Uptake of dissolved free amino acids by four cold-water coral species from the Mediterranean Sea

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ABSTRACT

Dissolved organic matter, which contains many compounds such as lipids, sugars and amino acids, is an important source of carbon and nitrogen for several symbiotic and asymbiotic tropical coral species. However, there is still no information on its possible uptake by cold-water coral species. In this study, we demonstrated that dissolved organic matter, in the form of dissolved free amino acids (DFAA), is actively absorbed by four cold-water coral species from the Mediterranean Sea. Although the uptake rates observed with 3 μM DFAA concentration were one order of magnitude lower than those observed in tropical species, they corresponded to 12–50% of the daily excreted-nitrogen, and 16–89% of the daily respired-carbon of the cold-water corals. Consequently, DFAA, even at in situ concentrations lower than those tested in this study, can supply a significant amount of carbon and nitrogen to the corals, especially during periods when particulate food is scarce.

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1. Introduction

Distribution and abundance of cold-water coral (CWC) species are strongly influenced by several abiotic factors such as seawater temperature and density, aragonite saturation horizon, presence of appropriate substratum, and water flow regimes (Dullo et al., 2008; Orejas et al., 2009; Roberts et al., 2009a). Enhanced flows limit coral smothering by sediment, and play a crucial role in food supply (Roberts et al., 2009b), which has been considered as one of the main biotic factors explaining CWC distribution (Frederiksen et al., 1992; Mortensen et al., 2001; Kenyon et al., 2003; Thiem et al., 2006; Davies et al., 2009). Due to the major role of feeding in the ecology and physiology of coral species (Szmant-Froelich and Pilson, 1984; Naumann et al., 2011), several studies have investigated the diet of CWCs, and have demonstrated that they feed on a variety of food sources including phytodetritus, phytoplankton, and mesozooplankton (Duineveld et al., 2004, 2007, 2012; Kiriakoulakis et al., 2005; Carlier et al., 2009; Dodds et al., 2009), mainly herbivorous calanoid and omnivorous or carnivorous copepods (Henrich and Freiwald, 1997; Freiwald, 2002; Dodds et al., 2009; Roberts et al., 2009b). Laboratory experiments have also shown that CWCs can actively capture both dead and live particles (Mortensen, 2001; Roberts and Anderson, 2002), such as

Artemia salina nauplii and adults (Purser et al., 2010; Tsounis et al., 2010). However, there is currently no information on their capacity to take up smaller particles, such as pico and nanoplankton, as well as dissolved organic matter.

Dissolved organic matter contains many biochemically identifiable classes of compounds, such as lipids, sugars and amino acids (Hansell and Carlson, 2001). Dissolved free amino acids (DFAA) have several origins, from phytoplankton exudates or consumption by zooplankton (Lee and Bada, 1977), to microbial decomposition of the sedimentary organic matter (Jørgensen, 1982; Bronk et al., 1994). Overall DFAA represent approximately 10% of the dissolved organic nitrogen in seawater (Sharp, 1983; Tada et al., 1998), and have been shown to be a potential food source for a wide range of marine invertebrates (e.g. Stephens and Schinske, 1961; Ferguson, 1982; Wright and Manahan, 1989; de Goeij et al., 2008a, 2008b). Although coral species are known to release dissolved organic matter into the seawater (Wild et al., 2008, 2010 and reference therein), some species are also known to actively take it up in the form of DFAA (Ferrier, 1991; Al-Moghrabi et al., 1993; Hoegh-Guldberg and Williamson, 1999; Grover et al., 2008). The fact that most animals have retained the capacity to take up dissolved organic matter indicates that it can be used as a source of nutrients and energy, especially when particulate food is scarce (Ferguson, 1982; Ambariyanto and Hoegh-Guldberg, 1999). The uptake of DFAA was indeed found to supply from 40% to 80% of the energetic requirements of some tropical coral species (Sorokin, 1981; Schlichter, 1982), while the

entire metabolic rates could be sustained by DFAA in a temperate sea anemone (Schlichter, 1978).

Based on previous observations made on coral species, and given that no study has yet been performed on CWCs, we investigated the capacity of the four main CWC species from the Mediterranean Sea to actively take up dissolved organic matter in the form of DFAA. Subsequently, after quantifying the coral respiration and nitrogen excretion rates, we estimated the possible contribution of DFAA to the daily respired-carbon and excreted-nitrogen (Jørgensen, 1979; Szmant-Froelich and Pilson, 1984). The final aim of this work was to increase our knowledge on the trophic ecology of CWC species, and the role they can play in the marine food webs.

2. Materials and methods

2.1. Coral collection and maintenance

Specimens of *Lophelia pertusa* (Linnaeus, 1758), *Madrepora oculata* Linnaeus, 1758, *Desmophyllum dianthus* (Esper, 1794), and *Dendrophyllia cornigera* (Lamarck, 1816) were collected in the Cap de Creus Canyon (northwestern Mediterranean Sea, 42° 23′ 20″N; 003° 18′ 50″E). Highly energetic hydrodynamic and sedimentary processes, mainly linked to cold dense shelf water cascading events, continuously occur in the Cap de Creus Canyon (Canals et al., 2006, 2009) with down-canyon current velocities up to 80 cm s^{-1}, maximum suspended sediment concentrations of 68 mg L^{-1}, and maximum fluxes of $52 \text{ g m}^{-2} \text{ s}^{-1}$ (Palanques et al., 2006, 2012). Cap de Creus Canyon therefore acts as a preferential conduit of the dense shelf waters and associated suspended organic particles towards the slope region (Palanques et al., 2006, 2012) which sustain a high biological productivity inside the canyon (Orejas et al. 2009; Canals et al., 2009). Coral samples were collected from 200 to 400 m depth by means of the Remotely Operated Vehicle (ROV) PHANTOM HD2+2 and the manned submersible JAGO (IFM-GEOMAR, Kiel, Germany), and maintained alive on board the RV 'García del Cid' during the cruises 'Deep Coral I_Coral4′ (July 2006) and 'HERMES IV_Coral8' (September 2007). Corals were then transported to the Centre Scientifique de Monaco (Monaco) and maintained in darkened 100 L tanks, with a continuous flow of Mediterranean seawater freshly pumped from 50 m depth at a rate of approximately 60 L h^{-1}. Water temperature was maintained close to in situ conditions ($12 \pm 1.0 \text{ °C}$) by chillers and 300 W heaters connected to independent temperature controllers. Two submersible pumps provided continuous water movement with a flow rate of 570 L h^{-1}. Corals were fed twice a week with frozen *Mysis* (Crustacea, Eumalacostraca) and *Cyclops* (Crustacea, Copepoda). The polyp surface (*PS*) of each coral specimen was determined by means of advanced geometry (Naumann et al., 2009) according to the equation $PS = \pi \cdot (r+R) \cdot a + \pi \cdot R^2$, where r and R represent respectively the basal and apical polyp radius, and a is the apothem, measured with a caliper (Rodolfo-Metalpa et al., 2006).

2.2. Calibration of DFAA measurements in the presence of ammonium

The protocol used to measure DFAA uptake rates by the CWCs was adapted from the one previously used on tropical symbiotic corals by Grover et al. (2008) and Tremblay et al. (2012), after Parsons et al. (1984). This fluorometric method is precise, simple, with no needs for high-performance liquid chromatography (HPLC), and therefore very useful for routine analysis. A fluorescent reagent, o-phthalaldehyde, reacts with the primary amines of the DFAA in the presence of 2-mercaptoethanol, and the fluorescent products can be easily measured using a fluorometer. This technique works perfectly when there is no ammonium in seawater, because ammonium reacts positively with the fluorometric reagent o-phthalaldehyde, leading in this case to an overestimation of DFAA concentrations (Tada et al., 1998). Conversely to tropical corals which take up ammonium (Muscatine and D'Elia, 1978), CWCs excrete large amounts of ammonium during a few hours of incubation, and the DFAA measurement should therefore be corrected. For this purpose and for each incubation trial (see Section 2.3), a set of 20 standards was prepared using 50 μm pre-filtered seawater and a combination of DFAA (in the form of an algal mix, see Section 2.3) (0, 1.0, 2.0 and 3.0 μM) and ammonium (0, 1.0, 3.45, 7.28, 9.62 μM) concentrations. Emission intensity of the fluorescence produced by the reaction with o-phthalaldehyde in each standard was quantified using a spectrofluorometer Xenius (Safas) following the protocol of Grover et al. (2008), after Parsons et al. (1984). Samples were excited at 342 nm wavelength, and emission intensity was measured at 452 nm. Photomultiplier voltage was set up to 600 V, and excitation and emission bandwidths to 10 nm. The R-language function Lm of the R software platform (R Core Team, 2012) was used to perform multiple linear regressions between the emission intensities (Y) measured for the 20 standards, and the DFAA (x_1) and ammonium ($x2$) concentrations ($Y = \beta_0 + \beta_1 \cdot x_1 + \beta_2 \cdot x_2$). Afterwards, the R-language function Summary was used to (1) test the adequacy of the obtained equations by means of an analysis of variance (ANOVA), (2) calculate the multiple regression coefficients of determination (R^2), and (3) assess the significance of DFAA and ammonium concentrations in the explanation of the emission intensity. The equations of the regression plane obtained for each incubation trial were used to determine the DFAA concentration, corrected for the presence of ammonium measured in each sample of the subsequent incubations.

2.3. Uptake of DFAA

Uptake rates of DFAA by the four studied CWCs were assessed using an algal mix containing 19 different amino acids (Algal Amino Acid Mixture, ULM-2314-1, Lot PR-19236, Larodan Fine Chemicals AB, 220 Sweden), whose composition is close to the natural seawater DFAA composition (Tremblay et al., 2012). All control and experimental incubations with CWC specimen were performed in five replicates using beakers filled with 200 ml seawater pre-filtered on 50 μm, maintained at a constant temperature of $12 \pm 0.5 \text{ °C}$ in a water bath, and enriched to a concentration of 3.0 ± 0.1 μM DFAA. Pre-filtration was performed on a 50 μm filter to keep the microbial community in all control and experimental incubations. Water movement inside each beaker was obtained by means of a teflon-coated magnetic stirrer, and incubations ran for 6 h. Seawater samples were taken from each beaker at the beginning and end of the incubations to quantify DFAA and ammonium concentration. Samples for DFAA analysis (5 ml) were sterile filtered (0.2 μm), and quantified as described above (see Section 2.2). Samples for ammonium analysis (5 ml) were sterile filtered (0.2 μm) and were kept frozen (-20 °C) until concentration of ammonium was determined by means of the spectrofluorometric method of Holmes et al. (1999) adapted by Godinot et al. (2011). DFAA concentrations were corrected for the presence of ammonium using the equations of the regression plane presented above (see Section 2.2). To assess if changes in DFAA concentration in the incubations with coral specimens can truly be explained by the uptake of the corals, three different control treatments were performed for each species. The first control consisted in five beakers only filled with pre-filtered seawater sampled in the CWCs' aquaria, to assess DFAA changes due to bacteria and other microorganisms present in seawater.

The second control consisted in five beakers filled with pre-filtered seawater, and each containing a dead coral skeleton to assess the potential DFAA adsorption on dead material. The third control consisted in assessing DFAA changes due to bacteria present on coral mucus (Wild et al., 2008). For this purpose five beakers filled with pre-filtered seawater, and each containing a coral nubbin, were incubated without DFAA addition, during 6 h. Nubbins were then removed, leaving the released mucus and its associated bacteria in the beakers. DFAA were then added to the beakers, which were again incubated during 6 h.

Mean variation in the DFAA measured from the control beakers was subtracted from those measured in the coral beakers, and DFAA uptake rates were derived from the variation over the 6 h incubation. All results, expressed as mean ± standard deviation, were normalized to the coral surface area, in order to be comparable with previous results obtained with other scleractinian coral species. Moreover, DFAA uptake rates were also converted to carbon and nitrogen (μmol) equivalents by taking into account the composition of the DFAA mix and the total amount of carbon and nitrogen contained in each amino acid. Normal distribution of the data was tested using the Kolmogorov–Smirnov test performed with the R-language function ks.test, whereas homogeneity of variances was tested by means of the Bartlett test performed with the R-language function bartlett.test. Since variances were not homogeneous, the significance of DFAA depletion rates in coral compared to controls, as well as interspecific differences in DFAA uptake rates were tested with the non-parametric Kruskal–Wallis test performed with the R-language function kruskal.test. When significant differences were observed, a posteriori non-parametric multiple comparisons for relative contrast effects were performed with the R-language function nparcomp which is available in the nparcomp library.

2.4. Coral respiration and excretion

Coral respiration and excretion rates were assessed in incubations performed with five specimens from each studied species. Each specimen was incubated in a 370 ml beaker, completely filled with 50 μm pre-filtered seawater, closed with a plastic membrane, and maintained at a constant temperature of $12 \pm 0.5\ °C$ in a water bath. A control beaker without coral was run in parallel. Water movement inside each beaker was obtained by means of a teflon-coated magnetic stirrer, and incubations ran for 6 h. Respiration rates were assessed by determining oxygen concentration from each beaker, at the beginning and end of the incubations, using a multiparametric sensor (Hach HQ 40b, precision $0.2\ mg\ L^{-1}$). Variation in the oxygen concentration measured from the control beaker was subtracted from those measured in the coral beakers, and respiration rates were derived from the recorded depletion of dissolved oxygen over the incubation. Oxygen consumption rates were converted to carbon equivalents (μmol) according to the equation $C\ respired = O_2\ consumed \cdot RQ$, where RQ is a coral-specific respiratory quotient equal to 0.8 mol C:mol O_2 (Muscatine et al., 1981; Anthony and Fabricius, 2000; Widdig and Schlichter, 2001; Ribes et al., 2003). Excretion rates were assessed by determining ammonium concentration in seawater samples (5 ml) taken from each beaker at the beginning and end of the incubations. Samples were sterile filtered (0.2 μm) and were kept frozen ($-20\ °C$) until concentration of ammonium was determined by means of the spectrofluorometric method of Holmes et al. (1999). All results were normalized to the coral surface area, and are expressed as mean ± standard deviation. After testing the normal distribution of the data and the homogeneity of variances with the R-language functions ks.test and bartlett.test, interspecific differences in respiration and excretion rates were tested by means of the non-parametric Kruskal–Wallis test performed with

the function kruskal.test, since variances were not homogeneous. When significant differences were observed, a posteriori non-parametric multiple comparisons for relative contrast effects were performed with the function nparcomp.

3. Results

3.1. Calibration of DFAA measurements in the presence of ammonium

Multiple linear regressions adequately represented the relationship between the relative fluorescence intensity measured, and the DFAA and ammonium concentrations (ANOVA, $p < 0.001$). Both DFAA (t test, $p < 0.001$) and ammonium (t test, $p < 0.001$) concentrations resulted to be significant in the explanation of the emission intensity. The regression plans obtained (Fig. 1 and Table 1) presented high regression coefficients of determination ($R^2 > 0.997$). Thus, the equation obtained for each incubation were confidently used to calculate the DFAA concentration, while correcting for the effect of the measured ammonium concentration.

3.2. Uptake of DFAA

Significant depletion of DFAA by coral nubbins compared to the controls was observed in *L. pertusa* (Kruskal–Wallis test, $p = 0.007$), *D. cornigera* (Kruskal–Wallis test, $p = 0.004$), *M. oculata* (Kruskal–Wallis test, $p = 0.006$), and *D. dianthus* (Kruskal–Wallis test, $p = 0.013$) (Table 2), whereas no significant differences were observed among the control treatments for all the species (Table 3). Normalized to the coral surface area, DFAA uptake rates were equivalent to $0.083 \pm 0.022\ \mu mol\ cm^{-2}\ d^{-1}$ for *L. pertusa*, $0.066 \pm 0.010\ \mu mol\ cm^{-2}\ d^{-1}$ for *M. oculata*, 0.075 ± 0.022 $\mu mol\ cm^{-2}\ d^{-1}$ for *D. dianthus*, and $0.050 \pm 0.010\ \mu mol\ cm^{-2}\ d^{-1}$ for *D. cornigera* (Fig. 2), without significant differences among the four species (Kruskal–Wallis test, $p = 0.063$). In terms of total carbon and nitrogen ingested (Table 2), these rates corresponded to 0.242–$0.400\ \mu mol\ C\ cm^{-2}\ d^{-1}$ and 0.060–0.100 $\mu mol\ N\ cm^{-2}\ d^{-1}$, respectively.

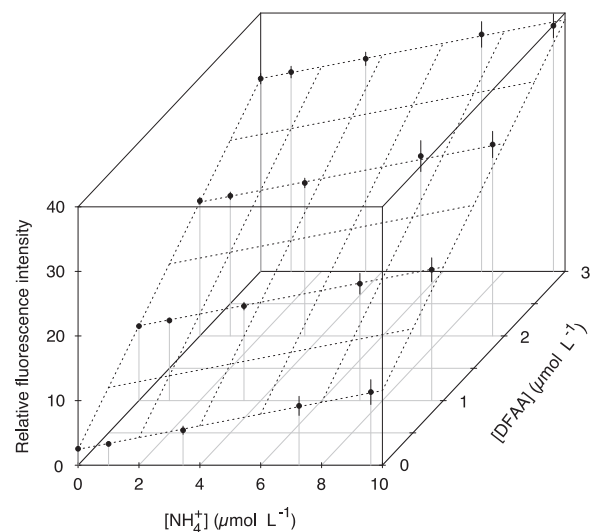**Fig. 1.** Emission intensity of the fluorescence (mean ± SD) measured in the 20 standards prepared for each incubation using 50 μm pre-filtered seawater and a combination of DFAA and ammonium concentrations. Regression plan obtained by multiple linear regressions, represents the relationship between the relative fluorescence intensity measured, and the DFAA and ammonium concentrations.

Table 1

Relative fluorescence intensity (RFI) produced by the reaction (see Section 2.2) in the standards prepared using a combination of DFAA and ammonium concentrations.

DFAA (μM)	Ammonium (μM)	RFI	
		Mean	SD
0	0	2.54	0.31
	1	3.30	0.27
	3.45	5.41	0.64
	7.28	9.18	1.48
	9.62	11.32	1.92
1	0	11.53	0.29
	1	12.41	0.38
	3.45	14.63	0.60
	7.28	18.09	1.60
	9.62	20.26	1.85
2	0	20.92	0.56
	1	21.70	0.59
	3.45	23.70	0.74
	7.28	27.86	2.40
	9.62	29.65	2.04
3	0	29.85	0.82
	1	30.86	0.90
	3.45	32.92	1.03
	7.28	36.69	1.98
	9.62	38.03	1.85

3.3. Coral respiration and excretion

Oxygen depletion observed in the incubation chambers (0.4–6.0 μmol L^{-1} h^{-1}) was clearly distinguishable from oxygen depletion in control chambers attributable to microbial respiration (< 0.3 μmol L^{-1} h^{-1}). Normalized to the coral surface area, respiration rates were equivalent to 0.47 $\pm$ 0.15 μmol C cm^{-2} d^{-1} for *L. pertusa*, 0.64 $\pm$ 0.14 μmol C cm^{-2} d^{-1} for *M. oculata*, 1.34 $\pm$ 0.31 μmol C cm^{-2} d^{-1} for *D. dianthus*, and 1.57 $\pm$ 0.32 μmol C cm^{-2} d^{-1} for *D. cornigera* (Fig. 3a), with significant differences among the four species (Kruskal–Wallis test, $p=0.002$), with *L. pertusa* and *M. oculata* respiring significantly less than *D. dianthus* and *D. cornigera* ($p < 0.001$). The observed uptake of DFAA covered the following amount of daily respired-carbon: 88.6 $\pm$ 21.2% for *L. pertusa*, 51.1 $\pm$ 6.8% for *M. oculata*, 27.4 $\pm$ 5.9% for *D. dianthus*, and 15.6 $\pm$ 2.5% for *D. cornigera*.

Changes in ammonium concentration observed in the incubation chambers (0.27–1.76 μmol L^{-1} h^{-1}) were clearly distinguishable from the changes observed in control chambers (< 0.15 μmol L^{-1} h^{-1}). Normalized to the coral surface area, ammonium excretion rates were equivalent to 0.37 $\pm$ 0.07 μmol N cm^{-2} d^{-1} for *L. pertusa*, 0.16 $\pm$ 0.02 μmol N cm^{-2} d^{-1} for *M. oculata*, 0.40 $\pm$ 0.15 μmol N cm^{-2} d^{-1} for *D. dianthus*, and 0.52 $\pm$ 0.20 μmol N cm^{-2} d^{-1} for *D. cornigera* (Fig. 3b), with significant differences among the four species (Kruskal–Wallis test, $p=0.011$), with *M. oculata* excreting significantly less than the other species ($p < 0.001$). The observed uptake of DFAA covered the following amount of daily excreted-nitrogen: 26.7 $\pm$ 3.9% for *L. pertusa*, 49.6 $\pm$ 5.1% for *M. oculata*, 24.0 $\pm$ 6.8% for *D. dianthus*, and 12.1 $\pm$ 3.2% for *D. cornigera*.

4. Discussion

This study assesses, for the first time, the capacity of four CWC species to take up dissolved organic matter in the form of DFAA from the surrounding waters. The effectiveness of corals for taking up DFAA depends on DFAA concentration and composition in seawater (Grover et al., 2008), which are both currently unknown

in CWC environments. The concentration tested in this study (3.0 μmol L^{-1}) is high compared to the values usually measured in open ocean (0.1–1.0 μmol L^{-1}, Lee and Bada, 1977; Henrichs and Williams, 1985; Keil and Kirchman, 1999) and tropical coral reefs waters (0.05–1.0 μmol L^{-1}, Ferrier, 1991; Hoegh-Guldberg, 1994; Ben-David-Zaslow and Benayahu, 2000 and references herein). However, CWCs are reported to be associated with areas of high surface primary productivity (Guinotte et al., 2006; Davies et al., 2008), which are also correlated with high DFAA concentrations (Poulet et al., 1985). During downwelling events, these surface waters can be rapidly advected to the bottom where CWC dwell (Roberts et al., 2009a), likely providing pulses of high concentrations of particulate and dissolved organic matter. In the Mediterranean Sea, downwellings occur simultaneously with spring phytoplankton blooms, advecting fresh organic matter to the submarine canyons of the Gulf of Lions and the Adriatic Sea (Palanques et al., 2006; Fabres et al., 2008; Canals et al., 2009), where dense CWC populations have been observed (Taviani et al., 2005; Orejas et al., 2009; Vertino et al., 2010; Gori et al., 2013). Interplays between topography and tides also lead to rapid downwellings of fresh organic matter to the CWC reefs in the Sea of the Hebrides (northeast Atlantic) (Davies et al., 2009; Roberts et al., 2009b; Duineveld et al., 2012). Moreover, near bottom waters (Ferguson, 1982) and interstitial sediment waters (North, 1975; Henrichs and Farrington, 1979; Jørgensen and Kristensen, 1980) contain significantly more DFAA (4–13 μmol L^{-1}) than that higher in the water column. This may increase the possibility for CWCs to be exposed to somewhat elevated DFAA concentrations, at least when sediment is removed by bioturbation or strong currents. Finally, DFAA concentrations may be increased adjacent to corals over very small spatial and temporal scales, for example during prey capture, when nematocyst discharges are believed to cause leakage of body fluids (including DFAA) from zooplankters (Ferrier, 1991).

Although DFAA concentrations surrounding CWCs remain to be precisely estimated, uptake rates of 3 μM DFAA, measured in this study, can be compared to those estimated with other coral species performed with the same DFAA concentration. Uptake rates by CWCs (from 0.050 to 0.083 μmol DFAA cm^{-2} d^{-1}) are thus one order of magnitude lower than those observed in tropical corals (from 0.10 to 0.82 μmol DFAA cm^{-2} d^{-1}; Ferrier, 1991; Hoegh-Guldberg and Williamson, 1999; Grover et al., 2008; Tremblay et al., 2012). This discrepancy might be explained by a different thermal environment, which affects the affinity of the DFAA carrier systems. Indeed, the transport of amino acids in corals is an active process, mediated by either Na-dependent or Na-independent carrier systems (Al-Moghrabi et al., 1993). Their kinetic parameters, such as the affinity constant, were shown to be dependent on both seawater temperature and coral trophic status (Al-Moghrabi et al., 1993; Shotwell et al., 1983). Temperature effect has also been clearly demonstrated with bacteria, with a negative correlation between temperature and the response time to DFAA enrichment (Shiah and Ducklow, 1994).

Concerning nitrogen excretion, rates measured for the CWC species (from 0.16 to 0.52 μmol N cm^{-2} d^{-1}) were similar to values observed in asymbiotic tropical corals (~0.50 μmol N cm^{-2} d^{-1}, Szmant et al., 1990), but lower than those observed in temperate species (1.17–2.65 μmol N cm^{-2} d^{-1}, Szmant-Froelich and Pilson, 1984). In deep waters, nitrogen excretion by corals is an obligatory step in resupplying pools of dissolved inorganic nitrogen (DIN) that sustain a high microbial activity in the vicinity of the corals (Wild et al., 2008). An intense coral–microbial interaction via coral mucus production has indeed already been highlighted (Wild et al., 2008), and the release of ammonium, directly usable by bacteria is thus an additional tool to maintain this dynamic microbial loop. There were notable differences in nitrogen excretion between the CWC

Table 2
Incubations performed for each studied coral species and control treatment. When coral nubbins were incubated, their weight, surface and number of polyps is reported. The observed variation in DFAA is reported for each incubation, and its equivalence in carbon and nitrogen was calculated for the corals incubations, by taking into account the composition of the DFAA mix and the total amount of carbon and nitrogen contained in each amino acid.

Species	Treatment	Replicates	Weight (g)	Surface (cm^2)	Number of polyps	Δ DFAA (µmol)	Equivalent ingestion	
							C (µmol)	N (µmol)
Lophelia pertusa	Corals	1	3.92	20.5	7	0.310	1.504	0.374
		2	2.23	11.8	8	0.281	1.364	0.340
		3	7.64	21.8	7	0.368	1.783	0.444
		4	1.29	7.8	3	0.197	0.957	0.238
		5	1.67	7.6	4	0.234	1.135	0.283
	Skeletons	1	1.56	7.4	5	0.014		
		2	1.48	5.6	6	0.020		
		3	2.36	7.9	8	0.006		
		4	5.91	13.9	8	0.013		
		5	3.05	12.5	6	0.034		
	Mucus	1	3.92	20.5	7	0.025		
		2	2.23	11.8	8	0.102		
		3	7.64	21.8	7	0.027		
		4	1.29	7.8	3	0.014		
		5	1.67	7.6	4	0.033		
	Seawater	1				0.030		
		2				0.013		
		3				0.012		
		4				0.011		
		5				0.034		
Madrepora oculata	Corals	1	1.64	7.3	18	0.142	0.690	0.172
		2	1.96	8.6	25	0.174	0.844	0.210
		3	2.46	13.3	24	0.181	0.877	0.218
		4	2.64	12.2	16	0.217	1.052	0.262
		5	1.87	9.9	27	0.161	0.779	0.194
	Skeletons	1	0.85	6.1	11	0.021		
		2	0.81	8.0	12	0.035		
		3	1.52	6.4	12	0.010		
		4	1.09	6.8	9	0.027		
		5	1.19	6.7	11	0.001		
	Mucus	1	1.64	7.3	18	0.011		
		2	1.96	8.6	25	0.005		
		3	2.46	13.3	24	0.002		
		4	2.64	12.2	16	0.004		
		5	1.87	9.9	27	0.003		
	Seawater	1				0.020		
		2				0.007		
		3				0.008		
		4				0.003		
		5				0.013		
Desmophyllum dianthus	Corals	1	5.57	15.7	1	0.389	1.888	0.470
		2	7.07	21.3	1	0.260	1.260	0.314
		3	4.42	12.9	1	0.313	1.518	0.378
		4	3.86	12.8	1	0.287	1.392	0.346
		5	7.30	18.3	1	0.285	1.381	0.344
	Skeletons	1	2.14	11.0	1	0.027		
		2	1.38	7.2	1	0.001		
		3	2.64	17.1	1	0.025		
		4	1.12	9.3	1	0.018		
		5	1.45	10.1	1	0.005		
	Mucus	1	5.57	15.7	1	0.010		
		2	7.07	21.3	1	0.011		
		3	4.42	12.9	1	0.021		
		4	3.86	12.8	1	0.031		
		5	7.30	18.3	1	0.012		
	Seawater	1				0.028		
		2				0.006		
		3				0.011		
		4				0.018		
		5				0.015		
Dendrophyllia cornigera	Corals	1	8.78	21.3	2	0.248	1.201	0.299
		2	4.93	13.7	1	0.134	0.648	0.161
		3	4.65	12.6	3	0.128	0.619	0.154
		4	2.06	8.2	1	0.111	0.536	0.133
		5	10.43	18.4	1	0.291	1.414	0.352
	Skeletons	1	2.11	9.9	1	0.043		
		2	6.39	14.9	2	0.105		
		3	0.66	4.7	1	0.006		
		4	2.00	9.3	1	0;001		

Table 2 (continued)

Species	Treatment	Replicates	Weight (g)	Surface (cm²)	Number of polyps	Δ DFAA (μmol)	Equivalent ingestion	
							C (μmol)	N (μmol)
		5	0.87	3.4	1	0.003		
	Mucus	1	8.78	21.3	2	0.008		
		2	4.93	13.7	1	0.010		
		3	4.65	12.6	3	0.014		
		4	2.06	8.2	1	0.018		
		5	10.43	18.4	1	0.018		
	Seawater	1				0.013		
		2				0.004		
		3				0.001		
		4				0.004		
		5				0.001		

Table 3

Nonparametric multiple comparison procedure of the DFAA variation in the incubations with coral nubbins and the control treatments (see Section 2.3); significant p-values are indicated with one (p-value < 0.05), two (p-value < 0.01), or three asterisks (p-value < 0.001).

| Species	Treatments	t	p-value	
Lophelia pertusa	Corals/skeleton	−9.76	< 0.001	***
	Corals/mucus	−9.76	< 0.001	***
	Corals/seawater	−9.76	< 0.001	***
	Skeleton/mucus	1.28	0.706	
	Skeleton/seawater	0.09	0.999	
	Mucus/seawater	0.99	0.879	
Madrepora oculata	Corals/skeleton	−9.76	< 0.001	***
	Corals/mucus	−9.76	< 0.001	***
	Corals/seawater	−9.76	< 0.001	***
	Skeleton/mucus	−1.06	0.831	
	Skeleton/seawater	0.94	0.891	
	Mucus/seawater	−1.33	0.658	
Desmophyllum dianthus	Corals/skeleton	−9.76	< 0.001	***
	Corals/mucus	−9.76	< 0.001	***
	Corals/seawater	−9.76	< 0.001	***
	Skeleton/mucus	0.273	0.999	
	Skeleton/seawater	−0.18	0.999	
	Mucus/seawater	0.192	0.999	
Dendrophyllia cornigera	Corals/skeleton	−9.76	< 0.001	***
	Corals/mucus	−9.76	< 0.001	***
	Corals/seawater	−9.76	< 0.001	***
	Skeleton/mucus	0.40	0.999	
	Skeleton/seawater	0.87	0.934	
	Mucus/seawater	1.92	0.275	
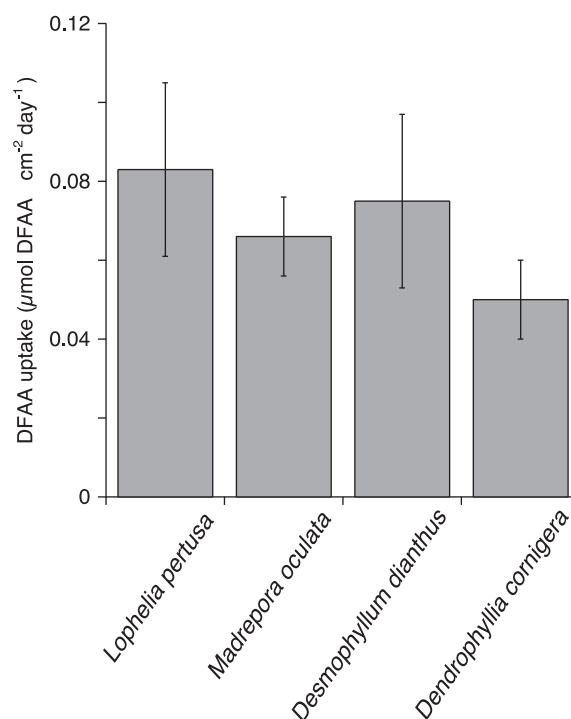
Fig. 2. Dissolved free amino acid (DFAA) uptake rates (mean $\pm$ SD) for the four studied species (at initial concentration of 3.0 ± 0.1 μM DFAA), expressed as μmol DFAA cm⁻² of coral surface d⁻¹.

species, with the lowest excretion rate for *M. oculata* (0.2 μmol N cm⁻² day⁻¹) and the highest rate for *D. dianthus* (0.6 μmol N cm⁻² day⁻¹), reflecting differences in species metabolism since ammonium is mostly formed by the catabolism of proteins (Wright, 1995). Indeed, respiration rates of *D. dianthus* measured in this study and in a previous one at a similar temperature (Naumann et al., 2011) were at least three times higher than in *M. oculata*. Therefore, depending on the species metabolism, 3 μM DFAA can account for 12–50% of the daily excreted-nitrogen. This contribution is in the range of previous estimations made on tropical corals for an equivalent DFAA concentration (20% of nitrogen requirements of *S. pistillata*, Grover et al., 2008 and from 8.5 to 14.7% of *P. damicornis*, Hoegh-Guldberg and Williamson, 1999). Concerning respiration rates, DFAA uptake can cover between 16 and 89% of the daily respired-carbon, depending on the species. This contribution was higher in the branched species (*L. pertusa* and *M. oculata*) than in the massive ones (*D. cornigera* and *D. dianthus*) due to the much higher respiration rates of the massive species, possibly related to their larger amount of tissue. Overall, DFAA contribution to the daily

respired-carbon in the CWCs is higher than estimations of 10% made for tropical corals (Grover et al., 2008; Tremblay et al., 2012), for a comparable DFAA concentration in seawater. However, it has to be noticed that respiration rates in this study (ranging between 0.47 and 1.57 μmol C cm⁻² d⁻¹) were much lower than those measured in tropical (3.8–27.0 μmol C cm⁻² d⁻¹, McCloskey and Muscatine, 1984; Szmant et al., 1990; Houlbrèque et al., 2003) and temperate coral species (3.3–16 μmol C cm⁻² d⁻¹, Szmant-Froelich and Pilson, 1984; Rodolfo-Metalpa et al., 2008; Tremblay et al., 2011), and even in *L. pertusa* from northeast Atlantic (7.2 μmol O₂ cm⁻² d⁻¹ at 11 °C, Dodds et al., 2007). Overall, respiration rates for *D. dianthus* in this study (1.34 ± 0.31 μmol C cm⁻² d⁻¹) were in the same order of magnitude than those reported for starved corals of the same species (~1.5 μmol C cm⁻² d⁻¹, Naumann et al., 2011), evidencing that corals in our experiment showed a reduced metabolic activity, possibly as a consequence of the low feeding frequency (twice a week). Consequently, during periods when particulate food is scarce, the observed uptake of DFAA could potentially cover a larger amount of carbon and nitrogen metabolic

(A)

(B)
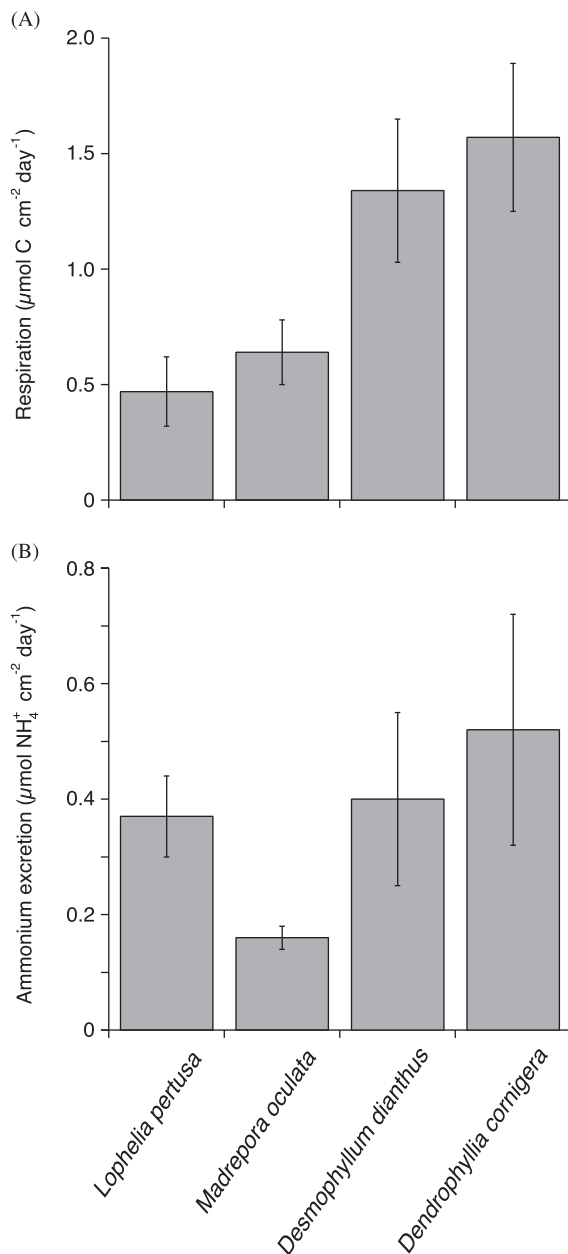
Fig. 3. Rates of (a) respiration and (b) ammonium excretion (mean $\pm$ SD) for the four studied species, expressed as μmol C and N cm^{-2} of coral surface d^{-1}.

requirements in the studied CWC species than in tropical or temperate species.

5. Conclusions

In conclusion, the data presented in this study have demonstrated uptake of DFAA by CWCs. For a comparable concentration of DFAA in seawater, CWC species presented much lower uptake rates compared to tropical corals, suggesting a possible role of temperature in the uptake of DFAA by scleractinian corals, or a different affinity in the DFAA carrier systems. Even so, due to the lower metabolism of CWCs, the contribution of 3 μM DFAA to daily respired-carbon and excreted-nitrogen was higher in CWCs than in tropical corals. DFAA concentration tested in this study is likely to be higher than those experienced by CWCs in the field. However, since potential CWC prey items are patchily distributed

and in addition may be diluted, uptake of DOM may supplement the diet of CWCs when particulate food is scarce (Szmant et al., 1990; Naumann et al., 2011).

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