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Nickel and ocean warming affect scleractinian coral growth

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ABSTRACT

The sensitivity of corals and their *Symbiodinium* to warming has been extensively documented; however very few studies considered that anthropogenic inputs such as metal pollution have already an impact on many fringing reefs. Thus, today, nickel releases are common in coastal ecosystems. In this study, two major reef-building species *Acropora muricata* and *Pocillopora damicornis* were exposed *in situ* to ambient and moderate nickel concentrations on a short-term period (1 h) using benthic chamber experiments. Simultaneously, we tested in laboratory conditions the combined effects of a chronic exposure (8 weeks) to moderate nickel concentrations and ocean warming on *A. muricata*. The *in situ* experiment highlighted that nickel enrichment, at ambient temperature, stimulated by 27 to 47% the calcification rates of both species but not their photosynthetic performances. In contrast, an exposure to higher nickel concentration, in combination with elevated temperature simulated in aquaria, severely depressed by 30% the growth of *A. muricata*.

1. Introduction

Seawater quality in coastal areas is changing in response to human activities (e.g. Cooper et al., 2009). Deforestation or mining operation increasing soil erosion, domestic and agricultural pollutions, and coastal dredging operations are more and more frequent. Mining activities, leading to sedimentation increase, metal and chemical inputs concern also many reef areas worldwide (e.g. Costa Rica, Panama, Red Sea, Thailand, Tuvalu, Puerto Rico) (Guzmán and Jiménez, 1992; Ali et al., 2011; Tanaka et al., 2013; Fujita et al., 2014; Whitall et al., 2014). Nickel and nickel compounds in particular have many industrial and commercial uses and the progress of industrialization has led to increased release of this metal into marine ecosystems (Cempel and Nikel, 2006; Wilburn, 2011). For instance, the largest nickel deposits and smelting activities are in Cuba, New Caledonia, Indonesia and Philippines (Elias, 2002; Mudd, 2010), and can be located very close to coral reefs (Carey, 1981). In particular, the soil of New-Caledonia, which is the fifth largest producer of nickel and owns many open-pit mines, is particularly rich in nickel. Soil leaching, especially during heavy rainfalls, greatly contributes to metal discharges into the lagoon (Fichez et al., 2005; Hédouin et al., 2006; Metian et al., 2008). As a

consequence, extended portions of fringing reefs in the New Caledonian lagoon build up in seawater enriched in metals (Moreton et al., 2009). While nickel concentration in seawater is generally around 0.1 to 0.5 $\mu\text{g L}^{-1}$ (Barceloux, 1999; Sutherland and Costa, 2002), it has been found to exceed 20 $\mu\text{g L}^{-1}$ in some places along New Caledonian coasts (Moreton et al., 2009) and up to 100 $\mu\text{g L}^{-1}$ in other highly polluted coastal areas (e.g. Chennai city, India; Shanmugam et al., 2007).

To the best of our knowledge, the effects nickel exposure might have on marine organisms in general, and corals in particular, have been poorly investigated. Only two studies assessed the effects of high nickel concentrations (equivalent to 1000 $\mu\text{g L}^{-1}$) on the fertilization success and planulae stage of two coral species (Goh, 1991; Reichelt-Brushett and Hudspeth, 2016). These studies bring data for the first life stages of corals and highlight a toxicity of nickel only at high concentrations, but no study deals with response on the effect of this metal on the different physiological mechanisms of adult corals at concentrations found in waters affected by anthropogenic inputs, estimated to be around 15–20 $\mu\text{g L}^{-1}$ (Moore and Ramamoorthy, 1984; Moreton et al., 2009). This paucity of data on the role/effect of nickel on corals is surprising because nickel is an essential trace metal for microorganisms and plants (Cempel and Nikel, 2006) and may play an important role in

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several enzymatic and metabolic reactions, either as a component or enzymatic activator (Eder and Kirchgessner, 1997; Markich et al., 2002). For example, it has been demonstrated that nickel is essential for the phytoplankton utilization of urea, an important nitrogen source in N-limited oceanic waters (Price and Morel, 1990; Morel et al., 2003; Dupont et al., 2008a) since nickel is the active metal center in urease enzyme (which catalyzes the dissociation of urea into ammonium and carbon dioxide). A nickel-superoxide dismutase (Ni-SOD) was also found in marine cyanobacteria, a dominant group of picophytoplankton in open ocean waters (Dupont et al., 2008a,b). In general, SOD enzymes play a critical role in protecting photosynthetic organisms from self and environmentally induced oxidative stress (Wolfe-Simon et al., 2006; Dupont et al., 2008b). As nickel is involved in the functioning of urease and SODs, two essential enzymes in coral metabolism, it is likely that its increased concentrations in seawater might modify their metabolism.

Local pollutions are not the only threat for coral reefs as global warming and ocean acidification are already altering marine ecosystems (Hoegh-Guldberg et al., 2007). In particular, the sensitivity of corals and their endosymbiotic dinoflagellates (*Symbiodinium* spp.) to warming has been documented extensively (e.g. Hoegh-Guldberg, 1999). *Symbiodinium* trap solar energy and nutrients, providing > 95% of the coral metabolic requirements (Falkowski et al., 1984), and favoring high coral calcification rates. When temperatures exceed summer maxima by 1 to 2 °C for 3 to 4 weeks, this symbiosis is broken and corals bleach (Fitt et al., 2001). Massive coral bleaching has increased in intensity and frequency in recent decades (e.g. Done et al., 2003; Donner et al., 2005; Hoegh-Guldberg, 1999; Hoegh-Guldberg et al., 2005; Hughes et al., 2017) although massive bleaching events have never been reported in New Caledonia until early February 2016.

To our knowledge, while two studies were interested in the combined impacts of metals and thermal stress on early life stages of corals (Negri and Hoogenboom, 2011; Kwok et al., 2016), only one study investigated physiological effects of metal exposure (copper) and temperature increase on their adult life stage (Nyström et al., 2001). The aim of our work was to investigate the effect of nickel alone and/or in combination with warming on the calcification rates and photosynthetic properties of two major reef-building corals: *Acropora muricata* and *Pocillopora damicornis*. Both corals were exposed *in situ* to ambient and elevated nickel concentrations, while in laboratory the combined effects of nickel enrichment and warming were tested on *A. muricata* only.

2. Methods

2.1. *In situ* experiment

In situ experiments were conducted at the end of December 2015 during the summer Austral season and during several consecutive sunny days. The study site was located at Ilot Maitre (22°19.702" S; 166°24.626" E) in the southwest New Caledonia lagoon at a depth of 3 m. Three colonies (15 cm long) of *Acropora muricata* and *Pocillopora damicornis* were sampled by scuba diving near the study site and immediately transferred into the benthic chambers where they were allowed to recover from sampling for around 1-h before the beginning of the experiment. Four transparent PVC benthic chambers of 6.4 L volume were used simultaneously: three chambers containing *A. muricata* or *P. damicornis* colonies and one without the coral colony to account for seawater microbial activity. Each chamber was connected to an YSI 600 OMS probe coupled to an optical dissolved oxygen 6150 module which recorded oxygen, temperature, salinity and depth every minute. Seawater was recirculated between the chamber and the probe at a water flow of 2 L min⁻¹ using an adjustable submersible pump alimented by waterproof batteries (see: Biscéré et al., 2015; Lorrain et al., 2015 for a detailed description of the system). Photosynthetically active radiation irradiance (I, μmol photons m⁻² s⁻¹) was measured

adjacent to the experimental chambers using quantum sensors (LI-193 SA coupled to a Li-1500 LI-COR). At the beginning of the incubations the chambers were hermetically closed; seawater was left recirculating within the system after which the incubation started. Between incubations, enclosures were left open for at least 30 min to restore ambient conditions.

For each species (*A. muricata* and *P. damicornis*), six successive incubations of 1 h were performed, from 09:00 am to 9:00 pm, to encompass the full range of daily irradiance levels, including dark. The first series of incubations has been realized at seawater natural nickel concentration (nickel concentration: 0.15 ± 0.02 μg L⁻¹). The second series of incubations have been performed on the same colonies but 20 mL of a 960 μg L⁻¹ nickel solution (NiNO₃, CPAchem, Bulgaria) have been injected in each chamber at the beginning of each incubation. Final nickel concentration into the chambers was equal to 2.71 ± 0.21 μg L⁻¹ (i.e. enriched). Between incubations, enclosures were left open for at least 30 min to restore ambient conditions.

2.1.1. Photosynthetic, respiration and calcification rates

Coral metabolic rates were measured at different light levels and in the dark. At the beginning and at the end of each incubations, seawater samples were collected with 450 mL syringes in each chamber for pH and total alkalinity (A_T) measurements. pH was measured immediately on board using a pH meter with a glass electrode (Methler 826 pH mobile) calibrated with Tris/HCl referenced solutions (Dickson et al., 2007). Samples were then filtered through 0.47 μm Whatman glass-fiber filters (GF/F) and stored in 250 mL bottles in the dark. Total alkalinity (μmol kg⁻¹) was further determined on 20 ml subsamples (3 replicates) by Gran automatic potentiometric titration (Radiometer, Titrilab TIM 865) using 0.01 M HCl and calculated from the Gran function applied to pH variations from 4.2 to 3.0 as mEq L⁻¹ from the slope of the curve HCl volume vs. pH. Titrations of A_T standards provided by A.G. Dickson (batch 121) were within 0.7 μmol kg⁻¹ of the nominal value.

Calcification rates at each light level and in the dark were estimated using the alkalinity anomaly technique (Chisholm and Gattuso, 1991) as the change in the A_T values during each incubation. The integrated calcification rates (from 9 am to 9 pm) expressed in μmol CaCO₃ cm⁻² h⁻¹ have been calculated for each coral species and nickel concentration. Oxygen production and consumption rates were measured by the oxygen sensor of the YSI probe, which was calibrated before each experiment against air saturated with moisture. Rates of photosynthesis (P) and respiration (R) were estimated by regressing oxygen data against time.

Rates of calcification, photosynthesis and respiration were calculated according to the equations described in Biscéré et al. (2015). For each colony, a Michaelis-Menten model (Lederman and Tett, 1981; Vermaat, 2009) was iteratively fitted using non-linear regression (Gauss-Newton algorithm) to P_{net} (μmol O₂ cm⁻² h⁻¹) vs. PAR irradiance (I, μmol photons m⁻² s⁻¹). This model generates three parameters: maximal gross photosynthesis (P_{max}), half saturation constant (K_m) and respiration (R). At the end of all the incubations coral colonies were frozen (-20 °C) for further analyses. Photosynthesis, respiration and calcification rates were normalized by unit surface skeleton (cm⁻²) measured using the wax-dipping method described in Stimson and Kinzie (1991).

2.2. *Aquaria* experiment

2.2.1. Coral collection and experimental setup

One hundred sixty terminal portions (i.e., nubbins) of branches (2-cm long) of *Acropora muricata* were cut using a plier from ten parent colonies on the fringing reef of Baie des Citrons (22°18.033"S; 166°26.166"E), 2 miles far from Ilot Maitre. After collection, nubbins were hung on nylon wires and suspended on the aquaria with the apex oriented toward the artificial light source. They were allowed to recover

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