Marine Pollution Bulletin 126 (2018) 93–100

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

High zinc exposure leads to reduced dimethylsulfoniopropionate (DMSP) levels in both the host and endosymbionts of the reef-building coral *Acropora aspera*

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ARTICLE INFO

Keywords:
Metal contamination
Ecotoxicology
Symbiodinium
Dose

ABSTRACT

Dimethylsulfoniopropionate (DMSP) is a biogenic compound that could be involved in metal detoxification in both the host and endosymbionts of symbiotic corals. *Acropora aspera*, a common reef-building coral of the Great Barrier Reef, was exposed to zinc doses from 10 to 1000 µg/L over 96 h, with zinc being a low-toxic trace metal commonly used in the shipping industry. Over time, significantly lower DMSP concentrations relative to the control were found in both the host and symbionts in the highest zinc treatment where zinc uptake by both partners of the symbiosis was the highest. This clearly indicates that DMSP was consumed or stopped being produced under high and extended zinc exposure. This drop in DMSP was first observed in the host tissue, suggesting that the coral host was the first to respond to metal contamination. Such decrease in DMSP concentrations could influence the long-term health of corals under zinc exposure.

1. Introduction

Stretching over 2300 km, the Great Barrier Reef (GBR) in Australia is the largest living reef system on the planet, with coral reefs being known for their exceptional biodiversity but also vulnerability (Harrison and Booth, 2007). However, Australian designated shipping routes cross the Great Barrier Reef (GBR) world heritage area, with 80% of the GBR marine park being available to ship navigation (GBRMPA, 2014). For this reason, the impact of shipping on coral reef communities of the GBR, through metal contamination and other boat discharges, needs to be assessed for appropriate government regulations to be implemented. Besides unpredictable oil spill events associated with accidental shipwrecks, the main two impacts of shipping activity to the reef are physical impacts or pollution from toxic antifouling paint. The former represents a constant threat for surrounding coral reefs through long-term contamination by toxic metals such as copper and zinc (Negri et al., 2002). Dredging of navigation channels for ports as well as inputs from sewage treatment plants, catchment runoffs, mining and ore processing that are associated with high human activity also increase metal bioavailability to corals (Reichelt and Jones, 1994).

Zinc is generally considered to have low toxicity for coral ecology, with other metals such as copper causing toxic responses at much lower concentrations (Reichelt-Brushett and Harrison, 1999, 2005). However, sensitivity to trace metals depends on a variety of physiological and biochemical factors that are specific to each species as well as external factors such as the duration of exposure (Hudspith et al., 2017). Like other essential elements, zinc at low concentrations contributes to the normal metabolism of most living organisms through its role as a co-factor in > 300 enzymatic reactions (Morel et al., 1994). For instance, in symbiotic corals zinc contributes to the acquisition of inorganic carbon from the surrounding seawater, which is a key step in the two major mechanisms of photosynthesis and calcification (Ferrier-Pagès et al., 2005). In fact, low zinc concentrations are often considered a limiting factor in phytoplankton growth (De La Rocha et al., 2000; Morel et al., 1994; Sunda and Huntsman, 1995). However, zinc can also have deleterious effects on the fertilization of corals gametes (Heyward, 1988; Reichelt-Brushett and Harrison, 2005) as well as on the settlement, metamorphosis and survival rate of coral larvae (Negri et al., 2002) when present in excess concentrations.

Dimethylsulfoniopropionate (DMSP) is known to be synthesised via various sulfur assimilation pathways through the uptake and reduction of sulfate from seawater and incorporation into sulfur-containing amino acids such as cysteine and methionine (Bromke et al., 2013; Stefels, 2000). In marine algae, methionine is involved in all biochemical

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http://dx.doi.org/10.1016/j.marpolbul.2017.10.070
Received 24 July 2017; Received in revised form 25 September 2017; Accepted 25 October 2017

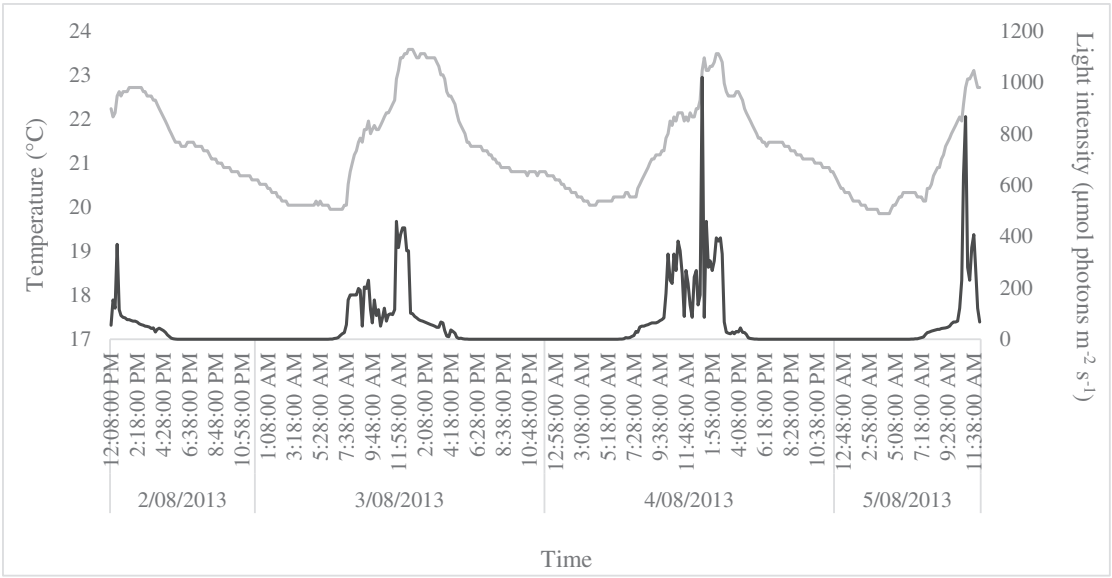

Fig. 1. Temperature and light conditions over the 96 h experiment.

pathways leading to DMSP synthesis (Gage et al., 1997; Greene, 1962; Kocsis et al., 1998), with cysteine being used for de novo biogenic production of methionine (Stefels, 2000). On the other hand, *Acropora* spp. depend on their symbionts for cysteine production as they lack the gene for the biosynthesis of this amino acid (Shinzato et al., 2011). In this coral genus, it seems that methionine is generated by the methylation of homocysteine without having to come from cysteine (Aguilar et al., 2017). Nevertheless, since cysteine is also known to be involved in detoxification processes in response to metal exposure in marine phytoplankton (Ahner et al., 2002), it is likely that DMSP production in the coral holobiont could be altered under metal contamination due to possible increased recruitment of cysteine from the symbionts for detoxification processes.

Reef-building corals represent a complex and unique platform for the production of DMSP and other dimethylated sulfur compounds based on the recent discovery that DMSP can be produced by both the endosymbiotic microalgae and animal host (Raina et al., 2013). Corals were found to possess orthologues of two diatoms key genes that code for enzymes involved in the biosynthesis of DMSP from methionine, with these genes most likely being acquired from the symbiosis by horizontal gene transfers. DMSP has also been found to be a biomarker of stress in corals as its concentrations often increased as a response to environmental stress exposure in reef corals (Deschaseaux et al., 2014). It is thus hypothesised that metal contamination could affect the production and partitioning of DMSP in both the host and endosymbiotic microalgae of *Acropora aspera*, a common symbiotic reef-building coral of the GBR.

Previous research has quantified DMSP in the reef-building coral *Montastraea franksi* and associated *Symbiodinium* under copper exposure (Yost et al., 2010), with the holobiont and symbiont fraction showing differential responses to metal exposure: DMSP levels generally decreased with copper dose in both fractions whereas DMSP levels exclusively increased in the symbiont at the highest copper dose. This indicated that biochemical changes can be overlooked when quantifying DMSP in the holobiont rather than in the different partners of the symbiosis. Here, DMSP was quantified in both partners of the symbiosis in response to zinc exposure at targeted concentrations of 10 µg/L, 100 µg/L and 1000 µg/L, which are considered as ecotoxicogically relevant (Reichelt-Brushett and Harrison, 1999, 2005). Zinc was selected as a low level toxicant in this study to ensure metal concentrations could be analytically measured in various low volume compartments and because we were exploring subtle sub-lethal biochemical changes and did not want to cause overt reactions and mortality. Zinc uptake was also quantified in both the animal and algal fractions after exposure. Since DMSP is often synthesised as a response to stress exposure, we hypothesised that DMSP levels would increase in both partners of the symbiosis with proportion to zinc uptakes.

2. Methods

2.1. Study design

On the 1st of August 2013, three coral colonies of *Acropora aspera* were collected from within the Scientific Research Zone of the Heron Island outer reef flat at low tide (0.7 m between 11:00 and 12:00). Coral colonies were transported to the Heron Island Research Station in floating Nally bins containing in situ collected seawater. Coral colonies were transferred into outdoor aquaria supplied with flow-through reef water. After 24 h acclimation, each colony was nubbinised in 32 coral fragments of approximately 5 cm in length. At time 0 (12:00 on 2nd of August), 4 nubbins from each colony (12 nubbins in total) were placed in 8 different plastic tubs corresponding to 4 different zinc treatments in duplicates: control (containing reef-flat seawater), and targeted zinc chloride ($ZnCl_2$) concentrations of 10 µg/L, 100 µg/L and 1000 µg/L. These zinc concentrations were considered as ecotoxicologically relevant, with the ANZECC/ARMCANZ trigger value for 99% protection of marine species currently being 7 µg/L. One set of duplicates was mainly kept for the determination of metal partitioning between the host and symbionts under zinc exposure whereas the other set of duplicates was primarily kept for the quantification of DMS + DMSP partitioning in both partners of the symbiosis. At time 0, 6 nubbins (3 for metal quantification and 3 for sulfur analysis) were also collected from each coral colony as “untreated controls” and were immediately snap-frozen in liquid nitrogen. Nubbins were then transferred into thick polyethylene (PE) plastic bags and kept at − 80 °C until tissue extraction and sample analysis. At times 24 h, 48 h, 72 h and 96 h, three nubbins corresponding to the three different colonies were removed from each treatment in duplicate (1 duplicate for metal quantification and 1 duplicate for sulfur analysis), immediately snap frozen in liquid nitrogen and kept at − 80 °C until tissue extraction and sample analysis.

Seawater was constantly aerated and shade cloth placed above outdoor aquaria at peak hours of sunlight to minimize photonic stress due to direct sunlight exposure. Light and temperature were measured every 10 min throughout the entire experiment using HOBO data