CHAPTER 9

Chemical Pollution on Coral Reefs: Exposure and Ecological Effects

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Abstract: In this chapter we review the effects of anthropogenically derived chemical pollutants on tropical coral reef ecosystems. A wide range of compounds, including pesticides, trace metals and petroleum hydrocarbons enter reef systems through various pathways and affect different reef species and/or life history stages. Tools for evaluation of chemical stress on coral reefs consist of molecular, biochemical, physiological and ecological bioindicators, providing information at organismal or community levels. This chapter collates and assesses available information on different chemical stressors in the marine environment and the effects on reef-building corals. Ecological effects from chemical stressors are strongly dependent on exposure characteristics. Three probable pollution scenarios are discussed and their individual properties evaluated. Short-term, pulse-like pollution events including oil spills or antifoulant deposition through ship groundings often have a direct and severe impact upon multiple trophic levels of the system. However, these events are typically localised and possibly irrelevant on an ecosystem-wide scale. In contrast, recurring pollution events such as input from river floods or chronic pollution from land runoff (e.g. sewage treatment effluent or herbicides), may exert subtle effects on lower trophic levels of the system, affecting species fitness and driving adaptation. Effects from recurring or chronic pollution are more likely to combine and interact with other environmental factors, but remain poorly understood. Over time, chronic sub-lethal stress may decrease resilience of reef organisms to other forms of environmental stress like elevated sea surface temperatures and ocean acidification.

CORAL REEF ECOSYSTEMS AND SYMBIOTIC PRODUCTION

Coral reefs are biogenic structures that often contribute significantly to the seaward section of tropical shorelines, buffering the coast from wave action and erosion [1]. Coral reefs are among the most biologically diverse and productive systems in the world and many coastal communities depend upon these economically and culturally important ecosystems as a source of income or resources. Coral reefs are crucial to tropical fisheries and tourism and provide many island populations with primary building materials. Reef-related tourism alone generates vast revenues in some parts of the world. For example, in the 1990s, an estimated $140 billion was generated by Caribbean reefs annually [2]. The ecological value of coral reefs is also enormous. There are over 600 species of calcifying corals, and these contribute directly to the habitat of thousands of species of tropical fish, algae and invertebrates. The physical protection offered by coral reefs enables formation and persistence of associated ecosystems such as seagrass beds and mangrove forests, allowing for the existence of essential habitats, hatching grounds and fisheries [3]. These ecosystems are in turn crucial for the reefs, as they play an important part in the sustenance of the marine foodweb by providing detrital matter that helps maintain the lower trophic levels of the reef food web, by functioning as a sediment trap limiting particulate matter reaching reefs and by acting as nursery grounds for many juvenile fish that find their way out to the reef in adulthood [1].

Reef-like structures have existed on earth for over 500 million years, with modern reefs developing around 250 million years ago [4]. Most coral reefs are located in tropical oceans within 30° of the equator with water temperatures ranging between 18 and 30 °C (Fig. **1**). Coral reefs are primarily formed by calcification processes of scleractinian (hard) corals and coralline algae, providing a structural basis for reef-dwelling organisms. The extraordinary productivity of coral reefs that may seem remarkable within a marine environment harbouring low nutrient concentrations can be explained by the photosynthetic contribution of intracellular microalgae to host tissues [5]. Reef building corals all host endosymbiotic dinoflagellates (zooxanthellae) of the genus *Symbiodinium*. Coral hosts profit from this mutualistic relationship by obtaining high-energy photosynthetic products in the form of sugars, amino acids, carbohydrates and small peptides from the algae, while the symbiotic algae receive inorganic plant nutrients, refuge and protection within the polyp tissues [5, 6]. The symbiosis serves the main purpose of

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restricting nutrient outflow into the surrounding oligotrophic water column, and as a result the host is endowed with substantially more energy than would otherwise be available to heterotrophs, enabling corals to extract calcium carbonate from surrounding waters and secrete it as a skeleton [6, 7]. As coral symbiosis based upon algal primary production is the engine driving coral reef ecosystems, stressors that interfere with photosynthetic processes could undermine the basis of this biologically and economically important marine habitat with serious consequences [8].

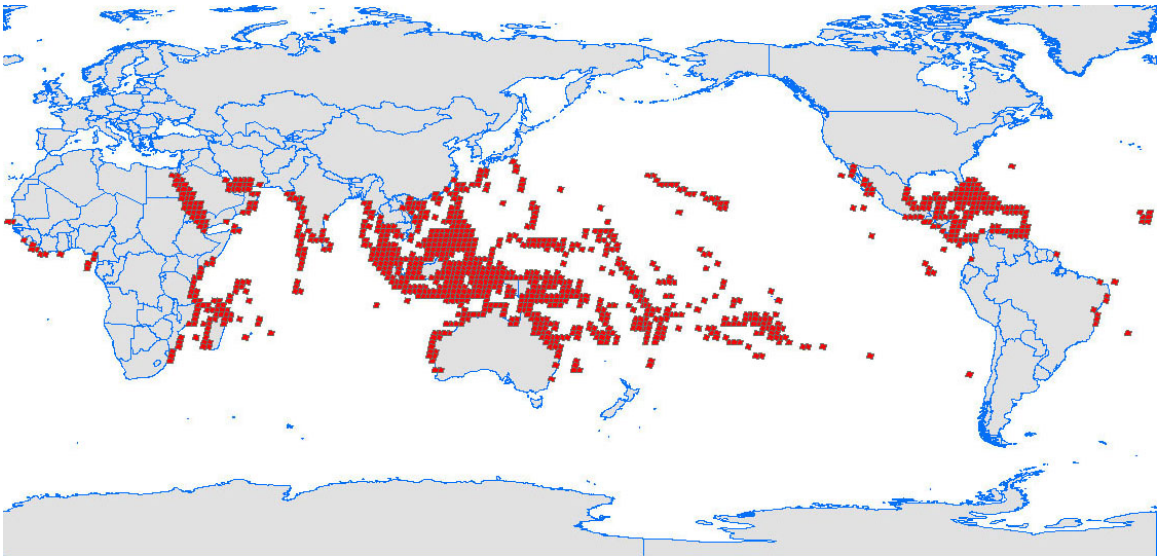

Figure 1: Distribution of coral reefs around the world (source: http://www.nasa.gov/).

Most scleractinian corals reproduce by broadcast spawning, with many species releasing eggs and sperm into the water column simultaneously at annual spawning events [9]. Typically fertilisation of the highly buoyant eggs is external and mobile planula larvae develop over 48–72 h [10]. The swimming planulae are usually competent to undergo settlement on the substratum followed by metamorphosis into a juvenile coral polyp after 96 h. Larvae of many coral species are known to require a biochemical inducer from their preferred settlement substratum, crustose coralline algae, to trigger metamorphosis [11]. Juvenile coral polyps are generally less than 2 mm long and are susceptible to predation as well as smothering by sediments and other stressors [12]. It takes between five and ten years for many coral species to become reproductive and these early life histories, including fertilisation, larval development, larval metamorphosis and the juvenile stage, are all critical for the long term resilience and health of coral reefs and their vulnerability to anthropogenic stress needs to be considered [13, 14].

Over the last century and a half in which intensive agriculture, fishing practises and industries evolved, an estimated 30% of coral reefs worldwide have been severely depleted. Between 50 and 70% of coral reefs are thought to be under direct and immediate threat from climate change and human activities [15, 16]. Coral reef cover in the Caribbean has been reported to have declined over 80% in the last 30 years [17]. Climate related changes in ocean acidity and temperature, nutrients and chemical pollution are the main proposed reasons for coral reef declines and cause for concern [12, 16, 18-21]. In this chapter we aim to critically review and collate information about the possible ecological effects of chemical pollution on coral reefs. We assess potential pollutants, sources and the likely exposure of reef-building corals and their zooxanthellate symbionts. Furthermore, we evaluate what trophic level is most likely to be affected by various pollutants and in what particular way an organism will be impacted. Finally, we aim to assess evidence that links exposure to effect and identifies gaps in our knowledge concerning the effects of anthropogenic pollutants on coral reefs.

SOURCES OF MARINE POLLUTANTS

Waterborne chemicals affecting tropical marine communities can have both point and non-point sources and may be transported to reefs from distant origins. Different classes of contaminants are often associated with a particular environmental compartment because of their physicochemical characteristics. Fig. (**2**) presents a conceptual model for

potential contaminant routes reaching reef ecosystems. Transport, dispersion, and ultimately biological effects of pollutants in marine systems depend on the persistence of these chemicals under tropical conditions and their bioaccumulation and biodegradation rates. Typically pollutants with a higher solubility in surrounding waters will find their way further offshore. Association of pollutants with particulate matter may increase environmental persistence. Because of the rapid sorption of many contaminants to sediments, it is not surprising the largest reservoirs of chemical stressors will be found in estuaries, wetlands or nearby urban centres. Nevertheless, suspended sediments transported in monsoonal flood-plumes have the potential of contaminating sites further offshore. Additionally, volatilisation from surface waters, transport through the atmosphere and redeposition elsewhere can deliver residues far from their original sites of application [22]. Biota carrying accumulated loads of persistent chemicals in their tissues can also transport pollutants between ecosystems and far from their application or deposition sites.

Terrestrial runoff from rivers and streams contaminated by agricultural, industrial or urban activities is usually the most important route for chemicals to enter marine waters (Table **1**). The array of potential contaminants is very wide from organics such as pesticide residues, pharmaceuticals and hydrocarbons to industrial waste products, metals and organometallic compounds. In highly protected areas such as Australia's Great Barrier Reef (GBR), chemicals of concern are contemporary pesticides; most specifically herbicides originating from farming activities [23]. In more highly populated regions such as south-east Asia, a much wider range of urban and industrial contaminants also threaten coral reefs.

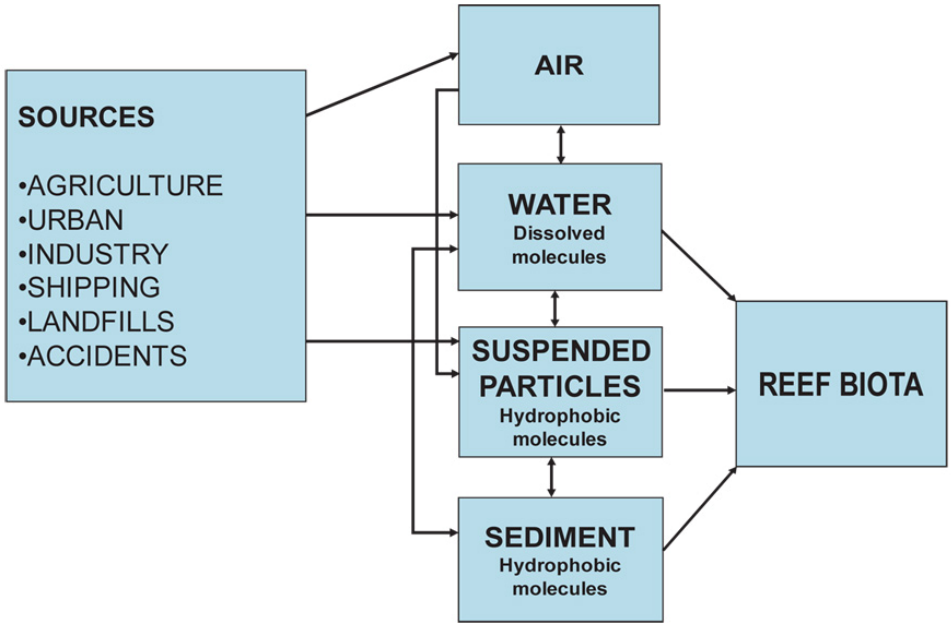

Figure 2: Conceptual model for pollutant pathways in marine systems.

Industrial activities including mining and smelting operations are sources of metals and dioxin-like compounds while shipping operations, refineries and oil extraction and explorative processes introduce hydrocarbons and trace metals to the marine environment [24, 25]. In addition, urban sources such as sewage outfalls, desalination plants and landfills can contribute considerably to contaminant loads [1]. The potential off-site movement of chemical residues depends on both physicochemical properties of compounds (e.g. partition properties) and environmentally specific factors such as hydrology, sediment composition, temperature and biological degradation parameters [26]. While the breakdown of organic compounds is likely to be more rapid in the tropics than in temperate regions, the warmer conditions may increase sorption of organics to particulates thereby increasing their persistence in some environmental compartments. Direct contamination of marine waters can result from inputs associated with boating (hydrocarbons and antifouling paint applications) or chemical-based fishing (e.g. cyanide, [27]). Oceanic waste disposal, ship groundings and incidental spills are additional routes of chemical pollution. Coral reefs are often in close proximity to shipping lanes where contaminated bilge water is disposed of or cargo is spilled. Dredging of channels can resuspend metals and other buried organic pollutants [28].

Table 1: Main contaminants, sources and concerns in regards to tropical coral reefs

Contaminant group	Representatives	Sources	Main concerns
Insecticides	DDT	Agricultural & urban runoff	Survival, reproduction, early life transitions & genetic effects. (Bioaccumulation for persistent OC pesticides)
	Dieldrin		
	Chlorpyrifos		
	Carbaryl		
	Permethrin		
Herbicides	Diuron	Agricultural & urban runoff, antifouling applications, ballast water discharge	Photosynthesis & calcification
	Atrazine		
	Hexazinone		
	Glyphosate		
Antifouling agents	Irgarol-1051	Shipping activities & marine structures	Photosynthesis & calcification Survival, reproduction, early life transitions & genetic effects
	Zn-pyrithione		
	TBT		
Industrial OCs	Dioxins	Thermal processes (atmospheric deposition) & terrestrial runoff	Bioaccumulation, reproduction in birds & mammals, metabolism & genetic effects
	PCBs		
	Furans		
Oil products & PAHs	Often unspecified mixtures	Shipping operations, industrial discharge, oil exploration, mining activities & spills	Bioaccumulation, survival, reproduction, metabolism, growth & genetic effects
Metals	Copper	Agricultural runoff, various urban and industrial sources, oil explorative activities & antifouling applications	Bioaccumulation, survival, reproduction, growth & behaviour
	Zinc		
	Mercury		
	Cadmium		

STRESS EVALUATION

Coral reef ecosystems are composed of a diverse community ranging from algae to mammals interacting in a variety of ways on multiple hierarchical levels. All reef organisms are likely to vary substantially in their sensitivity and response to individual pollutants or combinations thereof. Furthermore, susceptibility of species and possible stressor interactions may differ considerably depending on the life stage of exposed organisms. Even though reef-building corals are only one component of the ecosystem, they are considered fundamental for its existence and hence in this review we will concentrate on available knowledge and key questions related to potential effects of pollutants on reef-building corals and their endosymbionts.

The delicate interaction between coral host and *Symbiodinium* sp. is particularly vulnerable to water quality declines and stress from high sea surface temperatures and high light intensities [3, 12]. From a toxicological point of view, chemicals can affect the host animal, the algal symbionts, or both. While contact of host with contaminants is often direct, multiple membranes need to be crossed before a chemical can reach the intracellular algae. Studies in the past have suggested physiological differences between reef building organisms may determine their susceptibility to chemically induced stress. For example, branching coral species seem more vulnerable to some chemical contaminants than massive corals [29] and small-polyped species seem more susceptible to pollution stress than large-polyped corals [30]. Orientation, growth form, life stage, reproduction strategy, mucus production and lipid content are all factors that will determine how easily survival of a coral species is influenced by chemical stress [1]. A wide range of laboratory assays have been used to evaluate effects of chemical pollutants on corals over the past four decades. Table **2** provides an overview of assays used to evaluate stress in multiple life history stages of hard corals.

Prior to 2000 most studies focussed on the effects of metals, PAHs and older organochlorine pesticides on corals (for reviews see [1, 53]). Due to increased application of modern pesticides and altered pollution profiles, up to date information is now required on the distribution and impact of contemporary pollutants on coral reefs.

Table 2: Examples of bioassays and biomarkers used to assess the effects of contamination on scleractinian corals.

Life stage	Host or symbiont	Assay description	Reference(s)
Adult	Host & symbiont	Quantification of bleaching	[13, 31, 32]
	Host	Calcification rate	[33, 34]
		Lipid content	[31]
		Reproductive output	[31]
		Photopigment composition	[31]
Adult	Symbiont	Respirometry	[35, 36]
		Primary production	[35]
Adult and isolated symbionts	Symbiont	^{14}C fixation	[34]
Adult and juvenile	Symbiont	PAM fluorometry to estimate electron transport in photosystem II	[14, 31, 37-39]
Gametes	Host	Fertilization success	[13, 14, 40, 41]
Larvae	Host	Larval settlement and metamorphosis	[13, 14, 42-44]
Adult	Host & symbiont	Gene expression (various genes)	[45-49]
		Stress protein analysis: e.g. MnSOD, GPx, GST, HSPs, ubiquitin	[50-52]

The purposes of toxicity testing are to: (I) determine cause and effect relationships; (II) determine thresholds for lethal and sub-lethal effects to enable the derivation of water quality guidelines; and (III) to use this information in combination with exposure concentrations to evaluate and compare hazards and ideally determine the magnitude of risk potential and mitigation options. While the majority of toxicological data for most chemical contaminants originates from temperate studies and species, in the past decade there has been an increase in the number of studies examining the effects of relevant contaminants on tropical marine species, including corals. A number of assays have been developed to evaluate sublethal effects of chemical pollution on scleractinian corals. These are molecular, cellular and physiological diagnostic indicators that provide a means of assessing both qualitative and quantitative responses to a variety of pressures, individually and collectively. Organisms respond to environmental changes by regulating metabolic pathways to prevent physiological damage. These expressions precede population-level changes and are useful indicators if linked to specific physiological or ecological events [54].

Often a combination of pressures can result in mortality or impaired biological function. Thus, the evaluation of environmental effects on biological systems associated with anthropogenic pressures such as pollutants must begin with understanding causal linkages between stressor and effect [45]. For adult corals, biomarkers of effect include molecular (e.g. gene regulation), biochemical (e.g. lipid content), physiological (e.g. reproduction, calcification) and ecological (e.g. bleaching due to loss of symbionts, distribution) endpoints. The effects of contaminants on zooxanthellate photosynthesis have been assessed using respirometry, ^{14}C fixation and pulse amplitude modulated (PAM) fluorometry [55]. A technique is based on fluorescence quantum yield measurements as a function of photochemical efficiency of photosystem II (PSII) in phototrophic organisms, which can be applied *in situ* or in the laboratory (for a technical review, see [56]). The degree of quantum yield inhibition may be directly correlated to the stressor concentration the evaluated organism is exposed to [57].

Previous studies have demonstrated that different stressors may trigger similar responses through alternating pathways. For example, exposure to metals [32, 58], cyanide [27], herbicides [14], elevated temperature [59, 60] and ocean acidification [61] can all induce bleaching. It is also possible that a single stressor induces a variety of responses that can only be detected using an assortment of different biomarkers [62]. Molecular analysis of gene expression can evaluate the relative impact of stressors, by identifying specific responses to individual stressors [49, 54, 63]. The field of transcriptomics identifies transcription of certain genes at a given time and allows monitoring

of gene expression which in turn can identify gene function and mechanisms behind a particular biological response [47, 64], although establishment of a causal link between genomic and physiological responses has often proved difficult. In effect, while physiological or ecological endpoints indicate stress effects at organismal, population or even ecosystem levels, genetic tools may reveal subtle and specific effects at a genomic level, preceding physiological responses such as coral bleaching or impaired photosynthesis.

CHEMICAL STRESSORS ON CORAL REEFS

In this section we separately investigate organic biocides and industrial pollutants, metals and oil hydrocarbons. We will discuss pathways of exposure and summarise known effects on scleractinian corals. Finally, we will describe ecological impacts associated with these contaminants and evaluate risk posed to coral reef communities.

Pesticides and Antifouling Agents

The second half of the 20th century has seen a massive increase in population growth and a corresponding expansion of world food production. The acceleration of agricultural output can partly be ascribed to the introduction of organochlorine (OC) pesticides such as DDT, which eventually allowed for the shift from classical small-scale farming to industrialised agriculture. The use of pesticides in combination with fertilisers has been growing steadily and has become an essential element of modern agriculture, by preventing the spread of pests and exotic species and by enhancing growth potential, resulting in dramatic increases in crop yields. By the late 1960s environmental drawbacks arising from the use of DDT for agricultural purposes, or as a vector of disease control, became apparent and usage of selected persistent OC pesticides (e.g. DDT, HCB, dieldrin and chlordane) was slowly phased out in most developed countries. Other classes of pesticides such as organophosphates, carbamates, organotins and pyrethroids soon filled these roles. Today a wide variety of pesticides are used in specialised farming globally, with enormous annual application rates (see Chapter 4).

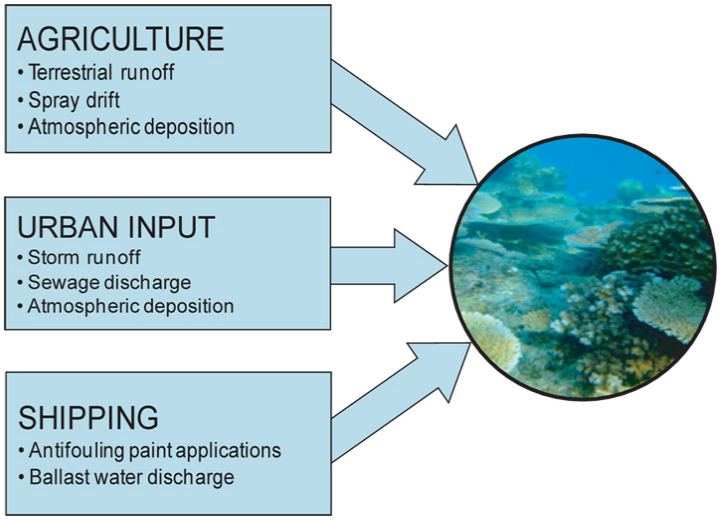

Figure 3: Sources of pesticides reaching coral reef sites.

Modern pesticides generally exhibit shorter half-lives in the environment when compared with their predecessors. Improved usage patterns and progressive application techniques, alongside highly specialised modes of action, make unintended side-effects to non-target species considerably less of a concern. However, care must be observed. The economies of countries in tropical areas are often based on agriculture, and depend on intensive use of pesticides to maintain and improve production [65]. As agricultural activities are primarily concentrated in river valleys and coastal plains, it is not surprising that agrochemical residues originating from terrestrial applications are ubiquitous in streams and rivers, eventually draining into estuaries and coastal seas [23, 66]. Multiple studies have shown that the main source of pesticides in nearshore coastal areas is agricultural application, transported via terrestrial runoff (especially during the monsoon season) [23, 67, 68]. The effects of pesticide residues are of great concern as many of these compounds and their breakdown products have been reported to influence both human and environmental health. It is

therefore vital to characterise transport and fate of pesticides and their toxicity to non-target organisms to confidently assess risk associated with application, especially in tropical areas where pesticide usage patterns are generally much higher than in temperate zones [65]. Fig. (**3)** provides an overview of sources of biocides reaching reef sites.

In addition to terrestrial sources, biocides are intentionally released into the marine environment by incorporation into antifouling paint formulations where they function to prevent unwanted growth of a wide range of algae and invertebrates on boats or other marine structures. The organometallic biocide tributyltin (TBT) proved the most effective antifouling ingredient in the latter half of the 20th century and is a common contaminant in harbours globally. Although normally uncommon on coral reefs, TBT as well as metal biocide 'boosters' have been identified in extreme concentrations on reef rubble following ship groundings [69, 70]. The use of TBT has been regulated internationally since 1990 due to recognition of its severe impact on aquatic ecosystems. In 2003 the International Maritime Organization (IMO) and their Marine Environment Protection Committee (MEPC) banned application of TBT as an antifouling agent on ships completely. As of 2008, TBT-based antifouling paints must be removed or covered with a sealer-coat [71]. Since the phasing out of TBT in antifouling paints alternative coatings have been developed often using copper or zinc in combination with an organic booster biocide. These are typically herbicidal in nature, as primary colonisation of hull surfaces by microalgae allows for a convenient base for subsequent attachment and growth of seaweeds and invertebrates. This may result in elevated environmental concentrations in areas of high yachting activity, particularly in and around harbours [72]. Studies have shown that biocide dissipation from hulls of vessels is a function of leaching and degradation rates, water movement, sorption and hull treatment amongst other parameters [73]. The same studies have suggested two of the most popular booster biocides in use, diuron and Irgarol 1051, to persist in the water column while other booster biocides disappear more rapidly [73]. Although some data are available on biodynamics of antifouling agents in temperate regions, there is little comparable information on bioavailability in tropical waters.

Until 2000, most studies on pesticides in tropical marine ecosystems focussed on OC compounds, as these persistent chemicals have dominated the pesticide market for decades in tropical areas. Studies also focused on estuaries, as this is likely to be where most terrestrially derived sediments settle and therefore where most concentrated reservoirs of persistent chemicals will be detected. Furthermore, studies focused on commercial species such as fish that may affect human health. Apart from a few studies performed in the Florida Keys [74-76], Bermuda [34] and the Philippines [77], most available data on pesticide dynamics on coral reefs are associated with the Great Barrier Reef. The limited studies assessing banned persistent organochlorine substances on coral reefs found concentrations to be generally low [74-76, 78] or declining [53, 79]. While organochlorine residues (e.g. endosulfan) have been measured in recent times [78-80], it is the current generation of pesticides that are of greatest concern to inshore marine ecosystems. Organophosphorous (OP) insecticides such as chlorpyrifos, or herbicides such as glyphosate (Round-up®); triazine herbicides such as atrazine, simazine, ametryn and Irgarol 1051; and urea herbicides such as diuron and tebuthiuron are some of the key pesticides that may affect coral reef biota [23, 67, 81].

Recent studies have found contemporary herbicides to be ubiquitous in waters nearby coral reefs [23, 34, 68, 81, 82]. Systemic herbicides are of particular ecological concern to coral reef systems, as these compounds are developed for quick environmental uptake through the root system of plants and are therefore relatively water soluble. A chemical's solubility will determine whether it mainly exists in the water column or is associated with suspended particulate matter, and therefore more likely to sink to the bottom. In contrast to the latter fraction that precipitates nearshore and becomes incorporated in the sediment, chemicals dissolved in the water column can travel greater distances and exert adverse effects far from their application sites. Thus, apart from a greater potential to reach reef sites, herbicide pollution can have severe consequences for ecosystems dependent on primary production. Herbicides target a wide range of physiological processes; however the herbicides most commonly detected on coral reefs are the photosystem II (PSII) herbicides (Table **3**). This class of herbicide acts by inhibiting electron transport through the photosystem in chloroplasts by reversibly binding to a specific electron-acceptor protein (D1-enzyme in PSII). These herbicides outcompete the normal ligand for binding sites on this highly conserved protein vital for plant photosynthesis [83]. The D1-enzyme also forms part of the photosystem in symbiotic zooxanthellae of corals and is likewise affected by herbicide exposure. As far as the holobiont (host combined with symbiont) is concerned, direct effects of herbicide exposure are a decrease in algal photosynthetic efficiency, limiting energy flow from symbiont to host [84]. Secondary effects of restricted electron flow in PSII include a build-up in reactive oxygen leading to oxidative stress [85], a process intensified by high illumination [86]. Further effects include disruption of

membrane structure and chlorosis, as well as aberrations in energy dynamics due to the reduced availability of photosynthetic products, ATP, NADPH and ferredoxin [87]. As inhibition of photosynthesis can lead to decreased algal production and eventually expulsion of symbionts (bleaching), herbicide contamination may disturb the fragile keystone algal-coral relationship so important for all ecological processes on coral reefs.

Impact

Table **3** provides an overview of studies on pesticide effects on hard corals. Little is known regarding the effects of OC pesticides on reef building corals. Suspected adverse effects caused by OCs range from carcinogenesis, interruption of neurological function, changes in cell metabolism and gene expression, to endocrine disruption and interference with reproduction [78].

In an early study on organochlorine pollution, McCloskey & Chesher [89] observed photosynthetic depression in a number of scleractinian corals after *in situ* exposure with DDT, dieldrin and a PCB with concentrations in the mg/L range. However, even these extremely high concentrations did not result in alterations of feeding behaviour, polyp expansion, sediment clearing or skeletal crystal formation. Olafson [90] explored bioaccumulative potential of OCs in reef biota and found DDT, chlordane and lindane able to accumulate in coral tissues. In a recent study assessing effects of short-term exposure (up to 96 h) to low insecticide concentrations on different life history stages of the branching coral *Acropora millepora*, endosulfan (OC), chlorpyrifos and profenofos (OPs) were found to affect photosynthetic performance and/or density of zooxanthellae within adult branches at relatively high concentrations [13]. In addition, profenofos-exposed branches expressed permanent tissue retraction. These neurotoxic insecticides did not inhibit fertilisation of gametes as may be expected from the absence of neurons in oocytes and sperm, yet larval metamorphosis was heavily impacted, with 50% effect concentrations (EC50) for inhibition of metamorphosis as low as 0.3-1.0 µg/L [13]. This study found the swimming behaviour of the larvae was not affected by the insecticides, an observation confirmed by Acevedo [91], who demonstrated that far greater concentrations of chlorpyrifos and carbaryl were required (mg/L) in order to cause mortality amongst larvae of the brooding coral *Pocillopora damicornis*. These findings suggest that the mode of impact of investigated insecticides in coral larvae involves specialised pathways instead of general neurotoxicity. In contrast, another study on adult colonies of *P. damicornis* demonstrated 50% mortality (LC50) to occur after 96-h exposure to 6 µg/L chlorpyrifos [92]. While most insecticides tested appear to negatively affect corals or their larvae, a recent study indicates that the "eco-friendly" larvicidal agent, *Bacillus thuringiensis* ssp *israelensis* (Bti), used extensively to control mosquitoes in the tropics, is harmless to coral larvae [93].

Herbicides readily penetrate coral tissues and rapidly (within minutes) reduce the photosynthetic efficiency of the endosymbiotic zooxanthellae. No apparent acute effects have been observed on host animals, fertilisation of gametes or metamorphosis of larvae after short-term exposures to diuron [14]. Nonetheless, bleaching of established recruits or adult coral branches is a common reaction to high concentrations or chronic exposures of PSII herbicides [14, 32, 94]. The dissociation of symbiosis is considered a sub-lethal stress response and a secondary effect most probably caused by oxidative stress in zooxanthellae as a result of chronic photoinhibition. Although the mechanism is still not entirely understood, the main hypothesis is that by expelling symbionts, host corals can reduce the number of damaged symbionts within their tissue, while at the same time limit their exposure to reactive singlet oxygen [86, 94].

In a study on the effects of the antifouling herbicide Irgarol 1051, Owen and co-workers [34] found isolated *in vitro* symbionts of *Madracis mirabilis* to cease incorporation of $H^{14}CO_3^-$ after 4-8 h exposures to concentrations as low as 63 ng/L. The effects of PSII herbicides on coral symbionts has flow-on effects to the host, which suffers a proportional decrease in the energy translocated as sugars to the animal tissue [84] and this can lead in the long term to reduced reproductive output [31]. Jones [86] reviewed the toxicological effects of various PSII herbicides on a range of coral life history stages and isolated symbiotic algae and argued the most sensitive endpoint to be inhibition of photosynthesis in algal symbionts. Overall, in directly comparable exposure experiments using adult branches of multiple coral species, concentrations of tested herbicides reducing effective quantum photosynthetic yield (10-h EC50) of symbiotic algae *in hospite* ranged over three orders of magnitude (Fig. **4**), while significant reductions in photosynthetic yields were observed at concentrations as low as 50 ng/L (Irgarol 1051), 200 ng/L (diuron) or 300 ng/L (ametryn) [38, 39, 86]. These outcomes were supported by tests on isolated symbionts [88] and can be directly compared with toxicological results on other aquatic primary producers, including tropical estuarine microalgae [95], seagrass [96], crustose coralline algae [97], marine diatoms [98] and freshwater algae [99]. Conversely, recovery of normal photosynthetic rates occurred quickly after placement in clean exposure medium [38, 39]. This would suggest short-term exposure to PSII herbicides inflicts no permanent damage.

Table 3: Toxicological studies on the effects of selected pesticides on different life history stages of scleractinian corals

Contaminant	Life stage	Toxicological endpoint[1]	Toxicological data (References)[2]
Herbicides			
Diuron	Adult	Bleaching	[39, 86]
		Symbiont density	[31]
		PSII inhibition	[14, 31, 38, 39, 77]
		Photopigment composition	[31]
		Lipid content	[31]
		Respiration	[77]
		Fecundity	[77]
	Juvenile	Bleaching	[14]
		Tissue retraction	[14]
		PSII inhibition	[14, 84]
		Energy acquisition	[84]
	Isolated symbionts	PSII inhibition	[39]
		$H^{14}CO_3^-$ incorporation	[88]
	Larvae	Metamorphosis	[14]
	Gametes	Fertilisation	[14]
Atrazine	Adult	PSII inhibition	[39]
	Isolated symbionts	$H^{14}CO_3^-$ incorporation	[88]
Irgarol 1051	Adult	Bleaching	[39]
		PSII inhibition	[39]
		$H^{14}CO_3^-$ incorporation	[34, 88]
	Isolated symbionts	$H^{14}CO_3^-$ incorporation	[88]
Simazine	Adult	PSII inhibition	[38]
	Isolated symbionts	$H^{14}CO_3^-$ incorporation	[88]
2,4-D	Adult	Tissue damage	[74]
		PSII inhibition	[77]
		Respiration	[77]
	Isolated symbionts	$H^{14}CO_3^-$ incorporation	[115]
Ametryn, hexazinone, Tebuthiuron, Ioxynil	Adult	PSII inhibition	[38]
Insecticides			
Endosulfan, chlorpyrifos, profenofos, carbaryl, permethrin	Adult	Symbiont density	[13]
		PSII inhibition	[13]
	Larvae	Metamorphosis	[13]
	Gametes	Fertilisation	[13]

1. For methodology and exposure durations please refer to text or original publications

2. For quantitative toxicological data please refer to text or original publications

However, chronic exposure experiments of coral branches to low (1-10 µg/L) diuron concentrations showed, apart from decreased photosynthetic rates, severe visible bleaching, partial colony mortality, two to five fold reductions in tissue lipid content and significant reductions in fecundity [31]. In laboratory exposures, the widely used non-PSII herbicide 2,4-D (a growth inhibitor) failed to exert any effect on colour, tissue extension, photosynthesis and metabolism of adult corals except in extremely high concentrations (100 mg/L) [74, 77]. When exposed to a formula containing a 2,4-D amine salt including a 'wetting agent' (dispersant) though, severe toxic effects were observed at 100 µg/L [74].

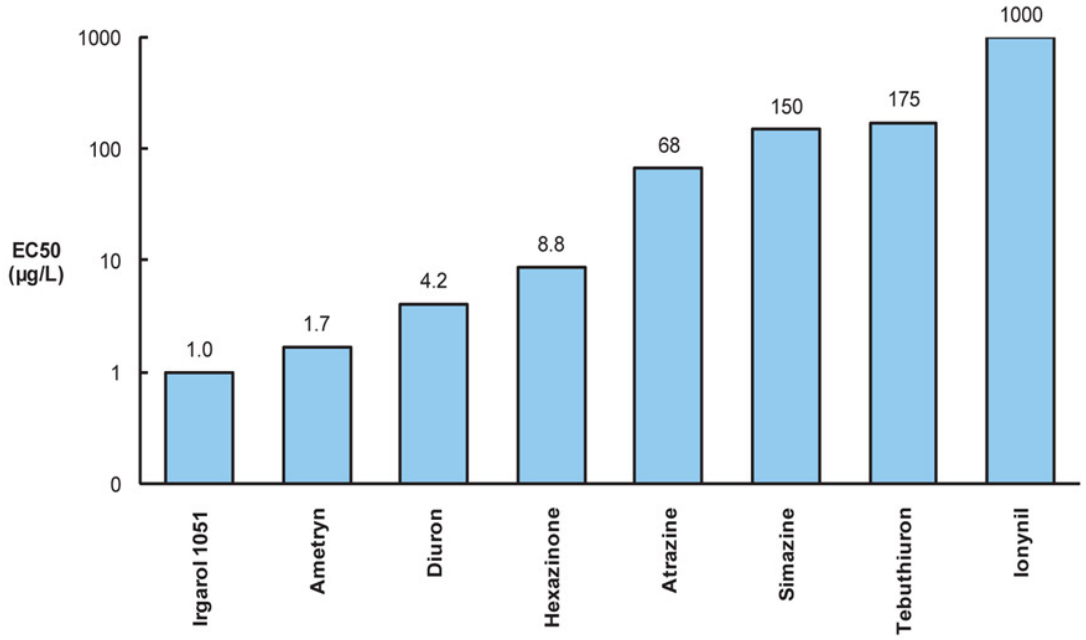

Figure 4: EC50 for several herbicides impacting effective quantum PSII yield of zooxanthellae symbionts in tissues of different coral species. Lower values indicate greater toxicity with susceptibility ranging over three orders of magnitude. Data after [38, 39, 86].

The historic focus on persistent pesticides has found that the key concern is associated with accumulation in the food chain and potential effects in air-breathing top predators. A key finding in the last decade relates to PSII herbicides. There is increasing evidence of widespread relatively low level exposure of inshore reefs (e.g. on Australia's GBR) to these chemicals. Concentrations are typically below levels at which adverse effects may occur on photosynthesis (e.g. diuron <20 ng/L). However, during major flow events concentrations have been determined in flood plumes near inshore reefs that are sufficiently high for effects to be detectable (diuron 0.1-1 µg/L) [23]. Furthermore, as flood plumes often carry elevated concentrations of several pollutants simultaneously, it is likely that nearshore reef systems are exposed to combinations of chemical stressors. Herbicides are commonly detected in complex mixtures within fresh and seawater systems. As multiple factors potentially interfere with the same physiological mechanism (e.g. PSII electron flow), additive or even synergistic toxic effects may occur [100]. A recent study has confirmed that the phytotoxicity of PSII herbicides commonly detected in GBR waters towards benthic microalgae is additive [101]. Overall, the margin of safety between observed concentrations and measurable effects is relatively small and the potential risks from chronic exposure remain unclear. Herbicide-induced interference with primary producers may exert a bottom-up pressure on the system, potentially decreasing reef resilience to other environmental stressors as elevated temperatures and ocean acidification.

Industrial Organochlorines: PCBs, Dioxins and Furans

Polychlorinated dibenzodioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) are ubiquitous organic contaminants. This group of chemicals is extremely persistent, has a tendency to bioaccumulate in biotic tissues and includes some of the most toxicologically potent compounds known (see Chapter 7). Although a global treaty aimed at the reduction and possible elimination of PCBs and dioxin-like chemicals has been established in 2001, significant concentrations are still found in marine environments worldwide. Evidence exists that distribution of PCBs, PCDD/Fs into the aquatic environment is mainly due to atmospheric deposition of volatilised molecules [102]. Despite many studies in temperate and arctic regions and extensive reporting on the concentrations and some effects of PCBs and PCDD/Fs in tropical marine species including mammals [103-105], fish [106, 107] and invertebrates [79, 107], to our knowledge no studies have been performed assessing concentrations in tropical waters or on coral reefs and effects on reef-building corals remain speculative. Dioxin-like substances bind to the aryl hydrocarbon receptor in vertebrates and invertebrates, resulting in interference with a broad range of cellular processes. Concentrations of these compounds in water are presumably too low to cause direct effects on primary producers and environmental impact is mostly

associated with bioaccumulation, affecting air-breathing organisms at the top of the food chain. Therefore, likely ecological impacts of dioxin-like pollutants on coral reefs will consist of top-down imbalance of the system.

Oil Hydrocarbons and PAHs

Crude oil, refined petroleum and their combustion products all contain aromatic hydrocarbons, including some polycyclic aromatic hydrocarbons (PAHs). These compounds enter the marine environment through anthropogenic processes or have naturally occurring sources (Table **1**) [53]. A study by Capone and Bauer [108] suggested that in the late 1980s an estimated average of 6 million metric tons of petroleum products were released into our oceans annually. As most petroleum products are hydrophobic in nature, the majority of aromatic hydrocarbons introduced into the marine environment will associate themselves with particulate matter and be deposited in the sediment [108], where these compounds tend to persist. Benthic filter feeders or sessile organisms are at risk through direct contact or ingestion of oil compounds. Straughan [109] argued the biological consequences of oil spills should be determined by the nature and interaction of a multitude of factors, including type of oil, dosage, remedial action, prior exposure, presence of other stressors, differences between biota and many physical environmental, climatic and seasonal factors. Generally, a mixed product containing a broad spectrum of hydrocarbons is released to the marine environmental where it may affect a variety of biological processes [108].

Table 4: Studies on the effects of selected oil products on different life history stages of scleractinian corals.

Contaminant	Life stage	Toxicological endpoint[1]	Toxicological data (References)[2]
Crude oil	Adult	$H^{14}CO_3^-$ incorporation	[110]
	Larvae	Metamorphosis	[42]
	Gametes	Fertilisation	[42]
Dispersed crude oil	Larvae	Metamorphosis	[42]
	Gametes	Fertilisation	[42]
Fuel oil 467	Gametes	Fertilisation	[120]
Dispersed fuel oil	Adults	Tissue damage	[121]
	Gametes	Fertilisation	[120]
2-stroke oil (vegetable)	Adult	Bleaching	[122]
		PSII inhibition	[122]
	Gametes	Fertilisation	[122]
2-stroke oil (mineral)	Adult	Bleaching	[122]
		PSII inhibition	[[122]
	Gametes	Fertilisation	[122]
Product formation water (PFW)[3]	Larvae	Metamorphosis	[42]
	Gametes	Fertilisation	[42]
	Symbionts	PSII inhibition	[123]
Drilling mud[3]	Adult	Mortality	[124]

1. For methodology and exposure durations please refer to text or original publications

2. For quantitative toxicological data please refer to text or original publications

3. PFW and drilling mud often contain toxic concentrations of both metals and hydrocarbons

The potential of oil contamination to coral reefs is high given their often intense commercial activity and proximity to shipping lanes [1]. Acute exposure of reef ecosystems to oil spills can occur through accidental discharge from ships or as a result of terrestrial runoff. Production platforms in the vicinity of coral reefs have the potential to contaminate surrounding waters through discharge of production formation water (PFW) [42], a complex mixture that may contain petroleum hydrocarbons, suspended solids, metals, naturally occurring radioactive materials, organic acids and inorganic ions amongst other substances [110]. Likewise, the drilling of oil-wells may introduce mud heavily contaminated with petroleum hydrocarbons and metals to the marine system, while refineries and pipelines are additional sources with potential for chronic pollution by oil products. Floating oil can be deposited on reef flats or to interfere with reproductive processes in buoyant gametes or larvae [111]. Large oil spills are often moderated by application of surface dispersants that dissolve slicks into smaller droplets. However, application of

dispersants is likely to increase hydrocarbon concentrations in the water column and thus increase exposure to benthic reef organisms [42, 112]. Several studies have demonstrated that a combination of dispersal and oil products expressed higher toxicity than either component separately [42, 113, 114], yet it has been suggested modern dispersants may be less toxic to marine biota [1]. Once in the water column petroleum hydrocarbons rapidly become associated with organic matter and suspended particles. Volatile components evaporate while non-volatile components are deposited into the sediment. This deposited fraction is unlikely to absorb, evaporate, dissolve or be biologically degraded [53, 108]. Oil products may also occur in globulised form dispersed through the water column and can settle onto the reef [115]. Aromatic hydrocarbons, either in the form of dispersed oil or as water soluble components, can be absorbed by coral tissues while oil globules can adhere to coral surfaces [116]. The high lipophilicity of aromatic hydrocarbons stimulates rapid passive uptake in the coral tissue, while detoxification can be slow [114, 117]. Residues of aromatic hydrocarbons have been reported to remain present in coral tissues months after exposure occurred [118], yet evidence suggests hydrocarbon deposits in sediments and coral tissues to be substantially reduced after two years at high-energy reef sites [76, 119].

Impact

Adult coral colonies can be killed or injured by direct contact with oil or drilling mud [116, 124, 125]. Filter feeders and benthic organisms that cannot escape the oil or contaminated sediments will typically see bioaccumulation of toxic compounds, genetic mutations and metabolic disorder in their tissues. Corals exposed to hydrocarbons have been shown to exhibit loss of zooxanthellae (bleaching), impaired reproduction and tissue damage [116]. Field studies on mud discharges during oil well drilling found decreased coral growth rates in *Montastraea annularis* exposed to the fluids, while several years of exposure found 70 to 90% reduction in coral cover within one hundred meters from the drilling site [126]. These findings were supported by laboratory studies predicting decreased growth, metabolic aberrations and nutritional abnormalities [127, 128]. More recently, Raimondi and colleagues [124] observed tissue mortality in adult cup corals after exposure to drilling muds. Bak [129] observed decreased coral cover, diversity and local recruitment after chronic exposure to refinery petroleum on a Caribbean reef and argued chronic pollution to have more severe effects than single spills, yet comparable detrimental effects were observed after an acute major oil spill in Panama [130]. Guzmán and co-workers [130] also observed a strong correlation between injured corals and oil residues in sediment during five years of monitoring coral health after this spill. Aromatic hydrocarbons have been shown to decrease photosynthetic performance of dinoflagellate symbionts in some corals [113, 131]. Jones and Heyward [123] observed decreased photochemical efficiency and subsequent expulsion of zooxanthellae by host corals exposed to PFW as a result of photoinhibition in the symbionts. Freshly isolated symbionts were affected at slightly lower concentrations [123]. Tissue retraction as an environmental stress response has been observed after exposure to low concentrations of oil or dispersed oil, but normal tentacular expansion recovered within a week [114]. It has also be hypothesized that aromatic hydrocarbons may cause a reduction in coral tissue lipid contents, thereby limiting fat reserves necessary for increased mucus production or proliferation of mucus secretory cells [116, 118, 121].

Decreased reproductive success of both brooding and broadcasting corals after oil exposure has been observed in a number of studies. Two histological studies showed impaired gonadal development [121, 132], while in field studies infertility and decreased egg size as a result of increased injury occurrence were observed five years after exposure to a major oil spill [132]. Loya and Rinkevich [111] noted oil products could induce premature expulsion of larvae in a brooding coral. More recently, Negri and Heyward [42] suggested that early life stages may be more sensitive to pollution than established adult colonies. In annual mass spawning events, as experienced in the Indo-West Pacific or Australian waters, gametes of broadcast spawning coral species are released in the water during brief synchronised events. The highly buoyant eggs float to the surface where fertilisation occurs [133]. If the spawning event coincides with an oil spill, an entire year of reproductive effort is threatened while adverse environmental conditions endure with implications for larval settlement and parent colonies. A number of laboratory studies have reported a dose-dependent inhibition of fertilisation, metamorphosis and settlement of broadcast spawning scleractinian corals after exposures to oil and/or dispersant [42, 120, 134]. For example, crude oil inhibited larval metamorphosis at 82 μg/L and this was reduced to 33 μg/L in the presence of a non-ionic dispersant [42]. Despite these findings, effects of oil (whether or not dispersed) on gamete fertilization, embryogenesis, larval metamorphosis and settlement are unknown for the majority of both broadcast spawning and brooding species.

Marine pollution from PAHs and oil hydrocarbons is associated with localized events as runoff from urban centres, oil exploration and extraction activities and accidental spills. These chemicals are highly hydrophobic and therefore

contamination typically remains relatively confined, although spillage from offshore drilling operations can travel for great distances. Likely effects on coral reefs will consist of overall disturbance of biological homeostasis by exerting effects over multiple trophic levels. The greatest impacts on coral reefs are likely to occur if hydrocarbons come into direct contact with coral spawn during mass reproduction or at low tide on shallow reefs. Interactions with other environmental stressors are improbable, as effects from spills and shipping incidents often cause severe mortality and will overwhelm subtle adverse effects from other factors.

Trace Metals and Metalloids

Metals are a physical component of rocks and soils and enter the environment through natural weathering and erosion processes. Many metals are biologically essential, yet most have the potential to become toxic above certain threshold concentrations [135]. Industrial activities such as mining and smelting as well as agricultural applications (*i.e.* organometallic pesticides and fertilisers) and urban waste have substantially contributed to the release of elevated quantities of trace metals into the environment [53]. Even though recognition of toxic potential and legislation in the past have seen a great reduction in metal output, environmental contamination continues. Terrestrial runoff and sediment-bound transport through freshwater streams and rivers eventually delivers these contaminants to estuaries and inshore seas (Fig. **1**). Metals are strongly associated with particulate matter and therefore not usually directly available to aquatic biota. However, particulate metals in sediments can be solubilised by acidic juices in the gut of sediment-feeding organisms, and thus become available for accumulation in biotic matrices through passive uptake across permeable surfaces such as gills or the digestive tract [136, 137]. Biological availability and solubilisation rates of trace metals from particulate matter are dependent upon a variety of environmental variables, including sediment cation exchange capacity and organic content, dissolved oxygen concentrations, pH, salinity, temperature and redox potential amongst other factors [53, 137, 138]. Furthermore, remobilisation and resuspension of sediments may return metals to the water column [28]. High environmental metal concentrations are generally restricted to locations adjacent urban centres, industrialised areas or sites draining areas of intensive agriculture [139]. Trace metals and metalloids have a multitude of applications and sources, yet the most abundant metals entering the environment in elevated quantities as a result of agricultural activities are copper (Cu) and zinc (Zn), used as constituents of fertilisers or biocides; arsenic (As), cadmium (Cd) and mercury (Hg) as components of some fungicides. Lead (Pb), nickel (Ni), aluminium (Al), manganese (Mn) and iron (Fe) often enter marine waters as the results of mining activities (as do As and Hg), industrial or urban waste discharges and runoff. Tin (Sn) has generally been introduced into the environment as a biocide, principally as constituent of antifouling paint formulations, e.g. tributyltin (TBT) [139, 140].

Numerous studies exist on metal contamination and effect on corals [141-144]. In adult corals, metals might be absorbed and occur in various capacities. As early as 1971, Livingstone and Thompson [145] found trace metals to be incorporated into the aragonite (a carbonate mineral) of coral skeletons. Quantification of these built-in skeletal metals is currently used as a biomarker that reflects environmental conditions during the coral's lifetime [146-148]. Trace metals can also be found in skeletal cavities [149], integrated within the organic matrix of coral skeletons [150], or absorbed onto exposed surfaces of the skeleton [151]. Besides skeletal inclusion, several studies have demonstrated trace metals present in coral tissue [33, 152, 153]. Pathways through which corals absorb metals may vary. Brown and colleagues [151] found that corals retract their tissue in response to environmental stress, and thus may be more susceptible to direct uptake of metals by exposed skeletal spines. Another responsive action to physical or chemical stress is the excretion of high quantities of mucus with a high affinity to bind metals that may actively reduce metal uptake [127]. Additionally, it has been suggested corals are able to regulate internal metal concentrations through the physiology of their zooxanthellae endosymbionts. Studies with related symbiotic organisms like sea anemones indicated zooxanthellae to be responsible for the majority of metal uptake and accumulation [154]. In response to elevated metal concentrations, zooxanthellae can enhance calcification rates. Furthermore, zooxanthellae may be involved in the active uptake of trace metals, accumulating higher concentrations of metals than do host coral tissues [58, 143, 145, 155]. Subsequent stress-induced expulsion of symbionts by the coral host may act as a regulatory response mechanism in reaction to high metal concentrations [32].

Impact

Bioavailability, physiological effects and fate of trace metals are highly dependent on the chemical form and oxidation state in which metals exist, as reflected by their toxicity [164]. Thus, it is of clear importance to distinguish between

individual metal species present in a particular biological compartment [165]. Once introduced in a biotic matrix, trace metals have the potential to affect nutrient cycling, cell growth and regeneration, as well as reproductive cycles and photosynthetic potential [1, 53]. Table 5 summarises a range of effects on corals caused by exposures to metals and organometallic compounds. Elevated levels of copper, zinc and tin in the effluent of a tin smelter in Thailand caused reduced growth and calcification rates in branching corals [33]. In a study considering corals in a Hong Kong estuary exposed to elevated concentrations of metals, pesticides, nutrients, sewage effluents and suspended sediments over a prolonged period of time, it was argued metals were mainly responsible for declines in coral cover, diversity, abundance and growth rates [30]. Laboratory exposure of the massive coral Porites lutea to elevated iron concentrations resulted in bleaching. It was noted that corals that had been pre-exposed to an iron-enriched environment responded in a less drastic way, suggesting development of some form of iron tolerance [58]. Jones [32, 94] found elevated copper concentrations to induce rapid bleaching in the branching corals Acropora formosa and Seriatopora hystrix, while no inhibition of photosynthetic efficiency of zooxanthellate endosymbionts was observed. The author suggested copper-induced bleaching to occur without affecting the algal photosynthesis but may be related to effects on the host coral. However, in a longer term exposure experiment on Plesiastrea versipora, low concentrations of copper were observed to reduce symbiont response to a host signalling factor regulating photosynthesis, while stress responses as inhibition of photosynthetic efficiency or bleaching were not detected [166]. To further emphasise the toxic effects of copper, two separate studies showed how copper has a detrimental effect on the metabolism of both the branching coral Pocillopora damicornis and the massive coral Porites lutea [35, 167].

Table 5: Toxicological studies on the effects of selected trace metals and metalloids on different life history stages of scleractinian corals

Contaminant	Life stage	Toxicological endpoint[1]	Toxicological data (References)[2]
Trace metals			
Copper	Adult	Mortality	[32, 156]
		Bleaching	[32, 157, 158]
		PSII inhibition	[86]
	Larvae	Mortality	[159]
		Metamorphosis	[44, 160]
	Gametes	Mortality	[144]
		Fertilisation	[40, 144, 160, 161]
Zinc	Gametes	Fertilisation	[40, 161]
Cadmium	Gametes	Fertilisation	[40]
Nickel	Larvae	Mortality	[162]
		Settlement	[162]
Iron	Adult	Bleaching	[58]
Organometallic substances			
TBT	Adult	Mortality	[156, 163]
		Bleaching	[163]
		PSII inhibition	[163]
	Juvenile	Mortality	[163]
	Larvae	Metamorphosis	[43, 160, 163]
		Settlement	[43]
MEMC	Adult	Symbiont density	[13]
		PSII inhibition	[13]
	Larvae	Metamorphosis	[13]
	Gametes	Fertilisation	[13]

1. For methodology and exposure durations please refer to text or original publications

2. For quantitative toxicological data please refer to text or original publications

In a recent study on the effects of an organometallic fungicide containing mercury on different life history stages in *Acropora millepora*, 2-methoxyethylmercuric chloride (MEMC) severely affected adult branches exposed to 10 µg/L MEMC. Branches bleached and some host tissue died at this concentration, while at a lower concentration (1 µg/L) polyps retracted and photosynthetic efficiency decreased [13]. Early life history stages proved very sensitive to MEMC exposure. Lowest observed effect concentrations (LOECs) inhibiting fertilisation of gametes and larval metamorphosis were established at 1 µg/L MEMC (EC50 values of 1.7 µg/L and 2.5 µg/L, respectively) [13]. In a series of experiments assessing effect of trace metals on fertilisation and settlement success of selected coral species, copper was found to be a highly effective inhibitor of fertilisation in all species tested, with fertilisation rates dropping proportionally with increasing copper exposure concentrations (EC50 = 15-40 µg/L Cu). Zinc, lead and cadmium were much less potent. However, high interspecific variety in sensitivity was observed [40, 41]. In contrast to the high sensitivity of hard-coral gametes to copper exposure, gametes of the soft coral *Lobophytum compactum* exhibited a surprising resistance to copper toxicity (EC50 = 261 µg/L) [168]. Settlement of *Acropora tenuis* larvae was significantly reduced at concentrations of 42 µg/L Cu (48-h EC50 = 35 µg/L) [44]. Negri and Heyward [160] found fertilisation of gametes and larval metamorphosis of *Acropora millepora* reduced when exposed to low concentrations of copper and TBT. Copper proved the most potent inhibitor of fertilisation in this study (4-h EC50 = 17.4 µg/L), while TBT proved more toxic towards larval metamorphosis (24-h EC50 = 2 µg/L). The same study also showed that surfaces coated with antifouling paints containing copper or TBT to inhibit both fertilisation and metamorphosis [160]. These findings were confirmed by Victor and Richmond [144] who exposed *Acropora* gametes in Guam to low copper concentrations and calculated 50% inhibition of gamete fertilisation after 12-h exposure to 11.4 µg/L Cu. TBT is known to inhibit protein synthesis [169] and some cnidarians such as the sea anemone *Aiptasia palliida* have been shown to exhibit reduced resistance to infection and decreased zooxanthellae densities after chronic exposure to very low concentrations (0.05 µg/L) of TBT [170]. In another study involving antifouling paint contamination, sediment polluted with a mixture of TBT, copper and zinc, all components of commercial antifouling formulations used until recently and acquired at a ship grounding site in Australia, demonstrated potential to interfere with normal larval behaviour [43]. Modern antifouling formulations that contain organometallic components used as replacements for TBT are often not exempt from environmental impact; e.g. the biocide zinc-pyrithione (Zpt) is detrimental to embryonic development of both sea urchins and mussels [171]; however, no toxicological studies of this compound have been performed on corals. In a review by Reichelt-Brushett and McOrist [143] on trace metal contamination within corals from around the world it was made evident that, although high interspecific, regional and temporal variance was observed, environmental concentrations of selected metals (especially copper) were often near or exceeding concentrations proven to exert detrimental ecological effects on scleractinian corals.

Trace metal pollution is often limited to areas adjacent to urban and industrial centres or near river deltas. As metals are relatively immobile in the marine environment, adverse effects are likely to be exerted on a localised scale. Copper and organometallic substances containing tin or mercury are significantly more potent than other trace metals and affect a broad range of variables in a variety of species. Consequences for the system will consist of both bottom-up and top-down effects. Chronic, low-level metal contamination may decrease resilience of marine organisms to other environmental stressors such as elevated temperatures, ocean acidification and other chemical pollutants.

Interactions of Multiple Stressors

Nearshore marine pollution often occurs in combination with other natural and anthropogenic sources of stress for resident biota. A globally changing climate results in increasing sea surface temperatures and ocean acidification, arguably the most important factors when considering stress on coral reefs [3, 19, 35]. Moreover, in the tropics monsoonal rainfall delivers vast amounts of fresh water, nutrients and suspended sediments to estuaries and inshore reef systems [172]. At this time of the year seawater temperatures approach thermal tolerance limits for many coral species [6, 60]. Thus, during flooding events, inshore coral reefs can potentially face combinations of low salinity, high turbidity, nutrient and pesticide exposures during episodes of thermal stress. It is assumed that nearshore corals and other sensitive organisms may be at extreme risk to combinations of stressors but little research has been performed to test this hypothesis.

Of the studies performed on stressor interactions, most have dealt with temperature in combination with an additional stress factor. Temperature affects physicochemical properties of membranes, including permeability, fluidity and diffusion rates. This will have a likely effect on a chemical's toxicity that is dependent on target site delivery [38]. Simultaneously, temperature can affect toxin solubility, speciation and (bio)degradation rates but also an organism's sensitivity to a

particular chemical [164]. The PSII herbicide diuron was observed to take longer at 20 °C than at 30 °C to reach a similar response in *Seriatopora hystrix*, while a decreasing sensitivity to the herbicide was observed at higher temperatures [38]. Another good illustrative example has recently been provided in a study where high dissolved inorganic nitrogen (DIN) concentrations were correlated with a decreased resilience of corals to high temperature [173]. Wooldridge and Done [174] subsequently proposed how combinations of high temperature and high DIN concentrations work synergistically and may be a causative mechanism for large-scale coral bleaching. However, the multiple sources of stress (often simultaneous events), coupled with the great complexity of marine ecosystems and their high variability obscures the establishment of simple causal relationships between stressors and observed effects, which greatly complicates assessment of tolerance, resilience and ecological implications of stress [175].

SYNTHESIS

Tropical coral reefs are among the most biologically diverse and productive ecosystems in the world but their continued existence is threatened by a number of factors. Despite their extended geological subsistence, coral reefs appear very sensitive to changes in environmental conditions. Elevated ocean acidity and surface temperature, high turbidity and nutrient concentrations through terrestrial runoff and chemical pollution from various sources are the main pressures exerted on modern reef systems and among the proposed reasons for global coral reef declines [16, 19]. Community alterations or a shift from autotrophy to heterotrophy will eventually affect the entire reef community, and could possibly change the dominant ecological process from calcium carbonate deposition to erosion [176]. Pressures on coral reef ecosystems are likely to increase further as a result of expanding coastal agricultural practises and industrialisation, population growth and climate change. While limiting the effects of climate change is a global challenge, management approaches to minimise the effects of pollution pressures on nearshore coral reefs can contribute towards sustainable exploitation of our marine resources; curbed inflow of suspended sediments, nutrients and chemical stressors are potential means to protect our reef systems in a shifting environment [12, 177, 178]. We have shown substantial differences exist in sensitivity and response of scleractinian corals and their zooxanthellae symbionts to various types of chemical pollutants. Furthermore, susceptibility may differ considerably depending on its life history stage. Evaluation of environmental stress on coral reefs caused by chemical pollution is constrained by the limited number of assessment endpoints, implying further research is required on a broad range of species and life history stages.

Chemical pollutants can enter and affect a reef ecosystem in a number of ways. The type of exposure often determines the severity and scale of effects. Coral reef ecosystems may be chronically exposed to combinations of stressors at low concentrations, regular pulses of chemical stressors and/or be subject to acute exposures of specific chemicals at relatively high concentrations during an accidental pollution event (Table **6**). While accidental pollution events (e.g. oil spills) will most likely overshadow effects from other potential stressors and have profound effects at all trophic levels, they are often localised, not harming the overall structure and function of large ecosystems. In contrast, regularly recurring pollution events such as input from river floods or chronic pollution from land runoff (e.g. sewage treatment effluent or herbicides) is more likely to affect a larger area and exert subtle effects, driving the system towards genetic and ecological adaptability. In these scenarios, the combined effects of chemical mixtures may significantly increase total toxicity and associated risk.

Low-level chronic pollution and recurring pollution events may interfere with the resilience of lower trophic levels instead of directly impacting the ecosystem. Because adverse conditions persist for prolonged periods of time, fitness of species is affected in the long run and this may lead to an increased vulnerability to various other stressors, including those related to climate change.

Ecological risk assessment requires knowledge of the spatial and temporal distributions of key stressors in relation to the most vulnerable tropical species. Exposure is a function of the intensity (magnitude), timing, frequency and duration of adverse conditions. Thus, ecological exposure scenarios may become very complex, especially when multiple stressors are involved and exert pressures on interconnected biological compartments. With all relevant information taken into consideration, it is not likely that the chemicals discussed in this chapter will significantly impact reefs single-handedly as concentrations are generally low or localised. For example, a number of chemical pollutants have been identified at low concentrations on the GBR, including the herbicide diuron and the insecticide chlorpyrifos (both primarily used in coastal agriculture), TBT and copper (antifoulants) and PAHs from oil spills and boating/shipping activities. The toxic effect concentrations of these pollutants and their observed annual mean

(background) and peak event (e.g. oil spill, ship grounding, flood plume) concentrations can be combined as risk quotients (effect concentration divided by environmental concentration), providing semi-quantitative estimates of risk and safety margins for GBR species (Table **7**).

Table 6: Profiles and likely effect for three types of pollution scenarios on coral reefs.

	Scenario I: Chronic pollution	**Scenario II: Recurring pollution events**	**Scenario III: Accidental pollution events**
Description	Non-pulse	Regular pulse	Random pulse
Input pathways	Terrestrial runoff Atmospheric deposition	Flooding events	Accidents
Area of concern	Large	Medium-Large	Localised
Timeframe	Chronic long-term disturbance	Moderate transient disturbance	Major short-term disturbance
Recovery phase	No	Limited	Yes
Stressor interactions	Very important	Very important	Less important as event overshadows environmental factors (although recovery may be affected)
Likely effects	Subtle and broad effects driving adaptation Bottom-up consequences on a large scale	Localised mortality Subtle and broad effects driving adaptation Bottom-up consequences on a large scale	Localised mortality Whole foodchain affected, limited large scale effects
Pollutant type	Agrochemicals Metals Antifouling agents Pharmaceuticals	Agrochemicals Metals	Oil hydrocarbons PAHs Antifouling agents

Table 7: Concentrations of environmentally relevant chemical pollutants on the Great Barrier Reef (GBR) linked to effect

Chemical (most sensitive endpoint)	**LOEC (µg/L)**	**Background inshore GBR (µg/L)**	**Event inshore GBR (µg/L)**	**Risk quotient LOEC/ background[1]**	**Risk quotient LOEC/ event[1]**	**Exposure scenario[2]**
diuron (PSII inhibition)	0.3-1	0.001-0.020	0.1-1	>15	>0.3	I - II
chlorpyrifos (metamorphosis)	0.4	0.0004	0.0007	>1000	>570	I - II
TBT (settlement)	0.4-0.9		<3.6 µg/g (sediment)[3]			I - III
copper (fertilisation)	10-100	0.2[4]		>50		I - II
PAHs (fertilisation)	2-30		0.1-0.65		>3	I - III
Total oil hydrocarbons (fertilisation)	2-30		10-2000		>0.001	III

LOEC=Lowest observed effect concentration.

[1] Higher numbers indicate a greater safety margin.

[2] Exposure scenario is indicative of which scenario is more likely for a given type of chemical to be relevant in terms of risk: (I) chronic exposure, (II) recurring event-style exposure, (III) random event-style exposure (Table **6**)

[3] Concentrations of TBT are usually specified in µg/g sediment; to our knowledge no information is available for water concentrations found in Australia.

[4] Concentrations found offshore in northern Australia[179].

Few chronic pollutants on the GBR approach concentrations that may cause harmful effects to corals as indicated by high risk quotients (>10 for background concentrations) for diuron, chlorpyrifos and copper. Only during short term events, such as in river plumes, does the concentration of diuron become great enough to affect corals (risk quotient = 0.3). However, risks posed by mixtures of pollutants may well exceed those presented by individual chemicals.

Despite the relatively low risk associated with chemical pollution on the GBR, evidence is emerging that pollution reduces the resilience of corals and other organisms to global climate change [174] and more research is required to document the increased sensitivity of corals to pollutants at elevated temperatures or under more acidic conditions. Even though the GBR is one of the most highly monitored regions in the tropics, risk quotients can only be calculated for a small number of pollutant types as indicated by data gaps in Table **7**. This general lack of data is more extreme in other tropical regions where other pollutant types may be more relevant and stronger links between regional water quality monitoring and relevant toxicological testing is required. In spite of our growing understanding of the effects of chemical stressors on reef species, there are still gaps in our knowledge which complicate assessment of ecological significance. To characterise a risk to an ecosystem, all relevant information concerning exposure and effect needs to be evaluated, including concentrations and distribution patterns of chemicals. Extremely limited information is available for concentrations of chemical pollutants on coral reefs worldwide. Obtaining these data is difficult as the majority of tropical coral reefs are situated in developing areas and most available data originates from more developed areas such as Florida or the GBR, where usage patterns of pesticides and discharge of industrial and urban waste is likely to differ from pollution patterns in South-east Asia, the Pacific islands or Africa.

Research in the last two decades has identified that coral reefs are threatened by a variety of stressors including elevated ocean temperatures, ocean acidification, overfishing, nutrient input and turbidity and that these pressures vary considerably between regions. While overfishing and nutrification are currently considered as the most detrimental local stressors to coral reefs, current knowledge on the distribution and effects of chemical pollutants is too limited to assess how these compare with other stressors. While laboratory studies have identified toxic thresholds of corals and other tropical organisms to a widening range of pollutants, further monitoring and research needs to be undertaken in several key areas:

- Stronger efforts need to be made linking pollution monitoring and ecotoxicological studies to better assess risk.
- More studies are required on the subtle effects of chronic pollution (Scenario I) and how low levels of pollution may inhibit the recovery of coral reefs from disturbance.
- Further experimental work needs to be undertaken to better understand the potential interactive effects between combinations of pollutants in flood plumes during tropical monsoons.
- Identification of which pollutants in a region may be reducing the resilience of corals and other organisms to climate change is essential to effectively target and develop management policies that may improve the survival prospects of our tropical coral reefs.

ACKNOWLEDGEMENTS

This work was supported by the Australian Government's Marine and Tropical Sciences Research Facility, implemented in North Queensland by the Reef and Rainforest Research Centre Ltd. JvD received financial support from the University of Queensland. Entox is a partnership between Queensland Health and The University of Queensland.

REFERENCES

[1} Peters EC, Gassman NJ, Firman JC, Richmond RH, Power EA. Ecotoxicology of tropical marine ecosystems. Environ Toxicol Chem 1997; 16(1): 12-40.

[2] Jameson SC, McManus JW, Spalding MD. State of the Reefs: Regional and Global Perspectives. US Department of State, Washington, DC; 1995.

[3] Hoegh-Guldberg O. Climate change, coral bleaching and the future of the world's coral reefs. Mar Freshwat Res 1999; 50: 839-866.

[4] Veron JEN. Corals of the World. Townsville, Australia: Australian Institute of Marine Science; 2000.
[5] Muscatine L. The role of symbiotic algae in carbon and energy flux in reef corals. Coral Reefs 1990; 25: 1-29.
[6] Lesser MP. Experimental biology of coral reef ecosystems. J Exp Mar Biol Ecol 2004; 300(1-2): 217-252.
[7] Hallock P. Coral reefs, carbonate sediments, nutrients and global change. In: Stanley JGD, Ed. The History and Sedimentology of Ancient Reef Systems. New York: Kluwer Academic Publishing/Plenum; 2001. pp. 387-427.
[8] Lesser MP, Farrell JH. Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. Coral Reefs 2004; 23(3): 367-377.
[9] Richmond RH, Hunter CL. Reproduction and recruitment of corals: comparisons among the Caribbean, the tropical Pacific, and the Red Sea. Mar Ecol Prog Ser 1990; 60: 185-203.
[10] Harrison PL, Wallace CC. Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z, Ed. Coral Reefs (Ecosystems of the World, 25). New York: Elsevier Science; 1990. pp. 133-207.
[11] Heyward AJ, Negri AP. Natural inducers for coral larval metamorphosis. Coral Reefs 1999; 18: 273-279.
[12] Fabricius K, De'ath G, McCook L, Turak E, Williams DM. Changes in algal, coral and fish assemblages along water quality gradients on the inshore Great Barrier Reef. Mar Pollut Bull 2005; 51(1-4): 384-398.
[13] Markey KL, Baird AH, Humphrey C, Negri AP. Insecticides and a fungicide affect multiple coral life stages. Mar Ecol Prog Ser 2007; 330: 127-137.
[14] Negri A, Vollhardt C, Humphrey C, *et al.* Effects of the herbicide diuron on the early life history stages of coral. Mar Pollut Bull 2005; 51(1-4): 370-383.
[15] Bellwood DR, Hughes TP, Folke C, Nystrom M. Confronting the coral reef crisis. Nature 2004; 429: 827-833.
[16] Wilkinson C, Ed. Status of Coral Reefs of the World. Townsville, Australia: Australian Institute of Marine Science; 2004.
[17] Gardner TA, Cote IM, Gill JA, Grant A, Watkinson AR. Long-term region-wide declines in Caribbean corals. Science 2003; 301: 958-960.
[18] Anthony KRN, Connolly SR, Hoegh-Guldberg O. Bleaching, energetics, and coral mortality risk: effects of temperature, light, and sediment regime. Limnol Oceanogr 2007; 52(2): 716-726.
[19] Baker AC, Glynn PW, Riegl B. Climate change and coral reef bleaching: an ecological assessment of long-term impacts, recovery trends and future outlook. Estuarine Coastal Shelf Sci 2008; 80(4): 435-471.
[20] Brodie JE, Mitchell AW. Nutrients in Australian tropical rivers: changes with agricultural development and implications for receiving environments. Mar Freshwat Res 2005; 56: 279-302.
[21] Hughes TP, Baird AH, Bellwood DR, *et al.* Climate change, human impacts, and the resilience of coral reefs. Science 2003; 301: 929-933.
[22] Nash RG, Hill BD. Modelling pesticide volatilization and soil decline under controlled conditions. In: Kurtz DA, Ed. Long Range Transport of Pesticides. MI, USA: Lewis Publishers; 1990. pp. 17-28.
[23] Lewis SE, Brodie JE, Bainbridge ZT, *et al.* Herbicides: a new threat to the Great Barrier Reef. Environ Pollut 2009; 157(8-9): 2470-2484.
[24] Loya Y, Rinkevich B. Effects of oil pollution on coral reef communities. Mar Ecol Prog Ser 1980; 3: 167-180.
[25] USEPA. Report to Congress on implementation of section 403(c) of the Federal Water Pollution Control Act. Washington, DC.; 1990 Contract No.: EPA 503/6-90/001.
[26] Kookana RS, Baskaran S, Naidu R. Pesticide fate and behaviour in Australian soils in relation to contamination and management of soil and water: a review. Aust J Soil Res 1998; 36(5): 715-764.
[27] Jones RJ, Steven AL. Effects of cyanide on corals in relation to cyanide fishing on reefs. Mar Freshwat Res. 1997; 48: 517-522.
[28] Reichelt AJ, Jones GB. Trace metals as tracers of dredging activities in Cleveland Bay-Field and laboratory studies. Aust J Mar Freshwat Res 1994; 45: 1237-1257.
[29] Stebbing ARD, Brown BE. Marine ecotoxicological tests with coelenterates. In: Persoone G, Jaspers E, Claus C, Eds. Ecotoxicological Testing for the Marine Environment. Bredene, Belgium: State University of Ghent and Institute of Marine Scientific Research; 1984. pp. 307-39.
[30] Scott PJB. Chronic pollution recorded in coral skeletons in Hong Kong. J Exp Mar Biol Ecol 1990; 139(1-2): 51-64.
[31] Cantin NE, Negri AP, Willis BL. Photoinhibition from chronic herbicide exposure reduces reproductive output of reef-building corals. Mar Ecol Prog Ser 2007; 344: 81-93.
[32] Jones RJ. Zooxanthellae loss as a bioassay for assessing stress in corals. Mar Ecol Prog Ser 1997; 149(1-3): 163-171.
[33] Howard LS, Brown BE. Metals in *Pocillopora damicornis* exposed to tin smelter effluent. Mar Pollut Bull 1987; 18: 451-454.
[34] Owen R, Knap A, Toaspern M, Carbery K. Inhibition of coral photosynthesis by the antifouling herbicide Irgarol 1051. Mar Pollut Bull 2002; 44(7): 623-632.

[35] Alutoin S, Boberg J, Nystrom M, Tedengren M. Effects of the multiple stressors copper and reduced salinity on the metabolism of the hermatypic coral *Porites lutea*. Mar Environ Res 2001; 52(3): 289-299.

[36] Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U. Temperature-induced bleaching of corals begins with impairment of the CO2 fixation mechanism in zooxanthellae. Plant Cell Environ 1998; 21(12): 1219-1230.

[37] Jones RJ, Hoegh-Guldberg O. Effects of cyanide on coral photosynthesis: implications for identifying the cause of coral bleaching and for assessing the environmental effects of cyanide fishing. Mar Ecol Prog Ser 1999; 177: 83-91.

[38] Jones RJ, Kerswell AP. Phytotoxicity of Photosystem II (PSII) herbicides to coral. Mar Ecol Prog Ser 2003; 261: 149-159.

[39] Jones RJ, Muller J, Haynes D, Schreiber U. Effects of herbicides diuron and atrazine on corals of the Great Barrier Reef, Australia. Mar Ecol Prog Ser 2003; 251: 153-167.

[40] Reichelt-Brushett AJ, Harrison PL. The effect of copper, zinc and cadmium on fertilization success of gametes from scleractinian reef corals. Mar Pollut Bull 1999; 38: 182-187.

[41] Reichelt-Brushett AJ, Harrison PL. The effect of selected trace metals on the fertilization success of several scleractinian coral species. Coral Reefs 2005; 24(4): 524-534.

[42] Negri AP, Heyward AJ. Inhibition of fertilization and larval metamorphosis of the coral *Acropora millepora* (Ehrenberg, 1834) by petroleum products. Mar Pollut Bull 2000; 41(7-12): 420-427.

[43] Negri AP, Smith LD, Webster NS, Heyward AJ. Understanding ship-grounding impacts on a coral reef: potential effects of anti-foulant paint contamination on coral recruitment. Mar Pollut Bull 2002; 44(2): 111-117.

[44] Reichelt-Brushett AJ, Harrison PL. The effect of copper on the settlement success of larvae from the scleractinian coral *Acropora tenuis*. Mar Pollut Bull 2000; 41(7-12): 385-391.

[45] Downs CA, Woodley CM, Richmond RH, Lanning LL, Owen R. Shifting the paradigm of coral-reef `health' assessment. Mar Pollut Bull 2005; 51(5-7): 486-494.

[46] Mitchelmore C, Schwarz JA, Weis VM. Development of symbiosis-specific genes as biomarkers for the early detection of Cnidarian-algal symbiosis breakdown. Mar Environ Res 2002; 54: 345-349.

[47] Morgan MB, Snell TW. Characterizing stress gene expression in reef-building corals exposed to the mosquitoside dibrom. Mar Pollut Bull 2002; 44(11): 1206-1218.

[48] Snell TW, Brogdon SE, Morgan MB. Gene expression profiling in ecotoxicology. Ecotoxicology 2003; 12: 477-485.

[49] Venn AA, Quinn J, Jones R, Bodnar A. P-glycoprotein (multi-xenobiotic resistance) and heat shock protein gene expression in the reef coral *Montastraea franksi* in response to environmental toxicants. Aquat Toxicol 2009; 93(4): 188-195.

[50] Black N, Voellmy R, Szmant AM. Heat shock protein induction in *Montastraea faveolata* and *Aiptasia pallida* exposed to elevated temperatures. Biol Bull 1995; 188: 234-240.

[51] Downs CA, Fauth JE, Robinson CE, *et al*. Cellular diagnostics and coral health: declining coral health in the Florida Keys. Mar Pollut Bull 2005; 51(5-7): 558-569.

[52] Sharp V, Brown BM, D. Heat shock protein (Hsp 70) expression in the tropical reef coral *Goniopora djiboutiensis*. J Therm Biol 1997; 22: 11-19.

[53] Haynes D, Johnson JE. Organochlorine, heavy metal and polyaromatic hydrocarbon pollutant concentrations in the Great Barrier Reef (Australia) environment: a review. Mar Pollut Bull 2000; 41(7-12): 267-278.

[54] Edge SE, Morgan MB, Gleason DF, Snell TW. Development of a coral cDNA array to examine gene expression profiles in *Montastraea faveolata* exposed to environmental stress. Mar Pollut Bull 2005; 51(5-7): 507-523.

[55] Ralph PJ, Schreiber U, Gademann R, Kuhl M, Larkum AWD. Coral photobiology studied with a new imaging pulse amplitude modulated fluorometer. J Phycol 2005; 41(2): 335-342.

[56] Schreiber U. PAM fluorometry and Saturation Pulse Method: an overview. In: Papageorgiou GCG, Ed. Advances in Photosynthesis and Respiration, vol 19; Chlorophyll a Fluorescence: A Signature of Photosynthesis. Dordrecht, Netherlands: Springer; 2004. pp. 279-319.

[57] Jones RJ, Kildea T, Hoegh-Guldberg O. PAM chlorophyll fluorometry: a new *in situ* technique for stress assessment in scleractinian corals, used to examine the effects of cyanide from cyanide fishing. Mar Pollut Bull 1999; 38(10): 864-874.

[58] Harland AD, Brown BE. Metal tolerance in the scleractinian coral *Porites lutea*. Mar Pollut Bull. 1989; 20: 353-7.

[59] Fitt WK, Warner ME. Bleaching patterns of four species of Caribbean reef corals. Biol Bull 1995; 189(3): 298-307.

[60] Jokiel PL, Coles SL. Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. Coral Reefs 1990; 8(4): 155-162.

[61] Anthony KRN, Kline DI, Diaz-Pulido G, Dove S, Hoegh-Guldberg O. Ocean acidification causes bleaching and productivity loss in coral reef builders. Proc Natl Acad Sci USA 2008; 105(45): 17442-17446.

[62] Downs CA, Mueller E, Phillips S, Fauth JE, Woodley CM. A molecular biomarker system for assessing the health of coral (*Montastraea faveolata*) during heat stress. Mar Biotechnol 2000; 2(6): 533-544.

[63] Morgan MB, Vogelien DL, Snell TW. Assessing coral stress responses at the level of gene expression. Environ Toxicol Chem 2001; 20(3): 537-543.
[64] Snape JR, Maund SJ, Pickford DB, Hutchinson TH. Ecotoxicogenomics: the challenge of integrating genomics into aquatic and terrestrial ecotoxicology. Ecotoxicology 2004; 67: 143-154.
[65] Ecobichon DJ. Pesticide use in developing countries. Toxicology 2001; 160: 27-33.
[66] Mueller JF, Duquesne S, Ng J, *et al.* Pesticides in sediments from Queensland irrigation channels and drains. Mar Pollut Bull 2000; 41(7-12): 294-301.
[67] Mitchell C, Brodie J, White I. Sediments, nutrients and pesticide residues in event flow conditions in streams of the Mackay Whitsunday Region, Australia. Mar Pollut Bull 2005; 51(1-4): 23-36.
[68] Packett R, Dougall C, Rohde K, Noble R. Agricultural lands are hot-spots for annual runoff polluting the southern Great Barrier Reef lagoon. Mar Pollut Bull 2009; 58(7): 976-986.
[69] Haynes D, Christie C, Marshall P, Dobbs K. Antifoulant concentrations at the site of the Bunga Teratai Satu grounding, Great Barrier Reef, Australia. Mar Pollut Bull 2002; 44(9): 968-972.
[70] Negri A, Marshall P. TBT contamination of remote marine environments: ship groundings and ice-breakers as sources of organotins in the Great Barrier Reef and Antarctica. J Environ Manage 2009; 90(Supp. 1): S31-S40.
[71] IMO. International convention on the control of harmful anti-fouling systems on ships: International Maritime organisation; 2001.
[72] Voulvoulis N, Scrimshaw MD, Lester JN. Occurrence of four biocides utilized in antifouling paints, as alternatives to organotin compounds, in waters and sediments of a commercial estuary in the UK. Mar Pollut Bull 2000; 40(11): 938-946.
[73] Thomas KV, Fileman TW, Readman JW, Waldock MJ. Antifouling paint booster biocides in the UK coastal environment and potential risks of biological effects. Mar Pollut Bull 2001; 42(8): 677-688.
[74] Glynn PW, Howard LS, Corcoran E, Freay AD. The occurrence and toxicity of herbicides in reef building corals. Mar Pollut Bull 1984; 15(10): 370-374.
[75] Glynn PW, Rumbold DG, Snedaker SC. Organochlorine pesticide residues in marine sediment and biota from the northern Florida reef tract. Mar Pollut Bull 1995; 30: 397-402.
[76] Glynn PW, Szmant AM, Corcoran EF, Cofer-Shabica SV. Condition of coral reef cnidarians from the northern Florida reef tract: pesticides, heavy metals, and histopathological examination. Mar Pollut Bull 1989; 20(11): 568-576.
[77] Råberg S, Nystrom M, Eros M, Plantman P. Impact of the herbicides 2,4-D and diuron on the metabolism of the coral *Porites cylindrica*. Mar Environ Res 2003; 56(4): 503-514.
[78] Haynes D, Muller J, Carter S. Pesticide and herbicide residues in sediments and seagrasses from the Great Barrier Reef world heritage area and Queensland coast. Mar Pollut Bull 2000; 41(7-12): 279-287.
[79] Negri AP, Mortimer M, Carter S, Müller JF. Persistent organochlorines and metals in estuarine mud crabs of the Great Barrier Reef. Mar Pollut Bull 2009; 58(5): 769-773.
[80] von Westernhagen H, Klumpp DW. Xenobiotics in Fish from Australian Tropical Coastal Waters, Including the Great-Barrier-Reef. Mar Pollut Bull 1995; 30(2): 166-169.
[81] Prange J, Haynes D, Schaffelke B, Waterhouse J. Reef Water Quality Protection Plan. Townsville: Great Barrier Reef Marine Park Authority; 2007 ISSN 1832-9225.
[82] Shaw M, Negri A, Fabricius K, Mueller JF. Predicting water toxicity: pairing passive sampling with bioassays on the Great Barrier Reef. Aquat Toxicol 2009; 95: 108-116.
[83] Tischer W, Strotmann H. Relationship between inhibitor binding of chloroplasts and inhibition of photosynthetic electron transport. Biochim Biophys Acta 1977; 460: 113-125.
[84] Cantin N, van Oppen M, Willis B, Mieog J, Negri A. Juvenile corals can acquire more carbon from high-performance algal symbionts. Coral Reefs 2009; 28(2): 405-414.
[85] Rutherford AW, Krieger-Liskay A. Herbicide-induced oxidative stress in Photosystem II. Trends Biochem Sci 2001; 26: 648-653.
[86] Jones R. The ecotoxicological effects of Photosystem II herbicides on corals. Mar Pollut Bull 2005; 51(5-7): 495-506.
[87] Moreland DE. Mechanisms of action of herbicides. Annu Rev Plant Physiol 1980; 31: 597-638.
[88] Owen R, Knap A, Ostrander N, Carbery K. Comparative acute toxicity of herbicides to photosynthesis of coral zooxanthellae. Bull Environ Contam Toxicol 2003; 70(3): 541-548.
[89] McCloskey LR, Chesher RH. Effects of man-made pollution on the dynamics of coral reefs. In: Miller JW, van der Walker JG, Waller RA, Eds. Scientists in the Sea. Washington, DC: US Department of Interior; 1971. pp. 229-237.
[90] Olafson RW. Effect of agricultural activity on levels of organochlorine pesticides in hard corals, fish and molluscs from the Great Barrier Reef. Mar Environ Res 1978; 1(2): 87-107.
[91] Acevedo R. Preliminary observations on effects of pesticides carbaryl, napthol, and chlorpyrifos on planulae of the hermatypic coral *Pocillopora damicornis*. Pac Sci 1991; 45: 287-289.

[92] Te FT. Preliminary investigations into the effects of Dursban registered insecticide on *Pocillopora damicornis* (Scleractinia: Cnidaria). J Mar Environ Eng 1998; 4: 189-199.

[93] Negri AP, Soo RM, Flores F, Webster NS. *Bacillus* insecticides are not acutely harmful to corals and sponges. Mar Ecol Prog Ser 2009; 381: 157-165.

[94] Jones RJ. Testing the 'photoinhibition' model of coral bleaching using chemical inhibitors. Mar Ecol Prog Ser 2004; 284: 133-145.

[95] Magnusson M, Heimann K, Negri AP. Comparative effects of herbicides on photosynthesis and growth of tropical estuarine microalgae. Mar Pollut Bull 2008; 56(9): 1545-1552.

[96] Chesworth JC, Donkin ME, Brown MT. The interactive effects of the antifouling herbicides Irgarol 1051 and diuron on the seagrass *Zostera marina* (L). Aquat Toxicol 2004; 66: 293-305.

[97] Harrington L, Fabricius KGE, Negri A. Synergistic effects of diuron and sedimentation on photosynthesis and survival of crustose coralline algae. Mar Pollut Bull 2005; 51(1-4): 415-427.

[98] Schreiber U, Mueller JF, Haugg A, Gademann R. New type of dual channel PAM chlorophyll fluorometer for highly water toxicity biotests. Photosynthesis Res. 2002; 74: 317-333.

[99] ElJay A, Ducruet JM, Duval JC, Pelletier JP. A high sensitivity chlorophyll fluorescence assay for monitoring herbicide inhibition of Photosystem II in the chlorophyte *Selenastrum capricornutum*: comparison with effect in cell growth. Arch Hydrobiol 1997; 140: 273-286.

[100] Bengtson-Nash SM, McMahon K, Eaglesham G, Mueller JF. Application of a novel phytotoxicity assay for the detection of herbicides in Hervey Bay and the Great Sandy Straits. Mar Pollut Bull 2005; 51: 351-360.

[101] Magnusson M. The impact of herbicide contamination on tropical microalgae of the Great Barrier Reef lagoon. Townsville, Australia: James Cook University; 2009.

[102] USEPA. Prevention, Pesticides and Toxic Substances. United States Environmental Protection Agency; 2009 [updated 2009; cited July 2009]; Available from: http: //www.epa.gov/.

[103] Haynes D, Müller JF, McLachlan MS. Polychlorinated dibenzo-p-dioxins and dibenzofurans in great barrier reef (Australia) dugongs (*Dugong dugon*). Chemosphere 1999; 38(2): 255-262.

[104] Buckland SJ, Hannah JA, Taucher E, Dawson S. Polychlorinated dibenzo-p-dioxins and dibenzofurans in New Zealand's Hector's dolphins. Chemosphere 1990; 20: 1027-1045.

[105] Jarman WM, Norstrom RJ, Muir DCG, *et al.* Levels of organocblorine compounds, including PCDDs and PCDFs, in the blubber of cetaceans from the west coast of North America. Mar Pollut Bull 1996; 32: 426-436.

[106] Lobel LMK, Davis EA. Immunohistochemical detection of polychlorinated biphenyls in field collected damselfish (*Abudefduf sordidus*; Pomacentridae) embryos and larvae. Environ Pollut 2002; 120(3): 529-532.

[107] Matthews V, Päpke O, Gaus C. PCDD/Fs and PCBs in seafood species from Moreton Bay, Queensland, Australia. Mar Pollut Bull 2008; 57(6-12): 392-402.

[108] Capone DG, Bauer JE. Environmental Microbiology. Oxford: Clarendon Press; 1992.

[109] Straughan D. Factors causing environmental changes after an oil spill. J Pet Sci Technol 1972; 24: 250-254.

[110] Burns K, Codi S. Non-volatile hydrocarbon chemistry studies around a production platform on Australia's northwest shelf. Estuaries Coast Shelf Sci 1999; 49: 853-876.

[111] Loya Y, Rinkevich B. Abortion effect in corals induced by oil pollution. Mar Ecol Prog Ser 1979; 1: 77-80.

[112] Lunel T, Ed. Dispersant effectiveness at sea. Proc 1995 Int Oil Spill Conf; 1995. American Petroleum Institute, publication No. 4620.

[113] Cook CB, Knap AH. Effects of crude oil and chemical dispersant on photosynthesis in the brain coral *Diploria strigosa*. Mar Biol 1983; 78: 21-27.

[114] Knap AH, Sleeter TD, Dodge RE, *et al.* The effects of oil spills and dispersant use on corals: a review and multidisciplinary experimental approach. Oil Chem Pollut 1983; 1: 157-169.

[115] Teal JM, Howarth RW. Oil spill studies: a review of ecological effects. Environ Manage 1984; 8: 27-44.

[116] Jackson JBC, Cubit JD, Keller BD, *et al.* Ecological effects of a major oil spill on Panamanian coastal marine communities. Science 1989; 243: 37-44.

[117] Kennedy CJ, Gassman NJ, Walsh PJ. The fate of benzo[a]pyrene in the scleractinian corals *Favia fragum* and *Montastrea annularis*. Mar Biol 1992; 113: 313-318.

[118] Burns KA, Knap AH. The Bahia Las Minas oil spill: hydrocarbon uptake by reef building corals. Mar Pollut Bull 1989; 20: 391-398.

[119] Burns KA. Hydrocarbon chemistry. Technical Report. New Orleans: U.S. Department of the Interior; 1993.

[120] Harrison PL. Oil pollutants inhibit fertilization and larval settlement in the scleractinian reef coral *Acropora tenuis* from the Great Barrier Reef, Australia. Sources, Fates and Consequences of Pollutants in the Great Barrier Reef and Torres Strait. Townsville, Australia: Great Barrier Reef Marine Park Authority; 1999.

[121] Peters EC, Meyers PA, Yevich PP, Blake NJ. Bioaccumulation and histopathological effects of oil on a stony coral. Mar Pollut Bull 1981; 12: 333-339.
[122] Mercurio P, Negri AP, Burns KA, Heyward AJ. The ecotoxicology of vegetable versus mineral based lubricating oils: 3. Coral fertilization and adult corals. Environ Pollut 2004; 129(2): 183-194.
[123] Jones RJ, Heyward AJ. The effects of Produced Formation Water (PFW) on coral and isolated symbiotic dinoflagellates of coral. Mar Freshwat Res 2003; 54: 153-162.
[124] Raimondi PT, Barnett AM, Krause PR. The effects of drilling muds on marine invertebrate larvae and adults. Environ Toxicol Chem 1997; 16: 1218-1228.
[125] Rinkevich B, Loya Y, Eds. Harmful effects of chronic oil pollution on a Red Sea scleractinian coral population. Proc 3rd Int Coral Reef Symp: Miami; 1977.
[126] Hudson JH, Robin DM. Effects of drilling mud on the growth rate of the reef building coral *Montastrea annularis*. In: Geyer RA, Ed. Marine Environmental Pollution. Amsterdam: Elsevier; 1980. pp. 455-470.
[127] Dodge RE, Szmant-Froelich A. Effects of drilling fluids on reef corals: a review. In: Duedall IW, Kester DR, Park PK, Ketchum BH, Eds. Wastes in the Ocean. New York: John Wiley & Sons; 1985. pp. 341-364.
[128] White DC, Nickels JS, Gehron MJ, Parker JH, Martz RF. Coral metabolic activity, nutritional status and microbial infection with exposure to drilling fluids. In: Duedall IW, Kester DR, Park PK, Ketchum BH, Eds. Wastes in the Ocean. New York: John Wiley & Sons; 1985. pp. 365-376.
[129] Bak RPM. Effects of chronic oil pollution on a Caribbean coral reef. Mar Pollut Bull 1987; 18: 534-539.
[130] Guzmán HM, Burns KA, Jackson JBC. Injury, regeneration, and growth of Caribbean reef corals after a major oil spill in Panama. Mar Ecol Prog Ser 1994; 105: 231-241.
[131] Neff JM, Anderson JW. Response of marine animals to petroleum and specific petroleum hydrocarbons. London: Applied Science Publishers; 1981.
[132] Guzmán HM, Holst I. Effects of chronic oil–sediment pollution on the reproduction of the Caribbean reef coral *Siderastrea siderea*. Mar Pollut Bull 1993; 26: 276-282.
[133] Arai T, Kato M, Heyward AJ, *et al.* Lipid composition of positively buoyant eggs of reef building corals. Coral Reefs 1993; 12: 71-75.
[134] Harrison PL. The effects of oil pollutants on fertilization rates in the scleractinian coral *Acropora tenuis*. Joint Scientific Conference on Science, Management and Sustainability of Marine Habitats in the 21st Century; 1994; Townsville, 1994.
[135] Kennish MJ. Ecology of Estuaries: Anthropogenic Effects. Boca Raton: CRC Press; 1992.
[136] Rainbow PS. Heavy metals in marine invertebrates. In: Furness RW, Rainbow PS, Eds. Heavy Metals in the Marine Environment. Boca Raton: CRC Press; 1990. pp. 67-80.
[137] Waldichuk M. Biological availability of metals to marine organisms. Mar Pollut Bull 1985; 16: 7-11.
[138] Brinkhuis BH, Penello WF, Churchill AC. Cadmium and magnesium flux in eelgrass *Zostera marina*. II. Metal uptake by leaf and root-rhizome tissues. Mar Biol 1980; 58: 187-196.
[139] Batley GE. Heavy metals and tributyltin in Australian coastal and estuarine waters. In: Zann LP, Sutton DC, Eds. The State of the Marine Environment Report for Australia Technical Annex: 2 Pollution. Townsville, Australia: Great Barrier Reef Marine Park Authority; 1995. pp. 63-72.
[140] Carnahan EA, Hoare AA, Hallock P, Lidz BH, Reich CD. Distribution of heavy metals and foraminiferal assemblages in sediments of Biscayne Bay, Florida, USA. J Coast Res 2008; 24(1): 159-169.
[141] Guzmán HM, García EM. Mercury levels in coral reefs along the Caribbean coast of Central America. Mar Pollut Bull 2002; 44(12): 1415-1420.
[142] Ramos AA, Inoue Y, Ohde S. Metal contents in *Porites* corals: anthropogenic input of river run-off into a coral reef from an urbanized area, Okinawa. Mar Pollut Bull 2004; 48(3-4): 281-294.
[143] Reichelt-Brushett AJ, McOrist G. Trace metals in the living and nonliving components of scleractinian corals. Mar Pollut Bull 2003; 46(12): 1573-1582.
[144] Victor S, Richmond RH. Effect of copper on fertilization success in the reef coral *Acropora surculosa*. Mar Pollut Bull 2005; 50(11): 1448-1451.
[145] Livingston HD, Thompson G. Trace element concentrations in some modern corals. Limnol Oceanogr 1971; 16(5): 786-796.
[146] Al-Rousan SA, Al-Shloul RN, Al-Horani FA, Abu-Hilal AH. Heavy metal contents in growth bands of *Porites* corals: record of anthropogenic and human developments from the Jordanian Gulf of Aqaba. Mar Pollut Bull 2007; 54(12): 1912-1922.
[147] David CP. Heavy metal concentrations in growth bands of corals: a record of mine tailings input through time (Marinduque Island, Philippines). Mar Pollut Bull 2003; 46(2): 187-196.

[148] Fallon SJ, White JC, McCulloch MT. *Porites* corals as recorders of mining and environmental impacts: Misima Island, Papua New Guinea. Geochim Cosmochim Acta 2002; 66: 45-62.
[149] Howard LS, Brown BE. Heavy metals and reef corals. Oceanogr Mar Biol Annu Rev 1984; 22: 195-210.
[150] Mitterer RM. Amino acid composition and metal binding capability of the skeletal protein of corals. Bull Mar Sci 1978; 28: 173-180.
[151] Brown BE, Tudhope AW, LeTissier MDA, Scoffin TP. A novel mechanism for iron incorporation into coral skeletons. Coral Reefs 1991; 10: 211-215.
[152] Bastidas C, García E, Eds. Metal concentrations in the tissue and skeleton of the coral *Montastrea annularis* at a Venezuelan Reef. Proc 8th Int Coral Reef Symp II; 1997.
[153] Esslemont G. Heavy metals in corals from Heron Island and Darwin Harbour, Australia. Mar Pollut Bull 1999; 38: 1051-1054.
[154] Harland AD, Nganro NR. Copper uptake by the sea anemone *Anemonia viridis* and the role of zooxanthellae in metal regulation. Mar Biol 1990; 104: 297-301.
[155] Marshall AT. Occurrence distribution, and localisation of metals in cnidarians review. Microsc Res Tech 2002; 56: 341-357.
[156] Henderson RS. Marine microcosm experiments on effects of copper and tributyltin-based antifouling paint leachates. San Diego: U.S. Navy Ocean Systems Centre; 1988.
[157] Evans EC. Microcosm responses to environmental perturbations. Helgolander Wiss Meeresunters 1977; 30: 179-191.
[158] Howard LS, Crosby DG, Alino P. Evaluation of some methods for quantitatively assessing the toxicity of heavy metals to corals. Coconut Island: Technical Report No. 37. Hawaii Institute of Marine Biology; 1986.
[159] Esquivel IF. Short term copper bioassay on the planula of the reef coral *Pocillopora damicornis*. Coconut island: Technical Report No. 37. Hawaii Institute of Marine Biology; 1983.
[160] Negri AP, Heyward AJ. Inhibition of coral fertilisation and larval metamorphosis by tributyltin and copper. Mar Environ Res 2001; 51(1): 17-27.
[161] Heyward AJ. Inhibitory effects of copper and zinc sulphates on fertilization in corals. Proc 6th Int Coral Reefs Symp, Townsville; 1988. pp. 299-303.
[162] Goh BPL. Mortality and settlement success of *Pocillopora damicornis* planula larvae during recovery from low levels of nickel. Pac Sci 1991; 45(3): 276-286.
[163] Smith LD, Negri AP, Philipp E, Webster NS, Heyward AJ. The effects of antifoulant-paint-contaminated sediments on coral recruits and branchlets. Mar Biol 2003; 143(4): 651-657.
[164] O'Donnel JR, Kaplan BM, Allen HE, Eds. Bioavailability of trace metals in natural waters. In: Aquatic Toxicology and Hazard Assessment. 7th Symp, American Society for Testing and Materials; 1985; Philadelphia.
[165] Allen HE. The significance of trace metal speciation for water, sediment and soil quality criteria and standards. Sci Total Environ 1993; 134(Supp. 1): 23-45.
[166] Grant AJ, Graham K, Frankland S, Hinde R. Effect of copper on algal-host interactions in the symbiotic coral *Plesiastrea versipora*. Plant Physiol Biochem 2003; 41(4): 383-390.
[167] Nyström M, Moberg F, Tedengren M, Eds. Natural and anthropogenic disturbance on reef corals in the inner Gulf of Thailand: physiological effects of reduced salinity, copper and siltation. Proc 8th Int Coral Reef Symp 2; 1997.
[168] Reichelt-Brushett AJ, Michalek-Wagner K. Effects of copper on the fertilization success of the soft coral *Lobophytum compactum*. Aquat Toxicol 2005; 74(3): 280-284.
[169] Allemand D, Tambutte E, Jaubert J. Organic matrix synthesis in the scleractinian coral *Stylophora pistillata*: role in biomineralization and potential target of the organotin tributyltin. J Exp Biol 1998; 201: 2001-2009.
[170] Mercier A, Pelletier E, Hamel J. Effects of butyltins on the symbiotic sea anenome *Aiptasia palleda* (Verrill). J Exp Mar Biol Ecol 1997; 215: 289-304.
[171] Bellas J, Granmo A, Beiras R. Embryotoxicity of the antifouling biocide zinc pyrithione to sea urchin (*Paracentrotus lividus*) and mussel (*Mytilus edulis*). Mar Pollut Bull 2005; 50(11): 1382-1385.
[172] Furnas MJ. Catchments and Corals: Terrestrial Runoff to the Great Barrier Reef. Townsville, Australia: Australian Institute of Marine Science; 2003.
[173] Wooldridge SA. A new conceptual model for the warm-water breakdown of the coral–algae endosymbiosis. Mar Freshwat Res 2009; 60(6): 483-496.
[174] Wooldridge SA, Done TJ. Improved water quality can ameliorate effects of climate change on corals. Ecol Appl 2009; 19(6): 1492-1499.
[175] Adams SM. Assessing cause and effect of multiple stressors on marine systems. Mar Pollut Bull 2005; 51(8-12): 649-657.
[176] Richmond RH. Coral reefs: present problems and future concerns resulting from anthropogenic disturbance. Am Zool 1993; 33: 524-536.

[177] Hughes TP, Rodrigues MJ, Bellwood DR, *et al.* Phase shifts, herbivory, and the resilience of coral reefs to climate change. Curr Biol 2007; 17(4): 360-365.

[178] Hutchings P, Haynes D, Goudkamp K, McCook L. Catchment to reef: water quality issues in the Great Barrier Reef Region - An overview of papers. Mar Pollut Bull 2005; 51(1-4): 3-8.

[179] Munksgaard NC, Parry DL. Trace metals, arsenic and lead isotopes in dissolved and particulate phases of North Australian coastal and estuarine seawater. Mar Chem 2001; 75: 165-184.