MBA.AC.UK

Sensitivity Assessment of Contaminant Pressures - Anthozoa – Evidence review

DATE: May 2023

AUTHOR: Watson, A. & Tyler-Walters, H.
Marine Life Information Network

Marine Biological Association

Recommended citation:

Watson, A. & Tyler-Walters, H., 2023. Sensitivity Assessment of Contaminant Pressures - Anthozoa – Evidence Review. MarLIN (Marine Life Information Network), Marine Biological Association of the UK, Plymouth, pp. 113. Available from https://www.marlin.ac.uk/publications

(Page left blank)

CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 Introduction

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

The resultant 'Anthozoa Evidence Summary'[1] spreadsheet and 'Evidence review' that follows benefited from improvements and resultant minor adjustments. 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[2] 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 'Anthozoa Evidence Summary' and the report that follows are defined in Appendix 1.

[1] https://www.marlin.ac.uk/sensitivity/contaminants
[2] https://pubchem.ncbi.nlm.nih.gov/

(Page left blank)

2 Evidence review overview

The evidence review was conducted inform the assessment of the sensitivity of the three main species of sea pen in British and Irish waters, namely *Virgularia mirabilis, Funiculina quadrangularis* and *Pennatula phosphorea* and their biotopes to contaminants. Preliminary searches revealed exactly 'zero' studies of the effects of contaminants on these sea pens. Therefore, the literature review was expanded to the Class Anthozoa. Articles that focused on the effects of contaminants on the symbiotic zooxanthellae within the tissues of coral and anemone species rather than effects on the host species were excluded during the screening process.

The initial searches (02 January 2023) resulted in ca 20,583 hits of which 12,486 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[3] Knowledgebase were used due to time constraints. The resultant references were screened for relevance based on the proposed REA protocol. Screening against the exclusion criteria reduced this number to fifty-nine articles[4], which were taken forward for detailed review. However, seven 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 'Anthozoa'.

Review stage	No. articles identified/retained	No. articles rejected/removed
Web of Science	20,583	
ECOTOX database	76	
Duplicates removed	9,870	12,486
Screening	120	9,750
Taken forward*	59	61
Not accessible	4	

[3] https://cfpub.epa.gov/ecotox

[4] The term 'article(s)' or 'study' are used for peer reviewed papers, reports, and other publications relevant to the review.

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

The detailed evidence extracted from fifty-two articles is provided in the 'Anthozoa Evidence Summary' spreadsheet and the supporting evidence and sensitivity assessments discussed below. All the technical terms used in the 'Anthozoa Evidence Summary' and the report that follows are defined in Appendix 1.

The review provided 178 worst-case ranked mortalities (hereafter referred to as 'results'[5]) from fifty-two articles. 'Metals' (27 articles, 39.89% of results) were the most studied contaminant within the reviewed articles, followed by 'Pesticide/Biocide' (10 articles, 16.85% of results), 'Hydrocarbons (Petrochemical)' (seven articles, 12.36% of results),' Organometals' (five articles, 8.99% of results) 'Dispersants' (five articles, 5.62% of results). Many articles examined multiple experimental studies, which provided a large number of results for Metals and Pesticide/Biocides.

Overall, the articles reviewed reported mortality ('Severe' to 'Some') in 45.51% of results, no mortality ('None') in 7.87% of results, and sublethal effects in 45.51% of results. The level of mortality or sublethal effect was 'unspecified' in the remaining 1.12% of results. Most of the

[5] 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.

sublethal effects (22.47%) were reported in the effects of ‘metals’ on Anthozoa (

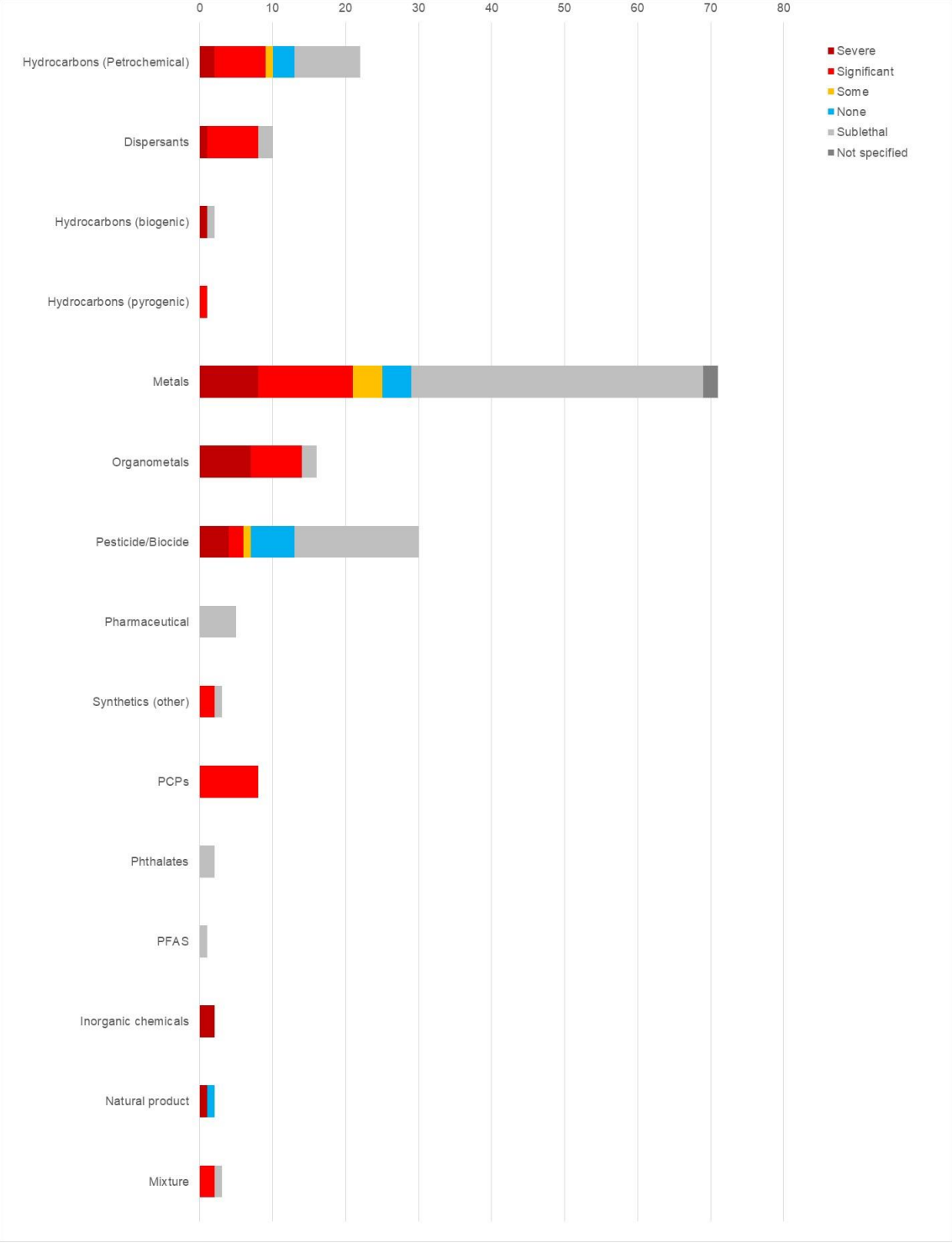

Figure 2.1).

The early life stages (embryos and planulae larvae) of Anthozoan species were the most studied life stage in the reviewed articles (50.56% of results) (Table 2.2). This may be because nineteen of the articles reviewed focused on sublethal effects of contaminants on the reproduction of species, for example, forty ‘worst–case’ results (22.47% of results) were sublethal fertilization effects.

Table 2.2. Number of ‘worst-case’ ranked mortality ‘results’ at different life stages reported from articles reviewed.

Life stage	No. of worst-case’ ranked mortality results
Early life stages (embryo, larvae/planulae)	90 (50.56%)
Adults and Juveniles	28 (15.73%)
Not reported	60 (33.71%)

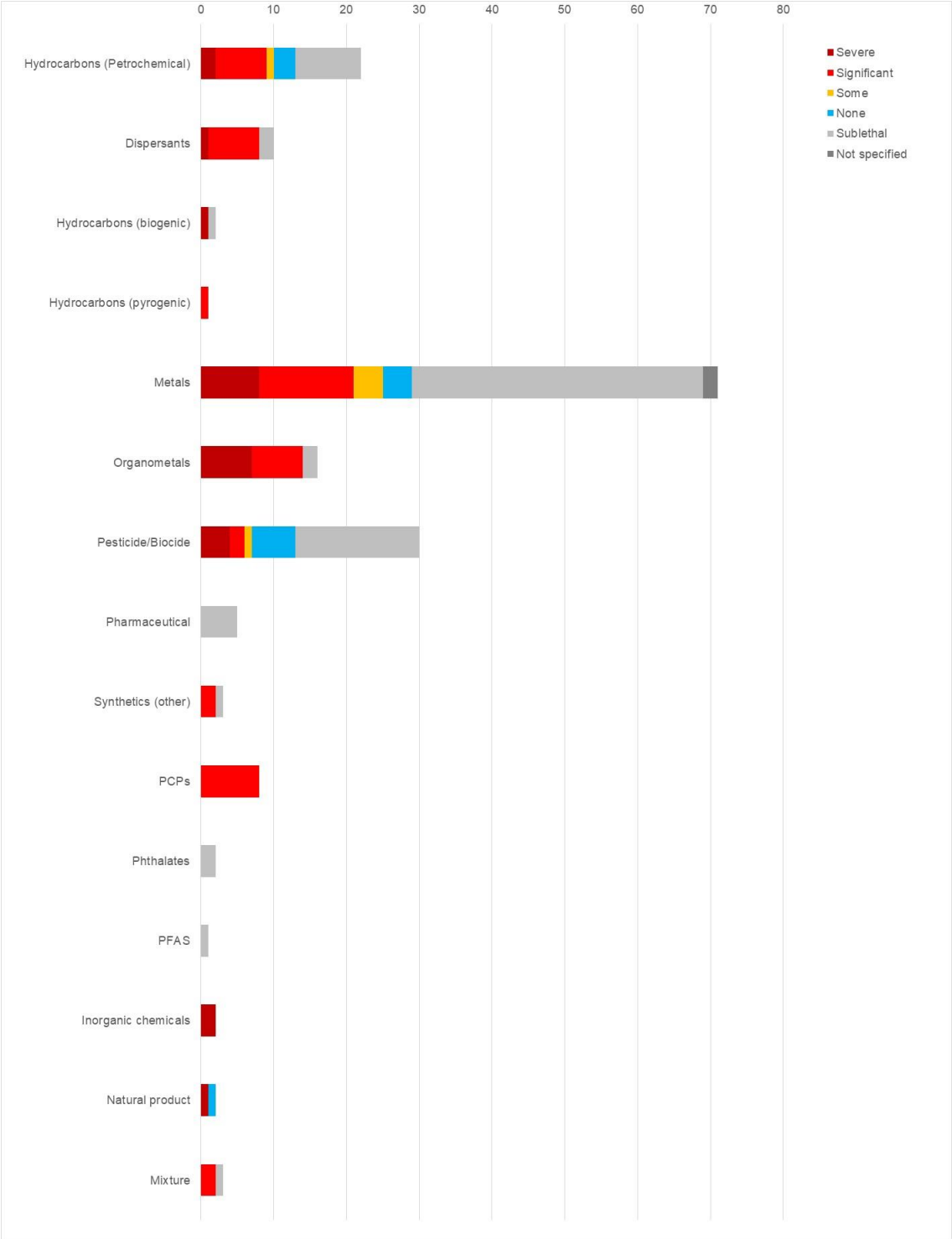

Figure 2.1. Count of worst-case ranked mortalities due to exposure to contaminants in Anthozoa. Mortality is ranked as follows: ‘Severe’ (>75%), ‘Significant’ (25-75%), ‘Some’ (<25%), ‘None’ (no mortality reported), and ‘Sublethal’ effects.

Twenty-three of the articles did not report the life stage (‘Not reported’) (33.71% of results) and it is assumed that the life stage examined was adults. Few studies looked at adults and juveniles (Figure 2.2). One article (Bao *et al.*, 2011) looked at both adults and juveniles, and was included in the adult and juvenile results.

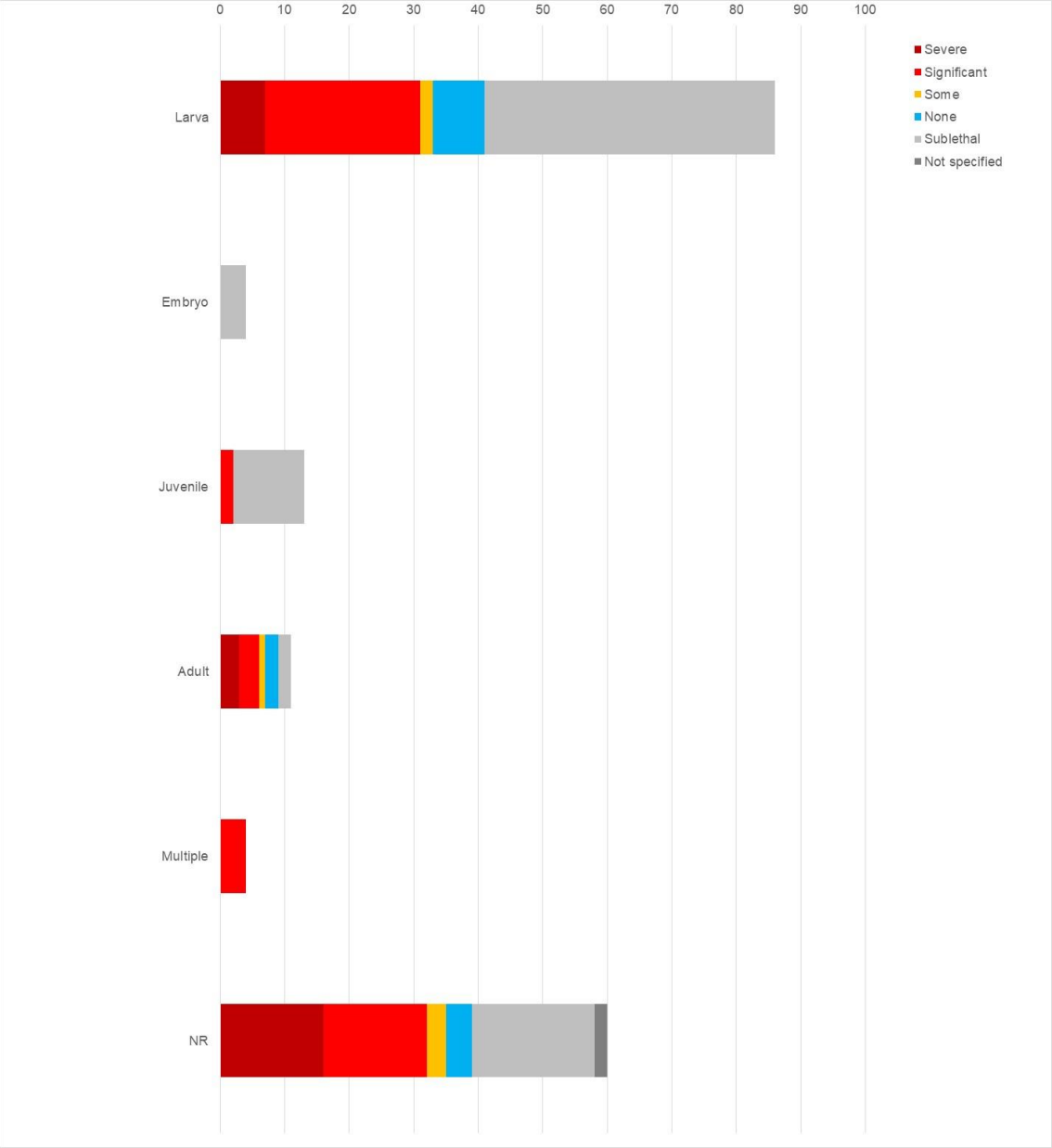

Figure 2.2. Count of worst-case ranked mortalities in at each life stage in Anthozoa. Mortality is ranked as follows: ‘Severe’ (>75%), ‘Significant’ (25-75%), ‘Some’ (<25%), ‘None’ (no mortality reported), and ‘Sublethal’ effects.

The studies reviewed were also categorised into broader taxonomic groups within the Class Anthozoa: Scleractinia (true corals) (61.80% of results), Actinaria (true anemones) (32.02% of results), Octocorallia (Soft corals) (5.62% of results) and Zoantharia (0.56% of results) (Table 2.3; Figure 2.3). These categories were introduced to examine any trends in response between the different orders of Anthozoa, especially the Octocorallia which includes sea pens.

The effects of ‘Metals’ was the most studied contaminant on Scleractinia, Actinaria, and Zoantharia groups. However, the effects of petrochemical hydrocarbons were mostly studied on Octocorallia species. Within the taxonomic groups, *Acropora millepora* was the dominant Scleractinia species studied (9.55% of results, *Aiptasia pulchella* was the most dominant Actinaria species studied (12.36% of results) and *Swiftia exserta* was the most dominant Octocorallia species studied (2.25% of results) (Table 2.3).

Table 2.3. The taxonomic group, species name and number of ‘worst-case’ ranked mortality ‘results’ reported from articles reviewed.

Taxonomic group (code)	**Species name**	**No. of worst-case’ ranked mortality results**
Actinaria (Ac)		
	Actinia equina	7
	Aiptasia pallida	4
	Aiptasia pulchella	22
	Aiptasia sp.	6
	Anemonia sulcata	2
	Anthopleura sp.	1
	Cereus pedunculatus	1
	Edwardsia elegans	1
	*Exaiptasia (*syn. *Aiptasia) pallida*	7
	Nematostella vectensis	4
	Sagartia elegans	1
	*Urticina (*syn. *Tealia) felina*	1

Taxonomic group (code)	Species name	No. of worst-case' ranked mortality results
Octocorallia (Oc)		
	Dentomuricea meteor	2
	Hypnogorgia pendula	1
	Lobophytum compactum	1
	Placogorgia spp.	1
	Swiftia exserta	4
	Thesea spp.	1
Scleractinia (Sc)		
	Acropora cervicornis	1
	Acropora formosa	1
	Acropora longicyathus	2
	Acropora microphthalma	4
	Acropora millepora	17
	Acropora surculosa	1
	Acropora tenuis	9
	Acropora tumida	6
	Acropora valida	1
	Favites chinensis	2
	Galaxea fascicularis	1
	Goniastrea aspera	12
	Goniastrea retiformis	1
	Montastraea annularis	1
	Montastraea cavernosa	1
	Montastraea faveolata	5
	Montipora aequituberculata	1
	Montipora capitata	1
	Montipora verrucosa	4
	Oxypora lacera	1
	Platygyra acuta	1
	Platygyra daedalea	2
	Platygyra ryukyuensis	2
	Pocillopora damicornis	16

Taxonomic group (code)	Species name	No. of worst-case' ranked mortality results
	Porites astreoides	8
	Porites cylindrica	3
	Porites divaricata	1
	Porites lichen	1
	Porites lutea	1
	Stylophora pistillata	3
Zoantharia (Zo)		
	Zoanthidae	1

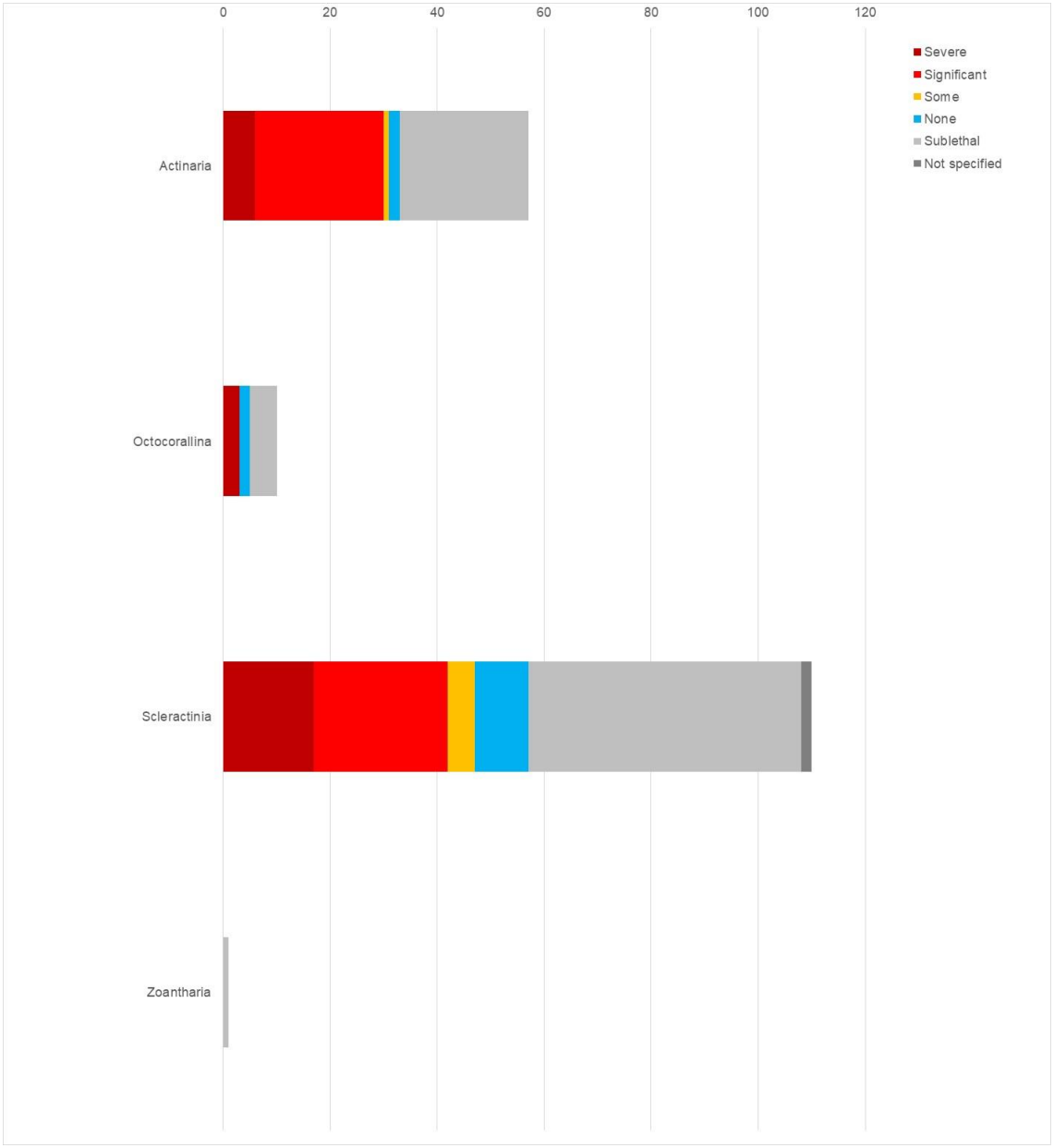

Figure 2.3. Count of worst-case ranked mortalities in each taxonomic group in Anthozoa. Mortality is ranked as follows: ‘Severe’ (>75%), ‘Significant’ (25-75%), ‘Some’ (<25%), ‘None’ (no mortality reported), and ‘Sublethal’ effects.

(Page left blank)

3 Hydrocarbons and PAHs

At total of thirty-five results were obtained from nine articles that studied the effect of hydrocarbons, PAHs, and dispersants on Anthozoa, of which 62.86% examined the effects of petrochemical hydrocarbons such as crude or fuel oils and/or their water accommodated fractions (WAF). Dispersants alone contributed 28.57% of the worst-case results and was the most studied contaminant type. Pyrogenic hydrocarbons and biogenic hydrocarbons contributed only 2.86% and 5.71% of the results respectively (Figure 3.1).

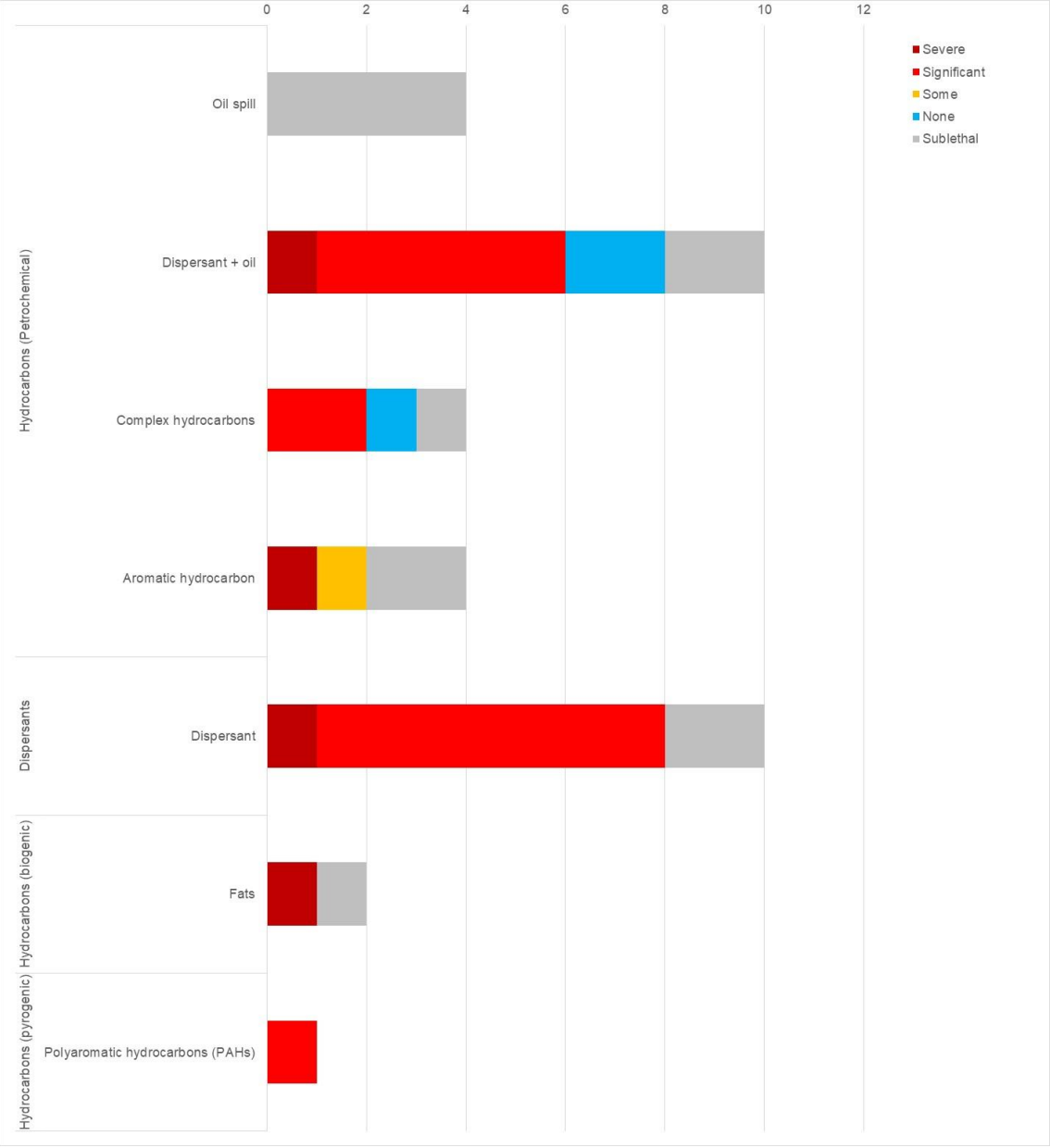

Figure 3.1. Count of ranked mortalities due to exposure to hydrocarbons in Anthozoa. Mortality is ranked as follows: ‘Severe’ (>75%), ‘Significant’ (25-75%), ‘Some’ (<25%), ‘None’ (no mortality reported), and ‘Sublethal’ effects.

Scleractinia corals were the most studied and contributed 51.43% of the results for ‘Hydrocarbons and PAHs’ (Figure 3.2). Despite this, the sea anemone *Actinia equina*, and the coral *Acropora millepora*, were the most studied species both of which contributed 14.29% of results in the review. Larvae were the most studied life stage with 45.71% of results. However, there was a higher percentage (48.57%) of results which had a ‘Not reported’ life stage.

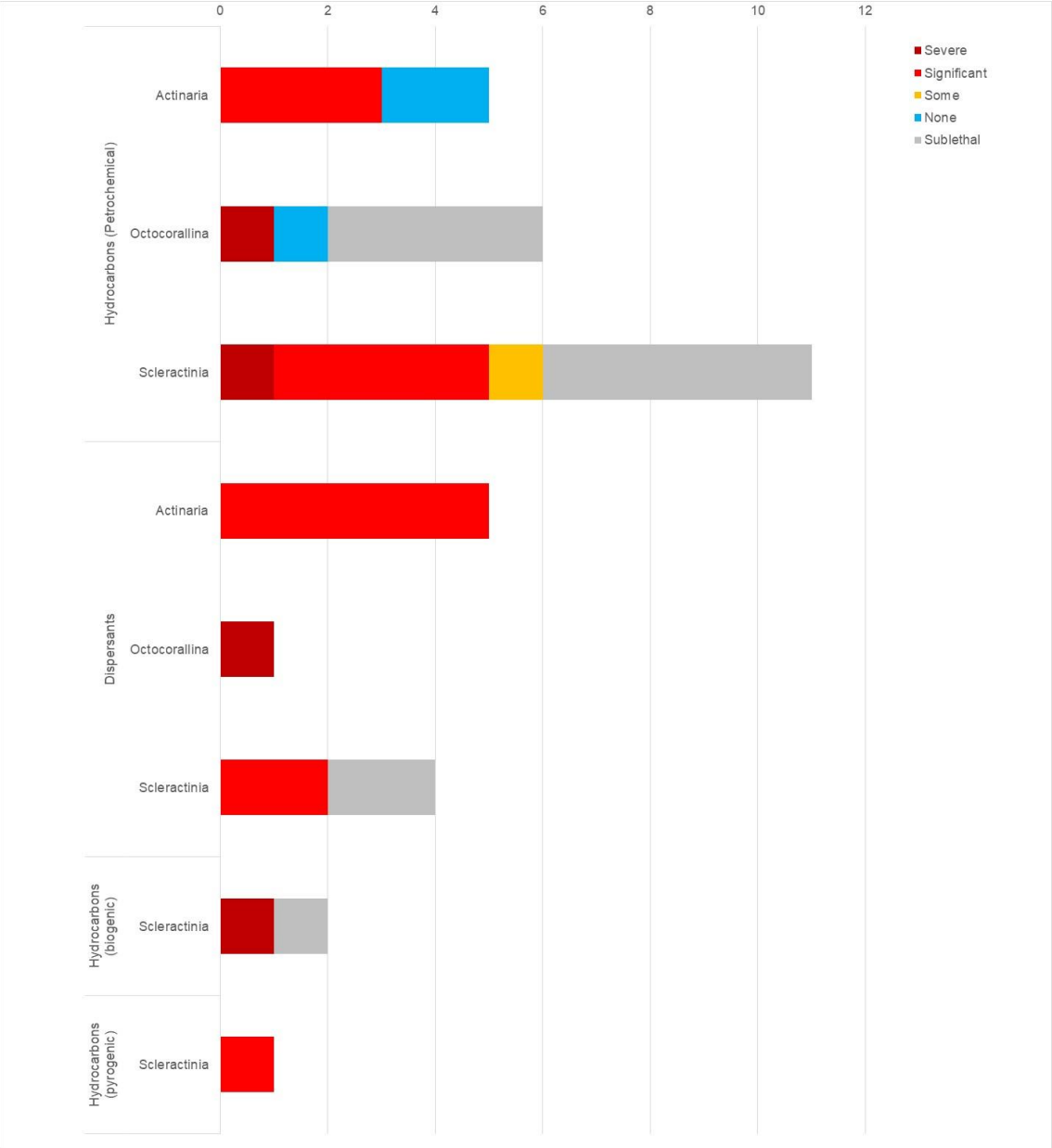

Figure 3.2 Count of worst-case ranked mortalities due to exposure to hydrocarbons in Anthozoan taxonomic groups, Scleractinia, Actinaria, and Octocorallia. Mortality is ranked as follows: ‘Severe’ (>75%), ‘Significant’ (25-75%), ‘Some’ (<25%), ‘None’ (no mortality reported), and ‘Sublethal’ effects.

3.1 Oil spills

The effect of an oil spill was reported in only one article. The evidence is summarised below.

Etnoyer *et al.* (2016) examined the health and condition of species of large gorgonian octocorals before and after a *Deepwater Horizon* (DWH) oil spill from Macondo well. They used the after-control-impact (BACI) research design, which used still image captures from remotely operated vehicles (ROVs). Three mesophotic reef sites near the Macondo well were investigated: Alabama Alps Reef (AAR), Roughtongue Reef (RTR), and Yellowtail Reef (YTR). In this field study, the reefs were surveyed in 1989, before the spill, and in 2010, 2011, and 2014 after the spill. The health assessment involved using image stills to identify the species and outline healthy and injured regions. The degree of injury (injury impact rank) was assigned based on a category scale where '0' was healthy (less than 1% injury), '1' is 1-10% injury, '2' is 10-50% injury and '3' is 50-90% injury and '4' indicated more than 90% damage. The degree and type of injury were recorded. Species of *Swiftia exserta, Hypnogorgia pendula, Thesea nivea, Thesea rubra, Placogorgia spp., Parmuricea* spp*.,* and *Muriceides cf. M. hirta* were assessed. The species composition at each reef varied, so the large discernible species (*S. exserta, H. pendula, Thesea spp.* and *Placogorgis spp.)* were combined in a single 'large gorgonian' group. The results found a significant change in gorgonian octocoral condition before and after the oil spill in sites near the well. After the spill, injury was 10.8 times higher than before the spill, with 6.9 times higher for more than 10% injury observed. Injury rate increased to 38% at YTR and RTR after the spill and increased to 50% at AAR. The percentage injury was 21% of *Placogorgis spp*., between 45 – 50% of *S. exserta*, between 50 – 55% of *H. pendula* and 62% of *Thesea spp*. Data on percentage injury of other octocoral species was extracted from Figure 5 (Etnoyer *et al.,* 2016).

A subsequent experiment examined the change in injury overtime from 2011 after the spill – 2014 by marking colonies initially after the spill and then re-photographing the marked colonies in 2014. Eighteen marked colonies included *Hypnogorgia* and *Swiftia* species. Eight colonies were healthy and 10 were injured after the spill. Colonies that were healthy in 2011 remained healthy but injured colonies worsened by 2014. Injuries included tissue loss, broken and bare branches, overgrowth, and toppling of colony. The study provided evidence of injury to at least four species of gorgonian octocoral from the DWH spill at Macondo wellhead. The authors noted that the degree of injury was substantial and that up to 50% of octocorals were affected.

3.2 Petroleum hydrocarbons – oils and dispersed oils

Seven articles that examined the effects of complex hydrocarbons (e.g., *Deepwater Horizon* water accommodated fractions), aromatic hydrocarbons and dispersant and oil mixtures. The relevant summaries are below, but Etnoyer *et al.* (2016) is described above.

Frometa *et al.* (2017) examined the effects of the *Deepwater Horizon* oil water-accommodated fractions (WAF), dispersant Corexit 9500, and the combination of both (chemically enhanced WAF, CEWAF) on the deep-water gorgonian octocoral *Swiftia exserta* from Florida's Southeast coast. The total polycyclic aromatic hydrocarbons (tPAH50) of CEWAF and WAF treatments were measured. Octocoral fragments were exposed to 0.056, 0.123, 0.151, 0.868, 1.70 mg/l of tPAH50 in WAF: 0.004, 0.015, 0.044, 0.123, 0.280 mg/l of tPAH50 in CEWAF and 6.25, 12.5, 25, 50, 100 mg/l of Corexit 9500 for 96 hours in a laboratory. The exposure in treatments was static for WAF and CEWAF concentrations but renewed every 24 hours in Corexit 9500 concentrations. The health of each fragment was assessed using health scoring from 0-5 based on live polyps and remaining tissue. A score of zero was given when there was no remaining tissue and fragments were dead. A score of 1-2 was given to fragments with less than 50% of live polyps and tissue. A score of 3 was given when around 50% of live polyps and tissue, and a score of 4-5 were given when fragments had more than 50% of live polyps and tissue remaining. The average score for each treatment was recorded and measurements were taken at 24-, 48-, 72-, and 96 hours. No significant differences were observed when fragments were exposed to WAF concentrations, and some fragments looked healthier than ones in the control group; all were given a health score of 4 or higher. Exposure to 0.280 mg/l tPAH50 resulted in polyp retraction. One fragment died in the 0.004 mg/l tPAH50 treatment, which was unexpected. The authors suggested this fragment was stressed prior to the experiment. Health declined significantly in 50 and 100 mg/l concentrations of Corexit 9500, after the first 24 hours. In addition, 100% mortality was seen at 48 hours in corals exposed to 100 mg/l Corexit 9500 and partial mortality was seen in corals exposed to 50 mg/l. The measured 96-hour LC50 value was 70.27 mg/l and the 96-hour LC10 value was 64.23 mg/l. Significant effects were observed in all treatments with CEWAF. Total (100%) mortality was seen within 72 hours when exposed to 0.868 mg/l tPAH50 and 1.70 mg/l tPAH50. The measured 96-hour LC50 value of Corexit in CEWAF was 41.04 mg/l and the measured 96-hour LC10 value was 40.28 mg/l. The authors concluded that exposure to Corexit 9500 and CEWAF caused more effects on the health of *S. exserta* than WAF exposure alone. They noted that the high concentrations that caused 100% were higher than what would be expected on the sea floor over a large area and their

study did not replicate the *Deepwater Horizon* oil spill conditions completely as there were other environmental factors that contributed to the effects caused by the spill.

Goodbody-Gringley *et al.* (2013) investigated the effects of the 2010 BP-operated *Deepwater Horizon* (DWH) oil spill on brooding and broadcast spawning coral *Porites astreoides* and *Monastraea faveolata* larvae in the Florida Keys. The larvae were exposed to multiple concentrations of BP Horizon oil; crude oil, weathered and oil water-accommodated fractions (WAFs), chemically enhanced water-accommodated fractions CEWAF (combination of oil and dispersant Corexit 9500) and Corexit 9500 alone in laboratory conditions. The effects were examined on behaviour, settlement, and survival. *P. astreoides* planulae were exposed to 35 mg of weathered DWH oil for 96 hours and larval settlement was measured every 24 hours. No settlement occurred in the planulae exposed to the oil after 72 hours and they had a greater mortality post-settlement. Their survival decreased by 42% in exposed treatments. In addition, the planulae were exposed to 4 mg of weathered DWH oil to monitor swimming behaviour over three days. During this test, larvae were observed in contact with the oil, but they did not settle. Significantly high mortality was observed in the exposed treatments after 24 and 72 hours.

Porites astreoides planulae were exposed to a constant 25, 50, and 100 ppm of Corexit 9500 for 72 hours (measurements were taken after 48 and 72 hours). Settlement was significantly reduced; results; mean settlement was 33% at 25 ppm, 13% at 50 ppm and 0% settlement at 100 ppm after 72 hours. There was a negative relationship with the mean survival rate of *P. astreoides* planulae decreasing to 67% at 25 ppm, 13% at 50 ppm, and 0% at 100 ppm after 72 hours. The 72-hour LC50 was 33.4 ppm Corexit 9500.

In addition, *P. astreoides* planulae were exposed to a constant 0.32, 0.33, 0.62 ppm of WAF for 72 hours (measurements were taken after 48 and 72 hours). Larval settlement and survival decreased significantly as the concentration of WAF increased. The mean settlement of larvae after 72 hours was 53% at 0.32 ppm and 0.33 ppm and 33% at 0.62 ppm. The mean larval survival after 72 hours was 73% at 0.32 ppm, 60% at 0.33 ppm, and 33% at 0.62 ppm. Survival was only reduced significantly at the highest concentration of WAF. The 72-hour LC50 was 0.51 ppm WAF.

P. astreoides planulae was exposed to 0.71, 4.28, 30.99 ppm of CEWAF (chemically enhanced water accommodated fraction) for a total of 72 hours. Larval settlement and mortality were observed after 48 and 72 hours. A negative relationship was found between survival and settlement with increasing concentration of CEWAF after 72 hours of exposure.

After 72 hours, the mean percent of settled larvae was 67% at 0.17 ppm, 20% at 4.28 ppm, and 7% at 30.99 ppm. The mean percent of surviving larvae was 67% at 0.17 ppm, 27% at 4.28 ppm, 7% at 30.99 ppm, and the 72-hour LC50 was 1.84 ppm CEWAF.

M. faveolata planulae were exposed to a constant 25, 50, 100 ppm of Corexit 9500 for 48 hours. Larval settlement was low after exposure to lowest concentrations. The mean settlement rate was 5% at 25 ppm and 0% at 50 and 100 ppm. The survival rates decreased significantly, with 9% survival at 25 ppm, and 0% at 50 and 100 ppm. The 48-hour LC50 19.7 ppm Corexit 9500. *M. faveolata* planulae were also exposed to a constant 0.65, 1.34, and 1.50 ppm of WAF DWH oil for 48 hours. The results were similar to *P. astreoides*. There was a significant decrease in settlement and survival of the larvae as the concentration increased. The mean settlement of larvae was 27% at 0.65 ppm, 16% at 1.34 ppm, and 5% at 1.5 ppm. The mean larval survival was 35% at 0.65 ppm, 20% at 1.34 ppm, 11% at 1.5 ppm and the 48-hour LC50 0.50 ppm WAF.

M. faveolata planulae were exposed to 14.73, 18.56, 35.76 ppm of CEWAF for 48 hours and a negative relationship was found between survival and settlement as the concentration of CEWAF increased. The mean settlement rate of the larvae and the larval survival rate were both 4% at 14.73 ppm, 0% at 18.56 ppm, and 1% at 35.76 ppm. The 48-hour LC50 was 0.28 ppm CEWAF.

M. faveolata planulae were also exposed to a spiked exposure; 0.49, 0.51, 0.84 ppm of WAF 0.86, 30.06, 42.08 ppm of CEWAF and 1000, 1500 ppm of Corexit 9500 for 96 hours. These treatments were diluted with filtered seawater 2ml/min for the duration of the 96 hours. Larval survival decreased as the concentration of WAF 500 increased. Survival decreased at all the concentrations WAF tested. The mean survival was 33% at 0.49 ppm, 27% at 0.51 ppm and 7% at 0.84 ppm. Survival also declined when planulae were exposed to 30.06 ppm (7% mean larval survival) and 42.08 ppm (0% survival) concentrations of CEWAF. The survival varied for planulae exposed to Corexit 9500. Lower concentrations also resulted in low survival rates. The authors reported 96-hour LC50 of 0.45 ppm for WAF, 0.12 ppm for CEWAF and 343.8 ppm of Corexit 9500.

Overall, Goodbody-Gringley *et al.*, (2013) concluded that survival and settlement of *P. astreoides* and *M. faveolat*a planulae decreased significantly when exposed to *Deepwater Horizon* crude oil, weathered oil, WAF, CEWAF and Corexit 9500.

Mercurio *et al.* (2004) studied the effects of biodegradable vegetable-derived lubricants (VDL- 1A, VDL-1B and VDL-2) and mineral - derived oils (MDL) water accommodated fractions (WAF) on the fertilization of *Acropora microphthalma* gametes and sublethal effects on the symbiosis in branchlets of *A. microphthalma* adults. The total hydrocarbon content (THC µg/l) of MDL, VDL – 1A, VDL- 1B and VDL-2 WAF was measured. Coumarin (benzopyran-2-one) was predominant in VDL-1A and VDL-1B but not in the newly formulated VDL-2 and naphthalene was predominant in MDL. *A. microphthalma* gametes were exposed to 0.40, 4.0, 40, 100, 200, 300, 400 (100% stock) THC µg/l of MDL and 0.60, 6.0, 60, 150, 300, 450, 600 (100% stock) THC µg/l of VDL-1A during the fertilisation experiment, in laboratory conditions at Orpheus Island, Queensland, Australia for four hours and fertilized eggs recorded. The highest exposure concentrations of MDL were more toxic than VDL-1A. However, fertilization was inhibited significantly at a lower concentration of VDL-1A. Fertilization decreased significantly from 200 µg/l of MDL (53%) and from 150 µg/l of VDL-1A (64%). The fertilization at the highest exposures decreased to 7.5% in 400 µg/l of MDL and 26% in 600 µg/l of VDL-1A. The authors suggested that both mineral-derived oils and vegetable–based lubricating oils are potentially toxic to gametes but only in high exposure concentrations. *A. microphthalma* colonies from Magnetic Island, Queensland, Australia, branchlets were used to take a visual observation of the adult corals health (included mucous production, coral polyp condition, bleaching and mortality) during adult coral exposure experiments. The effect/mortality was expressed as percentage of number of branchlet fragments measured. Branchlets were exposed to 24, 48, 95, 190, 380 (100% stock) THC µg/l of MDL; 37, 74, 149, 295, 590 (100% stock) THC µg/l of VDL-1A; 35, 70, 140, 280, 560 (100% stock) THC µg/l of VDL-1B and VDL-2 for 48 hours. Coral tissue remained a healthy colour for all treatments. The lowest concentration to cause significant mortality after 48 hours was 190 µg/l of MDL, 149 µg/l of VDL-1A, 140 µg/l of VDL-1B and 140 µg/l of VDL-2. Most of the branchlets (9 out of 12; 75%) had severe tissue necrosis and died in 295 µg/l of VDL-1A, 5 out of 12 (41.67%) branchlets died in 140 µg/l of VDL-1B, and one branchlet (8.3%) died in 140 µg/l of VDL-2. During exposure to MDL, 2 (16.67%) coral branchlets died at 190 µg/l MDL, therefore mortality only occurred at the highest exposure. The authors concluded that, after 48 hours, increasing lubricant concentrations caused clear and visual effects on adult *A. microphthalma,* but were more pronounced and showed more mortality due to exposure to VDL-1A and VDL-1B. The VDL-2 with no coumarin had the least toxic effect despite similarities amongst all VDLs. The authors concluded both mineral and vegetable derived lubricants have the potential to affect coral fertilization and coral branchlets, but vegetable derived lubricants are slightly more toxic.

Negri & Heyward (2000) examined the sublethal effects of water the accommodated fractions (WAF) of crude oil, the dispersant Corexit EC9527A and production formation water (PFW) on fertilization and larval metamorphosis from colonies of *Acropora millepora* at Lizard Island, Great Barrier Reef. *A. millepora* was exposed to PFW, crude oil, Corexit EC9527A (1% and 10% v/v) and combined WAFs (1 % v/v oil/dispersant and 10 % v/v oil/dispersant), in the laboratory and the total hydrocarbons (THC) were measured. Gametes were exposed to the contaminants for four hours in the fertilization assays. RPFW did not inhibit fertilisation in 0.0360 mg/l THC (equivalent to 10% of PFW). The highest concentration of PFW (0.0721 mg/l THC, 50% of PFW) was the minimum concentration, at which fertilization was inhibited significantly. The crude oil minimum concentration that inhibited fertilisation was 0.165 mg/l THC (10% of crude oil stock). In the combined 1% v/v oil/dispersant treatment, fertilization was inhibited at 0.225 mg/l THC and in 10% v/v oil/dispersant fertilization was inhibited at 0.0325 mg/l, while complete fertilization was observed at 0.325 mg/l (10% v/v). Corexit EC9527A significantly inhibited fertilisation at 10 mg/l. This concentration of Corexit EC9527A was higher than other contaminant concentrations that inhibited fertilization. Therefore, it was suggested that dispersants were more toxic to fertilization. Gametes were fertilized to produce 8-day-old larvae in the metamorphosis assays, and crustose coralline algae (CCA) species were used to induce metamorphosis in laboratory conditions. The 8-day larvae were exposed to contaminants and CCA for 24 hours. The lowest concentration of PFW (0.0360 mg/l THC, equivalent to 10% of PFW) significantly inhibited metamorphosis. Larval metamorphosis of *A. millepora* was more sensitive than fertilization to exposure to crude oil without dispersant, metamorphosis was significantly inhibited at 0.0824 mg/l THC of crude oil and completely inhibited at 0.165 mg/l THC of crude oil. Corexit EC9527A inhibited metamorphosis at between 5 and 10 mg/l THC, which suggested that crude oil and Corexit EC9527A were toxic to *A. millepora* metamorphosis.

Smith (1968) examined the effects oil and 3500 gallons of detergent sprayed on Porthleven Reef following an oil spill and clean-up with detergent. Field observations reported that *Actinia equina* and *Urticina (*as *Tealia) felina* were the most common and the most resistant animals. Some specimens of *Cereus pedunculatus*, *Sagartia elegans* and *Anemonia sulcata* were found dead and few survived. The exact mortality was not specified. Smith (1968) also examined the effects of BP 1002 from 0.2 – 100 ppm on intertidal species in laboratory conditions. The majority of *Actinia equina* specimens died in 24 hours at 25 ppm BP 1002 but some younger individuals survived. The majority of *Anemonia sulcate* specimens died in 24

hours at 50 ppm but some that looked dead later recovered. The exact mortality was not specified.

Te (1991) studied the effects of benzene and gasoline:oil mixtures on the settlement of *Pocillopora damicornis* planulae. The planulae were collected from coral heads from Kaneohe Bay, Hawaii and were studied in open and closed systems in a laboratory. The planulae were exposed to four treatments of unleaded gasoline with motor oil (SAE 40) diluted with seawater, at 5, 10, 50, 100 ppm, in an open system experiment for 13 days. Mortality was not recorded. The planulae were found to be resistant to the different concentrations as settlement was observed on the sides of the petri dishes of the 50-ppm treatment and all other treatments did not elicit a settlement response.

P. damicornis was also exposed to 1, 5, 20, and 100 ppm of the gasoline:oil mix for 13 days in a closed system experiment. The results revealed corallite formation on day 3 of exposure and 100% mortality observed at 100 ppm after 2 days. A third experiment exposed the larvae to 1, 5, 20, and 100 ppm treatments of benzene with settling plates and the same concentrations without settling plates for 10 days. The settling plates were used to increase surface area for settlement. Mortality was not recorded. No settlement occurred at 1 ppm benzene and no significant differences were observed between the other treatments. There was more settlement on the treatments with settling plates. It was concluded that the settlement of *P. damicornis* may be unsuitable to test these hydrocarbons.

3.3 Dispersants

The effects of dispersants alone were examined by five articles, Frometa *et al.* (2017), Goodbody-Gringley *et al.,* (2013), Negri & Heyward (2000) and Smith (1968) are detailed above.

Maggi & Cossa (1973; abstract only) examined the effects of five anionic synthetic detergents; Docusate, sodium p-dodecylbenzenesulfonate, Dobane 83, Dobane 25-35 and Dobane JN on 15 marine organisms including *Actinia equina.* After exposure for 48 hours, the LC50 values for *A. equina* were 15 mg/l Docusate and Dobane 83, 16.3 mg/l sodium p-dodecylbenzenesulfonate and Dobane JN, over 100 mg/l Dobane 25-35 and after exposure for 96 hours the LC50 values were 9.2 mg/l Docusate, 15 mg/l sodium p-dodecylbenzenesulfonate and Dobane JN, 13.3 mg/l Dobane 83, over 100 mg/l Dobane 25-31. The lethal concentrations for the most susceptible marine species studied during the experiment was near 1 mg/l.

3.4 Hydrocarbon (biogenic)

Mercurio *et al.,* (2004) examined the effects of vegetable derived lubricants. They suggested that both mineral-derived oils and vegetable–based lubricating oils were potentially toxic to gametes but only in high exposure concentrations (detailed above).

3.5 Polyaromatic hydrocarbons (PAHs)

The evidence is summarized below for the only article that examined the effects of polyaromatic hydrocarbons (PAHs) of pyrogenic origin.

Farina *et al.* (2008) examined the effects of mercury (Hg) and benzo(a)pyrene (B(a)P) on the survival of *Porites astreoides* larvae and biochemical responses. *P. astreoides* larvae from Cayo Paiclá, National Park Morrocoy, Venezuela were exposed to a control, 10 µg/l treatment of Hg and 10 µg/l treatment of B(a)P for 96 hours, with recordings on survivorship taken every 24 hours under laboratory condition. The *P. astreoides* survivorship rate of 98% (2% mortality) when exposed to Hg was similar to the control but significantly higher when compared to the 72% survivorship rate (28% mortality) of the larvae exposed to B(a)P of the same concentration. Mortality also occurred after 48 hours in larvae exposed to B(a)P. However, was earlier than the little mortality that occurred due to Hg exposure. Mercury exposure for 96 hours had no toxic effect. It was suggested that larvae born from adults in contaminated areas could be less sensitive and have a higher tolerance to the contaminant. The authors concluded there was a higher survival when *P. asteroides* larvae were exposed to mercury than B(a)P. Protein extractions were also performed during the study to determine the presence of biochemical markers in the larvae and show enzymatic responses to the contaminants. The results showed the total protein content and catalase (CAT) activity were similar in all treatments and unaltered by exposure to contaminants. Glutathione-S-transferase (GST) activity was not statistically different in control larvae exposed to Hg. However, GST activity was detected in one of three replicates exposed to B(a)P. Exposure to Hg did not significantly change the thiol content in *P. astreoides* larvae. However, there was a significant increase in free thiols after exposure to B(a)P, which suggested an oxidative stress response in larvae exposed to B(a)P. The authors were uncertain about how the enzymatic activity relates to tolerance of *P. asteroides* to contaminants.

4 Transitional metals and organometals

Eighty-seven results (ranked ‘worst-case’ mortalities) were obtained from twenty-eight articles that examined the effects of transitional metals and organometals on anthozoan species. The effects of transitional metals contributed to 81.61% of results, most of these results were sublethal (56.34% of results). Copper (Cu) was the most studied metal, accounting for 33% of results. Cadmium (Cd) (10.34% of results), zinc (Zn) (10.34% of results), lead (Pb) (8.05% of results), and nickel (Ni) (6.90% of results) were studied routinely (Figure 4.1). Tributyltin (TBT) and tributyltin oxide (TBTO) were the most studied organometal. Overall, sublethal effects were the most reported result (48.28%) of exposure to transitional metals and organometals in the articles examined.

All life stages studied exhibited effects from transitional metals and organometals. The early life stages (larvae and embryos) were the most studied life stage (51.72% of results). However, life stage was ‘Not reported’ in 31.03% of results. Adults and juveniles accounted for 17.24% of the results.

Scleractinia was the most studied taxonomic group (60.92% of results), followed by Actinaria (36.78% of results). Octocorallia and Zoantharia were both 1.15% of the results. *Aiptasia pulchella* provided most of the results (25.29%) and was the most studied species. *Goniastrea aspera* was the next well studied species and provided 13.79% of the results.

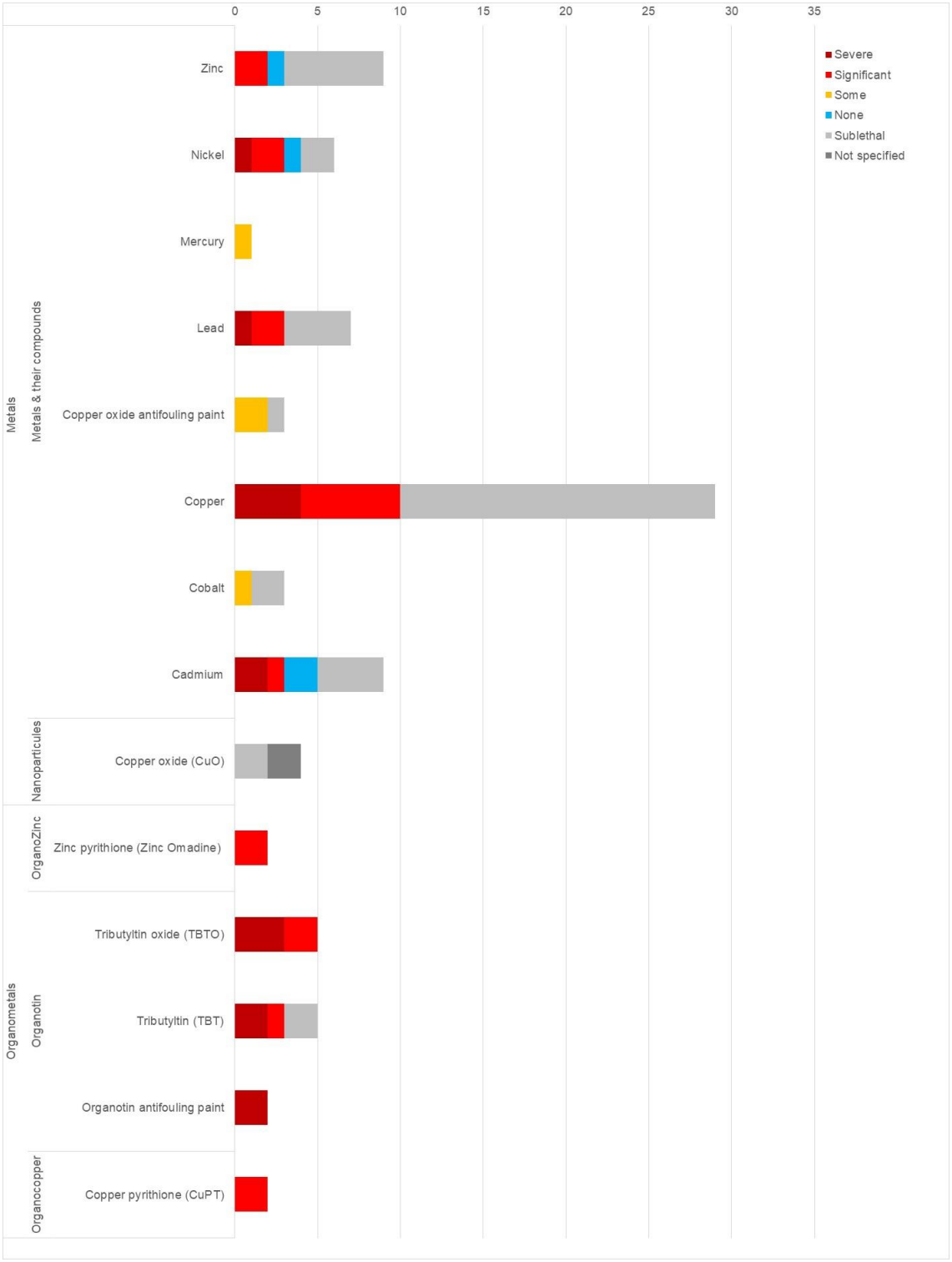

Figure 4.1. Count of worst-case ranked mortalities due to exposure to ‘Transitional metals’ and ‘Organometals’ in Anthozoa. Mortality is ranked as follows: ‘Severe’ (>75%), ‘Significant’ (25-75%), ‘Some’ (<25%), ‘None’ (no mortality reported), and ‘Sublethal’ effects.

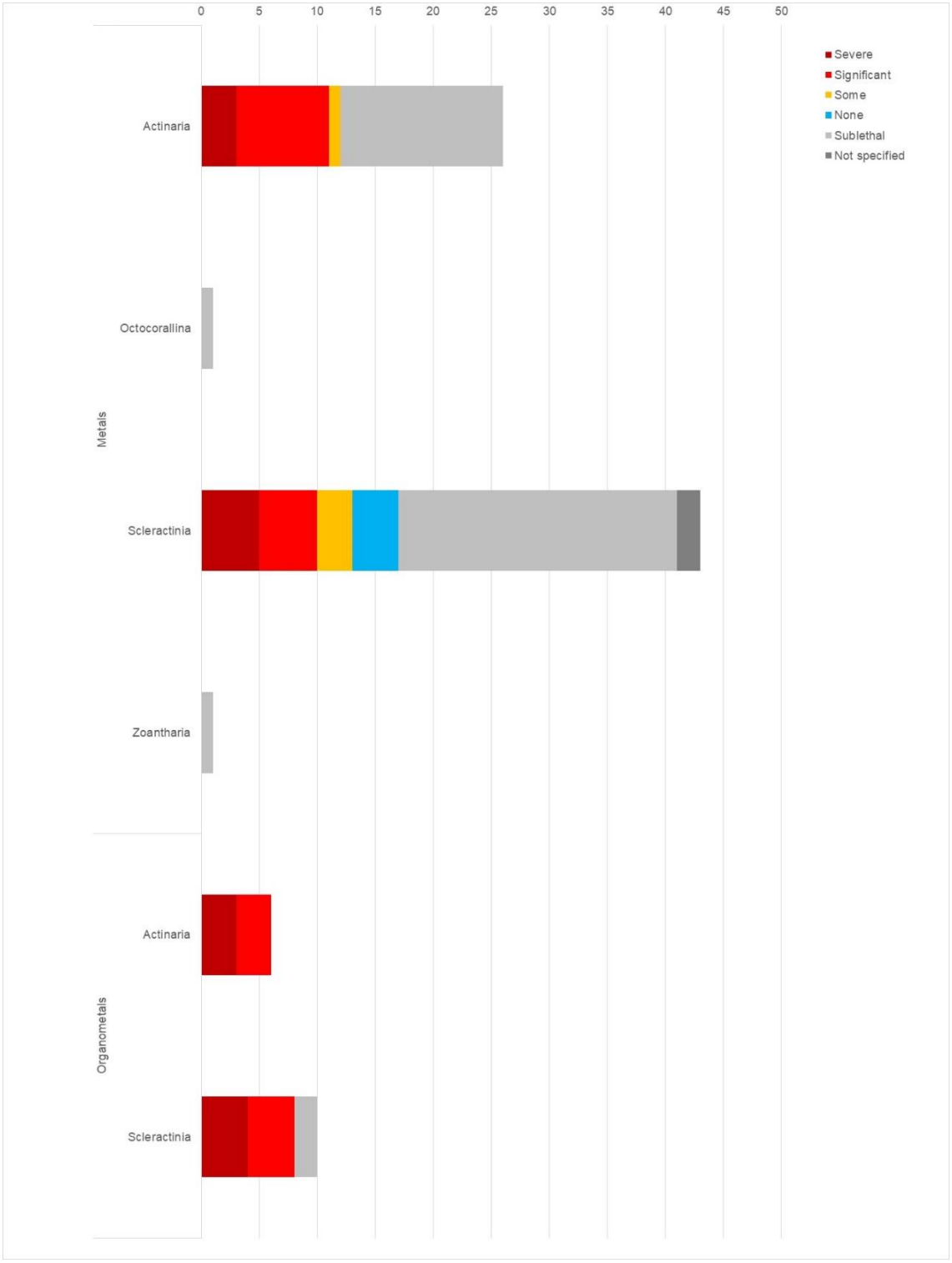

Figure 4.2. Count of ranked mortalities due to exposure to Transitional metals and Organometals in Anthozoan taxonomic groups; Scleractinia, Actinaria, Octocorallia and Zoantharia. Mortality is ranked as follows: ‘Severe’ (>75%), ‘Significant’ (25-75%), ‘Some’ (<25%), ‘None’ (no mortality reported), and ‘Sublethal’ effects.

4.1 Transitional metals

There were 27 articles reviewed that examined the effects of transitional metals. The relevant summaries are below, but Farina *et al.*, (2008) is detailed above.

Alutoin *et al.* (2001) examined the effects of copper (Cu) exposure and reduced salinity acting simultaneously on the metabolism of a hermatypic coral *Porites lutea*. The study took place in the field on a coral study area south of Sichang Island in the inner Gulf of Thailand. The effect on metabolism was measured in terms of primary production rate per chlorophyll *a* and respiration. There were five different treatments of exposure tested during the study: 10 µg/l and 30 µg/l exposure concentrations of copper in ambient 30 psu and 20 psu. The corals were kept in an outdoor facility, in exposure tanks for 14 hours. After the exposure period, the corals were placed in different experimental tanks to measure dissolved oxygen levels and temperature. The experimental tanks contained water from the exposure tanks. No significant effect on respiration was found amongst the treatments. The authors concluded that copper exposure primarily affected photosynthesis and not respiration rate. On the other hand, 30 µg/l copper at 20 psu and 30 psu reduced the net production rate in the corals. The lowest observed effect (LOEL), which significantly decreased production, was 30 µg/l at 30 psu. No change in net primary production was observed in 10 µg/l copper at 30 psu (NOEL). There was no effect on production rate at 10 µg/l Cu at 20 psu, which Alutoin *et al.* (2001) concluded was an antagonistic effect. In addition, a discolouration of the corals was observed. It was suggested that the higher copper concentration caused the discolouration due to coral polyps exposing the white naked skeleton. There was no colour loss to corals exposed to 10 µg/l Cu at 20 psu. It was concluded that corals exposed to high concentration of copper experienced sublethal effects through a reduction in production per chlorophyll *a,* while corals exposed to lower concentrations experienced no effects. This was a short-term study Therefore corals could be affected to the lower concentrations of copper if exposed for a longer time period.

Bao *et al.* (2011) examined the effect of tributyltin (TBT), copper (Cu) and five other antifouling booster biocides used as alternative antifouling paints, including Irgarol, Diuron, copper pyrithione (CuPT) and zinc pyrithione (ZnPT). The effects of these were tested on the multiple life stages of sea anemone *Aiptasia sp.* and the larvae of stony coral *Acropora tumida* 2-3 days after fertilization when they are about to enter the planula larval stage. Specimens were obtained in sub-tropic Hong Kong and exposed in a laboratory. Mortality was used as the endpoint for these species and only the LC50 results were discussed in the

paper. *Aiptasia sp.* and *Acropora tumida* were exposed to stock solutions of 1 g/l tributyltin oxide, 5 g/l Irgarol, 10 g/l diuron, 10 g/l ZuPT, 1 g/l CuPT and 1 g/l Cu diluted with seawater for a total of 96 hours. Results showed that *Aiptasia sp.* was more sensitive to TBT, with a LC50 at 9.4 µg/l after 96 hours, and less sensitive to ZnPT (LC50 at 410 µg/l), CuPT (LC50 at 2000 µg/l), and Diuron (LC50 at 19,000 µg/l). The lethal concentrations of Irgarol and Cu to *Aiptasia sp.* were not determined. Although the coral larvae were also exposed for 96 hours, mortality was high by the end of the first 24 hours of exposure, which suggested the larvae were more vulnerable during early development. Therefore, the 24-hour LC50 and LC10 values for *A. tumida. Acropora tumida* larvae, which have a high natural mortality, found the larvae were more sensitive to TBT, with LC50 at 7.5 µg/l and LC10 at 0.67 µg/l. In addition, LC50 and LC10 were observed at 28 and 0.24 µg/l of CuPT, 80 and 5.8 µg/l of Cu, 180 and 100 µg/l of ZnPT and 4800 and 91 µg/l of Diuron. Irgarol had a LC10 of 440 µg/l but the LC50 was not determined. The authors concluded that TBT was the most toxic biocide to *A. tumida* and *Aiptasia sp.* and all of the other organisms tested in the study.

Goh (1991) examined the effect of nickel (Ni) on planulae of *Pocillopora damicornis* from Kaneohe Bay, Hawaii. The planulae were exposed to 0.18 ppm, 1 ppm, 9.08 ppm, and 23.03 ppm under four time regimes, 12 hours, 24 hours, 48 hours, and 96 hours. Mortality and settlement success were recorded in a laboratory. Goh (1991) observed mortality for a 96-hour recovery period and settlement for a 17-day recovery period, after exposure. The control, 0.18 ppm at all exposure regimes, and 1 ppm concentration of nickel at 12 hours and 24 hours resulted in less than 5% mortality during recovery. There was 100% mortality observed at 23.03 ppm and 9.08 ppm nickel concentrations after 96 hours; these were the most lethal concentrations. After 48-hour exposure, the 23.03 ppm treatment was at 97.8% mortality and reached 100% 12 hours into recovery. Only, 50% mortality was observed during the recovery period after the 23-ppm treatment exposed for 12 and 24 hours, and 9 ppm treatment exposed for 12, 24 and 48 hours. At the end of the experiment, planulae mortality was below 50% in the 1 ppm treatment exposed for 48 and 96 hours. The authors concluded that the coral planulae were affected at threshold concentrations of 1 ppm and 9.08 ppm of nickel. They reported that these Ni concentrations might not be experienced in the natural environment, because nickel concentrations in uncontaminated water were reported at 1.8 ppb and between 2.5 and 15 ppm in contaminated coastal waters. The success of planulae settlement was also recorded in this study. The author found that settlement rates were less than 50% under the four exposure times and concentrations of 0.2

ppm and 1.0 ppm Ni. The author suggested that the settlement rates may have been affected by time and the glass slides used in the experiment were not effective settlement surfaces.

Hansen *et al.* (1996) examined the effects of cadmium and acid-volatile sulphide (AVS) contaminated marine sediments on the colonisation of benthic organisms. AVS can be used to predict the availability of cationic metals for benthic organisms living in sediment spiked by divalent metals. During the study, sediments were spiked with cadmium to gain cadmium/AVS ratios (0.1, 0.8, and 3.0) and colonization was studied for 118 days. The interstitial water concentrations were measured, and cadmium concentrations ranged between 3.0 – 174,000 µg/l. Results recorded one individual species of Cnidaria, *Edwardsia elegans* in the 0.1 cadmium/AVS ratio treatment and four *E. elegans* individuals in the 3.0 cadmium/AVS treatment. The species richness and abundance of other organisms recorded in this study decreased as cadmium concentration increased. However, this was not seen in *E. elegans* but was not discussed by the author.

Harter & Matthews (2005) conducted acute and chronic tests on the effect of cadmium chloride ($CdCl_2$) on the reproduction of *Nematostella vectensis* from Padilla Bay, Washington. *Nematostella vectensis* were tested in laboratory conditions and sexual fertilization was induced before exposure. Three acute experiments were conducted of which one was used to determine to suitable range of concentrations. Mature female *Nematostella vectensis* were exposed to 0.0 – 2.2 mg/l of cadmium chloride for 96 hours. The resultant 96-hour LC50 values were 2.11 mg/l ($CdCl_2$) in test one 1.78 mg/l ($CdCl_2$) in test two. Two chronic renewal experiments were also conducted. In the first experiment, mature females were exposed to 0.0 – 2.2 mg/l of $CdCl_2$ for 21 days and mortality measured every day. The resultant 21-day LC50 value was 0.31 mg/l ($CdCl_2$). The second chronic test measured egg production daily and anemone weight every 7 days. Mature females were exposed to concentrations 0.0, 0.075, 0.250, 0.500 mg/l for a total of 21 days. No significant differences in anemone weight were found in the control and 0.075 mg/l concentrations after 21 days, but the weight was significantly lower in 0.250 and 0.500 mg/l concentrations after the first 7 days. There were many zero egg counts recorded in the experiment because no females reproduced, which resulted in no significant differences. However, significantly fewer eggs were counted in the 0.500 mg/l treatments few females that did reproduce. The authors suggested that the results were a stress response, which caused an increase in reproductive frequency but inhibited total egg production. For instance, a total of 43,328 eggs were counted in the control treatment, but eggs were only present in 50% of the replicate treatments, and 22,253 eggs were counted in 0.500 mg/l treatment, but eggs were present in 78% of replicate treatments.

The authors concluded the responses and sensitivity of *N. vectensis* to cadmium was similar to other marine and estuarine species from similar prior studies.

Hedouin & Gates (2013) examined the effect of copper (Cu(II)) exposure on the fertilization success of coral *Montipora capitata* from Coconut Island, Kaneohe Bay, Oahu, Hawaii. The effects were examined in laboratory conditions on four different spawning nights (4th and 5th June, 3rd July, and 1st August) to determine if the time of spawning had an influence. Colonies of *M. capitata* typically release gamete (egg-sperm) bundles between June and August which were collected on the four different spawning nights. Each bundle was exposed to 0, 10, 20, 50, 100, 200 and 500 µg/l of dissolved copper for three hours. The number of fertilized eggs and egg size were determined, with fertilization success rate as the recorded endpoint. Fertilization rates ranged from 76% - 92%, in bundles collected in 4th and 5th June and 3rd July, when exposed to 10 µg/l, which was not significantly different to the control. However, at higher concentrations the copper significantly decreased fertilization success. The highest concentrations (200 µg/l and 500 µg/l) blocked the separation of the bundles and resulting in less than 10% success rate. The authors suggested that Cu affected the normal bundle breakdowns and that disassembly of the bundles occurred potentially before fertilization. The resultant 3-hour EC50 value was 17.5±0.8 µg/l of Cu on 5th June and 16.6±0.8 µg/l of Cu on 3rd July. The 3-hour EC50 was significantly higher on 4th June at 31.7±1.2 µg/l of Cu. On 4th June, the 3-hour EC10 value was 15.1±1.1 µg/l and 3-hour EC20 value was 19.4±1.2 µg/l. On 5th June, the 3-hour EC10 value was 9±1 µg/l and the 3-hour EC20 value was 11.3±0.9 µg/l. On 3rd July, the 3-hour EC10 value was 10.4±1.2 µg/l and the 3-hour EC20 value was 12.2±1.1 µg/l. On August 1st there was almost no fertilization observed in the control and other treatments. The authors suggested that this was a normal pattern because August is towards the end of the spawning period. Egg size increased over the spawning period, which is a typical trend. There was no significant different between egg size on 3rd July and 1st August. The authors concluded Cu has the potential to inhibit *M. capitata* fertilization and gametes were more resistant to Cu during the earlier spawning period (4th June).

Heyward (1988) examined the effects of copper (Cu) and zinc (Zn) on the fertilization of *Goniastrea aspera, Favites chinesis* and *Platygrya ryukyuensis* from colonies collected during the spawning period in Okinawa, Japan. Gametes were collected after spawning in laboratory conditions and the eggs and sperm were combined immediately in treatments before addition of contaminant. *G. aspera* was exposed to 0.1 - 1.0 mg/l of Cu and 0.5 - 1.0 mg/l of Zn for 6 hours. *F. chinesis* was exposed 80 minutes after insemination for 4.5 hours

and *P. ryukuensis* was exposed 4.5 hours after insemination. Fertilization was measured as the percentage of dividing embryos. The results found no embryos or egg loss by lysis (cell disintegration by rupture of the membrane cell wall) in the sub-sampled *P. ryukuensis* and *F. chinesis* after 5 hours of exposure. Cu inhibited fertilization in all species in treatments above 0.5 mg/l. Zn was less toxic to fertilization, but the author noted that only one concentration was tested. Exposure of *P. pryukensis* to 0.1 mg/l of Cu inhibited fertilization and *F. chinesis* was more affected at 1.0 µg/l of Zn. It was noted that few early planulae survived when fertilization occurred. The author concluded that refinements could be made to the study but that high levels of toxic contaminants could reduce fertilization and were able to induce stress and mortality.

Howe *et al.* (2012) examined the effect of copper concentrations on the anemone *Aiptasia pulchella* from the National Marine Science Centre seawater flow-through system in Coffs Harbour, New South Wales, Australia. *A. pulchella* reproduced in laboratory conditions and semi-outdoor conditions in recruitment tanks optimal for reproduction. The total number of pedal lacerates produced after 3, 12, and 20 weeks were recorded, and juveniles were used in toxicity tests. Acute toxicity tests with ten replicates and two chronic toxicity tests with five replicates were studied. During the analysis of data, LC50 values were measured using trimmed Spearman-Karber method to find LC50 values after the different exposure period (24, 48 and 96 hours). LC50 values were estimated when the criteria for the analysis were met to find LC50, LC5 and LC95 values after 96 hours, as a substitute for NOEL/NOEC values. EC50 values recorded for inhibition of reproduction. *A. pulchella* were exposed to initial concentrations of 30±8, 56±13, 82± 13, 111±17 and 136±28 µg/l of copper in an acute 96-hour static-renewal test. Mortality and anemone condition were observed at 0, 1, 6, 24, 48, 72, 96 hours. Complete tentacle retraction and loss of zooxanthellae was observed after 96 hours in all concentration treatments. Mortality was recorded in all treatments after 1-hour and the percentage mortality increased over the 96 hours. After 48 hours, 70 to 100% mortality was recorded. The LC50 values were low for all experiments and the overall mean 24-, 48-, and 96-hour LC50 ranged from 99±27 – 66±23 µg/l. The overall mean LC5, LC50, and LC95 values were 34±21, 64±23 and 142±72 µg/l. *A. pulchella* was also exposed to 6, 14, 30, 46 and 50 µg/g Cu and 5, 10, 18 and 22 µg/l Cu in a chronic 28-day test. Mortality, condition, and tentacle retraction were recorded every 48 hours, and live pedal lacerates numbers were recorded after 9, 12, 22 and 28 days. Severe or complete retraction of the tentacle and loss of zooxanthellae observed in all condition treatments after 28 days there was. After 12 days, 100% mortality was recorded in treatments 46 and 50 µg/l of copper and

there were no live lacerates in treatments 30 µg/l and above. Mortality was recorded in all treatments after 28 days. All remaining lacerates in lower concentrations showed severe tentacle retraction. The mean 9-, 12- and 28-day LC50 values ranged from 49 – 26 µg/l Cu and the mean 9-, 20- and 28-day EC50 ranged from 15-18 µg/l Cu. Inhibition of *A. pulchella* reproduction occurred at over 30 µg/l of copper. The authors concluded that *A. pulchella* mortality was consistent in both the acute and chronic exposure to copper and the response to copper was severe. The observed toxic sublethal effects were common and occurred rapidly within the first hour. Howe *et al.* (2012) concluded *A. pulchella* was acutely sensitive to copper when compared with other marine organisms and had relatively low LC/EC50 values.

Howe *et al.* (2014a) examined the effect of cadmium (Cd), cobalt (Co), copper (Cu), nickel (Ni) and zinc (Zn) on the asexual reproduction of *Aiptasia pulchella*, collected from the National Marine Science Centre, Charlesworth Bay. Asexual reproduction of *A. pulchella* occurs by 'pedal laceration' of small segments from the pedal disc tissue and this offspring develops into a juvenile after 8 days. *A. pulchella* were exposed to 0, 15±1, 28±3, 54±2, 107±3, 211±6 µg/l Cd; 4, 26±2, 131±5, 260±8, 515±18, 757±22 µg/l Co; 0, 4±1, 11±1, 19±3, 29±3, 40±2 µg/l Cu; 0, 50±2, 263±7, 513±18, 1014±33, 2022±52 µg/l Ni, and 8, 39±15, 140±18, 270±28, 507±50, 717±49 µg/l Zn for 28 days. The mean number of offspring (pedal lacerate) and developed juveniles produced by adults, adult health and lacerates condition were assessed. Recording mortality of lacerates was difficult and was only recorded where there were evident signs of tissue decomposition. Juveniles were recorded if they had eight tentacles. Results recorded for the EC50, EC10, and LOEC values of *A. pulchella* offspring and juvenile production at each metal can be seen in the evidence summary spreadsheet.

Overall, there was a significant decrease in the number of offspring produced by adults in all metals tested except for cadmium. High reproduction rates occurred in the controls and at some low metal concentrations. Cu was the most toxic metal, followed by high concentrations of Zn and Ni where the reproduction rates were distinctly lower at the higher concentrations. Concentrations more than 19 µg/l of copper showed significant decrease in offspring which was lower than seen in the other metals. It was noted that 26 µg/l of Co and 107 µg/l of Cd produced a higher (but not significantly) mean number of offspring compared to the control. However, obvious stress responses (tentacle retraction) were observed. Toxicity threshold for Co was not obvious by looking at the results. The highest mortality of adults was seen in zinc exposure (28-day LC50 388 µg/l) and nickel exposure (28-day LC50 1,439 µg/l). Further sub-lethal responses were observed during the experiments. Adults exhibited stress responses

(tentacle retraction, zooxanthellae loss) despite reproducing, and the authors suggested the increase in reproduction at the beginning of the experiment was an adaptive stress response. The authors noted that all tested metals caused a decrease in the total number of live offspring and developed juveniles after 28 days.

Howe *et al.* (2014b) examined the effects of cadmium (Cd), cobalt (Co), lead (Pb), nickel (Ni) and zinc (Zn) on *Aiptasia pulchella* from the National Marine Science Centre seawater flow-through system at Charlesworth Bay, New South Wales, Australia. *A. pulchella* were exposed to Cd between 361 and 2,775 µg/l, Pb between 38 and 1,547 µg/l, Ni between 688 and 15,530 µg/l, Zn between 4 and 5,922 µg/l and Co between 316 and 2,775 µg/l. During the experiment, there were five replicates of each metal that were tested 1-3 times (five tests for zinc and two for lead). *A. pulchella* was exposed for 96 hours, which was extended to 144 hours if low mortalities were observed. Mortality and retraction of the tentacle were measured at 1-, 6-, 12-, 18-, 24-hours and every 24 hours after that. Lethal response to the metals depended on concentration and time. There was little mortality measured in Co concentrations after 96 hours, but some mortality was observed after a longer 144-hour exposure period. However, this mortality was not over 50% so no LC50 was measured. Mortality was observed in *A. pulchella* exposed to all metals. The mean 96-hour LC50 values were 1,040 µg/l for Cd, 10,230 µg/l for Pb, 3,980 µg/l of Ni and 955 µg/l of Zn. The rest of the results are shown in the evidence summary spreadsheet. Severe tentacle retractions were used as the sub-lethal endpoint and a behavioural response of the anemone in the early exposure periods. Tentacle retraction was common and occurred quickly, after 24 hours exposure to all metals except Co. ‘Severe’ retraction did not occur until after 72 hours. The EC50 value was calculated in each test but not if there was a 100% effect. After 12 hours the EC50 values were 711 µg/l and 276 µg/l of Cd; 1,740 µg/l of Pb; 1,430, 3,280 and 2,380 of Ni; and 327 µg/l of Zn. The rest of the results can be seen in evidence summary spreadsheet. The authors noted it was not easy to define the degrees of ‘severe’ retraction. The authors concluded that Cd, Pb, Ni and Zn caused considerable mortality in *A. pulchella* after a 96-hour exposure.

Jones (1997) examined the effect of copper on the branch tips of *Acropora Formosa* from Magnetic Island, Australia in laboratory conditions. Three toxicity tests were conducted. Two tests exposed the branch tips to 5, 10, 20, 40, and 80 µg/l of copper for 48 hours and the third test exposed branch tips to 5 µg/l of copper for 96 hours. The densities of symbiotic zooxanthellae in the colonies varied amongst the three tests as the main focus of the paper was examine the variation of bleaching amongst colonies. The density of zooxanthellae,

pigment loss and rate of zooxanthellae release because of exposure were measured. Results from the two 48-hour toxicity tests observed changing of branch tip colour from natural brown to lighter colour but was observed to be less extensive in the second test. There were mortalities observed in high concentration of copper, in 80 µg/l of copper all tips had died between 24 and 36 hours (100% mortality) and in 40 µg/l of copper four of the five tips (80%) died. No mortalities were observed in test 2. The results from the 96-hour toxicity test found no significant difference in coral colour and no mortality was recorded. The author concluded *A. formosa* branch tips bleached as a result of increased copper exposure, this occurred as a result of loss of zooxanthellae and the varied densities of algal affected this sublethal stress response. It was noted that bleached tips recovered after the experiment, showing loss of zooxanthellae as a sublethal response. In test one, the mortalities observed could be explained as the algal densities were lower.

Kaiser *et al.* (2003) examined the effects of copper on the population growth of the rock anemone *Aiptasia* from a marine ornamental fish hatchery (Rhodes University, Grahamstown, South Africa). The study focused on behaviour, physical appearance, reproductive success, and mortality. After acclimation to laboratory conditions for 24 hours, adult *Aiptasia* were exposed to 0, 0.01, 0.1, 1.0, 10 mg/l of copper for 24 hours, followed by a 48-hour recovery period, in an acute experiment to identify appropriate concentration exposure range. In a chronic experiment, *Aiptasia* was exposed to 29±0.9, 51±2.8, 70±3.2, 106±3.6, 128±1.5, 154±2.9, 169±3.0, 199±3.5 µg/l of copper for six weeks, followed by a 2-week recovery period. The 6-week exposure period and 2-week recovery period were analysed separately. Behaviour responses were grouped into damage categories, which ranged from no effect to death. The resultant 48-hour LC50 value was 120±40 µg/l Cu. During the six-week exposure period damaged increased as the concentration of copper increased. The categories that described severe damage (polyp head retracting into base, base greatly reduced) and death were seen most often in 128 and 154 µg/l Cu treatments, which suggested more exposure caused more severe damage. Death amongst the anemone was seen from four weeks of exposure. Dead *Aiptasia* were found most often in treatments exposed to 128, 154, 169 and 199 µg/l Cu. Mortality did not increase in the 2-week recovery period. Recovery from damage occurred in the anemones exposed to range from 70 to 100 µg/l Cu, during the 2-week recovery period. The reproductive success of juveniles was examined as the adults reproduced during the eight-week period and were easy to identify by the fact that they do not grow to the size of adults during this same period. Rapid population growth was observed in the control treatment. However, copper inhibited reproduction

between 106 and 128 µg/l Cu, and higher copper concentrations were lethal. Population growth was slower even at the lower copper concentrations. There was a significant decrease in *Aiptasia* numbers in 154, 169 and 199 µg/l Cu, due to no reproduction and a significant number of deaths. The authors concluded copper inhibited reproduction and reduced survival of *Aiptasia* adults. It was suggested the success of the control treatment is partly due to the number of symbiotic zooxanthellae. The loss of zooxanthellae and deprivation of the individuals of nutrients were suggested as the main cause of the inhibition of reproduction and growth, and onset mortality.

Kwok & Ang Jr (2013) studied the combined effect of elevated temperature and copper on the survival of scleractinian coral*, Platgyra acuta* from north-eastern Hong Kong. Larvae were exposed to 40, 80, 120, and 200 µg/l of copper for a total of 96 hours (measurements were taken at 24, 48 and 96 hours at 27°C and 30°C). Swimming activity and mortality of the larvae were studied in laboratory conditions during the exposure period and a 48-hour depuration period. The number of living larvae was counted, and a 40-second video of swimming activity was taken. Mortalities occurred in all treatments, including the control, and the highest moralities (60-75% mortality) were seen at 200 µg/l of copper. There was significantly higher mortality seen in 80, 120, and 200 µg/l copper treatments compared to 40 µg/l. Only minor mortalities were seen at 40 µg/l of copper. The 24-hour IC50 value was 66.8 µg/l copper at 27°C and 58.5 µg/l copper at 30°C. The 48-hour IC50 value was 46.9 µg/l copper at 27°C and 59.3 µg/l copper at 30°C. The 96-hour IC50 value was 56.7 µg/l copper at 27°C and 67.4 µg/l copper at 30°C. In addition, larval swimming distance was lowest at 96 hours and at 200 µg/l. Larval swimming distance was significantly lower at 80, 120, and 200 µg/l of copper compared to the control and 40 µg/l. The 24-hour LC10 was 49.2 µg/l at 27°C and 41.6 at 30°C, and the 24-hour LC50 was 133.3 µg/l at 27°C and 108.7 µg/l at 30°C. The 48-hour, LC10 was 23.9 µg/l at 27°C and 40.8 at 30°C, and the 48-hour LC50 was at 96.5 µg/l at 27°C and 102.4 µg/l at 30°C. The 96-hour LC10 was 25.5 µg/l at 27°C and 26.8 at 30°C, and the 96-hour LC50 was at 93.3 µg/l at 27°C and 91.0 µg/l at 30°C. The mortalities and mobility results were not significantly different between temperature treatments, which suggested the larvae could tolerate thermal stress and the copper did not affect that thermal threshold of the larvae. After the 48-hour depuration period, the larvae swimming distances were higher but not statistically different. The lowest concentrations of copper had lower mortality and it was suggested that antioxidant defence mechanisms allowed larvae to survive the contaminant stress at low concentrations. Kwok & Ang Jr (2013) suggested the loss of swimming at high concentrations could be explained by loss of energy store and

ciliary action in cells. The authors concluded that mortality and swimming behaviour were negatively impacted by the presence of copper and increase in exposure time. However, the difference in temperature showed no significant effects.

Negri & Heyward (2001) examined the effect of tributyltin (TBT) and copper (as $CuCl_2$) on the metamorphosis of larvae and fertilization of gametes from scleractinian coral *Acropora millepora* colonies at Coral Bay, Ningoloo Reef, Western Australia. *A. millepora* was exposed to concentrations of $CuCl_2$ (between 10 – 100 µg/l), TBT chloride (between 10 – 100 µg/l), Cu based antifouling paint (540-565 g/l cuprous oxide and 40-50 g/l Diuron) and TBT/Cu based antifouling paint (20 g/l TBT oxide and 100-200 g/l cuprous oxide). The antifoulant paints were painted in 6 mm and 2 mm diameter circles on plastic sheets. During the 4-hour fertilization experiment, eggs and embryos were assessed using dissecting microscope. Both TBT chloride and Cu chloride inhibited the fertilization of gametes. The 4-hour IC50 value was 17.4±1.1 µg/l of Cu and 200± 31 µg/l of TBT. TBT was less toxic compared to Cu. The 6 mm diameter TBT/Cu based paint inhibited of fertilization 100% while the 6 mm diameter Cu-based paint inhibited at least 90%. The smaller 2 mm diameter circles of both paints inhibited fertilization by approx. 80%.

Metamorphosis of larvae is triggered by biochemicals on calcareous substrata (e.g., crustose algae) as larvae recognise the substrata as suitable for settlement and metamorphosis. Crustose algae were used in the metamorphosis experiment as a natural inducer to initiate metamorphosis. Metamorphosis was measured when free swimming or pear-shaped casually attached forms of larvae change into squat, firmly attached disc-shaped structures that has flattened oral aboral axis and septal mesenteries radiating obviously from the central mouth area. Attached larvae were counted. The 24-hour metamorphosis experiment provided an IC50 value of 110±20 µg/l of Cu and 2.0±0.3 µg/l of TBT. TBT was more toxic to larval metamorphosis compared to Cu. When exposed to the antifouling paints, the 6 mm diameter TBT/Cu based paint and Cu based paint inhibited 100% of metamorphosis. The smaller 2 mm diameter Cu based paint inhibited metamorphosis at around 30% and TBT/Cu based paint at less than 20%. It was also suggested that the effects of Cu and TBT may also affect recruitment and survival of the crustose algae, but this data was not reported in this study. The authors concluded the TBT chloride concentration that inhibited fertilization was much higher than the ANZECC and US EPA guidelines but that the copper chloride concentration that inhibited fertilization was close to the guidelines. The results of copper exposure on fertilization were similar with earlier findings and it was suggested that the sperm sensitivity to copper was the reason for the inhibition of fertilization as it can damage the sperm. Copper

chloride inhibited larval metamorphosis at concentrations 20 times higher than ANZECC guidelines and TBT inhibited three times higher than the ANZECC guideless but close to US EPA guidelines. It was also concluded that each type of antifouling paint inhibited fertilization and metamorphosis.

Nystrom *et al.* (2001) studied the combined effect of copper and temperature on the hermatypic coral *Porites cylindrical* metabolism. Branches of the coral from Cuenco Island, Hundred Islands, Philippines were exposed to increased temperatures for 24 hours in an initial exposure experiment (reported as “pre-exposed”). After a recovery period, corals “pre exposed” and “unexposed” to increased temperature were exposed to copper concentrations and increased temperature for a further 24 hours. The six treatments were two control (pre-exposed and unexposed), 11 µg/l copper unexposed, increased temperature (unexposed), increased temperature and 11 µg/l of copper (unexposed) and 11 µg/l copper pre-exposed. The sublethal stress indictors measured were changes in level of dissolved oxygen in light (net production) and dark respiration per chlorophyll-*a* and surface area. Qualitative results observed coral loss of colour in “unexposed” treatments exposed to copper and increased temperature, treatments with just increase temperature, and treatments of just copper. “Pre-exposed” corals showed a slight coloration loss after initial increased temperature exposure, completely recovered their colour after 5-day recovery and then lost colour, appearing pale, after the second exposure. The results showed a significant reduction in photosynthetic rate in treatments exposed to a combination of copper and heat and heat alone but no significant effect in copper alone treatments, which suggested no synergistic effect of copper and heat. In addition, there was a significant decrease in respiration rates in treatments exposed to a combination of copper and heat, copper alone, and increased heat alone. However, no significant difference in respiration rate was observed between ‘pre-exposed’ corals and the control. The authors concluded that high concentrations of copper might affect metabolism. However, 11 µg/l Cu alone did not affect the photosynthetic rate significantly. Their results suggested a combination of stressors have more effect on corals rather than an individual stressor acting alone, as photosynthetic rate reduced significantly in “pre-exposed” treatments. Furthermore, the authors concluded copper alone caused a significant decrease in coral respiration, but no significant response in “pre-exposed” corals. They noted that this was a short-term study, and a longer exposure period may have caused more stress response.

Reichelt-Brushett & Harrison (1999) examined the effects of copper, zinc, and cadmium on *Goniastrea aspera* gamete fertilization rate and the effects of cadmium on *Oxypora lacera*

gamete fertilization rate. Colonies were collected from Geoffrey Bay, Magnetic Island, Great Barrier Reef. Egg and sperm were exposed separately to 0, 2, 20, 100, 200, 500, and 1000 µg/l of copper, zinc, and cadmium for 30 minutes. Then dosed spermatozoa were added to dosed eggs for a 5-hour development period. Sealed vials were kept in a mesh bag, tied to a buoy in Geoffrey Bay. *G. aspera* fertilization rates at 2 µg/l of copper were not significantly different to control (93±4.03 % mean fertilization rate). However, higher concentrations of copper resulted in a significant decrease in fertilization rates; 41% fertilization rate at 20 µg/l Cu, and less that 1% fertilization at 200 µg/l Cu. The 5-hour EC50 was 14.5 µg/l Cu. The fertilization rate of *G aspera* was not significantly reduced by Zn exposure; 97% fertilization rate at 2 µg/l Zn, 96% at 20 µg/l, 98% at 200 µg/l and 98% at 500 µg/l Zn. Similarly, fertilization of *G. aspera* was not significantly reduced by cadmium exposure; 96% at 2 µg/l, 99 % at 20 µg/l, 96% at 100 µg/l and 99% at 200 µg/l) or the addition of a higher dose of cadmium at 1,000 µg/l to *O. lacera* (88% fertilization rate). The authors concluded that copper was the most toxic to fertilization of *G. aspera* and that fertilization was not obviously affected by cadmium and zinc. The results showed low concentrations of copper can cause disruptions to corals in the GBR. They noted that zinc was very abundant in the marine environment and, therefore, organisms developed bioregulation mechanisms to deal with different concentrations of zinc, which could explain why no effects were seen in this study.

Reichelt-Brushett & Harrison (2000) examined the settlement success of *Acropora tenuis* when exposed to copper. Terracotta ceramic tiles were placed in racks on an upper reef slope in Geoffery Bay, Magnetic Island in the Great Barrier Reef to use for settlement. In a pilot study (1994), *A. tenuis* larvae were exposed to 20 µg/l and 200 µg/l of copper, for 48-hour settlement period. They reported that 200 µg/l Cu inhibited settlement and caused mortality, but 20 µg/l Cu did not significantly inhibit settlement (NOEC). In the 1996 study, *A. tenuis* was exposed to 7.9, 17.3, 42, and 80.5 µg/l of copper for 48 hours. They found that 7.9 and 17.3 µg/l Cu had no significant effect on the mean percentage of larval settlement. The percentage of settlement decreased significantly at 42.0 µg/l and 80.5 µg/l Cu. The resultant 48-hour EC50 was 35 µg/l and NOEC was 20 µg/l Cu. The authors concluded that copper is toxic and can significantly affect *A. tenuis* larval settlement.

Reichelt-Brushett & Harrison (2004) examined the sublethal effects of lead and copper on larvae of *Goniastrea aspera* and *Platgyra daedalea* (pilot study). In the main experiment, colonies of *G. aspera* from Magnetic Island were collected prior to spawning and fertilisation occurred in tanks in laboratory conditions. Once larvae were five and six days old, they were exposed to copper chloride concentrations between 5 - 500 µg/l of and lead nitrate

concentrations between 100 – 10,000 µg/l and 500 – 20,000 µg/l for 72 hours. The same experiment was conducted on five- and six-day old larvae separately. The exposure larvae were kept in vials in bags tied to a buoy in Geoffery Bay and the survival of larvae was measured. Results found larval survival rates decreased with increasing copper concentrations. At 200 and 500 µg/l of copper concentration 100% mortality occurred between 6 – 72 hours. In exposure to low copper concentrations (10 and 50 µg/l), larval survival was significantly lower than controls after 48 – 72 hours. However, survival was not significantly different between concentrations lower than 50 µg/l. In exposure to lead larval survival also decreased as lead concentration increased but not as much as exposure to copper. In the higher lead concentration (20,000 µg/l) there was 100% mortality observed after 6 hours, this was a significant decrease compared to controls. There was 44% mortality observed at 10,000 µg/l after 72 hours. The 72-hour copper LC50 value was 34 µg/l (5-day old larvae) and 82 µg/l (6-day old larvae) and the 72-hour lead LC50 was 9,890 µg/l. The author concluded 10,000 and 20,000 µg/l of lead was critical to larvae survival.

In the pilot study *P. daedalea* larvae from Magnetic Island, Great Barrier Reef (GBR) were exposed to copper (as copper chloride) concentrations from 10 –200 µg/l and lead (as lead nitrate) concentrations from 1000 – 20,000 µg/l to examine the swimming distance of larvae (larvae motility). Results found motility decreased as copper and lead concentrations increases. In exposure to lower copper concentrations (10, 20, and 50 µg/l), the motility was significantly greater than at higher copper concentrations (100 and 200 µg/l). Larvae motility was significantly greater in controls than in the lowest lead concentration (1000 µg/l). The motility EC50 during the pilot study was 36 µg/l for copper and 1,950 µg/l of lead.

Following the pilot study, the motility experiment was modified for exposure to *G. aspera* larvae. In this experiment, the larvae were exposed to copper concentrations between 5 – 175 µg/l and lead concentrations between 10 – 7,500 µg/l. The results were similar to the pilot study, showing higher copper and lead concentrations reduce larval motility. The motility in lower concentrations of copper (5, 10, and 20 µg/l) slowly decreased and stabilized. Motility decreased in 30 µg/l and higher copper treatments and no movement (subsequent mortality) was observed from the beginning of the experiment. There was 100% larval mortality observed at 30 µg/l of copper after 72 – 96 hours of exposure and mortality occurred in 75 µg/l of copper after 12 hours of exposure, and 100 µg/l and higher copper concentrations after 6 hours. Similar results occurred in the lead exposure treatments as lower concentrations of lead stabilized overtime and higher concentrations decreased over the full time period. There was no 100% mortality observed in 96-hour lead exposures. The

EC50 values in the modified experiment were 22 µg/l of copper (48-hour LC50) and 2,230 µg/l of copper (72-hour LC50).

The author concluded both copper and lead affected larval survival of *G. aspera* and copper concentrations as low as 10 µg/l significantly reduced survival, which suggested that copper had more toxic effects at low concentrations. In addition, the study concluded that larval motility was inhibited by increasing copper and lead concentrations and leads to mortality.

Reichelt-Brushett & Harrison (2005) conducted multiple experiments (in 1995 and 1996) to examine the effects of trace metals; copper (Cu), lead (Pb), zinc (Zn), cadmium (Cd) and nickel (Ni), on the fertilization of scleractinian coral gametes from Magnetic Island and One Tree Island, Great Barrier Reef, Australia. Corals were collected before spawning in an annual spawning event. Gametes were exposed in laboratory conditions and were kept in sealed vials in mesh bags tied to a mooring buoy in the reef for the fertilization period. In each experiment, the sperm and egg of the coral were dosed with 5 ml of the appropriate metal concentration for 30 minutes and then spermatozoa were added to the eggs and examined after a 5-hour development period. The percentage fertilization was measured. In 1995, *Goniastrea aspera* was exposed to Cu, Pb and Ni concentrations and *Goniastrea retiformis* was exposed to Cu concentrations. In 1996, *Goniastrea aspera* was exposed to Cu, Pb and Ni, *Acropora tenuis* was exposed to Cu, Zn, Pb, Cd and *Acropora longicyathus* was exposed to Cu and Pb s. Cu exposure concentrations ranged from 0 – 250 µg/l, Pb from 0 – 1,000 µg/l, Zn from 0-5,000 µg/l, Ni from 0- 2,500 µg/l and Cd from 0- 10,000 µg/l.

Exposure of Cu to *G. reformis* had no significant effect on mean fertilization success of gametes exposed to the control, 2, 5 and 10 µg/l of Cu, and mean fertilization success was between 91 – 97%. There was a significant decline in mean fertilization success found in gametes exposed to 20 µg/l and above. The lowest fertilization success occurred at highest concentration, 250 µg/l. The 5-hour LC50 value was 24.7 µg/l and NOEC value was 10.0 µg/l. Different exposure concentrations of Cu were used on *G. aspera* compared to other species. No significant effect on mean fertilization success of gametes exposed to the control, 4.8 and 12.8 µg/l of Cu was found, and mean fertilization success was between 59 - 67%. There was a significant decline in mean fertilization success in gametes exposed to 20.4 µg/l and above. Fertilization success decreased as concentrations increased. The mean fertilization success was at 1% or less at 74.8 µg/l concentrations and above. The LC50 value was 18.5 µg/l and NOEC value was 12.8 µg/l.

No significant effect on mean fertilization success in gametes of *A. tenuis* was found after exposure to the control, 4.5, 7.4, 15.6, and 33.5 µg/l of Cu. The mean fertilization success was between 73 and 88%. There was a significant decline in mean fertilization success in gametes exposed to 41.9 and 66.6 µg/l, and there was a less than 5% success at 66.6 µg/l Cu. The 5-hour LC50 value was 39.7 µg/l and NOEC value was 33.5 µg/l Cu. No significant effect on mean fertilization success of *A. longicyathus* was found in gametes exposed to the control, 5 and 10 µg/l of Cu. There was a significant decline in mean fertilization success in gametes exposed to 23.6 µg/l and above. Concentrations of 60.5 µg/l Cu and higher had mean fertilization success rate of less than 3%. The 5-hour LC50 value was 15.2 µg/l and NOEC value was 15.3 µg/l Cu. *G. aspera* gametes were exposed to lead in 1995, but results found that the mean fertilization success was not less than 78% at the highest concentration of 1,000 µg/l. Therefore, in 1996, all species were exposed to higher concentrations of lead. *G. aspera* mean fertilization success significantly decreased at 6,409 µg/l Pb and higher concentrations. The 5-hour LC50 value was 2,467 µg/l and NOEC value was 5,455 µg/l Pb. The *A. tenuis* mean fertilization success rate significantly decreased at 1,982 µg/l of Pb, the 5-hour LC50 value was 1,801 µg/l and NOEC value was 790 µg/l. In *A. longicyathus* the mean fertilization success rate decreased significantly at 855 µg/l Pb. The 5-hour LC50 value was 1,453 µg/l and NOEC value was 451 µg/l Pb. *A. longicyathus* was the most sensitive species to lead exposure. Exposure of *A. tenuis* to zinc found a significant decrease in mean fertilization success of gametes exposed to 10, 100, and 1000 µg/l of Zn. The mean fertilization success was 70% at 10 µg/l of Zn, which reduced to 1% in 100 µg/l. No fertilization occurred in 5,000 µg/l Zn and the NOEC value was less than 10 µg/l Zn.

Exposure of *A. tenuis* to cadmium found a significantly lower mean fertilization success rate of gametes exposed to 5,000 µg/l of Cd (52% fertilization success) and 10,000 µg/l of Cd (53% fertilization success). The NOEC value was 2,000 µg/l. *G. aspera* gametes were exposed to the same concentrations of Ni in the 1995 and 1996 experiments. In 1995, there was no overall trend between increasing concentrations and a decrease in fertilization success. The mean fertilization success was above 83% in all treatments (5, 100, 1,000, 2,000 µg/l Ni). In 1996, a significantly low fertilization success rate was found at 100 µg/l of Ni and above, but the fertilization success did not decrease to less than 60%. The authors concluded the trace metals tested varied in effects on the coral species but, generally, the fertilization success was affected. Copper was the most toxic and all species experienced a similar negative effect on fertilization. Nickel and cadmium seemed to have less toxic effect on fertilization.

Reichelt-Brushett & Michalek-Wagner (2005) examined the effects of copper on fertilisation success in the soft coral *Lobophytum compactum*. Two experiments were conducted on coral from Bay Rock, on the Great Barrier Reef, during a mass coral spawning event in 2004, colonies were collected 10 days before spawning and exposed in laboratory conditions. Using the same procedure as Reichelt–Brushett & Harrison, (1999). The percentage of fertilization success was determined by the number of eggs counted in photographic images. In experiment 1, gametes were exposed to 6, 9, 16, 22, 36, 69, and 132 µg/l of copper. The fertilization success was 90% in the control and the fertilization percentage remained high for all exposed treatments. There was a significantly lower percentage of fertilisation success at 69 µg/l and 132 µg/l Cu. An EC50 was not calculated because fertilization success did not fall below 50%. The fertilization success was 68% at the highest concentration (132 µg/l Cu). The LOEC value was at 69 µg/l and the NOEC value was at 36 µg/l. In experiment 2, gametes were exposed to 18, 25, 39, 69, 117, 489, and 676 µg/l of copper. The fertilization success was 82% in the control and results showed a clear concentration-response curve. There was a significant difference in fertilization success at 117, 489, and 676 µg/l concentrations. The EC50 for fertilization success was 261 µg/l, the LOEC value was 117 µg/l, and the NOEC value was 69 µg/l. The authors concluded that the fertilization success of the soft coral species *L. compactum* was significantly more resistant to copper effects than other coral species that had been studied previously. Reichelt-Brushett & Michalek-Wagner, (2005) suggested this result could be because the species has two gametogenic cycles a year, where eggs develop for 21 months, which is longer than hard corals. Therefore, the eggs are exposed to copper in the marine environment for a longer period and may have developed biochemical detoxification mechanisms to adapt to different exposure concentrations of copper.

Rumbold & Snedaker (1997) conducted multiple sea-surface microlayer (SSML) bioassays on embryos of multiple species, including embryos of the coral *Montastraea favelota* from Key Largo, Florida. Embryos were exposed to copper sulphate ($CuSO_4$), sodium dodecyl sulphate (SDS) and Dibrom (O,O,-dimethyl-1- O-1,2,dibromo-2,2-dichloroethyl phosphate) for 24 hours in laboratory conditions. Endpoints for the coral larvae were recorded as active planulae, where directed or spinning movement was considered normal and active. The LOEC and 24-hour EC50 for copper exposure were 12.7 and 24.9 (8.9 – 70.3) µg/l Cu, respectively. The LOEC and 24-hour EC50 for SDS exposure were less than 4.0 mg/l, therefore, values could not be estimated. The results of *M. favelota* exposure to Dibrom were not available. The authors concluded that *M. favelota* was sensitive to copper and insensitive

to SDS. The results from other species bioassays indicated to the author that *M. favelota* were not as sensitive to the contaminants compared to the other species tested. It was noted that obtaining gametes is difficult and parameters were maintained to avoid added stress.

Sabdono (2009) examined the effects of copper on coral *Galaxea fascicularis* in Jepara coastal waters, Java Sea. Coral fragments were exposed to 0.001, 0.01, 0.1, 0, 1, 10 mg/l of copper in a range-finding experiment, for 48 hours, in laboratory conditions. Mortality was recorded based on percentage of bleaching. All coral fragments died (100% coral mortality) after 24 hours when exposed to 10 mg/l of copper and 8 out of 10 fragments (80% coral mortality) died when exposed to 1 mg/l of copper. After 48 hours, 100% mortality was observed when exposed to 1 mg/l and 50% coral mortality was observed at 0.1 mg/l of copper. No other concentrations observed deaths. Subsequently colonies were exposed to 0, 0.025, 0.050, 0.075 and 0.10 mg/l for 96 hours based on the range-finding experiment. Mortality was recorded when polyp tissue was not seen in calices. Results found 50% coral mortality was observed in colonies exposed to 0.025, 0.05, 0.075, and 0.10 mg/l of copper and the 96-hours LC50 value was 0.032 mg/l. The author concluded that *G. fascicularis* was more sensitive to heavy metals but can survive in higher concentrations for a longer time compared to other coral species. This study showed coral bleaching had the potential to assess stress.

Siddiqui *et al.* (2015) examined the sublethal effects of copper oxide nanoparticles (CuO NP) and copper chloride ($CuCl_2$) on *Exaiptasia pallida*, in laboratory conditions at Valdosta State University. Antioxidant enzymes and protein activity of catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and carbonic anhydrase (CA) were recorded as a sublethal effect because these are produced by organisms as a defence mechanism. *E. pallida* was exposed to control, 10 µg/l (dissolved conc. 4.43 µg/l), 50 µg/l (dissolved conc. 14.3 µg/l) and 100 µg/l (dissolved conc. 39.6 µg/l) of CuO NP for 21 days and exposed to a control, 50 µg/l (dissolved conc. 41.9 µg/l) and 100 µg/l (dissolved conc. 89.6 µg/l) of copper as $CuCl_2$ for 14 days. The antioxidant enzyme activity of *E. pallida* increased over 14 days in both copper types and stayed high until 21 days in CuO NP exposures.

CAT activity increased significantly for both types of copper in the first 4 days at 50 µg/l but was not increased significantly at 100 µg/l until 14 days of exposure. CAT activity in CuO NP treatments decreased to control levels after 21 days. The exposed *E. pallida* CAT activity was higher in CuO NP treatments than in $CuCl_2$. GPx activity increased with increasing copper exposure, over the 14 days exposed to $CuCl_2$ and 21 days exposed to CuO NP. *E.*

pallida GPx activity was increased significantly in 7 days when exposed to CuO NP. Activity then decreased to control levels in 10 µg/l treatments, increased and stabilised in 50 µg/l and increased continuously in 100 µg/l CuO NP treatments. GPx activity was 2 and 1.6 times lower in $CuCl_2$ treatment of the same concentration (50 µg/l and 100 µg/l respectively). *E. pallida* GR activity increased with increasing concentrations when exposed to CuO NP. A significant increase in GR activity was observed at 100 µg/l CuO NP after 14 days and in the lower concentrations (10 and 50 µg/l) activity increased in 3-7 days before decreasing to control levels after 21 days.

CA activity in both types of copper decreased in 14 days. The activity significantly increased in 3 days exposed to 10 µg/l of CuO NP. However, activity significantly decreased when exposed to 50 µg/l of CuO NP and slightly decreased at 100 µg/l after 14 days. CA activity decreased significantly in *E. pallida* exposed to 50 and 100 µg/l of $CuCl_2$ in 7 days. The authors reported no bleaching but observed a collapse, a change to dark coloured, curled tentacles and mucous release during the CuO NP exposure and the anemone decreased in size and lost tentacles during the $CuCl_2$ exposure period. *E. pallida* exposed to $CuCl_2$ were affected severely after 14 days and it appeared they would not survive 21 days. The authors concluded that the oxidative stress response, demonstrated by enzymes, was observed when *E. pallida* was exposed to both types of copper. Therefore, they suggested the response was dependent on the form of copper and concentration exposure. Exposure to CuO NP had greater oxidative stress response. The paper also focused on tissue copper accumulation, but this information was not included, however is important to note that increased enzyme activity coincided with an increase in tissue copper in the anemone.

Victor & Richmond (2010) examined the effect of copper on *Acropora surculosa* fertilization rates and gamete survival to the embryo stage. The reef building coral was collected from reef areas in Guam before the coral spawning period began. They were exposed to a stock solution of 4 mg/l of copper in seawater in a laboratory. Two colonies were exposed to 0, 10, 25, 50, 75, 100, and 200 µg/l of copper sulphate ($CuCO_4$) for 5 hours in each toxicity test on the rate of fertilization. The mean fertilization rate converted into percentages. The results showed a significant effect of copper on fertilization rate for all treatments. The rate was reduced to 90% at the lowest copper concentration treatment (10 µg/l). The fertilization rate was reduced to 50% in 25 µg/l Cu, less than 40% in 25 µg/l, less than 30% in 75 µg/l, less than 20% in 100 µg/l and less than 10% in 200 µg/l Cu. The toxicity test did not show what occurs after the fertilized egg continued to be exposed to copper. Therefore, another toxicity test was carried out to expose gametes to 0, 12, 30, 58, 86, 114, 216 µg/l of $CuCO_4$ for 12

hours. The results of this test also showed that copper had a significant effect on gametes. No gametes survived in concentrations above 58 µg/l (NR-LETH). Less than 50% of gametes survived at 12 µg/l (EC50) and less than 20% survived in treatments 30 µg/l and 53 µg/l of copper. The authors concluded that this paper presented the first quantitative results to show low concentrations of copper could disrupt successful reproduction on Guam's coral reefs and that the exposure duration would have a negative impact on success of larvae.

4.2 Organometals

There was a total of 16 results from organometals studied in five articles. Evidence summaries of Bao *et al.*, (2011) and Negri & Heyward (2001) are detailed above and the relevant article focusing on organometals alone is below.

Henderson & Salazar (1996) conducted multiple bioassay experiments on the effects of different antifouling tributyltin (TBT) leachates on a range of shallow water organisms in laboratory conditions in San Diego and Hawaii. In experiment 1, Henderson & Salazar (1996) studied the effect of leachates from copper antifouling paint (contained cuprous oxide) and TBT antifouling paint (contained TBT oxide and cuprous thiocyanate) on benthic organisms in Mokapu, Hawaii. The exposure concentrations were 5,600 ng/l of copper for copper paint and 900 ng/l TBT and 1,300 ng/l copper for TBT paint. The abundance of the brown anemone *Aiptasia pulchella*, the Bush coral *Pocillopora damicornis* and the Wart coral *Montipora verrucosa* were monitored. The results showed evidence that the abundance of all infauna studied (including the relevant species) reduced more drastically in TBT exposure tanks compared to copper exposure tanks. The decrease in abundance was 2.5x higher than decreases seen in copper exposed tanks. There were no live *A. pulchella* observed after the 93-day exposure to TBT whereas several hundred were observed in copper exposure tanks and the control. In *P. damicornis* all coral polyps died after 13 days and at 27 days only 11% of *Montipora verrucosa* coral polyps were alive in the TBT exposure tanks. The survival rate of both corals was significantly lower than the control treatment but significantly higher than coral exposed to TBT concentrations in the copper exposure tanks. The sublethal effects of the two coral species exposed to copper were different; *P. damicornis* polyps were normally distended and pigmented and *M. verrucosa* polyps retracted and observed to be stressed. In experiment 2, *Aiptasia pulchella*, *Pocillopora damicornis* and *Montipora verrucosa* from Mokapu, Hawaii were exposed to 510 – 1700 ng/l of TBT for 105 days. Low levels of toxic effects were observed at 510 ng/l after 28 days and several *A. pulchella* were present. Overall, a decrease in abundance was seen in all infauna, but was greatest in the highest

exposures (1,400 and 1,700 ng/l TBT). All *P. damicornis* and *M. verrucosa* corals died after 23 days of exposure, except for *M. verrucosa* at 510 ng/l, had 6% live tissue. The resultant LD50 values of 500 ng/l occurred at 17 days for *M. verrucosa* and 8 days for *P. damicornis.* High mortalities of the anemone and two coral species were observed in both experiments; in corals, these were dependent on TBT dose concentrations. The authors concluded that species abundance and density were significantly decreased by 500 ng/l and higher exposure concentrations of TBT, and this caused high mortalities within the first 2-3 weeks of the long exposure period.

Henderson (1988) examined the effects of Navy Fleet organotin antifouling paints on a series of experiments using flow through outdoor microcosm. Experiments were conducted at a microcosm facility in a laboratory on Mokapu peninsula, Oahu, Hawaii. Formula 121/63 (F121/63) copper toxicant paint, a self-polishing copolymer formula 4 (SPC-4) organotin paint and an organometallic copolymer paint (OMP-253) were the paints used in the study. The antifouling paint was applied to plexiglass panels placed in tanks, which would be removed during recovery phase. The copper, TBTO and dibutyltin chloride were measured in the water in each paint treatment tank. The mean dissolved copper concentration in F121/63 tank was 5.6 µg/l. The mean value yield of dissolved copper in SPC-4 was 1.3 µg/l and there was 1.7 µg/l of organotin measured in SPC-4. There was a 93-day exposure period and 104-day recovery period for F121/63 and SPC-4 treatments. Exposure to OMP-253, involved different microcosm tanks, ‘LoRes’ (low residence time) tanks, ‘HiRes’ (high residence time) tanks and HiOrg’ (increased organic levels, similar to LoRes). LoRes tanks were exposed to OMP-253 panels for two days, then removed for 73-day recovery period. The water from LoRes entered HiRes tanks which were then exposed for 105 days and a 105-day recovery phase. The mean concentrations of TBTO in all OMP-253 tanks ranged from 0.5 to 1.8 µg/l. In the first study, marine communities including *Aiptasia pulchella* and Zoanthids were exposed to the treatment microcosms and observations on abundance and distribution were recorded. Results found all *A. pulchella* species died between 23 and 42 days (100% mortality) when exposed to SPC-4 but were very abundant in the control and F121/63 tanks. Normal abundances of zoanthids were observed in SPC-4 tanks. In OMP-253 exposure *A. pulchella* had disappeared completely in treatments (90% mortality), but during recovery most *A. pulchella* had recolonized and abundances returned to normal. In a subsequent experiment *Pocillopora damicornis* and *Montipora verrucosa,* coral colonies from Kaneohe Bay were exposed to the same F121/63, SPC-4, and OMP-253 exposure tanks. The percentage of live polyp coverage, polyp retraction or distension and pigmentation were measured. Results

found, in SPC-4 exposure tanks 8 out of 12 colonies (66.7%) of *P. damicornis* were dead within one week and 5 – 90% remaining tissues had lost pigments from algal symbionts. After 2 weeks all *P. damicornis* colonies were dead. In SPC-4 exposure, the *M. verrucosa* colonies tissues were alive after one week, but polyps retracted and after 4 weeks only 10% of polyps were alive. In F121/63 exposure tanks survival of both corals was only slightly less than survival in the control (5% mortality). In OMP-253 exposure both coral species lost most pigmentation and polyp retracted. After 23 days, all P. damicornis colonies in LoRes and HiRes tanks, and *M. verrucosa* in LoRes tanks observed 100% mortality. The *M. verrcuosa* colonies in HiRes tank had 6% live tissue. The HiOrg treatment of OMP-253 observed 50% mortality in *M. verrucosa* polyps and 100% mortality in *P. damicornis* polyps.

Watanabe *et al.* (2006) examined the effects of Diuron (DCMU), tributyltin chloride (TBT-Cl) and Dichlorvos (DDVP) on *Acropora tenuis* juveniles in symbiotic free and symbiotic conditions. Colonies of *A. tenuis* were collected from Aka Island, Okinawa, Japan and fertilization and metamorphosis was initiated in laboratory conditions, and settled polyps were infected with *Symbiodinium* cells (PL-TS-1). Two experiments were carried out, exposing both aposymbiotic and symbiotic juveniles. In the first experiment, aposymbiotic juveniles were exposed to concentrations of DDVP (0.01, 0.1, 1, and 10 mg/l), DCMU (0.1, 1, 10, and 100 µg/l) and TBT-Cl (0.05, 0.2, 1, 5 µg/l) for 10 days and morphology abnormalities were recorded. Then PL-TS-1 cells were added to exposed polyps to examine the uptake of symbionts. The second experiment infected juveniles with PL-TS-1 cells first and the exposed them to same concentrations of DDVP, DCMU and TBT-Cl for 10 days. Microscopic observations of the abundance of symbionts, using level of tentacle pigmentation, were recorded during the study. Morphological abnormalities (detachment of soft tissue from skeleton) and mortality (complete lysis of soft tissues) were recorded.

After exposure to DDVP, detachment of soft tissue was observed in 52% of aposymbiotic juveniles at 10 mg/l, partial detachment of soft tissue was observed in 18% of corals at 1 mg/l and no abnormalities were observed at 0.01 and 0.1 mg/l. All aposymbiotic juveniles inhabited symbionts in DDVP concentrations, and 12 – 53% of symbiont inhabited juveniles exhibited partial detachment of soft tissue after exposure at all concentrations of DDVP. A reduction of pigmentation levels was observed in symbiotic inhabited juveniles suggesting loss of symbionts from the coral.

Aposymbiotic juveniles did not exhibit abnormalities or detachment of tissue or death after exposure to DCMU. However, one of the juveniles was found dead at 1 µg/l but this death is

unlikely to be due to DCMU, as juveniles exposed to higher concentrations appeared normal after exposure. Pigmentation in aposymbiotic juveniles were normal in 0.1, 1 and 10 µg/l concentrations but significantly reduced in 100 µg/l, which suggested DCMU impaired the ability an aposymbiotic juveniles to take up symbionts. In addition, no abnormalities were found in symbiotic juveniles after 0.1, 1 and 10 µg/l exposure to DCMU. In 100 µg/l of DCMU, the majority of symbiotic juveniles exhibited abnormalities; 28% of individuals had partial detachment of soft tissue and in 39% had spherical tissue aggregates, as zooxanthellae had been lost. Pigmentation in symbiotic juveniles reduced significantly after exposure to DCMU.

All aposymbiotic individuals were dead and exhibited detached soft tissue after exposure to the highest exposure concentration (5 µg/l) of TBT-Cl by day 4. Pigment levels were significantly reduced. No abnormalities were observed in aposymbiotic corals exposure to 0.05 and 0.2 µg/l TBT-Cl. In addition, 94% of symbiotic juveniles died in 5 µg/l of TBT-Cl, one individual tissue ball was observed, and 33% of juveniles exhibited partially detached tissue in 1 µg/l of TBT-Cl. Pigment levels were significant reduced in symbiotic juveniles. No abnormalities were observed at 0.2 µg/l f TBT-Cl.

The study concluded that symbiotic *A. tenuis* juveniles were more sensitive than in aposymbiotic condition to DDVP and DCMU, but not TBT-Cl.

(Page left blank)

5 Synthetic compounds – including Pesticides and Pharmaceuticals

A total of forty-nine results (ranked 'worst-case' mortalities) were obtained from seventeen articles that examined the effects of 'Synthetic compounds' on anthozoan species. Pesticides/biocides were most studied, with 61.22% of the results, within which 26.53% of the results came from effects on Herbicides (Figure 5.1). Ultraviolet (UV) filters, which are Personal care products (PCPs), were the also well studied synthetic compound (16.33% of results). Overall, most worst-case results were sublethal (53.06% of results). However, the PCPs results all reported 'Significant' mortalities.

All life stages studied were affected by 'Synthetic compounds'. The early life stages ('Larva' and 'Embryo') was the most studied life stage (57.14% of results), followed by 'Adults and Juveniles' (18.37% of results). There was a high percentage (24.49%) of results where the life stage was not 'Not reported'.

Actinaria (26.53% of results) and Scleractinia (73.47% of results) were the only taxonomic group studied in effects of 'Synthetic compounds (Figure 5.2). Overall, *Acropora millepora* was the most studied species (18.37% of results), followed by the well-studied *Pocillopora damicornis* (14.29% of results). *Exaiptasia pallida* (also known as *Aiptasia pallida* but reported in this study as new name) was the best studied Actinaria species (10.20% of the results).

5.1 Pesticides/biocides

A total of twenty-eight results were reported by 10 articles on the effects of Pesticide/Biocides on anthozoan species. The relevant summaries are below, but Bao *et al.,* (2011) and Watanabe *et al.* (2006) are detailed above.

Acevedo (1991) examined the effects of Carbaryl, naphthol and Chlorpyrifos on *Pocillopora damicornis,* a hermatypic coral collected from Kaneohe Bay, Oahu under laboratory conditions. The planulae of the coral were kept in 200 ml vials and exposed to 0.01, 0.1, 1, 10, 100 ppm of Chlorpyrifos, 1- naphthol and Carbaryl, for 96 hours in the shade. The number of planulae which were observed to be swimming and non-swimming were recorded every six hours. At concentrations of 0.01 ppm of all contaminants had no effect on the planulae. Concentrations 0.1, 1 and 10 ppm of Carbaryl and 1-naphthol had also no

noticeable effects. However, 100% of planulae were killed at 100 ppm 1-naphthol, and 70 – 90% of planulae were killed at 100 ppm Carbaryl.

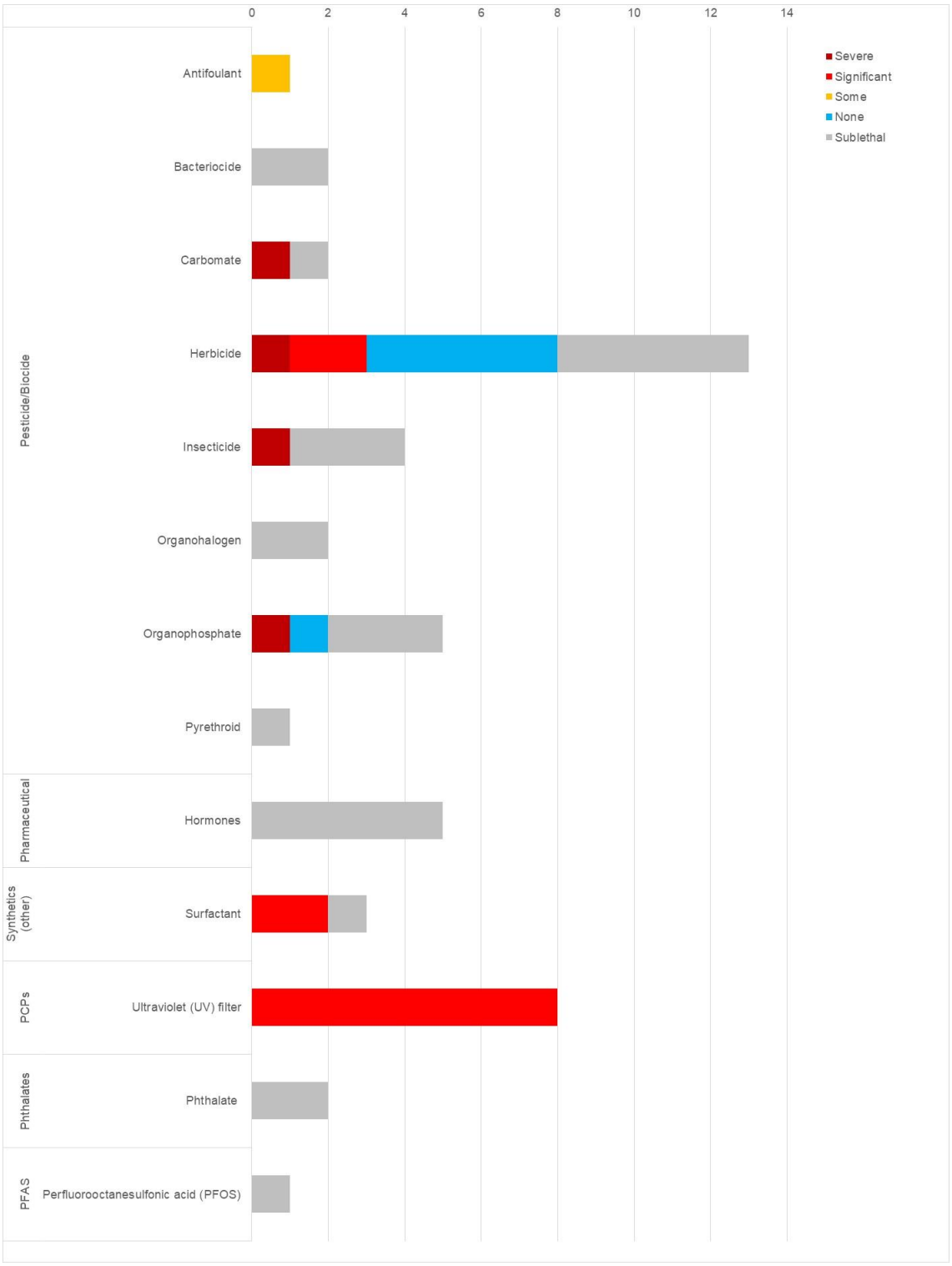

Figure 5.1. Count of worst-case ranked mortalities due to exposure to ‘Synthetic compounds’ in Anthozoa. Mortality is ranked as follows: ‘Severe’ (>75%), ‘Significant’ (25-75%), ‘Some’ (<25%), ‘None’ (no mortality reported), and ‘Sublethal’ effects.

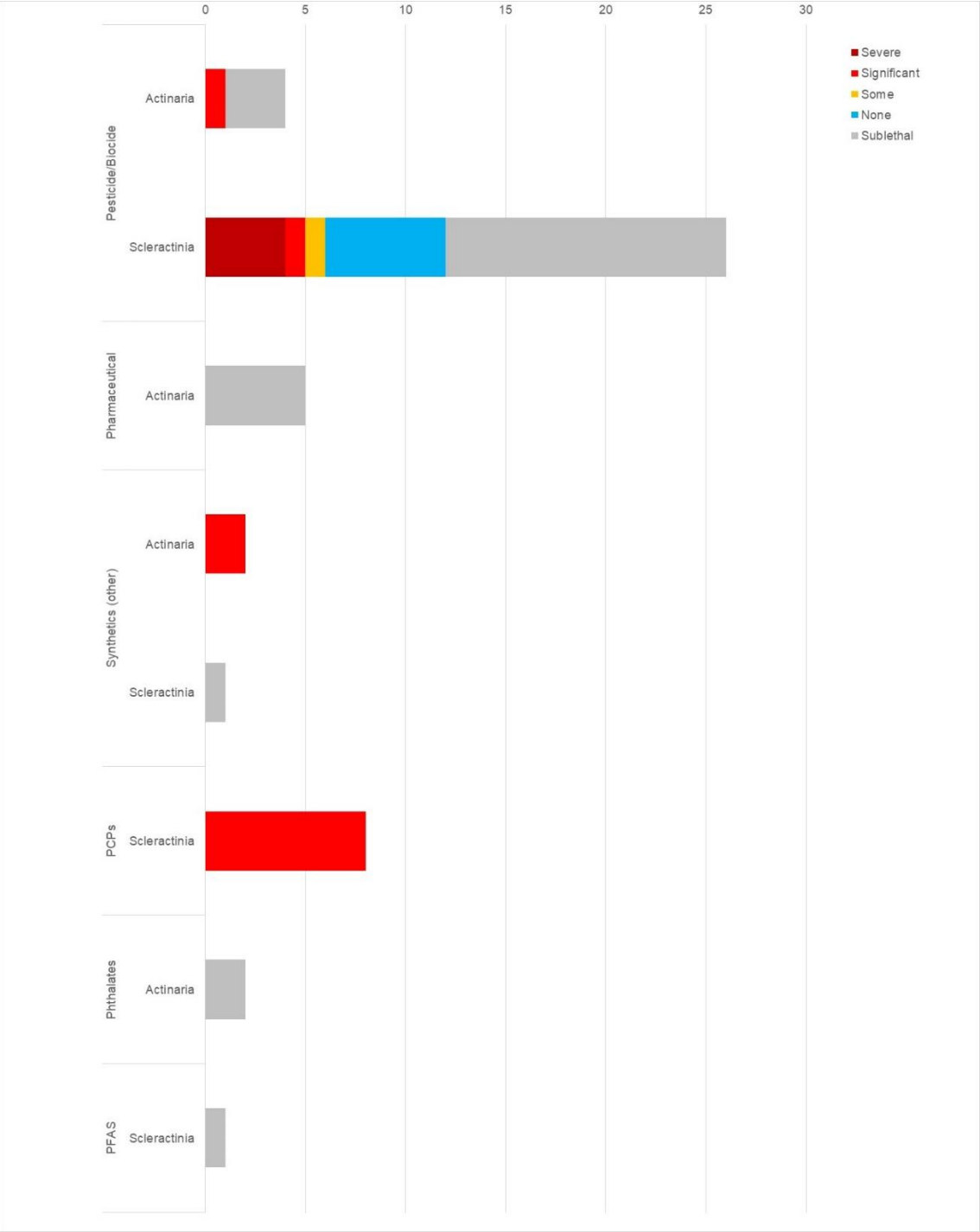

Figure 5.2. Count of ranked mortalities due to exposure to Synthetic compounds in Anthozoan taxonomic groups; Scleractinia and Actinaria. Mortality is ranked as follows: ‘Severe’ (>75%), ‘Significant’ (25-75%), ‘Some’ (<25%), ‘None’ (no mortality reported), and ‘Sublethal’ effects.

The motion planulae slowed down in the first 24 hours at 0.1 ppm of Chlorpyrifos and 50 to 100% were killed at 1 ppm. However, 100% of the coral planulae were killed at 10 ppm of Chlorpyrifos and no other experiments were conducted on higher concentrations. The author concluded that low concentrations of Chlorpyrifos had the strongest adverse effects on coral

planulae. The author noted that a concentration of 100 ppm of Carbaryl or 1-naphthol was unlikely to occur in the environment and the sample size of this study is small (10 planulae).

Cantin *et al.* (2007) studied the effects of the photosystem II (PSII) herbicide Diuron, which causes induced photoinhibition, on spawning coral *Acropora tenuis* and *Acropora valida* from Nelly Bay, Magnetic Island, Australia, and the brooding coral *Pocillopora damicornis* from Horseshoe Bay, Magnetic Island. The corals were exposed to 0, 0.1, and 10 µg/l Diuron for 53 days (*A. tenuis*), 67 days (*P. damicornis*) and 90 days (*A. valida*). The mean measured concentration between replicate tanks was 0.91±0.05 and 8.8±0.2 µg/l. The exposure treatments were examined in tanks outside. The mortality of colonies was measured as a result of tissue loss exposing the bare skeleton on coral branches or full colonies (full tissue loss on every branch in colony). Branches and reproductive polyps on the corals were sampled before exposure, 8-12 weeks into exposure period (prior to spawning or planulation) and after spawning or planulation to measure total lipid content as reproductive investment and the fecundity. The photosynthetic efficiency, pigment composition, and density of *Symbiodinium* spp. were also measured, but not recorded. All *A. tenuis* and *P. damicornis* colonies survived Diuron exposure. Bleaching of *A. tenuis* was not observed in both Diuron exposures and coincided with consistent densities of symbiotic *Symbiodinium* within the corals. Complete mortality was observed in 25% of *A. valida* colonies while the other colonies showed partial mortality in 10 µg/l exposure treatments of Diuron. Patchy bleaching and some fully bleached branches were observed in all colonies of *A. valida* exposed to 10 µg/l of Diuron prior to the mortality. Severe bleaching coincided with loss of *Symbiodinium* spp. in *P. damicornis* exposed to 10 µg/l of Diuron. Total lipid content significantly decreased in *A. tenuis* and *A. valida* species at 10 µg/l Diuron exposure treatments. A decrease in total lipid was also see in 1.0 µg/l treatments and continued to decrease after spawning. In addition, the lipid content also significantly decreased in *P. damicornis* before planulation and remained low. The colonies of *A. tenuis* exposed to 10 µg/l successfully spawned and no significant decrease in average number of eggs per poly or egg size. *A. valida* polyps exposed to 10 µg/l, resulted in a significant reduction in the number of eggs and polyp fecundity; there was no spawning observed in 10 µg/l Diuron. A concentration of 1.0 µg/l did not affect fecundity, which suggested no effect in the reproductive tissue *of A. valida. P. damicornis* did not release larvae and experienced bleaching when exposed to 10 µg/l Diuron. However, the branches still contained reproductive polyps, and had a significantly higher number of polyps in 1.0 µg/l treatments. The authors concluded that *A. valida* and *P. damicornis* were affected the most by diuron exposure, and showed severe bleaching,

mortality, and decreased reproduction. It was concluded that the photoinhibition by Diuron and the decrease in *Symbiodinium* spp. densities, resulted in a decrease in the amount of energy available for reproduction.

Markey *et al.* (2007) examined the effects of pesticides on gamete fertilization, larval metamorphosis and adult *Acropora millepora* broadcast spawning coral from High Island, central Great Barrier Reef Australia in order to study the effects of the contaminant on different coral life stages. Fertilization and larval metamorphosis may be more sensitive to contaminants than adults. The seven pesticides examined were insecticides Endosulfan, Chlorpyrifos, Chlorpyrifos oxide, Profenofos, Carbaryl, Permethrin and the fungicide 2-methoxyethylmercuric chloride (MEMC). Gametes were exposed to 0.3 – 30 µg/l of each toxicant during the fertilization experiment. Fertilisation was not significantly affected by the insecticides (LOEC and EC50 were more than 30 µg/l). However, exposure to fungicide MEMC reduced fertilization by 90±3% at 1.0 µg/l concentration and 1.1±1.8% at 3 µg/l concentration. The MEMC LOEC value was 1.0 µg/l and the EC50 value was 1.68±0.04 µg/l. All eggs ruptured at 30 µg/l. Six-day old larvae were exposed to 30, 100, and 300 µg/l of each toxicant for 18 hours during the metamorphosis experiment, and two crustose coralline algae species (*Nogoniolithon fosliei* and *Hydrolithon onkodes*) were added to each treatment to induce metamorphosis. All toxicants at all concentrations inhibited metamorphosis completely. This experiment was repeated with seven-day old larvae exposed to 3.0, 10 and 30 µg/l of each toxicant and 8-day old larvae exposed to 0.1, 0.3, and 1.0 µg/l of each toxicant. Larval settlement decreased by 60 and 100% in low 0.3 to 1.0 µg/l exposure concentrations after 18 hours. The LOEC values were 0.3 µg/l in Chlorpyrifos oxide and Profenofos concentrations, 1.0 µg/l in Endosulfan, Chlorpyrifos, Permethrin, and MEMC concentrations and 3.0 µg/l in Carbaryl concentrations. Larval swimming behaviour was not affected by insecticides, but larvae were often motionless when exposed to 3 µg/l and higher concentrations of MEMC. Adult *A. millepora* polyp retraction, visual bleaching and tissue mortality, dinoflagellate density, and photosynthetic yields of symbionts were measured. Adults were exposed to 1 and 10 µg/l of each toxicant for 96 hours. Tentacles were feeding in most pesticide exposures, apart from branches exposed to 10 µg/l of MEMC and Profenofos when tentacles retracted permanently after the exposure period. Paling (apparent visual bleaching) was observed in adults exposed to 1 µg/l of MEMC and 10 µg/l of Chlorpyrifos, Endosulfan, Profenofos, Permethrin, and MEMC. Dinoflagellate density decreased and tissue mortality occurred in adults exposed to MEMC. Photosynthetic yields of symbiotic algae reduced significantly in corals exposed to 10 µg/l of Endosulfan, Chlorpyrifos, and MEMC and

reduced when exposed to 1 µg/l of MEMC. The authors concluded that insecticides were highly likely to affect fertilization and metamorphosis of *A. millepora.* The coral was likely to experience even broader toxicity, in all life stages, from fungicide MEMC even at low concentrations. The result endpoint values for each contaminant can be found in evidence summary spreadsheet.

Negri *et al.* (2005) examined the effects of Diuron in laboratory experiments on different early life stages of brooding coral *Pocillopora damicornis* from Magnetic Island and two broadcast corals *Montipora aequituberculata* and *Acropora millepora* from Lizard Island. The early life stages (oocytes, sperm, larvae, and new recruits) and transitions including metamorphosis and fertilization were studied in all species. Concentrations of Diuron ranged from 0 –1000 µg/l. Gamete fertilization and larvae metamorphosis experiments were conducted on the broadcast spawning corals. Stock crustose coralline algae (CCA) was used to induce metamorphosis and were assessed after 24 hours. Results found no significant fertilization inhibition of Diuron to broadcast coral up to 1000 µg/l. Metamorphosis experiments were conducted on brooding coral larvae and assessed after 24 hours. Three experiments were conducted to test the effects of Diuron on coral recruits. The first was an indoor exposure measuring survival and bleaching where the larvae of the broadcast corals were induced to settle and metamorphose and, after settlement on cell culture plates, the recruits were exposed to Diuron for 96 hours and renewed every 24 hours. Symbiont density in *P. damicornis* was also measured. No significant fertilization inhibition in *A. millepora* or *M. aequituberculata* was observed at any Diuron concentrations. On the other hand, A. millepora metamorphosis was significantly inhibited by 300 µg/l and above concentrations of Diuron but no effect on metamorphosis was observed at concentrations up to 100 µg/l of Diuron. The A. millepora recruits exposed to 30 – 1000 µg/l of Diuron survived under low illumination and had no visible signs of stress. The *P. damicornis* recruit survival did not decrease in exposures up to 1000 µg/l of Diuron, but symbiotic dinoflagellate loss and bleaching were observed.

The second experiment was an outdoor exposure where *P. damicornis* recruits were exposed to Diuron for 96 hours under partial shading; tissue retraction from the skeleton and symbiont expulsion/bleaching were measured. Symbiont density was measured. The third experiment was a similar outdoor experiment as the second but the photosynthetic efficiency of *P. damicornis* was measured in recruits exposed to diuron for 96 hours. After 96 hours of exposure, results found all *P. damicornis* recruits survived at high illuminations. This was

correlated with a decrease in dinoflagellate numbers. Extensive bleaching was observed at low concentrations.

A further outdoor experiment was conducted on adult colonies of *A. millepora* and *P. damicornis* in the same conditions as the recruitment outdoor experiments. Branchlets of the corals were exposed to Diuron for 96 hours, renewed every 25 hours and the photosynthetic efficiency was measured. Diuron concentrations at 1.0 µg/l and above caused a rapid and significant decrease in the photosynthetic efficiency of symbionts in adults and in recruits of both *A. millepora* and *P. damicornis*. The authors concluded that *P. damicornis* recruits were the most sensitive life stage to Diuron because of the extensive bleaching at low concentrations.

Pridmore *et al.* (1992) examined the effects of Chlordane on an intertidal sand flat near Wiroa Island, Manukau Harbour, New Zealand in a two-month field study which identified sensitive species and their response time scale. The anemone *Anthopleura aureoradiata* was a dominant species identified in the study area. *A. aureoradiata* was exposed to 80 ng/cm^2 of technical Chlordane for 1, 3, 5, 13, 19, 44, 71, 112 tides in the experimental site. Tides at the site are semi-diurnal and used to measure time. *A. aureordiata* decreased in abundance to roughly half its initial density by tide 13. The reason for species decline was not known. The authors could not determine if mortality was due to Chlordane, behavioural changes, or both.

Raberg *et al.* (2003) examined the effects of pesticides, Diuron and 2,4-D on the hermatypic coral *Porites cylindrica* from Quenco Island, Hundred Islands archipelago. The coral was exposed to 10, 50 and 100 µg/l of Diuron and 10 and 100 mg/l of 2,4-D for 48 hours in laboratory conditions. The effect of dissolved oxygen concentrations was used to measure sublethal stress and gross primary production rate and respiration rate combined to measure net primary production. Net primary production and gross primary production of the coral decreased significantly due to exposure to 100 mg/l 2,4-D but respiration was not affected. In addition, net primary production and gross primary production were significantly decreased by exposure to 10, 50 and 100 µg/l of diuron but respiration rate was decreased significantly by 50 and 100 µg/l of diuron. The physiological state of photosynthetic organisms (zooxanthellae) was measured in this study but not recorded, as it is not relevant to this review.

Ross *et al.* (2015) examined the combined effect of temperature and two mosquito pesticides Dibrom (also known as Naled) and its degradation product dichlorvos (DDVP), and Permanone 30-30 (includes ingredient Permethrin) on *Porites astreoides* larvae from

Wonderland Reef, Florida. This laboratory study focused on physiological effects including, larval survival, settlement, post-settlement survival, and zooxanthellae release. Sublethal oxidative stress assays were also conducted in this study, but these were not reported (transcription and cellular). DDVP was measured because of the short half-life of Naled in seawater. The one-day-old larvae were exposed to 0, 0.1, 1.0 or 10.0 µg/l of Naled, Permethrin and DDVP at ambient temperature (26.9± 0.14°C) and an elevated temperature (30.50±0.12°C) for 18 to 20 hours. After exposure, the survival and settlement of larvae were measured with a further 48-hour settlement period and at 48 hours, the number of swimming larvae, and larvae that had settled and metamorphosed were recorded. Post settlement survival was measured in the field, with newly settled spat placed on a patch of reef in the east Looe Key research area. The results revealed that *P. astreoides* larvae survival was not affected by permethrin exposure, but survival was significantly decreased when exposed to 2.96 µg/l and higher concentrations of Naled. There was no significant effect on the larvae survival exposed to elevated temperature. Furthermore, no significant effect on the larvae settlement or post-settlement survival was observed when exposed to pesticides and elevated temperatures. The author concluded the exposure of the pesticides did not affect *P. astreoides* larvae settlement and post-settlement survival.

Tagatz *et al.* (1979) studied the biocides Surflo–B33 and paraformaldehyde (Aldacide) used in drilling muds for exploratory drilling effects on estuarine macrobenthic assemblages, including *Aiptasia pallida*. The abundance and density of the specimens that grew from larvae were measured in different treatments for seven weeks. The larvae were exposed to 41 µg/l and 819 µg/l of Surflo-B33 and 15 µg/l and 300 µg/l of Paraformaldehyde in laboratory conditions. The results showed a significant decrease in *Aiptasia pallida* juvenile abundance when exposed to Surflo-B33. The mean number of the specimens significantly decreased in treatments exposed to a higher concentration of Surflo-B33 as six were collected from the 41 µg/l of Surflo-B33 and none collected from 819 µg/l of Surflo-B33 after seven weeks. There was no significant decrease in abundance or the average number of the specimens in treatments exposed to Paraformaldehyde, as 11 *Aiptasia pallida* were collected from the 15 µg/l treatment 12 from 300 µg/l. The author concluded chlorophenol-type biocides could affect benthic settlement by planktonic larvae, and that Surflo-B33 had more toxic adverse effects on settlement. It was suggested that the decrease in juvenile density may be a direct effect of biocides, which could lead to secondary effects such as the reduction in recruitment and survival.

5.2 Pharmaceuticals

Morgan *et al.* (2022) examined the effects on endocrine disruptors on sea anemones *Exaiptasia diapana*, syn. *Aiptasia pallida*) in a molecular study that focused on genes of interest that can be linked to the exposure. The anemone was exposed to 20 ppb of testosterone (T), estradiol (E2), benzyl butyl phthalate (BBP), cholesterol and oxybenzone (BP-3) for four hours in laboratory conditions. The endocrine disruptors altered the transcription of genes associated with sterol transport, oogenesis, steroidogenesis, and Hedgehog signalling pathways[6].

5.3 Synthetics (other)

The 'synthetics (other)' category includes a range of chemicals that do not fit into other categories conveniently. Maggi & Cossa (1973) and Rumbold & Snedaker (1997), as described above, also studied the effects of surfactants, which fits into the 'synthetics (other)' category.

5.4 Personal Care Products (PCPs)

Two studies examined the effects of Ultraviolet (UV) filter, a Personal Care Products. The evidence is detailed below.

Downs *et al.* (2014) examined the effects of the sunscreen UV filter, benzophenone-2 (BP-2) on *Stylophora pitillata* planulae and toxicity to its cells. As the corals have photosynthetic symbiotic organisms, experiments were conducted in environment light (relevant natural light) and darkness in a laboratory. The cellular pathology, DNA damage, planular ciliary movement, planulae morphology, chlorophyll florescence (estimate coral bleaching) and mortality were measured. *S. pitillata* planulae were exposed to 246, 24.6, 2.46 ppm of BP-2 for eight hours in light, eight hours in darkness, a full 24-hour diurnal cycle and 24 hours in darkness. The four replicates had two controls, planulae were in exposure tanks containing all seawater (ASW) and ASW containing dimethyl sulfoxide (DMSO). In summary, results found an increase in bleaching with increasing concentrations of BP-2 in all replicates, because of chloroplast degradation in zooxanthellae, loss of zooxanthellae and its photosynthetic pigments. In light replicates cellular necrosis was dominant form of cell death

[6] Hedgehog signalling is a developmental pathway where cholesterol interacts with specific sterol binding proteins (Morgan *et al.,* 2022)

in tissues, and lysis occurred in both the epidermis and gastrodermis. There was a significant increase in levels of DNA-AP (abrasic) lesions in larvae exposed in light due to the increasing BP-2 concentrations and lesions were higher in BP-2 exposure in the dark. The no observed effect levels (NOEL) were difficult to record in the 8-hour exposure replicates because responses were similar. All of the planulae survived and they were not deformed in some concentrations. However, the 8-hour NOEL value was 246 ppm for live planulae which was not deformed and the NOEL value for DNA AP was 246 ppm in both light and darkness. The 24-hour NOEL value was 246 ppm in both light and darkness. The EC20, EC50 and LC50 values were estimated, and the full list can be seen in the evidence summary spreadsheet. The worst case 24-hour LC50 was 508 ppm. The author concluded BP-2 is a phototoxicant as it induced different pathologies in planulae exposed in light or darkness. The cells of corals were more sensitive than the planulae to increased BP-2 concentrations.

Downs *et al.* (2016) examined the effects of benzophenone-3 (BP-3, oxybenzone), a sunscreen ingredient on *Stylophora pitillata* planulae and cultured primary cells in laboratory conditions at the Inter-University Institute of Marine Science (IUI) in Israel. The experimental design was based on the Downs *et al.* (2014) experiment, with the same controls, replicates, and exposure times. The planulae were exposed to 228, 22.8, 2.28 mg/l and 228 22.8, 2.28 µg/l of BP-3. The planular ciliary movement, planulae morphology, chlorophyll florescence (estimate coral bleaching), mortality and DNA Abrasic (DNA AP) lesions were measured. In summary results found a significant decrease in ciliary movement and morphology as the natural elongated planulae shaped deformed into a “drew drop,” planulae appeared to be more sessile in all concentrations of BP-3. As the concentration of BP-3 increased there was an increase in bleaching observed, in the highest concentrations (228 µg/l) a bleaching response was observed as planulae lost zooxanthellae and/or photosynthetic pigments. The bleaching LOEC value was 2.28 µg/l in light and 22.8 µg/l in dark. Cell death and degradation of tissue was seen in the highest concentration of BP-3 (228 µg/l) in both light and dark, although cell deterioration was less severe in the dark. In addition, with increasing concentrations of BP-3 a higher level of DNA AP lesions were significantly higher in both light and dark conditions. The estimated 8-hour NOEC value for planulae survival and not deforming was 228 mg/l in both light and dark, and the 24-hour NOEC value for planulae survival in light was 2.28 µg/l and 22.8 µg/l in dark. The estimated NOEC for DNA AP was 22.8 µg/l in light and darkness. The EC20, EC50, and LC50 values were estimated using different statistical methods. The full list can be seen in the evidence summary spreadsheet. The worst-case 24-hour LC50 value was 873.4 µg/l in darkness. Based on this coral cell

toxicity assay, LC50 and LC20 values were provided to show differences in sensitivity to BP-3 exposure of other coral species in the Indo-Pacific and Caribbean reefs, which included *Pocillopora damicornis, Acropora cervicornis, Montastraea annularis, Montastraea cavernosa, Porites astreoides, and Porites divaricata*. The author concluded BP-3 as a phototoxicant which is toxic to the planulae in light and darkness, and coral cells were more sensitive to BP-3 than coral planulae.

5.5 Phthalates

Klein *et al.* (2021) examined the effects of environmental contaminants phthalates (phthalic acid esters (PAEs) and potassium nitrate) on the early exposure of the sea anemone *Nematostella vectensis* on embryo development and microbiome. Embryos were exposed to 1, 10 or 20 µM of potassium nitrate and 1, 10, or 20 µM of dioctyl phthalate. Molecular DNA extraction, cnidocyte staining, gene sequencing and regeneration experiments were conducted. Results found exposure to high concentrations of phthalates (20 µM and above) lead to possible high morbidity and toxicity. Body size defects and tentacle defects (few tentacle numbers and uneven length) of the embryos were observed in high concentrations. A decrease in cnidocytes used to capture food was also recorded. The author concluded early exposure to the contaminants had major effects on embryo development and growth, which could lead to death and decrease in *Nematostella* populations.

5.6 Perfluoroalkyl substances (PFAS)

Bednarz *et al.* (2022) examined the combined effect of perfluoroctane sulfonate (PFOS) concentrations and increasing temperature on the physiology of *Stylophora pistillata,* a Scleractinia coral, originally from the Gulf of Aqaba but cultured and studied in laboratory conditions. Coral nubbins were exposed in four treatment groups for 28 days; control, 100 ng/l of PFOS in ambient temperature (25°C), no PFOS in elevated 32°C and 100 ng/l of PFOS in elevated 32°C. Multiple different physiological parameters were measured including; the maximum non photochemical quenching (NPQmax), net photosynthesis (Pnet) and respiration rates (R) of corals. The photosynthetic efficiency of symbionts was measured by looking at the maximum quantum yield of photosystem II of corals. Results found NPQmax was affected by increase in temperature. However, there was combined effect of temperature and PFOS significantly decreased the photosynthetic efficiency and Pnet rates. No effects were observed on respiration rates and protein content in the coral. Oxidative stress endpoints were also measured; reactive oxygen species (ROS) lipid peroxidation (LPO) and total antioxidant capacity (TAC) of the corals were analysed as a proxy for oxidative stress

metabolism. The results showed a significant increase in LPO in PFOS at 25°C, PFOS at 32°C and elevated temperature alone treatments. TAC increased and ROS decreased after 28 days but this was not significantly different to the control. The results suggested a combination of increased temperature and PFOS did affect physiological changes but are not statistically different. The authors concluded that PFOS exposure and increasing temperatures significantly affected the physiology of *S. pistillata*, which suggested pollution might exacerbate ocean warming effects for corals. This study also focused on the bioconcentration and accumulation of PFOS in corals, this information was not included here.

6 Other substances

'Other substances' include a range of chemicals that do not fit into the other categories of contaminant. Neither do they group conveniently. Four articles fit into this category. These articles examined Scleractinia (48.86% of results), Actinaria (28.57% of results) and Octocorallia (28.57% of results). The Scleractinia species *Pocillopora damicornis, Porites lichen, Acropora millepora*, the Actinaria species *Exaiptasia pallida* (syn. *Aiptasia pallida*), and the Octocorallia species *Dentomuricea meteor* were studied. Juveniles and larvae were studied. However, 57.14% of results from 'Other substances' evidence did not report the life stage. 'Juveniles' was the most studied life stage (28.57% of results), followed by 'larvae' (14.29% of results).

6.1 Inorganic chemicals

Jones & Steven (1997) examined the effects of cyanide on the common reef coral *Pocillopora damicornis* and *Porites lichen* from One-Tree Island lagoon reef, southern limit of the Great Barrier Reef, Australia. In the toxicity tests, *P. damicornis* and *P. lichen* colonies were exposed to 0.2, 0.02, 0.002, and 0.0002 M cyanide for 1, 5, 10, 20 and 30 minutes to test the physiological effects. In the respirometry tests *P. damicornis* specimens were exposed to 1.0, 0.02, and 0.002 M of cyanide for 2.5, 5, and 7.5 minutes over three days in to test the effects of cyanide on respiratory rates. *P. damicornis* respiratory rates were determined during 10 to 20-minute incubations for 1-2 hours in respirometry chambers before and after exposure outside of these chambers. The results found both corals exhibited a change in colouration or 'bleaching' in response to concentrations of cyanide. The degree of colouration was dependent on the concentration. No discolouration occurred in corals exposed to 0.002 and 0.002 M. The reason for this response was the reduction in zooxanthellae or pigment concentration of zooxanthellae. In addition, no mortality was observed amongst *P. damicornis,* and corals exposed to 0.002 and 0.0002 M and *P. lichen* corals exposed to 0.002. All *P. lichen* colonies and *P. damicornis* explants died when exposed to 0.2 M of cyanide. The results from respiration tests found lower rates of oxygen consumption in corals exposed to cyanide. There was more than 90% inhibition of respiration in 0.2 M of cyanide. Lower doses (0.002 M) of cyanide caused 10-20% inhibition of the respiration rate. Despite these results, the respiration rates returned to pre-exposure levels within 0.5 – 2.0 hours.

6.2 Natural products

Carreiro-Silva *et al.* (2022) examined the effects of elevated suspended polymetallic sulphide (PMS) particles and suspended quartz particles on cold water octocoral *Dentomuricea meteor.* These particulates may be generated during deep-sea mining activities for PMS. *Dentomuricea meteor* was exposed to suspended particulate concentrations that ranged from 2-3 mg/l of PMS and 15-18 mg/l of quartz for 4 hours and monitored for 27 days. Colonies were collected from Condor Seamount and studied in laboratory conditions. Physiological changes (respiration and ammonium excretion rates) and coral mortality were recorded. High mortality was observed in corals exposed to PMS, that is, 13% mortality after 13 days, 80% after 20 days and 95% after 25 days. Small PMS particles covered and clogged the polyps of coral fragments. Corals exposed to the quartz particles experienced paling of tissues but darkening and sloughing of tissues in PMS exposure. Polyps were more retracted in corals exposed to PMS compared to quartz. The respiration rates of corals increased after 13 days of treatment exposure in exposure to PMS, but there was no significant difference in respiration of corals exposed to quartz. Respiration rate was 1.6 – 2 times higher in PMS than quartz. The ammonium excretion decreased in quartz treatment and did not significantly differ between exposure concentrations. However, ammonium released in PMS significantly varied between treatments and by day 13, excretion rates in PMS treatments increased and were five times greater than quartz. Analysis of trace metal content in the seawater and metal bioaccumulation by corals was also measured. The trace metal concentrations in the water column were 6.6 µg/l of copper in quartz and 22 µg/l in PMS treatment, 0.13 µg/l of magnesium in quartz and 0.26 µg/l of magnesium in PMS treatment and 0.045 µg/l of cobalt in quartz and 0.26 in PMS treatment.

6.3 Mixtures

Two articles examined the effects of effluents, which were unidentified and therefore categorised as ‘Mixtures’. Negri & Heyward (2000) (described above) examined the effects of Production Formation Water (PFW) on the coral *Acropora millepora*. Howe *et al.* (2015) summarised is below.

Howe *et al.* (2015) examined the effect of two unidentified effluents (from confidential sources), on survival and pedal lacerate development of the sea anemone *Exaiptasia pallida* (formally *Aiptasia pulchella* and synonymous with *Aiptasia pallida*). *E. pallida* juveniles were exposed to 6.3, 12.5, 25, 50, and 100% v/v of whole effluent 1 (WE1) and whole effluent 2 (WE2) in static - renewal toxicity tests for 96 hours (acute) and 12 days (chronic) and

renewed every 48 hours. Two different experimental designs were used in this study. In exposure to WE1, mortality was measured at 4, 42, 48, 72, 96 hours and in exposure to WE2, pre juvenile pedal lacerates were used to assess the lacerate development to a juvenile stage and measure general condition and mortality after 2, 24 and every 48 hours after, for 12 days. No mortality was observed after WE1 exposure at any concentrations until 72 hours where mortality was recorded in 100% v/v concentration treatments. *E. pallida* mortality was observed in both WE1 and WE2 at low concentrations within 96 hours, the 96-hour LC50 value was 40(30-54)% v/v of WE1 and the 12-day LC50 value was 30 (28-33)% v/v of WE2. The 8-day pedal lacerate development EC50 value was 7% v/v (3-11) of WE2 and showed high toxicity of the effluent to *E. pallida* development. The authors concluded toxicity was seen in mortality and lacerate development of *E. pallida* to the two unidentified whole effluents.

(Page left blank)

7 Sensitivity assessment

The effects of contaminants of Anthozoa were poorly studied, even in true corals (Scleractinia), when compared to other species groups, e.g., Polychaetes, Molluscs, and sea grasses. Nevertheless, the evidence was dominated by information from studies of true corals. In addition, the life history traits and population dynamics of the Anthozoa are poorly known. Also, they vary within and between different taxonomic groups, e.g., Actinaria, Octocorallia, Scleractinia, and Zoantharia. Hence, each taxonomic group is discussed separately.

No direct evidence on the effects of contaminants on the British sea pens (*Virgularia, Pennatula,* and *Funiculina*) was found. Therefore, their sensitivity assessments are based on examples of species in their taxonomic group (Octocorallia) or Anthozoa as a group.

7.1 Resilience and recoverability

Information on resilience (recovery rates) was taken from readily available sources such as MarLIN for temperate species and the IUCN Red list for tropical corals. The different life forms and habitats of the Anthozoa affect their recovery rates. No information was found to assess the recovery of Zoantharia.

7.1.1 Sea anemones (Actinaria)

The evidence on the life history, larval dispersal and, hence, resilience of many sea anemones is limited and varies between species. Therefore, the resilience assessment is based on the few examples available.

Metridium senile reproduces each year, and the planulae spend months in the plankton and are likely to disperse over in excess of 10 km from parent anemones Sebens (1985). New jetty piles at Lundy were colonized by their third year. Settled planulae or individuals produced by basal laceration are likely to grow rapidly. Bucklin (1987a) found that, for *Metridium senile* from California, individuals showed rapid growth to large sizes when fed at frequent intervals. Mean size grew steadily during the first eight months then levelled off. An increase from 5 cm^2 pedal disk area to 45 cm^2 occurred within 12 months. However, in the clearance experiments undertaken by Sebens (1985), he found that it took 5-10 years for *Metridium senile* to return to pre-clearance cover rates. In another study, Wahl (1985) found that *Metridium senile* returned to rock walls only one week after oxic conditions returned

following annual de-oxygenation events in the Inner Flensburg Fjord. Overall, resilience is assessed as **'Medium'** even where no nearby populations exist.

Little information was found to assess the resilience of *Urticina felina*. No evidence was found for longevity. Some individuals may be hundreds of years old, given their ability to regenerate (Francis, 1976). *Urticina felina* readily repairs damage to the body. Removal of tentacles by clipping does not alter the behaviour or *Urticina felina* and the tentacle regenerates within a few days (Mercier *et al.*, 2011). *Urticina felina* broods young internally, which when released settle close to the adult. Brooding prevents predation of juveniles, in areas of high wave action and water flow, counteracts removal and predation and supports the formation of aggregations of anemones in harsh environments (Kaliszewicz *et al.*, 2012). However, brooding does limit dispersal (Kaliszewicz *et al.*, 2012) and may inhibit recovery where a population is entirely removed. The large size, slow growth rate, and evidence from aquarium populations suggest that *Urticina felina* is long lived. Aquaria reports suggest that, protected from predation, animals can live for tens of years (P.G. Moore, pers. comm.). Dispersal ability is considered poor in the similar species *Urticina eques* (Solé-Cava *et al.* 1994). Adults can detach from the substratum and relocate but mobility is limited. Impacts that remove large proportions of the population over a wide area will effectively reduce the availability of colonists. However, *Urticina felina* colonized the ex-*HMS Scylla,* which was sunk purposely, to create an artificial reef in Whitsand Bay, in the second year of the vessel being on the seabed and had increased in numbers four years after (Hiscock *et al.*, 2010). Therefore, where resistance is 'Medium' then resilience is assessed as **'High'** based on reproduction and recolonization from the remaining population. But where resistance is 'Low' or 'None', recovery to pre-impact abundance may be delayed and resilience is assessed as **'Medium'** based on the observation of Hiscock *et al.* (2010).

Nematostella vectensis is a very small sea anemone that lives in the surface of muddy sediments in very sheltered conditions. It can reproduce both sexually and asexually. It grows rapidly in the laboratory and has a short generation time (2-3 months), although sexual reproduction in the wild is probably less frequent, probably annual, and does not occur in all populations (Hand & Uhlinger, 1992, 1994, 1995; Darling *et al.*, 2005). Asexual reproduction allows this species to generate clones that can rapidly colonize a suitable habitat. The rapid seasonal increases in abundance reported in UK lagoons are probably due to rapid asexual proliferation in response to food availability (Sheader *et al.*, 1997). But while it can occur in huge numbers its normal abundance is probably low (Hand & Uhlinger, 1994; Sheader *et al.*, 1997; Reitzel *et al.*, 2008). Sexual reproduction, coupled with asexual proliferation provides

this species with the ability to develop genetic clonal lines that are highly adapted to the local conditions (Darling *et al.*, 2004, 2009; Reitzel *et al.*, 2008). Local adaptation with varied levels of asexual reproduction and founder effects (bottlenecks due to die back in populations) partly explain the high levels of genetic differentiation seen between populations. However, numerous studies (Sheader *et al.,* 1997; Person *et al.*, 2002; Darling *et al.*, 2004, 2009; Reitzel *et al.*, 2008) concluded that the extraordinarily high levels of genetic differentiation was explained by low gene flow due to poor dispersal and/or bottlenecks and sporadic, discontinuous dispersal by random events and anthropogenic transport. Hence, if members of the population survive, resilience is probably **'High'**. However, if a population is removed or lost (i.e., resistance is 'None') then recolonization would depend on either human transportation (or intervention) or random, unpredictable, events and take anywhere from a few years to never. In this instance, resilience is assessed as **'Very low'**(negligible or prolonged recovery).

MES (2010) suggested that the genus *Cerianthus* would be likely to have a low recovery rate following physical disturbance based on long-lifespan (11-20 years) and slow growth rate. No specific evidence was cited to support this conclusion. The MES (2010) review also highlighted that there were gaps in information for this species and that age at sexual maturity and fecundity is unknown although the larvae are pelagic (MES, 2010).

No information of life- history and/or larval dispersal was available for *Actinia* spp., *Anemonia* spp., *Aiptasia* sp. *Cereus* spp., *Edwardsia* spp., *Exaiptasia* spp. and *Sagartia* spp. Overall, most anemones are hermaphrodite and produce short or long-lived planulae larvae, depending on species, and potentially poor dispersal. However, the ubiquity of many species, e.g., *Actinia* or *Anemonia* suggests that recruitment may be reasonable. In addition, asexual reproduction can allow the species to dominate areas rapidly if the food supply is good. Therefore, where resistance is ‘Medium’ then resilience is assessed as **‘High’** based on reproduction and recolonization from the remaining population. But where resistance is ‘Low’ or ‘None’, recovery to pre-impact abundance may be delayed and resilience is assessed as **‘Medium’** based on the assumption that *Metridium senile* is a good proxy for the other species above. However, the confidence in this assessment is ‘Low’.

7.1.2 Soft corals (Octocorallia)

Sea fans are sessile colonial cnidarians that grow erect from the substratum, with each colony formed of many small polyps, each with tentacles that may be either extended or retracted. Populations of *Swiftia pallida* are thought to be self-sustaining, with short-lived

larvae and limited potential for larval dispersal. It is thought that the colonization of the Shetland Islands has been prevented by geographical barriers (Hiscock *et al.*, 2001). Reproduction is likely to be annual and may be triggered by either summer high or winter low temperatures (Hiscock *et al.*, 2001).

The average number of eggs per polyp in other gorgonians increases with increasing colony size, although *Swiftia pallida* has not been specifically studied. The number of eggs released from larger colonies can be orders of magnitude higher than for smaller colonies (Beiring & Lasker, 2000). It has been suggested that when a large colony size is attained, more energy is available for reproduction because relative colony growth decreases (Beiring & Lasker, 2000). Growth rates for this species are unknown. However, the pink sea fan *Eunicella verrucosa* has highly variable growth. A population of *Eunicella verrucosa* at Lundy Island had growth rates of approximately 1 cm/year, which may be similar to *Swiftia pallida*. The lifespan of *Swiftia pallida* is estimated to be between 10 and 20 years (Hiscock *et al.* 2001). Little information was found on the recovery potential of this species. The ability to recolonize an area following mass mortality is likely to be restricted (Hiscock *et al.*, 2001).

Settlement of *Eunicella verrucosa* occurred in the fourth year after ex-*HMS Scylla* was placed on the seabed near Plymouth. Growth was initially rapid, and fans had reached 6 cm in height by the end of the first winter, and some were 17 cm high by the beginning of the next winter, often with several branches (Hiscock *et al.*, 2010). By 2017, many were about 20 cm high and extensively branched (Keith Hiscock, pers. comm.). There is no specific information on reproduction in *Eunicella verrucosa* but the larvae of *Eunicella singularis* are most likely lecithotrophic and have a short life (several hours to several days) (Weinberg & Weinberg, 1979). Recruitment in gorgonians is often reported to be sporadic and/or low (Yoshioka 1996; Lasker *et al.* 1998; Coma *et al.* 2006). *Eunicella verrucosa* has been known to colonize wrecks at least several hundred metres from other hard substrata but is thought to have larvae that settle near the parent (Hiscock, 2007). Growth rate can be highly variable. An increase in branch length of up to 6 cm was reported for some branches in one year but virtually none in others in Lyme Bay populations over a year (C. Munro, pers. comm. cited in Hiscock, 2007). In the morphologically similar *Paramuricea clavate* in the Mediterranean, Coma *et al.* (1995) described reproduction and the cycle of gonad development. Spawning occurred 3-6 days after the full or new moon in summer. Spawned eggs adhered to a mucus coating on female colonies; a feature that would be expected to have been readily observed if it occurred in *Eunicella verrucosa*. Maturation of planulae took place among the polyps of the parent colony and, on leaving the colony, planulae immediately settled on surrounding

substrata. It seems more likely that planulae of *Eunicella verrucosa* are released immediately from the polyps and are likely to drift (Hiscock, 2007). Coma *et al.* (2006) reported ongoing recovery in *Eunicella singularis* populations in the Mediterranean four years following a mass-mortality event. Although not recovered, Sheehan *et al.* (2013) noted that within three years of closing an area in Lyme Bay, the UK to fishing, some recovery of *Eunicella verrucosa* had occurred, with a marked increase compared to areas that were still fished.

Alcyonium digitatum colonies are likely to have a lifespan that exceeds 20 years as colonies have been followed for 28 years in marked plots (Hartnoll, 1998). Colonies that were 10-15 cm in height were aged at between 5 and 10 years old (Hartnoll, unpublished). Sexual maturity is predicted to occur, at its earliest, when the colony reaches its second year of growth. However, the majority of colonies are not predicted to reach maturity until their third year (Hartnoll, 1975). *Alcyonium digitatum* spawns from December and January. Gametes are released into the water where fertilization occurs. The embryos are neutrally buoyant and float freely for 7 days when they give rise to actively swimming lecithotrophic planulae which may have an extended pelagic life before they eventually settle (usually within 1 or 2 further days) and metamorphose to polyps (Matthews, 1917; Hartnoll, 1975; Budd, 2008). Larvae have been reported to survive for up to 35 weeks as non-feeding planulae and may favour the dispersal and eventual discovery of a site suitable for settlement (Hartnoll, 1975). *Alcyonium digitatum* can recruit onto bare surfaces within 2 years but may take up to 5 years to recover fully following significant mortality (Whomersley & Picken, 2003; Hiscock *et al.,* 2010).

No information on the other octocorals found the review was available. Overall, where the species population of the gorgonian corals (exemplified by *Swiftia* and *Eunicella* above) is severely impacted (i.e., resistance is ‘None’) then resilience is assessed as ‘**Low**’ (recovery within 10-25 years) (Readman & Durkin, 2016). However, where resistance is 'Low' or 'Medium', resilience is assessed as ‘**Medium**’ (recovery within 2-10 years). In the case of Alcyonacean soft corals (exemplified by *Alcyonium* spp.) resilience would be assessed as ‘**Medium’** (recovery in 2-10 years) if the population declined significantly (resistance of ‘None’ or ‘Low’) resilience. However, where resistance was assessed as ‘Medium’ or ‘High’ then resilience would be assessed as **‘High**’ (Readman & Williams, 2021).

7.1.3 Sea pens

Studies of oogenesis in *Funiculina quadrangularis* and *Pennatula phosphorea* in Loch Linnhe, Scotland, demonstrated that they were dioecious, with 1:1 sex ratio, highly fecund,

with continuous prolonged oocyte development and annual spawning (Edwards & Moore 2008; Edwards & Moore 2009). In *Pennatula phosphorea*, oogenesis exceeded 12 months in duration, with many small oocytes of typically 50 per polyp giving an overall fecundity of ca 40,000 in medium to large specimens, depending on size. However, <30% matured (synchronously) and were spawned in summer (July-August). Mature oocytes were large (>500 µm) which suggested a lecithotrophic larval development (Edwards & Moore, 2008). In *Funiculina. Quadrangularis* fecundity was again high, expressed as 500-2000 per 1 cm midsection, but not correlated with size, and again, only a small proportion of the oocytes (<10%) matured. Unlike *Pennatula phosphorea*, annual spawning occurred in autumn or winter (between October and January). Also, the mature oocytes were large (>800µm), which suggested lecithotrophic larval development (Edwards & Moore, 2009).

The lecithotrophic larval stage of *Funiculina quadrangularis* may result in a relatively long pelagic stage and high potential dispersal ability and may explain the high gene flow observed between colonies of *Funiculina quadrangularis* in two Scottish sea lochs (Wright *et al.*, 2015). Wright *et al.* (2015) found limited genetic population subdivision within and between populations of *Funiculina quadrangularis* in Loch Linnhe and Loch Duich. However, the high genetic diversity and unique genotypes supported the absence of asexual reproduction in this species (Wright *et al.*, 2015). No similar studies were available for *Virgularia mirabilis*, but Edwards & Moore (2009) noted that many sea pens exhibited similar characteristics. In a study of the intertidal *Virgularia juncea* fecundity varied with length (46,000 at 50 cm and 87,000 at 70 cm), eggs reached a maximum size of 200-300 µm in May and were presumed to be spawned between August and September (Soong, 2005). Birkland (1974) found the lifespan of *Ptilosarcus gurneyi* to be 15 years, reaching sexual maturity between the ages of 5 and 6 years; while Wilson *et al.* (2002) noted that larger specimens of a tall sea pen (*Halipteris willemoesi*) in the Bering Sea were 44 years old, with a growth rate of 3.6 - 6.1cm/year.

Hughes (1998a) suggested that patchy recruitment, slow growth, and long lifespan were typical of sea pens. Larval settlement is likely to be patchy in space and highly episodic in time, with no recruitment to the population for some years. Greathead *et al.* (2007) noted that patchy distribution is typical for sea pen populations. Hence, where a proportion of the sea pen population is removed or killed (resistance is ‘Medium’, ‘Low’ or ‘None’) then, although the species have a high dispersal potential and long-lived benthic larvae, larval recruitment is probably sporadic and patchy and growth is slow, which suggests that recovery will take many years. Therefore, resilience is assessed as **'Low'** (>10 years).

7.1.4 True corals (Scleractinia)

The recovery rates of tropical corals are outside the scope of this review. The age at first maturity of most reef-building corals is typically three to eight years (Wallace, 1999). The IUCN (e.g., Kitahara *et al.*, 2022) state “we infer that the average age of mature individuals of this species is greater than eight years. Based on average sizes and growth rates, we also infer that the average length of one generation is 10 years. Longevity is not known but is likely to be greater than 10 years” for many true corals. Many species of tropical reef corals have declined or are in decline. Therefore, we suggest that resilience is assessed as **‘Low’** if resistance is ‘Medium’ to represent their ability to repair and regrow, albeit slowly. But resilience is assessed as **‘Very low’** where resistance is ‘Low’ or ‘None’. However, the confidence in this assessment is ‘low’ due to the lack of evidence presented.

7.2 Sensitivity assessment – Hydrocarbons and PAHs

The count of ranked mortalities due to ‘Hydrocarbons and PAHs’ are summarized in Figure 3.1 and Figure 3.2. Table 7.1 below summarises the possible sensitivities of adults and larvae to Hydrocarbons and PAHs. Sublethal effects were the most reported effect (50% of results) on larvae and the results for larvae reported 43.75% ‘Significant’ mortalities and 6.25% ‘Severe’ mortalities. ‘Severe’ and ‘Some’ mortalities were reported in adults (both 50% of results). In the case of the studies that did not report the life stage, 47.06% of results reported ‘Significant’ mortalities and 23.53% of results were sublethal, 17.65% of results reported ‘no’ mortalities and 11.76% of results reported ‘Severe’ mortalities, depending on the contaminant type.

7.2.1 Oil spills

The effects of an oil spill on Anthozoa were only reported in two papers. It was unclear if the effects of the *Torrey Canyon* spill on Anthozoa (Smith 1968) were due to the oil and dispersant or oil alone. Etnoyer *et al.* (2016) examined the health and condition of species of large gorgonian octocorals before and after a *Deepwater Horizon* (DWH) oil spill. Although the octocoral reefs occurred at depth below the resultant oil slick, they suggested that the octocorals could come into contact with contaminants in the form of particulates or contaminated phytoplankton in the water column. Etnoyer *et al.* (2016) reported a significant increase in injury to a number of species of octocoral after the spill (38-50% of large gorgonians) but no direct mortality at the end of their study in 2014, four years after the spill. They suggested that the chance of recovery from injury was unlikely, which implies that

mortality may have occurred in the longer-term. **Therefore, although their paper reported only sublethal effects (resistance is 'High') it may be prudent to assess the resistance of Octocorals to oil spills as at least 'Medium', albeit with 'Low' confidence. Hence, resilience is assessed as 'Medium and sensitivity as 'Medium'.**

Table 7.1 Summary of count of worst-case ranked mortalities to 'Hydrocarbons and PAH' contaminants reported in the evidence review and resultant proposed sensitivity assessments for Anthozoan species in Taxonomic groups (Actinaria =Ac, Octocorallia = Oc, Scleractinia = Sc and Zoantharia = Zo) (N= None, VL= Very low, L= Low, M= Medium, High= High, and NS= Not sensitive)

Group /Type	Taxon. group	Species name	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Petrochemical											
Oil spill[7]											
	Oc	*Hypnogorgia pendula*					**1**	1	H	H	NS
	Oc	*Placogorgia* spp.					**1**	1	H	H	NS
	Oc	*Swiftia exserta*				**1**	1	2	H	H	NS
	Oc	*Thesea spp.*					**1**	1	H	H	NS
Complex hydrocarbons											
	Oc	*Swiftia exserta*				**1**			H	H	NS
	Sc	*Acropora millepora*					**1**	1	H	H	NS
	Sc	*Montastraea faveolata*		**1**				1	L	VL	H
	Sc	*Porites astreoides*		**1**				1	L	VL	H
Total				**2**		**1**	**5**	**8**	**L**	**VL**	**H**
Dispersant + oil											
	Ac	*Actinia equina*				**1**		1	H	H	NS
	Ac	*Anemonia sulcata*		**1**				1	L	M	M
	Ac	*Cereus pedunculatus*		**1**				1	L	M	M
	Ac	*Sagartia elegans*		**1**				1	L	M	M
	Ac	*Urticina* (syn. *Tealia) felina)*				**1**		1	H	H	NS
	Oc	*Swiftia exserta*	**1**					1	N	L	H
	Sc	*Acropora millepora*					2	2	H	H	NS

[7] See text.

Group /Type	Taxon. group	Species name	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
	Sc	*Montastraea faveolata*		1				1	L	L	H
	Sc	*Porites astreoides*		1				1	L	L	H
Total			1	5		2	2	10	L	L	H
Aromatics											
	Sc	*Acropora microphthalma*			1		1	2	M	L	M
	Sc	*Pocillopora damicornis*	1				1	2	N	VL	H
Total			1		1		2	4	N	VL	H
Petrochemical Total			2	7	1	3	9	22	N	VL	H
Dispersants											
	Ac	*Actinia equina*		4				4	L	M	M
	Ac	*Anemonia sulcata*		1				1	L	M	M
	Oc	*Swiftia exserta*	1					1	N	VL	H
	Sc	*Acropora millepora*					2	2	H	H	NS
	Sc	*Montastraea faveolata*		1				1	L	L	H
	Sc	*Porites astreoides*		1				1	L	L	H
Dispersants Total			1	7			2	10	N	VL	H
PAHs											
PAHs	Sc	*Porites astreoides*		1				1	L	L	H
Biogenic											
Fats	Sc	*Acropora microphthalma*	1				1	2	N	VL	H
Overall total			4	15	1	3	12	35	N	VL	H

7.2.1.1 Sea pens

No direct evidence of the effects of oil spills on sea pens was found. However, the evidence of the effects of the DWH spill on Octocorals suggests that sea pens, even at depth could be affected by an oil spill. The magnitude of the effect is likely to depend on the scale and duration of the spill, the depth of the sea pen biotope relative to the resultant slick, and the degree of vertical mixing in the water column. **Nevertheless, it may be prudent to assess the resistance of sea pens to oil spills as at least 'Medium', albeit with 'Low' confidence'. Hence, resilience is assessed as 'Low' and sensitivity as 'Medium'.** The

shallowest examples of the sea pen (SpnMeg) biotopes are likely to be more vulnerable to oil spills than deeper examples.

7.2.2 Petroleum hydrocarbons – oils and dispersed oils

Frometa *et al.* (2017) reported 'no' mortality in *Swiftia exserta* exposed to DWH WAF but 'Severe' mortality after exposure to dispersant and oil mixtures (CEWAF). They concluded that combinations of dispersants and oils were more toxic to octocorals than oils alone. Smith (1968) reported that *Actinia equina* and *Urticina (*as *Tealia) felina* were the most common and the most resistant animals on the shore after the oil spill and clean up, while some specimens of *Cereus pedunculatus*, *Sagartia elegans* and *Anemonia sulcata* were found dead and few survived. However, in the true coral examples (Table 7.1) mortality ranged from 'Severe' to 'None' depending on the study.

Overall, in most of the studies reviewed, the **resistance of true corals (Scleractinia) and Octocorals to petroleum hydrocarbons, oils, and dispersed oils would be assessed as 'Low' or 'None'** and, due to their **'Low'** resilience, **sensitivity would be assessed as 'High'** but with 'Low' confidence due to the limited number of studies examined. However, the resistance of Actinaria **to petroleum hydrocarbons, oils, and dispersed oils is dependent on species** (Table 7.1).

7.2.2.1 Sea pens

No direct evidence of the effects of petroleum hydrocarbons, oils, and dispersed oils on sea pens was found. However, the evidence of the effects on Octocorals suggests that sea pens may be sensitive. Therefore, **resistance is assessed as 'Low,' resilience as 'Low' and sensitivity as 'High'** but with 'Low' confidence due to the lack of direct evidence.

7.2.3 Dispersants

All but one of the species examined in the articles reviewed (*Acropora millepora*) were reported to experience either 'Severe' or 'Significant' mortality due to exposure to dispersants. Therefore, the **resistance of Anthozoa, as a group, to dispersants is assessed as 'None' or 'Low' depending on the species** (Table 7.1). Hence, **resilience is assessed as 'Low' or 'Very low' in the Octocorals and true corals but 'Medium' in the Actinaria**. Therefore, **sensitivity to dispersants is assessed as 'High' in Octocorals and true corals but 'Medium' in the Actinaria**, but with 'Low' confidence due to the limited number of studies examined.

7.2.3.1 Sea pens

No direct evidence of the effects of dispersants on sea pens was found. However, the evidence of the effects on Octocorals suggests that sea pens may be sensitive. Therefore, **the resistance of sea pens to dispersants is assessed as 'Low', resilience as 'Low' and sensitivity as 'High'** but with 'Low' confidence due to the lack of direct evidence.

7.2.4 Vegetable oils and PAHs

One study (Mercurio *et al.*, 2004) examined the effects of vegetable oils on *Acropora microphthalma* and one other (Farina *et al.*, 2008) examined the effect of the PAH B[a]P on *Porites astreoides.* 'Severe' or 'Significant' mortality was reported, respectively. Therefore, the resistance of true corals to vegetable oil or PAH exposure **may be 'Low' or 'None' and sensitivity may be 'High'** (Table 7.1). **This assessment may represent the sensitivity of other Anthozoa, including sea pens, but with only 'Low' confidence due to the limited number of studies found.**

7.3 Sensitivity assessment – Transitional metals and organometals

The count of ranked 'worst-case' mortalities due to 'Transitional metals and organometals' are summarized in Figure 4.1 and Figure 4.2 above. Table 7.2 below summarises the sensitivity of all life stages to metals and organometals. Mortalities that were not specified were omitted.

Sublethal effects were the most reported effect (63.64% of results) of 'Transitional metals and organometal' exposure in studies on larvae. Whereas 6.82% of results reported 'Severe' mortalities, 18.18% of results reported 'Significant' mortality, 2.27% reported 'some' mortalities and 9.09% reported 'no' mortality. However, in studies on adults, 'Significant' mortalities were the most reported (60% of results), followed by 'Severe' mortalities (40% of results). Sublethal effects were reported in all the results from studies on embryos and juveniles. Those studies that examined 'multiple' life stages reported 'Significant' mortality. In the case of the studies that did not report the life stage, 37.04% of results were 'Severe', 22.22% of results were 'Significant', 11.11% of results were 'some' and 22.22% of results were sublethal.

Table 7.2. Summary of count of worst-case ranked mortalities to 'Transitional metals and organometals' contaminants reported in the evidence review and resultant proposed sensitivity assessments for Anthozoan species in Taxonomic groups (Actinaria =Ac, Octocorallia = Oc, Scleractinia = Sc and Zoantharia = Zo) (N= None, VL= Very low, L= Low, M= Medium, High = High, and NS= Not sensitive)

Group/Type	Taxon. group	Species name	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Metals											
Metals & their compounds											
	Ac	*Aiptasia pallida*		**1**				1	L	M	M
	Ac	*Aiptasia pulchella*	**1**	6	1		10	**18**	N	M	M
	Ac	*Aiptasia sp.*		**1**			1	2	L	M	M
	Ac	*Edwardsia elegans*					**1**	1	H	H	NS
	Ac	*Nematostella vectensis*	**2**					2	N	VL	H
	Oc	*Lobophytum compactum*					**1**	1	H	H	NS
	Sc	*Acropora formosa*	**1**					1	N	VL	H
	Sc	*Acropora longicyathus*					**2**	2	H	H	NS
	Sc	*Acropora millepora*					**1**	1	H	H	NS
	Sc	*Acropora surculosa*	**1**					1	N	VL	H
	Sc	*Acropora tenuis*					**5**	5	H	H	NS
	Sc	*Acropora tumida*		**1**				1	L	VL	H
	Sc	*Favites chinensis*					**2**	2	H	H	NS
	Sc	*Galaxea fascicularis*	**1**					1	N	VL	H
	Sc	*Goniastrea aspera*	**1**	**1**		3	7	12	N	VL	H
	Sc	*Goniastrea retiformis*					**1**	1	H	H	NS
	Sc	*Montastraea faveolata*					**1**	1	H	H	NS
	Sc	*Montipora capitata*					**1**	1	H	H	NS
	Sc	*Montipora verrucosa*			**1**			1	M	L	M
	Sc	*Oxypora lacera*				**1**		1	H	H	NS
	Sc	*Platygyra acuta*		**1**				1	L	VL	H
	Sc	*Platygyra daedalea*		**2**				2	L	VL	H
	Sc	*Platygyra ryukyuensis*					**2**	2	H	H	NS
	Sc	*Pocillopora damicornis*	**1**		1			2	N	VL	H
	Sc	*Porites astreoides*			1			1	M	L	M
	Sc	*Porites cylindrica*					**1**	1	H	H	NS
	Sc	*Porites lutea*					**1**	1	H	H	NS
	Zo	Zoanthidae					**1**	1	H	H	NS
Total			**8**	**13**	**4**	**4**	**38**	**67**			

Group/Type	Taxon. group	Species name	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Nanoparticulates											
	Ac	*Aiptasia pallida*					1	1	H	H	NS
	Ac	*Aiptasia pulchella*					1	1	H	H	NS
Total							**2**	**2**	H	H	NS
Metals Total			**8**	**13**	**4**	**4**	**40**	**69**	**N**	**VL**	**H**
Organometals											
Organocopper											
	Ac	*Aiptasia* sp.		**1**				1	L	M	M
	Sc	*Acropora tumida*		**1**				1	L	VL	H
Total				**2**				**2**	**L**	**??**	**H**
Organotin	**Ac**	*Aiptasia pulchella*	**3**					3	N	M	M
	Ac	*Aiptasia* sp.		**1**				1	L	M	M
	Sc	*Acropora millepora*					**1**	1	H	H	NS
	Sc	*Acropora tenuis*					**1**	1	H	H	NS
	Sc	*Acropora tumida*		**1**				1	L	VL	H
	Sc	*Montipora verrucosa*	**1**	1				2	N	VL	H
	Sc	*Pocillopora damicornis*	**3**					3	N	VL	H
Total			**7**	**3**			**2**	**12**	**N**	**VL**	**H**
Organozinc	**Ac**	*Aiptasia* sp.		**1**				1	L	M	M
	Sc	*Acropora tumida*		**1**				1	L	VL	H
Total				**2**				**2**	**L**	VL	H
Total			**7**	**7**			**2**	**16**	**N**	**VL**	**H**
Overall total			**15**	**20**	**4**	**4**	**42**	**85**			

?? = uncertain

7.3.1 Transitional metals

The reported effects of transitional metals on Anthozoa varied between taxonomic group, species, the metal studied and its exposure concentration or duration (Table 7.2). Copper, nickel, cadmium, lead, and zinc were probably the most toxic with the largest number of ‘Severe’ or ‘Significant’ mortalities reported in the results (Figure 4.1).

Overall, the Actinaria were reported to experience ‘Severe’ or ‘Significant’ mortality due to transitional metal exposure but to be of ’Medium’ sensitivity due to their reasonable recovery rates. *Nematostella vectensis* is an exception due to its inability to disperse over long distances or between sites without human intervention (see above). **Therefore, while resistance is assessed as ‘Low’, resilience is probably ‘Medium’ and sensitivity is assessed as ‘Medium’,** albeit with ‘Low’ confidence.

The Scleractinia (true corals) also show a varied response to transitional metal exposure. However, **resistance is probably 'Low' or 'Very low'** based on the 'worst-case' mortality reported, the metals, and their dose. Hence, **resilience is assessed as 'Very low' and sensitivity as 'High'** albeit with 'Low' confidence due to the variation in response.

The Octocorallia and Zoantharia are each represented by single study, in which only sublethal effects were observed so, **no sensitivity assessment** for these groups is suggested.

7.3.1.1 Sea pens

No direct evidence of the effects of transitional metals on sea pens was found. Reichelt-Brushett & Michalek-Wagner (2005) reported fertilization success in the Octocoral *Lobophytum compactum* was more resistant to copper exposure than other species studies but also reported a significant decrease in fertilization success. A decrease in fertilization success due to copper might impair recruitment in sea pens. Overall, the effects of transitional metal exposure were varied but metals had the potential to cause 'severe' or 'Significant' mortalities in Anthozoa as a group. Therefore, **the worst-case resistance of Anthozoa and, hence, sea pens to transitional metal exposure is assessed as 'Low', resilience as 'Low' and sensitivity as 'High'** but with 'Low' confidence due to the lack of direct evidence and the variation in response.

7.3.2 Nanoparticulate metals

The effects of nanoparticulate copper oxide exposure on *Aiptasia* spp. were examined by Henderson & Salazar (1996) and Siddiqui *et al.* (2015). Only sublethal effects were reported. Therefore, the **sensitivity of Actinaria is assessed as 'Not sensitive' but with 'Low' confidence due to limited number of studies found**. The evidence is probably too limited to assess the sensitivity of sea pens species or other groups of Anthozoa.

7.3.3 Organometals

The Anthozoa (Actinaria and Scleractinia) were reported to experience 'Severe' or 'Significant' mortality from organometals (organozinc, organotin, and organocopper) exposure in 87% of the results reviewed. **Therefore, the resistance of Anthozoa as a group to organometal exposure is assessed as 'None.' The resilience of Actinaria is probably 'Medium', so sensitivity is assessed as 'Medium. However, the resilience of Scleractinia is probably 'very low' so sensitivity is assessed as 'High.'**

7.3.3.1 Sea pens

No direct evidence on the effects of organometals on sea pens was found. **Resistance is assessed as 'Low', based on the assumption that Anthozoa share similar hormonal and biochemical pathways. Hence, resilience is assessed as 'Low' and sensitivity as 'High'** but with 'Low' confidence due to the lack of direct evidence.

7.4 Sensitivity assessment – Synthetic compounds

The count of ranked 'worst-case' mortalities due to 'Synthetic compound' are summarized in Figure 5.1 and Figure 5.2 above. Table 7.3 below summarises the sensitivity of all life stages to 'Synthetic compounds.' Significant effects were the most reported effect (36% of results) in studies of larvae, but 12% 'Severe' mortality, 4% 'Some' mortality, 16% 'no' mortality and 32% 'sublethal' effects were reported in the remaining instances. Sublethal effects were reported in all the results from studies on embryos and juveniles. The studies of adults reported 50% 'no' mortality and 50% sublethal effects. Studies that examined 'Multiple' life stages reported significant mortality. In the case of the studies that did not report the life stage, 8.33% of results were 'Severe', 16.67% of results were 'Significant', and 75% of results were 'sublethal'.

7.4.1 Pesticides/biocides

'Pesticides/biocides' exposure was reported to result in mortality ('Severe', 'Significant' or 'Some' mortality) in only 23% of the worst-case results collated. The majority (77%) of studies reported 'no' mortality or sublethal effects (Table 7.3). In the case of the Actinaria, *Aiptasia* spp. was reported to experience significant mortality after exposure to 19 mg/l Diuron (Bao *et al.*, 2011) but only sublethal effects to paraformaldehyde or the bactericide Suflo-B33 (Tagetz *et al.,* 1979), while *Anthopleura* spp. was reported to experience only sublethal effects after exposure to Chlordane (Pridmore *et al.,* 1992). Similarly, the Scleractinia were reported to experience only sublethal effects from pesticide/biocide exposure, except in a few species/pesticide combinations.

Overall, the evidence suggests that pesticides/biocides may be toxic to some species of Anthozoa depending on the dose and life stage and that sensitivity should be assessed on a species-specific and chemical-specific basis. **Therefore, the 'worst-case' resistance of Actinaria as a group to pesticide/biocide exposure is potentially 'Low'** so that **resilience is probably 'Medium', and sensitivity is assessed as 'Medium. However, the 'worst-case' resistance of Scleractinia as a group to pesticide/biocide exposure is**

potentially 'None', **and as** t**he resilience of Scleractinia is probably 'Very low', sensitivity is assessed as 'High'**. But the confidence in the **assessment is 'Very low'**.

Table 7.3. Summary of count of worst-case ranked mortalities to 'Synthetic compounds' reported in the evidence review and resultant proposed sensitivity assessments for Anthozoan species in Taxonomic groups (Actinaria =Ac, Octocorallia = Oc, Scleractinia = Sc and Zoantharia = Zo) (N= None, VL= Very low, L= Low, M= Medium, High = High, and NS= Not sensitive)

Group/type	Taxon. group	Species name	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Pesticide/ Biocide											
Antifoulant	**Sc**	*Acropora tumida*			1			1	M	L	M
Bactericide	**Ac**	*Aiptasia pallida*					2	2	H	H	NS
Carbomate	**Sc**	*Acropora millepora*					1	1	H	H	NS
	Sc	*Pocillopora damicornis*	**1**					1	N	VL	H
Herbicide	**Ac**	*Aiptasia* sp.		**1**				1	L	M	M
	Sc	*Acropora millepora*				2		2	H	H	NS
	Sc	*Acropora tenuis*					2	2	H	H	NS
	Sc	*Acropora tumida*		**1**				1	L	VL	H
	Sc	*Acropora valida*	**1**					1	N	VL	H
	Sc	*Montipora aequituberculata*				**1**		1	H	H	NS
	Sc	*Pocillopora damicornis*				**2**	1	3	H	H	NS
	Sc	*Porites cylindrica*					2	2	H	H	NS
Insecticide	**Sc**	*Acropora millepora*					3	3	H	H	NS
	Sc	*Pocillopora damicornis*	**1**					1	N	VL	H
Organohalogen	**Ac**	*Anthopleura* sp.					1	1	H	H	NS
	Sc	*Acropora millepora*					1	1	H	H	NS
Organophosphate	**Sc**	*Acropora millepora*					1	1	H	H	NS
	Sc	*Acropora tenuis*					1	1	H	H	NS
	Sc	*Pocillopora damicornis*	**1**					1	N	VL	H
	Sc	*Porites astreoides*				**1**	1	2	H	H	NS
Pyrethroid	**Sc**	*Acropora millepora*					1	1	H	H	NS
Total			**4**	**2**	**1**	**6**	**17**	**30**			
Pharmaceutical											
Hormones	**Ac**	*Exaiptasia pallida*					5	5	H	H	NS
Phthalates											
Phthalate	**Ac**	*Nematostella vectensis*					2	2	H	H	NS
Synthetics (other)											
Surfactant	**Ac**	*Actinia equina*		2				2	L	M	M

Group/type	Taxon. group	Species name	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
	Sc	*Montastraea faveolata*					1	1	H	H	NS
Total				**2**			**1**	**3**			
PFAS											
PFOS	**Sc**	*Stylophora pistillata*					1	1	H	H	NS
PCPs											
UV filter											
	Sc	*Acropora cervicornis*		**1**				1	L	VL	H
	Sc	*Montastraea annularis*		**1**				1	L	VL	H
	Sc	*Montastraea cavernosa*		**1**				1	L	VL	H
	Sc	*Pocillopora damicornis*		**1**				1	L	VL	H
	Sc	*Porites astreoides*		**1**				1	L	VL	H
	Sc	*Porites divaricata*		**1**				1	L	VL	H
	Sc	*Stylophora pistillata*		**2**				2	L	VL	H
Total				**8**				**8**	**L**	**VL**	**H**
Overall Total			**4**	**12**	**1**	**6**	**26**	**49**			

7.4.1.1 Sea pens

No direct evidence on the effects of pesticides/biocides on sea pens was found. In addition, the reported effects of pesticides on the Anthozoa are species and chemical-specific. It is **precautionary to assume** that sea pens may be affected adversely by some pesticides, in the same way as some Anthozoa. Therefore, **the resistance of sea pens to pesticides/biocides** is assessed **as 'Low' as a precaution,** so resilience is **assessed as 'Low' and sensitivity as 'High' but with 'Low' confidence**. Further study is required.

7.4.2 Personal Care Products (PCPs)

The effects of UV filters (benzophenone-3 and benzophenone-2) on the planulae of true corals were examined by Downs *et al.* (2014, 2016). They reported significant mortality of planulae in all the species studied. Loss of planulae and hence recruitment may not be detrimental in the short term because true corals are long-lived. However, if unchecked it may result in population decline in the long term depending on the species. Therefore, **resistance is assessed as 'Low', resilience as 'Very low' and sensitivity as 'High'**.

7.4.2.1 Sea pens

No direct evidence of the effects of personal care products on sea pens was found. It is **precautionary to assume** that sea pens may be affected adversely by some **personal care products**. Therefore, **the resistance of sea pens to personal care products** is assessed **as 'Low' as a precaution,** so resilience is **assessed as 'Low' and sensitivity as 'High' but with 'Low' confidence**. Further study is required.

7.4.3 Pharmaceutical and other synthetics

The effects of 'Pharmaceutical', 'Phthalates', 'PFAS/PFAS' and other synthetics were examined by a limited number of studies in only four species. The evidence was not adequate to make an overall assessment of sensitivity, or to inform an assessment of the effects on sea pen species.

7.5 Sensitivity assessment – Other

The count of ranked 'worst-case' mortalities and sensitivity of juveniles and larvae in the 'Other' category are summarized in Table 7.4 below. Studies on juveniles reported significant effects and larvae reported sublethal effects. In the case of the studies with no reported life stage, 75% reported 'Severe effects' and 25% reported 'no' effects. However, they were only represented in four of the studies reviewed.

Exposure to cyanide was reported to result in 'Severe' mortality in both of the true corals examined. Therefore, it is assumed that cyanide is probably toxic to all Anthozoa, depending on concentration. Hence, resistance is assessed as '**None**'. Sensitivity assessment is dependent on resilience. Therefore, **sensitivity is assessed as 'Medium' in Actinaria, but 'High' in Octocorallia and Scleractinia. The sensitivity of cyanide to sea pens is probably also 'High'.**

The effects of wastewater or production formation water 'effluent' exposure (Negri & Heyward, 2000; Howe *et al.,* 2015) are shown in Table 7.4 and the 'Anthozoa evidence summary'. However, in both cases the active ingredients of the effluents were not specified. Hence, no assessment is attempted.

Carrerio-Silva *et al.* (2022) examined the effects of elevated suspended polymetallic sulphide (PMS) particles and suspended quartz particles on the cold water octocoral *Dentomuricea meteor.* These particulates may be generated during deep-sea mining activities for PMS. They reported 'Severe' mortality of the octocoral after exposure to PMS, together with an

increase in the concentration of metals associated with the particulates in the water column and the tissue of the octocoral.

Table 7.4 Summary of count of worst-case ranked mortalities to 'Transitional metals and organometals' contaminants reported in the evidence review and resultant proposed sensitivity assessments for Anthozoan species in Taxonomic groups (Actinaria =Ac, Octocorallia = Oc, Scleractinia = Sc and Zoantharia = Zo) (N= None, VL= Very low, L= Low, M= Medium, High = High, and NS= Not sensitive)

Group/type	Taxon. group	Species name	Severe	Significant	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Inorganic chemicals										
Cyanide	**Sc**	*Pocillopora damicornis*	**1**				1	N	VL	H
	Sc	*Porites lichen*	**1**				1	N	VL	H
Total			**2**				**2**			
Mixture										
Effluent	**Ac**	*Exaiptasia pallida*		**2**			2	L	M	M
	Sc	*Acropora millepora*				**1**	1	H	H	NS
Total				**2**		**1**	**3**			
Natural product	**Oc**	*Dentomuricea meteor*	1		1		2	N	L	H
Grand Total			**3**	**2**	**1**	**1**	**7**			

Therefore, the resistance of Octocorallia to suspended polymetallic sulphide (PMS) particles is assessed **as 'None', resilience as 'Low' and sensitivity as 'High'**. **No direct evidence of the effects of suspended polymetallic sulphide (PMS) particles on sea pens was found**. It is **precautionary to assume** that sea pens may be affected adversely. Therefore, **the resistance of sea pens to suspended polymetallic sulphide (PMS) particles** is assessed **as 'Low' as a precaution,** so resilience is **assessed as 'Low' and sensitivity as 'High' but with 'Low' confidence**. Further study is required.

(Page left blank)

8 Conclusions

This report presents the finding of a time limited Rapid Evidence Assessment (REA) of the effects of contaminants on Anthozoa. The aim of the review was to inform the assessment of the sensitivity of the three main species of sea pen in British and Irish waters, namely *Virgularia mirabilis, Funiculina quadrangularis* and *Pennatula phosphorea* and their biotopes to contaminants. The key findings are summarised below.

- A broad literature review of Anthozoa identified ca 20,650 articles that examined Anthozoa and/or contaminants but only 59 satisfied the inclusion criteria and were taken forward for evidence mapping.
- ‘Metals’ were the most studied contaminant within the reviewed articles, followed by ‘Pesticide/Biocide’, ‘Hydrocarbons (Petrochemical)’, ‘Organometals’ and ‘Dispersants’.
- The early life stages (embryos and planulae larvae) of Anthozoan species were the most studied life stage in the reviewed articles.
- Scleractinia (true corals) were most studied, followed by Actinaria (true anemones) while the evidence on Octocorallia (soft corals) and Zoantharia was limited to a small number of studies.
- The effects of ‘Metals’ was the most studied contaminant on Scleractinia, Actinaria and Zoantharia groups. However, the effects of petrochemical hydrocarbons were mostly studied on Octocorallia species.
- *Acropora millepora* was the dominant Scleractinia species studied, *Aiptasia pulchella* was the most dominant Actinaria species studied and *Swiftia exserta* was the most dominant Octocorallia species studied.
- Organometals were the most toxic contaminant group to Anthozoa, based on the relative proportion of ‘’Severe’ and/or ’Significant’ mortalities reported in the reviews. This group was followed by ‘transitional metals’, petrochemical hydrocarbons, dispersants, and pesticide/biocides.
- The toxicity of Personal Care Products (PCPs) stands out as all of the species studied were reported to experience ‘Significant’ mortality after exposure. However, only true

corals were examined, and further study is required before their toxicity to other Anthozoa (and sea pens) can be determined.

- Nevertheless, results for each species and contaminant studied were often derived from a small number of results (1-4), except for the anemone *Aiptasia* spp. and the corals *Acropora* spp. and *Goniastrea* spp..
- The evidence also demonstrated variation in the response of individual species or taxonomic groups (Actinaria, Octocorallia, and Scleractinia) to exposure to any given contaminant type or group.
- In addition, the life history traits and population dynamics of Anthozoa are poorly studied, and the resilience assessments varied between each taxonomic group and species. Therefore, the resultant sensitivity assessments were made for each taxonomic group rather than Anthozoa.
- Therefore, the confidence is the sensitivity assessments made is ‘Low’ because of the limited number of studies available and the lack of information on life history and recovery rates.
- **No direct evidence on the effects of any chemical contaminant on sea pens was found in the review**. Therefore, the sensitivity assessments made above are based on other species as ‘proxies’ and the assumption that species within the same taxonomic group and, hence, biochemistry and physiology, are likely to exhibit a similar response to any given chemical contaminant. Nevertheless, all the sea pen sensitivity assessments were made with ‘Low’ confidence.

9 Bibliography

9.1 Articles included in the evidence review

Acevedo, R., 1991. Preliminary observations on effects of pesticides Carbaryl, Naphthol, and Chlorpyrifos on planulae of the Hermatypic Coral Pocillopora damicornis. *Pacific Science*, **45** (3), 287-289. DOI http://hdl.handle.net/10125/1394

Alutoin, S., Boberg, J., Nyström, M. & Tedengren, M., 2001. Effects of the multiple stressors copper and reduced salinity on the metabolism of the hermatypic coral Porites lutea. *Marine Environmental Research*, **52** (3), 289-299. DOI https://doi.org/10.1016/S0141-1136(01)00105-2

Bao, V.W., Leung, K.M., Qiu, J.W. & Lam, M.H., 2011. Acute toxicities of five commonly used antifouling booster biocides to selected subtropical and cosmopolitan marine species. *Marine Pollution Bulletin*, **62** (5), 1147-1151. DOI https://doi.org/10.1016/j.marpolbul.2011.02.041

Bednarz, V.N., Choyke, S., Marangoni, L.F.B., Otto, E.I., Béraud, E., Metian, M., Tolosa, I. & Ferrier-Pagès, C., 2022. Acute exposure to perfluorooctane sulfonate exacerbates heat-induced oxidative stress in a tropical coral species. *Environmental Pollution*, **302**, 119054. DOI https://doi.org/10.1016/j.envpol.2022.119054

Beiring, E.A. & Lasker, H.R., 2000. Egg production by colonies of a gorgonian coral. *Marine Ecology Progress Series*, **196**, 169-177.

Bielmyer, G.K., Grosell, M., Bhagooli, R., Baker, A.C., Langdon, C., Gillette, P. & Capo, T.R., 2010. Differential effects of copper on three species of scleractinian corals and their algal symbionts (Symbiodinium spp.). *Aquatic Toxicology*, **97** (2), 125-133. DOI https://doi.org/10.1016/j.aquatox.2009.12.021

Bucklin, A., 1987. Growth and asexual reproduction of the sea anemone *Metridium*: comparative laboratory studies of three species. *Journal of Experimental Marine Biology and Ecology*, **110**, 41-52.

Cantin, N., Negri, A. & Willis, B., 2007. Photoinhibition from chronic herbicide exposure reduces reproductive output of reef-building corals. *Marine Ecology Progress Series*, **344**, 81-93. DOI https://doi.org/10.3354/meps07059

Carreiro-Silva, M., Martins, I., Riou, V., Raimundo, J., Caetano, M., Bettencourt, R., Rakka, M., Cerqueira, T., Godinho, A., Morato, T. & Colaco, A., 2022. Mechanical and

toxicological effects of deep-sea mining sediment plumes on a habitat-forming cold-water octocoral. *Frontiers in Marine Science*, **9**. DOI https://doi.org/10.3389/fmars.2022.915650

Coma, R., Linares, C., Ribes, M., Diaz, D., Garrabou, J. & Ballesteros, E., 2006. Consequences of a mass mortality in populations of *Eunicella singularis* (Cnidaria: Octocorallia) in Menorca (NW Mediterranean). *Marine Ecology Progress Series*, **331**, 51-60.

Darling, J.A., Kuenzi, A. & Reitzel, A.M., 2009. Human-mediated transport determines the non-native distribution of the anemone *Nematostella vectensis*, a dispersal-limited estuarine invertebrate. *Marine Ecology Progress Series*, **380**, 137-146.

Darling, J.A., Reitzel, A.M. & Finnerty, J.R., 2004. Regional population structure of a widely introduced estuarine invertebrate: *Nematostella vectensis* Stephenson in New England. *Molecular Ecology*, **13** (10), 2969-2981.

Darling, J.A., Reitzel, A.R., Burton, P.M., Mazza, M.E., Ryan, J.F., Sullivan, J.C. & Finnerty, J.R., 2005. Rising starlet: the starlet sea anemone, *Nematostella vectensis*. *BioEssays*, **27** (2), 211-221.

Downs, C.A., Kramarsky-Winter, E., Fauth, J.E., Segal, R., Bronstein, O., Jeger, R., Lichtenfeld, Y., Woodley, C.M., Pennington, P., Kushmaro, A. & Loya, Y., 2014. Toxicological effects of the sunscreen UV filter, benzophenone-2, on planulae and in vitro cells of the coral, *Stylophora pistillata*. *Ecotoxicology*, **23** (2), 175-191. DOI https://doi.org/10.1007/s10646-013-1161-y

Downs, C.A., Kramarsky-Winter, E., Segal, R., Fauth, J., Knutson, S., Bronstein, O., Ciner, F.R., Jeger, R., Lichtenfeld, Y., Woodley, C.M., Pennington, P., Cadenas, K., Kushmaro, A. & Loya, Y., 2016. Toxicopathological effects of the sunscreen UV filter, Oxybenzone (benzophenone-3), on coral planulae and cultured primary cells and its environmental contamination in Hawaii and the U.S. Virgin Islands. *Archives of Environmental Contamination and Toxicology*, **70** (2), 265-288. DOI https://doi.org/10.1007/s00244-015-0227-7

Etnoyer, P.J., Wickes, L.N., Silva, M., Dubick, J.D., Balthis, L., Salgado, E. & MacDonald, I.R., 2016. Decline in condition of gorgonian octocorals on mesophotic reefs in the northern Gulf of Mexico: before and after the Deepwater Horizon oil spill. *Coral reefs*, **35** (1), 77-90. DOI https://doi.org/10.1007/s00338-015-1363-2

Farina, O., Ramos, R., Bastidas, C. & Garcia, E., 2008. Biochemical Responses of Cnidarian Larvae to Mercury and Benzo(a)pyrene Exposure. *Bulletin of Environmental*

Contamination and Toxicology, **81** (6), 553-557. DOI https://doi.org/10.1007/s00128-008-9534-2

Francis, L., 1976. Social organization within clones of the sea anemone *Anthopleura elegantissima*. *The Biological Bulletin*, **150** (3), pp. 361-376.

Frometa, J., DeLorenzo, M.E., Pisarski, E.C. & Etnoyer, P.J., 2017. Toxicity of oil and dispersant on the deep water gorgonian octocoral *Swiftia exserta*, with implications for the effects of the Deepwater Horizon oil spill. *Marine Pollution Bulletin*, **122** (1-2), 91-99. DOI https://doi.org/10.1016/j.marpolbul.2017.06.009

Gissi, F., Stauber, J., Reichelt-Brushett, A., Harrison, P.L. & Jolley, D.F., 2017. Inhibition in fertilisation of coral gametes following exposure to nickel and copper. *Ecotoxicology and Environmental Safety*, **145**, 32-41. DOI https://doi.org/10.1016/j.ecoenv.2017.07.009

Goh, B., 1991. Mortality and settlement success of *Pocillopora damicornis* planula larvae during recovery from low levels of Nickel. *Pacific Science*, **45**, 276-286. DOI http://hdl.handle.net/10125/1393

Goodbody-Gringley, G., Wetzel, D.L., Gillon, D., Pulster, E., Miller, A. & Ritchie, K.B., 2013. Toxicity of *Deepwater Horizon* source oil and the chemical dispersant, Corexit® 9500, to coral larvae. *PLoS ONE*, **8** (1), e45574. DOI https://doi.org/10.1371/journal.pone.0045574

Hand, C. & Uhlinger, K.R., 1992. The culture, sexual and asexual reproduction, and growth of the sea anemone, *Nematostella vectensis*. *Biological Bulletin, Marine Biological Laboratory, Woods Hole,* **182**, 169-176.

Hand, C. & Uhlinger, K.R., 1994. The unique, widely distributed, estuarine sea anemone, *Nematostella vectensis*, Stephenson: A review, new facts, and questions. *Estuaries*, **17**, 501-508.

Hand, C. & Uhlinger, K.R., 1995. Asexual reproduction by transverse fission and some anomalies in the sea anemone *Nematostella vectensis*. *Invertebrate Biology*, **114**, 9-18.

Hansen, D.J., Mahony, J.D., Berry, W.J., Benyi, S.J., Corbin, J.M., Pratt, S.D., Di Toro, D.M. & Abel, M.B., 1996. Chronic effect of cadmium in sediments on colonization by benthic marine organisms: An evaluation of the role of interstitial cadmium and acid-volatile sulfide in biological availability. *Environmental Toxicology and Chemistry*, **15** (12), 2126-2137. DOI https://doi.org/10.1002/etc.5620151208

Harter, V.L. & Matthews, R.A., 2005. Acute and chronic toxicity test methods for *Nematostella vectensis* Stephenson. *Bulletin of Environmental Contamination and Toxicology*, **74** (5), 830-836. DOI https://doi.org/10.1007/s00128-005-0656-5

Hartnoll, R.G., 1975. The annual cycle of *Alcyonium digitatum*. *Estuarine and Coastal Marine Science*, **3**, 71-78.

Hartnoll, R.G., 1998. Circalittoral faunal turf biotopes: an overview of dynamics and sensitivity characteristics for conservation management of marine SACs, Volume VIII. *Scottish Association of Marine Sciences, Oban, Scotland, 109 pp.* [UK Marine SAC Project. Natura 2000 reports.] Available from: http://ukmpa.marinebiodiversity.org/uk_sacs/pdfs/circfaun.pdf

Hédouin, L. & Gates, R.D., 2013. Assessing fertilization success of the coral Montipora capitata under copper exposure: Does the night of spawning matter? *Marine Pollution Bulletin*, **66** (1), 221-224. DOI https://doi.org/10.1016/j.marpolbul.2012.11.020

Henderson, R.S., 1988. Marine microcosm experiments on effects of copper and tributyltin-based antifouling paint leachates. *Naval Ocean Systems Center, San Diego, California,* 51 pp.

Henderson, R.S. & Salazar, S.M., 1996. Flowthrough Bioassay Studies on the Effects of Antifouling TBT Leachates. In Champ, M.A. and Seligman, P.F. (eds.). *Organotin: Environmental fate and effects*, Dordrecht: Springer Netherlands, pp. 281-303.

Heyward, A.J., 1988. Inhibitory effects of copper and zinc sulphates on fertilization in corals. In Choat, J.H. *Proceedings of the 6th International Coral Reef Symposium*, Townsville, Queensland, Australia, pp. 299-303.

Hiscock, K., Southward, A., Tittley, I., Jory, A. & Hawkins, S., 2001. The impact of climate change on subtidal and intertidal benthic species in Scotland. *Scottish National Heritage Research, Survey and Monitoring Report*, no. 182., Edinburgh: Scottish National Heritage

Hiscock, K., Sharrock, S., Highfield, J. & Snelling, D., 2010. Colonization of an artificial reef in south-west England—ex-HMS 'Scylla'. *Journal of the Marine Biological Association of the United Kingdom*, **90** (1), 69-94. DOI https://doi.org/10.1017/S0025315409991457

Howe, P.L., Reichelt-Brushett, A.J. & Clark, M.W., 2012. Aiptasia pulchella: a tropical cnidarian representative for laboratory ecotoxicological research. *Environmental Toxicology and Chemistry*, **31** (11), 2653-2662. DOI https://doi.org/10.1002/etc.1993

Howe, P.L., Reichelt-Brushett, A.J. & Clark, M.W., 2014. Effects of Cd, Co, Cu, Ni and Zn on asexual reproduction and early development of the tropical sea anemone Aiptasia pulchella. *Ecotoxicology*, **23** (9), 1593-1606. DOI https://doi.org/10.1007/s10646-014-1299-2

Howe, P.L., Reichelt-Brushett, A.J. & Clark, M.W., 2014. Investigating lethal and sublethal effects of the trace metals cadmium, cobalt, lead, nickel, and zinc on the anemone Aiptasia pulchella, a cnidarian representative for ecotoxicology in tropical marine environments. *Marine and Freshwater Research*, **65** (6), 551-561. DOI https://doi.org/10.1071/mf13195

Howe, P.L., Reichelt-Brushett, A.J., Krassoi, R. & Micevska, T., 2015. Comparative sensitivity of the cnidarian *Exaiptasia pallida* and a standard toxicity test suite: testing whole effluents intended for ocean disposal. *Environmental Science and Pollution Research*, **22** (17), 13225-13233. DOI https://doi.org/10.1007/s11356-015-4513-8

Jones, R. & Steven, A., 1997. Effects of cyanide on corals in relation to cyanide fishing on reefs. *Marine and Freshwater Research*, **48**, 517-522. DOI https://doi.org/10.1071/MF97048

Jones, R.J., 1997. Zooxanthellae loss as a bioassay for assessing stress in corals. *Marine Ecology Progress Series*, **149** (1-3), 163-171. DOI https://doi.org/10.3354/meps149163

Kaiser, H., Chalmers, R. & Oellermann, L.K., 2003. The Relationship Between Copper-Related Damage and Population Growth of the Rock Anemone, Aiptasia spp. *Journal of Applied Aquaculture*, **14** (1-2), 53-67. DOI https://doi.org/10.1300/J028v14n01_04

Kaliszewicz, A., Panteleeva, N., Olejniczak, I., Boniecki, P. and Sawicki, M., 2012. Internal brooding affects the spatial structure of intertidal sea anemones in the Arctic-boreal region. *Polar biology*, **35** (12), pp.1911-1919.

Kitahara, M.V., Croquer, A., Vermeij, M. & Feingold, J., 2022. *Porites astreoides*. *The IUCN Red List of Threatened Species* 2022: e.T133680A166010213. Accessed on 30 March 2023.

Klein, S., Frazier, V., Readdean, T., Lucas, E., Diaz-Jimenez, E.P., Sogin, M., Ruff, E.S. & Echeverri, K., 2021. Common environmental pollutants negatively affect development and regeneration in the sea anemone *Nematostella vectensis* holobiont. *Frontiers in Ecology and Evolution*, **9**. DOI https://doi.org/10.3389/fevo.2021.786037

Kwok, C.K. & Ang, P.O., 2013. Inhibition of larval swimming activity of the coral (*Platygyra acuta*) by interactive thermal and chemical stresses. *Marine Pollution Bulletin*, **74** (1), 264-273. DOI https://doi.org/10.1016/j.marpolbul.2013.06.048

Lasker, H.R., Kim, K. & Coffroth, M.A., 1998. Production, settlement, and survival of plexaurid gorgonian recruits. *Marine Ecology Progress Series*, **162**, 111-123.

Maggi, P. & Cossa, D., 1973. Relative harmfulness of five anionic detergents in the sea. I. Acute toxicity with regard to fifteen organisms. *Revue des Travaux de l'Institut des Pêches Maritimes*, **37** (3), 411-417.

Markey, K.L., Baird, A., Humphrey, C. & Negri, A., 2007. Insecticides and a fungicide affect multiple coral life stages. *Marine Ecology Progress Series*, **330**, 127-137. DOI https://doi.org/10.3354/meps330127

Mercier, A., Sun, Z. & Hamel, J.-F., 2011. Internal brooding favours pre-metamorphic chimerism in a non-colonial cnidarian, the sea anemone *Urticina felina*. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**, 1-6.

Mercurio, P., Negri, A.P., Burns, K.A. & Heyward, A.J., 2004. The ecotoxicology of vegetable versus mineral based lubricating oils: 3. Coral fertilization and adult corals. *Environmental Pollution*, **129** (2), 183-194. DOI https://doi.org/10.1016/j.envpol.2003.11.008

MES, 2010. *Marine Macrofauna Genus Trait Handbook*. Marine Ecological Surveys Limited. http://www.genustraithandbook.org.uk/

Minchin, D., 1987. *Swiftia pallida* Madsen (Coelenterata: Gorgonacea) in Irish waters, with a note on *Pseudanthessiusthorelli* (Brady) (Crustacea: Copepoda) new to Ireland *Irish Naturalists' Journal*, **22**(5), 183-185

Morgan, M.B., Ross, J., Ellwanger, J., Phrommala, R.M., Youngblood, H., Qualley, D. & Williams, J., 2022. Sea anemones responding to sex hormones, Oxybenzone, and Benzyl Butyl Phthalate: Transcriptional profiling and in silico modelling provide clues to decipher endocrine disruption in cnidarians. *Frontiers in Genetics*, **12**. DOI https://doi.org/10.3389/fgene.2021.793306

Negri, A., Vollhardt, C., Humphrey, C., Heyward, A., Jones, R., Eaglesham, G. & Fabricius, K., 2005. Effects of the herbicide diuron on the early life history stages of coral. *Marine Pollution Bulletin*, **51** (1), 370-383. DOI https://doi.org/10.1016/j.marpolbul.2004.10.053

Negri, A.P. & Heyward, A.J., 2000. Inhibition of fertilization and larval metamorphosis of the coral *Acropora millepora* (Ehrenberg, 1834) by petroleum products. *Marine Pollution Bulletin*, **41** (7), 420-427. DOI https://doi.org/10.1016/S0025-326X(00)00139-9

Negri, A.P. & Heyward, A.J., 2001. Inhibition of coral fertilisation and larval metamorphosis by tributyltin and copper. *Marine Environmental Research*, **51** (1), 17-27. DOI https://doi.org/10.1016/S0141-1136(00)00029-5

Nyström, M., Nordemar, I. & Tedengren, M., 2001. Simultaneous and sequential stress from increased temperature and copper on the metabolism of the hermatypic coral Porites cylindrica. *Marine Biology*, **138** (6), 1225-1231. DOI https://doi.org/10.1007/s002270100549

Pearson, C.V.M., Rogers, A.D. & Sheader, M., 2002. The genetic structure of the rare lagoonal sea anemone, *Nematostella vectensis* Stephenson (Cnidaria; Anthozoa) in the United Kingdom based on RAPD analysis. *Molecular Ecology*, **11** (11), 2285-2293.

Pridmore, R.D., Thrush, S.F., Cummings, V.J. & Hewitt, J.E., 1992. Effect of the organochlorine pesticide technical chlordane on intertidal macrofauna. *Marine Pollution Bulletin*, **24** (2), 98-102. DOI https://doi.org/10.1016/0025-326X(92)90737-Q

Råberg, S., Nyström, M., Erös, M. & Plantman, P., 2003. Impact of the herbicides 2,4-D and diuron on the metabolism of the coral Porites cylindrica. *Marine Environmental Research*, **56** (4), 503-514. DOI https://doi.org/10.1016/S0141-1136(03)00039-4

Readman, J.A.J. & Durkin O.C. 2016. *Caryophyllia (Caryophyllia) smithii* and *Swiftia pallida* on circalittoral rock. In Tyler-Walters H. and Hiscock K. (eds) *Marine Life Information Network: Biology and Sensitivity Key Information Reviews*, [on-line]. Plymouth: Marine Biological Association of the United Kingdom. [cited 30-03-2023]. Available from: https://www.marlin.ac.uk/habitat/detail/386

Readman, J.A.J. & Williams, E., 2021. *Alcyonium digitatum* and faunal crust communities on vertical circalittoral bedrock. In Tyler-Walters H. and Hiscock K. *Marine Life Information Network: Biology and Sensitivity Key Information Reviews*, [on-line]. Plymouth: Marine Biological Association of the United Kingdom. [cited 30-03-2023]. Available from: https://www.marlin.ac.uk/habitat/detail/1097

Reichelt-Brushett, A.J. & Harrison, P.L., 1999. The Effect of Copper, Zinc and Cadmium on Fertilization Success of Gametes from Scleractinian Reef Corals. *Marine Pollution Bulletin*, **38** (3), 182-187. DOI https://doi.org/10.1016/S0025-326X(98)00183-0

Reichelt-Brushett, A.J. & Harrison, P.L., 2000. The Effect of Copper on the Settlement Success of Larvae from the Scleractinian Coral Acropora tenuis. *Marine Pollution Bulletin*, **41** (7), 385-391. DOI https://doi.org/10.1016/S0025-326X(00)00131-4

Reichelt-Brushett, A.J. & Harrison, P.L., 2004. Development of a Sublethal Test to Determine the Effects of Copper and Lead on Scleractinian Coral Larvae. *Archives of Environmental Contamination and Toxicology*, **47** (1), 40-55. DOI https://doi.org/10.1007/s00244-004-3080-7

Reichelt-Brushett, A.J. & Harrison, P.L., 2005. The effect of selected trace metals on the fertilization success of several scleractinian coral species. *Coral reefs*, **24** (4), 524-534. DOI https://doi.org/10.1007/s00338-005-0013-5

Reichelt-Brushett, A.J. & Michalek-Wagner, K., 2005. Effects of copper on the fertilization success of the soft coral Lobophytum compactum. *Aquatic Toxicology*, **74** (3), 280-284. DOI https://doi.org/10.1016/j.aquatox.2005.05.011

Reitzel, A.M., Burton, P.M., Krone, C. & Finnerty, J.R., 2007. Comparison of developmental trajectories in the starlet sea anemone *Nematostella vectensis*: embryogenesis, regeneration, and two forms of asexual fission. *Invertebrate Biology,* **126** (2), 99-112.

Reitzel, A.M., Chu, T., Edquist, S., Genovese, C., Church, C., Tarrant, A.M. & Finnerty, J.R., 2013. Physiological and developmental responses to temperature by the sea anemone *Nematostella vectensis*. *Marine Ecology Progress Series*, **484**, 115-130.

Reitzel, A.M., Darling, J.A., Sullivan, J.C. & Finnerty, J.R., 2008. Global population genetic structure of the starlet anemone *Nematostella vectensis*: multiple introductions and implications for conservation policy. *Biological Invasions*, **10** (8), 1197-1213.

Ross, C., Olsen, K., Henry, M. & Pierce, R., 2015. Mosquito control pesticides and sea surface temperatures have differential effects on the survival and oxidative stress response of coral larvae. *Ecotoxicology*, **24** (3), 540-552. DOI https://doi.org/10.1007/s10646-014-1402-8

Rumbold, D.G. & Snedaker, S.C., 1997. Evaluation of Bioassays to Monitor Surface Microlayer Toxicity in Tropical Marine Waters. *Archives of Environmental Contamination and Toxicology*, **32** (2), 135-140. DOI https://doi.org/10.1007/s002449900165

Sabdono, A., 2009. Heavy metal levels and their potential toxic effect on coral Galaxea fascicularis from Java Sea, Indonesia. *Research Journal of Environmental Sciences*, **3**, 96-102. DOI https://doi.org/10.3923/rjes.2009.96.102

Sebens, K.P., 1985. Community ecology of vertical rock walls in the Gulf of Maine: small-scale processes and alternative community states. In *The Ecology of Rocky Coasts: essays presented to J.R. Lewis, D.Sc.* (ed. P.G. Moore & R. Seed), pp. 346-371. London: Hodder & Stoughton Ltd.

Sheader, M., Suwailem, A.M. & Rowe, G.A., 1997. The anemone, *Nematostella vectensis*, in Britain: considerations for conservation management. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **7**, 13-25.

Sheehan, E.V., Stevens, T.F., Gall, S.C., Cousens, S.L. & Attrill, M.J., 2013. Recovery of a temperate reef assemblage in a marine protected area following the exclusion of towed demersal fishing. *Plos One*, **8** (12), e83883.

Siddiqui, S., Goddard, R.H. & Bielmyer-Fraser, G.K., 2015. Comparative effects of dissolved copper and copper oxide nanoparticle exposure to the sea anemone, *Exaiptasia pallida*. *Aquatic Toxicology*, **160**, 205-213. DOI https://doi.org/10.1016/j.aquatox.2015.01.007

Smith, J.E., 1968. *" Torrey Canyon" Pollution and Marine Life: A Report by the Plymouth Laboratory of the Marine Biological Association of the United Kingdom*. Marine Biological Association of the United Kingdom.

Solé-Cava, A.M., Thorpe, J.P. & Todd, C.D., 1994. High genetic similarity between geographically distant populations in a sea anemone with low dispersal capabilities. *Journal of the Marine Biological Association of the United Kingdom*, **74**, 895-902.

Tagatz, M.E., Ivey, J.M. & Oglesby, J.L., 1979. Toxicity of Drilling-Mud Biocides to Developing Estuarine Macrobenthic Communities. *Northeast Gulf Science*, **3**, 88-95. DOI https://doi.org/10.18785/negs.0302.04

Te, F.T., 1991. Effects of two petroleum products on Pocillopora damicornis planulae. *Pacific Science*, **45** (3), 290-298. DOI http://hdl.handle.net/10125/1395

Tkachenko, K.S., 2017. Coral reefs in the face of ecological threats of 21st century. *Biology Bulletin Reviews*, **7**, 64-83. DOI https://doi.org/10.1134/S2079086416050091

Topcu Eryalcin, N.E., 2017. Demographic Structure of Gorgonian (Anthozoa, Octocorallia, Holaxonia) Assemblages in the Bay of Saros. *Kahramanmaras Sutcu Imam University Journal of Natural Sciences*, **20** (4), 368-377. DOI https://doi.org/10.18016/ksudobil.280545

Victor, S. & Richmond, R.H., 2005. Effect of copper on fertilization success in the reef coral Acropora surculosa. *Marine Pollution Bulletin*, **50** (11), 1448-1451. DOI https://doi.org/10.1016/j.marpolbul.2005.09.004

Wahl, M., 1985. The recolonization potential of *Metridium senile* in an area previously depopulated by oxygen deficiency. *Oecologia*, **67**, 255-259.

Wallace, C.C. 1999. *Staghorn Corals of the World: a revision of the coral genus* Acropora. CSIRO, Collingwood.

Watanabe, T., Yuyama, I. & Yasumura, S., 2006. Toxicological effects of biocides on symbiotic and aposymbiotic juveniles of the hermatypic coral Acropora tenuis. *Journal of Experimental Marine Biology and Ecology*, **339** (2), 177-188. DOI https://doi.org/10.1016/j.jembe.2006.07.020

Weinberg, S. & Weinberg, F., 1979. The life cycle of a gorgonian: *Eunicella singularis* (Esper, 1794). *Bijdragen tot de Dierkunde*, **48** (2), 127-137.

Whomersley, P. & Picken, G., 2003. Long-term dynamics of fouling communities found on offshore installations in the North Sea. *Journal of the Marine Biological Association of the UK*, **83** (5), 897-901.

Yoshioka, P.M., 1996. Variable recruitment and its effects on the population and community structure of shallow-water gorgonians. *Bulletin of Marine Science*, **59** (2), 433-443.

9.2 Articles that could not be accessed during the review

Eisler, R., 1975. Acute toxicities of crude oils and oil-dispersant mixtures to Red Sea fishes and invertebrates. *Israel Journal of Zoology*, **24** (1-2), 16-27. DOI https://doi.org/10.1080/00212210.1975.10688408

Esquivel, I.F., 1986. Short-term copper bioassay on the planula of the reef coral Pocillopora damicornis. In Jokiel, P.L., Richmond, R.H. and Rogers, R.A. (eds.). *Coral reef population biology*: Hawaii Institute of Marine Biology Technical Report, pp. 465-472. No. 37].

Nystrom, M., Moberg, F. & Tedengren, M., 1997. Natural and anthropogenic disturbance on reef corals in the inner Gulf of Thailand: Physiological effects of reduced salinity, copper, and siltation. *Proceedings of the 8th International Coral Reef Symposium Vol. 2*, Smithsonian Tropical Research Institute, Panama, June 24-27, 1996, pp. 1893-1898.

Topcu Eryalcin, N.E., 2017. Demographic structure of gorgonian (Anthozoa, Octocorallia, Holaxonia) assemblages in the Bay of Saros. *Kahramanmaras Sutcu Imam University Journal of Natural Sciences*, **20** (4), 368-377. DOI https://doi.org/10.18016/ksudobil.280545

(Page left blank)

10 Appendix 1

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

Term	Definition
Reproduction	Measurements and endpoints to track the effect of toxicants on the reproductive cycle; includes behavioural and physiological measurements

Effect measurement

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

- Accumulation
  - Body burden
  - BCF
- Behaviour/Avoidance
  - Chemical avoidance
  - Substratum avoidance
- Biochemical (ECOTOX =1,641 entries)
  - Acyl-CoA oxidase activity
  - Acetylcholinesterase (AchE) activity
  - Acid phosphatase
  - Catalase (CAT)
  - Cytochrome P450 activity
  - Gamma-Glutamyl Transpeptidase
  - Glutathione disulphide
  - Glutathione peroxidase (GPX),
  - Glutathione reductase (GR),
  - Heat shock proteins
  - Lactate dehydrogenase
  - Lipid peroxidation,
  - Metallothioniens
  - MFO (BPH, CYP-dependent monoxygenase)
  - Multixenotoxicity resistance
  - NADPH-Neo tetrazolium Reductase activity

    - NF-E2-related factor 2 (Nrf2),
    - Superoxide dismutase (SOD)
- Cellular (ECOTOX has 143 entries)
    - DNA damage/Micronuclei/Adduct formation
    - Genotoxicity
    - Haemocyte counts population
    - Phagocytosis
    - Lysosomal membrane stability
    - Ovarian and spermatic follicles
    - Transmembrane sodium energy gradient
    - Transcriptomics
- Ecosystem processes
    - General
    - Reduced/Increased productivity (primary/secondary)
    - Community
- Growth/Development/Morphology
    - Abnormal development/larvae
    - Growth rate
    - Leaf/shoot/rhizome/root elongation
    - Leaf shape/morphology
    - Mortality (adult/larval)
    - Adult survival
    - Larval survival
- Physiology/Immunological/Injury/Intoxication
    - Byssal thread production
    - Clearance/filtration rate
    - Excretion rate
    - Larval swimming velocity/ability
    - Respiration rate
    - Condition indices
    - Photosynthetic efficiency
    - PSII function/damage
    - Scope for growth (SFG)
    - Valve gape
    - Population

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

Response site

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

- Community
- Digestive gland
- Embryo
- Gametes (oocytes and sperm)
- Gonad
- Haemocytes
- Larva
- Leaf/shoot
- Lysosomes
- Muscle tissue
- Rhizomes/roots
- Population

- Seedling
- Soft tissues
- Whole organism (assumes adult)

End points

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

- BCFD - Bioconcentration factor calculated using dry weight tissue concentration
- 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' ranked 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, many papers examined several different combinations of contaminant type and 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 distinct species (e.g., in seagrasses) then we would record twelve separate ‘worst-case’ mortalities.

MBA.AC.UK

Marine
Biological
Association