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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.

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

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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 g m^{-2} 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 ± 1.0 °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 ± 0.5 °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.