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Effect of nanoparticles on DMS consumption and DMSP concentration in *Aiptasia pallida*

Abstract

Wide use of nanoparticles (NPs) in day-to-day products increases the likelihood of NPs entering and effecting marine organisms as a result of direct emissions and indirect terrestrial runoff. The effects of NPs on dimethylsulfide (DMS) consumption and concentrations of dimethylsulfoniopropionate (DMSP) within the holobiont of cnidarians remain obscure. This study examines the effects of titanium dioxide (TiO_2 NP) and capped silver nanoparticles (cAg NP) at 50mg/L concentrations, on the microscopically observations of physiological changes to the tropical sea anemone, *Aiptasia pallida* (clone CC7), the concentrations of DMS and DMSP of its intracellular symbiont *Symbiodinium* sp. (A02 and B01) and its surface DMS consuming bacteria.DMS was quantified during NP exposure, and 24 and 96h post exposure using gas chromatography (GC). DMS production was found to be 2.5 fold higher in A02 compared to B01. However, concentrations of particulate DMSP were only observed to increase in the B01 strain when exposed to TiO_2 NP (22.90±1.55nmol $h^{-1}mg^{-1}$) compared to control (11.41±0.05nmol $h^{-1}mg^{-1}$). A common pattern was identified for the surface DMS consuming bacteria on *A. pallida*, regardless of *Symbiodinium* strain or NP treatment. During NP exposure DMS consumption was lower in all NP treatments (0.10±0.05nmol $h^{-1}mg^{-1}$) in comparison to the controls (0.19±0.19 nmol $h^{-1}mg^{-1}$). 24h post exposure, DMS consumption was higher (0.27±0.13 nmol $h^{-1}mg^{-1}$) compared to the controls (0.20±0.22nmol $h^{-1}mg^{-1}$), with DMS consumption returning to similar rates as controls in NP treatments post 96h exposure. Microscopical observations showed that cAg NP had the greatest adverse effect on both *Symbiodinium* strains, with approximately 40% decrease in cell density after 24h exposure, and loss of tentacles in *A. Pallida* being observed only in this NP treatment. This work illustrates the potentially negative impacts of NPs on DMS consumption and concentrations of DMSP in the holobiont of *A. pallida*, thus emphasising the need for more studies to be conducted on NP effects in the tropical marine environments.

Keywords: Nanoparticles, *A. Pallida*, Concentrations, *Symbiodinium*, $DMSP_t$, $DMSP_p$

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Abbreviations: NPs, Nanoparticles; DMS, Dimethylsulfide; DMSP, Dimethylsulfoniopropionate; FASW, Filtered Artificial Seawater; cAg NP, Capped Silver Nanoparticles; GC, Gas Chromatography; ROS, Reactive Oxygen Species

Introduction

Nanoparticles (NPs) are used in day-to-day products which can be released into the marine environments from manufacturing processes and poorly managed breakdown or decay of manufactured items. They enter the environment via water sources such as terrestrial run off mainly from coastal urban environments, therefore exposure to marine life it is inevitable. Our knowledge of NPs affect marine organisms is very limited. Studies have shown that NPs can have an antimicrobial effect, majority of studies have been conducted with human pathogenic bacteria,[1-3] whilst this is an attractive property of NPs, many non-target effects on bacteria that play crucial roles in environmental cycling, such as sulphur[4,5] could be negatively impacted, particularly in the tropical marine system where sulphur plays many biological roles.[6-8] Given the poor current understanding of the toxic effects of NPs on marine life, it is crucial for more research to be conducted in this field so such effects can be better forecasted. This study looks at how titanium dioxide Nanoparticles (TiO_2NP) and capped silver Nanoparticles (cAg NP) affects dimethylsulfide (DMS) consumption and production in the holobiont of *Aistasia pallida*, which is extensively studied for coral research[9] and observes physical indicators of NP stress. Results from this study consumption and production in the coral holobiont. This will create a better understanding of the potential risks to the holobiont associated with *A. pallida* when exposed to nanoparticles in aquatic environments.

This study concentrated on the affects of TiO_2NP and cAg NPs on the production of DMS by *Symbiodinium* and consumption of DMS by surface bacteria on the sea anemone species *Aiptasia pallida* as a holobiont. This study teases apart individual components of the holobiont and investigates how each interlink to balance between DMS consumption and DMSP concentration when exposed to NPs. The aim of this thesis is to investigate how NPs stress affects DMS consumption and DMSP concentration of the *Aiptasia pallid* holobiont.

The main hypothesis of this study is that DMS consumption will decrease in NP treatments with *A. pallida*, since NPs have a detrimental effect on bacteria.[10] Exposure to NPs should lead to elevated levels of DMS due to reduced bacterial consumption.[5] In parallel, this hypothesis further was hypothesised that because NPs produce Reactive Oxygen Species[11] less DMS will be produced by the symbiont to maintain higher levels of intercellular DMSP acting as antioxidants.[12] This thesis presents the first experimental data concerning the DMS response of the *A. Pallid* holobiont towards NP contamination.

Materials and methods

Making up filtered artificial seawater (fasw)

35g Reef salt (Ocean+ Pro formula, D-D the Aquarium Solution Ltd. Essex) was dissolved in 1 L of reverse osmosis water using

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a magnetic stirrer for 10 minutes. Salinity was checked using a refractometer (salinity at 34 - 36 ppt). The artificial seawater (ASW) was pumped into an autoclaved 1 L Duran via a 1 L Nagene filter unit with a 0.2um cellulose nitrate membrane filter (WhatmanTM) using a VRC - model 200 - 7.0 rotary vane vacuum pump.

Maintenance of *Aiptasia pallida*

Aiptasia pallida used in all experiments were derived from the CC7 anemone clone lineage obtained from the Pringle Lab. Anemones were maintained in filtered artificial seawater at 26°C with a light intensity of 30 $\mu Em^{-2} s^{-1}$a photoperiod of 12:12 hours (light: dark) and were fed once weekly with freshly hatched (24-48 hours) *Artemiasalina* after 24-48 hours of feeding, artificial seawater was changed over and any slime was scrubbed off as needed.

Maintenance of *Symbiodinium* culture

*Symbiodinium*strains A02 and B01 were grown in sterile 50ml of ASP-8A medium[13] in 75cm3NuclonTM treated flask (with blue filter cap) at 26°C in a light intensity of ~140$\mu Em^{-2} s^{-1}$ on a photoperiod of 12:12 hours (light: dark). Both strains were obtained from the Pringle Lab.

Nanomaterials

Capped silver nanoparticles (cAg NP) were capped with methoxy-polyethylene glycol, with a core diameter of 13nm ± 7nm. Titanium dioxide nanoparticles (TiO_2 NP) had a specified diameter of <25nm and obtain as a dry power (Sigma-Aldrich Chemie GmbH, Germany).

Analysis of DMS

The following equipment and configuration were used in this work. A Shimadzu GC-2010 Gas chromatography (GC) (Milton Keynes, UK) fitted with a 23m packed chromatography column (inner diameter 0.53mm; J&W Scientific), on-column flame and injector detector was used for gas sample analyse. The temperature settings were: injector 200°C, column 120°C and detector 250°C. The GC gases were: nitrogen 60 $cm^3 min^{-1}$ (carrier gas) and air 70 $cm^3 min^{-1}$ and hydrogen 50 $cm^3 min^{-1}$ (flame gases). All samples were run through trap and purge cryogenic enrichment to concentrate DMS gas, before being flushed into the column. Purge was off during all experimental runs. Data were collected and analysed using GC solutions software program (version 2.30)

DMS Calibration

The DMS concentration range produced during the *A. Pallida* exposure to nanoparticles were covered by setting up a series of known concentrations of DMSP in NaOH from stock solutions. These standards duplicated in the same size vials used for the experiment. Vials of 4.92ml total volume were used with the final volume of 2ml, with 150µl NaOH pipetted into 1850µl DMSP calibration stock. Production of DMS was initiated by shaking the vial. The vials equilibrated at 26°C for 6 hours. Each vial was run through the trap and purge cryogenic enrichment to concentrate the gaseous DMS sample before running it through the GC to produce a peak area corresponding to the DMS concentration in vial headspace. The calibration showed a linear detector response with 0 - 100nM DMS in the aqueous phase (Figure 1). The linear regression was used to obtain the DMS concentration from the peak areas produced by the cleavage activity of NaOH from DMSP.

Known concentrations of DMSP were added to 2ml of NaOH (headspace of 3ml). These underwent trap and purge cryogenic enrichment to concentrate the gaseous DMS sample. Samples were run through GC to produce a peak area corresponding to the DMS concentration in vial headspace. Two runs were done per known DMSP concentration (n = 2).

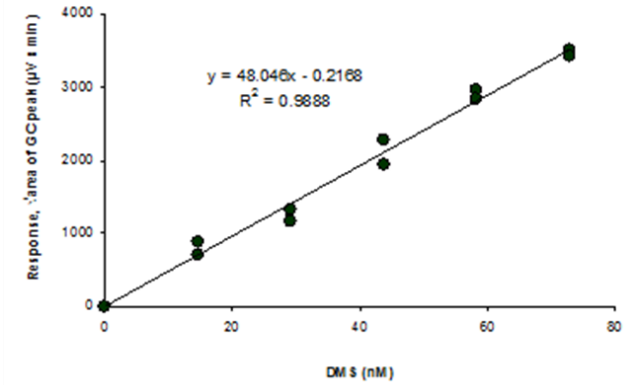

Figure 1 Results of an *in vitro* DMS headspace calibration.

Aquatic DMSP is cleaved by NaOH into DMS until the headspace DMS and aquatic DMS has come to an equilibrium, hence concentration of DMS in headspace should be equivalent to concentration of DMSP added (100nM) in control vials as the system is static. However in a dynamic system where there is consumption of DMS in solution by bacteria all rates measured are under estimations due to the lag of DMS in the air constantly diffusing into solution.

Different dms production between *Symbiodinium* cultures

A known volume of *Symbiodinium* was taken from culture and counted under a light microscope (Olympis BH-2) using a haemocytometer (Marienfeld, Germany). Cell counts were calculated to give the same number of cells per vial in each *Symbiodinium* strain, A02 and B01. This was left to incubate for 24 hours at 26°C. DMS production between the strains was analysed using the trap and purge cryogenic enrichment technique as previous described.

Calculating *Aiptasia pallida* biomass and DMS consumption

*A. pallid*were dislodged gently using a spatula, pipetted up and put into autoclaved vials with 2ml filtered artificial seawater (FASW) and left overnight to settle. Vials with only FASW were also prepared as controls. *A. pallida* were narcotized with 0.37M of $MgCL_2$ making them unresponsive to touch, and oral disc diameter was measured using a under a dark field lens on a Olympic stage microscopy (model S2X2-KLT). Using the composite equation described in[14] dry weight was calculated from the oral disc diameter, allowing for DMS consumption to be normalised accordingly.

Titanium (IV) dioxide (TiO_2NP) and capped silver nanoparticles (cAg NP) both at 50mg/L was used. 99% DMS (Sigma-Aldrich Chemie GmbH, Germany) was diluted to 100nM in 100ml of sterile ASW in volumetric flask and shaken well and left to equilibrate for 5minutes. 20µL pipetted into experimental vials (2ml in total) and immediately capped tightly. Nanoparticles and DMS pipetting were done in a systematic staggered matter (Table 1) with 10 minute intervals per vials (this allows for an equal incubation time between vials when run on the GC). Vials were incubated for 4 hours at 26°C with a light intensity of 30 $\mu Em^{-2} s^{-1}$.

Citation: Wijesinghe PS. Effect of nanoparticles on DMS consumption and DMSP concentration in *Aiptasia pallida*. *J Aquac Mar Biol.* 2015;2(5):228–235. DOI: 10.15406/jamb.2015.02.00044

Table 1 Shows the systematic 10 minute staggered time runs from 9am

T runs	Time	Experimental runs	Run in gas chromatography
T0 (start)	8	Control - Filtered ASW	Yes
T0 (start)	8.1	Control - Filtered ASW + DMS (100nM)	Yes
		Start of systematic staggered time run	
T0 (end)	9	Control - Filtered ASW	No
T0 (end)	9.1	Control - Filtered ASW + DMS (100nM)	No
T0	9.2	Filtered ASW + DMS (100nM) + *A. pallida*	No
T0	9.3	Filtered ASW + DMS (100nM) + *A. pallida*+ 50mg/L (TiO_2 NP or Ag NP)	No
		4 hours incubation	
T1 (end)	13	Control - Filtered ASW	Yes
T1 (end)	13.1	Control - Filtered ASW + DMS (100nM)	Yes
T1	13.2	Filtered ASW + DMS (100nM) + *A. pallida*	Yes
T1	13.3	Filtered ASW + DMS (100nM) + *A. pallida*+ 50mg/L (TiO_2 NP or Ag NP)	Yes

T0 (start) were the only vials that were run under the Gas Chromatography (GC) without an incubation period, this provides the true start values of DMS in vials accounting for potential loss by leakage or bacterial consumption. T0 (end) were measured by the GC 4 hours after incubation, thereby taking into account loss between T0 (start) - T0 (end). DMS consumption was then measure on an hourly basis by T0 (end) - T1. Note: these run were done in triplicates, table 1 only shows the summary of one run.

Total and particulate Dimethylsulfoniopropionate (DMSP) concentrations

Methodology for total dimethylsulfoniopropionate ($DMSP_t$) and particulate dimethylsulfoniopropionate ($DMSP_p$) extraction was adapted from[15] for *A. Pallida* were homogenized individually in 1ml of FASW in a pestle and mortar. Further 3ml of FASW was added to increase volume of homogenized material. Cell count on the total diluted homogenate aliquots were conducted immediately under a light microscope (Olympus BH-2) using a haemocytometer(Marienfeld, Germany), simultaneously measuring cell volume using a eyepiece graticule and calibrated ocular micrometer (it was assumed *Symbiodinium* were spherical, dividing cells were excluded; n = 30 cells per sampling counted). This was used to calculate the intracellular DMSP volume per cell ($DMSP_p$).

2ml of homogenate was filtered through a Whatman GF/F 25mm glass fibre at <100 mm Hg vacuum,[16] and placed in 10M of NaOH for 24 hours at 30°C, to allow of DMSP cleavage to calculate $DMSP_p$. Whilst $DMSP_t$ was calculated by pipetting 1.85ml of total homogenate into 0.15ml of 10M NaOH in 4.92ml vials and left for 24hours for headspace equilibration at 30°C, these were all analysed for DMS by injecting 0.30ml volume of headspace through the vial septum every 60 seconds in to the GC using a gas-tight syringe (GC setting were as previous described).

Data analysis

All data is presented as the mean ± standard deviation of the mean. Data were analyed using either an unpaired *t*-test or one way ANOVA when data was normally distributed, or a Kruskal-Wallis test if data failed normality. Significance was taken at $p<0.05$ using the statistical package R version 3.0.2. All graphical illustration were created using Sigma Plot 10. Some figures were produced using Microsoft Windows Paint version 6.3.

Results

DMS production between A02 and B01 *Symbiodinium* strains

Both A02 and B01 *Symbiodinium* strains were normalized to cell counts and incubated in at 26°C to identify which strain had produced more DMS. It was found that the A02 strain produced significantly greater volume of DMS per cell compared to the B01 strain. An unpaired t-test was conducted identified a very significant difference; $p<0.001$ (Figure 2).

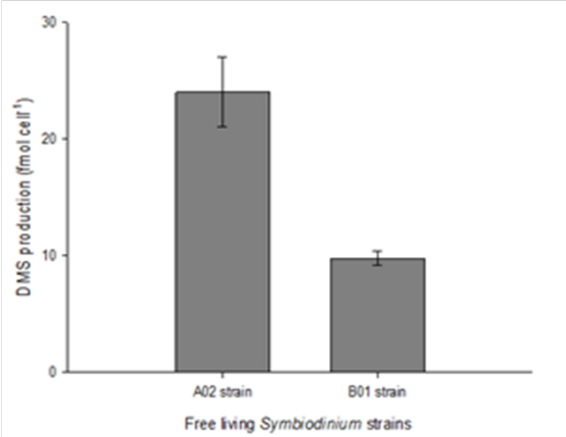

Figure 2 Difference in DMS production in free living *Symbiodinium* strains (normalized to cell number).

(All data is represented as the mean ± SD (n = 4). Data were analysed using Student's *t*-test ($p<0.05$).

Cellular density, diameter and volume between A02 and B01 *Symbiodinium* strains

Cell densities were normalized to dry weight. There was approximately ~40% decrease in cell densities in both strains when exposed to cAg NP treatments compared to control treatments. Cell densities in TiO_2NP treatments remained similar to control treatments. A02 control treatment strains had the smallest diameter of 8.0µm^3 and a corresponding cell volume of 269µm^3 cell-1, and B01 control treatment strain had the largest diameter of 10.2µm^3 and a corresponding cell volume of 556µm^3 cell-1. Cell diameter and volume decreased in both strains after exposure to either NP treatments (Table 2).

Citation: Wijesinghe PS. Effect of nanoparticles on DMS consumption and DMSP concentration in *Aiptasia pallida*. *J Aquac Mar Biol.* 2015;2(5):228–235. DOI: 10.15406/jamb.2015.02.00044

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Table 2 Average values for cell density, cell diameter and cell volume in whole organisms of *A. pallida* in symbiosis with two different strains of *Symbiodinium* sp. Shown are *Symbiodinium* per *A. pallida* means ± SD (n = 2)

Treatment type	*symbiodinium*strain	Cell density (cell mg^{-1})	Diameter (μm^3)	Cell Volume ($\mu m^3 cell^{-1}$)
Control		$1.0\times10^6 \pm 4.5\times10^5$	8.0 ± 0.42	269 ± 42.7
cAg NP	A02	$6.1\times10^5 \pm 1.9\times10^4$	7.1 ± 0.99	193 ± 78.7
TiO_2NP		$1.0\times10^6 \pm 1.9\times10^5$	7.5 ± 0.21	254 ± 20.5
Control		$1.3\times10^6 \pm 2.0\times10^5$	10.2 ± 0.28	556 ± 46.2
cAg NP	B02	$7.6\times10^5 \pm 4.6\times10^5$	9.7 ± 0.49	472 ± 72.4
TiO_2NP		$1.5\times10^6 \pm 4.7\times10^4$	9.2 ± 0.14	408 ± 18.8

Behavioural and microscopical observations

General observations suggested smaller *A. pallida* were more susceptible to NPs, and in some cases severely comprised, especially to cAg NP treatment. Mucus production and tentacle retraction was immediately observed in both *A. pallida* strains when exposed to TiO_2NP, with the majority of free floating TiO_2NP being trapped within the mucus (Figure 3E). After 24h being exposed to TiO_2NP some anemones were observed to have partially or full retracted tentacles (Figure 3C,3D) such behaviour was not specific to any one strain. On the other hand, cAg NP initiated mucus production and tentacle retraction notably later in comparison to TiO_2NP treatment. A far greater potent effect was observed after 24h of cAgNP exposure, *A. pallida* displayed extreme stressed symptoms such as: loss of tentacles, bleached tentacle tips, complete bleaching, expulsion of zooxanthellae and bloated body with a thickened epidermis layer (Figure 3G,3H,3I,3F,3D). Darkened areas (Figure 3C,3D,3F) were never observed on controls.

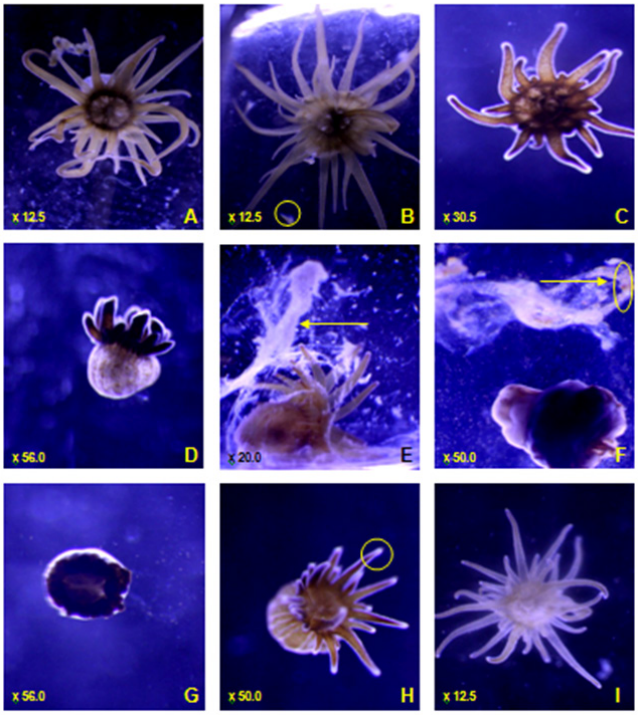

Figure 3 Common physical symptoms of *Aiptasia pallida*. (A) control *A. Pallida* (B) control *A. pallida* feeding on *Artemiasalina* (free swimming *Artemiasalina* circled) (C) partially retracted long tentacles (D) all tentacles retracted with thickened epidermis layer and bloated body (E) mucus production (F) mucus production with expulsion of zooxanthellae around *A. pallida* and trapped in mucus (circled), loss of tentacles, thickened epidermis layer (G) loss of tentacles (H) bleached tentacle tips (I) bleached *A. pallida*.

Note: magnification on bottom left of images.

DMS consumption after np treatment in surface bacteria

Trend was observed in consumption pattern for DMS consuming bacteria on *A. pallida*, regardless of *Symbiodinium* strain or NP treatment. Trend general showed a decrease in DMS consumption following exposure in either NP treatments when compared to the controls. This was followed by a peak in DMS consumption 24h post exposure when compared to the controls, with DMS consumption returning to similar rates as controls in NP treatments post 96h exposure.

A one-way ANOVA was conducted to compare the differences between NP treatments to control treatments on DMS consumption. There were no significant effects on either strain when treated with TiO_2NP (Figure 4A,4C). A significant response was found for cAg NP treatments on A02 *A. pallida*, $p<0.05$, a post hoc Tukey test identified a significant decrease in DMS consumed 96h post NP (0.02±0.01nmol $h^{-1}mg^{-1}$ dry weight) compared to control (0.10±0.05nmol $h^{-1}mg^{-1}$dry weight) $p<0.05$ (Figure 4B). Similarly, cAg NP on B01 *A. Pallida* had a very significant effect on DMS consumed $p<0.001$. Post hoc Tukey test revealed that DMS consumed significantly decreased during exposure (0.15±0.07nmol $h^{-1}mg^{-1}$dry weight)and 24h post exposure (0.37±0.08nmol $h^{-1}mg^{-1}$ dry weight) when compared to controls during exposure and 24h post exposure; 0.50±0.09nmol $h^{-1}mg^{-1}$ dry weight and 0.56±0.05nmol$h^{-1}mg^{-1}$ dry weight, respectively, $p<0.05$ (Figure 4D).

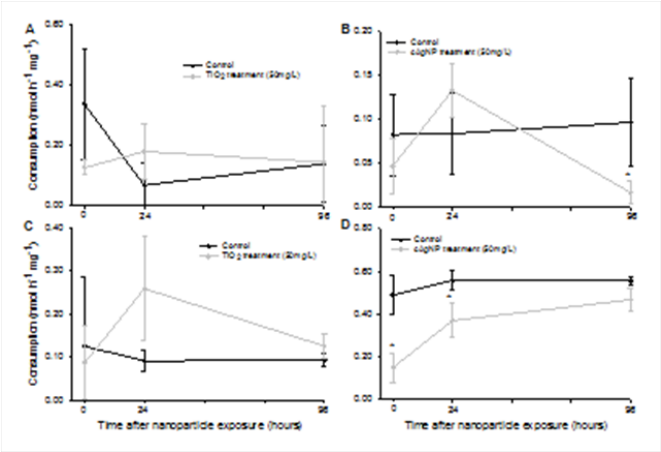

Figure 4 Rate of DMS consumption (normalized to dry weight) during 4h exposure (shown as 0 hours on the x-axes), and after 24 and 96h of exposure. (A) *A. Pallida* A02 with TiO_2 NP, (B) *A. Pallida* A02 with cAg NP, (C) *A. Pallida* B01 with TiO_2 NP, (D) *A. pallida*B01 with cAg NP.

Figure 4 shown are means ± SD (n = 3). Data were analysed using one way ANOVA followed by Post hoc Tukey test. Asterisks denote a significant difference from concurrent controls ($p<0.05$). Note the difference in scales along the y-axes.

Citation: Wijesinghe PS. Effect of nanoparticles on DMS consumption and DMSP concentration in *Aiptasia pallida*. *J Aquac Mar Biol.* 2015;2(5):228–235. DOI: 10.15406/jamb.2015.02.00044

Controls between treatments were found to have different rates of consumption, even between the same strains (Figure 4) after normalization to dry weight. Thus it was hypothesised surface area: volume ratio played a role in such differences. A Pearson's correlation was conducted to test the relationship between oral disc diameter of both strains and DMS consumption at 0 hours. A very significant negative relationship was identified between DMS consumption and oral disc diameter, $p<0.001$ (Figure 5).

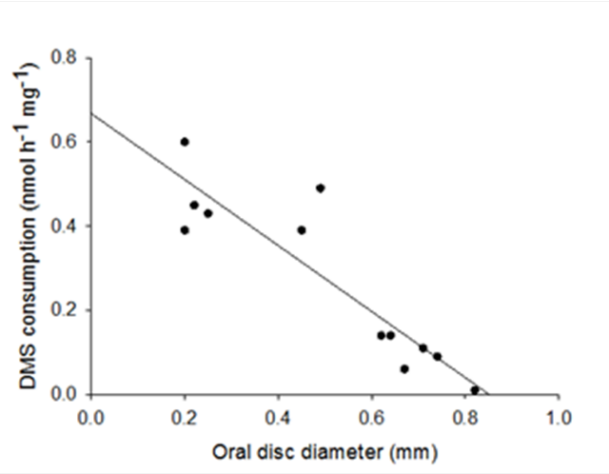

Figure 5 Comparison of oral disc diameter (mm) to DMS consumption (nmol h^{-1} mg^{-1}) for both control *A. pallid Symbiodinium strains*.

Data were analysed using Pearson's correlation. Significance was found to $p<0.001$.

DMSPp and DMSPt concentrations

$DMSP_t$ and $DMSP_p$ were normalized to dry weight. Concentrations of $DMSP_p$ were only observed to increase in the B01 strain when exposed to TiO_2 NP compared to control, however $DMSP_p$ remained similar to controls when treated with cAg NP. On the other hand A02 treated with either NP treatment decreased in $DMSP_p$ when compared to the control (Figure 6A). Furthermore, A02 $DMSP_p$ concentrations were greater than B01, this results correspond with results previously described from DMS production between free living strain A02 and B01 strains (Figure 2). Concentrations of $DMSP_p$ were generally observed to be lower in either NP treatment for both strains (Figure 6A). A one-way ANOVA was conducted to compare the difference between NP treatment to control treatments on DMS production in $DMSP_p$ and $DMSP_t$ between the two strain types. No significant response was found in either strain for $DMSP_p$ or $DMSP_t$.

All data is represented as the mean ± SD (n = 2 for $DMSP_p$, n = 3 for $DMSP_t$). Data were analyzed using one way ANOVA, no significant differences were found to $p<0.05$. Note: the scale difference between graphs.

Discussion

Behavioural and microscopical observations

A study on copper oxide NPs[16] found these NPs accumulated within the tissue of the host, however, there is no information on the observed physical symptoms of NPs on the host. Results of this study were very similar to the microscopically observed behaviours on *A. pallida*[17] and *A. Pulchella*[18] when exposed to trace metals such as cadmium, cobalt, lead, nickel, copper and zinc, suggesting that the mechanism behind trace metal and NP accumulation are likely to be the similar. Although, the biological reasons behind such behaviours were ambiguously explained. This study provided some suggestions to the biological reasons behind these behaviours. Studies have shown that cnidarian species such as corals produce mucus in turbid waters as a mechanism to remove sedimentation that has settled in the epidermis of the coral, consequently blocking light to the photosynthetic symbiont.[19] Instead of sedimentation, NPs that had settled on the epidermis of *A. pallida* may have triggered the same mucus producing mechanism (Figure 7).

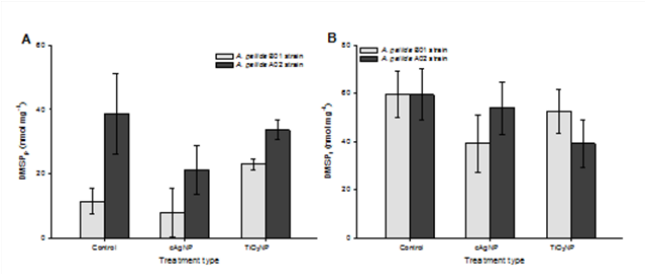

Figure 6 DMS produced in $DMSP_p$ and $DMSP_t$ in *A. pallida* to 24 hours of exposure to nanoparticle treatment. (A) $DMSP_p$ for control, cAgNP and TiO_2NP on B01 and A02 *A. pallida*, (B) $DMSP_p$ for control, cAgNP and TiO_2NP on B01 and A02 *A. pallida*.

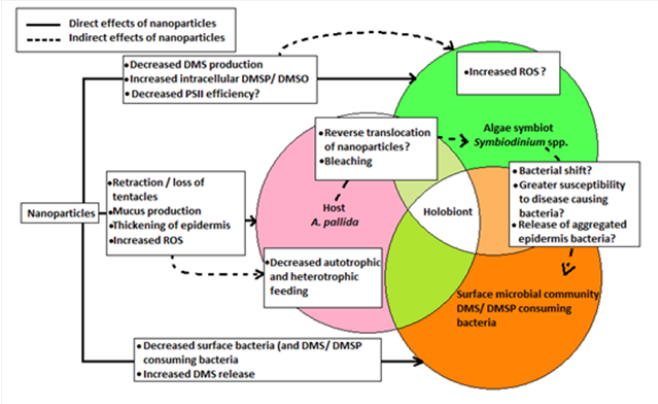

Figure 7 A schematic showing the direct and indirect effects of nanoparticles found in this study.

Physiological changes such as a thickened epidermis layer may be to reduce passive entry and accumulation of NPs by osmosis, and bloated body may be to decrease the surface area to volume ratio, thereby decreasing contact area for NP settlement. Tentacle retraction and in other more extreme cases loss of tentacles (which was only observed in cAg NP) may have been due to a greater accumulation of NPs in the symbiont due to its greater tolerates.[12] This is reinforced by observations of retracted tentacles to tributyltin (a biocide in anti-fouling paint) in symbiotic anemones compared to no retraction of tentacles in aposymbiotic anemones[20] suggested that the host anemones are able to reverse translocate tributylin into the its symbionts, therefore, the loss of tentacles is likely to be a "last resort" mechanism, in which majority of the NPs are reverse translocated from the host to tissue into the symbiont (Figure 7). Furthermore, retracted or loss of tentacles also reduces its surface area. No re-growth of tentacles were observed even after two weeks post NP exposure, thus it is likely that *A. pallida* was completely autotrophic at this stage (Figure 7). Another mechanism which supports reverse translocation of NPs and was observed is bleaching; the active expulsion of the symbiont by the host.

Additionally, no pedal laceration reproduction was observed during or after NP exposure, which may be due to the detrimental effects of

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NPs to juvenile marine organisms[18,21,22] which may be linked more to the surface area of juvenile organisms compared to adult organisms.

Effects of NPs on consumption of DMS and concentration of DMSP

There was a more pronounced rate of DMS production in the free living *Symbiodinium* strainA02 compared to B01 in controlled conditions. It was important to establish were there was a difference as this could have played a role in the amount of DMS consumed in surface bacteria. Furthermore, it has been observed that DMS was only produced by the symbiont in *A. pallida,*[4] however if *A. pallida* utilise DMS is unclear.

A decrease in DMS consumption pattern was observed immediately during exposure to TiO_2 NP or cAg NP when compared to control treatments. This suggested that DMS consuming bacteria were either inhibited for the process of DMS consumption or the antimicrobial effects of the NPs severely decreased the surface microbiome population (Figure 7 & 8). The latter argument has been supported by many other studies which have demonstrated that the antimicrobial properties have a negative impact on bacterial populations.[10,23] In contrast, 24h after TiO_2 NP or cAg NP removal, DMS consumption was observed to peak above across all treatments and strains. Reasons for such a dramatic peak in consumption remains obscure, however, it is unlikely that the bacterial population recovered to normal within 24h of NP removal. This is even more unlikely for DMS consuming bacteria, as such bacteria were observed to have a very long stationary phase when grown on either liquid or solid media (data not shown here). However, it is possible that the antimicrobial properties of the NPs exposed DMS bacterial strains that sensitive to it and possibly selecting for more resistant DMS consuming bacteria, which could have reached the exponential growth phase faster than normal due to the lack of competition and abundance availability of DMS.

The other potential explanation comes from observations of bacterial aggregates within the epidermis of the *A. pallida* (Figure 7). These bacteria were identified as a genus of bacteria called *Vibrio,*[24] although the reasoning behind why such bacteria may exist was not suggested. Interestingly, the *Vibrio* genera of bacteria were found to consume DMS or DMSP due to its successful isolation when cultured in DMS or DMSP only plates.[25] Although, this thesis only suggests possible hypothesis for the peak consumption of DMS, further examination on whether bacterial communities shifted before and after NP treatments may yield interesting results.

Total symbiont per mm2 was derived from.[26] Note: due to no significant differences found in $DMSP_t$ or $DMSP_p$ between NP treatments for either strain in this thesis, the gross production is assumed not to change between control and NP treatment. Furthermore, it is known that DMS only produced by the symbiont in *A. pallida,*[4] however if *A. pallida* utilise DMS is unclear.

96h post NP treatment saw DMS consume return to similar levels of control treatments. Two possible scenarios can be derived from this, first being the total recovery of all bacterial strains on the surface microbiome of *A. pallida*, therefore competing for space with DMS consuming bacteria which are likely to be out competed due to their slow stationary phase (data not shown). Second, the *Symbiodinium* within *A. pallida* are no longer stressed thus release of DMS levels are of similar rates to control treatments. Resulting in two input sources of DMS; the external experimental input of DMS and the actually DMS production of by the symbiont. The latter was thought to be an artefact of consumption explained by Michaelis-Menten kinetics,[27] however, rates of control consumption did not follow the trend described by Michaelis-Menten kinetics.

It was observed that control consumption rates differed between each other between the same strains even after being normalised to dry weight. It was hypothesised that the surface area to volume ratio may play a role in these differences as it does not follow a linear pattern, as expected when DMS consumption was normalised to dry weight. It was found that as oral disc diameter increased DMS consumption decrease, suggesting that the surface area to volume ratio is likely to be a better measurement of DMS consumption compared to dry weight normalisation with sea anemone experimentation. Furthermore, oral disc diameter can be used as a parameter for relative maturity of *A. pallida*, following the logic that the larger the size of the anemone is the older it is likely to be. Following this suggestion and the fact that more juvenile anemone would have a relatively greater surface area compared to adult anemones. A greater surface area allows for a greater exposure of NPs to juveniles. As previous mentioned no pedal lacteration reproduction was observed, therefore this thesis supports that the detrimental magnitude of NPs may change according to the life stages of *A. pallida*, other studies have found similar results of other juvenile marine organisms.[18,21,22]

Such results would suggest that general the type of nanoparticle (capped or uncapped, or type of metal) does not matter, as both had similar negative effects on the surface microbiome (DMS consuming bacteria). Whilst the host (*A. pallida*), was observed to lose tentacles only when exposed to cAg NPs, the general pattern of behaviour between both NPs used overlapped greatly. However, the symbiont's response between NP treatments were notable different. The general cell volume between strains in each NP treatment was similar to the control treatment. However, cell density in both strains decreased by approximately 40% in cAg NP when compared to control treatments, TiO_2 NP treatments did not change in comparison to controls. On the other hand a decrease in cell diameter was only observed in TiO_2 NP treatments when compared to controls. A possible reason for the symbiont's varied response to specific NPs may be due to the host acting as a buffer against complete exposure to NPs, which further explains the general uniform effects to both host and DMS consuming bacteria.

It is also possible that the decreased cell density in cAg NP maybe be due to the loss of tentacles. However, it is important to note that the NPs used in this thesis were engineered differently. TiO_2NP was not capped, therefore would either settle with time or be trapped within mucus produced by *A. pallida*. Ag NP were capped, preventing aggregation or effective removal by mucus. Therefore, it was more likely to interact with the host tissue and causing more damage; loss of tentacles, thus, cAg NP acts indirectly in the observed decrease of cell density (Figure 7).

There were no observed differences between control and NP treatments for $DMSP_p$ or $DMSP_t$ between either *Symbiodinium* strain. However, $DMSP_p$ showed the A02 strain was still the higher DMS producing strain, as $DMSP_p$ was higher in A02 compared to B01. Therefore, it can be assumed that DMS production by the symbiont remains the same regardless of NP treatment (Figure 8). Furthermore, other studies have suggested that NPs can produce reactive oxygen species (ROS) within algae and phytoplankton, in relation to this it has been observed that other ROS producing conditions such as UV light, CO2 and Fe limitation were observed to increase cellular DMSP and/ or its lysis to DMS in marine algal cultures[28] (Figure 7). Interestingly, $DMSP_t$ and $DMSP_p$ results from this study conflict with results from.[28] This is likely due to the duration of NP exposure, all

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marine algal cultures were exposed for 9-14 days in ROS producing conditions[28] whilst this study only exposed *A. Pallida* for 24h, thus it is likely that NP exposure was not long enough for ROS species to be produced hence the insignificant differences between and NP treatments. Further studies need to be done to investigate if algae species in a symbiotic relationship respond in a similar way to algae species on their own.

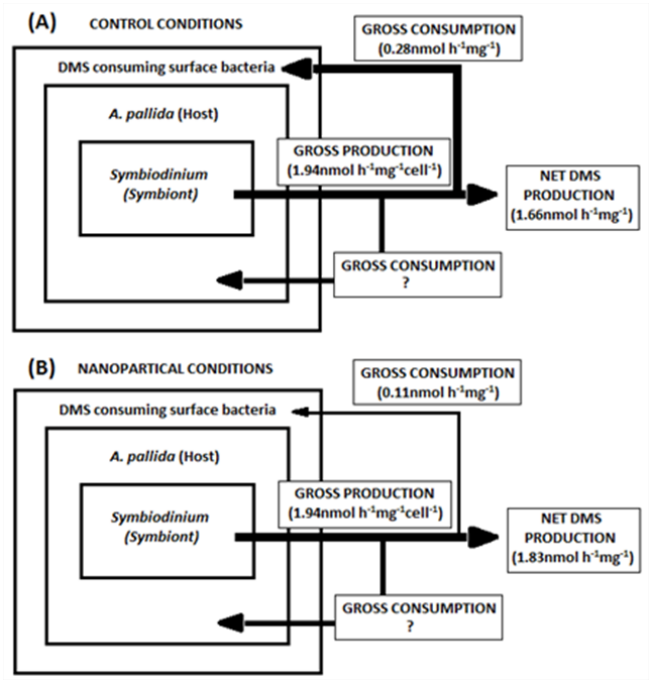

Figure 8 A schematic illustrating the average observed DMS consumption and production in both strains of *Symbiodinium* in control and both NP treatments.

Conclusion

In conclusion, this study provides evidence which supports the hypothesis that NP exposure to *A. Pallida* will have a detrimental effect on bacteria. It was hypothesized that NP exposure would increase DMSP due to ROS, however no increase of intercellular DMSP was observed after NP treatments. This is most likely due to the short term exposure of NPs (24h), which possibly could have not been long enough for ROS production to occur.

Directions of future research

The focus of this study was to assess the affect DMS consumption and DMSP concentrations in the holobiont of *A. pallida* after being exposed to TiO_2NP and cAg NP. However, it is important to gain an understanding of the effects of DMS consumption and production to environmentally-relevant concentrations of a range of NPs to the holobiont of *A. Pallid* and coral species. In addition, more studies need to be conducted to assess the long-term effects of NPs exposed at environmentally-relevant concentrations and investigate its effects over multiple generations.

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Conflicts of interest

None.

References

1. Choi O, Deng KK, Kim NJ, et al. The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. *Water research*. 2008;42(12):3066–3074.
2. Choi O, Hu Z. Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. *Environ sci technol*. 2008;42(12):4583–4588.
3. Kangwansupamonkon W, Lauruengtana V, Surassmo S, et al. Antibacterial effect of apatite–coated titanium dioxide for textiles applications. *Nanomedicine*. 2009;5(2):240–249.
4. Van Alstyne KL, Dominique III VJ, Muller–Parker G. Is dimethylsulfoniopropionate (DMSP) produced by the symbionts or the host in an anemone–zooxanthella symbiosis? *Coral Reefs*. 2009;28(1):167–176.
5. Yoch DC. Dimethylsulfoniopropionate: its sources, role in the marine food web, and biological degradation to dimethylsulfide. *Appl Environ Microbiol*. 2002;68(12):5804–5815.
6. Raina JB, Dinsdale EA, Willis BL, et al. Do the organic sulfur compounds DMSP and DMS drive coral microbial associations? *Trends microbial*. 2010;18(3):101–108.
7. Savoca MS, Nevitt GA. Evidence that dimethylsulfide facilitates a tritrophic mutualism between marine primary producers and top predators. *Proceedings of the National Academy of Sciences*. 2014;111(11):4157–4161.
8. Borell EM, Steinke M, Horwitz R, et al. Increasing pCO2 correlates with low concentrations of intracellular dimethylsulfoniopropionate in the sea anemone Anemoniaviridis. *Ecol evol*. 2014;4(4):441–449.
9. Lehnert EM, Burriesci MS, Pringle JR. Developing the anemone *Aiptasia* as a tractable model for cnidarian–dinoflagellate symbiosis: *the transcriptome of aposymbiotic A. pallida. BMC genomics*. 2012;13(1):271.
10. Poole JG. Impact of Engineered Nanoparticles on Aquatic Microbial Processes. PhD dissertation: University of Essex, Colchester. 2013.
11. Siddiqui S. Comparative effects of dissolved copper and copper oxide nanoparticles on sea Anemone *Aiptasia Pallida*. In 144th Annual Meeting of the American Fisheries Society. 2014.
12. Deschaseaux ES, Jones GB, Deseo MA, et al. Effects of environmental factors on dimethylatedsulfur compounds and their potential role in the antioxidant system of the coral holobiont. *Limnology and Oceanography*. 2014;59(3):758–768.
13. Ahles MD. Some Aspects of the Morphology and Physiology of Symbiodinium microadriaticum. PhD dissertation: Fordham University, New York,USA. 1967.
14. Clayton Jr WS, Lasker HR. Individual and population growth in the asexually reproducing anemone *Aiptasia pallida* Verrill. *Journal of experimental Marine Biology and Ecology*. 1985;90(3):249–258.
15. Yost DM, Mitchelmore CL. Determination of total and particulate dimethylsulfoniopropionate (DMSP) concentrations in four scleractinian coral species: a comparison of methods. Journal of Experimental *Marine Biology and Ecology*. 2010;395(1–2):72–79.

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16. Steinke M, Malin G, Turner SM, et al. Determinations of dimethylsulphoniopropionate (DMSP) lyase activity using headspace analysis of dimethylsulphide (DMS). *Journal of Sea Research*. 2000;43(3):233–244.

17. Main WP, Ross C, Bielmyer GK. Copper accumulation and oxidative stress in the sea anemone, *Aiptasia pallida*, after waterborne copper exposure. *Comp Biochem Physiol C Toxicol Pharmacol*. 2010;151(2):216–221.

18. Howe PL, Reichelt–Brushett AJ, Clark MW. Development of a chronic, early life–stage sub–lethal toxicity test and recovery assessment for the tropical zooxanthellate sea anemone Aiptasia pulchella. Ecotoxicol Environ Saf. 2014;100:138–147.

19. Christian Wild, Markus Huettel, Anke Klueter, et al. Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. *Nature*. 2004;428(6978):66–70.

20. Mercier A, Pelletier E, Hamel JF. Toxicological response of the symbiotic sea anemone Aiptasia pallida to butyltin contamination. *Marine Ecology Progress Series*. 1996;144(1–3):133–146.

21. Griffitt RJ1, Brown–Peterson NJ, Savin DA, et al. Effects of Chronic Nanoparticulate Silver Exposure to Adult and Juvenile Sheepshead Minnows (Cyprinodonvariegatus). *Environ Toxicol Chem*. 2012;31(1):160–167.

22. Zhu X1, Zhou J, Cai Z. TiO_2 nanoparticles in the marine environment: Impact on the toxicity of tributyltin to abalone (Haliotisdiversicolorsupertexta) embryos. *Environ Sci Technol*. 2011;45(8):3753–3758.

23. Adams LK, Lyon DY, Alvarez PJ. Comparative eco–toxicity of nanoscale TiO_2, SiO2, and ZnO water suspensions. Water Res. 2006;40(19):3527–3532.

24. Edward EP, Warren RJ, Joan SP, et al. Bacterial aggregates within the epidermis of the sea anemone Aiptasia pallida. *The Biological Bulletin*. 1989;177(1):130–140.

25. Raina JB, Tapiolas D, Willis BL, et al. Coral–associated bacteria and their role in the biogeochemical cycling of sulfur. *Appl Environ Microbiol*. 2009;75(11):3492–3501.

26. Fransolet D, Roberty S, Herman AC, et al. Increased Cell Proliferation and Mucocyte Density in the Sea Anemone Aiptasia pallida Recovering from Bleaching. *PLoS One*. 2013;8(5):e65015.

27. Cohen I, Neori A. Ulva lactucabiofilters for marine fishpond effluents. I. Ammonia uptake kinetics and nitrogen content. *Botanica Marina*. 1991;34(6):475–482.

28. Sunda W, Kieber DJ, Kiene RP, et al. An antioxidant function for DMSP and DMS in marine algae. *Nature*. 2002;418(6895):317–320.

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