A COMPARISON OF THE EFFECTS OF TAPIS CRUDE OIL AND DISPERSED CRUDE OIL ON SUBTIDAL *ZOSTERA CAPRICORNI*

K. G. Wilson and P. J. Ralph
Institute for Water and Resource Management, University of Technology, Broadway, Sydney, NSW, 2007, Australia

ABSTRACT

Oil spill mitigation managers need to know the effects of chemical dispersants on subtidal seagrass in order to determine the least net environmental impact of their actions. The decision-making process for chemical dispersant use in Australia, known as Net Environmental Benefit Analysis, is compromised in near shore areas due to a lack of information on dispersed oil impacts on subtidal seagrasses. This study aimed to determine the toxic effects of crude oil, dispersed and non-dispersed, on subtidal seagrass and to quantify the exposure amount.

Zostera capricorni plants were exposed to a range of concentrations of different oil and dispersant combinations in the field. Photosynthetic health was measured using Pulse Amplitude Modulated (PAM) fluorometry and chlorophyll pigment analysis. Oil concentration was calculated in relative oil units using Ultraviolet Fluoresence (UVF) spectrophotometry.

Limited photosynthetic impact was detected in Z. capricorni exposed to the water soluble fraction of the non-dispersed Tapis crude oil treatments. No significant photosynthetic impact was evident in the dispersed Tapis crude oil treatment even though the Total Petroleum Hydrocarbon (TPH) concentration in these treatments was higher than in the non-dispersed Tapis crude oil treatments. Plants from both treatments recovered following replenishment from the surrounding seawater. A substantial reduction of the total petroleum hydrocarbons within the mesoscosms over the 10 hour exposure period was evident and would likely suggest a rapid loss of the toxic mixture to the sediments rather than assimilation by the seagrass.

INTRODUCTION

Seagrasses are flowering aquatic plants, living fully submerged in seawater and are found throughout the world in coastal and estuarine zones (den Hartog 1970). Seagrasses are highly productive, remove sediment and nutrients from coastal waters, provide shelter and habitat for fish and invertebrates, and act to stabilise the substrate through their root system (Kuo 1982; Poiner *et al.* 1993). However, due to their near shore distribution, seagrass habitats are susceptible to oil spill contamination.

Oil contamination can have deleterious effects on intertidal organisms, including mangrove and intertidal seagrass habitats. In mangrove habitats, stranded oils become incorporated into the sediments and often lead to direct mortality from smothering and uptake into the pneumatophores and roots (Baca *et al.* 1996). Chemically dispersing an oil slick reduces the amount of floating oil from reaching such environments. However, in the process of dispersing an oil slick, the subtidal habitats are exposed to an increased amount of petroleum hydrocarbons. Whilst dispersants have resulted in successful outcomes in many oil spill situations for intertidal habitats (API 1999), it is the risk to subtidal organisms exposed to the increased petrochemical component in the water column that remains uncertain.

Dispersants are a combination of a surfactant in a solvent which work by increasing the interfacial tension between water and oil (API 2001), effectively displacing the oil slick from the water surface and moving it into the water column. Current day dispersants are less toxic than those previously used (API 2001). Studies indicate that low levels of dispersant contribute less to the toxicity of the mixture than the oil itself does (Lunel and Lewis 1999). However, conflicting results occur between different studies as to the relative toxicity of dispersed oil treatments than to oil alone.

When seagrasses absorb the water soluble fraction (WSF) of spilled oil the plants become more susceptible to other stress factors (Zieman *et al.* 1984). Studies of dispersed oil have shown deleterious impacts on marine vegetation, including subtidal seagrass meadows (Thorhaug 1988; Howard *et al.* 1989). Seagrasses have been found to absorb more aliphatic and aromatic oil fractions when the oil is dispersed, therefore increasing the toxicity (den Hartog 1984). Dispersants are thought to affect the waxy cuticle of the seagrass blade and in doing so, increase the penetrability of the dispersed oil to the photosynthetic apparatus, particularly the thylakoid membrane (Howard *et al.* 1989; Wolfe *et al.* 1998).

Previous research suggests that amongst seagrass species there is a wide diversity of responses to petrochemicals (Thorhaug 1988) and studies investigating the effects of chemically dispersed oil to subtidal species have shown conflicting results. Thorhaug *et al.* (1986) and Macinnis & Ralph (2003) found the addition of chemical dispersants to oil had less of an effect on subtidal seagrass than non-dispersed oil, whereas Hatcher and Larkum (1982) found the complete opposite in that the dispersed oil had a greater impact than the non-dispersed oil.

Oils contain thousands of different compounds and are described as one of the most complex and variable mixtures to study toxicologically (API 1999; Singer *et al.* 2000). Furthermore, once oil enters the marine environment a multitude of factors affect its chemical and physical composition. Couple this complex mixture with highly varying environmental conditions relating to geographical location, season, seawater temperature, wave action and irradiance, and it is clear that oil and dispersed-oil studies are quite multifaceted (API 1999).

The water soluble fraction (WSF) is the component which is incorporated into the water column from an overlying oil slick. Saeed and Al-Mutairi (2000) suggest that as little as 0.1 to 1% of

the original spilt oil is incorporated into the water soluble fraction. Aromatics are more soluble than aliphatics of similar molecular weight, so whilst they form a major component of the water soluble fraction, they are also lost rapidly (Zieman *et al.* 1984; Saeed & Al-Mutairi 2000). As the WSF is the component which has the potential to affect the subtidal organisms, the preparation of this solution for experimental work is also contentious (Singer *et al.* 2000; Ziolli & Jardim 2002).

One shortfall of many seagrass/oil investigations is that only nominal doses are reported, few studies have actually measured or described the other components, including polycyclic aromatic hydrocarbons (PAH) available in the experimental set-up. Hatcher and Larkum (1986) used ultraviolet fluorescence to quantify the total petroleum hydrocarbon concentration prior to and following exposure to the seagrass. They found that the dispersed oil treatments had significantly higher concentrations of hydrocarbons both prior to, and following the exposure period. Clearly, the amount and components within the water soluble fraction of the oil, dispersed and non-dispersed, has the potential to effect the toxicity to the seagrass differently.

Oil spill response managers need to know whether chemical dispersants increase the risk of petrochemical effects to subtidal seagrass. Uncertainty as to the least net environmental impact with regards to dispersant use and subtidal seagrass habitats has occurred in recent oil spill events in Australian waters. Different findings between studies as a result of different species, different oils, different geographical regions, seasons etc., do little to assist these managers.

This is the first in a series of experiments to address the issue of oil spills and dispersant usage in an effort to provide oil spill mangers the best information when faced with an oil spill situation around subtidal seagrass habitats. The aim of this study was to determine the effects of Tapis crude oil and dispersed Tapis crude oil on subtidal *Zostera capricorni*.

METHODS

Water soluble fraction

Five litres of filtered (0.45-µm) seawater brought to summer average seawater temperature (Sydney), 22º C (Booth 2007, unpublished data), was held in 5-L Erlenmeyer flasks, and placed on magnetic stirrers (150 rpm). A vortex was created prior to the addition of oil to decrease the amount of oil forced onto the inside flask wall (Singer *et al.* 2000). Tapis crude oil (Caltex Australia Petroleum) was added close to the water surface using a glass pipette. To produce a 1 % w/v solution, 50 g of oil was added to the 5 litres of seawater. During weathering, the room was darkened to reduce oil degradation and alteration by artificial lighting (Ali *et al*. 1995).

The flasks were stirred for 24 hours and allowed to settle for one hour. For the dispersed oil experiments, 5 g of Corexit© 9527 was added and the flasks stirred for a further 10 minutes prior to settling for one hour. The flask lids were removed, plastic tubing was inserted below the oil - water surface and the WSF was siphoned into amber glass bottles using a vacuum pump. The WSF was held at 4° C in darkness and used within two days (Singer *et al.* 2000).

Experimental design

Fifteen clear Perspex cylinders (400-mm high and 180-mm diameter, 10-L volume) were placed in *Zostera capricorni* meadows at Bonna Point (151° 11' E, 34° 00' S), Botany Bay, New South Wales during February 2007. The cylinders were pushed into the sediment to approximately 5 cm and secured with four pegs attached by ropes to the external cylinder wall. Specially designed Perspex lids sealed the cylinders.

Inside the exposure cylinder, healthy seagrass blades were held in position by specially designed leaf clips supported by plastic stakes. This allowed the blades to retain their natural orientation and enabled measurements to be taken from the same position on the leaf blade throughout the exposure day. An optic fibre was held in a 45º position relative to the leaf clip and extended outside the cylinder wall through a rubber blanking plug. This allowed for remote fluorescence measurements to be taken from outside of the cylinder. The cylinders and leaf clips were set up the day before the exposure day, at low tide, and left overnight.

Petrochemical treatments consisted of either Tapis crude oil alone or Tapis crude oil with dispersant (Corexit 9527©). For each experiment, five concentrations of the specific petrochemical were added (0.00, 0.05, 0.10, 0.20, 0.40 %). The WSF was added to the cylinders in different volumes specific to the concentration desired (ie., half the cylinder volume would give half the initial WSF concentration). In the field, petrochemicals were poured into a plastic bag which was then loosely tied. The bag was gently pushed into the top of the cylinder, the lid placed on the cylinder, and the bag extracted through a blanking plug in the chamber wall.

Fluorescence measurements, effective quantum yield of photosystem II (ΔF/Fm'), were taken using a Diving-PAM (Pulse Amplitude Modulated) fluorometry (Walz GmbH, Effeltrich, Germany). The Diving PAM settings were as follows: measuring light intensity, 10; saturating pulse intensity, 8; saturating pulse width, 8; and gain, 6. An initial effective quantum yield measurement was taken prior to the petrochemical exposure (0700 hrs) and then every 2 hours until 1700 hrs.

Chemical Analysis

A 2.5-L water sample was siphoned from each 10-L cylinder at the end of the exposure day (17:00h) for hydrocarbon analysis. For hydrocarbon preservation, 5 ml of methanol was added to each sample. Samples were refrigerated immediately upon returning to the laboratory and analysed within 28 days (APHA 1995).

Samples were extracted from solid phase extraction (SPE) cartridges (Oasis HLB 6cc). Cartridges were pre-treated with two, 5-ml aliquots of methanol, followed by two, 5-ml aliquots of milliQ water. Samples were passed through the cartridges at approximately 30 ml per minute and eluted with two, 5-ml aliquots of n-hexane. Sample bottles were further rinsed with two, 5-ml aliquots of n-hexane to ensure hydrocarbon removal. Samples were then made to 25-ml volumes in volumetric flasks with n-hexane. Controls of n-hexane alone and standard WSF samples were stored, extracted, eluted and analysed in the same manner as the samples were made up for total petroleum hydrocarbon (TPH) concentration determination and for quality control. Fresh standards were prepared when the correlation coefficients of existing batches were less than 95%.

The WSF samples were analysed for TPH using UV fluorescence with a spectrophotometer (Varian Cary Eclipse). For concentration determinations, the following spectrophotometer settings were used: excitation wavelength, 310 nm; emission wavelength, 360 nm; and excitation slits, 10 nm. The samples were read against the standard calibrations (UNEP 1992).

All glassware were soaked with Pyroneg ©, rinsed with distilled water, rinsed with milliQ water, and finished with three n-hexane (low aromatic hydrocarbons- Sigma Aldrich) washes.

Photosynthetic pigment analysis

Seagrass blades were collected from each treatment at the end of the exposure day (10 h) and following the recovery period (96 h) for pigment analysis. Samples were stored frozen prior to analysis.

Seagrass blades were gently scraped free of epiphytes, dried on paper towel and the surface area (mm^2) determined using a Leaf Area Meter (LI-3000A, Lincoln, USA). Samples were initially

placed in two ml of acetone in 15-ml amber glass bottles and refrigerated for an hour. Samples were then ground in 10 ml of 90% acetone in a mortar and pestle with a pinch of acid-washed sand (Denison 1994). Several drops of $MgCO_3$ were added and the samples were centrifuged and refrigerated (4 ºC) for 1 day prior to analysis. Absorbance was measured at 480, 647 and 664 nm, and all readings were corrected for turbidity scattering by subtracting the 750 nm absorbance. A spectrophotometer (LKB Introspect II UV/Vis, model 4050) with a spectral resolution of 1.00 nm was used for the absorbance measurements. Wellburn's (1994) extraction coefficient equations were used.

Statistical Analysis

Data was analysed using SSPS software. Repeated measures two-way analysis of variance (rmANOVA) tests were used to determine if significant differences were present at each time interval for the control and the five treatments. One-way ANOVAs were used to assess whether treatments differed significantly. Tukey's post hoc HSD tests were conducted to determine where a significant difference was detected. One-way ANOVAs were used to assess different treatments. Hydrocarbon analyses were statistically analysed using *t*-tests. Data was tested for normality and homogeneity of variance and arc-sine transformed where needed.

RESULTS

Tapis Crude Oil

Chlorophyll a fluorescence

The effect of the water soluble fraction (WSF) of the crude oil on the effective quantum yield (Δ*F/Fm'*) of *Zostera capricorni* was small. The effective quantum yield of the control and most concentrations varied little over the exposure day (Figure 1), however, the 0.40% treatment showed a decrease in Δ*F/Fm'* over this time. Significant differences were found at six (ANOVA, $p = 0.012$) and eight (ANOVA, $p = 0.012$) hour's exposure with these differences due to the 0.40% WSF treatment. Whilst the Δ*F/Fm'* of the 0.40% WSF treatment remained less than the control and the other treatments at the end of the exposure day, there was no significant difference between treatments at this time.

Limited variation in ΔF/Fm' was also shown between the control and treatments over the recovery days (Figure 1). No significant difference was found suggesting that the seagrass had recovered from the initial impact during the exposure day.

Chlorophyll pigment concentration

The total chlorophyll, chlorophyll *a* and chlorophyll *b* were not significantly different from the controls at 10 h and 96 h following exposure to the Tapis crude oil. All chlorophyll pigment concentrations decreased from 10 h to 96 h following exposure (Table 1).

Total petroleum hydrocarbon concentration

Ultraviolet fluorescence (UVF) analysis found that the pre-field exposure Tapis crude 1% water soluble fraction (WSF) was 91µg L^{-1}. This equated to 437 µg L^{-1} (4.8 L volume of 0.40%), 218 µg L^{-1} (2.4 L volume of 0.20%), 109 µg L^{-1} (1.2 L volume of 0.10%) and 55 µg L^{-1} (0.6 L volume of 0.05%) total petroleum hydrocarbons added to the chambers.

After 10 hours in the field chambers, the total petroleum hydrocarbon concentrations were less than 1µg L for all treatments (Figure 3).

Dispersed Tapis Crude (Corexit 9527)

Chlorophyll a fluorescence

Whilst the dispersed oil treatments showed variation in the effective quantum yield over the exposure day (Figure 2) no significant differences were found at any time. After four hours of petrochemical exposure, the 0.40 % treatment was markedly less than the other treatments and the control but recovered following this time. Similarly, no significant differences were found in treatments or the control at any time over the recovery days (Figure 2).

Chlorophyll pigment concentrations

Chlorophyll pigment concentrations showed few significant differences between 10 h and 96 h following dispersed Tapis exposure (Table 2). Significant differences were shown in the 0.00% WSF treatment for chlorophyll *a*, *b* and total (*a* + b) and in the 0.40% WSF treatment for chlorophyll *a* and *b*.

Total Petroleum Hydrocarbon concentrations

UV fluorescence (UVF) analysis found that the pre-field exposure Tapis crude and Corexit 9527 1% water soluble fraction (WSF) was 200 µg L^{-1}. This equated to 960 µg L^{-1} (0.40%), 480 µg L^{-1} (0.20%), 240 µg L^{-1} (0.10%) and 120 μg^{-1} L (0.05%) TPH added to the chambers.

After 10 hours in the field chambers, the TPH concentration was less than 10 µg L^{-1} for all treatments except for the 0.40% WSF treatment (Figure 3). Following 10 hours in the *in situ* chambers, the initial 0.40% WSF treatment had a TPH concentration of 12 µg L^{-1}.

DISCUSSION

Tapis Crude oil

The impact of the Tapis crude oil was evident only in the 0.40% WSF treatment and only during a few hours following exposure. This impact on the seagrass, however, was short-lived as no detectable impact was found in the final measurement on the exposure day. Macinnis and Ralph (2003) showed photosynthetic impact to occur almost immediately following petrochemical exposure, whereas in the current study the response was delayed for several hours. Thorhaug et al. (1986) also found a lesser impact from oil alone than dispersed oil, and that the difference amongst seagrass species were greater than differences amongst oil treatments.

Thorhaug et al. (1986) showed that mortality was evident only after 100 hours exposure period. In this study, and in Macinnis and Ralph (2003), a photosynthetic response was detected during a 10 hour exposure period. With current oil spill response capabilities, response teams can be activated at short notice, usually within 24 hours (AMSA 2000). To complement these rapid response measures, oil spill managers need to know what a likely scenario will be within a short period *and* prior to mortality. The chlorophyll *a* fluorescence technique used in this study provided a non-destructive monitoring tool of photosynthetic stress of the seagrass showing subtle physiological responses.

Whilst the pigment data did not display any differences, the samples were collected at the end of the exposure day. The final chlorophyll *a* fluorescence measurements (10 hrs exposure) agreed with the pigment data in that no impact on the seagrass pigment was evident at this time.

The WSF remaining following 10 hours exposure in the field dosing chambers was very low (< 10 µg L^{-1} TPH) for all treatments. Considering an initial dose of 400 µg L^{-1} TPH was added to the 0.40% WSF treatment chamber, a substantial loss of hydrocarbons occurred over the 10 hour exposure period. This loss of TPH over the exposure period is consistent with what should occur to oil once it enters the marine environment as many processes within the environment degrade and alter its composition (API 1999; Saeed and Al-Mutairi 2000). After dosing field chambers with Bass Strait crude oil, Hatcher and Larkum (1982) also found very low (< 10 µg L^{-1}) or undectable oil concentrations following a 24-hr field exposure. The degradation and movement of oil and its components once it enters the marine environment is complex (API 1999). This loss of WSF from the water column could be explained by many reasons. The oil may have adhered to the seagrass

itself, to the epiphytic organisms on the seagrass or other benthic organisms within the mesocosm, or it may have been assimilated into the sediments. Spaulding et al. (1994) stated that approximately 35 % of oil spilt during the *Braer* tanker spill was resident in the nearby sediments.

Dispersed Tapis Crude oil (Corexit 9527©)

The results from the chlorophyll *a* fluorescence and the chlorophyll pigment analyses data showing no significant difference at any time over the exposure and recovery days suggests that the dispersed oil had no detectable impact on the photosynthetic health of the seagrass. In comparison, Macinnis & Ralph (2003) and Hatcher and Larkum (1982) found a greater impact on subtidal *Z. capricorni* and *P. australis*, respectively, by non-dispersed oil than dispersed oil.

The 0.40% WSF treatment at the end of the exposure day still displayed a measurable amount (12 µg L^{-1}) of total petroleum hydrocarbons (TPH) in the field-dosing chambers; however, no impact to the seagrass physiology was detectable. Howard et al (1989) suggested that dispersants penetrate the waxy cuticle of the seagrass blade but the results from this study showed no impact to the photosynthetic output of the organism. Dispersants increase the bioavailable fraction of hydrocarbons in the water column (Wolfe et al. 1998), so it is unclear why this should result in a lesser impact to the seagrass than oil alone.

A major finding from this study is that the Tapis crude oil alone had a greater impact on *Z. capricorni* than the dispersed Tapis, yet the TPH concentration that remained following the exposure period was greater in the dispersed Tapis treatments. A study by Baca and Getter (1984) showed similar findings where crude oil had a greater impact than dispersed crude oil although the hydrocarbon concentration was greater in the dispersed treatments. It was suggested this was due to the high level of toxic components of benzene, toulene and C-2 benzenes in the water soluble fraction (Baca *et al.* 1996).

It is suggested that the chemical differences of the dispersed and non-dispersed treatments may be the underlying factor governing whether a photosynthetic impact occurs to the seagrass. It is quite clear that any future study investigating the impacts of dispersed- and non-dispersed oils need to not only quantify the total concentration of hydrocarbons used, but also detail the composition of hydrocarbon and other components within the treatments. It must be remembered, that in simulating a real oil spill the 'oils' both dispersed and non-dispersed are initially weathered to form the component that would potentially reach the subtidal seagrass, the water soluble fraction. This initial weathering process, for this study lasting 24 hours, would have resulted in a loss of some hydrocarbon components from the original, non-weathered or 'fresh', oil.

Dispersants have been found to severely impact the epiphytic community living upon the seagrass blade (Baca *et al.* 1996) even when the seagrass does not show any direct decrease in photosynthetic efficiency. Mobile organisms often try to escape, whilst sedentary organisms show an increase in morality due to the toxic impact of the mixture (Zieman *et al*. 1984). The dispersed oil treatments may have led to a reduction in epiphytic activity, potentially increasing the light levels available to the seagrass plant and leading to an increase in photosynthetic output.

Mesocosm experiments are the preferred method in simulating real field conditions but they are not without their complications. Nutrient concentrations, which play a significant role in the microbial degradation of hydrocarbons, can be greatly disrupted within mesocosms (Zieman *et al.* 1984). Temperature and flow variations within mesocosms can greatly alter these biological processes. Furthermore, as oils readily adhere to surfaces within mesocosm experiments, they are likely to not only adhere to the seagrass blades but also to the internal mesocosm walls. This adherence may not be equal between oil and non-dispersed oil and it is possible that this factor alters the quantity of petrochemical reaching the seagrass between the two treatments. The main question arising from these unknown factors is whether the oil is actually assimilated by the seagrass. If the oil is actually taken up by the seagrass this would strongly suggest that the seagrass can tolerate this level of oil contamination. These findings would have major implications in management decisions governing oil spill clean up situations.

CONCLUSIONS

Overall, this study found that dispersed Tapis crude oil has less of an impact on *Zostera capricorni* than Tapis crude oil alone. The impact of the Tapis crude oil alone was short-lived and the seagrass recovered even prior to the replenishment of the surrounding seawater. The results of this study provide further assistance to oil spill mitigation managers when dealing with the issue of whether to add dispersants to a crude oil spill over an important habitat area. Future studies in this area need to focus on two things: (1) they need to include a detailed analysis of the hydrocarbon components to identify the toxic components remaining in the WSF following simulated weathering and field exposure, and (2) they require a determination of the endpoint location of the WSF within mescosm experiments.

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TABLES AND FIGURES

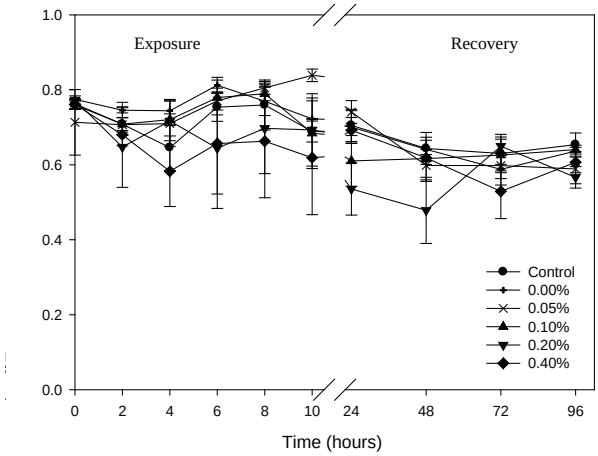

FIGURE 1: EFFECTIVE QUANTUM YIELD OF *ZOSTERA CAPRICORNI* EXPOSED TO DIFFERENT CONCENTRATIONS OF THE WATER SOLUBLE FRACTION (WSF) OF TAPIS CRUDE OIL OVER THE EXPOSURE AND RECOVERY DAYS. TIME ZERO IS PRE - PETROCHEMICAL EXPOSURE. AVERAGES ± STANDARD ERROR OF THE MEAN ARE SHOWN (*N* =3).

Chlorophyll *a*	10 h	96 h	Chlorophyll *b*	10 h	96 h	Total chlorophyll	10 h	96 h
Control	5.53±0.3	4.8±0.6	Control	2.7±0.1	1.3±0.7	Control	8.2±0.5	5.8±1.3
0.00%	5.28±0.8*	5.0±1.3*	0.00%	2.7±0.0	2.3±0.4	0.00%	8.0±0.8	7.3±1.7
0.05%	5.58±0.8	4.4±0.3	0.05%	2.7±0.7	1.8±0.2	0.05%	8.3±1.5	6.2±0.5
0.10%	6.11±0.4	5.0±0.5	0.10%	2.5±0.1	2.1±0.2	0.10%	8.6±0.5	7.1±0.8
0.20%	5.17±1.0	4.7±0.2	0.20%	4.6±1.0	2.0±0.2	0.20%	9.8±2.0	6.7±0.4
0.40%	4.52±0.1	4.2±0.7	0.40%	3.3±1.6	2.1±0.6	0.40%	7.9±1.8	6.3±1.4
ANOVA *F*	1.742	0.579	ANOVA *F*	0.532	0.528	ANOVA *F*	0.500	0.543

TABLE 1: PHOTOSYNTHETIC CHLOROPHYLL PIGMENTS IN *ZOSTERA CAPRICORNI* EXPOSED TO TAPIS CRUDE OIL. * DENOTES SIGNIFICANT DIFFERENCE ($P < 0.05$). AVERAGES ± STANDARD ERROR OF THE MEAN ARE SHOWN (*N* =3).

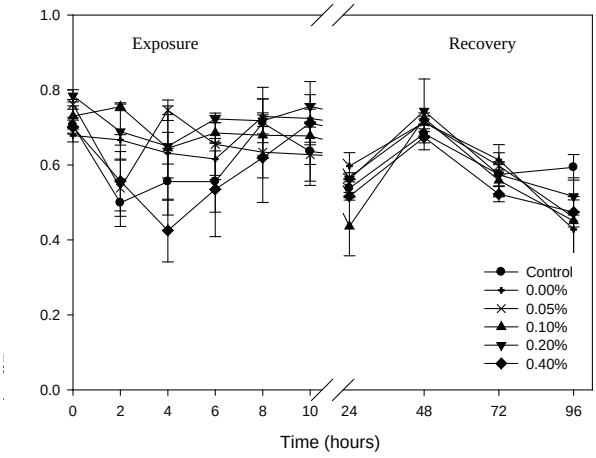

FIGURE 2: EFFECTIVE QUANTUM YIELD OF *ZOSTERA CAPRICORNI* EXPOSED TO DIFFERENT CONCENTRATIONS OF THE WATER SOLUBLE FRACTION (WSF) OF TAPIS CRUDE OIL AND DISPERSANT COREXIT 9527© OVER THE EXPOSURE AND RECOVERY DAYS. TIME ZERO IS PRE-PETROCHEMICAL EXPOSURE. AVERAGES ± STANDARD ERROR OF THE MEAN ARE SHOWN (*N* =3).

Chlorophyll *a*	10 h	96 h	Chlorophyll *b*	10 h	96 h	Total chlorophyll	10 h	96 h
Control	7.7±1.4	6.0±1.3	Control	3.0±0.7	3.9±0.8	Control	10.6±2.1	9.1±2.1
0.00%	6.4±0.8*	3.8±1.2*	0.00%	1.4±0.0*	2.2±0.7*	0.00%	7.8±0.9*	6.1±1.9*
0.05%	7.6±1.3	3.8±0.8	0.05%	2.9±0.1	3.4±0.7	0.05%	10.5±1.4	7.2±1.6
0.10%	7.4±1.6	3.2±0.4	0.10%	2.9±0.4	3.5±0.5	0.10%	9.0±1.9	6.7±0.9
0.20%	5.7±0.8	4.5±1.0	0.20%	2.2±0.7	3.9±0.5	0.20%	7.8±1.6	8.4±1.5
0.40%	9.1±0.6*	5.6±1.2*	0.40%	1.4±0.0*	4.2±0.4*	0.40%	10.6±0.6	9.8±1.6
ANOVA *F*	0.350	0.710	ANOVA	2.636	1.165	ANOVA	0.472	1.830

TABLE 2: PHOTOSYNTHETIC CHLOROPHYLL PIGMENTS IN *ZOSTERA CAPRICORNI* EXPOSED TO TAPIS CRUDE OIL AND COREXIT 9527. * DENOTES SIGNIFICANT DIFFERENCE (P < 0.05). AVERAGES ± STANDARD ERROR OF THE MEAN ARE SHOWN (*N* =3).

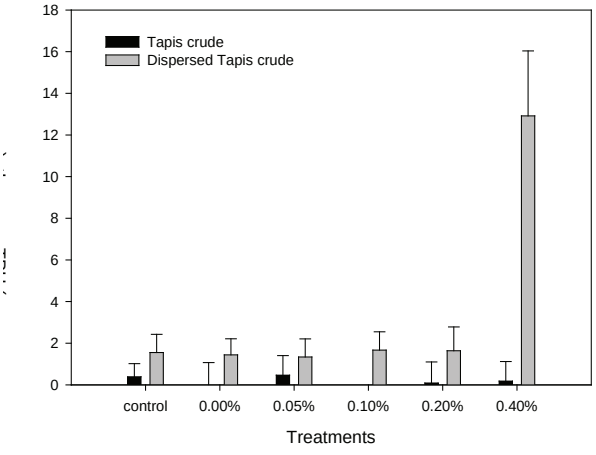

FIGURE 3: POST FIELD EXPOSURE TPH (TOTAL PETROLEUM HYDROCARBON) CONCENTRATION OF TAPIS CRUDE OIL ALONE AND DISPERSED (COREXIT 9527) TAPIS CRUDE OIL. MEAN ± S.E. ARE SHOWN.