Symbiosis-specific changes in dimethylsulphoniopropionate concentrations in *Stylophora pistillata* along a depth gradient

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Abstract

Scleractinian corals are prolific producers of dimethylsulphoniopropionate (DMSP), but ecophysiological mechanisms influencing cellular concentrations are uncertain. DMSP is often proposed to function as an antioxidant but interactions between specific host–symbiont genotype associations, plasticity in DMSP concentrations and environmental conditions that can either exert or alleviate oxidative stress are unclear. We used long-term (6 months) reciprocal transplantation of *Stylophora pistillata* hosting two distinct symbiont phylotypes along a depth gradient, clades A (< 20 m) and C (> 20 m), to assess the effect of change in depth (light intensity) on DMSP concentrations in relation to symbiont genotype and photoacclimation in corals between 3 and 50 m in the Gulf of Aqaba. Bathymetric distribution of total DMSP (DMSPt) per cell varied significantly while particulate DMSP (DMSPp) appeared to be unaffected by depth. Highest DMSPt concentrations in control corals occurred at 20 m. While 3 m transplants showed a significant increase in DMSPt concentration at 20 m and became affiliated with an additional genotype (C72), 50 m transplants largely persisted with their original genotype and exhibited no significant changes in DMSPt concentrations. DMSPt concentrations in transplants at both 3 and 50 m, on the other hand, increased significantly while all corals maintained their original symbiont genotypes. Photoacclimation differed significantly with transplantation direction relative to the controls. Symbionts in 3 m transplants at 20 m exhibited no changes in chlorophyll a (chl a) concentration, cell density or cell diameter while symbiont densities decreased and chl a concentrations increased significantly at 50 m. In contrast, symbiont densities in 50 m transplants remained unaffected across depths while symbiont diameters decreased. Chl a concentrations decreased at 20 m and increased at 3 m. Our results

indicate that DMSPt concentrations following changes in depth are not only a function of symbiont genotype but result from different acclimation abilities of both symbiotic partners.

Introduction

Dimethylsulfoniopropionate (DMSP) is a secondary metabolite that is produced and accumulated at high intracellular concentrations by many marine microalgae (Keller et al. 1989). It is the precursor of dimethylsulfide (DMS), whose oxidation products play a major role in the formation of clouds, cloud albedo, and thus in the regulation of global climate (Vallina and Simó 2007). Scleractinian corals maintain an obligate symbiosis with DMSP-producing dinoflagellates in the genus *Symbiodinium* (Hill et al. 1995) and are thought to make a substantial contribution to the amount of DMS entering the atmosphere (Broadbent and Jones 2004; Fischer and Jones 2012). However the production of DMSP is complex and cellular concentrations are influenced by a suite of environmental variables including salinity, nutrients, light, and temperature (Stefels 2000; Stefels et al. 2007). While the functional role of DMSP in macroalgae and free living phytoplankton is fairly well apprehended (Stefels 2000), fundamental questions remain concerning the biosynthesis, regulation and metabolic roles of DMSP in symbiotic cnidarians (Yost et al. 2012; Raina et al. 2013; Tout et al. 2015). Among various physiological functions (Stefels 2000; Stefels et al. 2007), DMSP and its enzymatic breakdown products are potent scavengers of reactive oxygen species (ROS) (Sunda et al. 2002). Because high concentrations of DMSP were observed in coral host and symbionts following exposure to oxidative stress inducing agents such as light, temperature or copper (Yost et al. 2010; McLenon and DiTullio 2012; Deschaseaux et al. 2014a,b) as well as in bleached corals (Jones et al. 2014), DMSP is thought to play an important role in the antioxidant system of the holobiont (the animal and

symbiont in combination). Exposure to high levels of visible light and ultraviolet radiation, which in clear water can penetrate to depths of 20 m (Fleischmann 1989), leads to the photodynamic production of ROS within the holobiont (Smith et al. 2005), which is thought to be the principal cause of coral bleaching (Weis 2008). Concentrations of antioxidant defences in corals are therefore often greatest in shallow-water corals and decrease with increasing depth (Shick et al. 1995; Banaszak et al. 1998; Richier et al. 2008).

The genus *Symbiodinium* currently comprises nine phylogenetic clades with numerous independently evolving lineages (Sampayo et al. 2009; Pochon and Gates 2010). Each symbiont is adapted to a particular light regime; this is thought to play a major role in structuring host species distributions over reef slopes (Iglesias-Prieto and Trench 1994; Warner and Berry-Lowe 2006; Finney et al. 2010) but a corresponding depth-related pattern of DMSP concentrations in different coral host–symbiont genotype associations has not been established so far (Yost et al. 2012). Some symbionts exhibit within-genotype variation in DMSP concentrations between different coral hosts (Yost et al. 2012), indicating that DMSP concentrations are a function of both the endosymbiont and coral host. To date, there have been no studies on interactions between specific host–symbiont genotype associations and the plasticity in DMSP concentrations in response to environmental conditions such as light intensity that can either induce or alleviate oxidative stress.

The coral *Stylophora pistillata* (Esper, 1797) in the Gulf of Aqaba hosts two distinct *Symbiodinium* from clades A and C, each of which displays well-defined vertical zonation in this region. Deep-water colonies (>20 m) host symbionts belonging to clade C (typical for low irradiance habitats), whereas colonies growing in shallow-water host clade A (typical for high irradiance habitats) (Rowan and Knowlton 1995; Winters et al. 2009). We used reciprocal

transplantation of *S. pistillata* along a depth gradient between 3 and 50 m in the Gulf of Aqaba to assesse the effect of changes in light intensity on DMSP concentrations in relation to symbiont genotype. Specifically we hypothesized that if transplanted corals remain affiliated with their respective symbiont genotypes, DMSP concentrations in deep-water corals hosting clade C *Symbiodinium* will increase with decreasing depth/increasing light intensity as they will be more sensitive to high light levels than corals hosting clade A *Symbiodinium* and thus be more prone to experience photo-oxidative stress. Conversely, we assumed that DMSP concentrations in shallow-water corals hosting clade A *Symbiodinium* will not be significantly greater than DMSP concentrations in deep-water corals as shallow-water *S. pistillata* in the Gulf of Aqaba is extremely bleaching resistant (Fine et al. 2013) suggesting that these corals are well adapted to high light conditions. DMSP concentrations in shallow-water transplants were expected to remain either unaffected or, because the production of DMSP in corals is thought to be linked to symbiont photosynthesis (Deschaseaux et al. 2014a), to decrease following transplantation to 50 m.

Materials and methods

Coral transplantation

Thirty fragments from independent colonies of *S. pistillata* (at least 10 m apart) were collected from depths of 3 and 50 m, and ten fragments were collected from 20 m from the waters in front of the Interuniversity Institute for Marine Sciences (IUI), Eilat, Gulf of Aqaba, Red Sea (29°30′N, 34°56′E) in May 2012 using Trimix SCUBA. Fragments were collected under a special permit from the Israeli Natural Parks Authority. The fragments (~7 cm length) were placed in black labeled plastic bags filled with ambient seawater and transported directly to the

laboratory. Under dim light conditions (< 50 µmol m^{-2} s^{-1}), each fragment was tied to a piece of nylon string allowing the fragments to hang freely in the water column. The fragments were labeled to identify colony number and original depth. The following day, all corals were transferred back to the sea to the depth from which they were initially collected and left to recover for one week, after which corals from 3 and 50 m were slowly acclimated to changing light regimes (average light intensities at 3, 20 and 50 m are 1700, 700 and 180 µmol m^{-2} s^{-1}) respectively (Mass et al. 2007).

The corals were kept hanging on a horizontal iron cross positioned parallel to the sea floor. The cross, lodged in the ground via a metal pole, protruded 70 cm into the water and corals were hung from each of the four arms 50 cm off the ground with sufficient space between each fragment to ensure that the corals did not touch each other. For the light acclimation a metal cross was installed at 3 m and then at 10 m increments down to 50 m depth.

Twenty fragments from 3 m were directly transplanted to a depth of 20 m while 20 fragments from 50 m were transplanted up to 40 m depth where they remained for a week. After that the fragments were reciprocally transplanted in 10 m intervals. Fragments from 3 m were acclimated to low light conditions over a period of 8 d, remaining at 30 and 40 m for 4 d respectively. Fragments from 50 m depth were acclimated to higher light conditions over a period of 24 d to avoid excessive stress or mortality of the corals as has been observed previously in 'upward' transplantations due to the sensitivity to acute high light exposure for many corals collected from low light conditions (Baker 2001; Cohen and Dubinsky 2015). Specifically, corals were acclimated to 30 and 20 m for 4 d each, after which they were acclimated to 10 m and finally to 3 m over 8 d at each depth.

After reciprocal transplantation was complete, coral fragments at 3 m comprised ten control fragments and ten transplanted fragments from 50 m, corals at 20 m comprised ten control corals from 20 m, ten transplants from 3 m and ten transplants from 50 m, and fragments at 50 m consisted of ten controls from 50 m and ten transplants from 3m. Algae were cleaned from the iron frames and nylon strings every fortnight. No algal growth occurred on the corals. All fragments were kept in the sea for 6 months until the end of November 2013, after which they were transported to the lab using black plastic bags with ambient seawater. Once ashore, the fragments were immediately frozen in liquid nitrogen and then stored at -80 °C pending tissue analyses.

Sample processing

Coral tissues were stripped from the skeletons under dim light using an airbrush and sterile filtered seawater (FSW, 0.2 µm) to obtain a slurry that was collected in a 5 mL Falcon tube. The slurry (20–50 mL) was homogenized for 10 s with an electric homogenizer (DIAX 100 homogenizer Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). Sub-samples were immediately removed for analyses of multiple indices using sterile transfer pipettes. We measured algal genotype, total DMSP (DMSPt = sum of algal and host DMSP) and particulate DMSP (DMSPp = algal DMSP), chlorophyll a (chl a), symbiont densities and cell sizes (diameter). The remaining coral skeleton was saved for surface area measurements using the paraffin wax technique (Stimson and Kinzie 1991).

Analyses of algal indices and algal genotype

To quantify symbiont numbers, diameters and chl a concentrations, 2 mL of homogenate was centrifuged (1900 g at 4 °C) and the algal pellets resuspended three times in FSW. Resuspended algae were used for chl a extraction in acetone (100%) at 4 °C in the dark for 24 h. Concentrations were determined spectrophotometrically (Jeffrey and Humphrey 1975). Symbiont densities were quantified from four replicate counts using a Neubauer hemocytometer. Densities and chl a were normalized to coral surface area. Cell diameters were determined microscopically with an eyepiece graticule from 30 replicate measurements per sample.

Nucleic acid extractions from five randomly chosen replicates were conducted using a modified Promega Wizard genomic DNA extraction protocol (LaJeunesse et al. 2003). Symbiont identity was characterized by denaturing gradient gel electrophoresis (DGGE) fingerprinting of the partial 5.8S and internal transcribed spacer region 2 (ITS2; LaJeunesse 2002). The region was amplified using a touch-down thermal cycle profile with the primers "ITS2clamp" and "ITSintfor2" (Lajeunesse and Trench 2000), and the PCR products resolved on denaturing gels (45–80% of 7 mol L^{-1} urea and 40% formamide) using a CBS Scientific system (Del Mar, CA) for 16 h at 95 V. The dominant band of the symbiont's DGGE profile was excised, reamplified, and cycle-sequenced to provide the ITS2 sequence that dominates the symbiont's genome.

DMSPt and DMSPp analyses

For the quantification of DMSPt, 1 mL of homogenate was added to 2 mL 0.5 mol L^{-1} NaOH in a gas-tight, screw-cap headspace vial. To isolate the algal component (DMSPp) from the homogenate, 1 mL of each sample homogenate was centrifuged (2900 g at 4 °C) for 20 min, the pellets resuspended in 1 mL FSW and immediately added to 2 mL 0.5 mol L^{-1} NaOH in a headspace vial. The supernatant of the control corals from 3 and 20 m was transferred into

another headspace vial containing 0.5 mol L^{-1} NaOH to analyze DMSP concentrations in the host tissue (DMSPh). DMSPt concentrations were compared to the sum of DMSPp and DMSPh concentrations to account for the potential loss of DMSP due to the conversion to DMS during centrifugation and handling. The addition of NaOH produces an alkaline hydrolysis that rapidly converts DMSP to equimolar concentrations of DMS, which can be quantified using gas chromatographic methods with direct injection of headspace (Steinke et al. 2011). Results were expressed as femtomole DMSPt and DMSPp per symbiont cell and as nmol cm^{-2} surface area.

Data analyses

Data were checked for homogeneity of variances using the Cochran's C-test and ln(x)-transformed if necessary before using one-way ANOVA. Student–Newman–Keuls (SNK) tests were used for post hoc multiple comparisons. At the end of the experiment two fragments were missing from the control corals at 20 m and one fragment from the control corals at 3 m. To avoid confounding effects due to unbalanced sample sizes all measured variables were analyzed using eight replicates. Data were analyzed using WinGMAV (EICC, University of Sydney, Australia). An additional regression analysis to test for correlations between DMSPp and DMSPt (normalized to surface area) and symbiont densities was performed and analyzed by ANOVA using JMP Pro 10.0.2 (SAS).

Results

Symbiodinium genotypes in *S. pistillata*

Three distinct symbionts belonging to clades A and C were found in *S. pistillata* fragments that were maintained at their native depths (Table 1). *Symbiodinium* A1 dominated all fragments at 3

and 20 m depths, while C167a and C168a were found in the fragments at 50 m. Except for some transplanted corals at 20 m, all transplanted fragments persisted in association with symbionts corresponding to their original collection depth. Three fragments transplanted from 3 to 20m possessed C72a-b, while one fragment transplanted from 50 to 20m possessed C169a-b.

Photoacclimation of *Symbiodinium* along the depth gradient

Symbiont densities, chl a concentrations and cell diameters in control corals and coral transplants varied significantly with depth (Table 2). Algae in control corals at 3 and 20 m occurred at similar densities but displayed a significant decrease at 50 m (Fig. 1a). This trend was paralleled by cell densities in corals transplanted from 3 to 20 and 50 m. By contrast, change in depth had no effect on symbiont densities in 50 m transplants. Chl a concentrations in control corals did not vary significantly between depths (Fig. 1b). Concentrations in 3 m transplants at 20 m were similar to the control corals while concentrations in 3 m transplants at 50 m exhibited a significant increase. Chl a concentrations in 50 m transplants changed significantly at both depths, with an increase in concentration at 3 m and a decrease at 20 m. The symbiont cell diameter in control corals significantly increased with increasing depth (Fig. 2). Correspondingly, algae in 50 m transplants exhibited a decrease in diameter with decreasing depth. By contrast, the cell diameter of symbionts in 3 m transplants remained unaffected by changes in depth.

DMSP concentrations

Potential loss of DMSPp due to centrifugation and handing was insignificant (Table 3). The bathymetric distribution of DMSPp and DMSPt varied significantly depending on the normalization index used (Table 2). Concentrations of DMSPt normalized to algal cell in control

corals were significantly higher at 20 m than at 3 and 50 m (Fig. 3a). DMSPt in 3 m transplants increased significantly at both 20 and 50 m relative to the control corals. Likewise there was a significant increase in DMSPt concentrations in 50 m transplants at 3 m. However DMSPt concentrations at 20 m were not significantly different from the controls at 50 m (Fig. 3a). DMSPt normalized to surface area exhibited a similar trend to that of symbiont densities (Fig. 3b). DMSPt concentrations in control corals at 3 and 20m were significantly higher than control corals at 50 m with the highest concentrations occurring at 20 m. DMSPt concentrations in 3m transplants paralleled those of the control corals while there were no significant differences in DMSPt between depths for 50 m transplants. No significant effects were detected for concentrations of DMSPp normalized to cell (Fig. 4a). In contrast, DMSPp normalized to surface area followed the pattern of the depth distribution of symbiont densities (Fig. 4b). Regression analyses revealed a significant correlation between DMSPt (nomalised to surface area) (r^2 = 0.60, ANOVA, $F_{1, 55} = 80.00$, $P < 0.01$; Fig. 5a) and DMSPp (normalized to surface area) (r^2 = 0.52, ANOVA, $F_{1, 55} = 57.72$, $P < 0.01$; Fig. 5b) and symbiont density.

Discussion

DMSP concentration and normalization index

To date, there is no clear consensus on which index best conveys DMSP concentrations in corals. Concentrations are frequently normalized to multiple indices, each of which can reveal a different mechanistic process that may influence DMSP concentrations in the holobiont (Yost et al. 2012; Deschaseaux et al. 2014a). Normalization to surface area can capture potential effects of symbiont densities. Normalization to cell, on the other hand, can reflect the ecophysiological effects on DMSP concentrations in the holobiont. Although DMSP is commonly perceived to be

produced by the algal symbionts (Van Alstyne et al. 2009), the location of DMSP production within the coral remains ambiguous. While concentrations of DMSP are found in the tissue of the host implying that DMSP either leaks or is translocated from symbiont to host (Yost et al. 2012), there is also evidence showing that juvenile corals lacking symbionts are able to synthesize DMSP directly (Raina et al. 2013). Our results show that regardless of the normalization index used, concentrations of DMSPt were consistently greater than concentrations of DMSPp, consistent with previous studies (Yost et al. 2010, 2012). The regression analyses, together with the fact that the bathymentric distribution pattern of DMSPt and DMSPp normalized to surface area closely paralleled that of the symbiont densities, indicate that accumulation of DMSP in the host tissue occurred in a cell density-dependent fashion. Normalization of the data to algal cell on the other hand demonstrated a clear effect of depth on DMSPt concentrations in *S. pistillata*, while DMSPp concentrations appeared to be unaffected by depth. This suggests that DMSP translocation from symbiont to host may be to some extent mediated by the coral animal. DMSPt and DMSPp in the following discussion refer to concentrations normalized to cell.

Depth distribution of DMSP

The depth distribution of DMSPt in control corals was in line with our predictions, showing that concentrations in corals at 3 m hosting clade A symbionts and in corals at 50 m hosting clade C symbionts did not differ significantly. In contrast, the high DMSPt concentrations measured in both the control corals and 3 m transplants at 20 m were unexpected.

Combined with chl a concentrations and symbiont densities of the control corals at 3 m, which provided no signs of bleaching, the data indicate that *S. pistillata* at 3 m was well adapted to high

levels of irradiance supporting the tenet of high bleaching resistance of this species in our study area (Fine et al. 2013). Compared to the shallow-water light environment, corals at 20 m are exposed to enormous variation in light intensity (Dishon et al. 2012). Such fluctuations may have rendered corals at this depth more susceptible to stress than shallow-water corals that were exposed to high but continuous levels of irradiance (Allahverdiyeva et al. 2015). Further indication that corals at 20 m experienced more fluctuation than those at 3 m is suggested by the fact that chl a concentrations in 50 m transplants were significantly lower at 20 m than at 3 m, and by the emergence of additional genotypes (Jones et al. 2008) in coral transplants which only occurred at this depth while transplants at 3 and 50 m maintained their original genotypes.

DMSP distribution in coral transplants and bathymetrically-driven association with *Symbiodinium*

DMSPt concentrations displayed a significant increase relative to the controls at each transplantation depth in all but deep-water transplants at 20 m. While increases in DMSPt at both 3 and 50 m occurred independently of symbiont genotype, high DMSPt concentrations in 3 m transplants at 20 m coincided with the emergence of an additional genotype. The presence of C72-a-b symbiont genotypes in the 3 m transplants is consistent with previous observations of the alternation and co-occurrence of symbiont types C72 and A1 in *S. pistillata* at this depth in the Gulf of Aqaba (Byler et al. 2013). Against this background it is intriguing that the 50 m transplants at 20 m exhibited no increase in DMSPt concentrations and remained largely affiliated with the same types of *Symbiodinium* as their 50 m controls. The relevance of the

emergence of the genotype C169-a-b in 50 m transplants remains to be determined, as it has not been observed in *S. pistillata* before and did not appear to be very abundant.

One reason for the contrasting DMSPt concentrations at 20 m may be related to intrinsic differences in the capacities to acclimatize to changing light levels between shallow- and deep-water holobionts. This is supported by the variation in symbiont population characteristics relative to the control corals at each transplantation depth. While shallow-water transplants at 20 m exhibited no changes in chl a concentration, cell density or cell diameter, symbionts in deep-water transplants decreased in cell diameter (hence volume) and contained significantly less chl a relative to the control corals. Differential acclimation abilities of coral hosts may have also accounted for the symbiont recombination in the 3 m transplants at 20 m relative to the control corals at 20 m, which were affiliated with *Symbiodinium* type A1 only. This is consistent with previous observations indicating that the ability of the algal symbionts to acclimate to specific light regimes does not occur in isolation but is influenced by morphological and/or physiological constraints of the coral host, which in turn affects physiological responses and thus relative stress tolerance of the holobiont (Goulet et al. 2005; Frade et al. 2008a,b). The fact that DMSPp did not change as a function of depth together with the observed variation in photoacclimation responses indicate that DMSPt concentrations were to some extent regulated by the host. Indeed, this agrees with observations by Yost et al. (2012) showing that DMSPt concentrations varied between different coral species hosting the same symbiont genotype.

Diverging photoacclimation strategies were also evident in coral transplants at 3 and 50 m, which maintained their original symbiont genopytes. The photoacclimation response of shallow-water transplants hosting clade A symbiont genotypes was similar to that of the control corals showing a classic pattern of photoacclimation to low light levels with a decrease in

symbiont numbers (Dustan 1982) and concomitant increase in chl a concentrations (Falkowski and Dubinsky 1981; Cohen and Dubinsky 2015) corroborating other observations for *S. pistillata* along the same depth gradient in the Gulf of Aqaba (Mass et al. 2007; Winters et al. 2009; Cohen and Dubinsky 2015). In contrast, in the current study, deep-water corals hosting clade C78 and C161a symbionts responded to high light intensity with a complete switch to C161a at 3 m. A switch in the relative dominance of genotype, referred to as symbiont shuffling, is thought to present an acclimation mechanism in response to changes in environmental conditions (Jones et al. 2008) as different genotypes vary markedly in their photophysiological characteristics (Iglesias-Prieto et al. 2004; Frade et al. 2008b). In contrast to 3 m transplants, photoacclimation in 50 m transplants did not entail changes in symbiont densities but a decrease in cell diameter and curiously, an increase in chl a concentrations at 3 m.

While DMSPt concentrations in deep-water transplants at 3 m conform to the notion that corals hosting clade C were more sensitive to high light than shallow-water controls hosting clade A, high chl a concentrations in these corals rebut this suggestion. A lesser-known function for DMSP, proposed by Stefels (2000), is that of an overflow mechanism for excess reduced sulphur and as a means to dissipate excess energy under conditions where carbon incorporation exceeds the rate of protein synthesis, a common phenomenon in shallow-water corals (Falkowski et al. 1984). Although copious amounts of fixed carbon are translocated from symbiont to host, very little of it is assimilated as the translocated products are deficient in nitrogen, which necessitates release of large quantities of carbon to the environment (Falkowski et al. 1984). Carbon translocation and secretion, however, differ with environmental conditions (Falkowski et al. 1984; Tremblay et al. 2014), as well as between host or symbiont genotypes (Davy et al. 1996; Loram et al. 2007). Differential amounts of released carbon due to specific symbiont–host

interactions and distinct photophysiological characteristics could therefore account for the high concentrations of DMSP in deep-water corals at 3 m relative to the controls.

An intriguing aspect of our results was the significant increase in DMSPt concentrations in shallow-water corals transplanted to 50 m which contrasted with our assumption that DMSP concentrations would decrease with a decrease in the potential to experience photo-oxidative stress (e.g., Shick et al. 1995). Likewise, our results contradict findings by Deschaseaux et al. (2014b) who observed no significant effect of light depletion on DMSPt concentrations over the course of 3 d. One possibility is that DMSPt concentrations in shallow-water transplants in our study did not change at 50 m and that the observed increase in DMSPt is merely an artifact resulting from a decrease in symbiont densities in transplants at 50 m. Although the increase in DMSPt in our study coincided with a significant increase in chl a concentration, it seems unlikely that these were linked to the high levels of DMSPt since, despite photoacclimation, the photosynthetic efficiency of symbionts generally decreases significantly with depth (Lesser et al. 2000; Titlyanov et al. 2001; Mass et al. 2007). An alternative explanation may be that the physiological function of DMSP may vary with depth. An important metabolic function of DMSP in both algae (Kirst 1996) and symbiotic cnidarians (Yancey et al. 2010) is that of an osmolyte. Though not very well investigated as osmoconformers, corals maintain high levels of compatible solutes (Stefels 2000; Mayfield and Gates 2007). However, the distribution of these solutes, including DMSP, is likely to change with depth because rates of carbon assimilation and quality of photoassimilates are not uniform across different depths (Falkowski et al. 1984; Muscatine et al. 1984; Alamaru et al. 2009), thus leading to changes in the relative concentration of each solute (Stefels 2000).

Regardless of the metabolic role of DMSP in *S. pistillata*, our results indicate that DMSPt

concentrations following changes in light intensity are not only a function of symbiont genotype but result from complex interactions between both symbiotic partners. As coral reefs are subjected to enormous environmental pressures via pollution (Fabricius 2005) and climate change (Hoegh-Guldberg et al. 2007), which influence light levels (Wooldridge 2008; Reopanichkul et al. 2009), symbiont population characteristics and genetic composition (Baker et al. 2008; Jones et al. 2008), our data present important considerations for ongoing efforts to elucidate how environmental variability can influence plasticity in DMSP concentrations in the coral holobiont.

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Figure legends

Fig. 1 Mean (n = 8, ± SE) (**a**) symbiont densities and (**b**) chlorophyll a concentrations in control corals (*grey*), corals transplanted from 3 to 20 and 50 m (T3, *striped*) and from 50 to 20 and 3 m (T50, *white*) in the Gulf of Aqaba. *Letters above error bars* indicate significant differences between groups (SNK tests, $p < 0.05$)

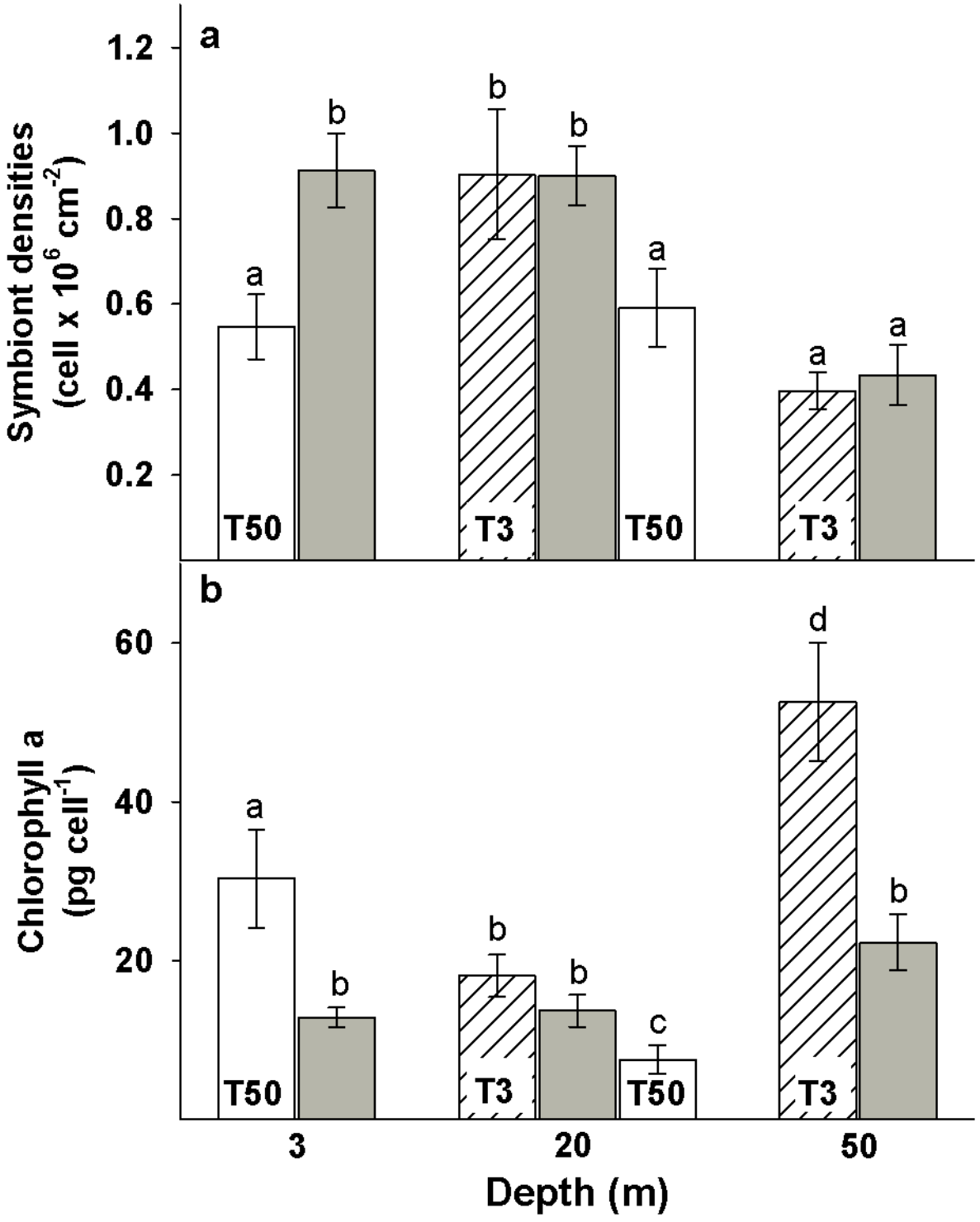
Fig. 2 Mean (n = 8, ± SE) diameter of symbionts in control corals (*grey*), corals transplanted from 3 to 20 and 50 m (T3, *striped*) and from 50 to 20 and 3 m (T50, *white*) in the Gulf of

Aqaba. *Letters above error bars* indicate significant differences between groups (SNK tests, $p < 0.05$)

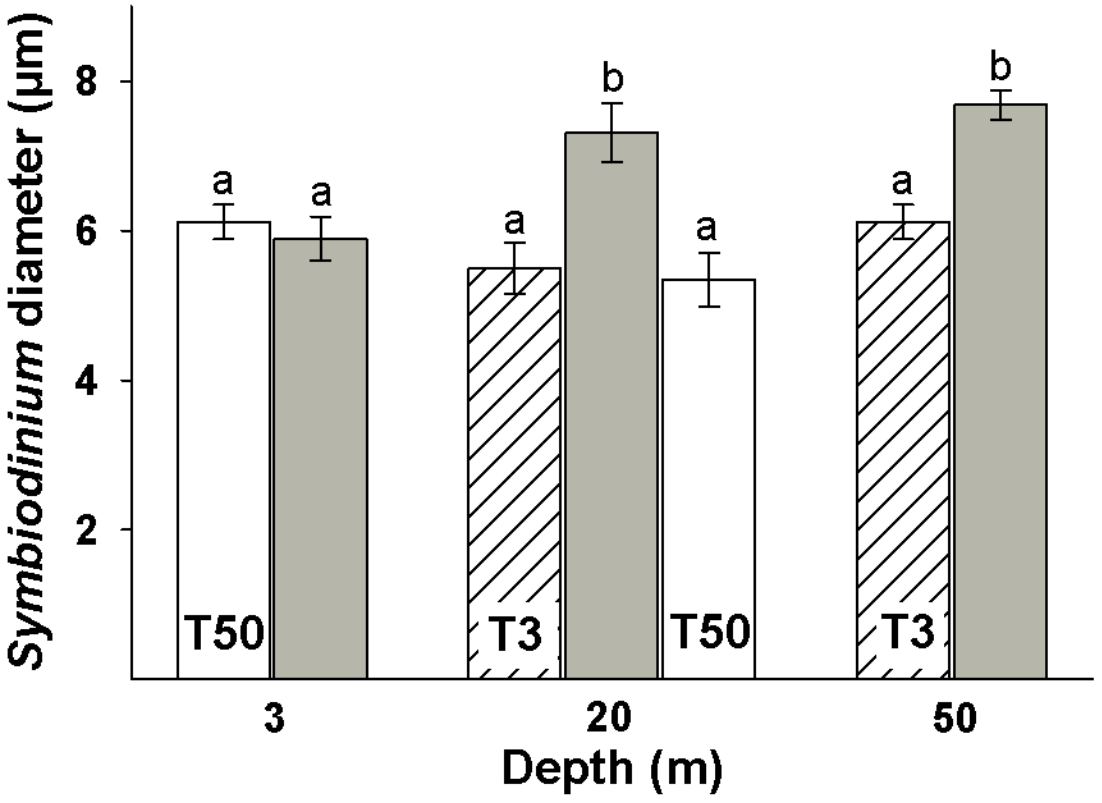
Fig. 3 Mean concentrations (n = 8, ± SE) of (**a**) DMSPt expressed as fmol $cell^{-1}$ and of (**b**) DMSPt expressed as nmol cm^{-2} in control corals (*grey*), corals transplanted from 3 to 20 and 50 m (T3, *striped*) and from 50 to 20 and 3 m (T50, *white*) in the Gulf of Aqaba. *Letters above error bars* indicate significant differences between groups (SNK tests, $p < 0.05$)

Fig. 4 Mean concentrations (n = 8, ± SE) of (**a**) DMSPp expressed as fmol $cell^{-1}$ and (**b**) DMSPp expressed as fmol cm^{-2} in control corals (*grey*), corals transplanted from 3 to 20 and 50 m (*striped*) and from 50 to 20 and 3 m (*white*) in the Gulf of Aqaba. *Letters above error bars* indicate significant differences between groups (SNK tests, $p < 0.05$)

Fig. 5 Regression analyses of the relationship between symbiont cell densities and (**a**) DMSPt and (**b**) DMSPp (n = 56)



Borell et al. 2016 - Figure 1



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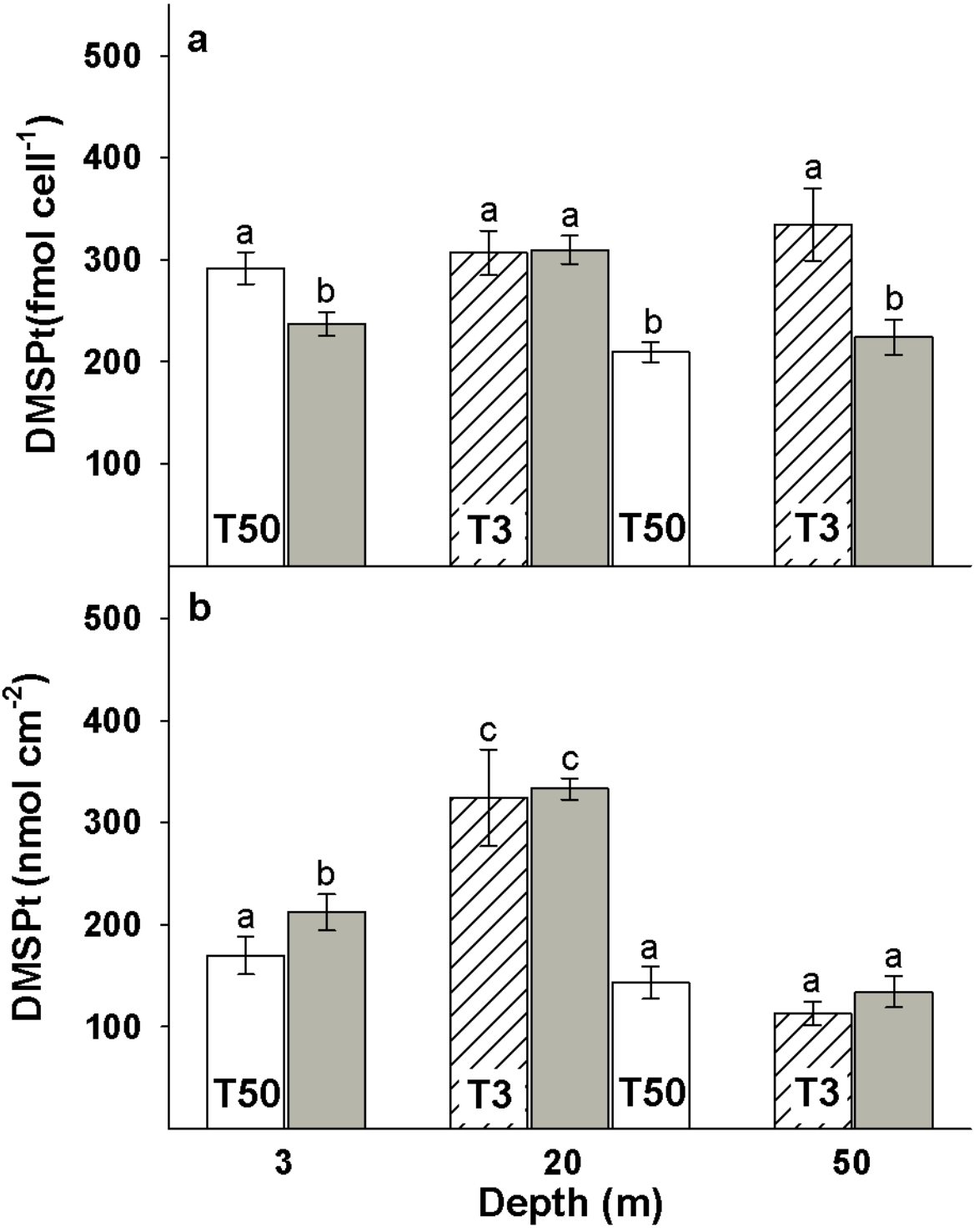
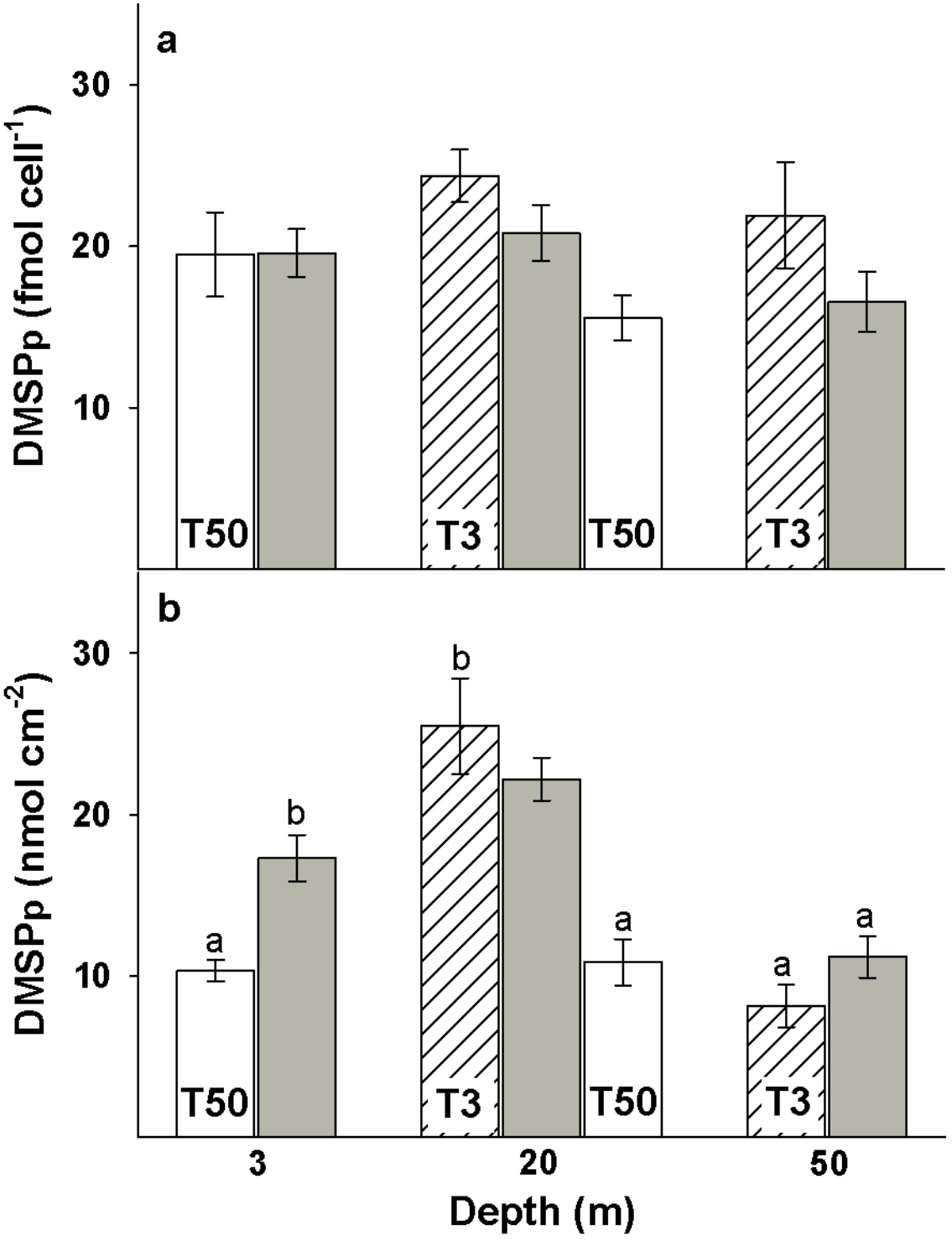
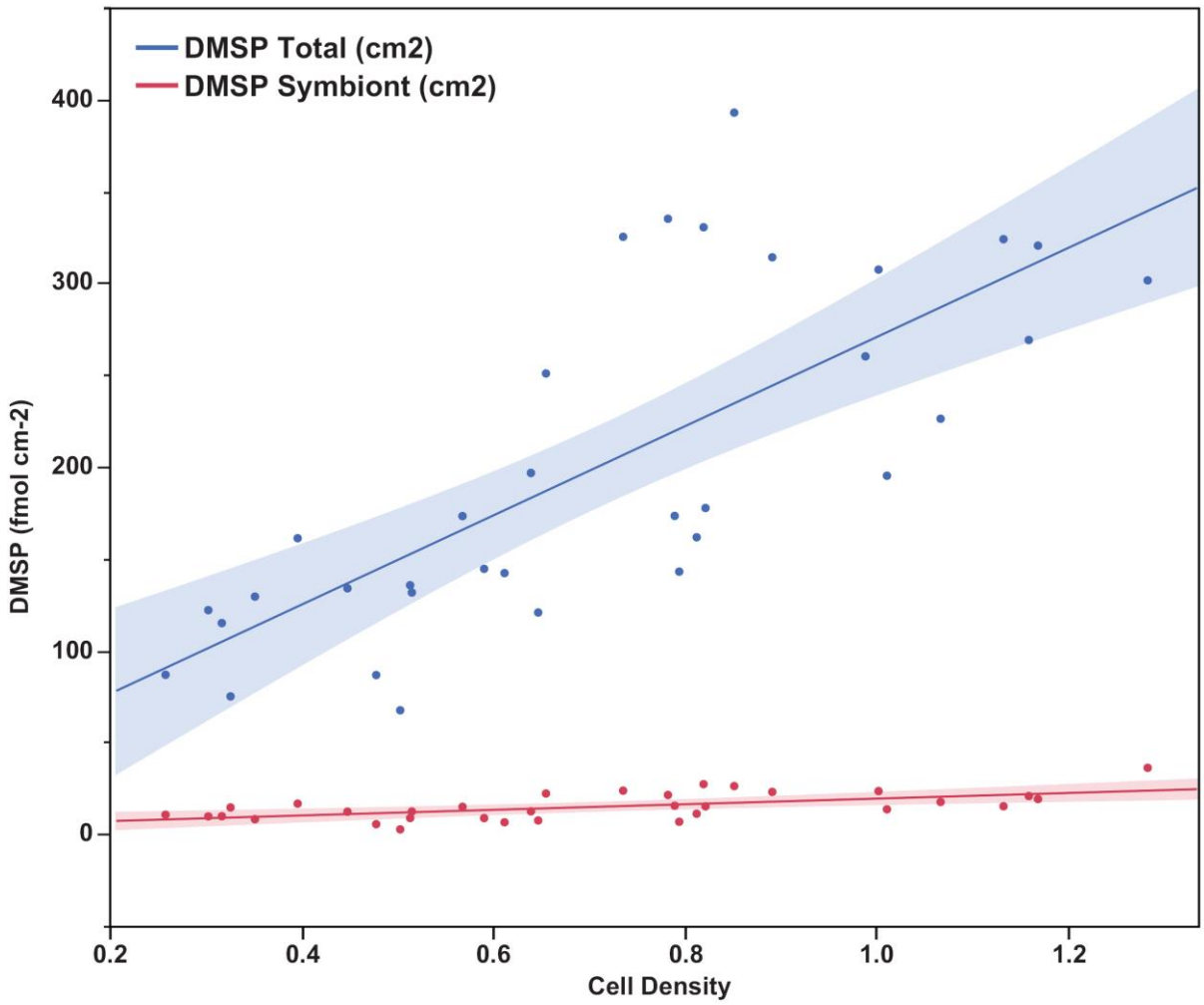


Borell et al. 2016 - Figure 3



Borell et al. 2016 - Figure 4



Borell et al. 2016 - Figure 5