RESEARCH ARTICLE

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

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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\textsubscript{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\textsuperscript{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.

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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 [Houbrèque and Ferrier-Pagès (Houbrèque 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, protozoan, 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; Houbrèque 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 Koff, 1995; Sebens et al., 1996; Sebens et al., 1998; Witting, 1999; Ferrier-Pagès et al., 2003; Houbrèque 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 analyses 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±19 μmol m⁻² s⁻¹ (12h:12h light:dark regime), nitrate 2±1 μmol l⁻¹, phosphate 0.03±0.01 μmol 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 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, Sachsen, 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 (Tecco 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 6h 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 Ultrorax X1030 homogenizer (Ingenieurbüro M. Zipperer GmbH, Döttingen, Germany) for 5 min in the 10 ml remaining volume at room temperature. For each sample, the Ultrorax 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 oxidation on a TOC-5050A auto analyzer (Shimadzu Scientific Instruments, Shimadzu Corporation, Kyoto, Japan) followed by detection of CO2 with a non-dispersive infrared analyzer (Skalar Analytical BV, Breda, The Netherlands). All data presented are means ± s.d. Graphs were plotted with SigmaPlot 11.0 (Systat Software, San Jose, CA, USA).

RESULTS

Throughout all of the 6h 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 Waminia 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 6h period (Fig. 2). Ingestion of nauplii was observed for only one of the three single polyps tested. In total, 27

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 PO43– 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 PO43– and C:P ratios (P<0.05). For TOC, TON and TOP we used one-way ANOVA followed by Bonferroni’s test. For PO43– 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).
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. 2F). 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 deeply depigmented 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<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).
slightly (by 0.5%), changes in capture rate due to a concentration calculated by the total average nauplii aggregate size, only dropped corrected for negative controls. Values are means ± s.d. (N=6).

Fig. 4. Loss of TOC, TON, TOP and PO4³⁻ 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).

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 depigmented 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 (Duermen, 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 Praet, 1980). The observed decrease in prey capture and release over time (Fig. 2), and the slow release of aggregates after 6h 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. pistilata (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 (Duermen, 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 6h 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 ¹⁵N 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 1. Nutrient ratios of Artemia nauplii

<table>
<thead>
<tr>
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<th>C:N</th>
<th>C:P</th>
<th>N:P</th>
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<tbody>
<tr>
<td>t=0</td>
<td>6.1±0.3</td>
<td>38.1±1.9</td>
<td>6.3±0.3</td>
</tr>
<tr>
<td>Control</td>
<td>6.0±0.4</td>
<td>38.6±2.8</td>
<td>6.5±0.3</td>
</tr>
<tr>
<td>Captured</td>
<td>7.5±2.1</td>
<td>51.2±20.1</td>
<td>6.7±0.7</td>
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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 6h incubation in a water bath (control) and after 6h incubation with Galaxea fascicularis colonies (captured). Values are means ± s.d. (N=6).
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: $X_{N} =(X_{Artemia, t=0} - X_{Artemia, t=6}) - X_{Artemia, control}) 0.08 P$, where $X_{N}$ is the amount of heterotrophically acquired carbon, nitrogen, phosphorus or PO$_4^{3-}$ expressed in $\mu$g per cm$^2$ tissue per day, $X_{Artemia}$ is the average amount of TOC, TON, TOP or PO$_4^{3-}$ expressed in $\mu$g 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 TableS1). The factor 0.08 corrects for intracoeloral polyp competition, as not all polyps in the context of a colony form aggregates (see supplementary material TableS2). 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 $\mu$g organic carbon, 15.2±0.0 $\mu$g organic nitrogen, 2.3±0.2 $\mu$g organic phosphorous and 0.5±0.8 $\mu$g inorganic phosphorus per cm$^2$ of tissue per day (Table 2). Following the same procedure, intracoelenteric feeding provides only 1.1±1.7 $\mu$g organic carbon, 0.2±1.7 $\mu$g organic nitrogen, 0.03±1.74 $\mu$g organic phosphorous and 0.01±1.91 $\mu$g 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 $\mu$g 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 $\mu$g C polyp$^{-1}$ day$^{-1}$), although they used a higher Artemia carbon content (0.93$\mu$g 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 zooxanthellate cold-water scleractinian coral Lophelia pertusa is able to take up a high theoretical maximum of 350±46.1 $\mu$g 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 $\mu$g Ccm$^{-2}$ tissue$^{-1}$ per day$^{-1}$ and a daily net photosynthetic production of 68.4 $\mu$g Ccm$^{-2}$ tissue$^{-1}$ day$^{-1}$ (Schutter, 2010) for G. fascicularis into account (based on a 12h:12h 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

<table>
<thead>
<tr>
<th>Species</th>
<th>Prey captured (ind cm$^{-2}$ day$^{-1}$)</th>
<th>TOC input ($\mu$g cm$^{-2}$ day$^{-1}$)</th>
<th>TON input ($\mu$g cm$^{-2}$ day$^{-1}$)</th>
<th>TOP input ($\mu$g cm$^{-2}$ day$^{-1}$)</th>
<th>Pi input ($\mu$g cm$^{-2}$ day$^{-1}$)</th>
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<tbody>
<tr>
<td>G. fascicularis; extracoelenteric</td>
<td>256±0</td>
<td>76.5±0.0</td>
<td>15.2±0.0</td>
<td>2.3±0.2</td>
<td>0.5±0.8</td>
</tr>
<tr>
<td>G. fascicularis; intracoelenteric</td>
<td>4±2</td>
<td>1.1±1.7</td>
<td>0.2±1.7</td>
<td>0.03±1.74</td>
<td>0.01±1.91</td>
</tr>
<tr>
<td>Stylorhapha pistillata; intracoelenteric</td>
<td>35</td>
<td>3.8</td>
<td>0.8</td>
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<td></td>
</tr>
</tbody>
</table>

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 $\mu$g cm$^{-2}$ tissue day$^{-1}$. We used a daily concentration of 10,000 nauplii$^{1+}$ and 5 cm$^{-1}$ 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).

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

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Prey captured (ind cm$^{-2}$ day$^{-1}$)</th>
<th>TOC input ($\mu$g cm$^{-2}$ day$^{-1}$)</th>
<th>TON input ($\mu$g cm$^{-2}$ day$^{-1}$)</th>
<th>TOP input ($\mu$g cm$^{-2}$ day$^{-1}$)</th>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
greatly exceed extracoelenteric 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. f. carteri 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. f. carteri 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 zoanthellate 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.

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REFERENCES

<table>
<thead>
<tr>
<th>Colony</th>
<th>Surface area (cm²)</th>
<th>Number of polyps</th>
<th>Polyp density (polyps cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48.3</td>
<td>254</td>
<td>5.3</td>
</tr>
<tr>
<td>2</td>
<td>37.1</td>
<td>264</td>
<td>7.1</td>
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<tr>
<td>3</td>
<td>47.6</td>
<td>326</td>
<td>6.8</td>
</tr>
<tr>
<td>4</td>
<td>54.7</td>
<td>310</td>
<td>5.7</td>
</tr>
<tr>
<td>Mean ± s.d.</td>
<td>46.9±0.9</td>
<td>288.5±34.9</td>
<td>6.2±0.9</td>
</tr>
</tbody>
</table>

Table S1. Surface area, number of polyps and polyp density of *Galaxea fascicularis* colonies.
**Table S2. Aggregate densities of* Artemia* nauplii on* Galaxea fascicularis* colonies**

<table>
<thead>
<tr>
<th>Colony</th>
<th>Aggregate density (aggregates polyp⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.11</td>
</tr>
<tr>
<td>2</td>
<td>0.08</td>
</tr>
<tr>
<td>3</td>
<td>0.05</td>
</tr>
<tr>
<td>4</td>
<td>0.06</td>
</tr>
<tr>
<td>Mean ± s.d.</td>
<td>0.08±0.03</td>
</tr>
</tbody>
</table>

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.