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Coral feeding on microalgae assessed with molecular trophic markers

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Abstract

Herbivory in corals, especially for symbiotic species, remains controversial. To investigate the capacity of scleractinian and soft corals to capture microalgae, we conducted controlled laboratory experiments offering five algal species: the cryptophyte *Rhodomonas marina*, the haptophytes *Isochrysis galbana* and *Phaeocystis globosa*, and the diatoms *Conticribra weissflogii* and *Thalassiosira pseudonana*. Coral species included the symbiotic soft corals *Heteroxenia fuscescens* and *Sinularia flexibilis*, the asymbiotic scleractinian coral *Tubastrea coccinea*, and the symbiotic scleractinian corals *Stylophora pistillata*, *Pavona cactus* and *Oculina arbuscula*. Herbivory was assessed by end-point PCR amplification of algae-specific 18S rRNA gene fragments purified from coral tissue genomic DNA extracts. The ability to capture microalgae varied with coral and algal species and could not be explained by prey size or taxonomy. Herbivory was not detected in *S. flexibilis* and S. *pistillata*. *P. globosa* was the only algal prey that was never captured by any coral. Although predation defence mechanisms have been shown for *Phaeocystis* spp. against many potential predators, this study is the first to suggest this for corals. This study provides new insights into herbivory in symbiotic corals and suggests that corals may be selective herbivorous feeders.

Keywords: coral feeding, heterotrophy, PCR, phytoplankton, specific primers

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Introduction

Symbiotic and asymbiotic corals are able to ingest a wide range of prey, from pico- and nanoplankton to mesozooplankton (see review by Houlbreque & Ferrier-Pagès 2009). For symbiotic corals that harbour photosynthetic dinoflagellates (zooxanthellae), ingested prey provides essential nutrients that cannot be supplied by the zooxanthellae. However, heterotrophic feeding may also account for a significant fraction of the fixed carbon, especially during bleaching events, or in deep and/or turbid areas when photosynthetic products are unavailable (Anthony & Fabricius 2000; Grottoli *et al.* 2006).

Most studies addressing coral heterotrophy have focused on the ingestion of zooplankton prey and have documented significant ingestion rates (e.g. Sebens *et al.* 1996, 1998; Palardy *et al.* 2008). In contrast, the ability of corals (subclass Hexacorallia) to ingest phytoplankton has been less thoroughly investigated, and therefore, the importance of herbivory in coral nutrition remains unclear for most species. Although the capacity of asymbiotic soft corals (order Alcyonacea) to feed on phytoplankton has been relatively well investigated (e.g. Fabricius *et al.* 1995a,b; Widdig & Schlichter 2001; Migne & Davoult 2002; Orejas *et al.* 2003; Lira *et al.* 2008), only a few studies have investigated herbivory in symbiotic corals (Sorokin 1973; Ribes *et al.* 1998; Tremblay *et al.* 2012; Seemann *et al.* 2013). Further, it is not known if selectivity occurs when feeding on microalgae and, if so, what are the mechanisms involved. This knowledge gap may be associated with methodological limitations. Current techniques to assess coral feeding include microscopy of dissected polyps and prey

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removal rates in feeding chambers. The first method is expected to be inaccurate for microalgae because such small prey items are difficult to detect quantitatively in the relatively large polyps, and microalgae may rapidly loose recognizable features such as fluorescence, flagella and cell shape after ingestion. The prey removal approach does not allow its detection in high prey concentrations when microalgae ingestion may be high and may be prone to several containment effects including trophic cascades as discussed in Nejstgaard *et al.* (2008).

In this study, we performed a qualitative assessment of the potential for symbiotic and asymbiotic corals to capture microalgae using molecular trophic markers. While these molecular tools have been successfully used to study marine invertebrate trophic interactions (e.g. Troedsson *et al.* 2007; Simonelli *et al.* 2009; O'Rorke *et al.* 2012a; Roura *et al.* 2012), they have only been used once to investigate coral feeding on zooplankton (Leal *et al.* This issue).

In order to assess the potential for coral herbivory, laboratory feeding studies were conducted with two symbiotic soft corals, three symbiotic and one asymbiotic scleractinian corals. Corals were offered five different microalgae separately as monospecific prey concentrations typical of bloom conditions. Prey capture was assessed using end-point PCR and prey species-specific primers targeted to the 18S rRNA gene of the different microalgae prey in order to address the following two initial hypotheses: (i) tested coral species are able to capture microalgae; and (ii) prey size and/or taxonomy determines coral success in capturing microalgae.

Methods

Corals

Two soft and four scleractinian coral species were investigated in this study: the tropical corals *Tubastrea coccinea*, *Heteroxenia fuscescens, Pavona cactus, Stylophora pistillata and Sinularia flexibilis* and the temperate coral *Oculina arbuscula* (Table 1). Coral species selection was based on the range of different coral types (scleractinian/soft corals and presence/absence of zooxanthellae) that was available either in laboratory cultures or by field access. With the exception of *O. arbuscula*, all corals were cultured at 26 °C in open flow-through aquaria supplied with natural seawater continuously pumped from a 50-m depth well (renewal rate of 50% per hour). The four tropical symbiotic species were maintained at an irradiance of 200 μmol photons/m^2/s (12 h light cycle/12 h dark cycle). The asymbiotic species, *T. coccinea,* was maintained in the dark. Feeding trials using these species were conducted at Centre Scientifique de Monaco. The temperate symbiotic species *O. arbuscula* was maintained under an irradiance of 100 μmol photons/m^2/s^{-1} (12 h light cycle/12 h dark cycle) at 24 °C in a recirculating system composed of a 200 L aquarium connected with a 100-L reservoir equipped with a protein skimmer and a biological filter. Partial water changes (20%) were performed weekly using freshly pumped filtered seawater. Experiments with *O. arbuscula* were conducted at the Skidaway Institute of Oceanography (Savannah, Georgia, USA).

Coral nubbins were prepared from three different colonies from each coral species by cutting their apical branches or, for soft corals, portions of several polyps. Coral nubbins were allowed to heal until tissue recovery was observed at the sites of fracture. The five symbiotic corals were not fed until the experimental feeding trials were performed (at least 2 weeks before the trials). The asymbiotic *T. coccinea* was fed twice a week with newly hatched *Artemia* sp. nauplii.

Microalgae

Five microalgal species from three phyla were utilized as prey during this study: the diatoms *Conticribra weissflogii* and *Thalassiosira pseudonana*, the cryptophyte *Rhodomonas marina,* and the haptophytes *Isochrysis galbana* and *Phaeocystis globosa* (see Table 2 for strain, source and cell size). Microalgae species selection was based on availability in cultures and what has been commonly used as model phytoplankton prey species. All algae were grown in semicontinuous batches, in *f*/2 media, 14 : 10 h light cycle and at 20 °C. All microalgae used in the experiments were single cell form in exponential growth phase.

Feeding experiments

Feeding experiments were performed in triplicate with each of the six coral species and each of the five microalgae prey species, respectively (6 coral species × 5 microalgae species × 3 replicates = 90 feeding trials). Each coral nubbin was individually incubated in 200-mL cylindrical feeding chambers. Preliminary feeding experiments conducted with newly hatched *Artemia* sp. nauplii, confirmed that feeding behaviour was not affected by the gentle semicircular mixing generated by a magnetic stirring plate (130 rpm) in the experimental feeding chambers. After the polyps were completely expanded, a single microalgae species was added at a final concentration of approximately 10^4 cells/mL. Corals were allowed to feed for 60 min, and after this period, each coral fragment was harvested and thoroughly rinsed three times in consecutive baths of freshly filtered seawater (Whatman GF/F filter, nominal pore size 0.7 μm) to remove any algae

Table 1 Coral species used in this study. All corals are tropical, except for the temperate *Oculina arbuscula*

Species	Family	Coral type	Distribution* (collection site)	Symbiotic status	Polyp size (diameter in mm)
Tubastrea coccinea	Dendrophylliidae	Scleractinian	Coral reefs worldwide (South-East Asia)	Asymbiotic	10–12
Heteroxenia fuscescens	Xeniidae	Soft	Red Sea and Eastern Africa (Red Sea)	Symbiotic	8–10 (1 mm mouth)
Oculina arbuscula	Oculinidae	Scleractinian	Mid and South Atlantic Bight (Georgia coast, USA)	Symbiotic	2–3
Pavona cactus	Agariciidae	Scleractinian	Indo-Pacific, Red Sea and Western Indian Ocean (Red Sea)	Symbiotic	0.2–0.3
Stylophora pistillata	Pocilloporidae	Scleractinian	Indo-Pacific, Red Sea and Western Indian Ocean (Red Sea)	Symbiotic	0.5–1
Sinularia flexibilis	Alcyoniidae	Soft	Indo-Pacific (Indo-Pacific)	Symbiotic	0.5

*Distribution according with Veron (2000).

Table 2 Microalgae used as prey in this study. All algae were grown in single-cell (solitary) form

Species	Algae group	Distribution*	Cell size (μm)	Source/Strain
*Conticribra weissflogii***	Diatom	Coastal waters in the Atlantic and Pacific Oceans	5–12	CCMP 1050
Thalassiosira pseudonana	Diatom	World's oceans	4–6	CCMP 1335
Rhodomonas marina	Cryptophyte	Northeast Atlantic	5–8	IFREMER (Brest, France)
Isochrysis galbana	Haptophyte	Northeast Atlantic	4–6	CCMP 1611
Phaeocystis globosa	Haptophyte	World's oceans	4–6	CCMP 628

*According with www.algaebase.org and www.marinespecies.org.
**Formerly known as *Thalassiosira weissflogii*.

remaining on the coral surface layer (Sebens *et al*. 1996; Ribes *et al*. 1998; Anthony 2000). Negative experimental feeding controls consisted of corals placed in feeding chambers without algal prey and processed exactly as fed animals. Positive experimental controls consisted of analysis of feeding chamber water after algal prey had been added. Water was collected and filtered onto 0.8-μm Supor (Pall Corp) filters and genomic DNA (gDNA) extracted. All experimental controls were conducted in triplicate. Coral tissue from scleractinian species was harvested using an air pick, while the polyps of soft corals were cut into small pieces. The air pick consisted of a 0.8-mm opening plastic pipette tip attached to a flexible tubing, collection bottle and a vacuum pump, as successfully used by Ferrier-Pagès *et al*. (2011) and others to harvest tissue from scleractinian corals.

DNA extraction, primer design and PCR

The gDNA was purified from coral tissues and microalgae using the DNeasy Blood & Tissue kit (Qiagen, Valencia, CA, USA), following the manufacturer's specifications. To maximize DNA yield, DNA was eluted twice from the DNeasy spin columns with 100 μL of elution buffer supplied by the manufacturer.

Prey-specific PCR primers used in this study are shown in Table 3. Unless otherwise noted, 18S rRNA-gene-targeted PCR primers used in this study were designed using the software package Primer3 (Rozen & Skaletsky 2000). Algal species sequence alignments [see Appendix S1 (Supporting information)] were constructed using the BioEdit sequence alignment editor (Hall 1999). Regions conserved in target organisms but divergent from other diatoms, cryptophytes and haptophytes were targeted for primer design. The specificity of each primer pair in detecting algal DNA was confirmed empirically in PCR assays against gDNA purified from all coral and algal species used in this study, and against *Artemia* sp. that was used to feed the asymbiotic corals. However, these primer sets were not specifically validated for use beyond this study and therefore should be used cautiously in any future studies. All oligonucleotides were synthesized and purified (standard desalting) by Integrated DNA Technologies (www.IDT.com).

Table 3 Primers used in this study and its product length and optimal annealing for each microalgae species

Microalgae	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Product length (bp)	Annealing temperature (°C)
Conticribra weissflogii	CTA TGC CGA CTC AGG ATT GG	ATG CAC CAC CAC CCA TAG AA	244	60
Thalassiosira pseudonana	CTA TGC CGA CTC AGG ATT GG	ATG CAC CAC CAC CCA TAG AA	244	50
Rhodomonas marina	GCG ACT CCA TTG GCA CCT TGT*	CAA TGT CTG GAC CTG GTA AGT	175	57
Isochrysis galbana	CCG ACT AGG GAT TGG AGG AT	ATT TAG CAG GCT GCG GTC TC	295	55
Phaeocystis globosa	GGC TAC TTC TAG TCT TGT AAT TGG A†	AAA GAA GGC CGC GCC†	194	56

*From (Troedsson *et al.* 2009).
†From (Nejstgaard *et al.* 2008).

Table 4 Number of times algal prey was detected per a total of three feeding experiments performed for each coral species and prey microalgae combination. Complete dataset archieved in Dryad (Leal *et al.* 2013)

Coral species	*Conticribra weissflogii*	*Thalassiosira pseudonana*	*Rhodomonas marina*	*Isochrysis galbana*	*Phaeocystis globosa*
Tubastrea coccinea	3	3	0	1	0
Heteroxenia fuscescens	0	0	3	0	0
Oculina arbuscula	2	0	0	2	0
Pavona cactus	0	0	3	2	0
Stylophora pistillata	0	0	0	0	0
Sinularia flexibilis	0	0	0	0	0

All PCRs were performed in 25 μL reaction volumes using prey-specific primers (Table 3). PCR was performed using an Applied Biosystems GeneAmp PCR System 9700 and the Qiagen Taq PCR Master Mix reagents (Qiagen, Valencia, CA, USA). Each reaction contained 12.5 μL of Qiagen Taq PCR Master Mix, 120 nM of each primer and template gDNA ranging from 200 to 600 ng/mL. This concentration was achieved using 2 μL of either undiluted or tenfold dilution (in water) of the gDNA purifications. Amplification conditions consisted of an initial denaturation step (10 min, 94 °C) followed by 35 3-step amplification cycles (denaturation: 30 s, 94 °C; annealing: 30 s, temperature described in Table 3; extension: 60 s, 72 °C) and by a final extension step (72 °C, 7 min). PCR grade water was used as template for negative control. PCR products were visualized by gel electrophoresis on a 2% agarose gel buffered in 1× TAE (0.04M Tris-Acetate, 1 mM EDTA, pH 8.0).

Results and discussion

End-point PCR was successfully used to detect microalgal small subunit (18S) rRNA gene fragments in total gDNA extracts collected from corals immediately after exposure to single species microalgae suspensions. Herbivory was detected in four of the six corals for at least one type of microalgae prey. *T. coccinea*, the only asymbiotic coral examined in this study, captured the largest range of prey types (three of five microalgae species). Herbivory was not detected in *S. flexibilis* and *S. pistillata* (Table 4). The haptophyte *I. galbana* was the microalgae detected in most corals (three of six coral species). In contrast, the closely taxonomically related and similar-sized (4–6 μm) microalgae *P. globosa* was not detected in any of the six tested corals. Although only three of the five symbiotic corals captured some microalgae, these results support the hypothesis that corals are able to capture microalgae, but indicate that herbivory can be variable across coral species and algal prey types. There was not a clear relationship between prey selection and algae prey size or prey taxonomy in the relatively small-sized phytoplankton tested here.

The question of whether zooxanthellate scleractinian corals are capable of feeding heterotrophically and specifically whether they may feed on microalgae has been a matter of speculation for some time. Three decades ago, Sorokin (1973) addressed this question reporting herbivory in *Pavona* sp. In a more recent study, Tremblay *et al.* (2012) reported that *S. pistillata* was able to graze on natural mixtures of pico- and nanoplankton. However, *S. pistillata* did not capture any of the microalgae in the present study. A possible explanation for these differences may be that this coral species is a selective feeder.

The results from this study support the possibility of selective microalgae grazing by zooxanthellate corals although the basis for such selectivity is unknown. For example, *P. cactus* and *O. arbuscula* were only able to capture some of the microalgae species tested (Table 4). A hypothesis that could explain selective feeding is a relationship between coral polyp and prey size. However, in this study, all microalgae were similarly sized (4–12 μm) and orders of magnitude smaller than the polyp width (0.2–12 mm) (Table 1). For example, *O. arbuscula*, a species with relatively large polyps (2–3 mm in diameter), and *P. cactus*, a species with smaller polyps (0.2–0.3 mm in diameter), were both able to capture the small (4–6 μm) microalgae *I. galbana*. Further, *R. marina*, *I. galbana* and *T. pseudonana* have very similar sizes (4–8 μm, Table 2), but contrasting results were observed among the different coral species provided with these prey (Table 4). Similarly, others have reported that polyp size does not limit zooplankton feeding or influence feeding rates (Sebens *et al.* 1996; Palardy *et al.* 2005, 2006). These observations suggest that coral grazing on microalgae and zooplankton is not simply a matter of physical capacity and that other factors are likely to be involved. An alternative hypothesis to size-based selectivity is prey mobility-based selectivity. However, this would not likely explain the striking difference between the two very similar-sized haptophyte species *I. galbana* and *P. globosa* (Table 4).

Another possibility may be a differential feeding preference by each coral species based on algal palatability. However, if this is the case, selectivity will likely be difficult to predict based on algal taxonomy alone. For example, *O. arbuscula* fed differently on two closely related diatom species (*T. pseudonana* and *C. weissflogii*) (Table 4). Similarly, the results from this study with the asymbiotic coral *T. coccinea* highlight the difficulty of predicting prey preference. As an asymbiotic coral species, *T. coccinea* is generally thought to be able to feed on phytoplankton (Fabricius *et al.* 1995a,b). However, in this study, we were not able to detect feeding by *T. coccinea* on *P. globosa* or *R. marina*. Selectivity based on algal palatability could explain negative results, as it is unknown whether these microalgae species are present in coral reefs and therefore may be considered artificial prey. On the other hand, besides *P. globosa,* all other algae selected for the experiments are widely used food algae that have never been found toxic. Thus, they would represent good model algae for generic tests of potential feeding. In addition, although these microalgae are mainly recorded in temperate environments, most of them have a relatively broad distribution (Table 2). However, the use of prey species that may be absent from tropical ecosystems is not likely to cause any bias, as corals are capable to feed on prey that are known to be absent from their natural habitat, such as *Artemia* cysts and nauplii (e.g. Helmuth & Sebens 1993; Sebens *et al.* 1998; Piniak 2002).

One possible mechanism for feeding selectivity could also be entrapment of algae cells in coral mucus, that is, false positives due to an incomplete wash process before extraction. However, our results do not support this, as we would expect the majority of tested algae species to be trapped by the most mucus-producing species if microalgae would passively be entrapped in the mucus. Further, while the highly mucus-producing *S. pistillata* and *S. flexibilis* did not show any positives for the microalgae, the much less mucous-producing *P. cactus* showed positive PCR products for two of the tested algae.

Consistent in this study was the avoidance of *P. globosa* as a prey item. Feeding-deterring mechanisms have been reported for *Phaeocystis* spp. for a wide range of potential predators (Nejstgaard *et al.* 2007). We therefore speculate that this may be due to feeding-deterring mechanism(s) as has previously been reported also for strains of single-celled *P. globosa* fed to copepod nauplii Dutz & Koski (2006). To our knowledge, this is the first-time potential feeding-deterring effects in *Phaeocystis* spp. have been suggested for corals.

In conclusion, this study contributes to the growing understanding that both symbiotic and asymbiotic corals have the capacity to capture phytoplankton. This finding has ecological consequences to coral reef ecology, as phytoplankton has been largely overlooked as a potential food source of symbiotic corals. Furthermore, symbiotic corals may now be considered in carbon budgets assessing phytoplankton grazing and benthic–pelagic coupling in coral reefs (Yahel *et al.* 1998), which may contribute to the understanding of the complex food-web dynamics of coral reef ecosystems. We further provide new insights into prey selectivity; specifically that prey selectivity may not be predictable based on prey size or taxonomy alone. However, future feeding-selectivity studies should expand the range of microalgae and corals species, which should encompass a wider range of prey and polyp sizes. Ultimately, coral herbivory may be investigated *in situ*, where the range of prey and predators is much larger. Therefore, the methodological approach using species-specific primers may only be useful when certain prey species are known to occur in the sampling site. An alternative approach to investigate coral herbivory in nature would be to use sequencing techniques with group-specific primers (see reviews by O'Rorke *et al.* 2012b; Pompanon *et al.* 2012).

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M.C.L., C.F.-P., J.C.N. and M.E.F. conceived the study and designed research; M.C.L., C.F.-P. and M.E.T. performed experiments; M.C.L. analysed data; C.F.-P., R.C., J.C.N. and M.E.F. contributed with reagents or analytical tools; M.C.L., C.F.-P., J.C.N., M.E.F., M.E.T. and R.C. wrote the study.

Data accessibility

Data from laboratory feeding experiments: doi:10.5061/dryad.3kf75.

Sequences alignment for primer design: Supplementary Information.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1. Sequence alignment used for primer design.