Impact of crude oil pollution on marine dimethyl sulfide production

Jessica M. Pimentel Almonte¹ and Zsuzsanna Balogh-Brunstad²

INTRODUCTION

Phytoplankton are photosynthetic microorganisms, which are commonly known for their position at the base of the oceanic food chain, followed by zooplankton. Despite their small size, phytoplankton are the ocean’s main primary producers and account for approximately half of global primary productivity. Thus, these species are responsible for carbon consumption and oxygen production, which is a great interest for modern society as the anthropogenic carbon dioxide production has been influencing global climate patterns (Zindler et al. 2014).

Phytoplankton have been known to play an additional critical role in global climate via production of dimethylsulfonylpropionate (DMSP) when under stress from stronger solar radiation. The DMSP in the ocean gets converted to dimethyl sulfide (DMS) by the resident bacteria before entering the atmosphere as aerosol particles, which has a negative feedback as it induces cloud formation (Arnold et al. 2013). The clouds reduce incoming radiation and keep the oceans cooler. Charlson et al. (1987) proposed a negative feedback loop that operates between ocean ecosystems and Earth's climate. The production of dimethyl sulfide by phytoplankton is responsive to variations in climate forcing, and that these responses act to stabilize the temperature of the Earth's atmosphere, it is called Charlson-Lovelock-Andreae-Warren hypothesis (CLAW; Andreae et al. 1995). The DMS cycle is critical for other species living in the oceans and sensitive to climate warming. The negative feedback on climate warming is greatly affected by the phytoplankton’s ability to produce DMSP (Arnold et al. 2013). Previous studies have shown that phytoplankton are able to increase their CO₂ consumption and increase their DMSP production during high stress from solar radiation and they are responsible for about half of the fossil fuel produced CO₂ sequestration in the oceans (Arnold et al. 2013; Petrou et al. 2016). Higher concentrations of intracellular DMSP was measured in phytoplankton with elevated temperature and CO₂ concentrations, which was also associated with higher metabolic rates and absorption of toxins (Marwood et al. 1999; Arnold et al. 2013; Ozhan et al. 2014). These results are all in line with CLAW, however, anthropogenic impact on the ocean (and on Earth) does not stop with increased CO₂ levels and elevated temperatures. Chemical and physical pollution caused by organic and inorganic compounds also greatly affect the ecosystems; many with unknown interactions, co-pollution impacts, and reactions (Echeveste et al. 2016; Speers et al. 2016). The response of phytoplankton to compounding effects of climate warming and pollution is still unknown, but is really important as the DMS production greatly balances the energy budget of Earth (Arnold et al. 2013).

Recently, with devastating results of oil spills on marine life, and events such as the Deepwater Horizon oil spill in the Gulf of Mexico on April 2010, the effects of oil on phytoplankton have been initiated (Ozhan et al. 2014). Huang et al. (2011) showed that varying concentrations of crude oil have significant effects on the growth of phytoplankton communities. In fact, the smaller concentrations (≤1.21 mg/L) were found to stimulate growth, whereas larger concentrations (≥2.28 mg/L) inhibited growth. In addition, the tolerance of phytoplankton to crude oil turned out to be species sensitive. Similar growth responses were observed among species when

¹Undergraduate Student Researcher, Department of Biology, Hartwick College Oneonta, NY.
²Associate Professor, Departments of Chemistry, Geology and Environmental Sciences, Hartwick College, Oneonta, NY.
temperature and time of exposure to crude oil or elevated temperatures were varied (Huang et al. 2011). The change in growth rate and the photosynthetic ability of phytoplankton indicates that the combination of oil pollution and increased radiation (temperature) could modify their ability to produce DMSP, which would have a direct effect on DMS production, thus on the negative climate feedback. Hing et al. (2011) tested the tolerance of three species to crude oil exposure and found high variation among the tested species with the lowest tolerance of 0.3 mg/L by Phaeodactylum tricornutum and the highest tolerance of 17.0 mg/L by Chlorella salina. Ozhan et al. (2014) found that small diatoms had a lower tolerance to crude oil than larger species, but the growth of the smaller ones can be stimulated by crude oil in some instances depending on geographical location or climate. These studies did not investigate the direct impact of crude oil on DMS production of the oceans, only the growth and the photosynthetic ability of phytoplankton under pollution and increased temperatures, and found highly variable responses. Thus, the effects of oil spills on DMS production are unknown.

This study focused on investigating the impact of crude oil on DMS production in the ocean and its relationship to phytoplankton growth. A negative correlation between crude oil concentrations and DMS production accompanied by phytoplankton growth was expected. To test this hypothesis phytoplankton samples from the Kiel Fjord, Baltic Sea, Germany were collected and varied concentrations of crude oil were added. DMS, isoprene, and carbon disulfide concentrations were measured and compared to control bottles daily. Isoprene was selected in addition to DMS because isoprene has shown to be produced by plants during heat stress (Sharkey et al. 2007). Carbon disulfide is also produced in small amounts by microorganisms and naturally found in ocean waters, thus contributing to the sulfur and carbon cycles (Watts 2000). Phytoplankton and bacteria counts were determined using FlowCam particle analyzer at the Biological Field Station of SUNY Oneonta, NY to assess population dynamics.

METHODS

Sample Collection

Ocean water samples were collected using large plastic containers off of the dock on the West Shore Campus, GEOMAR Helmholtz Centre for Ocean Research in Kiel Fjord, Germany in June 2016. The Kiel Fjord is located on the Baltic Sea and it is often overpopulated with jellyfish, which is an indicator of an unbalanced ecosystem due to the lack of their fish competitors (Javidpour et al. 2009). Mussel farming was established in this shallow water ecosystem in order to hinder eutrophication that can cause algae growth and increase anoxic levels (Schröder et al. 2014). The climate is oceanic in Kiel with an average high of 22.5°C and an average low of 14°C during summer months with frequent rains and cloud covers (Gandhi Sas, 2017).

Experiments

Two experiments were performed with identical setups: one with unfiltered and the other with filtered ocean water. Half of the ocean water samples were filtered using 0.20 μm Millipore filter membrane with slow suction to avoid cell rupture. The two experiments ran consecutively for 10 days because there were only a limited number of quartz bottles available. For both experiments, the water samples were distributed into sixty 250 mL quartz bottles without headspace and each bottle was randomly labeled for its oil concentrations, which included control 1, control 2, 1 mg/L,
and 5 mg/L. This setup allowed for 15 replicates of each treatment. After the crude oil was introduced into the appropriate bottles, each bottle was tightly capped avoiding headspace formation, and then randomly placed within the incubation bath. Two controls were used to monitor bottle effect. The incubation bath was placed outside to allow natural lighting, but it was constantly regulated to prevent overheating (Figure 1; Zindler et al. 2014).

Every 24 hours of the first 5 days of the experiment, a set of four bottles (control 1, control 2, 1 mg/L, and 5 mg/L) were randomly removed from the incubation bath. After the 5th day of the experiment, the sampling frequency was increased to every 12 hours in order to capture diurnal (night/day) changes in DMS production (Zindler et al. 2014; Marandino, personal communication). After collection, the temperature and salinity of each bottle were recorded prior to extracting volatiles for purge and trap (PT) and gas chromatography/mass spectrometry (GC-MS) analysis at the Biogeochemistry Laboratory of GEOMAR. The remaining samples of each bottle were used for determining phytoplankton and bacteria counts (Zindler et al. 2014). A 9 mL aliquot of each sample were divided into two 4.5 mL vials and prepared for bacteria and phytoplankton quantification, and preserved with 200 μl and 20 μl glutaraldehyde, respectively, then frozen at -80°C and shipped on dry ice to Oneonta, NY for FlowCam analysis.

Figure 1. The setup of the filtered experiment is imaged here (the unfiltered setup was the same). Quartz bottles filled with filtered ocean water were incubated in water baths outside under natural conditions with constant temperature control to prevent overheating.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The volatile gases present in each sample were extracted using the purge and trap (PT) method. First liquid nitrogen was used for trapping the volatiles during the purging for 15 minutes, then use of boiling water released the volatiles into the GC-MS (Vogt et al. 2008). The GC separated the compounds in the volatile mixture based on their mass dependent travel time (retention time) through the oven column with a constant temperature, and then the MS quantified each of the separated compounds (Poole and Poole 2012). Compounds with same retention time can be separately quantified by ionization and grouping by mass to charge ratios (de Hoffmann and Stroobant 2001). The collected samples were analyzed for carbon disulfide, isoprene, and dimethyl
sulfide content. The GC-MS (Agilent 7890A/5975C, Agilent Technologies, Inc., Santa Clara, CA, USA) measurements were conducted immediately after sample collection at GEOMAR, Kiel, Germany.

**FlowCam Analysis**

FlowCam (Fluid Imaging Technologies Inc., Scarborough, Maine, USA) analyzer uses flash illumination and color image capture of particles and it was developed for phytoplankton analysis (Dashkova et al. 2016). Using the accompanying analytical software, organisms can be grouped and identified by area, length, width, radius, shape, and fluorescence from captured images. After the samples were thawed and well mixed by several inversion of each vial, 1 mL was transferred into the intake tube of the FlowCam. Lower capture size limit was set as 2 µm for phytoplankton aliquots and no lower limit was set for bacteria aliquots. The sample runs were continuously monitored to avoid artifacts such as bubbles forming in the cells, repeated captures of same particles or large areas. Every time when artifacts developed the sample run was repeated after flushing the cell to obtain true counts of particles. Selected samples were run twice to check for consistency, and deionized water was also run for determining background counts for the flow cell. The FlowCam analytical work was conducted at the Biological Field Station of SUNY Oneonta, Cooperstown, NY.

**Data Analysis**

The GC-MS peak areas for carbon disulfide at a molar mass of 76 M, isoprene at a molar mass of 68 M, and dimethyl sulfide at a molar mass of 62 M were recorded. A sampling (internal) standard of known concentration was also analyzed throughout the sample analysis to monitor instrumental drift. A sampling and calibration standard ratio was determined for each sampling standard run by dividing the sampling standard peak area with the calibration standard peak area, then graphed (e.g. Figure 2) and the obtained polynomial equation was used to determine the relative ratio for a given compound. This value was determined for each sample run and used to correct the measured peak areas of each sample, obtaining “New PA”, eq. 1.

\[
\text{New Pa}_{\text{compound X}} = \frac{\text{Peak Area}_{\text{compound X}}}{\text{relative ratio}} \tag{eq. 1}
\]

The concentration of “compound X” (X = carbon disulfide, isoprene, or dimethyl sulfide) was calculated using eq. 2.

\[
[\text{Compound X}] = \frac{\text{New Peak Area}_{\text{compound X}} - \text{intercept}_{\text{standard X}}}{\text{slope}_{\text{standard X}}} \div 0.05 \div 10^6 \tag{eq. 2}
\]

Phytoplankton concentrations were determined from the particle counts (>2 µm) obtained from the FlowCam output and bacteria concentrations were determined from the difference between counts without lower limits and the counts larger than 2 µm. The counts were divided by the total volume analyzed, then corrected with the average error factor of each run to acquire concentration values. The error factor was set as the ratio between the software analyzed counts and the counts resulted from visual (manual) inspection of all captured images of a set of five selected samples per run.
Figure 2. This graph is an example of how the relative ratio was obtained for eq. 2. The ratio of the peak area of the DMS sampling (internal) standard to the peak area of the DMS calibration standard was graphed throughout the unfiltered experiment. A polynomial fit equation was used to determine the relative ratio for DMS for a particular sample run on the GC-MS.

RESULTS

Chemical Parameters Unfiltered Experiment

Temperature slightly increased and salinity stayed relatively constant through the experiment (Figure 3). A slight increase in temperature is occurred due to “greenhouse effect” of the transparent closed experimental box and the outdoor temperature changes.

Dimethyl sulfide (DMS) concentrations increased to 193-206 hours and then a decrease was observed in all treatments with or without added oil (Figure 4). After about 80 hours, differences between treatments increases, and the average DMS concentrations of the controls (5.73E-09±1.12E-09 mol/L) exceed the average concentrations of the oil treatments (4.84E-09±1.10E-09). However, there are no statistically significant differences between controls and oil treatments (p=0.148). During the second 5 days, a slight diurnal pattern developed with generally higher concentrations of DMS in the mornings than in the evenings.
Figure 4. DMS concentrations were measured over time in the unfiltered experiment using GC-MS. Differences increase over time with slightly lower DMS production with added crude oil. Each data point represents one sample bottle at the time of sampling.

Isoprene concentrations remained very low and consistent throughout the 10 days of the experiment and no differences were detected among treatments. The average concentration is $6.94 \times 10^{-11} \pm 1.22 \times 10^{-11}$ mol/L and no diurnal pattern was detected (Figure 5).

Figure 5. Isoprene concentrations through time in the unfiltered experiment. Low and consistent concentrations were observed for all treatments with GC-MS. Each data point represents one sample bottle at the time of sampling.
Carbon disulfide concentrations were also low and near or below the detection limit of the GC-MS (Figure 6). All sample values were essentially the same among all treatments and no patterns developed through time.

![Carbon Disulfide in Unfiltered Experiment](image)

Figure 6. Carbon disulfide concentrations through time in the unfiltered experiment. Very low values near or below the detection limit of the GC-MS were found for all samples in all treatments. Each data point represents one sample bottle at the time of sampling.

**Chemical Parameters Filtered Experiment**

Both the temperature and the salinity stayed constant through the experiment (Figure 7). Slight variation in temperature corresponded with the outdoor temperature changes.

![Average Temperature in Filtered Experiment](image) ![Average Salinity in Filtered Experiment](image)

Figure 7. Temperature (°C; A) and salinity (mg/L; B) change through the filtered experiment. Each point represents the average of a set of four samples pulled at a given time and error bars show standard deviation.

The average starting DMS concentration of the filtered experiment was 1.97E-09 mol/L and decreased for the following 190 hours. The lowest DMS concentrations were measured between 142 and 190 hours with an average of 4.83 ± 1.70E-10 mol/L. After 190 hours the DMS concentrations increased, but only a few bottles with oil past the initial DMS concentrations. Within the last 24 hours, the average concentration was 1.79E-09 ± 5.26E-10 mol/L (Figure 8), which is a
2.5-3 times lower concentration than in the unfiltered experiment (Figure 4). There was no difference between controls and oil treatments, and no diurnal patterns were detected.

![Dimethyl Sulfide in Filtered Experiment](image)

Figure 8. DMS concentrations were measured over time in the filtered experiment using GC-MS. No differences were detected between controls and oil treatments. DMS production decreased from the initial concentrations and only bounced back in the last 24 hours. Each data point represents one sample bottle at the time of sampling.

![Isoprene in Filtered Experiment](image)

Figure 9. Isoprene concentrations through time in the filtered experiment. Low concentrations were observed for all treatments with a slight increase in concentration and variance during the second half of the experiment. Each data point represents one sample bottle at the time of sampling.

Isoprene concentrations were low throughout the experiment, but a slight increase was observed in concentrations during the second half of the experiment in all treatments (Figure 9).
The variance among samples also increased, but the treatments remained statistically similar. The average isoprene concentrations were 5.18E-11 ± 5.40E-12 mol/L and 6.45E-11 ± 9.77E-12 mol/L for the first and the second halves of the experiment, respectively (Figure 9). No diurnal pattern was detected.

Carbon disulfide concentrations were low with relatively high variation among samples, but without a consistent trend (Figure 10). The average concentration of carbon disulfide was 2.44E-10 ± 5.65E-11 mol/L for all treatments.

Figure 10. Carbon disulfide concentrations through time in the filtered experiment. Low values were observed for all samples in all treatments and no differences are found between controls and oil treatments. Each data point represents one sample bottle at the time of sampling.

Phytoplankton and Bacteria Quantification

All treatments of the unfiltered experiment started with the same concentrations of phytoplankton and bacteria, about 4800 and 50000 per ml, respectively (Figures 11 and 12). Phytoplankton concentrations equally increased in all treatments until about 100 hours, then a decline was observed with high variation among treatments (Figure 11). There were no statistically significant differences among the treatments because of this high variation. However, it seems that the 1 mg/L added oil treatment promoted the phytoplankton growth between 130 and 200 hours (highest concentrations), while 5 mg/L added oil depressed the phytoplankton growth at the same time (lowest concentrations). During the last 48 hours of the experiment, the treatments become more similar and reached the level of the starting phytoplankton concentrations (Figure 11). Bacteria concentrations in the same experiment remained about 10 times higher than phytoplankton and exhibited similar growth pattern, steady increase during the first 100 hours then decline to the initial concentrations (Figure 12).

The water for the filtered experiment was passed through a 0.2 μm filter, thus it only contained bacteria initially at about 20000 per mL concentration (Figure 13), any larger particles and living organisms were filtered out. A significant increase in bacteria concentration was only
observed after 130 days in all treatments and the bacteria concentration fluctuated between 50000 and 100000 per mL. Added oil and the controls exhibited similarly large variation, thus no statistically significant difference is seen (Figure 13).

Figure 11. Phytoplankton concentrations as counts per mL over the course of the unfiltered experiment. Initial uniform growth was followed by a decline with high variation among and within treatments. Each data point represents one sample bottle at the time of sampling.

Figure 12. Bacteria concentrations as counts per mL over the course of the unfiltered experiment. Initial steady growth was followed by a decline with high variation among and within treatments. Each data point represents one sample bottle at the time of sampling.
Figure 13. Bacteria concentrations as counts per mL over the course of the filtered experiment. Low starting concentrations only significantly increased after about 130 hours, and variation between and within treatments also increased but remained higher for the second part of the experiment. Each data point represents one sample bottle at the time of sampling.

DISCUSSION

The expected outcomes of the study were that oil influences both the DMS production and the growth rate of phytoplankton and bacteria. We predicted an inverse relationship between oil concentrations and the response, because Huang et al. (2011) found a threshold oil concentration in the China Bay Sea that supported the idea of low concentration ($\leq 1.21$ mg/L) of oil is stimulating growth, and high concentration ($\geq 2.28$ mg/L) of oil is suppressing growth of phytoplankton. Our study in the Kiel Fjord did not support these observations as the controls and the two added oil concentration treatments behaved similarly through time in both experiments. The growth rate of phytoplankton initially increased and after about 130 hours decreased, but the oil treatments did not show a consistent and significantly different response from the controls (Figure 11). Seemingly, the higher oil concentration suppressed growth and the lower oil concentration stimulated growth for a short time period between 130 and 200 hours in the unfiltered experiment, but the results are inconclusive (Figures 11 and 12). The population of bacteria in the filtered experiment significantly increased after about 130 hours, but treatment differences were insignificant and variations within treatments were high (Figure 13). Temperature has been shown to cause a stimulating effect on phytoplankton growth and also on DMSP production (Arnold et al. 2013), so temperature and salinity were regulated and stayed relatively constant through time in both experiments the impact of these parameters on growth rate is ruled to be negligible (Figures 3 and 7). The 2016 summer had a high rate of phytoplankton production (NASA 2017), which did not reflect on the experiments. Several studies demonstrated that the oil pollution tolerance of the phytoplankton is species sensitive (Huang et al. 2011; Ozhan et al. 2014; Echeveste et al. 2016), thus, identical results cannot be expected. In addition, the environmental conditions and climate have shown to impact the response of phytoplankton to oil pollution (Echeveste et al. 2016), and the number of
available published field data analyses is low, so there is not enough information to draw globally relevant conclusions. To fully understand the growth response of phytoplankton to oil pollution multiple in-situ studies are needed worldwide.

The initial DMS concentration was about the same in both experiments, but in the filtered experiment the concentration decreased over time and only increased back to initial levels during the last 36 hours (Figure 8). In this experiment anything larger than 0.2 μm were filtered out which includes phytoplankton, so the lack of DMS production was expected. The unfiltered experiment DMS production increased after 110 hours to about 200 hours in all treatments (Figure 4). While there were no statistically significant differences among the treatments and the variation within treatments was high, the 5 mg/L oil treatment generally had the lowest DMS production followed by the 1 mg/L and the controls exceeded the DMS concentrations of the oil treatments in the unfiltered experiment (Figure 4), which correlate with Huang et al. (2011). There was no clear correlation between growth response of phytoplankton and the DMS concentrations in the unfiltered experiment, thus there must be other factors influencing DMS production that we might not have controlled for. Also, González et al. (2013) found that the initial composition of the phytoplankton community determines the degree of response to oil pollution, which this study did not investigate. In addition, the water in the bottles and in the clear water bath (Figure 1) may have stayed clearer than the natural ocean environment as sediment was excluded from the experiment, and such the light penetration could have been greater than naturally, which can induce phototoxicity of certain organic compounds found in the water and lower the phytoplankton activity (Marwood et al. 1999). Isoprene and carbon disulfide concentrations were low and unaffected by the added crude oil through the experiments, so they do not warrant discussion.

SUMMARY AND CONCLUSIONS

Crude oil pollution did not significantly impact the growth of native Kiel Fjord phytoplankton and production of DMS, isoprene and carbon disulfide by phytoplankton and associated bacteria in a 10-day long experiment. A slight growth stimulating effect of the 1 mg/L treatment, and a growth inhibiting effect of the 5 mg/L treatment was observed for a short time period of the unfiltered experiment, which coincided with differences in DMS production, but no clear trends were documented. This study support that the response of phytoplankton to oil pollution may depend on initial phytoplankton community, climate conditions, nutrient availability, light penetration and photo-oxidation of organic compounds. Further studies are needed to elucidate the global impact of oil pollution on phytoplankton growth and DMS production.

ACKNOWLEDGEMENTS

The authors thank the Emerson family for providing the funding of JMPA’s travel and research experience to Kiel through the Fred L. Emerson Foundation International Internship Scholarship at Hartwick College, Oneonta, NY. The authors are also thankful for Dr. Christa Marandino and Dennis Booge at GEOMAR Helmholtz Centre for Ocean Research, Kiel, Germany for their help, training, and allowing the use of their instrumentation and facilities to carry out the experiments and the GC-MS analytical work. The authors thank Holly Waterfield and Matthew Albright at SUNY
Oneonta Biological Field Station for training and allowing the use of the FlowCam Analyzer for determining algae and bacteria concentrations in the samples.

REFERENCES


