Assessing Organism and Community Responses

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Acronyms and Abbreviations

ANOVA	Analysis of variance
AF	Assessment factor(s)
EC50	Concentration of a toxicant that causes a measured negative effect to 50% of a test population
EC10	Concentration of a toxicant that causes a measured negative effect to 10% of a test population
EDA	Effects-directed analysis
HC1	Harmful concentration for 1% of species. Equivalent to the PC99
HC5	Harmful concentration for 5% of species. Equivalent to the PC95
LC50	Concentration of a toxicant that causes a 50% mortality to a test population
LOE	Line of evidence
NOEC	No observed effect concentration
PC99	The protective concentration for 99% of species
PC95	The protective concentration for 95% of species
POPs	Persistent organic pollutants
QSAR	Quantitative structure–activity relationship
SF	Safety factor(s)
SSD	Species sensitivity distribution
TIE	Toxicity identification evaluation
US EPA	United States Environmental Protection Agency
WET	Whole effluent toxicity test
WOE	Weight of evidence

3.1 Introduction

Many of the chemicals in the environment are naturally derived from compounds in plants, petroleum oils, or minerals in rocks. However, their chemical composition, concentration, and distribution through the environment have been altered by humans, usually as a result of an economic incentive (e.g. mining). Other chemicals are synthetic, produced in laboratories, and manufactured for specific uses. These manufactured chemicals are known as xenobiotics and include some fertilisers, pesticides, dyes, manufactured petroleum products, personal care products, and pharmaceuticals. What is common to all natural and Synthetic chemicals is that they are potentially toxic and likely to have come from a small geographic area or a limited number of sources. The chemicals are then redistributed in the environment through natural and anthropogenic activities, where organisms can intentionally or unintentionally be exposed to them. Some exposed species, and indeed some individuals, will be more sensitive than others, which can lead to adverse effects at the population level. When sensitive species are keystone or foundation species for a particular ecosystem, or enough species are affected, this can alter the structure and function of the exposed communities, having flow-on effects at the ecosystem level (Figure 3.1).

3.2 Ecotoxicology

Toxicity testing of organisms has been developing since the 1940s (Cairns Jr and Niederlehner 1994) because of the need to understand the effects of chemicals on organisms. Its application in environmental monitoring has grown rapidly (e.g. Auffan et al. 2014). In fact, the term **ecotoxicology**, which the field of study is now referred to as, was first used in 1969 by René Truhaut, defining it

» "*as a science describing the toxic effects of various agents on living organisms, especially on populations and communities within ecosystems*"

Ecotoxicology is a multidisciplinary science that combines chemistry, biology, ecology, pharmacology, epidemiology, and of course toxicology. It seeks to understand and predict the effects of chemicals on organisms and ecosystems and is constantly evolving as a discipline area (Sánchez-Bayo et al. 2011). Pollution studies use ecotoxicology as a tool to document the effects of pollutants at known concentrations on living organisms (Phillips 1977; Chapman and Long 1983) and to supplement conventional pollutant concentration data.

Ecotoxicology is used in a multiple **lines of evidence** (**LOE**) approach to risk assessment. This means that you use more than one source of information to support and understand the risk. Ecotoxicological experiments are most often conducted in laboratories under controlled conditions, and this chapter provides a guide

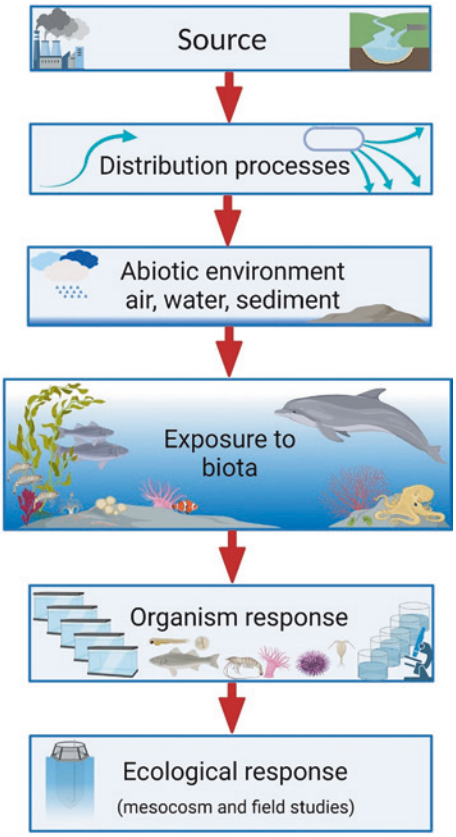

Figure 3.1 Transport of chemicals into biological systems. Chemicals come from a source and are distributed through the abiotic environment by air, water, and soil/sediment movement. Through this process, organisms are exposed to the chemicals and they too become part of the distribution process. Scientists use ecotoxicological experiments (see also Figure 3.3) to test the effects of exposure at the organism level and also at the ecosystems level using mesocosms and other multispecies assessments. *Image*: A. Reichelt-Brushett with Biorender.com

to how these experiments are performed. While the resulting information is limited by its lack of relevance to conditions in the environment, it provides important standard approaches for comparative assessment to help understand the relative sensitivity of different species and the relative toxicity of chemicals. Standard approaches to toxicity testing are explained in ▶ Section 3.2.3. Non-laboratory approaches for assessment under more relevant environmental conditions are discussed in ▶ Section 3.6.1.

The expansion of ecotoxicology to develop **species sensitivity distribution (SSD)** using toxicity data for a range of species and taxonomic groups (for detail see ▶ Section 3.4) increases the ecological relevance of laboratory-based toxicity results. The greater the number and diversity of species used in an SSD the greater the confidence in predicting concentrations that should protect any chosen percentage of species (see ▶ Section 3.4). Another means of gaining a better understanding of ecological interactions through ecotoxicological assessment is by using microcosm or mesocosm level studies in the field or laboratory (▶ Box 3.1).

Box 3.1. Microcosms and Mesocosm Studies in Ecotoxicology

There are many benefits of assessing toxicity using highly controlled single-species laboratory toxicity tests. Strictly controlled test conditions (e.g. temperature, photoperiod, contaminant dispersion, concentration, etc.) isolate a chosen contaminant (or contaminants) as the cause of any toxic effects. However, ecotoxicological experiments using enclosed experimental ecosystems (microcosms and mesocosm) provide considerably more ecologically relevant understanding of contaminant effects in the environment. They can also be used as a line of evidence along with single-species tests.

Microcosms are similar to standard toxicity tests, being generally conducted in a laboratory in small experimental vessels. The main difference is that microcosm studies involve exposing numerous interacting species to a contaminant, rather than a single species. This provides insight into contaminant effects in an environment with a much higher (and

more realistic) level of biological organisation and interaction. For example, a species may not be directly affected by a contaminant but suffer effects from ingesting organisms which have absorbed the contaminant. Or, a species may not be directly affected, but its population may be decimated by a predator that has lost another food supply (i.e. the population of another prey species has been affected by the contaminant).

mesocosm are similar to microcosms but are usually (though not always) conducted in the field and are much larger. mesocosm experiments incorporate natural abiotic effects on the toxicity of a contaminant (i.e. contaminants are exposed to the elements). mesocosm are as close as scientists can get to "replicating" the effects of contaminants in natural ecosystems without intentionally distributing contaminants into the environment. However, they are generally constrained by costs and logistics, limiting the number of replicates and interactions, and thus, the statistical confidence.

3.2.1 General Principles of Ecotoxicology

Ecotoxicologists are guided by four general principles, including the following:

- **You can only find what you are looking for**—toxicity tests can only provide targeted and specific information. For example, only the contaminants and responses of interest are measured, although other contaminants may be present and other responses may be occurring. This is particularly relevant in the context of the explosion of new and emerging contaminants, as well as the need to develop more sensitive and ecologically relevant test methods.
- **The dose determines the poison**—sediment and aquatic toxicity tests are only able to arrive at an effect concentration, rather than a dose. The consumption (dose) of a contaminant can generally not be quantified due to the multiple exposure routes including ingestion (in food, water, particulates, and sediment) and direct absorption from water or sediment. The influence of different types of exposure differs depending on the organism (e.g. behaviour, physiology, life stage, etc.), the contaminant (e.g. different substances may dissolve, bind to sediments or suspended particles, etc.), and the environmental conditions (e.g. physiochemistry, hydrological processes, etc.). For example, in a given environment, different species (or life stages of the same species) may be exposed to vastly different concentrations and types of contaminants due to their different feeding behaviours, preferred food sources, and detoxification and depuration abilities. Bioavailability is very important to consider here, since a high aqueous concentration does not determine toxicity if a substance is not bioavailable.
- **Toxicity can only be measured by living material** (e.g. organisms, cell lines, or enzymes). However, models such as quantitative structure–activity relationships (QSARs) and quantitative activity-activity relationships (QAARs) can be useful to predict potential toxicological effects. In QSARs, structural characteristics of chemicals are used to predict the toxicity (activity) of chemicals without toxicity data. In contrast, in QAARs, the toxicity (activity) of chemicals to one organism is used to predict the toxicity to another species.

Weight of Evidence

A **weight of evidence** (**WOE**) approach, which incorporates LOE has long been used in legal systems. It is a broad term that simply means that several pieces of evidence are considered together, rather than basing a decision on a single piece of evidence (Chapman et al. 2002). Court decisions should use a WOE approach, instead of relying on a single LOE, to provide **proof beyond a reasonable doubt**. According to the Federal Rules of Evidence in the USA (Annas 1994, 1999), court judges must consider the following:

- whether hypotheses used in experimental studies are testable or falsifiable;
- whether the relevant techniques/theories have been peer-reviewed and published;
- the potential rate of error in the methods; and
- if there is a general acceptance of the theory or method (similar to the point above).

More recently, a WOE approach has been used in the context of ecotoxicological risk assessments and is determined by multiple LOE. The LOE may include experimental (e.g. toxicity tests, contaminant characteristics) and observational (e.g. field assessments, physiological biomarkers) data, with each having its quality (e.g. were appropriate methods and analyses used) and extent (e.g. short/long term, local/regional/global scale) assessed. Qualitative LOE may also be considered in the WOE (e.g. best professional judgement) to help arrive at a prediction of the ecological risks based on the various LOE (e.g. Suter II 2016).

3.2.2 Factors Influencing Toxicity

The concentration of a chemical is one of the more obvious factors that will affect its toxicity. You may have heard the historic quote from Paracelsus (1493–1541), who expressed:

» *All substances are poisons; there is none that is not a poison. The right dose differentiates a poison and a remedy.*

However, many other environmental, physiological, biological, and chemical factors influence the toxicity of a chemical (e.g. Charition et al. 2010a; de Almeida Rodrigues et al. 2022). More specifically:

- **Temperature**—the toxicity of many substances differs with temperature, however, there is no general rule as to whether a higher or lower temperature will elicit a higher toxicity. For example, some metals are more toxic in tropical marine ecosystems than in temperate or polar regions, whereas for other metals this is reversed (Chapman et al. 2006). This makes the extrapolation of toxicity data from different climatic regions unreliable. This is one reason that some countries such as Canada and the USA have requirements to use toxicity data for endemic species. This approach is possible for North American and European countries because most of the test organisms used in toxicity tests originated from those regions. However, in other countries with smaller populations, different climates, and/or different ecosystems and species, this approach is not logistically possible, and instead toxicity data generated using non-endemic species must be relied upon.
- **pH**—affects solubility, and therefore bioavailability, of contaminants such as metals. Generally, a decrease in pH increases the toxicity of metals as they become more bioavailable. Since marine waters are buffered, they resist changes in pH (to a point).
- **Salinity**—can decrease the toxicity of chemicals by decreasing their aqueous solubility or changing their chemical form, although this depends on the type and concentration of other competing substances such as dissolved organic compounds (e.g. Hall and Anderson 2008).
- **Suspended sediment**—may increase toxicity (for example by providing a surface for contaminants to bind to, allowing uptake of contaminants by some organisms), but can also decrease toxicity (e.g. by decreasing the bioavailability of hydrophobic chemicals to aquatic organisms). This largely depends on the type of contaminant and the type of sediment. Suspended sediments may also act as a stressor in their own right.
- **Dissolved organic carbon**—may decrease toxicity by decreasing a chemical's aqueous solubility, and its ability to pass through membranes or bioavailability.
- **Previous exposure/resistance**—populations or strains of species may develop resistance to toxicants, so that considerable differences in sensitivity may exist within one species. For instance, species in areas with naturally elevated concentrations of metals may become more tolerant to those metals.
- **Organism life stage**—generally the early and oldest life stages are the most sensitive to the harmful effects of chemicals.
- **Duration of exposure**—generally a longer period of exposure will result in adverse effects at lower concentrations of a toxicant than the same effects measured after a short period of exposure.

3.2.3 Considerations for Planning Ecotoxicology Experiments

Experimental Procedures

As with all scientific experiments, ecotoxicology requires strict adherence to protocols including replication, quality control, statistical analyses, and interpretation of results. The assessment of the toxicity of a chemical usually starts with a **range finder** test. The purpose of these tests is to determine a broad range of concentrations of the chemical that include no effect through to 100% effect. For example, a range finder test might include concentrations of a test chemical including 0 (control), 0.001, 0.01, 0.1, and 1.0 mg/L. It is important that the amount or concentration of the chemical is measured using appropriate analytical equipment. If they are not measured, they are defined as nominal concentrations. Generally, only studies that have measured concentrations are publishable in scientific literature. Once the relevant EC50 (concentration of a toxicant that causes a measured negative effect to 50% of a test population) or LC50 (concentration of a toxicant that causes 50% mortality of a test population) has been determined from the range finder test, a **definitive test** is completed using concentrations in a much tighter range around the concentrations which induced the predetermined toxicological response (endpoint). Figure 3.2 shows the basic approach to replication in a static system and highlights that each replicate is wholly independent of the others, with the intention of avoiding pseudo-replication (e.g. many organisms in a single container for each test concentration). It is important to record the key physicochemical parameters of the media used in toxicity tests (e.g. dissolved oxygen content, temperature, pH, and water hardness) throughout the duration of the test. This information is important because these parameters may influence the speciation or behaviour and subsequent toxicity of the chemical in question or exert toxic effects in their own right. It is undesirable to have an additional stressor of declining water quality in combination with the chemical stressor of interest, unless the experiment is specifically designed to test the effect of multiple stressors.

Laboratory toxicity tests may be static (no renewal of the test solution), semi-static or static-renewal (renewal of the test solutions at set times, e.g. every 24 or 48 h for the duration of the test), or flow through (constant renewal of test solution for the duration of the

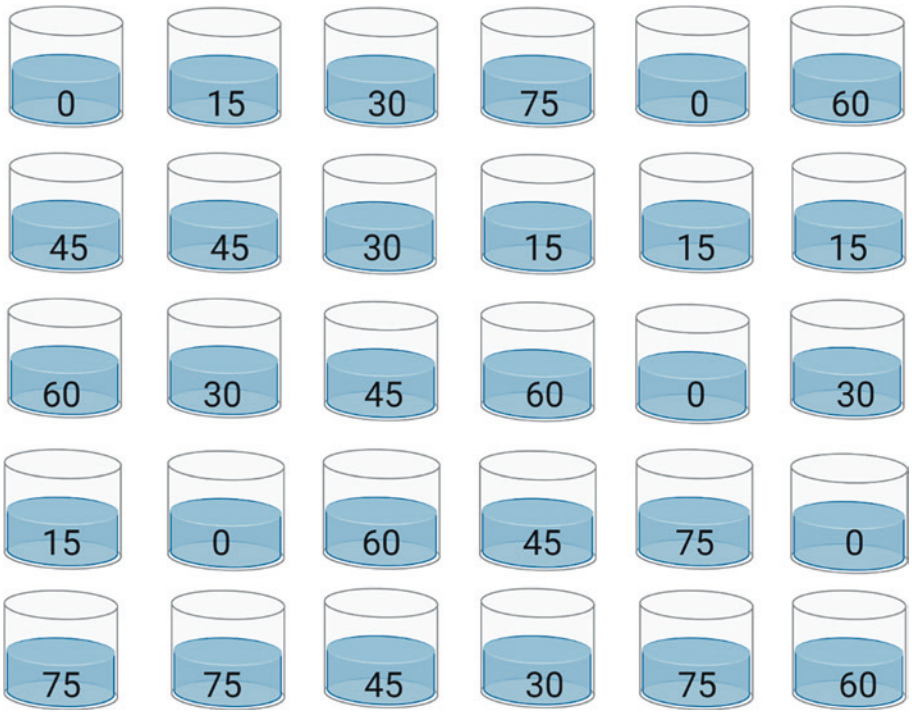

Figure 3.2 A typical experimental design for a replicated definitive ecotoxicological test. There are six treatments including a control (the concentrations, µg/L, are the numbers in the test containers), each with five replicates. A fixed number of test organisms are placed in each test chamber. The replicates of each concentration are randomly located in the testing room or incubator. The biological effect will be determined at set time intervals such as 0, 2, 24, 48, 72, and 96 h. These data will then be statistically analysed and the concentrations that cause biological effects of a certain magnitude are determined. *Image*: A. Reichelt-Brushett using Biorender.com

test). **Static systems** require simple equipment and are cost-efficient, however, they have the potential to provide inaccurate results due to changes in the concentration of contaminants during the test period, which may be absorbed and metabolised by test organisms, or be volatilised, degraded, and/or adsorbed onto the test container. The advantage of **semi-static** and **flow-through** test systems is that they maintain much more consistent chemical concentrations and minimise the accumulation of food, faeces, waste products (e.g. ammonia), algae, etc. in the test containers, which may influence the results. Flow-through tests, if maintained properly, provide the most consistent chemical concentrations. Another type of toxicity test is termed **pulse-exposure**, which, as the term suggests, exposes organisms to pulses of contaminant loads. This approach mimics an exposure regime that you might expect to see from rainfall and runoff events.

Test Endpoints

Ideally, standardised toxicity tests should have a well-defined, easily quantifiable endpoint (biological effect) that does not require a particularly high level of expertise to measure and interpret. Importantly, the duration of exposure needs to be considered and there are two main terms used to describe this. **Acute** toxicity tests are short-term tests that measure the lethal or sub-lethal effects of exposure to relatively high concentrations of chemicals. The duration which is considered short-term depends on the lifespan of the test organism. For example, durations of up to 96 h tests are commonly considered acute; however, many microorganisms (e.g. algae) double their cell number several times within this time frame, and hence 96 h is a chronic or longer-term exposure period for those species. **Chronic** toxicity tests are longer term and usually encompass a large period of the life cycle of the test organisms—typically of greater than 10% of the organism's lifespan (Newman 2010). Endpoints include both sub-lethal effects (e.g. reproduction, growth, population growth rate, and immobilisation) and lethal effects.

Lethality is the most basic test endpoint, whereby test organisms are determined as either dead or alive after a given exposure time, and the median lethal concentration (i.e. LC50) is calculated from the test data. While lethal endpoints are relevant to fish kills or exposure to high chemical concentrations, they are not ideal for deriving toxicant limits designed to protect the form and function of ecosystems (refer to ▶ Section 3.4).

Sub-lethal toxicity endpoints assess the effect of contaminants on a particular life stage and may be shorter (e.g. 1 h sea urchin fertilisation) or longer (e.g.

7-day fish growth) than acute lethal tests. Tests using these endpoints allow estimations of effect concentrations (EC values, e.g. EC50) rather than lethal concentrations (LC values). Traditional sub-lethal endpoints used for marine species include the following:

- growth (e.g. algae, juvenile and adult fish);
- germination (e.g. algae);
- fertilisation (e.g. sea urchin);
- early life-stage development (e.g. larval development in oysters, mussels, scallops); and
- behaviour (e.g. fish imbalance).

Considerable research effort has been (and continues to be) directed towards developing and standardising new toxicity tests with ecologically relevant sub-lethal endpoints. Examples of well-developed new test endpoints include the following:

- behaviour (e.g. coral larvae motility [Reichelt-Brushett and Harrison, 2004] and motor behaviour in fish [Harayashiki et al. 2019]);
- physiology (e.g. heart rate, neurotoxicity, and production of reactive chemical species);
- development (e.g. species-specific larval/juvenile development, larval malformation rate, heart rate, spontaneous movements, tail length, enzyme activities,biomarker genes and plant root elongation [Howe et al. 2014, Zhu et al. 2014, Rodriguez-Ruiz et al. 2014, van Dam et al. 2016])
- reproduction population growth (e.g. asexual reproduction rate and algal biomass change); and
- photosynthesis (e.g. in algae, plants, and symbiotic organisms such as corals).

Sediment Toxicity

Sediments are repositories for many contaminants, and for some contaminants, particularly hydrophobic contaminants, concentrations may be orders of magnitude higher in sediments than in the overlying waters. The metals and organic contaminants in sediment that are of most interest to ecotoxicologists are those that are available for uptake (i.e. they are bioavailable) by organisms exposed to sediments and/or sediment pore water. The presence of pollutants in aquatic sediments may cause toxic responses from benthic (sediment dwelling) organisms and bottom-feeding animals (e.g. prawns, some fish), and suspended sediments interfere with filter-feeding species such as bivalve molluscs. Most of the toxic effects result from toxicants dissolved in the interstitial water of the sediment, since animal gills are the prime sites of toxic action, although toxicants can be bioaccumulated from food and sediment ingestion. Additionally, organism interaction with sediments via feeding on the sediment, burrowing, and bioturbation may also change the local physicochemical conditions and alter the availability and/or toxicity of the contaminants (e.g. pH change through digestive acids and organic complexation through mucus secretion) (McConchie and Lawrence 1991; Han et al. 1996; Luoma 1996; Reichelt-Brushett and McOrist 2003). Sediment toxicity assessment is challenging because it is very hard to define the exposure/dose, and different sediment types influence the bioavailability of the contaminant and this availability will vary between the various compounds or complexes being tested (Chariton et al. 2010a). Some studies have investigated the status of tropical and temperate sediment toxicity although testing (e.g. Adams and Stauber 2008) and concluded that further tests for ecologically relevant species need to be developed. This is an ongoing field of research although there are some standard sediment toxicity test procedures established by the United States Environmental Protection Agency (US EPA) and Organisation for Economic Co-operation and Development (OECD) (Table 3.1).

3.2.4 Selecting Species for Toxicity Testing

Traditional Species

The majority of available standard toxicity test species are freshwater temperate species. This is because ecotoxicological work has traditionally been conducted in temperate regions in the Northern Hemisphere and because contamination of freshwater ecosystems has been recognised for longer than marine contamination. It is valuable to determine the toxicity of a number of different taxonomic groups to help represent the ecosystem composition and water and sediment exposure. Figure 3.3 shows a range of taxonomic groups that are commonly used in toxicity tests. Standard test methods have been developed throughout the world for different species that represent these taxonomic groups.

Novel Toxicity Test Species

A lot of research effort has been directed towards developing toxicity test methods for novel species, particularly **keystone or** foundation **species** of specific ecosystems, to increase the ecological relevance of results. This is particularly relevant for tropical marine species, which are under-represented in standard toxicological testing (e.g. van Dam et al. 2008). The following criteria are usually considered in species selection:

- suitability for culturing in laboratory conditions (e.g. tolerant of handling and laboratory culturing conditions, not particularly large);
- high reproduction rate and easily induced reproduction;
- ecological relevance (e.g. wide-ranging, ecologically relevant, representative species); and
- quantifiable toxicological responses.

Sometimes a species or taxonomic group will not meet all these requirements, and where keystone or foundation species for ecosystems are concerned, considera-

Table 3.1 Examples of whole sediment toxicity tests for marine and estuarine species

Type of organism	Species	Temperate/Tropical	Test endpoint	Acute/Chronic
Bacterium	*Vibrio fischeri*	Temperate	15-min luminescence	Acute
Microalga	*Entomoneis punctulata*	Temperate	72-h growth	Chronic
Amphipod	*Melita plumulosa*	Temperate	10-d survival	Acute
			28–42 d reproduction	Chronic
	Grandidierella japonica	Temperate	10-d survival 28-d growth	Chronic
	Corophium cola	Temperate	10-d survival and emergence	Acute
			14-d growth	Chronic
	Corophium insidiosum	Temperate	10-d survival	Acute
Crab	*Diogenes* sp.	Tropical	10-d survival	Acute
Bivalve	*Tellina deltoidalis*	Temperate	10-d survival	Acute
	Paphies elongate	Temperate	10-d survival	Acute
			28-d growth	Chronic
	Donax cuneate/Donax columbellia	Temperate/Tropical	10-d survival	Acute
Polychaete worm	*Australonereis ehlersi*	Temperate	10-d survival	Acute
	Ceratonereis aequisetis	Temperate	10-d survival	Acute

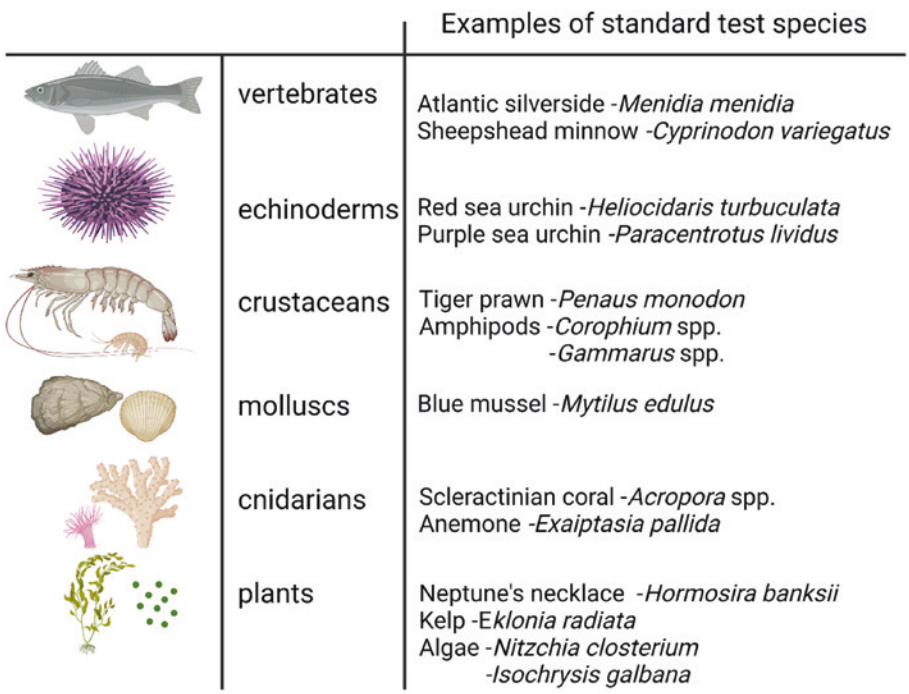

Figure 3.3 Examples of some standard taxonomic groups used in marine toxicity test species. *Image*: A. Reichelt-Brushett using Biorender.com

ble laboratory infrastructure and experimental design may need to be developed. Reef-building scleractinian corals are an example of foundation species that require intensive animal husbandry to maintain in aquarium conditions for ecotoxicology testing. Since most scleractinian corals are broadcast spawning and fertilisation occurs in the water, followed by metamorphosis (Figure 3.4), reproduction is considered a particularly sensitive stage of development to chemical exposure (e.g. Reichelt-Brushett and Harrison 2004;

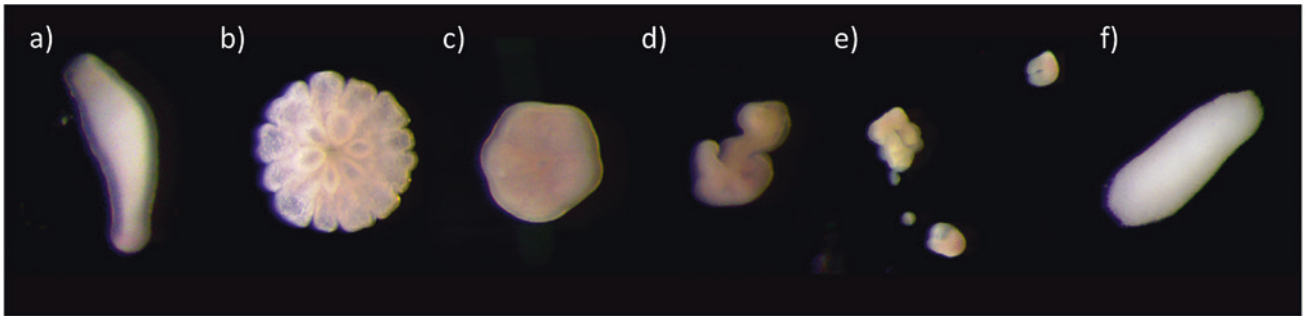

Figure 3.4 Examples of normal and abnormal larvae and recruits observed during larval *Acropora millepora* assays with exposure to total aromatic hydrocarbons (TAH). Morphologies observed included: **a** normal-sized planula larva (0–100 μg/L TAH), **b** fully metamorphosed recruit (0–100 μg/L TAH), **c** early-stage metamorphosed recruit (10–500 μg/L TAH), **d** severely deformed larvae undergoing fragmentation (10–500 μg/L TAH), **e** swimming larval fragments and deformed larvae undergoing fragmentation (10–500 μg/L TAH), and **f** larva-shaped mass of dead cells (>350 μg/L TAH). Examples extracted from photographs obtained using a Leica MS5 dissecting microscope with a 5.1 MP camera calibrated using the ToupView software. *Source*: Nordborg et al. 2021 with permission

Reichelt-Brushett and Hudspith 2016; Nordborg et al. 2021). External fertilisation is quite common among marine invertebrates, which results in gametes being directly exposed to chemicals in the water.

Animal Ethics Considerations

In 1959, the publication of the seminal book *The Principles of Humane Experimental Technique* by Russell and Burch encouraged scientific researchers using animals to "*remove the inhumanity*" of animal research by considering the **three Rs**—reduction (reduce the number of animals needed to obtain a given data set by controlling variability and optimising the design and analysis, so as to avoid repeating tests), refinement (techniques to minimise suffering), and replacement of animal use (use of non-animal alternatives wherever possible) (Russell and Burch 1959). These concepts aimed to minimise the unnecessary suffering of animals. In many countries, animal ethics approval must be acquired for research using animals although the definition of an **animal** may vary with jurisdiction. For example, in the Australian Code for the Care and Use of Animals for Scientific Purposes (NHMRC 2013), animals are defined as

> "*any live non-human vertebrate (that is,* fish, *amphibians, reptiles,* birds *and* mammals *encompassing domestic animals, purpose-bred animals, livestock, wildlife) and cephalopods*" [Box 3.2]).

Box 3.2: Global Horizon Scanning Project

The Global Horizon Scanning Research Prioritization Project was launched by the Society of Environmental Toxicology and Chemistry (SETAC) World Council. The purpose of the project was to identify research needs that are geographically specific and improve our understanding of the effects of different types of stressors on environmental sustainability (see ► https://globe.setac.org/ghsp-2017-recap/). Participants involved in the global research were asked to consider the following aspects when proposing their priority research needs:

- Does the research address important knowledge gaps?
- Can the research questions be answered by the implementation of a realistic research design which will enable the arrival at a factual answer that is not dependent on value judgements?
- Does it cover a temporal and spatial scale that could realistically be addressed by a research team?
- For research questions regarding impacts and interventions, does it contain a subject, intervention, and a quantifiable outcome?

Examples of proposed priority research needs in the Australasian region include the following:

"How can we identify and examine the environmental fate and toxicity of ingredients other than the stated 'active' components in commercial formulations individually and in chemical mixtures?" (Gaw et al. 2019 p. 74).

"How do we advance ecotoxicology testing to be more relevant to ecological systems?" (Gaw et al. 2019 p. 76).

Other proposed priorities for Australasia included the following:

- improving predictive risk assessment tools relevant to environmental exposure and toxicology;
- reducing and replacing animal testing;

- development of non-target analytical screening methods to identify priority contaminants in ecosystems which are exposed to complex mixtures;
- effects of multiple stressors;
- vulnerability of regional flora and fauna;
- improved management of ecosystems that are unique;
- stress from global trends (e.g. urbanisation, deforestation); and
- climate change related stress.

Priority research areas have also been identified in Europe (van den Brink et al. 2018), Latin America (Furley et al. 2018), and North America (Fairbrother et al. 2019).

Toxicity Identification Evaluation Analysis

Identification of sources of toxicity in sediment, water, or effluent samples provides information that can be used to develop methods to treat and reduce their toxicity, characterise priority substances in contaminated sites to guide remediation, identify the **active stressors** in an environmental sample to facilitate relevant ecological risk assessment, diagnose stressors that are impairing ecosystem function in watersheds and develop management strategies and policies to reduce the concentration of the stressors, and identify emerging contaminants (Burgess et al. 2013).

The **toxicity identification evaluations** (**TIEs**) framework was developed by the United States Environment Protection Agency (US EPA). This framework combines toxicity testing, physical and chemical separation procedures, and chemical analysis to identify and quantify toxicants in samples when the sample is toxic and the cause(s) of the toxicity is unknown (e.g. in complex mixtures such as effluents or environmental samples) (Figure 3.5). The resulting knowledge may enable the mitigation of the toxic component(s), for example by targeted treatment (i.e. removal or reduction of the source of toxicity.

In a TIE, potential sources of toxicity are systematically removed by treating the sample, and the remaining sample is re-tested to determine if its toxicity has decreased or remained the same. A decrease in toxicity indicates that the type of chemicals removed by the treatment is the cause of, or contributes to, the toxicity of the original sample. Potential sources of toxicity are removed by physical or chemical treatments (e.g. pH adjustment to remove acids or bases, aeration to remove volatile chemicals, filtering to remove particulates, passing through a cation-exchange column to remove cations, and passing through a C18 column to remove hydrophobic organic chemicals). Chemical separation and identification techniques are then used to identify the chemical or chemicals contributing to the toxicity based on the earlier results. Solutions of the identified chemicals at their concentrations in the original sample are then created and their toxicity determined. If they result in the same toxicity as the original sample, then the chemicals causing the toxicity have been identified. If the toxicity of the solutions is not as great as the original sample, then further TIE work is needed to identify other toxicants. Specialised techniques for TIEs need to be developed for individ-

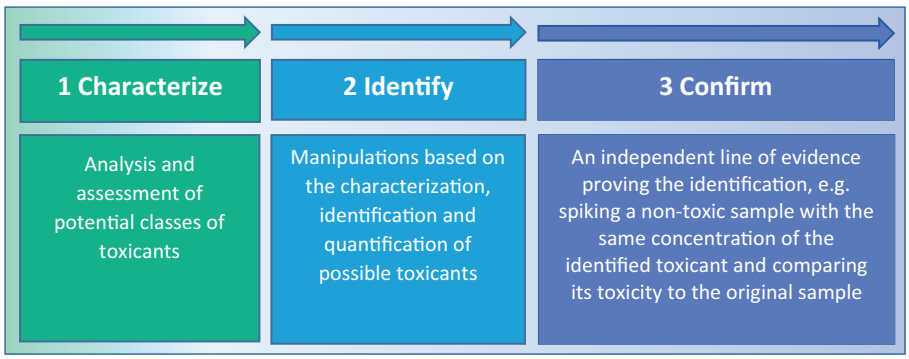

Figure 3.5 Summary of a toxicity identification, evaluation (TIE) process for sediments, effluents, and receiving waters. *Image*: A. Reichelt-Brushett and M St. J. Warne

Table 3.2 Primary differences between toxicity identification evaluations (TIEs) and effects-directed assessments (EDAs). Adapted from Burgess et al. 2013

Parameter	TIEs (in-vivo)	EDAs (in-vitro)
Toxicological endpoint	Whole organism: e.g. survival, reproduction, etc.	Genotoxicity, endocrine disruption, and mutagenicity
Targeted toxicants	All toxicants	Organic toxicants
Bioavailability	Is considered	Not considered, may be a source of inaccuracy due to how the compounds are extracted from the sample
Form of sample	Whole water, interstitial water, and sediment samples	Organic solvent extracts of sediment, water, interstitial water, biota, technical mixtures, and consumer products
Chemical analysis	Usually targeted analysis for suspected toxicants	Commonly non-targeted analyses and elucidation of structure
Specificity of toxicant identification	High for groups of contaminants and moderate for individual toxicants	High for individual toxicants
Relevance to natural exposure conditions	Primary goal of TIEs	Secondary goal of EDAs

ual contaminants and their degradation/transformation products, and hence much research effort needs to be directed towards developing techniques for isolating the effects of new and emerging contaminants (e.g. Dévier et al. 2011). Despite recent advances, it is not always possible to identify all the causes of toxicity in a complex sample.

Effects-Directed Analysis

A second tool used to identify chemicals causing toxicological effects in the environment is **effects-directed analysis** (**EDA**). EDA is an approach used to reduce the complexity of possible or actual toxicity while limiting the chance of overlooking significant chemicals that contribute to risks and effects (Brack et al. 2016). The general approach is to test the biological activity of a sample using responses from sub-cellular systems or whole organisms; samples are then fractionated (separated) and analysed to quantify and characterise the toxic components. This fractionation and effects assessment can be repeated to eliminate fractions that are not biologically active, enabling the isolation and identification of the toxic components (Brack et al. 2016). This method has some fundamental differences to TIEs and should be seen as complementary, rather than being interchangeable with TIEs (Table 3.2). Although there are many advantages of EDAs over TIEs, EDAs have some important limitations (e.g. only organic chemicals can be assessed and their bioavailability is not considered). Also, care must be taken when interpreting EDAs, as the techniques used to extract the toxicants may alter their bioavailability compared to natural conditions and so overestimate their toxicity (Burgess et al. 2013).

3.3 Current Status of Marine Ecotoxicology

The vast majority of aquatic ecotoxicological data is for freshwater species because humans have been aware of contamination of fresh water for much longer. We have had a much greater investment in fresh water, and hence pollution of freshwater ecosystems has historically been more relevant to us and more noticeable. As discussed in ▶ Chapter 1, it is only relatively recently in human history that we have become aware of the effects of pollution in marine ecosystems, and the vast majority of marine data is for temperate, Northern Hemisphere species, because that is where most ecotoxicology has been conducted (Lacher and Goldstein 1997).

3.3.1 Temperate Marine Ecotoxicology

As you can see from Table 3.1, most species used in sediment toxicity testing are temperate, and this is the same for aquatic toxicity tests. Because a lot of toxicity data exists for temperate marine species, and ecotoxicological risk assessment is much more relevant when a larger amount of data are available, considerable effort has been directed towards understanding whether temperate data can be applied for the ecosystem protection in other climatic regions. Research has illustrated that there are no predictable patterns in toxicity between temperate and tropical, or temperate and polar species (Chapman et al. 2006; Wang et al. 2014). Rather, it is evident that the relative toxicity depends on the contaminant (i.e. some metals are more toxic to tropical species than temperate species, and vice versa) (Kwok et al. 2007).

3.3.2 Polar Marine Ecotoxicology

Despite the remoteness and isolation of polar regions from the centres of anthropogenic activity, contamination is increasingly being identified in these regions, including in deep ocean sediments (e.g. Isla et al. 2018), benthic organisms, and in the tissues of organisms high in the food chain (e.g. polar bears and other mammals, large seabirds [e.g. Eckbo et al. 2019], and sharks [Ademollo et al. 2018]). While there are a few isolated point sources of contaminants (e.g. sewage and other waste from research stations, fuel, and oil), the primary concern and challenge is that contaminants are being transported to polar regions in ocean currents, in the atmosphere, (refer to ▶ Chapter 7 for more detail), and by trophic transfer. These dispersed contaminants include a wide range of persistent organic pollutants (POPs) (see Chapters 7 and 8), plastics (including micro- and nano-plastics, microfibres, and their degradation products) (e.g. Mishra et al. 2021) (see ▶ Chapter 9), and pesticides (see ▶ Chapter 7).

Unfortunately, to date, ecotoxicological risk assessments for polar environments are constrained by the very limited amount of regionally relevant toxicity data. Consequently, they are mostly derived from extrapolations of temperate and tropical toxicity data. There is a valid argument that extrapolation of data from other regions is better than no data at all; however, taxonomic compositions, chemical toxicity, and organism physiology are extremely different in the consistently low temperatures experienced in polar regions (e.g. Kefford et al. 2019). Obtaining the necessary toxicological data to enable the development of relevant water quality guidelines for these ecosystems is currently the subject of dedicated research effort (e.g. King et al. 2006; Gissi et al. 2015; Alexander et al. 2017; Koppel et al. 2017; Kefford et al. 2019; van Dorst et al. 2020).

Generally, polar species are more sensitive to long-term exposure to contaminants than tropical or temperate species. Chapman and Riddle (2005) suggest the following possible reasons for this:

- many species have relatively long lifespans and long development times (and so have a long time to accumulate contaminants);
- many species are relatively large, exhibiting gigantism, which may influence the response time (and so have a slower uptake of contaminants due to the low surface-area-to-volume ratio);
- slower metabolic rates and slow uptake kinetics (resulting in slower accumulation of contaminants, but also slower detoxification/depuration);
- less energy consumed (so less energy is available for detoxification/depuration); and/or
- high lipid content (so accumulate higher concentrations of lipophilic contaminants).

Some of these factors also affect the way that toxicity tests need to be conducted. For example, toxicity tests may need to be continued for longer time periods to account for slower metabolism and slower transition through different life stages, and different endpoints or assessments may be needed for lipophilic substances (Chapman and Riddle 2005).

3.3.3 Tropical Marine Ecotoxicology

Tropical marine ecosystems have a very different taxonomic composition, biodiversity, and physiology of organisms compared to temperate marine ecosystems. Some tropical marine ecosystems have extremely high levels of biological complexity, organisation, and diversity. For example, the tropical area known as the **Coral Triangle** (a marine region that spans parts of Indonesia, Malaysia, Papua New Guinea, the Philippines, the Solomon Islands, and Timor-Leste) is recognised as a global hotspot of biodiversity for corals and reef fishes (Allen 2007). Hence, there are more species that are susceptible to being exposed to contaminants, as well as more complex ecological interactions which further complicates risk assessment. Many tropical marine waters are oligotrophic (see ▶ Chapter 4), which provides less opportunities for contaminants to form complexes and so can result in higher bioavailability. Physiologically, organisms generally have a higher metabolism in warmer temperatures, which can increase either or both uptake and detoxification of contaminants. Degradation (biological and abiotic) might be expected to be enhanced in tropical marine systems compared to temperate and polar marine systems; however, research by Mercurio et al. (2015) found that five herbicides had half-lives of greater than 1 year in tropical marine water compared to earlier studies in temperate laboratories that reported half-lives of months.

Ecotoxicology in tropical marine environments is limited and there is a dearth of data on the dose–response characterisations of pollutants, particularly for early life stages. Lacher and Goldstein (1997) discussed the rapid increase in agricultural, urban, and industrial development in tropical regions. Peters et al. (1997) stressed that managers of tropical marine ecosystems have few tools to aid in decision-making and policy implementation and presented **conceptual models** as a future tool for the problem formulation phase of ecological risk assessment. Measurable responses to stressors, such as the concentrations of chemicals (i.e. ecotoxicological studies), are used within these models and are pertinent to the decisions that may be made to protect the environment (Peters et al. 1997). Since the study by Peters et al. (1997), some progress has been made in developing an understanding of the impacts of trace metals on tropical species (Chapman et al. 2006). However, there is a paucity of fully developed regionally relevant

marine toxicity testing methods for tropical marine systems (e.g. van Dam et al. 2008). Fortunately, research effort is growing in tropical marine ecotoxicology.

3.4 Using Ecotoxicological Data to Set Guideline Values

As the preceding text has shown, chemicals, if present at sufficiently high concentrations, can cause a diverse range of harmful effects. Largely as the result of some particularly disturbing pollution events in the United States of America, the United States Environmental Protection Agency (US EPA) started to develop maximum concentrations of chemicals in the water that are **safe** or provide a high degree of protection to aquatic ecosystems. Subsequently, numerous countries, states, and provinces have developed similar limits for chemicals in water, soil, sediment, and animal tissue in order to protect ecosystems. These limits are called guidelines, criteria, standards, or objectives, depending on the legal framework of the jurisdiction developing the limits. Although these terms are often used interchangeably, they have different meanings. Criteria and standards generally have some legal standing and if they are exceeded, this can lead to prosecution in courts of law. Guidelines do not have any legal standing but rather provide guidance on what is a safe concentration. Typically, if environmental concentrations are greater than a guideline concentration, then further work is required. This work can take several forms such as the development of management actions to decrease the concentration, amount, or type of chemicals released, or investigations to determine if the guideline is appropriate or if there are special conditions at the site that may increase or decrease the degree of protection provided. Criteria, standards, and guidelines are all based on the available scientific information, but scientific information is only one of the multiple factors that may be considered in deriving objectives. Other potentially relevant factors include costs and benefits, commercial considerations, and religious and cultural values. Objectives, criteria, standards, and guidelines are limits that reflect the management goals for a particular part of the ecosystem. For the remainder of this section, the term **limit** will be used generically to mean guidelines, objectives, criteria, and standards.

3.4.1 Deriving Limits

There are three main methods for deriving limits: background concentrations, assessment or safety factors (AF or SF), and SSDs.

Background Concentration Method

This method determines a fixed percentile (e.g. the median or 90th percentile) of the **background concentration** of a chemical and adopts that as the limit. While this is conceptually straightforward, it is often quite complex to obtain background concentrations, particularly in areas with a long history of human activity (e.g. in-shore regions near major urban developments). However, while they may not be relevant for particular sites, publications or databases of background concentrations are often available.

Assessment Factor Method

The **assessment factor** (**AF**) method requires a literature search for available data on the responses of marine organisms to toxicants. The data are then screened and assessed for quality, and inappropriate and/or low-quality data are removed. Then the lowest toxicity value is identified and divided by an AF to derive the limit. The magnitude of the AF depends on the amount and type of toxicity data that are available (Table 3.3). Basically, an AF of 10 is applied to account for the following: a lack of data, the difference between toxicity values from acute (short-term) and chronic (long-term) exposures, and differences in toxicity data from laboratory-based and field-based experiments (Table 3.3). This method is easy to understand, and the resulting limit will prevent any of the toxic effects reported in the literature, but it can lead to very low limits.

A key criticism of the AF approach is that there is little scientific justification for the magnitude of the AFs. A crit-

Table 3.3 Assessment factors applied to the minimum toxicity value depend on the type and amount of toxicity data available

Type of toxicity data	Assessment factor	Type of extrapolation
Chronic NOEC[a,b]	10	Field to laboratory
Acute EC50 or LC50[a]	100 (10 × 10)	Field to laboratory and acute to chronic
Acute EC50 or LC50 for 1 or 2 species	1000 (10 × 10 × 10)	Field to laboratory and acute to chronic and few to many

[a]Data are available for at least one species of algae, a crustacean, and a fish (OECD 1992). [b] No observed effect concentration is the highest concentration used in a toxicity test that does not cause a statistically significant effect compared to the control

ical assessment of the strengths and weaknesses of the AF methods is provided in Warne (1998). Experimentally determined AFs termed acute to chronic ratios (ACRs) have been developed to convert acute toxicity data to chronic data. However, while these are better than the above default AF of 10, they also have limitations (Warne 1998).

Species Sensitivity Distribution Method

The newer and currently preferred method for deriving limits is the SSD approach. This approach was developed in 1985 by Stephan and colleagues (Stephan et al. 1985) and has subsequently been extensively improved. All these SSD methods require a thorough search of the literature, followed by screening and assessing the quality of the toxicity data. The data that pass the screening and quality assurance process are then manipulated to obtain a single value to represent each species for which are data available (e.g. Saili et al. 2021). The data are ordered from highest to lowest toxicity (i.e. lowest to highest concentration at which toxic effects occur) and then given a ranking increasing from one. The cumulative frequency (a percent value) for each species is then calculated by:

$$\text{Cumulative frequency} = \text{rank}/(n+1) \times 100.$$

where n is the number of species for which toxicity data are available (i.e. the highest rank number). Thus, if there are toxicity data for 10 species, the cumulative frequency values for the first three species would be 9.09% (1/11×100), 18.18% (2/11×100), and 27.27% (3/11×100). The cumulative frequency values for each species are then plotted against the toxicity value representing the species and a statistical distribution is fitted to the data. Once the statistical distribution that best fits the data has been identified, that distribution is used to calculate the concentration that corresponds to protecting any selected percentage of species (conversely, the concentration that will permit a certain percentage of species to experience adverse effects). An example SSD for a hypothetical toxicant to marine species is presented in Figure 3.6.

The usual percentages of species selected to be protected for toxicant limits are 99 and 95%, and the usual limits of species that are permitted to be harmed are 1 and 5%. The concentrations that correspond to these levels of protection are termed the protective concentrations for 99 and 95% of species (i.e. PC99 and PC95, respectively) or the harmful concentrations for 1 and 5% of species (i.e. HC1 and HC5, respectively). While these are the most commonly used levels of protection, it is possible to calculate the concentration that corresponds to any percentage of species desired to be protected or harmed. Examples of the protocols for deriving limits are those used in Canada (CCME 2007) and Australia and New Zealand (Warne et al. 2018), and the software packages used to generate SSDs include ssdtools (Thorley and Schwarz (2018) and Burrlioz (CSIRO 2016). A critical assessment of the strengths and weaknesses of the SSD approach and the validity of its assumptions is presented in Warne (1998).

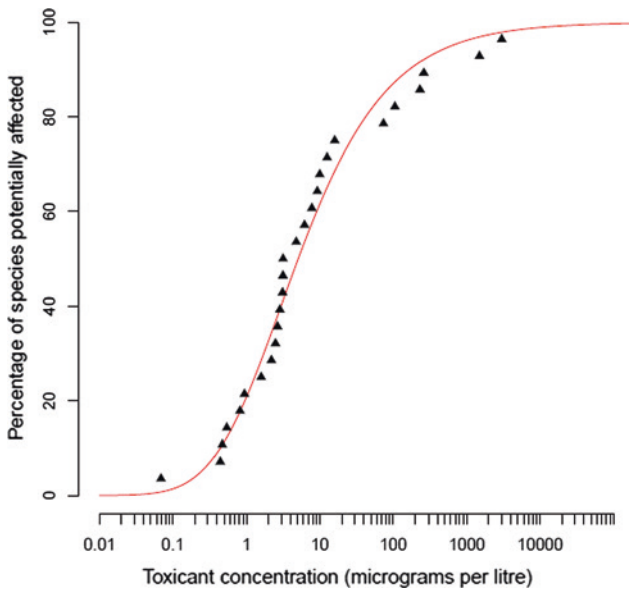

Figure 3.6 A cumulative frequency plot of the sensitivity of marine species (a species sensitivity distribution [SSD]) to a hypothetical toxicant. Each black triangle represents the concentration of the toxicant at which toxic effects commence for a species. *Image*: M St. J. Warne, output generated by Burrlioz V2

3.5 Limitations of Species Toxicity Studies

There are many limitations with toxicity studies and these are principally related to the fact that they are conducted in laboratories. Some of the limitations are as follows:

- The experimental conditions are highly standardised and controlled to minimise variation (i.e. not like the real world). For example, toxicity tests try to maintain the concentration of the test chemical for the duration of the test. This makes calculations of the toxicity simpler, but organisms in the environment are exposed to concentrations that change over time.
- The experimental conditions are usually optimal for the species, whereas that is often not the case in the environment and could lead to an underestimation of toxicity.
- The species used in toxicity tests have often been chosen because of their ease of being cultured in the laboratory or other pragmatic considerations. Such organisms may not be the most sensitive to chemicals. Rare and endangered species are seldom used in toxicity tests—yet these might be organisms that are important to protect.
- Toxicity test methods have only been developed for some species and are therefore biassed with many important organism types not being included, or there is a marked bias in the proportion of organism types with toxicity data.
- The duration of short-term (acute) tests is based on pragmatic considerations rather than biological reasons. For example, many acute tests are of 96 h duration to permit a toxicity test to be established and completed in a working week.
- Most toxicity tests only expose individuals of a single species and can therefore only measure the direct effects of chemicals on the test organism. Whereas, in the real-world, multiple species will be simultaneously exposed and both direct and indirect effects of chemicals on the test organisms can occur.
- Most toxicity tests only expose the test organism to a single chemical, whereas in reality, organisms are usually exposed to mixtures of chemicals (e.g. Warne et al. 2020). This can lead to underestimation or overestimation of the harmful effects caused by chemicals.

3.6 Assessing Responses from Organisms at the Community Level

So far in this chapter, we have focussed on single-species ecotoxicological assays, with these generally based on the exposure to one or a small number of toxicants under stable laboratory conditions. Ideally, these are designed to provide guidance about the exposure and the concentrations of contaminants at which toxicity commences which can then be used to derive chemical limits and protect marine environments. However, as discussed above (▶ Section 3.5), these approaches are not without limitations, and extrapolating laboratory-derived predictions about the adverse effects of contaminants at specific concentrations is fraught with ambiguity. This is not only because organisms are generally exposed to multiple stressors in the field (see ▶ Chapter 14) but also because environmental protection focuses on communities rather than individual species.

Even without pollutants, marine communities are complex and dynamic systems. Species migrate and immigrate, and interact with each other, sometimes favourably (e.g. symbiotic or mutualistic relationships), other times, less favourably (e.g. predation, parasitism, disease, and competitive displacement). Overlaying these complex interactions are a myriad of abiotic conditions (e.g. seasonality, substrate differences, temperature, depth, salinity, etc.). In many cases, these variables can also be stressors, albeit natural stressors. For example, while many organisms are accustomed to residing in relatively stable marine waters, living in estuaries is far more challenging, with marked tidal changes in salinity, temperature, pH, etc., and only a relatively small number of species are physiologically equipped to deal with such conditions. **Marine communities change over space and time**, and the challenge for scientists is being able to distinguish the effects of any contaminants over the natural variation. This will assist in determining whether the ecological impacts of the contaminants are significant and, therefore, whether the contamination is deemed pollution. Ideally, the assessment would identify which contaminant(s) are driving any observed changes. Here, we will discuss three approaches for examining the effects of contaminants on marine communities: in situ (field) surveys; experimental in situ studies; and community-level laboratory studies.

3.6.1 In situ Studies

Logically, the most common approach for examining the potential effects of contaminants on marine communities is by **in situ surveys**, also known as **field studies**. Given that marine ecosystems can encompass many different types of environments (e.g. seawalls, pelagic, coral, soft-substrate, intertidal, abyssal, etc.), each with its own range of communities, it is imperative to first establish which communities or assemblages (group of taxonomically related species, e.g. fish) should be targeted. This decision should be driven by a number of factors, including the following:

- whether a particular community or assemblage has a high conservation, socio-economic, or other values (e.g. key diet species of local people);
- the type of contaminant and its primary exposure pathway;
- accessibility, time, and cost to collect and process samples;
- relevant taxonomic and ecological expertise; and
- whether sufficient and representative community-level samples can be obtained.

Hence, it is essential that the targeted community is of ecological and ecotoxicological relevance to the potential pollutant(s).

While the types of marine communities captured in ecotoxicological studies are highly varied and can include rocky tidal platform communities, fish, and even the microbiome associated with particular host species (e.g. sponges [Glasl et al. 2017]), the most common approach is to examine the **macrobenthic communities** associated with soft-bottom sediments. This includes taxa such as polychaetes, amphipods, bivalves, and gastropods. This is because, firstly, sediments often contain far greater concentrations of contaminants than the water column and, thus, can pose a significant risk to the whole ecosystem. Secondly, macrobenthos interact with the sediment via consumption or residing within it, and therefore may experience multiple exposure pathways. Thirdly, macrobenthos are numerous and diverse, and therefore not only capture a wide variety of life strategies and sensitivities, but also are generally in numbers sufficient for robust statistical analysis. Fourthly, because of their size and historic use, they tend to be relatively easy to identify at the family level of taxonomic rank and higher. Fifthly, macrobenthic invertebrates are also generally relatively sessile, and therefore, their composition reflects the condition of the environment they were sampled in. Finally, and importantly, benthic communities are a crucial part of near-shore food webs, and consequently, changes in their composition may have cascading effects on other components of the system (e.g. fish) (Antrill and Depledge 1997; Fleeger et al. 2003).

While macrobenthic communities are typically the focus of in situ field studies, it is important to reiterate that the choice of targeted community in any in situ study is dependent on several factors and is by no means limited to macrobenthic invertebrates. In fact, there is an increasing trend to use DNA-based approaches such as metabarcoding to capture a far wider range of taxa than can be obtained using traditional means (Chariton et al. 2010b; Cordier et al. 2020; DiBattista et al. 2020).

Broadly speaking, two different approaches are most commonly used in in situ community surveys: reference/condition and gradient (Quinn and Keough 2002; Chariton et al. 2016). The first approach is a comparison of reference and condition sites. For example, the composition of the targeted communities from several relatively unmodified reference sites is compared to those from several sites exposed to the contaminant(s) of interest (e.g. sites with elevated copper derived from mine tailings). It is emphasised that **multiple sites** are required for each treatment, with this approach being founded on a **factorial design**, like those where an Analysis of Variance (ANOVA) can be applied. One of the biggest challenges of this approach is finding replicated reference locations, which is becoming increasingly difficult with the increasing loss of natural marine habitats. Careful consideration is required when choosing **reference sites**, as it is possible that other variables (e.g. grain size, seagrass cover, and unmeasured contaminants), and not the stressor of interest, may be driving any potential differences between the reference and impacted sites. This issue was highlighted in a comprehensive survey of North Carolina estuaries (Hyland et al. 2000) where the authors found that benthic assemblages were impaired in approximately one-quarter of sites (27%) even though no significant concentrations of contaminants were observed. This suggests that in these cases the communities were either being modified by natural variables, unmeasured contaminants, or a combination of the two.

In contrast to the factorial design which underpins the reference/condition sites approach, **gradient studies** aim to detect variability along a dominant pollution gradient. For example, sites are sampled at increasing distances from the deposition point for deep-sea tailings. Indeed, the approach can be used to capture multiple gradients, both natural and anthropogenic, with an increasing number of statistical tools becoming available which enable scientists to identify the proportion of variation in the community data which can be explained by the measured contaminants and other environmental variables (Chariton et al. 2010b). Gradient studies can be expensive and time-consuming, requiring sufficient environmental data (e.g. metals, pesticides, and natural stressors) and community data to capture the correlative patterns which underpin the gradient(s). However, if designed and implemented properly, they can provide key insights into how communities may be being shaped by both natural and anthropogenic variables, as well as their interactions. Preliminary or investigative studies that provide a reasonable understanding of the factors that may be at play will help in designing gradient studies.

At first glance, it would be logical to assume that either in situ approaches would result in determining whether a contaminant is causing the observed negative impairments to the marine community. However, this is not the case, as in situ studies are correlative and consequently cannot be used to state causality. For example,

even if there was a very strong correlation between sediment copper concentrations and benthic diatom communities, there may be other reasons for the observed trend, such as another unmeasured contaminant or natural variation. What in situ studies can tell us is that there is evidence that the contaminant is causing an adverse effect and is acting as a pollutant. Very much like a legal court case where no single LOE can be used to make a verdict, additional LOEs are required to state with a high level of certainty that the contaminant is causing an effect. This evidence may be obtained from ecotoxicological data such as described earlier in this chapter, bioaccumulation and biomarker studies, or via the use of manipulative experiments.

To reiterate, in situ studies are an important tool for helping to determine whether contaminants may be negatively impacting a marine community and therefore causing pollution. However, they cannot determine causality, and thus their findings must be used within the context that they are a correlative LOE. Additional information on the fundamental designs and analyses associated with in situ studies can be found in Underwood (1994), Quinn and Keough (2002), and Chariton et al. (2016).

3.6.2 Experimental In situ Studies

In order to validate correlative studies and to increase our understanding of how contaminants affect marine communities, the testing of models founded on cause and effect is essential. One way to do this is via in situ experiments. In **marine community ecotoxicological studies,** the two most common approaches are spike and translocation studies. **Spiked studies** are predominately sediment-based experiments that involve dosing a sediment with the contaminant of interest. Non-dosed (control) and dosed sediments are then transferred into containers and placed into the substrate unimpacted site(s) (e.g. Lu and Wu 2006; Birrer et al. 2018). The containers then remain in the sediment for sufficient time for them to be recolonized by the native biota, and comparisons between the compositions of the recolonized communities are used to determine whether the spiked sediment altered the species composition, and if so, at what concentrations effects were observed.

One of the challenges of spiked studies is ensuring that the contaminant remains bound to the sediment and does not alter the physicochemical properties of the sediments, minimising any differences between the controls and spiked sediments, apart from the toxicant of interest. Other additional limitations associated with this approach are that the endpoint is based on recolonized communities, and these may behave differently to established fauna; this approach is also limited to contaminants that bind to sediments, as hydrophilic contaminants will be released into the water column and be no longer present in the sediment.

Importantly, considerable resources are required to perform such studies, especially when you consider the quantity of sediment that may be required to fill, for example, 50×5-L containers. Furthermore, upscaling the approach to capture multiple contaminants at multiple concentrations is challenging. Imagine if you needed five containers of each treatment (replicates). If you just had a control and one contaminant at one concentration, 10 containers would be required. If you had one control and four concentrations (contaminant A, e.g. copper) this would require 25 containers (◘ Figure 3.7a). If you added another stressor (contaminant B, e.g. endosulfan) even at one concentration, you would need 5 control containers, 20 containers of contaminant A, 5 containers of contaminant B by itself, and 20 containers to capture each concentration of contaminant A plus contaminant B. That's 50×5-L containers (◘ Figure 3.7b), and as sediment often weighs around 2 kg/L, that is roughly 500 kg of sediment which needs to be manipulated. As you can see, if you wanted multiple concentrations of contaminant B, the experiment would get rather big and complex, not only requiring a lot of sediment to be spiked, but also an extraordinary large amount of effort to install, recover, and process the samples. Consequently, spiked studies are generally restricted to a limited number of treatments.

Translocation studies are similar to spiked studies in that they involve placing containers of sediments into a reference site; however, in this case, the sediments are sourced from the locations sampled in the field study. The aim is not specifically to identify if a specific contaminant is causing an effect, but rather to test whether there is something about the sediments per se which is causing the effect. That is, translocation studies are designed to remove the effect of location by translocating all the sediments to a single location, enabling a direct comparison between sediments obtained from multiple locations. One of the challenges of this process is keeping the sediments intact, including their contaminants, while simultaneously removing the biota. This can be done either by freezing or anoxia (Chariton et al. 2011; O'Brien and Keough 2013) and is essential, given that recolonisation is the endpoint and all sediments must start with the same de-faunated state (i.e. no organisms).

While by no means routine, both spike and translocation studies can provide an additional line of community-level information to complement in situ field studies and laboratory-based toxicity tests. Spiking experiments aim to provide experimental evidence of whether a specific contaminant has the capacity to alter community composition, as well as some insight about at what concentration this may occur. Translo-

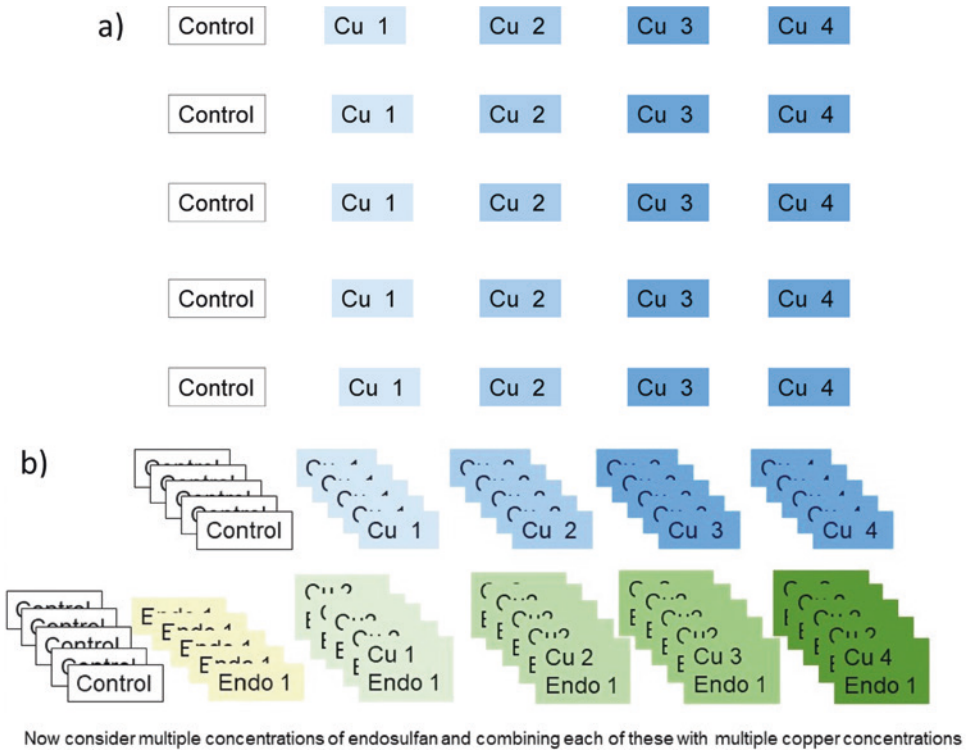

Figure 3.7 Representative experimental designs for in situ spiked sediment tests **a** for a single contaminant copper (Cu) and **b** for two contaminants, copper at four concentrations and one concentration of endosulfan (Endo). *Image*: A. Reichelt-Brushett

cation studies, on the other hand, remove the potential confounding influence of location. Both approaches are resource-intensive and thereby place constraints on the experimental design, often limiting their statistical power (Chariton et al. 2011).

3.6.3 Laboratory Studies

While in situ experiments can provide community-level responses under environmentally relevant conditions, they are not without limitations. Most notably, they are very much sediment focussed and not easily amendable to hydrophilic chemicals, chemicals with short-half-lives (e.g. some herbicides), or for exploring the toxicity of contaminants within the water column. In such cases, it may be more appropriate to expose whole communities to a contaminant of interest under laboratory conditions (e.g. in replicated aquaria). Under such conditions, the physicochemical properties of the water column as well as the concentration of the stressor can be controlled. Furthermore, the overlying waters can be continually renewed, ensuring that metabolic waste such as ammonia is removed and not impairing the health of the exposed communities.

The power of **laboratory community assays** was demonstrated by Gissi et al. (2019) who exposed the coral *Acropora muricata* to a range of dissolved nickel and copper doses. While the coral itself is a species, each fragment contains its own microbial assemblages. Consequently, in this study, the authors were able to gain experimental laboratory species-level data from the host (*A. muricata*) as well as community-level information by examining the host's external microbiomes. In the case of Gissi et al. (2019), the corals were wild-caught and allowed to acclimate for many weeks prior to exposure to the copper and nickel. While this helps ensure that the microbial communities are similar across all individuals, it does not infer that the microbial communities are the same as those which naturally reside on the corals at the site of collection, with other studies showing that marked differences in community structure can occur when transferring communities from the field to the laboratory (Ho et al. 2013; Chariton et al. 2014).

In a novel study by Ho et al. (2013), the authors examined the effects of the antibacterial agent triclosan on marine meiobenthic and macrobenthic communities. Their approach was to collect whole communities, including their residing sediment, and allow the whole sediment communities to acclimatise within a facility under a continual flow system that also supplied food. Instead of dosing the sediments, the authors placed a layer of the toxicant in a slurry on top of the community, and then 2 weeks later applied a clean sediment on top of this. The authors hypothesised that those ani-

mals which were alive would migrate through the clean sediment enabling them to be sampled, thereby providing a different community-level endpoint to the recolonized fauna obtained in in situ spiked and translocation experiments.

As in the case of in situ field experiments, laboratory-based community experiments can be logistically challenging and require significant resources and expertise. As a rule of thumb, replication is generally kept to a minimum, and designs incorporating the interactions between multiple stressors are generally avoided.

The data generated by both in situ field and laboratory-based community experiments can be used in SSDs to derive limits. The data could be used by itself or by combining it with more traditional single-species laboratory-based data (e.g. Leung et al. 2005).

3.7 Summary

Obtaining a comprehensive understanding of the effects of contaminants on marine organisms and communities requires a combination of both laboratory and field studies. Ecotoxicology is one of the LOEs that can be used in risk assessment. Experiments are most often conducted in laboratories under controlled conditions. As with all studies, ecotoxicology requires strict adherence to protocols including replication, quality control, statistical analyses, and interpretation of results. Physicochemical conditions need to be standardised throughout experiments and measured to ensure experimental conditions are suitable for organism survival. While this information is limited by its lack of relevance to changing conditions in the environment, it provides some important criteria for comparative assessment and is used in a multiple LOEs approach to develop guideline values for water and sediment quality. Studies of the effects of contaminants on community structure and function are more often based in situ. Due to the complex and dynamic nature of marine communities, thought must be given to the many variables that will influence toxicity.

3.8 Study Questions and Activities

1. What are the benefits of using sub-lethal endpoints to assess toxicity, as opposed to lethal endpoints?
2. What considerations must be given to chronic toxicity test procedures?
3. Describe what a species sensitivity distribution curve is and how it is used.
4. Explain why range finder experiments are used in ecotoxicology.-
5. Design a laboratory toxicity test to assess the effects of the pesticide imidacloprid on a marine species. Consider the species of interest to you, the experimental design, the duration of the exposure, what endpoint you will use, the concentration range you will use, how and when you will measure the test conditions (including the imidacloprid concentrations), and how you will interpret the results.
6. What are the advantages and disadvantages of in situ experiments?

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