 μg *Artemia*$^{-1}$) and 84.6% (0.0019±0.0008 μg *Artemia*$^{-1}$), respectively. For extracoelenteric zooplankton feeding alone, total estimated nutrient inputs for *G. fascicularis* colonies were 76.5±0.0 μg organic carbon, 15.2±0.0 μg organic nitrogen, 2.3±0.2 μg organic phosphorus and 0.5±0.8 μg inorganic phosphorus per cm^2 coral tissue per day. These values exceed calculations based on intracoelenteric feeding by up to two orders of magnitude. Our results demonstrate that extracoelenteric zooplankton feeding is a key mechanism of nutrient acquisition for a scleractinian coral. These results are of importance to coral aquaculture and our understanding of benthic–pelagic coupling on coral reefs.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/214/20/3351/DC1

Key words: *Galaxea fascicularis*, heterotrophy, extracoelenteric, *Artemia*, zooplankton, coral, scleractinian.

INTRODUCTION

Heterotrophy is vital to coral nutrition, as it supplies the coral and its symbiotic algae with essential elements such as carbon, nitrogen and phosphorus. For the scleractinian coral *Stylophora pistillata*, it has been found that heterotrophy increases tissue protein concentration and stimulates growth both directly, by enhancing calcification and organic matrix synthesis, and indirectly, by increasing photosynthetic rates [Houlbrèque and Ferrier-Pagès (Houlbrèque and Ferrier-Pagès, 2009) and references therein]. Furthermore, heterotrophy is an important source of nutrients during coral bleaching episodes, when autotrophy is virtually absent due to loss of symbiotic dinoflagellates (Grottoli et al., 2006). Heterotrophic sources for corals consist of dissolved organic matter, and detrital and live particulate organic matter including bacteria, protozoa, phytoplankton and zooplankton. Of these sources, zooplankton constitutes a significant proportion of the daily carbon and nitrogen input for scleractinians; up to 100% of the total organic carbon input (Grottoli et al., 2006) and approximately 49% of the total organic nitrogen input when high prey concentrations are used (Ferrier-Pagès et al., 2003; Houlbrèque and Ferrier-Pagès, 2009).

Scleractinian corals employ intricate mechanisms of zooplankton capture, which encompass tentacle movement combined with cnidocyte firing and subsequent mucociliary feeding to ingest immobilized prey (Sorokin, 1990). It has long been known that scleractinian corals may also digest prey externally, by expulsion of mesenterial filaments as a response to prey detection (Duerden, 1902; Carpenter, 1910; Matthai, 1918; Yonge, 1930; Yonge, 1973; Abe, 1938; Goreau et al., 1971; Lang, 1973; Logan, 1984; Lang and Chornesky, 1990; Goldberg, 2002). Mesenterial filaments may be extruded through any part of the polyp epithelium, after which prey is either ingested (Goldberg, 2002) or digested externally (Lang, 1973). Externally digested prey may contribute significantly to the daily carbon, nitrogen and phosphorus input to the diet of scleractinian corals, but this has never been quantified adequately. Until now, studies have resorted to particle analysis of the polyp coelenteron or prey clearance rate (Leversee, 1976; Dai and Lin, 1993; Webber and Roff, 1995; Sebens et al., 1996; Sebens et al., 1998; Witting, 1999; Ferrier-Pagès et al., 2003; Houlbrèque et al., 2004; Palardy et al., 2005; Grottoli et al., 2006; Osinga et al., 2008; Hii et al., 2009; Purser et al., 2010), with clear limitations. The first method only quantifies ingestion, excluding extracoelenteric digestion, which may be an important process in terms of number of prey items digested and nutrients assimilated. The alternative, prey clearance rate, takes both ingested and externally retained particles into account, but fails to reveal the dynamics of prey capture, (extracoelenteric) digestion and release, possibly obscuring realistic estimates of nutrient input from zooplankton. This is because (partially) digested and subsequently released particles are re-

counted in the water column and, therefore, are not quantified as captured and digested.

To quantify the dynamics of zooplankton prey capture, digestion and release for a scleractinian coral, we performed detailed video analyses of single polyps of the oculinid scleractinian *Galaxea fascicularis* feeding on nauplii of the brine shrimp *Artemia*. In addition, we employed carbon, nitrogen and phosphorus analysis of *Artemia* nauplii before and after capture by *G. fascicularis* colonies to estimate the quantitative role of (extracoelenteric) zooplankton feeding in the diet of a common Indo-Pacific scleractinian coral.

MATERIALS AND METHODS

Selected species and husbandry

For this study, the Indo-Pacific scleractinian coral *Galaxea fascicularis* (Linnaeus 1767) was used, which bears corallites that are usually less than 10 mm in diameter (Veron, 2000). All colonies were genetically identical to rule out genotype-specific effects. Corals were kept in a closed system of 400 l, with the following parameters (±indicates minimum–maximum deviations) salinity 35±0.5 g l^{-1}, temperature 26±0.5°C, pH 8.2±0.3, photon flux density 368 µmol m^{-2} s^{-1} (12 h:12 h light:dark regime), nitrate 2±1 mg l^{-1}, phosphate 0.03±0.01 mg l^{-1}, calcium 400±20 mg l^{-1} and magnesium 1300±50 mg l^{-1}. Water flow was provided by four Turbelle nanostream 6045 circulation pumps (Tunze Aquarientechnik GmbH, Penzberg, Germany) and an Eheim 1260 return pump (Eheim GmbH Co. KG, Deizisau, Germany), providing a total flow rate of 20,000 l h^{-1} or 5–10 cm s^{-1}. Single polyp clones were used for the photographic and video analysis. Single polyps were individually removed from a large parent colony using pincers, and were subsequently glued onto 7×7 cm PVC plates with epoxy resin (Aqua Medic GmbH, Bissendorf, Germany). Whole colonies were used for the carbon, nitrogen and phosphorus depletion studies. All single polyps and colonies were of the same genotype, because they all originated from a single parent colony.

Analysis of colony surface area, polyp number and polyp density

To determine mean colony surface area, polyp number and polyp density for *G. fascicularis*, we photographed colonies (N=4) and analyzed images using ImageTool 3.0 (The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA). Surface area was determined by using a ruler as a reference. Polyp numbers were scored and polyp densities were calculated from colony surface areas and polyp numbers.

Determination of aggregate density

To determine the mean aggregate density on *G. fascicularis* colonies, we incubated colonies (N=4) in a respirometric flow cell (Wageningen UR, Wageningen, The Netherlands) together with *Artemia* nauplii at a concentration of 4100 nauplii l^{-1}. Colonies were photographed at 6 h of incubation and images were analyzed using Adobe Photoshop 11.0.1. Aggregate numbers were scored and aggregate densities were calculated from colony surface areas and aggregate numbers.

Video analysis

For photographic and video analysis, single polyp clones of *G. fascicularis* (N=3) were incubated in a respirometric flow cell (Wageningen UR) with a volume of 3.5 l for 6 h. Water flow was created by a built-in model boat propeller, driven by a Maxon DC motor that was connected to a computer. Flow speed was set at 200 r.p.m. (5 cm s^{-1}), controlled by EPOS user interface software (version 2.3.1, maxon motor ag, Sachseln, Switzerland) (for details see Schutter, 2010). Water from the holding tank was used for the experiments to rule out artifacts resulting from changes in water chemistry. Temperature was kept at 26±0.5°C by means of a water jacket connected to a TC20 water cooler (Teco SRL, Ravenna, Italy). Photon flux density was set to holding tank intensity, 368 µmol m^{-2} s^{-1}, with a T5 fluorescent lighting fixture containing four 24 W T5 fluorescent tubes with a color temperature of 14,000 K (Elke Müller Aquarientechnik, Hamm, Germany). An HDR-CX505VE handycam (Sony Corporation, Tokyo, Japan) was used for recording still and moving images in high-resolution formats. *Artemia salina* nauplii were hatched from cysts (Great Salt Lake *Artemia* cysts, Artemia International LLC, Fairview, TX, USA) at a salinity of 25 g l^{-1} and a temperature of 28°C, and used immediately after hatching. Average nauplii size was 440 µm according to the manufacturer. A concentration of 10,000 nauplii l^{-1} was used for all experiments (N=3). Polyps were acclimated for 15 min before the start of every incubation. Each polyp was analyzed once. Capture and release of *Artemia* nauplii by the coral polyps was scored by analyzing videos after all experiments. Captured nauplii were defined as prey that attached to the polyp surface for at least 10 s. Released nauplii were defined as prey that detached from the polyp surface and remained in suspension for longer than 10 s. Aggregate formation was defined as a cluster of two or more nauplii attached to the polyp surface.

Carbon, nitrogen and phosphorus depletion

For the carbon, nitrogen and phosphorus depletion studies, the same setup was used as described above. *Galaxea fascicularis* colonies (N=6) with a mean of 449±22 polyps were used and incubated for 6 h in a respirometric flow cell. A concentration of 10,000 nauplii l^{-1} was used for all experiments. Colonies were acclimated for 15 min before the start of each incubation. Each colony was analyzed once. As a negative control, nauplii from each experiment's stock were incubated in a water bath at 26±0.5°C for 6 h to determine their inherent metabolism (mainly yolk sac consumption). Data on carbon, nitrogen and phosphorus lost due to this inherent metabolism were used to calculate net loss of nutrients after digestion. To determine the nutrient content of nauplii at the start of each experiment, nauplii were collected from the stock population, washed on a 150 µm filter mesh, quantified by multiplying the collected volume (500 µl) with that day's determined stock concentration and frozen shortly after hatching. The same procedure was carried out for the control samples, after 6 h of incubation in a water bath at 26°C. Approximately 2000 nauplii were collected during each experiment for both the start and control samples. After 6 h of incubation, nauplii from the digestion experiment were collected with plastic Pasteur pipettes. As *G. fascicularis* polyps retain most of their prey externally, aggregates of *Artemia* nauplii could easily be collected from the polyp surface. After collection, nauplii were transferred onto a 150 µm filter mesh and washed thoroughly with demineralized water. After washing, nauplii were quantified by counting all individuals under an M8 stereomicroscope (Wild Heerbrugg, Heerbrugg, Switzerland). All samples were transferred to 50 ml tubes (Greiner Bio One GmbH, Frickenhausen, Germany), resuspended in 50 ml demineralized water and frozen at –20°C until further analysis.

Carbon, nitrogen and phosphorus content analysis

To determine carbon, nitrogen and phosphorus depletion, several methods were used. All samples were thawed in water baths at 25°C

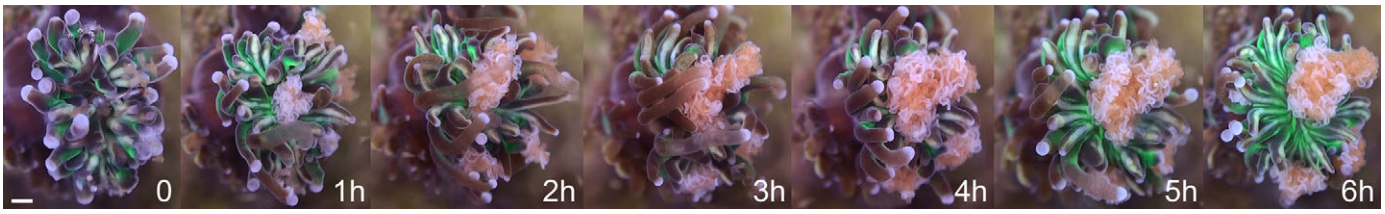

Fig. 1. Photographic time lapse series of *Artemia* nauplii aggregate formation during a 6 h incubation, representative for all individual *Galaxea fascicularis* polyps tested (*N*=3). Polyps displayed tentacle expansion and extrusion of mesenterial filaments (white strands). Aggregates increased significantly during the first 210 min, after which they stabilized. Scale bar, 500 μm.

and subsequently centrifuged at 1157 ***g*** at 4°C for 3 min. Forty milliliters of each supernatant was removed. Samples were homogenized with an Ultratorax X1030 homogenizer (Ingenieurbüro M. Zipperer GmbH, Dottingen, Germany) for 5 min in the 10 ml remaining volume at room temperature. For each sample, the Ultratorax was washed with demineralized water after homogenization to collect remaining *Artemia* tissue. Next, samples were centrifuged at 1157 ***g*** at 4°C and were adjusted to 20 ml final volume with demineralized water. Total carbon was measured by high temperature catalytic oxydation on a TOC-5050A auto analyzer (Shimadzu Scientific Instruments, Shimadzu Corporation, Kyoto, Japan) followed by detection of CO_2 with a non-dispersive infrared sensor. Inorganic carbon was measured by acidifying subsamples to a 25% phosphoric acid solution followed by NDIR detection of purged CO_2. Total nitrogen, total phosphorus, inorganic nitrogen (ammonium, nitrite and nitrate) and inorganic phosphorus (orthophosphate), were analyzed with a San^{++} continuous flow analyzer (Skalar Analytical BV, Breda, The Netherlands). All measured concentrations were converted to μg per *Artemia* nauplius, by taking volume (20 ml) and number of *Artemia* nauplii in each sample into account. Total organic carbon (TOC), total organic nitrogen (TON) and total organic phosphorus (TOP) were calculated by subtracting the inorganic from the total fractions.

Data analysis

Normality of data was tested by plotting the residuals of each data set *versus* predicted values, and by performing a Shapiro–Wilk test. Homogeneity of variances was determined using Levene's test. Residuals of TOC, TON, TOP and PO_4^{3-} depletion and N:P ratios were normally distributed ($P>0.05$), whereas those of C:N and C:P ratios were not ($P<0.05$). All depletion data showed homogeneity of variances ($P>0.05$), except those for PO_4^{3-} and C:P ratios ($P<0.05$). For TOC, TON and TOP we used one-way ANOVA followed by Bonferroni's test. For PO_4^{3-} we used one-way ANOVA followed by the Games–Howell test. For the C:N, C:P and N:P ratios we used a Kruskal–Wallis test followed by a Mann–Whitney test. A value of $P<0.05$ was considered statistically significant, except for Mann–Whitney where we used a critical value of $P<0.025$, based on a Bonferroni correction factor of 2. Statistical analysis was performed with SPSS Statistics 17.0 (IBM, Somers, NY, USA). Graphs were plotted with SigmaPlot 11.0 (Systat Software, San Jose, CA, USA). All data presented are means ± s.d.

RESULTS

Throughout all of the 6 h incubations, tentacles of *G. fascicularis* polyps were active and well expanded. Tentacles moved towards the *Artemia* nauplii that came in contact with the polyp, actively maneuvering nauplii onto the oral disk. During the last hour of incubation, a slight retraction of tentacles was visible (Fig. 1 and supplementary material Movie 1). Mucus excretion was apparent, which seemed to aid in prey capture. Several flatworms, possibly *Waminoa* sp., were also observed, moving slowly across the oral disk. At approximately 20 min, expulsion of mesenterial filaments through several areas of the polyp ectoderm and oral pore was clearly visible (Fig. 1 and supplementary material Movie 1). Within the first 30 min of the incubations, aggregates of *Artemia* nauplii started to appear on the polyp surface. These aggregates increased in size over time (Figs 1, 2 and supplementary material Movie 1). One to three aggregates per polyp were observed.

Galaxea fascicularis polyps captured and released significant amounts of *Artemia* nauplii during the incubations (Fig. 2). On average, a single polyp captured 558±67 nauplii and released 383±75 nauplii over the entire 6 h period (Fig. 2). Ingestion of nauplii was observed for only one of the three single polyps tested. In total, 27

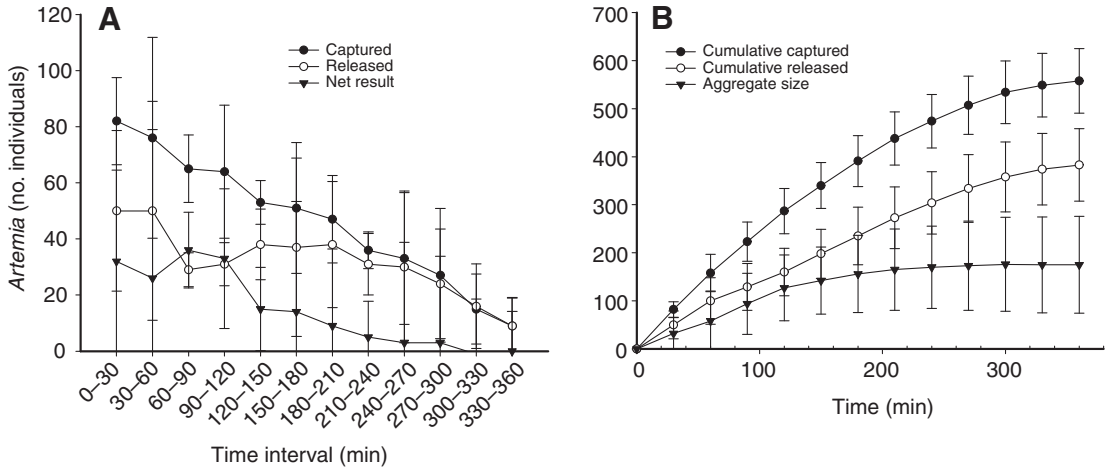

Fig. 2. Overview of *Artemia* nauplii capture and release dynamics of single polyps of *G. fascicularis*. (A) Numbers of captured and released *Artemia* nauplii, and their net result, shown in 30 min intervals. (B) Cumulative capture, release and accumulation (aggregate size). Values are means ± s.d. (*N*=3).

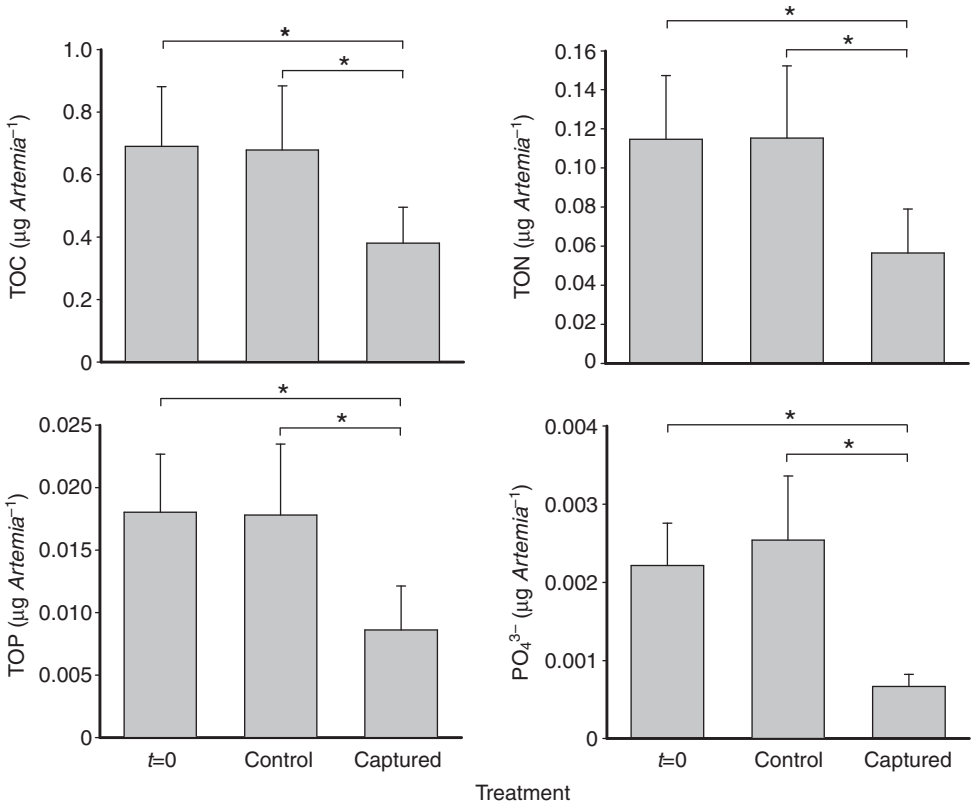

Fig. 3. Total organic carbon (TOC), total organic nitrogen (TON), total organic phosphorus (TOP) and inorganic phosphorus (orthophosphate, PO_4^{3-}) content expressed in µg per *Artemia* nauplius for three different treatments: *t*=0, *Artemia* nauplii shortly after hatching; control, 6 h incubations of *Artemia* nauplii in a water bath at 26°C; and captured, captured *Artemia* nauplii after 6 h of incubation together with *G. fascicularis* colonies. Values are means ± s.d. (*N*=6). Asterisks indicate significant differences between treatments (P<0.03, Bonferroni for TOC, TON and TOP; P<0.01, Games–Howell for PO_4^{3-}).

nauplii were ingested, which was only 4.1% of the total number (659) of captured nauplii at 360 min for that polyp. During the first 300 min, more nauplii were captured than released (Fig. 2A). This was reflected in the size of the aggregates that formed, which increased considerably to an average size of 165 nauplii (93.8% of maximum aggregate size) during the first 210 min (Fig. 2B and supplementary material Movie 1). As time progressed, the dynamics of prey capture and release leveled off. This was indicated by the decreasing amounts of nauplii captured and released per time interval (Fig. 2A), as well as the cumulative numbers of *Artemia* captured and released (Fig. 2B). The maximum average aggregate size was 176 nauplii, which was reached at 300 min. After 300 min, capture and release rates became similar and, as consequence, aggregate size did not increase further (Fig. 2B and supplementary material Movie 1). After 6 h, polyps slowly released aggregates, possibly by increasing mucus production (not shown).

The concentration of *Artemia* nauplii decreased from 10,000 prey l^{-1} to a minimum of approximately 9950 prey l^{-1} at 300 min, calculated by a maximum average aggregate size of 176 nauplii. This equaled a concentration decrease of 0.5%.

Colonies of *G. fascicularis* also retained aggregates of *Artemia* nauplii at the polyp surface during 6 h incubations. *Artemia* aggregates remained in intimate contact with protruded mesenterial filaments for several hours, suggesting extensive digestive processes (supplementary material Movie 1). Captured *Artemia* nauplii appeared fragmented and heavily depigmentated at the end of the incubations. Subsequent elemental analysis showed that the putatively digested *Artemia* nauplii were significantly depleted of TOC, TON, TOP and PO_4^{3-} (Fig. 3) when compared with nauplii that had not been captured. After 6 h of incubation with *G. fascicularis* colonies, captured *Artemia* nauplii were found to have a TOC content of 0.381±0.114 µg *Artemia*$^{-1}$, a TON content of 0.056±0.023 µg *Artemia*$^{-1}$, a TOP content of 0.009±0.004 µg *Artemia*$^{-1}$ and a PO_4^{3-} content of 0.0007± 0.0002 µg *Artemia*$^{-1}$ (Fig. 3). These values were significantly lower (Bonferroni for TOC, TON and TOP, $P \leq 0.03$; Games–Howell for PO_4^{3-}, P<0.01) than the values found for the negative controls (i.e. *Artemia* nauplii that had been incubated in seawater for 6 h), which were 0.678±0.206, 0.115±0.037, 0.018±0.006 and 0.0025± 0.0008 µg *Artemia*$^{-1}$ for TOC, TON, TOP and PO_4^{3-}, respectively (Fig. 4). No significant differences between the negative controls and freshly hatched *Artemia* nauplii (*t*=0) were found (Bonferroni for TOC, TON and TOP, P>0.05; Games–Howell for PO_4^{3-}, P>0.05). Inorganic nitrogen species (ammonium, nitrite and nitrate) could not be measured accurately because of very low concentrations and are therefore not shown. When taking the nutrient content of *Artemia* nauplii at the start of every experiment into account, and correcting for all negative controls, 43.1% (0.298± 0.148 µg *Artemia*$^{-1}$) of TOC was lost after 6 h of incubation with *G. fascicularis* colonies (Fig. 4). For TON, TOP and PO_4^{3-}, these values were 51.3% (0.059±0.028 µg *Artemia*$^{-1}$), 50.9% (0.009± 0.004 µg *Artemia*$^{-1}$) and 84.6% (0.0019±0.0008 µg *Artemia*$^{-1}$), respectively (Fig. 4).

The C:N ratios were 6.1±0.3 at *t*=0, 6.0±0.4 for the negative control and 7.5±2.1 for captured nauplii after 6 h incubation with *G. fascicularis* colonies (Table 1). For the C:P ratios, these values were 38.1±1.9, 38.6±2.8 and 51.2±20.1, respectively (Table 1). For the N:P ratios we found values of 6.3±0.3, 6.5±0.3 and 6.7±0.7, respectively (Table 1). The C:N ratio was not significantly different among the three groups of *Artemia* nauplii (Mann–Whitney, captured, control and *t*=0, P>0.025). The C:P ratio of captured nauplii did not differ significantly from the negative control (Mann–Whitney, P>0.025), but was significantly different from the *t*=0 group (Mann–Whitney, P<0.025) after 6 h incubation with *G. fascicularis* polyps. The N:P ratio did not differ significantly among the three groups of nauplii (Mann–Whitney, P>0.025).

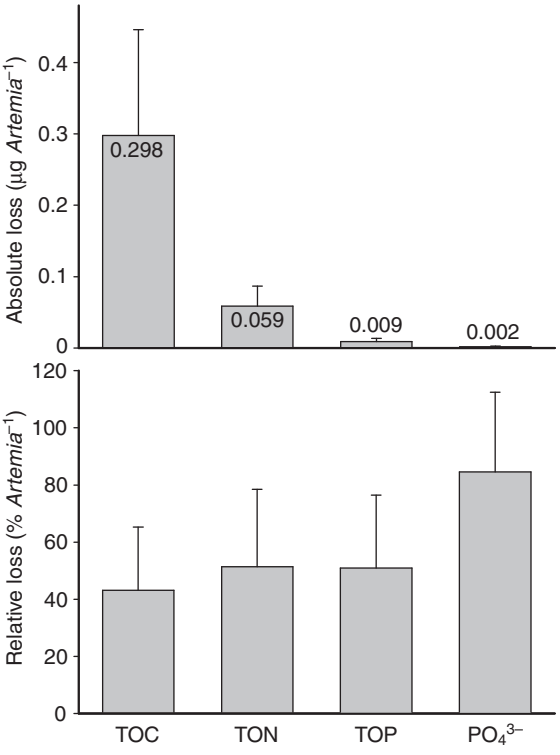

Fig. 4. Loss of TOC, TON, TOP and PO_4^{3-} content expressed in absolute (µg *Artemia*$^{-1}$) and relative (% *Artemia*$^{-1}$) values of captured *Artemia* nauplii after 6 h of incubation together with *G. fascicularis* colonies. All values were corrected for negative controls. Values are means ± s.d. (*N*=6).

Table 1. Nutrient ratios of *Artemia* nauplii

	C:N	C:P	N:P
t=0	6.1±0.3	38.1±1.9	6.3±0.3
Control	6.0±0.4	38.6±2.8	6.5±0.3
Captured	7.5±2.1	51.2±20.1	6.7±0.7

Carbon to nitrogen (C:N), carbon to phosphorus (C:P) and nitrogen to phosphorus (N:P) ratios for *Artemia* nauplii at the start of the experiments (*t*=0), after 6 h incubation in a water bath (control) and after 6 h incubation with *Galaxea fascicularis* colonies (captured). Values are means ± s.d. (*N*=6).

DISCUSSION

Our results show that the scleractinian coral *G. fascicularis* is capable of capturing large amounts of zooplankton prey within a time frame of several hours. The observed extrusion of mesenterial filaments and the clearly fragmented and depigmentated outer appearance of captured *Artemia* nauplii at the end of the incubations strongly suggest effective extracoelenteric digestion of zooplankton. This assumption is supported by frequent reports of cnidarian mesenterial filaments as digestive structures (Duerden, 1902; Carpenter, 1910; Matthai, 1918; Yonge, 1930; Yonge, 1973; Abe, 1938; Goreau et al., 1971; Lang, 1973; Logan, 1984; Lang and Chornesky, 1990; Goldberg, 2002), which may contain both digestive zymogen cells and absorptive cells (Yonge, 1930; Abe, 1938; Van Praët, 1980). The observed decrease in prey capture and release over time (Fig. 2), and the slow release of aggregates after 6 h of incubation, indicate satiation. This phenomenon, also found for the coral *Acanthogorgia vegae* (Lin et al., 2002), is what would be expected if feeding were indeed the process at hand. As the concentration of *Artemia* nauplii, calculated by the total average nauplii aggregate size, only dropped slightly (by 0.5%), changes in capture rate due to a concentration effect can be ruled out.

On average, our *G. fascicularis* polyps captured 93±0.12 nauplii h^{-1}, which is substantially higher than the results of a similar study by Hii et al. (Hii et al., 2009), who found a lower capture rate for *G. fascicularis* (50±30 nauplii polyp^{-1} h^{-1}) under similar conditions by using prey clearance rate. This indicates that prey clearance rate studies may indeed underestimate the amount of prey captured and digested. Intracoelenteric prey analysis is another commonly used method to quantify zooplankton capture, and is highly precise. However, all externally digested prey items are not quantified, which may represent a major fraction of nutrient input. Grottoli and colleagues found that *Montipora capitata* polyps increased their feeding rate whilst in a bleached status, in contrast to two other species that displayed no increased capture of zooplankton (*Porites compressa* and *P. lobata*) (Grottoli et al., 2006). According to the authors, this may lead to shifts in coral species composition on the reef within several decades from now, due to increased bleaching events and the heterotrophic advantage of species such as *M. capitata*. Taking extracoelenteric zooplankton feeding into account, however, could place this theory in a completely new perspective, as many species may be able to utilize this feeding mechanism. Although it could be argued that our observations are genotype or species specific, we found similar digestive behavior for a different genotype of *G. fascicularis* and a species with much smaller polyps, *S. pistillata* (approximately 1 mm in diameter) using video analysis (unpublished results). Extracoelenteric feeding has now been reported for many scleractinian coral species from various families including the Mussidae, Faviidae, Fungiidae, Meandrinidae, Astrocoeniidae, Pocilloporidae, Agariciidae, Siderastreidae, Poritidae and Oculinidae (Duerden, 1902; Carpenter, 1910; Matthai, 1918; Yonge, 1930; Yonge, 1973; Abe, 1938; Goreau et al., 1971; Lang, 1973; Logan, 1984; Lang and Chornesky, 1990; Goldberg, 2002). This shows that extracoelenteric zooplankton feeding is a common feeding mechanism amongst scleractinian corals bearing a wide variety of polyp sizes.

The assumption of significant extracoelenteric feeding is supported by analysis of organic carbon, nitrogen, phosphorus and inorganic phosphorus of *Artemia* nauplii captured and processed by *G. fascicularis* polyps, which showed clear depletion of the aforementioned nutrients (Figs 3, 4). The C:N, C:P and N:P ratios of captured nauplii did not differ significantly from the negative controls after 6 h incubation with *G. fascicularis* colonies, indicating that all elements were taken up in equal proportions. Proportional uptake of carbon and nitrogen may have been due to the fact that *Artemia* nauplii are rich in proteins, with a mean content of 52.2±8.8% (Léger et al., 1987), possibly resulting in concomitant uptake of both carbon and nitrogen from proteins. This theory is supported by the findings of Piniak and Lipschultz (Piniak and Lipschultz, 2004), who found for *Oculina arbuscula* and *O. diffusa* that approximately 90% of ingested ^{15}N from labeled *Artemia* nauplii comprised proteins, amino acids and nucleic acids. Proportional uptake of carbon and phosphorus may have been the result of phospholipid and nucleic acid removal from prey: the lipid content of *Artemia* nauplii is on average 18.9±4.5% (Léger et al., 1987), of which 19.1±0.2% are phospholipids (Navarro et al., 1991). Indeed, both Al-Moghrabi et al. (Al-Moghrabi et al., 1995) and Treignier et al. (Treignier et al., 2008) found an increase in the lipid content of scleractinian coral tissue after feeding with zooplankton, although they did not specifically address phospholipids. Substantial assimilation of organic nitrogen from zooplankton is supported by the literature, as this element is considered an important building

Table 2. Estimated nutrient inputs for *Galaxea fascicularis* colonies from zooplankton feeding

Species	Prey captured (ind cm^{-2} day^{-1})	TOC input (μg cm^{-2} day^{-1})	TON input (μg cm^{-2} day^{-1})	TOP input (μg cm^{-2} day^{-1})	Pi input (μg cm^{-2} day^{-1})
G. fascicularis; extracoelenteric	256±0	76.5±0.0	15.2±0.0	2.3±0.2	0.5±0.8
G. fascicularis; intracoelenteric	4±2	1.1±1.7	0.2±1.7	0.03±1.74	0.01±1.91
Stylophora pistillata; intracoelenteric[a]	35	3.8	0.8		

Estimated nutrient inputs (total organic carbon, nitrogen, phosphorus and inorganic phosphorus) for *G. fascicularis* colonies from both intracoelenteric and extracoelenteric zooplankton feeding, compared with previous literature estimates and expressed in μg cm^{-2} tissue day^{-1}. We used a daily concentration of 10,000 nauplii l^{-1} and 5 cm s^{-1} as the water flow rate. Corals were allowed to feed for 6 h. We used an average of 6.2±0.9 polyps cm^{-2} and a conservative mean aggregation density of 0.08±0.03 aggregates polyp^{-1} (see supplementary material Tables S1 and S2) to estimate nutrient input for whole *G. fascicularis* colonies. Values are means ± s.d. (*N*=6).

[a]Data are based on Ferrier-Pagès et al. (Ferrier-Pagès et al., 2003) and recalculated for similar conditions.

block for organic matrix synthesis and tissue growth (reviewed by Houlbrèque and Ferrier-Pagès, 2009). Assimilation of organic phosphorus may be important for maintenance and growth of coral tissue (Sorokin, 1973; D'Elia, 1977). Sorokin (Sorokin, 1973) demonstrated that corals are able to consume organic phosphorus in the form of planktonic bacteria (approximately 3 mg day^{-1}), although it is difficult to compare this value with our data as it is not expressed per unit of tissue surface area. The significant depletion of PO_4^{3-} of captured *Artemia* nauplii (Figs 3, 4) could have been due to uptake by symbiotic zooxanthellae. It is known that zooxanthellae reside in the coral gastroderm (reviewed by Furla et al., 2005; Stat et al., 2006), allowing efficient uptake of nutrients from digested prey by these symbiotic dinoflagellates. Moreover, it has been proposed that zooxanthellae may take up inorganic nitrogen from zooplankton directly, in the form of ammonium (NH_4^+) (Piniak and Lipschultz, 2004). As phosphate is an important inorganic nutrient for zooxanthellae (Deane and O'Brien, 1981; Jackson and Yellowlees, 1990; Belda et al., 1993), it is possible that the observed phosphate depletion of *Artemia* nauplii was due to assimilation by zooxanthellae.

When estimating heterotrophic nutrient input from feeding on zooplankton, it is important to take digestive efficiency into account. Previous studies have assumed a 100% assimilation of available carbon from zooplankton during intracoelenteric digestion (Fabricius et al., 1995; Sebens et al., 1996; Sebens et al., 1998; Houlbrèque et al., 2004; Grottoli et al., 2006; Purser et al., 2010), which may not be accurate. In this study, depletion of total organic carbon, nitrogen and phosphorous was only 43.1, 51.3 and 50.9%, respectively. However, as polyps of *G. fascicularis* continue to capture prey throughout the observed period, and taking a digestion time of 3 to 6 h into account (Lewis, 1982; Fabricius et al., 1995; Hii et al., 2009), collected *Artemia* aggregates may have represented a heterogeneous pool in terms of digestive status. Therefore, our measured nutrient depletions may reflect an average extracoelenteric feeding efficiency of *G. fascicularis* for *Artemia* nauplii. Another possible shortcoming of nutrient depletion measurements is that this method cannot distinguish between nutrients assimilated and those leaked into the surrounding environment. Therefore, tracer studies with stable isotopes, such as ^{13}C and ^{15}N, may provide even more detailed information about the efficiency of coral prey digestion and assimilation. Nevertheless, taking a certain proportion of refractory (i.e. resistant to digestion) organic material into account when estimating nutrient input from zooplankton seems important.

When assuming an average capture rate based on video analysis, average residence time of *Artemia* nauplii, and coupled to that an average digestive efficiency under environmental conditions as described above, nutrient input for *G. fascicularis* from zooplankton feeding can be calculated with the following formula: $X_H=[(X_{Artemia,t=0}-X_{Artemia,\text{captured}})-(X_{Artemia,t=0}-X_{Artemia,\text{control}})]0.08P$, where X_H is the amount of heterotrophically acquired carbon, nitrogen, phosphorus or PO_4^{3-} expressed in μg per cm coral tissue per day, $X_{Artemia}$ is the average amount of TOC, TON, TOP or PO_4^{3-} expressed in μg per *Artemia* nauplius, and *P* is the number of average prey items (*Artemia* nauplii) captured per cm^2 of coral tissue per day. One cm^2 of coral tissue, in this case, equals 6.2±0.9 polyps (see supplementary material Table S1). The factor 0.08 corrects for intracolonial polyp competition, as not all polyps in the context of a colony form aggregates (see supplementary material Table S2). The subscript *t*=0 indicates freshly hatched nauplii, control indicates an incubation for 6 h at 26°C without a *G. fascicularis* colony, and captured indicates captured nauplii during 6 h of incubation with a *G. fascicularis* colony. The assumption is made that all nutrients lost are assimilated. Based on our observations and by using the above formula, we calculate that for *G. fascicularis* colonies, extracoelenteric zooplankton feeding can provide 76.5±0.0 μg organic carbon, 15.2±0.0 μg organic nitrogen, 2.3±0.2 μg organic phosphorus and 0.5±0.8 μg inorganic phosphorus per cm^2 of tissue per day (Table 2). Following the same procedure, intracoelenteric feeding provides only 1.1±1.7 μg organic carbon, 0.2±1.7 μg organic nitrogen, 0.03±1.74 μg organic phosphorus and 0.01±1.91 μg inorganic phosphorus per cm^2 of tissue per day (Table 2). The obtained values for extracoelenteric feeding exceed calculations based on intracoelenteric feeding data for *G. fascicularis* by two orders of magnitude, and by one order of magnitude for *S. pistillata* (Ferrier-Pagès et al., 2003), underscoring the vital importance of extracoelenteric zooplankton feeding. Interestingly, a recent study by Hii et al. (Hii et al., 2009) revealed that *G. fascicularis* acquires 279±27.9 μg carbon per polyp per day under conditions similar to those of this study. This lies in the same order of magnitude as calculated for our study (166.3±0.5 μg C polyp^{-1} day^{-1}), although they used a higher *Artemia* carbon content (0.93 μg C individual^{-1}) and did not correct for refractory organic material, which is a significant factor to take into account. In addition, Purser et al. demonstrated that the azooxanthellate cold-water scleractinian coral *Lophelia pertusa* is able to take up a high theoretical maximum of 350.9±46.1 μg C polyp^{-1}day^{-1} from zooplankton feeding, even though polyp number per cm^2 for *L. pertusa* is much lower than that of *G. fascicularis* (Purser et al., 2010). When taking a dark respiration rate of 19.2 μg C cm^{-2} tissue day^{-1} and a daily net photosynthetic production of 68.4 μg C cm^{-2} tissue day^{-1} (Schutter, 2010) for *G. fascicularis* into account (based on a 12 h:12 h light:dark photoperiod), it becomes clear that when feeding at high daily prey concentrations, extracoelenteric zooplankton feeding is the major source of nutrient input and by itself easily meets the daily metabolic energy requirements for this species.

Our results put an entirely new perspective on heterotrophic nutrient input from zooplankton, as extracoelenteric feeding may

greatly exceed intracoelenteric feeding in terms of prey numbers digested and nutrients assimilated. Although external prey digestion may seem to have the disadvantage of nutrient leakage into the water column, it may be an energetically favorable process as coral polyps do not have to transport all prey items into the coelenteron by mucociliary feeding and muscle action. Even though we used high prey concentrations, which are four orders of magnitude higher than ambient *in situ* concentrations (Palardy et al., 2006), our results provide a well-founded estimation of maximum daily nutrient input from extracoelenteric zooplankton feeding for *G. fascicularis* under high prey concentrations. In the field, nutrient input from extracoelenteric feeding is likely to be much lower than found during this study; however, this is equally true for internal feeding, as both processes depend on prey capture rates. Prey capture rates, in turn, are strongly influenced by zooplankton concentration, and ingestion is indeed relatively low *in situ* (Johannes and Tepley, 1974; Palardy et al., 2006). It is therefore possible that even in the field, extracoelenteric feeding contributes a relatively large part to the daily heterotrophic nutrient input for scleractinian corals, even though overall feeding rates are low. Furthermore, we have observed that *Artemia* aggregates also form on *G. fascicularis* polyps when applying lower concentrations (1000 *Artemia* nauplii l^{-1}). Future studies should focus on determining thresholds for extracoelenteric zooplankton feeding in terms of prey size and concentration, both in captivity and *in situ*. In addition, quantifying daily nutrient input from extracoelenteric feeding for coral species *in situ* would provide more realistic insights into benthic–pelagic coupling on coral reefs.

In conclusion, our results demonstrate that under high prey concentrations, extracoelenteric zooplankton feeding is a key mechanism of daily nutrient acquisition for a zooxanthellate scleractinian coral, which is of importance to aquaculture efforts. In addition, our findings generate new thoughts about the nature and extent of benthic–pelagic coupling on coral reefs.

ACKNOWLEDGEMENTS

We thank Sietze Leenstra, Aart Hutten, Wian Nusselder, Sander Visser, Truus Wiegers-van der Wal and Ronald Booms for technical support. We thank Paul van Zwieten and Mark Verdegem for assistance with the statistical analysis, and André van Leeuwen and Wobbe Schuurmans for constructive discussions concerning the *Artemia* analysis. Finally, we thank two anonymous reviewers for critically evaluating and improving the manuscript. This work was funded by Wageningen University.

REFERENCES

Abe, N. (1938). Feeding behaviour and the nematocyst of *Fungia* and 15 other species of corals. *Palao. Trop. Biol. Stn. Stud.* **1**, 469-521.
Al-Moghrabi, S., Allemand, D., Couret, J. M. and Jaubert, J. (1995). Fatty acids of the scleractinian coral *Galaxea fascicularis*: effect of light and feeding. *J. Comp. Physiol. B* **165**, 183-192.
Belda, C. A., Lucas, J. S. and Yellowlees, D. (1993). Nutrient limitation in the giant clam–zooxanthellae symbiosis: effects of nutrient supplements on growth of the symbiotic partners. *Mar. Biol.* **117**, 655-664.
Carpenter, F. W. (1910). Feeding reactions of the rose coral (*Isophyllia*). *Proc. Am. Acad. Arts Sci.* **46**, 149-162.
D'Elia, C. F. (1977). The uptake and release of dissolved phosphorus by reef corals. *Limnol. Oceanogr.* **22**, 301-315.
Dai, C.-F. and Lin, M.-C. (1993). The effects of flow on feeding of three gorgonians from southern Taiwan. *J. Exp. Mar. Biol. Ecol.* **173**, 57-69.
Deane, E. M. and O'Brien, R. W. (1981). Uptake of phosphate by symbiotic and free-living dinoflagellates. *Arch. Microbiol.* **128**, 307-310.
Duerden, J. E. (1902). West Indian Madreporarian polyps. *Mem. Nat. Acad. Sci.* **8**, 399-597.
Fabricius, K. E., Genin, A. and Benayahu, Y. (1995). Flow-dependent herbivory and growth in zooxanthellae-free soft corals. *Limnol. Oceanogr.* **40**, 1290-1301.
Ferrier-Pagès, C., Witting, J., Tambutté, E. and Sebens, K. P. (2003). Effect of natural zooplankton feeding on the tissue and skeletal growth of the scleractinian coral *Stylophora pistillata*. *Coral Reefs* **22**, 229-240.
Furla, P., Allemand, D., Shick, J. M., Ferrier-Pagès, C., Richier, S., Plantivaux, A., Merle, P.-L. and Tambutté, S. (2005). The symbiotic anthozoan: a physiological chimera between alga and animal. *Integr. Comp. Biol.* **45**, 595-604.
Goldberg, W. M. (2002). Feeding behavior, epidermal structure and mucus cytochemistry of the scleractinian *Mycetophyllia reesi*, a coral without tentacles. *Tissue Cell* **34**, 232-245.
Goreau, T. F., Goreau, N. I. and Yonge, C. M. (1971). Reef corals: autotrophs or heterotrophs? *Biol. Bull.* **141**, 247-260.
Grottoli, A., Rodrigues, L. and Palardy, J. (2006). Heterotrophic plasticity and resilience in bleached corals. *Nature* **440**, 1186-1189.
Hii, Y.-S., Soo, C. L. and Liew, H. C. (2009). Feeding of scleractinian coral, *Galaxea fascicularis*, on *Artemia salina* nauplii in captivity. *Aquacult. Int.* **17**, 363-376.
Houlbrèque, F. and Ferrier-Pagès, C. (2009). Heterotrophy in tropical scleractinian corals. *Biol. Rev. Camb. Philos.* **84**, 1-17.
Houlbrèque, F., Tambutté, E., Richard, C. and Ferrier-Pagès, C. (2004). Importance of a micro-diet for scleractinian corals. *Mar. Ecol. Prog. Ser.* **282**, 151-160.
Jackson, A. E. and Yellowlees, D. (1990). Phosphate uptake by zooxanthellae isolated from corals. *Proc. R. Soc. Lond. B* **242**, 201-204.
Johannes, R. E. and Tepley, L. (1974). Examination of feeding of the reef coral *Porites lobata in situ* using time lapse photography. *Proc. 2nd Int. Coral Reef Symp.* 127-132.
Lang, J. C. (1973). Interspecific aggression by scleractinian corals. II. Why the race is not only to the swift. *Bull. Mar. Sci.* **23**, 260-279.
Lang, J. C. and Chornesky, E. A. (1990). Competition between scleractinian reef corals – a review of mechanisms and effects. *Ecosystems of the World* **25**, 209-252.
Léger, P., Bengtson, D. A., Sorgeloos, P., Simpson, K. L. and Beck, A. D. (1987). *Artemia Research and its Applications. Vol. 3. Ecology, Culturing, Use in Aquaculture*, 556 pp. Wetteren, Belgium: Universa Press.
Leversee, G. J. (1976). Flow and feeding in fan-shaped colonies of the gorgonian coral, *Leptogorgia*. *Biol. Bull.* **151**, 344-356.
Lewis, J. B. (1982). Feeding behaviour and feeding ecology of the Octocorallia (Coelenterata: Anthozoa). *J. Zool.* **196**, 371-384.
Lin, M. C., Liao, C. M. and Dai, C. F. (2002). Modeling the effects of satiation on the feeding rate of a colonial suspension feeder, *Acanthogorgia vegae*, in a circulating system under lab conditions. *Zool. Stud.* **41**, 355-365.
Logan, A. (1984). Interspecific aggression in hermatypic corals from Bermuda. *Coral Reefs* **3**, 131-138.
Matthai, G. (1918). On reactions to stimuli in corals. *Proc. Camb. Philos. Soc.* **19**, 164-166.
Navarro, J. C., Amat, F. and Sargent, J. R. (1991). A study of the variations in lipid levels, lipid class composition and fatty acid composition in the first stages of *Artemia* sp. *Mar. Biol.* **111**, 461-465.
Osinga, R., Charko, F., Cruzeiro, C., Janse, M., Grymonpre, D., Sorgeloos, P. and Verreth, J. A. J. (2008). Feeding corals in captivity: uptake of four *Artemia*-based feeds by *Galaxea fascicularis*. *Proc. 11th Int. Coral Reef Symp.* 149-153.
Palardy, J. E., Grottoli, A. G. and Matthews, K. A. (2005). Effects of upwelling, depth, morphology and polyp size on feeding in three species of Panamian corals. *Mar. Ecol. Prog. Ser.* **300**, 79-89.
Palardy, J. E., Grottoli, A. G. and Matthews, K. A. (2006). Effect of naturally changing zooplankton concentrations on feeding rates of two coral species in the Eastern Pacific. *J. Exp. Mar. Biol. Ecol.* **331**, 99-107.
Piniak, G. and Lipschultz, F. (2004). Effects of nutritional history on nitrogen assimilation in congeneric temp rate and tropical scleractinian corals. *Mar. Biol.* **145**, 1085-1096.
Purser, A., Larsson, A. I., Thomsen, L. and van Oevelen, D. (2010). The influence of flow velocity and food concentration on *Lophelia pertusa* (Scleractinia) zooplankton capture rates. *J. Exp. Mar. Biol. Ecol.* **395**, 55-62.
Schutter, M. (2010). The influence of light and water flow on the growth and physiology of the scleractinian coral *Galaxea fascicularis*. PhD thesis, Wageningen UR, Wageningen, The Netherlands.
Sebens, K. P., Vandersall, K. S., Savina, L. A. and Graham, K. R. (1996). Zooplankton capture by two scleractinian corals *Madracis mirabilis* and *Montastrea cavernosa* in a field enclosure. *Mar. Biol.* **127**, 303-317.
Sebens, K. P., Grace, S. P., Helmuth, B., Maney, E. J., Jr and Miles, J. S. (1998). Water flow and prey capture by three scleractinian corals, *Madracis mirabilis*, *Montastrea cavernosa* and *Porites porites*, in a field enclosure. *Mar. Biol.* **131**, 347-360.
Sorokin, Y. I. (1973). Role of microflora in metabolism and productivity of Hawaiian reefs. *Oceanology USSR* **13**, 262-267.
Sorokin, Y. I. (1990). *Ekosistemy Korallovykh rifov* (*Ecosystems of Coral Reefs*). Moscow: Nauka.
Stat, M., Carter, D. and Hoegh-Guldberg, O. (2006). The evolutionary history of *Symbiodinium* and scleractinian hosts – symbiosis, diversity, and the effect of climate change. *Perspect. Plant Ecol. Evol. Syst.* **8**, 23-43.
Treignier, C., Grover, R. and Ferrier-Pagès, C. (2008). Effect of light and feeding on the fatty acid and sterol composition of zooxanthellae and host tissue isolated from the scleractinian coral *Turbinaria reniformis*. *Limnol. Oceanogr.* **53**, 2702-2710.
Van Praët, M. (1980). Absorption des substances dissoutes dans le milieu des particules et des produits de la digestion extracellulaire chez *Actinia equina* L. (Absorption of substances dissolved in the environment, particles and products of extracellular digestion in *Actinia equina* (Cnidaria, Actiniaria)). *Reprod. Nutr. Dév.* **20**, 1393-1399.
Veron, J. E. N. (2000). *Corals of the World*, 1382 pp. Townsville, Australia: Australian Institute of Marine Science.
Webber, M. K. and Roff, J. C. (1995). Annual structure of the copepod community and its associated pelagic environment off Discovery Bay, Jamaica. *Mar. Biol.* **123**, 467-479.
Witting, J. H. (1999). Zooplankton capture and coral growth: the role of heterotrophy in Caribbean reef corals. PhD thesis, Northeastern University, Boston, MA, USA.
Yonge, C. M. (1930). Studies on the physiology of corals. I. Feeding mechanisms and food. *Sci. Rep. Gt. Barrier Reef Exped.* **1**, 13-57.
Yonge, C. M. (1973). The nature of reef building (hermatypic) corals. *Bull. Mar. Sci.* **23**, 1-15.

Table S1. Surface area, number of polyps and polyp density of *Galaxea fascicularis* colonies

Colony	Surface area (cm^2)	Number of polyps	Polyp density (polyps cm^{-2})
1	48.3	254	5.3
2	37.1	264	7.1
3	47.6	326	6.8
4	54.7	310	5.7
Mean ± s.d.	46.9±0.9	288.5±34.9	6.2±0.9

Table S2. Aggegrate densities of *Artemia* nauplii on *Galaxea fascicularis* colonies

Colony	Aggregate density (aggregates polyp^{-1})
1	0.11
2	0.08
3	0.05
4	0.06
Mean ± s.d.	0.08±0.03

Aggregate densities of *Artemia* nauplii on *G. fascicularis* colonies at 6 h incubation. We used a concentration of 4100 nauplii l^{-1} and 5 cm s^{-1} as the water flow rate. Corals were allowed to feed for 6 h.