

First experimental evidence of corals feeding on seagrass matter

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Abstract We present the first experimental evidence of a coral (*Oulastrea crispata*) ingesting and assimilating seagrass material. Tropical seagrass meadows export a substantial portion of their productivity and can provide an important source of nutrients to neighbouring systems such as coral reefs; however, little is known about the mechanisms of this link. To investigate whether seagrass nutrient uptake via coral heterotrophy is possible, we conducted a feeding experiment with seagrass particulate and dissolved organic matter. Using gut extractions and stable isotope analyses, we determined that *O. crispata* ingested ^{15}N -

enriched seagrass particles and assimilated the nitrogen into its tissue at a rate of $0.75 \mu\text{g N cm}^{-2} \text{h}^{-1}$. Corals took up nitrogen from dissolved matter at a comparable rate of $0.98 \mu\text{g N cm}^{-2} \text{h}^{-1}$. While other ecological connections between seagrass meadows and reef ecosystems are well known, our results suggest a previously unstudied direct nutritional link between seagrasses and corals.

Keywords Seagrass · Coral heterotrophy · Habitat connectivity · Nutrient transfer

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Introduction

The interconnectivity among seagrass meadows, coral reefs and mangrove forests is known to be important for maintaining the health of these key habitats (Mumby et al. 2004; Dorenbosch et al. 2007). Research into nutrient connectivity from tropical seagrass meadows to coral reefs and mangroves has focused mainly on faunal migration or fauna-associated nutrient transport (Dorenbosch et al. 2007; Unsworth et al. 2008). Nutrient export from seagrass meadows via faunal intermediates, however, is generally thought to be less important than the quantities transported directly in seagrass material (Hemminga et al. 1991). Seagrass litter is exported in large quantities from seagrass meadows to other habitats, where it can be a significant nutrient source (de Boer 2000). Estimates place the percentage of net primary productivity exported from seagrass meadows at up to 77.34 %, with an additional contribution from dissolved organic matter (DOM) leaching from living and dead plant material (see Heck et al. 2008 for review). However, little is known about the mechanisms of coral uptake of nutrients from seagrass-derived organic matter, which is puzzling given the possible significance of such a relationship.

Organic matter and nutrients from seagrasses can potentially subsidise the metabolic needs of corals and may even promote growth and reproduction. While much of these requirements are derived from autotrophic symbiotic algae, corals also feed on a variety of planktonic organisms, with heterotrophy contributing substantially to their energy budget (see Houlbrèque and Ferrier-Pagès 2009). Osinga (2012) recently demonstrated that growth in the coral *Pocillopora damicornis* increased after being fed the marine diatom *Tetraselmis suecica*, highlighting that corals can derive nutrients from ingested phytoplankton. This result portends that seagrass matter could be eaten by corals and contribute to their growth. The discovery of a brush border enzyme pathway in the stony coral *Stylophora pistillata*, a pathway which in vertebrates breaks down carbohydrates and peptides (Osinga 2012), further supports this notion. Given the large quantity of organic matter exported by seagrass meadows, their common occurrence near coral reefs in tropical regions (Short et al. 2007), and the propensity for corals to ingest particulate matter, it is entirely plausible that corals can and do acquire nutrients by feeding on seagrass material.

We investigated a previously untested source of nutrient uptake in corals, direct ingestion of seagrass particulate

matter, and compared it to the uptake rates of seagrass dissolved matter. We conducted a feeding experiment on the coral, *Oulastrea crispata*, and seagrass, *Halophila ovalis*, using a particle-suspension chamber. Through gut extractions and stable isotope analyses, we determined the extent to which corals assimilate nitrogen from seagrass material.

Materials and methods

Study species

In November 2012, 27 fragments (3–5 cm in diameter) from separate colonies of zebra coral, *O. crispata* (Fig. 1a), were collected from the south-west shoreline of mainland Singapore (1°18'44N, 103°59'35E). These fragments were acclimatised in a semi-indoor aquarium at the National University of Singapore for 1 week.

Approximately 800 g of seagrass *H. ovalis* was collected from a nearby island, Pulau Tekukor (1°13'54N, 103°50'12E), and maintained in the same facility. The seagrass was labelled daily for 1 week with ^{15}N -enriched

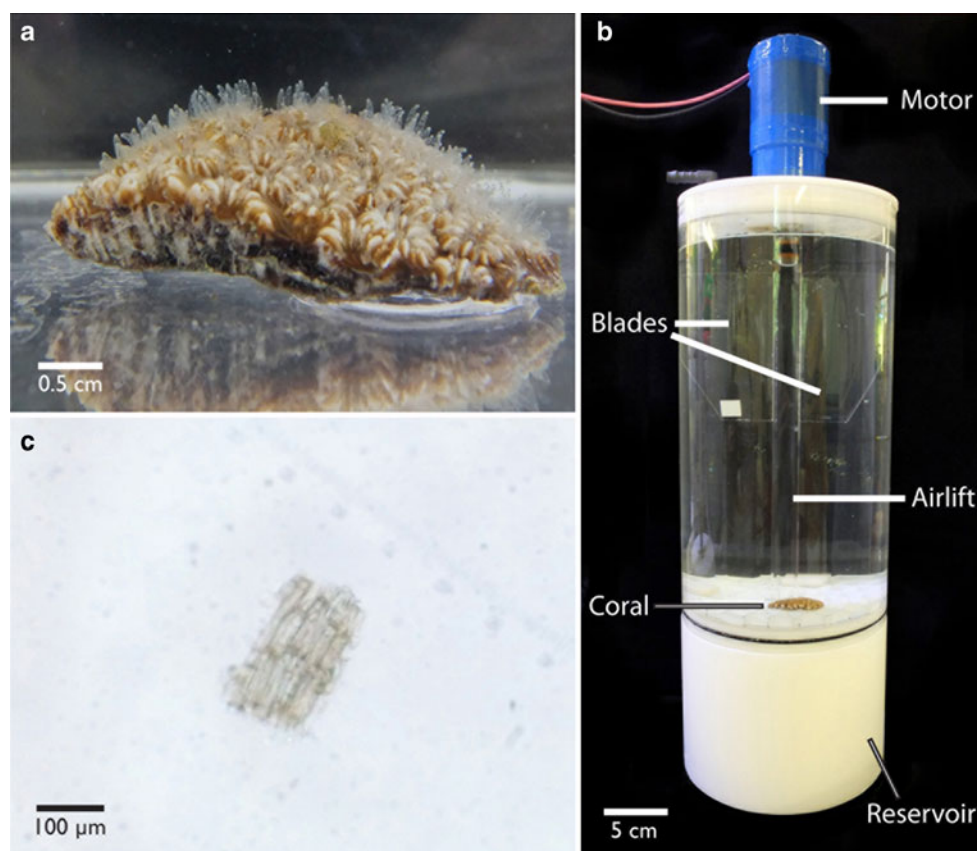


Fig. 1 **a** *Oulastrea crispata* fragment with tentacles extended, **b** feeding chamber with coral, **c** seagrass particle extracted from the gut of a specimen in the POM treatment

NH_4Cl (99 % ^{15}N , Cambridge Isotope Laboratories) to a concentration of $20 \mu\text{mol L}^{-1}$; and a doubled concentration of $40 \mu\text{mol L}^{-1}$ was applied 1 d before the experiment.

Preparation of seagrass POM and DOM

To create the feeding suspension of particulate organic matter (POM) and DOM, 200 g of rinsed seagrass (wet weight) was blended with 400 mL of sand-filtered seawater for 1 min. The suspension was sieved to obtain particulate sizes ranging from 75 to 500 μm for the POM, leaving the filtrate for the DOM solution. The POM was re-suspended in 400 mL of seawater, centrifuged for 5 min at 6,000 rpm and decanted to remove the dissolved matter in the supernatant. This was repeated three times to remove as much dissolved matter as possible, so as to ensure that the great majority of the isotopic label was from the seagrass particles only (see Anthony 1999). The residue was then re-suspended in 800 mL of filtered seawater for the experiment. The DOM filtrate was further passed through a 20- μm filter to remove more particles, and centrifuged three times for 5 min at 6,000 rpm, retaining only the supernatant each time. The POM and DOM solutions were refrigerated between each feeding experiment.

Coral feeding

Nine coral fragments were randomly assigned to individual feeding chambers filled with 5 L of sand-filtered seawater. The chambers, based on Davies et al. (2009), employed air-lifts to re-suspend particulate matter and rotating paddles to provide constant water flow (Fig. 1b). After 1 h of acclimatisation, 100 mL of the seagrass POM suspension was added to three randomly selected chambers, 100 mL of DOM was added to another three, and 100 mL sand-filtered seawater was added to the remaining three as the control. The average concentration of POM in the feeding chambers was 0.72 g L^{-1} (SD ± 0.01) (dry weight) and had an average atom % N of 0.02 % (SD ± 0.01). DOM concentration was 0.73 g L^{-1} (SD ± 0.01), and average atom % N was 0.08 % (SD ± 0.02). Corals were allowed to feed for 2 h before being rinsed to remove any residual label. They were then placed in seawater-filled individual 500-mL beakers until their polyps re-extended. Altogether, three rounds with three replicates of each treatment (POM, DOM and control) were conducted over a 2-d period ($n = 9$) in the semi-indoor aquarium with ambient photosynthetically active radiation and temperature conditions of 223–280 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and 27–29 °C, respectively.

Following Hii et al. (2008), 1-mL syringes with 25-gauge (Terumo®) needles were used to extract the gastrointestinal contents of ten polyps from each fragment. If any one of the ten polyps was found to contain seagrass

particles (recognised by the presence of rectangular cells), that particular fragment was considered to have a positive result for ingesting POM. The remaining polyps of each fragment were left to complete ingestion for an hour. To test whether the ^{15}N label was ingested and assimilated, the tissue was stripped into 20 mL of seawater using an air-pick and frozen. Samples were sent to the Royal Netherlands Institute for Sea Research for analyses of ^{15}N and total nitrogen (after zooxanthellae were removed by centrifugation) using a thermo Electron Flash EA 1112 analyser coupled to a Delta V isotope ratio mass spectrometer. In addition, samples of POM and DOM were analysed for ^{15}N content. Enrichment of the coral tissue in POM and DOM treatments was calculated following Veuger and Middelburg (2007). Values were divided by feeding time and coral surface area to obtain the uptake rate, expressed as N/cm^{-2} of coral/ h^{-1} . Rates between POM and DOM treatments were compared using a Student's t test. One sample of the POM treatment was spoiled during transport, so an unbalanced test was conducted using the uptake rates of the eight remaining samples.

Results and discussion

Gut extractions indicated that five out of the nine samples of the POM-fed *O. crispata* had seagrass particles in them (Fig. 1c); no particles were found in the DOM and control samples. The uptake rates for the corals through DOM and POM did not differ significantly (Student's t test, $p < 0.05$), with the corals in the POM treatment having a mean uptake rate of 0.75 (SE ± 0.30) $\mu\text{g N cm}^{-2} \text{ h}^{-1}$ and in the DOM treatment, 0.98 (SE ± 0.20) $\mu\text{g N cm}^{-2} \text{ h}^{-1}$. These results demonstrate for the first time that corals can assimilate ingested seagrass particles and absorbed dissolved seagrass matter and that the uptake rates through both mechanisms are comparable.

The importance of corals being able to assimilate nitrogen and other nutrients from seagrass material is clearly dependent on the extent to which this occurs in situ. Seagrass meadows export large quantities of fresh and detrital litter, and it is therefore probable that high concentrations of seagrass particles are transferred to adjacent reefs. Seagrass matter transported to neighbouring ecosystems can quickly fragment into smaller particles and leach their organic matter (Heck et al. 2008), which can lead to a lower nitrogen content in dead seagrass compared to the fresh matter used in this experiment. However, the sheer amount of the dead material exported suggests that the derived nutrients, both particulate and dissolved, could still subsidise the metabolic needs of corals on typically oligotrophic reefs. Granek et al. (2009) found that the natural isotope signature of corals revealed a substantial

organic matter contribution from *Thalassia testudinum* seagrass found in nearby meadows. The mode of the uptake in corals was not discussed, although heterotrophy was cited as one of the possible factors affecting nutrient incorporation. Direct ingestion could explain how organic matter from seagrass meadows becomes assimilated into coral tissue. This has further implications on the type of organic matter corals can break down. If corals are capable of digesting plant matter, it opens up the possible route for nutrient transfer from other vegetal sources, e.g., mangroves, marshes, algal beds and coastal forests.

The implications of a direct nutrient link from seagrass meadows to coral reefs are profound, as the availability of nutrients can strongly influence the structure and function of reef systems (Hallock and Schlager 1986). An increase in inorganic nutrient levels, as seen in eutrophic reefs, can have a direct negative effect on corals, depressing growth rates, larval settlement and reproduction (Koop et al. 2001). Inorganic nitrogen is preferentially taken up by symbiotic zooxanthellae and used to promote their own growth, reducing the amount of photosynthetic product translocated to the coral host—resulting in slower coral growth (Stambler and Stimson 1991). Others, however, have documented an increase in growth and reproduction with organic nutrient enrichment (Bongiorni et al. 2003), suggesting the effect of nutrients may be dependent on the overall concentration and chemical form (Ferrier-Pagès and Gattuso 2000). Corals have to supplement their phototrophic carbon diet with organic nitrogen-rich materials, such as zooplankton or DOM, as their capacity to assimilate inorganic nitrogen is low (Falkowski et al. 1984). As such, both particulate and dissolved nitrogen contributions from seagrass might promote coral growth without upsetting the balance between the zooxanthellae and their coral hosts.

Our experiments demonstrate that corals can assimilate nitrogen-based nutrients by feeding directly on seagrass particulate matter and/or absorbing leached DOM. Additional investigations are essential to understanding the mechanisms involved, especially the enzymatic pathway used in plant matter digestion and the extent to which ingestion of seagrass particles occurs in situ. The importance of the reciprocal relationships between coral reefs and their neighbouring habitats for conservation management has been emphasised many times. The potential nutrient transfer from seagrass meadows to coral reefs demonstrated here further underlines the interconnectivity between these critical ecosystems.

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