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Sensitivity Assessment of Contaminant Pressures - Oyster species - Evidence review.

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1 Introduction

An evidence review of the effects of contaminants on species of oyster was undertaken between August 2022 and January 2023. The evidence review followed the Rapid Evidence Assessment (REA) protocol developed previously (Tyler-Walters *et al.*, 2022).

The resultant 'Oyster Evidence Summary' spreadsheet (available here) and 'Evidence review' that follows benefited from improvements and resultant minor adjustments to the prior reviews (Tyler-Walters *et al.*, 2022). The 'Evidence summary' template was updated to improve data entry. The improvements included:

- the addition of both the reported and standardised values for the exposure concentrations of contaminants used (where available),
- the addition of both the reported and standardised values for the observed or effect concentrations of contaminants (where available), and
- use of 'common' or 'trivial' names for chemicals derived from the PubChem[1] database where possible, and
- the adoption of a standard 'summary narrative' writing style for consistency in reporting.

In addition, 'contaminant type' is recorded as the function of the chemical (e.g., herbicide, analgesic), rather than the structure of the chemical (e.g., organohalogen, organophosphate), if the information allows.

All the technical terms used in the ''Oyster Evidence Summary' and the report that follows are defined in Appendix 1.

[1] https://pubchem.ncbi.nlm.nih.gov/

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2 Evidence review overview

The literature review focused on the Ostreidae, i.e. true oysters such as *Ostrea* spp., *Crassostrea* spp., *Magallana gigas* (syn. *Crassostrea gigas*)[2] and *Saccostrea*[3] spp.. Pearl oysters and saddle oysters were excluded to keep the literature review manageable.

The initial searches (01-15 August 2022) resulted in ca 20,583 hits of which 15,547 were duplicates (Table 2.1) using the standard search strings developed previously (Tyler-Walters *et al.*, 2022). Only the Web of Science (WoS) science citation index and the ECOTOX[4] Knowledgebase were used due to time constraints. The resultant references were screened for relevance based on the REA protocol. Screening against the exclusion criteria reduced this number to 485 articles, which were taken forward for detailed review. However, 84 articles could not be accessed, even using inter-library loans. Only articles written in English or with readily available English translations were included.

Table 2.1. Results of literature review for 'oysters'.

Review stage	No. articles identified/retained	No. articles rejected/removed
Web of Science	20,583	
ECOTOX database	342	
Duplicates removed	5,378	15,547
Screening	485	4,893
Taken forward*	485	107
Not accessible	84	

* does not include further articles identified from the articles reviewed, or alternative sources

The detailed evidence extracted from 294 articles[5] is provided in the 'Oyster Evidence Summary' spreadsheet and the supporting evidence and sensitivity assessments discussed below. The terms used in the 'evidence summary spreadsheet' and the following report are defined in Appendix 1.

[2] Please note, although *Magallana gigas* is the current accepted name for the *Crassostrea gigas*, the species names are presented as studied in the original articles.
[3] Not found in the UK but included to keep the review inclusive.
[4] https://cfpub.epa.gov/ecotox
[5] The term 'article(s)' or 'study' are used for peer reviewed papers, reports and other publications relevant to the review.

Most of the articles (92%) examined *Crassostrea* spp. (includes *Magallana gigas*) while *Ostrea* spp. and *Saccostrea* sp. were examined by 3% and 5% of the articles, respectively. This is consistent with the findings of the review of bivalve toxicology studies by His *et al.* (2000) who also found that the European oyster *Ostrea* sp. was one of the least studied (3%) of the oyster or other bivalve species they reviewed. The Pacific oyster *Crassostrea gigas* was the most used in bioassays, followed by the American oyster *C. virginica* and then *Mytilus edulis*.

Another 84 articles could not be accessed. However, the relevant evidence for 43 articles was obtained from the ECOTOX database and included in the 'Oyster Evidence Summary' spreadsheet.

His *et al.* (2000) noted that bivalves are suitable for the bioassay of environmental quality because they are tolerant of a wide environmental range and relatively easy to rear in the laboratory. They also noted that early life stages (i.e. embryos, blastulae, trochophores, veligers, D-stage larvae, pediveligers, and spat) and embryos were more sensitive to the effects of contaminants than their adults. Therefore, it is a not surprising that most of the articles reviewed by His *et al.* (2000) and this study (44%), examined early life stages (Table 2.2).

Table 2.2. Number of 'worst-case' ranked mortality 'results' reported from articles reviewed. Note a separate 'worst case' ranked mortality is given for the 'endpoints' and evidence from each combination of contaminant type and species reported in each article.

Life stage	**No. of worst-case' ranked mortality results**
Gametes	168 (13%)
Early life stages (embryos, larvae, spat)	570 (44%)
Adults and juveniles (inc. yearlings etc)	311 (24%)
Not reported	233 (19%)

Most studies of early life stages report the effects of exposure as 'effect concentrations' (e.g., ECXX) where the effect measured is abnormal development or larval mortality. Few studies take the larvae through to adulthood. Therefore, in this study, ECXX endpoints for early life stages (embryos, larvae, and spat) are interpreted as lethal concentrations, on the assumption that abnormally developed larvae are unlikely to survive to settlement or

adulthood in the wild. The relative sensitivities of 'adults and juveniles', 'early life stages' or 'gametes' are presented separately, where appropriate.

The resultant review provided 1,282 results (worst-case ranked mortalities) from 294 articles. 'Pesticides/biocides' (28% of articles, 44% of results), 'Metals' (32% articles, 18% of results), and 'Petrochemical' hydrocarbons (9% of articles, 11% of results) were the most studied groups of contaminants, closely followed by 'Synthetics (other)' (10% of articles, 6% of results), Organometals (11% of articles, 4% of results), and Pharmaceuticals (5% of articles, 3% of results) (Figure 2.1). The number of results for 'Pesticides/biocides' and 'Metals' was due to a small number of articles that reported numerous experimental studies on many contaminants, for example Butler, 1962, 1963, 1964, 1965, 1965b; Davis, 1961; Davies & Herbert, 1969; Finch *et al.*, 2014; Garcia *et al.*, 2014; and Hansen, 1980.

Overall, the articles reviewed reported mortality ('severe' to 'some') in 50.6% of results, no mortality ('none') in 4.2% of results, and sublethal effects in 40.5% of results. The level of mortality or sublethal effect was 'unspecified' in the remaining 4.7% of results.

Most sublethal effects were reported in studies of the effects of 'pesticides/biocides' on oysters (Figure 2.1). Due to the high proportion of sublethal effects reported, only articles that reported mortality as an effect are documented in the body of the report, unless the number of studies for each chemical group is limited. All the evidence collated is included in the 'Oyster Evidence Summary' spreadsheet.

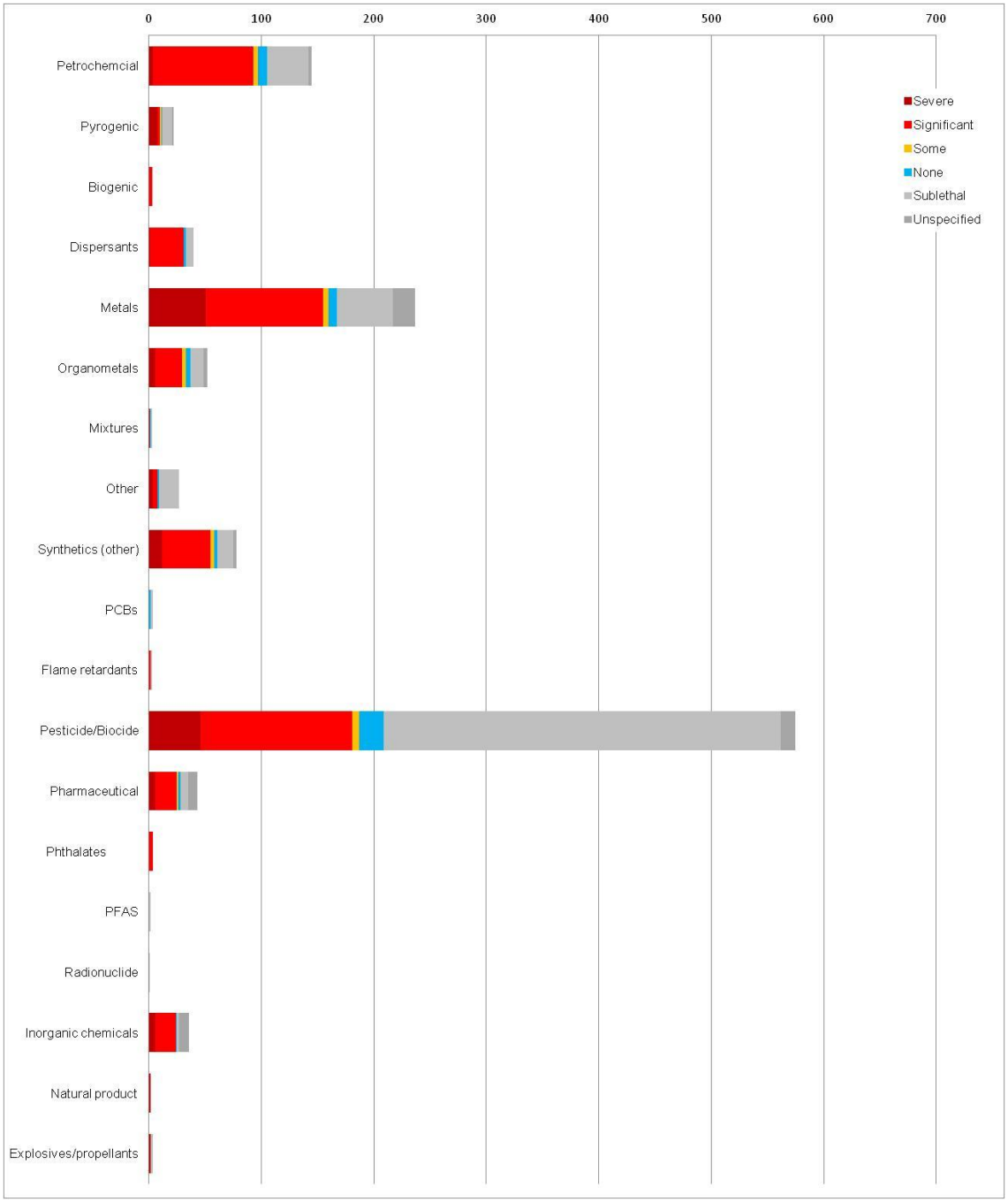

Figure 2.1. Count of worst-case ranked mortalities due to exposure to contaminants in oysters. Mortality is ranked as follows: 'Severe' (>75%), 'Significant' (25-75%), 'Some' (<25%), 'None' (no mortality reported), and 'Sublethal' effects.

3 Hydrocarbons and PAHs

A total of 205 results were obtained from 55 articles that studied the effect of hydrocarbons, PAHs and dispersants on oysters, of which 56% examined the effects of complex hydrocarbons such as crude or fuel oils and/or their water accommodated or saturated fractions (WAF/WSF) (Figure 3.1).

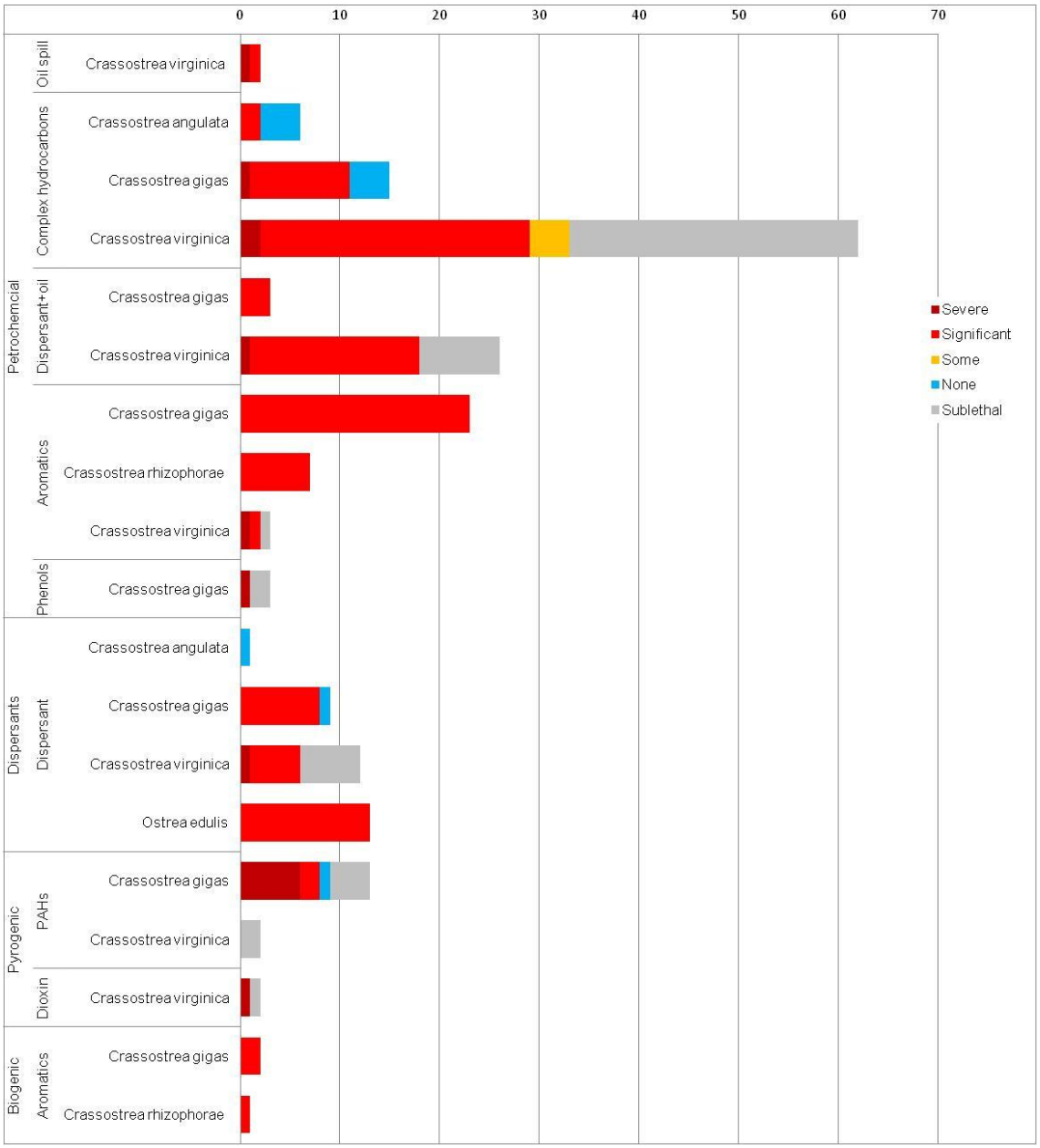

Figure 3.1. Count of ranked mortalities due to exposure to hydrocarbons in oyster species. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects.

The effects of pyrogenic hydrocarbons such as polyaromatic hydrocarbons (PAHs) and dioxins were examined by 22% of these articles. The effect of dispersants was examined by 18%, while biogenic hydrocarbons (i.e., toluene) were examined by only 3% of the articles. Lethal effects were reported in 69% (Figure 3.1) and sublethal effects in 28% of the cases reported.

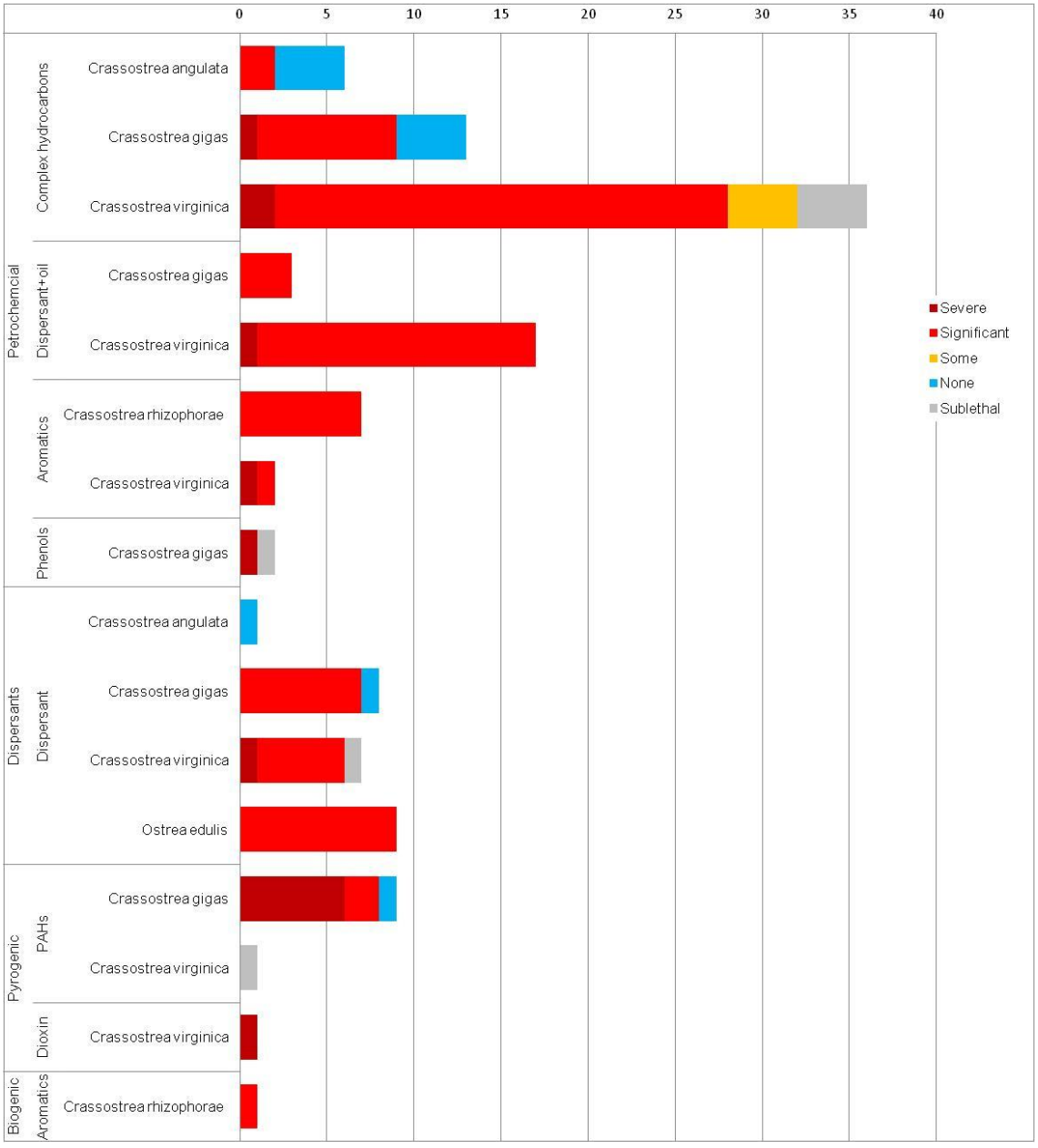

Figure 3.2. Count of ranked mortalities due to exposure to hydrocarbons in the early life stages of oyster species. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects.

Crassostrea spp. dominated the studies examined. *Ostrea* sp. was only reported in two studies, both of which examined the effect of dispersants. Also, the number of studies that

examined early life stages (60%) greatly exceeded studies of adults and juveniles (7%) or gametes (30%) (Figure 3.2).

3.1 Oil spills

The effect of an oil spill was only reported by two articles. The evidence is summarized below.

***Galeta* oil spill - April 1986 (crude oil).** Levings *et al.* (1994) investigated the long-term effects of *Galeta* oil spill (April 1986) on epibiota in mangrove fringe of the Bahas las Minas on the Caribbean cost of Panama. Between 1986 and 1991, the abundance of *Crassostrea virginica* was observed in oiled and unoiled sites. The cover of oysters at oiled sites was significantly reduced. Pre-spill (1981-1982) abundance ranged from 50 to 54% cover. In 1986, the mean abundance of oyster cover at three stations along an oiled channel averaged 27% and dead *Crassostrea* averaged 22%. In the last nine months of the first year following the spill, oyster abundance at oiled sites dropped to a mean cover of 6%. During this same period, oyster abundance at unoiled sites averaged 30% cover and dead oysters <4%. From years 2-5 post-spill, the abundance of oysters at five oiled sites was examined. The comparison of abundance at oiled and unoiled sites suggested that the oysters were negatively affected by the oil for at least five years (Levings *et al.*, 1994).

***Deepwater Horizon* – April 2010 (crude oil).** The effects of the *Deepwater Horizon* oil spill on intertidal oyster beds were reported by Powers *et al.* (2017). They assessed the impacts of shoreline oiling on eastern oysters *Crassostrea virginica* near the salt marsh edge at 187 sites in Louisiana and Mississippi Sound in 2013. Oyster habitat was 77% less abundant in areas that experienced heavy oiling, than in areas where no oil was observed. Areas near marshes, characterized by more moderate levels of oiling had 33% less oyster habitat than areas where no oil was observed. Similarly, the number of sites without any oyster habitat was higher in heavily and persistently oiled areas compared to areas where no oil was observed (56% vs. 24%) (Powers *et al.*, 2017). However, freshwater input contributed to the decline of subtidal populations (Murawski *et al.* 2021).

3.2 Petroleum hydrocarbons – oils and dispersed oils

Thirty-one of the articles examined the effects of petroleum oils (e.g. crude oil and fuel/bunker oils), and dispersed oils, and are summarized below.

Banks & Brown (2002) investigated the effects of hydrocarbons on fouling assemblages. Clay tiles were exposed to crude oil, a 10% water soluble fraction (%WSF) of crude oil, or artificial seawater, and placed out at two locations, in two seasons, and at two tidal levels in an estuary. *Crassostrea virginica* did recruit onto the tiles and the oysters grew to larger sizes on the oil-exposed tiles. However, laboratory trials with the same treatments showed settlement to be significantly depressed on the crude oil exposed tiles compared to the controls. In the laboratory trials, the survival and development of the oyster larvae were also assessed after 72 hours of exposure to crude oil. The survival of the oyster larvae was significantly decreased compared to the control and the metamorphosis was reduced.

Boulais *et al.* (2018) exposed *Crassostrea virginica* gametes, embryos, and larvae to sediment contaminated by oil from the *Deepwater Horizon* (DWH) spill to assess the effects on early development. Effective concentrations for fertilization inhibition were 40.6 µg tPAH50/l[6] and 173.2 µg tPAH50/l for 1-hour EC20 and EC50 values, respectively. Embryo exposure resulted in dose-dependent abnormalities (EC20 and EC50 values were 77.7µg tPAH50/l and 151 µg tPAH50/l, respectively) and reduction in shell growth (EC20 24-hour value of 1180 µg tPAH50/l). The development and growth of veliger larvae were less sensitive to sediment-associated PAHs compared to embryos. Fertilization success and abnormality of larvae exposed as embryos were the most sensitive endpoints for assessing the toxicity of oil-contaminated sediment.

Cardwell *et al.* (1979) investigated the effects of pharmaceuticals, surfactants, metals, and hydrocarbons on larval marine organisms. *Crassostrea gigas* larvae were exposed to the chemicals for a period of 48 hours to determine the lethal concentration that caused 50% mortality in embryos (LC50) and the effective concentration that caused 50% of the larvae to develop abnormally (EC50). Pacific oyster larvae were not very sensitive to ammonia and dissolved oxygen, but quite sensitive to various suspended solids, hydrogen sulphide, petroleum hydrocarbons, unbiodegraded linear alkylate sulfonates and deviations in pH and salinity to which the gametes were unacclimated. The exposure of oyster larvae to Kuwait crude or Alaskan crude resulted in LC50s of 380 µg/l and 5,800 µg/l respectively. Bivalve larvae were more sensitive to dodecyl sodium sulphate (DSS) and cadmium than the larvae of Dungeness crab (*Cancer magister*), spot shrimp (*Pandalus platyceros*), and Pacific herring, but of intermediate sensitivity to the pesticide Methoxychlor.

[6] tPAH50 = sum, or total, of 50 PAHs expressed in µg/l

Finch *et al.* (2016) examined the photo-enhanced toxicity of two weathered Macondo crude oils in the early life stages of the eastern oyster *Crassostrea virginica*, in static exposure experiments. The toxicity tests were conducted with sperm and three larval ages (2-4 hours post-fertilization (PF), 8-10 hours PF and 44-46 hours PF) to evaluate the photo-enhanced toxicity of low-energy water-accommodated fractions (WAFs) of two weathered Macondo crude oils collected from the *Deepwater Horizon* incident. Significant mortality was observed in larvae exposed to WAF of crude oils between >100 and 48.6 %WAF (see evidence summary for detail). Larvae exposed to oil WAFs under UV-filtered light demonstrated consistently higher survival and normal development than larvae exposed to WAFs under UV light.

Finch *et al.* (2018) examined the photo-enhanced toxicity of undispersed and dispersed weathered Macondo T crude oil to Pacific (*Crassostrea gigas*) and eastern oyster (*Crassostrea virginica*) larvae, in static exposure experiments. Larvae were exposed to CTC weathered crude oil (collected by the CTC barge) and Chemically Enhanced WAFs (CEWAFs[7]) of Corexit 9500 (dispersant), and a combination of weathered oil and Corexit 9500 at 100, 50, 25, 12.5, 6.25, and 0% for 48 hours in the presence or absence of UV light. Phototoxic effects were observed for larval Pacific oysters exposed to combinations of oil and dispersant, but not for oil alone. For larval Pacific oysters, exposure to combinations of oil and dispersant produced an EC50 of 53.4% WAF in the absence of UV and an EC50 of 34.1 %WAF in the presence of UV. For larval eastern oysters, phototoxic effects were observed for exposure to oil alone and combinations of oil and dispersant. For eastern oysters, the EC50 of oil alone was >100 %WAF in the absence of UV but was 31% %WAF in the presence of UV. Eastern oysters exposed to combinations of oil and dispersant had an EC50 of 35.3 %WAF in the absence of UV and an EC50 of 13.4 %WAF in the presence of UV. Corexit 9500 did not exhibit phototoxicity but resulted in significant mortality for Pacific oysters with EC50s of 59.3 (+UV) and 68.2 (-UV).

Garcia *et al.* (2020) investigated the sublethal effects of soluble fractions of crude oil alone (WAF) and crude oil in combination with Corexit 9500 dispersant (CEWAF) on oysters at three life stages. Veliger swimming, pediveliger settlement, and adult clearance rates were assessed after 24-hour exposures. Veliger swimming speeds were not significantly influenced by 24-hour exposures to WAF (10, 50 and 100 µl/l) or CEWAF (10 & 50 µl/l).

[7] CEWAFs (chemically enhanced WAF) are mixtures of oil and dispersant

There was a significant increase in the number of inactive veligers and pediveliger following WAF and CEWAF exposure compared to the control. Effects were seen from 50 µl/l for veliger larvae, and from 10 µl/l for pediveliger larvae. Pediveliger settlement was significantly affected at the highest concentration of both WAF and CEWAF, with reductions of 40 and 50% compared to controls. No significant differences were observed for WAF at the lowest tested concentration, but at the lowest tested concentration of CEWAF, there was a 20% reduction. When clearance rates were measured in the same oil/dispersant conditions in which the oysters had been exposed for 24 hours, decreases in clearance rates for adult oysters were observed compared to controls. The clearance rate of WAF-exposed oysters significantly decreased from 100 µl/l and the clearance rate of CEWAF-exposed oysters significantly decreased from 50 µl/l. Clearance rates measured in clean seawater (no oil or dispersants) following a 24-hour exposure generally showed no changes in clearance rates compared to the controls. Following the 24-hour exposures, the oyster's clearance rates were monitored for 33 days. WAF exposed oysters had a slightly lower clearance rate mean than the controls, however, there were significant reductions in clearance rates of CEWAF exposed oysters that persisted for 33 days after acute exposure.

Jasperse *et al.* (2018) investigated the effects of Corexit® 9500, oil, and a Corexit®/oil mixture on the clearance rates of the eastern oyster, *Crassostrea virginica*. Oyster feeding rates were significantly decreased following exposure to Corexit®, High Energy Water Accommodated Fractions (HEWAF), and CEWAF, with EC50s of 11,700, 3.5, and 1.2 µg/l respectively.

Langdon *et al.* (2016) examined the effects of chronic exposure of eastern oyster (*Crassostrea virginica*) larvae to the water-accommodated fractions (WAF) of fresh and weathered oils collected from the *Deepwater Horizon* incident, with and without additions of the dispersant Corexit 9500A, as well as to solutions of Corexit alone. This study conducted 10-day and 28-day toxicity tests with *C. virginica* larvae. The 28-day bioassays used CTC WAF, CTC CEWAF, and Corexit alone. The 28-day larvae tests were conducted under static-renewal conditions and new solutions were prepared every 48 hours for renewals. At the end of the 28-day exposures, endpoints were calculated based on survival, normal shell development, shell growth, and larval settlement. The 10-day bioassays were conducted with CTC WAF and CEWAF, Juniper WAF and CEWAF, and MASS WAF, MASS CEWAF, and Corexit alone. Ten-day tests were conducted using the same methods and test conditions as the 28-day test. At the end of the 10-day exposures, endpoints were calculated based on survival, proportion normal larvae development, and growth. In both the 10-day

and 28-day tests abnormal larvae were defined as those that were D-stage (or less developed) larvae with 90% tissue, or larvae in later stages of development with shell or tissue deformities and 90% tissue. Larvae with less than 90% tissue, regardless of size and development, were considered dead. Growth and settlement endpoints were more sensitive than larval survival and normal development after 10-day and 28-day exposures.

Laramore *et al.* (2014) examined the effects of Macondo Canyon 252 oil WAF and CEWAF on embryogenesis, larval development, growth, behaviour, and survival of *Crassostrea virginica*. Exposure concentrations were 0, 100, 200, 400, 800 and 1200 mg/l for WAFs, and 0, 6.25, 12.5, 25, 50, 100 and 200 mg/l for CEWAFs. The effects on embryogenesis were assessed by fertilization success. CEWAFs exposure caused reductions in fertilization success from 12.5 mg/l. However, WAFs had no impacts on fertilization success at the concentrations tested. Larval development was significantly influenced by both CEWAF and WAF exposures, with decreases in the number of developing larvae and an increase in the percentage of abnormal larvae. The swimming behaviour of larvae that had been exposed to concentrations of CEWAFs above 100 mg/l or WAFs from 200 mg/l was significantly influenced at 24-, 48-, 72-, and 96-hours following exposures. Impacts on the survival of the larvae were assessed at D-larval and eyed larval stages, CEWAF exposure had a significant effect on the survival at both stages of development, whereas WAFs only affected D-stage larvae. In D-stage larvae exposure, to CEWAF the LC50 for 24-, 48-, 72- and 96 hours were 177.6, 44.7, 33.8, and 24.8mg/l, respectively. In eyed-stage larvae, exposure to CEWAF the LC50 for 24-, 48-, 72- and 96 hours were 81.9, 44.2, 21.6 and 14.5 mg/l, respectively. In D-stage larvae, exposure to WAF, the LC50 for 24, 48, 72 and 96 hours were 1092.8, 554.2, 289.2, and 261.8 mg/l, respectively.

Renzoni (1973) investigated the effects of crude oil, derivatives, and dispersants on the larvae of *Crassostrea angulata* and *Crassostrea gigas*. Oyster eggs were exposed to the crude oil, derivatives, and dispersants at concentrations between 1 and 1000 ppm an hour after fertilization. After six hours of exposure, samples of eggs were taken to assess fertilization, embryogenesis, and larvae development. The fertilization, embryogenesis, and development of larvae were only low in the treatments with Crude oil (Venezuelan (Tiajuana, Maracaibo)) and Fuel oil No. 1 at the highest tested concentration. The swimming activity of the larvae was assessed after seven hours exposure. Crude oil (Venezuelan (Tiajuana, Maracaibo)) and Fuel oil No. 1 exposures had significantly fewer swimming larvae than the controls and other treatments.

Renzoni (1975) examined the effects of water soluble fractions (%WSF) crude oils from three locations (Kuwait, Alaska and Nigeria) on gametes, fertilization, and development in *Crassostrea virginica* and *Mulina lateralis*. WSF of oil (0.001. 0.01, 0.1 & 1 ml/l) were added to freshly mixed gametes before fertilization, to sperm before mixing with untreated eggs and eggs before mixing with untreated sperm. Fertilization, development, and larval survival were measured in both species for 48 hours and larval growth in *Mulina lateralis* for 14 days. The toxicity of WSF of oils to fertilization, development, and larval survival increased with increasing concentration. For example, larval survival was reduced by 45 to 57% at 1 ml/l WSF depending on the oil used. The Nigerian oil (WSF) was significantly more toxic that the other two oils, in all the experimental treatments.

Salehi *et al.* (2017) investigated the acute 48 h toxicity of Corexit 9500 and hyperbranched polyethyleneimine (HPEI) on larvae and early spat stages of the Eastern oyster, *Crassostrea virginica*. The oyster larvae of different size classes (0.1, 0.2, 0.3, 0.7, and 2 mm) were exposed to 3,130, 6,250, 12,500, 25,000, and 50,000 µg/l. For Corexit, 100% oyster mortality was detected for the <0.2 mm size classes at the two highest tested concentrations (25,000 and 50,000 µg/l). For the 0.3 and 0.7 mm size classes, >50% mortality occurred at the two highest tested concentrations (25,000 and 50,000 µg/l). HPEI exhibited mortality rates <30% for all concentrations for all oyster size classes except for the 0.1 mm class that had mortality up to 60%.

Sigler & Leibovitz (1982) examined the toxicities of three bilge cleaners and No. 2 fuel oil, as well as a detergent used to clean larval culture containers on the larvae of *Crassostrea virginica*. The three bilge cleaners and the one detergent were tested alone and in combination with No. 2 Fuel oil. No. 2 Fuel oil was also tested alone. Larvae were exposed to the contaminants for 96 hours and mortality was assessed every 24 hours during the 96-hour period. The concentrations that caused 50% mortality were calculated for each of the contaminants. For, No.2 fuel oil the 96-hour LC50 was 1,900 µg/l.

Vignier *et al.* (2015) investigated the impacts of *Deepwater Horizon* oil and associated dispersants on the early development of *Crassostrea virginica*. The effects of exposing gametes and embryos to dispersant alone (Corexit), mechanically High Energy Water Accommodated Fractions (HEWAF) and Chemically Enhanced WAF (CEWAF) *Deepwater Horizon* oil were evaluated. Fertilization success and the morphological development, growth, and survival of larvae were assessed. Gamete exposure reduced fertilization, caused abnormal development, reduced growth, and caused mortality. Similarly, embryo

exposure caused abnormal development, reduced growth, and caused mortality. Gametes exposed to Corexit had reduced fertilization success with an EC50 of 11,500 µg/l. Gamete and embryo exposure caused abnormal larvae with an EC20 of 7,390 µg/l for gamete exposure and an EC50 of 5,670 µg/l for embryo exposure. In addition, mortality was increased by Corexit gamete exposure with 96-hour LC50 of 2.7 µg/l. The 96-hour LC50 for Corexit exposure of embryos could not be calculated due to high control mortality. Gametes exposed to HEWAF had reduced fertilization success with an EC50 of 2,250 µg/l. Gamete and embryo exposure caused abnormal larvae with an EC50s of 267 and 342 µg/l, respectively. Mortality was increased by HEWAF exposure to gametes and embryos with 96-hour LC50s of 307 and 220 µg/l respectively. Gametes exposed to CEWAF had reduced fertilization success with an EC50 of 29.9 µg/l. Gamete and embryo exposure caused abnormal larvae with an EC50s of 14.9 and 15.6 µg/l, respectively. Mortality was increased by CEWAF exposure to gametes and embryos with 96 hour LC50s of 8.5 and 17.7 µg/l, respectively.

Vignier *et al.* (2016) investigated the lethal and sub-lethal effects of *Deepwater Horizon* oil and dispersant on oyster (*Crassostrea virginica*) larvae. Exposures to HEWAF, CEWAF, and Corexit were toxic to larvae, impairing growth, settlement success, and survival. Larval growth and settlement were reduced at concentrations of tPAH50[8] ranging from 1.7 to 106 µg/l for HEWAF and 1.1 to 35 µg/l for CEWAF. For Corexit, larval growth was reduced by 20% at concentrations of 3,500 and 10,700 µg/l. The concentrations that caused 50% mortality of larvae were 58,000 µg/l for Corexit, 715 µg/l for HEWAF and 41.8 µg/l for CEWAF.

Vignier *et al.* (2017) investigated the sensitivity of *Crassostrea virginica* spermatozoa and oocytes to dispersed oil. Spermatozoa and oocytes were exposed to HEWAF at concentrations of 25,000 and 500,000 µg/l, and CEWAF at concentrations of 50,000 and 100,000 µg/l for 30 minutes. Exposure to HEWAF caused no significant reduction in the number of viable sperm cells. CEWAF exposure at 50,000 µg/l did not cause any significant reduction in sperm viability. However, at 100,000 CEWAF sperm viability was significantly reduced. Oocytes were not affected by the 30 min exposure to any of the oil treatments. Fertilization success where both sperm and oocytes were exposed was significantly reduced by the highest dose of HEWAF, and by both doses of CEWAF. The fertilization success of

[8] tPAH50 = sum, or total, of 50 PAHs expressed in µg/l

oocytes exposed to 500 ppm of HEWAF and fertilized with control sperm was reduced to 46% but was not significantly lower in the 250 ppm HEWAF treatment. Oocytes exposed to 50 and 100 ppm CEWAF and fertilized with non-exposed sperm had significantly reduced fertilization success by 17.2% and 18.4% respectively. After 24 hours, 100% of embryos exposed to HEWAF or CEWAF were either abnormal or dead regardless of whether oocytes and sperm were both exposed, or only oocytes were exposed. Total (100%) mortality occurred in the 500 ppm (500,000 µg/l) HEWAF and 100 ppm (100,000 µg/l) CEWAF treatment groups.

Vignier *et al.* (2018) investigated the toxicity of *Deepwater Horizon* slick oil on the spat of the oyster *Crassostrea virginica*. The clearance rate and survival of oyster spat were evaluated. For the clearance rate measurements, the oyster spat were exposed to concentrations of HEWAF ranging from 100 to 2,000 mg/l for 24 hours. The clearance rate of the spat was significantly impacted at all concentrations of HEWAF. Mortality was assessed by exposing the spat to HEWAF for 10 days at concentrations between 7 and 3,450 µg/l. No significant differences in mortality between control and exposed treatments were observed and no clear dose-dependent mortality response was observed.

Vignier *et al.* (2019) investigated the effects of 14 days exposure to *Deepwater Horizon* oil on the growth, development, and survival of *Crassostrea virginica* larvae. After 14 days of exposure, larval growth and survival were negatively affected at concentrations as low as 1.6 µg/l. The EC50s for abnormal development and growth inhibition were 27.2 and 6.8 µg/l, respectively. For mortality, the 14-day LOEC and LC50 were 3.2 and 78 µg/l.

Volety *et al.* (2016) investigated the effects of *Deepwater Horizon* oil toxicity on the spermatozoa of *Crassostrea virginica*. The fertilization success of gametes was assessed by exposing both sperm and oocytes to HEWAF, CEWAF or dispersant for 30 minutes prior to fertilization. Fertilization success was determined one-hour post-fertilization and 100 embryos per treatment were examined for cell cleavage. CEWAF and HEWAF showed no effect on sperm viability at any of the tested concentrations. However, at the highest tested concentration of dispersant, sperm viability was decreased.

3.3 Dispersants

The effects of dispersants themselves were examined by 10 articles. Eight of the articles studied different types of Corexit alone or in combination with oils (see Renzoni, 1973; Vignier *et al.*, 2015, 2016; Langdon *et al.*, 2016; Volety *et al.*, 2016; Salehi *et al.*,

2017;Jasperse *et al.*, 2018; and Garcia *et al.*, 2020 above). Salehi *et al.*, 2017 also examined hyperbranched polyethylenimine (HPEI). Portman & Wilson (1971, unseen) and Woelke (1972, cited by His *et al.*, 2000) examined a variety of different dispersants or detergents used to clean bilge tanks. Refer to the oyster evidence summary spreadsheet for details.

3.4 Petroleum hydrocarbons – phenols

Nice *et al.* (2000, 2003) and Nice (2005) examined the effects of 4-nonylphenol of *Crassostrea* spp. Nonylphenol is used to manufacture antioxidants, emulsifiers, detergents, and lubricating oils.

Nice *et al.* (2000) investigated the effects of 4-nonylphenol (0.1, 1, 10, 100, 1000 and 10,000 µg/l) on the development of *Crassostrea gigas*. Development of the larvae was monitored over 72 hours at 8-hour intervals. 4-nonylphenol delayed development to D-shape, caused abnormal development, and caused a significant decrease in survival rate with 100% mortality at 1,000 µg/l.

Nice *et al.* (2003) investigated the long-term and transgenerational effects of nonylphenol exposure at a key stage in the development of *Crassostrea gigas*. Seven day old larvae were exposed to nonylphenol at 1 and 100 µg/l for 48 hours. After the 48-hour exposure, the larvae were rinsed and put into uncontaminated seawater to grow. At one-month post-fertilization, larvae that had developed into spat were transferred into a flow-through system to grow out. Growth and development of the oyster spat were monitored to adulthood, growth was monitored monthly, and at 10 months of age, the oysters were sexed. Transgenerational effects were monitored by observing the offspring produced from pre-exposed parents and unexposed parents. Up to 30% hermaphroditism was seen in oysters exposed to nonylphenol for a 48-hour period during early larval development. However, no hermaphrodites were present in the controls. Shell length increased with time, however, there was no significant difference in length between exposed and control individuals at any of the time intervals monitored from juvenile (spat) to adulthood. Transgenerational effects were observed. When individuals were crossed and the survival rate of offspring recorded 48-hour post-fertilization, the offspring from control parents had a significantly higher survival rate than offspring where at least one parent had been exposed to nonylphenol during larval development.

Nice (2005) exposed *Crassostrea gigas* juveniles to nonylphenol at concentrations of 1 and 100 µg/l for 72 hours during the period of gametogenesis. Growth was monitored at regular intervals until sexual maturity when sperm motility was assessed. The growth rate of *C. gigas* was unaffected by exposure to nonylphenol during gametogenesis. However, the number of individuals with motile sperm was significantly reduced. In the controls, 100% of the oysters had motile sperm. However, only 30% of the oysters from the 1 µg/l treatment and only 10% from the 100 µg/l treatment had motile sperm.

Davis & Herbert (1969) and Meyer (1987) examined the effects of phenols used as a pesticide. Da Cruz *et al.* (2007) examined a number of contaminants including 4-chlorophenol and phenol as pesticides on *Crassostrea* sp. Their results are included under 'pesticides/biocides below.

3.5 Petroleum hydrocarbons – others

Another five articles examined the effects of various petroleum products on the early life stages of *Crassostrea* sp, for example aliphatics such as pentane, octane, and heptanes, or aromatics such as benzenes, cyclopentane, cycloheptane, napthas, and xylenes.

Paixao *et al.* (2007) investigated the toxic effects of gasoline components on oyster (*Crassostrea rhizophorae*) embryos. Oyster embryos were exposed to different gasoline formulations as water-soluble fractions (WSF) at a range of concentrations (0%, 4.6%, 10.0%, 22.0%, 46.0%, and 100%), for 24 hours. Bioassays carried out on the eight different gasoline components aimed to correlate gasoline toxicity with the toxic potential of its components. End-points of EC50 (estimate concentrations which can cause abnormalities in 50% of the exposed population) were established. Principal component analysis showed that light naphtha (mean EC50: 41.34%), PGH stream (mean EC50:40.70%), XMEB (mean EC50:30.76%) and raw naphtha (mean EC50:27.50%) to be the least toxic components, while C9S (mean EC50:7.17%), C9DI (mean EC50: 9.97%), heavy naphtha (mean EC50: 11.58%) and toluene (mean EC50: 12.38%) were shown to be the most toxic to oyster embryos.

The details of the remaining studies (Chevron Chem. Corp, 2000; EPA/OTS, 1991; Thursby & Berry, 1987; and Legore, 1974) could not be accessed but their data is included in the oyster evidence summary spreadsheet and overall sensitivity assessment.

3.6 Polyaromatic hydrocarbons (PAHs)

The effects of polyaromatic hydrocarbons (PAHs) of pyrogenic origin were examined by 12 articles. The evidence is summarized below, except for Banks & Brown (2002) which is summarised above.

Choy *et al.* (2007) investigated the effects of brood stock exposure of benzo(a)pyrene (B[a]P) on the reproductive success, hatch rate, survival, growth, and development of their larvae. The brood stock was fed algae grown in 0, 50, 500, or 5,000 µg/l B[a]P for 28 days. After 28 days, 20 oysters were stripped and the egg solution was sampled for reproductive success, expressed as a percentage ratio of normal to total gametes. Then the eggs were fertilized to determine hatch rates, survival, growth, and development. Spat attachment was also assessed. None of the 500 and 5,000 µg/l oysters achieved reproductive success over 80% with more significantly adverse effects in the 5,000 µg/l oysters. The reproductive success of the 50 µg/l oysters was over 80%, similar to the controls. The hatching rates of the fertilized eggs were also significantly affected by B[a]P exposure. The hatching rates of the fertilized eggs of the control, 50, 500, and 5,000 µg/l oysters were 88.5, 86.0, 82.0, and 19.0%, respectively. The control survival of larvae in the first 8 days was 83%, with a significant mortality from day 10. The control survival was 21% on day 14. The survival of the 50 µg/l larvae was similar to that of the controls from day 0 to day 8 but survival was reduced thereafter. The survival of the 500 µg/l larvae was comparable to that of the controls over the first six days. However, there were significant differences in survival from day eight. The survival rate of the 500 µg/l larvae declined sharply, with 4% survival on day 14. The survival of the 5,000 µg/l larvae started to decline significantly from day four, reaching only 0.2% survival by the end of the experiment. The larval growth was measured as a shell length. The control and 50 µg/l larvae grew faster than the 500 and 5,000 µg/l larvae groups. Over the 14-day experiment, the net growth of the control, 50, 500 and 5,000 µg/l larvae were 0.159, 0.1349, 0.0333, and 0.0534 mm, respectively. The effect of B[a]P on late larvae development was assessed. The survival rates of the hatched larvae were 20.5, 11.5, 0.2, and 0%, for control, 50, 500, and 5,000 µg/l larvae, respectively. Developmental time to the eye-spotted larvae stage was 14 days for the control, 15 days for 50 larvae, and 19 days for 500 larvae. None of 5,000 larvae developed to the eye-spotted larvae stage. Spat attachment was influenced by the different exposures. The average numbers of spat attached on a collector were 14.3, 7.57, and 0.60 for control, 50, and 500 µg/l spats, respectively.

His *et al.* (1997) investigated the effects of hydrocarbon-polluted sediment on *Crassostrea gigas* metamorphosis and survival. *Crassostrea gigas* larvae were exposed to unfiltered and 0.45 µm filtered elutriates over two days. No effects on larval mortality were observed. However, the larval metamorphosis was significantly reduced.

Jeong & Cho (2005) examined the effects of parental and subsequent PAH exposure on sperm mobility, fertilization success and embryo development in *Crassostrea gigas* under laboratory conditions. Adults were exposed to a cocktail of PAHs (acenaphthene, acenaphthylene, banx(a)anthracene, benzo(a)pyrene, chrysene, dibenz(a,h)anthracene, fluoranthene, fluorene, phenanthrene, and pyrene) at 200 ppb (200 µg/l) for 30 days. Once ripe, gametes were extracted and exposed to 0, 50, 100 and 200 ppb of the PAH cocktail. Sperm movement was highly affected by parental and gametic exposure. Sperm motility was significantly reduced by parental exposure, while sperm linearity was significantly decreased by gametic exposure. Exposure to the PAHs during fertilization significantly reduced fertilization efficiency to 7.4 to 26.4% (compared with 51.1 to 68.6% in controls). As a result, D-larvae development was also significantly reduced. Jeong & Cho (2005) reported that eggs and sperm had been shown to accumulate more PAHs than somatic tissue in *Crassostrea gigas*, so that gonads were one of the most impacted organs. They concluded that PAH exposure could cause a breakdown in sperm motility and larval development (Jeong & Cho, 2005).

Kim *et al.* (2007) investigated the effects of polycyclic aromatic hydrocarbons (PAH) on *Crassostrea gigas*. The oysters were exposed to varying levels of PAH (0, 50, 100 and 200 µg/l) for 7 days. The filtration rate and respiration rate increased significantly at 50 µg/l PAH and decreased at 100 and 200 µg/l compared with the control. The scope for growth of the exposed oysters was similar to the control.

Lyons *et al.* (2002) examined the phototoxicity of pyrene and benzo[a]pyrene (B[a]P) to embryo-larval stages of the Pacific oyster *Crassostrea gigas*, in static exposure experiments. Embryos were exposed to B[a]P and pyrene at nominal concentrations of 1, 2.5, 25 and 100 µg/l under fluorescent lighting and at 0.25, 0.5, 1 and 5 µg/l under UV lighting for 48 hours. The effect on the proportion of embryos developing normally to the D-stage veliger stage was assessed. Embryos that either did not develop to the D-shell stage or D-shell larvae that had soft tissue deformations or shell malformations, were considered abnormal. The toxicity of both B[a]P and pyrene was enhanced by UV exposure. At 1 µg/l B[a]P, under fluorescent lighting abnormalities were at 19.2%. However, at the same concentration under UV lighting

abnormalities reached 83.7%. At 1 µg/l pyrene, under fluorescent lighting abnormalities were at 11.2%. However, at the same concentration under UV lighting abnormalities reached 73.5%.

Nogueira *et al.* (2017) investigated the effects of phenanthrene on the development of *Crassostrea gigas*. Embryos were exposed to concentrations between 0.37 to 6 µg/l phenanthrene for 24 hours to establish an EC50 of 1.91 µg/l. After the EC50 concentration was determined, a second experiment was carried out with two concentrations: 0.02 and 2.0 µg/l. Exposure to both concentrations of phenanthrene caused developmental deformities. A 10% decrease in normal larvae development occurred at the lowest tested concentration of 0.02 µg/l. But 80% abnormal development occurred at the highest tested concentration of 2 µg/l.

Wessel *et al.* (2007) investigated the relationship between embryotoxic and genotoxic effects of benzo[a]pyrene (B[a]P), 17 α-ethinylestradiol (EE2) and Endosulfan (ES) on *Crassostrea gigas* embryos. Embryotoxicity was evaluated by calculating the percentage of abnormal D-larvae obtained after 20 hours development following exposure to the contaminants. EE2 displayed no toxic effects on the embryos at the tested range of 0.02 to 1.7nM. For BaP, embryotoxicity was observed from the lowest tested concentration of 0.2nM. For ES, embryotoxicity was observed from 300nM.

Xie *et al.* (2017b) investigated the effects of benzo[a]pyrene (B[a]P) and 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) on the development of embryos and the survival of larvae. In the embryotoxicity tests, embryos were exposed to 10, 20, 40, 80, or 160 µg/l of B[a]P, or were exposed to 100, 200, 400, 800, or 1600 BDE-47 for 24 hours. In the larval survival tests, larvae were exposed to the same concentrations as used in the embryotoxicity testing but larvae were exposed for 96 hours. The 24-hour embryotoxicity EC50s for B[a]P and BDE-47 were calculated as 18.4 and 203.3 µg/l, respectively. Larvae mortality 96-hour LC50s were calculated as 26.8 µg/l for B[a]P, and at 244.5 µg/l for BDE-47.

3.7 Dioxins

Cooper *et al.* (2009) investigated the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the embryonic development of *Crassostrea virginica*. Oysters were injected with 0.1 ml of TCDD (at 2.0 or 20 pg/g wwt) into the adductor muscle, on day zero and on day 14. After 28 days, eggs and sperm were stripped from the oysters and allowed to fertilize. Larvae from exposed oysters had 0% survival to the D-larval stage, compared to the control that had

80.3%. Larvae spawned from non-exposed oysters and reared in 0, 2, and 10 pg/ml TCDD resulted in 76, 2.3, and 1.1% survival to the trochophore and D-larval stage. They concluded that reproduction in bivalve molluscs is highly sensitive to TCDD. They suggested that TCDD interfered with steroid and insulin metabolic pathways involved in gonad development. The resultant experimental body burdens (2-10 pg/g wet weight) altered gonad development and reduced veliger survival in the laboratory but were comparable to levels observed in field populations. Cooper *et al.* (2009) suggested their results might explain the lack of self-sustaining populations of bivalves in estuaries contaminated by TCDD.

3.8 Sensitivity assessment – Hydrocarbons and PAHs

The count of ranked mortalities due to 'Hydrocarbons and PAHs' are summarized in Figure 3.1, Figure 3.2 and in Table 3.1 below. The data presented in Table 3.1 include all life stages and articles where life stage were not reported or were unspecified (NR). Only 7.8% of the resultant ranked mortalities were derived from 10 articles that examined adults and juveniles for the effects of 'Hydrocarbons or PAHs', of which 62.5% resulted in sublethal effects, 31.25% in significant mortalities, and 6.25% in severe mortality. Another 30% of the results were from the 11 studies that included gametes, of which 48% resulted in significant mortality and 52% sublethal effects. Life stage was not reported in three studies, which only contributed 1.9% of the results. The remaining results (59.5%) were derived from studies that examined early life stages, that is, embryos, blastulae, trochophores, veligers, D-stage larvae, pediveligers, and spat. A comparison of Figure 3.1 and Figure 3.2 shows that the early life stages dominate the patterns of mortality shown by the evidence collected. This is consistent with His *et al.* (2000) who reported that sensitivity to contaminants is usually higher in early life stages in the order embryo >veliger >pediveliger >juvenile >adult.

3.8.1 Oyster recovery rates and resilience assessment

The decline in oyster numbers is a global phenomenon with an estimated 85% of oyster reefs lost globally (Bayne, 2017). In Europe, the abundance of *Ostrea edulis* declined from the 18th century. For example, the fishery became uneconomical in the Wadden Sea in1926, surveys in the Firth of Forth found no living oyster in 1957, and the population collapsed in Loch Ryan in 1930s. However, the Loch Ryan fishery recovered by 1976 (ca 1 Million oysters) because the fishery was artificially stocked and carefully managed locally (Bayne, 2017). Native oyster beds were considered scarce in Europe as early as the 1950s (Korringa, 1952; Yonge, 1960) and are still regarded as scarce today. The picture is similar in North America for both *Crassostrea virginica* and *C. gigas*.

Table 3.1. Summary of count of ranked mortalities to 'Hydrocarbons and PAH' contaminants reported in the evidence review and resultant proposed sensitivity assessments for oyster species (N= None, VL= Very low, L= Low, M= Medium, High = High, and NS= Not sensitive).

Group /Type	Species name	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Petrochemical										
Oil spill	*Crassostrea virginica*	**1**	**1**				**2**	**N**	**VL**	**H**
Complex hydrocarbons	*Crassostrea angulata*		2		4		6	L	L	H
	Crassostrea gigas	**1**	10		4		15	N	L	H
	Crassostrea virginica	**2**	27	4		29	62	N	VL	H
Total		**3**	**39**	**4**	**8**	**29**	**83**	**N**	**VL**	**H**
Dispersant + oil	*Crassostrea gigas*		**3**				3	L	L	H
	Crassostrea virginica	1	17			8	26	L	L	H
Total		**1**	**20**			**8**	**29**	**L**	**L**	**H**
Aromatics	*Crassostrea gigas*		23				23	L	L	H
	Crassostrea rhizophorae		7				7	L	L	H
	Crassostrea virginica	1	1			1	3	N	L	H
Total		**1**	**31**			**1**	**33**	**N**	**VL**	**H**
Phenols	*Crassostrea gigas*	**1**				**2**	**3**	**N**	**VL**	**H**
Petrochemical Total		**7**	**91**	**4**	**8**	**40**	**150**	**N**	**VL**	**H**[9]
Dispersants										
	Crassostrea angulata				1		1	H	H	NS
	Crassostrea gigas	1	8		1		10	L	L	H
	Crassostrea virginica	1	5			6	12	N	VL	H
	Ostrea edulis		13				13	**L**	**L**	**H**
Dispersants Total		**2**	**26**		**2**	**6**	**36**	**N**	**VL**	**H**
Pyrogenic										
PAHs	*Crassostrea gigas*	6	2		1	4	13	N	VL	H
	Crassostrea virginica					2	2	H	N	NS
PAHs Total		**6**	**2**		**1**	**6**	**15**	**N**	**VL**	**H**
Dioxin	*Crassostrea virginica*	**1**				**1**	**2**	**N**	**VL**	**H**
Pyrogenic Total		**7**	**2**		**1**	**7**	**17**	**N**	**VL**	**H**
Biogenic										
Toluene	*Crassostrea gigas*		2				2	L	L	H
	Crassostrea rhizophorae		1				1	L	L	H
Biogenic Total			**3**				**3**	**L**	**L**	**H**
Total		**15**	**122**	**4**	**11**	**53**	**205**	**N**	**VL**	**H**

[9] See text below

For example, in Chesapeake Bay, USA, oyster population decline has been documented since the 1880s. Fishing yield has dropped from ca 550 g/m^2 in 1884 to 22 g/m^2 in 1991, and current models predict a continued decline in the population (Bayne, 2017). The population dynamics of oyster populations are dependent on positive feedback between adult abundance and recruitment via the provision of reef habitat for the settlement of larvae (e.g. adult shell), and the growth of the height of the reef about the sediment and the supply of food (facilitated by current flow) (Bayne, 2017).

Therefore, we have assumed that the recovery rates (resilience) of oyster reefs are probably similar irrespective of the species, that is, *Crassostrea* spp. or *Ostrea* spp. However, no information on the recovery rates of *Saccostrea* reefs was found.

3.8.2 Oyster sensitivity assessment

The majority of studies examined the effects of contaminants on the early life stages of oyster species (see above). This is because early life stages have been identified as the most sensitive stages, and suitable for the bioassay on pollutants (His *et al.*, 2000). It is difficult to extrapolate a direct relationship between adult oyster or oyster bed mortality (or sensitivity) from early life stage mortality (or sensitivity). However, Bayne (2017) noted that the population dynamics of oyster populations are dependent on both larval recruitment and adult abundance. Therefore, we have assumed that the survival of oyster beds is dependent on recruitment, and that even short-term recruitment failure (e.g. due to a spill) could adversely affect the abundance of adult oysters**. Hence, where the evidence suggests that the resistance of early life stages is 'None' or 'Low' we have assessed the overall sensitivity of the oyster population as 'Low',** unless the evidence suggests otherwise.

3.8.3 Oil spills

Only two studies reported on the direct effect of oils spills on oyster beds. Levings *et al.*, (1994) reported that the *Galeta* oil spills resulted in a significant reduction in *C. virginica* is beds along the mangrove fringe, which lasted at least five years. Powers *et al.* (2017) reported a 'severe' reduction the abundance of C. virginica in intertidal beds after the *Deepwater Horizon* spill. However, any effect on subtidal beds was obscured by mass mortality caused by freshwater runoff. Therefore, the evidence suggests that direct oiling of oyster beds could cause 'severe' or 'significant' mortality amongst the oysters. Hence, **resistance is assesses as 'None', resilience as 'Very low' and sensitivity as 'High' for oysters as a group**. However, evidence of the direct effect of oiling on *Ostrea edulis* was

not found. In the UK, *Ostrea edulis* beds occur in the shallow subtidal (0-20 m) and rarely in shallows exposed at low tide. Therefore, the **resistance of *Ostrea edulis* beds is assessed a 'Medium'** to represent the chance that the most shallow extent of the biotopes might be exposed to an oil spill that coincided with the lowest tides. Hence, **resilience is assessed as 'Medium'** and **sensitivity to oils spills as 'Medium'** but with 'Low' confidence.

3.8.4 Petroleum hydrocarbons – oils and dispersed oils

The effects of various petroleum hydrocarbons as oils (e.g. crude, fuel, and diesel) and dispersed oils (e.g. CEWAFs) were examined by 31 separate articles on adult, juveniles, early life stages and gametes of oyster species. There was considerable variation in the types of oil or oil and dispersant mixtures studied, experimental design and hence results. For example, 3.6% of the results from the studies of the effects of complex hydrocarbons (crudes oils, WAF/WSF/HEWAF) on oysters reported 'severe' mortality, 47% reported 'significant' mortality, 4.8% 'some', 9.6% no mortality and 35% reported only sublethal effects (Table 3.1). However, if only early life stages are included, 5.4% report 'severe' mortality, but 65% report 'significant' mortality, 7% 'some', 14% no mortality and only 7% report only sublethal effects. The effects of dispersed oils (dispersant and oil mixtures) are similar. For example, 72% of the results of the effects of dispersed oils reported 'severe' or 'significant' mortality but 100% of the results from early life stages reported 'severe' or 'significant' mortality (Table 3.1). However, only one article examined the effects of dispersed oil on adults and reported only sublethal effects. Similarly, only two articles examined the effects of oils on oysters, both of which reported sublethal effects.

His *et al.* (2000) also noted that oils, detergents, and their mixtures were usually toxic to the early life stages of bivalves at concentrations in the order of 1 ppm of higher. His *et al.*, 2000 also noted that refined oils were more toxic than crude oils but rarely at environmentally realistic concentrations. Oils and detergents also inhibit settlement in *Crassostrea virginica* while oil inhibited settlement in *Ostrea edulis* at 1-2 ppt (Renzoni, 1973b; Smith & Hackney, 1989; His *et al.*, 2000).

Therefore, the **resistance of the early life stages of oyster species** to oils (WAF/WSF/HEWAFs) and dispersed oils **is assessed as 'None'**. Although limited evidence suggests that the adults may be resistant, we may assume that loss of larval stages would result in a decline in resident populations that are dependent on recruitment for their abundance (Bayne, 2017). No evidence on the effects oils and dispersed oils on *Ostrea*

edulis was found. Hence, **resistance of oyster beds is assessed as 'Low'** to represent the result loss in annual recruitment and potential population decline. Therefore, **resilience is assessed as 'Low' and sensitivity as 'High'** but with 'Medium' confidence due to the lack of evidence from adult populations.

3.8.5 Dispersants

The majority (72%) of the results of the effects of dispersants on oyster species reported 'significant' mortality, 5.5% 'severe' mortality, 5.5% no mortality and 16.7% reported only sublethal effects. The proportion of 'severe' and 'significant' mortality results increase to 7.7% and 80.7% respectively in early life stages. However, none of the results from adults and juveniles reported 'severe' mortality, but 66% reported 'significant mortality and 33% reported sublethal effects. Therefore, it appears that dispersants alone are more toxic to oyster species as adults, juveniles, or early life stages than complex hydrocarbons and dispersed oils. This is consistent with the finding of Woelke (1972; cited in His *et al.*, 2000).

Therefore, the resistance of oyster species to dispersants is assessed as 'None', resilience as 'Very low' and sensitivity as 'High'. However, *Ostrea edulis* may be an exception as only 'significant' mortality was reported in this species. Hence, **the resistance of *Ostrea edulis* and its beds to dispersants is assessed as 'Low', resilience as 'Low' and sensitivity as 'High'.**

3.8.6 Nonylphenol

Nice *et al.* (2000; 2003) examined the effect of nonylphenol on *Crassostrea gigas* larvae or gametogenesis (Nice, 2005). Nonylphenol reduced sperm production, caused hermaphroditism in some specimens after 48-hours exposures, and had transgenerational effects in which offspring had reduced survival if one parent was exposed to nonylphenol during larval development. However, 72-hour exposure of larvae to 1.0 mg/l nonyphenol resulted in 100% larval mortality (Table 3.1). **Therefore, the resistance of oyster species to nonylphenol is assessed as 'None', resilience as 'Very low' and sensitivity as 'High'.**

3.8.7 Others

The results for 'other' petrochemicals are dominated by aromatic hydrocarbons (Table 2.3) and detailed in the 'evidence summary spreadsheet'. Exposure of Crassostrea virginica embryos for 48-hours to 25 mg/l 2,4-Dimethylphenol was reported to result in 'severe' mortality in one study. Exposure to biphenyl was reported to result in sublethal effects in one

study. However, exposure to one or more of 19 aromatic petrochemicals (individually) was reported to result in significant mortality in five of the studies reviewed. Therefore, the sensitivity of the early life stages of oyster species to 2,4-Dimethylphenol is probably 'High' (resistance is 'None' and 'resilience 'Very low'), albeit at high concentrations. But oysters are probably 'Not sensitive' to biphenyl, albeit based on limited evidence. Overall, the early life stages and hence populations of oyster species are probably **of 'High' sensitivity (resistance and resilience are 'Low')** to aromatic petrochemicals depending on the individual chemical, the exposure concentration, exposure duration and life stage exposed.

3.8.8 Polyaromatic hydrocarbons (PAHs)

The results of exposure to PAHs were split evenly between 'severe' and sublethal effects, albeit based on only 12 articles. However, early life stages were more sensitive, with 64% of the results reporting 'severe' mortality', 18% 'significant and only 9% either no mortality or sublethal effects. PAH exposure was reported to result in reduced scope for growth in adults, reduced sperm motility and reduced fertilization rate and abnormal larval development (His *et al.*, 1997; Jeong & Cho, 2005; Choy *et al.*, 2007; Kim *et al.*, 2007; Wessel *et al.*, 2007; Nogueira *et al.*, 2017; Xie *et al.*, 2017b). The toxicity of PAHs also increased in light (UV exposure) (Lyons *et al.*, 2002). Therefore, the **resistance of oyster species to PAHs is assessed as 'High', resilience as 'Very low' and sensitivity as 'High'.**

3.8.9 Dioxins

Cooper *et al.* (2009) investigated the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the embryonic development of *Crassostrea virginica*. They reported 97-99% mortality in larvae at 2-10 µg/l TCDD ('severe' mortality) but only sublethal effects on adults. Cooper *et al.* (2009) suggested their results might explain the lack of self-sustaining populations of bivalves in estuaries contaminated by TCDD. Therefore, **resistance of oyster species to TCDD is assessed as 'None' in early life stages but 'High' in adults.** However, if Cooper *et al.* (2009) suggestion is correct, and TCDD contamination might result in population decline, **the resistance of oyster beds may be assessed as 'Low', resilience as 'Low' and sensitivity as 'High'** but with 'Low confidence as the evidence is based on a single study.

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4 Transitional metals and organometals

A total of 266 results (ranked 'worst-case' mortalities) were obtained from 151 articles that examined the effects of transitional metals and organometals on oyster species. *Crassostrea gigas* was the most studied species, which provided the most results from studies of metals (44%) and organotins (9.4%) (Figure 4.1). *Crassostrea virginica* was the next well studied species with 18.8% of results for metals and 4% for organotins but *Ostrea edulis* only appears in 2.3% of results for metals and 1.9% for organotins.

4.1 Transitional metals

The most studied metals were in the order copper (Cu) >cadmium (Cd) >zinc (Zn) >silver (Ag) =mercury (Hg). Unsurprisingly, tributyltin (TBT) and tributyltin oxide (TBTO) were the most studied organotins with 55% and 39% of the results respectively (Figure 4.2). However the majority of the effects of exposure to transitional metals reported (75%) resulted in mortality, 3% in 'no' mortality and 22% in sublethal effects. As above, most of the results on the effects of transitional metals were provided by studies of early life stage (54%), followed by adults and juveniles (23%) (Figure 4.3). The evidence is summarized below.

Abbe *et al.* (1994) examined the effects of silver on *Crassostrea virginica* under controlled conditions. Silver had no significant effect on the condition index of the oysters at concentrations of 2 and 7 µg/l over a 19-day exposure period.

Almeida *et al.* (1998) exposed *Crassostrea gigas* to two different concentrations of lead, 470 and 790 µg/l. The exposure experiment was run over a period of four months, with water renewals performed every two days. Results showed that the shells of oysters exposed to the higher concentration of lead grew less than in the other treatments. However, there were no significant differences between treatments for length or tissue weight.

Amiard *et al.* (2005) investigated the influence of chromosomal ploidy and metal exposure in *Crassostrea gigas*. Young oysters were exposed to silver 10 µg/l, Cadmium 10 µg/l or Copper 30 µg/l for 15 days. No significant changes to the condition index of the mussels were caused by genetic type or exposure conditions.

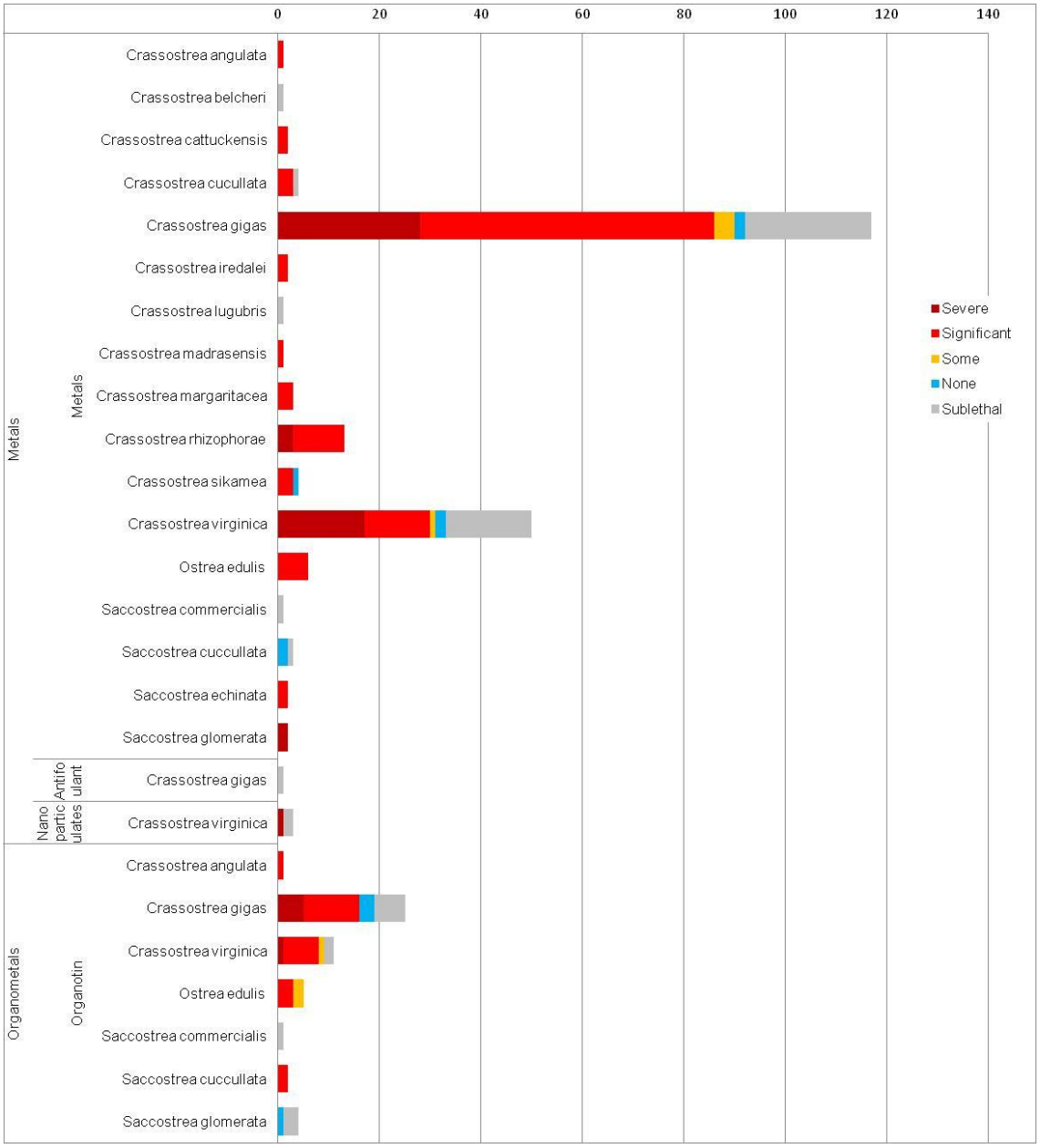

Figure 4.1. Count of ranked mortalities due to exposure to transitional metals and organotins in oyster species. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects.

Barrera-Escorcia *et al.* (2010) investigated the effects of cadmium exposure on the condition index and filtration rate of *Crassostrea virginica*. The oysters were either exposed to 0, 95 or 170 µg/L cadmium for 12 days, with daily renewals of cadmium. Cadmium exposure significantly reduced the filtration rate. Reductions in the condition index and increased mortality (ca 18%) were observed at the highest tested concentration of cadmium (170 µg/L).

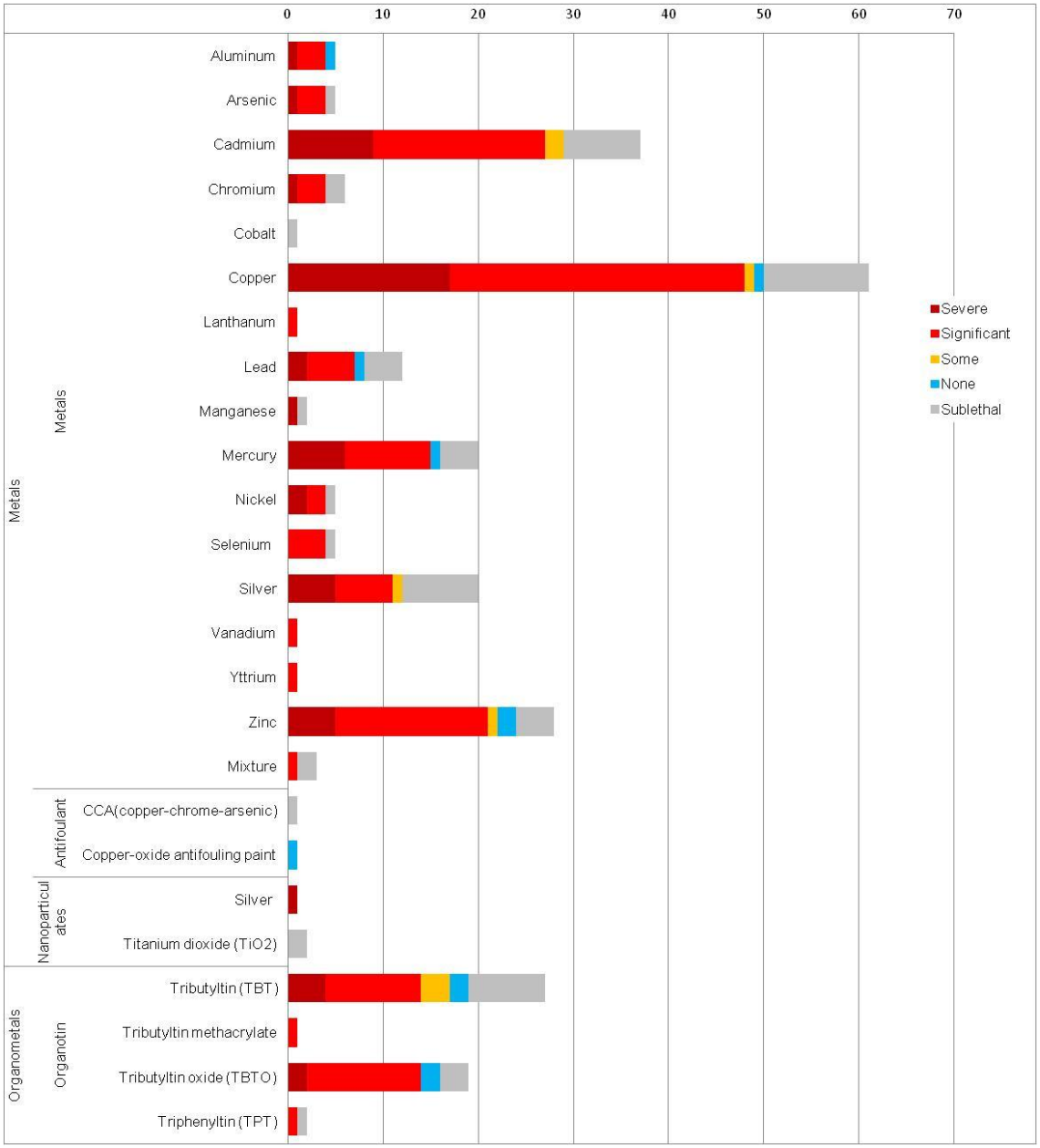

Figure 4.2. Count of ranked mortalities due to exposure to oyster species to the range of transitional metals and organotins examined. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects.

Beiras & His (1994) examined the effects of mercury (Hg) concentrations ranging from zero (control) to 1,024 µg/l upon embryogenesis, survival, growth, and metamorphosis of *Crassostrea gigas* (Thunberg) oyster larvae in static containers under laboratory conditions. Embryogenesis was abnormal in 50% of the individuals exposed to 11 µg/l. The 48-hour LD50 for D-shaped, umbonate and pediveliger larvae were 33, 115 and 200 µg/l respectively. The increase in LD50 with age was partially explained by the larval weight increase, although weight-specific tolerance to Hg was higher in smaller larvae. The metamorphosis rate was significantly reduced when competent pediveligers were exposed to 64 µg/l for 48 hours prior

to the addition of the metamorphosis inducer epinephrine. Growth was significantly reduced at 4 µg/l by a factor 0.7 and by a factor of 0.4 at 8 µg/l while acute mortality was not noted until 32 µg/l. Beiras & His (1994) noted that reduced growth and, hence, a prolonged time in the plankton at environmentally realistic concentrations of Hg, could adversely affect recruitment.

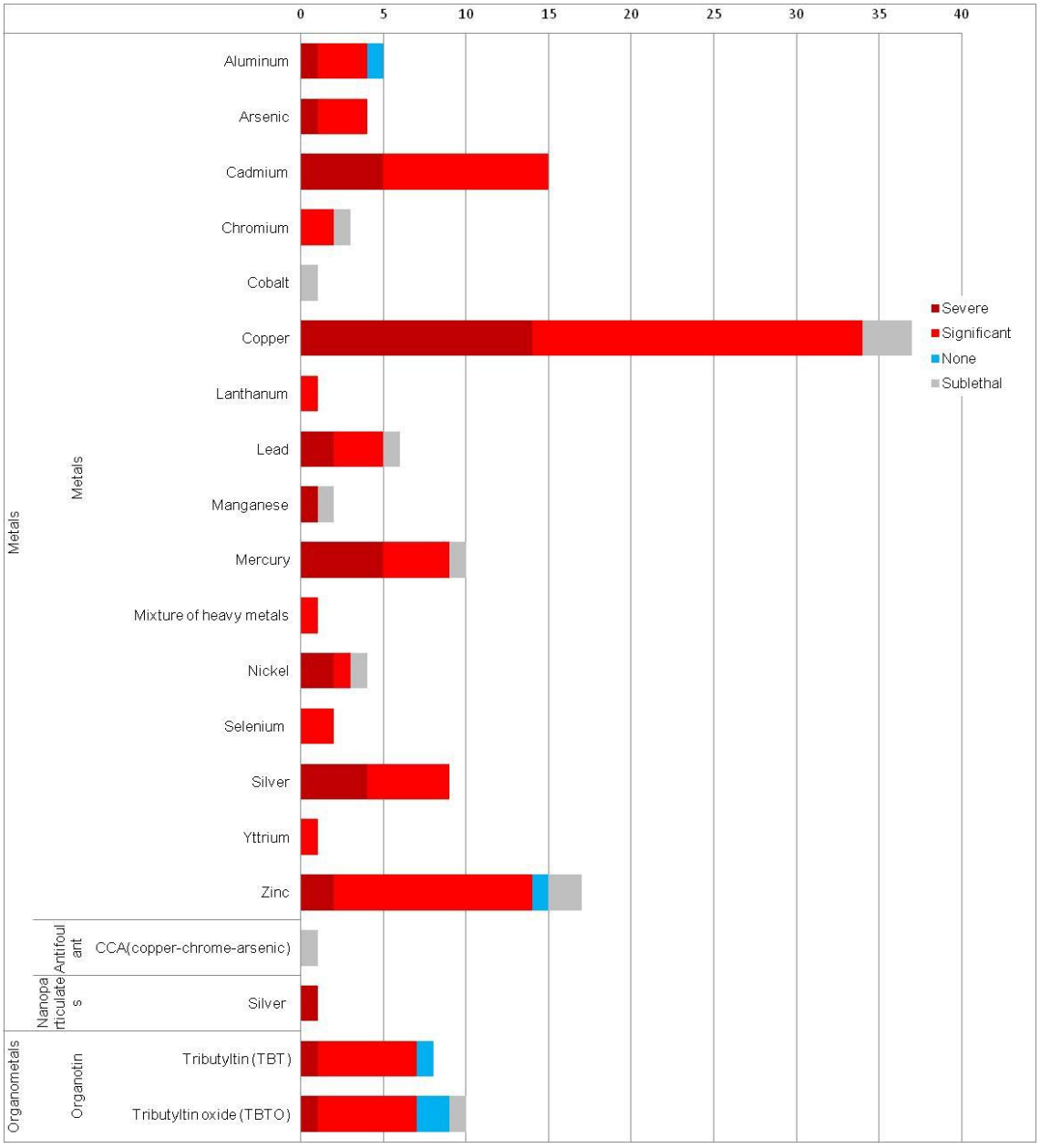

Figure 4.3. Count of ranked mortalities due to exposure to the early life stages of oyster species to the range of transitional metals and organotins examined. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects.

Berthet *et al.* (1992) investigated the toxicity of silver on bivalve molluscs over a 28-day period. The oyster *Crassostrea gigas*, was exposed to concentrations of silver between 1 and 100 µg/L. The mean lethal time (LT50) values for the concentrations 1-10, 100 and 1000 µg/L were 16, 8.7 and 4.5 days respectively. The lethal time decreased with increasing concentration of silver. *Crassostrea gigas* were more sensitive to silver exposure than *Scrobicularia plana* but less sensitive than *Mytilus galloprovincialis* and *Chlamys varia*.

Bookes *et al.* (2007) investigated the effects of dissolved organic carbon on the toxicity of copper to embryos of *Crassostrea gigas*. The oyster embryo-larval bioassay was used to determine toxicity. Embryos were exposed to copper chloride for 24 hours to assess the effects of the metal on larvae development. The exposure to copper caused significant effects with an increase in the number of abnormalities, the EC50 of copper ranged between 5.47 and 8.05 µg/L.

Bouilly *et al.* (2006) investigated the effects of cadmium exposure on *Crassostrea gigas*. Adults and juveniles were exposed to two different concentrations of cadmium (0.05 and 0.5 µg/L) for two months. After the two-month exposure period, adult oysters were crossed to assess hatch rates and larval growth. For adult oysters exposed to 0.0, 0.05, and 0.5 µg/L cadmium, mortality rates were 27.1%, 39.3%, and 17.9%, respectively. For juvenile oysters exposed to 0, 0.05, and 0.5 µg/L cadmium, mortality rates were 23.3%, 33%, and 15.7%, respectively. The hatch rate of oysters exposed to 0, 0.05, and 0.5 µg/L cadmium were 70.3%, 74.8%, and 94.5%, respectively. The offspring of exposed oysters did not show any significant differences in larval growth compared to the control treatment.

Boyden *et al.* (1975) investigated the effects of zinc on the success of settlement of the oyster *Crassostrea gigas*. The oyster larvae were exposed to concentrations of 125, 250, and 500 µg/l zinc for a period of five days before being transferred to clean water for five days. The mortality of the larvae for 125, 250 and 500 µg/l zinc was calculated at 14, 28, and 30% respectively. Settlement of the larvae was delayed in the zinc treatments compared to the control, with maximum settlement occurring two days earlier in the control tanks. The zinc exposure was detrimental to settlement success, with the total settlement in all the zinc treatments less than that in the controls.

Brereton *et al.* (1973) investigated the effects of zinc on the growth and development of *Crassostrea gigas*. Eggs were exposed to concentrations of zinc between 50 to 500 µg/L for two to five days, and then transferred to clean water for a further five days for analysis. In trial 1 (125, 250 & 500 µg/l Zn), the percentage of eggs developing into larvae after 48 hours

was 84% at 125 µg/L, 52% at 250 µg/l, and 10% at 500 µg/l. Abnormal development, delayed development and slow growth rates were present in all treatment groups. At 250 µg/L abnormal movement was evident and practically no growth took place. The larvae that did survive the 500 µg/L zinc treatment had retarded growth. In trial 2 (50,100,150 & 200 µg/L Zinc), the occurrence of abnormal behaviour and structure increased with increasing concentration of zinc. At 50 µg/L zinc has reduced effects on the larvae except for a few individuals that had rotational movement, pale in colour or were inactive by day six. At concentrations >150 µg/L zinc most of the larvae were affected showing abnormal development. Size and growth rate were affected from 150 µg/L zinc. After four days of exposure, the growth rate of larvae in the 100 and 150 µg/L zinc treatment showed a sharp decrease in growth. At 200 µg/L zinc, most of the larvae were considerably smaller than the other treatments. In conclusion, mortality, behaviour, structural development, size, and growth rate were all adversely affected in the presence of zinc.

Bryan *et al.* (1987) reviewed the long-term metal pollution of Restronguet Creek in the Falmouth Estuary. *Ostrea edulis* was able to survive in the lower reaches of Restronguet Creek, one of the most heavy metal polluted estuaries in the world, where metals from mining wastes reached concentrations several orders of magnitude above normal. For example, the copper levels in the Carnon River leading into the Creek were 100x (ca 593 µg/l Cu) the reported EC50 for abnormal embryo development in *Crassostrea gigas* (5.3 µg/l). Bryan *et al.* (1987) noted that *O. edulis* in the creek had been reported to be highly polluted, i.e. specimens were green since the 1880s, in 1927, and in specimens collected in 1980 (the present study). *Ostrea edulis* from the Falmouth estuary were shown to be able to detoxify metals (Cu and Zn) in amoebocytes. Bryan *et al.* (1987) concluded that this detoxification mechanism allowed *O. edulis* to survive in the lower reaches of the creek, while the cockle *Cerastoderma edule* was excluded from the creek.

Calabrese *et al.* (1973) investigated the toxicity of 11 metals to embryos of *Crassostrea virginica*. The concentrations at which 50% of the embryos did not develop over a 48-hour period were determined. Aluminium was non-toxic at the tested concentrations up to 7,500 µg/L (NR-ZERO: 7,500 µg/L). However, the other metals tested resulted in toxicity as follows: arsenic LC50: 7500 µg/L; cadmium LC50: 3800µ g/L; chromium LC50: 10,300 µg/L; Copper LC50: 103 µg/L; lead LC50: 2450 µg/L; manganese LC50: 16,000 µg/L; mercury LC50: 5.6 µg/L; nickel LC50: 1180 µg/L; silver LC50: 5.8 µg/L; and zinc LC50: 310 µg/L.

Calabrese *et al.* (1977) investigated the effects of silver and mercury on the survival of *Crassostrea virginica* through a series of exposure experiments. Observations showed that embryos were more sensitive than larvae. The concentrations of mercury and silver that caused 50% mortality in embryos (LC50) in 48 hours were 5.6 and 5.8, respectively (Calabrese *et al.*, 1973). However, the concentrations of mercury and silver that caused 50% mortality in larvae in 12 days were 12 and 25 µg/L, respectively.

Calabrese *et al.* (1977b) investigated the survival and growth of *Crassostrea virginica* under heavy metal stress. The concentrations at which 5% (LC5), 50% (LC50) and 95% (LC95) of the larvae died were determined, and the percentage of growth reductions at LC5 and LC95 were calculated. The order of toxicity of the metals was Hg >Ag >Cu >Ni with LC50 values of 12, 25, 32.8 and 1200 µg/L, respectively. The growth of the larvae was not reduced at LC5 values, but at LC50 values growth was significantly reduced.

Caplat *et al.* (2012) exposed adult *Crassostrea gigas* to zinc for two different time periods at two different concentrations. The oysters were exposed to either 530 µg/L zinc for 10 weeks or 10,200 µg/L zinc for 7 days. No mortality was observed in the long-term exposure, but 81.8% of the oysters died at the end of the short-term exposure.

Chen (1994) investigated the effects of copper on the respiration rate of *Crassostrea gigas* during 180-minute exposures. The respiration rates were affected at concentrations higher than 25 µg/L.

Chung (1980) investigated the lethal concentrations of copper, cadmium, chromium, and zinc, over a variety of durations. Oysters were exposed to the metals for 48, 96, 168 and 216 hours to establish the concentrations that caused 0%, 50% and 100% mortality within those periods. The order of toxicity of the metals to *Crassostrea rhizophorae* was cadmium >copper >chromium >zinc. The LC50 concentrations of the metals dramatically decreased as the period increased. For cadmium, the LC50s at 48, 96, 168 and 216 hours were >60,000, 5,000, 800 and 600 µg/L, respectively. For Chromium, the LC50s at 48, 96, 168 and 216 hours were >80,000, >80,000, 40,000 and 7000 µg/L, respectively. For copper the LC50s at 48, 96, 168 and 216 hours were >40,000, 40,000, 2,500 and <1000 µg/L, respectively. For zinc the LC50s at 48, 96, 168, and 216 hours were >80,000, >80,000, 40,000 and 1600 µg/L, respectively.

Coglianese (1982) investigated the effects of salinity on the toxicity of copper and silver on *Crassostrea gigas* embryos. Embryos were exposed to salinities of 16.5, 22.7 and 33ppt,

copper concentrations between 0 to 10 µg/L and silver concentrations between 0 to 18 µg/L for 48 hours. Exposure to salinities of 22.7 and 33 ppt had no significant effect on normal embryonic development, but significant effects were observed below 22.7 ppt. Analysis of variance indicated a significant interaction between salinity and metal concentration in increasing embryotoxicity. The interactive effect was manifested at low salinities where a large decrease in percentage normal embryonic development occurred at the lowest concentrations of copper and silver.

Coglianeset & Martin (1981) investigated the effects of metals copper and silver on the embryonic development of *Crassostrea gigas*. Copper concentrations were tested between 0 and 12 µg/L, and silver concentrations were tested between 0 and 18 µg/l. As the concentration of both silver and copper increased the percentage of abnormal larvae increased. Only 50% of the embryos developed normally at 10 µg/L copper. However, silver concentrations between 16 and 18 µg/l resulted in 50% normal development.

Connor (1972) investigated the effects of mercury on the survival of larvae and adult *Ostrea edulis* over 48 hours. An LC50 of 4200 µg/L was established for adults and an LC50 between 1 and 3.3 µg/L for larvae.

Cunningham (1976) exposed juvenile oysters (*Crassostrea gigas*) to 12-hour pulses of 10 or 100 ppb of mercuric acetate for up to 47 days in static containers in the laboratory. Shell height (from the hinge to the posterior margin) was measured to determine shell growth rate. Growth was inhibited by 77% at 100 ppb mercuric acetate and by 33% for 10 ppb compared to controls after 47 days. Shell growth rates were comparable to controls with 34 days (100 ppb) and 20 days (10 ppb) during a 162 day depuration period. Cunningham (1976) noted that shellfish can concentrate heavy metals in their tissue and that mortality is a poor indicator of mercury accumulation. Cunningham (1976) cited Cunningham & Tripp (1973) who reported that mercury residues in adult oysters of 27,960 (after exposure to 10 ppb) did not cause mortality higher than 6% but that exposure to 100 ppb resulted in 50% mortality when mercury tissue residue was 140,710 ppb.

De Campos *et al.* (2022) exposed juvenile *Crassostrea gigas* to silver at concentrations between 10 and 10,000 µg/L for 96 hours. The condition index of the oysters was significantly influenced with a LOEC of 100 µg/L. In addition, the survival in air test produced significant differences in survival time between the oysters in the control and those exposed to 10,000 µg/L.

Devos *et al.* (2012) investigated the effect of chronic zinc exposure on the spat of *Crassostrea gigas* from metamorphosis up to 10 weeks. The oyster spat was exposed to concentrations of zinc between 3.89 and 33 µM. Zinc exposure had significant effects on the growth rate of the oyster spat, as the dose of zinc increased the growth rate of the spat decreased. The zinc concentration that reduced the growth rate to 50% (EC50) was calculated at 7.55 µM. The survival of the spat was affected by zinc exposure concentrations of 3.89, 7.46, and 11.44 µM induced no significant mortality until 53 days of exposure. Mortality occurred significantly after 6 days at 33.0 µM, after 26 days at 15.5 µM, and after 53 days at 7.46 mM. At the highest tested concentration (33 µM) 100% mortality occurred within 20 days.

Elfwing & Tendengren (2002) investigated the effects of copper on oxygen consumption, ammonia excretion, clearance rate and absorption efficiency and scope for growth, on two intertidal oyster species, *Saccostrea cucullata* and *Crassostrea lugubris*, and one subtidal species, *C. belcheri*. The oysters were exposed for a short-term (12 hours) to 20 µg/L copper in static conditions. Significant differences were observed in the respiration rate of *Saccostrea cucullata*, and with the clearance rate and scope for growth of *C. belcheri*.

Fichet & Miramand (1998) investigated the effects of vanadium on the development and growth of *Crassostrea gigas*. Short-term (2-day) exposure experiments were conducted at 50, 100, 250, 500 and 750 µg/L vanadium. At the lowest tested concentration of 50 µg/L toxic effects of vanadium were observed to cause abnormal larvae development. At 500 and 750 µg/L 50% of the larvae developed abnormally. The growth of the larvae was not affected by the exposure to 750 µg/L of vanadium.

Fitzgerald *et al.* (2019) assessed the toxicity of cadmium and mercury on juvenile Eastern oysters, *Crassostrea virginica*, following four weeks of exposure to a low exposure designed to be a realistic field concentration, and a high exposure set as an elevated, yet sublethal concentration. The condition index in oysters exposed to cadmium decreased with increasing concentration. After four weeks of exposure to cadmium, the condition index was higher in the control and statistically lower in the highest cadmium exposure treatment. For mercury, after the four weeks of exposure, there were no differences between the condition index of the oysters in the control and those in either of the two mercury treatments.

Fowler *et al.* (1981) examined the effects of cadmium and selenium on the respiration of *Crassostrea virginica*, under flow-through laboratory conditions. Oysters were exposed to 400 µg/L of cadmium and selenium independently and as a mixture over a 14-day period, during

which time the respiration rate of the oysters was recorded at daily intervals. Selenium exposure did not affect the respiration rate of the oysters during the 14-day exposure. However, cadmium exposure increased respiration, the mixture of cadmium and selenium increased the respiration rate of the oysters but not as much as cadmium alone.

Gamain *et al.* (2016) investigated the effects of copper and the herbicide Metolachlor exposure at different salinities on the early development of *Crassostrea gigas*. The oyster embryos were exposed to copper at 1, 10, or 50 for 24 hours, there was significant dose dependent embryotoxicity. Embryotoxicity was observed from the lowest tested concentration at µg/L copper. At 10 µg/L copper, there was 54.4% abnormal D-larvae, at 50 µg/L there was 100% abnormal D-larvae. An EC50 of 7.35 µg/L was established. Oyster embryos were exposed to Metolachlor at 0.01, 0.10 or 1µg/L for 24 hours. There were dose-dependent increases in abnormal D-larvae following exposure to Metolachlor. The EC50 value was not calculated because the highest tested Metolachlor concentration did not exceed the 50% effect.

Gamain *et al.* (2017) investigated the effects of copper and Metolachlor exposure at different temperatures on the early development of *Crassostrea gigas*. The oyster embryos were exposed to copper at 1, 10 or 50 µg/L for 24 hours. There was significant dose dependent embryotoxicity with an average of 31, 54 and 95% at 1, 10 and 50 µg/L respectively for oysters' native to Arcachon Bay; and 42, 65 and 100% for the hatchery oysters. Oyster embryos were exposed to Metolachlor at 0.01, 0.10 or 1 µg/L for 24 hours. There were dose-dependent increases in abnormal D-larvae following exposure to Metolachlor. Mortality of oysters exposed to Metolachlor exceeded 25%, 40% and 50% when exposed at 0.01, 0.10, or 1 µg/L, respectively.

Gao *et al.* (2017) investigated the effects of different concentrations of copper on the survival of the Pacific oyster *Crassostrea gigas* during 96 h exposure experiments. The results showed that the LC50 of copper was 21.75 mg/L. Mortality of oysters increased with increasing copper concentration, with 96 hours of exposure causing 10%, 45%, 75%, 95% and 100% mortality occurred in the 15, 20.25, 27.34, 36.91 and 50 mg/l treatment groups, respectively. The control group was normal and had no death.

Geffard *et al.* (2001) investigated the effects of contaminated sediment exposure on spermiotoxicity and embryotoxicity of *Crassostrea gigas*. The toxicity of two marine sediments, one polluted by polycyclic aromatic hydrocarbons (PAH) and the other by heavy metals was investigated by exposing sperm and embryos to suspensions of sediment at 0,

0.5, 1, 2.5 and 5 g/L. Sperm were exposed for 30 minutes before eggs were added for two hours to assess fertilization success. Embryos were exposed to sediment suspensions for 24 hours before being assessed for developmental abnormalities. Fertilization success was not influenced by exposure with only 5% and 7.2% unfertilized eggs in the PAH and metal contaminated sediment, respectively. Abnormalities in larval development were observed in both PAH and metal contaminated sediment exposures with LC50s of 0.65 g/l for metal contaminated sediment (Bidassoa Estuary, whole sediment), and 0.72 g/l for PAH contaminated sediment (Arcachon, whole sediment).

Geret *et al.* (2002) investigated the influence of metal exposure on *Crassostrea gigas*. The oysters were exposed to silver, cadmium, copper, mercury and zinc at respectively 20; 200; 40; 20 and 1000 µg/l in seawater for a period of 4 or 21 days. No mortality was recorded during the four-day experiment. But, during the 21-day experiment, the mortality of oysters from exposure to silver, cadmium, copper, mercury, and zinc, were 4, 17, 13, 0 and 13%, respectively.

Glickstein (1978) investigated the acute toxicity of mercury and selenium to *Crassostrea gigas* embryos. Oyster embryos were exposed to mercury at 2,5 and 10 µg/l and to selenium at 10, 100, 1000 and 10,000 µg/L for 48 hours. Mercury concentration that caused abnormal development in 50% of the oyster embryos (EC50) during the 48-hour exposure was 5.7 µg/l but the 48-hour EC50 for selenium was greater than 10,000 µg/l.

Golding *et al.* (2015) exposed *Saccostrea echinata* embryos to aluminium for a period of 48 hours, the concentration that caused 50% abnormal development (EC50) was calculated at 410 µg/l. In addition, copper used as a reference contaminant had a 48-hour EC50 of 16 µg/l.

Harrison & Rice (1978) examined the effects of copper exposure on the survival of adult pacific oysters (*Crassostrea gigas*), under flow-through laboratory conditions. Adult oysters were exposed to copper chloride at concentrations between 180 to 1300 µg/L for up to 12 days. Survival of the oysters was monitored during exposure. Analysis of the data determined a 48-hour LC50 of 650 µg/L, a 96-hour LC50 of 430 µg/l and a 168-hour (7-day) LC50 of 30 µg/l.

Harrison *et al.* (1980) investigated the effects of copper on the development of *Crassostrea gigas* embryos. Oyster embryos were exposed to copper at concentrations between 5 and

100 µg/l for 48 hours. Abnormal development was observed at all tested concentrations with LC50 and LC100 values of 12 and 20 µg/L, respectively.

His & Robert (1981) investigated the effects of copper chloride on the fertilization, development, and survival of *Crassostrea gigas*. The first experiment assessed the effects on fertilization and subsequent development of gametes exposed to concentrations of between 5 and 100 µg/l. Fertilization was unsuccessful above 50 µg/L, and under 25 µg/L larval abnormalities increased with increasing concentration. Growth of the larvae was only assessed to treatments <25 µg/L, but no significant differences were observed. The second experiment exposed larvae to copper at concentrations between 25 and 500 µg/L. Total (100%) mortality occurred within seven days at concentrations of 100 µg/L and above. Significant mortality was also observed at 50 µg/L with 45% mortality within seven days. The behaviour of the surviving larvae was abnormal with abnormal rotational movements. At concentrations >100 µg/L, the effects on the growth of the larvae was significant as no evidence of growth was observed.

His & Robert (1982) investigated the effects of copper sulphate on the development of *Crassostrea gigas* larvae. Embryos were exposed to concentrations of copper between 10 to 500 µg/L for 24 or 48 hours, before being assessed for abnormalities. Significant differences compared to the control were observed at concentrations from 50 µg/l, with 100% abnormalities at 150 µg/l. The effects of copper on larval growth were also observed from two life stages. Embryos and 24-hour larvae were exposed to copper for 12 days. Significant differences in growth were observed in embryos exposed to 50 µg/L and in larvae exposed to 250 µg/L for 24 hours.

His *et al.* (1996) examined the toxicity of the effluent from an aluminium plant on *Crassostrea gigas* oyster embryogenesis (lethal effects) and larval growth (sublethal effects). Liquid and solid phases of the effluent were tested separately, and the effects of mixing during exposure were also evaluated. The effluent was highly toxic, causing abnormal embryogenesis at 0.03 to 1 g/l and reduced growth at 0.01 to 0.3 g/1. The solid fraction was more toxic than the liquid fraction. Mixing during exposure consistently increased both lethal and sublethal toxicity.

His *et al.* (1999) investigated the effects of metals and pesticides on the development of *Crassostrea gigas* larvae. Embryos were exposed to copper at 10, 25, 50, 100 and 150 µg/l. Copper had significant effects on the larvae, with increased abnormalities at concentrations up to 50 µg/L, and 100% of larvae abnormal at 100µg/l. His *et al.*, (1999) calculated an EC50

of 37µg/l copper. The concentrations of lead tested on embryos were 10, 25, 50, 100, 150, 300, 600 and 1200 µg/L. Lead had significant effects on larvae development. Abnormalities increased slowly at the higher concentrations, reaching 31.2% at 1200 µg/L. The insecticide Mercaptodimethur and herbicide Glyphosate were tested at 10, 25, 50, 100 and 200 µg/L. Neither Mercaptodimethur nor Glyphosate had any significant effects on the development of the larvae. The herbicide Dinoterbe was tested at 10, 25, 50, 100, 150, 200 and 250µg/L. Dinoterbe had significant effects on larvae development with significant effects at the lowest tested concentration (10 µg/l). An EC50 of 72.2 µg/l was established for Dinoterbe.

Knezovich *et al.* (1981) investigated the effects of copper on the development of *Crassostrea gigas* embryos with a 48-hour static exposure experiment. The embryos were exposed to copper at 5, 10, 15, 20, 40 and 100 µg/l. The resultant percentage of abnormal larvae produced from the copper exposures was 13.4, 42.7, 56.5, 97.6, 99.6 and 100%, respectively. The LC50 and LC100 were established at 12 and 20 µg/l, respectively.

Kumaraguru & Ramamoorthi (1978) report the toxicity of copper on *Crassostrea madrasensis*. The oysters were exposed to copper concentrations of 52, 62, 72, 82, 86, 92 and 112 µg/L, for seven days to establish the LT50s (median lethal time) for each concentration. The lethal concentration that caused 50% mortality over a 96-hour period was calculated at 88 µg/L copper.

Kumari & Nair (1992) exposed *Saccostrea cucullata* to 10 and 20 µg/L copper, and 50 and 100 µg/L zinc for a period of 60 days. No mortalities occurred during the 60-day exposures.

Lannig *et al.* (2006) investigated the effects of cadmium exposure and temperature on the basal metabolic rate (BMR) and mortality of *Crassostrea virginica*. The BMR significantly increased in cadmium exposed at both 20 and 28°C. After 30 days of exposure to 50 µg/L cadmium at 20°C, no mortality occurred. However, the combined exposure to cadmium and elevated temperatures of 28°C resulted in significantly higher mortality than temperature stress or cadmium stress alone. At 28°C, 25% and 45% of oysters died in control and cadmium-exposed groups, respectively.

Lannig *et al.* (2006b) investigated the effects of temperature and cadmium exposure on *Crassostrea virginica*. The oysters were exposed to 41.1 µg/l cadmium at 20, 24, and 28℃ for up to 40 days, but oysters in 28℃ control treatments did not survive more than 30 days. Cadmium exposure at 20 and 24℃ had no significant effects on valve closure, condition index, or survival. However, significant effects on oxygen consumption were observed from

cadmium exposure at all temperatures. Cadmium exposure at 28℃ had significant effects on the condition index with a 31% decrease after 20 days. Cadmium exposure at 28℃ significantly decreased survival with 46% mortality within 20 days, compared to the 28℃ control that experienced 25% mortality within 30 days of exposure.

Lin *et al.* (1992) examined the effect of metal contamination (Cd, Cu, and Zn) on glycine update and filtration rates in *Crassostrea gigas* under flow-through conditions in the laboratory. The filtration rate (volume of water completely cleared of colloidal carbon per unit time) by control oysters was 36.60 ml/g hr +/- 7.68. Filtration rates decreased with increasing concentrations of Cd^{2+} and Zn^{2+}. In 8-16 mg/l Cu^{2+}, filtration rates were significantly higher than the control, but in Cu^{2+} concentrations above 32 mg/l, filtration rates were lower than controls. The uptake rate of glycine from 1 µM solution was 37.79 µmol/g/hr. Metals inhibited glycine uptake in the order $Cu^{2+} > Cd^{2+} > Zn^{2+}$. In 128 mg/l Cu^{2+}, glycine uptake rate was reduced to 3.96 nmol/g/hr or 10.5% of control. The rate of glycine uptake by filter feeding bivalves is dependent on rate of water pumping rate. The volume specific glycine transport (amount of glycine transported/unit volume of seawater completely cleared of colloidal carbon) by control oysters in 1 µM glycine concentrations was 1.03 µmol/l. The volume specific glycine transport remained constant in increasing Zn^{2+} concentrations and declined in increasing Cu^{2+} concentrations. This suggested different effects of the metals on particle filtration and the epithelial amino acid carriers. The apparent volume specific glycine transport increased to 2.14, µmol/l in 128 mg/l Cd^{2+}. The authors concluded that this increase in volume specific transport suggested the oyster may take up cadmium complexed glycine (Lin *et al.*, 1992). No mortality was reported.

Losso *et al.* (2004) investigated the sensitivity of bivalve mollusc embryos exposed to three pesticides (Carbofuran, Atrazine, and Malathion) and copper. All the compounds were embryotoxic in *Crassostrea gigas*, with EC50 values of 22.5mg/l for copper, 10 mg/l for Malathion and less than 10 mg/l for Carbofuran and Atrazine.

MacInnes & Calabrese (1978) examined the effect of metal exposure on embryonic development in *Crassostrea virginica*. They examined the development of embryos for 48 hours while exposed to a range of concentrations of copper (Cu), zinc (Zn), mercury (Hg), and silver (Ag) at 20, 25 and 30°C. They also examined mixtures of copper and zinc and mercury and silver. The percentage of abnormal larvae (D-larvae) in experimental treatments was corrected for the percentage abnormal larvae in controls and used to determine EC50 for each metal and mixtures. They reported that Hg >Cu >Ag >Zn in toxicity

to embryo development. The toxicity of Zn, Cu, and Hg were not influenced by temperature but silver was more toxic at 20°C than at 25°C. Cu:Zn mixtures demonstrated a simple additive effect on toxicity but Hg:Ag mixtures were less than additive in toxicity (MacInnes & Calabrese, 1978).

MacInnes & Calabrese (1979) investigated the combined effects of salinity, temperature, and copper exposure on embryos and early larvae of *Crassostrea virginica*. The experiment used temperatures of 20, 25, and 30℃ and salinities of 17.5, 22.5, and 27.5‰. Copper concentrations of 0, 5, 10, and 20 µg/l were used for the embryos and 0, 30, 60, and 90 µg/L for the larvae. The capacity of the embryos to adapt to the temperature-salinity changes was impaired when exposed to 20 µg/l copper. The temperature had the greatest effect on the larvae when exposed to 30, 60, and 90 µg/l copper. The interaction between temperature and salinity was significant only at the higher levels of copper.

Mai *et al.* (2012) investigated the embryotoxicity and genotoxicity of two metals (copper and cadmium) and two pesticides (Metolachlor and Irgarol) on *Crassostrea gigas* larvae. Embryotoxicity was measured by calculating the percentage of abnormal D-shaped larvae. After 24 hours exposure, significant increases in the percentage of abnormal D-larvae were observed from 0.1 µg/L copper, 10 µg/L cadmium and 0.01 µg/L for both Irgarol and Metolachlor. EC50s of 12.5 µg/L copper, 212.3 µg/L cadmium and >10 µg/L for both Irgarol and Metolachlor were established.

Martin *et al.* (1981) investigated the toxicity of ten metals on the development of *Crassostrea gigas* embryos. The oyster embryos were exposed to metals through the completion of embryogenesis (48 hours), and the effects on abnormal development were monitored. The concentrations that caused 50% abnormal development (EC50) were 5.3 µg/L for copper, 6.7 µg/L for mercury, 22 µg/L for silver, 119 µg/L for zinc, 326 µg/L for arsenic, 611 µg/L for cadmium, 349 µg/L for nickel, 758 µg/L for lead, 4538 µg/L for chromium, and greater than 10,000 µg/L for selenium.

Metayer *et al.* (1990) investigated the toxicity of silver on *Crassostrea gigas*. The oysters were exposed to silver at 1, 10, 100 and 1000 µg/L in order to determine the time it took for 100% mortality to occur. In the 1 and 10 µg/L treatments, 100% mortality did not occur within the 16 days trial, therefore the NR-LETH was classed as greater than 16 days. At 100 µg/L, 100% mortality occurred at 8.7 days, and at 1000 µg/L 100% mortality occurred within 4.5 days.

Moreira *et al.* (2018) investigated the effects of Arsenic (As) on the embryo-larval development of *Crassostrea angulata* and *Crassostrea gigas* at different temperatures and salinities. Oyster embryos were exposed to 0, 30, 60, 120, 240, 480, 960, and 1920 µg/L arsenic at salinities of 20, 26, and 33, and temperatures of 20, 24, and 28℃ for 24 and 48 hours. As had a higher toxicity to *C. angulata* embryos than *C. gigas* with an EC50 10x lower than that of *C. gigas*. The As EC50 for *C. angulata* was 39.2 µg/l As (18.7 μg/1 As at 48-hour). Moreira et al (2018) noted that its EC50 was the lowest then recorded for an Crassostrea spp. species. The As EC50 for C. gigas was 452 µg/l As (24 °C, 33 salinity, 24-hour) and 663.5 µg/l) As (48-hour). The toxicity of As was influenced by salinity and temperature. However, salinity had the greatest effect on toxicity, especially in *C. gigas*.

Moreira *et al.* (2020) investigated the embryotoxic effects of lanthanum (La) and yttrium (Y) on *Crassostrea gigas* embryos. Embryos were exposed at concentrations of 2.5, 5.0, 10, 20, 40 and 160 µg/L of both lanthanum and yttrium, separately, for 24 and 48 hours. Both lanthanum and yttrium affected embryo development with a 24-hour EC50 of 6.7 µg/l and a 48-hour EC50 of 36.1 µg/l for lanthanum (La); and a 24-hour EC50 of 147 µg/L a 48-hour EC50 of 221.9 µg/l for yttrium (Y). Moreira *et al.* (2020) suggested that La was amongst the most toxic compounds to C. gigas embryos, while Y was ranked among compounds with intermediate toxicity, based on a comparison of toxicity thresholds in the literature.

Mottin *et al.* (2012) investigated the effects of chronic and acute zinc exposures on the Pacific oyster, *Crassostrea gigas*. In the chronic exposure, oysters were exposed to 0.53 mg/L zinc for 10 weeks. In the acute exposure, oysters were exposed to 10.2 mg/L for one week. At the end of the acute exposure experiment, 81.8% mortality was recorded. However, no mortality occurred in the 10-week chronic exposure.

Okazaki (1976) investigated the toxicity of copper exposure on *Crassostrea gigas*. The oysters were exposed to copper concentrations of 0.10, 0.25, 0.50, 0.75, and 1.00 ppm (mg/l) to determine the concentration at which 50% of the experimental population died after 96 hours of exposure. An LC50 of 0.56 ppm was established for the 96-hour exposure. Okazaki (1976) also ran a 14-day exposure at lower concentrations of 0.010, 0.025, 0.050, 0.075, and 0.100 ppm (mg/l). The LC50 for the 14-day exposure was estimated at 0.1 ppm (0.1 mg/l).

Park & Kim (1978) investigated the effects of short-term acute exposures to mercury, cadmium, and copper on *Crassostrea gigas*. The oysters were exposed for 6 days to

mercury, cadmium, and copper at concentrations of 2-10 mg/l, 2-32 mg/l, and 1 to 10 mg/l, respectively. Mercury was found to be the most toxic, then copper and then cadmium with 96-hour LC50s of 1.1 mg/l, 2.54 mg/l and 19.5 mg/l, respectively.

Phelps & Mihursky, 1986 investigated the settlement, development, and survival of *Crassostrea virginica* spat on natural vs. copper-enriched aufwuchs material (faunal and floral crusts). Oyster larvae (setting stage) were exposed to oyster shell fragments having copper-enriched aufwuchs. Copper-enrichment was achieved by allowing the aufwuchs to grow in copper-enriched water. The larvae showed a normal setting preference for bottoms and edges of shell surfaces but a slight decrease in the total set with increasing aufwuchs copper concentration. Settled oyster spat died or failed metamorphosis with LD50 = 534 µg/g copper aufwuchs.

Prael *et al.* (2001) examined the effect of leachates from wood treated with CCA (copper-chrome-arsenic), an antifoulant used to protect wood files from attack by borers. They compared the swimming speed of 2, 3, and 7-day-old larvae (observed in cuvettes) to those of larvae exposed for 5 mins to 20, 50, or 100 µl of leachate applied to the water surface. The early veliger larvae avoided a layer of concentrated leachate. Two-day-old larvae seemed unaffected but 3 and 7-day-old larvae swam two to three times faster in leachate than plain water and moved up and down more. The authors noted that the leachate concentrations used exceeded likely environmental levels but suggested that their study may form the basis of a bioassay (Prael *et al.*, 2001). No mortality was recorded.

Quiniou *et al.* (2007) exposed *Crassostrea gigas* to copper at concentrations between 20 and 80 µg/L in a laboratory for 24 hours. The number of abnormal larvae was assessed and an EC50 of 41.99 µg/L copper was established.

Ramachandran *et al.* (1997) exposed *Crassostrea iradalei* larvae to copper and cadmium during 48-hour exposure assays. Oysters were exposed to copper and cadmium concentrations between 5 and 200 and 500 and 2000 µg/l, respectively. The concentrations that caused 50% abnormal development (EC50) were calculated to be 81 µg/l for copper and 459 µg/l for cadmium.

Ringwood & Conners (2000) investigated the effects of metal exposure on the fertilization and development of *Crassostrea virginica*. The fertilization assays involved exposing sperm to a range of cadmium (1-8 mg/l) and copper (10-80 µg/l) concentrations for 1h before adding eggs and incubating for two hours to determine fertilization success. No effects on fertilization

success were observed in the cadmium treatments. However, fertilization was significantly reduced at concentrations of 40 µg/l Cu. For the development assays, after fertilization, the embryos were exposed to cadmium (1-4 mg/l) or copper (10-40 µg/l) treatments for 48 hours, before being assessed for abnormalities. Significant abnormalities were caused by both metals, with EC50s of 17.5 µg/l for copper and 2.25 mg/l for cadmium.

Roesijadi *et al.* (1996) investigated the effects of cadmium on the development of *Crassostrea virginica* larvae development. Newly fertilized eggs were exposed to 0.2 mM (36.66 µg/l) cadmium for 24 hours, compared to the control the exposed treatments had delayed development to the D-stage veliger larval stage.

Sanders *et al.* (1990) investigated the effects of silver on the growth of *Crassostrea virginica*. Adult oysters were exposed to silver at concentrations of 2, 5 or 7 µg/L for a period of 14 days. The growth of the oysters was significantly reduced in all treatments compared to the controls.

Shuster & Pringle (1969) examined the effects of metal exposure on the metal accumulation, growth, appearance and mortality of *Crassostrea virginica* in flow through tanks. Oysters were exposed to 0.1 and 0.2 ppm (mg/l) zinc, 0.1 and 0.2 ppm cadmium, 0.025 and 0.05 ppm copper, and 0.05 and 0.1 ppm chromium for 20 weeks. Oysters were removed periodically to determine body burden and shell growth. Oysters exposed to copper developed bluish-green bodies, increased the pigmentation of the mantle edge, but grew well with mortality rates only slightly higher than the controls. No statistical comparison was possible due to an accidental contamination of the controls, which were then rerun. Zinc and Chromium exposure also resulted in mortalities similar to those of the controls. Oyster exposed to cadmium became emaciated, had very little growth, lost the pigmentation of the mantle edge and suffered high mortalities, resulting in 100% mortality after 19 weeks of exposure to 0.2 ppm (200 µg/l) ad 84% after 20 weeks exposure to 0.1 ppm (100 µg/l) (Shuster & Pringle, 1969). An LT50 of 10 weeks due to exposure to 0.2 ppm (200 µg/l) can be extrapolated from Figure 4 (Shuster & Pringle, 1969).

Suryawanshi & Langekar (2006) examined the toxicity of zinc and cadmium exposures on *Crassostrea cattuckensis*. The oysters were exposed to concentrations of zinc or cadmium for 96 hours to establish LC0 and LC50 values. The observed LC0 and LC50 values were 6,500 and 9,500µg/L for zinc and 1,000 and 4,000 µg/L for cadmium, respectively. The calculated LC50 values were 9,420 for zinc and 3,820µg/L for cadmium.

Thurberg *et al.* (1974) investigated the effects of silver on the oxygen consumption of *Crassostrea virginica*. The oysters were exposed to 100, 500 or 1,000 µg/L of silver for 96 hours. No significant differences in oxygen consumption were observed.

Ward (1982) measured the filtration rate of adult *Saccostrea commercialis* exposed to 0, 10, 50, and 100 µg/l cadmium chloride in flow through tanks. Filtration rate was estimated using the uptake and hence clearance of ^{14}C labelled bacteria (*Escherichia coli*). Ward (1982) reported that filtration efficiency of the cadmium treated oysters was 19 to 27% lower than controls in the first hour of feeding. The efficiency decreased with increasing Cadmium concentration. Ward (1982) noted that natural and cultured populations of *Saccostrea commercialis* probably only feed for a few hours per day. He concluded that low concentrations of cadmium could cause substantial physiological stress in natural populations due to the sublethal reduction in feeding efficiency.

Watling (1978) investigated the effects of cadmium exposure on the growth and survival of *Crassostrea gigas* larvae and spat at different stages of development. Larvae and spat were exposed to a series of concentrations of cadmium chloride. Exposing five-day old larvae to 20, 40 and 100 µg/l cadmium for seven days resulted in 50, 75 and 85% mortality, and reductions in growth occurred. Exposing 16-day old larvae to 50, 100 and 200 µg/l cadmium for seven days resulted in 25, 35, and 60% mortality, and decreased growth rate. Exposing 24-day old spat to 100, 250 and 500 µg/l cadmium for 10 days resulted in 35, 37 and 79% mortality and growth reduction was observed from the lowest tested concentration. Exposing 3-month old spat to 250, 500 and 1,000 µg/l cadmium for five days, resulted in 10, 50 and 90% mortality, and reduced growth occurred in the 250 and 500 µg/l treatments, however, growth inhibition occurred at 1,000 µg/l.

Watling (1981) investigated the effects of metal exposure on the filtration rate and survival of *Crassostrea gigas* and *Crassostrea margaritacea*. For the filtration rate tests, adult oysters were exposed to copper, zinc, cadmium, and lead for an 8-hour period to establish EC50 values. For *Crassostrea margaritacea*, the EC50 values for copper, zinc, and cadmium were 90, 850 and 790 µg/L, respectively. For *Crassostrea gigas*, the EC50 values for copper, zinc, and cadmium were 60, 610 and 1,080 µg/L, respectively. The lethal toxicity tests were run over 4 and 21-day periods to establish the concentrations that caused 50% mortality within those timeframes. For *Crassostrea margaritacea*, the 96-hour LC50 values for zinc, cadmium, copper and lead were >2.5, >5, >1.5 and >5 µg/L, respectively. The 21-day LC50 values for *Crassostrea margaritacea* exposed to zinc, cadmium, copper and lead were all

>0.5 µg/L. For *Crassostrea gigas*, the 96-hour LC50 values for zinc, cadmium, copper and lead were >2.5, 0.8, 0.55 and >5 µg/L, respectively. The 21-day LC50 values for *Crassostrea gigas* exposed to zinc, cadmium and lead were >0.5 µg/L, and 0.45 µg/L for copper.

Watling (1982) investigated the effects of zinc, cadmium, and copper on the larval growth of three oyster species (*Crassostrea gigas*, *Crassostrea margaritacea* and *Crassostrea cucullata*). Larvae of two different development ages were exposed to the metals at concentrations between 0 and 100 µg/l for four days. A decrease in growth rate with increased metal concentration was observed for each metal and species. The dose that caused 50% mortality was calculated for each of the metals and species. For *Crassostrea cucullata* the LC50 of cadmium, copper and zinc were 80, 60 and >100 µg/l for 3-day old larvae, however, the LC50 for each of the metals were greater than 100 µg/l for 13-day old larvae. For *Crassostrea gigas* the LC50 of cadmium, copper and zinc were 85, 80 and >100 for 6-day old larvae, however, the LC50 for each of the metals were greater than 100 µg/l for 16-day old larvae. For *Crassostrea margaritacea* the LC50 of cadmium, copper and zinc were 75, 60 and >100 µg/l for 3-day old larvae, however, the LC50 for each of the metals were greater than 100 µg/l for 13-day old larvae.

Watling (1983) investigated the impacts of eight metals (cadmium, chromium, cobalt, copper, lead, manganese, nickel and zinc) at concentrations between zero and 50 µg/l, on the settlement, metamorphosis, growth, and mortality of *Crassostrea gigas* larvae. The settlement of the larvae was reduced by 35-45% by all of the metals except copper which appeared to promote settlement.

Weng & Wang (2014) investigated the effects of metal exposure on four different natural populations of oysters. Embryos were exposed to 2, 8, 15, 20, and 40 µg/l copper, or, 5, 10, 50, 100 and 200 µg/L zinc, for 24 hours to text embryotoxicity. Larvae were exposed to 5, 10, 20, 40 and 100 µg/L copper, or, 10, 50, 100, 200 and 400 µg/l zinc for 96 hours to examine larval growth and survival. Embryotoxicity was observed by all populations and metal exposures with EC50s between 10.2 and 26.8 µg/L for copper and 25.1 and 84.9 µg/l for zinc. Larval growth was influenced by metal exposure with EC50s of 11.1 to 34.2 for copper and 33.1 to 132.8µg/L for zinc. Survival was not significantly affected by zinc exposure at the highest tested concentration (400 µg/l) compared to the controls. However, the highest tested concentration (100 µg/l) of copper resulted in significant mortalities, with LT50s between 34.2 and 49.9 hours. The study demonstrated that the resistance of oyster offspring to copper and zinc was correlated with the level of metal pollution experienced by

the parent oysters. The oyster embryo and larvae produced by adult oysters from contaminated sites had higher tolerance to metal stress than those from the reference sites.

Wilson & Hyne (1996) investigated the toxicity of aluminium on the embryos of *Saccostrea commercialis*. The embryos were exposed to 50 to 800 µg/L aluminium for 48 hours. Aluminium concentrations of 150 µg/l and above caused significant decreases in the percentage of embryos that developed to the D-veliger stage. An EC50 of 225 µg/L aluminium was established for the effects on survival. All embryos developed abnormally at a concentration of 400 µg/l and above.

Worboys *et al.* (2002) exposed *Crassostrea gigas* to copper concentrations between 0.6 and 20 µg/L for up to 72 hours. EC50s were calculated at 32-, 40- and 48-hours using probit analysis and bootstrap regression. The EC50s at 32, 40 and 48 hours were 2.72, 11.75 and 4.46 µg/L, respectively, based on probit analysis. The EC50s at 32, 40 and 48 hours were 4.4, 9.49 and 8.86 µg/L, respectively, based on bootstrap regression analysis. Larval developmental rates during 0–32 hours were significantly inhibited by copper at all concentrations.

Xie *et al.* (2017) investigated the effects of cadmium and lead on the development of embryos and the survival of larvae. Embryos were exposed to 20, 100, 500, 2500 and 12,500µg/L of cadmium or lead for 24 hours to examine embryotoxicity. Larvae were exposed to 20, 100, 500, 2,500 and 12,500 µg/L of cadmium or lead for 96 hours to examine larval survival. Significant embryotoxicity was observed at the lowest tested concentration of cadmium (20 µg/L) and 100 µg/L lead or above. The 24-hour embryotoxicity EC50s for cadmium and lead were calculated at 272.2 and 660.3 µg/L, respectively. Larval mortality 96-hour LC50s were calculated at 353.3 and 699.5 µg/L, respectively.

Zaroogian *et al.* (1979) exposed adult *Crassostrea virginica* to lead at 1 or 3.3 µg/L for up to 20 weeks. No mortality was recorded, and lead appeared to have no adverse effect on larvae from parents that had been exposed to 1.0 and 3.3 g/kg lead.

Zaroogian & Morrison (1981) investigated the effects of cadmium on *Crassostrea virginica* larvae development, growth, and survival from parental exposures. Adult oysters were exposed to cadmium at 5 or 15 µg/l for a minimum of 33 weeks before being stripped of gametes for embryotoxicity testing. In the control treatment, >93% of the larvae developed normally within 48 hours. However, in the 5 µg/L treatment, where gametes from exposed oysters were fertilized together, 70% of the larvae developed abnormally. When gametes

from an exposed oyster were crossed with gametes from unexposed oysters, there were less than 10% abnormalities.

4.2 Organometals

Exposure to organotins resulted in mortality (67%), 'no' mortality (8%), or sublethal effects (24%). The results of the effects of organometals were 38% from early life stages and 32% from adults and juveniles (Figure 4.3). The evidence is summarised below.

Axiak *et al.* (1995) investigated the effects of tributyltin (TBT) on *Ostrea edulis*. Field investigations looked at the differences in the thickness of oyster shells from polluted and reference sites. The oysters from the reference site had approximately 50% higher shell thickness indices than those from the contaminated sites. The controlled laboratory tests looked at the cellular impacts and survival rates. No mortalities occurred in the 0.01 and 0.1 µg/l treatment groups. However, 20% mortality occurred in the 1 µg/l group.

Birch *et al.* (2013) surveyed *Saccostrea glomerata* abundance and size between 1995 and 2005 in a highly urbanised estuary. Oyster abundance and density increased in the upper estuary with a 300% increase over the ten-year period. The areas of high-shipping activity coincided with areas of maximum oyster increase, which suggested that the partial banning of TBT in 1989 might have played a major role in the increase of *Saccostrea glomerata* in this estuary.

Birch *et al.* (2014) documented a significant and widespread increase in the abundance of the Sydney rock oyster *Saccostrea glomerata*, in Sydney estuary (Australia) based on surveys of oyster density in the estuary in 1989 and annually from 1994 to 2006. Oyster density at six control sites located in nearby National Parks unaffected by boating and storm water discharges was compared to 17 study sites widely distributed within Sydney estuary. No oyster populations were evident in Sydney estuary in 1989. However, by 1994 oysters had colonized areas of the lower and central estuary and by 2002 densities were statistically similar to control sites. The timing of estuary-wide increases in oyster abundance suggests that the partial banning of tributyltin in 1989 for vessels under 25 m long may have played a major role in the increase of *Saccostrea glomerata* in this estuary.

Davies (1988) investigated the radius effects of tributyltin (TBT) compounds released by fish farms on the accumulation and growth of *Crassostrea gigas*. Batches of oysters were deployed in cages at a total of 27 locations approximately 0, 200, 500, 1,000, 2,000 and

5,000 metres from fish farms. A sample of ten oysters was collected from each of the cages on five occasions during the five-month experiment. Measurements were made of shell length, total weight, tissue weight, dry shell weight and thickness of the upper valve. Total tissue tin and TBT were also measured. At the end of the five-month deployment oysters located closer to the fish farms were in poorer condition than those at greater distances from the farms. Increased shell thickening was also observed in oysters located closer to the fish farms.

Dyrynda (1992) deployed juvenile *Crassostrea gigas* at TBT contaminated sites and relatively clean sites for six months. After six months, the oysters grown at the clean sites showed normal growth, whereas those grown at contaminated sites, showed the onset of abnormal thickening.

Gendron (1986) investigated the effects of tributyltin (TBT) exposure on the survival of *Crassostrea gigas*. The oysters were exposed to tributyltin oxide (TBTO) at 2, 5, 10, 20, 50 and 200 µg/L. At 200 µg/l TBTO, 100% mortality occurred within 17 days. At lower concentrations, the mortality rate was spread out over longer periods of time.

Henderson & Salazar (1996) examined the effects of tributyltin (TBT) exposures, ranging from 1.4 to 2500 ng/l on a range of shallow-water organisms, especially fouling communities, using long-term flow through experiments. The survival of *Crassostrea virginica* at <= 1800 ng/l was similar to controls but exposure to 2500 ng/l resulted in 50% mortality after 30 days and 82% after 57 days. At the end of the experiment (57 days), the condition index of the oysters was significantly reduced in treatments >=100 ng/l. But condition indices returned to near-control levels after 60 days. In a second series of experiments, the condition index of *Crassostrea virginica* were significantly lower after exposure to 82 ng/l TBT for 30 days and 57 days. After 122 days of exposure *Crassostrea virginica* accumulated TBT, leading to BCF values of 19,600 after 122 days and 36,300 after 181 days. Exposure of *Crassostrea gigas* to 1.4 to 29 ng/l for 148 days resulted in condition indices similar to controls but 13-29 ng/l TBT significantly reduced growth rate and mortality rates (24.5%) were only higher than controls when exposed to 29 ng/l TBT. Juvenile *Ostrea lurida* were observed on settlement panels exposed to 200 ng/l TBT for 68 days. Henderson & Salazar (1996) reported that only pacific oysters and bay mussels were affected by TBT concentrations <100 ng/l while the other bivalve species studied showed few significant effects in harbour water at <=200 ng/l.

Henderson (1986) investigated the effects of long-term exposure to tributyltin (TBT) on the condition index and survival of *Crassostrea gigas*. Oysters were exposed to TBT at

concentrations of 0.04, 0.1, 0.5, 1.8, and 2.5 µg/L for 60 days. Condition indices of oysters exposed to TBT concentrations of 0.1 µg/L and greater were significantly lower than the control. The survival rate of the oysters exposed to 1.8 µg/L or less was similar to the control survival rate. Oysters exposed to 2.5 µg/L TBT had 50% mortality after 30 days of exposure.

Higuera-Ruiz & Elorza (2011) examined the effects of tributyltin (TBT) on shell thickening and chambering in the oyster. Field and laboratory experiments were carried out to identify the differences in natural thickening caused by sedimentation and the thickening caused by TBT exposure. Reductions in shell growth, stagnation of the adductor muscle scar and thickening of the chambers were observed in TBT exposed oysters.

His & Robert (1987) investigated the effects of two antifouling paints on the oyster *Crassostrea gigas*. Adult oysters were cultivated for 13 months in trays, each containing 200 oysters. The wooden sides of two of the trays were painted either with organotin antifouling paint (TBT International) or with copper-oxide antifouling paint. The organotin paint reduced growth rate expressed as weight, length, and width, but did not affect shell height. It decreased the dry condition factor and shell density but did not affect the viability of embryos and larvae from exposed oysters. However, some decrease in larval growth rate was observed. The copper paint had no effect on oyster growth but lowered the condition factor compared to controls. Neither the viability of embryos or larvae nor larval growth was affected by the copper paint.

His (1996) investigated the effects of tributyltin on the embryogenesis and larval development of *Crassostrea gigas*. *Crassostrea gigas* eggs were fertilized in TBT contaminated water. At concentrations above 1 µg/L, fertilized eggs did not develop to the D-larvae stage. At 1 µg/L, the D-larvae stage was reached, however, all larvae were abnormal and died within a few days. At concentrations from 0.05 to 0.5 µg/L abnormalities and mortalities were high (>78% over 12 days) and larval growth was significantly affected. No significant differences compared to the controls were observed at 0.02µg/L.

Karande & Ganti (1994) investigated the concentration of tributyltin oxide (TBTO) required to cause 50% mortality of *Saccostrea cucullata* during acute and chronic exposures. Acute toxicity testing was conducted over 96 hours in static laboratory conditions, with daily renewals. The lethal concentration (LC50) found to cause 50% mortality within 96 hours was calculated at 25 µg/L. Chronic toxicity testing was conducted over 28 days in flow-through laboratory conditions. The lethal concentration (LC50) found to cause 50% mortality within 28 days was calculated at 10 µg/L.

Karande & Udhayakumar (1993) investigated the toxicity of tributyltin (TBT) on the oyster Saccostrea cucullata. The oysters were exposed over 96 hours and 28 days to determine the concentration that caused 50% mortality (LC50). The LC50 established for the 96-hour exposure was 25 µg/l, however, the 28-day exposure produced a lower LC50 of 10 µg/l.

Labare *et al.* (1997) investigated the effects of tributyltin (TBT) toxicity on oyster larvae. *Crassostrea gigas* larvae were exposed to TBT at 1, 5,10, 30 and 50 µg/L for up to 96 hours. Mortality only occurred after 24 hours at the highest tested concentration (50µg/L), with 90% mortality. No significant effects on larval activity were observed at concentrations below 5 µg/L. However, there were temporary reductions in activity at 5 and 10 µg/L. The number of larvae exhibiting normal swimming activity was reduced to 63% after 96 hours at 30 µg/L. Exposure to 50µg/L caused reductions in swimming activity at all of the tested time intervals, with almost complete mortality after 24 hours.

Lawler & Aldrich (1987) investigated the sublethal effects of bis(tri-n-butyltin)oxide (TBTO) on *Crassostrea gigas* spat. The oysters were exposed to 0.2, 0.1, 0.05, 0.02 and 0.01 µg/L TBTO. Significant effects on oxygen consumption, feeding rate, and growth rate were influenced by exposure. Significant effects were observed at concentrations from 0.05 µg/L for oxygen consumption and feeding rate, and for growth, significant effects were seen from 0.02 µg/L.

Li *et al.* (1997) exposed adult oysters to tributyltin oxide (TBTO) at concentrations of 3 and 6 µg/L for 14 days. The influence of TBTO on fertilization and embryonic development was investigated by obtaining gametes from the exposed oysters and crossing those gametes with gametes from the control oysters. The fertilization and development rates were calculated by counting the fertilized eggs at hour 2 and straight-hinge larvae at hour 24 post-fertilization. The insemination of contaminated eggs resulted in reduced fertilization rates and development rates, resulting in abnormal larvae. Insemination of uncontaminated eggs with contaminated sperm did not affect fertilization. No significant mortality, compared to controls, was found.

Meng *et al.* (2005) investigated the effects of tributyltin (TBT) on the survival of *Crassostrea gigas.* Oysters were exposed to concentrations of 0.08, 0.40, 2.00, 10.00 and 50.00 µg/L TBT, for up to 120 hours. The 48, 72, 96, and 120-hour LC50s were 44.6, 18.4, 17.9, and 14.3 µg/l TBT, respectively.

Nell & Chvojka (1992) investigated the effects of bis-tributyltin oxide (TBTO) and copper on the growth of *Saccostrea glomerata* and *Crassostrea gigas*. *Saccostrea glomerata* spat were exposed to TBTO at concentrations between 5 and 100 ng/l for a period of four weeks. No significant mortality occurred during this period. However, the weight of the spat was significantly influenced by exposure at all tested concentrations. As the concentration of TBTO increased, the weight gain of the spat decreased. *Crassostrea gigas* spat were exposed to TBTO at 5 to 20 ng/l over four weeks. The weight of the spat was significantly influenced by the TBTO exposure with reduced growth at all exposure concentrations. The effects of copper and TBTO exposure on weight gain of *Saccostrea glomerata* were assessed. The oysters were exposed to 8, 16, 32, and 64 ng/l copper with and without the addition of 20 ng/l TBTO for a period of four weeks. Weight gains decreased with increasing copper concentrations. The weight gain of the spat exposed to the two toxicants was reduced more than those exposed to only one.

Oliver *et al.* (1995, abstract only) exposed adult *Crassostrea virginica* to 0, 0.03 and 0.08 µg/l tributyltin oxide (TBTO) for 0, 2, 4 and 8 weeks and examined their haemolytic defence function. Haemocyte mobility was reduced after two weeks but after four and eight weeks no difference was reported between treatment and control specimens. Serum protein levels were significantly elevated in the 0.08 µg/l TBTO treatment compared to the 0, and 0.03 µg/l treatments after two weeks but after four and eight weeks a dose dependent reduction was observed. Serum lysozyme levels were significantly lower in 0.08 µg/l treatment compared to controls after eight weeks. However, haemocyte chemiluminescence (an indicator of creative oxygen intermediate production) may have been inhibited after eight weeks but chemiluminescence measurements were variable (Oliver *et al.*, 1995).

Osada *et al.* (1993) investigated the effects of acute toxicity of tributyltin oxide on *Crassostrea gigas*. Oyster embryos, larvae, and spat were exposed to TBTO at concentrations between 1 and 100 µg/L. Embryos exposed to TBTO for 24 hours had significant abnormalities from 1.8 µg/L and above, with an EC50 of 3 µg/L. Mortalities occurred at all of the developmental stages tested with 24-hour LC50 values of 7 µg/L for embryos, 15 µg/L for larvae and 35 µg/L for spat.

Phelps & Page (1997) conducted several experimental studies to examined shell thickening to evaluate TBT in estuaries. Study 1: The shell thickness index of oysters collected from five locations around the Sado estuary showed severe upper valve chambering with significant differences in the shell thickness index compared to oysters from a control site.

Study 2: Oyster spat were transplanted in cages at a number of sites across the Sado estuary for 4-5 months. Control cages were placed at Tavira estuary, with one cage containing a wooden panel painted with a TBT-based paint that released approximately 17.5 ng/L TBT a day. The transplanted spat at the Sado estuary nature reserve showed significant shell thickening compared to the oysters in the unexposed control. Spat exposed to the TBT painted panel at the control location showed significant shell thickening. Spat transplanted near the ship painting industry (Gas limpo) had high mortality but no growth/shell thickening. Study 3: Oyster spat were exposed to painted panels releasing approximately 25, 40, 60, and 86 ng per day. Half of the spat were collected after two months of exposure and the remaining spat were collected after four months of exposure to assess shell thickening, mortality, and TBT tissue concentration. Oyster spat exposed to TBT painted panels had increased mortality of up to 29% after four months of exposure. In addition, the spat had increased shell thickening and increased TBT tissue concentrations. Study 4: spat were transplanted to different Portuguese estuaries for four months to observe differences in growth rates, shell thickness and tissue TBT/DBT concentrations. The shell-thickening index of the oysters was lower when the TBT and DBT concentrations were higher.

Pickwell & Steinert (1988; abstract only) exposed oysters to organotin between 0.05 to 2.5 µg/L for 90 days in a flow-through system. They reported low (but unspecified) oyster mortality during the exposure period.

Roach & Wilson (2009) observed declines in the abundance of oysters in sites near a TBT disposal site. The oyster cover closest to the disposal site ranged from 0% to 5% while downstream and upstream populations ranged in cover from 25% to 50% and 5% to 25%, respectively.

Robert & His, 1981 exposed *Crassostrea gigas* eggs and 24-hour larvae to tributyltin acetate (TBT) at concentrations between 1, and 5 µg/l. Exposure to TBT killed embryos and larvae in a few days and prevented the growth of 24-hour old larvae.

Roberts *et al.* (1987) examined the effect of tributyltin (TBT) on the gender, sexual maturity, and fertilization efficiency in adult *Crassostrea virginica* in flow through tanks in the laboratory. A fibre glass sheet was treated with enough methyl methacrylate-tributyltin-methacrylate copolymer paint, and placed in the water flow to deliver a range of TBT concentrations from 0 to 1.0 ng/l. The resultant measured TBT treatments were 0, 66+/-15, 140+/-40, 580+/-230, and 1140+/-300 ng/l TBT. Adult oysters were exposed to each

separate treatment and gender and gonad development examined at two week intervals. Over 95% of adults survived in all treatments. Mortality (ca 20-25% extrapolated from Figure 4) was only observed when the TBT concentration increased markedly (to 1708 ng/l) between weeks two and four due to a temporary drop in water flow. Robert *et al.* (1987) reported that their TBT treatments had no significant effect on sex ratios, sexual maturity, or fertilization ability. They noted that this was contrary to finding in *Ostrea edulis* (Thain & Waldock, 1986) who reported that the proportion of females dropped to zero at a TBT concentration as low as 240 ng/l (Roberts *et al.*, 1987).

Scammell *et al.* (1991) investigated the effects of TBT-based paint exposure on *Saccostrea commercialis*. Oysters were collected from an area where two boats had been painted with TBT -based paints two weeks prior, with a leach rate of 4 µg/cm^2/day. The oysters were physically analysed for abnormalities and chemically analysed for TBT accumulation. The oysters sampled had a number of shell deformities which analysis of variance showed to be significant in the area closest to the boats. In addition, shell deformity was correlated with TBT accumulation.

Stephenson (1991) investigated the effects of TBT on the growth of oysters in field experiments. The results showed that the oysters in areas with higher concentrations of TBT in the water were considerably smaller than those from areas with less TBT.

Thain (1983) investigated the acute toxicity of tributyltin oxide (TBTO) to adult *Ostrea edulis* and both adult and larvae *Crassostrea gigas*. Oysters were exposed to a series of concentrations of TBTO over 48 and 96-hour periods to establish LC50s. For adult *Ostrea edulis*, the 48-hour LC50 was >300 µg/L and the 96-hour LC50 was 210 µg/L. For adult *Crassostrea gigas*, 48-hour LC50 was 1800 µg/L and the 96-hour LC50 was 290 µg/L. For *Crassostrea gigas* larvae, the 48-hour LC50 was 1.6 µg/L.

Thain & Waldock (1985) examined the effects of tributyltin oxide (TBTO) on the growth and survival of *Ostrea edulis* and *Crassostrea gigas*. The first experiment involved exposing the oysters to concentrations of TBTO at 0.24 and 2.6 µg/L for 45 days in a flow-through system. After the exposure, the weight, length, meat weight, and mortality were calculated. At 2.6 µg/L, mortality was high for both *Ostrea edulis* (70%) and *Crassostrea gigas* (90%). Weight gain, length increase, and final meat weight were significantly reduced compared to the control treatments. No mortality was reported for *Ostrea edulis* exposed to 0.24 µg/L TBT but *Crassostrea gigas* had high mortality of 60%. Weight gain, length increase, and final meat

weight were significantly reduced for *Crassostrea gigas* compared to the controls, but similar results to the control were observed for *Ostrea edulis*.

Thain & Waldock (1986) reviewed laboratory and field studies carried out between 1982 and 1984 on the impacts of TBT on oysters. TBT was linked to increased mortality, reductions in growth, and numerous shell abnormalities. The early stages of oyster development were more sensitive to TBT exposure than adult exposure.

Tsunemasa & Okamura (2011) investigated the effects of organometal and pesticide antifoulants on the embryos of *Crassostrea gigas*. Oyster embryos were exposed to Diuron, Irgarol 1051, Sea-Nine 211, and tributyltin (TBT) or triphenyltin (TPT) at concentrations between 1 and 1000µg/L for a period of 24 hours. The 10% lethal concentration (LC10) and the lethal concentration 50% (LC50) values were calculated for each of the contaminants. For Diuron and Irgarol 1051, no evidence of any influence on the development of the oyster eggs was found, even at the maximum concentration (1000 µg/L). In the Sea-Nine211 treatment, all of the oyster eggs in the 100 µg/L treatment died after 2 h. Sea-Nine211 produced 2 hour and 24 hour LC50 values of 28 and 17µg/L. For TBT, the 2-hour and 24-hour LC50s were 16 and 3.9 µg/L. For TPT, the 2-hour and 24-hour LC50s were 14 and 3.7 µg/L respectively.

Valkirs *et al.* (1987) investigated the effects of TBT on the growth and survival of *Crassostrea virginica*. The oysters were exposed to TBT at concentrations between 0.04 and 1.89 µg/L for 66 days. Abnormal growth was not observed at any of the concentrations. However, mortality occurred at concentrations of 0.73 µg/L and above, and the LC50 was 0.97 µg/L.

Waldock & Thain (1983) investigated the effects of tributyltin oxide (TBTO) on the shell thickening and growth of *Crassostrea gigas*. Oyster spat were exposed to 0.15 or 1.6 µg/L TBTO for 56 days. Spat exposed to 0.15 µg/L TBTO had reduced growth compared to the controls and had thickening of the upper shell valve. Spat exposed to 1.6 µg/L TBTO had severely inhibited growth. Mortalities were low with only two specimens dying in the 1.6 µg/L treatment.

4.3 Nanoparticulate metals

Only two articles examined nanoparticulate (NP) metals. Doyle *et al.* (2015) investigated the effects of titanium dioxide nanoparticles (UV-Titan M212 (Titan) and Aeroxide P25 (P25), on the ingestion, bioaccumulation, and depuration of the eastern oyster (*Crassostrea virginica*). The oysters were exposed to 1,000 µg/L titanium dioxide nanoparticles for periods of two and

six hours during feeding experiments. No significant differences were found in the clearance rates of oysters exposed to Titan or P25 compared to controls.

Ringwood *et al.* (2010) exposed oyster embryos to silver nanoparticles to observe the percentage of normal development after 48 hours exposure. Concentrations ranging from 0.0016 to 1.6 µg/L silver nanoparticles were used. Silver nanoparticles significantly impaired the normal embryonic development of oysters at the highest tested concentration of 1.6 µg/L.

4.4 Sensitivity assessment – Transitional metals and organometals

The count of ranked 'worst –case' mortalities due to 'Transitional metals and organometals' are summarized in Figure 4.2, Figure 4.3 and Table 4.1 below. The data presented in Table 4.1 include all life stages and articles where life stage was not reported or were unspecified (NR). Only 7.8% of the resultant ranked mortalities were derived from articles (10) that examined adults and juveniles for the effects of 'Transitional metals and organometals', of which 10% resulted in severe mortality, 31% in significant mortalities, and 44% in sublethal effects.

Another 8% of the results were from the 39 studies that included gametes, of which 36% resulted in significant mortality and 45% sublethal effects. Life stage was not reported in 73 studies, which only contributed 12% of the results. The remaining results (52%) were derived from studies that examined early life stages, of which 29%% resulted in 'severe' mortality, 58% in significant mortalities, 3.6% in no mortality and 9.4% in sublethal effects. A comparison of Figure 4.2 and Figure 4.3 shows that the early life stages dominate the patterns of mortality shown by the evidence collected.

4.4.1 Transitional metals

The evidence summarised in Table 4.1 reported 'severe' and significant' mortality in oyster species after exposure to a range of transitional metals. Overall, 23% of the results reported 'severe' mortality and 49% reported 'significant' mortality.

Table 4.1. Summary of count of ranked mortalities to 'Transitional metals and organometals' contaminants reported in the evidence review and resultant proposed sensitivity assessments for oyster species (N= None, VL= Very low, L= Low, M= Medium, High = High, and NS= Not sensitive).

Group/Type	Chemical name	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Metals										
	Aluminium	**1**	3		1		5	N	VL	H
	Arsenic	**1**	3			1	5	N	VL	H
	Cadmium	**9**	18	2		8	37	N	VL	H
	Chromium	**1**	3			2	6	N	VL	H
	Cobalt					1	1	H	H	NS
	Copper	**17**	31	1	1	11	61	N	VL	H
	Lanthanum		**1**				1	L	L	H
	Lead	**2**	5		1	4	12	N	VL	H
	Manganese	**1**				1	2	N	VL	H
	Mercury	**6**	9		1	4	20	N	VL	H
	Nickel	**2**	2			1	5	N	VL	H
	Selenium		**4**			1	5	L	L	H
	Silver	**5**	6	1		8	20	N	VL	H
	Vanadium		**1**				1	L	L	H
	Yttrium		**1**				1	L	L	H
	Zinc	**5**	16	1	2	4	28	N	VL	H
	Mixture		**1**			2	3	L	L	H
Metals Total		**50**	**104**	**5**	**6**	**48**	**213**	**N**	**VL**	**H**
Antifoulant										
	CCA (copper-chrome -arsenic)					1	1	H	H	NS
	Copper-oxide paint				1		1	H	H	NS
Antifoulant Total					**1**	**1**	**2**	**H**	**H**	**NS**
Nanoparticulates (NP)										
	Silver	**1**					1	N	VL	H
	Titanium dioxide (TiO2)					**2**	2	H	H	NS
NP Total		**1**				**2**	**3**	**N**	**VL**	**H**
Organometals										
Organotin	Tributyltin (TBT)	**4**	10	3	2	8	27	N	VL	H
	Tributyltin methacrylate		1				1	L	L	H
	Tributyltin oxide (TBTO)	**2**	12		2	3	19	N	VL	H
	Triphenyltin (TPT)		1			1	2	L	L	H
Organometals Total		**6**	**24**	**3**	**4**	**12**	**49**	**N**	**VL**	**H**
Total		**57**	**128**	**8**	**11**	**63**	**267**	**N**	**VL**	**H**

In adults and juveniles, 12% ‘severe’ mortality and 33% reported ‘significant’ mortality but 43% reported sublethal effects. However, ‘severe’ mortality was reported in 31% of the results from early life stages, and ‘significant’ mortality in 57% of the results.

There is considerable evidence to suggest that exposure to copper, cadmium, zinc, silver, mercury and lead could result in ‘severe’ or ‘significant’ mortality, although experimental designs and exposure concentrations vary. Several other metals were only included in a few studies. Exposure to cobalt was the only metal that was not reported to cause mortality in the articles reviewed. Overall, all life stages were reported to experience mortality after exposure to transitional metals (except cobalt). This agrees with His *et al.* (2000) who ranked the toxicity of metals and organometals to bivalve larvae as follows: tributyltin >mercury >silver >copper >zinc >nickel >lead >cadmium. Therefore, **resistance to transitional metals exposure in adult, juvenile, and early life stages of oyster species is assessed as ‘None’, resilience as ‘Very low’ and sensitivity as ‘High’.**

Curiously, CCA (copper-chrome-arsenic) affected the swimming of *Crassostrea gigas* larvae swimming but did not result in mortality when used as an antifoulant mixture (Prael *et al.*, 2001). Similarly, copper-oxide paint was reported to have only sublethal effects on adult *C. gigas* (His *et al.*, 1987).

Ostrea edulis was the least studied oyster species. The adults and juveniles of *Ostrea edulis* were reported to experience ‘significant’ mortality after exposure to nickel, mercury, and chromium. The early life stages of *Ostrea edulis* were only studied in one article, which only examined the effects of mercury, which in turn resulted in ‘significant’ mortality. Bryan (1984) reported at 48-hour LC50 for Hg of 1-3.3 ppb in *Ostrea edulis* larvae compared with a 48-hour LC50 for Hg of 4200 ppb in adults.

Ostrea edulis was able to survive in the lower reaches of Restronguet Creek, one of the most heavy metal polluted estuaries in the world, where metals from mining wastes reached concentrations several orders of magnitude above normal (Bryan *et al.*, 1987). Bryan *et al.* (1987) noted that *O. edulis* in the creek were highly polluted, that is, specimens were green since the 1880s, in 1927, and in specimens collected in 1980 (Bryan *et al.*, 1987). *Ostrea edulis* from the Falmouth estuary were shown to be able to detoxify metals (Cu and Zn) in amoebocytes. Bryan *et al.* (1987) noted that Cu and Zn were accumulated in the tissues of *Ostrea edulis*, estimates ranging from ca 1000 to ca 16,500 µg/g dry weight. Bryan *et al.* (1987) concluded that this detoxification mechanism allowed *O. edulis* to survive in the lower reaches of the creek.

Ostrea edulis is, therefore, resistant of high levels of Cu and Zn and is able to survive in the lower reaches of Restronguet Creek, where other species are excluded by the heavy metal pollution. Larval stages may be less resistant, but larval recruitment must be high enough for a population of oysters to survive for ca 123 years in the lower reaches of Restronguet Creek and the Falmouth estuaries. Bryan *et al.* (1987) does not clarify their abundance/density in the creek. However, it appears that *Ostrea edulis* is capable of localized adaption to transitional metal contamination, in particular, from copper and zinc.

Therefore, the **resistance of *Ostrea edulis* to transitional metals is assessed as 'Low',** with the possible exception of Cu and Zn and the understanding that long-term exposure could result in localised adaption. Hence, **resilience is assessed as 'Low' and sensitivity as 'High',** albeit with 'Low' confidence.

4.4.2 Organometals

Overall, 12% of the results of exposure to organotins reported 'severe' mortality, 49% 'significant' mortality, 6% 'some', 8% no mortality and 24.5% sublethal effects (Table 4.1). In adults and juveniles, 'severe' mortality was reported in 6% of results, 'significant' in 31% and sublethal in 43% of results. However, in early life stages, 11% of the results of exposure to organotins reported 'severe' mortality, but 66.7% 'significant' mortality, 16.7% no mortality and 5.5% sublethal effects. The evidence suggests that early life stages are more sensitive than adults. In the five studies that examined *Ostrea edulis*, organotin exposure was reported to result in 'significant' or some 'mortality'. **Therefore, the resistance of oyster species to organotins is assessed as 'None', resilience as 'Very low' and sensitivity as 'High'**.

4.4.3 Nanoparticulate metals

Short-term (2-6 hours) exposure of *Crassostrea virginica* to Titanium dioxide (TiO_2) did not result in negative effects (Doyle *et al.*, 2015). However, 48-hour exposure of *C. virginica* embryos to silver nanoparticles significantly impaired development (Ringwood *et al.*, 2010). Therefore, **the resistance of oyster species to TiO_2 is assessed as 'High' but exposure to silver nanoparticulates may be 'Low'. Hence, resilience is assessed as 'Low' and sensitivity as 'High**' but with 'Low' confidence due to the lack of evidence.

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5 Synthetic compounds – including Pesticides and Pharmaceuticals

A total of 690 results (ranked 'worst-case' mortalities) were obtained from 127 articles that examined the effects of 'Synthetic compounds' on oyster species. Pesticides/biocides were most studied, with 82% of the results, followed by 'Synthetics(other)' (10%) and Pharmaceuticals (5%). *Crassostrea virginica* was the most studied species with 70% of the overall results (mainly under pesticides/biocides), followed by *Crassostrea gigas* with 24.5% of the results overall. *Ostrea edulis* was only reported in a few studies of 'Synthetics(others)' and represented only 0.3% of the results overall (Figure 5.1).

5.1 Pesticides/biocides

Where possible the pesticides/biocides were categorised by their function or target, for example herbicides, insecticides, rodenticides, or arcarides. The majority of results (worst-case ranked mortalities) for the effects of pesticides/biocides were from studies of herbicides (32.8%), organohalogens (20.4%), organophosphates (16.6%), and insecticides (11.4%) (Figure 5.2). *Crassostrea* spp. dominated the studies, but *Ostrea* spp. did not appear in the studies examined. Only, 8% of the results reported 'severe' mortality, 24.6% 'significant', 1.2% 'some', 3.8% 'none' while 62% of the studies reported sublethal effects. But, in the 157 results from studies that examined early life stages, 20.1% reported 'severe' mortality, 68.8% 'significant', 2.6% 'some', 1.9% 'none' mortality and 6.5% sublethal effects (Figure 5.3). Conversely, in the 202 results from studies that examined adults and juveniles, 88% of the reported effects were sublethal, 4.5% reported no mortality, but only 1.5% reported 'some' mortality, 2.5% reported 'significant and 1.5% 'severe' mortality. The evidence is summarised below.

Akcha *et al.* (2012) investigated the embryotoxic effects of Diuron, Glyphosate and Roundup exposure on gametes and embryos of oysters. For the embryotoxic testing, Glyphosate and Roundup were tested at 0.5, 1, 1.5, 2.5, and 5 µg/L, and Diuron was tested at 0.05, 0.10, 0.25 and 0.5 µg/L. Oyster embryos were exposed to each of the herbicides at each of the concentrations for 24 hours, enabling the embryos to develop to the D-shell stage. Following the exposures, the larvae were assessed for abnormalities. From the three replicated bioassays conducted, only one of the Glyphosate experiments showed Glyphosate to have embryotoxic effects at concentrations at or above 2.5 µg/L. There were significant differences between the assays, with assay 2 differing from assays 1 and 3. However, the

results from *a posteriori* Tukey test revealed exposure to Glyphosate at 5 µg/L led to a significant increase in abnormalities (Akcha *et al.*, 2012).

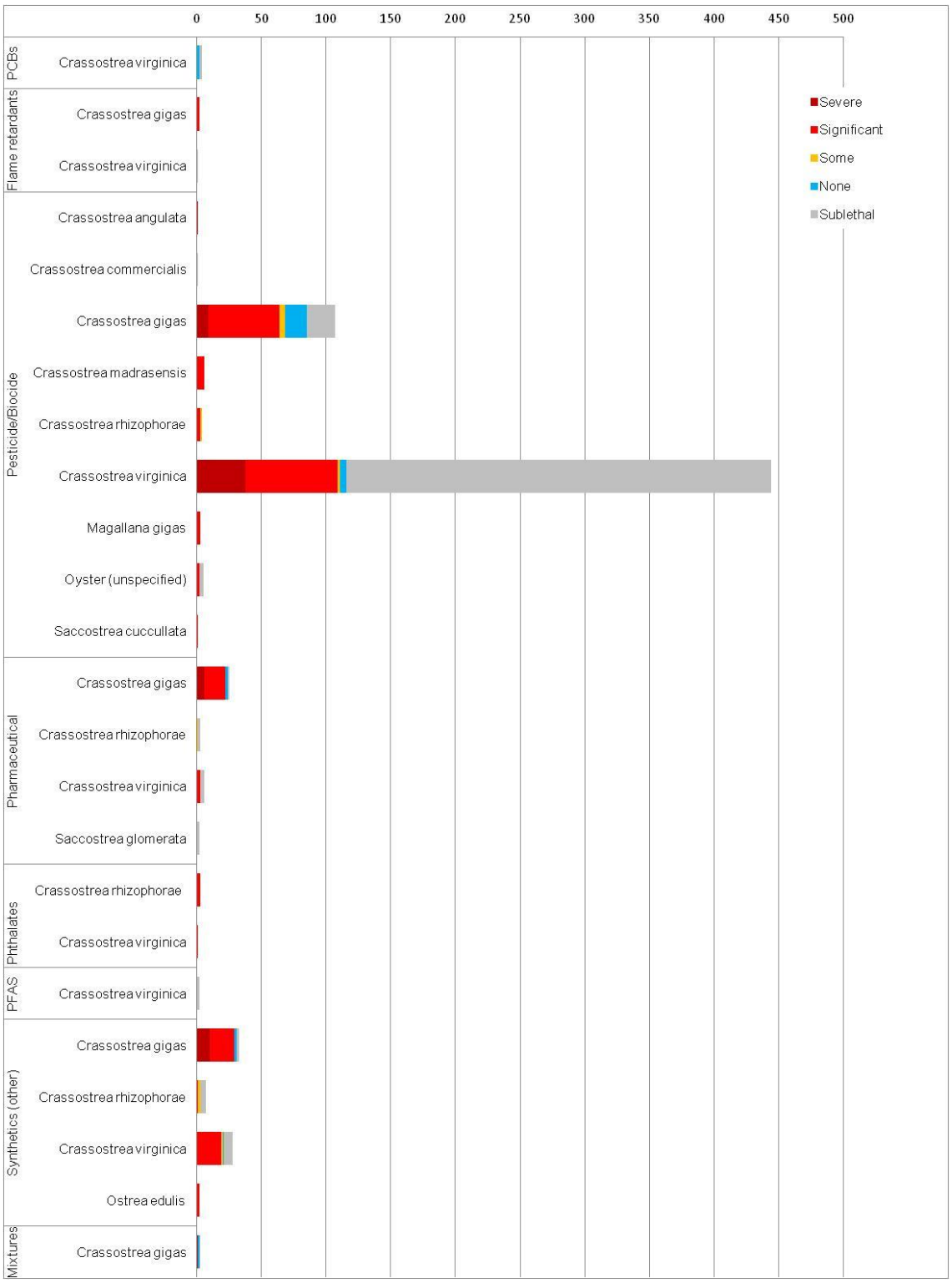

Figure 5.1. Count of ranked mortalities due to exposure to 'Synthetic compounds' in oyster species. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects.

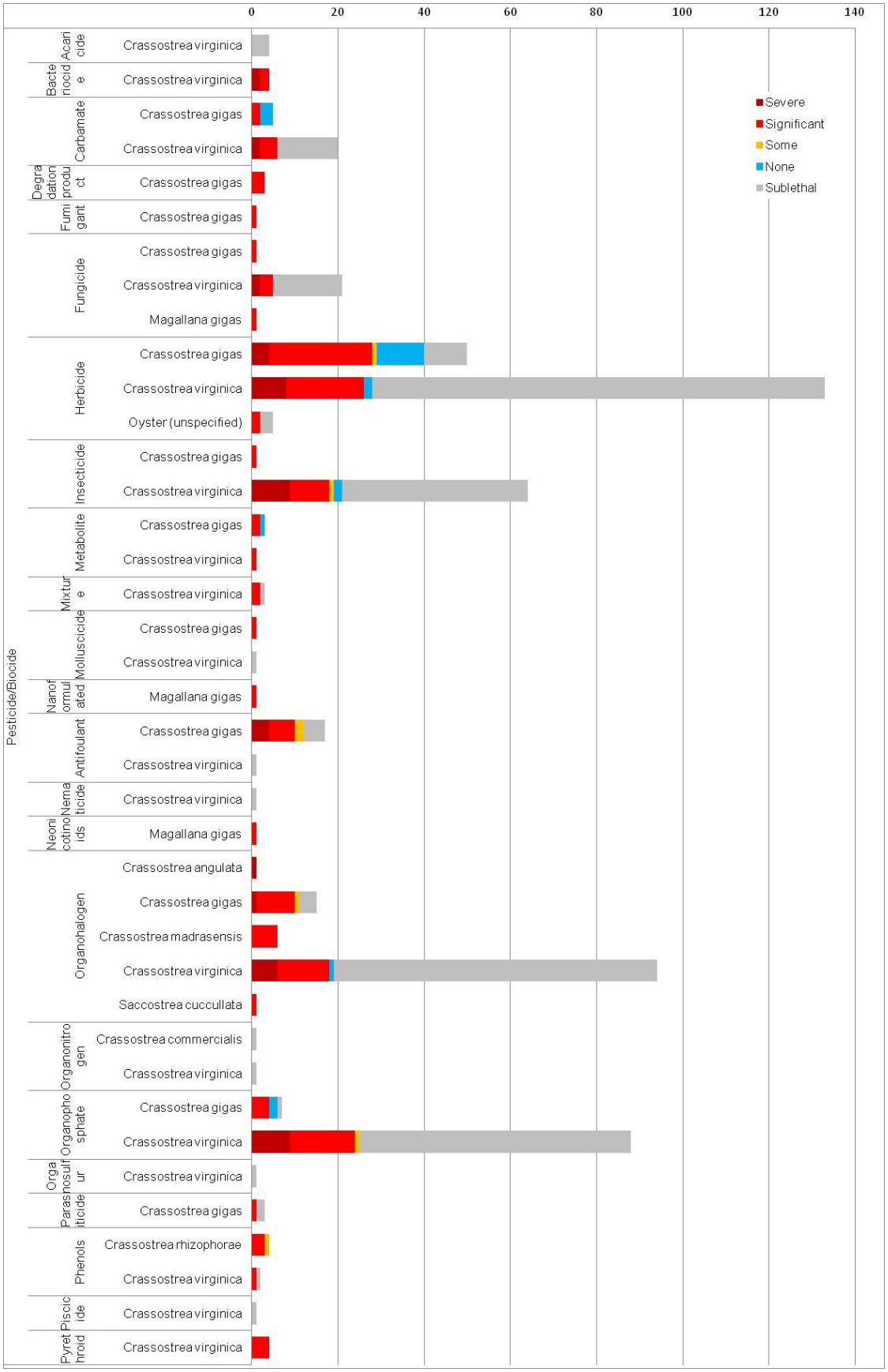

Figure 5.2. Count of ranked mortalities due to exposure to ‘pesticides/biocides’ in oyster species. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects.

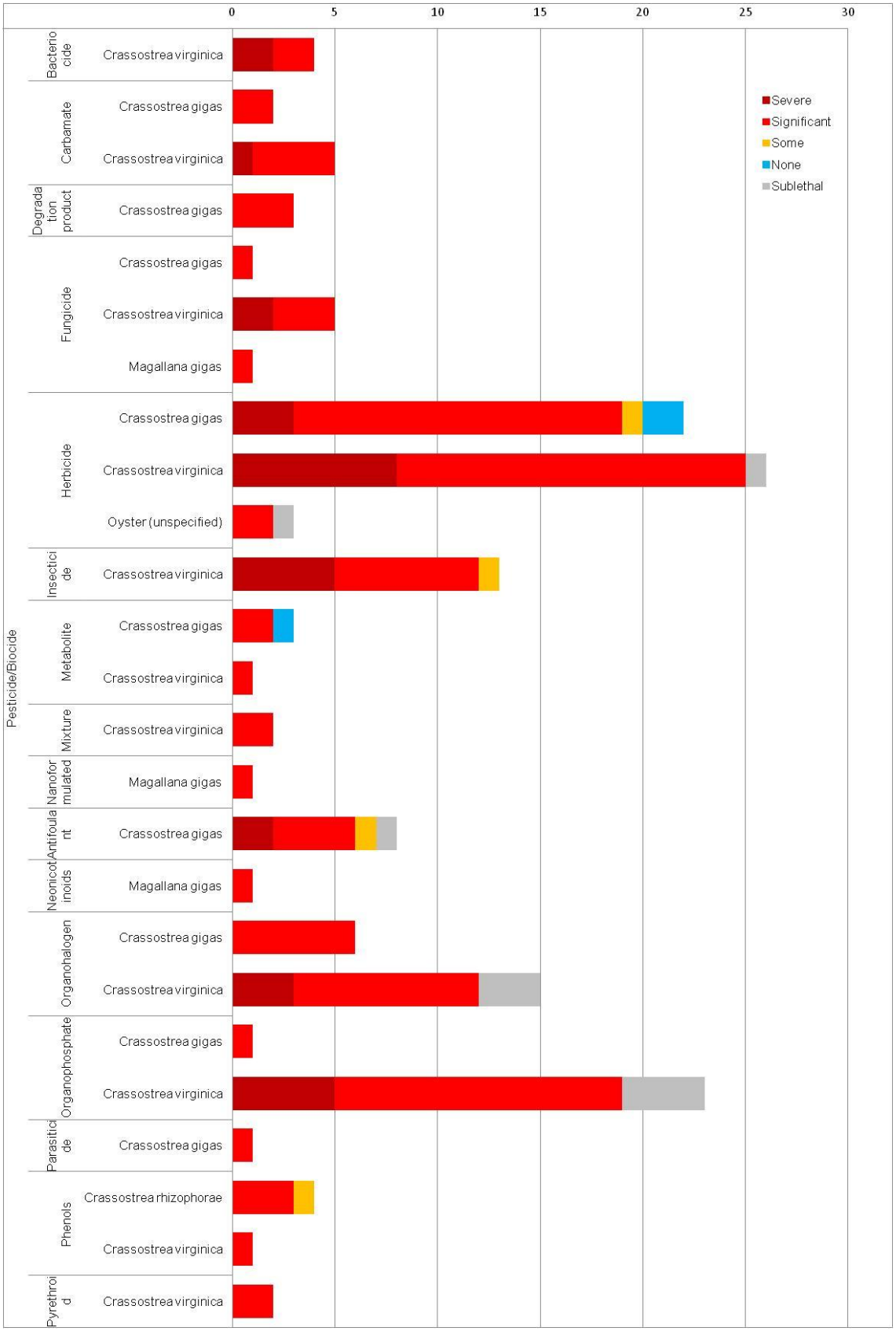

Figure 5.3. Count of ranked mortalities due to exposure to ‘pesticides/biocides’ in the early life stages of oyster species. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects.

Akcha *et al.* (2012) reported that Roundup had no effect on oyster development at the concentrations tested, whereas Diuron affected embryo-larval development significantly from the lowest tested concentration of 0.05 µg/L. The impacts of herbicide exposure on spermatozoa viability of the oysters were assessed at the same concentrations as used for the embryotoxicity study, with the exception of Diuron, which was tested at an additional concentration of 1.5 µg/l. The spermatozoa were exposed to the herbicides for an hour prior to being assessed for the reduction in sperm viability. No impacts on sperm viability were observed throughout the study.

Anguiano *et al.* (2007) investigated the effects of Lindane exposure on the filtration and survival rate of *Crassostrea gigas*. The oysters were exposed to concentrations of between 1000 to 10,000 µg/L Lindane for 12 days with daily renewal. The filtration rate of the oysters was significantly affected at all tested concentrations. Lindane caused oyster mortality, with the 12-day LC50 calculated at 2,220 µg/L. The lowest concentration that caused mortality was 300 µg/L after 11 d of exposure.

Anguiano *et al.* (2009) studied the effects of pesticides on *Crassostrea gigas*. *Crassostrea gigas* were exposed to Oxamyl, Carbofuran, and Dichlorvos at concentrations between 0.01 to 200 µM for a period of four days, and oysters were also exposed to Lindane at concentrations between 1-2.5 µM for a period of twelve days. The oysters exposed to Oxamyl, Carbofuran and Dichlorvos exhibited zero mortality during the treatment. However, those exposed to Lindane at 2.5 µM had a mortality rate of 10%.

Behrens *et al.* (2016) investigated the effects of Diuron, DCPMU (3-(3,4-Dichlorophenyl)-1-Methylurea), DCPU (1-(3,4- Dichlorophenyl) Urea) and 3,4-DCA (3,4-Dichloroaniline) on the early development of *Crassostrea gigas*. The standardized embryo-larvae bioassay (AFNOR XP-T-90-382) was used, exposing larvae to concentrations of the chemicals between 0.002 and 0.5 µg/l. The embryotoxicity on the D-larvae development of the oysters was assessed. 3,4-DCA did not show any signs of embryotoxicity up to concentrations of 5 µg/l. However, Diuron, DCPMU and DCPU, caused the embryotoxic effects. For Diuron, significant abnormalities were measured at 0.01 µg/l. For DCPMU, embryotoxicity was observed from 0.01 µg/l. For DCPU, an increase in larvae abnormalities was observed from exposure to 0.05 µg/l.

Bolton-Warberg *et al.* (2007) investigated the toxicity of the organophosphate Dichlorvos on *Crassostrea virginica* during a 96-hour exposure period. The oysters were exposed to

concentrations of Dichlorvos between 1 and 1000 mg/l. The 1 mg/L treatment group experienced 0% mortality, the 100 mg/l treatment had 60% mortality, and the 1000 mg/l treatment has 100% mortality, producing an LC50 of 31.62 mg/l.

Borthwick & Schimmel (1978) exposed *Crassostrea virginica* embryos to Pentachlorophenol at 3.2, 10, 32, and 100 µg/l for 48 hours. Pentachlorophenol was toxic to the embryos causing 50% abnormal development at 40 µg/l.

Bouilly *et al.* (2003) studied the impact of Atrazine exposure on *Crassostrea gigas* over a two-month period. Atrazine at the concentration of 100.3 µg/l had no significant effect on survival with low mortality at 5.3%.

Boutet *et al.* (2004) focused their research on the characterization and expression of mRNA sequences. However, 0% mortality was reported from *Crassostrea gigas* being exposed to 2µg/l of Glyphosate.

Brain *et al.* (2021) investigated the effects of Atrazine on the survival and growth of *Crassostrea virginica*. The oysters were exposed to Atrazine at concentrations between 1,000 to 17,000 µg/l for 96 hours. At the end of the 96-hour exposure, no mortality was observed at any of the treatment levels tested. Shell growth was reduced by 0.55%, 15%, and 16% in oysters exposed to 1000, 9200, and 17,000µg/L Atrazine, respectively. However, no statistically significant difference was determined at any treatment level. Therefore, Brain *et al.* (2021) reported a NOEC of 17,000 µg/l.

Bringer *et al.* (2021) investigated the effects of Chlortoluron on the valve activity and growth of *Crassostrea gigas*. Juvenile oysters were exposed to 30 µg/L Chlortoluron for 24 days. Exposure to Chlortoluron showed a significant increase in valve opening amplitude and a decrease in valve micro-closures. Shell growth and valve opening duration were unaffected by Chlortoluron exposure. No mortality was observed during the exposure period.

Bringer *et al.* (2021b) investigated the toxicity of six pesticides on the embryo-larval development of *Crassostrea gigas*. Newly fertilized oyster embryos were exposed for 24 h at 24°C to different concentrations of six different pesticides: Glyphosate, Roundup, Isoproturon, Nicosulfuron, Chlortoluron and Boscalid. All of the six tested pesticides induced a significant increase in larval malformations and developmental arrests. All pesticides

except Glyphosate and Isoproturon affected larval growth. Roundup[10], Nicosulfuron, Chlortoluron and Boscalid also affected the swimming behaviour of the D-larvae, with a significant decrease in maximum swimming speed. Comparison of the LOEC of each pesticide led to the following toxicity classification: Boscalid >Chlortoluron =Nicosulfuron > Glyphosate > Roundup > Isoproturon, with LOEC values of 0.0028; 0.015; 0.017; 0.11; 0.3 and 0.78 µg/l respectively.

Butler *et al.* (1962) examined the effect of pesticide exposure on the activity and shell growth of yearling and adult oysters, under flow through laboratory conditions. Shell activity (during opening, closing, pumping) was measured. Exposure to 0.1 ppm (0.1 mg/l) Toxaphene resulted in a 70% decrease in activity after four weeks while 0.1 ppm Dieldrin reduced activity by 50% after five weeks. No growth was observed in small oysters exposed to 0.1 ppm Toxaphene after two weeks, while Dieldrin inhibited their growth from the start of the experiment. But once the exposure to Dieldrin was stopped, activity in large oysters and growth in small oysters returned to control levels within three weeks. In growth experiments, small oysters were exposed to Alfrin, Chlordane, Dichlorobenzene, DDD, DDT, Dieldrin, Endrin, Heptachlor, Rotenone, Sevin, and Toxaphene for 24 hours. In all cases, growth rates were reduced by at least 35% and at most 100%. The minimum effective concentrations for each pesticide were recorded. Butler *et al.* (1962) concluded that each of the 11 pesticides tested was toxic and would reduce growth severely, if present in the environment at the stated minimum concentrations for an extended period.

Butler (1964) exposed juvenile *Crassostrea virginica* to numerous concentrations of pesticides to establish the concentrations of pesticides that caused 50% reductions in shell growth of juvenile oysters during 96-hour exposure experiments. The solubility of the pesticides limited the maximum concentration that could be tested under the test conditions. Therefore, in some cases EC50 values were not determined.

Butler (1965b) reported the toxicity of a range of pesticides on a number of marine species, including *Crassostrea virginica*, using flow-through laboratory tests. Butler (1965b) noted that growth rates in oysters made a good index of pesticide toxicity. The resultant EC50 (causing a 50% decrease in oyster growth rate) are listed. Butler (1965b) also noted that oysters could accumulate DDT to levels 70,000x greater than the 0.1 ppb in surrounding water, and

[10] Roundup is a propriety formulation of Glyphosphate

that fatty sperm and eggs contained high residues. Oyster also accumulated other pesticides such as Endrin, 2,4-Esters but to a smaller extent (Butler, 1965).

Chueycham *et al.* (2021) investigated the toxic effects of DDT on *Saccostrea cucullata*. The oysters were exposed to DDT at concentrations of 0, 10, 50, 100, 500, 1,000 and 2,000 µg/L for 96-hours and the LC50 (96-hour) was 891.25 µg/L.

Da Cruz *et al.* (2007) investigated the effects of surfactants, metals, and hydrocarbons on the early life stages of *Crassostrea rhizophorae*. Embryos were exposed to the chemicals for a period of 24 hours to determine the effective concentration that caused 15 and 50% of the larvae to develop abnormally. The mean concentrations that caused 15 & 50% abnormalities were: 4-Chlorophenol: 12.06 & 20.97 mg/l, phenol: 28.75 & 55.38 mg/l, dodecyl sodium sulphate: 0.690 & 1.360 mg/l, potassium: 25.13 & 35.56 mg/l, zinc 4.05 & 14.97 µg/l, silver: 1.47 & 3.03 µg/l, and cadmium: 114.03 & 282.5 µg/l.

Davies & Herbert (1969) examined the effects of a variety of pesticides (52 insecticides, herbicides, bactericides, fungicides, algicides) and four solvents on the development of *Crassostrea virginica* embryos, and the survival and growth of the larvae. The oyster embryos were exposed to the pesticides for periods of up to 48 hours before being assessed for abnormal development. The oyster larvae were exposed for up to 14 days, for assessment of growth and survival. They found that most of the compounds affected embryonic development more than survival or growth of larvae. However, some drastically reduced growth of larvae at concentrations that had relatively little effect on embryonic development. They suggested that variation in toxicity of pesticides to bivalve larvae was large enough to enable some pesticides to be used to control pests without adversely affecting the commercial species.

Davis (1961) investigated the effects of twelve pesticides on the eggs and larvae of *Crassostrea virginica*. The oyster eggs were exposed to the pesticides for periods of up to 48 hours before being assessed for abnormal development. The oyster larvae were exposed to the pesticides for up to 14 days, and the growth length and survival of the larvae were observed (see ‘evidence summary’ spreadsheet).

De Canales *et al.* (2009) exposed *Crassostrea angulata* to Lindane at a concentration of 16 µg/l for 15 days. Mortality did not occur during the 15-day exposure.

Di Poi *et al.* (2018) investigated the toxicity of five emerging pollutants on the development and metamorphosis of *Crassostrea gigas*. The tested pollutants were the biocides methylparaben (MP) and triclosan (TCS), a pesticide degradation product (AMPA), and the pharmaceuticals Venlafaxine (VEN) and Carbamazepine (CBZ). Embryotoxicity tests were conducted over 36 hours, following the standardized AFNOR XP-T-90-382 procedure. T he percentages of normal development were counted and the EC50s (effective concentration that affects 50% of the population) were calculated for each of the contaminants. The order of toxicity was VEN, TCS, MP, CBZ, AMPA with EC50s of 310, 340, 7,880, 22,830 and 76,900 µg/L, respectively.

Dinnel *et al.* (1983) investigated the toxicity of five metals (copper, lead, zinc, and cadmium) and four pesticides (DDT, Dieldrin, Endosulfan and Endrin) on sperm cells and embryos of *Crassostrea gigas*. Oyster sperm were exposed to each of the contaminants for 60 minutes before eggs were added to the solution for fertilization. Fertilization success was classified by the presence of the fertilization membrane. All the tested contaminants showed toxicity by reducing egg fertilization success, with pesticide EC50s of 214.8 µg/L for Endosulfan, 124.5 µg/L for Endrin, 0.4 µg/L for DDT and 51.8 µg/L for Dieldrin. For the metals, the EC50s were 11,900 µg/L for cadmium, 12.1 µg/L for copper, 5,500 µg/L for lead, 28.8 µg/L for silver, and 44.36 µg/L for zinc. Oyster embryos were exposed to each of the contaminants for 48 hours before being assessed for abnormal development. Embryos failing to reach the veliger stage or those without a reasonably developed shell were considered abnormal. All of the tested contaminants caused abnormal development of larvae, with EC50s of 55 µg/L for Endosulfan, 152.4 for Endrin, >4.6 µg/L for DDT and 22.9 µg/L for Dieldrin. For the metals the EC50s were >120 <1100 µg/L for cadmium,6.1µg/L for copper, 680µg/L for lead, 19 µg/L for silver and 206.5 µg/L for zinc.

Ewere *et al.* (2019) investigated the effects of the neonicotinoid Imidacloprid exposure on *Crassostrea commercialis*. The feeding rate of the oysters was determined following the exposure to Imidacloprid at 125, 250, 500, 1000 and 2000 µg/l. The filtration rate of the oysters was reduced on day one at 2000 µg/l. However, the reduction in filtration rate was not consistent as no significant difference in filtration rate was observed at that concentration over the following three days. The oyster filtration rate was also reduced on day four at 500 and 1000 µg/l. The filtration rate of the exposed oysters did not drop less than 50% of the control.

Falkenberg *et al.* (2017) investigated the effects of Avemectin on the reproductive life-stages of the Pacific oyster, *Crassostrea gigas*. The study assessed the effects of Avemectin on sperm motility, fertilisation success and larval development at 1000, 500, 100, 10 and 1µg/L. All three measures of oyster reproduction showed a statistically significant, negative dose-dependent response to Avemectin. Sperm motility was significantly reduced only at the highest tested concentration of Avemectin (1000 µg/L), whereas fertilisation success was significantly reduced at the two highest Avemectin concentrations (500, 1000µg/L), and larval development was significantly reduced at 100µg/L, and completely inhibited at 500 and 1,000 µg/L. The LOECs were 1000, 500, and 100 µg/L for sperm motility, fertilization success, and larval development respectively. Dose-response curves produced EC50 estimates for sperm motility, fertilization success and larval development of 934, 1,076.26 and 140 µg/L, respectively.

Garcia *et al.* (2014) investigated the toxicity of two pyrethroids (Resmethrin and Permethrin), an organophosphate (Naled), and a juvenile growth hormone mimic (Methoprene) on *Crassostrea virginica*, during acute and chronic exposure periods. Acute (96-hour) exposures were used to monitor larvae mortality at concentrations between 0.12 and 10 mg/l. For larval oysters, Naled, Permethrin, and Resmethrin concentrations at 3.33 mg/L caused significant mortality compared to the control. Naled exposure caused 38% mortality at 3.33 mg/L and 57 % mortality at 10 mg/L. Permethrin and Resmethrin exposures caused 14% mortality at 3.33 mg/L and approximately 32% mortality at 10 mg/L. Mortality at the highest Methoprene concentration tested (10 mg/L) was 11%. The 96-hour LC50 value determined for Naled was 8.26 mg/L. Acute (96-hour) and chronic exposures (21-day) were used to monitor the mortality of juvenile oysters exposed to the contaminants. There was a significant increase in mortality from the acute exposures, compared to the control. Naled exposure caused 24% mortality at 5 mg/L and 81% mortality at 10 mg/L. No significant mortality occurred with acute exposure to Permethrin, Resmethrin, or Methoprene at any of the concentrations tested.

Chronic exposure to Permethrin increased toxicity reducing the LC50 from >10 mg/l to 4.21 mg/L, with significant chronic effects observed from 2.5 mg/L. Methoprene also exhibited a significant increase in toxicity with chronic exposure with the LC50 decreasing from >10 mg/l to 1.32 mg/l, with significant effects on mortality observed at 0.625 mg/l. Chronic exposure to Naled increased toxicity decreasing the LC50 from 7.84 mg/L to 1.14 mg/l, with significant effects on mortality observed at 0.625 mg/l. Chronic exposure to Resmethrin reduced the acute LC50 from > 10 mg/l to 9.49 mg/l. The swimming activity of larval oysters after acute

exposure was significantly reduced by exposure to Permethrin and Methoprene at 3.33 mg/L and by Naled and Resmethrin at 1.11 mg/L. The 96-hour EC50 values determined for the effect of Naled, Resmethrin, Methoprene and Permethrin on the swimming activity in larval oysters were 0.60, 0.93, 0.99, and 2.33 mg/L, respectively. The growth of larval oysters after chronic exposure was significantly reduced at all concentrations of Resmethrin and Permethrin, and at all concentrations of Methoprene except 5mg/l. Oysters exposed to >2.5 mg/L Naled were significantly smaller than the control. The weight of larval oysters after chronic exposure was significantly reduced at all concentrations of Permethrin, Methoprene and Naled. Resmethrin concentrations >1.25 mg/l significantly decreased oyster weight. The 21-day EC50 values determined for the effect of Naled, Methoprene and Permethrin on the weight of larval oysters were 0.63, 0.59, and 4.13 mg/l, respectively.

His & Seaman (1993) investigated the effects of twelve pesticides on the larvae of *Crassostrea gigas*. The pesticides tested were Isoproturon, Chlorotoluron, Metoxuron, 2-4 D (Quinoxonc), Bromoxynil, Carbetamide, Mecoprop, Lindane, Fenitrothion, Parathion methyl, Carbofuran, and Metaldehyde. The pesticides were tested by exposing eggs during fertilization to the following concentrations 25, 50, 100, 250, 500, 1,000, 2,500, 5,000 and 10,000 µg/l, except Lindane (10 to 1,000 µg/l) and Fenitrothion (5 to 2,500 µg/l). After nine days, larvae mortality and shell heights were assessed. The results are shown in Table 5.1and the evidence summary spreadsheet.

Table 5.1. Effects of twelve pesticides on the growth and survival of *Crassostrea gigas* larvae (His & Seaman, 1993).

Pesticide	Larval growth EC10 (µg/l)	Larval mortality EC50 (µg/l)
Lindane	130	170
Mecoprop	130	4,200
2,4-D	No effect	No effect
Fenitrothion	190	No effect
Parathion methyl	87	7,200
Carbofuran	460	6,900
lsoproturon	250	370
Chlorotoluron	600	No effect
Metoxuron	9,000	No effect
Bromoxynil	800	7,000

Pesticide	Larval growth EC10 (µg/l)	Larval mortality EC50 (µg/l)
Carbetamide	4,200	9,300
Metaldehyde	690	7,400

His *et al.* (1996b) examined the size, weight, mortality, and condition of *Crassostrea gigas* oysters that had been cultured in cages coated with a novel antifouling material consisting of a polymer film based on a hydrogel which had been loaded with the benzalkonium chloride (BCl). The size, weight, mortality, and condition of adult oysters and growth rate of larvae obtained from BCl-exposed adults were equal to the controls.

Iliff *et al.* (2019) investigated the effects of Carbaryl on oyster reef community composition by using slow-dissolving plaster blocks containing Carbaryl and travertine tiles to simulate the hard-bottom microhabitat of an oyster reef. The dissolution blocks were replaced every 3–5 days. Sessile and epibenthic organisms were allowed to colonize the tiles for 28 days. After 28 days, the taxa colonized on the tiles were recorded. The colonization of *Crassostrea virginica* was unaffected by Carbaryl exposure when compared to the control.

Kennedy (1984) investigated the effects of the herbicide Hexazinone on the development of *Crassostrea virginica*. In the 48-hour exposure test at concentrations of 560 and 1,000 ppm none of the oyster embryos developed normally. However, at 320 ppm or lower no reductions in the number of normally developed embryos were observed. Therefore, the 48-hour EC50 was greater than 320, but less than 560 ppm.

Kuchovská *et al.* (2021b) investigated the effects of Imidacloprid, propiconazole, and nanopropiconazole on the development and behaviour of the Pacific oyster, Magallana gigas. Oyster embryos were exposed for 24, 30, and 42 hours to concentrations between 0.02 to 200,000 µg/L of the pesticides and to nanoPRO. No developmental abnormalities were observed after exposure to environmental concentrations detected in Arcachon Bay (the maximal detected concentrations of Imidacloprid and propiconazole were 174 ng/L and 29 ng/L, respectively). However, abnormal development was observed at higher concentrations with EC50s of 2930 and 2260 µg/L for propiconazole and nanoPRO. The EC50 of Imidacloprid exceeded 200,000 µg/L (200 mg/L). Imidacloprid did not affect larval swimming behaviour. propiconazole affected larval movement and decreased the average larvae swimming speed, while nanoPRO increased the maximal larvae swimming speed.

Lauth *et al.* (1996) investigated the effects of the insecticide Azinphosmethyl on *Crassostrea virginica* over 96 hours. In the tested low-dose series (<2.46 µg/l) no mortality occurred. However, 100% mortality occurred in the high dose series (<8.13 µg/l).

Le Bris *et al.* (1995) exposed *Crassostrea gigas* to Dichlorvos at 1 and 0.1 mg/l for six hours. No significant mortality was observed but all bivalves were open due to the relaxation of the adductor muscles.

Lowe *et al.* (1971) examined the effects of three pesticides (DDT, Parathion and Toxaphene) on growth in cultivated *Crassostrea virginica* in the laboratory using a flow through system. In expt. 1, oysters were exposed to DDT, Parathion and Toxaphene as a mixture. In 96-hour exposures the growth of juvenile oysters was inhibited by DDT at 10 ppb (10 µg/l), Toxaphene at 100 ppb and above, and by parathion at 1000 ppb (1000 µg/l) and above. A mixture of all three pesticides inhibited growth at 100 ppb (after 96 hours exposure). Exposure of adult oysters to the mixture for 48 weeks reduced growth significantly compared to controls only after 22 weeks, but by about 10% after 36 weeks. No significant mortality compared to the controls was reported. Oysters reared in the mixture exhibited tissue changes in the kidneys, visceral ganglion, gills, digestive tubules, and tissues beneath the gut. They also developed a fungal infection, which the authors suggested was due to a reduction in their natural defences (Lowe *et al.*, 1971). In expt. 2, oysters were exposed to each insecticide separately. No significant effects on growth were reported after 12 weeks exposure. The oyster accumulated relatively high levels of DDT and Toxaphene but eliminated them during a 3-month depuration phase (Lowe *et al.*, 1971).

Mai *et al.* (2013) investigated the effects of the pesticides Irgarol, Diuron and Metolachlor on the fertilization success, offspring quality and embryotoxicity of *Crassostrea gigas*. Embryotoxicity was measured as the percentage of abnormal D-shaped larvae after embryos were exposed to the pesticides for 24 hours. Fertilization success was measured by calculating the number of fertilized oocytes following sperm cells and oocytes being exposed to pesticides for 30 minutes. Offspring quality was assessed by calculating the number of abnormal D-larvae produced following sperm cells and oocytes being exposed. After a 24-hour exposure to the pesticides, there were significant increases in the percentage of abnormal D-larvae. The EC50 values were 2332 µg/L for Diuron, 196 µg/L for Irgarol, and 672 µg/L Metolachlor. A significant decrease in the fertilization rate was observed after sperm exposure to pesticide concentrations with LOECs of 0.01 to 0.04. The highest tested concentrations caused the percentage of fertilization success to decrease by 85.8% for

Metolachlor, 85.5% for Irgarol and 80.5% for Diuron. The fertilization rate decreased approximately to 85%, 86% or 80% when oocytes were exposed to the highest concentrations of Metolachlor, Irgarol or Diuron, respectively. The offspring of sperm exposed to Metolachlor, Irgarol and Diuron showed a significant increase in abnormal D-larvae. At the highest concentration tested the percentage of abnormal larvae reached 25% for Metolachlor, 29% for Irgarol and 37% for Diuron. The offspring from exposed oocytes significantly increased the occurrence of abnormal larvae from 0.1µg/L Metolachlor, 0.4µg/L Diuron and 1µg/L Irgarol. In the treatment where both sperm and oocytes were exposed to the pesticides, significant increases in abnormal larvae were observed at concentrations as low as 0.001. At the highest tested concentrations, the percentage of abnormal development was 32.5, 33.5, and 40.8% for Metholachlor, Irgarol and Diuron, respectively.

Mai *et al.* (2014) investigated the embryotoxicity and genotoxicity of the pesticide Metolachlor and its degradation products on *Crassostrea gigas* embryo development. Embryotoxicity was measured by calculating the percentage of abnormal D-shaped. Embryos were exposed to concentrations between 0.001 µg/l and 10 µg/l for 24 hours. After the 24-hour exposure, significant increases in the percentage of abnormal D-larvae had occurred at concentrations from 0.01µg/L Metolachlor and 0.1µg/L for Metolachlor ethane sulfonic acid and Metolachlor oxanilic acid.

Meyer (1987) tested the toxicity of numerous (ca 127) pesticides or other biocides on *Crassostrea virginica*. The oysters/embryos/larvae were exposed to pesticides/biocides for 48 to 96 hours. The endpoints were LC50s or EC50s. The EC50 endpoint for oyster embryos and larvae was abnormal development, and the endpoint for juvenile and adult oysters was shell deposition. Refer to the evidence summary spreadsheet for details.

Moraga & Tanguy (2000) investigated the effects of five pesticides on the survival of *Crassostrea gigas*. The oysters were exposed to Atrazine, Isoproturon, Alachlore, Metolachlore and Diuron at 0.1 and 0.2mg/l for a period of 45 days. A mortality rate of less than 1% was recorded in the different experimental groups exposed to Alachlore, Metolachlore, and Diuron. Atrazine and Isoproturon caused mortality rates of approximately 60% to 70% at pesticide concentrations of 0.1 and 0.2 mg/L after 45 days of exposure.

Mottier *et al.* (2013) investigated the effects of glyphosate-based herbicides on embryo-larval development and metamorphosis in the Pacific oyster, *Crassostrea gigas*. The study assessed the toxicity of glyphosate, its by-product, aminomethylphosphonic acid (AMPA) and two commercial formulations, Roundup Express® (REX) and Roundup Allées et Terrasses®

(RAT). The development of abnormalities was assessed after 48-hour exposures and the success of metamorphosis was examined in pediveliger larvae exposed to the contaminants for 24 hours. For glyphosate and AMPA concentrations between 0.1 to 100,000 µg/L were used, and for REX and RAT concentrations between 0.1 and 10,000µ g/L were used. No mortalities were observed at any of the concentrations of glyphosate or AMPA during the embryo-larvae exposure tests, whereas in the REX and RAT treatments no embryos or larvae could be observed from exposures to 10,000 µg/L. The EC50 values were 28,315 and 40,617 µg/L for glyphosate and AMPA, respectively, and 1,133 and 1,675µg/L for REX and RAT, respectively. The metamorphosis pediveliger larvae tests produced EC50 values that exceeded 100,000µg/L for glyphosate and AMPA but were lower for REX and RAT with EC50s of 6,366 and 6,060 µg/L, respectively. The pediveliger larvae mortality tests produced LC50s of greater than 100,000 µg/L for glyphosate and AMPA, and LC50s of 8,502 and 7,934µg/L for REX and RAT.

Mottier *et al.* (2014) investigated the effects of acute exposures to Mecoprop, Mecoprop-p and their biodegradation product (2-MCP) on two larval stages of the Pacific oyster, *Crassostrea gigas*. Embryotoxic effects were assessed on veliger larvae after 36 hours exposures, and the effects of the three substances were evaluated on 21-day-old pediveliger larvae by calculating metamorphosis rates after 24 h exposures. The results of the embryotoxicity assay indicated that 2-MCP) was more toxic (EC50: 10,810.22 µg/L) than the parent compounds (EC50 Mecoprop: 42,553.55 µg/L; EC50 Mecoprop-p: 78,853.12 µg/L). The results of the metamorphosis assay also indicated that 2-MCP was more toxic (EC50 7,199.79 µg/L) than the parent compounds (EC50s >100,000).

Mottier *et al.* (2015) investigated the effects of glyphosate exposure on juvenile oysters (*Crassostrea gigas*). The oysters were exposed to 0.1, 1 and 100 µg/L glyphosate for 56 days. No mortalities occurred during the experiment. The growth, length and condition index of the oysters were not influenced by any of the exposure treatments.

Onduka *et al.* (2022) investigated the effects of three antifouling biocides on Pacific oyster (*Crassostrea gigas*) embryos and larvae. The three tested biocides were Diuron, Irgarol 1051 (Irgarol), and 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT). Embryotoxicity tests were carried out over 24 hours at a variety of concentrations, all three of the biocides caused abnormal larvae development, with LOEC of 20.3µg/L for Irgarol, 22.7 µg/L for Diuron and 0.003µg/L for DCOIT. The effects of DCOIT exposure on larvae settlement were assessed,

and the percentage of the settled larvae was found to decrease with increasing concentration of DCOIT.

Parrish *et al.* (1973) investigated the effects of Dieldrin exposure on the shell growth of *Crassostrea virginica*. Oysters were exposed to Dieldrin at concentrations of 1, 3.2, 10, and 32µg/L for 96 hours. Shell growth was reduced by Dieldrin exposure with an EC50 of 31.20 µg/L.

Parrish *et al.* (1976) investigated the effects of chlordane on the shell deposition of *Crassostrea virginica* during a 96-hour exposure period. Oysters were exposed to chlordane at concentrations between 4.2 and 42 µg/L. Shell deposition was inhibited by exposure to measured concentrations >4.7 µg/L and an EC50 of 6.2 µg/L was established.

Pennington *et al.* (2004) exposed *Crassostrea virginica* to Endosulfan for 96 hours establishing an LC50 of more than 1.64 µg/L.

Rajendran *et al.* (1989) investigated the toxicity of the pesticides DDT, Lindane and Endosulfan on Crassostrea madrasensis. Median lethal concentrations at 24, 48, 72, 96 and 120 hours were established for each of the pesticides. The increase in the exposure time decreased the concentration required to kill 50% of the test organisms. For DDT the 24, 48, 72, 96, and 120-hour LC50s were 24.82, 16.19, 15.75, 9.36 and 9.28 µg/l, respectively. For Lindane the 24, 48, 72, 96 and 120-hour LC50s were 38.31, 37.17, 30.57, 26.36 and 25.09 µg/l, respectively. For Endosulfan the 24, 48, 72, 96 and 120-hour LC50s were 27.24, 20.54, 18.09, 14.13 and 12.58 µg/l, respectively.

Rajendran & Venugopalan (1986) examined the effects of three organochloride pesticides (DDT, Endosulfan, and Lindane) on adult *Crassostrea madraensis* collected from the Vella estuary. Ten adult oysters were exposed to serial dilutions of each pesticide, in a continuous flow-through system, for 96 hours to determine the 96-hour LC50s. Another ten oysters were then exposed to sublethal concentrations (at 100th of the 96-hour LC50) for 96 hours to examine their ability to bioaccumulate the pesticides. Rajendran & Venugopalan (1986) determined the following 96-hour LC50s, DDT 9.36+/-7.75-11.3 ppb, Endosulfan 14.13+/-10.79-18.52 ppb, and Lindane 26.36+/-24.36-28.52 ppb. They noted that these pesticides were quite toxic to oysters when compared to reported values for other bivalves. They reported that treated oysters exhibited abnormal behaviour in the higher test concentration rather than the lower concentrations (not given). Their siphons were extended but, eventually, the oysters were paralyzed and were not able to retract their siphons even in

response to mechanical stimulus. They also reported that the pesticides were accumulated by 10 to 100 times that of the control, in the order DDT >Lindane >Endosulfan.

Robert *et al.* (1986) investigated the effects of Atrazine-Simazine on the survival, development, and growth of *Crassostrea gigas*. Oyster embryos were exposed to Atrazine-Simazine for 24 hours before being assessed for developmental abnormalities. At concentrations <1000µg/L abnormalities were less than 5%. At concentrations of 2500 and 5000µg/L abnormalities were <20. But at 10,000 µg/L abnormalities were at 75%. For ten days following exposure, the mortality and growth of the oyster larvae were assessed. At concentrations above 500µg/L, the growth rate of the larvae was affected. From day six, 100% mortality occurred in the 7,500 and 10,000 µg/L treatments. From day 8, 100% mortality occurred in the 2,500 and 5,000 µg/L treatments. At concentrations of less than 2500µg/L mortality was low throughout the treatment.

Schimmel *et al.* (1977) investigated the effects of the organochlorine insecticide, Toxaphene on the shell growth of *Crassostrea virginica*. The oysters were exposed to 3.1, 9, 22, 36, or 77 µg/L Toxaphene for 96 hours. The concentration of Toxaphene estimated to reduce shell deposition by 50% (EC50) was 16 µg/L. No mortality occurred at any of the concentrations during the exposure period.

Schimmel *et al.* (1978) examined the effects of sodium pentachlorophenol (Na-PCP) on a number of estuarine species, including *Crassostrea virginica*, using flow-through laboratory experiments. They measured the effect of Na-PCP exposure on shell deposition rates in *Crassostrea virginica*. However, the experiment was extended to 192 hours rather than the standard 96 hours as the growth rates of the controls and experimental groups were reduced by unusually low water temperatures during the study. Nevertheless, they reported an acute toxicity of 192-hour EC50 (shell deposition) of 76.5 µg/l Na-PCP. Na-PCP reduced shell deposition in oysters at concentrations >= 34 µg/l during 192-hour exposure. They also noted that oysters accumulated Na-PCP between 41 and 78 times the water concentration after 28 days but depurinated themselves after four days in clean water (Schimmel *et al.*, 1978).

Seguin *et al.* (2017) investigated the sub-lethal effects of Roundup Express® (REX) and Polyethoxylated long-chain alkylamines (POEAs) on juvenile *Crassostrea gigas*. The oysters were exposed to sub-chronic exposures (35 days) to three concentrations (0.1, 1, and 100 µg/l) of REX or POEAs. Low mortality rates were calculated but no significance was observed. No significant differences were seen in growth weight or condition index.

However, at day 35 shell growth lengths were significantly reduced from the lowest tested concentration (0.1 µg/L) of both substances.

Stewart *et al.* (1967) examined the effect of Sevin and its decomposition produce 1-naphthol on various marine species, including the development of *Crassostrea gigas* larvae, in static conditions, in the laboratory. Five replicates or 10,000 larvae were exposed to Sevin or 1-naphthol for 48 hours, and the percentage of abnormal larvae counted, relative to controls. No exposure concentrations were given but the EC50s were determined from logarithmic plots. The mean 48-hour EC50 (50% abnormality) for Sevin was 2.2 mg/l and for 1-naphthol was 0.8 mg/l. Stewart *et al.* (1967) also reported the effects of Sevin and 1-naphthol on mud shrimp, ghost shrimp, shore crab, Dungeness crab, the blue mussel, cockle clam shine perch, English sole and three-spine stickleback. Stewart *et al.* (1967) reported that Seven was more toxic to crustaceans than molluscs and fish, while 1-naphthol was more toxic to mollusc and fish. Sevin was ca 30-300 times more toxic to crustaceans than 1-naphthol.

Tagatz et al (1976) exposed *Crassostrea virginica* to 0.01 to 0.083 µg/L of Mirex for 10 weeks. The mortality of the oysters was not influenced by exposure to Mirex.

Tripp (1974) investigated the effects of abate and dibrom exposure on *Crassostrea virginica*. Adult oysters were exposed to abate and dibrom at 1000 and 10,000µg/L. Mortality was low (<10%) at 1,000 and 10,000 µg/l abate and at 1000 dibrom. However, mortality was significantly high (25.5%)at 10,000 µg/L dibrom.

Tsunemasa & Okamura (2011) investigated the effects of organometal and pesticide antifoulants on the embryos of *Crassostrea gigas*. Oyster embryos were exposed to Diuron, Irgarol 1051, Sea-Nine 211, and Tributyltin (TBT) or Triphenyltin (TPT) at concentrations between 1 and 1,000 µg/L for a period of 24 hours. The 10% lethal concentration (LC10) and the lethal concentration 50% (LC50) values were calculated for each of the contaminants. No evidence of any influence on the development of the oyster eggs was found for Diuron and Irgarol 1051, even at the maximum concentration (1000 µg/L). In the Sea-Nine211 treatment, all of the oyster eggs in the 100 µg/L treatment died after 2 hours. Sea-Nine211 produced 2-hour and 24-hour LC50 values of 28 and 17 µg/L. For TBT, the 2-hour and 24-hour LC50s were 16 and 3.9 µg/L. For TPT, the 2-hour and 24-hour LC50s were 14 and 3.7 µg/L respectively.

Tsunemasa *et al.* (2013) investigated the toxicity of triphenylborane pyridine (TPBP) and triphenylborane octadecylamine (TPBOA) on *Crassostrea gigas* embryo development. For

TPBP, in the 100 µg/L treatment, 100% of the eggs died within two hours. In the 50 and 20 µg/L treatments, 100% of eggs died within 24 hours. In the 10 µg/L treatment, approximately 50% of the eggs died within 24 hours, the other 50% developed abnormally. In the 1 µg/L treatment, 85% of the eggs survived, and half of the eggs that survived developed into D-shape embryos but had delayed development and mantle deformity. In the 0.1 µg/L treatment, almost all of the embryos survived with 70% of the embryos developing into D-shape embryos and the other 30% had delayed development. The 2-hour and 24-hour LC50 for TPBP was calculated at 7.5 and 6.3 µg/L, respectively. For TPBOA, in the 100 µg/L treatment, 100% of the eggs died within two hours. In the 50 µg/L treatment, 100% of eggs died within 24 hours. In the 20 µg/L treatment, 80% of the eggs died within 24 hours and the remaining 20% developed abnormally. In the 10 µg/L treatment, approximately 30% died with the remaining 70% developing abnormally. In the 1 µg/l treatment, 90% of the eggs survived, with 10% of those surviving embryos showing deformed or delayed development. In the 0.1 µg/L treatment, almost all of the embryos survived with 10% of those embryos showing deformities. The 2 and 24-hour LC50 for TPBOA was calculated at 23 and 10 µg/L, respectively.

The U.S. Environmental Protection Agency (1981) investigated the toxicity of the pesticides acephate, aldicarb, carbophenothion, DEF, EPN, ethoprop, methyl parathion, and phorate on *Crassostrea virginica* larvae. Static acute toxicity tests were conducted to determine the 48-hour EC50 values that caused abnormal development in oyster larvae. The resultant 48-hour EC50s were: acephate 150,000 µg/L; Aldicarb 8,800 µg/L; carbophenothion 99 µg/L; DEF 197 µg/L; EPN 2,200 µg/L; ethoprop 16,000 µg/L; methyl parathion 12,000 µg/L; and for phorate was 900 µg/L.

Ward & Ballantine (1985) investigated the toxicity of Atrazine on the development of *Crassostrea virginica*. Embryos were exposed to Atrazine to establish the concentration required to cause 50% abnormal development of larvae during a 48 hour exposure, the EC50 value established was >3000 µg/l.

Wirth *et al.* (2004) investigated the effects of the insecticide Fipronil on the growth and survival of *Crassostrea virginica*. The oysters were exposed to Fipronil at concentrations between 0.172 and 7.61 µg/L for 28 days. Fipronil was not observed to have any effects on the survival or growth of the oysters.

5.2 Pharmaceuticals

A total of 36 results (worst-case ranked mortalities) were reported by the 18 articles that examined the effects of pharmaceuticals on oyster species. *Crassostrea* spp. were examined by the majority of studies but *Ostrea* spp. were not reported in the studies reviewed (Figure 5.4). 'Severe' mortality was reported in 16.6% of the results, 53% reported 'significant', 2.8% 'some', 5.5% 'no mortality, and the remaining 22% only reported sublethal effects. However, the five articles that examined adult and juvenile oysters did not report any mortality (22% of results) or only sublethal effects (88% of results). Conversely, the 11 articles that examined early life stages reported 'severe' mortality in 22% of the results, significant in 70%, 'some' in 3.7%, 'none' in 3.7% and sublethal effects in 3.7% of the results (Figure 5.5). The evidence is summarised below.

Andrew *et al.* (2010) exposed *Saccostrea glomerata* to 0, 6.25, 12.5, 25 or 50 ng/l of the human hormone 17a-ethynylestradiol (EE2) under controlled conditions. The condition index of the oysters declined in the 12.5 ng/l, 25 ng/l and 50 ng/l EE2 treatments by 8.5, 7.7, and 12.6%, respectively after four days. However, the oysters recovered with no significant differences between treatments after 21 and 49 days.

Cardwell *et al.* (1979) investigated the effects of pharmaceuticals, surfactants, metals, and hydrocarbons on larval marine organisms. *Crassostrea gigas* larvae were exposed to the chemicals for a period of 48 hours to determine the lethal concentration that caused 50% mortality in embryos (LC50) and the effective concentration that caused 50% of the larvae to develop abnormally (EC50).

Cardwell *et al.* (1979) reported that Pacific oyster larvae were not very sensitive to ammonia and dissolved oxygen, but quite sensitive to various suspended solids, hydrogen sulphide, petroleum hydrocarbons, unbiodegraded linear alkylate sulfonates and deviations in pH and salinity to which the gametes were unacclimated. The exposure of oyster larvae to Kuwait crude or Alaskan crude resulted in LC50s of 380 µg/l and 5,800 µg/l respectively. Bivalve larvae were more sensitive to dodecyl sodium sulphate (DSS) and cadmium than the larvae of Dungeness crab (Cancer magister), spot shrimp (Pandalus platyceros), and Pacific herring, but of intermediate sensitivity to the pesticide Methoxychlor.

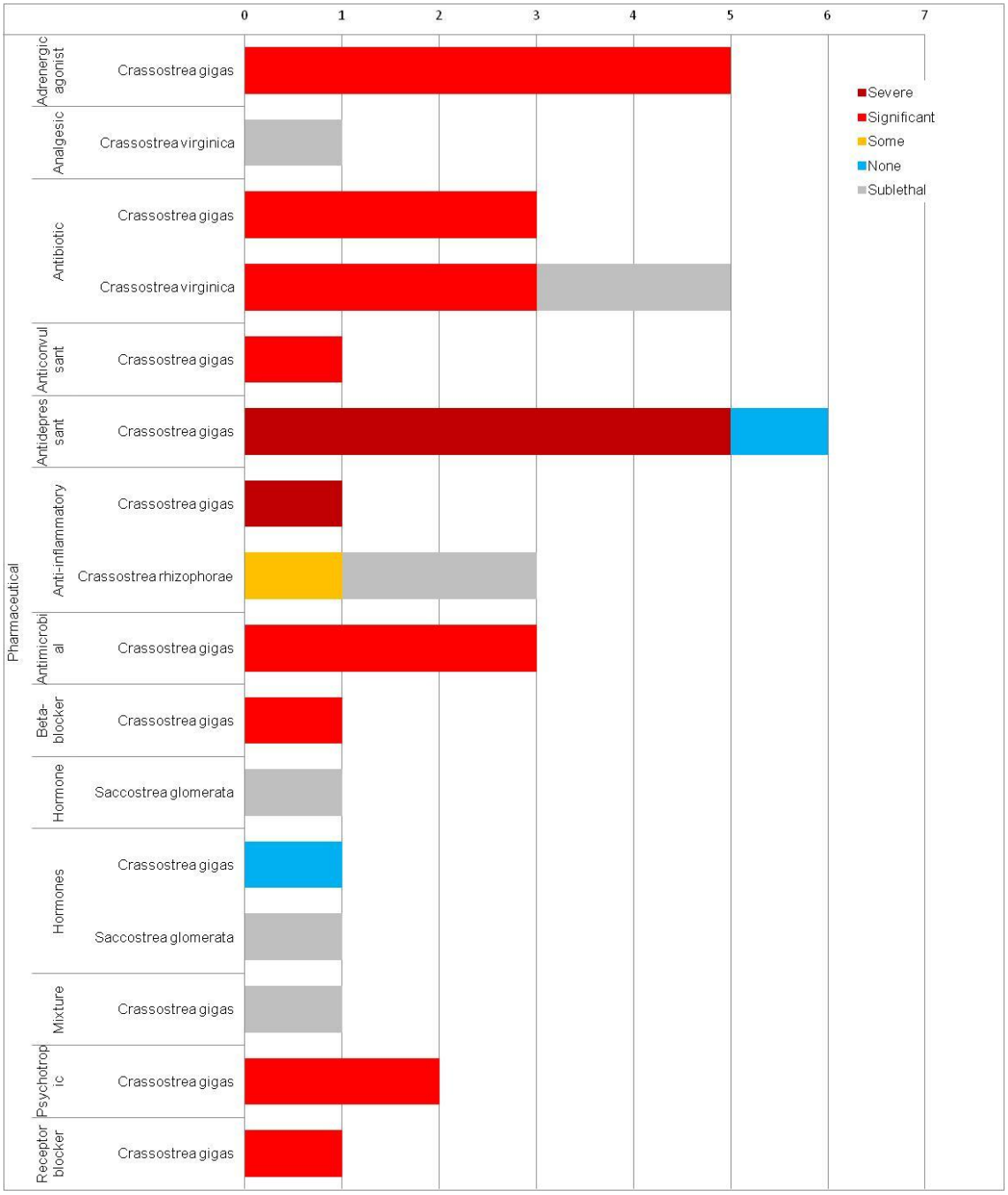

Figure 5.4. Count of ranked mortalities due to exposure to 'pharmaceuticals' in oyster species. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects.

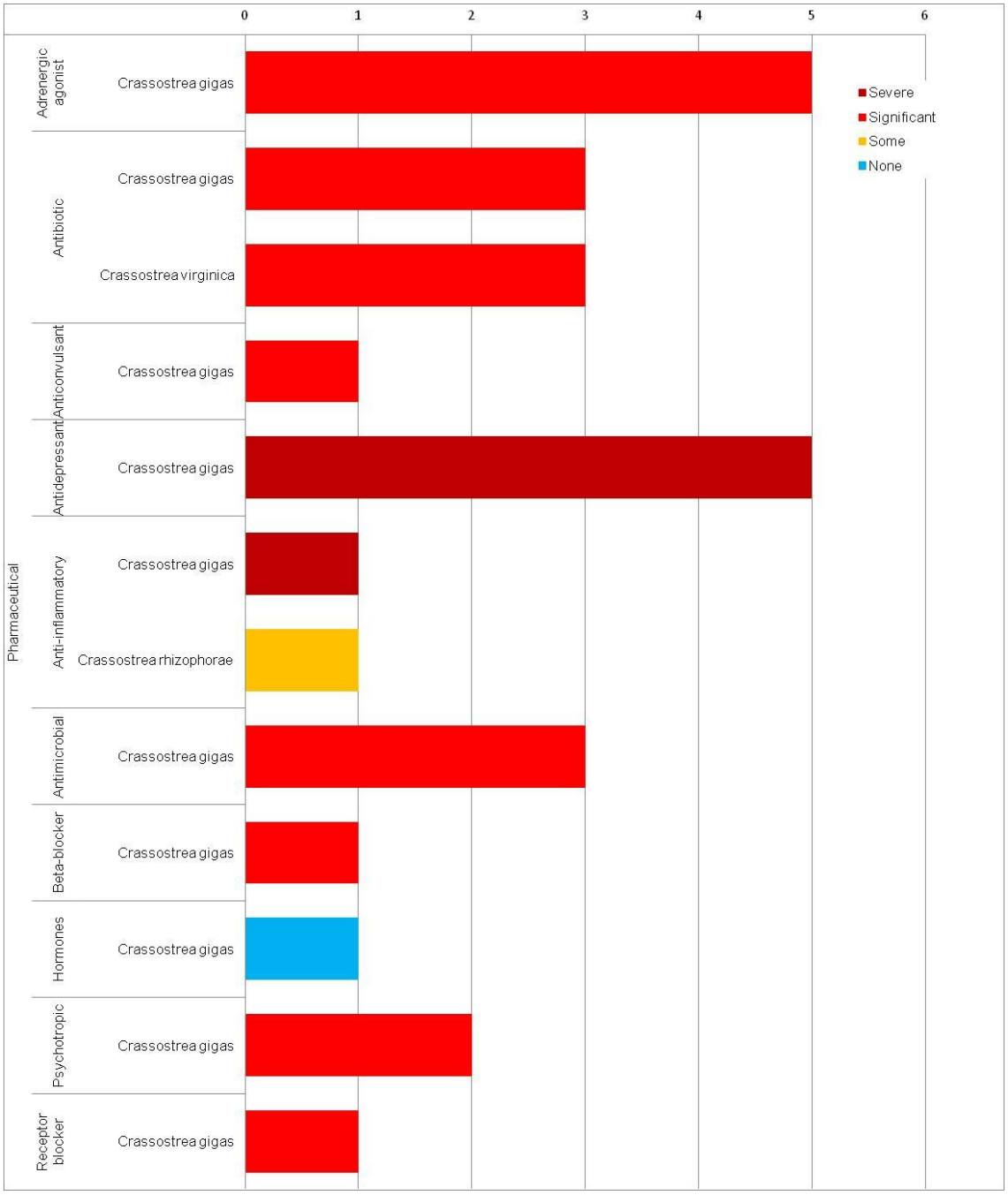

Figure 5.5. Count of ranked mortalities due to exposure to 'pharmaceuticals' in the early life stages of oyster species. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects.

Chao *et al.* (1994) examined the effects of cryoprotectants on the survival of embryos of *Crassostrea gigas*. Oyster embryos were exposed to the cryoprotectants, acetamide, dimethyl sulfoxide (DMSO), ethylene glycol (EG) and propylene glycol (PG), at concentrations from 1 to 5 M to test the toxicity tolerance of oyster embryos at different developmental stages after 60 -240 mins. Embryos were directly exposed to the desired concentrations of each treatment and 5 min equilibration time was allowed before returning to

seawater. Living embryos were determined 10 hours after fertilization by counting the embryos with active rotary motion. Oyster embryos were tolerant to low concentrations of the cryoprotectants tested in the range from 1M to 2 M for all developmental stages. Early-stage embryos were more vulnerable to high-concentration (4 M and 5 M) of cryoprotectants than late-stage embryos. For DMSO, the toxicity increased with concentration from 1 to 5 M. No significant difference in survival between the embryos treated with DMSO below 3 M were observed. Significant decreases in survival occurred at concentrations of 4 M and above. For Ethylene glycol, no significant difference in survival between the embryos treated with 1 and 2 M EG was observed. However, a transition from high survival to low survival was observed when the concentration reached 3 M, especially for the early-stage embryos. For PG, a significant transition from a high survival rate to a low survival rate occurred at the concentration of 4 M. PG seemed to be more toxic to early-stage oyster embryos at high concentrations. For embryos treated with 5 M PG, there were no survivals for the early-stage embryos. Survival of oyster embryos treated with acetamide was similar to PG. Embryos treated with 5M acetamide had relatively low survivals. Late-stage embryos appeared to be more tolerant to acetamide at high concentrations.

Coon *et al.* (1987) investigated the effects of 15 pharmaceuticals on the metamorphosis of *Crassostrea gigas*. Larvae were exposure to the pharmaceutical for 24 to 48 hours to assess if the pharmaceuticals influenced larvae development by inducing or inhibiting metamorphosis. The concentrations of pharmaceutical that caused 50% induction or 50% reduction in metamorphosis of oyster larvae were established. See ‘evidence summary spreadsheet for details.

Di Poi *et al.* (2014) investigated the toxicity of five antidepressant drugs on embryo–larval development and metamorphosis success in the Pacific oyster, *Crassostrea gigas*. The toxicity of Fluoxetine, Sertraline, Clomipramine, Amitriptyline, and Duloxetine was examined at a range of concentrations from 0.1 to 100,000 µg/l on two early life stages in the Pacific oyster. The toxicity was quantified in D-shaped larvae after 36 hours of exposure, and in 21-day-old pediveliger larvae after 24 hours of exposure using the percentage of normal larval development and the metamorphosis rate as endpoints, respectively. The embryotoxicity assays reported EC50 values for Fluoxetine, Sertraline, Clomipramine, Amitriptyline and Duloxetine at 191.5, 67.1, 157.3, 185.7 and 162.5 µg/l, respectively. No normal D-larvae were observed after exposure to Duloxetine or Sertraline at concentrations of 250 µg/l and no normal D-larvae were observed for the other contaminants at concentrations of 400 µg/l.

Di Poi *et al.* (2016) investigated the effects of the antidepressant Fluoxetine on juvenile oysters (*Crassostrea gigas*). The oysters were exposed to Fluoxetine at 0.001, 0.1 or 10 µg/l for 28 days. During the experiment, no mortality occurred in any of the treatments, however, at the two highest tested concentrations (0.1, 10µg/l) Fluoxetine stimulated shell growth in a transient manner.

Ehrhart & Granek (2021) transplanted juvenile oysters at varying distances near wastewater treatment plant outfalls and at oyster aquaculture control sites to assess small-scale spatial variation in contaminant uptake and oyster condition. The condition index of the oysters was lower at wastewater sites compared to aquaculture sites.

Islam *et al.* (2020) examined the embryotoxic impacts of exposure to environmentally relevant concentrations of the synthetic estrogen, 17a-ethinylestradiol (EE2), to male and female parents (50 ng/L) and their offspring (5 and 50 ng/L) in *Saccostrea glomerata*. Adult oysters were exposed to 50 ng/L EE2 for 25 days. After the exposure period the oysters were stripped and embryos were produced by fertilizing exposed male (M) and exposed female (F) gametes (FTMT), crossing where only one sex was exposed (FCMT & FTMC), and the crossing of controls (FCMC). Fertilization success and the proportion of individuals to reach D-veliger stage, along with swimming capabilities were measured. On day 2, post-fertilization larvae from each treatment-cross were exposed to 5 and 50 ng/L EE2, and a solvent control. On day 9, the survival of D-veliger larvae and shell length of D-veligers were assessed to quantify both parental as well as offspring exposure effects. No significant effects of parental exposure on fertilization success, proportions of early larval morphs and unfertilised eggs were observed. Offspring impacts were evidenced in terms of developmental delays, with decreased percentages of D-veligers, along with a reduction of swimming capabilities of larvae at two days post-fertilization when both parents had been exposed to 50 ng/L EE2. No significant parental effects were found on the survival of larvae at 9 days post-fertilization, but retardation of shell growth was observed in larvae in treatments where both parents had been exposed to 50 ng/L EE2. Larval exposure from 2 to 9 days post-fertilization caused declines in survival and reduction of shell length in larvae at both 5 and 50 ng/L EE2 across all parental exposure treatments. Parental EE2 exposure affected offspring in terms of retardation of larval development, and subsequent offspring exposure to EE2 further exacerbated the impacts on development (Islam *et al.*, 2020).

Nascimento *et al.* (2005) investigated the effects of the cryoprotectants dimethyl sulfoxide, propylene glycol and methanol on the gametes and larvae of Crassostrea rhizophorae.

Gametes and larvae were exposed to the cryoprotectants for 10, 20, and 30 minutes at a range of concentrations of 5-20%. The effective concentration that caused 15% abnormalities in the population for 24 hours was calculated. The EC15s for 10-minute sperm exposure were 3.83, 13.44, and 4.18%, for dimethyl sulfoxide, propylene glycol, and methanol, respectively. The EC15s for 10-minute oocyte exposure were 6.23, 7.89, and 2.81% for dimethyl sulfoxide, propylene glycol, and methanol, respectively. The EC15s for 10-minute larvae exposure were 10.72, 16.03, and 12.47% for dimethyl sulfoxide, propylene glycol, and methanol, respectively.

Wessel *et al.* (2007) investigated the relationship between embryotoxic and genotoxic effects of benzo[a]pyrene (B[a]P), 17α-ethinylestradiol (EE2) and Endosulfan (ES) on *Crassostrea gigas* embryos. Embryotoxicity was evaluated by calculating the percentage of abnormal D-larvae obtained after 20 hours development following exposure to the contaminants. EE2 displayed no toxic effects on the embryos at the tested range of 0.02 to 1.7 nM. For B[a]P, embryotoxicity was observed from the lowest tested concentration of 0.2 nM. For ES, embryotoxicity was observed from 300 nM.

5.3 Synthetics (other)

The ‘synthetics (other)’ category includes a range of chemicals that do not fit into other categories conveniently. Hence, several of the chemicals included under this category only appeared in one or two studies.

At total of 69 results were obtained from 35 studies reported the effects of ‘Synthetics (other)’ of oyster species. Surfactants were the most studied group (53.6% of results), followed by detergents (10%), glycols (11.6%) and alcohols (5.8%) (Figure 5.6). The relevant evidence is summarised below. The evidence from Butler, 1962, 1964, 1965, 1965b; Cardwell *et al.*, 1977, 1979; Chao *et al.*, 1994; Da Cruz *et al.*, 2007; Davies & Herbert, 1969; Meyer, 1987; Nascimento *et al.*, 2005; Nice *et al.*, 2000; Seguin *et al.*, 2017; Sigler & Leibovitz, 1982; and Zaroogian, 1981 are presented in the preceding sections.

Cardwell *et al.* (1977) investigated the effects of dodecyl sodium sulphate (SDS) on the development and survival of *Crassostrea gigas*. SDS concentrations causing 50% of the larvae to develop abnormally (EC50) and the concentrations causing 50% larval mortality (LC50) within 48 hours were estimated from the means of the multiple replicate tests. The

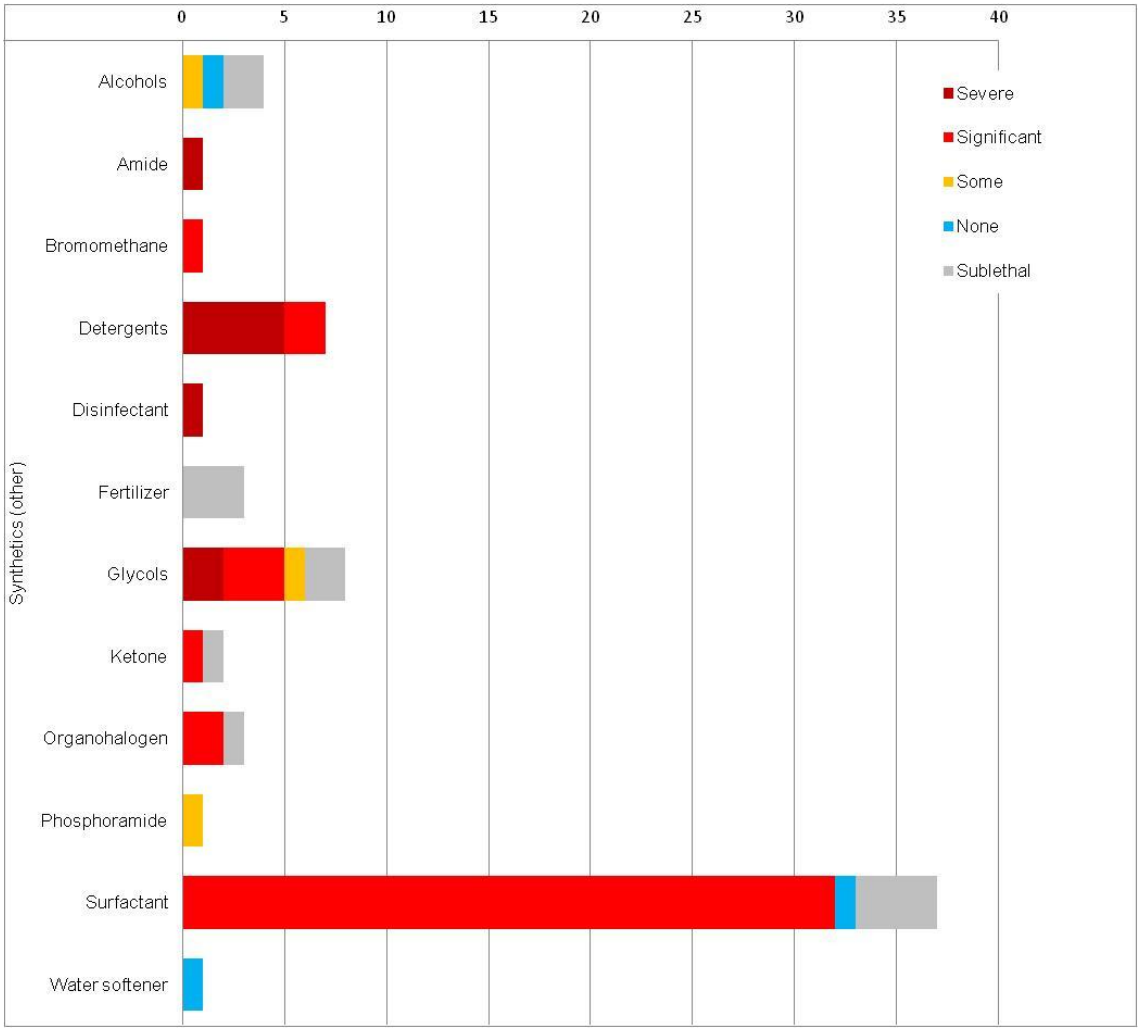

Figure 5.6. Count of ranked mortalities due to exposure to 'Synthetics(other)' in oyster species. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects.

Chapman & McPherson (1993) examined the effects of mine effluent on amphipods below the ice at Little Cornwallis Island, Northwest Canada. They also examined the effects of zinc, lead, sodium dodecyl sulphate (SDS) and cadmium chloride ($CdCl_2$) on the larval development of *Crassostrea gigas* to provide information on the relative toxicity of the mine effluent. Exposure to 0.4-3.7 mg/l Zn resulted in >95% larval abnormality so a lower concentration was used to determine the EC50. This gave an 48-hour EC50 of 0.2 mg/l Zn and a NOEC of 0.1 mg/l. Initial tests with lead also gave >95% abnormality but retesting with lower concentrations gave a 48-hour EC50 of 0.38-0.55 mg/l. Exposure to SDS gave a 48-hour EC50 of 1.8 and 2.8 mg/l SDS, and 48 hour EC50 of 0.8 to >1.0 mg/l $CdCl_2$.

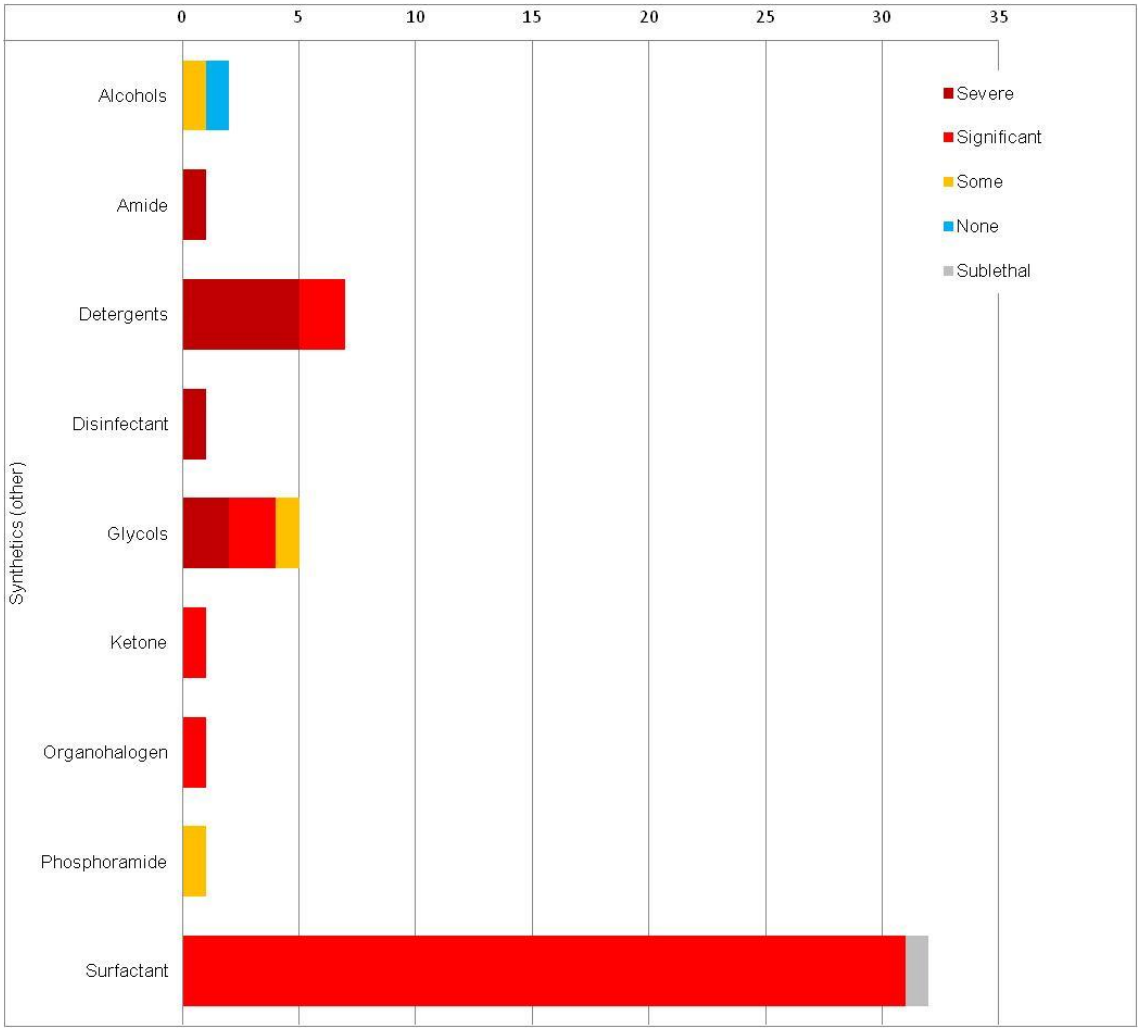

Figure 5.7. Count of ranked mortalities due to exposure to 'Synthetics (other)' in early life stages of oyster species. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects.

Devillers *et al.* (2002) exposed *Crassostrea gigas* embryos to ethylene glycol monobutyl ether (EGBE) and ethylene glycol monobutyl ether acetate (EGBEA) at concentrations between 1 to 10,000 mg/L for 48 hours. At concentrations up to 100 mg/l, no significant difference in the number of abnormal larvae occurred for either contaminant. However, at 1,000 and 10,000 mg/L 100% abnormal development occurred for both contaminants.

Devillers *et al.* (2002) reported the effects of Ethylene glycol monobutyl ether (EGBE) on various aquatic organisms' survival, growth, and reproduction. Citing MBA (1984) LC50 values of 181, 160, 114 and 89.4 mg/l for *Crassostrea virginica* larvae exposed to EGBE for 24, 48, 72 and 96 hours, respectively.

Ostroumov (2003) investigated the effects of the surfactants sodium dodecylsulphate (SDS), and tetradecyltrimethylammonium bromide on the filtration rate of *Crassostrea gigas*. Exposure to the surfactants reduced the filtration rates of the oysters.

Pittinger *et al.* (1992) investigated the effects of succinate tartrates on the survival and shell deposition of *Crassostrea virginica*. Concentrations between 190 to 900 mg/l did not affect the survival or shell deposition during a 96-hour exposure.

Schneider *et al.* (1979) investigated the effects of hexamethylphosphoramide on the development of *Crassostrea virginica*. Concentrations up to and including 5,140 mg/l hexamethylphosphoramide did not interfere with the normal development of the embryos to the veliger stage, during a 48-hour exposure. Oyster mortality during the 33-day exposure was initially low but after day 28, significant mortality occurred in the 10 and 100 mg/l treatment groups.

Smith (1968) reported the effects of a number of detergents on oyster larvae based on experiments carried out at the Cefas laboratories, Conway. Developing eggs were exposed to a range of detergents (Polyclens, BP1002, Houghton Solv. 122, Slip-clean, Dasic and Gamlen) at 0, 0.5, 1.0, & 3.0 ppm for 24 hours and the proportion of swimming D-larvae examined. All of the detergents tested were toxic to oyster larvae at 3 ppm with Polycleans and Houghton Solv 112 causing 100% mortality at 0.5 ppm and Dasic at 3.0 ppm. In other experiments with *Ostrea edulis* larvae, exposure to the detergents Kudos, Slix, Polyclens, Gamlen, Teepol, and Houghtosol halved the normal rate of larval development at concentrations ranging from 2.5-7.5 ppm (2.5-7.5 mg/l).

Stewart *et al.* (1979) investigated the toxicity of by-products of oxidative biocides on oyster larvae. *Crassostrea virginica* larvae were exposed to bromate, bromoform, and chloroform, at 0.05, 0.1, 1.0, and 10.0 mg/l for 48 hours. Mortality was observed after the 48-hour exposure period. Larval mortality occurred at all concentrations of the three substances.

5.4 Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) were examined in only four articles reviewed. *Crassostrea virginica* was the only oyster species examined in those studies. Overall, the studies reported either no mortality or sublethal effects alone.

Duke *et al.* (1970) investigated the effects of PCBs on the shell growth rate of *Crassostrea virginica*. The oysters were exposed to PCBs at 1, 10 and 100 µg/L for a period of 96 hours.

The exposure to PCB caused the shell growth rates to decrease with complete growth inhibition at 100 µg/l. However, the oysters did survive and the shell growth rate equalled that of the controls after three weeks in uncontaminated water.

Hansen *et al.* (1974) investigated the toxicity of Aroclor 1016 on the growth of *Crassostrea virginica*. The oysters were exposed to Aroclor 1016 at 1, 10, and 100 µg/l for a period of 96 hours. The shell growth of the oysters was inhibited by exposure, with 1, 10, and 100 µg/l Aroclor 1016 causing a 10, 38, and 93% reduction in shell growth.

Lowe *et al.* (1972) investigated the effects of the polychlorinated biphenyl Aroclor 1254 on *Crassostrea virginica*. Young oysters were exposed to Aroclor 1254 at 1 and 5 µg/l. In oysters exposed to 5 µg/l for 24 weeks, the growth rate was significantly reduced. The growth rate of the oysters exposed to 1 µg/l for 30 weeks was not significantly affected. The mortality of the oysters was not influenced by either treatment.

5.5 Flame retardants

The effects of flame retardants were examined in only two studies, of which one only could be accessed. The Great Lakes Chemical corporation (1989, unseen) reported that the flame retardant 4,4'-(1-Methylethylidene)bis[2,6-dibromophenol] affected shell deposition in immature *Crassostrea virginica* but did not report mortality.

Xie *et al.* (2017b) investigated the effects of the polyaromatic hydrocarbon (PAH) benzo[a]pyrene (B[a]P) and the flame retardant 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) on the development of embryos and the survival of larvae in *Crassostrea gigas*. In the embryotoxicity testing embryos were exposed to 10, 20, 40, 80, or 160 µg/l of B[a]P, or were exposed to 100, 200, 400, 800 or 1600 BDE-47 for 24 hours. Larvae were exposed to the same concentrations as used in the embryotoxicity testing but larvae were exposed for 96 hours. The 24-hour embryotoxicity EC50s for B[a]P and BDE-47 were calculated as 18.4 and 203.3 µg/l, respectively. Larvae mortality 96-hour LC50s were calculated as 26.8 µg/l for B[a]P and at 244.5 µg/l for BDE-47.

5.6 Phthalates

Phthalates or phthalate esters were examined by only three articles. The evidence from Meyer (1987) and Zaroogian (1981) are summarised above. Zaroogian (1981) reported sublethal effects on *Crassostrea virginica* embryos exposed to benzyl butyl phthalate, while

Meyer (1987) reported abnormal development (and mortality) of *C. virginica* larvae exposed to benzyl butyl phthalate, with a 48-hour EC50 of 780 µg/l.

De Araujo *et al.* (2015) determined the chemical composition and the toxicity of lightsticks recently activated, compared to lightsticks one year after activation, and to lightsticks collected on beaches. The effects of lightstick content on embryos of *Crassostrea rhizophorae* after 24 hours of exposure was assessed at various concentrations (0.32, 0.56, 1, 1.76, and 2.24% WSF). The effects of WSF of dibutyl phthalate (DBP) and dimethyl phthalate (DMP) were also examined at final concentrations of 10%, 18%, 32%, and 56% WSF. The value of the WSF-effective concentration (24-hour EC50) that caused abnormal development of larvae was 0.35% for new light sticks but, after one year of activation, the toxicity of the light stick was even higher at 0.65%. Similarly, the 24-hour EC50s that caused abnormal development in larvae were 7.42% exposed to DBP and 13.07% exposed to DMP.

5.7 Perfluoroalkyl substances (PFAS)

The effects of perfluoroalkyl substances (PFAS) on oysters were reported in only two studies. Drottar & Krueger, (2000; not accessed) reported that the perfluorooctanesulfonic acid (PFOS) 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluoro-1-octanesulfonic acid (potassium salt) affected shell deposition in *Crassostrea virginica* resulting in a 96-hour EC50 of 3 mg/l.

OECD (2002) reported the static acute (short-term) toxicity of potassium perfluorooctanesulphonate to *Crassostrea virginica* over a 96-hour exposure period. Shell growth inhibition was calculated as the percentage reduction in shell growth relative to mean shell growth in the negative control. The potassium perfluorooctanesulphonate 96-hour EC50 for the Eastern oyster was determined to be >3.0 mg/L, the highest concentration tested and the practical limit of solubility in unfiltered seawater. The 96- hour no effect concentration (NOEC) was 1.9 mg/l.

5.8 Sensitivity assessment – Synthetic compounds

The count of ranked ‘worst –case’ mortalities due to ‘Synthetic compounds’ are summarized in Figure 5.6 and Figure 5.7 above. Table 5.2 and

Table 5.3 below demonstrate considerable difference in the sensitivity of adults and juveniles when compared to early life stages. Sublethal effects were reported for 87.5% of the results from studies of adults and juveniles. Whereas, 19% of the results for early life stages reported ‘severe’ mortality, 70.6% ‘significant’ mortality, 3% ‘some’ mortality, 2% ‘no’ mortality

and only 4.6% reported sublethal effects. In the case of gametes, 14.75% of the reported results were 'severe', 34.4% 'significant', 3% no effect and 47.5% sublethal effects, albeit based on only 16 articles.

Table 5.2. Summary of count of ranked mortalities of adult and juvenile oysters to 'Synthetic compounds' reported in the evidence review and resultant proposed sensitivity assessments (N= None, VL= Very low, L= Low, M= Medium, High = High, and NS= Not sensitive).

Group	Type	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Pesticide/Biocide	Acaricide					4	4	H	H	NS
	Carbamate				2	5	7	H	H	NS
	Fungicide					13	13	H	H	NS
	Herbicide				6	50	56	H	H	NS
	Insecticide		2			33	35	L	L	H
	Mixture					1	1	H	H	NS
	Molluscicide					1	1	H	H	NS
	Antifoulant			1			1	M	M	M
	Nematicide					1	1	H	H	NS
	Organohalogen	1	4	1		34	40	N	VL	H
	Organonitrogen					1	1	H	H	NS
	Organophosphate	2	1	1	1	33	38	N	VL	H
	Organosulphate					1	1	H	H	NS
	Phenols					1	1	H	H	NS
	Pyrethroid		2				2	L	L	H
Total		**3**	**9**	**3**	**9**	**178**	**202**	**N**	**VL**	**H**
Pharmaceutical	Antibiotic					1	1			
	Antidepressant				1		1	H	H	NS
	Hormone					1	1	H	H	NS
	Hormones					1	1	H	H	NS
	Mixture					1	1	H	H	NS
Total					**1**	**4**	**5**	**H**	**H**	**NS**
Synthetics (other)	Fertilizer					1	1			
	Organohalogen					1	1	H	H	NS
	Surfactant				1	2	3	H	H	NS
Total					**1**	**4**	**5**	**H**	**H**	**NS**
Polychlorinated biphenyls (PCBs)	PCBs				**1**	**1**	**2**	**H**	**H**	**NS**
Flame retardants	Organobromide					**1**	**1**	**H**	**H**	**NS**
Overall total		**3**	**9**	**3**	**12**	**188**	**215**	**N**	**VL**	**H**

Table 5.3. Summary of count of ranked mortalities of early life stages of oysters to 'Synthetic compounds' reported in the evidence review and resultant proposed sensitivity assessments (N= None, VL= Very low, L= Low, M= Medium, High = High, and NS= Not sensitive).

Group	Type	Severe	Significant	Some	None	Sublethal	Total	Resistance	Recovery	Sensitivity
Pesticide/Biocide										
	Bacteriocide	2	2				4	N	VL	H
	Carbamate	1	6				7	N	VL	H
	Degradation product		3				3	L	L	H
	Fungicide	2	5				7	N	VL	H
	Herbicide	11	35	1	2	2	51	N	VL	H
	Insecticide	5	7	1			13	N	VL	H
	Metabolite		3		1		4	L	L	H
	Mixture		2				2	L	L	H
	Nanoformulated		1				1	L	L	H
	Antifoulant	2	4	1		1	8	N	VL	H
	Neonicotinoids		1				1	L	L	H
	Organohalogen	3	15			3	21	N	VL	H
	Organophosphate	5	15			4	24	N	VL	H
	Parasiticide		1				1	L	L	H
	Phenols		4	1			5	L	L	H
	Pyrethroid		2				2	L	L	H
Total		**31**	**106**	**4**	**3**	**10**	**154**	**N**	**VL**	**H**
Pharmaceutical										
	Adrenergic agonist		5				5	L	L	H
	Antibiotic		6				6	L	L	H
	Anticonvulsant		1				1	L	L	H
	Antidepressant	5					5	N	VL	H
	Anti-inflammatory	1		1			2	N	VL	H
	Antimicrobial		3				3	L	L	H
	Beta-blocker		1				1	L	L	H
	Hormones				1		1	M	M	M
	Psychotropic		2				2	L	L	H
	Receptor blocker		1				1	L	L	H
Total		**6**	**19**	**1**	**1**		**27**	**N**	**VL**	**H**
Synthetics (other)										
	Alcohols			1	1		2	M	M	M
	Amide	1					1	N	VL	H
	Detergents	5	2				7	N	VL	H
	Disinfectant	1					1	N	VL	H
	Glycols	2	2	1			5	N	VL	H

Group	Type	Severe	Significant	Some	None	Sublethal	Total	Resistance	Recovery	Sensitivity
	Ketone		1				1	L	L	H
	Organohalogen		1				1	L	L	H
	Phosphoramide			1			1	M	M	M
	Surfactant		31			1	32	L	L	H
Total		**9**	**37**	**3**	**1**	**1**	**51**	**N**	**VL**	**H**
Phthalates										
	Mixture		1				1	L	L	H
	Phthalate		2				2	L	L	H
	Phthalate esters		1				1	L	L	H
Total			**4**				**4**	**L**	**L**	**H**
Flame retardants										
	Organobromide		**2**				**2**	**L**	**L**	**H**
Overall total		**46**	**168**	**8**	**5**	**11**	**238**	**N**	**VL**	**H**

5.8.1 Pesticide/biocides

As above, adults and juveniles were more resistant to pesticide/biocide exposure than early life stages (Table 5.2 and Table 5.3). For example, 5.8% of the results of exposure of adults and juveniles to pesticides/biocides reported ‘severe’ or ‘significant’ mortality compared to 88% that reported sublethal effects, whereas 89% reported ‘severe’ or ‘significant mortality after exposure of early life stages, compared to only 6% that reported sublethal effects. Over 200 different pesticide/biocides, their metabolic or degradation products were catalogued divided amongst 22 different functional (e.g. herbicide, insecticide) or structural (e.g. organohalogen, organophosphate) groups. Therefore, it is not possible to discuss the sensitivity of each pesticide/biocide reviewed.

Overall, there is considerable evidence to suggest that adult and juveniles are resistant of most pesticides, with the exception of some insecticides, organophosphates and organohalogens (Table 5.2) but that early life stages (e.g. larvae) are sensitive to a wide range of pesticides/biocides. Therefore**, the resistance of the early life stages oyster species to pesticides/biocides is assessed as ‘None’**. Hence, the **resistance of oyster beds is assessed as ‘Low’, resilience as ‘Low’ and sensitivity as ‘High’** on the assumption that loss of recruitment would lead to population decline.

5.8.2 Pharmaceuticals

'Severe' mortality was reported in 16.6% of the results of exposure to pharmaceuticals, 53% reported 'significant', 2.8% 'some', 5.5% 'no mortality, and the remaining 22% only reported sublethal effects. However, the five articles that examined adult and juvenile oysters did not report any mortality (22% of results) or only sublethal effects (88% of results). Conversely, the 11 articles that examined early life stages reported 'severe' mortality in 22% of the results, 'significant' in 70%, 'some' in 3.7%, 'none' in 3.7% and sublethal effects in 3.7% of the results (Figure 5.5; Table 5.2, and Table 5.3). Overall, 28 separate pharmaceuticals were reported in the review but most of the chemicals were only tested in a single study (see 'Evidence summary spreadsheet).

The **resistance of the early life stages in oyster species to pesticides/biocides is assessed as 'None'.** However, the 'worst-case' resistance of adults and juveniles is probably 'High' (no mortality and/or sublethal effects). Therefore, the **resistance of oyster beds is assessed as 'Low' based** on the assumption that loss of recruitment would lead to population decline. Hence, **resilience is assessed as 'Low' and sensitivity as 'High'**.

5.8.3 Synthetics (other)

Overall, 13% of the results of exposure to 'synthetics (other)' reported 'severe' mortality, 59.4% reported 'significant mortality, 4.3% 'some' mortality, 4.3% no mortality and 18.8% reported sublethal effects (Figure 5.6; Table 5.2). No mortality or only sublethal effects were reported for adults and juveniles. However, the early life stages were less resistant to their effects. For example, 17.6% of the results reported 'severe' mortality, 72.5% reported 'significant' mortality, 5.8% 'some' mortality, 1.9% reported no mortality and 1.9% reported sublethal effects (Figure 5.7; Table 5.3).

Detergents and surfactants were the most toxic to larvae, which agrees with the finding of His *et al.* (2000). The exposure of *Ostrea edulis* larvae the detergents Kudos, Slix, Polyclens, Gamlen, Teepol, and Houghtosol halved the normal rate of larval development at concentrations ranging from 2.5-7.5 ppm (2.5-7.5 mg/l) (Smith, 1968). Renzoni, (1973b; cited by His *et al.*, 2000) also reported significant mortality in *Ostrea edulis* larvae exposed to tetrapropylene benzene sulphonate (with an LC50 of 2 mg/l).

The remaining 'Synthetic(other)' chemicals were reported by only a few studies and varied in their sensitivity (see Table 5.2 and Table 5.3), although most types of chemical would be assessed as 'High' sensitivity.

The **resistance of the early life stages in oyster species to synthetic (others) is assessed as 'None'.** However, the 'worst-case' resistance of adults and juveniles is probably 'High' (no mortality and/or sublethal effects). Therefore, the **resistance of oyster beds is assessed as 'Low' based** on the assumption that loss of recruitment would lead to population decline. Hence, **resilience is assessed as 'Low' and sensitivity as 'High'**.

5.8.4 Polychlorinated biphenyls (PCBs)

The effects of polychlorinated biphenyls (PCBs) were examined in only four studies (see above). Exposure to either Aroclor 1016, 1254 or PCB 1254 did not result in mortality and/or only resulted in sublethal effects. No studies of the effects of PCBs on oyster larvae were found. Therefore, **the resistance of oyster species to PCBs is assessed as 'High', resilience as 'High', and sensitivity as 'Not sensitive'.**

5.8.5 Flame retardants

Only two studies examined the effects of brominated flame retardants on oysters (Great Lakes corporation, 1989; Xie *et al.*, 2017b). Sublethal effects (on shell deposition) were reported in immature oysters (Crassostrea sp.) but BDE-47 caused abnormal development of embryos and significant mortality in larvae. **Therefore, the resistance of early life stages to brominated flame retardants is probably 'Low' but immature oysters is 'High'**. Hence, the **resistance of oyster beds is assessed as 'Low' based** on the assumption that loss of recruitment would lead to population decline. Hence, **resilience is assessed as 'Low' and sensitivity as 'High'**. However, confidence in the assessment is 'Low' due to the disagreement in effect between the limited number of studies.

5.8.6 Phthalates

The effects of phthalates were only examined in embryos and larvae. All three of the studies reported significant mortality and/or abnormal development in embryos and larvae to the phthalates studied. Therefore**, the resistance of early life stages to phthalates is probably 'Low'**. Hence, the **resistance of oyster beds is assessed as 'Low' based** on the assumption that loss of recruitment would lead to population decline. Hence, **resilience is assessed as 'Low' and sensitivity as 'High'**. However, confidence in the assessment is 'Low' due to the disagreement in effect between a limited number of studies.

5.8.7 Perfluoroalkyl substances (PFAS)

Perfluoroalkyl substances (PFAS) was examined in two studies (Drottar & Krueger, 2000; OECD, 2002) neither of which specified the life stage of *Crassostrea virginica* examined. Both studies reported sublethal effects at the concentrations tested. The OECD (2002) suggested a 96-hour NOEC of 1.9 mg/l. Therefore, **resistance is assessed as 'High', resilience as 'High' and sensitivity as 'Not sensitive',** albeit with 'Low' confidence due to the limited number of studies reviewed.

5.9 Radionuclides

The effects of exposure to tritium were reported by one article (Nelson, 1971; not accessed). Nelson (1971) reported mortality in *Crassostrea gigas* larvae after exposure to 0.000001 Ci/l[11] to 0.01 Ci/l Tritium but did not specify the larval stage or the level of mortality observed. Another paper that examined the effects of radioactive isotopes of chromium, strontium, zinc and yttrium on oyster larvae (Nelson, 1968) could also not be accessed. Therefore, **resistance is assessed as 'Low' as a precaution** but with 'Low' confidence because the level of mortality was not specified. Hence, **resilience is assessed as 'Low' and sensitivity as 'High'.**

5.10 Other substances

'Other substances' include a range of chemicals that do not fit into the other categories of contaminant. Neither do they group conveniently. Therefore, the results of individual chemicals are tabulated in Table 5.4. A total of 60 results ('worst-case' ranked mortalities) were obtained from 27 articles that examined the effects of 'other substances' on oyster species. *Crassostrea virginica* and C. gigas were the most studied species but *Ostrea* sp. were not examined in the studies reviewed. Early life stages represented 55% of the results, while gametes represented 31%, adults and juveniles 5%, and life stage was not specified in another 8% of results. The evidence is summarised below.

[11] Ci = Curie – a non-SI unit of radioactive decay.

Table 5.4. Summary of count of 'worst-case' ranked mortalities of oysters species to 'Other substances' reported in the evidence review and resultant proposed sensitivity assessments (N= None, VL= Very low, L= Low, M= Medium, High = High, and NS= Not sensitive).

Group	Chemical name	Severe	Significant	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Inorganic chemicals	Bromine chloride (BrCl)		1			**1**	L	L	H
	Calcium hypochlorite		1			**1**	L	L	H
	Chloramine	1				**1**	N	VL	H
	Chlorine	2	9			**11**	N	VL	H
	Chlorine/Bromine		1			**1**	L	L	H
	Sodium bromate	1	2			**3**	N	VL	H
	Sodium hypochlorite	1				**1**	N	VL	H
	Fluoride		1			**1**	L	L	H
	Phosphoric acid	1				**1**	N	VL	H
	Potassium chloride (KCl)		1	1		**2**	L	L	H
	Sodium azide				1	**1**	H	H	NS
	Sodium cyanide		1			**1**	L	L	H
	Sodium sulphide		1		1	**2**	L	L	H
Total		**6**	**18**	**1**	**2**	**27**	**N**	**VL**	**H**
Natural product									
	Starch	1				**1**	?	?	?
	Tannic acid		1			**1**	?	?	?
Explosives/propellants									
	Picramic acid	1			1	**2**	N	VL	H
	Picric acid	1			1	**2**	N	VL	H
Total		**2**			**2**	**4**	**N**	**VL**	**H**
Other									
	Collected light stick content		1			**1**	L	L	H
	Light stick content after one year		1			**1**	L	L	H
	New crumb rubber granulates	1			3	**4**	N	VL	H
	New light stick content		1			**1**	L	L	H
	New oyster-farming rubber bands	1			3	**4**	N	VL	H
	New Tires		1		3	**4**	L	L	H
	Used crumb rubber granulates	1			3	**4**	N	VL	H
	Used oyster-farming rubber bands	1			3	**4**	N	VL	H
	Used Tires			1	3	**4**	M	M	M
Total		**4**	**4**	**1**	**18**	**27**	**N**	**VL**	**H**
Overall total		**13**	**23**	**2**	**22**	**60**			

5.10.1Inorganic chemicals

Most of the studies examined chemicals used in chlorination as a form of disinfectant. The evidence is summarised below.

Bellance & Bailey (1977) reported a bioassay completed by the Virginia Institute of Marine Science that established an LC50 of 5 µg/L for *Crassostrea virginica* larvae exposed to chlorine for 48 hours in static testing.

Capuzzo (1979) investigated the effects of temperature on the toxicity of chlorine and chloramine to *Crassostrea virginica* in a flow-through system. The oyster larvae were exposed to chlorine or chloramine for 30 minutes at either 20 or 25℃, after the 30-minute exposure the toxicant was removed from the solution and the temperature was reduced down to the acclimation temperature. The mortality of the larvae was assessed 48 hours after exposure. The temperature was shown to enhance the toxic effects of the chemicals. The LC50 and LC100 values for exposure to chlorine at 20℃ were 120 and 1400 µg/l, respectively but at 25℃ the LC50 and LC100 values for chlorine were 80 and 860 µg/l, respectively. The LC50 and LC100 values for exposure to chloramines at 20℃ were 10 and 480 µg/l, respectively but at 25℃ the LC50 and LC100 values for chloramine were <10 and 160 µg/l, respectively.

Chien & Chou (1989) examined the effects of chlorine exposure on the development of *Crassostrea gigas* under various temperatures and salinities, at different stages of development. Fertilized eggs at the first polar stage and four larval stages (blastula, trochophore, veliger, and D-larva) were exposed to combinations of five concentrations (0 to 2520 µg/l) of chlorine, at four temperatures (22, 25, 28℃) and three salinities (18, 26, 34 ppt) for one hour. Chlorine exposure to all of the tested stages had lethal impacts on the larvae. In general, the resistance to chlorine increased with salinity, with lower LC50s observed at lower salinities. Larval sensitivity to chlorine generally increased with higher exposure temperatures.

Crecelius (1979) examined the effects of ozonization of seawater on the production of bromate. Crecelius (1979) reported that ozonization of seawater converted of all bromide to bromate within 60 mins. Ozonization of sodium chloride solution did not result in significant oxidants while sodium bromide solution resulted in both bromide and bromate. Nevertheless, they concluded that the levels of bromate produced by chlorination or ozonization of power plant cooling waters were not acutely toxic to *Crassostrea gigas* larvae, fish, shrimp, and

clams by comparison with toxicity figures determined in their laboratory. Crecelius (1979) reported that 1.0 mg/l bromate resulted in 90% mortality in *Crassostrea virginica* larvae, and 30 mg/l bromate caused abnormal development in 50% of *Crassostrea gigas* larvae during the first 48-hour of larval development (48-hour EC50/LC50 of 30 mg/l bromate). However, no experimental details were provided (Crecelius, 1979).

Roberts *et al.* (1975) examined the toxicity of chlorine in estuarine water to a range of marine species, using both flow-through and static tank systems. They examined oyster larvae and juveniles (*Crassostrea virginica*), *Mercenaria mercenaria* larvae, *Acartia tonsa* (copepod), *Palaemonetes pugio* and fish (*Menidia menidia, Syngnathus fuscus*, and *Gobiosoma bosci*). They noted that molluscan larvae and copepods were the most sensitive species with a 48-hour LC50 of less than 0.005 ppm.

Roberts & Gleeson (1978) exposed *Crassostrea virginica* larvae to bromine chloride (BrCl) during 48-hour exposure assays. The concentration that caused 50% mortality (LC50) was calculated at 210 µg/l bromine chloride. In addition, to the larvae tests, juvenile oysters were exposed to BrCl for 96 hours to assess the impacts on shell growth. The 96-hour EC50s for the shell growth were 100 and 160 µg/l.

Roosenburg *et al.* (1980) examined the effects of chlorine on two larval stages of *Crassostrea virginica*. Straight-hinge veliger larvae were exposed to concentrations of 10, 50, 100 and 200 µg/l chlorine for 6, 12, 24 and 36 hours, and to 50, 100, 200 and 300 µg/l for 8, 24, 48, 72 and 96 hours. Pediveliger larvae were exposed to 50, 100, 200 and 300 µg/l chlorine for 6, 24, 48, 72 and 96 hours. Mortality increased with increasing concentration in both larval stages. Straight hinge veliger larvae were more sensitive than pediveliger larvae with between 83-100% mortality at the highest tested concentration at 96 hours. Pediveliger larvae had between 32.4-46.1% mortality under the same conditions.

Scott & Middaugh (1978) investigated the seasonal toxicity of chlorination on *Crassostrea virginica*. Bioassays were conducted in the fall (45-day exposure), winter (75-day exposure) and spring (60-day exposure). Adult oysters were collected, accumulated, and exposed to sodium hypochlorite at nominal concentrations of 5.6, 3.2, 1.8, and 1 mg/L. During each of the seasonal bioassays survival, condition index, gonadal index, and faecal production were assessed. Total (100%) mortality occurred in all of the treatments at the highest nominal concentration. However, the winter assay had a delay in mortality with 100% mortality occurring on day 70, compared to day 22 for fall and day 32 for spring. The condition index

of the controls was higher than the exposed oysters. Similarly, the gonadal index was significantly higher in the control oysters.

Stewart *et al.* (1979) investigated the toxicity of by-products of oxidative biocides on oyster larvae. *Crassostrea virginica* larvae were exposed to bromate, bromoform, and chloroform, at 0.05, 0.1, 1.0, and 10.0 mg/l for 48 hours. Mortality was observed after the 48-hour exposure period, at all concentrations of the three substances larval mortality occurred.

All but one of the studies above examined the effect of chlorination, bromination, or ozonization of oyster larvae. All the studies reported 'severe' or 'significant' larval mortality at the concentrations tested. In addition, adult *Crassostrea virginica* experienced a reduction in condition due to exposure to chlorination and 100% mortality at 5.6 mg/l. **Therefore, resistance would be assessed as 'None', resilience as 'Very low, and sensitivity as 'High', especially in larvae.**

Fluoride. Cardwell *et al.* (1979) (see above) examined the effects of a large number of different chemicals on the larvae of *Crassostrea gigas*. Fluoride was reported to result in a 48-hour EC50 (abnormal development) of 58 mg/l and a 48-hour LC50 (larval mortality) of >100 mg/l. **Therefore, resistance to fluoride exposure would be assessed a 'Low', resilience as 'Low' and sensitivity as 'High'**. However, this assessment is based on a single study.

Phosphoric acid. The effects of phosphoric acid on oyster larvae were reported by Daugherty (1951) and Kunigelis & Wilbur (1987). Kunigelis & Wilbur (1987) could not be accessed and the level of mortality was not specified. Daugherty (1951) reported 100% mortality in C. virginica after 28-hour exposure to 1,500 mg/l but the article could not be accessed for further detail. . **Therefore, resistance would be assessed as 'None', resilience as 'Very low, and sensitivity as 'High', especially in larvae.** However, this assessment is based on a single study that used a high concentration (1,500 mg/l). It might represent the effects immediately after and in close proximity to a spill and confidence in the assessment is 'Low'.

Potassium chloride (KCl) was included in two studies. Da Cruz *et al.* (2007) reported that the exposure of Crassostrea rhizophorae embryos to potassium (as KCl) for 24-hours to resulted in abnormal larval development, and a 24-hour LC15 of 25.13 mg/l and a 24-hour LC50 35.56 mg/l. Nell & Holliday (1986) examined the use of KCl and $CuCl_2$ to stimulate larval settlement in *Saccostrea commercialis* larvae, in static containers in the laboratory.

Settlement was stimulated by 8-12 mM KCl (160 - 210 µg/l) and no mortality was observed. Therefore, low concentrations of KCl were used to induce larval settlement while high concentrations (mg/l) were reported to cause abnormal larval development. **Hence, resistance would be assessed as 'Low', resilience as 'Low' and sensitivity as 'High'.** However, the assessment is based on a single study using high concentrations of KCl so the confidence is assessed as 'Low'.

Caldwell *et al.* (1975) investigated the effects of **hydrogen sulphide** on the survival and development of *Crassostrea gigas*. The tests were run over a four day period, the longer the oysters were exposed to hydrogen sulphide the lower the concentration was to cause 50% mortality. The LC50 at 24, 48, and 96 hours were 3,300, 2,600, and 1,400 µg/l, respectively. **Therefore, resistance to sulphide exposure is be assessed as 'Low', resilience as 'Low' and sensitivity as 'High'.**

Okubo & Okubo, 1962 (cited by His, 2000) reported a 48-hour EC50 (abnormal development) of 32-100 µg/l in *Crassostrea gigas* embryos after exposure to sodium cyanide. Therefore, the r**esistance** of the early life stages of *C. gigas* to sodium cyanide would be assessed as **'Low', resilience as 'Low' and sensitivity as 'High'.**

5.10.2Natural products

Daugherty (1951) reported that exposure to 'starch' at a concentration of 3 g/l resulted in 100% mortality in *Crassostrea virginica*. Unfortunately, Daugherty (1951) could not be accessed and it is unclear how the starch was administered or how the effect was caused so no sensitivity, assessment is made. Similarly, Cardwell, 1979b (cited by His *et al.*, 2000) reported that exposure of the fertilized eggs of *Crassostrea gigas* to tannic acid resulted in abnormal development and a 48-hour EC50 of >10 mg/l. Unfortunately, Cardwell, 1979b (cited by His *et al.*, 2000) could not be accessed and it is unclear how the tannic acid was administered or how the effect was caused so **no sensitivity assessment is made**.

5.10.3Explosives

Goodfellow *et al.* (1983, 1983b) examined the lethal and sublethal effects of picric acid and picramic acid on oysters as they were potential contaminants from industrial effluents and the manufacture of explosives. Goodfellow *et al.* (1983) investigated the acute toxicity of picric acid and picramic acid on *Crassostrea virginica*. The 144-hour LC5Os for picric and picramic acid were 254.9 and 69.8 mg/l, respectively. No growth EC50s and shell deposition EC5Os showed that both contaminants caused adverse effects at much lower concentrations than

indicated by the LC50s. For example, the 144-hour shell deposition EC50s were 27.9 mg/l for picric acid and 5.6 mg/l for picramic acid.

Goodfellow *et al.* (1983b) investigated the effects of picric acid and picramic acid on the growth of *Crassostrea virginica*. Exposure to 0.45 and 0.05 mg/l (450 and 50 µg/l) picric acid and 0.24 and 0.02 mg/ (240 and 20 µg/l) picramic acid showed significant inhibition of shell deposition during the 42 days of exposure. In addition, discolouration of the nacre layer of the shell and body mass was observed after exposure to both contaminants by the end of the 42-day trial.

Exposure to picric or picramic acids in the water column was reported be lethal to C. virginica. **Therefore, resistance is assessed as 'None', resilience as 'Very low, and sensitivity as 'High'.**

5.10.4Lightsticks

De Araujo *et al.* (2015) determined the chemical composition and the toxicity of lightsticks that were recently activated, compared to lightsticks one year after activation and to lightsticks collected on beaches. The effects of lightstick content on embryos of *Crassostrea rhizophorae* after 24 hours of exposure was assessed at various concentrations (0.32, 0.56, 1, 1.76, and 2.24% WSF). The value of the WSF-effective concentration (24-hour EC50) that caused abnormal development of larvae was 0.35% for new light sticks but, after one year of activation, the toxicity of the light stick was even higher at 0.65%. **Therefore, resistance would be assessed a 'Low', resilience as 'Low' and sensitivity as 'High'**. However, this assessment is based on a single study.

5.10.5Rubber

Tallec *et al.* (2022) investigated the chemical toxicity of different types of new and used rubber products (tires, crumb rubber granulates, aquaculture rubber bands) on early life stages of the Pacific oyster, *Crassostrea gigas*. Leachates were obtained from the products at 0.1, 1, and 10 g/L. Sperm and embryos were exposed to leachates at 0.1, 1, and 10g/l for one hour before being assessed for viability. The effect on fertilization was assessed by combining gametes with the different concentrations of each of the leachates for 1.5 hours before assessing the fertilization yields. The impacts on the development of larvae were assessed by exposing embryos to leachates at 0.1, 1, and 10 g/l for 36 hours. Abnormal D-larvae were classed as those with morphological malformations or those which had developmental arrest during embryogenesis. The viability of oyster sperm were not

significantly affected by exposure to leachates from new tires, used tires, new crumb rubber granulates, used crumb rubber granulates, and used oyster-farming rubber bands at any of the concentrations (0.1, 1 and 10g/l) tested when compared with the control treatments. However, significant reductions in the percentage of live spermatozoa were observed at the highest tested concentrated leachate (10 g/l) from new oyster-farming rubber bands. The viability of oyster oocytes was not significantly affected by exposure to any of the leachates at any of the tested concentrations. The fertilization yield was not significantly affected by exposure to leachates from new tires, used tires, new crumb rubber granulates, used crumb rubber granulates, or used oyster-farming rubber bands when compared with the control treatment. However, significant reductions in fertilization yield were observed at the highest tested concentration of leachate (10 g/l) from new oyster-farming rubber bands. Embryo-larvae development was significantly reduced by 53% by exposure to new-tire leachate at 10 g/l. Embryo-larval development was completely inhibited at the highest tested leachate concentration (10 g/L) of new crumb rubber granulates, used crumb rubber granulates, and used oyster-farming rubber bands. Embryo-larval development was completely inhibited at 1g/l of new oyster-farming rubber bands leachate.

Therefore, the resistance of embryos and larvae to rubber leachates would be assessed as ‘None’, resilience as ‘Very low, and sensitivity as ‘High’.

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6 Toxicity

The MarESA approach bases sensitivity assessment on the 'worst-case' scenario. Contaminant sensitivity assessment is based on the 'worst-case ranked mortality' reported with any one given article/study. Therefore, it is difficult to compare relative sensitivity across species, taxonomic and/or chemical groups. Hence, we have attempted to rank the toxicity of chemicals and chemical groups to 'oyster species'.

The GESAMP (2013) acute toxicity hazard evaluation endpoints applied to the evidence dataset complied in this review. These endpoints form part of the GESAMP Hazard Profiles complied on chemical substances transported by shipping (Table 6.1).

Table 6.1. Revised GESAMP rating scheme for acute aquatic toxicity (GESAMP, 2013)

Description	LC/LL50, EC/EL50, IC/IL50 (mg/l)
Non-toxic	>1000 mg/l
Practically non-toxic	>100 – ≤1000 mg/l
Slightly toxic	>10 – ≤100 mg/l
Moderately toxic	>1 – ≤10 mg/l
Highly toxic	>0.1 – ≤1 mg/l
Very highly toxic	>0.01 – ≤0.1 mg/l
Extremely toxic	≤0.01 mg/l

The endpoints were applied to experimental endpoints of LC/LD50, EC/ED50, or IC/ID50 derived from 48-, 72- or 96-hour exposures, only. As a result, only a small subset of the data was included, that is, 438 out of the 7900 rows of data (5%) in the evidence summary spreadsheet. The acute toxicity endpoints for all life stages are shown in Table 6.2 and Figure 6.1.

It should be noted that our use of the GESAMP endpoints is not consistent with their use by GESAMP and should not be compared to the data they provide. The GESAMP acute toxicity endpoints were chosen for convenience.

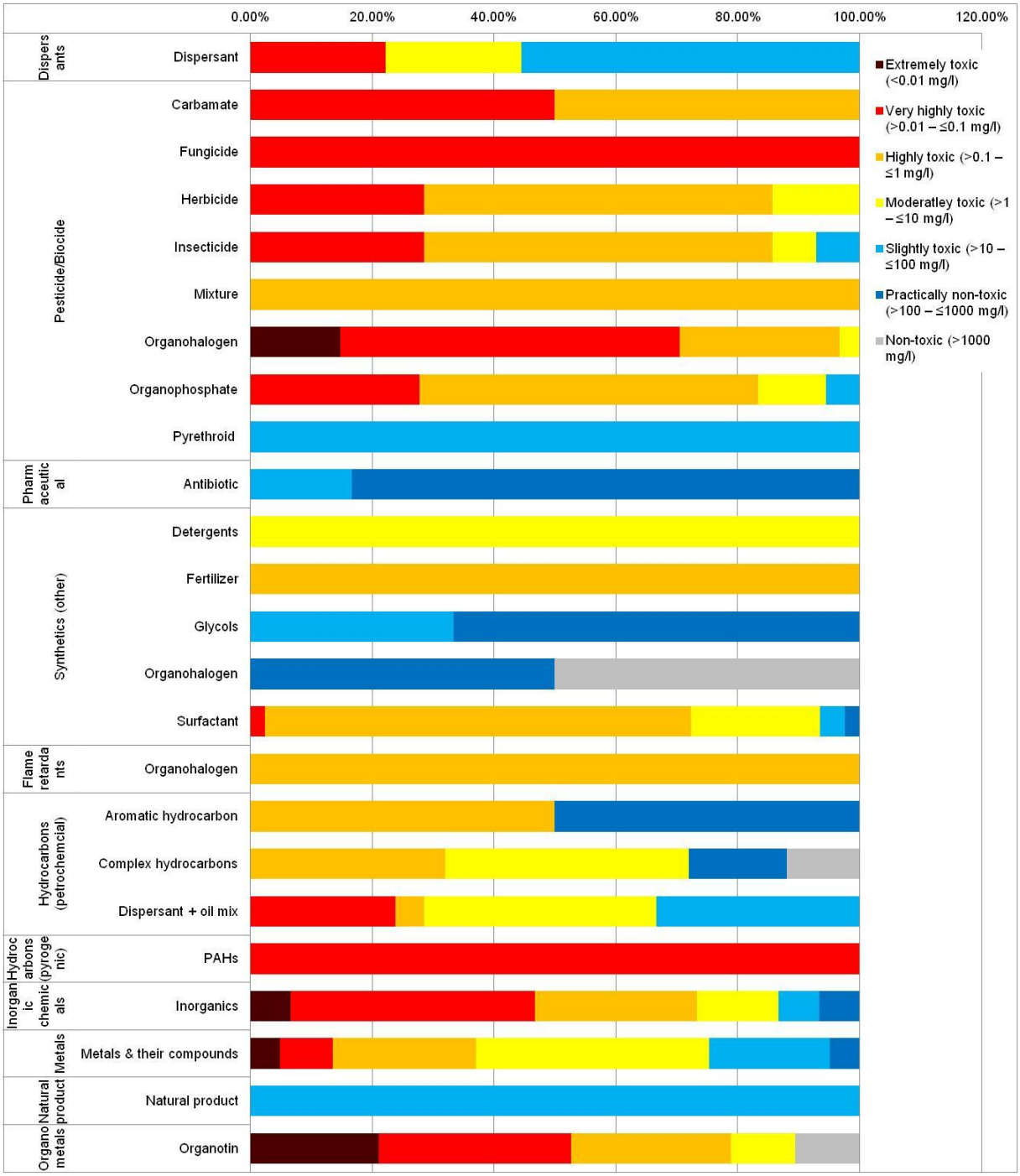

Figure 6.1. Relative ‘acute toxicity’ of different chemical groups to oyster species based on the application of GESAMP endpoints to the evidence collated in the review.

Table 6.2. GESAMP acute toxicity endpoints in oyster species based on the experimental studies of the major contaminant groups/types reviewed.

Contaminant Group	Contaminant type	Extremely toxic	Very highly toxic	Highly toxic	Moderately toxic	Slightly toxic	Practically non-toxic	Non-toxic	Total
Dispersants	**Dispersant**		**2**		**2**	**5**			**9**
Pesticide/Biocide	Carbamate		**1**	1					2
	Fungicide		**3**						3
	Herbicide		**6**	12	3				21
	Insecticide		**4**	8	1	1			14
	Mixture			1					1
	Organohalogen	**9**	**34**	16	2				61
	Organophosphate		**5**	10	2	1			18
	Pyrethroid					2			2
Pesticide/Biocide Total		**9**	**53**	**48**	**8**	**4**			**122**
Pharmaceutical	**Antibiotic**					**1**	**5**		**6**
Synthetics (other)	Detergents				4				4
	Fertilizer			3					3
	Glycols					1	2		3
	Organohalogen						1	1	2
	Surfactant		**3**	86	26	5	3		123
Synthetics (other) Total			**3**	**89**	**30**	**6**	**6**	**1**	**135**
Flame retardants	**Organohalogen**			**1**					**1**
Hydrocarbons (petrochemical)	Aromatic hydrocarbon			1			1		2
	Complex hydrocarbons			8	10		4	3	25
	Dispersant + oil mix		**5**	1	8	7			21
Hydrocarbons (petrochemical) Total			**5**	**10**	**18**	**7**	**5**	**3**	**48**
Hydrocarbons (pyrogenic)	**PAHs**		**1**						**1**
Inorganic chemicals	**Inorganics**	**1**	**6**	**4**	**2**	**1**	**1**		**15**
Metals	**Metals**	**4**	**7**	**19**	**31**	**16**	**4**		**81**
Organometals	**Organotin**	**4**	**6**	**5**	**2**			**2**	**19**
Natural product	**Natural product**					**1**			**1**
Grand total		**18**	**83**	**176**	**93**	**41**	**21**	**6**	**438**

There is no space to list all the chemicals examined in experimental studies here but their experiment endpoints are listed in the ‘oyster evidence summary’, where possible. Evidence from adults and juveniles make up only 14% of this subset of data. ‘Extremely toxic’ was only

reported for 1.6% of the endpoints (all from pesticides), 38% were ‘very toxic’ (mainly due to organometals and pesticides) and 31% ‘highly toxic (mainly due to organometals and pesticides). Early life stages represented 61% of this subset of data. Extremely toxic’ was only reported for 3% of the endpoints (mainly due to organotin), 7.8% were ‘very toxic’ (mainly due to organotin and inorganic chemicals), 44.5% ‘highly toxic (mainly due to synthetics (other), metals, and pesticides) and 27.8% were ‘moderately toxic’ (mainly due to pesticides, hydrocarbons, metals and synthetics(other). However, toxicity varied across the chemicals examined (see evidence summary spreadsheet).

Nevertheless, Figure 6.1 suggests that organotins, some metals, some inorganic chemicals (chlorine and chlorination), organohalogen pesticides and other pesticides, dispersants, dispersant and oil mixtures were amongst the most toxic to oyster species.

7 Conclusions

This evidence review (and associated evidence summary spreadsheet) complied evidence from ca 294 articles/studies, on the effects of several hundred different chemicals on four genera of true oyster (*Crassostrea* inc. *Magallana, Ostrea,* and *Saccostrea*). The conclusions of the study are summarised below.

- *Crassostrea* spp. were the most studied species in the review. Few studies examined *Ostrea* spp..
- Early life stages were the most studied life stages, presumably because they were identified as the most sensitive stages and suitable for bioassay (His et al., 2000).
- Few studies examined adult and juveniles and fewer still examined oyster populations.
- 'Pesticides/biocides', 'Metals', and 'Petrochemical' hydrocarbons were the most studied groups of contaminants, closely followed by 'Synthetics (other)', Organometals, and 'Pharmaceuticals'.
- 'Severe' or 'significant' mortality and, hence, 'High' sensitivity' was reported for most of the chemical groups reviewed, except for PFAS and PCBs.
- However, 'mortality' due to exposure to separate chemicals varied between studies and the chemical studied.

This study has assumed that 'high' sensitivity of early life stages of oysters leads to 'High' sensitivity of oyster beds, due to poor recruitment. This may not be true in all cases (e.g. Restronguet Creek), and should be considered a 'precautionary' approach. An examination of 'acute toxicity' (as defined by GESAMP) gave similar results.

Overall, this review agreed with His *et al.* (2000) who concluded that tributyltin was the most toxic compound yet tested on bivalve larvae (with EC50 as low as a few ng/l), followed with some heavy metals (especially silver, mercury and copper) with EC50 between a few ppb and 100 ppb. His *et al.* (2000) suggested that chlorine and organochloride pesticides may also have EC50 <100 ppb while detergents and petroleum products were less toxic.

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Appendix 1. Evidence summary – terms and definitions

The evidence extracted (or mapped) is 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.

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

Term	Definition
	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
- ECXX– Effect concentration at XX percentile
- ICXX - Inhibition concentration at XX percentile
- IDXX - Inhibition dose at XX percentile
- LCXX– Lethal concentration at XX percentile
- LDXX – Lethal dose at XX percentile
- LTXX – 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.

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