FINAL REPORT:

EFFECTS OF OIL AND DISPERSED OIL ON TEMPERATE SEAGRASS: SCALING OF POLLUTION IMPACTS

By Kim Wilson
Professor Peter Ralph
Plant Functional Biology & Climate Change Cluster (C3)
Department of Environmental Sciences
University of Technology, Sydney

Executive Summary

This report is a summary of research conducted into the effects of oil and dispersed oil on temperate seagrass using a range of *in situ* and laboratory experiments on whole plants and seagrass leaf blade sections. Apart from assessing the effects of oil and dispersed oil on seagrass between seasons, locations, and morphologically different species, the research also investigated whether laboratory results could be indicative of those obtained *in situ.* Petrochemical treatments consisted of whole plants exposed for ten hours with a four day recovery period to a range of concentrations of the water accommodated fraction (WAF) of oil alone (Tapis crude, IFO-380 fuel oil), dispersant alone (Corexit 9527, Ardox 6120, Slickgone LTSW, Corexit 9500) and dispersed oil. Experiments were conducted in both the laboratory and *in situ*. Photosynthetic health was monitored by assessing the effective quantum yield of photosystem II ($\Delta F/F_m$') and chlorophyll *a* pigment concentrations, whilst semi-quantitative methods of total petroleum hydrocarbon (TPH) concentration were used to determine the percent TPH remaining in the water column following the exposure period.

In most cases, the non-dispersed oils, Tapis crude and IFO-380, had less of an impact to both *Zostera capricorni* and *Halophila ovalis* than the dispersed oil treatments. Winter *in situ* experiments found slightly greater reductions of $\Delta F/F_m$' in *Z. capricorni* in most treatments compared with summer *in situ*, but generally there was minimal impact. *Zostera muelleri* exhibited a stimulatory response to both non-dispersed and dispersed

Tapis crude oil in Corio Bay, Victoria (summer *in situ* only). Laboratory whole plant experiments found *Z. capricorni* was for the most part less resilient to Tapis crude oil (non-dispersed and dispersed) treatments than *H. ovalis* whereas, with exposure to IFO-380 fuel oil (non-dispersed and dispersed) *H. ovalis* was less resilient than *Z. capricorni*. Quite severe, and, or prolonged, photosynthetic stress was evident in both *Z. capricorni* and *H. ovalis* when exposed to most of the dispersant alone treatments (Corexit 9527, Ardrox 6120and Corexit 9500), however the Slickgone LTSW alone treatment caused only a very short-lived response which was only evident in *H. ovalis*. The results of the laboratory whole plant experiments, conducted under Sydney summer water temperature conditions, reflect those observed in the summer *in situ* experiments rather than those observed in winter *in situ*; suggesting laboratory experiments can guide what the field response could be, given temperature is an important consideration. $\Delta F/F_m$' appeared a more reliable indicator than that achieved with the chlorophyll *a* pigment analyses. These results suggest that assessments of seagrass health in laboratory experiments can in some cases be representative of that observed *in situ* when similar experimental conditions are maintained. However, large differences in the percent TPH recovered between *in situ* and laboratory experiments suggests microbial activity and sediments played a substantial role in the partitioning of oils in these experiments. This requires further investigation.

The findings of this study are that non-dispersed oil, in general, leads to less photosynthetic stress to *Zostera capricorni* and *Halophila ovalis* compared with the addition of a chemical dispersant. When the addition of a chemical dispersant is deemed necessary to protect other resources in the area, the seagrass may still recover depending on the dispersant used.

Introduction

The effects on subtidal seagrass from the application of dispersants to oil spills remain unclear (AMSA 2008). Whether to apply a chemical dispersant to an oil spill is usually a "trade-off" between the relative importance of subtidal resources and shoreline habitats.

Dispersant application is often sought when sensitive shoreline resources (e.g. mangrove habitats, nesting bird colonies) are at a clear risk of oil contamination if the stranded oil were to be simply left to degrade and weather naturally. Chemically dispersing the oil into the water column may therefore be justified to decrease the risk of the oil coming closer to shore and to reduce the oil's overall persistence in the environment. In dispersing the oil spill, however, the hydrocarbon concentration in the water column is greatly increased and concurrently increases the potential risk to subtidal organisms, like seagrasses (NRC 2005; AMSA 2008).

Seagrass meadows are extremely productive environments (Hemminga & Duarte 2000; Mateo *et al*. 2006). They uptake nutrients from surrounding waters, act to stabilise the sediment and provide habitat and shelter for many species including those of commercial and recreational importance (Kirkman 1997; Hemminga & Duarte 2000). Declines in seagrass habitats have been reported worldwide as a result of natural events and human-induced stress (Larkum & West 1990; Kirkman 1997; Short & Wylie –Echevaria 2000; Green & Short 2003). Any controllable anthropogenic pressures need to be prevented or minimised where possible to reduce further degradation of these critical habitats.

Subtidal seagrass inhabit areas where oil spills commonly occur, in nearshore and inshore environments. The loss of 270 tonnes of heavy fuel oil from the *Pacific Adventurer* near the coast of Cape Moreton (QLD) in 2009; the breaching of the hull of the *Global Peace* (2006) in Port Curtis, Gladstone Harbour (QLD); and the spill of 95 tonnes of light crude oil from the *World Encouragement* in Botany Bay (1979) all occurred within close proximity to subtidal seagrass meadows. In the case of Botany Bay and Cape Moreton, at least part of those seagrass meadows were, or now are, encompassed within RAMSAR wetlands of international significance (DEWHA 2010), highlighting both the extreme importance of the seagrass and the risk posed to these areas.

The body of evidence to suggest that seagrasses are impacted by oil is largely derived from when there has been a direct contact with the above ground biomass of the plant, the blades (Jacobs 1988). Even so, unless the oil is retained within the seagrass meadow for a

sustained duration most studies report no long-term impacts to the meadow (Zieman *et al.* 1984; Jacobs 1988). Oil by itself affects seagrasses through the adsorption of the water accommodated fraction (WAF) which leads to a reduction in tolerance to other stress factors (Zieman *et al.* 1984). Smothering, fouling and asphyxiation are some of the effects that have been documented from oil contamination (Blumer 1971; Cintron *et al.* 1981). Seagrass blades have become bleached, blackened, yellowed and detached from the plant following direct oil contamination (Chan 1973; den Hartog & Jacobs 1980; Jackson *et al.* 1989; Dean *et al.* 1998;) whilst other effects from direct contact include a decrease in the density of shoots and flowering shoots (Chan 1973; den Hartog & Jacobs 1980; Dean *et al*. 1998). Zieman *et al.* (1984) suggests that in most cases the system has the ability for recovery however; damage to the rhizome-sediment structure may result in irreversible damage as the sediment stability becomes compromised. Subtidal seagrass can still be subjected to, and impacted by, direct contact with oil that has not been chemically dispersed. For example, subtidal beds of *Thalassia* and associated fauna were decimated following a crude oil spill in Puerto Rico (1973) (Naduau & Berquist 1977). Strong weather conditions caused the entrainment of oil into the subtidal seagrass beds. Incidentally, the crude oil was considered to be of low toxicity, yet the impacts to the seagrass were so severe that even the rhizome layer was affected.

Seagrasses have been shown 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, to increase the penetrability of the dispersed oil to the photosynthetic organs, particularly the thylakoid membrane (Howard *et al.* 1989; Wolfe *et al.* 1998). Dispersed oil is also more susceptible than non-dispersed oil to microbial breakdown, which can lead to a greater oxygen demand by the microbes (Fingas 2001; NRC 2005). A reduction in the oxygen in the seagrass community may impact on the seagrass system (Zieman *et al.* 1984) as seagrasses have a high respiratory demand to support their large non-photosynthetic underground biomass.

Research outcomes from petrochemical exposure to seagrass range from dispersed oil posing a greater threat than non-dispersed oil; dispersed oil posing less of a threat than non-dispersed oil; and that neither oil nor dispersed oil negatively impact seagrass. This disparity amongst previous research findings may simply be an artifact related to the experimental differences between the studies relating to different methodologies, different exposure regimes, different species investigated, different petrochemicals exposed and different temperature conditions of the experiments. Morphological variety in seagrass is vast and species resilience to petrochemical impacts is likely to reflect this. Thorhaug *et al*. (1986) showed clear differences in the response of different species of tropical seagrass to petrochemicals but to date, other research has largely been conducted on single species (e.g. Baca & Getter 1984; Hatcher & Larkum 1984; Ralph & Burchett 1998; Macinnis & Ralph 2003). Different species of seagrass clearly respond differently to stressors, including that imposed by petrochemical pollution. Most studies, however, do not incorporate this into their research design likely due to the increased logistical effort of conducting multi-species analyses, specifically with aquatic macrophytes (Kuster & Altenburger 2007).

There is also disparity between results obtained from real spill events, *in situ* experiments and laboratory experiments particularly with the effects to subtidal seagrass (e.g. Macinnis & Ralph 2003). Field assessments are logistically intensive in time, cost and effort and as such fewer field assessments can be conducted compared with laboratory experiments. Laboratory experiments have been shown to overestimate the effects of real spills and *in situ* experiments, and many environmental variables are difficult to replicate in the laboratory such as light attenuation (Clark & Noles 1994; Hemminga & Duarte 2000; Macinnis & Ralph 2003). The integration of both *in situ* and laboratory experiments may help in better understanding the effects of petrochemicals on seagrass and the disparity in research findings.

Finally, most studies assessing the effects of oil and dispersed oil on seagrass have focused on crude oil (e.g. Hatcher & Larkum 1984; Thorhaug *et al.* 1986; Baca *et al*. 1996; Ralph & Burchett 1998; Macinnis & Ralph 2003). Considering the recent spate of

fuel oil (rather than crude oil) spill incidents in Australian waters (e.g. *Pacific Adventurer* 2009, *Global Peace* 2006), an understanding of the effects of these oils to subtidal seagrass is required if mitigation procedures are to be successful.

Aims

The major aims of this project were to:
1) determine the extent to which subtidal seagrass is affected by non-dispersed and dispersed crude and IFO-380 oil and at what concentrations;
2) determine the relative effects of different temperate species of seagrass to oil and dispersed oil; and
3) determine whether *in situ* experiments can be replicated in laboratory experiments.

Methods

Field Sites and Species

Collection of seagrass (and *in situ* experiments) were performed under a New South Wales Department of Primary Industry Scientific Research Permit (Permit numbers: P06-09/0010).

Two field sites were used for the *in situ* component of the study. The primary field site was Bonna Point, along the eastern shores of Botany Bay, New South Wales, Australia. Bonna Point was also the site for seagrass collection for the laboratory experiments. A smaller subset of experiments was also conducted in Corio Bay, along the south-western shores of Port Phillip Bay, Victoria, Australia. Only two treatments (non-dispersed and dispersed Tapis crude oil) were conducted in this location.

The *in situ* component comprised the assessment of *Z. capricorni* in summer and winter in Botany Bay; and *Z. muelleri* in Corio Bay in summer only. The laboratory experiments entailed the analysis of *Z. capricorni* and *Halophila ovalis* under summer conditions.

Exposure Regime

Seagrass were exposed to the water accommodated fraction (WAF) of oil, dispersed oil and dispersant alone, *in situ* using specially designed mesocosms, and in the laboratory using glass aquaria. Mesocosms and tanks were both approximately 12 L in volume. After a ten-hour exposure period, *in situ* mesocosms were removed allowing replenishment of 'fresh seawater'; whilst in the laboratory experiments, the aquaria were drained, and refilled with 'fresh' seawater. The seagrass were then further monitored over a four-day recovery period.

Photosynthetic assessment

Photosynthetic stress of the seagrass was monitored by chlorophyll *a* fluorescence using Pulse Amplitude Modulated (PAM) techniques and also via analyses of the chlorophyll *a* pigment concentrations. Chlorophyll *a* fluorescence, specifically, the effective quantum yield of Photosystem II ($\Delta F/F_m'$) was measured using a Diving-PAM and Mini-PAM (Walz, Germany) for *in situ* and laboratory measurements, respectively. The $\Delta F/F_m'$ provides information regarding the photosynthetic activity, thereby providing valuable information regarding the physiological health of the seagrass.

A 2 mm fibre optic was held in position close to the seagrass blade via specially designed leaf clips. The fibre optic extended outside of the exposure chamber (mesocosm/ aquaria) enabling remote PAM measurements to be taken without disturbing the experiment. Photosynthetic activity ($\Delta F/ F_{m'}$) was monitored every two hours during the exposure day, followed with once daily $\Delta F/ F_{m'}$ measurements for the next four days, the recovery period. Leaf samples were collected for the determination of chlorophyll *a* pigment concentrations at the end of the exposure day and also at the conclusion of the recovery period.

Water Accommodated Fraction

The water accommodated fraction (WAF) was prepared similar to Singer *et al*. (2000) and Macinnis and Ralph (2003). To produce a 1.00 % w/v solution, 50 g of oil was added to 5 litres of seawater in Erlenmeyer flasks, and stirred for 24 hours on magnetic stirrers. For the dispersed oil treatments, 5 g of dispersant was added and the water stirred for a further 10 minutes. The dispersant alone treatments were created by adding 5 g of dispersant to 5 litres of seawater and, consistent with the other treatments, stirred for 24 hours. All treatments were allowed to settle for one hour following the stirring stage. The WAF was siphoned into amber glass bottles stored at 4° C in darkness and used within two days (Singer *et al.* 2000). Petrochemical treatments, less than and including the 1.00 % WAF were created using the 1.00 % WAF with an appropriate amount of seawater for dilution. A 2.00 % WAF was created under a different loading regime, whereby 100 g of oil was added to 5 L of seawater.

Semi-quantitative analysis of the WAF (UVF) was performed prior to exposure, and at the end of the exposure period to assess the percentage total petroleum hydrocarbon (TPH) remaining in the water column for each experiment. Quantitative analysis (Gas Chromatography techniques) of the 1.00 % w/v WAF (pre-exposure) was also undertaken to provide details of the actual composition of each treatment. These quantitative analyses were performed by a commercial laboratory, Sydney Environmental and Soil Analysis Laboratory (SESL) (NATA accreditation number 2901). These results are provided in the Appendix.

Petrochemical Treatments

The number and concentrations of the petrochemical treatments differed between experiments. More treatments and higher concentrations could be conducted under laboratory conditions than what could be conducted in the field. Petrochemical treatments included Tapis crude oil and IFO-380 oil, non-dispersed and dispersed, and dispersant alone treatments. Dispersants used were Corexit 9527, Ardrox 6120, Slickgone LTSW

and Corexit 9500. Concentrations ranged up to 0.40 % WAF in the field and up to 2.00 % WAF in the laboratory experiments.

Results/ Discussion

All chlorophyll *a* fluorescence data ($\Delta F/F_m'$) from the field and laboratory experiments have been synthesised into Tables 1 and 2 for summary purposes and to enable a comparison of the results. To differentiate between the levels of impact, the magnitude of decline in $\Delta F/F_m'$ from the control has been classified as "Low" (< 0.15), "Medium" (0.15 – 0.30) and "High" (> 0.30). A "High" level effect (> 0.30 units $\Delta F/F_m'$) represents a reduction of greater than 50 % of the photosynthetic efficiency of the seagrass. The classification does not take into account where the seagrass recovered from a photosynthetic impact from the petrochemical treatment but where recovery, or lack of, occurred, it is highlighted in the text. The timing of impact details any sampling time within the exposure and recovery periods (where applicable) when a treatment significantly decreased in $\Delta F/F_m'$ from the control. The range of concentrations that caused these significant decreases in $\Delta F/F_m'$ is also included and is derived from the total petroleum hydrocarbon (TPH) concentration pre–exposure as detected by Gas-Chromatography Mass Spectrometry analyses (Appendix). The 2.00 % water accommodated fraction (WAF) samples are represented as being greater than (>) the TPH of the 1.00 % WAF treatment. As the 2.00 % WAF treatment was created using a different loading regime, the TPH concentration cannot be calculated from the 1.00 % WAF treatment (Singer *et al.* 2000). Stimulation of growth (hormetic responses) occurred in most treatments and as these did not result in a negative impact during the experiment they have simply been marked with an *. For example, "No negative impact *" denotes that the $\Delta F/F_m'$ of the seagrass may have increased above the control at some sampling time from at least one concentration, at no time did any of the samples exhibit a decrease in $\Delta F/F_m'$ from the control.

Field Assessment

Treatments:

- *Tapis crude oil: Oil alone; Oil + Corexit 9527; Corexit 9527 alone*
- *IFO-380 oil: Oil alone; Oil + Slickgone LTSW; Slickgone LTSW alone*
- *Concentrations: 0.00, 0.05, 0.10, 0.20, 0.40 % WAF*

Neither the oil, dispersed oil or dispersant alone treatments resulted in a high level impact to the seagrass in summer or winter (Tables 1 & 2). No treatment led to an impact of greater than 0.2 units $\Delta F/ F_m'$ from the control. The initial $\Delta F/ F_m'$ was greater than 0.6 units in all samples. This means that no treatment exhibited a response greater than 30 % inhibition, with most showing a maximum response of far less than 20 % (0.1 units of photosynthetic activity below the control).

The magnitude of decrease in $\Delta F/ F_m'$ of *Z. capricorni* (Botany Bay) in the dispersed oil treatments (crude and IFO-380) was greater than the decrease in the oil alone or dispersant alone treatments (Tables 1 & 2), but this was only marginally so (within 0.1 units $\Delta F/ F_m'$). In some petrochemical treatments, the $\Delta F/ F_m'$ of the seagrass was actually enhanced. The results also showed that where negative responses were detected in the different petrochemical treatments, they were only in the highest WAF concentrations, mostly in the 0.40 % WAF concentration, and in some experiments also in the 0.20 % WAF concentration. Most importantly, the seagrass showed complete recovery following any negative effect.

There was some evidence of seasonal variation between the summer and winter results for *Z. capricorni* but again, due to the lack of any great decrease in $\Delta F/ F_m'$, this was only minor (Tables 1 and 2). The magnitude of decrease in $\Delta F/ F_m'$ in the winter experiments was often greater than that observed in the summer experiments. This was true for the crude alone, IFO-380 alone, IFO-380 dispersed with Slickgone LTSW, and the Corexit 9527 alone treatments. However, these differences were generally within 0.1 units $\Delta F/ F_m'$ of their summer counterparts. The small seasonal variation is unlikely to affect oil spill decision making, but is worth consideration for further investigation.

Zostera muelleri in Corio Bay (Victoria) was not negatively impacted by the petrochemical treatments (Table 1). The seagrass actually displayed a significant increase in $\Delta F/ F_m$' in most of the concentrations for both non-dispersed and dispersed crude oil. The chlorophyll *a* pigment concentration of the seagrass exposed to the crude alone treatment also showed an increase, but not until 96 hours following the initial exposure. The light intensities and temperatures subjected to *Z. muelleri* in Corio Bay, were likely to have been far greater than those experienced by *Z. capricorni* in the deeper waters of the Botany Bay experiments and are considered to have played a major role in this stimulation of growth. This is interesting, but should not influence the spill response decision.

Total petroleum hydrocarbon concentrations decreased to below detection limits by the end of the exposure period in all non-dispersed oil (crude and IFO-380) treatments. Only the greatest WAF treatments (0.20, 0.40 % WAF) of the dispersed crude and IFO-380 treatments displayed any measurable concentrations at the end of the exposure period, with generally no greater than 30 % remaining. There were slightly greater concentrations of both dispersed oils recovered in winter than in the summer.

Laboratory Assessment

Treatments:

- *Tapis crude oil: Oil alone; Oil + Corexit 9527; Oil + Ardrox 6120; Corexit 9527 alone; Ardrox 6120 alone*
- *IFO-380 oil: Oil alone; Oil + Slickgone LTSW; Oil + Corexit 9500; Slickgone LTSW alone; Corexit 9500 alone*
- *Concentrations: 0.00, 0.20, 0.40, 1.00, 2.00 % WAF*

Crude oil: non-dispersed, dispersed, dispersants alone

The crude alone treatment did not negatively impact *Z. capricorni* or *H. ovalis* (Table 1). This was supported by both the assessment of the chlorophyll *a* fluorescence data ($\Delta F/F_m$') and the chlorophyll pigment analyses for both species. Considering that

laboratory experiments often exaggerate the effects of oil and yet even the highest concentrations (2.00 % WAF) of the crude oil did not evoke a significant response from either species is an important finding for management.

The concern with dispersing an oil slick and the associated increased hydrocarbon content in the water column was somewhat supported by the results of these experiments (Table 1). The toxicity of the dispersed crude oil to *Z. capricorni* was specifically related to the actual dispersant used and the petrochemical loading in the water column. Whilst, both Corexit 9527 dispersed and Ardrox 6120 dispersed crude oil treatments produced negative impacts to *Z. capricorni*, the Ardrox 6120 dispersed treatment had a more sustained negative impact to the seagrass. For *H. ovalis*, $\Delta F/F_m$' impacts were only detected within the first four hours, followed by full recovery, when exposed to either of the dispersed crude treatments. These laboratory experiments suggest that dispersed crude oil is more toxic to *Z. capricorni* and *H. ovalis* than non-dispersed Tapis crude oil; and that Ardrox 6120 dispersed crude oil is slightly more toxic than Corexit 9527 dispersed crude oil.

The dispersant alone treatments, unlike most of the impacts from the dispersed oil treatments, resulted in significant negative impacts to the seagrass even from the lower concentrations (0.20 and 0.40 % WAF) (Table 1). Both species showed a significant impact from the Ardrox 6120 alone treatments, far more than the Corexit 9527 alone treatments. *Zostera capricorni* was impacted greater than *H. ovalis* from the dispersant alone treatments as no significant differences were detected in *H. ovalis* with the Corexit 9527 exposure. In the case of Corexit 9527 exposure to Z. *capricorni*, the impact was far greater than the dispersant-oil combination. The results from this study suggest that spill response managers need to exercise care not to over apply dispersants to disperse a slick, as in some cases the dispersant alone treatment was actually more toxic than the oil-dispersant combination as in the case of the Corexit 9527 and *Z. capricorni*.

IFO-380 oil: non-dispersed, dispersed, dispersant alone

Zostera capricorni showed no impacts from the IFO-380 oil alone treatment (Table 2); however, H. ovalis exhibited a high level (> 30 % decline in $\Delta F/F_m'$) although short-lived (up to six hours) impact (Table 2). Where a response was detected in the dispersed IFO-380 oil treatments it was generally far greater than that which occurred from the non–dispersed IFO-380 oil.

The effects of the two dispersed IFO-380 oil treatments varied between treatments and also between species (Table 2). *Zostera capricorni* was impacted only by the Corexit 9500 dispersed IFO-380 oil treatment whereas *H. ovalis* was impacted by both dispersed IFO-380 oil treatments. There were also differences in the timing of the response of the seagrass. The effects to *Z. capricorni* from the Corexit 9500 treatment occurred most notably following the removal of the chambers, whereas to *H. ovalis* this effect occurred almost immediately. The high level impact to *H. ovalis* (Table 2) from the Slickgone LTSW dispersed IFO-380 oil over the exposure period was clearly different to the response of *Z. capricorni* which was not impacted negatively by the treatment (Table 2).

The dispersant alone treatments also resulted in differences between treatments within the one species and differences between species response from the same treatment (Table 2). *Halophila ovalis* was impacted more severely than *Z. capricorni* when exposed to the same dispersant treatment. The Slickgone LTSW alone treatment caused no detectable impact to *Z. capricorni* and a very short-lived impact to *H. ovalis* (eight hours exposure only). The Corexit 9500 alone treatment led to high level (> 30 % reduction in $\Delta F/F_m'$) impacts to both species. For *Z. capricorni* this level of impact was only detectable during two of the recovery days (24 and 48 hours). *Halophila ovalis*, however, exhibited this level of impact throughout most of the exposure and recovery days.

Most treatments displayed a high percentage recovery of total petroleum hydrocarbons suggesting minimal breakdown in these laboratory experiments over the exposure period. However, where a greater loss of TPH was evident in some treatments it appeared to correlate with a greater photosynthetic impact to the seagrass. The Tapis dispersed with Ardrox 6120 treatment showed a greater loss of TPH and resulted in greater

photosynthetic stress to both species than the Corexit 9527 dispersed treatment. Similarly, both dispersed IFO-380 oil treatments also showed substantial loss of TPH over the exposure period which was also supported by an increased photosynthetic impact to both seagrass species. Dispersant alone treatments were also analysed with this semi-quantitative method with the results suggesting minimal loss of TPH over the exposure period. The results of these dispersant alone treatments should be treated with caution, however, as they may reflect backscattering of the UV light within the sample rather than any hydrocarbons. Further research into the use of oil-in-water fluorometers for treatments containing dispersants would increase the confidence of results with samples containing dispersants.

Conclusions/ Recommendations

There were clear differences in the response of the three species analysed in this study when exposed to the petrochemicals; this supports similar findings by Thorhaug *et al.* (1984). In the field experiments, the stimulation of growth in *Z. muelleri* in Corio Bay (VIC) is suggested to be partly due to the difference in the light attenuation and water temperature compared to Botany Bay (NSW). In the laboratory assessment, the morphological differences between *Halophila ovalis* compared with *Zostera capricorni* were considered to have played a significant role in the species resilience to the petrochemicals. The shape of the blade and the positioning of the seagrass in the water column (paddle-shaped blade in *H. ovalis* and close to the sediment; strap-like blade in *Z. capricorni* and higher in the water column) are two possible morphological differences that may have led to these differences.

Seasonal variation in the response of Z. *capricorni* to these petrochemicals appears slight, but likely, and supports other research findings on seasonal variation in plant sensitivity from oil pollution (eg. Pezeshki *et al.* 2000) and to other toxicants (Brun *et al.* 2002). Future research into the effects of water temperature into seagrass response to petrochemicals may shed more light on this factor, and also the enhancement of growth in *Z. muelleri*.

Table 1 Summary table of magnitude of stress ($\Delta F/F_m'$), timing of impacts and effective concentrations in Z. *muelleri, Z. capricorni* and *H. ovalis* from exposure to the crude oil treatments (non-dispersed, dispersed and dispersant alone). * not significantly different to control. na treatment was not performed under those conditions. See text for further explanation.

	Field Experiments			*Laboratory Experiments*	
	Z. muelleri	*Z. capricorni*	*Z. capricorni*	*Z. capricorni*	*H. ovalis*
Treatment	Summer	Summer	Winter	'Summer'	'Summer'
Crude	No negative impact *	No negative impact *	**Low 6-8, 48 h 2.4-4.8 mg L^{-1}**	No negative impact	No negative impact
Crude + C9527	No negative impact *	**Medium 4, 24 h 20-40 mg L^{-1}**	**Medium 4 h 40 mg L^{-1}**	**Low 6-8 h >101 mg L^{-1}**	**Medium 2h >101 mg L^{-1}**
Crude + Ardrox 6120	na	na	na	**Medium 8-72 h >105 mg L^{-1}**	**Medium 2h >105 mg L^{-1}**
C9527	na	No negative impact	**Medium 4 h 64 >128 mg L^{-1}**	**Medium 2, 6-8, 24-96 h 32 >317 mg L^{-1}**	**Medium 48-96 h >317 mg L^{-1}**
Ardrox 6120	na	na	na	**Medium 4-48, 96 h 92 >230 mg L^{-1}**	**High 2-4, 24-96 h 92 >230 mg L^{-1}**

Table 2 Summary table of magnitude of stress ($\Delta F/F_m'$), timing of impacts and effective concentrations in *Z. capricorni* and *H. ovalis* from exposure to the IFO-380 treatments (non-dispersed, dispersed and dispersant alone). * not significantly different to control. na treatment was not performed under those conditions. See text for further explanation.

	Field Experiments		*Laboratory Experiments*	
	Z. capricorni	*Z. capricorni*	*Z. capricorni*	*H. ovalis*
Treatment	Summer	Winter	'Summer'	'Summer'
IFO-380	No negative impact *	**Low** **2-4, 48 h** **1 mg L^{-1}**	No negative impact *	**Medium** **2-4 h** **1 >3 mg L^{-1}**
IFO-380 + Slickgone LTSW	No negative impact *	**Medium** **2-4 h** **80 mg L^{-1}**	No negative impact	**High** **4-8, 24, 96 h** **80 >200 mg L^{-1}**
IFO-380 + C9500	na	na	**Medium** **6-48 h** **200 >500 mg L^{-1}**	**Medium** **2-6, 10-24 h** **200 >500 mg L^{-1}**
Slickgone LTSW	No negative impact *	No negative impact *	No negative impact	**Medium** **8 h** **>150 mg L^{-1}**
C9500	na	na	**High** **24-48 h** **67 >167 mg L^{-1}**	**High** **2-48, 96 h** **>167 mg L^{-1}**

Clear differences were detected in this study regarding the amount of TPH recovered between the field and laboratory experiments. In the *field* experiments, there was minimal, if any, TPH recovered following the exposure period in most treatments which contrasted with the relatively high levels of TPH recovered following the same duration of exposure in the laboratory experiments. As sediments and microbial activity play a major role in the breakdown of petrochemicals (Leahy & Colwell 1990; Fingas 2001), a reduction of TPH found in these in laboratory experiments (Clark & Noles 1994) has led several authors to suggest that an increase in photosynthetic stress to organisms in laboratory experiments may be derived from a reduced rate of petrochemical breakdown by these microorganisms (eg. Macinnis & Ralph 2003). In the whole plant laboratory experiments in the current study, there would have been fewer microbes due to the filtered seawater and reduced amount of sediment when compared with the *in situ* experiments. Considering these factors, it is likely that the smaller loss of TPH in the laboratory experiments compared to the *in situ* experiments is due to the reduced microbial activity and sediments in the laboratory experiments.

Major findings from this study and recommendations for oil spill management:

- Even the highest concentrations (2.00 % WAF) of the crude oil did not evoke a significant response from either *Z. capricorni* or *H. ovalis.*
- In most cases the addition of dispersant to either Tapis crude or IFO-380 oil increased the stress response from the seagrass. It appears that it would be preferable, where possible, to not disperse either of these oils over regions of subtidal meadows of *Z. capricorni* or *H. ovalis*.
- Certain dispersants were more toxic to specific species of seagrass.
- Corexit 9527 dispersed crude oil appeared slightly less toxic than Ardrox 6120 dispersed crude oil to both *Z. capricorni* and *H. ovalis* and would therefore be considered slightly more favourable.
- Slickgone LTSW dispersed IFO-380 oil (and Slickgone LTSW alone) was less toxic to *Z. capricorni* than the respective Corexit 9500 treatments and would be a more suitable dispersant when this species is concerned.

- *Halophila ovalis* may be severely impacted by both dispersants and recommendations are to not disperse with either Slickgone LTSW or Corexit 9500. This species, however, is considered to recover rapidly from stress events, therefore where the species occurs in the same meadow as *Z. capricorni*, it may be more beneficial to focus response efforts on *Z. capricorni*.
- Most dispersant alone treatments caused photosynthetic stress to the seagrass, and in some cases this was greater than the dispersed oil and the oil alone treatments. It is strongly recommended that care is exercised not to over apply dispersants to an oil slick in the vicinity of either *Z. capriconi* or *H. ovalis*.

In summary, few treatments led to severe and, or, prolonged impacts to the seagrass investigated in this study. Where a photosynthetic impact to the seagrass was detected this was followed by full recovery in all field experiments and in the majority of the laboratory experiments. This finding implies that these species are quite resilient to the petrochemicals used under the experimental conditions of the study.

As the dispersed treatments did in most cases result in a photosynthetic impact to some degree, the results further suggest that when possible, it is better to not disperse over an area of subtidal seagrass when either Tapis crude oil or IFO-380 is spilt. However, when the addition of chemical dispersant is deemed appropriate to protect other resources within the area the seagrass may still recover depending on the dispersant used.

Acknowledgements

This project was funded through The National Plan to Combat Pollution of the Sea by Oil and other Noxious and Hazardous Substances and an Australian Research Council (ARC) Linkage Grant (LP05616333). The project was co-ordinated by the University of Technology, Sydney, the Australian Maritime Safety Authority and the National Plan Environmental Working Group.

References

Australian Maritime Safety Authority (2008). National Plan to Combat Pollution of the Sea by Oil and other Noxious and Hazardous Substances. Annual Report: 2007-2008. Viewed January 2010, <http://www.amsa.gov.au/Marine_Environment_Protection/National_plan/Annual_Reports/AR_2007-2008/NatPlanAnrep07-08.pdf>.

Baca, B. J. and Getter, C. D. (1984). The toxicity of oil and chemically dispersed oil to the seagrass *Thalassia testudinum*. *In Oil spill chemical dispersants: research, experience and recommendations* (T. E. Allen ed.). American Society for Testing & Materials, Philadelphia (pp314-323).

Baca, B. J., Snedaker, S. C., Dodge, R. E., Knap, A. H. and Sleeter, T. D. (1996). The effects of crude oil and dispersed crude oil of tropical ecosystems: long-term seagrass, mangrove, and coral studies. *Oceans* **1**, 469-486.

Blumer, M. (1971). Scientific aspects of the oil spill problem. *Environmental Affairs* **1**, 54-73.

Brun, F. G., Hernández, I., Vergara, J. J., Peralta, G. and Pérez-Lloréns, J. L. (2002). Assessing the toxicity of ammonium pulses to the survival and growth of *Zostera noltii*. *Marine Ecology Progress Series* **225**, 177-187.

Chan, G. L. (1973). A study of the effects of the San Francisco oil spill on marine organisms. In *Proceedings of the Joint Conference on Prevention and Control of Oil Spills*, pp. 741-759. American Petroleum Institute, Washington, DC.

Cintron, G., Lugo, A. E., Marinez, R., Cintron, B. B. and Encarnacion, L. (1981). Impact of oil in the tropical marine environment. Technical Publication, Division of Marine Research, Department of Natural Resources, Puerto Rico.

Clark, J.R. and Noles, J.L. (1994). Contaminant Effects in Marine/ Estuarine Systems: Field Studies and Scaled Simulations. In: Graney, R.L., Kennedy, J.H. and Rodgers, J.H. Jr., Lewis (Eds.) *Aquatic Mesocosm Studies in Ecological Risk Assessment*. Lewis Publishers, Boca Raton, pp. 47-68.

Dean, T.A., Stekoll, M.S., Jewett, S.C., Smith, R.O. and Hose, J.E. (1998). Eelgrass (*Zostera marina* L.) in Prince William Sound, Alaska: Effects of the Exxon Valdez oil spill. *Marine Pollution Bulletin* **36**(3), pp. 201-210.

den Hartog, C. (1984). *Seagrasses of the World*. North Holland Publishing Company, Amsterdam.

den Hartog, C. and Jacobs, R. P. W. M. (1980). Effects of the Amoco Cadiz oil spill on an eelgrass community at Roscoff (France) with special reference to the mobile benthic fauna. *Helgolaender Meeresuntersuchungen* **33**, 182-191.

Department of Environment, Water, Heritage and the Arts (2010). *Australian Ramsar Wetlands*, Australian Government Department of Environment, Water, Heritage and the Arts, viewed January 2010, <http://www.environment.gov.au/cgi-bin/wetlands/alphablist.pl>.

Fingas, M. F. (2001). *The basics of oil spill cleanup 2nd edition*. (Charles, J. (Ed.)). Lewis Publishers, Washington, D.C.

Green, E. P. and Short, F. T. (2003). *World Atlas of Seagrass.* University of California Press, London, England.

Hatcher, A.I. and Larkum, A.W.D. (1982). The effects of short term exposure to Bass Strait Crude Oil and Corexit 8667 on benthic community metabolism in *Posidonia Australia* Hook. f. dominated mircrocosms. *Aquatic Botany* **12:** 219-227.

Hemminga, M. and Duarte, C. (2000). *Seagrass Ecology*. University Press, Cambridge, UK.

Howard, S., Baker, J.M. and Hiscock, K. (1989). The effects of oil and dispersants on seagrasses in Milford Haven. In: Dicks, B. (Ed.) Ecological Impacts of the Oil Industry. Pp. 61-98, John Wiley and Sons Ltd, Chichester.

Jacobs, R.P.W.M. (1988). Oil and the seagrass ecosystem of the Red Sea. Oil and Chemical Pollution **5**, pp. 21-45.

Jackson, J. B. C., Cubit, J. D., Keller, B. D., Batista, V., Burns, K., Caffery, H. M., Caldwell, R. L., Garrity, S. D., Getter, C. D., Gonzales, C., Guzman, H. M., Kaufman, K. W., Knap, A. H., Levings, S. C., Marshall, M. J., Steger, R., Thompson, R. C. and Weil, E. (1989). Ecological effects of a major oil spill on Panamanian coastal marine communities. *Science* **243**(4887), 37-44.

Kirkman, H. (1997) *Seagrasses of Australia*, Australia: State of the Environment Technical Paper Series (Estuaries and the Sea). Department of the Environment, Canberra.

Kuster, A. and Altenberger. R. (2007). Development and validation of a new fluorescence-based bioassay for aquatic macrophyte species. *Chemosphere* **67** (1), 194-201.

Larkum, A.W.D. and West, R.J. (1990). Long-term changes of seagrass meadows in Botany Bay, Australia. *Aquatic Botany* **37**, 55-70.

Leahy, J. G. and Colwell, R. R. (1990). Microbial degradation of hydrocarbons in the environment. *Microbiological Reviews*, **54**, 305-315.

Macinnis-Ng, C. M. O. and Ralph, P. J. (2003). *In situ* impact of petrochemicals on the photosynthesis of the seagrass *Zostera capricorni*. *Marine Pollution Bulletin* **46**: 1395-1407.

Mateo, M.A., Cebrián, J., Dunton, K. and Mutchler, T. (2006). Carbon Flux in Seagrass Ecosystems. In: Larkum, A.W.D., Orth, R.J. and Duarte, C.M. (Eds.), *Seagrasses: Biology, Ecology and Conservation.* Springer, Netherlands, pp. 159-192.

Nadeau, R. J. and Berquist, E. T. (1977). Effects of the March 18, 1973, oil spill near Cabo Rojo, Puerto Rico on tropical marine communities. In *Proceedings of the 1977 Oil Spill Conference*, pp. 535-538, American Petroleum Institute. Washington, DC.

National Research Council (2005). *Oil spill Dispersants. Efficacy and Effects*. National Academies Press, Washington, DC.

Pezeshki, S. R., Hester, M. W., Lin, Q. and Nyman, J. A. (2000). The effects of oil spill and clean-up on dominant US Gulf Coast marsh macrophytes: a review. *Environmental Pollution* **108**, 129-139.

Ralph, P. J. and Burchett, M. D. (1998). Impact of petrochemicals on the photosynthesis of *Halophila ovalis* using chlorophyll fluorescence. *Marine Pollution Bulletin* 36(6), 429-436.

Short, F. T. and Wylie-Echevaria, S. (2000). Global seagrass declines and effects of climate change. In: Sheppard, C. (ed) *Seas at the Millennium: An Environmental Evaluation*, Vol. 3. Elsevier Science, Amsterdam, pp 10-11.

Singer, M.M., Aurand, D., Bragin, G.E., Clark, J.R., Coelho, G.M. and Sowby, M.L. (2000). Standardization of the preparation and quantification of water-accommodated fractions of petroleum for toxicity testing. *Marine Pollution Bulletin* **40**(11), 1007-1016.

Thorhaug, A., Marcus, J. and Booker, F. (1986). Oil and dispersed oil on subtropical and tropical seagrasses in laboratory studies. *Marine Pollution Bulletin* **17** (8): 357-361.

Wolfe, M.F., Schlosser, J.A., Schwartz, G.J.B., Singaram, S., Mielbrecht, E.E., Tjeerdema, R.S. and Sowby, M.L. (1998). Influence of dispersants on the bioavailability and trophic transfer of petroleum hydrocarbons to primary levels of a marine food chain (1998). *Aquatic Toxicology* **42**, 99. 211-227.

Zieman, J.C., Orth, R., Phillips, R.C., Thayer, G. and Thorhaug, A. (1984). The effects of oil on seagrass ecosystems. In: Cairns, J. Jr. and Buikema, A. L. Jr. (Eds.) *Restoration and Management of Marine Ecosystems Impacted by Oil.* Butterworth, Boston, pp.37-64.

Further Reading

Wilson, K. G. (2010). The effects of oil and dispersed oil on subtidal seagrass: Scaling of pollution effects. PhD Thesis, University of Technology, Sydney.

Wilson, K. G. and Ralph, P. J. (2008). The effects of Tapis crude oil on subtidal *Zostera capricorni*. *Proceedings of the 2008 International Oil Spill Conference*, Savannah, Georgia.

Appendix

Chemical analysis of the water accommodated fraction

The total petroleum hydrocarbon (TPH) concentration (Σ C_6 to C_{36}) for the 1.00 % crude alone water accommodated fraction (WAF), pre-exposure, was 12 mg L^{-1}(Fig. 1). Compared to the other treatments, this TPH was quite low. However, it was comprised of approximately 80 % of highly volatile, light weight hydrocarbons in the C_6 to C_9 range, by far the highest percentage composition of C_6 to C_9 hydrocarbons in any of the treatments; crude or IFO-380. The addition of the dispersants to the crude oil increased the TPH in the WAF by almost ten-fold. The Corexit 9527 and the Ardrox 6120 dispersed oil treatments showed very similar TPH, (101 and 105 mg L^{-1}, respectively) but did differ somewhat in their composition. The TPH within the dispersant Corexit 9527 alone and Ardrox 6120 alone treatments was high, 317 and 230 mg L^{-1} respectively. The high TPH concentration in the dispersant alone treatments compared with the non-dispersed and dispersed oil treatments is likely partially due to the one hour settling time of the treatments prior to siphoning; with a resurfacing of some of the oil and dispersed oil.

Toluene was the most abundant of the BTEX hydrocarbons within the non-dispersed and dispersed crude treatments (Fig. 2). Interestingly, the composition of the BTEX hydrocarbons, albeit slightly greater in the dispersed crude treatments, was very similar to the non-dispersed crude treatment. The dispersant alone treatments showed only minimal concentrations of the BTEX hydrocarbons.

The TPH within the IFO-380 WAF treatment was low compared to all other treatments 3 mg L^{-1} (Fig. 3). Both dispersed IFO-380 treatments increased the TPH within the WAF greatly, with the Corexit 9500 dispersed treatment increasing the amount by almost 200 times greater than the non-dispersed IFO-380 treatment (Fig. 3). The TPH within the Slickgone LTSW alone and Corexit 9500 alone treatments were 150 and 167 mg L^{-1} respectively (Fig. 3).

Low levels of BTEX hydrocarbons were detected in the IFO-380 oil treatments compared to the crude oil treatments (Fig. 4). BTEX hydrocarbons represented about 20 % of the total TPH of the IFO-380 alone WAF treatment with 82 % of the BTEX total made up of toluene and xylene (Fig. 4) The concentration of BTEX components within the water accommodated fraction decreased with the addition of the dispersants, but only slightly. The dispersant alone treatments were compromised of twice as much BTEX components than their respective dispersed IFO-380 treatments.

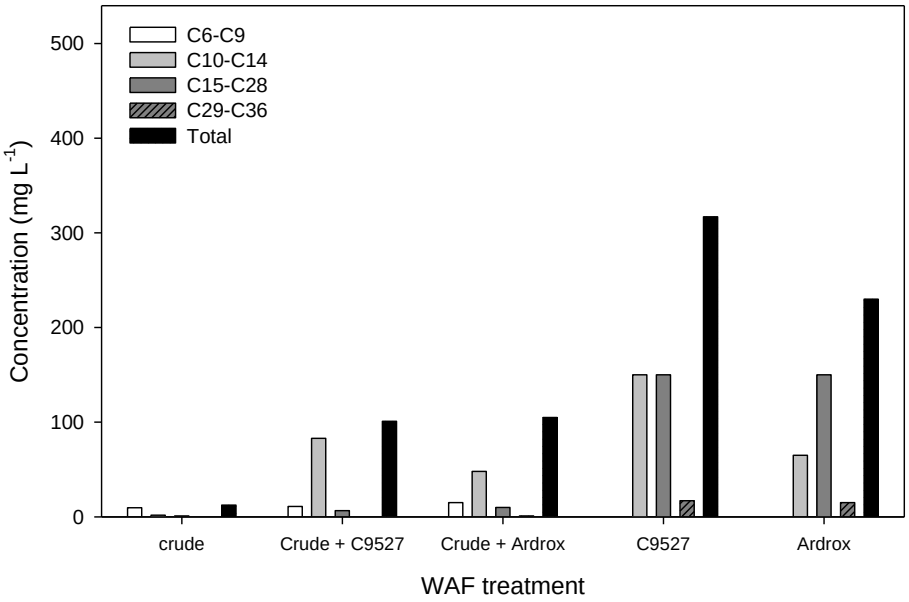

Figure 1: Carbon chain length fractionation per treatment and total petroleum hydrocarbon concentration (mg L^{-1}) within the crude, crude + Corexit 9527, Crude + Ardrox 6120, Corexit 9527 alone, Ardrox 6120 alone WAF treatments pre-exposure (*n* = 1).

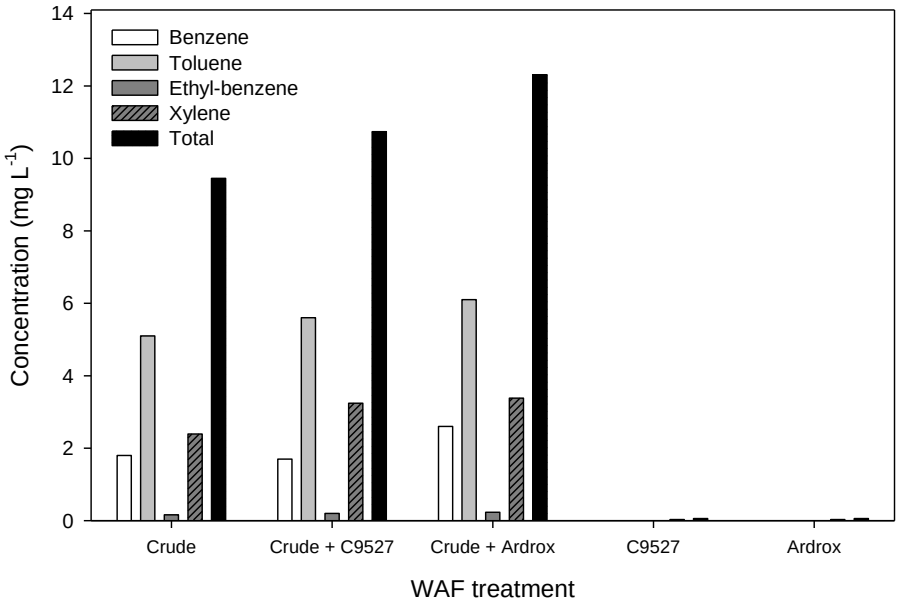

Figure 2: BTEX composition (mg L^{-1}) within the crude, crude + Corexit 9527, Crude + Ardrox 6120, Corexit 9527 alone, Ardrox 6120 alone WAF treatments pre-exposure (*n* =1).

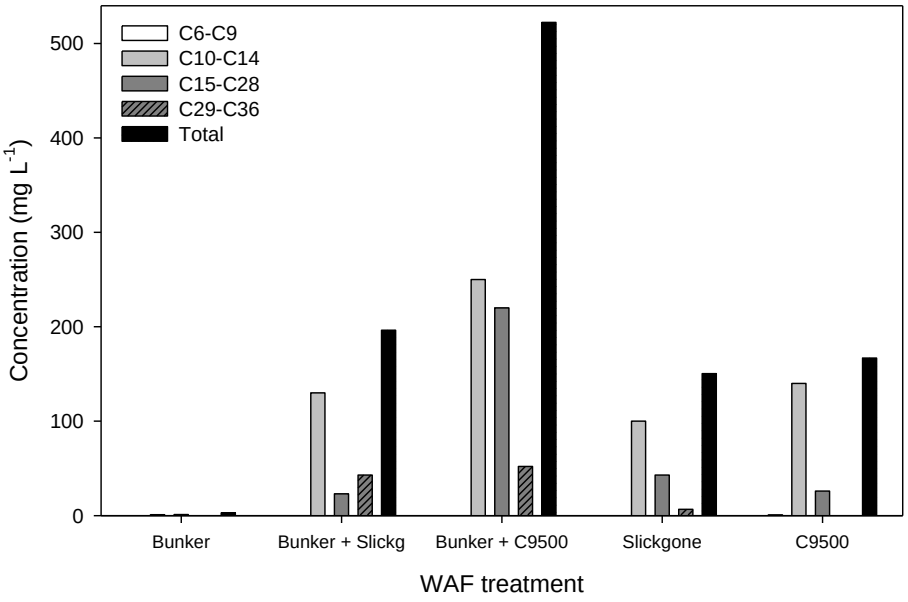

Figure 3: Carbon chain length fractionation per treatment and total petroleum hydrocarbon concentration (mg L^{-1}) within the IFO-380, IFO-380 + Slickgone LTSW, IFO-380 + Corexit 9500, Slickgone LTSW alone and Corexit 9500 alone WAF treatments pre-exposure (*n* = 1).

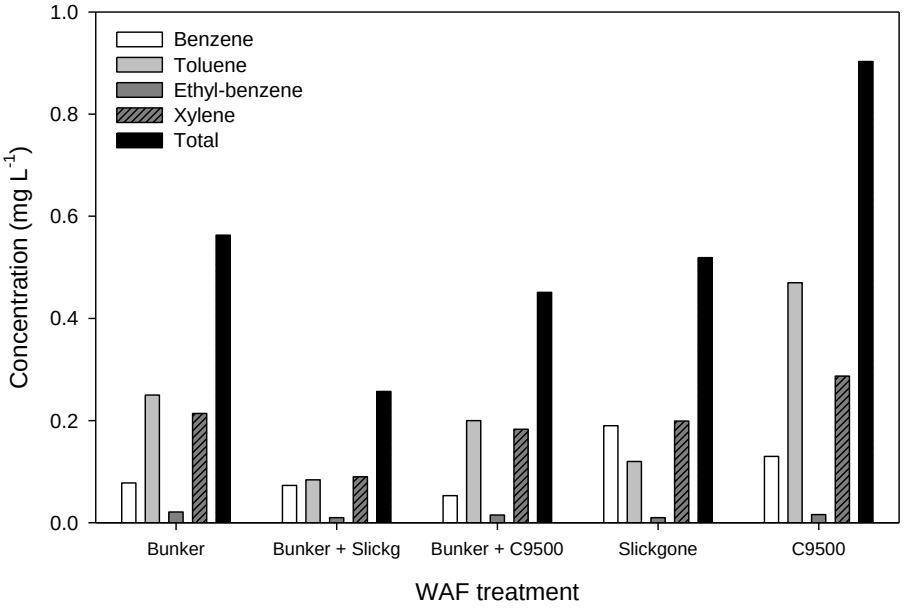

Figure 4: BTEX composition (mg L^{-1}) within the IFO-380, IFO-380 + Slickgone LTSW, IFO-380 + Corexit 9500, Slickgone LTSW alone and Corexit 9500 alone WAF treatments pre-exposure (n =1).