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Dimethylsulfoniopropionate (DMSP) content and antioxidant capacity in the host and endosymbionts of the sea anemone *Entacmaea quadricolor* are influenced by the host phenotype

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ABSTRACT

Dimethylsulfoniopropionate (DMSP) is a key biogenic compound in marine algae and symbiotic cnidarians. Besides its role as a precursor of the climatically active dimethylsulfide, DMSP is a putative antioxidant through its capacity to scavenge reactive oxygen species. Here, the production of DMSP concurrently with the antioxidant capacity (AOC) among the host and endosymbionts of two colour phenotypes of the sea anemone *Entacmaea quadricolor,* that provides essential habitat for anemonefish, was investigated for 9 days under heat stress (3.2 °C and 6.3 °C above control). Although visual signs of bleaching were observed on day 3 in all of the anemones at high temperature, cell density and chlorophyll *a* content did not vary among temperatures over time. The maximum quantum yield of endosymbionts decreased over time in medium and high temperature treatments in both phenotypes, indicating slight photoinhibition under thermal stress. Temperature had no or little effect on AOC and DMSP concentrations in the host and endosymbionts in both phenotypes, suggesting adaptation of subtropical *E. quadricolor* and associated symbionts to short-term abrupt changes in temperature, which is a regular occurrence in this region. However, host and endosymbiont AOC and DMSP concentrations were, at least partly, driven by the host phenotype, with overall greater AOC and lower DMSP concentrations being found in pink compared with green anemones. These results, along with stronger photoinhibition in the green phenotype under thermal stress suggest that green anemones could be more vulnerable to environmental pressure than the pink phenotype. As our oceans continue to warm, the differing responses of the colour phenotypes may influence their relative abundance on reefs, and have implications for reproductive success as the phenotypes correspond to different sexes. Furthermore, the selective disappearance of one phenotype could adversely affect anemonefish that preferentially associate with more sensitive colour morphs.

1. Introduction

Coral reefs around the world are rapidly deteriorating as a consequence of increasing sea surface temperatures, ocean acidification, rising sea levels, and other anthropogenic disturbances (Hoegh-Guldberg, 2011; Hoegh-Guldberg et al., 2007). The extent of decline is predicted to become widespread under future climate change scenarios (IPCC, 2013), with likely repercussions for the entire reef community (Hughes et al., 2003; Pandolfi et al., 2003). Bleaching is a well-documented consequence of environmental stress on reef corals, and is defined as a breakdown of the host-*Symbiodinium* association that occurs through the expulsion of the algal symbionts or a loss in photosynthetic pigments from within algal cells (Brown, 1997; Lesser, 2011) and can result in host mortality (Glynn, 1983; Harriott, 1985). In addition to corals, bleaching also occurs in other cnidarians such as sea anemones that provide essential habitat for 28 species of anemonefish (Fautin and Allen, 1997; Hill and Scott, 2012; Hobbs et al., 2013; Pontasch et al., 2014; Scott and Hoey, 2017). Due to the obligate nature of the symbiosis, habitat loss associated with sea anemone bleaching has severe consequences for the fish as they cannot survive in the wild without their hosts (Fautin and Allen, 1997).

Bleaching has been associated with an excessive build-up of reactive

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oxygen species (ROS), also known as oxidative stress (Downs et al., 2002; Lesser, 1997, 2011). ROS can cause damage to lipids, proteins and DNA, leading to cell impairment and eventually to algal expulsion as the host's final defence against oxidative damage (Lesser, 2011). Although ROS such as superoxide radicals (O_2^-) and hydrogen peroxides (H_2O_2) are naturally synthesised during respiration and photosynthesis in symbiotic cnidarians (Weis, 2008), environmental stressors such as exposure to elevated temperature, UV radiation and pollutants may exacerbate their production and lead to the formation of more reactive ROS such as singlet oxygen ($^1O_2$) and hydroxyl radicals (HO·) in host and algal cells (Dykens et al., 1992; Krueger et al., 2014; Lesser, 2006; Suggett et al., 2008). In response, antioxidants are produced by both partners of the symbiosis to detoxify ROS (McGinty et al., 2012; Shick et al., 1995; Yakovleva et al., 2004). Although cnidarians and their symbionts have developed adaptive antioxidant responses through the preferential up-regulation of certain antioxidants (Csaszar et al., 2009; Hawkins et al., 2015; Krueger et al., 2014), the complexity of the antioxidant machinery of the host-*Symbiodinium* association and the mechanisms underlying its activation remain understudied.

Dimethylsulfoniopropionate (DMSP) and its breakdown products dimethylsulfide (DMS) and dimethylsulfoxide (DMSO) are sulfur compounds found in various types of marine algae including *Symbiodinium* (Steinke et al., 2011; Yost et al., 2012; Yost and Mitchelmore, 2009). Among other biological functions these sulfur compounds are believed to act as antioxidants by being particularly reactive towards ROS such as HO· and $^1O_2$ (Sunda et al., 2002; Wilkinson et al., 1995). Other roles include climate regulation (Charlson et al., 1987; Quinn and Bates, 2011), osmoregulation (Reed, 1983), thermoregulation (Stefels, 2000), chemical defence against grazers (Van Alstyne et al., 2001) and chemoattraction (Seymour et al., 2010). Although DMSP production in cnidarians has mainly been attributed to the symbionts (Van Alstyne et al., 2006; Yost et al., 2012), a recent study demonstrated that the coral host could also be a source of DMSP (Raina et al., 2013).

The production of DMSP under thermal stress has been quantified in both symbiotic corals (Deschaseaux et al., 2014b; Fischer and Jones, 2012; Raina et al., 2013) and cultured symbionts (Deschaseaux et al., 2014a; McLenon and DiTullio, 2012), with increasing temperature being one of the major threats to coral reef organisms, especially to symbiotic cnidarians. Studies on the relative DMSP concentrations in the host and *in-hospite* symbionts in corals have demonstrated the partitioning of DMSP between algal symbionts and the host and their differential response to environmental stress (Yost et al., 2012; Yost et al., 2010; Yost and Mitchelmore, 2010). Here we measured DMSP concentrations and its partitioning between the host tissue and associated *Symbiodinium* during thermal stress in the sea anemone *Entacmaea quadricolor* collected from the Solitary Island Marine Park (SIMP) on the east coast of Australia. This region has been identified as a climate change hot spot (Hobday et al., 2006; Poloczanska et al., 2007; Ridgway, 2007), and contains a high-density assemblage of *E. quadricolor*, which provides habitat for three species of anemonefish (Richardson et al., 1997; Scott et al., 2011).

In this study, we worked on two different colour phenotypes of *E. quadricolor* that showed different pigmentation (either pink or green) at the tip of their tentacles (Fig. 1), which are likely to correspond to male and female anemones, respectively (Scott and Harrison, 2009). We hypothesised that: i) thermal stress would trigger DMSP production in both the host and endosymbionts as a response to oxidative stress; ii) that increased DMSP concentrations in either the host or symbionts would correlate with an up-regulation of the antioxidant capacity (AOC); iii) that DMSP concentrations would fluctuate similarly in both partners of the symbiosis; and iv) that DMSP production and partitioning in *E. quadricolor* would not vary between anemone phenotypes in response to thermal stress.

2. Materials

2.1. Collection

Thirty-six *E. quadricolor* were collected at approximately 18 m from North Solitary Island (29°55′S, 153°23′E), SIMP, New South Wales, Australia. These consisted of two different colour phenotypes: i) red column, dark brown tentacles with green tips, and ii) orange column, light brown tentacles with white pigmentation below pink tips (Fig. 1); referred to as 'green' (n = 18) and 'pink' (n = 18) phenotypes, respectively. Sea anemones were transported to the National Marine Science Centre, Coffs Harbour in 70-L tubs, and kept in a 3000-L tank with flow-through seawater (10 L min^{-1}, 19–20 °C) for 4 weeks before the start of the experiment. Light intensity had an average daily maximum of approximately 50 µmol photons m^{-2} s^{-1} to avoid light-induced bleaching. Anemones were fed to satiation with prawn flesh every 14 d, with the last feeding occurring 4 d before the start of the experiment.

2.2. Experimental design

Anemones were transferred at 1200 h on the 11 September 2012 (day 1) into 36 separate 15-L plastic tubs that were supplied with flow-through filtered seawater (5 µm Filtaflo sediment filters) at 600 mL min^{-1} for the 9-d experiment. Each tub was randomly assigned to one of three temperatures: 21.3 °C (control), 24.5 °C (medium – 3.2 °C above control) and 27.6 °C (high – 6.3 °C above control), and had an equal number of phenotypes. Medium and high temperatures represented ecologically relevant and extreme temperature treatments, being 1.5 °C below and 1.6 °C above maximum summer seawater temperatures, respectively. Seawater was heated to the desired temperature in three 3000-L header tanks equipped with thermostats. Experimental temperatures were gradually increased to reach target temperatures 48 h after the start of the experiment (day 3). Temperature was monitored in the tubs every 10 min using three haphazardly placed Thermochron iButton temperature loggers (Maxim, USA) per treatment, which were calibrated against a high precision mercury thermometer. Shade cloth was placed above the tanks to attenuate sunlight to 25% of incoming solar radiation and to simulate light intensity at the collection location. Light was monitored every 5 min using underwater Odyssey light loggers (Dataflow Systems, New Zealand) and calibrated against a Li-1400 photometer with a 2π Li-192SA quantum sensor (Lincoln, USA). Light intensity had an average daily maximum of 451 ± 9.27 µmol photons s^{-1} m^{-2} during the experiment, which was higher than under acclimation conditions; however, since this difference in light intensity was uniform for all anemones and all temperature treatments, significant differences between phenotypes and temperatures were still considered interpretable. Visual signs of bleaching and stress were monitored daily at 1100 h.

2.3. Separation and sub-sampling of the host and symbiont fractions

At 1200 h on days 1, 3, 5 and 9, three tentacles from each anemone were removed using forceps and scissors and placed into 15 mL centrifuge tubes. Samples were snap frozen in liquid nitrogen and then transferred to a − 20 °C freezer until processing, which occurred within 2 h of collection. Tissue processing and sub-sampling were performed on ice. Tissue was suspended in 6 mL of 75 mM sodium phosphate buffer (pH 7.4, 4 °C) and homogenized for 10 s using an Ultra Turrax homogenizer (IKA, Germany). Tissue lysates were centrifuged at 4500 × g for 10 min to separate the algal symbionts from the host tissue. The supernatant was separated from the algal pellet into a new centrifuge tube, and centrifuged twice more at 4500 × g for 5 min with the tubes being replaced between spins to ensure maximal removal of remaining symbionts from the host fraction. Simultaneously, the initial algal pellets were resuspended and homogenized in 6 mL of sodium