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# The toxic effects of petrochemicals on seagrasses. Literature review


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## 1. Abstract

Oils are derived from storage facilities, refineries, ports, harbours and storm water run off, while dispersants are used in cleanup operations. Both these petrochemicals can lead to reduced fitness of seagrasses. The primary phytotoxic effect of oil is induced by the absorption of the water soluble fraction (WSF). This causes a reduction in tolerance to other stress factors such as sublethal quantities of hydrocarbons which are incorporated into algae or macrophyte tissue. The hydrophobic nature of oil molecules allows them to concentrate in membranes of aquatic plants and hence the thylakoid membrane (an integral component of the photosynthetic apparatus) is susceptible to oil accumulation. Thus, a major symptom of oil toxicity involves (thylakoid) membrane oxidation impacting on photosynthesis. The type of oil spilled has different effects on different aquatic plants, and the use of dispersants can also cause an impact. There is an urgent need for a comprehensive Australian-based study to assess the relative toxicities of different oils and dispersants and the varying sensitivities of Australian seagrass species.

## 2. Pollution of seagrasses by non-petrochemical compounds

Ecotoxicology studies using seagrasses are rare (Lytle and Lytle, 2001) and studies of petrochemical impacts on seagrasses are largely confined to observations of spill events or physiological studies. Declines in seagrass distribution have been continuing over the last 30 years and the factors associated with seagrass death remain poorly understood (Short and Wyllie-Echeverria, 1996). In particular, the role of pollutants in seagrass degradation has received inadequate attention (Kirkman and Kirkman, 2000). The proximity of seagrasses to developed areas results in frequent exposure to a variety of pollutants (besides petrochemicals), including nutrients, heavy metals, herbicides, mixed effluents and heat pollution.

Eutrophication is seen as the cause for some of the largest and most devastating human induced disturbances to seagrasses (Hamdorf & Kirkman, 1995). The input of nutrients through effluents from sewage treatment plants, fish farming cages (Delgado et al., 1999), factories and runoff from fertilised agricultural land often reduces water quality. A further concern for seagrasses growing in polluted areas is that light attenuation in the water column will be increased and light levels for

seagrass will not be sufficient to maintain growth. Insolation, water turbidity and tidal range together determine the range of seagrass growth (Dennison & Alberte, 1985; Kenworthy & Fonseca, 1996). Epiphytic algal blooms induced by eutrophication reduce light reaching seagrass leaves, thereby decreasing photosynthesis and threatening seagrass survival (Hamdorf & Kirkman, 1995; McGlathery, 2001). As algal species are often fast-growing and have a relatively small amount of non-photosynthesising tissue to support in comparison to seagrasses, they can therefore successfully out-compete seagrasses very quickly (McGlathery, 2001). Eutrophication also causes low seagrass shoot density, low leaf area indices and low biomass (Tomasko & Lapointe, 1991; Kinney and Roman, 1998; Delgado et al., 1999), as well as altering the growth and morphology of leaves (Dawson & Dennison, 1996; Udy & Dennison, 1997), changing the chlorophyll *a:b* ratio (Lee & Dunton, 1997) and reducing standing crop (Neverauskas, 1987), thereby reducing the overall vigour of seagrasses.

Ammonium levels increased by eutrophication can be toxic to seagrasses (Brun et al., 2002). After a two week period in high ammonium concentrations (125 μM), *Zostera marina* became necrotic and after five weeks, the plant size was reduced and some plants had died, demonstrating growth inhibition (van Katwijk et al., 1997). Ammonium toxicity is one of the underlying causes of *Z. marina* loss in eutrophicated areas, furthermore, it may prevent recovery and hamper revegetation attempts (van Katwijk et al., 1997).

Siltation associated with nutrient inputs also smothers seagrass (Higginson, 1970; Hillman et al., 1990). Together with the addition of septic tank effluent and leached plant fertilisers, sedimentation has severely increased the light attenuation of the water and the content of dissolved nutrients of Tuggerah Lakes, New South Wales (Higginson, 1970), a trend common all over the world where human development surrounds lakes and estuaries (e.g. Nienhuis, 1983; Silberstein et al., 1986; Hillman et al., 1990; Batuik et al., 1992; Duarte, 1995; Olesen, 1996).

In addition to nutrients, effluent from urban and industrial areas contains a number of different compounds which have the potential to act synergistically to decrease the health of seagrasses. Effluent may be associated with increased loadings of dissolved organic carbon, poor water colour and other toxic substances, depending on the source (Neverauskas, 1987; Livingston et al., 1998). Effluent that contains organic matter of anthropogenic origin has been implicated for elevated sulfide levels

in sediments in Florida Bay: while below-ground tissue may be tolerant to high sulfide levels, in combination with high temperature and salinity, these high sulfide levels can contribute to die-back of the seagrass *Thalassia testudinum* (Koch & Erskine, 2001). As well as highlighting the impact of sediment sulfides, that study demonstrated the importance of investigating multiple stressors in finding reasons for seagrass decline.

Heated water effluent can kill seagrasses. Water heated 5°C above ambient temperatures by an electricity generation plant killed *Thalassia* in Tampa Bay Florida between 1959 and 1963 (Phillips, 1982). Thorhaug et al. (1978) suggested that heated water effluent should never be greater than 3°C above the summer water temperature to prevent stress in *Thalassia* communities. Similarly, community structure of both seagrasses and the fauna inhabiting the meadow was altered near the outfalls from thermal power stations in Lake Macquarie, NSW (Robinson, 1987).

Impacts of other contaminants associated with industrial and agricultural activity such as heavy metals and herbicides have found that current sediment and water-column concentrations are unlikely to have a physiological effect (Scarlett et al., 1999; Prange and Dennison, 2000; Macinnis-Ng and Ralph, 2002; Macinnis-Ng and Ralph 2003b). More conclusive evidence is required to discount the importance of additive impacts and multiple pulses of these contaminants (Macinnis-Ng and Ralph, 2004).

Thus, effluent and discharges as well as accidental spills and chronic exposure to every-day contaminants (such as fuel in marina areas) can affect seagrass physiology in a number of ways. Pollution inputs can disturb sediment and water quality, leading to later problems with seagrass growth (Livingston et al., 1998). Despite the adverse effects of pollution, Short & Wyllie-Echeverria (1996) noted that there was very little research into the effect of toxic compounds on seagrasses. Since this publication, more information has become available (for example, Ralph & Burchett, 1998a; Ralph & Burchett, 1998b; Scarlett et al., 1999a; Brun et al., 2002; Macinnis-Ng and Ralph, 2003; Macinnis-Ng and Ralph, 2004). Nevertheless, Kirkman & Kirkman (2000) assert more work is necessary. Moreover, Lytle & Lytle (2001) noted that toxicity testing using aquatic macrophytes remains in its infancy, further demonstrating that we do not yet fully understand the impacts of pollutants on seagrasses.

The remainder of this review focuses on the existing knowledge of the effects of petrochemicals on seagrasses, in particular the oil used as substrate for fuel synthesis, and the dispersants used to deal with inadvertent spillage of these oils.

## 3. Sources of petrochemicals

Petrochemicals (including both oils and dispersants) enter aquatic environments on a daily basis. Oils derive from storage facilities, refineries, ports, harbours and storm water run off, while dispersants are used in cleanup operations. Once dissolved in the water column, the vertical transport of emulsified oil apparently proceeds via adsorption to suspended particles which then settle to the sea floor (Ohwada et al. 2003).

### 3.1. Process of contamination by petrochemicals

Petrochemicals are introduced into the marine environment as a result of hazardous materials disposal, leakage from oil storage facilities, activities in refineries, ports and harbours and in stormwater run-off (Thorhaug 1992). Petrochemical contamination can also be through natural geological processes such as seepage (Thorhaug 1992). Since seagrasses are commonly found along the coastal fringe, they are always under threat from oil spills. Many large oil spills have been carefully monitored for long-term impacts on seagrasses, such as the *Exxon Valdez* spill (Dean et al. 1998) and the 1991 Gulf War (Burns et al. 1993) and have shown few lasting impacts.

The primary phytotoxic effect of oil is induced by the absorption of the water soluble fraction (WSF). This causes a reduction in tolerance to other stress factors such as sublethal quantities of hydrocarbons which are incorporated into algae or macrophyte tissue (Zieman et al. 1984). The hydrophobic nature of oil molecules allows them to concentrate in membranes of aquatic plants and hence the thylakoid membrane (an integral component of the photosynthetic apparatus) is susceptible to oil accumulation. Thus, (thylakoid) membrane oxidation impacting on photosynthesis is a symptom of oil toxicity (Ren et al. 1994; Marwood et al. 1999). The type of oil spilled has different effects on different aquatic plants, and the use of dispersants can also cause an impact (Thorhaug 1988).

Dispersants used to clean up oil spills can also pose a threat to seagrass both alone and in combination with the oil. Dispersants encourage the oil to spread and

increase the bioavailable fraction of oil by increasing the concentration and variety of petroleum-derived hydrocarbons in the water column (Yamada et al. 2003) and altering the interaction of these compounds with biological membranes (Wolfe et al. 1998).

### 3.2. Cases of petrochemical contamination

Investigations of recent spills (*Exxon Valdez*; Dean et al. 1998) have found no overall impact on seagrass biomass, density, flowering or seed production one year after the spill. No difference was found between oiled and reference sites. Similarly, as a result of the Gulf War (1991) vast areas of the coastline of Saudi Arabia were heavily oiled; however, one year after the initial event, the productivity of oiled and un-oiled seagrass meadows was the same (Burns et al. 1993). These are typical results for oil spills and reflect the general lack of field data to support any substantial long-term impact of petrochemical contamination. Australian seagrasses have not been properly investigated however so the conclusions should be treated with caution.

## 4. Toxic effects on seagrass

Generally, when seagrasses are exposed to petrochemicals, sub-lethal quantities are incorporated into the tissue, causing a reduction in tolerance to other stress factors (Zieman et al. 1984). Smothering of seagrass occurs when oil is stranded on areas of intertidal seagrass, leading to reduced growth rates, blackened leaves and mortality (Howard et al. 1989). This is only a problem for intertidal seagrasses. Oil appears to mainly affect flowering, and if plants are able to spread through apical meristem growth, flowering impacts are not a significant problem over the medium term for a well-established meadow (Dean et al. 1998). The *Exxon Valdez* oil spill in 1989 caused seagrass blades below oiled beaches to be bleached white and ultimately killed (Juday and Foster 1990). Another study of this spill noted a decrease in the density of leaves and flowering shoots of *Z. marina* after the event (Dean et al. 1998). Dispersants consist of a surfactant in a carrier or solvent (Hatcher and Larkum 1982). Dispersants can be toxic in their own right, but the solvent can also encourage the breakdown of the waxy cuticle, allowing greater penetration of oils into seagrass leaves and increasing phytotoxicity. A recent study showed that toxicity of biogenic surfactants was intermediate to that of synthetic surfactants (Edwards et al. 2003).

### 4.1. Ecophysiology of petrochemicals impact on seagrass

The primary phytotoxic effect of oil is induced by the absorption of the water-soluble fraction (WSF). The toxic components of petrochemicals are thought to be the poly-aromatic hydrocarbons (PAH), which are lipophilic, so they are able to pass through lipid membranes and tend to accumulate in the thylakoid membranes of the chloroplasts (Ren et al. 1994).

Some species are more sensitive to oil exposure than others (Thorhaug et al. 1986), although the reasons for this remain unclear. It is difficult to compare results from studies testing different oils and dispersants on different species (Hatcher and Larkum 1982; Thorhaug et al. 1986; Thorhaug 1988; Thorhaug, 1992; Ralph and Burchett 1998b). Any one of these variables could be solely responsible for the variations in the results, or a complex interaction of the three factors could be involved. Further controlled experiments are needed to understand the role of species sensitivity versus petrochemical toxicity.

### 4.2. Mixtures of oils and dispersants

Dispersants used to ameliorate oil spills increase the bioavailable fraction of oil by spreading petroleum derived hydrocarbons throughout the water column and altering the interaction of these compounds with biological membranes (Wolfe et al., 1998). Dispersants consist of a surfactant in a carrier or solvent (Hatcher & Larkum, 1982), which allows the toxic surfactant to penetrate the waxy protective coating of the seagrass blade, thereby further impacting on the cellular membranes and chloroplasts (Howard et al., 1989). Hatcher & Larkum (1982) found that a mixture of oil and dispersant placed more stress on a *Posidonia australis* community than oil alone, demonstrated by a decrease in photosynthetic oxygen production. The oil-treated mesocosms recovered whilst the oil- and dispersant-treated mesocosms did not. In contrast, Ralph & Burchett (1998) found that laboratory-cultured *Halophila ovalis* was reasonably tolerant of petrochemical exposure and there was little difference between exposure to oil, oil + dispersant and dispersant alone. $\Delta F/Fm'$ was shown to be a sensitive indicator of the onset of petrochemical stress and there was some degree of recovery after exposure to the petrochemicals over a five-day period (Ralph & Burchett, 1998). Macinnis-Ng and Ralph (2003) found the seagrass *Zostera capricorni* was more sensitive to oil alone than a mixture of oil and the new generation dispersant, VDC, possibly due to increased volatilisation of oil (Burridge

& Shir, 1995). Yet, a mixture of oil and dispersant can more toxic than oil alone (Hatcher and Larkum, 1982), have a similar toxicity to oil alone (Ralph and Burchett, 1998) or be less toxic than oil alone (Macinnis-Ng and Ralph, 2003). Therefore, further research is required to clarify anomalies in the influence of dispersant on oil toxicity, considering factors such as bioavailability (Wolfe et al., 1998) and volatilisation of oil (Burridge and Shir, 1995).

### 4.3. Recovery from petrochemical exposure

There are numerous examples of oil spills having no significant impact on the seagrasses after several years. One year after the Gulf War oil spill, leaf morphology and growth indicators suggested three species of seagrass remained in good health (Kenworthy et al. 1993). Dean et al. (1998) found that *Z. marina* showed little detrimental effects one year after the *Exxon Valdez* oil spill. These types of impact assessments are usually based on comparing the impacted site to a reference site, as the region is rarely surveyed before the spill. This limits the scope of interpretation of this style of investigation. Furthermore, we do not have a full understanding of the condition of seagrasses in the interim period. If they are struggling to survive, further stress such as a storm or another spill may have severe consequences. A better understanding of the physiological processes of recovery is required to ensure additional influences do not degrade the health of seagrasses.

## 5. Detecting oil toxicity with chlorophyll *a* fluorescence

During recent years there has been rapid progress in the interpretation and practical use of chlorophyll *a* fluorescence (Lichtenhaler et al., 1986; Krause & Weis, 1991; Roháček & Bartak, 1999). This has allowed much needed research into plant stress physiology, ecophysiology and phytotoxicity because it has produced quantitative, non-invasive and rapid techniques to assess photosynthesis in intact leaves, making it suitable for *in situ* measurements (van Kooten & Snel, 1990). In particular, recent seagrass studies using chlorophyll *a* fluorescence have looked at *in situ* photosynthesis (Ralph et al., 1998; Beer & Björk, 2000), depth limits (Longstaff et al., 1999; Iizumi, 2000), nitrogen impacts (Alcoverro et al., 2001), desiccation stress (Seddon, 2000) and pollution impacts (Scarlett et al., 1999a; Haynes et al., 2000b; Prange & Dennison, 2000; Ralph, 2000). Furthermone, Miles (1991) and

Brack & Frank (1998) saw great potential in using chlorophyll *a* fluorescence in plant ecotoxicology testing because it is a powerful, yet technically simple analysis.

Stress conditions can reduce the rate of photosynthesis, block photosynthetic electron transport or disturb the pigment-protein apparatus (Miles, 1991; Maxwell & Johnson, 2000). When light energy is absorbed by the antennae of a plant, some is used for photochemical reactions but a proportion is emitted as heat or fluorescence and these energy de-excitation pathways are proportional. If the functional state of the photosynthetic apparatus changes, the amount of fluorescence emitted also changes. This information can be used to quantify a stressor. Furthermore, the change in fluorescence will be different depending on which part of the photosynthetic apparatus is being influenced. This may be the absorption of light, the electron transport chain, the generation of proton-motive force or the inhibition of ATPase (Brack & Frank, 1998; Maxwell & Johnson, 2000). In this way, chlorophyll *a* fluorescence can be used to discriminate between different classes of pollutants or to determine the mechanism of toxicity.

Chlorophyll *a* fluorescence may be particularly effective for indicating oil toxicity in algae because Marwood et al. (2001) found a similarity between the impact of hydrocarbons on growth and chlorophyll *a* fluorescence responses, leading them to suggest a mechanistic link between these end points. Toxic impacts of oils were detected using Fv/Fm and ΔF/Fm' in seagrass (Ralph and Burchett, 1998; Macinnis-Ng and Ralph, 2003), freshwater macrophytes (Huang et al, 1997; Babu et al., 2001; Marwood et al., 2001), phytoplankton (Marwood et al., 1999) and corals (Jones and Heyward, 2003). Although ΔF/Fm' was the more sensitive parameter, the overall decline in both ΔF/Fm' and Fv/Fm suggest that the oil and dispersant caused the inactivation of photosystem II reaction centres due to oxidation or degradation of D1 proteins (Marwood et al., 2001).

Accepted methodologies for marine angiosperm ecotoxicological need to be developed. There are numerous endpoints which could be used to identify oil impacts. Growth has been used to assess phytotoxicity of oils in microalgae (Marwood et al. 2001) but detecting changes in seagrass growth requires much longer experiments. Enzyme production can be used as an indicator of a toxic response (MacFarlane and Burchett, 2001) but complex biochemical processes can be difficult to interpret. Oxygen evolution can be indicative of photosynthetic production but methodologies

are complicated and not well-suited to field experimentation with sufficient replication. Chlorophyll pigments appear to be insensitive to petrochemical pollution (Ralph and Burchett, 1998; Macinnis-Ng and Ralph, 2003). Protein biomarkers have the potential to indicate oxidative stress in many marine organisms (Downs et al. 2002), so should be developed for seagrass toxicology. Similarly, chlorophyll *a* fluorescence has shown promise as an indicator of toxic impacts in seagrasses. The mechanistic link between growth and chlorophyll *a* fluorescence indicates the biological significance of this end-point (Marwood et al., 2001).

### 5.1. Mode of toxicity and detection

Understanding toxicity mechanisms in seagrasses can assist in predicting the impacts of mixtures or multiple stressors. It appears that petrochemicals impact on photosystem I, not photosystem II, since initial photosynthetic activity downstream of PSII ($F_Q/F_M$) was more sensitive than Fv/Fm. Because of the intimate association between the two photosystems, the blockage in electron transport ultimately resulted in photo-oxidative stress on photosystem II (Huang et al., 1997).

ΔF/Fm' is a more sensitive indicator of photosynthetic stress caused by oil and dispersant than Fv/Fm (Macinnis-Ng and Ralph 2003), which implies non-photochemical quenching has an integral part in the light-adapted stress response (similar to Ralph & Burchett, 1998a and Marwood et al., 2001). This is supported by declining Fm and Fm' (Macinnis-Ng and Ralph, 2003) in the laboratory, particularly in the recovery period which indicates increased non-photochemical quenching in the form of heat dissipation or fewer available photosystem II reaction centres per unit area (Huang et al., 1996). In a laboratory-based experiment with aged crude oil, Fo and Ft increased, indicating incomplete oxidation of the photosystem II reaction centres (Schreiber et al., 1994) since a greater proportion of captured light is dissipated as fluorescence. Such a response is similar to that found in field experiment (Macinnis-Ng and Ralph 2003).

The response of Fm' in field experiments conducted by Macinnis-Ng and Ralph (2003) was variable and inconsistent. It was suggested that exposure to petrochemicals may have had no direct impact on electron transport around photosystem II (Marwood et al., 2001) and that the toxic effect was on photosystem I that causing back-pressure on photosystem II. Further, they observed an overall decline in both ΔF/Fm' and Fv/Fm and suggested that the inactivation of photosystem

II reaction centres that was caused by oil and dispersant was due to oxidation or degradation of D1 proteins (Marwood et al., 2001). The lack of a response (in ΔF/Fm' and Fv/Fm) in the laboratory and field oil + dispersant mixture experiments implied that the photosystem II reaction centres were performing normally (Macinnis-Ng and Ralph 2003). A recommendation arising from this study was that the use of the dispersant VDC is likely to decrease the on-going impact of oil exposure on *Z. capricornii*.

While recovery was clearly observed using fluorescence techniques, examination of pigment contents after cessation of exposure to petrochemicals is not so obvious. Recovery was not so clear when the chlorophyll pigments are considered (Macinnis-Ng and Ralph, 2003). In reduced irradiance conditions often experienced in laboratory experiments, the rate of photosynthesis may be reduced resulting in lower concentrations of pigment breakdown products being released (Karydis, 1982). In the field, however, carotenoid concentration and chlorophyll *a:b* ratio of *Z. capricornii* after exposure to aged crude oil have been observed as significantly different after the recovery period (Macinnis-Ng and Ralph, 2003). Similarly, laboratory exposures to dispersant can result in depressed chlorophyll *a* and *b* and total chlorophyll concentrations and the chlorophyll *a:b* ratio (Macinnis-Ng and Ralph, 2003), while specimens exposed to oil + dispersant in the laboratory recovered fully after 96 h. The difference between the chlorophyll *a* fluorescence and chlorophyll pigment results suggests that chlorophyll pigments vary substantially according to a variety of conditions.

Clearly there are complex factors that influence the toxicity of oil and dispersants and a combination of the two in both laboratory and field studies. One way forward is to focus on the impact of specific oils and dispersants on particular species of seagrass. Evidently, laboratory-based studies may not provide all the information we need to assess the impact of petrochemicals on marine angiosperms as there is evidence that laboratory samples (at least of *Z. capricornii*) may be more severely impacted than samples in the field.

### 5.2. Recent studies

When chlorophyll *a* fluorescence is used as an endpoint, laboratory-based studies of the photosynthetic impact of petrochemicals on seagrasss may overestimate the damage. In contrast to *in situ* exposures of *Z. marina* to aged crude oil, where the

seagrass were heavily impacted but did experience recovery, laboratory-based exposures showed reduced impacts but treated plants did not recover to their pre-exposure condition (Macinnis-Ng and Ralph, 2003). While these authors found that dispersant applied *in vivo* had a limited impact during the exposure period and clearly increased stress on the seagrasses during the recovery period, field samples were not impacted by the dispersant and the combination of oil and dispersant was less toxic than either of the two substances when alone, suggesting an antagonistic effect. This is in conflict with Thorhaug et al. (1986) who found that dispersed oil had a greater effect on the growth of *Halodule wrightii, Syringodium filiforme* and *Thalassia testudium* than oil alone. Similarly, Hatcher & Larkum (1982) found that stress levels in *Posidonia australis* were more severe when the oil was dispersed, in comparison to oil alone. Furthermore, Ralph & Burchett (1998a) found that oil + dispersant had an initial impact on ΔF/Fm' in *Halophila ovalis* but some recovery occurred after 96 hours of constant exposure to the mixture. The impact of oil + dispersant was more similar to that of dispersant alone than oil alone, and indeed, full recovery did not occur.

There are several problems with comparing results between different studies. The first is that different species are used. Thorhaug et al. (1986) (the only study to use more than one species of seagrass) demonstrated that the impact of oil is species-specific, but it is unclear whether this is a product of physiological or morphological differences. Nonetheless, these authors report that dispersed oil was more toxic than oil alone for all three seagrass species, although the toxicity occurred to different degrees. The second problem is that different oils and dispersants are often used in the different studies. Different oils are generally differently dispersed; the heavier the oil the more difficult it is to disperse (Thorhaug, 1992). Furthermore, different dispersants clearly act differently on the oil and Thorhaug (1988) found that response to different dispersant types varies greatly. The three previous studies mentioned all used a form of Corexit (Thorhaug et al. (1986) and Ralph & Burchett (1998a) both used Corexit 9527 and Hatcher & Larkum (1982) used Corexit 8667), while Macinnis-Ng and Ralph (2003) used a dispersant known as Dispersant VDC (SCOA) supplied by Shell Australia. The mixture is made up of 60% biodegradable anionic and non-ionic surfactants and 40% ethylene glycol monobutyl ether. Clearly more research is needed to clarify the impacts of different compounds and sensitivity of different seagrass species.

Lastly, the effectiveness of emulsions is reliant on temperature, where warmer temperatures generally enhance immiscibility (Thorhaug, 1992). In previous studies, temperatures have not been reported but the fact that they have been conducted in different parts of the world suggests that temperature differences may have contributed to the different results.

Petrochemicals are highly lipophilic so they tend to accumulate on organic membranes in the chloroplast which have a high lipid content (especially the thylakoid membrane) in preference to remaining in the cytoplasm (Ren et al., 1994). Greater penetration of the waxy coating (Howard et al., 1989) would lead to greater accumulation which would logically suggest that oil alone would be less toxic than oil + dispersant since penetration would be greater with the addition of dispersant, yet the conflicting results suggest there are other factors determining the overall toxicity of the mixture.

As well as allowing penetration of the waxy coating of seagrass leaves, the use of dispersants also increases the bioavailable fraction of petroleum hydrocarbons by increasing their water solubility (Wolfe et al., 1998). Therefore, an oil + dispersant mixture should be more toxic to all living organisms, however, results from a variety of studies do not necessarily support this concept. Epstein et al. (2000) found dispersed oil was dramatically more toxic than oil alone to coral larvae. Mitchell & Holdway (2000) found that Corexit + oil was less toxic to *Hydra* than oil alone but more toxic than dispersant alone. Wolfe et al. (1998) and Wolfe et al. (2001) found dispersant had no significant effect on the bioavailability and transfer of naphthalene to the rotifer *Brachionus plicatilis* and the topsmelt *Atherinops affinis*. Burridge & Shir (1995) found that germination rate of the macroalga *Phyllospora comosa* was enhanced by the addition of dispersants to crude oil and suggested this was due to increased volatilisation of solvents from the dispersant mixture, perhaps indicating that hormesis was occurring (Calabrese & Baldwin, 2002).

### 5.3. Field versus laboratory studies

Petrochemicals exposed to light become photomodified, which generally involves longer chain hydrocarbons breaking down into smaller chains. Photomidified petrochemicals can be more toxic than unaltered compounds, due to the new oxygenated compounds are usually more soluble in water than the parent compounds (Ren et al., 1994). Furthermore, photosentitisation of the organism in high light can

increase toxicity (Ren et al., 1994; Huang et al., 1997). The processes of photomodification and photosentitisation cannot be easily replicated in the laboratory and hence, field studies are particularly important for petrochemical studies.

Comparisons of the chlorophyll pigment content for dispersant and oil + dispersant obtained in laboratory and field assays have shown laboratory samples to be more sensitive than field samples, although field samples may appear to be more sensitive to oil alone (Macinnis-Ng and Ralph, 2003). The factors influencing the different responses to petrochemicals in the laboratory and the field might include light levels, contact time and the presence of bacterial infauna. Although higher light levels in the field may accelerate the degradation of oil and dispersant (rendering it less toxic), photomodified poly-aromatic hydrocarbons may in some instances be more toxic than those remaining intact (Ren et al., 1994; Huang et al., 1997). Notwithstanding this, ultraviolet radiation has been found to be the only spectral region that has induced an increase in the toxicity of petrochemicals (Ren et al., 1994).

Interpretation of the relative toxicities of oil and dispersant and their mixtures is problematic, particularly when comparing the chlorophyll *a* fluorescence data with the chlorophyll pigment data. Despite this, the dispersant laboratory chlorophyll pigment data provided in Macinnis-Ng and Ralph (2003) supports chlorophyll *a* fluorescence data by showing a delayed response to dispersant toxicity, demonstrated by a decline in chlorophyll *a*, chlorophyll *b* and total concentrations and the chlorophyll *a:b* ratio. The reason for the delay is unclear but the authors suggest it may be related to a reduced rate of photosynthesis in the laboratory due to lower light conditions (Masini et al., 1995), which may mean that the stress response took longer to take effect.

Laboratory assays are subject to a variety of confounding effects. An artefact that may arise from laboratory oil-exposure experiments is that seagrass samples may be in contact with the oil for a longer period because the slick floating on the surface of the exposure tanks is in contact with seagrass leaves (Macinnis-Ng and Ralph, 2003). An additional complication is that factors such as oil droplet size are thought to influence toxicity (Howard et al., 1989). The toxic compounds such as benzene, toluene and xylene found in oil are easily biodegraded by many marine micro-organisms (Atlas, 1995) and it is likely that populations of microbes in the field are generally larger and more active. Furthermore, abiotic limiting factors such as

molecular oxygen, phosphate and nitrogen concentration (Atlas, 1995) are likely to be more restricting in the laboratory. While these factors do not explain the reported reduced toxicity of dispersants *in situ* (Macinnis-Ng and Ralph, 2003), they may account for some of the reduction in oil toxicity.

Factors which are less likely to contribute to differences between field and laboratory derived results include exposure history and the amount of volatilisation (Burridge & Shir, 1995). There is a greater chance for gas (such as napthalene) to escape from laboratory tanks exposed to air rather than the fully submerged and enclosed field chambers.

## 6. Conclusions

Marine macrophyte ecotoxicology is an comparatively new field. For effective management of seagrasses threatened by oil spills, a comprehensive study of Australian seagrasses is needed. This study should address the sensitivity of different species to a range of oils and dispersants so effective management strategies can be developed. A mixture of laboratory and field studies would provide the most ecologically relevant data. With the tools currently available for assessment of seagrass condition, experimental studies will provide highly relevant data for confident management decisions.

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