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Food selectivity and processing by the cold-water coral *Lophelia pertusa*

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Abstract. Cold-water corals form prominent reef ecosystems along ocean margins that depend on suspended resources produced in surface waters. In this study, we investigated food processing of ^{13}C and ^{15}N labelled bacteria and algae by the cold-water coral *Lophelia pertusa*. Coral respiration, tissue incorporation of C and N and metabolically derived C incorporation into the skeleton were traced following the additions of different food concentrations (100, 300, 1300 µg C L^{-1}) and two ratios of suspended bacterial and algal biomass (1 : 1, 3 : 1). Respiration and tissue incorporation by *L. pertusa* increased markedly following exposure to higher food concentrations. The net growth efficiency of *L. pertusa* was low (0.08 ± 0.03), which is consistent with its slow growth rate. The contribution of algae and bacteria to total coral assimilation was proportional to the food mixture in the two lowest food concentrations, but algae were preferred over bacteria as a food source at the highest food concentration. Similarly, the stoichiometric uptake of C and N was coupled in the low and medium food treatment, but was uncoupled in the high food treatment and indicated a comparatively higher uptake or retention of bacterial carbon as compared to algal nitrogen. We argue that behavioural responses for these small-sized food particles, such as tentacle behaviour, mucus trapping and physiological processing, are more likely to explain the observed food selectivity as compared to physical–mechanical considerations. A comparison of the experimental food conditions to natural organic carbon concentrations above CWC reefs suggests that *L. pertusa* is well adapted to exploit temporal pulses of high organic matter concentrations in the bottom water caused by internal waves and downwelling events.

1 Introduction

Cold-water corals have a global distribution in the deep sea and are typically found at locations with high bottom-water velocities, such as continental margins, seamounts and mid-ocean ridges (Roberts et al., 2009; Davies and Guinotte, 2011; Yesson et al., 2012). Some cold-water corals are scleractinians and produce a three-dimensional carbonate structure, which provides settlement, refuge and feeding ground for many associated organisms (Henry and Roberts, 2007; Kutti et al., 2015). As a result, these reef communities are diverse, have high biomass and consume up to 20 times more organic carbon per square meter as compared to surrounding soft-sediment communities (Van Oevelen et al., 2009; White et al., 2012; Cathalot et al., 2015; Rovelli et al., 2015).

The main reef-building coral species in the North Atlantic Ocean is the branching coral *Lophelia pertusa*, which is a passive suspension feeder that uses tentacles to "catch" particles from the water column. Field observations on stable isotopes and fatty acids suggest that *L. pertusa* feeds on a broad range of food sources including particulate suspended matter, bacteria, phytoplankton and zooplankton (Kiriakoulakis et al., 2005; Duineveld et al., 2007; Sherwood et al., 2008; Dodds et al., 2009). Laboratory studies have confirmed the uptake of suspended particles, bacteria, phytoplankton and zooplankton by cold-water corals (Purser et al., 2010;

Mueller et al., 2014; Orejas et al., 2016). Recently, *L. pertusa* was also shown to take up dissolved organic matter in the form of free amino acids (Gori et al., 2014; Mueller et al., 2014) and to fix inorganic carbon into its biomass, supposedly through chemo-autotrophic activity of associated microbes (Middelburg et al., 2015). This flexibility in resource utilization clearly indicates an opportunistic feeding strategy (Mueller et al., 2014; Orejas et al., 2016).

In natural reefs the diversity of organic matter sources is high (Jensen et al., 2012) and it is presently unclear whether cold-water corals exhibit selective resource utilization or feed proportionally to resource availability. Moreover, organic matter supply to cold-water reefs is temporally variable due to seasonality in organic matter production in the surface ocean and the dynamic physical environment in which cold-water reefs occur (Duineveld et al., 2007; Davies et al., 2009; Findlay et al., 2013; Hebbeln et al., 2014; Mohn et al., 2014). Freshly hatched *Artemia salina* nauplii, which are often used as food in aquarium studies of scleractinians, were increasingly taken up by the cold-water coral *L. pertusa* with increasing concentration in the incubation vessel (Purser et al., 2010), indicating that *L. pertusa* responds to changes in food supply. In order to advance our understanding of cold-water coral physiology, we must better understand resource partitioning within the energy budget of the organism. For the cold-water coral *Desmophyllum dianthus* it was shown that zooplankton contributed to various components of the energy budget, including calcification, respiration and mucus release, following food withdrawal for 1 week (Naumann et al., 2011). The slow (i.e. months) response time of *L. pertusa* to changing food conditions renders this approach less effective in directly linking food uptake to physiological processing (Larsson et al., 2013).

The aims of this study are twofold. Firstly, we wanted to assess whether the cold-water coral *Lophelia pertusa* exhibits selective uptake when exposed to a mixed diet. Secondly, we aimed to quantitatively elucidate the energy budget of *L. pertusa* following feeding on different food quantities. To this end, we investigated food uptake, food selectivity and subsequent processing with a novel dual isotope labelling technique using mixed diets of ^{13}C-labelled algae/^{15}N-labelled bacteria and ^{15}N-labelled algae/^{13}C-labelled bacteria. This approach provided the high sensitivity needed to eliminate long-term incubations and allowed us to trace not only uptake, but also subsequent processing of algal and bacterial carbon and nitrogen. This experimental mixed diet study better represents the diversity of food available under natural coral reef conditions than traditional single food source studies and enables the quantitative tracing of individual food sources.

2 Materials and methods

2.1 Experimental design

Our dual isotope tracer design involved exposing separate coral fragments either to a food mixture of ^{13}C-labelled algae (^{13}C-Algae) + ^{15}N-labelled bacteria (^{15}N-Bacteria) or to a mixture of ^{15}N-labelled algae (^{15}N-Algae) +^{13}C-labelled bacteria (^{13}C-Bacteria) (Fig. 1a). Uptake, respiration and calcification rates are subsequently summed to obtain total C or N uptake and processing (i.e. by dividing rates with the fractional ^{13}C or ^{15}N enrichment of each food source, see below). Three food concentrations were tested in this study: 8.3 ($n = 2$ per food mixture), 25 ($n = 3$ per food mixture) and 108 ($n = 3$ per food mixture) µmol C L^{-1} (Fig. 1b). Replication in this study was limited due to collection restrictions for *Lophelia pertusa* from the Tisler Reef. The bacterial-C to algal-C ratio was 1:1 in the 8.3 and 25 µmol C L^{-1} treatment, but due to technical issues, appeared to be 3 : 1 in the 108 µmol C L^{-1} exposure.

2.2 Sampling location and maintenance

Corals were collected at the Tisler Reef, located 70 to 155 m deep in the Skagerrak, at the Norwegian-Swedish border. The Tisler Reef is located at a sill, which connects the Kosterfjord deep trough with the open Skagerrak. The current velocity at the reef varies from 0 to 50 cm s^{-1}, with peaks in excess of 70 cm s^{-1}, and the bottom-water temperature varies normally between 6 and 9 °C throughout the year (Lavaleye et al., 2009; Wagner et al., 2011), though peaks in excess of 12 °C have been observed in recent years (Guihen et al., 2012). The particulate organic carbon (POC) concentration at the reef varies between 3.6 and 8.9 µmol C L^{-1} and the depositional POC fluxes average 38 mmol C m^{-2} d^{-1} (Wagner et al., 2011).

Fragments of the cold-water coral *Lophelia pertusa* were collected from a depth of around 110 m using the remotely operated vehicle Sperre Subfighter 7500 DC. Fragments were placed in cooling boxes filled beforehand with cold seawater (7–8 °C) and transported within a few hours to the laboratory at the Sven Lovén Centre for Marine Sciences in Tjärnö, Sweden. After arrival, the coral fragments were clipped to approximately the same size (7.90 ± 2.12 g dry weight (DW) fragment^{-1}; 14.1 ± 2.4 polyps fragment^{-1} as mean ± SD) and were subsequently acclimated for 6 weeks in aquaria (∼ 20 L) placed in a dark thermo-constant room (7 °C). Sand-filtered (1–2 mm particle size) bottom water from 45 m depth out of the adjacent Koster fjord (salinity 31) was continuously flushed through the aquaria (∼ 1 L min^{-1}). Experience at the station and our earlier experiments showed that the sand-filtered water still contains sufficient organic particles, so that no extra food was provided during the acclimation period (Mueller et al., 2014).

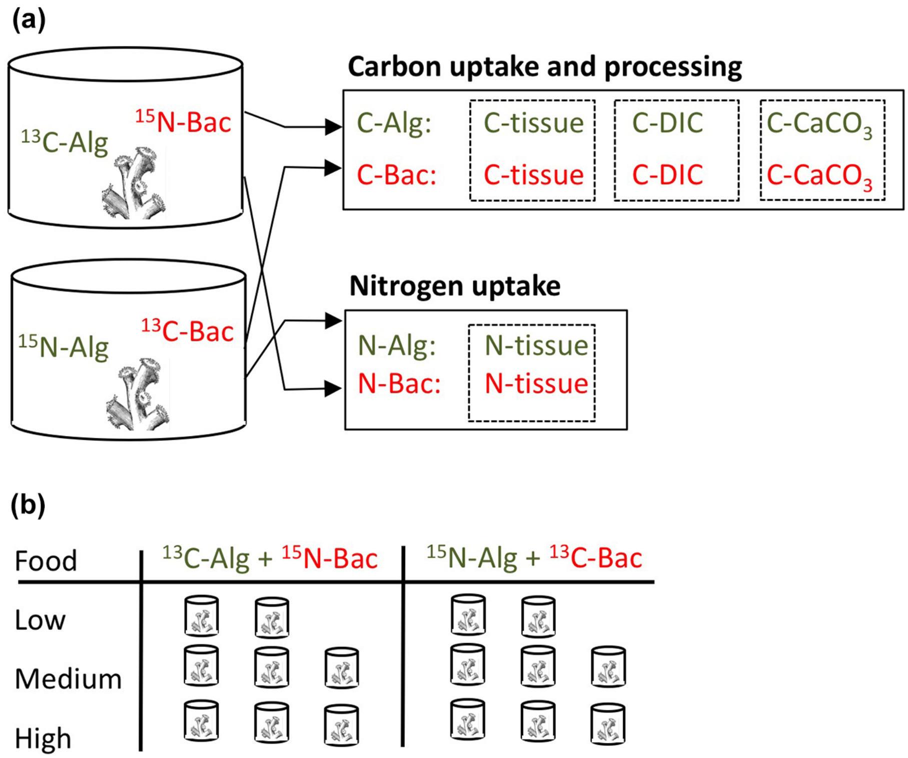

Figure 1. Experimental design of the dual-isotope labelling study. **(a)** Different coral fragments were exposed to a food mixture of ^{13}C-labelled algae (^{13}C-Alg) + ^{15}N-labelled bacteria (^{15}N-Bac) or to a mixture of ^{15}N-labelled algae (^{15}N-Alg) + ^{13}C-labelled bacteria (^{13}C-Bac). The N uptake in tissue (N-tissue), C uptake in tissue (C-tissue), respiration to dissolved inorganic carbon (C-DIC) and C incorporation into the skeleton (C-$CaCO_3$) was calculated for each incubation from the ^{13}C and ^{15}N transfer (see Methods). **(b)** Full experimental design with eight incubations for the ^{13}C-Alg + ^{15}N-Bac treatment and eight incubations for the ^{15}N-Alg + ^{13}C-Bac treatment, partitioned over a low, medium and high food concentration exposure.

2.3 Preparation of isotopically labelled algae and bacteria

^{15}N-labelled algae were cultured axenically in F/2 culture medium adjusted after Guillard (1975). The culture medium was prepared by replacing 80 % of the $NaHCO_3$ (^{13}C-Algae) or 70 % of the $NaNO_3$ (^{15}N-Algae) with its heavy isotope equivalent (Cambridge Isotopes, 99 % ^{13}C-$NaHCO_3$, 99 % ^{15}N-$NaNO_3$). Subsequently, a sterile inoculum of the diatom *Thalassiosira pseudonana* (∼ 5 µm) was added. After a 3 week culture period with a 12 h light–dark cycle, the culture had reached a cell density of 3–4 × 10^6 cells mL^{-1}. The diatoms were concentrated by centrifugation at 450 g and the concentrate was rinsed three times with 0.2 µm filtered seawater to remove residual label and the algal suspension was kept frozen until further use.

Bacteria (±1 µm diameter) were cultured by adding a few mL of natural seawater from the Eastern Scheldt estuary (Netherlands) to M63 culture medium adjusted after Miller (1972). To obtain ^{13}C or ^{15}N isotopically labelled bacteria, 50 % of glucose (3 g L^{-1}) or 50 % of NH_4Cl (1.125 g L^{-1}) was replaced by its heavy isotope equivalent (Cambridge Isotopes, 99 % ^{13}C-glucose, 99 % ^{15}N-NH_4Cl) in the culture medium. After 3 days of culturing in the dark, bacteria were concentrated by centrifugation (14 500 g), rinsed three times with 0.2 µm filtered seawater to remove residual label and the bacterial suspension was stored frozen until the start of the experiment.

Subsamples of the algae ($n = 3$) and bacteria ($n = 3$) were measured for ^{13}C, ^{15}N, C and N (see below). The algae used in the experiment had a molar C : N ratio of 7.8 ± 0.5, 44 at %^{13}C and 65 at %^{15}N, while bacteria had a C : N ratio of 4.8 ± 0.2, 58 at %^{13}C and 47 at % of ^{15}N.

2.4 Experimental procedure

Prior to the start of the experiment, 10 L incubation chambers were filled with 5 µm of filtered bottom water from the nearby Koster fjord and placed in a temperature-controlled room that was maintained at 7 °C. Each coral fragment was inserted into an elastic silicone tube, which was mounted on an acrylic plate to allow easy fixing onto the chamber base and to ensure that the fragments retained an upright position. During the experiment, a continuous level of turbulence and water circulation was maintained by a motordriven paddle wheel in the upper part of the incubation chamber (speed = 2 rpm).

The corals were exposed to the isotopically labelled food for 12 h per day during 10 consecutive days (i.e. the "feeding period"). A food suspension dosage of a few millilitres was given at the beginning of each day during the feeding period with the respective concentration and ratio of ^{13}C bacteria / ^{15}N algae and ^{13}C algae / ^{15}N bacteria (see above and Fig. 1). After 12 h of exposure to the food dosage, the chambers were flushed with 5 µm filtered Koster fjord water(140 mL min^{-1}) for 12 h to remove food particles, avoid accumulation of waste products and renew the O_2 supply. Corals for background isotope measurements (controls) were incubated in parallel under 'acclimatization' conditions, i.e. only exposed to sand-filtered seawater.

After the last flushing period on day 10, the incubation chambers were closed for 48 h to measure the production of ^{13}C dissolved inorganic carbon (^{13}C-DIC) as a proxy for respiration (Moodley et al., 2000). Filtered (GF/F) water samples were taken for DIC analysis before (control) and after the incubation period and stored in a 10 mL headspace vial. Biological activity was stopped by adding 10 µL $HgCl_2$ to the vials. The vials were closed with an aluminium cap fitted with a rubber septum and stored upside down for further analysis. Calculations based on literature respiration rates (Dodds et al., 2007) and pilot experiments indicated that the expected changes in pH and oxygen and ammonium concentration during the incubations are limited, so that no negative affect on coral or sponge physiology was expected. Coral fragments were stored frozen (−20 °C) at the end of the incubation for further analysis.

2.5 Sample analysis

Coral fragments were freeze-dried, weighed and subsequent ground with a ball mill for 20 s (MM 2000, Retsch; Haan, Germany). This ground coral material, comprised of skeleton and organic tissue, was measured for the incorporation of isotopic tracers in the skeleton and tissue (following Tanaka et al., 2007; Mueller et al., 2013). Around 30 mg of a coral sample was transferred to silver measuring boats and measured for C content and ^{13}C at % using a Thermo Electron Flash EA 1112 analyzer (EA) coupled to a Delta V isotope ratio mass spectrometer (IRMS). Another 30 mg of ground coral was transferred to pre-combusted silver boats and gently decalcified by acidification by placing them in an acidic fume for 3 to 4 days to remove most of the inorganic C. The ground coral was then further acidified by stepwise addition of HCl with increasing concentration (maximum concentration 12 mol L^{-1}) until the inorganic skeleton was removed (as evidenced by the absence of bubbling after further acid addition) (Mueller et al., 2013). After acidification, the samples were analysed on the EA-IRMS for C and N content and ^{13}C and ^{15}N at % in the organic fraction. Incorporation of ^{13}C into the inorganic skeleton, as proxy for calcification (sensu Tanaka et al., 2007), was obtained by subtracting the ^{13}C in the organic fraction from the total ^{13}C in the ground coral material. Note that this calcification proxy only tracks the incorporation of "metabolically derived" carbon, as the ^{13}C needs to be liberated by metabolism from the organic resource (algae or bacteria) before it can be incorporated. Calcification based on metabolically derived C may only be a small fraction of total calcification, but it can still be used as a tracer to detect changes in calcification (Mueller et al., 2013).

In the headspace vials taken for DIC analysis, a headspace of ∼ 3 mL was created by injecting N_2 gas through the vial septum (Mueller et al., 2013). Samples were acidified with 20 µL of concentrated H_3PO_4 to transform DIC into gaseous CO_2. A 10 µL sample of the headspace was injected into the EA-IRMS for analysis of CO_2 concentration and at % of ^{13}C-CO_2.

The incorporation of ^{13}C and ^{15}N in coral tissue and ^{13}C in $CaCO_3$ is the excess (E) ^{13}C or ^{15}N in a sample and is calculated as $E = F_{\text{experiment}} - F_{\text{background}}$, in which F represents the at % of ^{13}C or ^{15}N (i.e. $^{13}C / [^{12}C + ^{13}C]$ and $^{15}N / [^{14}N + ^{15}N]$, respectively) in an experimental or background sample. Hence, E is the above-background at % of ^{13}C or ^{15}N and positive values indicate transfer of isotope from the original algal or bacterial source to the coral. The excess values are multiplied with the C or N content in the ground coral material (i.e. µmol C g^{-1} DW and µmol N g^{-1} DW, respectively) and divided with the at % enrichment of the specific food source to obtain total incorporation rate during the "feeding period" in µmol C g^{-1} DW and µmol N g^{-1} DW. Incorporation rates throughout the paper are expressed as the daily rates by dividing total incorporation with the length of the feeding period (i.e. 10 days). Total respiration is calculated similarly, in which excess values of DIC are multiplied with the DIC concentration (µmol C L^{-1}) and chamber volume (10 L). Daily respiration rates are calculated over the length of the incubation period (2 days) and normalized to the coral weight (g DW) in an incubation chamber.

2.6 Data analysis

Selective uptake of algae or bacteria was assessed with the Chesson index (Chesson, 1983):

$$\alpha_i = \frac{r_i / n_i}{\sum_j r_j / n_j}, \tag{1}$$

in which α_i is the selectivity index for resource i, r_i is the uptake of resource i expressed as C or N uptake g^{-1} DW d^{-1}, n_i is the initial concentration of resource i in the incubation chamber and j is the total number of resources ($j = 2$ in this study). The Chesson index was calculated per food concentration tested and allows indicating selective uptake as the uptake is normalized to the respective food concentration, i.e. the algae vs. bacterial uptake is normalized for the dif-

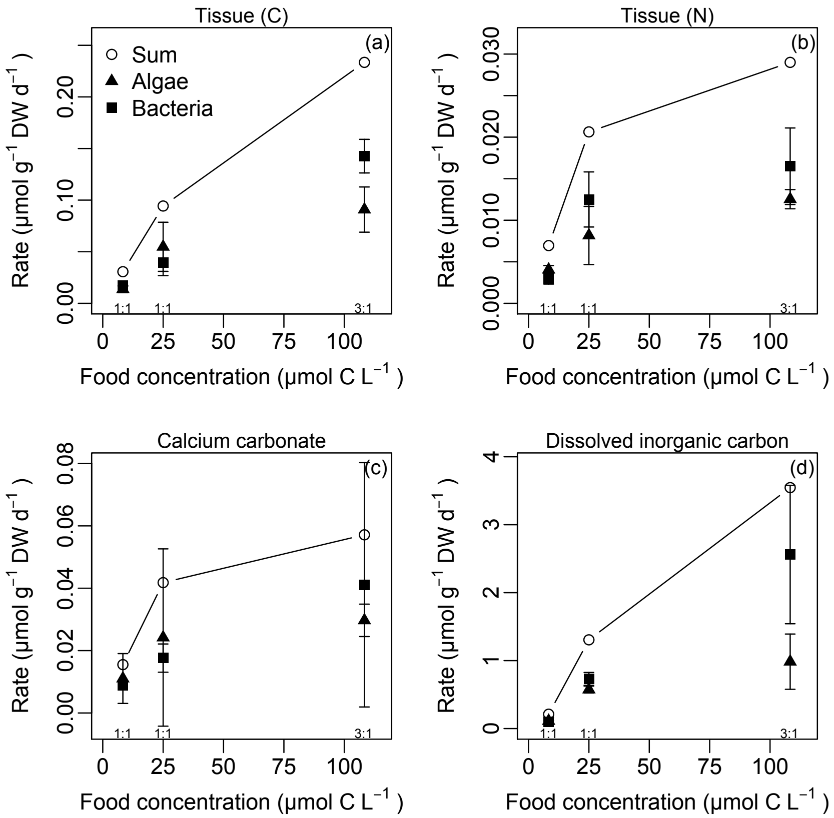

Figure 2. Food processing by the cold-water coral *L. pertusa* at different food concentrations and compositions. **(a)** Carbon assimilation in tissue, **(b)** nitrogen assimilation in tissue, **(c)** carbon incorporation into coral skeleton, **(d)** carbon respiration. The mean ± range is shown for the low food concentration treatment ($n = 2$). The ratios 1 : 1 and 3 : 1 in the sub-panels indicate the ratio of bacteria : algae in the respective food concentration treatment.

ferences in their availability. The selectivity indices sum to 1 and in the present experiment a selectivity index of 0.5 implies no selectivity, > 0.5 indicates "positive" selectivity (i.e. higher uptake than proportional availability) and < 0.5 indicates "negative" selectivity.

A net growth efficiency (NGE) was calculated from the C incorporation into tissue rate and the respiration rate as: NGE = tissue incorporation/(tissue incorporation + respiration). We also explored resource stoichiometric utilization by comparing the C : N ratio of food uptake from the algal and bacterial resources with the C : N ratio of the food in the incubation chamber (algal–bacterial mixture has a C : N of 5.9 in the low and medium food treatment and 5.3 in the high food treatment). Data are presented as mean ± SD, except where stated otherwise, and figures were made in R (R Development Core Team, 2015). The bacteria : algae ratio was not constant over the food concentrations tested (see "Experimental design" above); therefore, we refrained from statistical comparisons and instead discuss the trends in the data.

3 Results

3.1 Tissue incorporation and processing of food sources

Both bacterial and algal C and N were incorporated into the coral tissue and their incorporation rates increased with increasing food concentrations (Fig. 2a, b). Incorporation of algal C increased from 0.013 to 0.09 µg C g^{-1} DW d^{-1} and bacterial C increased from 0.017 to 0.14 µg C g^{-1} DW d^{-1} over the food concentration range (Fig. 2a). The incorporation rate into the carbonate skeleton tended to increase with food concentrations, but the estimated rates were associated with a high variability (Fig. 2c). Respiration of algal C increased substantially from 0.11 to 0.98 µg C g^{-1} DW d^{-1} and bacterial C increased from 0.10 to 2.6 µg C g^{-1} DW d^{-1} with increasing food concentration (Fig. 2d). The higher bacterial uptake and processing in the highest food treatment could be the result of the (unintended) higher bacteria : algae ratio in this treatment as compared to the other two treatments (3 : 1 vs. 1 : 1, respectively). A total of 2.6 ± 0.6, 4.8 ± 0.8 and 3.6 ± 1.4 % of the total added organic carbon was recovered in the investigated pools with increasing food concentrations(low, medium, high) respectively.

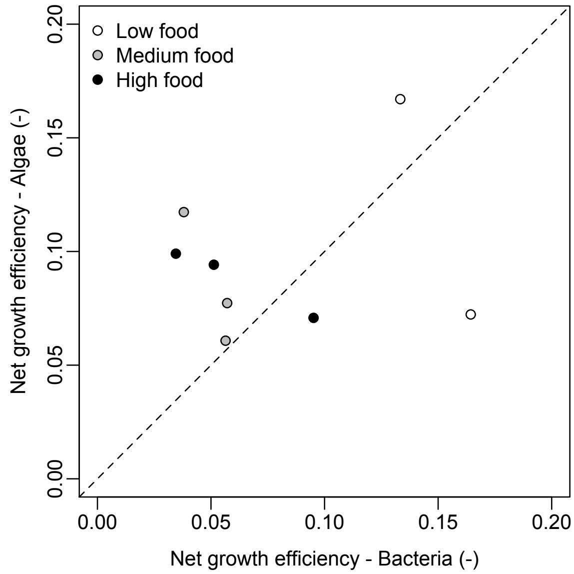

Figure 3. The net growth efficiency of *L. pertusa* when feeding on algae vs. bacteria. The colours represent the low, medium and high food concentrations, as in Fig. 2.

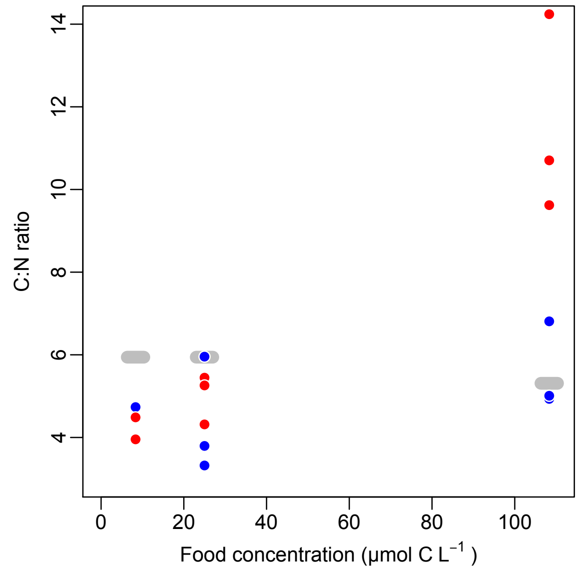

Figure 4. Resource stoichiometry in the experiment, presented as the C : N ratio of the resource vs. the C : N ratio in the coral tissue after the incubation. The grey bar is the C : N ratio of the suspended food (i.e. algae + bacteria), the blue dots are the C : N ratios of the coral tissue in the treatment ^{13}C-algae + ^{15}N-bacteria and the red dots are the C : N ratios of the coral tissue in the treatment ^{15}N-algae + ^{13}C-bacteria.

The incorporation rates into tissue were low compared to respiration losses (Fig. 3), resulting in low net growth efficiencies (NGEs) of 0.08 ± 0.03, independent of food concentration or type (Fig. 3).

The stoichiometric comparison shows that at low food concentrations the C : N incorporation into the coral tissue was equal to or lower than the bulk tissue C : N ratio of the coral (6.3 ± 1.5, mean ± SD) and the offered suspended food (i.e. ^{13}C-algae + ^{15}N-bacteria and ^{15}N-algae + ^{13}C-bacteria, Fig. 4). In the high food concentration treatment, however, the C : N incorporation into the coral tissue was evidently different between the two treatments (Fig. 4). The C : N values ranged from 4.9 to 6.8 in the ^{13}C-algae + ^{15}N-bacteria treatment and from 9.6 to 14.2 in the ^{15}N-algae + ^{13}C-bacteria treatment, indicating uncoupled processing of the available C and N.

3.2 Food selectivity

We found the mean C-based Chesson index for bacteria ranging from 0.56 to 0.35 over the food treatments and for algae ranging from 0.44 to 0.65 (Fig. 5a). When food concentration and food uptake are expressed in N-equivalents, the mean Chesson index ranged from 0.30 to 0.61 for bacteria and from 0.39 to 0.70 for algae (Fig. 5b). There was a clear tendency for selective uptake of algae at the higher food concentration treatment.

4 Discussion

4.1 Concentration-dependent food uptake and processing by *Lophelia pertusa*

Higher suspended food concentrations resulted in increased assimilation and respiration rates by *L. pertusa*, indicating that food uptake and metabolism is tightly coupled to food availability. This is consistent with observations by Purser et al. (2010) and Larsson et al. (2013) showing higher respiration and removal rates of zooplankton with increased particle concentration. Interestingly, food capture rates in Purser et al. (2010) and metabolic activity in this study start to saturate at a food concentration above 100 µmol C L^{-1}. POC concentrations above cold-water coral (CWC) reefs vary between 1 and 11 µg C L^{-1} (Kiriakoulakis et al., 2007; Wagner et al., 2011), which implies that *L. pertusa* is well adapted to exploit temporal pulses of high organic matter concentrations in the bottom water caused by internal waves and downwelling events such as observed in the Mingulay Reef complex(Davies et al., 2009), Tisler Reef (Wagner et al., 2011) and the Logachev Mounds at Rockall Bank (Duineveld et al., 2007; Soetaert et al., In press).

In contrast to assimilation and respiration rates, calcification rates increased less pronouncedly with increasing food concentration and were associated with a high variability. Hennige et al. (2014) found a short-term response in respiration rates by *L. pertusa* to higher pCO$_2$ conditions, but calci-

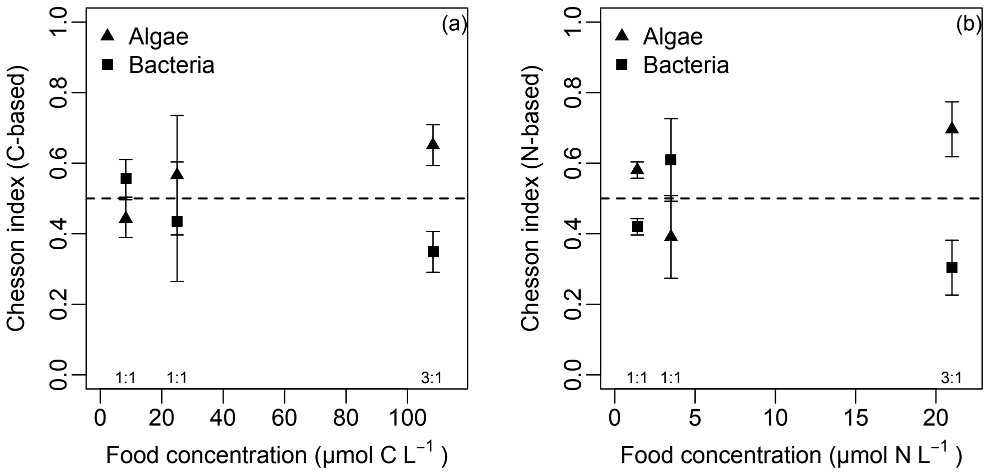

Figure 5. The Chesson index of *L. pertusa* for feeding on bacteria and algae expressed in **(a)** carbon and **(b)** nitrogen. The mean ± range is shown for the low food concentration treatment ($n = 2$). The ratios 1 : 1 and 3 : 1 in both sub-panels indicate the ratio of bacteria : algae in the respective food concentration treatment.

fication rates were not significantly affected. Similarly, Larsson et al. (2013) did not find a response in skeletal growth of *L. pertusa* after long-term exposure (months) to different food concentrations. Hence, it seems that the response time of calcification acts on a longer timescale than does food availability. Also, for tropical coral it is known that calcification processes can be less responsive to environmental conditions than tissue growth (Anthony and Fabricius, 2000; Tanaka et al., 2007; Tolosa et al., 2011). One explanation why a longer time period is needed before a response in calcification to altered food conditions can be measured may be the relatively low metabolic costs related to calcification in *L. pertusa* (McCulloch et al., 2012; Larsson et al., 2013). However, Naumann et al. (2011) did measure significantly higher calcification in fed compared to unfed specimens of the CWC *Desmophyllum dianthus*, but *D. dianthus* is a faster growing species and may therefore respond more rapidly to food availability.

The net growth efficiency (NGE) is the percentage of assimilated organic carbon that is transferred into biomass. Hence, a high NGE means that a food source is efficiently shunted into biomass. We are not aware of NGE estimates for cold-water corals in the literature. The NGEs of *L. pertusa* in our study ranged from 4 to 17 %, and these values are low compared to values of > 50 % for zooplankton (Anderson et al., 2005), a taxonomic group for which NGE is well studied. The shallow-water anemone *Anthopleura elegantissima*, taxonomically closely related to corals, also has substantially higher NGEs ranging from 30 to 60 % (Zamer, 1986). The NGE is positively correlated with growth rate for the sponge *Halichondria panicea* (Thomassen and Riisgard, 1995) and the bivalve *Mytilus edulis* (Kiørboe et al., 1981), and we therefore speculate that the low NGE of the cold-water coral *L. pertusa* is related to its slow growth rate (Roberts et al., 2009). The NGE tends to be higher when *L. pertusa* was feeding on algae compared to bacteria (Fig. 3), but these estimates are associated with a high variability. Hence, although it is known that the NGE can depend on food quality and quantity (Anderson et al., 2005), additional research is necessary to determine that relation for cold-water corals.

4.2 Food-composition dependent uptake by *L. pertusa*

Food assimilation at the lower two food concentrations responded proportionally to resource stoichiometry (Fig. 4) and food composition (Fig. 5), which indicates that *L. pertusa* is an opportunistic and seemingly unselective feeder. This opportunistic feeding strategy is consistent with uptake of various organic resources in aquarium experiments (Gori et al., 2014; Mueller et al., 2014; Orejas et al., 2016) and inferences from natural abundance stable isotope and fatty acid compositions from field-collected CWC (Duineveld et al., 2007; Dodds et al., 2009). Interestingly however, at higher food concentrations it appears that *L. pertusa* feeds selectively on algae (Fig. 5) and the C : N uptake or retention becomes unbalanced, with a comparatively higher uptake or retention of bacterial carbon as compared to algal nitrogen in the ^{15}N-algae + ^{13}C-bacteria treatment (Fig. 4). This conclusion is tentative because the high food concentration treatment contained proportionally more bacteria than algae (see Sect. 2.1), but these results do suggest that *L. pertusa* assimilates food in proportion to availability at comparatively lower food concentrations. Whereas, when food is in ample supply, *L. pertusa* starts to feed preferentially on algal organic matter and differentially process the carbon and nitrogen derived from its resources.

Our data do not allow identification of which mechanisms drive the observed food selectivity. The algal cells (5 µm) are a factor of 5 larger in diameter than bacteria, meaning food size may be a trigger that induces selective behaviour. Consistently, Tsounis et al. (2010) found that several CWCs, amongst other *L. pertusa*, fed at higher rates on adult *Artemia salina* compared to the smaller-sized *A. salina* nauplii. Shimeta and Koehl (1997) conducted a theoretical analysis of selective feeding by passive suspension feeders and found particle selection to be a function of encounter, retention and handling. For the particles considered in this study, i.e. bacteria and algae that are substantially smaller than the feeding tentacles, Shimeta and Koehl predict that encounter rates increase with particle size, while particle retention is likely to be independent of particle size. Sole mechanical predictions for the food handling stage provide only part of the story because behavioural choices may play an important role as well (Shimeta and Koehl, 1997). In this study, a role for behavioural and/or physiological responses is suggested by the uncoupling of C and N processing at a higher food concentration. Behavioural triggers that may increase encounter and retention, include enhanced polyp extension in the presence of the preferred food source or environmental conditions (Orejas et al., 2016) and trapping of food particles with the aid of mucus secretion (Mortensen, 2001). Zetsche et al. (2016) recently showed that mucus from *L. pertusa* is produced in small amounts and occurs localized in response to different stimuli. When exposed to *A. salina* nauplii, mucus strings and so-called "string balls" were observed to enhance food trapping. Given the comparatively small particle size used in this study and the uncoupling of C and N processing at higher food concentrations, we suggest that behavioural responses and subsequent physiological processing are more likely to explain the observed food selectivity as compared to the physical–mechanical considerations.

5 Data availability

The data underlying this manuscript are published in PANGAEA under https://doi.pangaea.de/10.1594/PANGAEA.865313.

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References

Anderson, T. R., Hessen, D. O., Elser, J. J., and Urabe, J.: Metabolic stoichiometry and the fate of excess carbon and nutrients in consumers, Am. Nat., 165, 1–15, 2005.

Anthony, K. R. N. and Fabricius, K. E.: Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity, J. Exp. Mar. Biol. Ecol., 252, 221–253, 2000.

Cathalot, C., Van Oevelen, D., Cox, T., Kutti, T., Lavaleye, M., Duineveld, G., and Meysman, F. J. R.: Cold-water coral reefs and adjacent sponge grounds: Hotspots of benthic respiration and organic carbon cycling in the deep sea, Front. Mar. Sci., 2, 1–12, doi:10.3389/fmars.2015.00037, 2015.

Chesson, J.: The estimation and analysis of preference and its relationship to foraging models, Ecology, 64, 1297–1304, 1983.

Davies, A. J. and Guinotte, J. M.: Global habitat suitability for framework-forming cold-water corals, PLoS One, 6, e18483, doi:10.1371/journal.pone.0018483, 2011.

Davies, A. J., Duineveld, G. C. A., Lavaleye, M. S. S., Bergman, M. J. N., Van Haren, H., and Roberts, J. M.: Downwelling and deep-water bottom currents as food supply mechanisms to the cold-water coral *Lophelia pertusa* (Scleractinia) at the Mingulay Reef complex, Limnol. Oceanogr., 54, 620–629, 2009.

Dodds, L. A., Roberts, J. M., Taylor, A. C., and Marubini, F.: Metabolic tolerance of the cold-water coral *Lophelia pertusa* (Scleractinia) to temperature and dissolved oxygen change, J. Exp. Mar. Biol. Ecol., 349, 205–214, 2007.

Dodds, L. A., Black, K. D., Orr, H., and Roberts, J. M.: Lipid biomarkers reveal geographical differences in food supply to the cold-water coral *Lophelia pertusa* (Scleractinia), Mar. Ecol. Prog. Ser., 397, 113–124, 2009.

Duineveld, G. C. A., Lavaleye, M. S. S., Bergman, M. I. N., De Stigter, H., and Mienis, F.: Trophic structure of a cold-water coral mound community (Rockall Bank, NE Atlantic) in relation to the near-bottom particle supply and current regime, Bull. Mar. Sci., 81, 449–467, 2007.

Findlay, H. S., Artioli, Y., Navas, J. M., Hennige, S. J., Wicks, L. C., Huvenne, V. A. I., Woodward, E. M. S., and Roberts, J. M.: Tidal downwelling and implications for the carbon biogeochemistry of cold-water corals in relation to future ocean acidification and warming, Glob. Change Biol., 19, 2708–2719, 2013.

Gori, A., Grover, R., Orejas, C., Sikorski, S., and Ferrier-Pages, C.: Uptake of dissolved free amino acids by four cold-water coral species from the Mediterranean Sea, Deep-Sea Res. Pt. II, 99, 42–50, 2014.

Guihen, D., White, M., and Lundälv, T.: Temperature shocks and ecological implications at a cold-water coral reef, Marine Biodiversity Records, 5, 1–10, 2012.

Guillard, R. R. L.: Culture of Phytoplankton for Feeding Marine Invertebrates, in: Culture of Marine Invertebrate Animals, edited by: Smith, W. L. and Chanley, M. H., Springer US, 1975.

Hebbeln, D., Wienberg, C., Wintersteller, P., Freiwald, A., Becker, M., Beuck, L., Dullo, C., Eberli, G. P., Glogowski, S., Matos, L., Forster, N., Reyes-Bonilla, H., and Taviani, M.: Environmental forcing of the Campeche cold-water coral province, southern Gulf of Mexico, Biogeosciences, 11, 1799–1815, doi:10.5194/bg-11-1799-2014, 2014.

Hennige, S. J., Wicks, L. C., Kamenos, N. A., Bakker, D. C. E., Findlay, H. S., Dumousseaud, C., and Roberts, J. M.: Short-term metabolic and growth responses of the cold-water coral *Lophelia* *pertusa* to ocean acidification, Deep-Sea Res. Pt. II, 99, 27–35, 2014.

Henry, L. A. and Roberts, J. M.: Biodiversity and ecological composition of macrobenthos on cold-water coral mounds and adjacent off-mound habitat in the bathyal Porcupine Seabight, NE Atlantic, Deep-Sea Res. Pt. I, 54, 654–672, 2007.

Jensen, S., Bourne, D. G., Hovland, M., and Murrell, J. C.: High diversity of microplankton surrounds deep-water coral reef in the Norwegian Sea, Fems Microbiol. Ecol., 82, 75–89, 2012.

Kiørboe, T., Møhlenberg, F., and Nøhr, O.: Effect of suspended bottom material on growth and energetics in Mytilus edulis, Mar. Biol., 61, 283–288, 1981.

Kiriakoulakis, K., Fisher, E., Wolff, G. A., Freiwald, A., Grehan, A., and Roberts, J. M.: Lipids and nitrogen isotopes of two deep-water corals from the North-East Atlantic: inital results and implication for their nutrition, in: Cold-water corals and ecosystems, edited by: Freiwald, A. and Roberts, J. M., Springer-Verlag, Berlin Heidelberg, 2005.

Kiriakoulakis, K., Freiwald, A., Fisher, E., and Wolff, G. A.: Organic matter quality and supply to deep-water coral/mound systems of the NW European Continental Margin, Int. J. Earth Sci., 96, 159–170, 2007.

Kutti, T., Fossa, J. H., and Bergstad, O. A.: Influence of structurally complex benthic habitats on fish distribution, Mar. Ecol. Prog. Ser., 520, 175–190, 2015.

Larsson, A. I., Lundälv, T., and van Oevelen, D.: Skeletal growth, respiration rate and fatty acid composition in the cold-water coral Lophelia pertusa under varying food conditions, Mar. Ecol. Prog. Ser., 483, 169–184, 2013.

Lavaleye, M., Duineveld, G., Lundälv, T., White, M., Guihen, D., Kiriakoulakis, K., and Wolff, G. A.: Cold-water corals on the Tisler reef: Preliminary observations on the dynamic reef environment, Oceanography, 22, 76–84, 2009.

McCulloch, M., Falter, J., Trotter, J., and Montagna, P.: Coral resilience to ocean acidification and global warming through pH up-regulation, Nature Climate Change, 2, 623–633, 2012.

Middelburg, J. J., Mueller, C. E., Veuger, B., Larsson, A. I., Form, A., and van Oevelen, D.: Discovery of symbiotic nitrogen fixation and chemoautotrophy in cold-water corals, Sci. Rep., 5, 17962, doi:10.1038/srep17962, 2015.

Miller, J. H.: Experiments in molecular genetics, Cold Spring Harbor Laboratory, 1972.

Mohn, C., Rengstorf, A., White, M., Duineveld, G., Mienis, F., Soetaert, K., and Grehan, A.: Linking benthic hydrodynamics and cold-water coral occurrences: A high-resolution model study at three cold-water coral provinces in the NE Atlantic, Prog. Oceanogr., 122, 92–104, 2014.

Moodley, L., Boschker, H. T. S., Middelburg, J. J., Herman, P. M. J., De Deckere, E., and Heip, C. H. R.: The ecological significance of benthic Foraminifera: ^{13}C labelling experiments, Mar. Ecol. Prog. Ser., 202, 289–295, 2000.

Mortensen, P. B.: Aquarium observations on the deep-water coral *Lophelia pertusa* (L., 1758) (Scleractinia) and selected associated invertebrates, Ophelia, 54, 83–104, 2001.

Mueller, C. E., Lundälv, T., Middelburg, J. J., and van Oevelen, D.: The symbiosis between *Lophelia pertusa* and *Eunice norvegica* stimulates coral calcification and worm assimilation, PLoS One, 8, e58660, doi:10.1371/journal.pone.0058660, 2013.

Mueller, C. E., Larsson, A. I., Veuger, B., Middelburg, J. J., and van Oevelen, D.: Opportunistic feeding on various organic food sources by the cold-water coral Lophelia pertusa, Biogeosciences, 11, 123–133, doi:10.5194/bg-11-123-2014, 2014.

Naumann, M. S., Orejas, C., Wild, C., and Ferrier-Pages, C.: First evidence for zooplankton feeding sustaining key physiological processes in a scleractinian cold-water coral, J. Exp. Biol., 214, 3570–3576, 2011.

Orejas, C., Gori, A., Rad-Menéndez, C., Last, K. S., Davies, A. J., Beveridge, C. M., Sadd, D., Kiriakoulakis, K., Witte, U., and Roberts, J. M.: The effect of flow speed and food size on the capture efficiency and feeding behaviour of the cold-water coral *Lophelia pertusa*, J. Exp. Mar. Biol. Ecol., 481, 34–40, 2016.

Purser, A., Larsson, A. I., Thomsen, L., and van Oevelen, D.: The influence of flow velocity and food concentration on *Lophelia pertusa* (Scleractinia) zooplankton capture rates, J. Exp. Mar. Biol. Ecol., 395, 55–62, 2010.

R Development Core Team: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2015.

Roberts, J. M., Wheeler, A., Freiwald, A., and Cairns, S.: Cold-water corals, The biology and geology of deep-sea coral habitats, Cambridge University Press, Cambridge, 2009.

Rovelli, L., Attard, K., Bryant, L., Flogel, S., Stahl, H., Roberts, J., Linke, P., and Glud, R.: Benthic O_2 uptake of two cold-water coral communities estimated with the non-invasive eddy correlation technique, Mar. Ecol. Prog. Ser., 525, 97–104, 2015.

Sherwood, O. A., Jamieson, R. E., Edinger, E. N., and Wareham, V. E.: Stable C and N isotopic composition of cold-water corals from the Newfoundland and Labrador continental slope: Examination of trophic, depth and spatial effects, Deep-Sea Res. Pt. I, 55, 1392–1402, 2008.

Shimeta, J. and Koehl, M. A. R.: Mechanisms of particle selection by tentaculate suspension feeders during encounter, retention, and handling, J. Exp. Mar. Biol. Ecol., 209, 47–73, 1997.

Soetaert, K., Mohn, C., Rengstorf, A., Grehan, A., and van Oevelen, D.: Ecosystem engineering creates a direct nutritional link between 600-m deep cold-water coral mounds and surface productivity, Sci. Rep., 6, 35057, doi:10.1038/srep35057, 2016.

Tanaka, Y., Miyajima, T., Koike, I., Hayashibara, T., and Ogawa, H.: Imbalanced coral growth between organic tissue and carbonate skeleton caused by nutrient enrichment, Limnol. Oceanogr., 52, 1139–1146, 2007.

Thomassen, S. and Riisgard, H. U.: Growth and energetics of the sponge *Halichondria panicea*, Mar. Ecol. Prog. Ser., 128, 239–246, 1995.

Tolosa, I., Treignier, C., Grover, R., and Ferrier-Pages, C.: Impact of feeding and short-term temperature stress on the content and isotopic signature of fatty acids, sterols, and alcohols in the scleractinian coral Turbinaria reniformis, Coral Reefs, 30, 763–774, 2011.

Tsounis, G., Orejas, C., Reynaud, S., Gili, J. M., Allemand, D., and Ferrier-Pages, C.: Prey-capture rates in four Mediterranean cold water corals, Mar. Ecol. Prog. Ser., 398, 149–155, 2010.

Van Oevelen, D., Duineveld, G. C. A., Lavaleye, M. S. S., Mienis, F., Soetaert, K., and Heip, C. H. R.: The cold-water coral community as hotspot of carbon cycling on continental margins: a food web analysis from Rockall Bank (northeast Atlantic), Limnol. Oceanogr., 54, 1829–1844, 2009.

van Oevelen, D., Mueller, C. E., Lundälv, T., and Middelburg, J. J.: Food selectivity and processing by the cold-water coral Lophelia pertusa, Royal Netherlands Institute for Sea Research, Dataset #865313, 2016.

Wagner, H., Purser, A., Thomsen, L., Jesus, C. C., and Lundälv, T.: Particulate organic matter fluxes and hydrodynamics at the Tisler cold-water coral reef, J. Mar. Syst., 85, 19–29, 2011.

White, M., Wolff, G. A., Lundälv, T., Guihen, D., Kiriakoulakis, K., Lavaleye, M., and Duineveld, G.: Cold-water coral ecosystem (Tisler Reef, Norwegian Shelf) may be a hotspot for carbon cycling, Mar. Ecol. Prog. Ser., 465, 11–23, 2012.

Yesson, C., Taylor, M. L., Tittensor, D. P., Davies, A. J., Guinotte, J., Baco, A., Black, J., Hall-Spencer, J. M., and Rogers, A. D.: Global habitat suitability of cold-water octocorals, J. Biogeogr., 39, 1278–1292, 2012.

Zamer, W. E.: Physiological energetics of the intertidal sea anemone *Anthopleura elegantissima*, Mar. Biol., 92, 299–314, 1986.

Zetsche, E.-M., Baussant, T., Meysman, F. J. R., and van Oevelen, D.: Direct Visualization of Mucus Production by the Cold-Water Coral *Lophelia pertusa* with Digital Holographic Microscopy, PLoS One, 11, e0146766, doi:10.1371/journal.pone.0146766, 2016.