Marine Life Information Network
Marine Biological Association of the United Kingdom

MARLIN – MARINE LIFE INFORMATION NETWORK

SENSITIVITY ASSESSMENT OF CONTAMINANT PRESSURES - APPROACH DEVELOPMENT, APPLICATION, AND EVIDENCE REVIEWS

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To:

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

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EXECUTIVE SUMMARY

Phase 1 of the project explored the range of chemical contaminants likely to affect marine habitats, examined the use of environmental standards (i.e. EQS, ERLs etc) and 'mixing zones' as potential benchmarks of chemical contamination, and oil and chemical spills response guidance as benchmarks for incidental spills and discharges.

Phase 1 concluded that it was difficult to see how a quantified value or scenario would function as a quantified benchmark for sensitivity assessment. The mechanisms whereby any individual species is exposed to any individual chemical is complex, and varies depending on the behaviour of chemicals in the environment, their mode of action and toxicity, as well as the nature of the receiving environment. Phase 1 concluded that:

1. a 'weight of evidence' approach was used and sensitivity to exposure to any given chemical assessed based on the reported levels of resultant mortality, as used for pressures such as 'abrasion', 'penetration' and 'introduction of non-native species';
2. sensitivity assessments were supported by a description of the relevant evidence on the method/route of exposure, and evidence from laboratory studies (e.g. LC/EC_{50}s) and observational studies where available;
3. the chemical behaviour of the chemical included in our pressure groupings ('hydrocarbons', 'synthetics', 'transitional metals', and 'others') were recorded/examined to identify those unlikely impact benthic species (e.g. 'evaporators'), and those likely to have physical (e.g. smothering, clogging) and/or chemical effects (e.g. toxicity);
4. sensitivity to physical and chemical effects were scored separately where needed, e.g. oil spills;
5. detailed Rapid Evidence Assessments (REAs) were used to record the details (meta-data) of the evidence used to support sensitivity assessment, the evidence summaries', in a separate spreadsheet;
6. a meta-analysis to 'rank' marine benthic species or taxonomic groups by their responses to chemical contaminants should be investigated; and
7. the resultant dataset should be provided online as an additional resource to SNCBs.

Phase 2 trialled the 'weight of evidence' approach and applied the standard REA methodology (Collins *et al.*, 2015) using *Mytilus* spp. as the test species and a focus on 'Hydrocarbons & PAHs'. Phase 3 completed the *Mytilus* spp. REAs for 'Transitional Metals' and 'Synthetic compounds' and included REAs for the 'contaminant' pressures in *Zostera* spp. and seagrasses. The results were as follows.

1. A list of chemical contaminants likely to occur in the marine environment was developed and attached to this report.
2. The existing pressure definitions for each of the contaminant pressures ('hydrocarbons', 'synthetics', 'transitional metals', and 'others') were revised.
3. A detailed REA protocol was developed and applied to the test species.
4. Detailed evidence for each of the contaminant pressures are provided in the relevant 'evidence summary' spreadsheets that accompany this report for both *Mytilus* spp. and Seagrasses.

5. Detail summaries of the relevant evidence on the effects of a range of contaminant types on both *Mytilus* spp. and Seagrasses were provided with the report.
6. The likely resistance, and hence sensitivity, of both *Mytilus* spp. and Seagrasses to the different contaminant types within each contaminant pressure were presented.

The development of the approach, the use of the REA protocol, evidence summaries, and resultant sensitivity assessments are discussed.

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1 Introduction

MarLIN (Marine Life Information Network) was tasked with assessing the sensitivity of benthic marine habitats in UK waters to contaminant (pollutant) pressures for inclusion in the MarESA (Marine Evidence-based Sensitivity Assessment) database and website. The MarESA approach is summarized on the MarLIN website (https://www.marlin.ac.uk/sensitivity/sensitivity_rationale) and explained in detail in MarESA Guidance Manual (Tyler-Walters *et al.*, 2018). All the terms used in sensitivity assessment, are defined and explained in detail in the above 'Manual' and summarized in the following report where needed.

The following report outlines the methodology used to develop an approach prior to its testing, its subsequent amendment, and application to example habitats or species. The resultant 'Rapid Evidence Assessments' are presented, together with recommendations for further development prior to the application of the proposed approach to further species and habitats.

1.1 Aim of the project

The project aimed to support an improved understanding of benthic habitat sensitivity (or risk), based on marine biotopes of the UK MHC (UK Marine Habitat Classification) and EUNIS (European Union Nature Information System) Habitat classifications. Sensitivity assessments are used to underpin MPA condition assessments and advice on operations, for example, they are used within the Natural England's (NE) designated sites system and the Scottish FeAST (Feature Activity Sensitivity Tool) tool to indicate MPA (Marine Protected Area) feature sensitivity to marine activities and associated pressures.

The current MarESA (Marine Evidence-based Sensitivity Assessment) approach ranks the 'relative' sensitivity of marine species and habitats against a standardised and specified quantitative or qualitative 'benchmark' level of exposure for each pressure assessed. All pressures are clearly defined based on the ICG-C (Intercessional Correspondence Group on Cumulative Effects) (OSPAR, 2011).

The benchmarks are based on those developed by MarLIN and MB0102 (Tillin *et al.,* 2010; Tillin & Tyler-Walters, 2014a&b) (see Tyler-Walters *et al.*, 2018). All sensitivity assessments are made against a standardised 'benchmark' level of each pressure. For example for temperature changes the benchmark used is 'a 5°C increase in temp for a one month period, or 2°C for one year'.

The MarESA dataset includes the following 'Contaminant' pressures:

- 'Transition elements & organometals' contamination;
- 'Hydrocarbon & PAH' contamination;
- 'Synthetic compound' contamination;
- 'Introduction of other substances' (solid, liquid or gas), and
- 'Nutrient enrichment'.

The benchmarks for the contaminant pressures are set to existing chemical/pollution standards levels, that is, 'Compliance with all AA EQS, conformance with PELs, EACs, ERLs' for chemical contaminants and 'Compliance with WFD criteria for good status' for 'Nutrient enrichment'. However, because these environmental standards have been set with the intention of avoiding environmental harm, nothing is therefore 'sensitive' to these pressures when 'compliance with all environmental standards' is used as a 'benchmark'.

The MarLIN Steering Committee[1] wanted these assessments updated to enable their use in identifying sensitive habitats within MPAs or other areas where unregulated events and pollution incidents may occur that exceed the EQS thresholds, for example an accidental chemical or oil spill, or discharge of excess fertilizer, so that SNCBs can advise on management and site recovery. MarLIN was, therefore tasked with developing new pressure definitions and benchmarks for these contaminant pressures.

There is a strong need to ensure we integrate with other existing work on contaminants, so an expert group and workshop was formed to support this project. We were aware of existing work undertaken on chemical thresholds and assessments by groups such as the UK TAG CTT and CSSEG. However, the MarLIN work did not aim to develop new standards or guidelines and the sensitivity benchmarks do not relate to protection goals. Sensitivity assessments are used to provide an indication of biotope sensitivity to a range of different pressures.

1.2 Project methodology

The project was divided into a series of phases or tranches of work.

- Phase 1 – Scoping study, discussion, and development of approach
- Phase 2 – Test of the proposed approach on example habitat/species
- Phase 3 – Application of the approach to an example habitats/species

The outputs of each phase were discussed and amended by the MarLIN Contaminants Expert Group[2] and the MarLIN Steering Committee. The project was initiated on 3rd December 2019 with a first 'experts' workshop and developed over the following timeline:

- initial contaminants pressures workshop 3rd December 2019;
- scoping study based on first contaminants workshop (above);
- work commenced in earnest with the second contaminants workshop in 16th November 2020;
- further study of 'Allowable zones of Effects' and the 'Tiered' approach to spills (oils and chemical) and pollution incidents;
- agreed list of chemicals to be included (scope) circulated 11th January 2021;
- further recommendations on benchmarking circulated on 20th January 2021;
- Steering Committee meeting to discuss recommendations, with invited experts, 10th February 2021;
- Phase 2 of the project – the 'testing of the approach' phase was completed and reported to the last Steering Committee and 'Contaminants' Expert group' in September 2021;
- a further test of the literature review was undertaken in October 2021;
- Phase 3 of the project focused on the completion of the *Mytilus* spp. Rapid Evidence Assessment (REA) for 'Metals' and 'Synthetics', and an additional REA for *Zostera* spp., Based on the recommendations received and the further literature review;
- the 'Metals' section of the *Mytilus* REA drafted and circulated for comment (February 2022);
- the 'Synthetics' section of the *Mytilus* REA and the *Zostera* REA is completed (end March 2022), and

[1] The MarLIN Steering committee is composed of representatives of Dept. For Environment, Food and Rural Affairs (Defra), Dept. of Agriculture and Rural Affairs (DAERA), Joint Nature Conservation Committee (JNCC), Marine Scotland (MS), Natural England (NE), Natural Resources Wales (NRW), and NatureScot.

[2] Composed of representatives of the MarLIN Steering Committee and invited experts from Cefas (Centre for Fisheries and Aquaculture Sciences), SEPA (Scottish Environmental Protection Agency), EA (Environment Agency), academia and consultancy.

- the final report circulated for comment (early April 2022).

In the report below, each Phase of the project is presented separately, to illustrate the different approaches discussed and how the proposed approach to assess the sensitivity of marine habitats to ‘contaminant’ pressures was developed.

2 Phase 1. Development of the approach

A short 'scoping study' was conducted based on the outcomes of the contaminants pressures benchmark workshop (3rd December 2019). The 'scoping study' followed up the advice provided during the workshop.

2.1 Initial scoping study

The scoping study report (May 2020) aimed to highlight the potential scale of the task and the information needs required to create the contaminant pressure benchmarks and modify (if required) the current MarESA sensitivity approach for the assessment of the sensitivity of marine habitats (biotopes) to a range of contaminants. The report also identified several questions about the proposed application of the assessments that needed to be addressed by the SNCBs and competent agencies before the work begins.

Summary of findings

The main suggestions and questions that arose from the scoping study and are discussed in detail below.

1. We need to agree the range of chemical contaminants to be addressed within the project, and agree the specification for the project.
2. The categories of chemicals (i.e. hydrocarbons, synthetics, heavy metals, and others) should be checked and revised if necessary.
3. The pressure 'Introduction of other substances' requires a clear definition.
4. The current benchmark 'compliance with all environmental standards' does not provide information for management decisions and should be excluded.
5. Further expert input from the competent agencies is required to set benchmarks for:
    a. concentrations within 'mixing zones' (e.g. 10x, 100x, 1000x EQS); and
    b. spills, especially chemical spills other than oil.
6. Bioaccumulation is best assessed as part of the level of exposure (under point 6 above) and a separate benchmark is not required.
7. The habitats (biotopes) should be grouped by similar structural / functioning species (or species group) to streamline the literature review.

2.1.1 Range of chemical contaminants

How many chemical contaminants do we 'want' or 'need' to address?

Ideally, we 'wanted' to examine the effects of any chemical contaminant that was known to or had the potential to adversely affect marine life and, hence, marine habitats (biotopes). Alternatively, we may only 'need' to assess those chemicals that are identified as PBT (Persistent, Bioaccumulative, or Toxic) and listed as Priority Chemicals under relevant Directives (PSD[3], WFD[4], MSFD) or conventions (OSPAR[5][6]). There are 45 chemicals listed on the PSD list, 29 on OSPAR's list of priority chemicals and ca 264 on

[3] PSD (Priority Substances Directive) - https://ec.europa.eu/environment/water/water-framework/priority_substances.htm
[4] WFD (Water Framework Directive) - https://ec.europa.eu/environment/water/water-framework/index_en.html
[5] OSPAR - Chemicals for priority action - https://www.ospar.org/work-areas/hasec/chemicals/priority-action
[6] OSPAR –List of substances of potential concern - https://www.ospar.org/work-areas/hasec/chemicals/possible-concern/list

OSPAR's list of chemicals of potential concern. However, the OECD AOP (Adverse Outcome Pathways) Portal[7] suggests there are ca 100,000+ chemicals in the environment (not limited to aquatic or marine).

We assumed that we would need to include 'legacy' chemicals (e.g. heavy metals, pesticides, and antifoulants) as well as emerging chemicals (e.g. pharmaceuticals, novel biocides, novel antifoulants, and nanoparticulates). However, we suggested that 'algal toxins', derived from harmful algal blooms (HABs) should be addressed under a separate pressure.

How do we group chemical contaminants?

At present, we have separate 'pressure definitions' for:

- 'Hydrocarbons and PAHs';
- 'Synthetic compounds (inc. pesticides, antifoulants, pharmaceuticals)';
- 'Transition elements and organo-metals';
- 'Radionuclides';
- 'Introduction of other substances (solid, liquid, gas)'; and
- 'Nutrient enrichment'.

These categories are based on the OSPAR Intercessional Correspondence Group on Cumulative Effects (ICG-C) (OSPAR, 2011) categories and mirror chemical categories used in the lists of priority substances and substances of potential concern.

Therefore, we suggested that we continue to use these categories but with a few minor changes.

1. TBT and other antifoulants should be included under 'synthetics' and not 'metals' (as decided at the expert workshop in December 2019).
2. 'Introduction of other substances' requires a clear definition.

The 'Introduction of other substances' pressure is unclear at present. It refers to 'produced waters' e.g. from the oil and gas industry but otherwise acts as a 'catch-all' for contaminants that do not fit anywhere else.

Literature review

Clearly, the greater the number of pressures, and the range of chemicals under each pressure we address, the larger the literature review required and the greater the number of papers documented as the basis for each assessment; and the longer the time required to complete the assessments.

In practice, MarLIN operates a 'tiered' approach to literature review. For example, in any one habitat, for each of the species (or species group) 'that are indicative of sensitivity'[8] within each biotope and/or particular habitat (rocky shores, seagrass beds, etc), we search for:

1. direct evidence of adverse effects of each category of contaminant in turn (i.e. hydrocarbons, PAHs, metals etc.);
2. direct evidence of adverse effects of each chemical within each category (e.g. Pb, Cd, Hg etc.).
3. evidence of adverse effects of each chemical or category of chemical on the taxonomic group or functional groups within the habitat (e.g. Crustacea, Mollusca, Echindodermata etc.), and

[7] OECD (Organisation for Economic Co-operation and Development) - AOP (Adverse Outcome Pathways) Portal - https://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm

[8] See section 4 of the MarESA manual for definition - https://www.marlin.ac.uk/assets/pdf/MarESA-Sensitivity-Assessment-Guidance-Rpt-Dec2018.pdf.

4. evidence of adverse effects of each chemical or category of chemical on similar habitats in the UK, NEA (North East Atlantic) or worldwide.

Priority is given to evidence of direct effects of a particular contaminant, or category of contaminant on the species or habitat of interest, as reported in the literature. For example, the results of significant oil spills on temperate rocky shores are well documented (e.g. *Exxon Valdez*, *Sea Empress*, *Amoco Cadiz* spills). Reports of the effects of pollution incidents on relevant habitats, and experimental exposures will be the priority source of evidence, although may be limited to legacy contaminants.

Where information on particular species and habitats is lacking then we will use proxies, such as the reported effects of contaminants on similar species or taxonomic groups.

The recent work on Adverse Outcome Pathways (AOPS) may be particularly useful. AOPs identify MIEs (Molecular Initiating Events) by which the chemical in question interacts with common biochemical pathways within an organism that results in one or more adverse effect. The AOP process promises to be able to identify chemicals of similar structure and, hence, similar MIEs to inform their likely effect on organisms. Similarly, where biochemical pathways are conserved within taxonomic groups, the AOPs provide evidence on which to assess their potential risk to a range of organisms (Gunnarson *et al*., 2008, 2019; Hutchinson *et al.,* 2013; Coady *et al.,* 2018).

In May 2020, there were ca 284 AOPs for individual chemicals, which documented 2087 MIEs, in the OECD AOP Knowledge base (https://aopkb.oecd.org/). Hopefully, we can mine that database for relevant information. A short glance through a random sample suggests that the number of test organisms, on which the AOPs are based, is small. However, that is true of most toxicity studies.

Similarly, the detailed dossiers[9] on the development of Environmental Quality Standards (EQS) for a range of chemicals may also be a useful information resource.

An indication of the scale of the literature review is given in Table 2.1, which lists the number of literature items returned in one abstracting journal (Aquatic Science and Fisheries Abstracts; ASFA) using simple search terms based on the contaminant categories and classes chemicals on the priority chemical lists (see above). Clearly, some results would require further specific terms while others require further investigation, and the results are only based on a single abstracting journal. We routinely use Web of Science (a science citation index), Google Scholar, and other sources. However, Table 2.1 suggests that we could expect to sift through several thousand (perhaps many thousands) literature sources to review the effects of the suggested range of chemicals on the range of habitats in the MarESA dataset (see below).

Questions and issues

The following questions need to be discussed with the MarLIN Steering Committee and /or 'expert group' in order to help specify the scope of work for the 'contaminants project'.

1. Do we need to review the effects of every possible chemical within the present categories?
2. Is there a minimum list of chemicals we 'need' to address?
3. Is there a list of 'likely suspects' based on case work within the SNCBs and competent authorities (EA, SEPA, and MMO) on which we 'need' to focus?
4. Are there any chemicals on the priority lists we can ignore, as they are unlikely to affect coastal or marine environments?
5. Are we happy to exclude 'algal toxins' derived from HABs from the project?
6. How do we define 'introduction of other substances'?

[9] https://ec.europa.eu/environment/water/water-dangersub/lib_pri_substances.htm

Table 2.1. The number of literature items discovered (May 2020) using the listed search terms in Aquatic Science and Fisheries Abstracts (ASFA) with a date range of 1960-2020 is listed. Multiple search terms are separated by '/' as are the number of 'hits'. Please note the numbers are indicative, and large numbers of 'hits' would be reduced using more specific terms and low numbers of 'hits' would be routinely investigated further.

Pressure	Search terms	ASFA 'hits'
Transition elements & organo-metal contamination.	Metals	184,098
	Organometals	89[10]
	Pb (Lead)	89,208
	Cd (Cadmium)	45,560
	Hg (Mercury)	38,142
Hydrocarbon & PAH contamination.	Hydrocarbons	85,032
	PAHs / Polyaromatic hydrocarbons	10,294 / 1,961
	Oil / Petroleum	286,530 / 133,128
	LPG / Liquefied petroleum gas	2,959 / 3,321
Synthetic compound contamination (incl. pesticides, antifoulants, pharmaceuticals).	Pesticides/biocides	45,304 / 2,112
	Antifoulants	361[11]
	TBT / Tri-butyl tin	29 / 29[12]
	Pharmaceutical / 'Pharmaceutical AND toxicity'	26,848 / 857
	PCBs / Polychlorinated biphenyl	12,002 / 11,514
	Organohalogens	318
	Organochlorines	2,640
	Organophosphates	3,937
	Phenols	23,900
	Hormones	40,033[13]
	Oestrogens/Estrogens	7,426 / 7,426
	Endocrine disruptors	4,523
Introduction of other substances (solid, liquid or gas)[14]	Barite / 'Barium AND contamination'	4,101 / 87
Radionuclide contamination	Radiation	158,290
	Radionuclides	6,205
Nutrient enrichment	Eutrophication / 'Eutrophication AND marine' Nitrogen Phosphate	46,760 / 5,563 187,096 78,846

2.1.2 Pressures and benchmarks

At the expert consultation workshop in December 2019, it was agreed that a suite of benchmarks for each of the pressure categories would be pursued. These were:

- Compliance with environmental standards (e.g. EQS) (the present benchmark);
- Permitted localised levels of chemical exposure i.e. within 'mixing zones';

[10] Further investigation required
[11] Further investigation required
[12] Further investigation required
[13] Mainly hormonal function rather than hormones as a contaminant
[14] This category lacks a clear definition, at present.

- Exposure to chemicals due to accidental spills; and
- Exposure to increased levels of contaminant due to bioaccumulation.

'Nutrients' was an exception. It was decided to use the WFD categories (i.e. 'Good', 'Poor', 'Bad') as the basis of the benchmarks. No further discussion is required in this report.

Compliance with environmental standards (e.g. EQS)

This is the current benchmark used for all contaminant pressures. It results in a default assessment of 'Not sensitive' because the environmental standards are set at levels that are thought not to cause any adverse effect on marine life. However, the contaminant pressures are reported as 'Not assessed' to avoid any confusion and the implication that the species or habitats are not sensitive to the contaminants, rather than 'Not sensitive as defined under the benchmark' (i.e. the EQS).

The expert consultation workshop suggested that this benchmark was retained. However, we would suggest it is excluded because it provides no information on which to base a management decision. Sensitivity assessments are not 'absolute' values but are 'relative'. The sensitivity assessment approach aims to identify those species and habitats that are more sensitive than others to a given pressure so that the need for management action (intervention, protection, mitigation etc.) can be prioritised. If all habitats and species are scored the same (in this instance 'Not sensitive') then none can be prioritised and the assessment serves no purpose.

Permitted localised levels of chemical exposure i.e. within 'mixing zones'

'Mixing zones' allow the effluent or discharges from a given activity and/or outfall to 'mix' in the water column and dilute the effluent or discharge to safe limits, that is, to the relevant EQS once outside the defined 'mixing zone'. However, the presumption is that the permitted levels of chemical may exceed the relevant EQS within the 'mixing zone'. Detailed information on the identification of 'mixing zones' is given by European Commission (2010) and duplicated in guidance of thermal plumes (BEEMS, 2011). The process is complicated and several modelling techniques are suggested (SEPA, 2013).

In summary, the identification of 'mixing zones' should take into account:

- the physical and chemical nature of the effluent / discharge;
- the physical and chemical nature of the receiving environment (inc. freshwater, lakes, transitional or saline waters);
- the flow rate, speed and buoyancy of the effluent/discharge (inc. solids or sediment content);
- the flow rate or hydrography of the receiving environment (inc. bed topography), and
- the ratio of the concentration of the chemical(s) of concern to its (their) EQS(s); amongst others.

In short, the 'mixing zones' and the permitted concentrations of chemicals within them will be specific to the activity under consideration, the type and content of the effluent / discharge, and its location.

Sensitivity assessments are not 'site specific' but benchmarks are designed to represent the most likely 'level of effect' of a given pressure. The expert workshop suggested that we could set this benchmark at 10 and/or 100x the EQS.

The European Commission (2011) guidance on the derivation of EQS lists 'Assessment factors' (AFs), 'Assessment factors' typically range from 5 to 10,000 depending on the receiving environment (freshwater or saltwater, or sediment), the type of chemical, and the confidence on the source of toxicity data and its relevance to the receptor (water column, sediment or biota). 'Assessment factors' are used to account for uncertainty in the evidence by reducing the EQS value (concentration) by increasingly large AFs as uncertainty increases. In theory, we could back calculate the EQS and use the

AF relevant to saltwater and marine environments to suggest a range of benchmarks between 5x to 10,000x the EQS. Alternatively, we could use the MAC-EQS (Maximum allowable concentration – EQS).

There is growing evidence for the effects of chemicals on '–omics' (i.e. the transcriptome, proteome, or metabolome) of organisms (Veldhoen *et al.,* 2012). The limited evidence so far examined suggests that '-omics' detect the effects of low levels of chemicals in the environment, and may act as an early warning and improve chemical risk assessment. However, most of the effects are sublethal and would not be incorporated into sensitivity assessment, where the existing 'resistance' scale is defined by mortality within the population. The exception is where the chemicals adversely affect reproduction or development and, hence, lead to population decline.

Questions and issues

If we decide to use multiples of the EQS, it means that we would create a separate benchmark for each individual chemical within the priority substances list or for chemicals for which an EQS has been determined. However, EQSs do not exist for every chemical that might be of concern. Also, we could not apply this approach to categories of chemicals (see section 1) nor to 'proxies' based on evidence from similar taxonomic groups.

1. What range of multiples of the relevant EQS (10x, 100x, 1000x) are representative of the concentrations permitted in 'mixing zones'?
2. Are the range of chemical concentrations typically permitted in 'mixing zones' publically available?
3. Can the relevant competent authorities advise on typical chemical concentrations permitted and the size (i.e. extent, area) of 'mixing zones'?

Further expert input is required from the SNCBs and other competent bodies (EA, SEPA, and MMO). Also, if we pursue this approach we must make sure that it is both transparent and understandable by agency staff and comparable to existing guidance on water and sediment quality standards. We will also need to incorporate information on the different pressure categories of chemicals and 'proxies' for taxonomic groups.

2.1.3 Exposure to chemicals due to accidental spills[15]

Current oil spill guidance identifies three tiers to describe the size and scope of the response (API, 2014; IPIECA, 2015; Alison Brand pers. comm.).

Tier 1: Minor spills, including incipient spills that are quickly controlled, contained and cleaned up using **local** (onsite or immediately available) equipment and personnel resources. A Tier 1 spill would typically be resolved within a few hours or days.

Tier 2: Moderate spills requiring activation of **significant regional oil spill response resources**. A Tier 2 spill response may continue for several days or weeks.

Tier 3: Major spills requiring activation of large quantities and multiple types of response resources including those from **out of the region, and possibly international sources**. A Tier 3 spill response may continue for many weeks or months.

However, the tier levels are typically **not associated with the volume of oil spilled**. Nor is the type of oil spilt specified. It is the overall impact of the spill, not the quantity alone that dictates the types and amounts of resources required and the duration of cleanup operations (Alison Brand pers. comm.).

[15] Spills refer to accidental releases of chemical and not controlled deliberate releases or discharges. We will need to adopt or create a definition.

The EAs' CICS (Common Incident Classification Scheme) (EA, 2016) provides guidance on the definition of an incident, the physical response to the incident and categories of potential and actual impact. The guidance is generic and not limited to oil or other spills but any incident. The environmental impact categorisation is split into four categories (EA, 2016).

- Category 1 – major, serious, persistent and/or extensive impact or effect on the environment, people and/or property
- Category 2 – significant impact or effect on the environment, people and/or property
- Category 3 – minor or minimal impact or effect on the environment, people and/or property
- Category 4 – substantiated incident with no impact.

There is close similarity between these categories and the resistance scale used in MarESA. However, the scheme provides little information on the size and nature of the spills because it is a designed as a framework with which to make decisions. Specific operational guidance mentioned in the CICS document was not available.

Questions and issues

The effects (immediate damage and recovery) of large-scale oil spills are well documented (e.g. *Exxon Valdez, Sea Empress, Amoco Cadiz* etc.). However, it is likely that the effects of small-scale local spills are less well studied, outside experimental studies. Nevertheless, we could develop a number of descriptive, qualitative benchmarks to describe the scale and extent of an oil spill for use as a qualitative benchmark, similar to those suggested above. We would also need to include oil types (as different types or fractions of oil have different toxicities). However, we are not aware of detailed studies of other chemical spills at present and further research is required.

1. Are detailed studies of the effects of minor, local oil and fuel spills available?
2. Can the competent authorities provide information on the typical size and nature of local oil spills?
3. Can the competent authorities provide information on the typical size and nature of local chemical spills (other than oils) encountered in their casework?
4. Are there any chemical or categories of chemicals that are unlikely to be released as a 'spill' and can be excluded from this pressure?

Further expert input is required from the SNCBs and other competent bodies (EA, SEPA, and MMO). Information from the relevant agencies on typical size and nature of spills, especially local spills, and their effects will focus the literature review and ensure compatibility between the sensitivity assessment approach adopted and their standard approaches. Information is existing operational guidance may also focus the review.

2.1.4 Exposure to increased levels of contaminant due to bioaccumulation

The European Commission (2010) clearly define the terms relevant to bioaccumulation. They define bioaccumulation via three terms.

- BCF (bioconcentration factor) of a compound is defined as the ratio of the concentration of the chemical in the organism and in water at equilibrium;
- BMF (biomagnification factor) is defined as the ratio between the uptake of a contaminant from food and its removal by depuration, excretion and metabolism; and
- bioaccumulation factor (BAF) can be expressed for simplicity as the steady state (equilibrium) ratio of the substance concentration in an organism to the concentration in the surrounding medium (e.g. water).

The potential for bioaccumulation and 'secondary poisoning of predators' is assessed as part of the process to derive an EQS, especially in biota. Therefore, we suggested that a separate benchmark for bioaccumulation was probably not required.

In practice, we can use the benchmark levels under section 2.1.2 above and the agreed 'multiples of the EQS' and treat 'bioaccumulation' as another pathway to exposure to the chemical of interest. Therefore, the potential for any given chemical to bioaccumulate through the food chain, especially for top predators will be taken into account in the literature review and subsequent sensitivity assessment.

2.1.5 Presentation of benchmarks and evidence

At present, we have a single benchmark and one evidence section for each of the categories of contaminant pressures listed.

- 'Hydrocarbons and PAHs';
- 'Synthetic compounds (inc. pesticides, antifoulants, pharmaceuticals)';
- 'Transition elements and organo-metals';
- 'Radionuclides';
- 'Introduction of other substances (solid, liquid, gas)'; and
- 'Nutrient enrichment'.

In the recent climate change project (Garrard & Tyler-Walters, 2020), we defined two or three benchmarks for each climate change pressure, depending on the climate change 'scenario' discussed. We then presented a single summary of the relevant evidence and provided a separate sensitivity assessment against each benchmark in the evidence text. This is our preferred approach. We can easily use headers in the evidence text if required. The only issue is that the volume of evidence could be high for some organisms, and the evidence sections could become lengthy. The alternative would be to separate sections of the assessment, e.g. compliance (but see above), 'mixing zones' and 'spills' so that we have (potentially) three sections for each of the existing categories. However, this may cause difficulties for Natural England and others for ingesting the dataset into their systems.

2.1.6 Habitat (biotope) sensitivity assessment

The MarESA dataset includes ca 400 separate habitats (biotopes). In each case, we address six contaminant categories (inc. 'Nutrients') and the above discussion suggests that we will have at least three benchmarks, probably more for each category. For example, the discussion above could result in one or more benchmarks of permitted releases within mixing zones, another for oil spills, and another for other substance spills. We have already proposed the development of three benchmarks for Nutrient, based on 'Good', 'Poor', and 'Bad' status under WFD. If we assume three benchmarks per pressure category, we could undertake 2,328 new and/or updated literature reviews and, at least 6,984 assessments. The literature reviews might be extensive (see section 1.3).

Therefore, we propose to 'group' habitats (biotopes) by their dominant structural/functional species groups and, hence, by their potential response to different chemical contaminants. For example, fucoid dominated shores are characterized by fucoids (*Fucus* spp., *Ascophyllum nodosum*) and their community dynamics are structured by the physical environment and grazers, e.g. molluscs (*Patella* spp., *Littorina* spp.) and amphipod and isopod crustaceans.

We would identify a range of structurally and functionally important species for review, across the range of marine habitats, grouped by habitat (biotope). We would then assess the sensitivity to contaminants for each group of habitats (biotopes). We could then 'cascade' the sensitivity assessment scores through the relevant ' children biotopes' in the dataset quickly. Of course, there will be exceptions and

unusual biotopes that require specific attention. Some biotopes may be deemed 'not relevant' to oil spills due to their depth and absence of mixing due to wave action. Nevertheless, we may need to review over 100 dominant structural/functional species (or species groups).

2.2 Spills (oil and chemical) and Allowable Zones of Effect (AZEs),

It was decided to examine the Tier approach to oil/chemical spills as a basis for a set of benchmarks (Nov. 2020). We also examined the AZEs (Allowable Zones of Effects) around Aquaculture installations as a special case of a 'mixing zone'.

Summary of findings

1. Quantified values were suggested to describe the three Tiers of oil and chemical spills.
2. Allowable Zones of Effect (AZEs') are special types of 'mixing zones' used in aquaculture in particular.
3. We could create a benchmark based on the maximum allowable exceedance of environmental standards, i.e. EQSx10; EQS's are extremely precautionary so that it is likely that all species will be ranked as 'not sensitive' at the benchmark level.
4. But we would need to create a separate benchmark for every chemical included in the study, assuming that environmental standards already exist for all of them.
5. However, it is difficult to see how a quantified value or scenario would function as a quantified benchmark for sensitivity assessment, given the variation in physical and chemical behaviour of substances that could be spilt or released into the marine environment, and the resultant variation in exposure experienced by benthos.

2.2.1 Chemical spills in the marine environment

A review of oil and other chemical spills in the marine environment was undertaken between Nov 20201 and Jan 2021 to inform the discussion of possible pressure definitions and their benchmarks.

Spills from oil & gas installations

OGUK (UK Oil and Gas Industry Association Limited) (2019) reported that the in 2011-2018 the average accidental oil release from oil and gas installations was 0.58 tonnes. However, oil releases of >50 t made up only 0.23% of incidents but ca 75% of the total mass released since 2011 (OGUK, 2019). Baker *et al.* (1995) suggested minor spills were "say 30-40 t".

The size of chemical spills was significantly lower, with an average of 2.59 t per release although a single release of 247 t was recorded in 2018 (OGUK, 2019). High and medium hazard category chemicals contributed 2% and 6.7% of the total mass of chemicals released 2011-2018 respectively. The OGUK (2019) also note that both accidental release of either oil or chemicals were significantly lower than the total amount released under permit; 0.16% for chemicals, and 0.7% for oil.

OGUK (2019) used <>50 t to report on accidental oil spills but provided no similar spill 'size' for chemicals, which were reported based total amount spilt per year from 2011-2018 based on the OCNS[16] hazard ranking category rather than the size of individual spills. OSPAR (2014) used <1 t and >1 t of oil spill to report against the number of both chemical and oil spills in 2003-2012, as does the PON[17] reporting system.

[16] OCNS (Offshore Chemical Notification Scheme) - https://www.cefas.co.uk/data-and-publications/ocns/
[17] PON (Petroleum Operations Notice) - https://www.gov.uk/guidance/oil-and-gas-environmental-alerts-and-incident-reporting

Spills from vessels

Some examples of oil and chemical spills are shown in Table 2.2 to give an indication of the range different spills sizes and chemicals spilt (Neuparth *et al.,* 2012; EMSA, 2013; Cunha *et al.,* 2015; Cedre Spill guide[18]). The Cedre database of spills worldwide categorizes spill into 10-100, 100-1000, 1000-10,000, 10,000-100,000 & >100,000 m^3 (rather than tonnes). Many of the spills reported by e.g. EMSA (2013) were not quantified, or were only qualified by no. bags, boxes, or drums and are not listed in Table 2.2, and only data up to 2012 is included.

Table 2.2**.** Examples of maritime incidents that resulted in spills (where the nature of the spill is reported); mixed cargoes e.g. from ferries or container ships excluded, as are spills quantified by no. bags, boxes, or drums (Source Neuparth *et al.,* 2012; EMSA, 2013, Cedre Spill guide).

Vessel	Date	Spill size	Spilt substance(s)	Chemical behaviour
Amoco Cadiz	1978	220,880 t	Light crude oil	Floater
Sea Empress	1996	72,000 t	Light crude oil	Floater
Prestige	2002	63,000 t	Heavy fuel oil	Floater
Fu Shan Hai	2003	66,000 t	Potash	Dissolver
Exxon Valdez	1989	32,000 t	Crude oil	Floater
Erika	1999	19,000 t	Heavy fuel oil	Floater
Cape Horn	2003	14,000 t	Methanol	Dissolver
Kemira Gas	2008	8,500 t	Liquefied ammonia	Gas/dissolver
Ece	2006	8,000 t	Phosphoric acid	Dissolver
Adamandas	2003	8,000 t	Deoxidized iron ore balls	Evaporator[19]
Susie	2008	8,000 t	Phosphine	Evaporator
Balu	2001	8,000 t	Sulphuric acid	Dissolver
Kira	1996	7,600 t	Phosphoric acid	Dissolver
Kairo	1997	6,240 t	Lead tetraethyl	Sinker
Martina	2000	6,000 t	Hydrochloric acid	Dissolver
Patricia S.	2008	4,800 t	Metal shavings	Gas/evaporator
Ievoli Sun	2000	3,998 t 1,027 t 996 t	Styrene Methyl-ethyl-ketone Isopropanol	Floater
Scaieni	1991	3,057 t	Ammonium nitrate	Dissolver
Grape one	1993	3,000 t	Xylene	Floater/evaporator
Camadan	2002	2,900 t	Phosphate granules	Dissolver
Ocean spirit	1988	2,850 t	Lead concentrate	Sinker
Nordfrakt	1992	2,352 t	Lead sulphur	Sinker
Dina	2001	2,340 t	Calcium fluoride	Unknown
Dogruyollar IV	1998	2,020 t	Zinc and lead concentrates	Sinker/dissolver
Val Rosandra	1990	1,800 t	Propylene	Gas/evaporator
Lina Star	2000	1,150 t	Sodium carbonated	Unknown
Jambo	2003	1,000 t	Zinc chloride	Unknown
Allegra	1997	900 t	Vegetable oil	Persistent floater
Bow eagle	2002	800 t	Ethyl acetate	Evaporator
Junior M	1999	700 t (packs)	Ammonium nitrate	Dissolver
Anna Broere	1988	547 t 500 t	Acrylonitrile Odecyl benzene	Dissolver
MSC Napoli	2007	300 t	Oil[20]	Floater
Albion Two	1997	114 t	Calcium carbide[21] (in barrels)	Dissolver

[18] Cedre Database of spill incidents and threats in waters around the world - http://wwz.cedre.fr/en/Resources/Spills
[19] re-oxidize in water, creating heat and hydrogen
[20] also carried 1600 tonnes of dangerous goods

Vessel	Date	Spill size	Spilt substance(s)	Chemical behaviour
			Camphor ammonia anhydrous	
Dana Optima	1984	16 t	Dinitrobutylphenol (Dinoseb)	Unknown
BG Dublin	2010	11.48 t	Sodium bromate	Dissolver
Ena II	2004	6 t	Sulphuric acid	Dissolver
Kilgas Centurion	2001	1 t	Propane	Gas/evaporator
Wilson Mosel	2011	0.850 t (bulk and big bags)	Ferrosilicum	Gas/evaporator
MT Trans Arctic	2010	0.5 t	Benzene	Evaporator/dissolver/floater
FS Odin	2005	insignificant	Styrene monomer	Floater/evaporator

However, Table 2.2 shows a range of possible chemical spills from <1 t to 220,880 t, of which, the oil spills were the largest presumably because crude and fuel oils are transported in the largest vessels. However, the range and scale of chemical spills in the marine environment is large and the behaviour of the spilt chemical in the environment is variable (see below) (Neuparth *et al.,* 2011, 2012; Cunha *et al*., 2015).

Oil and chemical spill scenarios

The three-tiered approach (API, 2014; IPIECA, 2015; Alison Brand pers. comm.) and the four categories of incidents used by EAs' CICS (Common Incident Classification Scheme) (EA, 2016) define the scale of spills ('minor', 'moderate' or 'major') based on the level of effort required to mitigate, control and clean-up the oil spill. The CICS is not limited to oil and guidance on the definition of an incident, the physical response to the incident and categories of potential and actual impact.

Both schemes are designed to provide a framework on which to base a decision of the action required but neither provide clear guidance on the size/scale of the spill.

It is possible to quantify the terms 'minor', 'moderate', and 'major' based on the reviews of spills from OGUK (OGUK, 2019) and OSPAR (2014) and the scales suggested by Baker *et al*. (1995). The following broad scenarios could be suggested for both oil and chemical spills based on the three-tiered approach (the CICS's category 4 relates to an incident with no impact and it not used below).

> **Tier 1:** Minor oil spills (<50 tonnes), including incipient spills that are quickly controlled, contained and cleaned up using **local** (onsite or immediately available) equipment and personnel resources. A Tier 1 spill would typically be resolved within a few hours or days. For example, spills from burst hoses or from open valves during loading or unloading of tankers, or operational failures in drainage systems, bulk transfer systems, hydraulics, diesel, production and storage systems.
>
> **Tier 2:** Moderate oil spills (50 - <1000 t) requiring activation of **significant regional oil spill response resources**. A Tier 2 spill response may continue for several days or weeks. For example, spills caused by minor collisions between vessels or vessels and docks.
>
> **Tier 3:** Major oil spills (>1000 t) requiring activation of large quantities and multiple types of response resources including those from **out of the region, and possibly international sources**. A Tier 3 spill response may continue for many weeks or months. For example, spills from major collisions, explosions, or blowouts.

[21] calcium carbide reacts with water to create flammable acetylene gas

However, the 'significance' of a spill of any particular size depends on:

- the nature of the chemical spilt,
- its chemical behaviour,
- its toxicity (see below),
- and the location of the spill, that is,
- in harbour/port,
- inshore or offshore,
- the sea state (mixing),
- and the nature of the receiving environment, e.g. intertidal rock or sediment, shallow rock or sediment or deepwater.

Several systems exist to classify chemicals transported or used in the marine environment depending on their toxicity and fate in the marine environment, e.g. MARPOL, IMDG (IMO Dangerous Goods Code), and GESAMP[22] (2019) and Cefas' OCNS system. OSPAR and the EU also list priority substances and substrates of concern. However, substances are identified as 'hazardous or noxious' substances (HNS) based on their:

- persistence in the environment;
- toxicity;
- bioaccumulation, and
- possible carcinogenic effects (Neuparth *et al.*, 2012).

However, the exposure of marine species or habitat is also affected by the chemicals behaviour in the environment, which is classified as:

- gases;
- evaporators;
- floaters;
- floaters persistent;
- dissolvers;
- sinkers, or
- combination of these attributes (GESAMP, 2019; Neuparth *et al.*, 2012; Cuhna *et al.* 2015).

Some chemicals, e.g. heavy metals, react differently depending on the local salinity and/or temperature of the receiving water body while others e.g. complex organics, degrade into other chemicals that may be more or, less toxic than their parent. For example, temperature effected the rate at which 'evaporators' dissipate or degrade, and the solubility of 'sinkers', while the effect of salinity was marginal but, in simulations, wave and currents affected the near bottom concentration of the chemical examined (aniline) (Factors Affecting Marine Emergency and Response Research', FAMERR, 2016[23]). However, temperature and salinity also affect toxicity of the chemicals spilt (FAMERR, 2016).

[22] GESAMP – Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection
[23] https://www.itopf.org/fileadmin/uploads/itopf/data/Documents/RDaward/FAMERR_overview.pdf

Hence, we need to consider:

- the 'physical' effects i.e. smothering, suffocation, clogging of gills and/or feathers;
- the 'chemical' effects i.e. toxicity of the chemicals and its degradation products (perhaps also chemical reactions in water); and
- the route of exposure of the species of interest i.e. through absorption through skin or respiratory surface (gills/lungs) or ingestion via particulates or food.

Therefore, it is unlikely that any given size of spill (defined by tonnage, or volume) would provide a clear indication of the level of chemical exposure (defined in terms of concentration or duration) that any one species or habitat would experience because of the spill.

While the environmental effects of major oil spills and a few chemical spills are well documented (Neuparth *et al.,* 2011, 2012; Cunha *et al*., 2015), little information on the effects of chemical spills was found in this preliminary Phase 1 review. For example, the spill databases document the size and nature of spill, ship name, date, and location but not environmental effects. It is, therefore, unclear how much evidence exists (outside the well-documented oil spills) against which to assess sensitivity.

2.2.2 Allowable Zones of Effect (AZEs)

Allowable Zones of Effect (AZEs) are specific types of 'Mixing Zones' used, in particular, to address emissions from aquaculture such as shellfish and fin-fish farms in Scotland (SEPA, 2005, 2019a,b,&c). Technically the AZEs are defined as "the area (or volume) of sea bed or receiving water in which SEPA will allow some exceedence of a relevant Environmental Quality Standard (EQS)". This is the same for 'mixing zones' whereby the 'mixing zone' is designed to be an area over which an emission is diluted to acceptable (EQS) levels (European commission, 2010).

In Scotland, the regulations are being updated (SEPA 2019a,b,&c) so that the size and shape of the AZE is determined by hydrographic modelling of the receiving waters. The current AZEs incorporate as near-field 'zone' within 25 m of fin-fish cages and a far-field 'zone' within 100 m of the cages, but the modelled zones will vary depending on the receiving environment. Within the AZEs, the emissions/discharge is regulated so that it meets the relevant EQS at the edge of the AZE. The AZEs are not permitted to overlap marine protected (or other designated) areas and EQS is not permitted to exceed 10xEQS within the AZEs (Jack Bloodworth pers. comm.). However, at present only a limited number of chemical discharges, namely, in-feed anti-parasitics, and directly controlled within AZEs (SEPA, 2005).

The discharges from shellfish and finfish farms in Scotland are recorded and available online via the Scotland's Aquaculture[24] website and SEPA's SPRI[25] website, which provide details on the main emissions in terms of amount of pollutant released. However, no information on the exact concentrations in the water column or sediment was readily available.

As discussed for 'mixing zones', the issue remains how we could use environmental quality standards as benchmarks because they are set at extremely precautionary levels, based on the most sensitive species studied, with an additional 'assessment factor' designed to account for the quality of the evidence used. Hence, even if we used an EQSx10 concentration as a benchmark, the value may still be so low that no species would be assessed as sensitive. Nevertheless, it should be noted that environmental standards are based on evidence derived from a limited number of 'sentinel' species and/or taxonomic groups so that it is possible that species that are more sensitive exist.

[24] Scotland's Aquaculture- http://aquaculture.scotland.gov.uk/default.aspx
[25] SEPA SPRI - https://www2.sepa.org.uk/SPRIPA/Search/ByIndustry/Criteria.aspx

In addition, the adoption of a value equivalent to EQSx10 would be arbitrary, and EQS are derived using Assessment factors (AF) that range from 1-10,000 depending on the quality of evidence available and/or biological concentrations (BCF) factors from 1-ca 20,000.

Also, if we adopted a quantified benchmark based on an EGS we may not capture the sensitivity of species exposed to higher concentrations due to bioaccumulation. For example, if we adopted a benchmark expressed in µg/l or µg/kg body weight but the concentrations observed in top predators, and implicated in their mortality, were measured in mg/kg body weight, then they would not be considered sensitive as the concentration of chemical implicated in mortality was several orders of magnitude higher than the benchmark. However, top predators have been shown to be susceptible to chemical pollution, due to bioaccumulation.

2.3 Phase 1 - Conclusions

At present, it is difficult to see how a quantified value or scenario would function as a quantified benchmark for sensitivity assessment. The mechanisms whereby any individual species is exposed to any individual chemical are complex, and vary depending on the behaviour of chemicals in the environment, their mode of action and toxicity, as well as the nature of the receiving environment as explained above.

2.3.1 'Weight of evidence' approach

Therefore, we suggest that we adopt a 'weight of evidence' approach similar to that adopted for qualitative benchmarks that describe a pressure or process. This is the approach used for other pressures such as 'abrasion', 'penetration' 'removal of non-target species', and 'introduction of non-indigenous species', where the level of resistance is determined by the levels of damage or disturbance documented in the evidence. In these cases, there is the danger that the sensitivity assessments do not compare 'like' with 'like' and care is taken to record the evidence used in detail.

For qualitative benchmarks, resistance is assessed against the available evidence for the effects of the pressure on the species or community of interest. For example:

- evidence of mass mortality of a population of the species or community of interest (either short or long term) in response to a pressure benchmark will be ranked as 'Low' resistance;
- evidence of reduced abundance, or extent of a population of the species or community of interest (either short or long term) in response to a pressure benchmark will be ranked as 'Medium' resistance;
- evidence of sub-lethal effects or reduced reproductive potential of a population of the species or community of interest will be assessed as 'High' resistance.

We anticipate that there will be two main types of evidence – 'observational' and 'experimental'. Observational evidence will come from reports of the effects of pollution incidents, e.g. oil spills or observed effects along transects from discharges, e.g. mine discharges, sewage effluents etc. In such cases, the actual concentrations of the chemicals may not be reported.

Experimental evidence will come from laboratory studies used to derive 'lethal' or 'effect' concentration 'end points' (e.g. LC_{50}, EC_{50}, and NOEC[26] etc.) in particular species.

In both cases, we will document and record the relevant concentrations to which species are exposed (where reported), their effects, and the level of mortality experienced (e.g. LC_{50}, EC_{50}, and NOEC etc.) in laboratory studies, and/or the level of mortality experienced in the field e.g. 'severe', 'significant', or 'some' in line with the current resistance scale.

[26] LC/EC_{xx} =Lethal or Effect Concentrations at given percentile and NOEC = No Observable-Effect-Concentration.

In addition:

- where ‘exposure’ to a ‘contaminant’ results in loss of the reproductive potential that results in a decline in the population, then the effects on reproductive output would be considered as a source of ‘mortality’;
- incidental oil and chemical spills will be treated as single events or exposures;
- operational discharges (e.g. from offshore/inshore installations, shipping, mariculture), effluent discharges (e.g. from permitted outfalls), runoff, sewage effluents, sediment contamination, and leaks from accidentally or deliberately dumped barrels or containers will be treated as periodic or ongoing ‘exposures’ for the purpose of assessment; and
- chemicals that behave as ‘gases’ and ‘evaporators’ at the point of release will probably not affect marine species and will be excluded from the assessments unless they have known underwater releases.

Where appropriate we will provide separate sensitivity assessment for ‘physical’ and ‘chemical’ effects. This can be achieved as two sets of sensitivity assessments, as we have for climate change pressures. This approach is probably most relevant to hydrocarbons and particularly the difference in physical effect of oils i.e. smothering, suffocation, clogging of gills and/or feathers and their chemical toxicity. Both petroleum-based and vegetable-based (e.g. sunflower, palm) oils may cause smothering, bind sediment, or clog the digestive tract when spilt (Cuhna *et al.,* 2015).

2.3.2 Additional and alternative approaches

Evidence reviews

We propose that we create a detailed database of the relevant evidence (reports/papers and their findings), as used in Rapid Evidence Assessments (REA; Collins *et al.*, 2015) and Systematic Reviews, together with our usual narrative (explanatory text) used to support MarLIN/MarESA sensitivity assessments. As a result, we will create a database of relevant evidence on the effects of chemical contamination on marine species that can be updated and used to support subsequent meta-analysis and advice on operations. Further statistical analysis or meta-analysis (see below) could allow us to ‘rank’ the ‘resistance’ (and hence ‘sensitivity’) of species and/or taxonomic groups empirically. The resultant dataset could also be updated and maintained as another resource for advice on operations.

The MarESA evidence review required is likely to be extensive (as outlined in the scoping report May 2020). A more detailed Rapid Evidence Assessment or Systematic Review would be more time-consuming by definition but also more versatile and informative and allow for empirical statistical analysis, where the evidence allows.

Species Sensitivity Distribution (SSD)

The Species Sensitivity Distribution (SSD) is a statistical process that uses the LC/EC_{50} concentrations for acute toxicity or $NOEC/EC_{10}$ for chronic toxicity to rank species by toxicity to any given chemical, using log-normal plots of cumulative distributions (Posthuma *et al.,* 2002; de Zwart, 2005; de Zwart *et al.*, 2009). They have been used to predict the no-effect concentrations of specific chemicals or the HC_5 (5th percentile hazardous concentration) in the derivation of EQS. The HC_5 predicts an environmental concentration below which an ‘a priori’ acceptable small proportion of species (i.e. 5%) would be affected (de Zwart, 2005). As a risk estimate, the SSD can provide an estimate of the number of species

exposed to a concentration causing an adverse effect, because the NOEC is exceeded (de Zwart, 2005). The US EPA CADDIS[27] suite provides standard SSD generator software for download.

SSDs could be used to 'rank' a large number of species/taxonomic groups by their toxicity to specific chemicals. But the technique requires experimental data on LC/EC_{50}s, and/or NOEC/EC_{10}s based on quality, comparable, laboratory studies, which may not be available for all the species of interest in sensitivity assessment.

Also, the use of SSDs would require an evidence review of a large number of marine benthic species before the SSD was created. Ideally, an evidence review of all the species 'indicative of sensitivity' required in sensitivity assessment of the relevant 300+ biotopes would need to be carried out for all the chemicals in the scope of the study, before the SSDs could be used to rank species by their response to each chemical.

Meta-analysis

A meta-analysis is a statistical examination of the information on the reported effects of a particular chemical (or other stressor) on a wide range of species. For example, Vaquer-Sunyer & Duarte (2008) examined 872 papers that experimentally examined oxygen thresholds in 206 marine benthic species. They used statistical techniques (including cumulative distributions similar to SSD) to examine the range of median lethal and sub-lethal oxygen concentrations and median lethal time across taxonomic groups. An extract of their results (Fig 3) is copied below as an illustration.

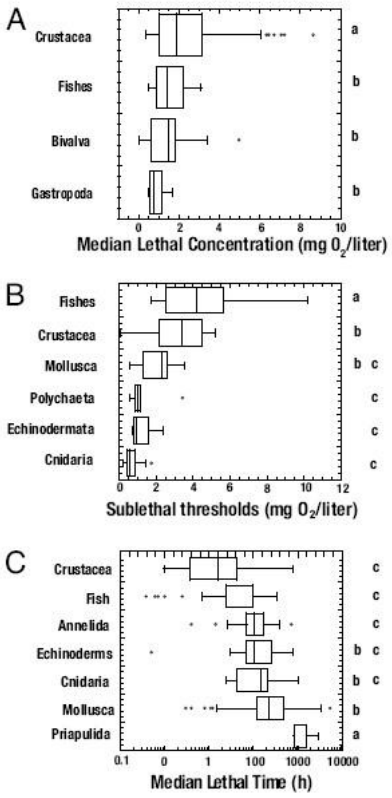

Fig. 3. Box plot showing the distributions of oxygen thresholds among taxa for (*A*) LC_{50} (mg O_2/liter), (*B*) SCL_{50} (mg O_2/liter), and (*C*) LT_{50} (h). The letters indicate the results of the Tukey HSD test, whereby the property examined did not differ significantly for taxa with the same letter.

Their paper demonstrates the potential for their technique to rank taxonomic groups and potentially species by their 'resistance' and, hence, sensitivity to a single stressor. However, it should also be noted

[27] https://www.epa.gov/caddis-vol4/caddis-volume-4-data-analysis-download-software

that their sample of 872 papers represented the papers in the ca 6000 on the subject of hypoxia that provided adequate data for the analysis.

As with the SSD approach above, the evidence review would need to be completed for a large number of marine benthic species for all the relevant chemicals before the analysis could be undertaken.

2.4 Phase 1 - Recommendations

Therefore, it was suggested that:

1. a 'weight of evidence' approach was used and sensitivity to exposure to any given chemical assessed based on the reported levels of resultant mortality, as used for pressures such as 'abrasion', 'penetration' and 'introduction of non-native species';
2. sensitivity assessments were supported by a description of the relevant evidence on the method/route of exposure, and evidence from laboratory studies (e.g. LC/EC50s) and observational studies where available, which is current practice;
3. the chemical behaviour of the chemical included in our groupings ('hydrocarbons', 'synthetics', 'transitional metals', 'other') was recorded/examined to identify those unlikely to impact benthic species (e.g. 'evaporators'), and those likely to have physical (e.g. smothering, clogging) and/or chemical effects (e.g. toxicity); and
4. sensitivity to physical and chemical effects was scored separately where needed, e.g. oil spills.

Furthermore, it was suggested that:

5. detailed Rapid Evidence Assessments were used to record the details (meta-data) of the evidence used to support sensitivity assessment in a separate dataset;
6. a meta-analysis to 'rank' marine benthic species or taxonomic groups by their responses to chemical contaminants should be investigated; and
7. the resultant dataset should be provided online as an additional resource to SNCBs.

We suggest that the meta-analysis could be an important addition, and allow us to 'rank' species and taxonomic groups using statistical techniques if the evidence allows. However, such ranking would not be possible until the evidence review was completed for the majority of biotopes so that biotopes could not be assessed piecemeal, i.e. in small groups but would need to be assessed together at the end of the review. If we adopt the 'weight-of-evidence' approach alone, we could assess biotopes as the evidence review proceeded. In both cases, the evidence review required is likely to be extensive.

Nevertheless, collating detailed meta-data on the evidence reviewed would be a powerful addition to the process and allow subsequent meta-analysis and a more defensible ranking of relative sensitivity of marine benthic species to chemical contamination.

2.5 Revised pressure definitions

The 2014 pressure definitions (see Tyler-Walters *et al.*, 2021) were revised to reflect the proposed scope of the literature review discussed above and detailed below.

Most organic molecules have a hydrocarbon backbone. Therefore, some chemicals may fit into the 'hydrocarbons' pressure or the 'synthetic chemical' pressure. At present, biogenic and petroleum-based hydrocarbons and their direct products are included under 'hydrocarbons and PAHs' while chemicals that have been 'manufactured' from other components for use in industry have been included under 'synthetics'. Further consultation on this split is required.

Organometals (e.g. TBT) are technically 'synthetic'. It was suggested that they should be included under 'Synthetic contaminants' rather than 'Transitional metals' (see 2.1.1 above). However, the

organometals compounds were routinely returned in the preliminary literature searches for 'metals'. Therefore, they are retained under 'metals' on the presumption that the 'metal' ion is the active, toxic, component, made more biologically available by its organic component.

2.5.1 Hydrocarbon & PAH contamination.

The existing pressure definition has been revised to separate physical and chemical effects.

Revised pressure definition.

Increases in the levels of these compounds compared with background concentrations. Naturally occurring compounds, or complex mixtures of two basic molecular structures:

- straight chained aliphatic hydrocarbons (relatively low toxicity and susceptible to degradation), and
- multiple ringed aromatic hydrocarbons (higher toxicity and more resistant to degradation).

These fall into three categories based on source (includes both aliphatic and polyaromatic hydrocarbons):

- biogenic hydrocarbons (from plants & animals);
- petroleum hydrocarbons (from natural seeps, oil spills and surface water run-off); and
- pyrogenic hydrocarbons (from combustion of coal, woods, and petroleum).

Ecological 'chemical' consequences include taint, acutely toxicity, carcinomas, and/or growth defects.

In addition, hydrocarbons may have 'physical' as well as 'chemical' (toxic) effects on marine species. Physical effects include smothering, suffocation, and clogging of feathers, breathing apparatus, or the digestive tracts of species at the air/water boundary, on rocks or in the sediment, they inhabit.

Guidance notes

Petroleum-based and vegetable-based (e.g. sunflower, palm) oils and other 'persistent floaters' can spread out over the surface of the water, smother, suffocate and clog feathers, breathing apparatus or the digestive tracts of species (e.g. mobile species) that cross or inhabit the air/water boundary. In addition, petroleum-based and vegetable-based oils may smother rock surfaces and/or bind and smother sediment, including the resident species, if they come ashore. Petroleum-based and vegetable-based oils may also release potentially toxic chemicals (Cuhna *et al.,* 2015).

Therefore, we propose **we assess and score the physical effects separately from the chemical or toxicological effects**, with an emphasis on petroleum-based and vegetable-based oils, that is, 'persistent floaters'.

2.5.2 Synthetic compound contamination (incl. pesticides, antifoulants, pharmaceuticals).

The existing pressure definition has been revised to outline the different groups of chemicals included under this pressure.

Revised pressure definition.

Increases in the levels of these compounds compared with background concentrations. Synthetic compounds are manufactured for a variety of industrial processes and commercial applications.

Chlorinated compounds and other organohalogens are often persistent and often toxic; includes:

- Polychlorinated biphenols (PCBs)
- Brominated flame-retardants

- Chemical precursors and solvents

Pesticides vary greatly in structure, composition, environmental persistence, and toxicity to non-target organisms, many of which are also organohalogens or organophosphates; includes:

- insecticides
- herbicides
- rodenticides
- fungicides
- parasiticides
- antifoulants

Pharmaceuticals and 'Personal Care Products' (PPCPs) originate from veterinary and human applications and include a variety of products:

- over the counter medications
- fungicides
- chemotherapy drugs and animal (e.g. fin-fish) therapeutics, such as growth hormones and oestrogens
- UV-filters e.g. from sun screens

Due to their biologically active nature, high levels of consumption, known combined effects, and their detection in most aquatic environments pharmaceuticals have become an emerging concern. Ecological consequences include physiological changes (e.g. growth defects, carcinomas).

Dispersants (used to disperse oils spills) are often mixtures of distillates, surfactants, and other ingredients.

This category also includes:

- Other synthetic and organic esters,
- Phthalate esters, and
- Synthetic musks; which may also be PBT[28]s.

Guidance notes

At present, this category includes a number of alcohols such as ethanol and methanol that are transported in bulk as well as some such as 1-Dodecanol and Isononanol that are PBTs. A number of synthetic chemicals that do not fit into other categories are also included as 'synthetic (others').

Dispersants are included here as they are mixtures of chemicals, i.e. 'synthetic mixtures' designed to break up oil spills.

Exposure to most of these synthetic compounds will probably be via the water column or adsorbed onto particulates. Some may be 'floaters' but further research is required to determine if we need to identify 'physical' and 'chemical' effects separately.

2.5.3 Transitional elements & organometal (e.g. TBT) contamination.

The existing pressure definition has been revised to outline the different groups of chemicals included under this pressure.

Revised pressure definition.

The increase in transition elements levels compared with background concentrations, due to their input from land/riverine sources, by air or directly at sea.

[28] PBTs – Persistent, Bioaccumulative, or Toxic substances

For marine sediments the main elements of concern are:

- Arsenic,
- Cadmium,
- Chromium,
- Copper,
- Mercury and organic mercury compounds,
- Nickel and its compounds,
- Lead and organic lead compounds, and
- Zinc.

However, the following may also be released into the marine environment:

- Aluminium
- Barium
- Cobalt
- Iron
- Molybdenum
- Selenium
- Tin
- Tungsten, and
- Vanadium.

Organo-metallic compounds such as the butyl tins (Tri butyl tin and its derivatives) can be highly persistent and chronic exposure to low levels has adverse biological effects, e.g. Imposex in molluscs. The use of other organo-metalloids, such as organo-copper and organo-zinc compounds, has increased due to the ban on organo-tins.

Nanoparticulate metals such a Zinc oxide (ZnO), Iron oxide (FeO), Copper oxide (CuO), Titanium (n-TiO_2), Gold, and Silver nanoparticulate metals are included.

Guidance notes

Although the organometalloids are synthetic, they are included here on the presumption that the metal ion is the active toxic component of the compound. Note, mercury, and lead form organic compounds naturally in the environment.

Engineered Nanomaterials (ENMs) include nanoparticulate metals (e.g. ZnO, FeO, CuO, n-TiO_2, Ag, and Au), other inorganic nanomaterials (e.g. Quantum Dots, SiO_2), and organic nanomaterials such as fullerenes and carbon nanotubes (Rocha *et al.*, 2015). Nanoparticulate metals are included here while non-metallic nanomaterials may be considered under the 'Introduction of other substances' pressure below.

2.5.4 Introduction of other substances (solid, liquid or gas)

The existing pressure definition has been revised to outline the different groups of chemicals included under this pressure**.**

Revised pressure definition.

The 'systematic or intentional release of solids, liquids, or gases …' (from MSFD Annex III Table 2) is considered e.g. in relation to produced water from the oil industry. It should therefore be considered in parallel with the other contaminants' pressures (P1, P2, and P3).

This pressure includes compounds released as operational discharges, produced waters or spills from maritime (offshore/ inshore) installations (e.g. oil & gas, renewables), mariculture, shipping and harbours etc. that are not assessed elsewhere. This pressure includes:

- Inorganic chemicals that vary in their physical or chemical effects, e.g.
  - Chemicals transported in bulk that may be spilt e.g. acetic acid, phosphoric acid, sulphuric acid, sodium hydroxide;
  - Chemicals in drilling waste or produced waters e.g. barite, calcium carbonate, potash, zinc oxide;
- Natural products with varied uses, e.g. molasses (transported in bulk) but also glycerins, formalin etc.
- Fin-fish food supplements – e.g. carotenoids, copper sulphate
- Releases from munitions dumps
  - Chemical warfare agents
  - Explosives/propellants

Guidance notes

This pressure can include a large list of chemicals of mixed ecological effect or none. At present, chemical warfare agents and explosives are included, based on legacy munitions dumps. However, their effects are varied and localized to the vicinity of the dump (hopefully) and may not be a significant concern.

Also, the list of 'natural products' may be reduced to focus on only those with localized toxicity. Several of the natural products are manufactured from natural occurring compounds or synthesized commercially and may need to be placed under the 'synthetics' pressure. Chromium trioxide and copper thiocyanate are inorganic chemicals used as antifoulants but are included under the 'Transitional metals' pressure.

Cuhna *et al.* (2015) also highlighted spills of non-toxic sinkers, such as coal, wheat, rice, sugar cane, copra, and cocoa beans. Spills of such items are likely to smother benthos and/or cause localized nutrient enrichment. They are not included under 'contaminants' as they are non-toxic and 'smothering' and 'nutrient' and 'organic enrichment' are addressed under other pressures.

2.5.5 Nutrient enrichment

The existing pressure definition was retained but the benchmark was amended.

Pressure definition.

Increased levels of the elements nitrogen, phosphorus, silicon (and iron) in the marine environment compared to background concentrations. Nutrients can enter marine waters by natural processes (e.g. decomposition of detritus, riverine, direct, and atmospheric inputs) or anthropogenic sources (e.g. wastewater runoff, terrestrial/agricultural runoff, sewage discharges, aquaculture, atmospheric deposition).

Nutrients can also enter marine regions from 'upstream' locations, e.g. via tidal currents to induce enrichment in the receiving area. Nutrient enrichment may lead to eutrophication (see also organic enrichment).

Adverse environmental effects include deoxygenation, algal blooms, changes in community structure of benthos and macrophytes.

Revised pressure benchmark

"A decrease in the one rank of nutrient status of a water body (as defined by WFD), that is, from High to Good, Good to Moderate, Moderate to Poor for a period of a year".

Where habitats are defined by eutrophic or nutrient enriched status (e.g. the *Beggiatoa* biotope) then sensitivity will be assessed against an increase in nutrient status.

Nutrient status is defined as follows in the "Water Framework Directive (Standards and Classification) Directions (England and Wales) 2015[29].

Table 16

Dissolved inorganic nitrogen standards for coastal water (salinity 32), or part of such water, (coastal waters categorised by type in accordance with paragraph 3 of Schedule 2)				
Mean dissolved inorganic nitrogen concentration (micromoles per litre) during the period 1st November to 28th February				
	Dissolved inorganic nitrogen concentration (micromoles per litre)			
Type	*High*	*Good*	*Moderate*	*Poor*
	Mean for the period 1st Nov to 28th Feb			
Clear	12(i)	18(i)	27 (i)	40.5(i)
		99 percentile standard for the period 1st Nov – 28th Feb		
Intermediate turbidity	12	70	105	157.5
Turbid	12	180	270	405
Very turbid	12	270	405	607.5

(i) The standard refers to the concentration of dissolved inorganic nitrogen at a mean salinity of 32 for the period of 1st November to 28th February.

Table 17

Dissolved inorganic nitrogen standards for transitional water (salinity 25), or part of such water, (transitional waters categorised by type in accordance with paragraph 3 of Schedule 2)				
Mean dissolved inorganic nitrogen concentration (micromoles per litre) during the period 1st November to 28th February				
	Dissolved inorganic nitrogen concentration (micromoles per litre)			
Type	*High*	*Good*	*Moderate*	*Poor*
	Mean for the period 1st Nov to 28th Feb			
Clear	20(i)	30(i)	45(i)	67.5(i)
		99 percentile standard for the period 1st Nov to 28th Feb		
Intermediate turbidity	20	70	105	157.5
Turbid	20	180	270	405
Very turbid	20	270	405	607.5

(i) The standard refers to the concentration of dissolved inorganic nitrogen at a mean salinity of 25 for the period of 1st November 28th February.

Guidance notes

The above tables were taken from the 'Directions' for 'England and Wales' 2015. Further advice is required on the standards in Scottish waters and offshore waters.

[29] https://www.legislation.gov.uk/uksi/2015/1623/pdfs/uksiod_20151623_en_auto.pdf

2.6 Detailed scope of the literature review

The 'scope' of the literature review will determine the amount of evidence available and collated and, hence, the time required and cost. The 'scope' is divided between the contamiant pressure, and the relevant groups and types of chemical to address ('Contaminants Chemicals Groups' March 2022 spreadsheet), the potential source activities (Table 2.3), and the 'species' and 'taxonomic groups' to address (Appendix 1).

Chemical compounds by pressure.

A preliminary list of chemical compounds has been collated in the detailed 'Contaminants Chemicals Groups' March 2022 spreadsheet attached and summarized below.

The intitial list was prioritised based on the following sources:

- WFD list of priority substances (WFD-P);
- OSPAR list of priority substances (OSPAR-P);
- OSPAR lists of substances of possible concern (OSPAR-PC(A-D);
- List of priority HNS (Hazardous and Noxious Substances) released into the marine environment (Neuparth *et al.*, 2011) (HNS-P);
- Review of chemical contaminants entering the marine environment from sea-based sources (Tornero & Hanke, 2016); and
- Review of HNS (Hazardous and Noxious Substances) involved in marine spill incidents (Cuhna *et al.*, 2015).

Additional information was obtained from:

- HNS-MS - data base, documents the physico-chemical properties of major HNS transported from or to the ports of Antwerp, Rotterdam, Hamburg, Nantes and Bordeaux (https://www.hns-ms.eu/); and the
- ECHA (European Chemical Agency) Candidate list of SVHCs (Substances of Very High Concern) (https://echa.europa.eu/candidate-list-table).

The initial list of potential PBTs, HNS and other chemicals of concern was expanded during the evidence reviews undertaken in Phase 2 and 3. The list should not be considered exhaustive. Not all of the 768 chemicals listed as HNS-MS are included. Not all chemicals and products listed under the Marine Dangerous Goods code are listed. It is not clear on the status of the ECHA SVHCs at present although some, e.g. PFASs, are listed as PBTs.

The 'Contaminants Chemicals Groups' spreadsheet includes the following information (where available):

- Pressure name;
- Contaminant group and type;
- Chemical name / Common name / Commercial name;
- Other names (where relevant);
- CAS number;
- Priority - where the chemical is listed under priority lists shown above;
- SEBC - Standardised European Behaviour Classification (in progess);
- Potential maritime sources (in progress, based on Tornero & Hanke, 2016);

- Functional group/use (if known); and
- Additional ecotoxicological data based on HNS-MS/GESAMP (incomplete).

The general terms 'groups' and 'types' of chemicals will form the basis of the literature review, although individual chemicals listed under WFD/OSPAR/HNS will also be used a search terms in their own right. The chemicals are listed using their common/commercial names or full chemical name; however, chemicals often have numerous synonyms. There are numerous dispersant mixtures in use in European waters and only a few are included as examples. However, dispersants will be researched as a group.

Activity specific terms

A list of 'process-based' search terms was provided to identify evidence on the effects of specific activities that may be sources of chemical contaminants (Table 2.3).

Species 'indicative of sensitivity'

A list of ca 85 species identified as 'indicative of sensitivity' in the existing sensitivity assessments of biotopes within the littoral and sublittoral biotope classification is shown in Appendix 1. These species or their taxonomic group will be the focus of the research about the potential effects of the range of 'contaminants'. In practice, we suspect that most of the evidence will be based on proxies or examples of species within the higher taxonomic groups (e.g. Phylum, Class, Order, or Family) or congeners.

In addition, we will also look at habitat characteristics that may affect benthic exposure to contaminants and, especially, their physical effects. For example:

- Littoral or Sublittoral zone;
- Depth
- Wave exposure and tidal streams;
- Sediment type (e.g. mud, muddy sands, sandy mud, sands, coarse, or mixed);
- Rock and boulders vs. soft rocks (e.g. chalk, clay, peats);
- Vertical aspect, overhangs and caves; and
- Under boulders.

The above list is of particular relevance to the physical effects of oils (petrochemical or biogenic).

Contaminant pressure specific terms.

The general terms (e.g. contaminant groups or type), together with specific chemicals identified as above are listed, by pressure, in the attached 'Contaminant-Chemical-Groups-March2022' spreadsheet'.

The list of chemical groups currently (April 2022) includes ca 639 separate entries. The chemicals are grouped by pressure, contaminant group, type, and chemical name (Table 2.4).

Table 2.3. Maritime/Coastal activity and process-based search terms (preliminary).

Primary term	Secondary terms
Shipping (commercial & recreational)	Vessels / Tankers / Freight
Spills (Accidental/Incidental)	Shipping (commercial & recreational) Inshore/offshore installations Harbours/Ports/Berths/Moorings/Bunkering Chemical spills Oil spills Spills of containers/barrels (oil and chemical)
Operational discharges	Shipping (commercial & recreational) Bilge water Ballast tanks Oil & gas installations Renewables/wind farms (inc. cable installation, support vessels) Mariculture Harbours/Ports/Berths/Moorings/Bunkering
Antifouling paints	Shipping (commercial & recreational) Mariculture/Aquaculture/Fin and shellfish) Offshore renewable Harbour/Port infrastructure (e.g. buoys pontoons etc.)
Mariculture/Aquaculture (fin-fish, shellfish)	Chemotherapeuticals/Medicines Antibiotics Parasiticides/Biocides Anaesthetics Disinfectants Food supplements Antifoulants
Oil & gas exploration/ production	Drilling wastes/muds/cuttings Produced waters Decommissioning Cables/Pipelines
Inshore/Offshore renewable (inc. cables)	Antifoulants Construction/decommissioning Sediment remobilization
Dredging and dumped spoil	Aggregate dredging Channelization Harbours/Ports
Inshore discharges/outfalls	Power stations Industrial effluents Sewerage effluents (inc. human pharmaceuticals)
Runoff	Agricultural runoff (e.g. hormones/pesticides/nutrients) Urban runoff Mine effluents/waste runoff
Munitions dumps	Chemical warfare agents Explosives/propellants
Ship wrecks	

Table 2.4. Number of chemicals identified within each contaminant group (as of March 2022)

Contaminant group	No. chemicals identified
Hydrocarbon (biogenic)	11
Hydrocarbons (Petrochemical)	63
Hydrocarbons (pyrogenic)	52
Mixtures	16
Metals	34
Organometals	19
Flame retardants	14
Esters	4
Perfluoroalkyl substances (PFAS)	3
Personal Care Product chemicals (PCPs)	6
Pesticide/Biocide	139
Pharmaceutical	99
Non-phthalate plasticizer	1
Phthalates	7
Polychlorinated biphenyls (PCBs)	9
Synthetic musk	2
Synthetics (other)	96
Inorganic chemicals	16
Natural product	14
Chemical warfare agent	22
Explosives/propellants	13
Total	**639**

3 Phase 2 – Test of the proposed approach

The aims of Phase 2' of the contaminants study were:

- examine the potential scale of the 'contaminants' literature review on one or more 'test' habitats or species;
- determine the practicability of using the 'Rapid Evidence Assessment' (REA) method to improve sensitivity assessment reviews, and to
- test the 'weight of evidence' approach, suggested in phase 1, for assessing resistance and, hence sensitivity.

Blue mussel beds (littoral and sublittoral) were chosen as the case study. Hence, *Mytilus edulis* was chosen as the main subject of review, as the biogenic, structural species in blue mussel beds (e.g. LS.LBR.LMus or SS.SBR.SMus.MytSS). In addition, *Mytilus* spp. are well studied. Therefore, it was hoped that *Mytilus* spp. would give a good indication of the potential scale of the literature review and the time taken to undertake the REA.

Two other species were examined briefly, the gravel sea cucumber *Neopentadactlya mixta* as the important characteristic species of SS.SCS.CCS.Nmix, and *Tubularia indivisa* as an example faunal turf species that dominates several faunal turf biotopes (e.g. CR.HCR.FaT.CTub).

3.1 Methodology

Phase 2 involved the following tasks:

1. Development of the Rapid Evidence Assessment protocol (REA);
2. Literature review and screening;
3. Development of a template spreadsheet to summarise and extract (or map) the evidence from the articles identified for the review; and
4. Evidence review and sensitivity assessment.

The evidence review was based on the Defra/NERC Quick Scoping Reviews and Rapid Evidence Assessments guidance[30] (Collins *et al.*, 2015) together with examples of relevant REAs and Systematic reviews (Johnston & Roberts, 2009; Johnston *et al.,* 2015; Randall *et al.*, 2015; Collier *et al.*, 2016; Mayer-Pinto *et al.,* 2020).

The REA process involves the following steps (summarized from Collins *et al.*, 2015).

- Develop protocol (including the details of the evidence review question(s) and methodology)
- Search for evidence (using the search strategy and methodology in the protocol)
- Screen the search results using relevancy (inclusions and exclusion) criteria outlined in the protocol
- Extract evidence relevant to the evidence review question(s) – and create a 'map of the evidence'
- Critically appraise the evidence and its relevance to the 'review question'.
- Synthesize the results.
- Communicate the results

[30]https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/560521/Production_of_quick_scoping_reviews_and_rapid_evidence_assessments.pdf

In Phase 2, the process outlined above was followed as far as possible with minor alterations due to the time constraints and the aims of the project at this stage.

3.2 Development of the REA protocol

The Rapid Evidence Assessment (REA) protocol was drafted during Phase 2, based on Collins *et al.* (2015) with reference to Johnston & Roberts, 2009; Johnston *et al.,* 2015; Randall *et al.*, 2015; Collier *et al.*, 2016; Mayer-Pinto *et al.,* 2020). The resultant REA protocol was applied in Phase 2 and amended based on the results below. The protocol is presented in Section 4 below.

3.3 Search for evidence

An initial search was conducted in Web of Science (WoS) using the chemical and activity-based keywords listed in the protocol and '*Mytilus*'. The exact search strings and their results are given in the 'Chemical-search-strings' spreadsheet (attached). Another 552 references were extracted from the US EPA's ECOTOX database[31].

The initial searches resulted in 9,197 hits of which 6,412 were duplicates. Therefore, only WoS and ECOTOX were used at this trial stage due to time constraints. All 'hits' were downloaded into and managed in Endnote (X20.1). The resultant 3,337 references were screened for relevance based on the proposed REA protocol (see below).

3.4 Screening

The resultant list of articles was then subject to a two-stage screening process (Collins *et al.,* 2015) against the inclusion and exclusion criteria (see protocol below). The results are summarized in Table 3.1.

Table 3.1. Summary of literature search and screening

REA procedure		No. hits / articles	No. excluded
Identification	Papers identified in database searches	9,749	
	Papers after duplicates removed	3,337	6,412
Screening (stage 1)	Papers after screen by title and abstract	2,684	653
Screening (stage 2)	Papers screened by full-text	664	NA**
Evidence review	Papers subject to detailed evidence review	144	520

** (note – the scope was reduced to 'hydrocarbons' only in Phase 2)

Stage 1 involved a look at the title the articles and a quick look at the abstract. Stage 2 involved an examination of the abstract, introduction, and possibly conclusions of the articles based on a speed-reading of the article. At this point, screening is intended to exclude those articles that are definitely not relevant to the 'review question'.

3.5 Trial evidence review

Stage 2 screening resulted in 664 articles relevant to the 'review question' (see protocol below). Therefore, the scope of the evidence review was limited to 'Hydrocarbons and PAHs' due to the time constraints on Phase 2.

The resultant 144 articles (Table 3.1) were subject to detailed review. At this stage, several review articles and additional papers were added to the list based on evidence cited within other papers. A further 25 articles were excluded at this stage after reading of the paper in detail. The resultant evidence review of *Mytilus* spp. and the pressure 'Hydrocarbons and PAHs' is presented in Section 6.

[31] US EPA Ecotoxicology database- https://cfpub.epa.gov/ecotox/

3.6 *Neopentadactyla mixta* and *Tubularia* spp.

Preliminary literature reviews were undertaken for two other species, the gravel sea cucumber *Neopentadactlya mixta* and the turf forming, fouling, hydroid *Tubularia* spp. both were subject to the search terms listed in 'Chemical-search-strings' spreadsheets against the relevant genus name.

The WoS searches for '*Neopentadactlya*' and contaminants returned exactly 'zero' hits. Therefore, the search was expanded, and the search strings entered into Google scholar. The searches returned 427 hits. However, only 31 remained after the initial screening, removal of duplicates left six potential articles, all of which were rejected as not relevant to contaminants.

The WoS searches for '*Tubularia* spp.' and contaminants returned 'zero' hits. Therefore, the search was expanded, and the search strings entered into Google scholar. The searches returned 4,816 hits, of which 663 were probably relevant after initial screening. However, only 104 hits remained after duplicates were removed, which may provide useful evidence.

In the preliminary searches, no evidence on the effect of contaminants on *Neopentadactyla* spp. was found. Therefore, sensitivity assessment would be based on the larger review of the effects of contaminants on echinoderms as a whole. Similarly, the searches found 104 potential reports on the effects of contaminants on '*Tubularia* spp. '. However, the breadth of contaminants mentioned in these 104 articles was limited. We suspect that additional information based on 'hydroids' in general would be needed to complete the sensitivity assessments of all of the contaminants pressures.

3.7 Conclusions

The aims of this phase of the 'contaminants' study (Phase 2) was to gauge the breadth and scale of the literature review, investigate the REA methodology, and test the 'weight of evidence' approach to sensitivity assessment.

1. *Mytilus* spp. was chosen deliberately as it was a well-studied species. However, the size of the literature review was larger than expected so much so that it had to be curtailed in order to fit into the ca one month of staff time budgeted for Phase2. To make this task manageable, only one bibliographic database was used (WoS), augmented by articles from the ECOTOX database, and then the focus of the literature review was restricted to only one genus *Mytilus* (rather than similar bivalves) and one pressure, 'Hydrocarbons & PAHs'.
2. We would need to use more than one bibliographic database (e.g. ScienceDirect, ASFA, and Google Scholar) in order to ensure 'good' coverage in future, and to follow standard REA procedures. WoS seemed to be inclusive from the mid 1990s but missed many seminal papers from the early 90s, the 80s, and 70s, which we added because we already had accessed them in prior sensitivity reviews. Due to the time restraints, we were not able to follow up many potential leads from the papers found nor pursue interlibrary loan services.
3. *Neopentadactlya mixta* was chosen deliberately as an example of a poorly studied species. In this case, we expanded the searches to include Google Scholar. No relevant hits were found. As expected, we would need to use proxies from similar taxonomic grounds, e.g. Holothuroidea, or Echinodermata to assess this species. As echinoderms are sentinel species used in toxicological studies, the Echinodermata review is also likely to be large.
4. *Tubularia* spp. was chosen as a potentially intermediate species. Again, WoS discovered nothing, and the review was expanded to Google Scholar, which revealed numerous potentially useful hits. But again, its sensitivity assessment is likely best served by a review of the effects of contaminants on 'Hydroids' as a group, which may be large.
5. The Rapid Evidence Assessment (REA) approach provided a transparent documentation of the literature review process and allowed us to provide a detailed overview of the evidence extracted

(e.g. Figures 1 &2) and potentially, to compare studies. The evidence summary demonstrated the prevalence of studies of sub-lethal effect but mainly the considerable variation in studies and their experimental design.

6. However, the detailed audit trail at the screening Stage 2 is an additional time-constraint as is the evidence summary (map). It may have added 30-50% additional time to the literature review process, although it would still have taken weeks to read and process the over 140 studies that included relevant evidence on the effects of 'hydrocarbons' on *Mytilus* spp.
7. The initial screening (stage 1) took one person ca 8 days to complete, screening stage 2 two people ca two weeks to complete and the evidence summary two persons ca one month to complete (although one was also pursuing additional leads), and not including writing up the sensitivity assessment (see above).
8. The results of Phase 2 suggest that we would need ca one person month for each of the remaining pressures ('Synthetics' and 'Metals') but less for 'Others' in *Mytilus* spp. Collins *et al.* (2015) suggest that one REA could take 5-8 months and cost £20-50K but that assumes a tightly focused REA with a one 'review question' while we are looking to review many species in many habitats.
9. We could put time limits on the literature review or limit ourselves to the first two thousand, but we risk missing important evidence. Similarly, if we limit ourselves by date we will miss key evidence, especially as in the case of *Mytilus* many of the acute toxicity studies date to the 70s and 80s. However, such limits may not conform to REA standards.
10. The results from Phase 2 suggest that WoS is poorly key worded and catalogued if a search for the phylum returns fewer results than a species within the phylum. Both WoS and Google scholar are only Science-Citation Indices and Google scholar is limited to materials in the public domain inc. grey literature.

3.8 Further test of literature review

The literature review approach was tested on a wider range of species to see if the number of 'hits' in the searches from *Mytilus* spp. were much higher than could be expected for most species. It was suggested that we examine additional bivalves (e.g. oysters), seagrass, and sea pens and examine the possibility of using high-level taxonomic groups.

Therefore, we applied the same search strings developed in Phase 2 to:

- Seagrass – *Zostera* spp.
- Sea pens – *Funiculina* spp., *Pennatula* spp., and *Virgularia* spp.
- Oysters – *Crassostrea* spp., *Ostrea* spp. and *Magallana* sp.,
- *Abra* spp., and
- Echinoderms.

In addition, we expanded the searches to the relevant Class and Phylum. In Echinoderms, we included species used to indicate sensitivity for a range of biotopes (see Appendix 1). We also include SCOPUS[32], another science citation index with a different range of journals than Web of Science (WoS).

Each of the search strings developed for *Mytilus* (see Section 5) were applied in turn across each of the bibliographic databases examined. However, we used common sense to determine the best search strings to use. For example, if the least restrictive search string gave an unmanageable number of 'hits' (e.g. several hundred) then a more restrictive (focused) string was used. Conversely, if the number of

[32] SCOPUS –www.scopus.com

'hits' was very low (e.g. <10) or zero then a less restrictive search string was used (see spreadsheet). Some species resulted in zero hits for most searches. In these cases, (e.g. the sea pens*, Leptometra, Ocnus* etc.) then the broad search (ALL=Taxon) was used to capture everything listed for that species in the bibliographic database. Furthermore, the search was also expanded to include Google Scholar in the hope of finding grey literature not included in the more academic science citation indices.

The results (hits) for *Abra, Zostera* and seagrass were also downloaded into Endnote, the number of duplicate results checked, and the remaining unique records subject to Level 1 screening (title and abstract only) against the inclusion and exclusion criteria developed in Phase 2.

Results and discussion

The results are summarized in Table 3.2, which shows the total number of hits (articles discovered) by all searches. Where known (see below) the number of unique articles is shown in brackets.

The ECOTOX[33] database records every chemical tested and different endpoints and test methods used from each article recorded so that any one article can have multiple entries. Hence, Table 3.2 records the number of 'hits' together with the number of unique articles in brackets. However, the focus of the ECOTOX database means that most (if not all) of the articles will be directly relevant to the effects of each chemical on each species but may not examine habitat or ecosystem effects.

SCOPUS routinely returned the most hits. However, it also had the highest rate of duplicate records. For example, for *Zostera* and seagrass 57% of records obtained via Web of Science (WoS) were duplicates while 87% of the records obtained for *Zostera* alone were duplicates. It was not sensible to download the 53,526 hits returned by SCOPUS for seagrasses. In *Abra*, the duplicate rate was 44% for WoS and 73% for SCOPUS. SCOPUS often returned duplicates in the same search. A 'by eye' appraisal of the search results from SCOPUS suggested that many of the 'hits' would also be irrelevant and excluded from the study.

Seagrass and *Zostera* returned several thousand hits from WoS but after removal of duplicates and level 1 screening, this number would be reduced to **only 403** potentially relevant articles, including those from ECOTOX. Fortunately, the term 'seagrass' also included several other species of seagrass in the articles discovered so that the review should include the effects on similar species if not *Zostera* itself. We did not search for the Class or Phylum, as 'flowering plants' were likely to return an impractical number of irrelevant hits. Similarly, SCOPUS returned an impractical number of hits (ca 71,101) even with an 87% duplicate rate. Nevertheless, it would take several weeks to interrogate ca 400 papers.

Mollusca and bivalves returned ca 49,000 hits across the entire group from WoS. *Mytilus* was the largest contributor with *Crassostrea* close behind. Based on our experience with *Mytilus*, this may result in several hundred relevant articles for detailed review. ECOTOX alone identified ca 610 articles for *Mytilus* and 340 for *Crassostrea*, which are probably relevant. There are a total of 36 bivalve species and 12 gastropods listed in Appendix 1.

Sea pens returned the fewest hits. In this case, the effects of contaminants on UK sea pens would need to be informed by a wider search of the effects on other Anthozoans and Cnidarians, and/or from review articles. Few of the articles on the sea pen species themselves might be relevant to the study. However, if we expand the search to include e.g. Anthozoa and Cnidaria we will probably need to examine at least 82-182 articles in detail.

Echinoderms was a varied group, with the sea urchins returning the most 'hits' and the crinoids and holothurians the least. We would probably need to review this group at the Phylum level, with perhaps the exception of *Paracentrotus* and *Strongylocentrotus,* whose larvae are used as a test species for chemical effects. ECOTOX alone provided **ca 300** articles, which are probably relevant.

[33] US EPA Ecotoxicology database- https://cfpub.epa.gov/ecotox/

Table 3.2. Total count of 'hits' for multiple search strings across multiple taxa i.e., Phyla, Class and example component species. The component species are those listed as characteristic within marine habitats. Notes: no. of hits given, no. of unique in brackets where known. ECOTOX hits in the database includes multiple entries/values from each article; ** too many to download in a single file.

Taxon / group	WoS	SCOPUS	ECOTOX	G. Scholar
Phylum Trachaeophyta	Too large			
Class Magnoliopsida	Too large			
Seagrass*	**2,639**	**53,526**	124 (12)	
Zostera	**1,201**	**17,575**	552 (32)	
Total for group	**3,837**	**71,101**		
Phylum Mollusca	**6,694**	305,316	40,000**	
Class Bivalvia	**14,446**	319,082	27,000**	
Oyster*	**11,038**	270,103	44	
Family Ostreidae	86	23,633	**4,271**	
Ostrea spp.	484	27,785	0	
Crassostrea spp.	**7,141**	151,943	**4,327 (340)**	
Magallana spp.	60	953	0	
Mytilus spp.	**9,197 (2,785)**		**6,564 (610)**	
Abra spp.	218 (101)	6,222 (1,231)	20(1)	
Total for group	**49,394**		**Ca 40,000**[34]	
Phylum Cnidaria	420	21,347	**2,012 (182)**	30,231
Class Anthozoa	107	26,208	930 (82)	13,379
Sea pen*	375	1,885	0	654
Virgularia spp.	29	284	0	195
Pennatula spp.	57	653	0	2,594
Funiculina spp.	27	225	0	366
Total for group	**1,015**		**Ca 2,000**[3]	
Phylum Echinoderm	**1,126**		**3,600 (307)**	
Class Ophiuroidea	28		38 (8)	
Brittle star*/Brittlestar*	103		36 (6)	
Amphipholis	13		0	
Amphiura	84		16 (1)	
Ophiocomina	45		0	
Ophiothrix	20		0	
Ophiura	12		1 (1)	
Class Crinoidea	16		47 (3)	
Crinoid*	95		0	
Antedon	95		47 (3)	
Leptometra	12		0	
Class Asteroidea	69		195 (42)	

[34] ECOTOX is catalogued by taxonomy

Taxon / group	WoS	SCOPUS	ECOTOX	G. Scholar
Starfish	405		145 (32)	
Asterias	434		194 (42)	
Marthasterias	12		4 (1)	
Crossaster	34		0	
Class Echinoidea	99		0	
Sea Urchin / Heart Urchin	**2,429**		**2,828(237)** / 21(1)	
Brissopsis	10		0	
Echinocardium	21		21 (1)	
Echinus	31		1074 (84)	
Psammechinus	49		141 (7)	
Paracentrotus	**1,158**		761 (78)	
Strongylocentrotus	331		**1,438 (152)**	
Class Holothuroidea	77		21 (6)	
Sea Cucumber	536		21 (6)	
Holothuria	189		17 (5)	
Leptosynapta	65		5 (1)	
Ocnus	21		0	
Neopentadactyla	4 (0)		0	
Total for group	**7,948**		**Ca 3,600**[3]	

Notes – 'sea pen' also returns articles on the use of 'pens' in the sea.

At this point, we have not looked at the Arthropoda and Crustacea or Annelida and Polychaeta or macroalgae, as they are likely to be a well-studied taxa while smaller groups (e.g. Bryozoa) may be much less studied. Table 3.3 lists the major taxonomic groups ranked the number of 'hits' on ECOTOX alone. Unsurprisingly the molluscs and crustaceans are likely to be the largest groups to review.

In short, even where any individual species is poorly studied, we would probably need to examine several hundred articles in detail for their taxonomic groups in order to answer or study question and report on the effects of contaminants on species and ultimately habitats. There are a few exceptions e.g. Brachiopods and sponges, although we may need to look for proxies with similar metabolic pathways and, hence, response to chemicals (e.g. pharmaceuticals).

3.9 Recommendations

The Rapid Evidence Assessment (REA) protocol provides a recognised approach to the critical appraisal of evidence. However, the literature reviews and assessment task is potentially large and, given the scope of the study and our time constraints, we need to simplify our Rapid Evidence Assessment (REA) approach and focus our effort.

Therefore, the following changes to the REA protocol and evidence summaries were suggested in Phase 3.

1. The detailed Level 1 and 2 screening used by the standard REA protocol is simplified. The inclusion and exclusion criteria developed in Phase 2 to identify relevant articles for examination are used. However, a detailed record of the inclusion/exclusion criteria against each article is not kept.

Table 3.3. Phyla and taxonomic groups ranked by the number of entries returned from ECOTOX. No. of unique references in brackets.

Taxon	ECOTOX (hits)
Green algae[35]	40,539
Mollusca	40,000
• Bivalvia	27,000
• Gastropoda	12,845
Crustacea	30,911
• Decapoda	23,888
Echinodermata	3,600 (307)
Polychaeta	3,068 (269)
Cnidaria	2,012 (182)
• Hydrozoa	993 (89)
• Anthozoa	930 (82)
Red algae	879 (86)
Brown algae	708 (91)
Bryozoa	281 (19)
Porifera	196 (21)
Lichens	48 (1)
Brachiopoda	7 (3)

2. The number of duplicates obtained by the searches is not recorded.
3. Emphasis is given to the results from ECOTOX for individual species/taxa and WoS for wider ecosystem/habitat wide effects.
4. Review articles are used to speed up the literature review where possible.
5. The SCOPUS bibliographic database is not used, unless no other database reveals results.
6. Google Scholar is used to fill gaps, e.g. in grey literature, where other databases reveal few 'hits', that is, in otherwise poorly studied group and species.
7. Relevant articles are tabulated and summarized (mapped) in detail to summarise toxicological 'end points' (e.g. LC_{50}, EC_{50}, NOEC, LOEC, etc.), physiological effects, mortality rates, and habitat effects in tabular form to support sensitivity assessments.
8. Relevant articles are summarized in narrative form for inclusion in sensitivity assessments.

Nevertheless, the task is large and we need to prioritise the time available. For example:

9. Examine habitats dominated by a single species, that is, biogenic habitats (e.g. horse mussel beds, blue mussel beds, flame shell beds, serpulid reefs, *Leptometra* aggregations, seagrass etc.), or
10. Focus on one or two dominant taxonomic groups within habitats, e.g. bivalves or polychaetes that dominate many sedimentary habitats.

[35] Probably includes microalgae as well as macroalgae

4 Phase 3 – Application of the approach to an example habitats/species

Phase 3 was taken forward based on the recommendations from Phase 2. Phase 3 included:

- Revision of the REA protocol;
- Revision of the Evidence summary spreadsheet template;
- Completion of REA Evidence reviews of the effect of contaminants on *Mytilus* spp. and resultant sensitivity assessments; and
- Completion of REA Evidence reviews of the effect of contaminants on *Zostera* spp. and resultant sensitivity assessments.

Mytilus spp. was chosen as the biogenic species, indicative of the sensitivity of blue mussel beds and their relevant biotopes. *Zostera* spp. was chosen biogenic species, indicative of the sensitivity of UK seagrass beds (Zmar and Znol). However, evidence of 'seagrasses' worldwide was included to ensure that the review covered the range of effects and contaminants outlined in the scope of the review.

4.1 Modification to the REA protocol

The REA protocol was modified based on the recommendations above (section 3.9) and the experience of Phase 2. In particular:

- Articles on bioaccumulation and body burden were excluded, unless they also documented any other effects on the species or population of interest; and
- Articles on the use of the species as bioindicators (and biochemical/enzymatic biomarkers) were excluded, unless they also documented any other effects on the species or population of interest.

4.2 Modification to the 'Evidence summary' spreadsheet

The 'evidence summary' spreadsheet template was modified to remove fields that we did not use in the REA for 'Hydrocarbons and PAHs' in *Mytilus* spp. and to record individual 'end points' in more detail. In particular:

- Fields related to bioaccumulation, and body burden were removed;
- All of the 'effects' or 'end points' (e.g. LC_{50}, EC_{50}, LOECs etc) reported in the articles examined are recorded; and
- A 'worst-case' effect or mortality, together with the narrative summary, for each individual article and/or individual contaminant studied within each article is recorded, on which to base the sensitivity assessment.

The changes to the 'evidence summary' also allowed us to import ECOTOX data directly into our spreadsheet to save time on data entry. We also streamlined the fields and adopted ECOTOX definitions where appropriate (see REA protocol).

4.3 Phase 3 – Evidence reviews and sensitivity assessments

The revised, current, protocol is discussed in Section 5 below, and the resultant 'Evidence reviews' and sensitivity assessments in Sections 6 & 7.

5 Rapid Evidence Assessment (REA) - protocol

The 'Contaminants' Rapid Evidence Assessment (REA) protocol was designed to collate and synthesis the evidence required to inform the assessment of the resistance to, and hence sensitivity of, marine habitats and species to the MarESA 'Contaminant' pressures; 'Hydrocarbons and PAHs'; 'Transition elements and organo-metals'; 'Synthetic compounds (inc. pesticides, antifoulants, pharmaceuticals)' and 'Introduction of other substances (solid, liquid, gas)'.

5.1 Background

Resistance assessment is based on the evidence collated in the literature review on the effects of each pressure (or activity that results in a given pressure) on the key elements of the feature (physical habitat and species that contribute to sensitivity). Resistance assessment considers the following for each pressure in turn:

- reported evidence on the direct effect of a given pressure on the key elements of the feature, compared to the benchmark level of pressure;
- the resultant levels of damage on the key elements, e.g. extent of damage to habitat, loss of population size or abundance, changes in diversity, loss or reduction in abundance of one of more species groups;
- reported evidence on the direct effect of a given pressure on similar habitats, species, or functional groups, and/or
- 'proxies' are used to inform the assessment of the likely effect of a pressure on the key elements of the feature, in the absence of direct evidence.

Wherever possible, direct evidence of the effect of a given pressure on the 'key elements of the feature' (habitat and/or the species) is used as the basis of the assessment of resistance. Where the evidence quantifies the magnitude, extent or frequency of the pressure then the evidence can be compared directly with the benchmark. Similarly, if the pressure is qualified in the evidence then it can be compared with the relevant benchmark. The quality of the evidence and its applicability to each pressure assessment is ranked using the 'confidence assessment' scale (Tyler-Walters *et al.*, 2018).

In some cases, where evidence is lacking, it is possible to use 'proxies' against which a resistance assessment can be made. For example, congeners or members of the same taxonomic Class or even Phylum may be suitable 'proxies' for the physiological or toxicological effects of one or more chemical groups. Similarly, chemicals that have the same mode of action or act on the same metabolic pathway may be proxies for other chemicals that are not studied in detail.

The resultant 'resistance' assessments are combined with a species or habitat 'resilience' assessment, reviewed separately, to determine an overall sensitivity assessment (Tyler-Walters *et al.*, 2018).

Phase 1 of the project (section 2) concluded that quantified benchmarks were impractical so that a 'weight of evidence' approach was the most practical way to assess resistance to the effects of contaminants on marine habitats and their species. It also concluded that the REA approach was a useful approach to improve and standardise the literature review process.

5.2 Current REA protocol

The evidence review process was based on the Defra/NERC Quick Scoping Reviews and Rapid Evidence Assessments guidance[36] (Collins *et al.*, 2015) together with examples of relevant REAs and Systematic reviews (Johnston & Roberts, 2009; Johnston *et al.,* 2015; Randall *et al.*, 2015; Collier *et al.*, 2016; Mayer-Pinto *et al.,* 2020).

The REA process involves the following steps (summarized from Collins *et al.*, 2015).

- Develop protocol (including the details of the evidence review question(s) and methodology)
- Search for evidence (using the search strategy and methodology in the protocol)
- Screen the search results using relevancy (inclusions and exclusion) criteria outlined in the protocol
- Extract evidence relevant to the evidence review question(s) – and create a 'map of the evidence'
- Critically appraise the evidence and its relevance to the 'review question'.
- Synthesize the results.
- Communicate the results

5.3 Defining of the 'review question'

The 'Contaminants' (REA) review aimed to provide the information required to assess the likely effect of any given 'contaminant' pressure on a range of marine habitats and their associated species.

MarESA resistance assessment is based on effects that result in:

- the loss of or reduction in population size, extent, or abundance of one or more species groups within the habitat,
- the loss of diversity, and/or
- damage to the extent or function of the habitat (see above).

These are likely to result from:

- the direct mortality of adults and their loss from the habitat/species population,
- the direct mortality of larvae, juveniles or other propagules so that recruitment is reduced/prevented, or
- direct or indirect effects on reproduction and recruitment resulting in population decline.

Resistance assessment, in MarESA, is predicated on evidence of 'mortality', 'population decline, and/or habitat modification. In most cases, it is assumed that the 'contaminant' pressures will affect habitats via their effects on individual species. The exceptions are the physical effects of oils and the ecosystem-wide effects of nutrient enrichment.

Therefore, the evidence requirements can be expressed as the following **'review question'**:

'Does exposure of taxon 'a' to contaminant 'x' result in:

1. **the direct mortality of adults and their loss from the habitat or population,**
2. **the direct mortality of larvae, juveniles or other propagules so that recruitment is reduced/prevented,**

[36] https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/560521/Production_of_quick_scoping_reviews_and_rapid_evidence_assessments.pdf

3. **or direct or indirect effects on reproduction and recruitment resulting in population decline of one or more species in the habitat of interest.**

Therefore, the 'Contaminants' REA evidence reviews concentrate on the evidence required to answer the above question. The term 'taxon' is used to denote the relevant taxonomic level of unit, e.g. Species, Genus, Family, Order, Class, or Phylum.

5.4 Scope (inclusions and exclusions)

The proposed scope is outlined below and summarized in the following PICO table (Table 5.1).

1. **Marine benthic habitats** - include all (ca 400+ biotopes) identified by the UK Marine Habitat Classification (UKMHC; JNCC, 2015) from the supralittoral to the sublittoral including habitats in the shallow shelf seas (<200 m deep) and deep-sea (>200 m deep) (Connor *et al.*, 2004; Parry *et al.*, 2015; JNCC, 2015).
2. The focus is on marine, estuarine and transitional water habitats as listed in the UKMHC (JNCC, 2015)
3. **Marine benthic species** refers to the agreed list of species 'indicative of sensitivity'[37] (Appendix 1), together with their congeners, co-familial and/or members of the same taxonomic group including Class and Phylum where required.
4. **Geographic range** –United Kingdom habitats and species, although relevant information from the coasts of the North East Atlantic and other temperate regions will be included where required. Where evidence is lacking, information from similar species and habitats in the temperate southern seas, or tropics will be included.
5. **Biogeographic range** – marine temperate species and habitats in the northern hemisphere. Where evidence is lacking, information from similar species and habitats in the temperate southern seas, or tropics will be included.

Information on the effects of contaminants on many marine species may be poorly studied. Therefore, the species range may be expanded to include similar congeners, members of the same family or Phylum. At present the emphasis in on marine benthic species and their propagules/larvae/juveniles. Therefore, demersal and pelagic mobile species are excluded, in particular, fish, marine reptiles and marine mammals. Phyto- and Zooplankton are also excluded, except where they include the larval or juvenile stags of the benthic species of interest.

The biogeographic and geographic range may be expanded to include evidence from the southern hemisphere and/or tropics. The emphasis is on UK marine and brackish water species and habitats. Freshwater species are excluded except where they can be used a 'proxies' for species of the same taxonomic group or that share the same AOP/MIE for one or more chemicals of interest.

6. **'Contaminant'** refers to those groups of chemicals and individual chemicals listed in the agreed table of contaminants (Section 2.6; Contaminant Chemicals Groups' March 2022 spreadsheet). At present, the agreed list excludes macro-plastics, micro-plastics and other marine debris. Chemicals that evaporate if spilt (evaporators) are also excluded.
7. **'Nutrients'** and 'organic enrichment' are excluded because these pressures have already been subject to MarESA sensitivity assessment.
8. **'Exposure'** - the following potential routes of 'exposure' to contaminants are included**:**

- physical contact – e.g. smothering/clogging by oils;

[37] As defined in the MarESA Guidance Manual 2018 (Tyler-Walters *et al.*, 2018).

- physical ingestion – e.g. of oils or particulates;
- ingestion and/or absorption from water i.e. the water column or interstitial water;
- ingestion /absorption from food including contaminants adsorbed onto organic or inorganic particulates, or
- absorption from the substratum e.g. sediment.

At present, inhalation by birds, reptiles, or mammals is excluded, as these mobile species are not included in the study.

Table 5.1. PICO elements and summary of relevant inclusion and exclusion criteria.

		Inclusion criteria	Exclusion criteria
Population	Marine benthic habitats and their component species	All species on agreed list, plus congeners, co-familial Members of same taxonomic groups e.g. Order, Class, Phyla	Mammals, reptiles, birds, fish; phytoplankton (unless a macroalgal propagule); viruses; zooplankton (unless a relevant larval stage).
Intervention (Exposure)	Physical smothering/ingestion/clogging Ingestion/absorption via water Ingestion/absorption via sediment/substratum Ingestion/absorption via food	Agreed list of chemicals Nanoparticulate / Engineered Nanomaterials	Air borne gases Evaporators that disperse at water surface Plastics/Microplastics Non-toxic spills – e.g. coal, wheat, grains etc.
Comparator	Examination of the effect of a contaminant, compared to a control on a species or habitat of interest Examination of the effect of a contaminant before or after a spill or incidental release into a habitat or on a species population of interest	Quantitative experimental controlled laboratory studies inc. randomized control and non-randomized control studies Quantitative experimental, controlled, in situ (field) studies/survey inc. randomized control and non-randomized control studies Quantitative observational studies/survey of before and after spills/incidents, case-controls Quantitative observational studies of long-term effects Quantitative or qualitative reviews – literature reviews, systematic reviews.	Anecdotal observations
Outcome	**Species** Toxicity (mortality of adult/larval/propagule) Larval/juvenile abnormalities Physical (smothering,	Direction of effect (i.e. increase or decrease) Qualification or quantification of effect Lethal effect concentrations	Accumulation studies e.g. bioaccumulation, bioindicator studies (except if they explain/result in mortality and population decline) Biochemical (except if they

		Inclusion criteria	Exclusion criteria
	suffocation, clogging) Toxicity (reproductive impairment; endocrine disruption) Toxicity (effect on growth, repair) Behavioural response (resulting in population decline (e.g. due to mating failure), feeding behaviour resulting in mortality or reduced fecundity/recruitment or loss from site of interest) **Habitat** Physical/chemical habitat modification resulting in recruitment failure Physical modification of the habitat (smothering) Change in species diversity, population extent, species abundance and community composition (i.e. biotope) Changes in trophic interactions (e.g. abundance of grazers), productivity,	(e.g. LC_{50}, PEC) No-effect concentrations (e.g. NOEC, PNEC) Sub-lethal effects	explain/result in mortality and population decline) Cellular/molecular studies (transomics and genomics) (except if they explain/result in mortality and population decline) Physiology or behaviour (except if they explain/result in mortality and popn decline) Studies of population genetics, ecology, autoecology, taxonomy, socio-economics, or non-contaminant –based 'pressures'

9. **Maritime activities –** the review includes operational and incidental spills, operational releases, and discharges from maritime activities (offshore and inshore), as well as activities that discharge into water courses that ultimately reach marine waters (Table 5.2).

The review will prioritize releases in the marine environment but will also need to include freshwater (riverine) inputs. Exposure from aerosol deposition is excluded except where the aerosol is known to dissolve in water and becomes available to benthic species.

5.5 Search for evidence

Peer-reviewed and grey literature is searched using:

- Web of Science (WoS; Core Collection: Citation Indexes) 1970-present, and
- US EPA's ECOTOX database.

In addition:

- Review articles, including systematic reviews, are used to speed up the literature review where possible.
- The SCOPUS bibliographic database is not used, unless no other database reveals results.

- Google Scholar is used to fill gaps, e.g. in grey literature, where other databases reveal few 'hits', that is, in otherwise poorly studied group and species.

Additional evidence will be obtained from the references lists of the literature discovered, together with relevant review articles and reports.

Table 5.2. Maritime/Coastal activity and process-based search terms (preliminary).

Primary term	Secondary terms
Shipping (commercial & recreational)	Vessels / Tankers / Freight
Spills (Accidental/Incidental)	Shipping (commercial & recreational) Inshore/offshore installations Harbours/Ports/Berths/Moorings/Bunkering Chemical spills Oil spills Spills of containers/barrels (oil and chemical)
Operational discharges	Shipping (commercial & recreational) Bilge water Ballast tanks Oil & gas installations Renewables/wind farms (inc. cable installation, support vessels) Mariculture Harbours/Ports/Berths/Moorings/Bunkering
Antifouling paints	Shipping (commercial & recreational) Mariculture/Aquaculture/Fin and shellfish) Offshore renewable Harbour/Port infrastructure (e.g. buoys pontoons etc.)
Mariculture/Aquaculture (fin-fish, shellfish)	Chemotherapeuticals/Medicines Antibiotics Parasiticides/Biocides Anaesthetics Disinfectants Food supplements Antifoulants
Oil & gas exploration/ production	Drilling wastes/muds/cuttings Produced waters Decommissioning Cables/Pipelines
Inshore/Offshore renewable (inc. cables)	Antifoulants Construction/decommissioning Sediment remobilization
Dredging and dumped spoil	Aggregate dredging Channelization Harbours/Ports
Inshore discharges/outfalls	Power stations Industrial effluents Sewerage effluents (inc. human pharmaceuticals)
Runoff	Agricultural runoff (e.g. hormones/pesticides/nutrients) Urban runoff Mine effluents/waste runoff
Munitions dumps	Chemical warfare agents Explosives/propellants
Ship wrecks	

Additional information on toxicology will be obtained from the HNS database and/or the AOP wiki. The Marine Biological Association's (MBA) library catalogue will be used to fill gaps, especially in grey literature and literature prior to 1980, if needed.

A date range of '1970 to present' is used. However, the range may be extended to 1960s for information on oil spills and experimental oil spills. The MarLIN Steering Group or relevant MBA experts will be approached for relevant un-published evidence, where required.

Key words and search strings

Key words based on the pressure name, contaminant groups, contaminant type (Section 2.6; 'Contaminant Chemicals Groups' March 2022 spreadsheet), and marine activities list (see Additional evidence will be obtained from the references lists of the literature discovered, together with relevant review articles and reports.

Table 5.2) were used to develop a suite of standard 'search strings' against each taxon (Appendix 2).

Each of the search strings developed for taxa or habitat type (Appendix 2) were applied in turn across each of the bibliographic databases examined (WoS, SCOPUS, Google Scholar). The search for evidence is designed to be as inclusive as possible so that no potential source of evidence is overlooked. However, we used common sense to determine the best search strings to use.

For example, if the least restrictive search string gave an unmanageable number of 'hits' (e.g. several hundred) then a more restrictive (focused) string was used. Conversely, if the number of 'hits' was very low (e.g. <10) or zero then a less restrictive search string was used (see spreadsheet). Some species resulted in zero hits for most searches. In these cases, (e.g. the sea pens*, Leptometra, Ocnus* etc.) then the broadest search (ALL=Taxon) was used to capture everything listed for that species in the bibliographic database.

ECOTOX has its own search tools that enable the user to specify individual species or broad taxonomic groups (e.g. Molluscs), chemicals, effects, and 'end points'. But, the ECOTOX is a specialist database that records available toxicological information from a wide range of species and habitats so that the majority of 'hits' obtained in ECOTOX are directly relevant to the 'review question'. The following search parameters were used in the ECOTOX database:

- Species name– taxon name, and/or
- Species group (e.g. Molluscs, Crustaceans, etc.) if required to broaden the search
- Chemicals – set to 'All'
- Endpoints – set to 'All'
- Publications - – set to 'All'
- Test conditions – set to 'All'
- Effect groups –, 'Biochemical', 'Cellular', 'Behavioural', 'Ecosystem', 'Growth', 'Multiple', 'Mortality', 'Physiology', 'Population', and 'Reproduction' groups are included but the 'Accumulation' group is excluded.

Search results

The number of the search results (hits) is recorded together with the date of the literature review. The resultant citations were downloaded from the relevant bibliographic database into Endnote (X20.1). The results of the ECOTOX searches were downloaded in Excel format, dated, and copied into the relevant 'Evidence summary' spreadsheet.

5.6 Screening

The resultant list of articles was then subject to a two stage screening process (Collins *et al.,* 2015) against the inclusion and exclusion criteria.

1. Stage 1 involved a look at the title the articles and a quick look at the abstract.
2. Stage 2 involved an examination of the abstract, introduction, and possibly conclusions of the articles based on a speed-reading of the article.

Stage 1 is intended to remove articles captured by the search strings that are obviously not relevant to the study. Stage 2 screening is intended to exclude those articles that are definitely not relevant to the 'review question'.

Collins *et al.* (2015) recommended that the results of Stage 2 were recorded together with reasons why each article was included or excluded based on the inclusion/exclusion criteria. However, this record was omitted due to the time constraints.

The following types of articles were included during screening:

- Papers that examine effects (sub-lethal, lethal, population) of one/more contaminant on the species or habitat of interest;
- Papers that examine effects (sub-lethal, lethal, population) of one/more contaminant on another similar species where no information on specific contaminants on the species of interest was found;
- Papers that might be relevant or link to relevant evidence but are unclear from title/abstract, or only title available;
- Review articles that pointed to other relevant evidence; and
- Evidence of sub-lethal effects on reproduction/scope of growth as it has the potential for population decline.

The following exclusions were made during screening:

- Methodological papers e.g. design of assays, biomarkers and their application;
- Metabolic/proteomics/genomics of the effects of chemicals;
- Marine biotoxins – i.e. from algal blooms/HABs;
- Novel chemicals of pharmaceutical potential extracted from species of interest;
- Human pathogens (e.g. *E.coli*, Strep and viruses) accumulated by mussels;
- Articles not relevant the taxon or habitat of interest – unless they were the only mention of chemical of interest in dataset and may function as 'proxies';
- Evidence on effects of shellfish poisoning or shellfish contamination on humans;
- Faecal pollution; and
- General physiology or genetics i.e. not related to the effect of contaminants.

5.7 Evidence extraction, mapping and appraisal

The evidence extracted (or mapped) was limited to fields likely to be relevant to sensitivity assessment or to categorise the 'level of effect' recorded in each article. The extensive systematic map suggested by Randall *et al.* (2015) was felt to be too onerous.

5.7.1 Evidence summary – terms and definitions

The field names and standard terms used within the 'Evidence summaries' were developed during Phase 2 and 3, based on terms used by the US EPA ECOTOX database or MarLIN glossary, or adapted from the literature review, wherever possible or relevant. Not reported (NR) is used wherever the relevant data/evidence is not reported or specified in the evidence. The field names and relevant standard terms follow.

Short citation

Standard short form of citation for article/paper/book/ report etc.

Study type

Outline of the type of study adapted from ECOTOX definitions:

Term	Definition
Field (obs)	Observation in the field e.g. effect of spills, physical disturbance
Field (expt)	Field based study, e.g. in situ mesocosm, field based experimental design exposed and control plots/quadrats/transects
Laboratory	Experimental or observational study conducted under laboratory conditions
Mesocosm	Experimental or laboratory studies conducted within mesocosms either based in the laboratory or the field
Review	Review article (paper/report). Reviews used as sources of evidence and only novel data in reviews included, originals articles examined for detail
Survey	Survey of multiple site presence/absence/abundance etc. of chemical or species

Note –chemical analysis requires access to a laboratory but is not included within the study type.

Chemical names and groups

'Contaminants group', 'contaminant type', 'contaminant name' and 'CAS number' from the agreed 'Contaminant Chemicals Groups' March 2022' spreadsheet. Two versions of 'contaminant name' are listed:

- 'Contaminant name' reported by the article cited, and
- 'Contaminant synonym' used by ECOTOX or others, if available and different from 'contaminant name'.

Species name

The name of the species studied as reported in the original article. Relevant synonyms, based on WoRMS, are used in the report text.

Life stage studied

Terms defined in MarLIN glossary

- Adult
- Juvenile
- Larvae
- Embryo
- Egg
- Sporophyte
- Gametophyte
- Multiple

Exposure concentration

The experimental concentrations the samples were exposed to, where available, and expressed in reported units and µg/l where possible.

Exposure type

Definitions of the type or route of exposure to the contaminant, adapted from ECOTOX.

Term	Definition
Environmental	Field and incidental exposures, includes via the water column or sediment
Environmental (sediment)	Optional where sediment concentration are paramount (e.g. sedimentary communities)
Flow-through	Continuous or frequent flow through test chamber with no recycling
Food	Introduced via food
Lentic	Static water without measurable flow e.g. lakes, ponds, lagoons
Pulse	Intermittent or fluctuating dosing
Renewal	Without continuous flow of solution, but with occasional renewal of test solutions after prolonged periods, e.g., 24 hours
Spill	Incidental spills
Static	Toxicity tests with aquatic organisms in which no flow of test solution occurs; solutions may remain unchanged throughout the duration of the test.
Tidal	Affected by tides

Study duration

The length of the study and reported by article in hours, days, months or years etc.

Exposure Duration (ECOTOX definition)

The Exposure Duration is the time of actual exposure to the chemical and is expressed as ‘days’. In cases where the observation time is the only duration reported, it is assumed that the Exposure Duration is equivalent to the longest observation time (field: Observed Duration).

For most field studies the ‘Exposure’ and ‘Study Duration’ are identical because it is difficult to determine when the exposure ends. For lab studies the ‘Exposure’ and ‘Study Duration’ may be different, such as when effect measurements were reported from a post-exposure period. For lab studies with injection, topical, or dietary (e.g. intraperitoneally or by gavage) exposure, ‘Exposure and Study Duration’ are typically the same.

For a fluctuating or intermittent dosing experiment, the total exposure time is recorded. In some instances, a biological, or qualitative, time is used, such as an exposure time reported as "until hatch", "growing season" or "after the nth egg has been laid".

Effect group (definitions from ECOTOX)

Term	Definition
Accumulation	Measurements and endpoints that characterize the process by which chemicals are taken into and stored in plants or animals; includes lethal body burden
Behaviour/Avoidance,	Activity of an organism represented by three effect groups - avoidance, general behaviour, and feeding behaviour
Biochemical (inc. enzyme(s), hormone(s))	Measurement of biotransformation or metabolism of chemical compounds, modes of toxic action, and biochemical responses in plants and animals; includes three effect groups - biochemical, enzyme and hormone effects
Cellular/ Histology/ Genetic	Measurements and endpoints regarding changes in structure and chemical composition of cells and tissues of plants or animals as related to their functions; includes three effect groups -cellular, genetic and histological effects
Ecosystem process	Measurements and endpoints to track the effects of toxicants on ecosystem processes; includes microbial processes
Growth/ Development/ Morphology	Category encompasses measures of weight and length, and includes effects on development, growth, and morphology
Mortality	Measurements and endpoints where the cause of death is by direct action of the chemical
Multiple	Measurements related to multiple or undefined effect.
No Effect	The author reported an end point but not a specific effect
Physiology/ Immunological/ Injury/ Intoxication	Measurements and endpoints regarding basic activity in cells and tissues of plants or animals; includes four effect groups - injury, immunity, intoxication and general physiological response
Population	Measurements and endpoints relating to a group of organisms or plants of the same species occupying the same area at a given time
Reproduction	Measurements and endpoints to track the effect of toxicants on the reproductive cycle; includes behavioural and physiological measurements

Effect measurement

A description of the effect measured. These are likely to vary between different taxonomic groups. The ECOTOX database includes many more categories than listed below for some of the 'effect groups'; the numbers are given in brackets. Examples of standard 'effect measurement' terms, organized by 'effect group', include:

- Accumulation
  - Body burden
  - BCF
- Behaviour/Avoidance
  - Chemical avoidance
  - Substratum avoidance
- Biochemical (ECOTOX =1,641 entries)
  - Acyl-CoA oxidase activity
  - Acetylcholinesterase (AchE) activity

    - Acid phosphatase
    - Catalase (CAT)
    - Cytochrome P450 activity
    - Gamma-Glutamyl Transpeptidase
    - Glutathione disulphide
    - Glutathione peroxidase (GPX),
    - Glutathione reductase (GR),
    - Heat shock proteins
    - Lactate dehydrogenase
    - Lipid peroxidation,
    - Metallothioniens
    - MFO (BPH, CYP-dependent monoxygenase)
    - Multixenotoxicity resistance
    - NADPH-Neo tetrazolium Reductase activity
    - NF-E2-related factor 2 (Nrf2),
    - Superoxide dismutase (SOD)
- Cellular (ECOTOX has 143 entries)
    - DNA damage/Micronuclei/Adduct formation
    - Genotoxicity
    - Haemocyte counts population
    - Phagocytosis
    - Lysosomal membrane stability
    - Ovarian and spermatic follicles
    - Transmembrane sodium energy gradient
    - Transcriptomics
- Ecosystem processes
    - General
    - Reduced/Increased productivity (primary/secondary)
    - Community
- Growth/Development/Morphology
    - Abnormal development/larvae
    - Growth rate
    - Leaf/shoot/rhizome/root elongation
    - Leaf shape/morphology
- Mortality (adult/larval)
    - Adult survival
    - Larval survival
- Physiology/Immunological/Injury/Intoxication
    - Byssal thread production
    - Clearance/filtration rate
    - Excretion rate
    - Larval swimming velocity/ability
    - Respiration rate
    - Condition indices
    - Photosynthetic efficiency
    - PSII function/damage
    - Scope for growth (SFG)
    - Valve gape

- Population
  - Abundance/biomass
  - Condition
  - Cover/canopy
  - Distribution/extent
  - Diversity
  - Population decline (general)
- Reproduction
  - Fecundity
  - Gametogenesis reduction
  - Gonad index
  - Fertilization success/failure
  - Recruitment success
  - Settlement
  - Sexual maturity (rate/age)
  - Sex ratios
  - Imposex

Response site

The part (or type) of the organism where the effect (response) is measured (or observed). ECOTOX has 594 entries, which vary between taxonomic groups. We should expect to add terms as we tackle more taxonomic groups but use ECOTOX definitions where possible. For example:

- Community
- Digestive gland
- Embryo
- Gametes (oocytes and sperm)
- Gonad
- Haemocytes
- Larva
- Leaf/shoot
- Lysosomes
- Muscle tissue
- Rhizomes/roots
- Population
- Seedling
- Soft tissues
- Whole organism (assumes adult)

End points

List of observed end points reported by the articles examined, used for consistency with ECOTOX data, but also includes population level effects due to environmental exposure, spills etc. For example:

- BCFD - Bioconcentration factor calculated using dry weight tissue concentration
- EC_{XX}– Effect concentration at XX percentile
- IC_{XX} - Inhibition concentration at XX percentile
- ID_{XX} - Inhibition dose at XX percentile
- LC_{XX}– Lethal concentration at XX percentile
- LD_{XX} – Lethal dose at XX percentile
- LT_{XX} – Lethal time at XX percentile

- LOEC/L – Lowest Observable-Effect-Concentration/Level: lowest dose (concentration) producing effects that were significantly different (as reported by authors) from responses of controls (LOEAL/LOEC)
- NOEC/L – No Observable-Effect-Concentration/Level: highest dose (concentration) producing effects not significantly different from responses of controls according to author's reported statistical test (NOEAL/NOEC)
- Mortality (e.g. after spills)
- NR-LETH – 100% Mortality
- NR-ZERO – 0% Mortality
- Population loss
- Population decline
- Recruitment failure

Endpoint concentrations

ECOTOX provides a single concentration or range (with or without confidence intervals) for each Endpoint. ECOTOX lists the confidence intervals as a range (min, max). In the 'Evidence summary' different End point concentrations (or ranges) are listed separately. Lethal (100%) is included where papers give a concentration resulting in 100% mortality, which is one endpoint recorded by ECOTOX.

Concentrations are expressed as mg/l (ECOTOX) and/or µg/l.

Mortality (%) reported

The percentage mortality reported in the articles examined, where available.

Ranked mortality

The mortality reported in the articles examined is 'ranked' according to the MarESA resistance scale. For example:

Ranked mortality	Resistance
Severe (>75%)	None
Significant (25-75%)	Low
Some (<25%)	Medium
None (reported)	High
Sublethal	High
Unspecified	Unspecified

Unspecified = mortality is reported but not quantified or no detail provided

Quality/Applicability of Evidence – based on MarESA scales

Summary of evidence

The relevant evidence from the articles is summarized in narrative form, using the standard MarESA format description of evidence.

'Worst-case' mortality

The reported 'end points' and evidence from each article is expressed as a 'worst-case' ranked mortality for each contaminant examined in each article. For example, where the specimens are exposed to a range of concentrations of one chemical and several 'end points' (e.g. EC_{50}, LC_{50}) determined, the 'worst-case' or greatest mortality is reported.

Please note, some papers examined several different combinations of contaminant type and seagrass species. Therefore, the 'worst case' mortality is recorded for each unique species vs. contaminant combination within each paper but not for every experimental permutation. For example, if a paper

studied three metals and one herbicide, then we would report the four 'worst case' mortalities rather than every mortality or effect from every concentration tested. However, if the papers examined the same combination on three different species (e.g. in seagrasses) then we would record twelve separate 'worst-case' mortalities.

5.8 Synthesis and communication

The aim of the study is a REA to inform a sensitivity assessment of each of the contaminant pressures against each habitat or the species indicative of sensitivity within each habitat. The resultant sensitivity assessment(s) is presented below. However, the REA approach allows us to qualify the evidence-base as a whole.

The key points from the REA are summarized in report format (see Sections 6&7 below) based on the summary narratives and analysis of the collated evidence. The detailed 'Evidence summaries' are provided in the attached spreadsheets. Only evidence relevant to the 'review question' (the effects of contaminants) on the taxon or habitat interest was recorded in the attached 'Evidence summary'. The evidence is separated into the pressure categories, 'Hydrocarbons and PAHS', 'Transitional metals (inc. organometals)', and 'Synthetics compounds (inc. pesticides, antifoulants, and pharmaceuticals)' and the 'Introduction of other chemicals'.

The results will be disseminated via the MarLIN/MarESA sensitivity assessment web pages.

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6 *Mytilus* spp. - Evidence review

The initial searches (01-15 June 2021) resulted in 9,197 hits of which 6,412 were duplicates. Therefore, only WoS and ECOTOX were used due to time constraints. The resultant 3,337 references were screened for relevance based on the proposed REA protocol (see Section 5). Stage 1 and stage 2 screening against the exclusion criteria reduced this number to 664 articles, which were taken forward for detailed review.

6.1 *Mytilus* spp. - Hydrocarbons and PAHs

The 143 articles relevant to the effects of 'Hydrocarbons and PAHs' and subject to detailed review are shown in 'Mytilus-evidence-summary-Mar2022.xls' (attached). Several review articles and additional papers were added to the list based on evidence cited within other papers. A further 25 articles were excluded at this stage after reading of the paper in detail.

- The majority of papers (ca 70%) were excluded at stage 2 screening because they examined bioaccumulation of contaminants, focused on the use of blue mussels (*Mytilus* spp.) to monitor or detect contaminants, or the use of numerous biomarkers to detect contaminants rather than the effect of contaminants on the mussels themselves.
- Another 25 papers were excluded at stage 3, after closer inspection of the papers, as they discussed monitoring studies, bioaccumulation, or biomarkers but, importantly, provided no information on the effect of hydrocarbon contaminant on *Mytilus* spp.
- Only 46 papers (7%) of those screened at stage 2 could not be accessed (at this stage).
- Most papers examined the effects of PAHs (27%); while the most commonly examined hydrocarbon contaminants were crude oils (22%), oil spills[38] (12.5%), fuel oils (12.5%), or multiple types of hydrocarbons (16%).
- While most papers used standard techniques to determine body burdens and detect a wide range of hydrocarbons, there was considerable variation in experimental design between studies. Therefore, it is difficult to compare results between studies.

Resistance assessment, as defined under MarESA, is based on the level of mortality reported in the evidence compiled in the literature review, as stated in the 'review question'. Evidence of lethal or sub-lethal effects was recorded in the evidence review. Where mortality was reported, the level of mortality was ranked using the resistance scale as 'severe', significant', 'some' or 'none.

Hydrocarbons were reported to cause a 'lethal' response in only 25% of the articles examined (Figure 6.1**.** Number of articles examined that reported lethal and sub-lethal effects to a range of hydrocarbon contaminants in *Mytilus* spp. (NR= not reported).). Most of the articles (70%) only reported and/or examined sub-lethal effects.

Where a lethal response was reported, only four articles (3.5%) reported 'Severe' mortality, but 12 (11%) reported 'Significant' mortality, and 12 (11%) reported 'Some' mortality (Figure 6.2). No mortality was reported in only 22% of the articles examined.

'Severe' mortality was only reported in four articles, two concerning exposures to crude oil, one to lubricant oil and one exposure to the water accommodated fraction (WAF) of fuel oil. Significant mortality was reported due to exposure to oil spills (two articles), crude oil (three articles), and fuel oil (six articles). Although PAHs were the most studied group of hydrocarbons, 'some' mortality was only reported in two articles, and the remaining 28 articles reported sub-lethal effects.

[38] Oil spills included instances of crude and fuel oils of different grades

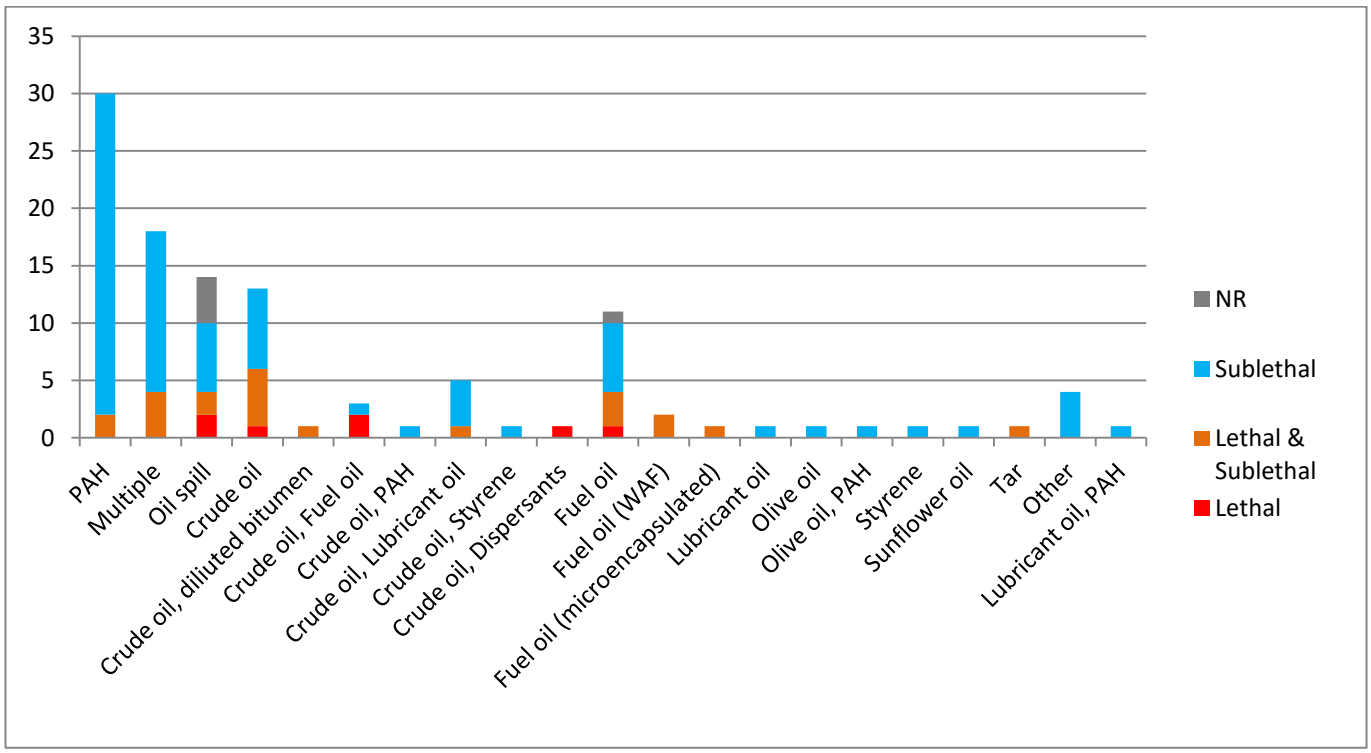

Figure 6.1. Number of articles examined that reported lethal and sub-lethal effects to a range of hydrocarbon contaminants in *Mytilus* spp. (NR= not reported).

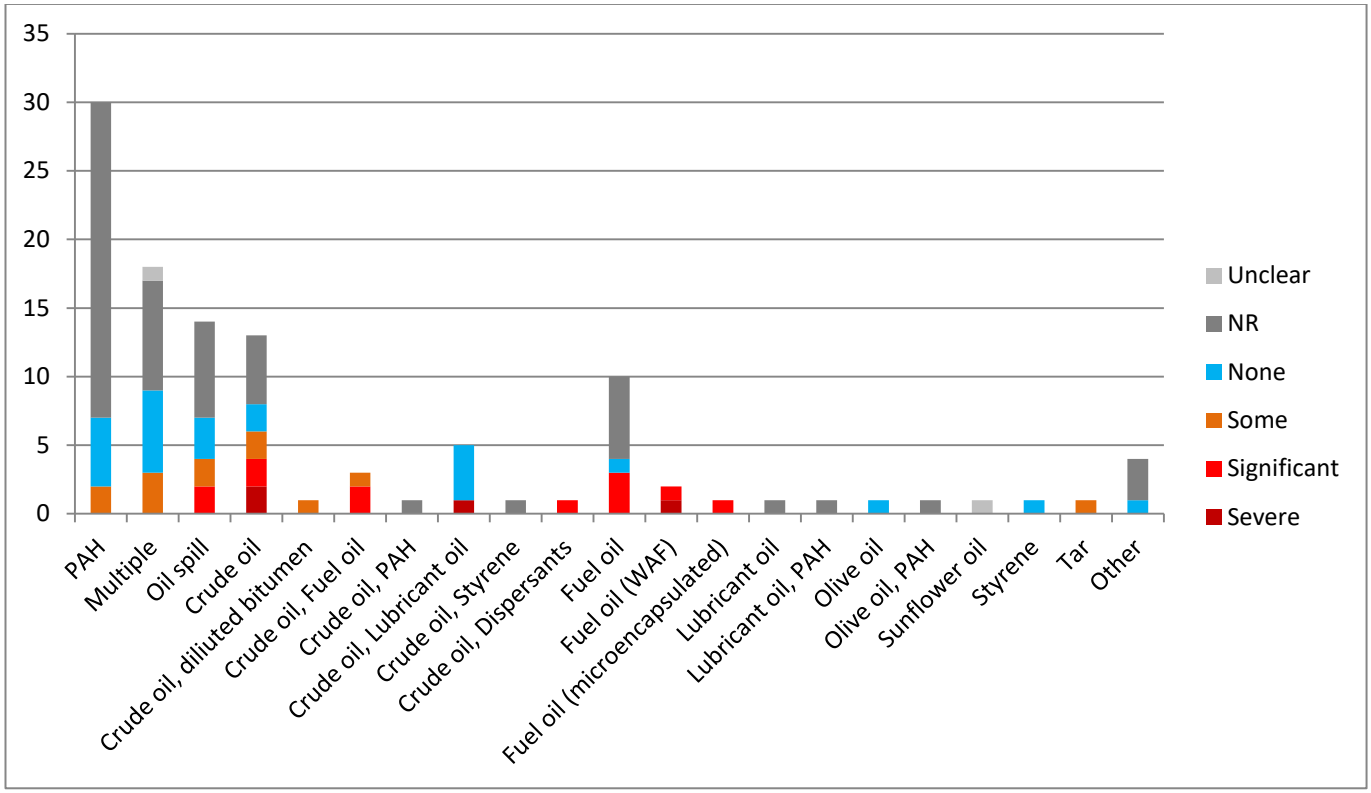

Figure 6.2. Count of ranked mortality (from 'severe' to 'none') in articles examined to a range of hydrocarbon contaminants in *Mytilus* spp. (NR= not reported).

Mortality was sometimes unclear or not mentioned in the studies examined. Mortality was 'not reported' in 50% of studies overall. However, most studies only examined sub-lethal effects.

A range of 'sub-lethal' effects were examined in the articles reviewed (Table 6.1). Most of the sub-lethal effects related to the use of mussels as biomarkers and few were informative about sensitivity.

Overall, the evidence collated demonstrates that hydrocarbons can cause direct mortality in adult and larval *Mytilus* spp. However, the variation in the experimental design makes it difficult to rank the relative mortality and/or resistance to hydrocarbons.

Table 6.1. Range of sub-lethal effects examined in the articles reviewed.

Sub-lethal effect	No. articles
Physiology	36
Accumulation	29
Mortality	29
Immunotoxicity	21
Histology	17
Genotoxicity	16
Toxicology	12
Proteomics	9
Reproduction	8
Feeding behaviour	8
Histochemical	7
Behaviour	7
Community	6
Other	15

The following evidence summaries and sensitivity assessments are based on the evidence collated in the literature review with an emphasis on evidence of mortality or sub-lethal effects that could result in population effects.

6.1.1 Oil spills.

***Torrey Canyon* 1967 (crude oil).** Smith (ed., 1968) reviewed information on the effects of the *Torrey Canyon* oil spill in 1967. *Mytilus edulis* was reported as missing from Porthleven, although Smith (1968) noted that it was more characteristic of the exposed north coast where they were found to be quite resistant of oil alone and moderate doses of detergent but “not intense” treatment. Mussels survived in Booby's Bay which was subject to heavy oil but no detergent treatment. Oil was noted amongst small mussel shells on rocks from which it had been washed off. Mussels were found behaving normally and alive in rock pools which had a film of oil at Portreath even though their mantle cavity contained globules of oil.

***Sea Empress* 1996 (crude oil)**. Crump *et al.* (1999) examined a set of permanent quadrats in Manorbier and West Angle Bay, Pembrokeshire, before and after the *Sea Empress* oil spill. At Manorbier, large numbers of small mussels were reported amongst crevices and cracks in the middle shore. Crump *et al.* (1999) noted that 'chocolate mousse' coated large areas of the rocky platform but tended to collect in gullies and crevices. Manorbier is a steep rocky, wave exposed, shore so most of the quadrats were clear of oil by the 28th February, 13 days after the spill. Coralline algae showed significant bleaching but there was no clear evidence of mortality or ill effects on the other organisms surveyed in their quadrats.

Moore (1997) examined rocky shore transects set up in Milford Haven shortly after the Sea Empress oil spill. Moore (1997) noted a slight increase in *Mytilus* abundance across sites between 1995 (pre-spill) and 1996 (after spill) but suggested that it was natural variation unlinked to the oil spill. Morrell (1998) examined permanent transects at Dale Fort set up after the Sea Empress oil spill. There was little

obvious effect on *Mytilus edulis* in Castel Beach Bay and Monkhaven where numbers were stable, except for one site at Monkhaven where the mussels were lost ca 11 months after the spill. However, the cause was uncertain.

Roston & Bunker (1997) survey sublittoral epibenthic rock communities, 16 months after the *Sea Empress* oil spill. One site included a subtidal *Mytilus edulis* bed habitat at 8-10 m. Samples were taken for hydrocarbon contamination measurement and revealed 12-17 ppm (although dwt or wwt not mentioned). Overall, all the habitats examined (inc. *Mytilus edulis*) were in normal condition and showed no effects of the oil spill.

***Exxon Valdez* 1989 (crude oil).** Highsmith *et al.* (1996) surveyed intertidal communities affected by the *Exxon Valdez* oil spill between spring 1990 and summer 1991, ca 1-2 years after the spill (Dec. 1989). *Mytilus trossulus* abundance and biomass were significantly reduced at most oiled sites studied compared to reference sites. Where the biomass was higher on oiled sites this was attributed to the survival of large individuals over smaller individuals. Highsmith *et al.* (1996) suggested that recovery had started at some Prince William Sound's sheltered rocky and estuarine shore, with no significant difference between oiled and reference sites by summer 1991. However, at coarse sediment and estuarine sites in Cook Inlet and Kenai Peninsula the differences were still significant, indicating that recovery was still in progress.

Conversely, Thomas *et al.* (1999) found no significant trends in byssal thread production or condition index between mussels from polluted sites and mussels from clean sites. However, mussels collected from oiled sites had significant PAH concentrations in their tissues compared to mussels from the reference sites.

Babcock *et al.* (1998) reviewed the effects of oiling from the *Exxon Valdez* on blue mussel beds (*Mytilus trossulus*) in Prince William Sound. Many of the oiled mussel beds in the Sound were not subject to cleaning due to their commercial value. Babcock *et al.* (1998) reported that the presence of the mussel bed retained the oil contaminant within the underlying sediment, preventing its breakdown or removal by wave action. As a result, the mussels remained contaminated and a threat (via consumption) to wildlife. Mussel condition was adversely affected by oil but some physiological measures in mussels contaminated for 3-4 years were not correlated with oil concentration. No mortality of mussels on the beds was reported, either directly related to oiling after the spill or to PAH contamination.

***Erika*, 1999 (heavy fuel oil).** Amat *et al.* (2004) demonstrates a genotoxic event on the digestive glands of the mussels living in the coast impacted by the Erika spill. Immediately after the Erika accident (December 1999 and January 2000), a very high amount of DNA adduct could be observed even in the reference site. Unfortunately, no data on the occurrence of adducts before the spill was available.

***Prestige* 2002 (heavy fuel oil)**. Peteiro *et al.* (2006) investigated whether the *Prestige* oil spill effected the growth of *Mytilus galloprovincialis* seed collected from three different populations along the coast of northwest Spain three months after the spill (February 2003). The results showed the mussels from the area most affected by the spill to have significantly less growth in terms of weight. In addition, the percentage of mussels classified as 'large' from the population most affected by the spill was significantly less than the other two populations. No significant difference in growth or biochemistry was noted in the mussel seed collected in 2004, which suggested the absence of sub-lethal effects in the offspring of mussels exposed to the spill (Peteiro *et al.,* 2006). However, Labarta *et al.* (2005) found mussel seed from sites with the greatest oil impact had the lowest survival performance in air, with the lowest survival rates observed from a site that exhibited the highest PAH values.

***Hebei Spirit* 2007 (crude oil).** Jung *et al.* (2015) examined the effects of the *Hebei Spirit* oil spill on intertidal habitats in Korea, nine months after the spill. *Hebei spirit* spilt ca 10,900 t crude oil in poor weather with strong wave action. After nine months, the density of all Mollusca, including *Mytilus galloprovincialis* was significantly reduced at impacted sites compared to controls. Donaghy *et al.*

(2016) found mussels from two polluted sites following the *Hebei Spirit* oil spill to have a significantly lower condition indices than the control site.

***Kimya* 1991 (sunflower oil).** Mudge *et al.* (1993) examined the levels of fatty acids in *Mytilus edulis* around Anglesey after the *M.V. Kimya* spill of sunflower oil in January 1991 at Bodorgan Head. Components of the sunflower oil were incorporated into flesh of mussels around Anglesey but enhanced levels of linoleic acid (18:2 w6) (the best indicator of sunflower oil contamination) was limited to a 3 km wide area centred on the wreck. They concluded that mussels were able to metabolise fatty acids from the spill. A large mortality of mussels occurred at Aberffraw in June 1991, but it could not be attributed to the sunflower oil conclusively.

Others. The *Esso Bernica* spill (Sullom Voe, 1978) had little effect of intertidal communities in un-cleared areas (Rolan & Gallagher, 1991). The *Braer* spill (Shetland 1993) removed grazers (although it may have also been due to the use of dispersants), and no change in *Mytilus* abundance was reported (Newey & Seed, 1995).

Sensitivity assessment (oil spills)

Little evidence on the direct physical effects of oil (smothering, or clogging) on *Mytilus* spp., was found and few studies examined blue mussel beds, except in Babcock *et al.* (1998) and Rostron & Bunker (1997). The evidence suggests that *Mytilus* spp. can be relatively tolerant of direct oiling (in the absence of dispersants or other cleaning treatments) and survived oil spilt by the *Torrey Canyon* and *Sea Empress*. In particular, blue mussel beds in Prince William Sound (Babcock *et al.*, 1998) survived direct oiling and continued exposure to oil retained in the sediment underneath the mussel beds for 3-4 years, although their condition was impaired. However, *Mytilus trossulus* abundance in other intertidal habitats was significantly reduced after the *Exxon Valdez* spill (Highsmith *et al.,* 1996). In addition, a significant reduction in *Mytilus galloprovincialis* abundance was also noted after the *Hebei Spirit* spill in Korea (Jung *et al.,* 2015). Hence, the effect of oil spills on *Mytilus* spp. and blue mussel beds is likely to be dependent on the type of oil spilt, the local habitat, and wave conditions at the time of spill. Therefore, resistance is assessed as '**Low'** to represent the potential for mortality. Resilience is probably '**Medium**' so sensitivity to oil spills is assessed as '**Medium**'.

6.1.2 Petroleum hydrocarbons (oils)

Lethal effects of exposure to hydrocarbons were only reported in 25% of the articles examined. Only 11 of the papers examined provided details of LC_{50}, LT_{50}, or EC_{50} values based on laboratory studies. The lethal effects of petroleum oils (e.g. crude oil, fuel oils, and lubricant oils) are summarized below and reported LC_{50} or LT_{50} values for petroleum hydrocarbons (i.e. oils) are shown in Table 6.2. Information on the effect of oil contamination on 'scope for growth' (SFG) (Widdows & Johnson, 1988; Widdows & Donkin, 1992) or other condition indices is also included.

- Saco-Alvarez *et al.* (2008) exposed developing *Mytilus galloprovincialis* larvae to WAF of Prestige oil and Marine fuel oil under light and dark conditions. The EC_{50} (50% larval abnormalities 48 hours) was 13% WAF irrespective of light regime, while the EC_{50} was 20% Marine WAF in the light treatment and >100% in darkness EC_{50}. Undiluted Marine WAF only caused a 20% decrease in mussel normal larvae.
- Swedmark *et al.* (1973) examined the exposure of *Mytilus edulis* to several dispersants, dispersant and fuel oil mixtures and Oman crude oil. The LC_{50} after 96 hr exposure to Oman crude oil and 48 hr recovery in clean seawater was >1000 ppm.

Table 6.2. Examples of LC_{50}, LT_{50}, or EC_{50} values for the effects of oils on *Mytilu*s spp..

Contaminant	Exposure Conc.	Exposure duration	Life stage	LC/EC_{50}	Short Reference
Oman Crude oil	350, 650, 1000 ppm	96 hours	Adults	LC_{50} 96 hr =<1000 ppm	Craddock, 1977; Swedmark *et al.*, 1973
Crude oil	NR	48 hours (embryogenesis), 5 days (mortality)	Larvae, Embryos (blastula / trochophore) (20 hours)	EC_{50} 48h embryogenesis = 2000 µg/l, LC_{50} 5days = 2-4 ppt	His *et al.*, 2000
Crude oil weathered and non-weathered Fresh oil: Mass, Q4000 Weathered oil: CTC, Juniper Corexit 9500A	NR	48 hours	Larvae	LC_{50} Fresh oil: Mass >100% WAF LC_{50} Fresh oil: Q4000 >100% WAF LC_{50}Corexit 9500A 25-45% WAF LC_{50} Mass + Corexit 9500A 20-35% WAF LC_{50} Q4000 + Corexit 9500A 20-30% WAF	Stefansson *et al.*, 2016
Tar	60, 80, 100, 120, 140, and 160 mg/l (acute toxicity test) 10, 40, 60 mg/l (condition test)	96 hours (acute toxicity test) 17 days (condition test)	Adults	LC_{50} =139.84 mg/l Tar	Alonso *et al.,* 2019
Diesel oil (microencapsulated), copper	5,10,30,50,100, 500 µg/l (larvae) 200, 600, 1000, 1300, 5000 µg/l (adults)	10 days larvae, 30 days adults	Adults, Larvae	EC_{50} (30 days) = 800 µg/l; LC_{50} (30 days) adults ca 5000 µg/l diesel oil (microencapsulated)	Strømgren & Nielsen, 1991; His *et al.*, 2000

- Schmutz *et al.* (2021) examined the effects of oil spill exposure under ice on caged mussels (*Mytilus edulis*), during the spawning season, and their resultant larvae in experimental mesocosms, using crude oil and diluted bitumens. Mussels were exposed to Heidrun and North Sea crude oil, and Cold Lake Blend and Access Western Blend diluted bitumen from Canadian oil sands. Bioaccumulation of PAHs was detected three days after exposure. Higher concentrations were associated with the crude oil (5.49 +/- 0.12 µg/g dwt) than both diluted bitumens (0.51 +/- 0.03 or 0.91 +/- µg/g dwt). Clearance rates were significantly reduced by Heidrun crude oil and Cold Lake Blend diluted bitumen. Cellular stress (lysosomal stability) was highest under each oil treatment, and byssus thickness was significantly lower under each oil treatment. However, there was good recovery and the negative effects on some biomarkers disappeared one month afterwards. Mortality was not excessive and never more than 15% in each treatment (Schmutz *et al.*, 2021). Gametogenesis and larval development were affected for a longer period. Gonad development was lower in oil treatments but showed no recovery after a month. Spawning was induced several weeks after treatment. Embryogenesis and larval development were severely affected, with larval development lagging five days behind the controls.

- Cajaraville *et al.* (1992) examined the effect of WAF of two types of crude oil and one type of commercial lubricant oil on the physiology of *Mytilus galloprovincialis*. A few mussels died in the crude oil treatments, irrespective of dose. However, exposure to the refined lubricant oil resulted in 100% mortality after 49 days at the high dose (40% WAF in seawater) and 77 days at the intermediate dose (6% WAF). They noted that Ural oil WAF was more toxic than Maya oil WAF due to higher concentrations of aromatic hydrocarbons in Ural oil WAF, although the lubricant oil had the highest concentration of aromatic hydrocarbons. Overall, exposure to the crude and refined oil WAFs significantly reduced growth of shell and flesh, and affected health, reproduction and survival.
- Alonso *et al.* (2019) estimated the LC_{50} (96-hour) of tar at 139.84 mg/l in *Mytilus galloprovincialis*. Alonso *et al.* (2019) also observed gonadal condition index of mussels exposed to 60 mg/l tar to be significantly reduced after 10 days, and after 17 days, the gonadal condition index of mussels in the 10 and 40 mg/l treatments was also reduced. Histological findings showed spermatogenesis disruption and alterations of somatic and germinal cells as a direct effect of treatment.
- Børseth *et al.* (1995) found oil, oil dispersants, formaldehyde, and benzene to cause the sodium gradient across cell membranes to drop and caused the death of some test organisms. Phenol had an anaesthetic effect (at 100 mg/l) but the depression in sodium gradient was not significant, and mussels recovered. Benzene and formaldehyde significantly decreased the sodium gradient. The oil and dispersant mixtures also significantly reduced sodium gradient. However, Boreth *et al.* (1995) stated that prior experiments found that exposure to benzene and formaldehyde at the stated levels for 5 days resulted in mortality and remarked that exposure to oil and dispersants at slightly higher concentrations or longer durations caused mortality but gave no supporting data.
- Ikävalko *et al.* (2006) investigated the use of cotton grass as oil sorbent in marine environmental protection. The addition of diesel to static tank experiments resulted in 100% mortality of *Mytilus* spp. and *Dreissena* spp. when exposed to diesel without the addition of cotton. However, the final concentration of diesel was not given.
- Lowe & Pipe (1987) examined the effect of diesel oil WAF (27.4 +/- 7.2 (Low) ppb and 127.7 +/- 28.3 (High) ppb total diesel oil hydrocarbons) on reproduction and survival in *Mytilus edulis* collected in summer when food reserves were high and in autumn when they were low. All treatments, including controls experienced mortality due to starfish predation and spawning stress between Jan-June. But in the following 80 days (June-Sept), mortality was highest in the High oil treatment (71% in mussels collected with low food reserves, and 27% in mussels with high food reserves), less in the Low oil treatment (14.5% and 12.6% as above) and zero in controls, which suggested that condition and season were factors in mortality from oil exposure.
- Stefansson *et al.* (2016) examined the toxicological effects of non-weathered and weathered crude oil from the *Deepwater Horizon* incident on the development of marine bivalve (*Mytilus galloprovincialis, Crassostrea gigas, Mercenaria mercenaria*) and echinoderm larvae. Weathered oils had no toxic effect on developing larvae. However, fresh oil had adverse effects on developing larvae. There was no significant difference in EC_{10} values between echinoderm and bivalve larvae. The average EC_{10} (abnormal development) values for the larval species exposed to these WAFs were 67±22 mg/l total PAH (46±18% WAF) and 66±19 mg/l total PAH (57±22% WAF) for fresh oil samples. Stefansson *et al.* (2016) gave LC_{50} values for *Mytilus* larvae of ca 130[39] µg/l TPAH and ca 140 µg/l TPAH for fresh oils but state that these values were higher than the highest concentration tested.
- Strømgren & Nielsen (1991) found microencapsulated diesel oil to reduce spawning frequencies of *Mytilus edulis* by 40-45% of the control at 1,000 and 1,300 µg/l. At 5,000 µg/l exposure, the spawning frequencies were negligible. The mortality of *Mytilu*s adults was around 40% when

[39] Approximate value extracted from graph/figure in text

exposed to 5,000 µg/l microencapsulated diesel oil, with the LC_{50} (30-day) corresponding to about 5,000 µg/l. However, larval mortality rose steeply until 20-30% mortality at 50 µg/l and was 100% at 500 µg/l. The LC_{50} value (10-day) for larvae was found to be 30-35 µg/l. Larval growth was significantly reduced at 10 µg/l, with an EC_{50} (10-day) of 24-30 µg/l; an order of magnitude less than the EC_{50} for growth of juvenile mussels (ca 1000 µg/l; Strømgren & Reisen, 1988 unseen). Therefore, they suggested diesel oil was, more toxic to larvae than juveniles. However, variation in larval mortality was higher between batches than variation in growth.

- Bokn *et al.* (1993) examined the effect of diesel oil WAF on littoral rocky shore communities in flowing water mesocosms, each including five steps to simulate tidal levels. The communities were allowed to establish in the mesocosms for 32 months prior to the experiment. Mesocosms were exposed to controls, High WAF (129.4 µg /l (mean)), and Low WAF (30.1 µg/l mean) for 24 months, and the communities were examined at three monthly intervals. *Mytilus* communities were the worst affected across the mesocosm. Their cover decreased to zero at all tidal levels within the High WAF mesocosm and the upper two tidal levels by the end of the study in the Low WAF mesocosm. At the lower tidal levels in the Low WAF mesocosm, the population of *Mytilus* was reduced to one individual by the end of the study. The population of *Mytilus* increased slightly in the control mesocosms. Bokn *et al.* (1993) noted that the decline in *Mytilus* cover corresponded to a decrease in byssal attachments and increased susceptibility to starfish predation.
- Baussant *et al.* (2011) exposed *Mytilus edulis* to dispersed crude oil (0.015-0.25 mg/l) for seven months across its entire gametic cycle to simulate the effect of produced water discharges from North Sea oil installations. Reduced fertilization success was observed when both adult mussels and gametes were exposed to 0.25 mg/l oil and only 60% of the eggs were fertilized. Larval development was affected by parental exposure to oil, causing abnormal growth. Adult and larvae exposure to oil resulted in a significantly smaller larva. Also, if only the adults or only the larvae were exposed to the oil, the larvae grew bigger than those in the adult larvae exposure, but the larvae were still significantly smaller than the control. There was a concentration-dependent increase in the volume density of atretic[40] oocytes in female mussels exposed to oil; females exposed to 0.25 mg oil/l had significantly higher volume density of atretic oocytes than control females. However, after spawning, the volume density of atretic oocytes was low and no differences between experimental groups were observed. When both adult mussels and their embryos were exposed to 0.25 mg oil/l, a significantly higher level of DNA strand breaks in the embryos one day post-fertilization was found. Overall, the study indicated a decrease in potential reproductive success and recruitment by mussels exposed to dispersed crude oil for months at 0.25 mg/l but Baussant *et al.* (2011) noted that 0.25 mg/l dispersed oil was probably restricted to with the first 0.5 km of a discharge point of North Sea oil platforms.
- Counihan (2018) used ecologically relevant concentrations of oil (10 ppm crude oil) and dispersant based on concentrations measured in dispersed oil field trials and after oil spills. Counihan (2018) observed 5% *Mytilus trossulus* mortality in treatments with non-dispersed crude oil and treatments with crude oil and Corexit 9500 (3.75%), however mortality in the treatments were low. After seven days, mussels in all the treatments had significantly thinner shells than the controls and after 21 days mussels in all treatments exhibited evidence of genetic damage, tissue loss and a continued stress response.
- His *et al.* (2000) reviewed the use of bivalve larvae as a monitor or biomarker for contaminants. In one example, His *et al.* (2000) reported a 48-hour EC_{50} embryogenesis of 2,000 µg /l, and 5-day LC_{50} of 2-4 ppt in *Mytilus edulis* larvae exposed to crude oil (Luca & Le Roux, 1975, cited in His *et al.*, 2000).

[40] Degenerated and reabsorbed oocytes

- Gomiero *et al.* (2015) found *Mytilus galloprovincialis* sampled from three gas field sites in the Adriatic in the summer months had a significant decrease in survival in air compared to the reference organisms. However, no significant difference was observed in winter collections.
- Widdows *et al.* (1982) used three experimental approaches to examine the effect of WAF crude oil on mussels. Experiment 1 examined the effect of hydrocarbons in food, while Experiment 2 examined hydrocarbon absorption and Experiment 3 examined long-term exposure. They reported that 30 µg/l WAF decreased feeding rate significantly and that 30-36 µg/l elevated respiration rate by 30%. Oxygen consumption increased after seven days and remained elevated for five months in the long-term experiment. In Experiment 1, mussels had negative SFG after 28 days. Overall, there was a correlation between the decline in SFG and increase in tissue aromatic concentrations, with negative SFG at ca >7 µg/g wwt of mussel tissue. Widdows *et al.* (1982) also noted that the 30-36 µg/l WAF concentrations used were comparable to levels found in the environment (e.g. the Thames in 1980) but that very high concentrations (5-1,000 mg/l) were required to elicit a lethal response in *Mytilus edulis* (see Craddock, 1977).
- Widdows *et al.* (1987) exposed *Mytilus edulis* to WAF diesel oil (125 +/- 28 µg/l High oil and 28 +/- 7 µg/l Low oil in tidal, flowing seawater mesocosms for eight months. Both low and high oil conditions resulted in a significant decrease in SFG mainly due to a reduction in feeding and food absorption. SFG was severely reduced in high oil conditions, resulting in weight loss as the mussels used tissue reserves. Widdows *et al.* (1987) reported a direct relationship between declining SFG and log of hydrocarbon concentration. SFG became negative at ca >30 µg/l WAF. However, no mortalities were reported in the eight-month exposure period, and all mussels had recovered 55 days after return to untreated seawater. They noted that mussels from the high oil treatment depurinated hydrocarbons and recovered faster than those exposed to the low oil treatment.
- Craddock (1977) reviewed the evidence of acute toxicity of marine organisms to petroleum. Craddock (1977) reported:
    - 0-100% mortality in *Mytilus californianus* collected from four locations, exposed to 10,000 ppm of the soluble and emulsified fractions of Santa Barbara crude oil for 48-56 hours; the larger mussels were the most susceptible;
    - 28.4% or 24.4% mortality in *Mytilus galloprovincialis* larvae exposed to 1,000 ppm of Venezuelan Crude oil or No. 1 fuel oil respectively;
    - 66% mortality in *Mytilus edulis* exposed to 10% Outboard motor effluent for 24 hours (and nine days holding);
    - a LC_{50} (96-hour) < 1,000 ppm in *Mytilus edulis* exposed to Oman crude oil. Note - <350 ppm lowest conc. affecting byssal activity and shell closure, <1,000 ppm lowest conc. affecting shell closure; Crude oil less toxic than emulsions;
    - an EC_{50} (loss of attachment and formation of byssus) in *Mytilus edulis* of 17 ppm WSF No. diesel oil after 24 hours and 15.6 ppm after 48 hrs; and
    - an EC_{50} (failure to reattach to substratum) in *Mytilus edulis* of 16.6 ppm after 24 hours and 15 ppm after 48 hours exposure to No. 2 diesel oil (layered on the surface and stirred constantly).

Sensitivity assessment (oils). Refined oils (e.g. lubricant and fuel oils) were reported to be more toxic than crude oils. Widdows *et al.* (1982) also noted that the 30-36 µg/l WAF concentrations used in their experiments were comparable to levels found in the environment (e.g. the Thames in 1980) but that very high concentrations (5-1,000 mg/l) were required to elicit a lethal response in *Mytilus edulis* (see Craddock, 1977). Overall, the evidence suggests (10% of articles on the effects of oils) that exposure to

oils or their water saturated (WSF) or water accommodated fraction (WAF) can result in 'severe' mortality (>75%) while another 30% of the articles report significant (25-75%) mortality depending on the type of oil and its concentration. Therefore, resistance is assessed as **'None'**. Resilience is probably **'Low'** so that sensitivity to petroleum-based oils is assessed as **'High'**.

In their review, Widdows & Donkin (1992) note that one reason mussels are good sentinels for pollution is because they are relatively tolerant of, but not insensitive, to a range of environmental conditions and contaminants. Furthermore, they noted that adults were >10-fold more sensitive than larvae to copper (Cu), petroleum hydrocarbons and sewage sludge. Widdows & Donkin (1992) suggested that LC_{50} values in *Mytilus* gave a false impression of high tolerance because adult bivalves were able to close their valves and isolate themselves from extreme (potentially lethal) conditions for long periods (i.e. days).

6.1.3 Polyaromatic hydrocarbons (PAHs)

Only three papers that examined the effects of PAH exposure reported 'some' mortalities, although not direct mortality but rather as LT_{50}s based on their 'survival in air'. Donkin *et al.* (1989) reported the EC_{50} (on feeding rates) for a range of PAHs. The remaining 27 papers concentrated on a range of sub-lethal effects as biomarkers of contamination (see below).

- Giannapas *et al.* (2012) exposed *Mytilus* spp. to phenathrene (0.1 mg/l), anthracene (0.1 mg/l) or a mixture (0.2 mg/l) for 7 days. They found that mussels exposed to PAHs to have reduced ability to survive in air with LT_{50} values in the range of 3-4 days, while control treatment mussels had an LT_{50} values of 7 days. The mussels from the PAH treatments also had lower cell viability, increased lysosomal acid phosphatise activity, high frequencies of micronuclei, and other abnormalities in haemocytes.
- Blanco-Rayon *et al.* (2020) examined the PAH and metal burden and suite of biomarkers in *Mytilus galloprovincialis* collected from two sites, Arriluze (highly polluted) and Plentzia (relatively clean) in the Bay of Biscay. They used LT_{50} in air examine the stress of the mussels. Although a highly polluted site, the mussels from Arriluze had a higher survival in air than those form Plentzia in all seasons. They suggested that a better nutritional state (of the Arriluze mussels) masked the negative effects of the pollutants.
- Eertman *et al.* (1993) examined 'survival in air' (LT_{50}) of *Mytilus edulis* transplanted to various sites in Dutch coastal waters for seven days. They concluded that increased tissue levels of PAHs and PCBs were correlated with decreased ability to 'survive in air'.
- Donkin *et al.* (1989) examined the effects of hydrophobic organic chemicals on the feeding rates of *Mytilus edulis*. Toxicity was confirmed by the concentration in mussel tissue required to reduce feeding rate by 50% (TEC_{50}). The concentration of contaminant in the water required to reduce clearance rates by 50% was also recorded. All of the contaminants in the study were found to reduce feeding rates.
- Widdows *et al.* (1995) examined scope for growth and body burden of contaminants (inc. hydrocarbons) in *Mytilus edulis* collected from North Sea coasts of the UK from Shetland to Whitstable. They reported a general increase in stress (reduced SFG) from the cleaner waters to north Scotland to the south of England. In the majority of the 26 coastal sites and 9 offshore sites, 90% of the decline in SFG was explained by PAHs. Polar organics, probably of natural origin, contributed to the decline at some sites.
- Widdows *et al.* (2002) examined SFG and various contaminant levels in mussels from 38 sites around the Irish Sea. A decline in SFG was associated with increased levels of contaminants. They reported that 50-80% of the decline in SFG was due to PAHs from fossil fuels and oil spills. TBT made a minor

contribution to the decline in SFG while the metal concentrations at their sites tested were not high enough to have a significant effect.

- Granby & Spliid (1995) examined the concentrations of a range of hydrocarbons in *Mytilus edulis* around the Danish coast. They found a significant negative correlation between the condition index the total PAH concentrations and paraffin-naphthene (p-n-)-hydrocarbon concentrations in their tissues.

Sensitivity assessment (PAHs). Only a few articles demonstrated 'some' mortality (<25%) due to exposure to PAHs, and then indirectly, as a result of stress and subsequent reduction in the specimen's ability to survive in air. Similarly, Widdows and others (1995, 2002) demonstrated a decrease in condition or SFG due to PAH exposure and body burden. However, most articles examined (93%) only reported sub-lethal effects (Figure 1). Therefore, resistance is assessed as **'Medium'** to represent the 'worst-case' potential of PAHs to cause indirect mortality due to reduced condition and/or stress. Resilience is probably '**Medium**' so sensitivity to PAHs is assessed as '**Medium**'.

6.1.4 Others

- Sabourin & Tullis (1981) found Benzo [*a*] pyrene (B[*a*]P) (10 ppm), benzene (50 ppm) and toluene (100 ppm) to significantly reduce the heart rates of *Mytilus californianus*. Additionally, significant declines in the rate of oxygen consumption occurred for 50 ppm benzene, 10 and 100 ppm toluene and 1 ppm B[*a*]P. **Mortality was only observed in the 100 ppm toluene treatment** but mortality was not quantified.
- Smith *et al.* (2001) observed the feeding rates of *Mytilus edulis* to reduce significantly when exposed to 6-cyclohexyltetralin or 7-cyclohexyl-1-propyltetralin, with a linear relationship between exposure concentration and body burden observed.
- Mamaca *et al.* (2005) exposed adult *Mytilus edulis* to 0.2 mg/l of styrene for 7 days in a flow through laboratory experiment. No mortality was observed in the test specimens, but lysosomal membrane activity was significantly reduced, and DNA damage was significantly increased compared with controls.
- Danellakis *et al.* (2011) found olive oil mill wastewater (OMW) at the concentration of 1, 0.2, 0.1, and 0.01% v/v to have no effects on the survival of *Mytilus galloprovincialis* over a period of four days. However, high frequencies of either micronuclei or other abnormalities tested were found in haemocytes of mussels exposed to 0.01 or 0.1% (v/v) OMW. A concentration dependent increase in levels of DNA damage were detected in haemocytes. In addition, a significant inhibition of Acetylcholineesterase (AChE) activity was observed in the haemolymph and in the gills of mussels in the treatment groups. The treatment groups also showed significant increases in metallothionein activity and lipid peroxidation.

The evidence on 'other' forms of hydrocarbons was limited. Evidently, toluene is potentially toxic to *Mytilus* spp., while benzene, olive oil mill wastewater, styrene and 'tetralins' were reported to have sub-lethal effects at the concentrations studied.

6.1.5 Sub-lethal effects

Overall ca 70% of the articles examined only reported sub-lethal effects of the effects of hydrocarbons in *Mytilus* spp. This was because many studies examined the effects of contaminants on immunotoxicity, genotoxicity, proteomics, and other biomarkers, or examined exposure to field relevant concentrations of contaminants. Many of the biomarkers examined indicated 'stress' in the exposed mussels. However, it was difficult to understand if the resultant 'stress' would result in changes in the population. Further research is required on how relevant 'sub-lethal' effects are to sensitivity

assessment, except where mortality, loss of condition, SFG, or changes in reproduction are shown (above) to affect the population adversely. Therefore, sub-lethal effects are not discussed further.

6.1.6 Sensitivity assessment - Hydrocarbons and PAHs.

In their review, Widdows & Donkin (1992) note that (one reason) mussels are good sentinels for pollution is because they are relatively tolerant of, but not insensitive, to a range of environmental conditions and contaminants. Furthermore, they noted that adults were >10-fold more sensitive than larvae to copper (Cu), petroleum hydrocarbons and sewage sludge. Widdows & Donkin (1992) noted that lethal responses give a false impression of high tolerance since the adults can close their valves and isolate themselves from the environment for days. They suggested that sub-lethal effects e.g., shell growth and 'scope for growth' (SFG), were more sensitive indicators of the effects of contaminants.

The evidence review suggests that exposure to hydrocarbon contamination can cause mortality in *Mytilus* spp., which is in some cases significant or even severe. The degree of mortality, or absence of mortality, depends on the type of hydrocarbon (crude or refined oils, oil saturated water fractions, PAHs, or refined products) to which the species is exposed, how they are exposed (through oil spills, effluents, the sediment, or food supply e.g. algae), the concentration of the contaminant and the duration of exposure, as well as seasonal influences on the species' condition, especially spawning and reproduction.

Therefore, the 'weight of evidence' based on reported 'severe' (>75%) and 'significant' (25-75%) mortality due to hydrocarbon contamination suggests an overall **'worst case'** resistance assessment of '**None'**. Resilience is probably '**Low**' so sensitivity to petroleum-based oils is assessed as '**High**'. However, it should be noted that the evidence reviewed also documented several occasions in which blue mussels and blue mussel beds had survived significant oiling and most evidence (70% of the articles examined) of exposure to hydrocarbons was reported to result in sub-lethal effects, although it was not clear how detrimental sub-lethal effects or 'stress' is to the species survival. Hence, confidence in the assessments is **'Medium'**.

6.2 *Mytilus* spp. - Transitional metals and organometals

A total of 133 articles were selected from 2,533 articles. These 133 articles focused on the physiological effects of metal exposure on *Mytilus* spp. of which 15 articles focused on the effects of nanoparticulates metals and 18 articles looked at the effects of organometals. The range of 'ranked mortalities'[41] reported in the 133 papers examined is shown in Figure 6.3.

In general, the evidence suggested that longer exposure times were required to understand the true impacts of metal exposure on *Mytilus*, as mussels can close their shells for days. Hence, short-term exposures (e.g. < 48 hrs) may underestimate sensitivity. This agrees with Widdows & Donkin (1992) who suggested that LC_{50} values in *Mytilus* gave a false impression of high tolerance because adult bivalves were able to close their valves and isolate themselves from extreme (potentially lethal) conditions for long periods (i.e. days). Different life stages had different sensitivities. This also agrees with Widdows & Donkin (1992) who noted that adults were >10-fold more sensitive than larvae to copper (Cu), petroleum hydrocarbons and sewage sludge.

However, it was difficult to describe many other general trends in metal toxicity due to the variation in the experimental conditions (e.g. laboratory or field), and especially duration between studies and the different toxicities of the variation metals and their compounds used. Therefore, the results of the review are presented separately for each metal and its compounds.

[41] Mortality is 'ranked' based on the MarESA resistance scale, i.e. some (<25%), significant (25-75%), and severe >75%, and None (observed), with 'sublethal' included as an additional category.

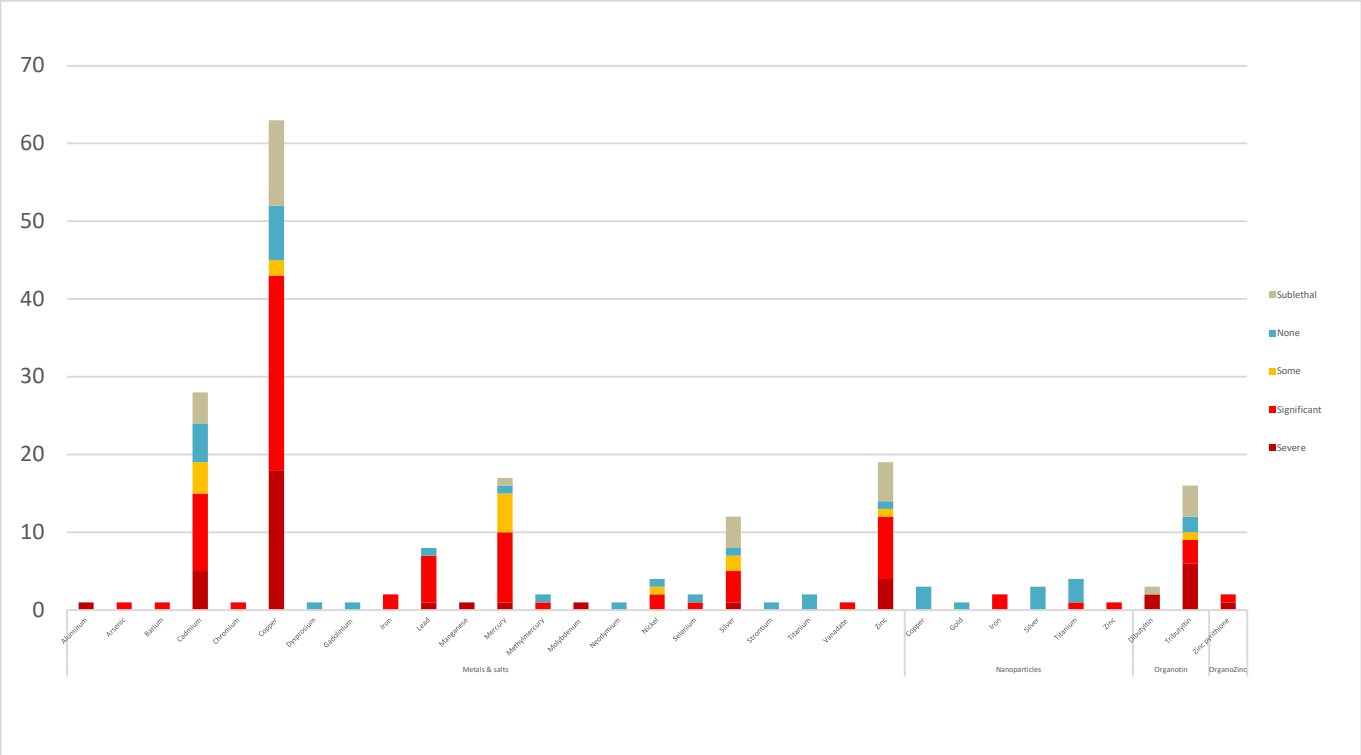

Figure 6.3. Count of ranked mortality (from 'severe' to 'none') in the articles examined for a range of metal contaminants in *Mytilus* spp..

6.2.1 Copper

The effects of copper exposure on *Mytilus* spp. have been well studied with multiple papers investigating the lethality of copper at different concentrations and life stages. A total of 52, of the 133 papers screened, document the lethality of copper, 29 of those looked at the effects on adults, 20 looked at the effects on embryo/larvae stages, and three papers looked at the effects on juveniles (Figure 6.4).

The results from the papers show significant differences in the concentrations at which mortality occurred. The variation in the concentrations that resulted in mortality typically correlated with the exposure duration. For example, Al-subiai *et al.* (2011) observed no mortality of *Mytilus edulis* adults exposed to 50 µg/l (0.05 mg/l) copper for five days. However, Martin (1979) exposed *Mytilus edulis* adults to 50 µg/l (0.05 mg/l) copper for a period of 20 days resulting in complete mortality. The results clearly demonstrate that lower doses of copper can be just as lethal as higher concentrations when the mussels are exposed over a longer period. For example, Martin (1979) exposed groups of *Mytilus edulis* individuals to a variety of concentrations of copper between 0.02 µg/l – 3 µg/l (0.00002 – 0.003 mg/l) for a period of 50 days. Complete mortality occurred at the highest dose within 6 days and at the lowest dose, complete mortality had occurred by day 40.

The development of embryo/larvae life stages of *Mytilus* was shown to be more sensitive to copper than adult stages. Copper has been shown to have toxic effects on the embryo/larvae stages of *Mytilus* development with 100% development abnormality occurring at 10 µg/l with a 48-hour exposure (Yaroslavtseva & Sergeeva, 2007). In *Mytilus californianus,* larval mortality occurred at 6.5 µg/l copper (Hall *et al.,* 2020). In addition, concentrations of copper as low as 0.5 µg/l copper caused the abnormal development of larvae. The experiments showed copper to have dose-dependent effect on embryo-larval development characterized by an increase in abnormal D-larvae with increasing metal concentration (Boukadida *et al.*, 2016).

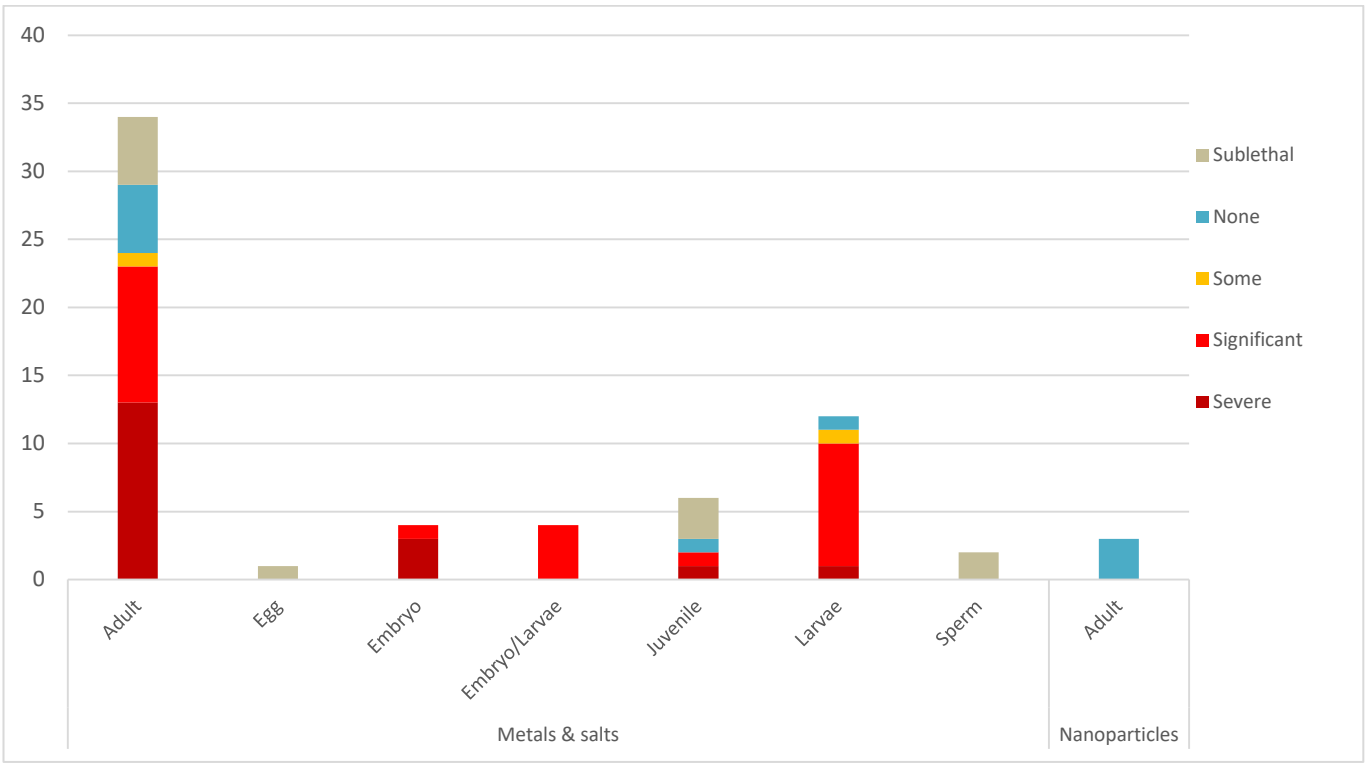

Figure 6.4. Count of ranked mortality (from 'severe' to 'none') in the articles examined for inorganic copper and its compounds in *Mytilus* spp..

6.2.2 Cadmium

Cadmium has the second greatest number of articles reporting lethal effects from exposure, with 24 of 133 screened papers reporting effects on survival. Significant to severe mortality was observed in all life stages of *Mytilus spp*. from exposure to cadmium (Figure 6.5).

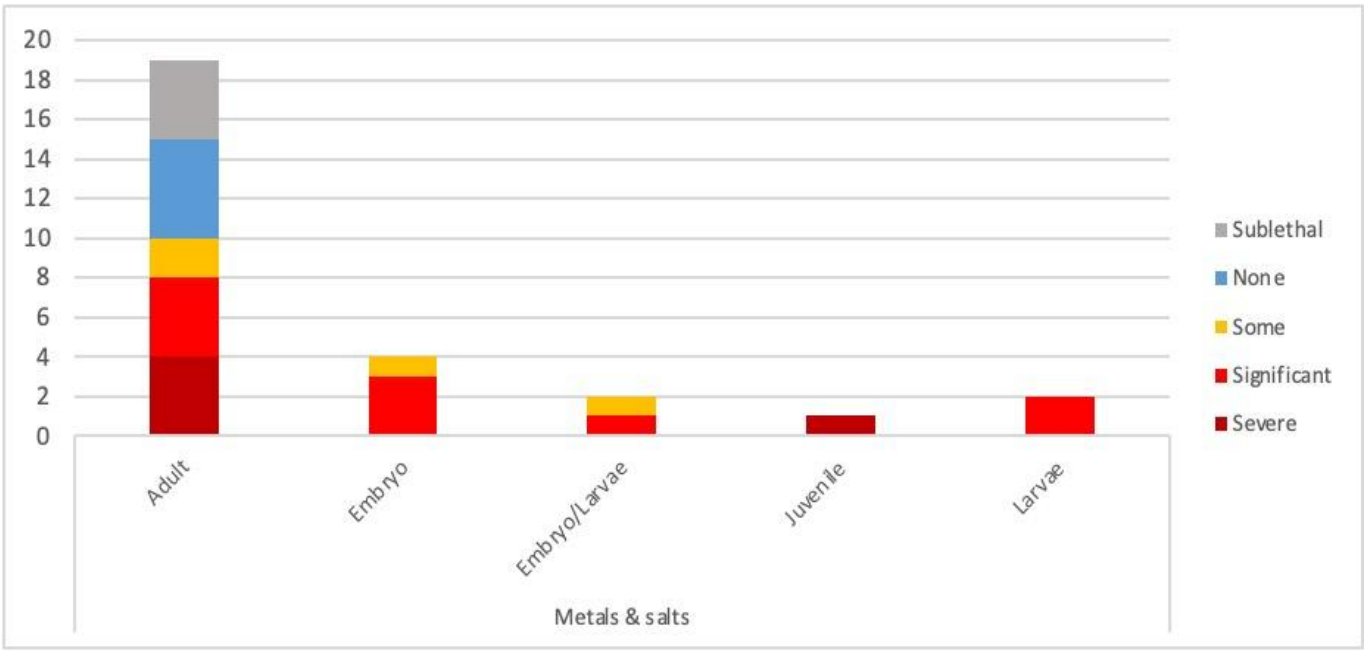

Figure 6.5. Count of ranked mortality (from 'severe' to 'none') in the articles examined for inorganic cadmium and its compounds. (NR= not reported).

The evidence is summarized below.

- Amiard-Triquet (1986) exposed *Mytilus edulis* adults to a large range of concentrations of cadmium over a 16-day period. The cadmium concentration that caused 50% mortality in 96 hours (LC_{50}, 96 hr) was 1550 µg/l (1.55 mg/l). At a concentration of 2500 µg/l (2.5 mg/l) cadmium 100% mortality occurred within 8 days.
- Chalkiadaki *et al.* (2014) exposed *Mytilus galloprovincialis* adults to various concentrations of cadmium (0.5, 1, 2.5 & 20 mg/l) for 20 days with 10 days depuration period. During the experiment, all the mussels exposed to 2.5 mg/l (2500 µg/l) cadmium died within 10 days, and those exposed to 20 mg/l cadmium all died within five days. The mussels exposed to 0.5 and 1 mg/l (500 and 1000 µg/l) cadmium all survived until the end of the experiment with no mortalities.
- Eisler (1971) investigated the acute toxicity of cadmium on a variety of marine organisms including the blue mussel, *Mytilus edulis*. The concentration required to kill half of the population of mussels decreased with time, as follows, 24-hour LC_{50} >200 mg/l; 48-hour LC_{50} 165 mg/l, and 96-hour LC_{50} 25 mg/l.
- Vlahogianni & Valavandis (2007) conducted short-term toxicity tests with cadmium on *Mytilus galloprovincialis* adults establishing a 24-hour LC_{50} of 1700 µg/l (1.7 mg/l) but observing 0% mortality at 100 µg/l (0.1 mg/l).
- Coles *et al.* (1995) found the survival of *Mytilus edulis* adults not to be affected by a seven-day exposure to 40 µg/l and 4 µg/l (0.04 and 0.004 mg/l) cadmium.
- Bebbianno & Langston (1992) found no mortality of *Mytilus galloprovincialis* during a 40-day exposure to cadmium at concentrations of 400 µg/l (0.4 mg/l)
- Bebbianno & Serafim (1998) found no mortality of *Mytilus galloprovincialis* to occur during a 40-day exposure to 100 µg/l (0.1 mg/l) cadmium.
- Talbot *et al.* (1976) determined lethal doses of cadmium exposure on *Mytilus edulis* adults over a period of 200 days. The dose required to cause 50% mortality of the population decreased with increasing exposure time. At 30 mg/l cadmium, 50% mortality occurred within 96 hours, however at a lower concentration of 0.5 mg/l (500 µg/l), 50% mortality occurred within 200 days.
- Myint & Tyler (1982) found cadmium to suppress *Mytilus edulis* gametogenesis at a concentration of 50 µg/l (0.05mg/l) during the early stages of gonad development but did not affect the survival of adults during a 70-day exposure experiment at -1.5 to 0°C, or during a 28-day exposure at 18°C.
- Ahsanullah (1976) determined the acute toxicity of cadmium exposure on *Mytilus edulis* adults, establishing an LC_{50} of 1620 µg/l (1.62 mg/L).
- Nelson *et al.* (1988) exposed *Mytilus edulis* juveniles to cadmium at a variety of concentrations over a 96-hour period to find the lethal concentration; a LC_{50} of 960 µg/l (0.96 mg/l) was established.
- Martin *et al.* (1981) studied the toxicity of cadmium on the embryo development of *Mytilus edulis* and established the 48-hour EC_{50} of cadmium to be 1200 µg/l (1.2 mg/l).
- Beiras & Albentosa (2004) investigated the inhibitory effects of trace metals on the embryos of *Mytilus galloprovincialis*. Cadmium exposure caused an increase in the percentage of abnormal larvae with a dose response effect. A 48-hour EC_{50} 1925 µg/l (1.925 mg/l) cadmium was established, with the lowest observed effect concentration (LOEC) of 500 µg/l (0.5 mg/l).
- Balbi *et al.* (2014) investigated the effects of Cd^{2+} exposure on *Mytilus galloprovincialis*. Adult mussels were exposed to 100 µg/l (0.1 mg/l) for a period of 96 hours, during which time no mortality occurred. Embryo/larvae development was significantly affected by Cd^{2+} inducing a significant

decrease in the percentage of normal D-larvae (-41% compared to controls), including larvae held up in the trochophore or pre-veliger stages and malformed larvae.

- Pavicic *et al.* (1994a) observed the toxic effects of cadmium, zinc, and mercury on the development and growth of *Mytilus galloprovincialis* larvae. Mercury was the most toxic followed by zinc and then cadmium. The combined exposure to zinc and cadmium simultaneously resulted in an antagonistic effect with a higher percentage of normally formed larvae and reduced growth inhibition in comparison to the effects of the metals individually. Cadmium caused significant decreases in growth at concentrations above 2200 µg/l (2.2 mg/l). All three metals caused the abnormal development of veliger larvae with increasing metal concentration causing a higher percentage of abnormal larvae.
- Prato & Biandolino (2007) investigated the toxicity of copper, cadmium, and mercury individually and combined on *Mytilus galloprovincialis* using the embryotoxicity tests. The results showed all the metals to have significant effects on the larval development with the lowest tested concentrations of contaminant causing a significant impact on larvae development. The EC_{50} and LOEC of cadmium were calculated at 21 µg/l (0.021 mg/l) and 6.25 µg/l (0.00625 mg/l), respectively. The toxicity of the metals on larvae development showed an antagonistic effect for each combination of metals.
- Annicchiarico *et al.* (2007) exposed *Mytilus galloprovincialis* larvae to five concentrations of cadmium in the range of 3.125 to 500 µg/l (0.003125 to 0.5 mg/l) to determine the lethal concentration during a 48-hour exposure period. A 48 hr LC_{50} of 590 µg/l (0.59 mg/l) was reported.

6.2.3 Zinc

The effects of zinc exposure on *Mytilus* spp. have been reasonably well studied with 15 articles from the selected 133 articles investigating the lethality of zinc at different concentrations and life stages. Significant mortality has been observed in all life stages of *Mytilus* spp. in these articles (Figure 6.6).

The evidence is summarized below.

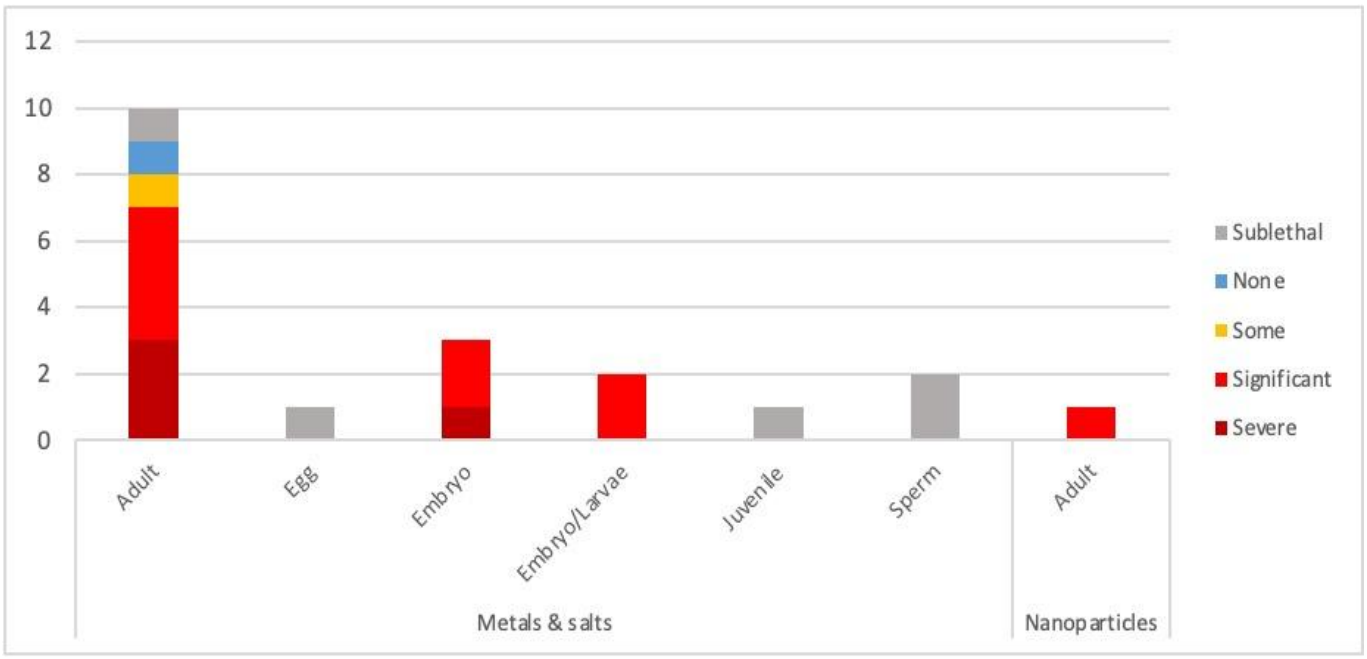

Figure 6.6. Count of ranked mortality (from 'severe' to 'none') in the articles examined for inorganic zinc and its compounds. (NR= not reported).

- D'silva & Kureishy (1978) exposed *Mytilus viridis* to zinc which caused 50% mortality at a concentration of 140 µg/l (0.14 mg/l) and 100% mortality at 250 µg/l (0.25 mg/l) zinc within 48 hours.

- Burbidge *et al.* (1994) found both particulate (elemental) Zinc and soluble Zinc ($ZnCl_2$) to be lethal to *Mytilus edulis* at 10 µg/l (0.01 mg/l) causing 100% mortality after 12 days. At concentrations of 2 µg/l (0.002 mg/l) particulate zinc did not cause any mortalities during the 12-day period; however, soluble zinc did cause some mortalities.
- Amiard-Triquet (1986) exposed mussels to a large range of concentrations of zinc over a 16-day period. The concentration of zinc that caused 50% mortality in 96 hours (LC_{50}) was >5000 µg/l (>5 mg/l). Complete (100%) mortality occurred after 16 days at 5000 µg/l (5 mg/l) zinc.
- Abel (1976) investigated the effects of pollutants on the filtration rate of *Mytilus edulis*. A reduction in filtration rates occurred with increasing concentrations of zinc. The LC_{50} was estimated to be 7,800 µg/l (7.8 mg/l) after 96 hours.
- Hietanen *et al.* (1988) found the LC_{50} value of zinc on *Mytilus edulis* to be 20.8 mg/l during a 41-day exposure. Throughout the experiment, the LC_{50} value changed in relation to time with lower concentrations causing the same percentage of mortalities as higher concentrations over a longer period.
- Cotter *et al.* (1982) investigated the effects of zinc on the survival of *Mytilus edulis* at different temperatures and salinities. The results showed zinc to cause mortality at a faster rate at 22°C and 35% salinity, than at lower temperatures and salinities.
- Myint & Tyler (1982) found no mortalities of *Mytilus edulis* adults occurred during a 70-day exposure to 200 µg/l (0.2 mg/l) zinc at -1.5 to 0°C, or during a 28-day exposure at 18°C.
- Nadella *et al.* (2009) assessed the embryo-larvae toxicity of zinc on mussel *Mytilus trossulus* during a 48-hour development test. Zinc caused abnormal larvae development with an established EC_{50} of 150 µg/l (0.15 mg/l) and an EC_{20} of 99 µg/l (0.099 mg/l).
- Martin *et al.* (1981) tested the toxicity of zinc on the embryos of *Mytilus edulis*. A 48-hour EC_{50} value of 175 µg/l (0.175mg/l) zinc was established for abnormal development.
- Beiras & Albentosa (2004) investigated the inhibitory effects of trace metals on the embryos of *Mytilus galloprovincialis*. Zinc exposure caused an increase in the percentage of abnormal larvae with a dose response effect. A 48-hour EC_{50} between 160-320 µg/l (0.16-0.32 mg/l) zinc was established.
- Pavicic *et al.* (1994b) observed the toxic effects of zinc on the development and growth of *Mytilus galloprovincialis* larvae. Zinc exposure caused abnormal development of veliger larvae with increasing metal concentration causing a higher percentage of abnormal larvae. A 48-hour EC_{50} of 145 µg/l (0.145mg/l) zinc was established.
- Ahsanullah (1976) determined the acute toxicity of zinc exposure on *Mytilus edulis* adults, establishing an LC_{50} of 2500 µg/l (2.5 mg/l) zinc in static exposure trials, and LC_{50}s of 3600 & 4300 µg/l (3.6 & 4.3 mg/l) in flow through exposure trials.

6.2.4 Mercury

The effects of mercury toxicity on *Mytilus* spp. have been assessed in several scientific articles. Severe mortality has been reported in juvenile mussels, and significant mortality has been reported in adult and embryo/larvae life stages (Figure 6.7).

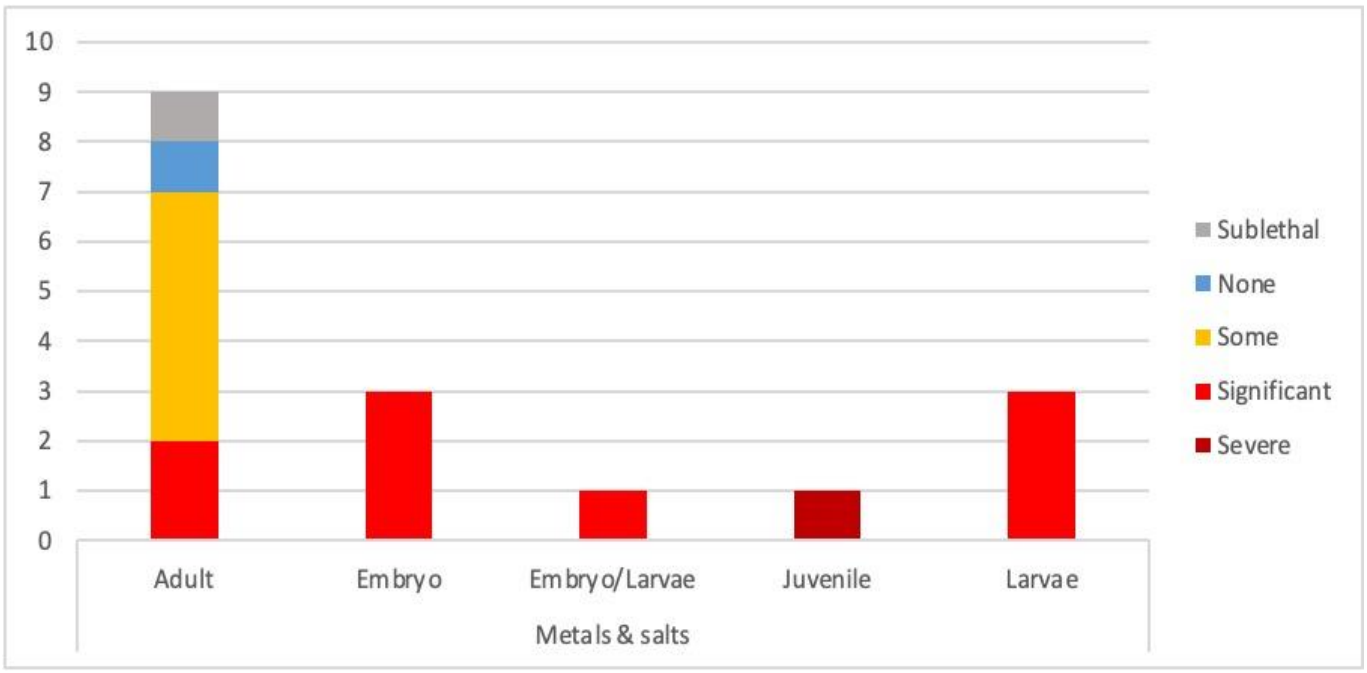

Figure 6.7. Count of ranked mortality (from 'severe' to 'none') in the articles examined for inorganic mercury and its compounds.

- Micallef & Tyler (1990) found that exposure of *Mytilus edulis* to 50 µg/l (0.05mg/l) mercury for five days did not affect survival during that period. However, Micallef & Tyler (1978) found mercury at concentrations of 2500 µg/l (2.5 mg/l) to cause 50% population mortality of *Mytilus edulis* within 96 hours. A long-term 98-day study by Domouhtsidou & Dimitriadis (2000) observed a concentration of 100 µg/l (0.1 mg/l) mercury to cause significant mortality (54.2%) of adult *Mytilus galloprovincialis*.
- Nelson *et al.* (1988) exposed *Mytilus edulis* juveniles to mercury at a variety of concentrations over a 96-hour period to find the lethal concentration. The established LC_{50} and LC_{95} were 161 µg/l (0.161 mg/l) and 284 µg/l (0.284mg/l) respectively.
- Martin *et al.* (1981) studied the toxicity of mercury on the embryo development of *Mytilus edulis* and established the 48-hour EC_{50} of mercury to be 5.8 µg/l (0.0058 mg/l).
- Beiras & His (1995) investigated the effects of mercury on *Mytilus galloprovincialis* embryos and the growth and survival of larvae at different stages of development. Larval growth was significantly reduced at 4 µg/l (0.004mg/l) mercury and an EC_{50} of 10 µg/l (0.01 mg/l) mercury was established for abnormal growth. Embryos were more sensitive to mercury exposure than the larvae with an EC_{50} of 10 µg/l (0.01 mg/l). The results showed the D-shaped larval stage to be the most sensitive larval stage, followed by early umbonate, late umbonate and then eyed larvae stage, with LC_{50}s of 51, 164, 322, 383 µg/l (0.051, 0.164, 0.322 and 0.383 mg/l) respectively.
- Beiras & Albentosa (2004) investigated the inhibitory effects of mercury on the embryo development of *Mytilus galloprovincialis*, establishing a 48-hour EC_{50} of 2 µg/l (0.002 mg/l).
- Pavicic *et al.* (1994) observed the toxic effects of mercury on the development and growth of *Mytilus galloprovincialis* larvae. Mercury exposure caused the abnormal development of veliger larvae with increasing concentration causing a higher percentage of abnormal larvae. The established 48-hour EC_{50} for mercury was 3.5 µg/l (0.0035mg/l).
- Prato & Biandolino (2007) investigated the toxicity of mercury on *Mytilus galloprovincialis* development using the embryotoxicity test. The results showed mercury to have significant effects on larval development. The lowest tested concentration of 0.4 µg/l (0.0004mg/l) mercury caused a

significant impact on larvae development; and the 48-hour EC_{50} was established at 1 µg/l (0.001 mg/l).

- Annicchiarico *et al.* (2007) exposed *Mytilus galloprovincialis* larvae to five concentrations of mercury in the range of 6.25-100 µg/l (0.00625–0.1 mg/l) to determine the lethal concentration during a 48-hour exposure period; an LC_{50} of 10 µg/l (0.01 mg/l) was established.
- The toxicity of methylmercury exposure on *Mytilus edulis* was monitored in two research papers. Dorn (1976) exposed mussels to concentrations of methylmercury acetate between 400 and 2800 µg/l (0.4 and 2.8 mg/l) during a 48-hour period and reported that the feeding rate of *Mytilus edulis* decreased in response to increasing concentration. However, no significant mortalities were recorded during the 48-hour exposure period. Pelletier (1988) exposed *Mytilus edulis* to methylmercury complexes at concentrations of 3 µg/l for 32 days and reported significant mortalities of 30-67%.

6.2.5 Silver

The effects of silver toxicity on *Mytilus* spp. have been assessed in several articles. Severe mortality was observed in juvenile mussels, and significant mortality was reported in adults and embryo/larvae life stages (Figure 6.8).

- Boukadida *et al.* (2016) investigated the toxic effects of silver concentrations (0.1, 1, 3, 10, 30 µg/l) on *Mytilus galloprovincialis* larvae development at different temperatures (18, 20, 22 or 24°C). The results showed a dose-dependent effect on embryo-larval development characterized by an increase in the rate of abnormal D-larvae with increasing silver concentration. Significant embryotoxicity was observed at the lowest tested concentration of silver (0.1 µg/l) with 19.7% of abnormal D-larvae. At 30 µg/l of silver, there was 100% larval abnormality. The 48-hour EC_{50} for silver at 18°C of was calculated at 6.58 µg/l.
- Metayer *et al.* (1990) observed the toxicity of silver on *Mytilus galloprovincialis* adults at three different concentrations. For each of the tested concentrations 1-10, 100 and 1000 µg/l (0.001-0.01, 0.1, and 1 mg/l) LT_{50} values were calculated. At the highest concentration of 1000 µg/l (1 mg/l), 50% mortality occurred at 3.3 days, at 100 µg/l (0.1 mg/l) silver 50% mortality occurred at 4.6 days and at 1 µg/l (0.001mg/l) 50% mortality was >16 days.
- Berthet *et al.* (1992) observed no mortality (0%) in adult *Mytilus galloprovincialis* after exposure to 20 µg/l silver for 28 days.
- A long-term 98-day study by Domouhtsidou & Dimitriadis (2000) found a concentration of 100 µg/l (0.1 mg/l) silver to cause significant mortality (51.4%) of adult *Mytilus galloprovincialis*.
- Nelson *et al.* (1988) exposed *Mytilus edulis* juveniles to silver at a variety of concentrations over a 96-hour period to find the lethal concentrations and determined a LC_{50} of 159 µg/l (0.159mg/l) silver.
- Martin *et al.* (1981) studied the toxicity of silver on the embryo development of *Mytilus edulis* and established the 48-hour EC_{50} of 14 µg/l (0.014 mg/l) silver.

6.2.6 Lead

The effects of lead exposure on *Mytilus* spp. has been studied by multiple papers. The lethal effects of lead were reported in most of the articles examined, although not all the papers reported direct mortalities. Abnormal larval development has been included as a lethal effect as abnormal development may be expected to lead to recruitment failure and population decline. There was an

almost 50/50 split in the papers that assessed the toxicity of lead on adult mussels or on early larvae development stages (Figure 6.9).

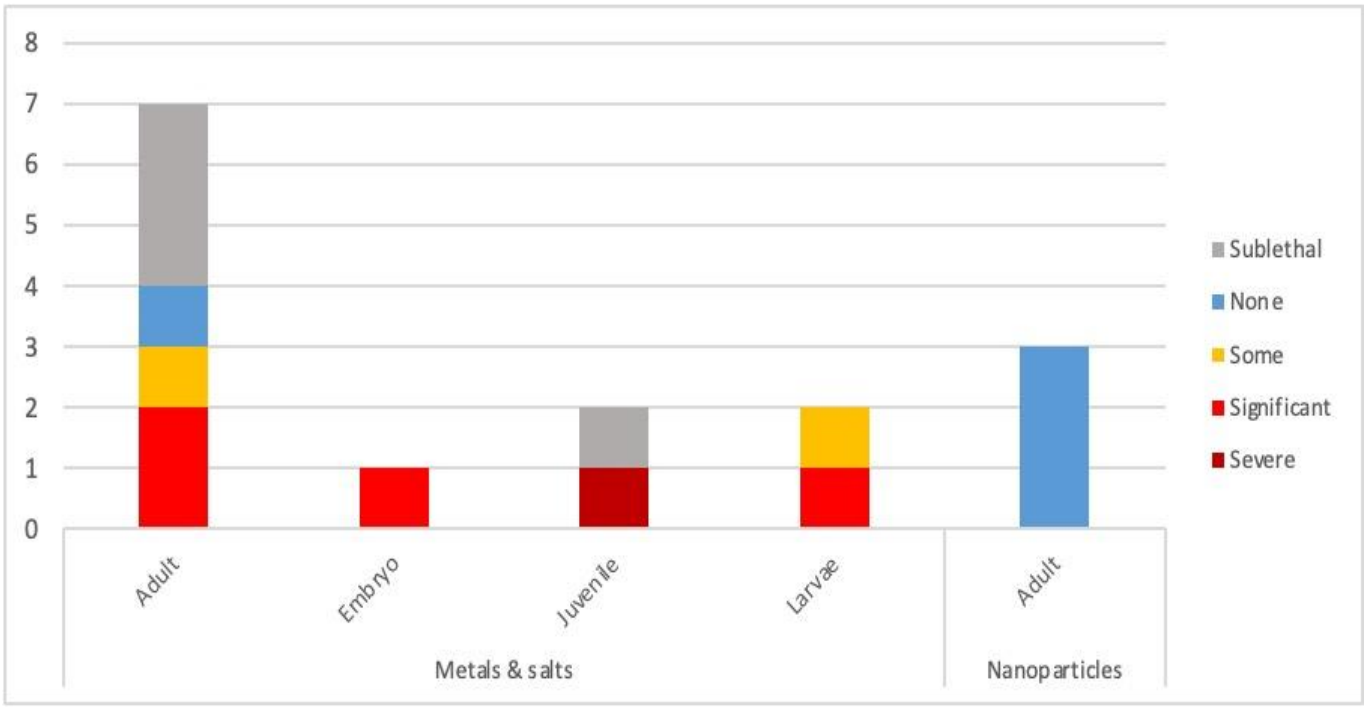

Figure 6.8. Count of ranked mortality (from 'severe' to 'none') in the articles examined for inorganic silver and its compounds.

Adults

- Freitas *et al.* (2019) observed no mortalities from exposure of *Mytilus galloprovincialis* to 50 µg/l (0.05mg/l) lead at a variety of temperatures and salinities for a period of 28 days.
- Vlahogianni & Valavandis (2007) conducted short term lead toxicity tests on *Mytilus galloprovincialis* over 24 hours and 10 days. The observed concentration required to cause mortality in 50% of the test population during a 24-hour exposure was calculated as LC_{50} 4500 µg/l (4.5 mg/l) lead. Additionally, Vlahogianni & Valavandis (2007) observed no mortalities to occur during a 10-day exposure to 150 µg/l (0.15 mg/l) lead.
- However, a long-term study by Domouhtsidou & Dimitriadis (2000) found a lower concentration of 100 µg/l (0.1 mg/l) lead to cause significant mortality (48.5%) of *Mytilus galloprovincialis* during 98-day exposure trials.
- Talbot *et al.* (1976) determined the lethal dose of lead on *Mytilus edulis* adults during a long-term exposure experiment. Talbot *et al.* (1976) observed a clear correlation in the exposure duration and concentration required to cause mortality to 50% of the test population, as follows: LD_{50} 20 mg/l 40 days; LD_{50} 30 mg/l 30-40 days; LD_{50} 10-20 mg/l 50-100 days; and LD_{50} 10 mg/l 100-200 days. The results showed that the lethality of lead depends on the exposure concentration and exposure duration

Embryo/larvae

The results from the examined papers have shown that *Mytilus* embryos and larvae are more sensitive to lead exposure than adult specimens.

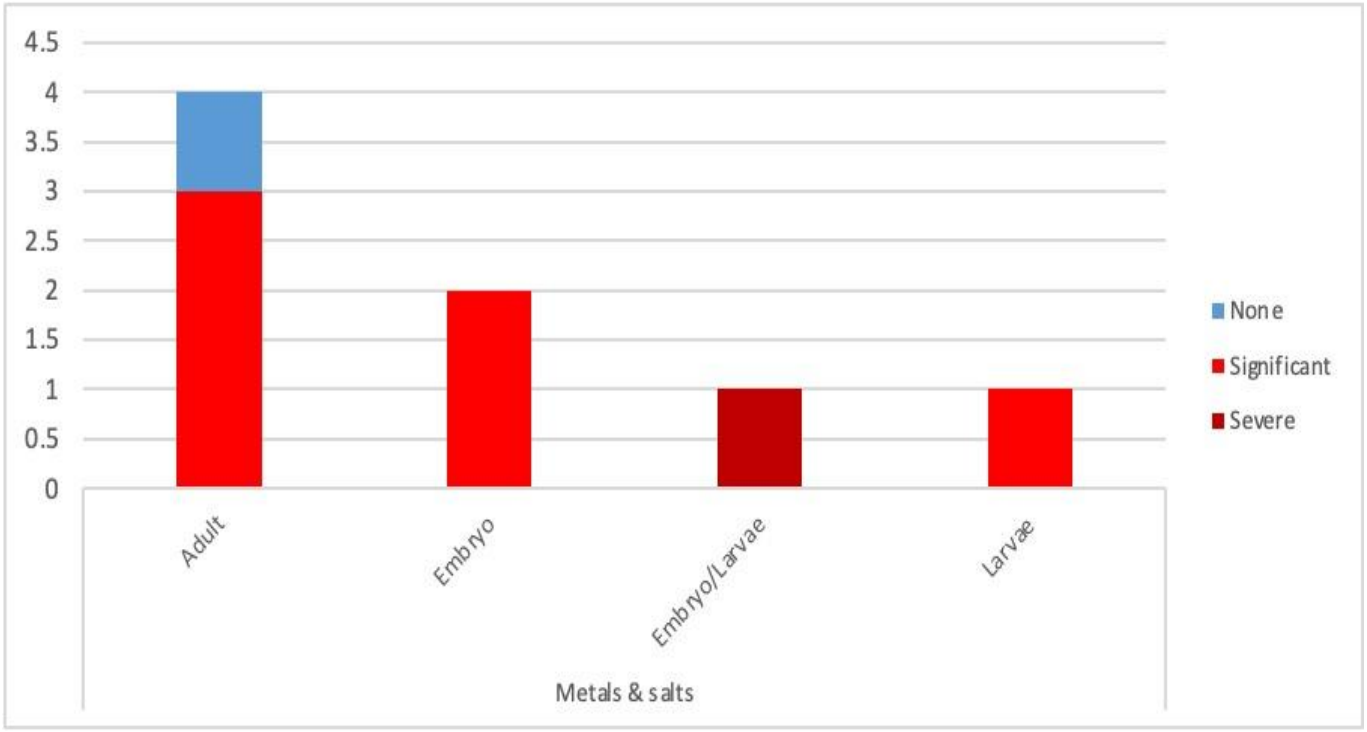

Figure 6.9. Count of ranked mortality (from 'severe' to 'none') in the articles examined for inorganic lead and its compounds.

- Hrs-Brenko *et al.* (1977) investigated the effects of different concentrations of lead at different salinities and temperatures on embryo development of *Mytilus galloprovincialis*. The embryonic development of *Mytilus galloprovincialis* was inhibited depending on the salinity and temperature. The percentage of embryonic development two days after fertilization decreased with increasing concentrations of lead. Lead exposure caused undeveloped larvae, abnormal larvae, and mortality of larvae with the percentage of effect depending on concentration, exposure time, temperature, and salinity. During the first 48 hours of exposure, the mortality rate of the larvae at all concentrations was very low, however, after 96 hours, the mortality rate had significantly increased, as follows:
    - at 100 µg/l (0.1 mg/l) lead embryo development ranged between 8.86-95.60%;
    - at 250 µg/l (0.25 mg/l) lead embryo development ranged between 0-87%;
    - at 500 µg/l (0.5 mg/l) lead embryo development ranged between 0-46.54%; and
    - at 1000 µg/l (1 mg/l) lead embryo development ranged between 0-22.36%.
- Lussier *et al.* (1999) observed 476 µg/l (0.476 mg/l) lead to cause a 50% reduction in larvae hatch rates. Similarly, Martin *et al.* (1981) reported 50% of *Mytilus edulis* larvae to have abnormal development when exposed for 48 hours to 476 µg/l lead. In addition, Beiras & Albentosa (2004) investigated the inhibitory effects of lead on the embryos of *Mytilus galloprovincialis* and established a 48-hour EC_{50} of 221 µg/l (0.221mg/l).

6.2.7 Nickel

The effects of nickel on *Mytilus* spp. have been investigated by a limited number of papers.

- Stromgren *et al.* (1982) observed concentrations up to 200 µg/l (0.2mg/l) nickel did not significantly affect the behaviour or growth rate of *Mytilus edulis* when compared to the control.

- Nadella *et al.* (2009) assessed the embryo-larvae toxicity of nickel on mussel *Mytilus trossulus* during a 48-hour development test. Nickel caused abnormal larvae development with an established EC_{50} of 150 µg/l (0.15mg/l) and an EC_{20} of 82 µg/l (0.082 mg/l).
- Martin *et al.* (1981) investigated the effects of nickel on *Mytilus edulis* larvae development during a 48-hour experiment, and established the 48-hour EC_{50} of nickel to be 891 µg/l (0.891 mg/l).
- Chalkiadaki *et al.* (2014) exposed *Mytilus galloprovincialis* adults to various concentrations of nickel (0.5, 1, 2.5 & 20 mg/l) for 20 days with 10 days depuration period. No mortalities occurred during the exposure period even at the highest concentration of 20 mg/l nickel.
- Deforest & Schlekat (2013) reviewed species sensitivity of chronic nickel toxicity. The toxicity of nickel on *Mytilus galloprovincialis* larval development was conducted by exposing embryos to nickel in static 48-hour toxicity tests. Four tests were conducted using natural seawater collected from four different locations with dissolved organic carbon (DOC) concentrations between 12,000 µg/l (1.2 mg/l) and 2700 µg/l (2.7 mg/l) and salinities between 29.5 and 30.1‰. The EC_{10}s (259, 228, 256 & 350 µg/l nickel) were based on normal larval shell development. The results showed no clear differences in the toxicity of nickel depending on DOC or salinity.

6.2.8 Titanium

Only two research papers investigated the effects of titanium exposure on *Mytilus* spp. Monteiro *et al.* (2019a&b) found titanium at concentrations below 100 µg/l (0.1 mg/l) to not significantly impact the survival of *Mytilus galloprovincialis* adults, during 14-day exposures.

6.2.9 Iron

Three research papers investigated the effects of iron on *Mytilus* spp.

- Kadar *et al.* (2010) found soluble Fe at concentrations of 0.08, 0.8, and 8 mg/l did not affect the development of *Mytilus galloprovincialis* larvae significantly in natural seawater at pH 8.1 during a 48-hour exposure. However, at pH 7 and pH 6 the percentage of normally developed D-shelled larvae reduced drastically, and the percentage of delayed embryos increased.
- Vlahogianni & Valavandis (2007) conducted short-term 24-hour toxicity exposure of *Mytilus galloprovincialis* adults to 1, 4, 6, 8, and 10 mg/l iron. A 24-hour LC_{50} value of >6 mg/l iron was established. In addition, no mortality occurred during a 10-day exposure to 0.15 mg/l iron.
- Pagano *et al.* (1996) studied the toxicity of iron on the early development, fertilization, and offspring quality of *Mytilus galloprovincialis*. Pagano *et al.* (1996) observed severe embryotoxicity when mussel embryos were reared in Fe (III) at concentrations above 10^{-6} M. At the highest tested concentration of iron 10^{-4} M, 25% larvae abnormality occurred. Iron exposure did not affect the fertilization success of sperm or cause a significant increase in the percentage of abnormal larvae following sperm exposure to iron.

6.2.10 Selenium

The toxicity of selenium on *Mytilus* spp. was examined in two research papers. Martin *et al.* (1981) investigated the effects of selenium on *Mytilus edulis* larvae development, establishing the 48-hour EC_{50} to cause abnormal larvae development of selenium to be >10 mg/l. Micallef & Tyler (1990) investigated the effects of selenium on *Mytilus edulis* adults, and reported no mortality after exposure to 50 µg/l selenium during a five-day exposure period. However, significant reductions in filtration rates of *Mytilus edulis* were observed.

6.2.11 Other metals

Aluminium Pagano *et al.* (1996) studied the toxicity of aluminium on the early development, fertilization, and offspring quality of *Mytilus galloprovincialis*. Pagano *et al.* (1996) found severe embryotoxicity when mussel embryos were reared in $Al_2(SO_4)_3$ at concentrations $\geq 10^{-6}$M. At a concentration of $3x10^{-6}$ M, most larvae developed abnormally with only 2 to 4% normal D-larvae development. At concentrations between 10^{-5} and 10^{-4} M only abnormal larvae developed. Aluminium exposure did not affect fertilization success or cause a significant increase in the percentage of abnormal larvae following sperm exposure to aluminium, except for the 10^{-4} M treatment group.

Arsenic. Martin *et al.* (1981) established the 48-hour EC_{50} of arsenic causing abnormal embryo development to be >3 mg/l.

Barium. Spangenberg & Cherr (1996) investigated the impacts of barium on the development of *Mytilus californianus* embryos and larvae. Barium significantly affected the development of larvae at concentrations between 200 & 800 µg/l (0.2 & 0.8 mg/l), causing abnormal and delayed development following 48 hours of exposure to barium. The NOEC determined was 0.1 mg/l barium and the EC_{50} was determined at 1890 µg/l (1.89 mg/l) barium.

Chromium. Martin *et al.* (1981) established the 48-hour EC_{50} of chromium causing abnormal embryo development to be 4469 µg/l (4.469 mg/l).

Dysprosium. Freitas *et al.* (2020a) found 28-day exposures to a variety of concentrations of dysprosium up to 40 µg/l (0.04 mg/l) did not affect the survival of *Mytilus galloprovincialis.* However, metabolic and oxidative effects did occur.

Gadolinium. Henriques *et al.* (2019) found concentrations of gadolinium up to 120 µg/l (0.12 mg/l) not to affect the survival of *Mytilus galloprovincialis* during a 28-day study. However, exposure to gadolinium did affect the biochemical performance of *Mytilus galloprovincialis*.

Manganese. Morgan *et al.* (1986) found the metal manganese to cause abnormal *Mytilus* larvae development and cause mortality during a 48-hour exposure period. The larvae were exposed to concentrations between 1 and 560 mg/l and an EC_{50} of 30 mg/l manganese was established. At 320 mg/l, 100% of larvae developed abnormally and survival was 1%. At the highest tested concentration of 560 mg/l 100% larval mortality occurred.

Molybdenum. Morgan *et al.* (1986) found the metal molybdenum to cause abnormal *Mytilus* larvae development and caused mortality during a 48-hour exposure period. The larvae were exposed to concentrations between 1 and 560 mg/l and an EC_{50} of 147 mg/l molybdenum was established. At 320 mg/l, 76.9% of larvae developed abnormally and survival was 7%. At the highest tested concentration of 560 mg/l, survival was 1% and 100% larvae abnormality occurred.

Neodymium. The survival of *Mytilus galloprovincialis* was not affected when exposed to a variety of concentrations of neodymium up to 40 µg/l (0.04 mg/l) for a period of 28 days (Freitas *et al.*, 2020b).

Strontium. Spangenberg & Cherr (1996) investigated the impacts of strontium on the development of *Mytilus californianus* embryos and larvae. Strontium did not affect the development of larvae at concentrations up to 20 mg/l.

Vanadium. Miramand & Unsal (1978) found an exposure concentration of 6500 µg/l (6.5 mg/l) vanadate to cause 50% mortality of *Mytilus galloprovincialis* within nine days of exposure.

6.2.12 Organometals

Tributyltin. The effects of tributyltin exposure on the survival of *Mytilus* spp. were investigated by multiple research papers.

- Guolan & Young (1995b) observed the effects of 60 days exposure to tributyltin chloride on *Mytilus edulis*. The results showed that exposure concentrations between 0.02 and 0.05 µg/l TBT did not affect the survival of the mussels during the 60-day period.
- Beiras & Bellas (2008) investigated the effect of TBT on the inhibition of embryo development of *Mytilus galloprovincialis*, using the percentage of normal larvae as the end point. The lowest observed effect concentration (LOEC) and the concentration that caused a 10% reduction in the percentage of morphologically normal larvae was 0.2 µg/l TBT, and the concentrations that caused a 50% reduction in the percentage of morphologically normal larvae was 0.377 µg/l TBT.
- Stenalt *et al.* (1998) studied the effects of tributyltin on *Mytilus edulis* larvae and post larvae over a 15-day period. The effects of TBT on mortality, growth, and settlement were assessed. The mortality of larvae increased in response to increasing TBT concentration, with an established LC_{50} of 0.254 µg/l.
- Lapota *et al.* (1993) found tributyltin to affect the growth and survival of *Mytilus edulis* larvae. The survival of larvae exposed to TBT ranged between 52 to 58%. The survival for the 0.006, 0.05, and 0.13 µg/l treatments were 80, 86 and 65%, respectively. By day 33, the mean survival had decreased to 58% in 0.006 mg/l treatment and 52% in the 0.05 and 0.13 µg/l treatments.
- Mazzei *et al.* (2015) tested the toxicity of TBT on the motility of *Mytilus galloprovincialis* sperm. The results showed dose-dependent sperm motility alteration, with the lowest tested concentrations of 0.0001 mg/l (0.1 µg/l) causing reductions in motility. At concentrations between 0.001-1000mg/l, the motility of the sperm was completely inhibited within 60 minutes. In addition, the exposure to TBT caused changes in sperm morphology with the sperm tail forming a hook shape.
- Beaumont & Budd (1984) investigated the effects of tributyltin on the mortality of *Mytilus edulis*. The results showed low concentrations of TBT to be lethal to larvae, with concentrations of TBT (0.1 µg/l) found in the natural environment to cause 50% mortality rates within 15 days and to cause surviving larvae to be moribund and grow significantly more slowly than the controls.
- Salazar *et al.* (1987) ran two test groups exposing *Mytilus edulis* juvenile to six different concentrations of TBT (0.04, 0.05, 0.07, 0.08, 0.16, and 0.2 µg/l) over a period of 56 and 196 days. None of the treatments affected the survival of the mussels during the trial period.
- Salazar & Salazar (1989) tested the impacts of different concentrations of TBTO on *Mytilus edulis*. At a concentration of 3 µg/l TBTO mortality did not occur during the 10-day trial period. However, at a concentration of 76 µg/l TBTO 100% mortality occurred within seven days.
- Jha *et al.* (2000) found tributyltin oxide concentrations between 0.56 and 5.65 µg/l to be toxic to the embryo-larval stages of *Mytilus edulis*, causing mortality and abnormal development with increased concentration causing increased mortality/abnormality.
- Dixon & Prosser (1986) observed clear evidence of dose-dependent reduction in survival for mussel larvae exposed to TBTO. *Mytilus edulis* larvae were exposed to 0.05, 0.1, 0.5, 1, and 5 µg/l TBTO for a period of 96 hours, during which time 14, 44, 54, 79 and 97% mortality occurred, respectively.
- Valkirs *et al.* (1987) monitored the mortality of *Mytilus edulis* over a period of 66 days to establish a reliable LC_{50} value. The results of the exposure treatment produced an LC_{50} of 0.97 µg/l TBT, which is considerably lower than 96-hour LC_{50} data reported in literature for this species and contaminant. Valkirs *et al.* (1987) stated the importance of long-term bioassay testing for assessment of realistic environmental toxicity levels, particularly with slow-acting toxicant such as tributyltin.

Dibutyltin. The effects of dibutyltin exposure on the survival of *Mytilus* spp. were investigated by two research papers. Lapota *et al.* (1993) observed dibutyltin exposures to affect the growth and survival of *Mytilus edulis* larvae. The survival of the larvae in the 2, 20, and 200 µg/l treatments were 88, 85, and

62% respectively. By day 33, survival rates were 83% in 2 µg/l treatment, 76% in the 20 µg/l treatment and 1% in the 200 µg/l treatment.

Mazzei *et al.* (2015) tested the toxicity of DBT on the motility of *Mytilus galloprovincialis* sperm. The results showed dose-dependent sperm motility alteration, with the lowest tested concentrations of 0.0001 mg/l (0.1 µg/l) causing reductions in motility. At concentrations between 0.001-1000 mg/l, the motility of the sperm was completely inhibited within 60 minutes. In addition, the exposure to DBT caused changes in sperm morphology with the sperm tail forming a hook shape.

Zinc pyrithione is an organometal that is used as an anti-fouling agent. The toxicity of zinc pyrithione on *Mytilus edulis* was investigated by two papers at two different life stages. Avelelas *et al.* (2017) investigated the effects of zinc pyrithione on adult mussels and reported a 96-hour LC_{50} at 211.3 µg/l (0.21 mg/l) while complete mortality occurred at 500 and 1000 µg/l (0.5 and 1 mg/l).

Bellas *et al.* (2005) investigated the effects of zinc pyrithione on the development of *Mytilus edulis* larvae, finding significant toxicity effects on the embryonic development at low concentrations 3.6 nM (EC_{10}). Normal development was found to be completely inhibited at 24 nM and the concentration required to cause 50% abnormal larvae development was calculated at 8 nM (48-hour EC_{50}).

6.2.13 Nanoparticulate metals

Titanium nanoparticulates. Four papers investigated the effects of nanoparticulate titanium on *Mytilus* spp. Balbi *et al.* (2014) exposed *Mytilus galloprovincialis* adults to 0.1 mg/l n-TiO_2 for a period of 96 hours, during which time no mortality occurred. Similarly, Canesi *et al.* (2014) exposed *Mytilus galloprovincialis* adults to 0.1 mg/l n-TiO2 for a period of 24 hours, during which time no mortality occurred. Also, Canesi *et al.* (2010) found exposure concentrations between 0.05-5 mg/l nano-titanium not to affect the survival of *Mytilus galloprovincialis* adults during a 24-hour exposure period.

Balbi *et al.* (2014) observed the effects of n-TiO_2 on larval development. Larvae were exposed to n-TiO2 for 48 hours, which did not significantly affect larval development. However, Libralato *et al.* (2013) observed titanium (n-TiO_2) exposure to cause embryotoxicity to *Mytilus galloprovincialis*, producing abnormal larvae. Titanium dioxide was tested at concentrations between 0.5-64 mg/l in light and dark exposure conditions. The results from the experiment showed non-linear regression producing two EC_{50} values per exposure. The maximum ecotoxicological effects were detected at 4 and 8 mg/l. The lowest observed effects were detected at 0.5 mg/l.

Zinc oxide nanoparticulates. Hanna *et al.* (2013) exposed *Mytilus galloprovincialis* adults to nanoparticulate zinc oxide for a period of 12 weeks. The mussels were split into size groups and exposed to 0.1, 0.5, 1, and 2 mg/l Zinc. The mean survival of the mussels in the control group was similar to the large and small mussel groups at all concentrations, except for the groups at the highest exposure concentration. After six weeks of exposure to 2 mg/l zinc large mussels had 91% survival and small mussels had 59% survival, but after 12 weeks of exposure, survival was down to 62% in the large group and 23% in the small group.

Copper oxide nanoparticulates. The effects of copper oxide nanoparticles were investigated in five articles. The survival of *Mytilus galloprovincialis* was not influenced when exposed to 0.01 mg/l copper oxide over 15-day periods (Gomes *et al*., 2011; Gomes *et al*., 2012; Gomes *et al*., 2013b; Gomes *et al*., 2014a). In addition, Hu *et al*. (2014) found that concentrations between 0.4 – 1 mg/l copper oxide nanoparticles did not affect the survival of *Mytilus edulis* during a one-hour exposure.

Iron oxide nanoparticulates. The effects of iron nanoparticles were investigated in two articles. Kadar *et al.* (2010) found neither nano-Fe nor soluble Fe concentrations to affect the development of *Mytilus* larvae significantly in natural seawater at pH 8.1. However, at pH 7 and pH 6, the percentage of normally developed D shelled larvae drastically reduced, and the percentage of delayed embryos increased. Furthermore, Kadar *et al.* (2011) found sperm exposure to zero-valent iron to affect the

development of *Mytilus* larvae significantly, indicated by the decrease in normal D-larvae. At the highest tested concentration of 10 mg/l, D-larvae were reduced to below 40%. In addition, sperm fertility was reduced by zero-valent iron exposure.

Gold nanoparticulates. Tedesco *et al.* (2010) exposed *Mytilus edulis* adults to nanoparticulate gold for a 24-hour period. No mortality occurred during the 24-hour period.

Silver nanoparticulates. The survival of *Mytilus galloprovincialis* adults was not influenced by exposures to 0.01 mg/l silver nanoparticles over 15-day periods (Gomes *et al.,* 2013a&b; Gomes *et al.,* 2014b).

6.2.14 Sensitivity assessment – Transitional metals and organometals

The number of articles that report mortalities due to metal, organometals, and nanoparticulate metals in *Mytilus* spp. are summarized in Figure 6.3 and in Table 6.3 and Table 6.4 below. Relevant resistance ranks and resultant sensitivities are shown in Table 6.3 and Table 6.4 based on the weight of evidence and 'worst-case' approach outlined above.

The majority of the evidence examined copper, followed by cadmium, zinc, silver, and mercury (Figure 6.3; Table 6.3). The evidence suggests that *Mytilus* adults and juveniles have a **'High'** sensitivity to copper, cadmium, mercury and silver and a **'Medium'** sensitivity to iron, lead, methylmercury and neodymium. The confidence in those assessments is probably **'Medium'** due to the volume of evidence examined.However, it is also clear that there is considerable variation in response to metal exposure, due in part to the variation in the experimental studies, and especially the concentration and exposure duration used.

Less evidence for the remaining metals and especially the organometals and nanoparticulate metals was found, and in some cases, the sensitivity assessment is based on one or two papers (e.g. nanoparticulate Zinc, or tributyltin oxide). While the articles present are all 'High' to 'Medium' quality and directly applicable, it may be prudent to treat these assessments with more caution and assess their confidence as **'Low'**.

The number of articles that reported the effects of metals on larvae and embryos alone is also dominated by studies on the effect of copper (Table 6.4).). The evidence suggests that *Mytilus* larvae and embryos are highly sensitive to copper, lead, and zinc, plus molybdenum and manganese although the last two are based on single papers. There is also evidence that organotins result in 'severe' (>75%) mortality in larvae and embryos.

Across the entire contaminant group, there is evidence that several metals, one nanoparticulate metal, and some organometals have been reported to cause 'severe' (>75%) mortalities in adult and juvenile mussels. Hence, an overall assessment of **'High**' sensitivity to metal contamination may be given based on the '**worst-case' scenario**.

Table 6.3. Summary of count of ranked mortalities reported in evidence review on the effects of metals in *Mytilus* spp. and resultant proposed sensitivity assessments for **adults and juveniles only**. (NS= Not sensitive, N= None, L= Low, M= Medium, H =High)

		Mortality (worst case reported)							Assessment		
Group	**Contaminant**	**Severe**	**Significant**	**Some**	**None**	**Sublethal**	**NR**	**Total**	**Resistance**	**Resilience**	**Sensitivity**
Metals & compounds											
	Cadmium	**5**	4	2	5	4	1	21	**N**	**L**	**H**
	Copper	**14**	11	1	6	9		41	**N**	**L**	**H**
	Dysprosium				**1**			1	H	H	**NS**
	Gadolinium				**1**			1	H	H	**NS**
	Iron		**1**					1	L	M	M
	Lead		**3**		1		1	5	L	M	M
	Mercury	**1**	2	5	1	1		10	**N**	**L**	**H**
	Methylmercury		**1**		1			2	L	M	M
	Neodymium				1			1	L	M	M
	Nickel				**1**		1	2	H	H	**NS**
	Selenium				**1**	1		2	H	H	**NS**
	Silver	**1**	2	1	1	4		9	**N**	**L**	**H**
	Titanium				**2**			2	H	H	**NS**
	Zinc	**3**	4	1	1	3	1	13	**N**	**L**	**H**
Metals & compounds (total)		**24**	**28**	10	23	22	4	111	**N**	**L**	**H**
Nanoparticulate metals											
	Copper				**3**			3	H	H	**NS**
	Silver				**3**			3	H	H	**NS**
	Titanium				**1**			1	H	H	**NS**
	Zinc		**1**					1	L	M	M
	Titanium dioxide				**2**			2	H	H	**NS**
	Gold				**1**			1	H	H	**NS**
Nanoparticulate metals (total)			**1**		10			11	**L**	**M**	**M**[42]
Organotin											
	Dibutyltin					**1**		1	H	H	**NS**
	Tributyltin			**1**	1	2		4	M	M	M
	Tributyltin oxide	**1**				1		2	**N**	**L**	**H**
Organotin (total)		**1**		1	1	4		7	**N**	**L**	**H**[43]
OrganoZinc											
	Zinc pyrithione	**1**						1	**N**	**L**	**H**
Grand Total		**26**	**29**	**11**	**34**	**26**	**4**	**130**	**N**	**L**	**H**[44]

[42] Based on a single paper while the remaining articles reported no observed mortalities so that 'Not sensitive' may be the more appropriate assessment, based on existing evidence.

[43] Based on a single paper while the remaining articles reported some or no mortalities or only sublethal effects so that 'Medium' sensitivity may be the more appropriate assessment, based on existing evidence.

[44] Across the entire group there is evidence that metals, one nanoparticulates and some organometals have been reported to cause severe mortalities in adult and juvenile mussels.

Table 6.4. Summary of count of ranked mortalities reported in evidence review on the effects of metals in *Mytilus* spp. and resultant proposed sensitivity assessments for **embryos and larvae only**. (NS= Not sensitive, N= None, L= Low, M= Medium, H =High)

		Mortality (worst case reported)							Assessment		
Group	Contaminant	Severe	Significant	Some	None	Sublethal	NR	Total	Resistance	Resilience	Sensitivity
Metals & salts											
	Arsenic		**1**					1	L	M	M
	Barium		**1**					1	L	M	M
	Cadmium		**6**	2				8	**L**	**M**	**M**
	Copper	**4**	14	1	1			20	**N**	**L**	**H**
	Lead	**1**	3					4	**N**	**L**	**H**
	Manganese	**1**						1	**N**	**L**	**H**
	Mercury		**7**					7	L	M	M
	Molybdenum	**1**						1	**N**	**L**	**H**
	Nickel		**2**	1				3	L	M	M
	Selenium		**1**					1	L	M	M
	Silver		**2**	1				3	L	M	M
	Strontium				**1**			1	H	H	**NS**
	Zinc	**1**	4					5	**N**	**L**	**H**
	Chromium		**1**					1	L	M	M
Metals & salts (total)		**8**	**42**	5	2			**57**	**N**	**L**	**H**
Nanoparticulate metals											
	Iron		**2**					2	L	M	M
	Titanium		**1**					1	L	M	M
Nanoparticulate metals (total)			**3**					3	**L**	**M**	**M**
Organotin											
	Dibutyltin	**1**						1	**N**	**L**	**H**
	Tributyltin		**3**					3	L	M	M
	Tributyltin oxide	**3**						3	**N**	**L**	**H**
Organotin (total)		**4**	**3**					**7**	**N**	**L**	**H**
OrganoZinc											
	Zinc pyrithione		**1**					**1**	L	M	M
Grand Total		**12**	**49**	**5**	**2**			**68**	**N**	**L**	**H**

6.3 *Mytilus* spp. – Synthetics

A total of 70 articles were selected from 2494 articles. These 70 articles focused on the physiological effects of exposure to synthetic contaminants on *Mytilus* spp. The range of ranked mortalities reported in the 70 papers examined is shown in Figure 6.10.

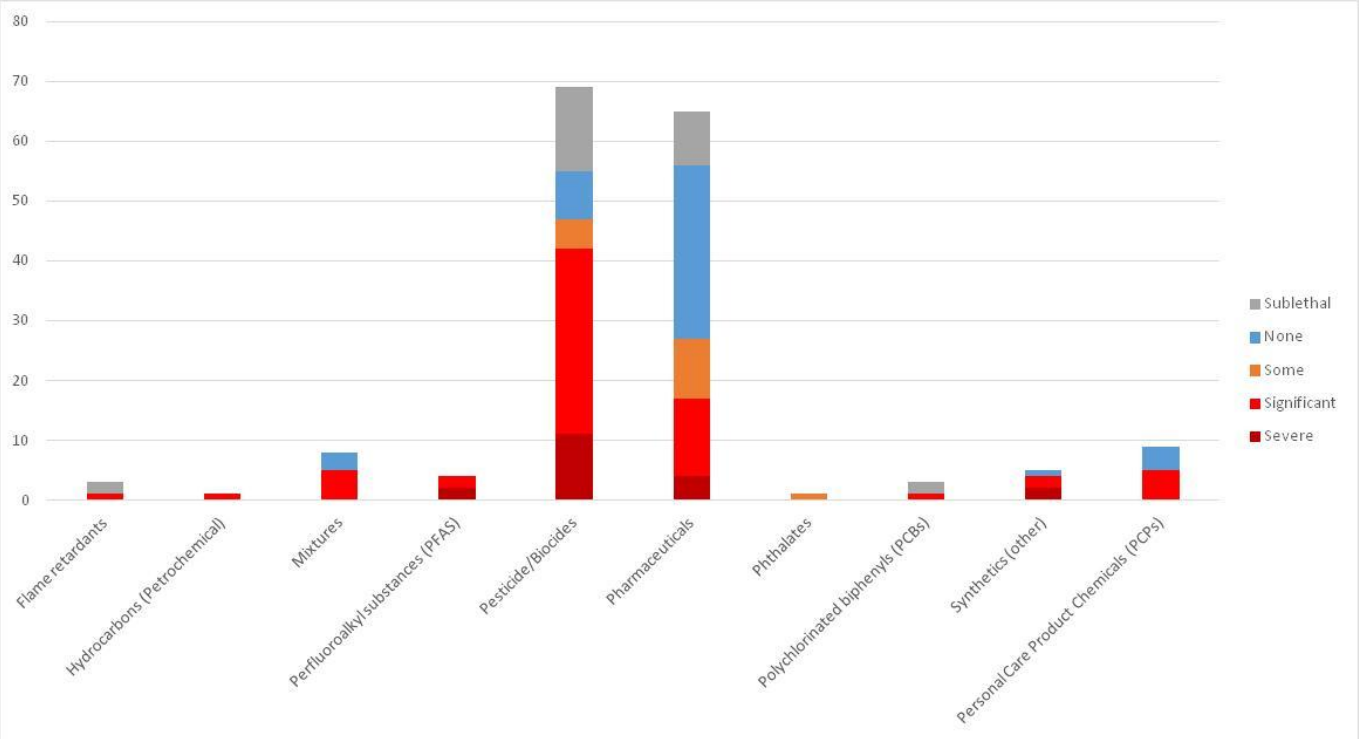

Figure 6.10. Count of ranked mortality due to exposure to synthetic contaminants in *Mytilus* spp.. Mortality is ranked as follows: 'Severe' (>75%), 'Significant' (25-75%), 'Some' (<25%), 'None' (no mortality reported), and 'Sublethal' effects. Note some articles are included more than once because they examined several different combinations of contaminant.

6.3.1 Flame retardants

Only two of the 70 articles selected examined the physiological effects of flame retardants on *Mytilus* spp.

- Barón *et al.* (2016) exposed *Mytilus galloprovincialis* adults to Decabromodiphenyl ether (56, 100, and 200 µg/l) and Dechlorane Plus (5.6, 56, and 100 µg/l) for a period of six days in semi-static exposure conditions. Exposure to either of the chemicals at the tested concentrations did not significantly affect clearance rates after six days of exposure.
- Fabbri *et al.* (2014) investigated the effects of eight contaminants on *Mytilus galloprovincialis* embryotoxicity during a 48-hour exposure experiment. The effects of different compounds representative of endocrine disrupting chemicals were tested in a wide concentration range (0.01,0.1,1,10,100,1000 µg/l). The flame retardant Tetrabromo bisphenol A showed a dose-dependent increase in the percentage of abnormal larvae in response to increasing concentration. Established NOEC, LOEC, and EC_{50} values were as follows 0.01 µg/l, 0.1 µg/l, and 5.52 µg/l.

6.3.2 Hydrocarbons

Fabbri *et al.* (2014) investigated the effects of eight contaminants on *Mytilus galloprovincialis* embryotoxicity during a 48-hour exposure experiment. The effects of different compounds representative of endocrine disrupting chemicals were tested in a wide concentration range (0.01, 0.1,

1, 10, 100, 1000 µg/l). The hydrocarbon Bisphenol A showed dose-dependent increase in the percentage of abnormal larvae in response to increasing concentration. The established NOEC, LOEC, and EC_{50} values were as follows 0.01 µg/l, 0.1 µg/l, and 3.68 µg/l.

6.3.3 Mixtures

Only one of the articles examined the physiological effects of contaminant mixtures on *Mytilus* spp.

Dispersants

Swedmark *et al.* (1973) investigated the effects of oil dispersants on the survival of *Mytilus edulis* after 96 hours of exposure and after 96 hours of exposure with 48 hours recovery in clean seawater. In addition, the effect of the dispersants on byssal activity and shell closure was investigated. Exposure to BP 1100X, Corexit 8666 or Fina-sol OSR2 did not cause any significant mortality during a four-day exposure period. However, exposure to Berol TL 188, BP1100, Fina-Sol SC and Nonylphenoxy polyethoxy ethanol caused significant mortality of *Mytilus edulis* during the four-day exposure period (Table 6.5).

Table 6.5. Range of 96-hour LC_{50}s, with and without 48 hours recovery in *Mytilus edulis* exposed to a range of dispersants (Swedmark *et al.*, 1973).

Dispersant	LC_{50} (96 hours) (mg/l)	LC_{50} (96-hour) after 48 hours recovery (mg/l)
BP 1100X:	>688	>688
Corexit 8666	>940	>940
Fina-sol OSR2	>700	>700
Berol TL 188	800	400
BP1100	>1000	250
Fina-Sol SC	>110	90
Nonylphenoxy polyethoxy ethanol	12	10
Polyclens TS 7	>984	>984
Berol TL 198	>1050	>1050
Corexit 7664	>1000	NR

6.3.4 Perfluoroalkyl substances (PFAS)

Only two of the selected articles examined the physiological effects of perfluoroalkyl substances (PFAS) on *Mytilus* spp.

- Hayman *et al.* (2021) investigated the toxicity of perfluorooctanoic sulphonate (PFOS) and perfluorooctanoic acid (PFOA) on larval development and survival. For PFOS the EC_{20} and EC_{50} that resulted in abnormal larvae development were 0.94 and 1.1 mg/l, respectively. Complete (100%) mortality occurred at >2mg/l PFOS, with LC_{20} and LC_{50} values determined at 0.93 and 1.07 mg/l, respectively. For PFOA the EC_{20} and EC_{50} that resulted in abnormal larvae development were 3.47 and 12 mg/l, respectively. Complete (100%) mortality occurred at 52 mg/l PFOA with LC_{20} and LC_{50} values determined to be 3.18 and 9.98 mg/l.
- Fabbri *et al.* (2014) investigated the effects of eight contaminants on *Mytilus galloprovincialis* embryotoxicity during a 48-hour exposure experiment. The effects of different compounds representative of endocrine disrupting chemicals were tested in a wide concentration range (0.01,0.1,1,10,100,1000 µg/L). The tested perfluoroalkyl substances showed a dose-dependent increase in the percentage of abnormal larvae in response to increasing concentration. For both

perfluorooctanoic acid (PFOA) and perfluorooctanoic sulphonate (PFOS) the established NOEC and LOEC values were 0.01 µg/l and 0.1 µg/l. The perfluoroalkyl substances PFOA and PFOS showed significant increase in abnormal larval development from 0.1 mg/l (17% and 27%, respectively). Maximal effects were observed at 100 mg/l (about 40% and 50%, respectively) with no further increase in percentage of abnormal development at higher concentrations.

6.3.5 Personal Care Product chemicals (PCPs)

Only four articles examined the effects of Personal Care Product (PCPs) on *Mytilus* spp..

- Gomez *et al.* (2012) investigated the bioconcentration of two pharmaceuticals (benzodiazepines: Diazepam and Tetrazepam) and two personal care products (UV filters: Octocrylene and 2-Ethyl-hexyl-4-methoxycinnamate) in *Mytilus galloprovincialis*. Significant mortality did not occur during the experiments and the condition index of the exposed mussels was not significantly different from the controls.
- Paredes *et al.* (2014) investigated the toxicity of four UV filters, 2-Ethyl-hexyl-4-methoxycinnamate (EHMC), 4-Methylbenzylidene-camphor (4-MBC), Benzophenone-3 (BP-3) and Benzophenone-4 (BP-4) on the development of *Mytilus galloprovincialis* larvae. The most toxic UV filter was 4-MBC (EC_{50} 587.17 µg/l), then, with similar toxicity, EHMC (EC_{50} 3118.19 µg/L) and then BP-3 (EC_{50} 3472.59 µg/l). BP-4 was not toxic at the tested concentrations, with an EC_{50} of >10,000 µg/l.
- Giraldo *et al.* (2017) investigated the effects of UV Filters Ethylhexyl Dimethyl p-Aminobenzoic Acid and Octocrylene on *Mytilus galloprovincialis* development. Both contaminants caused abnormal larvae. The established NOEC, LOEC, and EC_{50} of Ethylhexyl Dimethyl p-Aminobenzoic Acid were 25, 100, and 130 µg/l, respectively. The established NOEC, LOEC and EC_{50} of Octocrylene were 20, 40 and >650 µg/l, respectively.
- Bordalo *et al.* (2020) exposed *Mytilus galloprovincialis* to two UV filters (Benzophenone-3 and dimethyl sulfoxide) at concentrations between 0.01 to 1 µg/l. Neither contaminant caused mortalities during the 96-hour exposure trial.

6.3.6 Pesticide/Biocide

Twenty-seven of the selected articles examined the physiological effects of pesticides on *Mytilus* spp. Organohalogens (27%), carbamate (18%) and organophosphate (15%) were the most studied contaminants. A total of 15 (56%) of the 27 articles reported lethal effects (Figure 6.11). The evidence is *summarized* below for articles that reported 'end points'.

- Adema & Vink (1981) investigated the toxicity of Dieldrin, pentachlorophenol and 3,4-dichloroaniline on the survival of *Mytilus edulis*. The established LC_{50} values for Dieldrin at 14 and 30 days of exposure were >200 µg/l and 180 µg/l, respectively. The LC_{50} values for pentachlorophenol at 4, 7, and 14 days exposure were 18,000 µg/l, 950 µg/l and 750 µg/l, respectively. The LC_{50} values of 3,4-dichloroaniline at 4, 7, and 21 days exposure were 9,500, 8,000, and 6,500 µg/l respectively.
- Armstrong & Millemann (1974) investigated the effects of the insecticide Sevin and 1-naphthol on six different development stages of *Mytilus edulis*. All of the development stages were affected by the insecticide and 1-hour EC_{50} values were established from the numbers of normal and abnormal development. The most sensitive developmental stage was the stage following fertilization, at the time of appearance of the first polar body. Thereafter, sensitivity decreased as age increased. The EC_{50} values of 1-naphthol were only determined for the unfertilized egg and the first polar body with values of 24.5 and 5.2 mg/l, respectively. The EC_{50} values of Sevin on the larval developmental stages ranged from 5.3 to 24 mg/l, and the EC_{50} of Sevin on the unfertilized egg was 20.7 mg/l.

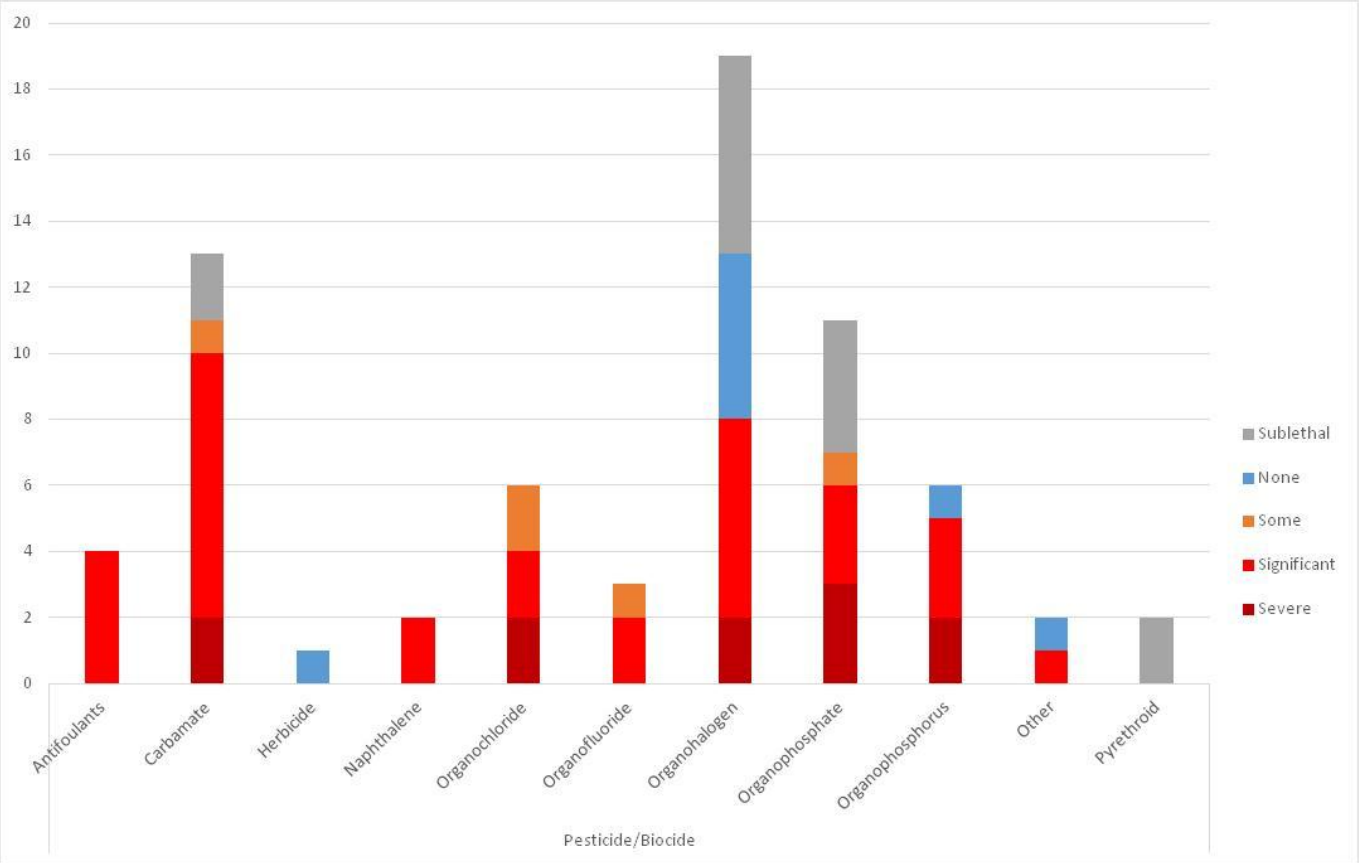

Figure 6.11. Count of ranked mortality due to exposure to pesticides or biocides in *Mytilus* spp.. Mortality is ranked as follows: 'Severe' (>75%), 'Significant' (25-75%), 'Some' (<25%), 'None' (no mortality reported), and 'Sublethal' effects. Note some articles are included more than once because they examined several different combinations of contaminant.

- Ayad *et al.* (2011) reported that concentrations of Cypermethrin up to 0.8 mg/l did not affect the time of survival in air of *Mytilus galloprovincialis* during 24 hours aerial exposure.
- Beiras & Bellas (2008) investigated the toxicity of the biocides Chlorpyrifos and Lindane on the inhibition of embryo development of *Mytilus galloprovincialis*, using the percentage of normal larvae as the end point. The EC_{10} and EC_{50} values were 79 and 154 µg/l for Chlorpyrifos, and 1,413 and 1,990 µg/l for Lindane.
- Bellas (2006) investigated the effects of antifouling biocides on the development of *Mytilus edulis*. Toxicity was quantified in terms of the EC_{50} (median effective concentration) and EC_{10} reducing embryogenesis success, larval growth, and larval settlement by 50% and 10% respectively. Chlorothalonil produced EC_{10} and EC_{50} values of 4.5 and 8.8 µg/l, whilst 'Sea- Nine 211' values were 7.1 and 11 µg/l. Dichlofluanid and Tolylfluanid showed similar toxicity, with EC_{10} values of 52 and 49 µg/l and EC_{50} values of 81 and 74 µg/l, respectively. Irgarol 1051 was the least toxic biocide with EC_{10} and EC_{50} of 797 and 1,540 µg/l. Sea-Nine 211 and Chlorothalonil were approximately 6–7 times more toxic than Dichlofluanid and Tolylfluanid, and 170 times more toxic than Irgarol 1051.
- Ernst & Doe (1989) compared the toxicity of the insecticide Fenitrothion flowable and Fenitrothion liquid technical formulations on the survival of *Mytilus edulis* during a 96-hour exposure, with an additional 96 hours in clean water to observe any delayed mortality. Significant mortalities occurred with LC_{50} values of 15 mg/l Fenitrothion flowable and 18.8 mg/l Fenitrothion liquid.
- Ernst *et al.* (1991) investigated the effects of Chlorothalonil (Bravo 500) toxicity on the survival of *Mytilus edulis* adults. They established a 96-hour LC_{50} value of 5.9 mg/l Chlorothalonil.

- Freitas *et al.* (2019b & 2019c) investigated the effects of Triclosan and Diclofenac on *Mytilus galloprovincialis* at different salinities during a 28-day exposure. No mortalities occurred throughout their experiments.
- Gowland *et al.* (2002) investigated the effects of Cypermethrin (Excis), at concentrations between 10 to 1,000 µg/l, on the aerial survival and shell closure of *Mytilus edulis*. Excis did not significantly affect the aerial survival of the exposed mussels compared to the controls. However, shell closure increased with increasing concentration of Excis.
- Karagiannis *et al.* (2011) investigated the effects of the herbicide Atrazine on the survival and byssus thread production of *Mytilus galloprovincialis*. The effects of Atrazine were monitored over 21 days at concentrations between 1 to 10 mg/l. Complete (100%) mortality occurred within five days at concentrations between 5 to 10 mg/l. In addition, byssal thread production was stopped. At the lower tested concentrations of 1 and 2 mg/l significant mortality occurred (32.5 and 60.83% respectively). The byssal thread production of the mussels in the 1 and 2 mg/l treatment was significantly reduced compared to the controls. After the 21-day exposure, the surviving mussels were then exposed to air and LT_{50} values were established. The control group had an LT_{50} of 5.14 days whilst the mussels that had been exposed to 1 and 2 mg/l Atrazine had LT_{50}s of 2.77 and 1.98 days, respectively.
- Lucu *et al.* (1980) investigated the toxicological effects of biocide Slimicide C-30 on the developmental stages of *Mytilus galloprovincialis*. The 96-hour EC_{50} was 0.07 mg/l.
- McHenery *et al.* (1997) investigated the effects of Dichlorvos exposure on *Mytilus edulis*. After 24 hours at concentrations of 3 mg/l and above, the mussels lost the ability to retract mantle fringes and close the valves of their shells, with an EC_{50} of 1.69 mg/l. Dichlorvos also affected the survival of *Mytilus edulis*, with a 24-hour LC_{50} of 8.2 mg/l.
- Pena-Llopis *et al.* (2002) investigated the toxicity of Fenitrothion and established 96-hour LC_{50} and LC_{85} of 8.4 and 12.1 mg/l respectively.
- Rao (1981) investigated the effects of two insecticides, gamma-hexachloran (Lindane) and Sevin (Carbaryl), on the survival of *Mytilus galloprovincialis* at 1, 2, 4, 6, 8, and 10 mg/l over seven days. The results indicated that the smaller mussels were more susceptible than larger mussels to both toxicants. At concentrations of 8 and 10 mg/l Lindane, total (100%) mortality occurred on day six for the small mussels and on day seven for the larger mussels. Sevin was less toxic as 10 mg/l caused 30% mortality of small mussels and 15% mortality of large mussels after seven days.
- Rao & Mane (1979) investigated the effects of Carbofos on the survival and respiration of *Mytilus galloprovincialis*. The survival of the mussels in the treatments between 2 and 12 mg/l decreased with increasing concentration of contaminant. All of the mussels in the 10 and 12 mg/l treatments were dead after seven days. But 100% survived at 0.5 and 1 mg/l Carbofos after the seven-day exposure. The oxygen consumption of the mussels varied depending on mussel size, exposure time and exposure concentration. At 1 mg/l, the small mussels initially expressed a rapid 19.4% increase in oxygen consumption compared to the control, followed by respiration suppression over the following four days, producing a 22.29% difference to the control. At 6 mg/l, the respiration of the small mussels was suppressed from the first day of exposure. At 1 mg/l, the large mussels had several peaks in respiration on the 1st, 3rd, 5th, and 6th day with increases in oxygen consumption of 88.60, 108.82, 34.71, and 11.56%, respectively. On the 2nd and 4th day, the respiration rate was less than the controls by 61.5 and 30.34%. The respiration of the large mussels initially decreased by 54.3% on the second day at 6 mg/l, followed by a threefold increase in respiration on the third day, that was then followed by reduced respiration with a difference of 69.9% of the control by the sixth day.

- Serrano *et al.* (1995) investigated the toxicity of five pesticides on the survival of *Mytilus galloprovincialis*. The mussels were exposed to the pesticides at concentrations of 1, 3.2, 5.6, 10, 32, and 56 mg/l. Methidathion, Chlorfenvinphos, and Chlorpyrifos caused significant mortality and inhibited byssal thread production. The LC_{50} values of Methidathion, Chlorfenvinphos, and Chlorpyrifos were calculated at 30.1, 26.3, and 22.5 mg/l, respectively. However, Dimethoate and Phosmet did not cause significant effects on mortality or byssal thread production.
- Liu & Lee (1975) investigated the toxicity of the insecticides Sevin, Methoxychlor, and Malathion, and the herbicides Treflan and 2,4-D on *Mytilus edulis*. The survival and byssus thread attachment were assessed in adult mussels, in addition to embryo shell development, larval growth, and metamorphosis. 96-hour LC_{50} values were calculated for each of the contaminants based on the survival of adults, and 48-hour EC_{50} values were calculated based on larval developmental abnormalities (Table 6.6).

Table 6.6. 96-hour EC_{50}s and LC_{50} determined for a range of pesticides in *Mytilus edulis*.

Pesticide	96-hour EC_{50} (mg/l)	96-hour LC_{50} (mg/l)
Sevin	1.5	22.7
Methoxychlor	>0.075	>0.092
Malathion	13.4	
Treflan	>0.12	>0.42
2,4-D	211.7	259

6.3.7 Pharmaceuticals

A total of 28 articles examined the effects of pharmaceuticals on *Mytilus* spp. Adrenergic agonists (21%) and Analgesics (NSAIDs) (24%) were the most studied, followed by beta-blockers (12%), chemotherapy agents (8%), antidepressants (8%) and antihyperlipidemic agents[45] (8%) (Figure 6.12). The majority (70%) of the articles reported lethal effects. The evidence is summarized below.

- Capolupo *et al.* (2018) investigated the impacts of three pharmaceuticals (Propranolol[46] (PROP), 17-α ethinylestradiol[47] (EE2), and Gemfibrozil[48] (GEM) on gamete fertilization and embryonic development in early life stages of *Mytilus galloprovincialis*. Concentrations comparable to or higher than environmental concentrations were used; PROP (0.5, 5, 50 µg/l), EE2 (0.005, 0.05, 0.5 µg/l), and GEM (0.050, 0.500, 5 µg/l). PROP did not affect gamete fertilization at the concentrations tested. However, inhibitory effects on fertilization of 24% and 17.6% were observed at environmental levels of EE2 (0.500 µg/l) and GEM (5 µg/l), and EC_{10} values of 0.142 and 2.4 µg/l, respectively, were determined. The 48-hour embryotoxicity exposure to all three pharmaceuticals caused the onset of morphologically abnormal larvae. The development of normal larvae was reduced by 18.5% in the 50 µg/L PROP treatment. Significant reductions of 19.9, 29.5, and 32.0% normal larvae development was observed at 0.005, 0.05, 0.5 µg/l EE2, with an EC_{10} of 0.0025 µg/l. The percentage of normally developed larvae was reduced by 23.3% at 0.5 µg/l GEM.
- Estevez-Calvar *et al.* (2017) investigated the effects of the antidepressant Sertraline on the development of *Mytilus galloprovincialis* embryos. The results showed that Sertraline significantly affected the development of mussel larvae with an EC_{50} of 206.80 µg/l.

[45] Antihyperlipidemic agents are pharmaceuticals designed to reduce the level of lipids or lipoproteins in the blood.
[46] A beta-blocker designed to reduce blood pressure
[47] A human hormone used in birth-control medication
[48] A antihyperlipidemic agent

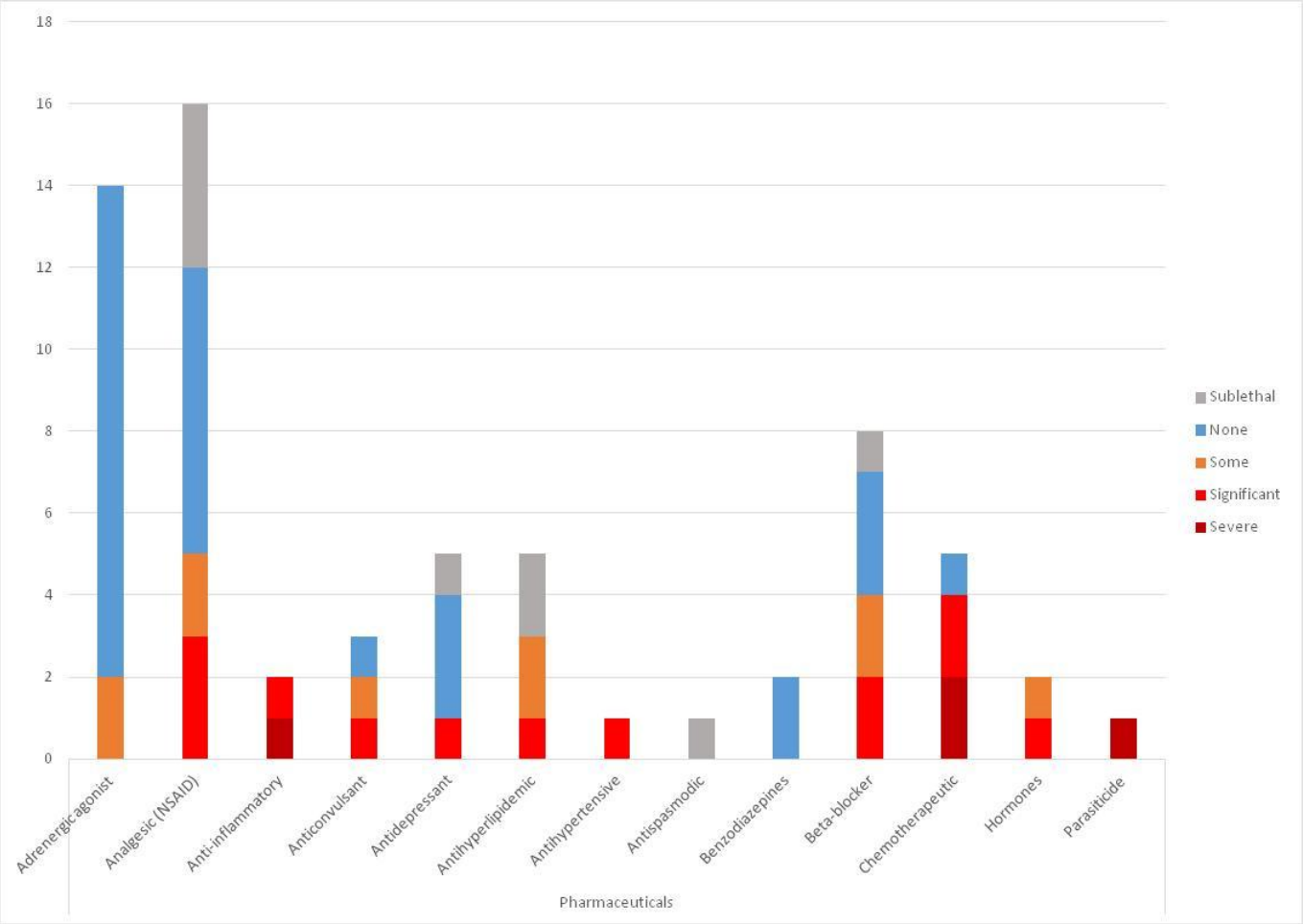

Figure 6.12. Count of ranked mortality due to exposure to pharmaceuticals in *Mytilus* spp.. Mortality is ranked as follows: 'Severe' (>75%), 'Significant' (25-75%), 'Some' (<25%), 'None' (no mortality reported), and 'Sublethal' effects. Note some articles are included more than once because they examined several different combinations of contaminant.

- Fabbri *et al.* (2014) investigated the effects of eight contaminants on *Mytilus galloprovincialis* embryos during a 48-hour exposure experiment. The effects of different compounds representative of endocrine disrupting chemicals were tested in a wide concentration range (0.01,0.1,1,10,100,1000 µg/l). The pharmaceuticals Ibuprofen[49] and Bezafibrate[50] showed dose-dependent increase in the percentage of abnormal larvae in response to increasing concentrations. The NOEC and LOEC values for Ibuprofen were calculated at 10 and 100 µg/l, respectively. The mussel embryos were more sensitive to Bezafibrate producing NOEC and LOEC values of 1 and 10 µg/l, respectively. Diclofenac[51] significantly affected mussel larval development at low concentrations 0.001 µg/l, but produced an inverted U-shaped dose response curve, with no effects at the highest tested concentration. The Diclofenac NOEC was calculated to be 0.001 µg/l.
- Franzellitti *et al.* (2019) investigated the toxicity of Carbamazepine[52] and Propranolol on the embryo/larvae stages of *Mytilus galloprovincialis* development. They tested a range of concentration between 0.01 and 1000 mg/l using a 48-hour embryotoxicity assay. The results showed both pharmaceuticals to significantly affected embryo development from environmentally

[49] Analgesic (NSAIDs)
[50] An antihyperlipidemic agent
[51] Analgesic (NSAIDs)
[52] An anticonvulsant used to treat epilepsy and neuropathic pain

realistic concentrations of the chemicals. The EC_{50}s of Carbamazepine and Propranolol were calculated at 0.82 and 1.34 µg/l respectively.

- Politakis *et al.* (2018) exposed *Mytilus galloprovincialis* to 25 µg/l Buscopan[53] plus and Mesulid[54] for seven days before exposing the mussels to air, to determine the observed stress on stress response. Both Buscopan and Mesulid exposed mussels had significantly reduced LT_{50} values (3–4 days) compared to the controls (5-6 days).
- Yang *et al.* (2011) investigated the effects of neurotransmitter blockers (Amitriptyline, Atenolol, Butoxamine, Chlorpromazine, Idazoxan, and Rauwolscine) and Tetraethylammonium (TEA) on larval metamorphosis in *Mytilus galloprovincialis*. Mortality only occurred in the TAE and Rauwolscine treatments during the 96-hour study period, with low mortality of <5%. Larval metamorphosis was not inhibited by 10^{-3} M TEA or at any of the tested concentrations of Rauwolscine, Atenolol, and Butoxamine. However, Chlorpromazine and Amitriptyline inhibited larval metamorphosis. The metamorphosis of the larvae was inhibited by 50% at 1.6 x 10^{-6} M Chlorpromazine and 6.6 x 10^{-5} M Amitriptyline. Idazoxin also inhibited metamorphosis with an IC_{50} of 4.4 x 10^{-3} M.

6.3.8 Phthalates

Only one of the selected articles examined the physiological effects of phthalates on *Mytilus* spp. Sif *et al.* (2016) exposed *Mytilus galloprovincialis* to two treatment exposures of potassium hydrogen phthalate (KHP) at 250 mg and 500 mg/kg of mussel for the first treatment period for 21 days before exposing the same mussels to 750 mg and 1000 mg/kg for the second treatment period for 21 days. Mortality rates significantly increased from 0-7% to 10-21% during the second exposure period. Exposure to KHP had negative effects on the growth of the mussels with more significant effects for the larger mussels. Significant differences were also observed between the control and exposed mussels condition index.

6.3.9 Polychlorinated biphenyls (PCBs)

A total of three articles examined the effects of polychlorinated biphenyls on *Mytilus* spp.

- Eertman *et al.* (1993) investigated the effects of 1 µg/l PCBs (technical mixture Clophen A50, Bayer, Leverkusen) on the survival of *Mytilus edulis* in air. The survival time of PCB exposed mussels was significantly less than the controls. However, there was no difference in the survival time between mussels exposed to PCBs for three or four weeks.
- Eertman *et al.* (1996) exposed *Mytilus edulis* adults to 0.284 µg/l PCB 126 for a period of seven weeks before performing aerial exposure experiments to determine LT_{50} values for their survival in air. Mortalities were low (<5%) during the seven weeks exposure period. However, 50% mortality (LT_{50}) occurred within 5.2 days during the aerial exposure trial, which was significantly lower compared to the control.
- Roberts (1975) investigated the effects of PCBs (Aroclor 1254, Aroclor 1242) and pesticides (Carbaryl, Endosulfan, and Trichlorphon) on byssus formation and attachment in *Mytilus edulis*. The results showed several of the tested contaminants to cause reductions in byssal attachment.

[53] An antispasmodic
[54] Analgesic (NSAIDs)

6.3.10 Synthetics (other)

Organohalogens

- Cui *et al.* (2021) found that 10 µg/l of polyfluoroalkyl phosphate diester did not affect the growth or mortality of *Mytilus galloprovincialis* during a 72-hour exposure.
- Adema & Vink (1981) investigated the toxicity of 1,1,2-trichloroethane on the survival of *Mytilus edulis*. The established LC_{50} values for 1,1,2-trichloroethane at 4, 7 and 14 days of exposure were 110, 80, and 65 µg/l, respectively.

Fatty alcohol

Granmo & Jorgensen (1975) investigated the effects of long-term exposure to a non-ionic surfactant (Tallow alcohol decaethyleneglycolether) on the fertilization and development of *Mytilus edulis*. Spawning ability was not affected after five months exposure to 500, 100 and 1500 µg/l. However, fertilization was reduced compared to the control and larvae development was inhibited or delayed. Gametes from exposed parents were more sensitive to surfactant exposure than those from the control. Larvae from contaminant exposure parents had increased mortality compared to larvae from unexposed parents. Larvae from pre-exposed parents had 100% mortality at 1 mg/l surfactant, but at the same concentration larvae from unexposed parents had high survival and showed normal development.

Alcohol

Helmstetter *et al.* (1996) investigated the toxicity of 1 to 10% methanol on *Mytilus edulis.* The mortality and behaviour of the mussels was not affected at the lowest concentration of 7,950 mg/l (1% methanol). However, at concentrations of 15,900 mg/l and above the survival and behaviour of the mussels were significantly influenced. An LC_{50} of 15,900 mg/l, equivalent to 2% methanol was determined. At the two highest tested concentrations of 5 and 10% methanol (39,750 & 79,500 mg/l) 100% mortality occurred within 13.5 hours.

6.3.11 Surfactants

Hansen *et al.* (1997) investigated the physiological effects of the detergent linear alkylbenzene sulphonate (LAS) on blue mussel larvae in laboratory and mesocosm experiments. *Mytilus edulis* larvae were exposed to concentrations between 0 to 39 mg/l LAS. In the laboratory experiments, the larvae showed 50% mortality at 3.8 mg/L LAS after a 96-hour exposure. In addition, swimming speed and helix track diameter (swimming characteristics) were decreased with LAS exposure, with significant affects at 0.8 mg/l LAS. The grazing rate of the larvae was strongly influenced by LAS exposure, showing an EC_{50} of 1.4 mg/l. The growth rate of the larvae was significantly affected by LAS exposure and showed decreased growth in response to increased concentrations. A statistically significant reduction in growth was observed at 6.5 mg/l LAS. The growth rate of the larvae reduced to half at 0.82 mg/l LAS over nine days. During the mesocosm experiment, the larval population decreased in abundance within two days at concentrations as low as 0.08 mg LAS/L, because of significant mortality, but also due to settling. The settling success rate was reduced at the same LAS concentration 0.08mg/l as that at which mortality was observed to increase significantly. Also, the larvae showed delayed metamorphosis and reduced shell growth from LAS exposure.

Eisler *et al. (*1972) investigated the effects of sodium nitrilotriacetic acid (a chelating agent) on the survival of *Mytilus edulis.* They determined 24, 96, 168-hour LT_{50}s of >10,000, 6,100 and 3,400 mg/l respectively.

6.3.12 Pesticides, Pharmaceuticals, and Personal Care Products (PCPs)

Fabrello *et al.* (2021) investigated the toxicity of a mixture of glyphosate (an herbicide), 17a-ethinylestradiol (a synthetic estrogen), and amyl salicylate (a fragrance) on *Mytilus galloprovincialis*. Mussels were exposed for seven days to two realistic concentrations of the mixture (0.01 and 0.1 µg/l) before survival in air tests were performed. The results showed no significant differences in the survival time in air between the controls and the two tested concentrations.

6.3.13 Sensitivity assessment – Synthetic compounds

The number of articles that reported mortalities due to synthetic compounds in *Mytilus* spp. are summarized in Figure 6.10 and in Table 6.7 and Table 6.8 below. Relevant resistance ranks and resultant sensitivities are shown in Table 6.7 and Table 6.8 based on the weight of evidence and 'worst-case' approach outlined above.

In general, the evidence suggested that longer exposure times were required to understand the effects of exposure to synthetic contaminants on *Mytilus*, as mussels could close their shells for days. Hence, short-term exposures (e.g. <48hrs) may underestimate sensitivity. This agrees with Widdows & Donkin (1992) who suggested that LC_{50} values in *Mytilus* gave a false impression of high tolerance because adult bivalves were able to close their valves and isolate themselves from extreme (potentially lethal) conditions for long periods (i.e. days).

The majority of articles reported a lethal response of exposure to synthetic compounds in *Mytilus* spp. A total of 57% of ranked mortalities reported in the evidence review were lethal ('Severe', 'Significant' or 'Some'), while 27% reported no mortality ('None') and 16% reported sub-lethal effects.

The majority of the articles examined pesticides/biocides and pharmaceuticals (Figure 6.10). A total of 15 (56%) of the 27 articles that examined pesticides reported lethal effects. The majority of the evidence suggested that pesticides resulted in lethal effects in adults and juvenile *Mytilus* spp. but that larval and embryos were probably more sensitive. Therefore, we can suggest that *Mytilus* spp. probably has a **'High'** sensitivity to pesticide exposure, with a few exceptions. The confidence in the assessment is assessed as **'Medium'** because of the number of articles examined and the consistency in the response.

However, 19 (70%) of the articles that examined pharmaceuticals reported lethal effects (Figure 6.12). The most lethal responses were shown by the larvae and embryos rather than adults and juveniles. Therefore, we can suggest that *Mytilus* spp. probably has a **'High'** sensitivity to the pharmaceuticals examined especially in the larvae and developmental stages. The confidence in the assessment is assessed as **'Medium'** because of the number of articles examined and the consistency in the response.

The evidence on other synthetic contaminant types is more limited. The flame retardant Tetrabromo bisphenol A (TBBPA) caused mortality and abnormal development in larvae (Fabbri *et al.*, 2014) while another two flame retardants had no significant effects on adults (Barón *et al.*, 2016). Different types of surfactant caused lethal responses in larvae, embryos and in adults. PFAS exposure caused mortality in larvae and embryos but no studies on the effects on adults were found.

Nevertheless, the results shown in Table 6.7 and Table 6.8 suggest that *Mytilus* spp. is probably sensitive to a number of synthetic compounds, especially in early development or as larvae. Therefore, the sensitivity of *Mytilus* spp. to the 'Synthetic compounds' examined is assessed as **'High'** (resistance is **'None'** and resilience is **'Low'**) especially in larvae and developmental stages. Overall, the confidence in the assessment is probably **'Medium'** because of the number of articles examined and the consistency in the response.

Table 6.7. Summary of count of ranked mortalities reported in evidence review of the effects of synthetic compounds on *Mytilus* spp. and resultant proposed sensitivity assessments in **adults and juveniles only**. (N= None, L= low, M= Medium, H =High, NS= Not sensitive).

		Mortality (worst case reported)							Assessment		
Group	**Contaminant**	**Severe**	**Significant**	**Some**	**None**	**Sublethal**	**NR**	**Total**	**Resistance**	**Resilience**	**Sensitivity**
Flame retardants											
	Organohalogen					2		2	**H**	**H**	**NS**[55]
Mixtures											
	Dispersants		**4**		3			7	L	M	M
	Pesticide/Biocides, Pharmaceuticals, PPCPs						1	1	H	H	**NS**
Mixtures (total)			**4**		**3**		**1**	**8**	**L**	**M**	**M**
Personal Care Product Chemicals (PPCPs)											
	Ultraviolet (UV) filter				**4**			4	**H**	**H**	**NS**
Pesticide/Biocide											
	Carbamate	**1**	1			2		4	**N**	L	H
	Organochloride	**1**	1					2	**N**	L	H
	Organofluoride		**1**					1	L	M	M
	Organohalogen	**2**	4		4	6		15	**N**	L	H
	Organophosphate	**2**	2			4		8	**N**	L	H
	Organophosphorus	**2**	3		1		2	8	**N**	L	H
	Other				**1**			1	H	H	**NS**
	Pyrethroid					2		2	H	H	**NS**
Pesticide/Biocide (total)		**8**	**12**		**6**	**14**	**2**	**42**	**N**	**L**	**H**
Pharmaceuticals											
	Analgesic (NSAID)			**2**	7	4		13	M	M	M
	Anticonvulsant			**2**	1			3	M	M	M
	Antidepressant				2	1		3	H	H	**NS**
	Antihyperlipidemic					2		2	H	H	**NS**
	Antispasmodic					1	1	2	H	H	**NS**
	Benzodiazepines				2			2	H	H	**NS**
	Beta-blocker		**1**	1		1		3	L	M	M
	Chemotherapeutic		**1**		1			2	L	M	M
Pharmaceuticals (total)			**2**	**5**	**13**	**9**	**1**	**30**	**L**	**M**	**M**
Phthalates											
	Phthalates			1				1	M	M	M[56]
Polychlorinated biphenyls (PCBs)											
	PCBs		**1**			2	1	4	**L**	**M**	**M**
Synthetics (other)											
	Alcohol	**1**						1	**N**	L	H
	Surfactant	**1**						1	**N**	L	H
	Organohalogen		**1**		1			2	L	M	M
Synthetics (other) (total)		**2**	**1**		**1**			**4**	**N**	**L**	**H**
Grand Total		**10**	**20**	**6**	**27**	**27**	**5**	**95**	**N**	**L**	**H**

[55] Based on one article
[56] Based on one article

Table 6.8. Summary of count of ranked mortalities reported in evidence review of the effects of synthetic compounds on *Mytilus* spp. and resultant proposed sensitivity assessments **in embryos and larvae only**. (N= None, L= low, M= Medium, H =High, NS= Not sensitive).

		Mortality (worst case reported)							Assessment		
Group	**Contaminant**	**Severe**	**Significant**	**Some**	**None**	**Sublethal**	**NR**	**Total**	**Resistance**	**Resilience**	**Sensitivity**
Flame retardants											
	Organohalogen		**1**					1	**L**	**M**	**M**[57]
Hydrocarbons (Petrochemical)											
	Phenols		**1**					1	**L**	**M**	**M**
Perfluoroalkyl substances (PFAS)											
	Perfluorooctanesulfonic acid (PFOS)	**1**	1					2	**N**	L	H
	Perfluorooctanoic acid (PFOA)	**1**	1					2	**N**	L	H
Perfluoroalkyl substances (PFAS) (total)		**2**	**2**					**4**	**N**	**L**	**H**
Personal Care Product Chemicals (PPCPs)											
	Ultraviolet (UV) filter		**5**				1	6	L	M	M
Pesticide/Biocide											
	Antifoulants		**4**					4	L	M	M
	Carbamate	**1**	7	1				8	**N**	L	H
	Naphthalene		**1**					1	L	M	M
	Organochloride	**1**	1	2				4	**N**	L	H
	Organofluoride		**1**	1				2	L	M	M
	Organohalogen		**2**					2	L	M	M
	Organophosphate	**1**	1	1				3	**N**	L	H
	Other		**1**					1	L	M	M
Pesticide/Biocide (total)		**3**	**17**	**5**				**25**	**N**	**L**	**H**
Pharmaceutical											
	Adrenergic agonist			**2**	12			14	M	M	M
	Analgesic (NSAID)		**2**					2	L	M	M
	Anti-inflammatory	**1**	1					2	**N**	L	H
	Anticonvulsant		**1**					1	L	M	M
	Antidepressant		**1**		1			2	L	M	M
	Antihyperlipidemic		**1**	1				2	L	M	M
	Antihypertensive		**1**					1	L	M	M
	Beta-blocker		**1**	1	2			4	L	M	M
	Chemotherapeutic	**2**	1					3	**N**	L	H
	Hormones		**1**					1	L	M	M
	Parasiticide[58]	**1**						1	**N**	L	H
Pharmaceutical (Total)		**4**	**10**	**4**	**15**			**33**	**N**	**L**	**H**
Synthetics (other)											
	Surfactant		**1**					1	L	M	M
Grand Total		**9**	**37**	**9**	**15**		**1**	**71**	**N**	**L**	**H**

[57] Based on one article
[58] An anti-malarial drug

7 Seagrasses – 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 7.1 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 7.1). 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.

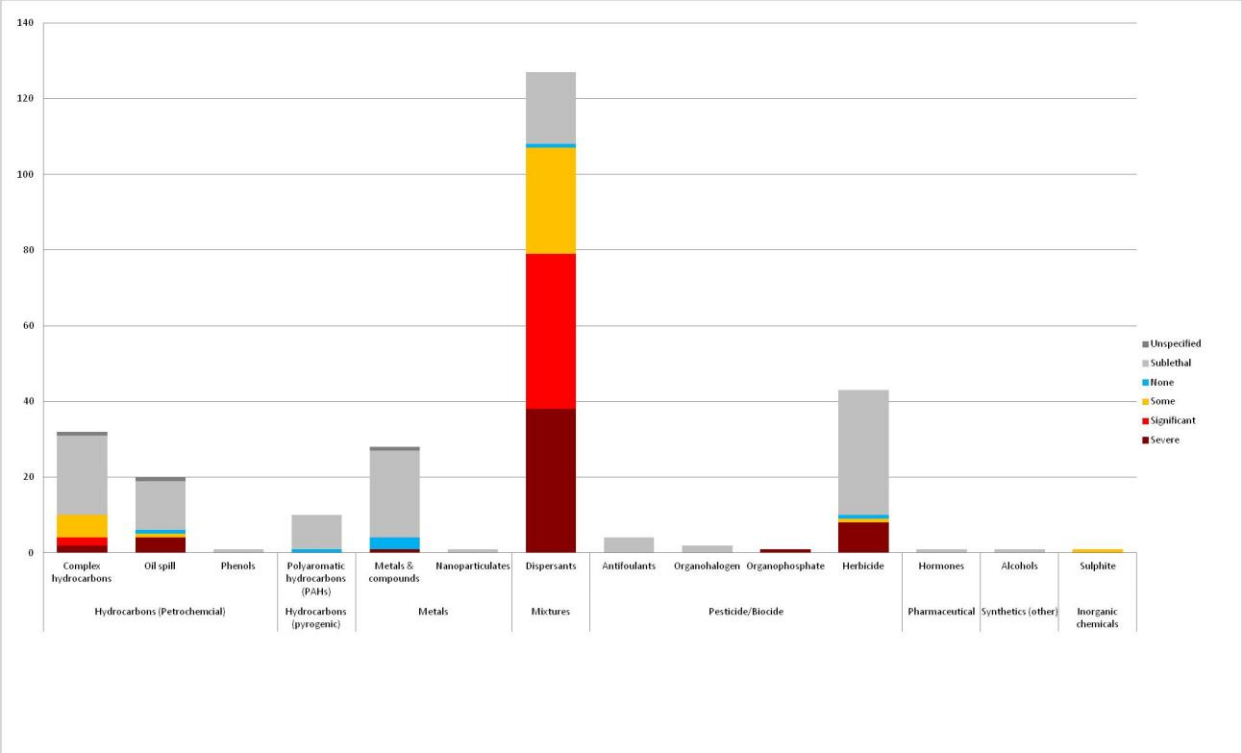

Figure 7.1. Count of ranked mortalities due to exposure to contaminants in seagrasses. Mortality is ranked as follows: 'Severe' (>75%), 'Significant' (25-75%), 'Some' (<25%), 'None' (no mortality reported), and 'Sublethal' effects. Note some articles are included more than once because they examined 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 7.2 and Figure 7.3). 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 7.1. 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	

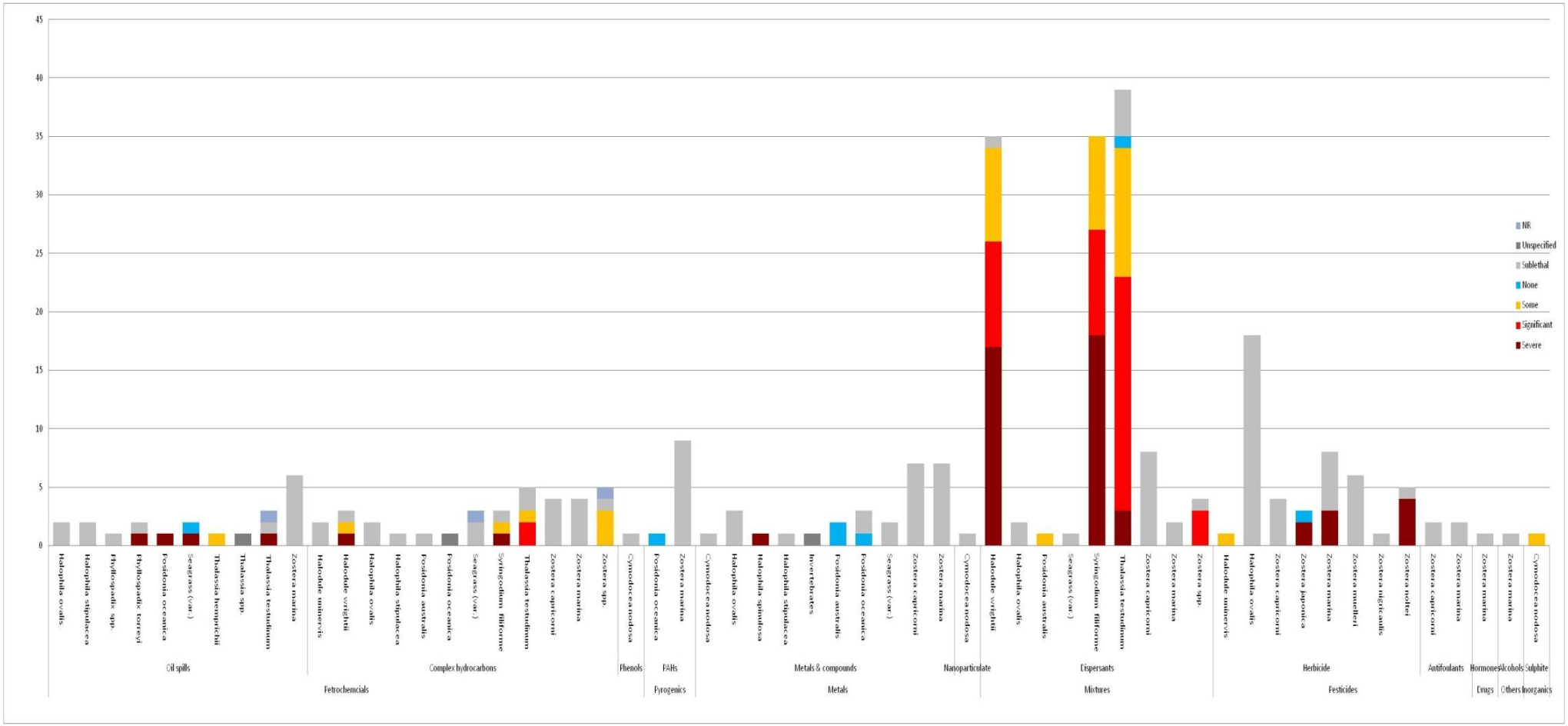

Figure 7.2. Count of ranked mortalities due to exposure to contaminants in seagrass species. Mortality is ranked as follows: 'Severe' (>75%), 'Significant' (25-75%), 'Some' (<25%), 'None' (no mortality reported), and 'Sublethal' effects. Note some articles are included more than once because they examined several different combinations of contaminant type and seagrass species.

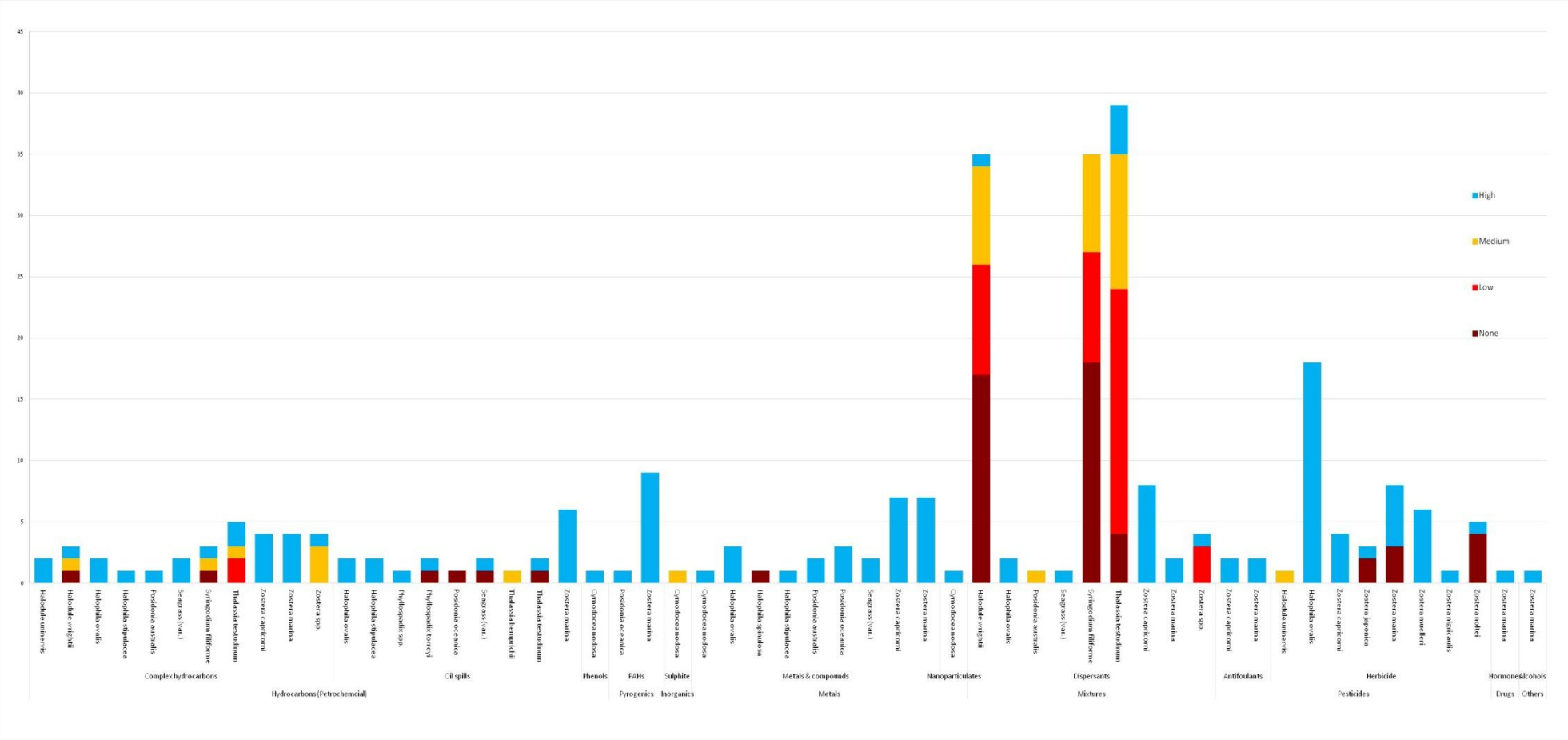

Figure 7.3. Count of ranked mortalities due to exposure to contaminants in seagrass species. Mortality is ranked by resistance as follows: 'None' (>75%), 'Low' (25-75%), 'Medium' (<25%), and 'High' (no mortality or only sublethal effects reported). Note some articles are included more than once because they examined several different combinations of contaminant type and seagrass species.

7.1 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 7.4) 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.

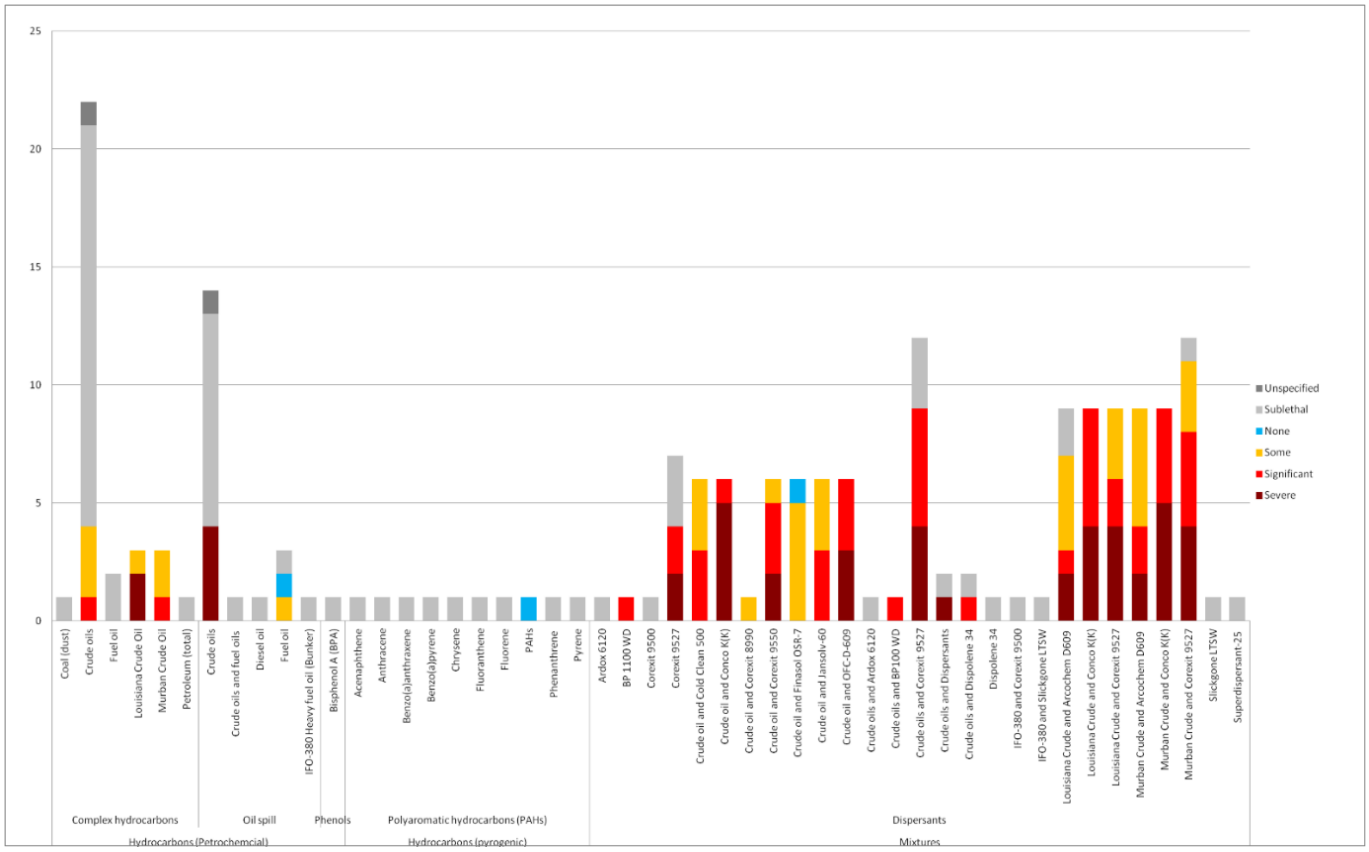

Figure 7.4. Count of ranked mortalities in seagrasses (across all species examined) due to exposure to hydrocarbons, dispersants, and dispersant/oil mixtures. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects. Note some 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 7.5 and Figure 7.6).

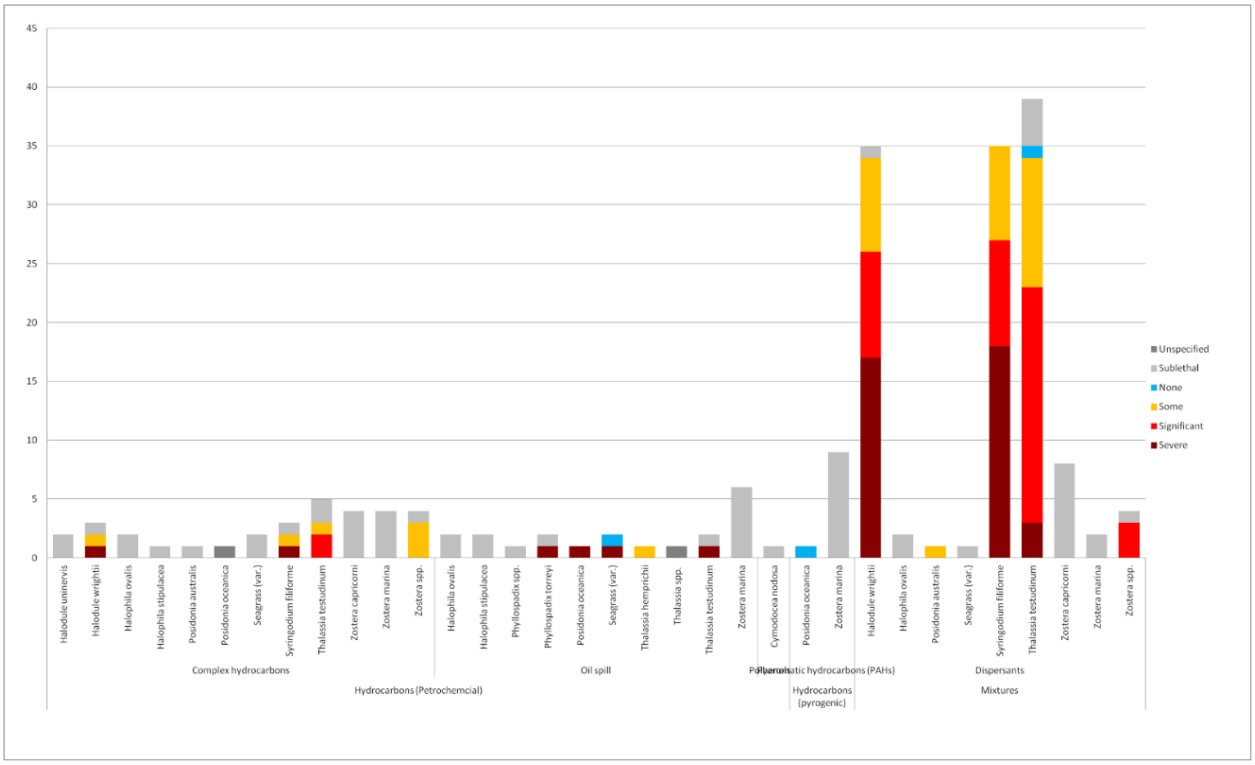

Figure 7.5. Count of ranked mortalities due to exposure to hydrocarbons in seagrass species. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects. Note some articles are included more than once because they examined several different combinations of contaminant type and seagrass species.

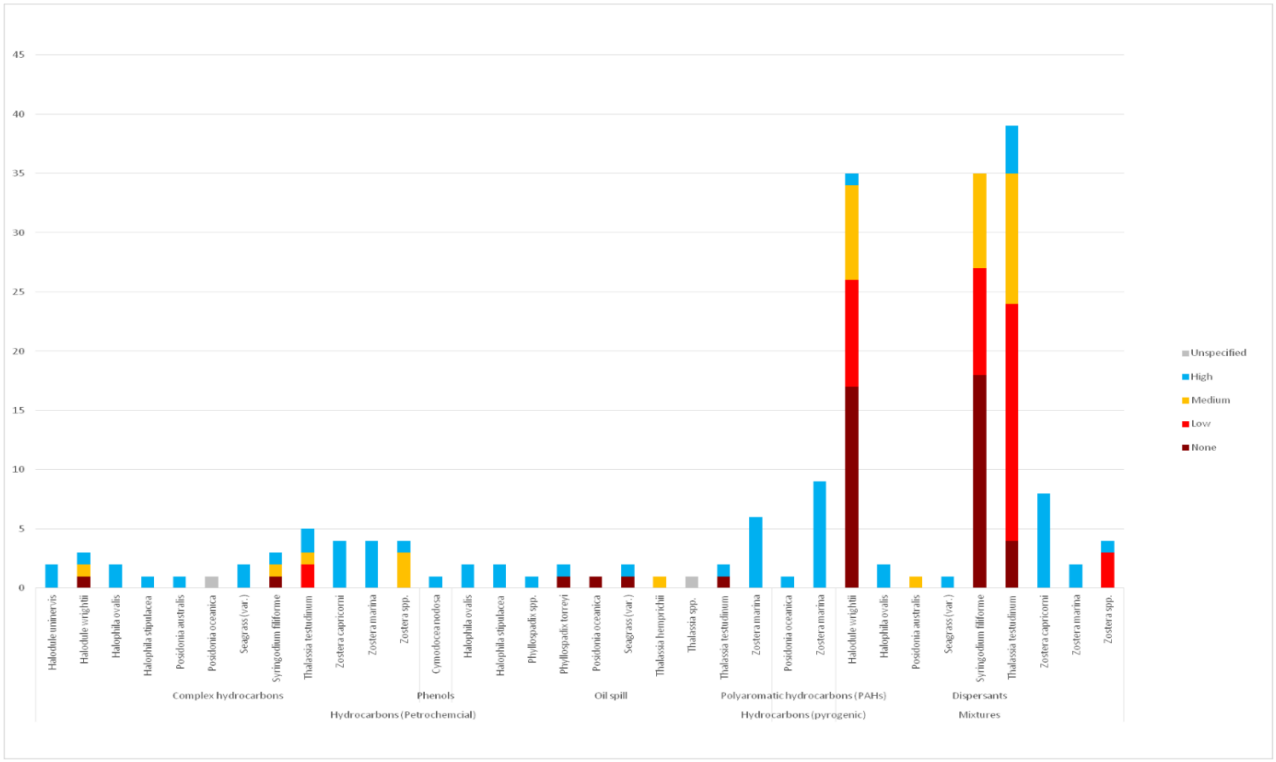

Figure 7.6. Count of ranked mortalities (expressed as resistance) due to exposure to hydrocarbons in seagrass species. Resistance is ranked as follows: 'None' (>75%), 'Low' (25-75%), 'Medium' (<25%), 'High' (no mortality and/or only sublethal effects reported). Note some articles are included more than 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*.

7.1.1 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.

7.1.2 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*[59] 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% after 18 months. 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

[59] *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.*

oil spills on the 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.

7.1.3 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).

7.1.4 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.

7.1.5 Sensitivity assessment (Hydrocarbons and PAHs)

The number of articles that report mortalities due to Hydrocarbons and PAHs' are summarized in Figure 7.4 and in Table 7.2 below.

Sensitivity assessment – Oil spills

The effects of the oil spills on seagrass meadows were inconsistent and variation was reported 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 results of oil spills 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 spilt 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). Jacobs (1980) noted a larger algal bloom than in previous years after the *Amoco Cadiz* spill in Roscoff, probably as a result of increased nutrients (from dead organisms and breakdown of oil) and the reduction of algal grazers. However, herbivores recolonized and the situation returned to 'normal' within a few months.

Table 7.2. 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).

			Worst case mortality						Assessment		
Group	Type	Species name	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience[60]	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**[61]
	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

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

Group	Type	Species name	Worst case mortality						Assessment		
			Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience[60]	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 the reported effects on *Zostera marina* after exposure to spilled crude oil and fuel oil were sublethal. However, the impact on 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 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. were exposed to crude oil in a field experiment (Howard *et al.*, 1989). However, these *Zostera* spp. were most likely the intertidal species *Zostera noltei* or the shallow extent of *Zostera marina* (as syn. *Zostera angustifolia*), which were more likely to have been into direct contact with the oil, and to experience 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 7.2) 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' mortality 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 in cover within the first week that resulted in a decrease in cover from 55% to 15% after 18 months (Howard *et al.,* 1989).

Technically, the worst-case sensitivity of seagrass, as a group, would be assessed as **'High'** (Table 7.2) 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 (Faganeli *et al.*, 1997; Mauro *et al.,* 2013). In these papers, environmental exposure to 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 is assessed as '**Not sensitive**'.

7.2 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 $N\text{-}NO^{3-}$ to 2 µM $P\text{-}PO_4^{3-}$) 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).

7.2.1 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.

7.2.2 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.

7.2.3 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 7.1 and in Table 7.3 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 7.3. 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**[62]	**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**[63]
		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.

[62] Resilience for *Zostera* spp. is assumed to be the same as the biotopes Zmar or Znol or unknown for other seagrasses.
[63] 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'**.

7.3 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 7.7).

7.3.1 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.

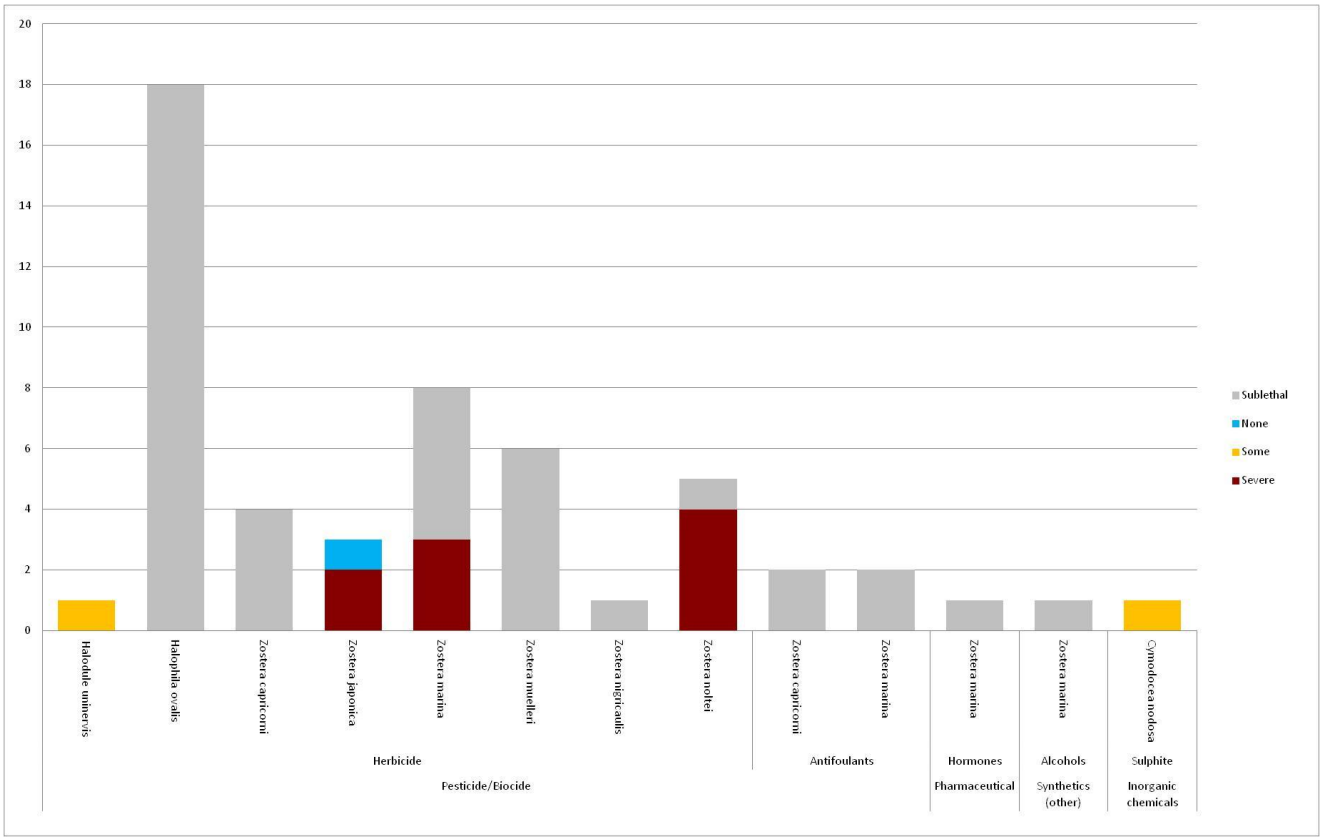

Figure 7.7. Count of ranked mortalities due to exposure to 'synthetic compounds' in seagrass species. Mortality is ranked as follows: 'Severe' (>75%), 'Significant' (25-75%), 'Some' (<25%), 'None' (no mortality reported), and 'Sublethal' effects. Note some articles are included more than once because 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.

7.3.2 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.

7.3.3 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.

7.3.4 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 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.

7.3.5 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 7.7 and in Table 7.4 below.

Table 7.4. 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[64]	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

[64] 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 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'**.

8 Discussion

The report outlines the process behind the development of an approach to assess the resistance (and hence sensitivity) of marine species and habitats to the 'contaminants' pressures, and its subsequent application to a number of test species indicative of the sensitivity of marine habitats. The species chosen were *Mytilus* spp. for blue mussel beds' and *Zostera* spp. for seagrass beds.

8.1 Literature review

The scale of the literature review is potentially huge and, therefore, time consuming, especially for 'sentinel' species and well studied groups. Collins *et al.* (2015) suggest that one REA could take 5-8 months and cost £20-50K but that assumes a tightly focused REA with a one 'review question'. However, the scope of the contaminants project is much larger as we plan to examine the effects of multiple types of contaminants on many species in many habitats.

1. At present, the scope includes ca 600 different chemicals across four pressures and ca 20+ groups of contaminants and ca 80+ different species indicative of the sensitivity of benthic marine habitats (ca 390+ biotopes) from all the major groups (Order, Class, Phylum) of marine benthic invertebrates, macroalgae, and flowering plants.
2. The literature search strategy was effective and returned numerous 'hits'. We are reasonably confident that we found most of the literature relevant to the effects of contaminants of the species examined. However, several articles were not accessible in the time available, and only articles available in English (inc. those translated into English) were examined.
3. In Phase 2, the initial screening (stage 1) took one person ca 8 days to complete, and stage 2 screening took two people ca 2 weeks to complete and the evidence summary took two persons ca one month to complete not including writing up the sensitivity assessment. The REA protocol was simplified and detailed record of the Stage 2 screening removed to save time based on our experience in Phase2.
4. The review was further focused and evidence on bioindicators and bioaccumulation excluded. The majority of papers on bioaccumulation focused on the use of the relevant species as a bioindicator and little, if any, information on the effect of the accumulated chemicals on the species was given.
5. Nevertheless, Phase 3 took another five months of staff time, between two staff, to complete for both the remaining pressures for *Mytilus* and reviews the effects of contaminants on seagrasses. The time constraints rest with the number of articles that need to be obtained, checked, evidence recorded and summarized.
6. Both *Mytilus* spp. and *Zostera* spp. were well studied examples. In *Mytilus* spp., the search strategy and inclusion/exclusion criteria were applied strictly to minimize the number of papers reviewed. In *Zostera* spp., other seagrass species were included to ensure a good coverage of contaminant types.
7. Phase 2 demonstrated that the search strategy would need to be modified to depending on the species or species group of interest. For example, little information was found on 'sea pens' in the initial search and the search would need to be expanded to Anthozoa or Cnidaria. Similarly, the search for *Neopentadactlya* and *Tubularia* would need to include Echinodermata and Hydrozoa respectively.
8. However, it remains difficult to predict how many of the articles returned by the search strings (Appendix 2) will be directly relevant to the 'review question' until the screening exercise is completed.

Recommendations

9. The literature search strategy needs to be tailored to the taxonomic group or species of interest.
10. Commercial and other well studied species should be researched separately. However, it may also be practical and cost/time effective to research entire taxonomic groups, for example, sea urchins, crinoids, isopods, amphipods, crabs, oysters, scallops, Anthozoa, and Hydrozoa (as suggested by section 3.8). However, it would not be possible to assess sensitivity until the group was completed.
11. Alternatively, the literature search (and hence evidence review) could focus on species specific to a priority list of habitats, for example, biogenic habitats (e.g. horse mussel beds, blue mussel beds, flame shell beds, serpulid reefs, *Leptometra* aggregations, seagrass etc.). This approach would allow sensitivity assessment to be made on a piecemeal basis but risk missing evidence from other taxonomic groups that may modify the assessments.
12. Alternatively, the literature search (and hence evidence review) could focus on one or two dominant taxonomic groups within priority habitats, e.g. bivalves or polychaetes that dominate many sedimentary habitats.

8.2 The Rapid Evidence Assessment (REA) protocol and its application

The REA approach is more transparent and systematic but requires more staff time to complete that the current MarESA sensitivity assessments. The inclusion/exclusion criteria were focused on the information required to assess 'review question', 'resistance' and, hence, sensitivity.

13. The REA approach provided a transparent documentation of the literature review process and allowed us to provide a detailed overview of the evidence extracted and, potentially, to compare studies. However, the detailed audit trail is a time-constraint (as above).
14. The evidence summaries (spreadsheets) record information on the study types, experimental approach, exposure concentrations, and resultant 'end points', where available. The summaries also include a narrative to capture more information and any relevant conclusions. The narrative also captures information from field observations, the effects of incidental spills and from review articles. Lastly, the evidence summaries record the 'ranked mortalities' and 'worst-case ranked mortalities' used in resistance assessment.
15. There is considerable variation in experimental design between the studies, so that it is difficult to compare the 'end points' (for example, EC/LC_{50}s) for any one chemical between or within the species examined. Therefore, it is difficult to 'rank' the toxicity of any chemical in the species examined. However, the detail recorded on toxicology may be useful to compare with pollution incidents or proposed effluent releases.
16. Surprisingly few articles examined the effect of the contaminant in the field at the population level. For example, numerous articles investigated the effects of herbicides on seagrasses and implied that reduced photosynthesis and growth could impair their survival in the field but did not examine population effects further. Similarly, in *Mytilus* spp., many articles described sublethal effects, without any evidence of effects on the population.
17. A meta-analysis or statistical analysis (e.g. SSD) of the evidence gathered to date has not been attempted. This would require further work.

Recommendations

18. We suggest that a meta-analysis could be an important addition, and allow us to 'rank' species and taxonomic groups using statistical techniques if the evidence allows, for example SSDs. However, such ranking would not be possible until the evidence review was completed for the majority of

biotopes so that biotopes could not be assessed piecemeal, i.e. in small groups but would need to be assessed together at the end of the review

19. Nevertheless, the detailed meta-data on the evidence reviewed may be powerful addition to the process and allow subsequent meta-analysis and a more defensible ranking of relative sensitivity of marine benthic species to chemical contamination.

20. We also suggest that the 'evidence summaries' could be provided as an additional online dataset on the effects of contaminants on marine species and habitats.

8.3 Sensitivity assessment

21. Phase 1 concluded that it was difficult to see how a quantified value or scenario would function as a quantified benchmark for sensitivity assessment. The mechanisms whereby any individual species is exposed to any individual chemical is complex, and varies depending on the behaviour of chemicals in the environment, their mode of action and toxicity, as well as the nature of the receiving environment as explained above.

22. The 'weight of evidence' approach was used to assess sensitivity of *Mytilus* spp. and seagrasses to the contaminant pressures. Several assessments are given, for individual containment groups, for example, oil spills, oils, PAHs, transitional metals, organometals, pesticides/biocides, or pharmaceuticals. An overall assessment is also provided for each pressure.

23. The resistance assessments are based on the 'worst-case' 'ranked mortality' for each chemical examined in each article reviewed. That is, if exposure to hydrocarbons was reported to result in 'severe' mortality then resistance is assessed as 'None', if 'significant' mortality was reported then resistance is assessed as 'Low' and so on using the standard MarESA 'resistance' scale.

24. It should be noted that an LC_{50} is equivalent to a report of 'significant' mortality. In a few studies, it might be possible to extract and NR-ZERO or NR-LETH (100% mortality) from the graphs presented but this is time-consuming and possibly inaccurate (or at least imprecise).

25. In undertaking the resistance assessment priority was given to studies that reported mortality (or survival), or reproductive or physiological effects (e.g. SFG, condition indices) that could result in population level effects. Studies that only focused on sub-lethal effects were not discussed.

26. The summary narratives for each article and the resultant resistance/sensitivity assessments are in standard MarESA style, that is, a summary of the evidence used and summary sensitivity assessments. However, the content varies depending on the number of articles that needed to be reviewed. For example, the narratives used in the *Mytilus* 'transitional metals' section are short because on the large number of studies examined.

27. Reporting of the 'worst-case' sensitivity may exaggerate species (and hence habitat) sensitivity without context. This is because acute toxicity may result from exposure to high concentrations of a contaminant in a laboratory that may itself not represent concentrations likely to occur in the environment, although spills may be an exception. However, reporting the 'worst-case' scenario probably remains the most 'transparent' and 'non-biased' approach.

28. The summary tables of resistance and sensitivity provided in each of the evidence reviews demonstrate the variation in potential sensitivity between different types of contaminant and/or species. For example, *Zostera* spp. appeared to be amongst the least sensitive species of seagrass to the effects of oil spills and oils, in the absence of dispersants. Similarly, the larval and developmental stages of *Mytilus* spp. were, in general, more sensitive to contaminants than adults and juveniles.

29. The summary tables also suggest that a single overall sensitivity assessment for any single contaminant pressure may obscure the range of sensitivities revealed in the evidence review.
30. Each sensitivity assessment also includes a confidence score but it is based on expert judgment rather than the standard MarESA approach. Each article included in the evidence review is scored on its 'Quality' and 'Applicability' to the study in the evidence summaries. The majority of articles were of 'High' and the remainder of 'Medium' quality. Similarly, all articles included in the study were directly applicable (High or Medium applicability) as any other studies were excluded at the Screening stage. Therefore, the overall confidence in the final assessments was based on 'expert judgement', itself based on the number of articles that agreed on the effects observed or reported in the evidence review.

Recommendations

31. The content of the summary narrative should be standardised.
32. The 'worst-case' ranked mortality is a transparent approach, to identify the potential effect of a pressure on a marine habitat or species. However, we should ensure that range of sensitivities exhibited by different species to different chemicals is also presented.
33. A standardised method to score the confidence in the overall sensitivity assessment requires further work.

HTW, EW, MJM, KAL 2022-06-07

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Appendix 1**.** List of 'species indicative of sensitivity' used as the focus of sensitivity assessment of littoral and sublittoral biotopes, excluding the deep-sea.

Phylum	Taxon group	Common term/species names
Porifera		**Sponges**
		Amphilectus (Esperiopsis) fucorum
		Chelonaplysilla noevus
		Ciocalypta penicillus
		Clathrina coriacea
		Cliona celata
		Dercitus bucklandi
		Dysidea fragilis
		Halichondria bowerbanki
		Halichondria panacea
		Haliclona oculata
		Hemimycale columella
		Hymeniacidon perlevis (syn. perleve),
		Leucosolenia spp.
		Pachymastia fucorum
		Pachymatisma johnstonia
		Phakellia ventilabrum
		Polymastia boletiformis
		Polymastia mamillaris
		Pseudosuberites sp.
		Raspailia ramosa
		Scypha ciliata
		Spongosorites sp.
		Stelligera rigida
		Stelligera stuposa
		Stryphnus ponderosus
		Suberites ficus
		Suberites spp.
		Tethya aurantium
Cnidaria		
	Hydrozoa	**Hydroids, white weeds, sea firs**
		Cordylophora caspia
		Eudendrium arbusculum
		Halecium halecinum
		Hartlaubella gelatinosa
		Hydrallmania falcata
		Nemertesia antennina
		Nemertesia ramosa
		Obelia dichotoma
		Obelia geniculata
		Obelia longissima
		Plumularia setacea

Phylum	Taxon group	Common term/species names
		Sertularia argentea
		Sertularia cupressina
		Sertularia cupressina
		Thuiaria thuja
		Tubularia indivisa
	Anthozoa	**Anemones, sea fans, sea pens, corals**
		Octocorals
		Cup corals
		Cold-water corals
		Actinothoe sphyrodeta
		Alcyonium coralloides
		Alcyonium digitatum
		Alcyonium glomeratum
		Caryophyllia inornatus
		Caryophyllia smithii
		Cerianthus lloydii
		Corynactis virdis
		Edwardsia timida
		Eunicella verrucosa
		Funiculina quadrangularis
		Halcampa chrysanthellum
		Hoplangia durotrix
		Leptopsammia pruvoti,
		Metridium dianthus (syn. senile)
		Pachycerianthus multiplicatus
		Parazoanthus spp.
		Pennatula phosphore
		Protanthea simplex
		Sagartia elegans
		Sagartiogeton undatus
		Swiftia pallida
		Urticina felina
		Virgularia mirabilis
Mollusca		
	Gastropoda	**Gastropods, snails, slugs, limpets**
		Barnea candida
		Crepidula fornicata
		Gibbula cineraria
		Hiatella arctica
		Hydrobia ulvae
		Littorina spp.
		Littorina littorea
		Littorina saxatilis
		Nucella lapillus

Phylum	Taxon group	Common term/species names
		Patella ulyssiponensis
		Patella vulgata
		Philine quadripartite (syn. asperta)
	Bivalvia	**Bivalves, mussels, clams, scallops**
		Venerids
		Abra alba
		Abra nitida
		Abra prismatica
		Abra spp.
		Astropecten irregularis
		Cerastoderma edule
		Cerastoderma glaucum
		Chamelea gallina
		Dosinia lupinus
		Ennucula tenuis (syn. Nuculoma tenuis)
		Ensis spp.
		Fabulina fabula
		Glycymeris glycymeri
		Goodallia triangularis
		Kurtiella bidentata (syn. Mysella bidentata)
		Limaria hians
		Limecola (syn. Macoma) balthica
		Macomangulus tenuis
		Modiolus modiolus
		Moerella (now Tellina) spp.
		Musculus discors
		Mya arenaria
		Myrtea spinifera
		Mytilus edulis
		Mytilus edulis
		Ostrea edulis
		Parvicardium ovale
		Pecten maximus
		Petricolaria pholadiformis
		Phaxas pellucidus
		Pholas dactylus
		Pseudamussium septemradiatum
		Scrobicularia plana
		Spisula elliptica
		Spisula subtruncata
		Thyasira spp.
		Timoclea ovata
		Venerupis corrugate (syn. senegalensis

Phylum	Taxon group	Common term/species names
Annelida		
	Polychaeta	**Bristleworms, ragworms, fanworms, spoon worms**
		Ampharete falcata
		Amythasides macroglossus
		Aonides paucibranchiata
		Aphelochaeta marioni
		Aphelochaeta spp.
		Arenicola marina
		Capitella capitata (agg.)
		Chaetozone setosa
		Chaetozone spp
		Cirratulus cirratus
		Cirriformia tentaculata
		Eteone longa
		Glycera lapidum
		Glycera spp.
		Hediste diversicolor
		Hesionura elongata
		Heterochaeta costata
		Heteromastus filifirmis
		Lagis koreni
		Lanice conchilega
		Laonice bahusiensis
		Levinsenia gracilis
		Lumbrineris gracilis
		Magelona mirabilis
		Magelona spp.
		Maldane sarsi
		Maldanid polychaetes
		Manayunkia aestuarina
		Maxmuelleria lankesteri
		Mediomastus fragili
		Melinna palmata
		Microphthalmus similis
		Nephtys cirrosa
		Nephtys hombergii
		Ophelia rathkei
		Ophryotrocha dubia
		Owenia fusiformis
		Paramphinome jeffreysii,
		Paranais litoralis
		Paraonis fulgens
		Poldora spp.
		Polydora ciliata
		Prionospio fallax

Phylum	Taxon group	Common term/species names
		Protodorvillea kefersteini
		Protodriloides spp.
		Protodrilus spp.
		Protomystides bidentata
		Pseudomystides limbata
		Pygospio elegans
		Sabella pavonina
		Sabellaria alveolata
		Sabellaria spinulosa
		Scolelepsis spp.
		Scoloplos armiger
		Serpula vermicularis
		Spio filicornis
		Spio spp.
		Spiophanes bombyx
		Spiophanes spp.
		Spirobranchus (syn. Pomatoceros) triqueter
		Spirorbids
		Streblospio shrubsolii
		Syllid polychaetes
		Travisia forbesii
	Oligochaeta	**Oligochaetes**
		Baltidrilus costata (formerly Heterochaeta costata
		Enchytraeidae oligochaetes
		Limnodrilus hoffmeisteri
		Tubifex tubifex
		Tubificid oligochaetes
		Tubificoides benedii
		Tubificoides spp.
Arthropoda	**Crustacea**	
	Cirripedia	**Barnacles**
		Balanus crenatus
		Balanus perforatus
		Chthalamus montagui
		Chthalamus stellatus
		Chthamalus spp.
		Semibalanus balanoides
		Verruca stroemia
	Tanaidacea	**Tanaids**
		Apseudes latreilli
	Decapoda	**Crabs, shrimps**
		Callianassa subterrranea
		Calocaris macandreae
		Nephrops norvegicus

Phylum	Taxon group	Common term/species names
	Cumacea	**Cumaceans**
		Diastylis bradyi
		Eudorellopsis deformis
		Iphinoe trispinosa
	Amphipoda	**Amphipods, sand hoppers**
		Ampelisca spinipes
		Ampelisca spp.
		Bathyporeia elegans
		Bathyporeia pilosa
		Bathyporeia spp.
		Corophium arenarium
		Corophium volutator
		Crassicorophium crassicorne (syn. Corophium crassicorne
		Echinogammarus incertae sedis planicrurus
		Gammarus salinus
		Haustorius arenarius
		Orchestia spp.
		Pontocrates arenarius
		Pontocrates spp.
		Talitrus spp.
		Urothoe brevicornis
	Isopoda	**Isopods, sea slaters, gribbles**
		Eurydice pulchra
	Mysida	**Mysids**
		Gastrosaccus spinifer
		Neomysis integer
Bryozoa		**Bryozoans, sea mats, horn wracks**
		Alcyonidium diaphanum
		Bugula spp
		Bugulina spp.
		Cellaria fistulosa
		Cellaria spp.
		Cellepora pumicosa
		Conopeum reticulum
		Crisularia plumosa
		Crisularia spp.
		Einhornia crustulenta
		Eucratea loricata
		Flustra foliacea
		Parasmittina trispinosa
		Pentapora foliacea (Syn. fascialis)
		Porella compressa
		Scrupocellaria spp.

Phylum	Taxon group	Common term/species names
		Securiflustra securifrons
Brachiopoda		**Brachipods, lamp shells**
		Novocrania (syn. Neocrania) anomala
Echinodermata		**Echinoderms**
	Ophiuroidea	**Brittlestars**
		Amphipholis squamata
		Amphiura brachiata
		Amphiura chiajei
		Amphiura filiformis
		Ophiocomina nigra
		Ophiothrix fragilis
		Ophiura albida
		Ophiura spp.
	Crinoidea	**Feather stars**
		Antedon spp.
		Leptometra celtica
	Asteroidea	**Starfish, sea stars, cushion stars**
		Asterias rubens
	Echinoidea	**Sea urchins, heart urchins**
		Brissopsis lyrifera
		Echinocardium cordatum
		Echinus esculentus
		Psammechinus miliaris
		Paracentrotus lividus
	Holothuroidea	**Sea cucumbers**
		Neopentadactyla mixta
		Ocnus planci (or Ocnus brunneus).
Chordata		**Chordates**
	Leptocardii	**Lanclets**
		Branchiostoma lanceolatum
	Ascidiacea	**Ascidians, sea-squirts**
		Ascidia conchilega
		Ascidia mentula
		Ascidiella aspersa
		Ascidiella scabra
		Ascidiella scabra
		Ascidiella spp.
		Ciona intestinalis
		Clavelina lepadiformis
		Dendrodoa grossularia
		Molgula manhattensis
		Polyclinum aurantium

Phylum	Taxon group	Common term/species names
		Styela gelatinosa
Rhodophyta		**Red seaweeds/algae**
		Ahnfeltia plicata
		Antithamnion spp.,
		Calliblepharis ciliata
		Callithamnion spp.
		Callophyllis laciniata
		Ceramium spp.
		Chondus chrispus
		Corallina officinalis
		Cryptopleura ramosa
		Delesseria sanguinea
		Encrusting corallines
		Furcellaria lumbricalis
		Gelidium pusillum
		Gracilaria gracilis
		Griffithsia devoniensis
		Hildenbrandia rubra
		Hypoglossum hypoglossoides
		Lithophyllum spp.
		Lithothamnion corallioides
		Lithothamnion glaciale
		Maerl
		Mastocarpus stellatus
		Membranoptera alata
		Odonthalia dentata
		Osmundea pinnatifida
		Palmaria palmata
		Phycodrys rubens
		Phyllophora crispa
		Phyllophora spp
		Phymatolithon calcareum
		Plocamium cartilagineum
		Plumaria plumosa
		Polyides rotunda
		Polyides rotundus
		Polysiphonia fucoides
		Porphyra purpurea
		Pterothamnion plumula
		Rhodothamniella floridula (syn) Audouinella purpurea
		Trailliella (Bonnemaisonia hamifera)
Chlorophyta		**Green seaweeds/algae**
		Chrysophyceae and Haptophyceae
		Blidingia spp.
		Chaetomorpha linum

Phylum	Taxon group	Common term/species names
		Chondrus crispus
		Cladophora liniformis
		Cladophora spp.
		Codium spp.
		Epicladia perforans (syn. Entocladia perforans)
		Pseudendoclonium submarinum
		Rhizoclonium riparium
		Ulothrix spp.
		Ulva spp.
		Urospora spp.
Ochrophyta		**Brown and yellow-green seaweeds/algae**
		Alaria esculenta
		Ascophyllum nodosum
		Ascophyllum nodosum ecad mackayi
		Bifurcaria bifurcata
		Chorda filum
		Cystoseira spp.
		Desmarestia spp.
		Dictyopteris polypodioides syn. membranacea
		Dictyota dichotoma
		Fucus ceranoides
		Fucus distichus
		Fucus serratus
		Fucus spiralis
		Fucus vesiculosus
		Halidrys siliquosa
		Himanthalia elongata
		Laminaria digitata
		Laminaria digitata
		Laminaria hyperborea
		Laminaria ochroleuca
		Pelvetia canaliculata
		Pleurocladia lacustris syn. Pilinia maritima
		Saccharina latissima
		Saccorhiza polyschides
		Sargassum muticum
Tracheophyta		**Flowering plants, seagrasses,**
		Phragmites australis
		Potamogeton pectinatus
		Ruppia maritima
		Zostera marina
		Zostera noltei
Fungi	**Lichens**	
		Anaptychia runcinata (as fusca)

Phylum	Taxon group	Common term/species names
		Caloplaca spp.
		Lecanora sp.
		Lichina pygmaea
		Ochrolechia parella
		Prasiola stipitata
		Ramalina siliquosa
		Rhizocarpon richardi
		Tephromela atra var. atra
		Verrucaria maura
		Verrucaria mucosa
		Xanthoparmelia pulla
		Xanthoria parietina

Appendix 2. List of standard search strings used to query the 'Contaminants' literature. *=wildcard; AND/OR = Boolean operators

Search string
ALL=(taxon)
ALL=((marine OR estuar* OR coast*) AND (contamin* OR pollut*) AND taxon)
ALL=(hydrocarb* AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND hydrocarb* AND taxon)
ALL=(("polyaromatic hydrocarbons" OR PAH*) AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND ("polyaromatic hydrocarbons" OR PAH*) AND taxon)
ALL=(("polychlorinated biphenyls" OR PCB*) AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND ("polychlorinated biphenyls" OR PCB*) AND taxon)
ALL=((Pesticid* OR Biocid*) AND taxon)
ALL=((Pesticide OR Biocide) AND (marine OR estuar* OR coast*) AND taxon)
ALL=((Pesticide OR Neonicotinoid) AND (marine OR estuar* OR coast*) AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND (Pesticid* OR Biocid*) AND taxon)
ALL=(organohalogen* AND taxon)
ALL=((marine OR estuar* OR coast*) AND organohalogen* AND taxon)
ALL=((marine OR estuar* OR coast*) AND dispersant* AND taxon)
ALL=(Organophosphat* AND taxon)
ALL=((marine OR estuar* OR coast*) AND organophosphat* AND taxon)
ALL=(organochlor* AND taxon)
ALL=((marine OR estuar* OR coast*) AND organochlor*AND taxon)
ALL=(Pharmaceutic* AND taxon)
ALL=((Pharmaceutic*) AND (marine OR estuar* OR coast*) AND (taxon))
All=(Chemotherapeut* AND taxon)
ALL=(Antifoul* AND taxon)
ALL=("flame retard*" AND taxon)
ALL=((marine OR estuar* OR coast*) AND (brominated flame retard*) AND taxon)
ALL=(Hormon* AND taxon)
ALL=((Human hormon*) AND (marine OR estuar* OR coast*) AND taxon)
ALL=(endocrine disrupt* AND taxon)
ALL=((marine OR estuar* OR coast*) AND ("endocrine disrupt*") AND taxon)
ALL=((Antibiotic AND Antimicrobial) AND Taxon)
ALL=((Antibiot* AND Antimicrob*) AND (marine OR estuar* OR coast*) AND taxon)
ALL=(Phthalat* AND Taxon)
ALL=((Phthalat*) AND (marine OR estuar* OR coast*) AND taxon)
ALL=(Dioxin AND taxon)
ALL=((Dioxin) AND (marine OR estuar* OR coast*) AND taxon)
ALL=(("Polychlorinated dibenzodioxin*" OR PCDD*) AND Taxon)
ALL=((Polychlorinated dibenzodioxins OR PCDDs) AND (marine OR estuar* OR coast*) AND taxon)
ALL=(("Polychlorinated dibenzofuran*" OR PCDF*) AND Taxon)
ALL=(("Polychlorinated dibenzofuran*" OR PCDF*) AND (marine OR estuar* OR coast*) AND taxon)
ALL=(("Perfluorooctanesulfonic acid*" OR PFOS*) AND Taxon)

ALL=(("Perfluorooctanoic acid" OR PFOA*) AND (marine OR estuar* OR coast*) AND taxon)
ALL=(("Perfluorooctanoic acid*" OR PFOA*) AND Taxon)
ALL=(("Perfluorooctanoic acid" OR PFOA*) AND (marine OR estuar* OR coast*) AND taxon)
ALL=(metal*) AND (marine OR estuar* OR coast*) AND (Taxon)
ALL=((metal*) AND (marine OR estuar* OR coast*) AND (Toxic*) AND (taxon))
ALL=((marine OR estuar* OR coast*) AND transition* metal AND taxon)
ALL=((marine OR estuar* OR coast*) AND transition* metal AND Toxic* AND taxon)
ALL=(Aluminium AND taxon)
ALL=(Antimony AND taxon)
ALL=(Cadmium AND taxon)
ALL=(Cadmium AND taxon AND toxic*)
ALL=((marine OR estuar* OR coast*) AND Cadmium AND taxon AND toxic*)
ALL=(Barium AND taxon)
ALL=(Selenium AND taxon)
ALL=(Selenium AND taxon AND toxic*)
ALL=(Tin AND toxic*)
ALL=(Tin AND taxon)
ALL=(Tin AND toxic* AND taxon)
ALL=(organometal* AND taxon)
ALL=(organometal* AND toxic* AND taxon)
All=(organotin* AND taxon)
All=(organotin* AND taxon AND toxic*)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND ship* AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND spills)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND spill* AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND spill* AND taxon AND toxic*)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND oil* AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND shipwreck AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND Aquacultur* AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND Maricultur* AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND "offshore renewables" AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND dredg* AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND aggregates AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND effluent* AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND oil AND gas AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND (drill* AND waste*) AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND drill* AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND runoff AND taxon)
ALL=((contamin* OR pollut*) AND (marine OR estuar* OR coast*) AND discharg* AND taxon)