Marine Life Information Network

Marine Biological Association of the United Kingdom

MARLIN – MARINE LIFE INFORMATION NETWORK

# SENSITIVITY ASSESSMENT OF CONTAMINANT PRESSURES

# SEAGRASSES (INC. *ZOSTERA* SPP.) – EVIDENCE REVIEW

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Authors:

H. Tyler-Walters, E. Williams, M.J. Mardle, & K.A. Lloyd

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To:

Joint Nature Conservation Committee, Defra, DAERA, Natural England, Natural Resources Wales, NatureScot, and Marine Scotland

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# 1 Seagrasses (inc. *Zostera* spp.) – Evidence review

The initial literature review (12-20th October 2021), based on the standard search strings (see section 5) returned 6964 (6004 from SCOPUS & 955 from WoS) citations on *Zostera* spp. and other seagrass genera, that is, *Cymodocea, Halodule, Halophila, Heterozostera, Phyllospadix, Posidonia, Syringodium*, and *Thalassia.* Citations on seagrasses other than *Zostera* spp. were included to increase the number of chemical contaminants covered by the review. Screenings against the inclusion/exclusion criteria (at Stages 1&2) reduced this number to 131 articles. Further examination of these articles yielded further relevant material so that 186 articles were taken forward to detailed evidence review. The more detailed reading of the articles excluded another 65 and another 25 articles could not be accessed so that 96 articles were included in the final evidence review, of which, 75 articles contained detailed evidence suitable for mapping.

The detailed evidence extracted is provided in the attached 'Seagrass Evidence Summary' spreadsheet and the supporting evidence and sensitivity assessments discussed below. Lethal and effect-based end points reported in the evidence review are summarized in Table 1.1 and all end points reported are included in the 'Seagrass Evidence Summary' spreadsheet'.

'Hydrocarbons (petrochemical)', 'Dispersants and oil mixtures', 'Herbicides', and 'Metals' were the most studied contaminants across all seagrass species reported (Figure 1.1). However, the number of results from the 'Dispersants and oil mixtures' category is skewed because of a few (8) articles two of which (Thorhaug *et al.*, 1986; Thorhaug & Marcus, 1987) examined multiple species, oil and dispersant combinations.

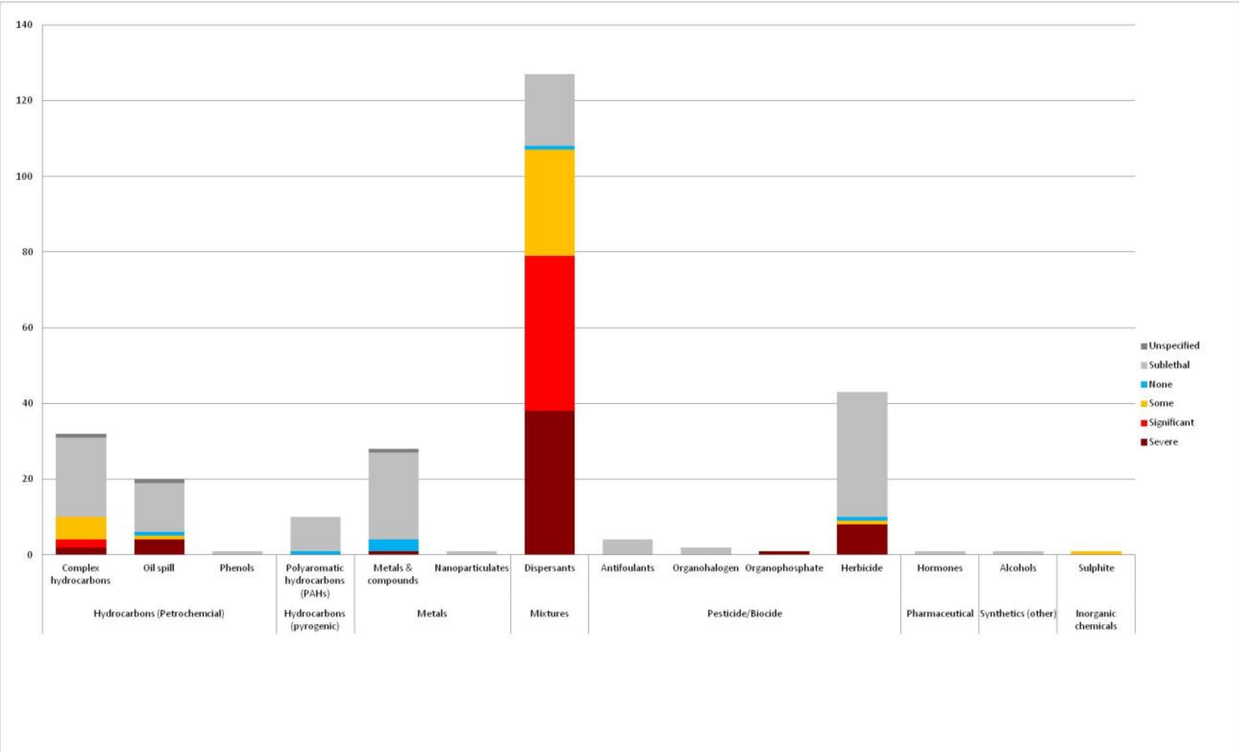


Figure 1.1. Count of ranked mortalities due to exposure to contaminants in seagrasses. Mortality is ranked as follows: 'Severe' (>75%), 'Significant' (25-75%), 'Some' (<25%), 'None' (no mortality reported), and 'Sublethal' effects. Note some articles are included more than once because they examined several different combinations of contaminant and seagrass species.

The majority of studies (69%) examined the effects of contaminants on ‘tropical’ species rather than *Zostera* spp. (31%) (Figure 1.2 and Figure 1.3). However, all species are included in the review as proxies for seagrass in general and any *Zostera* spp. specific effects identified in the text.

Table 1.1**.** Summary of lethal ‘end points’ reported in seagrass species exposed to contaminants.

| Group | Contaminant | Species name | Obs. (days) | No. doses | End point | Conc. (rpt) | Conc. (min) | Conc. (max) | Conc. (unit) | Short citation |
|---|---|---|---|---|---|---|---|---|---|---|
| Hydrocarbons (Petrochemical) | | | | | | | | | | |
| | Crude oils | *Thalassia testudinum* | 4 | 1 | $LC_{50}$ | 3.8 | | | ppm | Baca & Getter, 1984 |
| Hydrocarbon (mixtures) | | | | | | | | | | |
| | Dispersants | | | | | | | | | |
| | Corexit 9527 | *Thalassia testudinum* | 4 | 1 | $LC_{50}$ | 200 | | | ppm | Baca & Getter, 1984 |
| | Crude oils and Dispersants | *Thalassia testudinum* | 4 | 1 | $LC_{50}$ | 202.4 | | | ppm | |
| | Crude oils and dispersants | *Thalassia testudinum* | 4 | 1 | NR-LETH | 200 | | | ppm | |
| | Crude oils and dispersants | *Thalassia testudinum* | 0.5 | 1 | NR-LETH | 850 | | | ppm | |
| | Crude oils and Corexit 9527 | *Thalassia testudinum* | 4.2 | 1 | $LD_{50}$ | | 125 | | ml /100L | Thorhaug *et al.*, 1986 |
| | Crude oils and Corexit 9527 | *Halodule wrightii* | 4.2 | 1 | $LD_{50}$ | | 75 | | ml /100L | |
| | Crude oils and Corexit 9527 | *Syringodium filiforme* | 4.2 | 1 | $LD_{50}$ | | 75 | | ml /100L | |
| Metals | | | | | | | | | | |
| | Copper | *Halophila spinulosa* | 6 | 1 | NR-LETH | | | | | Prange & Dennison, 2000 |
| Pesticide/Biocide | | | | | | | | | | |
| | Atrazine | *Zostera marina* | 21 | 1 | NR-LETH | 1 | | | mg/L | Delistraty & Hershner, 1984 |
| | Atrazine | *Zostera marina* | 21 | 1 | $LC_{50}$ | 0.365 | 0.22 | 0.606 | mg/L | |
| | Atrazine | *Zostera marina* | 21 | 1 | $LC_{50}$ | 0.54 | 0.229 | 1.274 | mg/L | |
| | Atrazine | *Zostera marina* | 21 | 1 | $LC_{50}$ | 0.1 | 0.045 | 0.221 | mg/L | |
| | Atrazine | *Zostera marina* | 21 | 1 | $LC_{50}$ | 0.367 | 0.221 | 0.609 | mg/L | |
| | Atrazine | *Zostera marina* | 21 | 6 | $LC_{01}$ | 0.038 | | | mg/L | Hershner *et al.*, 1982 |
| | Atrazine | *Zostera marina* | 21 | 6 | $LC_{01}$ | 0.035 | | | mg/L | |
| | Atrazine | *Zostera marina* | 21 | 6 | $LC_{01}$ | 0.002 | | | mg/L | |
| | Atrazine | *Zostera marina* | 21 | 6 | $LC_{01}$ | 0.035 | | | mg/L | |
| | Atrazine | *Zostera marina* | 21 | 6 | $LC_{50}$ | 0.54 | | | mg/L | |
| | Atrazine | *Zostera marina* | 21 | 6 | $LC_{50}$ | 0.07 | | | mg/L | |
| | Atrazine | *Zostera marina* | 21 | 6 | $LC_{50}$ | 0.367 | | | mg/L | |
| | Atrazine | *Zostera marina* | 21 | 6 | $LC_{50}$ | 0.365 | | | mg/L | |
| | Atrazine | *Zostera marina* | 21 | 6 | $LC_{50}$ | 0.1 | | | mg/L | |
| | Atrazine | *Zostera marina* | 21 | 6 | NR-LETH | 1 | | | mg/L | |
| | Atrazine | *Zostera marina* | 21 | 6 | NR-LETH | 1 | | | mg/L | |
| | Atrazine | *Zostera marina* | 21 | 6 | NR-LETH | | 1.04 | 1.07 | mg/L | |

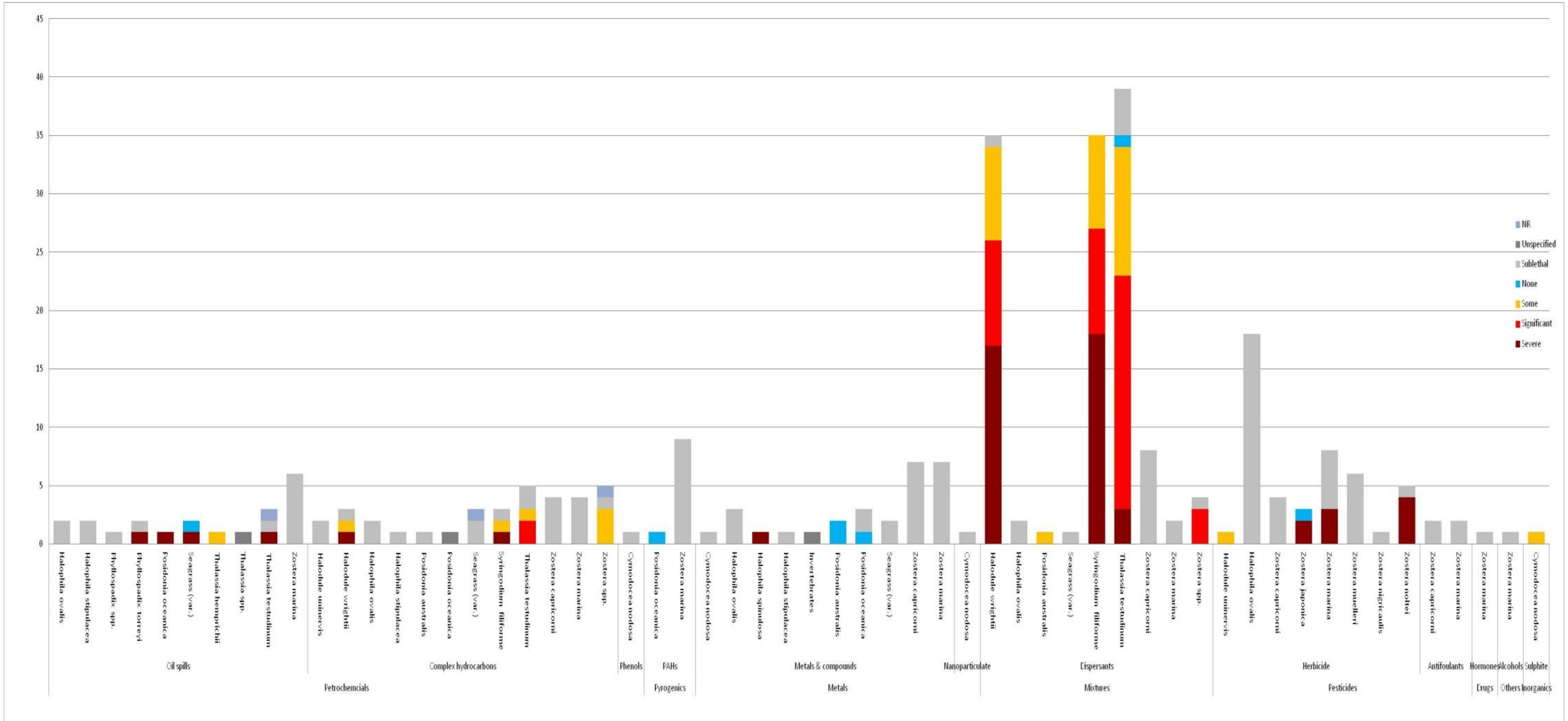


Figure 1.2. Count of ranked mortalities due to exposure to contaminants in seagrass species. Mortality is ranked as follows: 'Severe' (>75%), 'Significant' (25-75%), 'Some' (<25%), 'None' (no mortality reported), and 'Sublethal' effects. Note some articles are included more than once because they examined several different combinations of contaminant type and seagrass species.

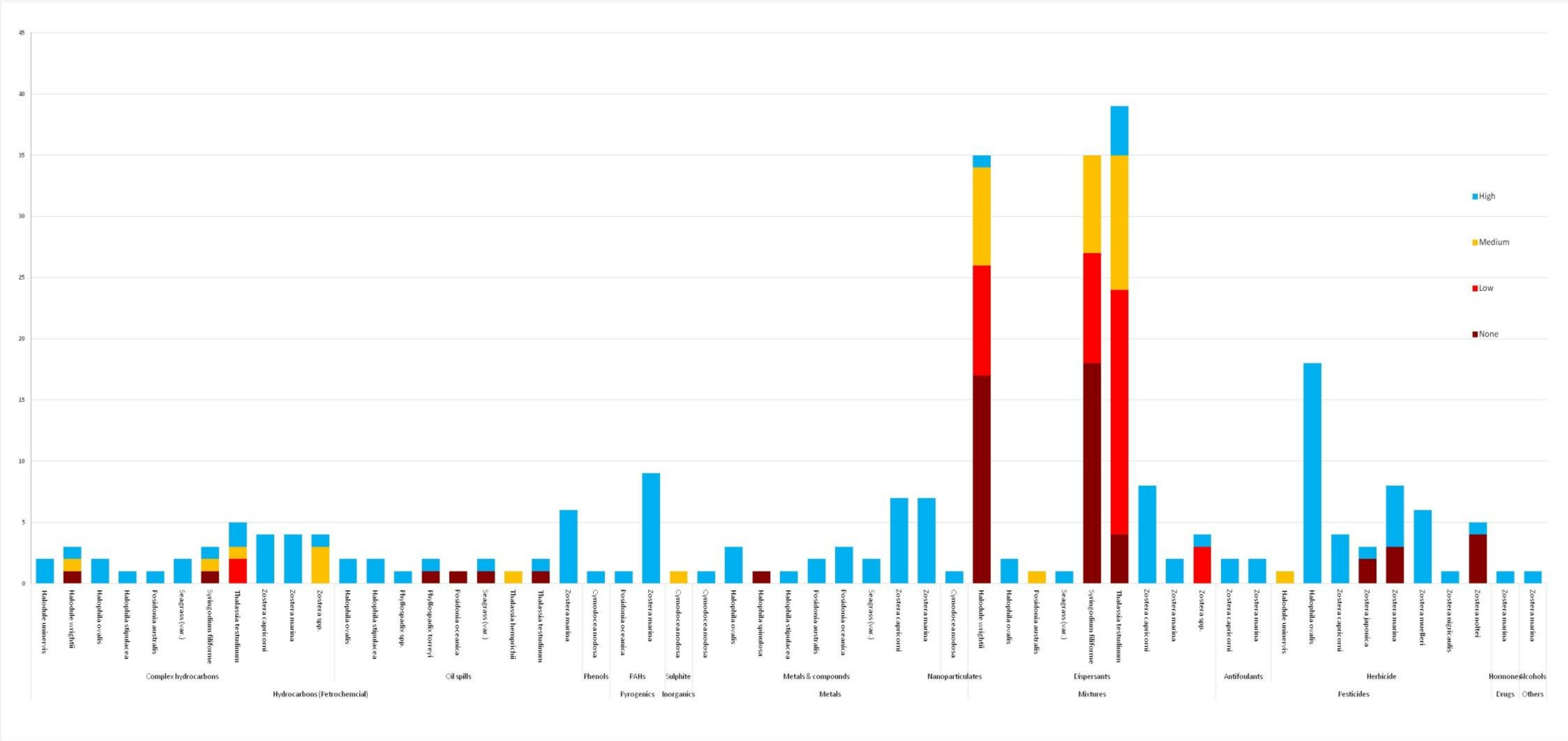


Figure 1.3. Count of ranked mortalities due to exposure to contaminants in seagrass species. Mortality is ranked by resistance as follows: 'None' (>75%), 'Low' (25-75%), 'Medium' (<25%), and 'High' (no mortality or only sublethal effects reported). Note some articles are included more than once because they examined several different combinations of contaminant type and seagrass species.

## 1.1 Seagrasses – Hydrocarbons and PAHs

The hydrocarbon evidence review examined the exposure to oil (crude oil, fuel oil and diesel oil), dispersants, dispersed oil (oil and dispersant mixture), the water accommodating fraction (WAF) and water soluble fraction (WSF). The effects of the exposure of seagrass species to hydrocarbons, PAHs, and dispersants was examined in 42 articles. Petrochemicals were the most examined group with 36 articles on the effects of both oil spills (17 articles) and experimental exposure (19 articles). Only 12 articles examined for dispersants and dispersed oil mixtures, however, this group reported the most results overall (67%). 'Hydrocarbons' and 'dispersants' were reported to cause a 'lethal' response in 64.2% of examined results (Figure 1.4) and 'severe' mortality was reported in 23.2% of cases. Mortality was sometimes unclear or not mentioned in the examined studies and the remaining 33.1% of results, reported or examined sublethal effects only.

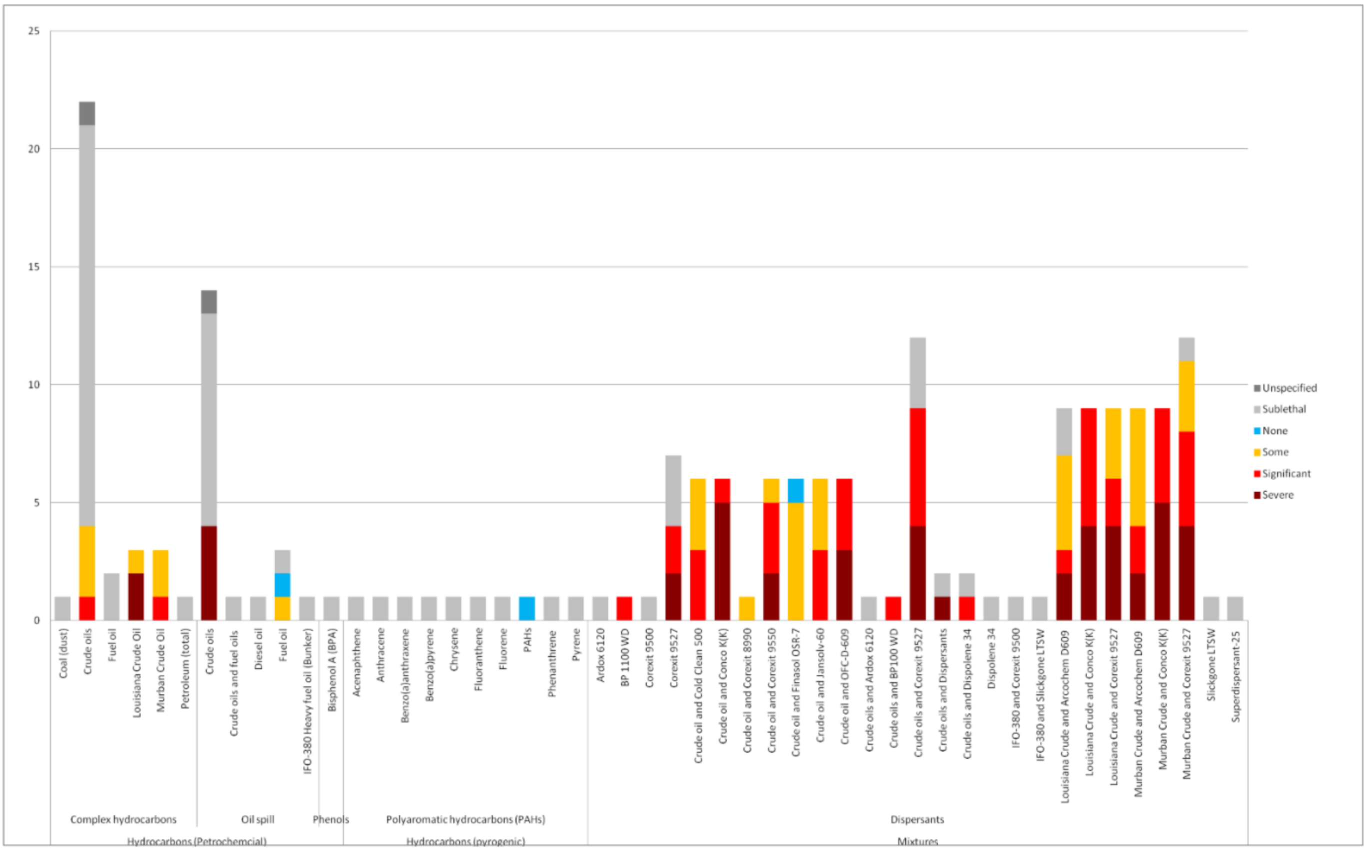


Figure 1.4. Count of ranked mortalities in seagrasses (across all species examined) due to exposure to hydrocarbons, dispersants, and dispersant/oil mixtures. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects. Note some articles are included more than once because they examined several different combinations of contaminant type and seagrass species.

The effects of crude oil were the most reported with 23.3% of results on seagrass, of which 33.3% were from oil spills and 66.6% were from experimental exposure to crude oil. Of these papers, 14.2% of reported mortality as 'severe' and 61.9% reported sublethal effects. Dispersed oil caused the greatest mortalities to seagrass. A lethal response was reported in 80.1% of dispersed oil treatments and 29.8% resulted in 'severe' mortality. Lethal effects were seen in 38.5% of the treatments where seagrass was exposed to dispersants alone. All other dispersants recorded only sublethal effects (61.5%). No mortality was reported due to the exposure to PAHs or Phenols.

Lethal effects were reported in 65.2% of the examined species after exposure to complex hydrocarbons and dispersant/oil mixtures (Figure 1.5 and Figure 1.6).

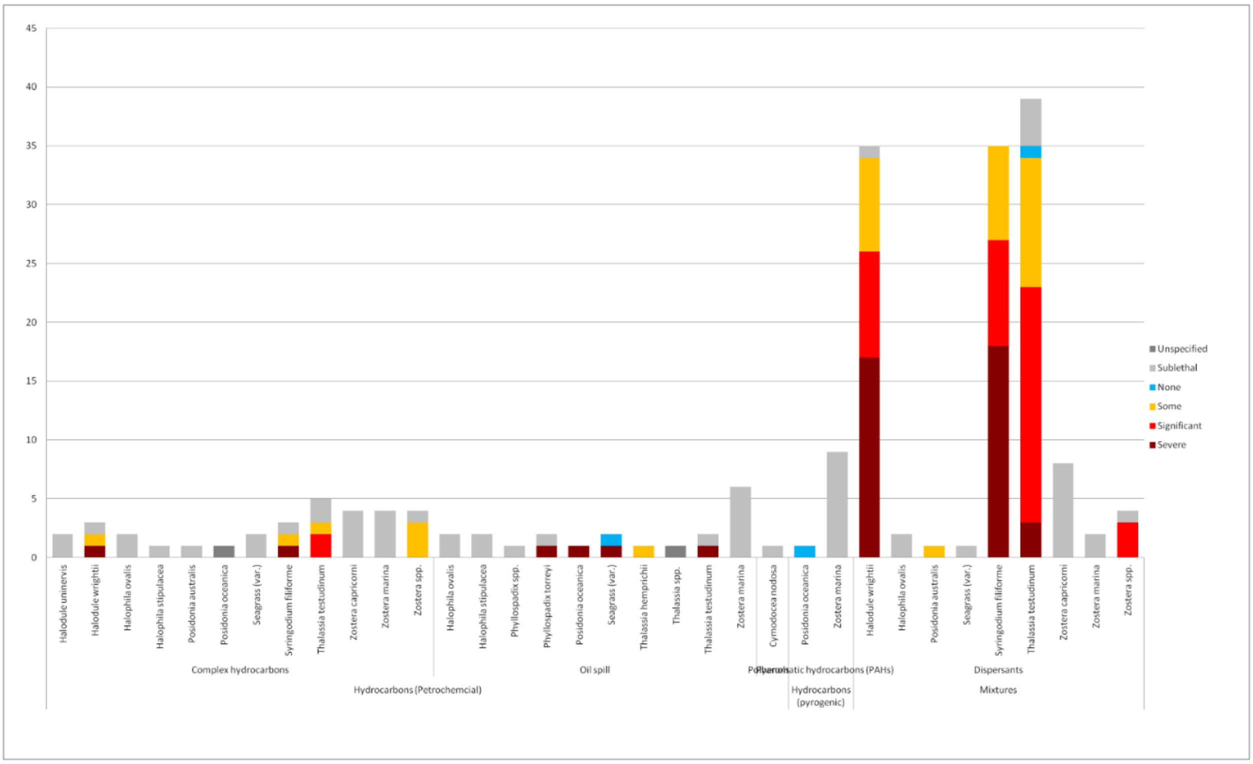


Figure 1.5. Count of ranked mortalities due to exposure to hydrocarbons in seagrass species. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects. Note some articles are included more than once because they examined several different combinations of contaminant type and seagrass species.

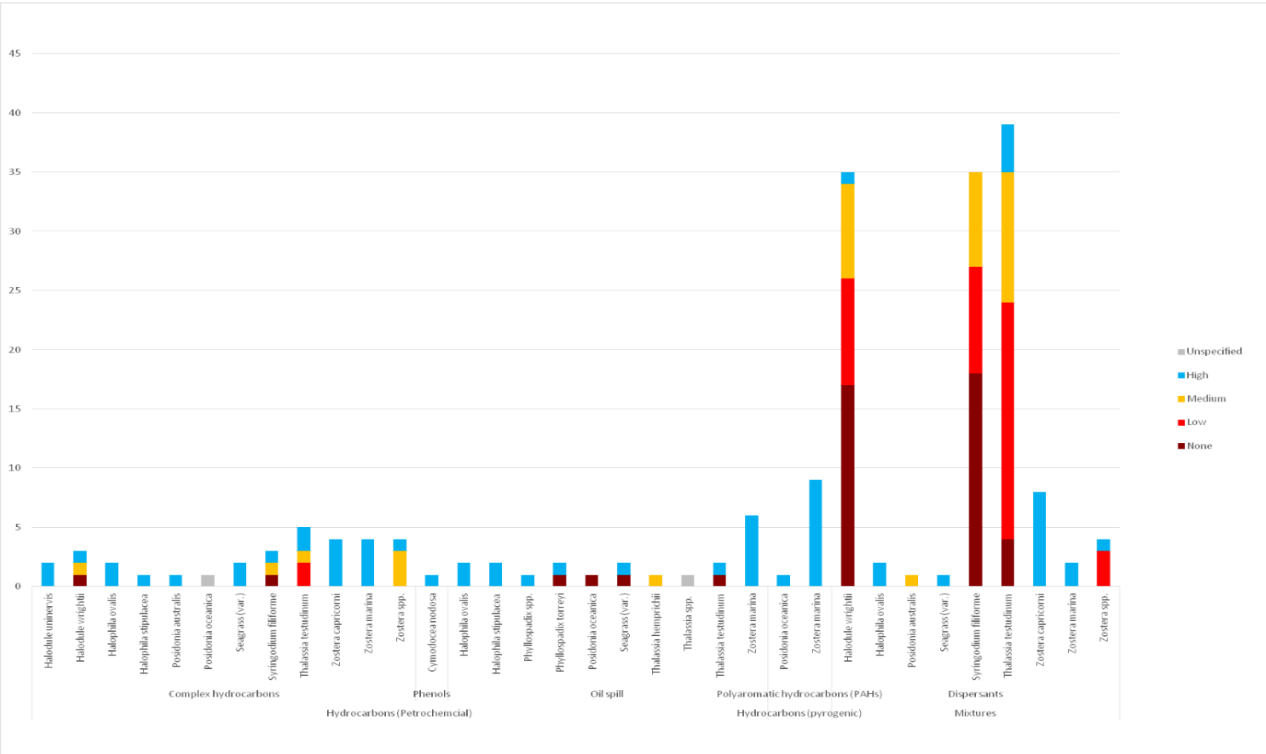


Figure 1.6. Count of ranked mortalities (expressed as resistance) due to exposure to hydrocarbons in seagrass species. Resistance is ranked as follows: 'None' (>75%), 'Low' (25-75%), 'Medium' (<25%), 'High' (no mortality and/or only sublethal effects reported). Note some articles are included more than once because they examined several different combinations of contaminant type and seagrass species.

Of these species, 'Severe' mortality was reported in five of the examined species: *Halodule wrightii*, *Syringodium filiforme*, *Thalassia testudinum*, *Phyllospadix torreyii,* and *Posidonia oceanica*.

Overall, *Halodule wrightii* and *Syringodium filiforme* had the most reported 'severe' cases of mortalities caused by exposure to oil and dispersed oil, which were found to be toxic to these species. Across all studies, 9.47% of 'severe' mortalities were *Halodule wrightii* and 10% of 'severe' mortalities were *Syringodium filiforme*.

### *1.1.1 Oil spills*

In the 14 articles that recorded the effects of oil spills on seagrasses, lethal effects were reported in five papers. Four species of seagrass were reported to experience 'severe' mortality after exposure to crude oil and one species experienced 'some' mortality after exposure to crude oil. The remaining nine papers only reported the range of sublethal effects.

***Amoco Cadiz* 1978 (Crude oil and Bunker fuel).** Den Hartog & Jacobs (1980) examined the *Amoco Cadiz* oil spill off the coast of Brittany, France on the seagrass community in Roscoff, France. The spill released 216,000 tons of crude oil and 4,000 tons of bunker fuel creating a slick that covered portions of well-studied *Zostera marina* beds in Roscoff. The eelgrass showed little impact from the oil spill. However, in the weeks after the oil spill there was evidence of blackening/burnt leaves that were lost. The loss of leaves was part of the normal seasonal pattern of the species but was accelerated due to the oil. Only these short-term effects were observed and the general structure of the seagrass remained normal. Species of Cumacae, Tanaidacea, and Echinodermata found in the seagrass habitat were reduced by the impacts of the oil spill but recovered within a year, although 21 species of Amphipoda were lost. However, the flat intertidal seagrass did provide a buffer between the oil slick and the substratum, which may explain why only the Amphipoda and some Polychaeta were affected. The seagrass buffer effect becomes evident when the invertebrate mortality was compared to those seen in open exposed beaches where the mortality was significantly greater. Den Hartog & Jacobs (1980) noted that the effects of the oil spill were more likely to damage the associated communities than the seagrass itself

Jacobs (1980) examined the effects of the *Amoco Cadiz* oil spill on a community of *Zostera marina* at Roscoff. The benthic fauna of *Zostera marina* community was investigated from October 1977 to April 1979. The oil spill occurred in March 1978. The direct effects on eelgrass were only seen locally and temporarily in the first weeks after the spill when many plants had blacked/burnt leaves. The spill resulted in a change of the faunal composition, with a decrease seen in many species including some herbivores. The lack of herbivores led to a greater algal bloom than seen in previous years and only recovered once the herbivores returned. Jacobs (1980) concluded that *Zostera marina* showed only a temporary decreased condition of the leaves because of the oil spill. In addition, all species recovered in the habitat within a year, apart from amphipods.

***Cosco Busan* 2007 (Bunker fuel oil).** *Cosco Busan* Oil Spill Trustees (2012) assessed the damage of the *Cosco Busan* oil spill on the seagrass species *Zostera marina* in San Francisco Bay. Several sites were assessed throughout the bay. Side-scan sonar surveys were used to measure the seagrass beds. Measurements of photosynthetic activity, rhizome node production, and phenolic compound analysis were conducted. The results for all the tests were inconclusive for impacts specific to oiled beds vs. un-oiled beds. Their results suggested that there was little impact on the seagrass habitat in this area despite the seagrass being oiled. There was no report of mortality or any significant impacts on the seagrass.

***Deepwater Horizon* 2010 (Crude oil).** *Deepwater Horizon* Natural Resource Damage Assessment Trustees (2016) reviewed the ecosystem effects of the *Deepwater Horizon* oil spill on the surrounding environment using the information from Constentio-Manning (2015; not seen) report on the effects of the *Deepwater Horizon* spill on seagrass beds. The *Deepwater Horizon* oil spill damage report stated that 109 hectares (1.09 $km^2$) of seagrass beds were destroyed in the Chandeleur Islands. There was a reduction in seagrass measured in five areas of the Chandeleur Islands following the oil exposure. Persistent loss, classified by the absence of seagrass for two persistent years of monitoring, was identified as 112 acres of seagrass beds. Delayed loss, classified by loss of the seagrass beds a year or more after the oil spill, resulted in 150 acres of loss. A total loss of 271 acres of seagrass beds was reported over the two-year monitoring period. They estimated recovery time for this area to be 1 to 10 years.

***Exxon Valdez* 1989 (Crude oil).** Dean *et al.* (1998) examined the effects of the *Exxon Valdez* Oil Spill on the seagrass *species Zostera marina* in Alaska from 1990 to 1995. Samples were collected along 30 m transects adjacent to the coast. The density of *Zostera marina* shoots, blades, flowering shoots were lower at the oiled sites than the reference sites. Flowering shoots were twice as dense at the reference sites than the oiled sites and, at one of the oiled sites, there were no flowers in the sampled quadrats (62% lower at oiled sites). Mean shoot densities were 24% lower at oiled sites than at the reference sites. There were no differences between the oiled and references sites for the above ground biomass of the seagrass and the mean seed densities did not differ either. The seed germination rates were higher at one of the oiled sites but the seeds these seedlings produced had higher mitosis abnormalities than the reference sites. There was no evidence of mortalities found at the seagrass sites and the lack of flowers did not show any long terms effects on the population. Overall, the injury to the seagrass was slight and did not persist for longer than a year after the spill when the hydrocarbon levels decreased. The lower densities and inflorescence at oiled sites were associated with the higher levels of hydrocarbons in the sediment.

***Haven* Oil spill 1991 (Crude oil).** Peirano *et al.* (2005) examined the effect of climate, invasive species, and anthropogenic impacts on the growth of *Posidonia oceanica*. They investigated the effects of the *Haven* oil spill (April 1991), near Genoa, Italy, on the local seagrass meadows. In the experiment, they found that none of the rhizomes was older than eight years old while in another location the seagrass had a maximum age of 19 years old. This meant that there were high mortality rates caused by the deposition of oil and sediment residue leading to suffocation. The impact was limited to time and space and only affected the one seagrass bed, nearest to the spill. Overall, the oil caused a large mortality event in the seagrass meadow. However, the Peirano *et al.* (2005) indicated that the seagrass meadow was able to recover.

Sandulli (1994) also investigated the conditions of *Posidonia oceanica* meadows after the *Haven* oil spill. ROV line transects were used to evaluate the macrostructural characteristics of the meadows as well as measure the percentage cover, density and leaf area index. General degradation was seen due to anthropogenic pressures, presumably preceding the *Haven* oil spill, and was common to all the meadows studied. The only signs of contamination directly related to the presence of oil residues were observed in the Arenzano meadow, at about 10 m in depth where there was a large area of the dead and vegetated matte area.

**San Francisco Bay 1971 (Bunker Fuel oil).** Chan *et al*. (1973) examined the effects of a tanker collision causing 840,000 gallons of Bunker C fuel to be spilt into San Francisco Bay. The marine communities in the area were examined including the seagrass species *Phyllospadix scouleri*. Pre-oil and post-oil observations were compared on the Duxbury reef to assess the impact of the oil spill on the marine organisms. Ten metre transects were taken, assigned a percentage of oiling and assessed. The tide pool examined was found to be saturated with oil causing the outer tips of the seagrass blades to die. However, after the spill the growth throughout the spring and summer was normal. In the late summer period, algal growth (*Urospora penicilliformis*) appeared to be heavier than in previous years after the oiling, which may have been a result of the mortality of grazers. Overall, there was little impact on the seagrass community and no reported mortality.

**Santa Barbara 1969 (Crude oil).** Foster *et al*. (1971b) examined the impact and effects of the oil spill in the Santa Barbara Channel on intertidal organisms, including the seagrass species *Phyllospadix torreyi*. Seagrass in the intertidal was heavily coated with oil, which took up, held the oil, and caused the blades to stick together. The exposed parts of the plants that had been oiled turn brown and disintegrated. They recorded that 50-100% of the exposed blades in transects were damaged in the intertidal areas affected by the oil spill. This varied from 30-50% at one site, 50-60% at another site, and the highest leaf mortality being 90-100% of the exposed blades. The subtidal and extremely low intertidal plants were relatively undamaged, as they did not come into direct contact with the oil. Foster *et al.* (1971b) concluded that the intertidal population of the seagrass was affected by the oil spill but that damage should not be long-term and the seagrass had the potential to recover quickly.

**Panama 1986 (Crude oil).** Jackson *et al*. (1989) examined the effect of two consecutive oil spills to the east of the Caribbean entrance to the Panama Canal, with 3.2 million litres of crude oil spilled from the *Witwater* wreck and at least 8 million litres of crude oil spilled from a ruptured storage tank. Community structure and mortality of the intertidal and subtidal seagrass meadows of *Thalassia testudinum* were examined around the coast of Isla Largo Remo. In heavily oiled intertidal reef flats, there was up to 100% mortality of seagrass, shown by the oil-covered dead leaves and dead but intact rhizome mats, which washed onto the shore. However, the subtidal seagrass survived in all locations affected by the spill, despite the oil turning the leaves brown and the seagrass being heavily fouled by algae for several months after the spill in areas of heavy oiling. One cause of the subtidal damage to the seagrass may have been due to the small amount of dispersant used on the oil causing the hydrocarbons to mix into the water. Four taxonomic groups of invertebrates in the seagrass communities were also affected by the spill. There was a significant decrease in the numbers of amphipods, tanaids, brachyurans, and ophiuroids. However, bivalves and gastropods showed no differences in abundance before and after the spill.

**Gulf War 1991 (Crude oil).** Kenworthy *et al*. (1993) examined the distribution, species composition, abundance, and productivity of seagrass in oil-contaminated bays along the northeastern coast of Saudi Arabia approximately one year after the Gulf War oil spills. During the Gulf War it is estimated that 0.5 to 8 million barrels of oil were released into the Gulf washing onto the embayments of Ras Tanequib, Dawhat al Musallamiya and Dawhat ad Daft. Two approaches were used to examine the impact of oils: a gradient study comparing inshore (oiled) and offshore (non-oiled) sits, and a comparative study using either the same species in these locations and other locations in the Gulf or other ecologically important seagrass species in other locations not immediately affected by oil. The Braun-Blanquet (1965) scales were used to demonstrate the impact on the frequency, density and abundance of the oiled and non-oiled sites for *Halodule unnerves* at the Dawhat ad Dafi and Dawhat al-Musallamiya sites. The biomass of *Halodule ovalis* at the oiled Jinnah Island site (34 g dwt/m$^2$) was similar to the non-oiled outer bay at al Musallamiya (39 g dwt/m$^2$). Specific leaf productivity for *Halodule uninervis* in a heavily oiled shallow site was a range of 0.94-0.250 g dwt/m$^2$/day or an average yield of 2.2%/day, which was similar to other reported rates for healthy populations of *Halodule* species. Heavily oiled inner and mid bays showed leaf densities between 1,530 and 2,533 leaf pairs per square metre for *Halodule ovalis*, which was similar in *Halodule stipulacea*. Leaf morphology and indicators of vegetative growth suggested that all three species were healthy, despite the recent history of oiling. Three of the four seagrass species known in the Gulf were growing in the heavily oiled embayments from the Gulf War. Therefore, Kenworthy *et al*. (1993) concluded that the seagrass along the north coast of Saudi Arabia was not experiencing long-term degradation or damage one year after the Gulf War oil spills.

**Taklong Island National marine reserve 2006 (Bunker fuel oil).** Nievales (2008) examined the changes to the mixed seagrass meadows dominated *Thalassia hemprichii* (but composed of *Thalassia hemprichii, Enhalus acoroides*, *Cymodocea rotundata*, *Cymodocea serrulata*, *Syringodium isoetifolium*, *Halophila ovalis*, *Halodule uninervis* and *Halodule pinifolia*) in Taklong Island National marine reserve after an oil spill. The shoreline at chosen sample site had heavy oiling for at least three weeks after the spill whereas the control site had no observed oil present following the spill. The assessment of the seagrass included percentage cover, species composition, blade density per species, shoot density and above ground biomass, which were recorded using three 50 m transects parallel to the shore. There was no account of the seagrass meadows being smothered or covered with oil. However, the spill did result in a decrease in the percentage cover of the seagrass from 28.2% pre-spill to 18.6% a year after, decreasing to 15% two years after the spill. Seagrass cover ranged from 11% to 29% at the oiled site and 18% to 38% at the non-oiled site. The above ground biomass of the seagrass recorded at the oiled site was consistently lower than the non-oiled site for 10 months after the spill. The biomass ranged from 21 to 120 g dwt/$m^2$ at the oiled site and 34 to 164 g dwt/$m^2$ at the non-oiled site. Blade density was also less at the oiled site. However, this was only short-term and recovered within one year of the spill. The shoot densities showed a 30% reduction at the oiled site and ranged from 156 to 491 shoots/$m^2$ at the oiled site and 220 to 538 shoots/$m^2$ at the non-oiled site. Nievales (2008) concluded that oil had an overall negative impact on the seagrass meadows, which was apparent within a year of the oil spill. The lowered biomass and percentage cover then persisted after two years of monitoring.

**Port of Gladstone 2010 (Fuel oil).** Taylor & Rasheed (2011) examined the effects of a small (25 tonnes) heavy fuel oil spill in the Port of Gladstone in Jan 2006, on seagrass meadows (mixed *Zostera capricorni*, *Halodule uninervis,* and *Halophila spp*.). The seagrass meadows were subject to a long-term monitoring program. They concluded that the oil spills did not affect the seagrass meadows significantly. Initial declines in seagrass biomass in the first month were mirrored by unaffected beds in the area and probably due to other climatic and human effects. The lack of effect was probably because the spill occurred at high neap tide so that the seagrass was not directly exposed until 2-3 days later when most of the volatile (and presumably most toxic) components had evaporated.

**Cabo Rojo 1973 (Crude oil).** Nadeau & Bergquist (1977) examined the effects of the March 1973 oil spill near Cabo Rojo, Puerto Rico on tropical marine communities, including *Thalassia* communities. The tanker spilled 37,000 barrels of Venezuelan crude oil into the coastal waters and 24,000 bbl (barrels of oil) of oil washed ashore at Cabo Rojo, contaminating sandy beaches, turtle grass, and rocky shore communities. The oil spill caused mortality in the seagrass community, killing both invertebrates and the seagrass. The subtidal community became exposed to oil entrained into the water column by surf action, which caused the leaves to become brown or black. *Thalassia* died and was removed by wave action, and led to the exposure of extensive areas of denuded vegetation and rhizome matrix. However, the scale of the mortality was not quantified by the authors. In January 1974, year after the oil spill, the *Thalassia* has begun to grow and by 1976, the *Thalassia* flats had renewed plant growth with coral-sand deposition. The invertebrate population had also declined in the seagrass beds. There was a lower diversity and abundance at the oiled *Thalassia* beds compared to the control with a reduction in sea urchins, chitons, and hermit crabs. Dead and moribund invertebrates were observed on the shoreline adjacent to the *Thalassia* seagrass beds. However, these species had recovered by the subsequent visits in 1974 and 1976.

**Moyia Bay 1998 (diesel oil).** Gab-Alla (2001) examined the effects of diesel oil pollution on *Halophila stipulacea* in the Sharm E, Moyia Bay in the Red Sea. The total biomass of the seagrass (g dwt/$m^2$), and density (a modified Braun-Blanquet (1965) cover-abundance scale) were examined. The samples were obtained using 0.25 $m^2$ quadrats at randomly selected samples at each site. Three oiled sites and five non-oiled sites were compared. The best comparison between sites was site 1 (non-oiled) and site 2 (oiled) due to a lower density and abundance found at other non-oiled sites caused by other environmental conditions in the bay. The percentage cover between sites 1 and 2 for percentage cover, shoot density, and biomass for the seagrass were not significantly different. The Braun- Blanquet (1965) cover-abundance scale showed that the three oiled areas and site 1, the non-oiled area, had the highest frequency, abundance, and density of seagrass. Gab-Alla (2001) concluded that the results of the cover and sexual growth in the plants showed that the seagrass plants in the oiled area remain healthy. However, the oil spill did adversely affect the invertebrate population inhabiting the seagrass with a decrease from 21 species at non-oiled sites to seven species at oiled sites.

### *1.1.2 Petroleum hydrocarbons*

Only four of the articles examined provided details of $LC_{50}$, $EC_{50,}$ or NOEC values based on laboratory studies. The lethal and sublethal effects of petroleum oils (e.g. crude oil and fuel/Bunker oils), dispersed oil, and dispersants are *summarized* below.

- Baca & Getter (1984) examined the effects of crude oil and dispersants in the laboratory on the seagrass *Thalassia testudinum*. Laboratory static bioassay experiments were used to assess the potential damage that could be caused by an oil spill in tropical waters. They used a 12-hour single-dose experiment that mimicked the natural system of tides washing the seagrass and a 96-hour experiment to assess the 96-hour $LC_{50}$. The dispersed oil was prepared in a 1:10 dispersant/oil solution and the mortality of a plant was recorded as the degradation of the meristem as seagrasses cannot recover from this. The Prudhoe Bay WSF resulted in a greater toxic effect than the dispersed oil, most likely because it contains large components (88%) of Benzene, toluene and C-2 benzene. The WSF oil had a lethal concentration of 3.8 ppm, which was the lowest of all the treatments. Despite the addition of dispersants to the oil, increasing the concentration of hydrocarbons in the water by 50 times, the dispersed oil had a higher lethal concentration of 202.4 ppm. The 12-hour treatments show that the same lethal concentrations after 96-hour exposure were sublethal if the treatment was flushed after 12 hours instead. Exposure to the 96-hour treatment resulted in mortality and sublethal effects in survivors after seven days of monitoring. In the dispersed oil treatment (measured concentration of 177 ppm), 60% of plants that had survived the seven-day exposure had yellow leaves, 8% had brown leaves, and 32% still had green leaves. The WSF oil treatment (3.8 ppm) had 28% yellow leaves, 12% brown leaves, and 60% green leaves and the dispersant-only treatment (200 ppm) had 0% yellow leaves, 70% brown leaves, and 30% green leaves. After 14 days of observation, some of these plants went on to die. Baca & Getter (1984) noted that, dieback and bleaching occur due to the intrusion of relatively fresh submerged oil and the toxic effects diminish as the oil weathers.
- Baca *et al.* (1996) examined the effects of a worst-case scenario exposure of Prude bay crude oil and dispersed oil on the short and long-term survival, abundance, and growth of seagrass *Thalassia testudinum*. After application, the sites were monitored constantly for 24 hours, visited periodically over two years and then again 10 years after the first exposure. The growth rates of seagrass showed considerable variation between sites exposed to oil, dispersed oil and the control site. After exposure to oil the growth rate of the seagrass had decreased in the month after exposure, however, recovery was seen within a year. There was however little effect on the seagrass growth rate after exposure to dispersed oil, with values continuing to increase after exposure. Despite variation being recorded there were no significant differences recorded in plant density in post-treatment and pre-treatment values for either the oil or dispersed oil. The effects to the seagrass were only minor with little long-term effects on the seagrass and the associated organisms after the oil treatment. Baca *et al.* (1996) did not quantify seagrass loss or mortality. Populations of sea urchins, *Echinometra lacunter* and *Lytechinus variegatus*, were counted using the line intercept method. Both species were affected by treatments with crude oil and dispersed crude oil. The population of *Echinometra lacunter* was reduced to less than half and virtually disappeared at the dispersed oil treatment site within the next 30 days. The population of *Lytechinus variegatus* was reduced by almost 90% at the oil only site and disappeared at the dispersed oil site following treatment. Numbers were still fluctuating throughout the two years after the treatment. However, after 10 years, numbers were back at the pre-treatment levels.

- Ballou *et al.* (1987) examined the effect of oil and dispersed oil on subtidal *Thalassia testudinum* beds. The sites were studied eight months and one week before treatment and continued 20 months after the treatments were applied. The sites were enclosed within an oil spill containment boom and 715 l of dispersed oil was released over 24 hours to achieve 50 ppm of petroleum hydrocarbons in the water to simulate the worst-case scenario of a large-scale oil spill. The recorded concentration of hydrocarbons in the water column at the dispersed oil site was 684 ppb. A total of 953 l of untreated crude oil was released on the other site and remained within the boomed area for two days, which resulted in an exposure of 1 $l/m^2$ and an overall concentration of hydrocarbons of 44 ppb. There were no significant differences seen in the growth rates between both treatments and the control in the first 3 months after the treatments. The blade areas for the oil and dispersed oil treatment were equal in pre-treatment recordings. The dispersed oil treatments had a larger recorded blade area than the oil treatment in all post-treatment results. However, these were both lower than the control site. There was a decline in the density of the seagrass following the treatment at both the oil only and the dispersant sites. Dispersants caused a decrease in population from 816.7 plants/$m^2$ to 673.3 plants/$m^2$ after four months. However, after seven months the density had recovered to a level greater than the pre-spill levels (922 plants/$m^2$). At the oil-only site, there was a decrease from 666.7 plants/$m^2$ to 488.0 plants/$m^2$ after seven months, which only showed signs of increase back to pre-spill levels after 12 months (692 plants/$m^2$). Both treatment sites showed a decrease in sea urchin abundance. After the exposure to the dispersed oil, the population reduced drastically with no live urchin were seen at the site four months after the treatment; however, recovery happened within one year. After exposure to oil, a slight decrease in sea urchin abundance was seen which did not show signs of recovery until seven months after the exposure when a large increase in abundance was seen. In the replicated worst-case spill scenario, there were no significant effects on the growth rate of the seagrass caused by exposure to either oil or dispersed oil. There was only a gradual but significant reduction in the seagrass density.

- Berry *et al.* (2016) examined the effect of coal dust (<0.63 um) on a coral, a fish, and the seagrass *Halodule uninervis* in flow through, laboratory studies to simulate the effect of a coal dust spill. They exposed samples to pulses of 0-275 µg/l coal dust. Although the coal dust was contaminated with heavy metals, it had no significant effect on the heavy metal concentration on the water in the study tanks. Coal dust coated the seagrass leaves and other surfaces of the pots in which the seagrass was grown. Leaf extension and shoot density were significantly reduced over time. Leaf extension was the most affected in treatment ≥73 mg/l coal dust after 14 and 28 days (LOEC) and growth was inhibited by 6.7-45% after 14 days and by 31.1 and 49.5% after 28 days. They estimated an $IC_{10}$ 42 mg/l coal dust after 14 days and 12 mg/l coal dust after 28 days, and an $IC_{50}$ 275 mg/l after 28 days. Shoot density increased at 38 mg/l coal dust but reduced significantly at ≥78 mg/l after 28 days (28-day LOEC) with a net loss of shoots. However, they concluded that the effects were probably due to light attenuation caused by the coating by coal dust.

- Costa *et al.* (1982) examined the before and after effects of two types of American fuel oils (American Petroleum Institute (API) Reference III and Baytown, Texas Exxon (BTE) refinery oil) on the weight, rhizomes, and leaf growth of eelgrass *Zostera marina* seedlings. Unpolluted sediment was collected and mixed with 0.0 and 3.0 mg of API oil and 0.0, 0.2, 1.0, 2.1, 6.2 mg/g of BTE oil. Seedlings were planted, immersed in the sediment mixed with oil after 12 days, and harvested 3 weeks later. At 0.2 mg/g of oil to sediment, leaf production was 60% below the control and weight increase was 40% below the control. At 1.0 mg/g of there was 50% less leaf production and inhibition of root and rhizome growth. Above 2.1 mg/g the rhizomes deteriorated, leaves were shed and many plants senesced. In the API oil experiment, chlorophyll-*a* concentration decreased by 60%. Costa *et al.* (1982) concluded that oil-contaminated sediment could affect the distribution and abundance of *Zostera marina*.

- Durako *et al.* (1993) examined the photosynthetic and respiratory response of leaf tissue in three species of seagrass: *Halophila ovalis, Halophila stipulacea,* and *Halodule uninervis*. These seagrasses were exposed to weathered Kuwait crude oil at a concentration of 1% aqueous solution for 12-18 hours. Photosynthesis vs. irradiance (PI) responses were measured and exhibited typical light saturation kinetics. In the short-term exposure, the respiration rates were not significantly affected. In addition, no significant differences in PI characteristics or respiration were detected among the species. No mortality was reported. Durako *et al.* (1993) concluded that crude oil would have a very limited effect on the subtidal seagrass communities and therefore the Gulf war oil spill would have a greater impact on intertidal communities.
- Hatcher & Larkum (1982) examined the effect of Bass Strait crude oil and Corexit 8667 on a seagrass mesocosm from March to August 1979. The oxygen consumption and leaf turnover of the seagrass *Posidonia australis* were recorded. Measurements were taken before, during, and after the 7-day treatment period. Four mesocosms were reviewed from March to August 1979. Two of the mesocosms received 450 ml of oil, one received 450 ml of oil and 8 ml of dispersant, and one received 450 ml of oil and 274 ml of dispersant, which completely dispersed the oil slick. Leaf turnover of *Posidonia australis* was not significantly affected by the oil or dispersant. Post-treatment mean daily leaf emergence and mortality rates did not differ significantly from pre-treatment rates in any microcosm. Photosynthetic oxygen production showed an immediate decrease at the addition of the treatments, due to an increase in respiration. The dispersant treatment microcosms exhibited an oxygen deficit in the light immediately following treatment, and the dark respiration rates increased two to three-fold over the control rates during the following two days. In August, 40 days after treatment, oxygen production rates and P/R ratios in the oil-treated microcosms were higher than rates measured before treatment. Hatcher & Larkum (1982) concluded that more severe stress is placed on the *Posidonia australis* dominated benthic community by oil and dispersant mixed than by oil alone. The seagrass recovered from the stress and the plants continued to grow at pre-treatment rates. There were no negative effects to the seagrass described in this paper.
- Howard *et al.* (1989) examined the results of studies on the effects of crude oil and crude oil treatment with dispersants on *Zostera*[1] conducted in Milton Haven by reviewing Holden & Baker (1980) and Howard (1986). Holden & Baker (1980) treated 1 $m^2$ plots with dispersant, oil, oil then dispersant or a premixed oil and dispersant and recorded the percentage cover change over 18 months. In all the single treatments, there was a reduction of *Zostera* when compared to the control but no differences between treatments. A second experiment used a successive application of the same treatments. The results from the second experiment showed that the successive application had no more impact than the single application in all but one treatment. Successive application of the premixed oil and dispersant treatment was particularly damaging and resulted in the complete elimination of the species in one plot. Howard (1986) used two 35 m transects parallel to the shore with 15 1 $m^2$ plots chosen to support the greatest densities of *Zostera*. One of five treatments of dispersant, oil, oil then dispersant or a premixed oil and dispersant, and control were randomly assigned to each plot. The results from all treatments, except the premixed oil and dispersant treatment, showed little temporal change in cover. However, the premixed oil and dispersant treatment showed a significant decrease within the first week that resulted in a decrease in cover from 55% to 15%. Howard *et al.* (1989) concluded that smothering by crude oil alone visually had little impact on the seagrass following the removal by tidal action. However, the oil did inhibit or reduce the growth and dispersal of the seagrass. The greatest potential impact of oil spills on the

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[1] *Zostera* was used as a general term throughout the Horward *et al.* (1989) review due to the difficulty in correctly distinguishing the seagrass species *Zostera noltei* and *Zostera angustifolia*. *Zostera angustifolia* is now thought to be synonym of *Zostera marina.*

intertidal *Zostera* bed is from the stranding of dispersant-treated oils. Plots treated with the premixed oil and dispersant mix suffered leaf blackening and high rates of mortality. This is due to the ability of the oil-dispersant mix to break down or penetrate the protective waxy layer covering the leaf, resulting in leaf mortality. Therefore, they suggested that oil treatment must be avoided if the stranding of the treatment mix cannot be avoided.

- Macinnis-Ng & Ralph (2003) exposed *Zostera capricorni* to crude oil, dispersant (VDC) (at 0.25% and 0.1%) and mixtures of both in the laboratory and in the field (using in situ chambers) for 10 hours followed by a four-day recovery period. In the laboratory, both oil and dispersants caused an initial decline in photosynthesis while mixtures did not. *In situ* samples were less sensitive and dispersants and mixtures did not cause a decline in photosynthesis. Oil caused an initial decline *in situ* but the plants had recovered after four days. Little effect on chlorophyll-*a* was observed.
- Ralph & Burchett (1998b) examined the impact of petrochemicals on the photosynthesis of *Halophila ovalis* using chlorophyll fluorescence. *Halophila ovalis* showed tolerance of exposure up to 1% (w/v) of Bass Strait Crude oil, a dispersant (Corexit 9527), and a mixture of the oil and dispersant. Florescence, PSII efficiency, and quantum yield were measured, with quantum yield being the most sensitive assessment. When exposed to 100%, 50% and 25% crude oil the PSII photochemical efficiency was lower than the control for all three concentrations and the quantum yield showed a significant decline within the first hour of treatment. For both the dispersants and crude oil dispersant mix at 100%, 50% and 25% there was a decrease in quantum yield within the first hour. However, there were signs of recovery after 72 hours. PSII photochemical efficiency was lower than the controls. Dispersants caused a significant decrease in chlorophyll-*a* & *b* and carotenoid in all treatments. There was a decrease in the pigments in the crude oil and the oil dispersant mix treatment but these were not found to be significant. Ralph & Burchett (1998) concluded that the petrochemicals had a limited impact on the photochemical processes of *Halophila ovalis*. They noted that a petrochemical spill alone might not cause a significant impact on a seagrass meadow. However, in combination with reduced light, the meadow may be threatened. In addition, oil pollution generally has the greatest impact on intertidal communities. Therefore, salt marshes, mangroves, and corals were more at risk (Den Hartog, 1984). Intertidal seagrasses are affected by physical contact with oil slicks whereas subtidal seagrass is more likely to be exposed to dispersed droplets. Seagrasses have been found to absorb more aliphatic and aromatic oil fractions when the oil is dispersed, therefore increasing the toxic damage. The dispersants have also been found to be more toxic than the oil itself. Mixed oil and dispersants are generally more toxic to seagrasses as it acts like a solvent, affecting the waxy epidermal coating of the leaf blade allowing the toxic components to access the cellular membrane and the chloroplasts (Ralph & Burchett, 1998b).
- Scarlett *et al.* (2005) examined the toxicity of the dispersants Superdispersant-25 and Corexit 9527 on the seagrass *Zostera marina*. This was measured by examining the chlorophyll fast fluorescence JIP transient measurements. The seagrass was exposed to five concentrations of the dispersants (0, 80, 130, 200, 320, 500 ppm) for 24 and 48 hours with a 24-hour recovery time. For all parameters, the lowest exposure of 80 ppm reduced the photosynthetic efficiency and resulted in an NOEC less than 80 ppm for both dispersants. Performance index (PI) was the most sensitive parameter with an $EC_{50}$ of 386 ppm for Superdiseperasnt-25 and Corexit 9527. The performance index is a combination of several JIP-test parameters and has been shown to be a highly sensitive measure that is correlated strongly with other measurements of plant health. Corexit was significantly more toxic at all parameters of 130 ppm and above. The leaves turned brown and started to detach at 200 ppm leaving only the more protected inner leaves. In the 24-hour recovery period, where the seagrass was washed and put in clean saltwater, the seagrass exposed to Superdispersant-25 showed signs of recovery as PI rose from 0.88 to 1.07 at 80 ppm. The Corexit exposed seagrass did not recover and mean PI values fell during the recovery period. Scarlet *et al.* (2005) noted that both dispersants had

a toxic effect and disrupted PSII during exposure. The leaves of *Zostera marina* have a thin cuticle that may afford a degree of protection from the dispersants, although it is clear that 24 hours was sufficient for photosynthesis to be affected. Mortality was not reported, as it was not considered a practical parameter to measure plant mortality by the authors.

- Thorhaug *et al.* (1986) examined effect of crude oil, dispersants, and an oil dispersant mixture on three seagrass species: *Thalassia testudinum, Halodule wrightii,* and *Syringodium filiforme*. Two types of crude oil, Louisiana Crude oil and Murban crude oil and one dispersant Corexit 9527 were used and specimens exposed in 100 litre outdoor laboratory tanks with 15 specimens per treatment. Exposure times were 5, 10, or 100 hours. *Thalassia testudinum* showed greater mortality the longer it was exposed to the oil. The largest recorded mortality in the species *Thalassia testudinum* was caused by exposure to a high concentration of dispersed Louisiana oil (500 ml oil and 50 ml dispersant) for five hours resulting in 47% mortality. Exposure of *Thalassia testudinum* to dispersed Murban oil at a lower concentration (12 ml dispersant mixed with 125 ml oil) for a longer period of 100 hours resulted in 40% mortality. *Halodule wrightii* and *Syringodium filiforme* were statistically the same across all the treatments. For these species, 12.5 ml in 100 l seawater of both oil dispersant oil mixes at 100 hours experienced 100% mortality. *Halodule wrightii* experienced 100% mortality at 500 ml dispersed oil with 5 hours exposure. The results showed that *Halodule wrightii* and *Syringodium filiforme* had an $LD_{50}$ of 75 ml in dispersed oil in 100 l of water for 100 hours of exposure. *Thalassia testudinum* was more tolerant with an $LD_{50}$ of 125 ml of dispersed oil in 100 l of seawater for 100 hours of exposure. Dispersants alone had a greater effect on *Halodule wrightii* and *Syringodium filiforme* than on *Thalassia testudinum* showing species differ in their tolerances. The difference in effect and mortality was found to be greater between the species than between the type of oil used. Thorhaug *et al.* (1986) noted that dispersed oil had a greater impact on seagrass growth and mortality than oil alone even when oil is at higher concentrations and has longer treatment periods. This may be due to an increased amount of hydrocarbons within the water column surrounding the seagrass blades when the oil is dispersed, rather than oil floating on a surface. Dispersants alone had a significant impact on *Halodule wrightii* and *Syringodium filiforme*, but not on *Thalassia testudinum*.

- Thorhaug & Marcus (1987) examined the effects of three dispersants (Corexit 9527, Arcochem D609, and Conco K(K) with two types of oil (Louisiana Crude and Murban Crude) on three tropical seagrass species (*Thalassia testudinum*, *Syringodium filiforme*, and *Halodule wrightii*). The treatments included dispersed oil, oil only, dispersant only, and a control. Many variables were tested that included changing the concentration of dispersed oil, time of exposure, type of oil and dispersant. In the first treatment, the seagrass was exposed to7.5 ml dispersant mixed and 75 ml oil in 100 l seawater for five hours. In this treatment, both Louisiana oil and Murban oil dispersed with Conco K(K) caused mortalities of approximately 70% in *Syringodium filiforme* and *Halodule wrightii*. In comparison the two other dispersants, Corexit 9527 and Arcochem D609, exposed to7.5 ml dispersant mixed and 75 ml oil in 100 l seawater for five hours resulted in mortality percentages less than 30%. The highest mortality (>70%) is seen in the *Syringodium filiforme* and *Halodule wrightii* when exposed to all dispersed oil mixtures at 7.5 ml dispersant to 75 ml oil in 100 l seawater for 100 hours. In this treatment, Murban oil and Louisiana oil mixed with Conco K(K) resulted in the most mortality with 100% mortality recorded in both *Syringodium filiforme* and *Halodule wrightii*. Exposure to 12.5 ml dispersant mixed with 125 ml oil for five hours resulted in 70-80% mortality in *Syringodium filiforme* and *Halodule wrightii*. Thorhaug & Marcus (1987) concluded that Corexit (0-87% mortality) and Arcochem (0-100% mortality) were less toxic than Conco K(K) (45-100% mortality) to all of the seagrass species tested. They also showed that *Syringodium filiforme* and *Halodule wrightii* were less tolerant to dispersed oil than *Thalassia testudinum*. It was also evident that a longer exposure time and larger concentration of oil and dispersant resulted in greater mortalities in the seagrasses.

- Thorhaug & Marcus (1987b) examined the effects of seven different dispersants mixed with Louisiana crude oil (Corexit 9527, Corexit 9550, OFC-D609, Conco K(K), Jansolv-60, Cold Clean 500, and Finasol OSR-7) on three tropical species of seagrass (*Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme*). There were two concentrations of the oil dispersant mixes, 75 ml and 125 ml of oil at a 10:1 ratio of oil to dispersant. For each treatment, 15 plants were exposed for 100 hours. They found that Finasol OSR-7, was the least toxic dispersant, followed by Jansolv-60, Cold Clean 500, Corexit 9550, Corexit 9527, and OFC-D-609. Conco K(K) was the most toxic and caused the most mortality. The *Thalassia testudinum* mortality was lowest for the 75 ml oil dispersant mix with 0-16% mortality. The 125 ml oil mixes had a mortality range of 7-26%. The most toxic dispersants were OFC-D-609 and Conco K(K) and resulted in 88% and 65% mortality respectively when the oil concentrations were combined. *Thalassia testudinum* was the most resilient seagrass. The *Syringodium filiforme* mortality was lowest for Finasol OSR-7, Jansolv-60, and Cold Clean 500 where mortalities ranged from 7-18% in the 75 ml treatment and from 10-30% in the 125 ml treatment. Conco and OFC-D-609 were the most toxic dispersant for *Syringodium filiforme* with an overall mortality of 84-100%. The *Halodule wrightii* mortality was lowest for the dispersants Finasol OSR-7 and Cold Clean 500. The most toxic dispersants were Corexit 9550, Corexit 9527, OFC-D-609 and Conco K(K). Mortality ranged from 7% to 21% when dispersants were combined with 75 ml of oil, and from 10% to 27% when combined with 125 ml of oil.
- Wilson & Ralph (2008) investigated the effects of Tapis crude Oil and dispersed crude oil on the subtidal seagrass *Zostera capricorni*. Perspex cylinders were pushed into the sediment of the *Zostera* meadows in New South Wales and treated with Tapis crude oil alone or Tapis crude oil mixed with dispersant (Corexit 9527). Five concentrations of the specific petrochemical were added (0.00, 0.05, 0.10, 0.20, and 0.40%) for each treatment. Seagrass blades were collected from each treatment at the end of the exposure day (10 hours) and following the recovery period (96 hours) for pigment analysis. The Tapis crude treatment did not result in any significant decrease in chlorophyll concentrations of the seagrass although all chlorophyll pigment concentrations decreased from 10 hours to 96 hours. There was a significant decrease in the effective quantum yield seen at 6-hour and 8-hour exposure at the 0.40% WSF (437 µg/l) treatment. In the dispersed Tapis crude oil treatment, there were no significant differences found in the chlorophyll-*a* fluorescence. Differences in the pigments of chlorophyll *a* & *b* were seen between the control and the 0.40% WSF (960 µg/l). Overall, the only impact in the crude oil treatment was at the 0.40% WSF concentration and these effects were short lived after the 10-hour exposure, as no differences were seen in the recovery period. In the dispersed oil treatment, the chlorophyll-*a* fluorescence and the chlorophyll pigment analyses showed no significant difference at any time suggesting that the dispersed oil had no detectable impact on the photosynthetic health of the seagrass.
- Wilson & Ralph (2012) examined the stress that petrochemicals have on the seagrass *Zostera capricorni*. The seagrass quantum yield of photosystem II (PSII) was measured after exposure to the water accommodated fraction (WAF) of dispersed and non-dispersed Tapis crude oil and fuel oil (IFO-380) for five hours. The crude oil treatment had a total petroleum hydrocarbon concentration of 12 mg/l. The crude oil treatment caused a small but significant decline in quantum yield of PSII, which occurred during the first four hours of exposure. The higher concentrations, seen particularly in the 2.0% WAF, significantly reduced the quantum yield during the first few hours. Dispersants resulted in an increase in the total petroleum hydrocarbons (TPH) in the WAF, which correlated with a greater physiological impact on seagrass health. The crude oil Corexit mix resulted in a TPH concentration of 101 mg/l and the crude oil Ardrox mixture of 105 mg/l. In both treatments, the photosynthetic efficiency significantly decreased in all concentrations after three hours. The fuel oil (IFO-380) TPH concentrations were low compared to other treatments measured as 3 mg/l. Therefore, leaf-blades displayed minimal stress in the quantum field of PSII during the experimental period. The fuel oil dispersant mix had a TPH of 196 mg/l in the Fuel oil and Slickgone LTWS

treatment and 522 mg/l in the fuel oil Corexit 9500 treatment. The Corexit fuel oil mix resulted in the greatest decrease in the quantum yield of all the experiments with fluctuation seen within one hour and a sharp and significant decrease after four hours. The chlorophyll-*a* concentration in the Slickgone treatment with the higher concentrations (1 and 2%) were significantly lower than the controls. The Corexit 9500 treatment also showed a significant decrease in chlorophyll-*a* in the 2% WAF treatment of the seagrass. The other treatment either showed small increases or no differences. Wilson & Ralph (2012) noted that photosynthetic efficiency was found to be sensitive to petrochemical exposure. However, there was minimal recoverable impact when only exposed to oil. Similarly, in the most concentrated IFO-380 treatment, there was a significant decrease in quantum yield of PSII but this was less than that seen in the dispersed oil treatment. Dispersants are thought to penetrate the waxy cuticle of the seagrass blade leading to a decreased tolerance of the seagrass to other stress factors (Zieman *et al.*, 1984; Howard *et al.*, 1989). The dispersant only treatments were found to be less toxic to the seagrass, suggesting the combination of oil and dispersant was the cause of the greatest damage to photosynthetic efficiency. Wilson & Ralph (2012) also noted that the concentrations tested on the seagrass in this study were reported to be realistic of that following actual spills, with the higher concentrations used being a worst-case scenario.

### 1.1.3 Polyaromatic Hydrocarbons (PAH)

Only two articles examined the effects of PAH exposure. Neither reported any evidence of mortality within the seagrass meadows examined.

- Faganeli *et al.* (1997) examined the effects of motorway pollution on the coastal sea, including some seagrass communities. The areas consisted of small sandy bottom seagrass meadows with predominantly *Posidonia oceanica, Zostera marina*, and *Cymodocea nodosa*. Pyrogenic PAH is normally introduced into the coastal marine environment as runoff. The levels of PAH were tested in the sediment along the coast of the Bay of Koper and the concentrations of PAH were higher in the two sites that were exposed to the runoff of the motorway. However, it was found that the offshore concentrations were higher than the near shore. Overall, the seagrass communities did not show any sign of degradation or any differences from the uncontaminated northern shoreline of the Bay. In addition, within the seagrass communities, there were no significant differences found in the fauna within the seagrass communities between sites, showing that the motorway discharge of PAH had no impact on these either.
- Mauro *et al.* (2013) examined the condition of a *Posidonia oceanica* bed in a lagoon exposed to human impacts for ca 40 years. They reported that the bed did not show any sign of regression, and may have been extending seaward, even though the sediment was contaminated with PAHs and metals. Mercury and PAHs exceeded ERLs while Cu was close to its ERL.

Huesmann *et al*. (2003) reported that *Zostera marina* increased the biodegradation of PAHs from crude oils in marine sediments, and contributed to the recovery process of the community after exposure (Huesmann *et al*., 2003; cited in Lewis & Devereux 2009).

### 1.1.4 Others

Malea *et al*. (2020) examined the effect of Bisphenol A (BPA) exposure on the growth of *Cymodocea nodosa* under laboratory conditions. Samples were exposed to 0.03, 0.1, 0.3, 0.5, 1, and 3 µg/l BPA in aquaria and the water renewed every two days for 10 days. The elongation rate of leaves, rhizomes, and roots was measured every two days. Growth of all plant parts was not significantly different from controls at 0.03 to 0.3 µg/l but decreased with increased BPA concentrations above those values. Juvenile leaves were more resistant than adult leaves and rhizomes but showed inhibition at lower concentration but at a lower extent than adult leaves or rhizomes. They reported an NOEC of 0.1 µg/l and an LOEC of 0.3 µg/l for all parts of the plants after 10 days. $EC_{50}$ values were lower for rhizomes than adult leaves and highest for juvenile leaves. They suggested that the higher toxicity for rhizomes might indicate the uptake route for BPA. Malea *et al*. (2020) noted that the LOEC, NOEC and $EC_{50}$ levels for *Cymodocea nodosa* were lower or the lowest reported for other aquatic organisms, and that BPA should be considered to be 'very toxic' to *Cymodocea nodosa* (where the EEC guidance terms 'very toxic = $EC_{50}$ <1 mg/l).

The evidence on 'other' forms of hydrocarbons was limited. The exposure to seagrass to the phenol Bisphenol A (BPA) reported sublethal effects at the concentrations studied.

### *1.1.5 Sensitivity assessment (Hydrocarbons and PAHs)*

The number of articles that report mortalities due to Hydrocarbons and PAHs' are summarized in Figure 1.4 and in Table 1.2 below.

#### Sensitivity assessment – Oil spills

The effects of the oil spills on seagrass meadows are inconsistent and variation was seen between seagrass species and oil types. Studies have shown some seagrass meadows to be tolerant to oil spill exposure and others have resulted in severe mortality.

*Zostera marina* is tolerant to oiling (in the absence of dispersants or other cleaning treatments). All reported effects on *Zostera marina* after exposure to spilled crude oil and fuel oil were sublethal. Only sublethal, short-term damage was reported in the form of a decline in abundance in shoots, blades, and flowering shoots in the *Exxon Valdez* oil spill and blackened/burnt leaves in the *Amoco Cadiz* oil spill.

Other species are less tolerant. 'Severe' mortality was reported in 20% of the oil spill results and is recorded in the species *Phyllospadix torreyi, Posidonia oceanica, Thalassia testudinum* and in unspecified Seagrass *(var.)* located in the Gulf of Mexico*,* after exposure to spilled crude oil. 'Some' mortality was also seen in *Thalassia hemprichii* after the fuel oil Taklong Island National marine reserve oil spill. In addition, the *Deepwater Horizon* oil spill report also recorded large-scale seagrass mortality/population loss but did not quantify the scale of losses. Sublethal effects were reported in 65% of the results on oil spill damage to seagrass. These ranged from reduced growth rates, bleaching, decreased density of shoots, reduced flowering success (Den Hartog & Jacobs 1980; Jacobs 1980; Dean *et al*. 1998; Keesing *et al*., 2018), blackening leaves, leaf loss (Den Hartog & Jacobs 1980; Jacobs 1980; Keesing *et al*., 2018) and reduced growth rate (Kenworthy *et al*., 1993).

Due to the low solubility of oil, subtidal seagrass species, such as *Zostera marina*, are exposed only to the water accommodating fraction (WAF) of oil or dispersed oil droplets meaning they are less susceptible to damage than intertidal seagrass beds that experience physical contact with oil leading to greater amounts of damage and mortality (Lopez, 1978; Zieman *et al.* 1984; Zieman & Zieman, 1989; Fonseca *et al.* 2017; Keesing *et al.* 2018). Other factors influencing the effect of oil on seagrass include seagrass species, oil type, intensity, duration, and circumstance of the exposure (Keesing *et al*., 2018).

Seagrass situated near an oil refinery in Milford Haven showed no chronic sensitivity or long-term effects to the exposure to the oil effluent. However, this may have been due to little penetration of the effluent (Hiscock, 1987, cited in; Holt *et al.*, 1995, 1997). In addition, oil spills can cause indirect effects and mortalities to seagrass communities. Heavy oiling can lead to an increase in algal growth resulting in heavy fouling that persists for several months after an oil spill has occurred due to the mortality of grazers (Jackson *et al*. 1989).

Table 1.2**.** Summary of count of ranked mortalities to 'Hydrocarbons and PAH' contaminants reported in the evidence review and resultant proposed sensitivity assessments for seagrass species, with specific reference to *Zostera* spp. (N= None, VL= Very low, L= Low, M= Medium, High = High, and NS= Not sensitive).

| Group | Type | Species name | Worst case mortality: Severe | Significant | Some | None | Sublethal | Total | Assessment: Resistance | Resilience[2] | Sensitivity |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Hydrocarbons (Petrochemical)** | | | | | | | | | | | |
| | **Oil spill** | | | | | | | | | | |
| | | *Halophila ovalis* | | | | | 2 | 2 | H | ?? | NS |
| | | *Halophila stipulacea* | | | | | 2 | 2 | H | ?? | NS |
| | | *Phyllospadix spp.* | | | | | 1 | 1 | H | ?? | NS |
| | | *Phyllospadix torreyi* | **1** | | | | 1 | 2 | N | ?? | H |
| | | *Posidonia oceanica* | **1** | | | | | 1 | N | ?? | H |
| | | *Seagrass (var.)* | **1** | | | 1 | | 2 | N | ?? | H |
| | | *Thalassia hemprichii* | | | **1** | | | 1 | M | ?? | M |
| | | *Thalassia testudinum* | 1 | | | | 1 | 2 | H | ?? | NS |
| | | ***Zostera marina*** | | | | | 6 | 6 | H | H | NS |
| | **Oil spill Total** | | **4** | | **1** | **1** | **13** | **19** | **H** | **H** | **NS** |
| | **Complex hydrocarbons** | | | | | | | | | | |
| | | *Halodule uninervis* | | | | | 2 | 2 | H | ?? | NS |
| | | *Halodule wrightii* | **1** | | 1 | | 1 | 3 | N | ?? | H |
| | | *Halophila ovalis* | | | | | 2 | 2 | H | ?? | NS |
| | | *Halophila stipulacea* | | | | | 1 | 1 | H | ?? | NS |
| | | *Posidonia australis* | | | | | 1 | 1 | H | ?? | NS |
| | | *Seagrass (var.)* | | | | | 2 | 2 | H | ?? | NS |
| | | *Syringodium filiforme* | **1** | | 1 | | 1 | 3 | N | ?? | H |
| | | *Thalassia testudinum* | | **2** | 1 | | 2 | 5 | L | ?? | H |
| | | ***Zostera capricorni*** | | | | | 4 | 4 | H | H | NS |
| | | ***Zostera marina*** | | | | | 4 | 4 | H | H | NS |
| | | ***Zostera spp.*** | | | **3** | | 1 | 4 | M | M | M |
| | **Complex hydrocarbons Total** | | **2** | **2** | **6** | | **21** | **31** | **M** | **M** | **M**[3] |
| | **Phenols** | | | | | | | | | | |
| | | *Cymodocea nodosa* | | | | | 1 | 1 | **H** | **??** | **NS** |
| **Hydrocarbons (Petrochemical) Total** | | | **6** | **2** | **7** | **1** | **35** | **51** | **M** | **M** | **M** |
| **Hydrocarbons (pyrogenic)** | | | | | | | | | | | |
| | **Polyaromatic hydrocarbons (PAHs)** | | | | | | | | | | |
| | | *Posidonia oceanica* | | | | 1 | | 1 | H | ?? | NS |
| | | ***Zostera marina*** | | | | | 9 | 9 | H | H | NS |
| | **Polyaromatic hydrocarbons (PAHs) Total** | | | | | **1** | **9** | **10** | **H** | **H** | **NS** |
| **Hydrocarbons (Mixtures)** | | | | | | | | | | | |
| | **Dispersant (inc. dispersed oils)** | | | | | | | | | | |
| | | *Halodule wrightii* | **17** | 9 | 8 | | 1 | 35 | N | ?? | H |
| | | *Halophila ovalis* | | | | | 2 | 2 | H | ?? | NS |
| | | *Posidonia australis* | | | **1** | | | 1 | M | ?? | M |
| | | *Seagrass (var.)* | | | | | 1 | 1 | H | ?? | NS |

[2] Resilience for *Zostera* spp. is assumed to be the same as the biotopes Zmar or Znol or unknown for other seagrasses.
[3] Based on one article

| Group | Type | Species name | Worst case mortality: Severe | Significant | Some | None | Sublethal | Total | Assessment: Resistance | Resilience[2] | Sensitivity |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Syringodium filiforme* | **18** | 9 | 8 | | | 35 | N | ?? | H |
| | | *Thalassia testudinum* | **3** | 20 | 11 | 1 | 4 | 39 | N | ?? | H |
| | | ***Zostera capricorni*** | | | | | 8 | 8 | **H** | **H** | **NS** |
| | | ***Zostera marina*** | | | | | 2 | 2 | **H** | **H** | **NS** |
| | | ***Zostera* spp.** | | **3** | | | 1 | 4 | **L** | **M** | **M** |
| | **Dispersants Total** | | **38** | **41** | **28** | **1** | **19** | **127** | **N** | **??** | **H** |
| **Hydrocarbons (Mixtures) Total** | | | **38** | **41** | **28** | **1** | **19** | **127** | **L** | **??** | **H** |
| **Total** | | | **44** | **43** | **35** | **3** | **63** | **188** | **N** | **??** | **H** |

Overall based on the 'worst case' scenario **for oil spills** the resistance is assessed as '**None**' for seagrasses as a group. Resilience is probably '**Low**' so sensitivity to petroleum-based oil spills is assessed as '**High**'. But the above evidence also suggests that *Zostera* spp. (and by inference *Zostera* dominated habitats), are **'Not sensitive'** to oil spills (in the absence of dispersants or other cleaning treatments). The confidence in the assessment is probably 'High' because all of reported effects on *Zostera marina* after exposure to spilled crude oil and fuel oil were sublethal. However, the impact to the community living in the seagrass is often greater than the impact on the seagrass itself (Jacobs, 1988; Holt *et al.,* 1995, 1997).

### Sensitivity assessment – Petroleum hydrocarbons (oils)

The reported results to the exposure of petroleum oils on seagrass suggest that 6.4% of cases resulted in 'Severe' mortality (>75%) while another 6.25% of the articles report 'significant' (25-75%) mortality and 18.75% of articles reported 'some' (<25%) mortality depending on the species of seagrass, type of oil and its concentration.

The majority of the reported effects of oil on seagrass were generally sublethal (64.5%). These include reduced photosynthetic efficiency, loss of leaf pigmentation, reduced growth rate and leaf loss. Exposure to oil was reported to cause 'severe' mortality in only 6.4% of the results. The result of exposure differed depending on the type of oil used. Louisiana crude caused 'severe' mortality in all reports of exposure of the seagrasses *Syringodium filiforme* and *Halodule wrightii.* Murban crude was less toxic to seagrass than Louisiana crude, causing only 'some' damage to these species. Hence, oils from various sources have different levels of toxicity on seagrass and, therefore, may explain some of the different results. Fuel oil was reported to only cause sublethal effects on seagrass (Costa, 1982; Wilson & Ralph, 2012). However, both and Zieman & Zieman (1989) and Keesing *et al*. (2018) noted that refined oils, diesel and bunker fuels were more toxic than crude oil. The exposure of seagrass to the simulated coal dust spill resulted in only sublethal effects.

The differences seen between species were greater than that seen between oil types. 'Severe' and 'significant' mortality were reported more often in the tropical species *Syringodium filiforme* and *Halodule wrightii* and *Thalassia testudinum* than *Zostera marina* and *Zostera capricorni* where exposure only led to sublethal effects. There was 'Some' mortality reported when *Zostera* spp. was exposed to crude oil in a field experiment, however, these were reported to most likely be the intertidal species *Zostera noltei* or the shallow extent of *Zostera marina* (as syn. *Zostera angustifolia*) that are more likely to have been into direct contact with the oil, which resulted in more damage than subtidal species (Howard *et al.*, 1989).

Technically, the worst-case sensitivity of seagrass, as a group, would be assessed as **'High'** (Table 1.2) based on the response of tropical species. Native *Zostera* spp. are probably less sensitive and a sensitivity of **'Medium'** is suggested **in the intertidal** based on the evidence presented by Howard *et al.* (1989), while subtidal species (and beds) are probably **'Not sensitive'**. Confidence in the assessment is **'Low'** due to the variation in effect shown in the evidence.

### Sensitivity assessment - Dispersants

Across six dispersant treatments recorded, only two dispersants (BP 1100 WD and Corexit 9527) were reported to cause lethal effects. Corexit 9527 was the most lethal dispersant. Two records of 'severe' mortality in *Syringodium filiforme* and *Halodule wrightii* were recorded and two records of 'significant' in *Thalassia testudinum*. There was one report of 'significant' mortality in *Zostera* spp. after exposure to BP 1100 WD. All other responses were sublethal. Therefore, sensitivity to dispersants is assessed as **'Medium'** for *Zostera* spp. and **'High'** for seagrasses as a group. However, confidence is assessed as **'Low'** because of the variation in response between species, and the limited number of dispersants examined in the evidence review.

### Sensitivity assessment - Dispersed oils

Overall, the reported results on the exposure to dispersed oils suggest that 29.8% of cases could result in 'Severe' mortality (>75%) while another 33.3% of the articles reported 'Significant' (25-75%) mortality and 24.6% of articles reported 'Some' (<25%) mortality depending on the species of seagrass, type of oil, dispersant and the concentration of both.

Dispersed oil was reported to have a variety of effects on seagrass from 'no observed' mortality to 100% mortality. Dispersed oil was more toxic than both oil and dispersant treatments alone with 89% of dispersed oil exposure resulting in a lethal effect on the seagrasses. Different dispersant oil mixtures had various levels of toxicity. The most toxic recorded dispersant mixed with crude oils was ConcoK(K), which had the highest number of results of 'severe' and 'significant' mortality (Thorhaug & Marcus 1987b).

Dispersants can break down the waxy epidermal coating on the leaves allowing the toxic components to access the cellular membrane. This allows for greater absorption of aliphatic oil fractions which increases the toxic damage and leads to a decreased tolerance to other stress factors (Zieman *et al*., 1984; Howard *et al*., 1989; Ralph & Burchett, 1998b; Wilson & Ralph, 2012). In addition, Wilson & Ralph (2012) noted that the addition of dispersants increases the total petroleum hydrocarbon (TPH) concentration in the water column from 12 mg/l to 101 mg/l in crude oil and 3 mg/l to 522 mg/l in fuel oil. These were considered realistic to those reported in oil spills with the higher concentrations being 'worse-case' scenarios (Wilson & Ralph, 2012). However, they resulted in no recorded mortality in *Zostera capricorni.* No mortality was also recorded in *Zostera marina* and *Halophila ovalis* after exposure to dispersed oils, which only experienced sublethal effects. Sublethal effects were mostly short-term negative impacts on the photosynthetic efficiency and decreased pigmentation of leaves after exposure. However, some species of seagrass were less tolerant of exposure to dispersed oil. The tropical species of seagrasses showed a low resistance to dispersed oil exposure with 'severe' mortality reported in 2.6% the results of exposure in *Thalassia testudinum*, 14.9% in *Syringodium filiforme* and 14% in *Halodule wrightii* (Thorhaug *et al.* 1986; Thorhaug & Marcus, 1987; Thorhaug & Marcus, 1987b).

However, Howard (1986) reported that treatment of *Zostera* spp. (probably *Zostera noltei* or lower shore intertidal *Zostera marina*) with premixed oil and dispersant treatment showed a significant decrease within the first week that resulted in a decrease in cover from 55% to 15% (Howard *et al.,* 1989).

Technically, the worst-case sensitivity of seagrass, as a group, would be assessed as **'High'** (Table 1.2) based on the response of tropical species. Native *Zostera* spp. are probably less sensitive depending on the exposure. **Intertidal** *Zostera noltei* and lower shore intertidal *Zostera marina* beds may exhibit a **'Medium'** sensitivity to dispersed oils based on the evidence presented by Howard *et al.* (1989), while subtidal species (and beds) are probably **'Not sensitive'**. Confidence in the assessment is **'Low'** due to the variation in effects shown in the evidence.

#### Sensitivity assessment – Polyaromatic hydrocarbons (PAHs).

The evidence on the effects of PAH contaminants on seagrass was limited with only two relevant papers found. In these papers, environmental exposure of PAH was recorded but no mortality or sublethal effects were reported. Therefore, the resistance is assessed as '**High**' and resilience as '**High**', so that the sensitivity of seagrasses to PAH exposure assessed as '**Not sensitive**'.

## 1.2 Seagrasses – Transitional metals and organometals

The effect of the exposure of seagrass species to metals was examined in 29 papers, only one of which examined nanoparticulate metals. The literature review identified many other papers that looked at bioaccumulation of metals in seagrasses or seagrass bed sediments but these are excluded from the scope of the study. The effects of exposure to Copper, Zinc, Cadmium, and Lead were the most studied, while the effects of Chromium, Mercury or Iron were limited to four articles. However, 'No mortality' or 'Sublethal' responses were reported in 93% of the articles examined, and a 'Severe' mortality was only reported in one article and only in *Halophila spinulosa*.

The evidence is summarized below.

- Govers *et al.* (2014) conducted a global meta-analysis of the accumulation of trace metals in seagrasses together with local case studies in the Caribbean Islands of Curaçao and Bonaire. They demonstrated that seagrasses were useful bioindicators of metals contamination worldwide. The Mediterranean (and *Posidonia oceanicia*) was the most studied region while Cobalt and Mercury were the least well-studied metals. They reported that seagrasses were metal accumulators with a 100-1000 fold range in concentrations of all individual metals. Metals concentrations varied seasonally with lower levels in the growing season than the dormant winter season. They also reported that leaf concentrations of metals were 2-4 fold increased in polluted sites compared to unpolluted sites. Govers *et al.* (2014) noted that many trace metals were naturally abundant in seagrass beds but that high concentrations may be toxic to seagrass (MacNinnis & Ralph, 2002; Prange & Dennison, 2000; Ralph & Burchett, 1998). Trace metal accumulation may also affect photosynthesis in seagrass (Conroy *et al.*, 1991; MacFarlane & Burchett, 2001(mangroves); Prange & Dennison, 2000) or inhibit metabolism (Ralph & Burchett, 1998) and may result in reduced growth or dieback (Clijsters & Van Assche, 1985).
- Hamoutene *et al.* (1996) examined the effect of cadmium exposure (5, 10, 20 µg/l) on extracted etiolated leaf tissue from *Posidonia oceanica* under laboratory conditions. There was significant inhibition of lipid perioxidation in samples from Iles de Lerins at all concentrations but not in samples from Villefranche-sur-mer. EROD (7-ethoxyresorufin O-dealkylase) activity was reduced at 10 µg/l Cd in samples from Iles de Lerins, but in Villefranche-sur-mer samples, it was reduced at 5 & 10 µg/l Cd but zero at 20 µg/l. Glutathione S transferase was not affected at 5 & 10 µg/l but increased at 20 µg/l Cd. The authors suggested glutathione might be involved in protecting the plant from adverse effects of the metal.
- Lafratta *et al.* (2019) demonstrated that seagrass beds (*Posidonia australis*) in the upper Spencer Gulf, South Australia, provided a sink for heavy metals and an archival record of heavy metal pollution from the upstream Pb-Zn smelter works since the 1890s. The concentrations of Pb, Zn, and Cd had increased 9-fold since the onset of operation. Yet, the seagrass beds within 70 km of the

smelter had accumulated 7-15% of the smelter emissions in their soils (sediments) over the previous 15 years.

- Lyngby & Brix (1984) examined the uptake of metals (Cu, Cd, Cr, Zn, Pb & Hg) into the tissues of *Zostera marina* and their effect on growth under laboratory conditions. They exposed plants to 0.1, 0.5, 5, & 50 µM concentrations. *Zostera marina* accumulated metals by 1850 times the concentration in water. Stems and leaves accumulated metals in the order Zn >=Cu >Cd >Hg >=Pb, while Hg was accumulated in roots. They also noted a significant reduction in growth rates due to exposure to metals, and reported that their toxicity was in the order Hg >Cu >Cd >=Zn >Cr & Pb. For example, a significant reduction in growth occurred at 5 µM Cd after 12 days and at 50 µM Cd after 8 days, and was only 50% of controls after 19 days. Significant reduction in growth occurred after 5 days at 5 µM Cu, and 2 days at 50 µM Cu. Plants turned black within hours at 50 µM Cu and similar visible effects occurred at 5 µM Cu after 2 days, although no significant effects were observed at 0.5 µM Cu. Exposure to mercury was more marked. Growth was reduced 45% and 18% of controls after 19 days at 5 and 50 µM Hg respectively, and plants exhibited similar visible effects to those caused by Cu. Exposure to 50 µM Zn significantly reduced growth after two days but lower concentrations had no significant effects. Pb and Cr had no significant effects on growth. However, the authors noted that the metals concentrations used to reduce growth in seagrass in their study were probably much higher than those observed in natural or polluted waters.
- Macinnis-Ng & Ralph (2002) exposed *Zostera capricorni* to a range of metals *in situ* using specialist field chambers. The plants were dosed with 0.1 and 1 mg/l of each metal for 10 hours and monitored for a 4-day recovery period. The results varied but Cu and Zn depressed photosynthesis during the 10-hour exposure period. Those exposed to Zn recovered in 4 days but those exposed to Cu did not. Cadmium and lead did not affect chlorophyll *a* fluorescence.
- Macinnis-Ng & Ralph (2004a) exposed *Zostera capricorni* to double pulses of the herbicide Irgarol 1051 and copper in the field using specialist experimental chambers. They examined the effects on photosynthetic efficiency (quantum yield) and chlorophyll concentration after exposure to 10 hours of toxicant, followed by 4-day recovery, and then another 10-hour pulse of toxicant. Photosynthesis in leaf clippings were examined at 2, 10, and 96-hour periods. Marked reduction in photosynthesis was noted after single pulse of Irgarol at 100 µg/l. However, samples showed some recovery even after the second dose of Irgarol. Copper (5 mg/l) inhibited photosynthesis during both exposure periods and caused a decline in chlorophyll concentrations. Samples were able to recover from the first pulse but not the second due to damage to the PSII apparatus and interference by copper with enzymes responsible for chlorophyll production. They reported that double pulses of either toxicant inhibited photosynthesis more than single pulses. They also noted that a single pulse of copper followed by a recovery period and a pulse of Irgarol was more damaging than Irgarol followed by copper. But the cumulative effects of copper and Irgarol on chlorophyll concentration were limited and were similar to control leaves at the end of the experiment (8 days).
- Macinnis-Ng & Ralph (2004b) used *in situ* chambers to examine the effect of copper and zinc exposure on photosynthesis and chlorophyll concentration in *Zostera capricorni*, in three sites with different background levels of metal contamination. Samples were exposed to 0.1 and 1 mg/l Cu or Zn for 10 hours and photosynthesis was examined at 2, 10, and 96 hours. Photosynthetic efficiency (quantum yield) in samples from the pristine site was significantly reduced by 1 mg/l Cu after two and 96 hours while samples from contaminated sites were not significantly different from control after 96 hours. Chlorophyll concentrations were also significantly reduced in samples from the pristine site at 1 mg/l. However, Zn had no significant effects on photosynthesis or chlorophyll concentration at any site. They reported that seagrasses from the pristine site were more sensitive than those from contaminated sites. However, the tolerance of seagrasses from contaminated sites

was not explained by background concentrations of metals in sediments or their accumulation in the leaves or roots of the seagrasses.

- Maestrini *et al.* (2002) examined the effect of 15-day exposure to 1uM mercury ($Hg(NO_3)_2$) on DNA in the shoots of *Posidonia oceanica* under laboratory conditions. The shoots accumulated mercury during the exposure. They reported that mercury treated shoots lost ca 48% of A-T rich DNA sequences from their extracted DNA compared to controls. Maestrini *et al.* (2002) noted that the A-T rich DNA sequences were probably repetitive DNA in the genome. However, the direct cause or effects were unclear and no mortality was reported.
- Marin-Guirao *et al.* (2005) compared the metal contaminated *Cymodocea nodosa* seagrass beds with uncontaminated reference areas in Mar Menor lagoon, Spain. The seagrass accumulated metals (Zn, Pb, and Cd) but there were few differences in seagrass metrics between sites. However, there were differences in the macroinvertebrate community.
- Mauro *et al.* (2013) examined the condition of a *Posidonia oceanica* bed in a lagoon exposed to human impacts for ca 40 years. They reported that the bed did not show any sign of regression, and may have been extending seaward, even though the sediment was contaminated with PAHs and metals. Mercury and T∑-PAHs exceeded ERLs while Cu was close to its ERL.
- Mishra *et al.* (2020) examined the sediment burden and tissue accumulation of heavy metals in two seagrasses (*Posidonia oceanica, Cymodocea nodosa*) at six $CO_2$ seeps in Italy and Greece. They reported that seep sites had higher levels of heavy metals than reference sites. Seagrasses had higher than sediment levels of Zn & Ni in *Posidonia* and Zn in *Cymodocea*, especially in roots. Copper levels were high at one site, at which seagrass was abundant yet showed low levels of copper. At other sites, the low pH increased the accumulation of heavy metals, e.g. Zn. They concluded that differences in heavy metal bioavailability and toxicity between sites affected the relative abundance of seagrasses between those sites.
- Mohammadi *et al.* (2019) examined the effect of copper stress on gene expression (transcriptomics) in *Zostera muelleri* exposed to 250 and 500 µg/l copper (CuCl2) for seven days. They mapped the relative expression of genes and metabolic pathways in response to copper exposure and suggested potential biomarkers of copper stress in *Zostera*. No mortality or other sublethal effects were examined or reported.
- Papathanasiou *et al.* (2015) examined the effect of different irradiance levels, nutrients (phosphate and nitrate) and copper concentrations on photosynthetic efficiency (effective quantum yield) and leaf/shoot elongation over eight days in the laboratory in *Cymodocea nodosa*. Samples were collected from one area impacted by effluent from wastewater treatment and crude oil desulphurization plant. Two other sites were chosen for their un-impacted 'good' environmental status. Quantum yield increased at high nutrient levels (30 µM N-$NO^{3-}$ to 2 µM P-$PO_4^{3-}$) but was only significant in samples from oligotrophic sites. Irradiance affected quantum yield irrespective of site and phosphate concentration but high levels were reported in low light conditions. Quantum yield was affected in samples from all sites above 1.6 µM Cu but only the highest concentrations (4.7 and 7.9 µM Cu) affected quantum yield significantly. The highest copper concentrations (4.7 and 7.9 µM) only affected samples from the most contaminated sites significantly. Samples from the uncontaminated sites tolerated copper exposure.
- Prange & Dennison (2000) examined trace metals in five seagrass species from an urban and an industrial site on the coast of Queensland. They reported that *Zostera capricorni* leaf and rhizome tissue has concentrations of metals in the order Fe > Al >Zn > Cr > Cu, but that Al did not seem to bioaccumulate in the seagrass. They examined exposure of *Halophila ovalis, Halophila spinulosa*, *Halodule uninervis, Cymodocea serrulata,* and *Zostera capricorni* to 1 mg/l Fe and 1 mg/l Cu (in the presence of EDTA) for 12 days under laboratory conditions. They measured photosynthetic

efficiency, amino acid levels, and leaf and rhizome/root metal accumulation. Iron only affected *Halophila* spp. while copper affected all the seagrasses examined. The effect of copper varied between the seagrasses. *Halophila* spp. showed an increase and decrease in photosynthetic efficiency, but *Halophila serratus* also showed premature leaf death within 24 hours and plant death after 6 days exposure to 1 mg/l Cu. Photosynthetic efficiency in *Zostera capricorni* decreased but recovered after transfer to fresh seawater (after 12 days). Copper exposure reduced photosynthetic efficiency in *Halodule uninervis* but did not affect *Cymodocea serrulata*. *Zostera capricorni* and *Halodule uninervis* showed significant declines in amino acid levels on exposure to copper. Prange & Dennison (2000) noted that toxicity was dependent on the species ability to accumulate or exclude copper. *Zostera capricorni* exhibited a 20-fold decrease in amino acids and a significant decrease in photosynthetic efficiency in response to 1 mg/l copper. *Halophila* spp. accumulated Cu into tissue more than the other species but was not affected significantly. *Cymodocea serratula* was shown to exclude copper but demonstrated no effect on PSII function and only accumulated copper in the root/rhizome.

- Ralph & Burchett (1998b) examined the effect on Cu, Zn, Cd, and Pb (at 1, 5, or 10 mg/l) on photosynthesis in *Halophila stipulacea* under laboratory conditions for 96 hours. Cadmium at all three concentrations caused a rapid decline in quantum yield in the first hour, stabilized by 48 hours but declined further after 72 and 96 hours, especially at 10 mg/l. PSII efficiency declined after 72 hours. Cadmium did not affect chlorophyll *a:b* ratio or total chlorophyll after five hours at all concentrations but the chlorophyll *a:b* ratio increased after 5 hours at 10 mg/l. Copper resulted in leaf loss (premature senescence) after 48 hours at 1 & 5 mg/l so that those experiments were terminated. Quantum yield declined in all treatments after five hours. Quantum yield declined by 18% and 48% after 96 hours in 5 and 10 mg/l copper respectively. PSII declined in a similar way but was less sensitive to copper. Chlorophyll content was similar to controls at 1 and 5 mg/l Cu but declined significantly in 10 mg/l Cu. Lead exposure had a limited effect on fluorescence with no significant effects on quantum yield or PSII efficiency. However, photosynthetic pigments were affected with significantly lower chlorophyll *a* & *b* and total concentrations at 10 mg/l Pb. Zinc significantly reduced fluorescence, especially at 10 mg/l. Quantum yield declined at all concentrations in one hour, stabilised at five hours, but continued to decline after 24 hours at 5 & 10 mg/l Zn, but showed signs of recovery at 1 mg/l. PSII efficiency was similar but less sensitive. Chlorophyll *a* & *b* and total concentrations were lower at 10 mg/l Zn but significantly lower at 1 & 5 mg/l. Chlorophyll *a:b* ratios were increased at 5 & 10 mg/l Zn. Ralph & Burchett (1998b) concluded that all the metals tested exhibited toxicity, which increased with concentration and exposure duration. Fluorescence (especially quantum yield) was the most sensitive marker. They also noted that toxicity was linked to uptake and suggested that Cu and Zn exhibited the highest toxicity because, as essential trace metals, they are activity taken up while Cd and Pb were excluded. They suggested that the relative toxicity was Cu > Zn >Cd > Pb based on weight or Zn > Cu > Cd > Pb based on molarity. Nevertheless, Cu was more toxic than Zn based on the lethal response at lower molarity.
- Wahsha *et al.* (2016) examined the sedimentary concentrations of heavy metals (inc. Cd, Cu, Fe, Mn, Ni, Pb, Zn) from two areas populated by seagrass beds, one control and one polluted by phosphate mine wastes in the Gulf of Aqaba. They examined the leaf morphology and cell structure of *Halophila stipulacea* from each site. They found that leaves from the polluted site exhibited massive changes in cell organization in the epidermis, mesophyll, and vascular bundles, including swelling of the outer epidermis, chloroplast degradation, and cell necrosis. They reported that the morphological changes were correlated with the levels of contamination in the sediment.
- Wang *et al.* (2019) examined the effects of water and sediment parameters on the restoration of *Zostera marina* seagrass bed (transplanted seedlings) compared with a natural population. They

examined water and sediment concentrations of heavy metals, nutrients, organic carbon, and total petroleum. In the natural population, biomass/shoot and shoot height were not correlated with any of the parameters measured but shoot density was negatively correlated with $Cu^{2+}$ concentration in sediment and N/P ration and root:shoot ratios were negatively correlated with $As^{2+}$ concentration in sediment. Total biomass was significantly positively correlated with nutrient levels ($[NO^{2-}]$ & $[PO_4^{3-}]$) but negatively correlated with sediment $Cu^{2+}$ and total petroleum levels. In the restored bed biomass/shoot, total biomass, and N/P ratio was not correlated with any chemical parameter. Shoot density was negatively correlated with water column total petroleum, but root:shoot ratio was significantly positively correlated with water column $NH^{4+}$ and shoot density with water column total petroleum and $Hg^{2+}$ concentration in the sediment. Wang *et al.* (2019) concluded that both the natural and restored beds had similar growth characteristics but that differences in chemical parameters may affect long-term growth and restoration.

- Ward (1984) transplanted samples of the mobile fauna of seagrass bed in southern Australia from a low metal contaminated site to a site subject to heavy metal effluents from a lead smelter. The fauna were placed in cages and monitored for mortality for three weeks. They reported that the fish *Neodax* spp. and isopod *Cymodocea longicaudata* were acutely affected by Cd, Cu, Pb, or Zn in the effluent, yet both species are known to occur in the contaminated seagrass beds. A third species, the fish *Helotes sexlineatus* was not acutely affected but had previously been found to exhibit a lower abundance at the contaminated site. Nevertheless, they concluded that the acute toxicity of metals played a minor role in structuring the seagrass faunal community.
- Ward (1987) examined density, standing crop, metals, epibiota, and leaf growth in seagrass (*Posidonia australis*) at three sites in Spence Gulf, South Australia. Site A was heavily contaminated by wastes from a smelter, while sites B and C were eight and 16 km southwest. Density and standing crop was highest at site C and lowest at site A, although the differences were not always significant. Site A generally exhibited a lower biomass of epibiota than sites B or C. Metals were concentrated in leaves in the order Zn >Cd >Mn >Pb. However, the concentrations of Cd, Cu, and Zn in epibiota were lower than the leaves but Mn and Ni were higher. Growth of leaves (estimated over 10 days) was lowest at Site A, higher at site B and highest and site C. They suggested that seagrasses were suitable as sentinel accumulators but that accumulation varied with season. They concluded that *Posidonia australis* was not sensitive to heavy metals as it maintained its distribution in highly contaminated areas with sediment concentrations of Cd, Pb, and Zn of 22, 312 and 1,300 µg/l respectively. They noted than that Cd and Zn were not toxic to *Halodule wrightii* (Pulich, 1980). They also reported that the levels of metals in sediment in their study area were an order of magnitude lower than those found to reduce growth in *Zostera marina* (Lyngby & Bix, 1984; Ward *et al.*, 1984). Note, Ward *et al.* (1984) is not Ward (1984) and the latter could not be accessed).

### *1.2.1 Organometals*

**Organometals** were only examined by two articles.

- Francois *et al.* (1989) reported that tributyltin (TBT) was taken up and concentrated by *Zostera marina*. The rate of TBT decomposition in the plant was slower than that of dibutyltin, and monobutyltin was released from the plant. No sublethal effects or mortality was reported.
- Levine *et al.* (1990) examined the accumulation and distribution of $C^{14}$-labelled TBT in mesocosms containing seagrass (*Thalassia testudinum*). Mesocosms were dosed periodically for 24 hours and harvested after 3 or 6 weeks. They reported that TBT was rapidly removed from seawater by sediment and seagrass leaves. Absorption was short-lived and 20-30% of that absorbed remained, while ca 50% was present as a degradation products. They suggested that seagrass beds could concentrate TBT and process it to degradation products, but also act as a vector to the food chain.

- Williams *et al.* (1994) reported that *Zostera marina* was known to accumulate TBT but no damage was observable in the field.

### 1.2.2 Nanoparticulate metals

**Nanoparticulate metals** were only examined by one article. Malea *et al.* (2019) examined the effects of nanoparticulate Zinc oxide (ZnO NP) on photosynthesis in *Cymodocea nodosa* in the laboratory. Preliminary experiments revealed that 1 and 3 mg/l ZnO NP had no effect on PSII function. Therefore, samples were exposed to 5 and 10 mg/l, which were 7-13 times the levels reported in water environments. The effects were monitored for 4, 12, 24, 48 and 72 hours and the test solutions and water changed every 24 hours. PSII function was disturbed after 4 hours and became severe after 12 hours at 10 mg/l ZnO NP. After 24 hours at 10 mg/l the samples showed a hormetic response (signs of adaptation or acclimation). However, after 48 and 72 hours, the resultant photo-protection was reduced and energy loss increased. The authors suggest that the effects at 72 hours were due to increased Zn uptake at 10 mg/l compared to 5 mg/l. No mortality was reported.

### 1.2.3 Sensitivity assessment – Transitional metals and organometals

Seagrasses were reported to be relatively tolerant of heavy metals contamination, accumulate metals in their tissues, act as useful bioindicators of heavy metals in the environment, and trap heavy metals in seagrass bed sediments (Lyngby & Brix, 1984; Ward, 1987; Williams *et al.*, 1994; Davison & Hughes, 1998; Prange & Dennison, 2000; Govers *et al.*, 2014). The tissue accumulation varied between the heavy metals, season, and species of seagrass tested.

The number of articles that report mortalities due to metal, organometals, and nanoparticulate metals are summarized in Figure 1.1 and in Table 1.3 below.

*Halophila serratus* was the only seagrass species reported to exhibit mortality due to exposure to copper under laboratory conditions (6 days at 1 mg/l Cu) (Prange & Dennison, 2000). The remaining articles reported 'toxicity' in terms of sublethal effects, primarily on photosynthetic efficiency (e.g. effective and maximum quantum yield, fluorescence, or photosystem II (PSII) function, photosynthetic pigment ratios, and growth (e.g. leaf extension). Ralph & Burchett (1998b) suggested that the relative toxicity was Cu >Zn >Cd > Pb based on weight or Zn >Cu >Cd >Pb based on molarity. Nevertheless, Cu was more toxic than Zn based on the lethal response at lower molarity. They also suggested that Cu and Zn were the most toxic as they were essential trace metals in plant metabolism and hence actively taken up, while Cd and Pb were less toxic as they were excluded. Toxicity increased with exposure time and concentration but most papers noted that the concentrations studied were higher than those reported in the environment (e.g. Lyngby & Brix, 1984; Ward, 1987).

There was also some evidence that prior exposure to heavy metals affected the toxic response, for example, Macinnis-Ng & Ralph (2004b) noted that seagrasses (*Zostera capricorni*) from their pristine site were more sensitive than those from contaminated sites.

Few articles examined the effect on seagrass beds and their associated community. The reduction in photosynthetic efficiency and growth demonstrated in the evidence would be expected the cause stress on seagrasses and had the potential to cause loss at the population level this was not demonstrated in the evidence. For example, Marin-Guirao *et al.* (2005) compared the metal contaminated *Cymodocea nodosa* seagrass beds with uncontaminated reference areas in Mar Menor lagoon, Spain and found but few differences in seagrass metrics between sites. However, there were differences in the macroinvertebrate community.

Table 1.3**.** Summary of count of ranked mortalities to 'Transitional metals and organometal' contaminants reported in the evidence review and resultant proposed sensitivity assessments for seagrass species, with specific reference to *Zostera* spp. (NS= Not sensitive)

| | | | **Worst case mortality** | | | | | | **Assessment** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Group** | **Contaminant** | **Species name** | **Severe** | **Significant** | **Some** | **None** | **Sublethal** | **Total** | **Resistance** | **Resilience**[4] | **Sensitivity** |
| **Metals & compounds** | | | | | | | | | | | |
| | Cadmium | *Halophila ovalis* | | | | | 1 | 1 | High | High | **NS** |
| | | *Posidonia oceanica* | | | | | 1 | 1 | High | High | **NS** |
| | | ***Zostera capricorni*** | | | | | **1** | 1 | **High** | **High** | **NS** |
| | Chromium | ***Zostera marina*** | | | | | **1** | 1 | **High** | **High** | **NS** |
| | Copper | *Cymodocea nodosa* | | | | | 1 | 1 | High | High | **NS** |
| | | *Halophila ovalis* | | | | | 1 | 1 | High | High | **NS** |
| | | *Halophila spinulosa* | **1** | | | | | 1 | **None** | **??** | **High**[5] |
| | | *Seagrass (var.)* | | | | | 1 | 1 | High | High | **NS** |
| | | ***Zostera capricorni*** | | | | | **3** | 3 | **High** | **High** | **NS** |
| | | ***Zostera marina*** | | | | | **2** | 2 | **High** | **High** | **NS** |
| | Iron | *Seagrass (var.)* | | | | | 1 | 1 | High | High | **NS** |
| | Lead | *Halophila ovalis* | | | | | 1 | 1 | High | High | **NS** |
| | | ***Zostera capricorni*** | | | | | **1** | 1 | **High** | **High** | **NS** |
| | | ***Zostera marina*** | | | | | **1** | 1 | **High** | **High** | **NS** |
| | Mercury | *Posidonia oceanica* | | | | | 1 | 1 | High | High | **NS** |
| | | ***Zostera marina*** | | | | | **1** | 1 | **High** | **High** | **NS** |
| | Zinc | ***Zostera capricorni*** | | | | | **2** | 2 | **High** | **High** | **NS** |
| | | ***Zostera marina*** | | | | | **2** | 2 | **High** | **High** | **NS** |
| | Various | *Halophila stipulacea* | | | | | 1 | 1 | High | High | **NS** |
| | | *Posidonia australis* | | | | 2 | | 2 | High | High | **NS** |
| | | *Posidonia oceanica* | | | | 1 | | 1 | High | High | **NS** |
| **Metals & compounds Total** | | | **1** | | | **3** | **23** | **27** | High | High | **NS** |
| **Nanoparticulate metals** | | | | | | | | | | | |
| | Zinc oxide | *Cymodocea nodosa* | | | | | 1 | 1 | High | High | **NS** |
| **Nanoparticulates Total** | | | | | | | **1** | **1** | High | **High** | **NS** |
| **Total** | | | **1** | | | **3** | **24** | **28** | High | High | **NS** |

Mauro *et al.* (2013) examined the condition of a *Posidonia oceanica* bed in a lagoon exposed to human impacts for ca 40 years and found that the bed did not show any sign of regression, and may have been extending seaward, even though the sediment was contaminated with PAHs and metals. Wang *et al.* (2019) concluded that both the natural and restored *Zostera marina* beds had similar growth characteristics but that differences in chemical parameters (metals, petroleum, and nutrients) may affect long-term growth and restoration. And Ward (1984) concluded that the acute toxicity of metals played a minor role in structuring the seagrass faunal community.

[4] Resilience for *Zostera* spp. is assumed to be the same as the biotopes Zmar or Znol or unknown for other seagrasses.
[5] See text

Similarly, Ward (1987) reported that seagrass (*Posidonia australis*) beds exhibited the lowest density, standing crop and leaf growth at a site contaminated by smelter effluent in Spence Gulf, South Australia when compared with sites further away from the effluent discharge. But the differences were not always significant. *Posidonia australis* was not sensitive to heavy metals as it maintained its distribution in highly contaminated areas. Lafratta *et al.* (2019) also reported *Posidonia* beds surviving downstream of smelter effluent in Spence Gulf, South Australia and accumulating heavy metals in the sediment over a 15-year period.

Therefore, the weight of evidence presented suggests that seagrasses are probably 'Highly' resistant and, hence, **'Not sensitive'** to heavy metal contamination, especially those concentrations reported in the environment. *Halophila spinulosa* is an exception when exposed to high concentrations (1 mg/l for 6 days) of copper. Technically, the response of *Halophila spinulosa* could be interpreted as the 'worst-case' scenario. But the overall weight of evidence suggests it was an exception, and it is unwise to extrapolate this to the entire dataset based on one observation in a single study. Nevertheless, studies of *Zostera* spp. dominated the evidence review (50% of records) so that the sensitivity assessment is probably representative of *Zostera* spp. All the papers examined were of High quality, and 'High or Medium' applicability and all (except one) did not report mortality. Therefore, confidence is assessed as **'Medium'**.

## 1.3 Seagrasses – Synthetic compounds

The effect of the exposure of seagrass species to synthetics was examined in 23 articles; only one of which examined pharmaceuticals (the human hormone MCPA) and one examined methanol, as it was used as the solvent for the herbicides that were the focus of the study (Hershner *et al.*, 1982). Pesticides were the most studied group (96%) and herbicides the most studied type of contaminant amongst them (92% of records). The majority of articles reported sublethal effects (78%) or no mortality (one article) while 'some' mortality was reported in one article and 'severe' mortality in five articles (17% of records) (Figure 1.7).

### *1.3.1 Seagrass – pesticides/biocides*

A total of 21 articles examined the effects of pesticides on seagrasses, of which 17 (81%) examined herbicides, in particular, herbicides that affect the photosystem II of plants or the Acetyl coenzyme A carboxylase (ACCase) of grasses. The evidence is summarized below.

- Bester (2000) examined the concentration of several triazine herbicides (Atrazine, Propazine, Trebutylazine, Prometryn) and their metabolites in sediments along the East Friesian coast of the Dutch Wadden Sea, and compared their concentrations with the condition (destroyed/total decline; sparse/diminished or healthy) of the *Zostera noltei* seagrass beds. Bester (2000) reported that the condition of the seagrass beds decreased with increasing herbicide concentration (expressed as a sum of their individual concentrations) and that high concentrations were observed where the seagrass beds were destroyed. However, further statistical analysis was required to demonstrate a correlation (Bester, 2000).
- Brackup & Capone (1985) examined the effects of acute doses of environmental pollutants metals (Ni, Hg, and Pb as chlorides), naphthalene, and pesticides on nitrogen fixation (acetylene reduction) by bacteria associated with the rhizomes and root of *Zostera marina*. Ni & Pb resulted in significant inhibition at 100 ppm, while Hg exhibited inhibition above 10 ppm. Chlordecone (kepone), naphthalene, Aldicarb, and pentachlorophenol (PCP) resulted in significant inhibition, although PCP was the strongest effect. Toxaphene had no significant effect. However, the study examined the effect on nitrogen fixation by bacteria associated with the *Zostera* and it is unclear how their findings relate to the sensitivity of *Zostera* to the tested pollutants.

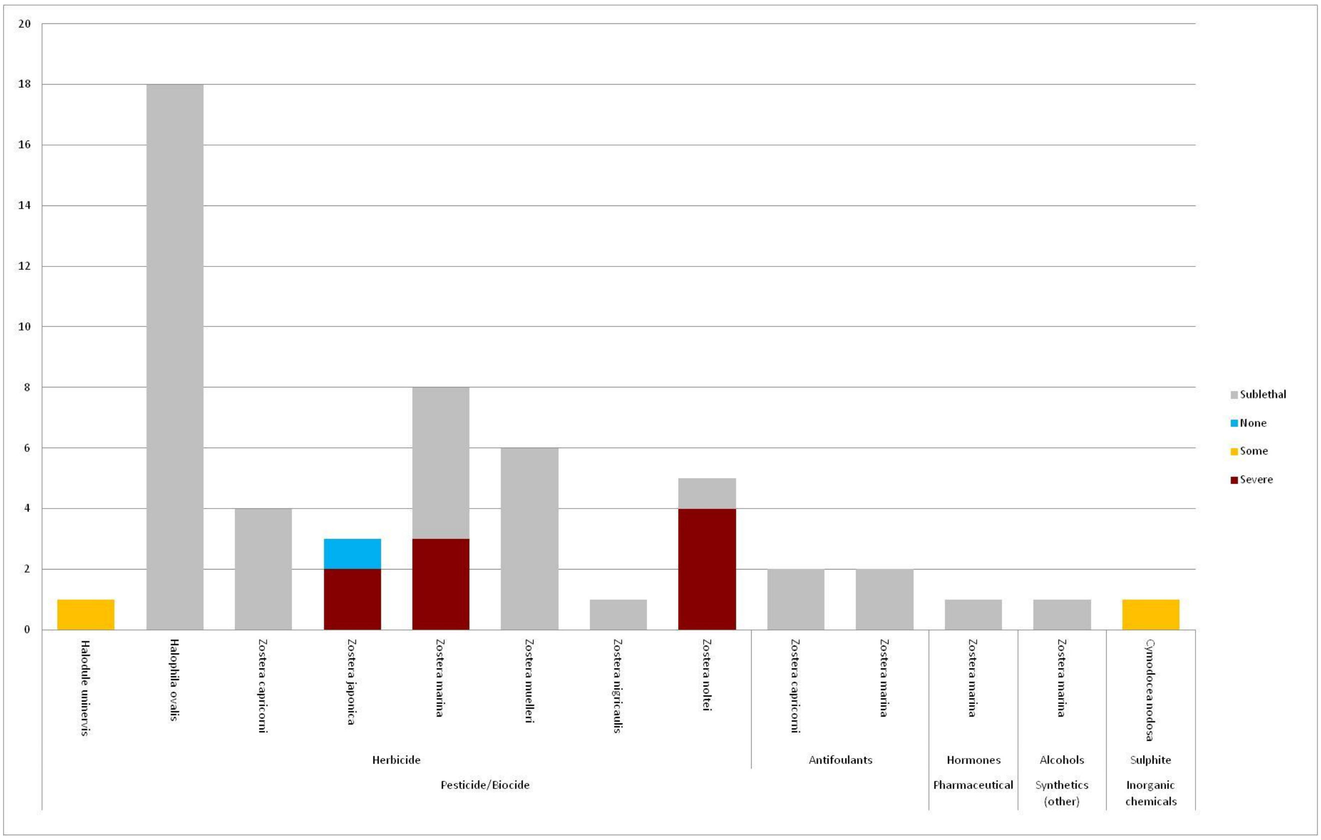


Figure 1.7. Count of ranked mortalities due to exposure to 'synthetic compounds' in seagrass species. Mortality is ranked as follows: 'Severe' (>75%), 'Significant' (25-75%), 'Some' (<25%), 'None' (no mortality reported), and 'Sublethal' effects. Note some articles are included more than once because they examined several different combinations of contaminant type and seagrass species.

- Carve *et al.* (2018) examined the effects of the grass (Poaceae) specific herbicide Fusilade Forte (Fluazifop) used to control Spartina, on *Zostera nigricaulis*. Fluazifop is an acetyl coenzyme-A carboxylase (ACCase) inhibitor. *Zostera* was exposed to 0.01- 10 mg/l Fluazifop under laboratory conditions for seven days followed by a seven-day recovery period. *Zostera nigricaulis* was resistant to its primary mode of action (ACCase inhibition) at ≥10 mg/l for seven days, but it did demonstrate significant physiological effects after seven days at ≥0.1 mg/l, such as a 72% reduction in photosynthetic pigment concentration and elevated lipid peroxidation.
- Chesworth *et al.* (2004) exposed *Zostera marina* to two herbicides, used in antifouling paints, under laboratory conditions. They examined photosynthesis rates and growth rates (as increases in leaf biomass). Irgarol 1051 was more toxic than Diuron with a LOEC for photosynthesis reduction of 0.5 µg/l and 1.0 µg/l, and an $EC_{50}$ of 1.1 and 3.2 µg/l respectively. A 40% reduction in photosynthesis occurred at 25 µg/l Diuron, while 25 µg/l Irgarol resulted in an 80% reduction, although it was not significantly different from 5 µg/l. The reduction in photosynthesis was most marked at lower concentrations. Growth was significantly reduced at 1 µg/l Irgarol and 5 µg/l Diuron. The application of the herbicides as mixtures did not result in significant further reduction in photosynthesis but the reduction was significant at lower concentrations. However, growth was further significantly reduced when Irgarol was added to Diuron but only at the lower concentrations. Overall, the LOEC for the mixtures was reduced to 0.5 µg/l. Chesworth *et al.* (2004) suggested that Irgarol was ca 3 times more toxic than Diuron but also noted that its effect plateaued over the 10-day experiment whereas Diuron did not, suggesting that Diuron was slower acting. They noted that the LOEC for significant reductions in photosynthesis and growth for both herbicides was 0.5 µg/l, which is lower than documented environmental levels. They also noted that the herbicides have environmental half-lives of 100 days, significantly longer than their 10-day study. They suggested

that *Zostera marina* in the vicinity of marinas and harbours could experience 50-65% reduction in photosynthesis and growth if exposed to reported levels of Irgarol and Diuron respectively (based on levels in Hythe Marina in 2001), more if exposed as mixtures (Chesworth *et al.*, 2004).

- Correll & Wu (1982) exposed submerged vascular plants (*Zostera marina, Potamogeton pectinatus, Zannichellia palustris,* and *Vallisneria americana*) to Atrazine in sediment under laboratory conditions. They reported that photosynthesis was inhibited in *Zostera marina* and *Potamogeton pectinatus* by 650 µg/l Atrazine but stimulated by 75 µg/l. They suggested that sensitivity to Atrazine in these plants was best determined after long-term exposure of 30-40 days.
- Delistraty & Hershner (1984) examined the effect of Atrazine on the biochemistry (AMP, ADP, and ATP), adenylate energy charge, productivity and mortality in *Zostera marina*, under laboratory conditions. They reported that total adenylates were reduced at 10 ppb and 100 ppb after six hours, while net productivity was reduced at 100 ppb but not 10 ppb. Long-term (21 days) exposure resulted in growth inhibition at 0.1 - 10 ppb and 50% mortality occurred at 100 ppb and 100% mortality was observed at 1000 ppb after 21 days.
- Diepens *et al.* (2016) examined the effects of mixtures of herbicides (15% Atrazine, 15% Irgarol, 15% diuron, and 55% S-metolachlor) at environmentally relevant levels on three biomarkers in *Zostera noltei*, photosynthetic efficiency, gluthionone reductase activity and photosynthetic pigment composition after 6, 24, and 96 hours. Exposure to the herbicide mixtures resulted in a slight but not significant reduction in glutathione reductase activity. Short-term exposure to the mixtures significantly affected photosynthetic efficiency and pigment composition, with ca 100% inhibition of photosynthesis at the two highest concentrations (100 & 1000 µg/l). $EC_{10}$ and $EC_{50}$ values decreased as the duration of exposure increased. Pigment composition was affected after six hours with a NOEC of 1 µg/l. An $EC_{10}$ at 2 µg/l was reported for photosynthetic efficiency. They concluded that there were no potential short-term impacts of the mixtures studied along the French Atlantic coast but noted that chronic effects at low concentrations of pesticides were likely to reduce the resilience of seagrass beds to other pressures.
- Flores *et al.* (2013) examined the effect of four herbicides (Diuron, Tebuthiuron, Atrazine, and Hexazione) on photosynthetic efficiency in four species of Australian seagrasses, inc. *Zostera muelleri*. Photosystem II (PSII) inhibition was measured. The time taken to for exposure to each herbicide to reach maximum inhibition (90%) was estimated in 24-hour experiments. Subsequent dose response experiments were based on 72-hour experiments to determine $IC_{10}$, $IC_{20}$, and $IC_{50}$ values for the four herbicides in *Zostera muelleri* and *Halodule uninervis*. All four herbicides caused 90% inhibition within four hours although the response rate to Hexazione was slower. Diuron was the most potent inhibitor of photosynthesis. Inhibition of PSII would eventually result in starvation in the affected plants. However, no significant reduction in growth rate was observed, probably due to the short duration of the study (Flores *et al.*, 2013). The authors noted that Diuron and Tebuthiuron inhibited photosynthesis by 20% and Atrazine and Hexazione by 10% at concentrations below those set for environmental protection in the Great Barrier Reef management plan (GBRMPA 2010; Flores *et al.*, 2013).
- Gao *et al.* (2011) examined the effect of Atrazine on seedlings and adult plants for four weeks under controlled conditions in outside aquaria. That reported that Atrazine significantly reduced plant fresh weight and chlorophyll concentration at 10 µg/l and resulted in 86.67% mortality at 100 µg/l. Mortality occurred in the controls (ca 9%) and at 1 µg/l (ca 15%) and 10 µg/l (ca 48%) but was only significant at 100 µg/l (ca 86%). All concentrations of Atrazine (2, 4, 8, 16, 32, 64 µg/l) significantly depressed photosynthesis within two hours in short-term experiments, and remained depressed at a lower level in adult plants. They concluded that Atrazine was more toxic to seedlings than to adults.

- Haynes *et al.* (2000) examined the effect of Diuron on photosynthesis in three Australian seagrass species, including *Zostera capricorni*, in five-day exposure studies. Exposure to 10 and 100 µg/l Diuron inhibited photosynthesis within two hours of exposure in all three species. Photosynthesis was significantly depressed after five days exposure to all concentrations of Diuron (0.1-100 µg/l) in *Halodule ovalis* and *Zostera capricorni* but only at the higher concentrations (10-100 µg/l) in *Cymodocea serratula*. Exposure to 10 and 100 µg/l inhibited photosynthesis by 50-75% in all three species and exposure to 0.1 and 1 µg/l inhibited photosynthesis by 10 and 30% in *Halodule ovalis* and *Zostera capricorni* respectively after five days. Inhibition remained after five days recovery from exposure to 10 and 100 µg/l Diuron (Haynes *et al.*, 2000).
- Hershner *et al.* (1982) studied the effects of Atrazine on *Zostera marina* in the Virginia waters of Chesapeake Bay, USA, using a mixture of field survey, *in situ* and greenhouse studies. They reported that field exposure was less than 1 ppb Atrazine and even in worst-case situation exposure to >1 ppb was short-term (1 week or less). Field experiments showed that 1000 ppb Atrazine reduced productivity in *Zostera* (measured as oxygen production) but that 100 ppb or less did not provide statistically significant results. Long-term exposure to Atrazine (21 days) in greenhouse experiments resulted in morphological effects at >60 ppb but, again, there was considerable variation between treatments. Short-term (six hours) Atrazine exposure reduced adenylate concentrations but 21 days exposure to 0.1, 1, and 10 ppb resulted in sublethal stress (change in adenylate concentrations). They suggested that *Zostera* could withstand >21 days exposure to low concentrations of Atrazine (≤10 ppb) but higher levels (100 & 1000 ppb) caused physiological changes.
- Macinnis & Ralph (2003) examined the effect of photosynthesis efficiency in *Zostera capricorni* exposed to three herbicides (Atrazine, Diuron, Irgarol) under controlled conditions in the laboratory and in the field. Photosynthesis was severely impacted by all three herbicides in the laboratory after 10 hours at both of the concentrations studied (10 and 100 µg/l), and most treatments did not recover after four days. In the field, Diuron and Irgarol severely affected photosynthesis whereas samples recovered completely from Atrazine exposure at the same concentrations.
- Major *et al.* (2004) examined the effects of a herbicide (isopropylamine salt or Glyphosphate) used to control *Spartina* on *Zostera japonica* in Willapa Bay, Washington, USA. *Spartina* clones were treated with mowing and single hand-spray application of herbicide, another two hectares were treated from the air, and the effect on *Zostera* shoot density and abundance adjacent to the treated areas monitored for one year. They reported that single hand spraying of *Spartina* did not affect *Zostera* at two sites and at the third site, shoot densities were consistent across the treatments. Aerial spraying reduced shoot density and percentage cover at two of three distances from treatment but that the reductions were greater in controls. They concluded that the potential threat to *Zostera* from *Spartina* itself was greater than that from the control measures.
- Negri *et al.* (2015) exposed seagrasses (*Halodule uninervis* and *Zostera muelleri*) to 0.3-7.2 µg/l Diuron, in a flow through system, for 79 days followed by a 14-day recovery period in uncontaminated water. They examined the effects on photosynthesis, PSII function, carbon assimilation, energy reserves, and growth. Photosynthetic efficiency was significantly inhibited and PSII was inactivated in both species at 0.3 µg/l Diuron during the 11-week exposure. No significant effect on total chlorophylls was observed. However, significant mortality and reductions in growth were only observed at 7.2 µg/l Diuron. However, there was significant reduction chlorophyll *a:b* ratios in both species: 12% at 1.7 µg/l and 19% at 7.2 µg/l in *Halodule uninervis*, and 10% at 7.2 µg/l in *Zostera muelleri*. Growth was reduced by 22% in *Halodule uninervis* and 23% in *Zostera muelleri* after 11 weeks at 7.2 µg/l Diuron but was not significantly different from controls after the 2-week recovery period. Shoot mortality was highly variable but Negri *et al.* (2015) noted a 22% reduction in shoots of *Halodule uninervis* at 7.2 µg/l for 11 weeks and 33% in *Zostera muelleri* at 1.7 µg/l for 11 weeks. Negri *et al.* (2015) reported that the health of the seagrasses was significantly impaired after

prolonged exposure to lower concentrations. They noted that carbon assimilation was reduced (C:N ratio dropped at 0.6 µg/l Diuron, and delta $C^{13}$ was reduced in leaves at 1.7 µg/l Diuron) and energy reserves (as starch) were approx. halved at and above 1.7 µg/l Diuron. Photosynthetic capability recovered after two weeks, except in samples from the highest concentration (7.2 µg/l) that exhibited chronic damage to PSII. They concluded that, although seagrasses may survive prolonged exposure to Diuron, exposure to ≥0.6 µg/l Diuron resulted in impacts to their energetic status that could increase their vulnerability to other stressors.

- Nielsen & Dahllof (2007) examined the effects of two pesticides (Glyphosphate and Bentazone) and the artificial auxin hormone MCPA on *Zostera marina* under controlled conditions. Glyphosphate had no significant effect on relative growth (length) or chlorophyll *a:b* ratios between 0.1 - 100 µM after three days but 10 µM (neither higher nor lower) did significantly stimulate growth by weight. Bentozone significantly reduced growth by weight at 10 µM, reduced chlorophyll *a:b* ratio above 0.1 µM, and reduced the RNA:DNA ratio at 10 µM. No significant effects for MCPA were observed. Two experimental mixtures of all three substances significantly reduced growth (by 57-65%) relative to control and strongly reduced both chlorophyll *a:b* and RNA:DNA ratios. Nielsen & Dahllof (2007) concluded that the herbicides and MCPA affected *Zostera* but that the effect of mixtures was greater, as mixtures reduced all the measured end points by nearly 50% of the controls.
- Patten (2003) examined the effect of herbicides (Imazapyr and Glyphosphate) on *Zostera japonica* in the field. Direct spraying of dry plants resulted in mortality. Imazapyr had the greatest impact with a reduction in cover of ca 0-98% after spraying with 0.84 or 1.68 ae kg/ha on a dry canopy (at sites higher on the shore), while Glyphosphate caused a 0-72% reduction in cover after spraying with 3.63 or 14.4 ae kg/ha. Plants were sprayed with 7.5 ae kg/ha Glyphosphate or 1.68 Imazapyr ae kg/ha in separate plots in separate experiments. However, the herbicides had no observable effect on cover when sprayed onto wet seagrass plants and the seagrass had recovered cover within 12 months. The author concluded that the potential effect of over spraying herbicides (used in the treatment of *Spartina*) on *Zostera japonica* was minor and short-term (Patten, 2003).
- Scarlett *et al.* (1999) examined the effect of the pesticide Irgarol 1051 on the growth rate and photosystem II synthetic efficiency in *Zostera marina* in laboratory conditions. *Zostera* was exposed to concentrations of Irgarol 1051 from 0 to 25 µg/$dm^3$. A comparison of leaf specific biomass ratios were used to assess the growth rate. A significant reduction in growth rate was found in specimens exposed to concentrations of Irgarol 1051 equal to equal to or above 10 µg/$dm^3$. The dry leaf $EC_{50}$ value was interpolated to be 1.1 µg/$dm^3$. Fluorescence induction kinetics was used to assess photosynthetic efficiency, which was significantly reduced by about 10% at 0.18 µg/$dm^3$with a 10-day $EC_{50}$ value of 2.5 µg/$dm^3$ and a 36-day $EC_{50}$ value of 0.2 µg/$dm^3$. Scarlet *et al.* (1999) concluded the loss of the photosynthetic efficiency could potentially lead to an energetic cost for the plants ability to cope with other stressors with plants situated close to marinas or in areas of high boat density leading to higher concentrations of Irgarol 1051 due to have the be the most affected. In areas with constant high exposure to Irgarol 1051, *Zostera* beds are likely to become damaged and cause stress in plants.
- Schwarzschild *et al.* (1994) examined the effect of Atrazine on *Zostera marina* via root/rhizome exposure in the laboratory. No significant effects on chlorophyll content, growth, or mortality were reported at Atrazine concentrations of 0-2.5 mg/l for 40 days. Concentrations were increased but no significant effects were seen on growth in rhizomes/roots at 7.5 mg/l after 15 days. But in static whole plant experiments, no new growth was observed ≥1.9 mg/l after 10 days. They reported, but did not specify, mortality in whole plants at ≥1.9 mg/l Atrazine. They concluded that *Zostera marina* was not susceptible to groundwater exposure to Atrazine and that Atrazine was not responsible for the declines of seagrass seen in Chesapeake Bay. They noted that the concentrations used in their

experiments were much higher than those likely to be found in the environment and that *Zostera* leaves/shoot were more susceptible to Atrazine than its rhizomes/roots.

- Wilkinson *et al.* (2015) examined the effect of 10 photosystem II (PSII) inhibiting herbicides individually and in mixtures on *Halophila ovalis* under laboratory conditions. They determined the acute toxicity of the herbicides (Diuron, Fluometron, Tebuthiuron, Atrazine, Ametryn, Metribuzin, Simazine, Prometryn, Bromacil, and Hexazinone) and mixtures (50:50 v/v Atrazine/Diuron; 10%v/v all ten herbicides) based on the inhibition of photosynthesis after exposure to 0-1000 µg/l for 24 and/or 48 hours. The herbicides showed a range of toxicities and inhibited photosynthesis by 50% at concentrations between 3.5 µg/l (Ametryn) and 132 µg/l (Fluometuron). After 24 hours, Diuron was the most potent and Fluometron the least. Maximum inhibition of PSII was reached within 24 hours except for Ametryn, Metribuzin, Prometryn, and Hexazinone, which took 48 hours to reach maximum inhibition. Binary mixtures of Atrazine and Diuron and mixtures of all 10 herbicides tested were largely additive in effect. They noted that inhibition of photosynthesis efficiency in turn led to reduced growth and mortality in seagrass. They concluded that low concentrations of PSII herbicides had the potential to affect ecologically relevant end points in seagrass.

### 1.3.2 Seagrass – pharmaceuticals

Only one paper (Nielsen & Dahllof, 2007 above) reported on the effects of a pharmaceutical on seagrasses, the artificial auxin hormone MCPA on *Zostera marina.* No significant effects for MCPA were observed.

### 1.3.3 Seagrass – other synthetics

Jebara *et al.* (2021) examined the concentrations of phthalate plasticizers (PAEs) and non-phthalate plasticizers (NPPs) in the water, sediment, seagrass and fish along the Tunisian coast. NPPs were more abundant than PAEs with DEHP and DEHT the most common. Sediment was more contaminated than water. Seagrass accumulated the plasticizers (DEHT = 9.11 and 23.2 µg/g and DEHP = 0.762 and 1.77 µg/g). *Posidonia oceanica* and the fish *Sparus aurata* had a low capability to accumulate plasticizers. The highest concentration was close to human sources, depending on coastal currents and varied with season due to runoff. The study focused on bioaccumulation and no mortality was observed or examined.

### 1.3.4 Seagrasses – inorganic chemicals

Portillo *et al.* (2014) examined the effects of sodium metabisulphite (SMBS), used to disinfect reverse osmosis systems, in the hypersaline effluent of a desalination plant in the Canary Islands on the adjacent *Cymodocea nodosa* seagrass bed. They examined the dispersal in the field and the effects in the laboratory. Seedlings reared in the lab were exposed to 0 or 100 ppm SMBS at normal (36 psu) and hypersaline (39 psu) conditions in 40 min pulses, once a week for 25 days. The increase in salinity did not significantly affect seedling survival. However, SMBS exposure significantly affected seedling survival and numbers decreased by 9-13%. SMBS also had a major effect on leaf elongation rates and proportion of necrotic leaf surface, accounting for 63-67% of total variance. Increased salinity significantly reduced leaf elongation rates (7.3%) and increased necrotic tissue (38.9%), while SMBS treatments consistently reduced leaf elongation rates (11-15%) and increased necrotic tissue (38-56%). Total mean surface area of shoots was 13.6% lower at 36.8 psu than 39 psu. SMBS caused a significant (22.7%) decrease in mean total leaf surface area at 36.8 psu with no additive difference at 39 psu. Portillo *et al.* (2014) concluded that the 39 psu salinity explained the exclusion of seagrass from the vicinity of the brine discharge in the field as *Cymodocea nodosa* was limited by the 39 psu isoline. They also concluded that exposure to SMBS affected significantly the survival and vitality of seagrass seedlings, probably as SMBS reduces the pH and dissolved oxygen concentration of the water column, and that its effect was greater under hypersaline conditions.

### *1.3.5 Sensitivity assessment – Synthetic compounds*

The effects of herbicides were examined in 92% of the results in the evidence review of pesticides and the antifoulant (pesticide) Irgarol was examined in the remaining 8% of results. The number of articles that report mortalities due to synthetic contaminants are *summarized* in Figure 1.7 and in Table 1.4 below.

Table 1.4**.** Summary of count of ranked mortalities to synthetic contaminants reported in the evidence review and resultant proposed sensitivity assessments for seagrass species, with specific reference to *Zostera* spp. (N= None, VL= Very low, L= Low, M= Medium, High = High, and NS= Not sensitive).

| **Count of Worst case mortality** | | | **Worst case mortality** | | | | | **Assessments** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Group** | **Contaminant** | **Species name** | **Severe** | **Some** | **None** | **Sublethal** | **Total** | **Resistance** | **Resilience[6]** | **Sensitivity** |
| **Pesticide/Biocide** | | | | | | | | | | |
| | **Herbicide** | *Halodule uninervis* | | **1** | | | 1 | M | ?? | M |
| | | *Halophila ovalis* | | | | 18 | 18 | H | ?? | NS |
| | | ***Zostera capricorni*** | | | | 4 | 4 | H | H | NS |
| | | ***Zostera japonica*** | **2** | | 1 | | 3 | N | VL | H |
| | | ***Zostera marina*** | **3** | | | 5 | 8 | N | VL | H |
| | | ***Zostera muelleri*** | | | | 6 | 6 | H | H | NS |
| | | ***Zostera nigricaulis*** | | | | 1 | 1 | H | H | NS |
| | | ***Zostera noltei*** | **4** | | | 1 | 5 | N | L | H |
| | **Herbicide total** | | **8** | **1** | **1** | **33** | **43** | N | VL | H |
| | **Antifoulants** | ***Zostera capricorni*** | | | | 2 | 2 | H | H | NS |
| | | ***Zostera marina*** | | | | 2 | 2 | H | H | NS |
| | **Antifoulants Total** | | | | | **4** | **4** | | | |
| **Pesticide/Biocide Total** | | | **9** | **1** | **1** | **39** | **50** | N | VL | H |
| **Pharmaceutical** | | | | | | | | | | |
| | **Hormones** | ***Zostera marina*** | | | | 1 | 1 | H | H | NS |
| | **Hormones Total** | | | | | **1** | **1** | H | H | NS |
| **Pharmaceutical Total** | | | | | | **1** | **1** | H | H | NS |
| **Synthetics (other)** | | | | | | | | | | |
| | **Alcohols** | ***Zostera marina*** | | | | 1 | 1 | H | H | NS |
| | **Alcohols Total** | | | | | **1** | **1** | H | H | NS |
| **Synthetics (other) Total** | | | | | | **1** | **1** | H | H | NS |
| **Inorganic chemicals** | | | | | | | | | | |
| | **Sulphite** | *Cymodocea nodosa* | | 1 | | | 1 | M | ?? | M |
| | **Sulphite Total** | | | 1 | | | 1 | | | |
| **Inorganic chemicals Total** | | | | 1 | | | 1 | | | |
| **Total** | | | **9** | **2** | **1** | **41** | **53** | N | VL | H |

[6] Resilience is based on that of Zmar or Znol biotopes for Zostera marina and Zostera noltei respectively. The resilience of other Zostera spp. is assumed to be the same as Zmar for assessment purposes. Resilience is unknown for other seagrass genera (??), in which case the worst-case sensitivity is presented.

Herbicides are released into the water column via spraying and via runoff from agriculture or land management. In a couple of studies (Patten, 2003, Major *et al.*, 2004) the articles examined the effect of herbicides used to control *Spartina* in the past. Both studies concluded that the effect of the herbicide was limited and the potential effect of *Spartina* on seagrass beds was worse.

It is not surprising that most papers examined the effects of herbicides on photosynthesis and, hence, growth in seagrasses, as many herbicides specifically target the PSII of plants. The effects varied with concentration, duration of exposure, type of herbicide, seagrass species and mode of application. Nevertheless, 76% of the reported effects were sublethal, 'some' mortality was only reported in a single article and 'severe' mortality in seven articles (18% of reported effects). Therefore, the resistance to herbicides is probably '**None'** based on the examples of 'severe' mortality reported in the evidence review. Hence, an overall sensitivity of **'High'** is suggested for herbicides and pesticides in general. In addition, 72% of the reported effects of herbicides examined *Zostera* spp. and all the 'severe' mortality results were from studies of *Zostera* spp. Therefore, the assessment is probably made with **'High'** confidence.

This assessment agrees with Bester (2000) who reported high concentrations of pesticides in areas of the German Bight where seagrass beds had been destroyed, with the caveat that further experimental evidence was required, and that other contaminants might have been involved. However, several authors suggested that the sublethal effects on photosynthesis and growth would probably render the seagrass vulnerable to other adverse effects.

The remaining evidence on the effect of pharmaceuticals, and other synthetics was each limited to a single article in the review. *Zostera marina* was reported to be not affected by exposure to methanol but only as a control in a study on the effects of herbicides (Hershner *et al.*, 1982). The pharmaceutical study did not report any effect of the artificial auxin hormone on *Zostera marina*. However, no evidence on the effect of human pharmaceuticals or maricultural or agricultural chemotherapeutics was found. Therefore, *Zostera marina* is probably **'Not sensitive'** to these contaminants but with **'Low'** confidence due to the limited evidence recovered.

The one remaining study (Portillo *et al.*, 2014) examined the effect of a disinfectant (SMBS) in the effluent for a desalination plant on *Cymodocea nodosa* seagrass bed. They also concluded that exposure to SMBS effected significantly the survival and vitality of seagrass seedlings, probably as SMBS reduces the pH and dissolved oxygen concentration of the water column, and that its effect was greater under hypersaline conditions. But it was the hypersaline conditions (39 psu) that excluded the seagrass from the vicinity of the discharge.

Overall, resistance to the effect of 'synthetics' contaminants on *Zostera* spp. is assessed as **'None'** so that *Zostera* spp. beds (Zmar and Znol) are assessed as **'High'** sensitivity, although the weight of evidence is based on the effect of pesticides and, in particular, herbicides. The evidence on other types of synthetic contaminants is limited so that overall confidence is assessed as **'Medium'**.

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