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RESEARCH ARTICLE

First evidence for zooplankton feeding sustaining key physiological processes in a scleractinian cold-water coral

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SUMMARY

Scleractinian cold-water corals (CWC) represent key taxa controlling deep-sea reef ecosystem functioning by providing structurally complex habitats to a high associated biodiversity, and by fuelling biogeochemical cycles *via* the release of organic matter. Nevertheless, our current knowledge on basic CWC properties, such as feeding ecology and key physiological processes (i.e. respiration, calcification and organic matter release), is still very limited. Here, we show evidence for the trophic significance of zooplankton, essentially sustaining levels of the investigated key physiological processes in the cosmopolitan CWC *Desmophyllum dianthus* (Esper 1794). Our results from laboratory studies reveal that withdrawal (for up to 3 weeks) of zooplankton food (i.e. *Artemia salina*) caused a significant decline in respiration (51%) and calcification (69%) rates compared with zooplankton-fed specimens. Likewise, organic matter release, in terms of total organic carbon (TOC), decreased significantly and eventually indicated TOC net uptake after prolonged zooplankton exclusion. In fed corals, zooplankton provided 1.6 times the daily metabolic C demand, while TOC release represented 7% of zooplankton-derived organic C. These findings highlight zooplankton as a nutritional source for *D. dianthus*, importantly sustaining respiratory metabolism, growth and organic matter release, with further implications for the role of CWC as deep-sea reef ecosystem engineers.

Key words: deep sea, cold-water coral, feeding ecology, respiration, calcification, organic matter release, carbon budget, *Desmophyllum dianthus*, Mediterranean.

INTRODUCTION

Coral reef ecosystems in the deep ocean are principally engineered by a few scleractinian cold-water coral (CWC) species that construct complex 3D reef framework habitats for a high associated biodiversity, and fuel reef biogeochemical cycles *via* suspension feeding and the continuous release of readily degradable particulate organic matter (POM) and dissolved organic matter (DOM) (Wild et al., 2008; Roberts et al., 2009; van Oevelen et al., 2009). Despite their accepted ecological significance to reef-associated fauna, our current understanding of CWC ecophysiology, particularly feeding ecology, is still in its infancy. This knowledge gap mainly results from the difficultly in accessing remote deep-ocean habitats, thus limiting the scope of non-invasive *in situ* studies, for instance on species-specific feeding behaviour. In addition, laboratory investigations on CWC physiology are rare because of the cost of acquisition at sea and the highly demanding maintenance in aquarium facilities (Olariaga et al., 2009). As a result, there is a significant lack of information regarding major food and energy sources fuelling basic but essential physiological processes, such as respiration, calcification and organic matter release, of these paramount deep-sea reef ecosystem engineers.

Unlike most of their tropical and temperate counterparts from shallow coastal waters, CWC are not associated with symbiotic dinoflagellates (i.e. zooxanthellae), which can provide substantial shares of coral metabolic demand by photosynthesis (Muscatine et al., 1981), but are believed to thrive exclusively from heterotrophic suspension feeding on POM (e.g. zooplankton) efficiently captured from surrounding waters (Purser et al., 2010; Tsounis et al., 2010). There is consensus based on studies employing lipid biomarkers, stable isotopes and *in situ* video surveys that zooplankton represents a potential dietary component for the dominant cosmopolitan CWC species (Kiriakoulakis et al., 2005; Dodds et al., 2009; van Oevelen et al., 2009; Purser et al., 2010; Tsounis et al., 2010). However, none of these studies have investigated physiological processes of live CWC specimens with respect to the availability of zooplankton food sources, and thus there is no direct evidence for the trophic significance of zooplankton feeding by CWC available to date.

This study investigated the influence of zooplankton feeding on key physiological processes in the cosmopolitan CWC *Desmophyllum dianthus* (Esper 1794) by laboratory experiments, principally aiming to (1) quantify rates of coral respiration, calcification and organic matter release with respect to zooplankton availability, and (2) evaluate the trophic significance of zooplankton as an organic C and energy source for scleractinian CWC. Our findings reveal the general trophic utilisation of zooplankton-derived organic compounds by CWC, and provide the first evidence for the principal trophic significance of zooplankton feeding in fuelling and sustaining levels of key physiological processes in these paramount deep-sea reef ecosystem engineers. This physiological evidence provides fundamental information for further research on

CWC habitats at the ecosystem level, as ecophysiological knowledge of engineering species is still scarce but ultimately essential to promote our understanding and sustainable management of deep-sea reef ecosystems.

MATERIALS AND METHODS

Coral collection and maintenance

Specimens of *D. dianthus*, known in the Mediterranean as *D. cristagalli* (Milne Edwards and Haime 1848), were collected alive within the South Malta Coral Province (35°30.506′N 14°06.230′E to 35°31.228′N 14°05.698′E, 467–632 m depth; and 35°30.720′N 14°06.561′E to 35°30.803′N 14°06.511′E; 452–585 m depth) using an epibenthic sledge on board the RV Urania during the cruise MARCOS (April 2007). This cosmopolitan species occurs at a depth of between 7 and 4000 m (Roberts et al., 2009), forms solitary polyps of 5–10 cm in height (diameter 1.5–3.0 cm) and has been documented as 'pseudocolonial' and a primary reef framework-constructor in the Pacific (Squires, 1965). Corals were transported to the laboratory and maintained in four identically equipped and darkened 100 l flow-through aquarium systems (Fig. 1A) located at the Monaco Scientific Centre (Monaco). Mediterranean subsurface seawater freshly pumped from 50 m depth was supplied at a flow-through rate of approximately 1 l min^{-1}, while water current speed created by aquarium pumps inside the systems (range 2–10 cm s^{-1}) was adjusted to optimum conditions reported for CWC zooplankton capture (Purser et al., 2010). Temperature close to *in situ* conditions (Freiwald et al., 2009) was established by cooling systems (Teco SeaChill TR 20, Ravenna, Italy) and 300 W heaters (Visi-Therm, Aquarium Systems Newa, Sarrebourg, France) connected to independent temperature controllers (West 6100, Kassel, Germany) regulating temperature at 12.0±0.2°C. Corals were fed 5 times per week (once per day) with frozen zooplankton (i.e. adult *Artemia salina*), and acclimatised under the above controlled conditions for 34 months prior to the experiments described herein.

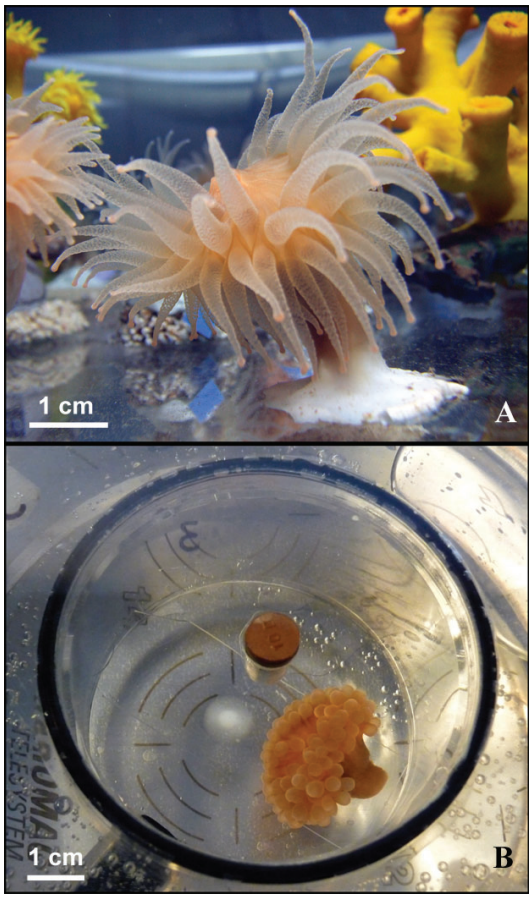

Fig. 1. Mediterranean *Desmophyllum dianthus* corals maintained under laboratory conditions. (A) Polyp in cold-water coral cultivation tank prior to zooplankton-exclusion treatment (photograph: A. Olariaga). (B) Polyp in respiration chamber during closed-cell incubation experiments.

Experimental design

One month before initial physiological measurements, 35 *D. dianthus* polyps of similar skeletal dry mass (±8% difference) were selected and transferred from the maintenance tanks into a 30 l darkened flow-through aquarium system. Selection by similar skeletal mass served to ensure comparability of CWC calcification rates with respect to age variability, assuming a close correlation of *D. dianthus* skeletal mass and age (Maier et al., 2009). Culture conditions were identical to those of the maintenance systems, except that the seawater supply was pre-filtered (pore size 50 µm). Pre-filtration prevented the introduction of additional zooplankton organisms from ambient seawater, which had previously been identified as the predominant POM-derived CWC dietary component in deep-sea reef habitats (Carlier et al., 2009). Supplementary zooplankton feeding was modified to a controlled daily supply of 4 *A. salina* adults (hereafter called zooplankton) per coral; a conservative measure considering solitary *D. dianthus* polyps are able to capture 8.48±2.97 *A. salina* per hour (Tsounis et al., 2010). *Artemia salina* adults served as close substitutes for locally occurring zooplankton taxa because of their similar composition and stimulatory effect on key coral physiological processes (Treignier et al., 2008; Tolosa et al., 2011). Zooplankton was pipetted onto protruded polyps, and subsequent capture and ingestion were closely monitored to ensure food intake. To determine daily organic C supply by zooplankton, the particulate organic C (POC) content of acidified (100 µl of 2 mol l^{-1} hydrochloric acid) and subsequently dried (40°C, 48 h) adult *A. salina* (N=12) was analysed. POC analysis was carried out using a Perkin Elmer 2400 Series II CHNS/O elemental analyser (Perkin Elmer, Waltham, MA, USA). Mean daily zooplankton-derived POC intake (i.e. 128±13 µmol POC day^{-1}) was calculated using certified glycine standards (K-factor; 32.00% C), and normalised to skeletal dry mass (46±5 µmol POC g^{-1} day^{-1}, mean ± s.d.). Feeding was suspended 24 h before physiological measurements of corals under fed conditions to rule out excretion of undigested particulate food items and any specific dynamic action effect during the period of incubation. Following initial measurements of corals under fed conditions, zooplankton feeding was suspended for a maximum of 3 weeks and measurements were repeated in a weekly time series (after 1, 2 and 3 weeks) to investigate the effects of zooplankton exclusion on coral respiration, calcification and organic matter release. To increase resolution at the beginning of this exclusion period, zooplankton-fed and unfed corals (1 week) were incubated in two incubation runs including five individual polyps each (results of respective runs were pooled). For 2 and 3 week unfed corals, one incubation experiment each including five individual polyps was carried out.

Physiological measurements

Integrated measurements of coral respiration, calcification and organic matter release rates were carried out by closed-cell incubation in temperature-controlled acrylic respiration chambers (N=6; volume 240 ml, water-jacketed), each equipped with an O_2 Clark electrode connected to a 6-channel recording system (Model

928; Strathkelvin Instruments, North Lanarkshire, UK). Corals were transferred individually without aerial exposure onto a glass slide support inside a pre-rinsed (3 times with purified and deionised water plus 3 times with incubation medium) incubation chamber filled with 50 µm pre-filtered seawater (one polyp per chamber). Pre-filtered seawater was used to prevent the introduction of zooplankton and subsequent feeding during incubations and to reduce the variability of measured total organic carbon (TOC) concentrations due to the sampling of scattered particles larger than 50 µm. Incubations in darkened coral chambers (N=5) and one control chamber, filled only with pre-filtered seawater, lasted for 6 h and were carried out at a chamber temperature of 12.0±0.1°C (Fig. 1B). Stirring inside the chambers was accomplished using glass-coated magnetic stir bars. Respiration rates were derived from depletion of dissolved O_2 recorded over the period of closed-cell incubation. Rates of coral calcification (i.e. skeletal growth) were determined by the total alkalinity (TA) anomaly technique assuming a consumption of 2 moles of alkalinity for every mole of calcium carbonate produced (e.g. Langdon et al., 2010). Organic matter release was measured by concentration differences of TOC in the incubation medium, and expressed as TOC net flux between the incubated coral and its surrounding seawater.

Before and after incubations, seawater subsamples were drawn by syringe from each chamber to determine TA, NH_4^+ and TOC concentrations of the incubation medium. The time of each sampling was recorded so measured concentration changes could be related to the period of incubation. Samples for TA analysis (60 ml) were sterile filtered through MQ pre-soaked (48 h) polyethersulfone (PES) membrane filters (0.2 µm pore size), treated with a saturated solution of the poison mercury chloride (20 µl) and kept refrigerated (4°C) pending analysis (no later than 7 days). TA was determined in 3–5 replicate Gran titrations with 0.1 mol l^{-1} HCl using a Titrando 888 titrator (Metrohm, Filderstadt, Germany). TA values were corrected for changes in NH_4^+ concentration in treatment and control chambers (Jacques and Pilson, 1980). NH_4^+ subsamples (10 ml) were sterile filtered (MQ pre-soaked PES filters, 0.2 µm pore size), immediately frozen (–20°C) and analysed by spectrofluorimetry (no later than 3 days). Tested concentrations of other inorganic nutrients (i.e. nitrate, nitrite and phosphate) potentially affecting TA values were below detection limit by an autoanalyser (Axflow, Stockholm, Sweden). TOC subsamples (17 ml, N=3 per chamber and sampling) were transferred into pre-combusted (450°C, 5 h) glass vials, acidified with phosphoric acid (20%, 250 µl) to pH<2 and kept frozen (–20°C) until analysis by high temperature catalytic oxidation using a Shimadzu TOC-VCPH analyser (Shimadzu Corporation, Kyoto, Japan; CV maximum ≤1.5%, i.e. ±1 µmol C l^{-1}, referenced by the CRM program of the Hansell Research Lab, USA; http://yyy.rsmas.miami.edu/groups/biogeochem/CRM.html).

Data analysis

For calculation of respiration, calcification and TOC net flux rates, differences in O_2, TA and TOC concentrations measured from the control chamber were subtracted from those measured in the coral chambers and the results were normalised as described below. Prior to normalisation, O_2 consumption rates were converted to C equivalents, as C respired (µmol)=O_2 consumed (µmol) × RQ, where RQ is a coral-specific respiratory quotient (i.e. 0.8) previously applied in studies on zooxanthellate as well as azooxanthellate tropical and temperate anthozoans (Muscatine et al., 1981; Widdig and Schlichter, 2001; Ribes et al., 2003). As corals were entirely covered by living tissue, all physiological parameters could be normalised to the very similar polyp-specific skeletal surface area and/or by polyp skeletal dry mass. Skeletal surface area (21.3±2.6 cm^2, mean ± s.d.) was quantified by advanced geometric techniques involving individual measurements of particular morphological sections of the coral polyps and subsequent computation using specific approximation factors. These factors were derived from comparison with techniques employing 3D reconstruction by computer tomography (Naumann et al., 2009). Skeletal dry mass (1.8±0.1 g, mean ± s.d.) was derived from calculation of the respective buoyant mass measured by precision balance (AT261, accuracy 0.1 mg; Mettler Toledo, Giessen, Germany) with a weight-below hook. After each incubation, polyps were weighed in a temperature-controlled (12.0±0.1°C) glass beaker filled with ambient seawater. Temperature and salinity were recorded to calculate seawater density, and skeletal aragonite density (mean 2.835 g cm^{-3}) was derived from individual skeletal micro-density measurements of eight dead *D. dianthus* polyps (Davies, 1989). Skeletal dry mass was corrected for the contribution of organic tissue biomass (5.8±2.3%, mean ± s.d.) derived from the relationship of ash-free dry mass (AFDM) to bulk dry weight examined for 10 additional zooplankton-fed corals. The percentage contribution of organic tissue biomass to bulk dry mass also allowed estimation of organic C flux into tissue growth by successive buoyant mass measurement of fed corals and computation assuming a comparable tissue AFDM organic C content, as previously reported for tropical Scleractinia and marine benthic macrofauna (41% and 40%, respectively) (Kang, 1999; Schutter et al., 2010).

Physiological process rates obtained from zooplankton-fed and unfed corals were analysed statistically using SPSS© software packages (v. 14.0, build 2005, IBM, New York, NY, USA). After confirming equal variances (Levene test) and normal distribution (Kolmogorov–Smirnov test), all results were analysed by one-way ANOVA and subsequent *post hoc* analysis appropriate for each specific parameter (respiration: Gabriel; calcification and TOC net flux: Games-Howell).

RESULTS

Respiration

O_2 consumption attributable to coral respiration was detected throughout all incubation experiments (3–17 µmol l^{-1} h^{-1}) and clearly distinguishable from background seawater microbial O_2 consumption measured in control chambers (0.2–1.0 µmol l^{-1} h^{-1}). Initial respiration rates of zooplankton-fed corals amounted to 3.0±0.8 µmol C cm^{-2} coral surface area day^{-1} (Fig. 2A) equivalent to 28±6 µmol C g^{-1} skeletal dry mass day^{-1} (both mean ± s.d.). Analysis of *D. dianthus* respiration rates revealed a significantly negative effect of zooplankton exclusion (one-way ANOVA, $F_{3,26}$=5.33, P<0.01). Respiration rates showed a remarkable negative trend (mean values 20% lower) after just 1 week. This trend continued after 2 and 3 weeks of exclusion, reaching 62% and 49% of that of zooplankton-fed corals, respectively (Fig. 2A). However, this decline became statistically significant only after 3 weeks of zooplankton exclusion (P=0.003).

Calcification

Changes in TA concentration were measurable after 6 h in coral incubation chambers (12–172 µmol kg^{-1}), while differences in seawater control chambers (1–5 µmol kg^{-1}) ranged within the limits of analytical precision (Langdon et al., 2010). NH_4^+ release by *D. dianthus*, used to correct of TA values, ranged from 1.7 to 14.4 µmol l^{-1} (control corrected), equivalent to release rates of 0.5±0.1 and 0.3±0.1 µmol NH_4^+ cm^{-2} coral surface area day^{-1} (means ± s.d.) for zooplankton-fed and 3 week unfed corals,

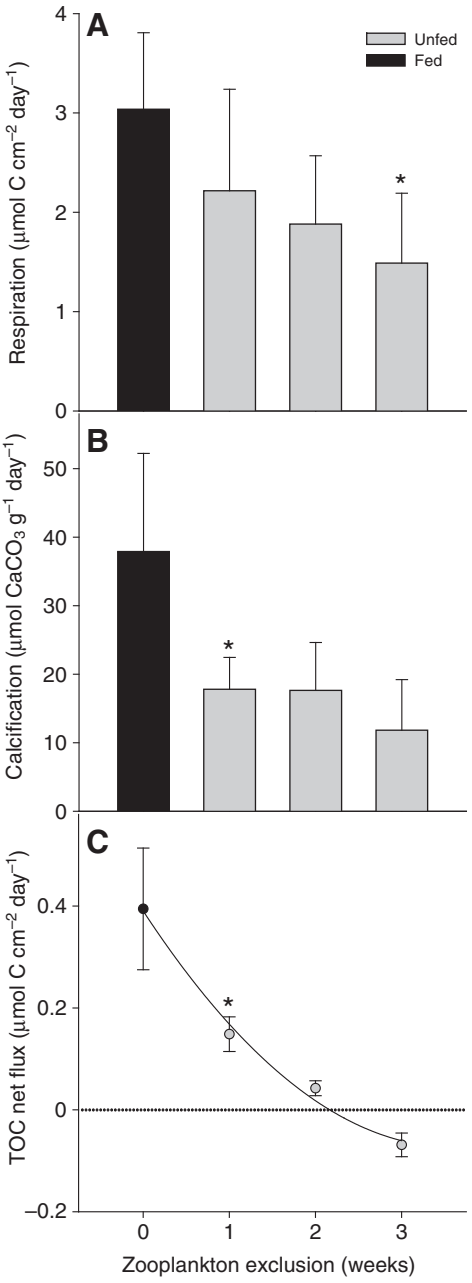

Fig. 2. Rates of key physiological processes in the scleractinian cold-water coral *D. dianthus* under zooplankton-fed and unfed conditions. (A) respiration, (B) calcification, (C) total organic C (TOC) net flux. Values are presented as means ± s.d. normalised to coral surface area (A,C) or skeletal dry mass (B). Asterisks indicate the first instance of a significant difference (*P<0.01) in unfed corals compared with initial (zooplankton-fed) conditions.

respectively. Calcification of zooplankton-fed *D. dianthus* was found to accrete 38±14 µmol $CaCO_3$ g^{-1} skeletal dry mass day^{-1} (Fig. 2B). As for coral respiration, exclusion of zooplankton had a significantly negative effect on coral calcification rates (one-way ANOVA, $F_{3,26}$=11.22, P<0.01). Calcification declined rapidly in unfed corals, showing a significant decrease (~53%) after 1 week (P=0.007). While remaining constant at this level after 2 weeks, calcification reached a minimum (31% of fed conditions) after 3 weeks in unfed corals (Fig. 2B). Integrated in bulk coral growth, the gain in tissue biomass of zooplankton-fed *D. dianthus* was estimated to average 5±1 µmol C g^{-1} day^{-1}.

Organic matter release

TOC concentration differences in coral incubation media were detectable throughout all experiments ranging from 2.1 to 16.3 µmol TOC l^{-1} after 6 h, while changes measured from control chambers (0.1–0.9 µmol TOC l^{-1}) stayed below analytical precision levels. TOC net flux between zooplankton-fed corals and the incubation medium was positive for all measured corals (range 0.21–0.59 µmol TOC cm^{-2} day^{-1}, mean ± s.d. 3±1 µmol TOC g^{-1} day^{-1}), indicating net release of particulate and/or dissolved organic compounds (Fig. 2C). This TOC net release was significantly affected by the exclusion of zooplankton (one-way ANOVA, $F_{3,26}$=44.85, P<0.01), decreasing substantially (by 38%) after 1 week (P=0.001), while continuing its significant decline after 2 weeks (P=0.024). Finally, measurements after prolonged zooplankton exclusion (3 weeks) provided evidence for significant TOC net uptake by *D. dianthus* (P<0.001; Fig. 2C).

DISCUSSION

Effect of zooplankton feeding on key physiological processes

This study provides the first information on key physiological process rates of CWC derived from interconnected laboratory measurements of respiration, calcification and organic matter release. This information is also unique in representing the only physiological data obtained from living specimens of the cosmopolitan CWC *D. dianthus* maintained under zooplankton-fed and unfed conditions. Our findings demonstrate that a substantial decrease in CWC calcification and organic matter release rates as a result of zooplankton exclusion is evident after a relatively short time period of 1 week. After prolonged (3 weeks) exclusion, respiration rates measured in *D. dianthus* also show a significant decline. This not only indicates the general trophic utilisation of zooplankton-derived organic compounds by *D. dianthus* but also provides evidence for the principal trophic significance of zooplankton feeding in fuelling and sustaining levels of CWC key physiological processes.

Our respiration rates for *D. dianthus* represent one of the very few data sets available for CWC to date (Dodds et al., 2007; van Oevelen et al., 2009) and provide the only information on respiratory metabolism for CWC in the Mediterranean sea, a temperate region where CWC are believed to thrive at their upper thermal threshold (12–14°C) (Freiwald et al., 2009). Respiration rates of zooplankton-fed *D. dianthus* averaged 3.0±0.8 µmol C cm^{-2} day^{-1}, but showed a continuous negative trend after short-term zooplankton exclusion. This negative trend became a significant decline (by 51%) after prolonged (3 weeks) zooplankton exclusion, thus clearly demonstrating the trophic significance of zooplankton in supporting CWC respiratory metabolism. This is confirmed by lowered NH_4^+ excretion rates found in unfed corals as a result of declining coral respiration (see Results). When recalculated to O_2 consumption and normalised by skeletal dry mass, respiration rates of fed *D. dianthus* (36.3±9.7 µmol O_2 g^{-1} day^{-1}) were substantially higher (5 times) than previous results (~7.2 µmol O_2 g^{-1} day^{-1}) from laboratory studies carried out on Atlantic specimens of the CWC *Lophelia pertus*a at elevated temperature (11°C) (Dodds et al., 2007). However, our recent studies on respiration of *L. pertusa* originating from the Mediterranean (M.S.N., unpublished) indicate that this difference probably reflects species-specific metabolism, possibly accompanied by an increase due to the higher experimental temperature within the present study (12°C) (Dodds et al., 2007). *Desmophyllum*

dianthus respiration rates are remarkably lower (~59%) than rates obtained for tropical Scleractinia in the dark (e.g. *Stylophora pistillata*: 4.0–7.4 μmol C cm^{-2} day^{-1}) (Houlbrèque et al., 2003), indicating a significantly reduced metabolic activity in CWC compared with their warm water counterparts. This may result from the positive correlation of coral respiration to increases in ambient temperature, as previously shown for symbiotic tropical and non-symbiotic cold-water Scleractinia (e.g. Edmunds, 2005; Dodds et al., 2007). In this context, respiration rates measured at tropical temperatures for zooxanthellate and azooxanthellate specimens of the temperate coral *Astrangia poculata* under zooplankton-fed and unfed conditions (fed 12.1–12.7 μmol C cm^{-2} day^{-1}, and unfed 3.1–3.5 μmol C cm^{-2} day^{-1}; non-symbiotic to symbiotic range) reveal only a very minor influence of dinoflagellate symbiosis, but emphasise the significantly positive metabolic effect of zooplankton feeding (Jacques and Pilson, 1980).

Previous studies on CWC growth have employed various techniques, e.g. linear skeletal extension, buoyant mass or radioactive $^{45}Ca^{2+}$ incorporation (Orejas et al., 2008; Orejas et al., 2011a; Orejas et al., 2011b; Maier et al., 2009). To our knowledge, this study represents the first opportunity to compare these earlier data sets with calcification rates obtained by the established and now widely applied TA technique. Calcification rates measured here for zooplankton-fed *D. dianthus*, expressed as % day^{-1} (0.1–0.3% day^{-1}), fall in the range of results previously obtained for scleractinian CWC (0.1–1% day^{-1}) (Maier et al., 2009; Orejas et al., 2011b), and are very similar (0.16±0.03 μmol $CaCO_3$ cm^{-2} h^{-1}) to rates generated for a temperate zooxanthellate species at comparable temperature and reduced zooxanthellae activity during the winter season (e.g. *Cladocora caespitosa*: 0.10–0.12 μmol $CaCO_3$ cm^{-2} h^{-1}) (Rodolfo-Metalpa et al., 2010), or to calcification rates by temperate azooxanthellate coral species (e.g. *A. poculata*: 0.13 μmol $CaCO_3$ cm^{-2} h^{-1}) (Jacques et al., 1983). However, compared with zooxanthellate tropical species (e.g. *Montastraea faveolata*: 0.58–8.11 μmol $CaCO_3$ cm^{-2} h^{-1}, dark to light calcification range), *D. dianthus* skeletal growth accounts for only 2% and 27% of light and dark calcification, respectively (Colombo-Pallotta et al., 2010). While this large difference probably reflects the effect of light-enhanced calcification in zooxanthellate corals (Allemand et al., 2011), contrasting findings of similar growth rates for the CWC *Madrepora oculata* in comparison to some zooxanthellate tropical (i.e. *Galaxea fascicularis*) or Mediterranean species (Orejas et al., 2011a) highlight the significance of zooplankton as an energy source for shallow and CWC taxa (Ferrier-Pagès et al., 2003; Houlbrèque et al., 2003; Ferrier-Pagès et al., 2011). This is further emphasised by the rapid and substantial decline in calcification rates observed here for *D. dianthus* as a result of zooplankton exclusion. Nonetheless, our findings also attest that *D. dianthus* calcification is functioning even after prolonged (3 weeks) zooplankton exclusion (at 69% reduced rate), which implies that, similar to other tropical, temperate and CWC species, different energy sources, e.g. tissue biomass (Barnes and Lough, 1993), uptake of nano- and micro-plankton (e.g. Orejas et al., 2003; Houlbrèque et al., 2004) and/or DOM (e.g. Grover et al., 2008) may serve to sustain calcification at low levels during the absence of zooplankton. Recently, this has been suggested for growth rates of temperate zooxanthellate corals in response to combined effects of darkness and zooplankton exclusion (Hoogenboom et al., 2010). Insufficient energy supply due to zooplankton exclusion may further promote a preferred investment in coral tissue biomass instead of skeletal growth, possibly reflected here by declining calcification rates (Anthony et al., 2002).

Organic matter release rates of zooplankton-fed *D. dianthus* measured in terms of TOC net release (0.4±0.1 μmol C cm^{-2} day^{-1}, mean ± s.d.) are in the lower range known for tropical Scleractinia (Naumann et al., 2010; Wild et al., 2010a) but, surprisingly, substantially lower than values reported for the CWC *L. pertusa* (~9.6 μmol TOC cm^{-2} day^{-1}) in the NE Atlantic (Wild et al., 2008). This substantial difference may suggest species-specific organic matter release rates, as found for tropical zooxanthellate corals (Naumann et al., 2010), possibly reflecting specific feeding strategies and physiological energy allocation, or be indicative of regional food quality and abundance. TOC net release by zooplankton-fed corals accounts for approximately 7% of daily zooplankton POC intake, but decreases significantly after just 1 week of zooplankton exclusion. This rapid decline implies that organic matter release is significantly affected by the availability of zooplankton as an energy source, and that organic compounds and/or energy derived from zooplankton are involved in the synthesis and exudation of coral-derived organic C compounds (e.g. as mucus) in *D. dianthus*, and perhaps scleractinian CWC in general. In addition, our finding of significant TOC net uptake (<50 μm) after prolonged zooplankton exclusion (3 weeks) accompanied by a decline in respiration and calcification rates suggest that uptake of DOC or non-zooplankton POC sources (Houlbrèque et al., 2004; Grover et al., 2008) is insufficient to sustain respiratory metabolism and skeletal growth at levels comparable to those of zooplankton-fed *D. dianthus*.

Budget of zooplankton-derived organic C in *D. dianthus*

Ingestion of zooplankton by fed *D. dianthus* polyps (46±5 μmol POC g^{-1} day^{-1}, see Materials and methods) represents ~1.6-fold of the corals' daily C demand in terms of respiration, which implies that zooplankton feeding can fully sustain respiratory metabolism. Respiratory organic C consumption thus constitutes a major fraction (48–73%; mean 61%) of daily zooplankton POC intake, while growth estimates of coral tissue biomass range from 10% to 14% (mean 12%). Continuous organic C release by *D. dianthus* into surrounding waters represents an additional C sink of 5–9% (mean 7%), thus adding up to ~80% (range 63–94%) explained fate for daily zooplankton-derived organic C intake. An additional ~20% fraction remains to be balanced by potential excretion of non-digested particulate zooplankton components, or more likely may reflect an underestimation of the actual organic C content of *D. dianthus* tissue. Coral respiration, as the potential major sink for zooplankton-derived organic C and thus a significant source of dissolved inorganic C (DIC) in the form of respiratory CO_2, may further importantly sustain the calcification process in this CWC. The chemical origin of DIC components predominantly responsible in calcium carbonate accretion is still under discussion for CWC (Adkins et al., 2003; Blamart et al., 2005), as for coral calcification in general (Allemand et al., 2011). However, if we assume a 70% contribution of respiratory CO_2 to calcium carbonate deposited by *D. dianthus* calcification (i.e. 27±10 μmol C g^{-1} day^{-1}), as previously reported for tropical Scleractinia (Furla et al., 2000), this rate nearly balances respiratory CO_2 production in zooplankton-fed corals (i.e. 28±6 μmol C g^{-1} day^{-1}), which strongly suggests respiration as a predominant DIC source for calcification in the CWC *D. dianthus*. This underlines the trophic significance of zooplankton-derived organic C by highlighting its secondary influence on physiological functioning in this CWC species.

Ecological implications

Respiration, calcification and organic matter release represent key physiological processes in scleractinian CWC, whose functioning

affects the physiological state of each solitary coral polyp and every single coral colony, but on a larger scale can also influence entire deep-sea reef habitats, where CWC control ecosystem functioning. The evolution of reef frameworks and dynamics of reef biogeochemical cycles are directly linked to the functioning of coral key physiological processes. Respiration and calcification allow for the deposition of large-scale carbonate frameworks acting as deep-sea hot spots for biomass, biodiversity and C cycling (Roberts et al., 2009; van Oevelen et al., 2009), while the release of organic matter in the form of C-rich POM and DOM (Wild et al., 2010b) to surrounding waters stimulates microbial activity in the direct reef vicinity, providing a vector for C and nutrient recycling *via* the microbial loop (Wild et al., 2008). In addition, CWC organic matter exudates are suggested to include compounds active in coral calcification and reef organomineralisation processes promoting reef framework stabilisation and growth (Freiwald and Wilson, 1998; Reitner, 2005). The physiological significance of zooplankton feeding revealed by the present study for the CWC *D. dianthus* can thus be projected to the ecosystem scale, as zooplankton-derived energy may eventually fuel CWC deep-sea reef ecosystem engineering. In this regard, additional physiological studies on feeding ecological aspects of the main cosmopolitan reef framework building CWC species (i.e. among others *L. pertusa*, *M. oculata* and *Oculina varicose*), accompanied by *in situ* observational studies on CWC zooplankton feeding and spatial distribution, may provide further insight into vital trophic pathways in deep-sea CWC habitats.

To advance our understanding of the role of zooplankton as a significant CWC food source, *in situ* investigations focusing on the ecology as well as on daily and seasonal vertical migrations of deep zooplankton populations associated to CWC habitats are required. These studies are still rare because of the difficulties associated with sampling zooplankton at greater depths, in particular close to the benthic boundary layer. Only very few recent studies have looked at the population and spatial dynamics of deep zooplankton communities (Koppelmann et al., 2003; Koppelmann and Weikert, 2007; Guidi-Guilvard et al., 2009). As a result, our current knowledge of deep-sea zooplankton ecology is still largely limited to more general aspects, such as zooplankton community composition (e.g. Hopkins et al., 1992). One key approach to understand the role of zooplankton as a significant food source for CWC could be diel vertical migrations observed for mesopelagic zooplankton species. As previously argued by Roberts and colleagues (Roberts et al., 2009), diel vertical migrations of specific components of the mesopelagic zooplankton community may constitute a substantial food supply mechanism for benthic deep-sea ecosystems, and especially for reef-dwelling CWC. The closer investigation of these potential trophic pathways represents currently one of the most challenging research topics dealing with benthic–pelagic coupling processes in deep-sea reef habitats.

Though occurring in deep oceanic zones, CWC reef ecosystems are strongly impacted by direct and indirect environmental threats of anthropogenic origin, such as bottom trawling and global climate change (Roberts et al., 2009). To increase our knowledge of the dynamics and essential habitat preconditions for CWC ecosystem development and also to assess the resilience and recovery capacity of CWC systems following disturbance, ecophysiological studies, such as the present one, must be carried out to provide fundamental information on crucial ecological aspects, such as growth, respiration or CWC reproduction. Further research is clearly needed to improve our understanding of basic processes playing a role in CWC ecophysiology, particularly in future scenarios in which primarily ocean acidification and warming are predicted to negatively influence CWC key physiological processes, such as calcification (Guinotte et al., 2006; Guinotte and Fabry, 2008; Maier et al., 2009). In addition to investigating deleterious climate change impacts, such as ocean acidification, on CWC physiology alone, there is a pressing need to study these impacts for specific groups within the zooplankton community representing potential CWC prey organisms (e.g. copepods). Negative physiological consequences for zooplankton prey organisms may result in decreasing prey availability, and consequently exacerbate the effect of climate change on key physiological processes acting in CWC, with further implications for deep-sea reef ecosystem functioning. However, this assumption requires further investigation, as our current knowledge of climate change effects, e.g. of ocean acidification, on the physiology of zooplankton organisms is still very limited (e.g. Fabry et al., 2008).

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