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Use of Microplate Respirometry to Measure Respiration of Individual Adult Coral Polyps Exposed to Deepwater Horizon Oil

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UNIVERSITY OF MIAMI

USE OF MICROPLATE RESPIROMETRY TO MEASURE RESPIRATION OF
INDIVIDUAL ADULT CORAL POLYPS EXPOSED TO DEEPWATER HORIZON
OIL

By

Brittany A. Jensen

A THESIS

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Master of Science

Coral Gables, Florida

December 2016

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Use of Microplate Respirometry to
Measure Respiration of Individual
Adult Coral Polyps Exposed to
Deepwater Horizon Oil

Abstract of a thesis at the University of Miami.

Thesis supervised by Professor Andrew Baker.

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The Deepwater Horizon (DWH) blowout of 2010 exposed many coastal and marine ecosystems in the Gulf of Mexico (GoM) to the acute and chronic effects of crude oil. To date, no studies have investigated the toxicity of DWH oil on shallow reef-building corals despite the relative proximity of some reef sites to the DWH (Flower Garden Banks, TX) and the potential for downstream oil transport to other more distant sites (Pulley Ridge and Florida Keys, FL). In this study, we investigated the effect of acute DWH oil exposure on reef corals, both alone and in combination with elevated temperature, to determine whether seasonal warming and/or climate change might interact with toxicity. We used a novel 24-well optical fluorescence oxygen-sensing system (microplate respirometer) to measure respiration rates of individual polyps of the common Caribbean coral, *Siderastrea siderea*, in response to a 48 h exposure to 4% of a 1g/L HEWAF dilution of surface oil from the DWH blowout at 25°C and 30°C, and used an Imaging Pulse Amplitude Modulated Fluorometer (I-PAM) to measure

photochemical efficiency of algal symbionts (*Symbiodinium* spp.). Experimental polyps were first acclimated to the experimental chambers for 7 days at 25°C until the baseline respiration rates stabilized. Temperatures in the heated treatment were then raised to 30°C over a 3-day period and all of the corals (including controls at 25°C) were exposed to oil on day 11. After 48 h of oil exposure corals were returned to clean seawater and maintained at 25°C or 30°C for 48 h of respiration measurements during recovery and an additional two weeks of visual monitoring. Overall, we found that respiration rates only significantly increased in corals exposed to both oil and high temperature, and these corals showed significant morbidity after 48 h of recovery (and high rates of mortality after a further two weeks). Changes in photochemical efficiency followed similar trends as respiration, suggesting oil affected both the coral host and its algal symbionts. These findings suggest that oil exposure may be more damaging to corals during the warmer summer months, but are likely conservative because we did not include the dispersant (Corexit) as a factor in the study, which has also been shown to be toxic to marine organisms (Incardona, 2004; Shafir et al., 2007; Goodbody-Gringley, 2013; Pie, 2015). This is the first study to have investigated the combined effects of DWH oil and elevated temperature on shallow reef-building corals, and is also the first to use a microplate respirometer to measure the respiration of individual coral polyps.

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Chapter 1

Background

Coral reefs are among the most diverse ecosystems on the planet and the most threatened. This is due to many factors; all driven by the human population growth, especially the urbanization of coastlines in the tropics (Burke, 2011). Runoff from cities into coastal systems is not only increasing the amount of nutrients in the water, but also toxic chemicals like PAHs (polycyclic aromatic hydrocarbons), which are toxic compounds found in oil (Incardona, 2004). Rising ocean temperatures are another factor increasing stress levels in these ecosystems. Elevated temperatures have also been correlated with an increase in the disease rate and susceptibility in corals (Boyett et al. 2007; Bruno et al. 2007; Ross et al., 2013). Consequently the combination of stress with acute exposure to PAHs (in the event of an accident such as an oil spill or blowout) can often exacerbate organismal response and result in more severe bleaching and/or mortality.

This study examined the response of the common Caribbean coral *Siderastrea siderea* to a 4% HEWAF dilution of surface oil from the DWH blowout at two temperatures, 25°C and 30°C. By investigating potential synergistic effects between stressors this approach can help inform sound science for policy makers to understand potential negative impacts, especially in the event of unexpected accidents or events, and work to mitigate them.

There have only been a few major oil spills in past 35 years: the Red Sea (1975), Bahia Las Minas in Panama (1986), Exxon Valdez off the coast of Alaska

(1989), and Deepwater Horizon (DWH) in the Gulf of Mexico (2010). At the time it occurred, the Exxon Valdez spill in 1989 was the largest spill in history, and consequently received a lot of publicity. Some of the research that resulted from the Exxon Valdez spill helped policy makers change laws that now require tankers carrying oil to be double hulled (Review, 1997). The main focus of this research was on the effects of oil and PAHs on larger mobile marine vertebrates. It showed how oil could cause high mortality in marine mammals, inhibit respiration in a species of green algae, and can affect corals ability to reproduce (Loya and Rinkevich 1979; Peters et al., 1981; Kwong-yu and Chiu, 1984; Guzmán and Holst, 1993; Loughlin, 1994; Harrison 1999; Peterson et al., 2003). Oil exposure can also cause corals and other organisms to modify their behaviors (Reimer, 1975; Wyers, 1986). PAHs found in oil bioaccumulate in coral tissue, slow coral growth rates, and disrupt the chemical cues that larvae need in order to successfully recruit to reefs (Loya, 1975; Firman, 1995; Sabourin et al., 2012; Ko et al., 2014).

The DWH spill in 2010 was unique in many ways: it originated at depth (which changed the composition of PAHs that were present), and a chemical dispersant was applied during the spill. Since this spill occurred at depth the oil experienced more weathering and the PAHs that normally would evaporate in a surface spill remained in the oil as it traveled through the water column: these two factors changed the toxicity of the oil (Incardona, 2004; Carls & Meador, 2009; Reddy, 2012)

Due to the location of the spill many embryonic and larval fish were exposed to oil, which has been shown to impair cardiac development in later life stages (Incardona, 2004). Another study showed that oil and dispersants decreased the activity of blue crab larvae, with larvae becoming relatively immobile after only 24 hours of exposure (Pie, 2015). The chemical dispersant, Corexit® 9500, was a new feature of this spill, and was used to help break up the oil and keep it in the water column or fall to the ocean floor so that natural currents could act to disperse it. The fact that dispersants and oil tend to sink in the ocean increases the chances of corals being exposed, since they are benthic sessile organisms. Relatively little is known about toxicity of Corexit® 9500, but, in general, toxicity of oil and dispersants has been shown to be variable and dependent on species, life stage, duration of exposure, and temperature (George-Ares and Clark, 2000). It has been the key focus in many recent studies due to the recent use to disperse the oil in DWH in the Gulf of Mexico. All studies to date that have looked at the combined effects of oil and dispersants concluded that dispersants (or the combination of oil and dispersants) was more toxic than the oil alone. This has been true for adult corals, coral larvae, and also the algal symbionts (*Symbiodinium spp.*) of corals (Peters, 1981; Cook and Knap 1983; Shafir et al., 2007; Goodbody-Gringley, 2013).

After the DWH blowout, oil was found in sediments to a distance up to 11 km away, with higher concentrations found in the surface layers of sediments, and lower concentrations found in area with high densities of coral (White et al., 2012; Hsing et al., 2013). Many of the deep-water corals affected in this area

were covered with oil flocculent, which was a direct cause of mortality for the corals around the well (Hsing et al., 2013). Oil accumulation on the corals also explains why concentrations of oil found in sediments near corals were lower than areas far from corals. Because corals, and especially deep corals, are long-lived and have low metabolic and growth rates (Bak, 1987) the full effects of the DWH spill may not be seen for years or decades to come (Montagna et al., 2013; Fisher et al. 2014; Prouty, 2016).

Many corals, including *S. siderea*, broadcast spawn larvae a few nights a year during the summer months; however these larvae must be able to recruit to substrate in order to grow into adults (Edmunds et al., 2001). PAHs accumulating in the sediments may change the cues larvae need to settle, thus reducing recruitment rates to suitable reefs, which could lead to a decline in survivorship of future larvae (Goodbody-Gringley, 2013). Even if they are able to spawn after oil exposure *Porites astreoides* and *Orbicella (Montastraea) faveolata* were found to have higher rates of larval mortality before and after settlement due to oil and dispersant exposure (Goodbody-Gringley, 2013). Both settlement and survivorship decreased with increasing concentrations of fresh weathered oil and when exposed to the combination of oil and Corexit® 9500 (Goodbody-Gringley, 2013).

Oil is a big industry; Louisiana and many other states that border the Gulf of Mexico depend on oil, which is why there is a very high density of marine oil wells located just off the coasts of Louisiana, Alabama, and Georgia. Florida's economy is more dependent on tourism, so there is currently a ban on offshore

oil drilling within 125 miles of the coastline. This ban is designed to protect the shallow reefs of the Florida Keys from oil spills that may occur in the region. However, the Gulf of Mexico has a major current system running through it, called the Loop Current, which potentially connects waters of Texas, Louisiana, and Alabama with Floridian waters that are protected from drilling activities. Drifters used to determine the route of the DWH oil have shown that some surface oil did enter the Loop Current, but due to the behavior of the current at the time of the spill it was not transported south to Florida's reefs (Liu et al., 2011). Wind currents were directed northward at the time of the spill, while water currents were running eastward. This prevented a large portion of the oil from entering the Loop Current and being transported south (Le Hénaff et al., 2012).

The possibility of a future spill occurring in the Gulf of Mexico is high. Our study only looked at the acute effects of oil on corals, but if a spill were to occur, corals could be exposed to oil or a combination of oil and dispersant for much longer. Therefore, it is important to know what types of interactions and synergistic effects oil will have on the shallow reef corals that are already experiencing the stress of rising ocean temperatures. Many multiple stressor tests have been done in the past, and elevated temperature is commonly one of the stressors tested, along with elevated levels of nutrients and salinities (Muthiga and Szmant, 1987; Nyström et al, 2001; Nordemar et al., 2003).

This experiment used surface slick oil prepared in a high energy water accommodated fraction (HEWAF) at ecologically relevant concentrations to investigate the effects of the Deepwater Horizon oil spill on the respiration rates

of *Siderastrea siderea*, a common Caribbean reef coral (Bejarano et al., 2013; Diercks et al., 2010; Wade et al., 2010). Measuring respiration rates of individual coral polyps has never been done before; so one aspect of this experiment was to see if the microplate respirometer could achieve this. In addition, four additional coral species were tested (*Orbicella faveolata*, *Porites astreoides*, *Acropora cervicornis*, and *Siderastrea siderea*) were also tested as adult polyps and the larvae of one species *Porites astreoides* were also tested as part of the preliminary work for this thesis (Serrano et al., in preparation). This means that future work can be undertaken comparing respiration rates between different life stages and species of corals.

An Imaging Pulse Amplitude Modulated Fluorometer (I-PAM) was used to measure photochemical efficiency (Walz GmbH, Germany). Coral depend on their algal symbionts (*Symbiodinium* spp.) and their expulsion from coral hosts during stress-induced “bleaching” events is a critical threat to coral reefs worldwide, principally as a result of climate change. Depending on stressor, bleaching can either be due to an animal stress response and/or algal stress response (Baker and Cunning 2015). Photochemical efficiency (F_v/F_m) was measured throughout the experiment assess how photosynthesis changes in response to oil exposure (Cook and Knap, 1983). I tested the hypothesis that oil exposure would affect photochemical efficiency, either alone or in combination with heat stress, indicating that oil was toxic to both algal symbionts as well as their coral hosts.

Chapter 2

Preliminary Trials and Troubleshooting

Maintaining health at small size

The microplate respirometer can be used with a variety of microplates each of which consists of up to 24 wells ranging in size from 150 μL to 650 μL in volume. This places an upper limit on the size of the experimental organisms that can be used in each plate. This experiment used the largest (650 μL) well plate and a 6mm drill bit to core multiple species of corals including *Porites astreoides*, *Orbicella faveolata*, *Acropora cervicornis*, and *Siderastrea siderea*. To assess their suitability for experiments at this small size, I first assessed the survivorship of each species was after coring with the 6mm drill bit. To do this, corals were drilled and then immediately placed in small cups with mesh sides that provided water exchange from the surrounding tank. However, mortality rate of these cores was high within days of coring. Higher survivorship was found in a second trial, which attached cores to a substrate in an upright position. This was achieved by using Reef Glue to secure each polyp to a Perler bead and each bead to a peg on a Perler plate. To overcome the positive buoyancy of these Perler plates they were in turn also secured to a small limestone tile to remain submerged.

During the first trial cores were drilled, removed immediately from the parent colony, and transferred to the indoor facility. We found a significant decline in mortality rates when we instead left polyps in their parent colony in the flow-through tanks at the University of Miami Experimental Hatchery for 2-3 days

before being snapping them out of the parent colony and transferring them to the indoor facility.

We found that when *O. faveolata* or *P. astreoides* was cored too deeply the core would split in two. However, cores of *S. siderea* cores were hard to extract if they were not cored deeply enough. Consequently, when extracting the core we angled our effort and applied pressure in a more downward fashion (rather than sideways) to decrease the chances of the polyps cracking or breaking in two. Of the colonies tested, the *O. faveolata* cores, at this size, experienced higher mortality rates after the initial drilling compared to the *S. siderea* cores, even when left in the parent colony for acclimation after drilling. There were multiple attempts where >50% of the *O. faveolata* cores died two to three days after the initial coring. Different tools were used to isolate polyps from each species: *O. faveolata* and *S. siderea*, could both be cored using a drill bit, whereas *P. astreoides* only needed a hammer and handheld 6mm corer and *A. cervicornis* required a Dremel tool to isolate polyps. The Dremel was used to cut a transect of each branch into disc shapes that had one layer of polyps (“coin slices”), and then a razor blade was used to isolate single polyps. In future, a Dremel, rather than a razor blade, is recommended to cut both the discs and isolate a single polyp of *A. cervicornis* to ensure uniformity in polyp size. This is because survivorship is higher when there is more skeleton attached to the polyp, which is hard to achieve with a razor blade.

Preliminary trials using all four of these species were run in the microplate with positive results. Survivorship of each species at this small size, and variation

in the data collected from each run determined which species would be best for repeated respiration measurements in a longer experiment. Both *O. faveolata* and *S. siderea* were an optimum size for the trials, but *S. siderea* adapted better to survive at this size.

Polyp volume

The first preliminary trials testing the respiration rates of *O. faveolata* after oil exposure corrected for the volume of coral, but still had a very large standard error. Due to the slight variation in the size of each polyp, the volume of the polyp was determined, using calipers, and subtracted from the total volume in the well to correct for the amount of water available to each polyp. This method was used for all of the species except for *A. cervicornis* trials due to their non-cylindrical shape. These *A. cervicornis* polyps were also significantly smaller than any of the other polyps used and the volume they displaced was minimal in the 650 μ L wells, so it was not corrected for in the *A. cervicornis* trials. However, the main reason behind the decision to not use *A. cervicornis* was that the majority of the polyps bleached after a week of being in ambient conditions. Volume determination was used in all of the preliminary trials as well as the final trial. Since the standard error was so high on the first preliminary trial for oil-exposed *O. faveolata* we were unable to use the data. However, when setting up this preliminary trial, it was observed that one of the colonies (colony C) was more stressed before drilling took place than the two other colonies. When looking at the results there was no response detected in colonies A or B, but there was a

decline in respiration rates in colony C (Figure 1). The decline in respiration rates after exposure may have been due to the added stress the colony was experiencing prior to the start. For this reason we decided to do a multiple stress test, including temperature and oil, in our final experiment.

Determining respiring tissue mass

Initial trials tested polyps in a sacrificial manner, but there was high variation between each polyp's respiration rates. Multiple methods were tested to reduce the amount of variation between the polyps. Initial trials used the weight of the entire polyp to correct for respiration. This was achieved by using a paper clip to hold each polyp on a buoyancy scale then corrected for the wet weight, using the following equation:

$$(\text{Brass dry weight}) / (\text{Brass wet weight}) = (\text{Polyp dry weight}) / (\text{Polyp wet weight})$$

This method did reduce the variation, but the reduction was not significant. The next hypothesis we tested was to isolate the respiring tissue on each polyp. Previous studies showed that coral tissue may be denser in some areas of a colony and can be different between species, which could have an effect on the respiration rate (Edmunds, 2005). Three different methods to measure the tissue mass on each polyp were tested.

The first method used HCl and refrigerated EDTA to dissolve calcium carbonate skeleton, both of which have been previously used in for this test

before on different organisms. One polyp was submerged in HCl and one polyp was submerged in EDTA then refrigerated for 24 h. The calcium carbonate skeleton that was exposed to HCl dissolved after 24 h, the tissue was then dried and weighed. The EDTA was unsuccessful at dissolving the skeleton, even after a week of refrigeration. Although the trial using HCl was successful, concerns were raised about the amount of tissue being degraded by such a strong acid. This was a major factor to be considered due to the small amount of tissue on each polyp before the acid was added.

The second method tested was a blasting technique. This method has been used on bigger cores in our lab to isolate tissue for cell counts. This technique used an airbrush to blast tissue off each polyp into a plastic bag that was then transferred to a pre-labeled and pre-weighed 2 μ L centrifuge tube. Each tube, containing a mixture of seawater and coral tissue, was then placed in a heat block at 72°C for 2 days to ensure each tube was completely dried. When the tube was completely dry the tube and the polyp tissue was re-weighed.

$$(\text{Final tube + tissue weight}) - (\text{Initial empty tube}) = \text{Mass of tissue}$$

The concerns for this method were the same as the HCl trial. The initial volume of tissue being blasted was small, and so transferring the solution (tissue and seawater) increased the amount of tissue lost twofold.

The third method tested used a heated NaOH treatment to break down the tissue, which only left dry skeleton. The tissue and skeleton were weighed before

exposure to NaOH and heat stress. Two different trials were run; the first patted each polyp dry before weighing to remove excess liquid and the second dried the cores in an oven overnight before the first weighing. After each polyp was weighed they were then submerged in 1 mL of NaOH and placed in an oven overnight. After 24 h in the oven each polyp, devoid of tissue, was removed, allowed to cool, and re-weighed.

$$(\text{Initial skeleton} + \text{tissue}) - (\text{Final skeleton}) = \text{Mass of tissue}$$

This method optimized the accuracy of the tissue weight, but polyp variation was still too large for this method to be used.

Due to the difficulty of accurately measuring the mass of the respiring tissue on such small polyps the design of this experiment used a pairwise approach, thus eliminating the need to determine the tissue mass. However, the requirement for this pairwise design was daily repeated measures over a defined length of time. This required a species that could survive for an extended period of time at a small size. Due to the high mortality rate in *O. faveolata* after the initial drilling, *S. siderea* was chosen for this experiment.

Use of glassware

Oil exposures had to be done in glass or metal containers because previous studies have shown that some types of plastics retain oil after being exposed (Broje and Keller, 2007). This would affect the total amount of PAHs

present in the oil sample during exposures and could create variability in our data. This also meant that perler plates and beads could not be used in the experiment. To resolve this problem we used circular glass aquaria and glass petri dishes. Each glass petri dish was identified by engraving with a Dremel rather than labeling with a Sharpie or tape (which that may interact with oil).

Chapter 3

Experiment

Introduction

Coral reefs worldwide are suffering from the combined effects of climate change nutrient pollution, overfishing, and ocean acidification (Langdon et al., 2005; Fabricius, 2005; Faxneld, 2011; Etnoyer et al. 2016) Local anthropogenic impacts, such as oil spills, exacerbate these impacts and can represent potential tipping points that kill otherwise hardy corals that have proved resistant to other stressors. One way of measuring impacts caused by exposure to these additional impacts is by measuring oxygen consumption, a fundamental measure of stress physiology. However oxygen consumption can be difficult to measure accurately in small organisms with low oxygen demand. There are many techniques of measuring the respiration rates of corals including oxygen electrodes, the use of an YSI in a closed system, or by using the diffusion boundary layer (Davies, 1980; Porter et al., 1999; Edmunds, 2005; Nordemar et al., 2003; Faxneld et al., 2010). All of these systems require large fragments of corals to reduce the noise and variability created by the instruments when smaller fragments are used. In this study, we used a microplate respirometer that allowed us to run highly replicated trials measuring the respiration rates of individual polyps, providing an in-depth look at how individual coral polyps respond to stress. This new technology could potentially be used to measure variation among polyps within the coral colony that cannot be assessed when measuring larger fragments. This method also allows the measurement of oxygen consumption in individual coral

larvae, allowing them to be directly compared to adult colonies.

The massive starlet coral, *Siderastrea siderea*, is a common shallow water reef-building coral that provides structure and protection to dynamic reef ecosystems (Jones, 1994). Its resilience to a wide range of stressors, including temperature and salinity, may help explain its prevalence on Caribbean reefs, and may make it a good candidate for an indicator species on degraded reefs of the Caribbean (Macintyre, 1969; Muthiga and Szmant, 1987; Banks et al., 2007; St. Gelais et al., 2016). This study used *S. siderea* to investigate the effect to a 4% of a 1g/L HEWAF dilution of surface oil from the DWH blowout on respiration and photochemical efficiency at two temperatures, 25°C and 30°C.

The 2010 DWH blowout leaked roughly 4 million barrels of crude oil into the Gulf of Mexico over a 3-5 month period, making it the largest oil spill to date (Crone & Tolstoy, 2010; McNutt et al., 2012; BP, 2015). Drifters used to determine the route of the DWH oil showed that some surface oil did enter the Loop Current, but due to the behavior of winds and water currents at the time of the spill it was not transported south to Florida's reefs (Liu et al., 2011; Le Hénaff et al., 2012). The Loop Current is the major current running through the GoM and if the spill had occurred at another time it is possible that the shallow reefs around the Florida Keys could have been affected. Seismic surveys from the Bureau of Ocean Energy Management support that currents did play a role in transporting oil, because deep reef sites up to 11 km away from the DWH blowout site showed signs of being affected (Fisher et al., 2014). These surveys observed the physical accumulation of oil on the corals over time, but due to

these corals slow growth rates the full effects of the oil may be ongoing (White et al., 2012; Etnoyer et al., 2016; Hsing et al., 2013; Peters et al. 1981).

How the DWH oil affects shallow reef corals remains unclear. The DWH blowout released crude oil, which is made up of a composite of different formations of polycyclic aromatic hydrocarbons (PAHs), at depth and under high energy into the GoM. The toxicity and composition of the PAHs found within a spill can vary greatly depending on the location of the spill and the amount of weathering that occurs (Incardona, 2004; Carls & Meador, 2009). This was a unique case study because the oil was released at depth, so the PAHs that would normally evaporate quickly from a surface spill were present as the oil traveled to the surface, thus changing its toxicity and composition (National Research Council, 2003; Reddy, 2012). Accumulations of some PAHs, from spills on the surface and at depth, have been found in sediments and coral tissue. This bioaccumulation creates not only immediate threats to coral health, but also to the organisms that depend on corals as a food source (Peters et al., 1981; Firman, 1995; Sabourin et al., 2012; Ko et al., 2014).

Crude oil has effects on various aspects of the metabolic processes of corals. Specifically, it is known to affect adult coral fecundity and fertilization, and has been shown to reduce the size of the female gonads, and may even cause them to release premature larvae (Loya and Rinkevich 1979; Peters et al., 1981; Guzmán and Holst, 1993; Harrison 1999). There are also long-term effects on the coral tissue leading to degeneration of cells and tissue deterioration (Peters et al., 1981). Corals in the Pacific genus *Pocillopora* showed an open mouth

response to oil exposure that lasted an extended period of time, but it was unclear if this behavior was due to tissue damage or constant stimulation (Reimer, 1975). Other behavioral responses in corals to oil have also been observed, such as mesenterial filament extrusion, tentacle and tissue contraction, and localized tissue rupture. However, it was unclear if these behavioral traits are short-term or long lasting (Wyers, 1986). Growth rates have also been shown to decline as a result of oil exposure, which can lead to lower success rates when competing with other organisms for space and lower resistance to grazing activities (Reimer, 1975). After an oil spill in Bahia Las Minas, Panama in 1986 oil did not have an effect on the growth rate of *Siderastrea siderea*, but growth rates *were* negatively impacted when the rainy season started, suggesting that low salinities in combination with previous oil exposure stressed the coral more than either stressor in isolation (Guzmán et al., 1990).

Multiple stressors are the norm, not the exception, in the natural environment, and consequently many studies are looking at their interactions and/or synergistic effects. Many studies have investigated the effects of elevated temperature when combined with different concentrations of nutrients, salinities, or sedimentation, but few have looked at the combined effects of oil and elevated temperature. Interestingly, respiration rates in corals tend to increase in response to elevated temperature alone, yet tend to decrease when combined with elevated salinities or nutrients (Muthiga and Szmant, 1987; Porter et al., 1999; Nyström et al, 2001; Nordemar et al., 2003; Faxneld et al., 2010, 2011). In

general, multiple stress tests on marine systems have shown synergistic or additive interactions between stressors (Crain, 2008), more work needs to show if the effects of oil and elevated temperature are synergistic or just additive. The same goes for the combined effects of oil and Corexit. The majority of studies that looked at the combined effects of oil and dispersants concluded that dispersants or the combination of oil and dispersants was more toxic than the crude oil alone in both adult corals and coral larvae (Shafir et al., 2007; Goodbody-Gringley, 2013). A preliminary microplate trial of oil exposure on adult *Orbicella faveolata* coral polyps (Chapter 2) also showed greater decline of coral respiration rates if they are initially stressed, due to handling and environmental conditions during post coring recovery, prior to the addition of oil compared to the response when initial stress levels are low (Figure 1). This is one of the reasons we decided to combine temperature stress with the oil exposure.

Many types of stress can also lead to coral “bleaching”, defined as the loss of the algal symbionts, or a reduction in their per-cell pigment concentrations (Baker and Cunning, 2016). Toxins such as oil, cyanide, or heavy metals have been shown to cause bleaching although the specific mechanisms involved for each stressor may be very different (Baird, 2009; Baker and Cunning, 2016). Some toxins, such as the herbicide Diuron (DCMU) cause bleaching as a result of an “algal stress response”, which causes the expulsion of damaged algae from the host (Jones, 2005). Other stresses appear to cause bleaching as a result of an “animal stress response”, in which the coral host is principally affected. In this study we used an Imaging Pulse Amplitude Modulated Fluorometer (I-PAM) to

measure the photochemical efficiency of the *Symbiodinium*, to help determine the effect of oil at both ambient and elevated temperatures. We monitored respiration rates and photochemical efficiency of individual polyps of adult *S. siderea* before and after exposure to a 4% HEWAF dilution of surface oil from the DWH blowout at 25°C and 30°C over a 7-day acclimation period, 3-day warming period, 2-day exposure period, and 2-day recovery period (Figure 2). We hypothesized that oil exposure would increase respiration rates as a result of organism stress, and that respiration rates would increase further still in response to the additional stress of higher temperature (30°C vs. 25°C).

Materials and Methods

Coral collection/acclimation

Three colonies of *Siderastrea siderea* were collected from Emerald Reef (25° 40.450' N, 80° 5.920' W) in the northern Florida Keys under permit SAL-14-1182B-SRP, and held in outdoor flow-through tanks at the University of Miami Experimental Hatchery for at least 12 months prior to being cored for experiments. Replicate cores (N=40) were then taken from each colony using a 6mm diameter core bit attached to a drill press. Cores were allowed to recover for 3 days in the outdoor tanks, and then relocated to an indoor experimental facility at 25°C under custom spectrum LED lights (1347 lux) for 2 days prior to the start of the experiment, the first week of the experiment was then used to gather acclimation respiration measurements. Replicate cores from each coral colony were randomly assigned to one of four experimental groups consisting of

two treatments and two controls. Each group of cores (6-8 cores per colony, N=18-20 per group) was attached to glass petri dishes using Reef Glue (manufacturer). These glass petri dishes provided a substrate for handling the cores as a group during experimentation and minimized individual handling stress. Each group of cores was maintained in an individual circular glass aquarium filled with 14L of ultraviolet sterilized seawater and maintained in a temperature-controlled seawater tank at 25°C. Experimental cores were allowed to acclimate to these conditions until respiration rates (measured on days 1-4) were stable, after 4 days. They were then maintained for 2 more days prior to the start of the experiment. After each microplate trial the cores that had been tested were fed Reef Chili in a separate container of UV sterilized seawater then randomly placed back into the glass aquaria within their treatment group.

Preparation of water accommodated fractions

On day 7 temperature was increased for the experimental cores by two degrees to 28°C, then was raised two more degrees on day 8 to 30°C in the two treatment aquaria. Respiration was measured daily in both the treatment and the controls, which were maintained around 25°C. Days 9 and 10 were used to measure the effects 30°C temperatures had on the treatment polyps. Oil was then added to all of the polyps on day 11 (no respiration measurements were taken) and new oil was added day 12 (Figure 2).

The oil used in this treatment was surface oil, acquired under the RECOVER grant, collected from skimming operations by British Petroleum for

testing purposes, and subsequently transferred under the chain of custody to the University of Miami (Sample ID: G017-B0617-001, G017-B0618-002, G017-B0618-003, & G017-B0619-004) This experiment used a 4% HEWAF (High-Energy Water Accommodated Fraction) dilution, which was prepared on the day of use at a measurement of 1 g of oil per 1L of UV sterilized seawater. It was then blended on low for 30 seconds using a Waring CB15 blender. Blended oil emulsions were immediately poured into a separatory funnel and were allowed to settle for one hour. This was considered a 100% WAF and the bottom 90% was collected and diluted with 10-14L of UV sterilized seawater to create a 4% dilution each glass aquaria.

Coral Exposures

When each set of dilutions was complete the corals were immediately put back to designated set of aquaria to start the exposure time that lasted a total of 48 h. New HEWAF dilutions were made after 24 h to ensure that the corals did not experience the added stress of dissolved oxygen levels dropping below normal levels of 32 ppm or 6.00 mg/L in the glass aquaria, or a change in the composition of the oil. Before oil was added to the glass aquaria they were rinsed out to decrease the chance of microbial growth that could affect the polyps and add noise the microplate trials. After 48 h the corals were moved to new glass aquaria filled with 14L of UV sterilized seawater to recover. The temperatures the polyps were in during exposure were maintained through recovery.

Water Chemistry Analysis

Samples for total sum analysis of PAH were collected in a 250 mL amber glass bottle immediately before and after the 24 and 48 h exposures (a total of 4 samples were taken). These samples were stored overnight in a 4°C freezer then shipped to ALS Environmental (Kelso, WA) for analysis via gas chromatography/mass spectrometry-selective ion monitoring (GC/MS-SIM; based on EPA method 8270D). Water quality measurements including temperature, pH, dissolved oxygen, and salinity were measured daily. Temperature and DO were taken with an YSI optical probe (YSI, Inc., Yellow Springs, OH), pH was measured using a PHM201 meter with a glass electrode (Radiometer, Copenhagen, Denmark), and salinity was measured using a refractometer.

Oxygen Consumption Trials

Microplate trials were run at the same time each day for 15 days in an environmentally controlled room, so that the temperature remained at 25°C. Sensors that corresponded with each well, measured real time oxygen concentration every 15 seconds until the trial is complete. Plates were run every day with the exception of day 11, when the first oil exposure was prepared and started.

Before each microplate trial the polyps from one group were dark acclimated for 10 minutes in a clean container of UV sterilized seawater while the

microplate was set up. During set-up the microplate was carefully placed in a flow-through water bath chamber then the coral polyps were quickly added to their predetermined 650 μ L well. A silicone membrane was carefully laid over the plate and secured down for an airtight seal. The water bath and plate are then placed on top of the SDR reader and attached to a pump that pushed water through the water bath; this ensured that the water temperature in the wells remained constant throughout the experiment. For the heated treatments a separate aquarium was used that contained a heater that maintained the temperature at 30°C, so the microplate trials ran at the elevated temperature. Each plate also had 4-6 control wells where no polyp was added to test for background respiration. The analysis of oxygen consumption took the initial 10% decline of oxygen (pO₂ in % air saturation). Each well had a corresponding graph in which this O₂ decline over time was visually plotted. Due to their varying size each polyp was exposed to slightly different volumes of water, so the final data was corrected for the volume displaced by each polyp. The final data was expressed in $\mu\text{mol polyp}^{-1} \text{min}^{-1}$.

Chlorophyll fluorometry

Imaging Pulse Amplitude Modulated Fluorometer (I-PAM, Walz, Effeltrich, Germany) was used to measure the ability of the symbiont community within the tissue of each polyp to photosynthesize. Since the corals needed to be dark acclimated before the I-PAM was used, they were immediately reattached to the petri dishes and measured following the microplate trials on days 1, 6 (when

respiration rates were consistent), and then days 9-15 excluding day 11 (oil preparation). A measuring light was used to mark each polyp and a saturating pulse was taken that recorded each polyp's maximum quantum yield of photosystem II.

Statistical Analysis

Data was presented as means \pm standard error of the mean (SEM). An outlier test was first run on all of the respiration rates collected and as a result three polyps were eliminated from the entire trial. For both the respiration data and the IPAM data the data were log transformed then SigmaPlot 13.0 was then used to run repeated two way ANOVA's that tested the differences for statistical significance. A $P < 0.05$ determined statistical significance.

Results

PAH Concentration and Composition of HEWAF preparations

The goal of the experiment was to keep the concentration of oil and Σ PAH consistent over the 48 h exposure period, while measuring natural depletion every 24 h. Initial and Final Σ PAH measurements were similar when looking at 24 h oil exposure and 48 h exposure, suggesting the goal was achieved. The geometric mean of the percent composite of the 50 PAHs tested for after 24 h oil exposure was 14.37 mg L^{-1} and 48 h oil exposure had a geometric mean of 14.25 mg L^{-1} . This suggests that the polyps were exposed to the same composition of Σ PAHs during the entire 48 h exposure. The distribution of Σ PAH

was similar during each day of exposure and was dominated by tricyclic forms of PAHs (Figure 3).

Oxygen Consumption Trials

There was a clear effect of colony at the ambient temperatures (25°C), but not at higher temperatures (30°C). Specifically, a post hoc pair wise test of this colony effect in the ambient treatment showed that the only significant difference was between Colonies A and C (Figure 4). Respiration rates were relatively stable when the temperature remained constant at 25°C, but showed an increasing trend as temperature increased. However, this increase was not significantly different from control (25°C) respiration rates (repeated two-way ANOVA).

Respiration rates also tended to increase after 48-h of oil exposure, but were only found to be significant at 30°C (Figures 4 and 5). There was no significant difference in respiration rates prior to oil exposure, even at higher temperatures (30°C). There was a trend of respiration rates decreasing after 24 h of oil exposure, which was also seen in a preliminary trial with individual polyps of *Orbicella faveolata* (Chapter 2), but this was not found to be significant.

Chlorophyll fluorescence

Initial chlorophyll fluorescence (I-PAM) data were collected once the respiration rates stabilized (Day 6) and then daily from the start of oil exposure until the end of recovery (Day's 9-15 and then a final measurement on day 23).

The average measurements at the control temperature (25°C) had a slight increase after 48 h of oil exposure whereas at the higher temperature (30°C) the measurements took a dip after 24 h then increased again after 48 h. These trends followed the respiration trends for both temperature points (Figures 6 and 7). When run through the same pair wise test as the respiration data multiple time points were found to be significantly different, most were significant when compared to the third recovery time point. Due to the complexity of the interactions the significant interactions are listed in Tables 1 and 2.

Discussion

Oxygen consumption

The initial decrease in respiration rate after 24 h of exposure to oil at 30°C was not significant and was followed by the increase in respiration rates after 48 h (Figure 3), suggests that *Siderastrea siderea* may have experienced an initial “shock” causing initially lower respiration rates after oil was added at 30°C, but it was able to compensate after 48 h for the added combinatorial stress of oil and higher temperature. This shock could have been caused by behavioral responses from the added stress, as was observed in *Diploria strigosa*, which retracted its tissues in the presence of oil (Wyers, 1986). The increase in respiration rates after 48 h exposure to oil was only significant at 30°C, although there was an increasing trend observed at 25°C after 48 h, suggesting that longer exposure times may eventually have resulted in higher respiration rates even at lower temperatures. Additional studies need to be done to test this

hypothesis and determine to what extent corals might be able to compensate for oil exposure over time. The fact that the geometric means of the Σ PAH are consistent throughout the experiment suggests that temperature did not affect the composition of the oil, indicating that elevated temperature and oil increased respiration rates due to metabolic effects at higher temperatures, rather than thermal effects on the oil. Previous studies have observed seasonal shifts in the concentrations of zooxanthellae inhabiting coral tissue, with highest concentrations in winter months and lowest in the late summer months (when temperatures are higher) (Fitt, 2000; Lesser, 2006). The fact that photochemical efficiency levels fluctuated with respiration suggests that the stress of temperature and oil was on the entire coral holobiont.

Although respiration rates in this experiment were only measured for two days of post-exposure recovery, they already decreased to pre-exposure levels during this time. This could have been due to the fact that the added stress of oil had been removed or because the exposure period was so short. This is supported by previous studies that have shown exposure to oil has long-term detrimental effects on many metabolic processes in corals (Reimer, 1975; Loya and Rinkevich 1979; Peters et al., 1981; Wyers, 1986; Guzmán and Holst, 1993; Harrison 1999), which could be exacerbated by the elevated temperatures. Longer recovery studies need to investigate if respiration recovers over time or if it remains higher than its baseline at higher temperatures after exposure.

These interactions between high temperature and oil exposure suggests that warmer temperatures increase the susceptibility of corals to oil exposure, as

has been indicated by previous multiple stressor research (Nyström et al, 2001; Nordemar et al., 2003; Boyett et al. 2007; Bruno et al. 2007; Faxneld et al., 2010, 2011; Ross et al., 2013;). This finding may have important implications for the toxicity of oil to corals exposed at different times of the year (with summer exposures being more detrimental than winter exposures) or by climate change (with corals experiencing warmer baseline temperatures and increased high-temperature anomalies also being more susceptible to oil).

Work has shown that bioaccumulation of the PAHs in coral tissues happens (Sabourin et al., 2012; Ko et al., 2014), but it is unclear if temperature has an effect on this accumulation. Bioaccumulation of PAHs in coral tissues may be expected to be greater in the warmer treatment due to faster metabolic rates and the Q10 effect and elevated temperature may also influence latent effects this accumulation has on the polyps. This may help explain the higher respiration rates during oil exposure at 30°C, as well as slower recovery times at this temperature. It has been shown that long-term effects of these accumulated PAHs affect future reproductive success of exposed corals; fewer larvae are produced, or are spawned prematurely, or even have trouble recruiting to reefs due to the accumulation of PAHs in the sediments (Shafir et al., 2007; Sabourin et al., 2012; Goodbody-Gringley, 2013; Ko et al., 2014).

Photochemical efficiency

The photochemical efficiency data, collected using I-PAM, showed trends that mirrored changes in respiration rates with various time point interactions that

were significant. The majority of these were when a time point during the exposure was compared to the recovery time points. This suggests that the oil exposure affects the recovery of a coral and increases its chances of morbidity. Previous work has shown that 30°C was not sufficient to cause heat stress-induced photoinhibition on these corals nor was oil alone able to affect photosynthesis, but the combination of oil and elevated temperature may be sufficient to induced photoinhibition (Cook and Knap 1983; Warner et al., 1996). The fact there are fewer significant time points at the higher temperature suggests the combination of elevated temperature and oil pushed the coral to a stress threshold, thus the reaction to any stress variation is lower. Previous studies have shown that oil or the combination of oil and another stressor have various effects on corals that may be evident immediately (Reimer, 1975; Peters et al., 1981; Wyers, 1986). This supports the data that show low photochemical efficiency during recovery and high-observed morbidity and mortality two weeks after oil exposure. Since the photochemical efficiency trends follow the respiration data it is unclear if the stress on the host animal is causing bleaching, if it is stress on the algal symbiont, or if it is the combination of stress on both the host and symbiont. Previous studies have indicated bleaching as a result of oil exposure is primarily an animal stress response, rather than an algal stress response, but more work must be done to study the combined effect of elevated temperature and oil have on the coral holobiont (Baird, 2009; Baker and Cunning, 2016). This experiment only tested a 48 h oil exposure at an ecologically relevant concentration, but if an oil spill were to occur the conditions

have the potential to be more damaging. The duration the corals would be exposed to the oil would be on the timeline of weeks not days and the concentration of oil could be higher especially if the spill occurred near the reef. This experiment also did not take into consideration the addition of dispersants that could be added to clean up the oil. Previous research has shown that the addition of a chemical dispersant increases the toxicity of oil to any organism that comes in contact with it (Peters, 1981; Cook and Knap 1983; George-Ares and Clark, 2000; Incardona, 2004; Shafir et al., 2007; Goodbody-Gringley, 2013; Pie, 2015;). This would suggest that if this study were repeated with dispersants the level of stress response would be much higher.

Further Research

Due to the location of oil resources in the GoM, and the importance of larval transport as a means of connecting coral reefs in the northern GoM (e.g., Flower Gardens) with reefs further south (e.g., Florida Keys), future studies should test the effect of crude oil on coral larvae that may be exposed to oil within the dispersal phase. The microplate respirometer tested here represents an important technical development that might help with these assessments. It would also allow for direct species comparisons at the larval and adult level. It could also be used to look at developmental changes between adult corals and larvae within the same species. These tests could be run using any combination of stressors and would provide a more in depth look at how corals develop and change throughout their life.

Since coral larvae use currents to find new reefs to recruit to it is important to look at how oil and dispersants would affect them, since they would be coming in direct contact in the water column. Evidence of this was seen when oil exposure was found on deep reefs up to 11 km away from the Deepwater Horizon blowout site (White et al., 2012; Hsing et al., 2013), which indicated that currents play a role in delivering oil to sites that are not immediately within a spill or blowout site. The use of drifters showed that the Loop Current, the major current system found in the Gulf of Mexico, did transport oil from the DWH, but due to the behavior of the winds and water current during the time of the spill the oil was not transported south to Florida (Liu et al., 2011; Le Hénaff et al., 2012). However, the Loop Current normally travels into the Gulf on its the western side and loops around to join the Gulf Stream, flowing through the Florida Straits after wrapping around the lower Florida Keys. Consequently, the potential for a future spill or blowout in the Gulf of Mexico to affect shallow reefs in Florida may be higher than suggested by the transport during the DWH event.

If an oil spill did occur in our natural environment it is not likely that the oil would stay in the system for only 48 h. Therefore it is important to look at longer exposure and recovery times for both stressors (temperature and oil). This could help tease out the role and mechanism each factor plays to cause coral stress. It could also provide information that could determine what was being affected by the stressor, the animal host or the algal symbiont. These trials could be repeated to test minimum exposures required for coral bleaching and how respiration changes during these longer exposures. Extended recovery times

would also determine how long corals take to fully recover from oil exposure, and whether smaller doses have long term chronic effects as well. These trials could also be repeated for different species and life stage comparisons, which could give us information about which corals are hardier. This could provide us with information we need to create future policies to protect these corals that are more resistant to stress.

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Figures

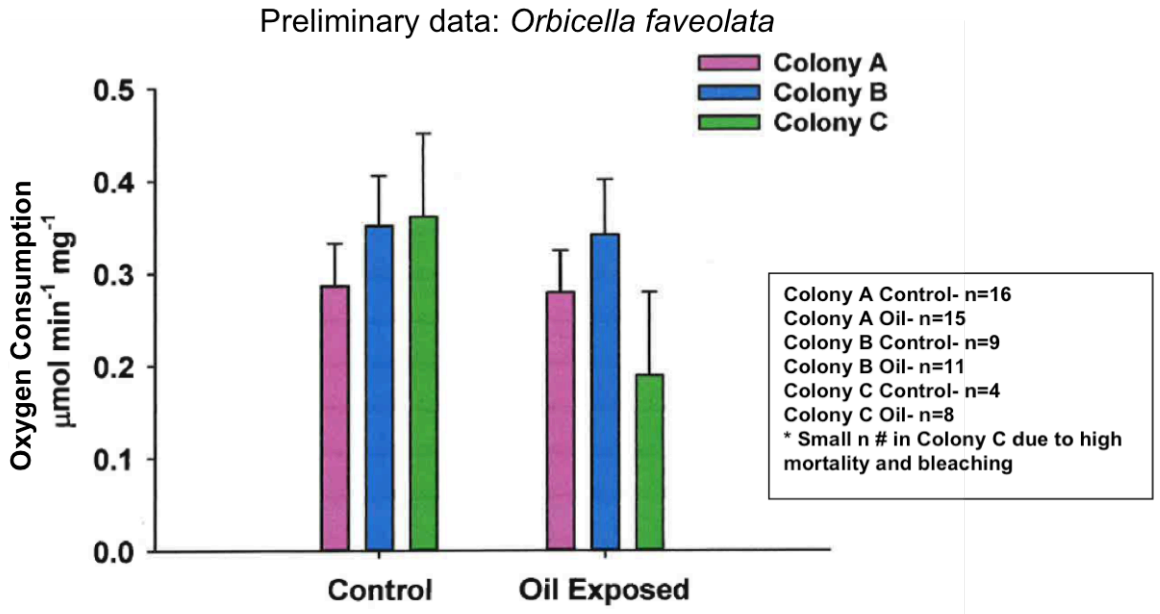


Figure 1. Preliminary trial with 3 colonies from *Orbicella faveolata*.

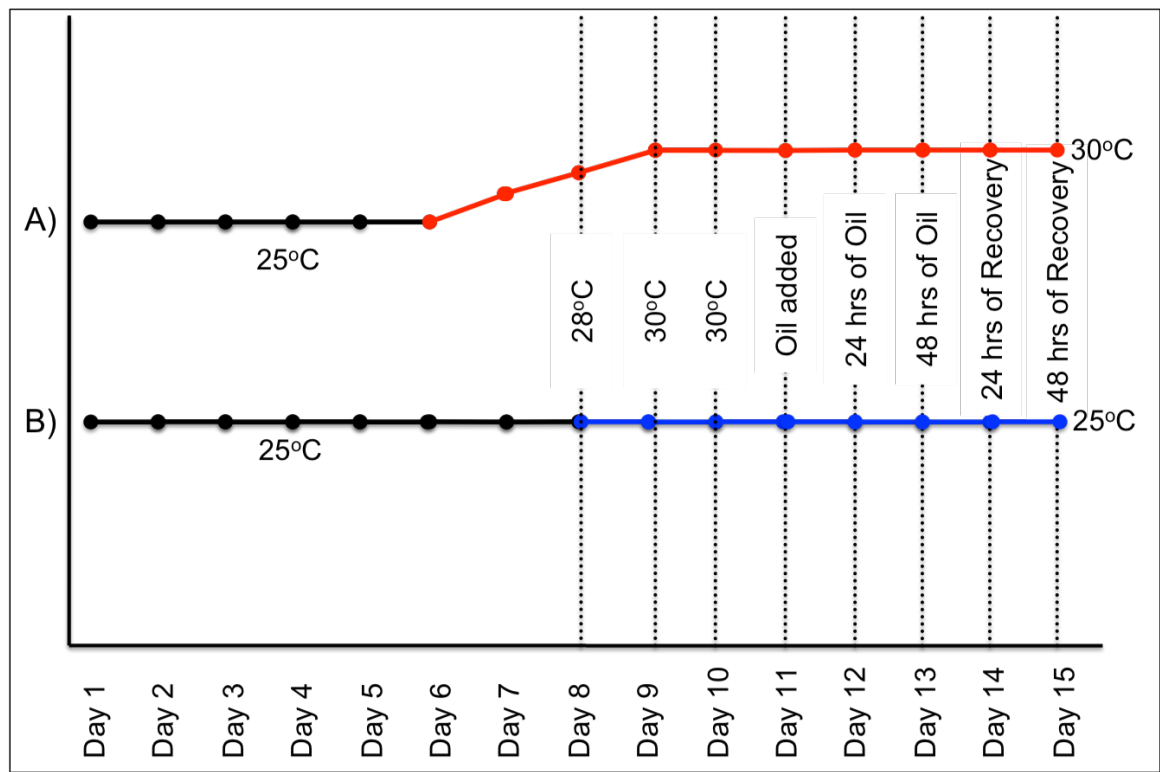


Figure 2. Experimental Design

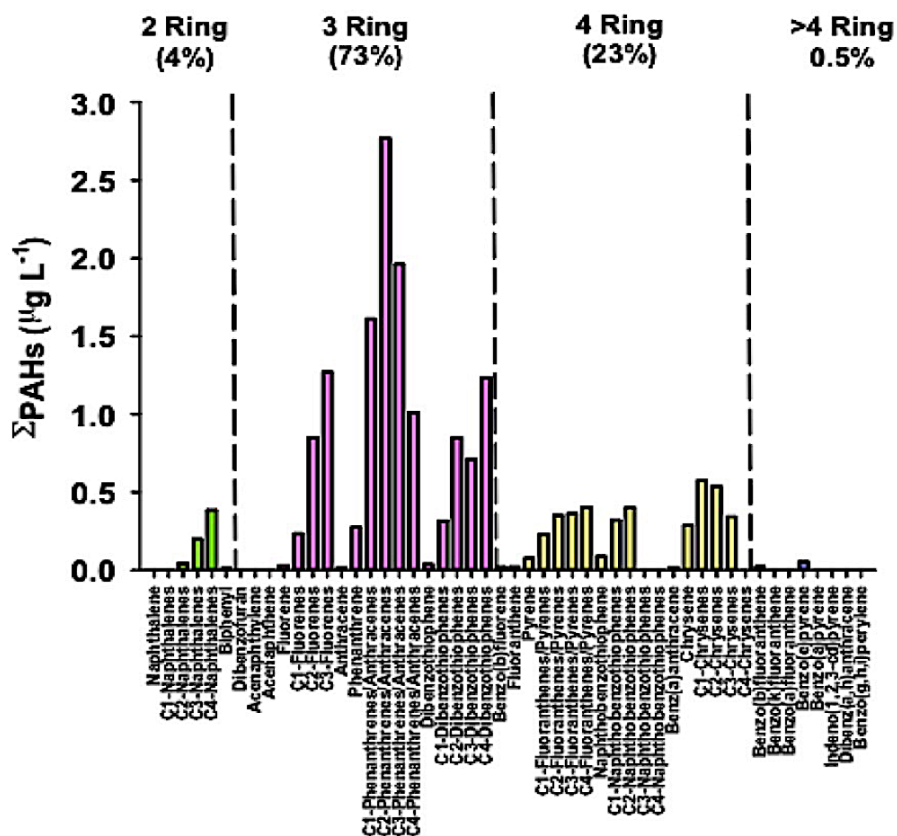


Figure 3. The Σ PAH breakdown of the oil sample used in this experiment. All four samples had a similar composition, so only one graphical representation is shown.

Colony A vs. Colony C P=0.023 (n=40)

25 Degrees

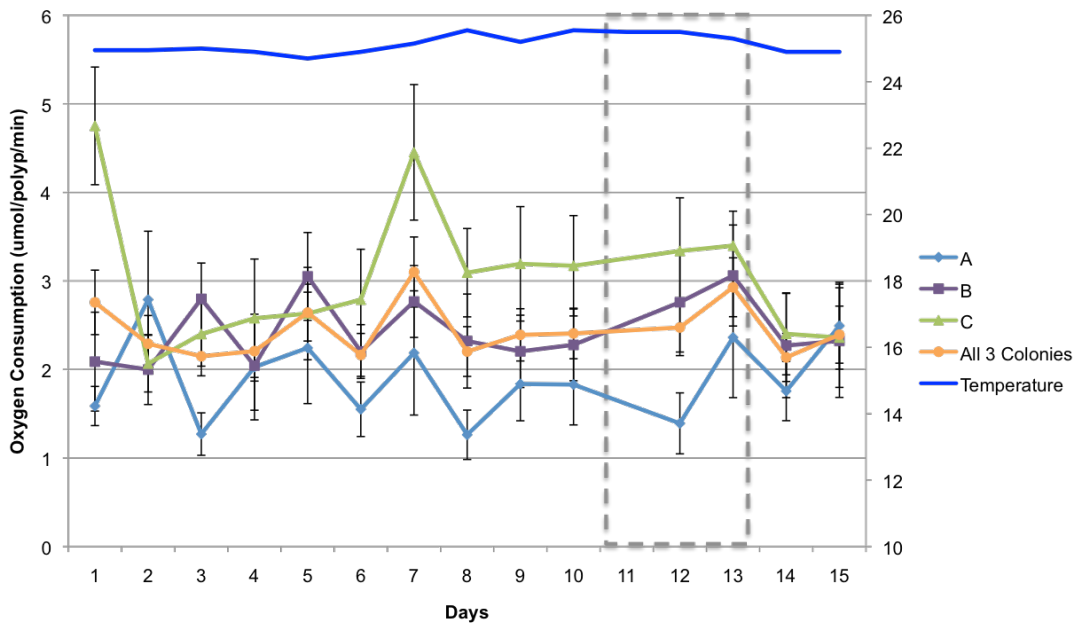


Figure 4. The means of each colony were analyzed each day. Days 8-10 (Control), Day 11(Oil was added, Day 12 (24 hr Oil), Day 13 (48 hr Oil), Days 14-15 (Recovery)

Day 10 vs. Day 8 P=0.007 (n=40)

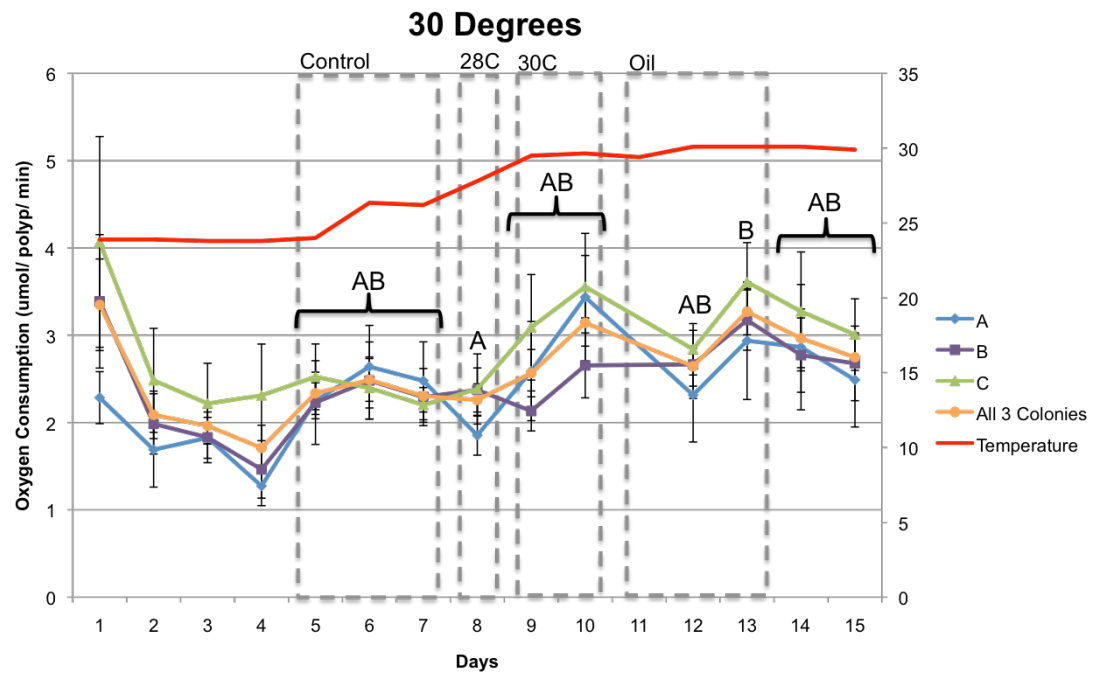


Figure 5. The means of each colony were analyzed each day. Days 5-7 (Control), Day 8 (28oC), Days 9-10 (30oC), Day 11(Oil was added, Day 12 (24 hr Oil), Day 13 (48 hr Oil), Days 14-15 (Recovery)

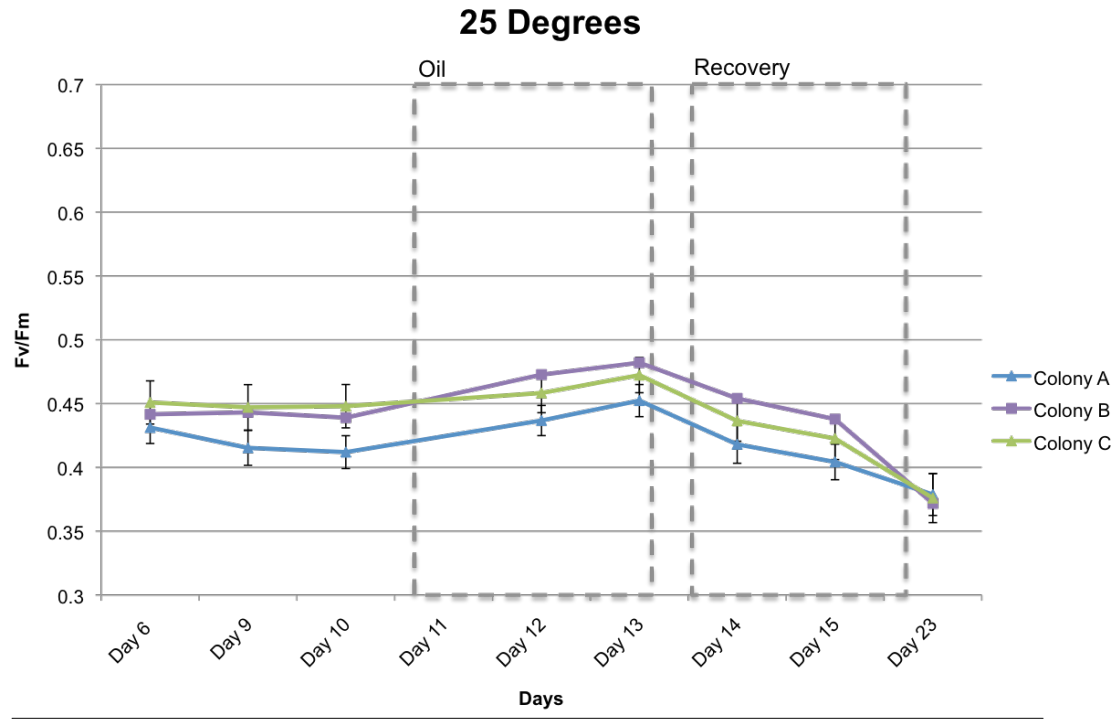


Figure 6. The mean photochemical efficiency data for each colony throughout the experiment looking at ambient temperature and oil exposure.

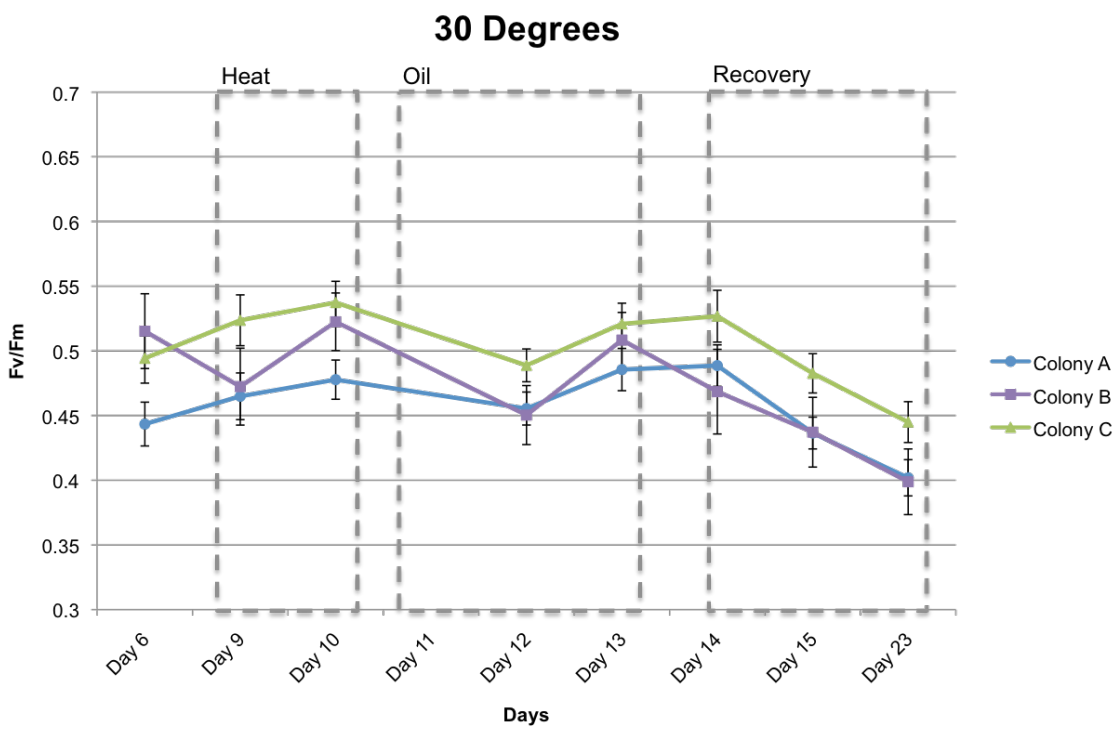


Figure 7. The mean photochemical efficiency data for each colony throughout the experiment looking at elevated temperature and oil exposure.

Tables

Colony	Timepoint A	Timepoint B	P value
Colony A	Ambient A	Recovery C	0.001
	Oil B	Recovery A	0.003
	Ambient B	Recovery C	0.004
	Oil A	Recovery B	0.005
	Control	Recovery B	0.042
	Oil B	Ambient A	<0.001
	Oil B	Ambient B	<0.001
	Oil B	Recovery B	<0.001
	Control	Recovery C	<0.001
	Oil A	Recovery C	<0.001
	Oil B	Recovery C	<0.001
	Recovery A	Recovery C	<0.001
	Colony B	Oil A	Ambient B
Oil A		Control	0.005
Oil A		Ambient A	0.008
Oil B		Recovery A	0.015
Oil B		Control	<0.001
Oil B		Ambient A	<0.001
Oil B		Ambient B	<0.001
Oil A		Recovery B	<0.001
Oil B		Recovery B	<0.001
Ambient A		Recovery C	<0.001
Ambient B		Recovery C	<0.001
Control		Recovery C	<0.001
Oil A		Recovery C	<0.001
Oil B		Recovery C	<0.001
Recovery A		Recovery C	<0.001
Recovery B		Recovery C	<0.001
Colony C	Oil B	Recovery A	0.002
	Oil A	Recovery B	0.003
	Control	Recovery B	0.041
	Oil B	Recovery B	<0.001
	Ambient A	Recovery C	<0.001
	Ambient B	Recovery C	<0.001
	Control	Recovery C	<0.001
	Oil A	Recovery C	<0.001
	Oil B	Recovery C	<0.001
	Recovery A	Recovery C	<0.001
	Recovery B	Recovery C	<0.001

Table 1. Time point comparisons at the ambient temperature where the photochemical efficiency was significantly different

Colony	Timepoint A	Timepoint B	P Values
Colony A	Heat B	Recovery C	0.002
	Heat A	Recovery C	0.025
	Recovery A	Recovery C	<0.001
	Oil B	Recovery C	<0.001
Colony B	Heat B	Recovery B	0.014
	Control	Recovery B	0.037
	Oil B	Recovery C	<0.001
	Heat B	Recovery C	<0.001
	Control	Recovery C	<0.001
Colony C	Recovery A	Recovery C	0.002
	Heat A	Recovery C	0.004
	Oil B	Recovery C	0.006
	Heat B	Recovery C	<0.001

Table 2. Time point comparisons at the elevated temperature where the photochemical efficiency was significantly different