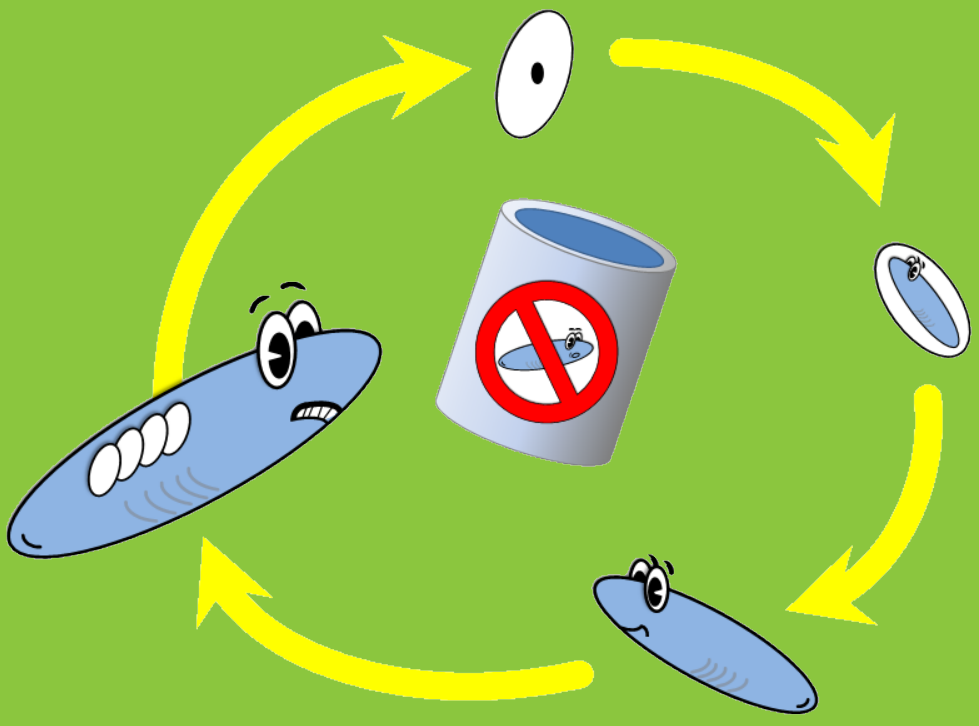
Making Sense of Chemical Stress

Applications of Dynamic Energy Budget Theory in Ecotoxicology and Stress Ecology



Tjalling JAGER

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Alternatively, refer to one of the papers in the open literature. E.g., [123] for the general concepts, [121] for toxicants in the standard DEB animal model, [136] for mixture toxicity in the scaled standard model, [137] for the classical DEBtox model, [127] for the basic DEBkiss model, or [106] for the updated DEBtox model based on DEBkiss.

If you spot errors (spelling, grammar or conceptual), or want to provide other feedback on the book, please contact me by email (see address below).

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Preface

Who should read this book?

Have you ever asked yourself why the effects of toxic chemicals depend on the exposure time? Or asked why stress effects on growth and reproduction are so different in the same organism, even though these responses must be linked in some causal way? Did you ever wish to *understand* toxic effects, so that you can make an educated prediction of effects under other conditions? Or do you want to understand why toxic effects depend on the presence of other factors such as temperature, food density, and life stage? This book offers a framework to address those questions by taking a radically different approach than what is common in ecotoxicology and stress ecology: by simplifying biological reality to an enormous extent. In this book, I will present a 'mechanistic' or 'biology-based' treatment of chemical effects. The main focus lies on one particularly useful framework for the interpretation of toxic effects, namely Dynamic Energy Budget (DEB) theory, and more specifically, the formulation by Bas Kooijman in 2010 [147]. Even if you are not convinced that this theory is the way to go for your particular problem, knowledge of the concepts behind it allows you to examine your (and other people's) data and models more critically.

This is not a cookbook with recipes for how to derive *the* toxicity of a chemical from your test data. First and foremost, it is an open invitation to start thinking about toxic effects on organisms as the result of underlying processes; processes in time. We need to treat the individual organism as a system. Furthermore, this book is an invitation to focus on the generalities that link all species and all toxicants, instead of losing ourselves in the details that make them unique. Recognising and understanding the dominant processes governing the toxic response is invaluable for understanding the effects of toxicants in a laboratory test. This understanding, in turn, is crucial to compare effects between species and between chemicals, to discover meaningful patterns, and to make science-based predictions for the real environment, under conditions far removed from those in the laboratory. However, I also want to show you how stress in general (and toxicants in particular) can help to provide insight into the basic structure of metabolic organisation in organisms.

This book covers a lot of fields: biology, (eco)toxicology, chemistry, modelling and statistics. I will not dive into any of these fields in great depth; the message is in their interconnection. There is, as far as I know, no education to properly prepare you for a multi-disciplinary arena such as this. For this reason, I attempted to write this book for a broad audience, assuming no specific background knowledge, and keeping it math-free. However, training in science and in abstract thinking is needed to fully appreciate all of the concepts presented (and some knowledge of ecotoxicology and general biology would help), and more training would be needed to effectively apply the models.

Why a book?

Simply because a book like this did not exist. Ecotoxicological textbooks do not address the questions I raised in the beginning of this preface, and if they touch upon these subjects, they stick to descriptions. The DEB book of Bas Kooijman [147] has a chapter on toxicant effects. However, that chapter is a tough read as it contains a lot of detail in a highly condensed form. Furthermore, the book presents DEB theory over its full width of application, which will deter many an ecotoxicologist. There exists a dedicated booklet on 'DEBtox' [150], presenting a DEB-based analysis for standard toxicity tests: acute survival, juvenile fish growth, *Daphnia* reproduction, and algal population growth. However, it is out of print and is more a collection of scientific papers than a coherent treatise. Furthermore, it presents the equations as such, without paying much attention to explaining the underlying concepts.

Since 2002, I have been working on toxicants in DEB theory, and have tried to explain what I was doing in a considerable number of papers, lectures and courses. I noticed that there is quite a learning curve to DEB theory. Even though the basic concepts of the theory are truly simple, and can be explained in ten minutes, they constitute an extreme abstraction of living systems. Such a level of abstraction is hardly part of the biological scientific tradition, and might lead to feelings of discomfort in the unsuspecting. Furthermore, even though the concepts are simple, following them to their logical consequences is not. Application of the theory almost always requires mathematics, enhancing the feelings of discomfort in many among the audience. Discomfort easily leads to disbelief. In a mathematical model, it is relatively easy to hide a few *ad hoc* factors to get a good fit to a set of data. Of course, all models are simplifications, and thus 'wrong', but how can you be sure that a model is actually useful for some purpose?

Models (at least, all useful ones) follow from assumptions. In fact, they should follow *uniquely* from a set of assumptions. Once you accept these assumptions, and given a correct implementation, you should also accept the model and its implications. If you do not accept the model predictions, it is wise to scrutinise the assumptions. The purpose of this book is thus to clarify the assumptions underlying DEB models for the analysis of toxic effects, with a high level of transparency. Once these assumptions are clear, it will be easier to interpret the model's fit to actual data sets, and the predictions made from them. For most biologists and ecotoxicologists, math is not helpful to explain something, and probably even a hindrance. To apply DEB models in ecotoxicology, you do not need to be good at math, but you do need a firm grip on the concepts and assumptions.

For the underlying math, I like to refer you to other books and papers. A list with links and/or free downloads is offered at the book's support page (see below). This page also offers a download of the separate 'technical background document' for this e-book. The main part of this document was written in 2012, and has now become pretty out-dated. Nevertheless, it still contains some potentially useful insights and derivations that may be useful for those wanting to dive deeper into DEB-based TKTD modelling. For a more general introduction into modelling, and the math and stats needed for TKTD modelling, I advise my e-book on 'Mechanistic modelling essentials' [109] (not free but friendly priced). For an in-depth treatise on reserve-less DEBkiss models, including equations and derivations, you can consult the dedicated e-book [108] (which can be downloaded for free).

Limitations of this book

To limit the size of this book, and to allow for a more coherent discussion of concepts, I will limit myself to applications involving heterotrophic organisms (mainly animals), and more specifically invertebrates (and, to some extent, fish). The reasons to select this group is that I personally have most experience with them, and the data sets that are routinely collected for these organisms are often directly amenable to a DEB-based treatment. I realise that by limiting myself to a selection of organisms, I neglect one of the most important achievements of DEB theory: the unification of all living organisms into a single, coherent, quantitative theory. Certainly, there are very good examples of DEB application to stressor effects in other groups of organisms such as toxicity in algae [155, 41] and birds [231], and tumour induction and growth in mammals [237]. However, a treatment of these developments might distract from the general message that I want to convey.

Support on the web

The supporting website for this book is http://www.debtox.info. Here, you will find software (as toolboxes for Matlab) to perform the calculations, lists of publications that apply these concepts, and information on courses. This site also contains the dedicated support page for this e-book (http://www.debtox.info/book.html), which hosts/links documents that presents the mathematical formulations, their derivations, as well as alternative formulations to explore. In addition, I maintain a version log on this web page to keep track of the development of the book.

For more DEB-related information, check out the website of the department of Theoretical Biology: http://www.bio.vu.nl/thb/deb. Even though the department no longer exists, the website is still there. More up-to-date information can be obtained from the DEB portal: https://debportal.debtheory.org/docs/.

What's that thing on the cover?

The creature on the cover, and in several figures in the book, is a PHylogenetically Indeterminate Life form, or 'Phil' for short. Phil is inspired by the creature that graces the cover of the third edition of the DEB book [147], and is used to illustrate general principles without focusing on specific species. In fact, a cartoon is a model: a simplification of a complex real system, brought back to its essence. Using a cartoon organism instead of a real one thus fits extremely well with the message I want to convey.

Notes for the update to version 2.0

The update to version 2.0 of the book involved a rather major reshuffling of the text. The conceptual switch that was made was to put 'damage' into a central position (whereas this concept has been largely ignored for a long time in a DEBtox context). This reflects the developments for survival modelling with GUTS [114], which has now also been adopted in DEBkiss [108], and more recently in updates of simplified (DEBtox2019, [106]) and complete (stdDEB-TKTD, [121]) DEB-based TKTD models. Not only is it a good idea to strive for harmonisation between the different modelling frameworks, I also feel that considering damage is essential for TKTD models in general. The

'old' models are still in there, but they form a special case of the overarching general model. This reshuffling and rewriting mainly affected the old chapters on toxicokinetics and toxicodynamics.

Acknowledgements

Firstly, I am indebted to Bas Kooijman whose work on DEB theory laid the foundation on which this book is built. Furthermore, I would like to thank all of my former colleagues at the department of Theoretical Biology, and the other DEB and TKTD enthousiasts around the world, for their inspiring papers, presentations and discussions. Thanks to LaTeX for providing the platform to write this book, and thanks to WikiPedia (http://www.wikipedia.org) for many interesting facts.

Warnings

I noticed that some of my e-books are offered through other websites, after removal of the title page. Please download this e-book from Leanpub, which is the only place where it is legitimately offered (even for free). That will ensure that you have a complete and correct version, and that you are notified as soon as a new version is available.

This book *will* inevitably contain errors. If you spot one, please let me know so that I can include corrections in updates of this book. I do not accept liability or responsibility for any damage or costs incurred as a result of these errors.

Chapter 1

Setting the scene

This is a book about the effects of chemical stress on organisms. It is an attempt to construct a general framework to quantitatively understand, and ultimately predict, the biological effects of chemicals over time. In writing this book, I realised that I need to be more specific about the things I want to discuss. Such a limitation is necessary for me to maintain focus in my discussion (which is difficult enough as it is), and for the reader to understand why it is helpful (in my opinion even inevitable) to work in an energy-budget framework. Different choices in scope would lead (and have already led) to very different books. Even though "effects of chemical stress on organisms" sounds like a well-demarcated research area, I do not think it is.

1.1 Limiting the scope

The world is full of things, and all material things are made of chemicals. Unfortunately, we cannot divide chemicals into toxic and non-toxic ones. Paracelsus (1493-1541) was right on the mark when he wrote: "All things are poison and nothing is without poison, only the dose permits something not to be a poison." Many chemicals are required by organisms for their normal functioning (nutrients). I will not talk about nutrients explicitly, but restrict the discussion to chemicals that are not part of the organism's 'normal' functioning, or are present in levels exceeding the requirements for such functioning. I realise that this definition of 'chemical stress' is a bit vague, but it will have to do for now. Even though the focus lies on chemicals, this book has a lot to offer for researchers interested in non-toxicant environmental stress (e.g., food, temperature, disease or pH stress), because the same principles can often be applied. Most of the time, I will be talking about the effects of a single toxicant in isolation. However, it is good to realise that organisms are always exposed to a mixture of chemicals; even in an experimental toxicity test, inevitably, other chemicals will be present in the test medium (although usually at non-toxic levels). In the real world, mixture exposure is the norm, although experimental testing and risk assessment mainly focus on single chemicals.

There are many million species of organism, so clearly, I want to restrict myself in the biological scope too. The concepts I present are equally valid for all forms of life on this planet (and likely also on others), but I will only work out the case for animals. More specifically, the focus will be on multi-cellular ectotherms. Even though this group represents only a small fraction of the total number of species on the planet,

they have something special. They are popular species in chemical-stress research, they form a group that is homogeneous enough to be described by the same basic model structure (as I will discuss in Chapter 2), and furthermore, the data sets that are routinely collected for these organisms are often directly amenable to the type of analysis that I will present. The last reason is a personal one: my experience with such critters is greater than the other forms of life, which makes it easier for me to write this book.

Another important set of restrictions is in the organisation levels that I will treat. Chemical effects start at the molecular level, and work their way up to the ecosystem, and even global scale. I will focus on the effects on an individual's life cycle, and thus on life history traits or endpoints[1] such as growth, development, reproduction and survival. The individual level is of key interest as it is possible to work with mass and energy balances, and because individuals are the units of natural selection and the building blocks of populations and ecosystems [105]. I will make some excursions to lower and higher levels of organisation, but the individual will be the basis. This implies that I will not deal (explicitly) with effects at the molecular and tissue level, and not with effects on ecosystems, even though there are clear links with the individual level (in fact, it is the individual level that connects these two worlds). In this book, the focus is on understanding and predicting the effects of chemicals on individual-level traits over time, over the entire life cycle of the individual (in principle, from egg to death).

The final restriction I pose myself is that I want to provide a general framework. That is, not specific for a chemical, species or effect. Making a model that accurately predicts the effects of chemical A on trait B of species C is very nice, but the number of different combinations of A, B and C is quite large. In my opinion, there is a need for generalisation as we cannot ever hope to test all the relevant permutations. The intellectual challenge in this book is to provide a framework that applies to all A, B and C within the restrictions posed above. In the case studies, it will become clear that biology often defies a strict generalisation, and more specific auxiliary assumptions will creep in.

With these restrictions in mind, I hope that the subsequent sections in this chapter, and my observations on current research in fields dealing with chemical stress, can be placed in its proper perspective.

1.2 Many faces of chemicals stress

Chemical stress is not something that humans have introduced on this planet; it is as old as life itself. The earth's mantle contains a range of compounds that can affect organisms negatively (for example metals and sulphur compounds). The appearance of free oxygen in the atmosphere (produced by photosynthesis in bacteria), some 2.4 billion years ago, is thought to have caused a mass extinction among the anaerobic[2] organisms dominating before that time. Incomplete combustion of organic matter is accompanied by the release of a range of particularly toxic organic chemicals such as polycyclic aromatic hydrocarbons and dioxins. Organisms themselves also produce all kinds of (sometimes very complex) chemical compounds, and put them to cunning use to aid

[1] In ecotoxicology, the term 'endpoint' is often used to denote a life-history trait that is observed to see if it responds to toxicant exposure.

[2] Anaerobic organisms function without the need for oxygen. For many of them, oxygen is in fact deadly.

their own survival. In this section, I will put 'chemical stress' in a broad perspective, providing examples of naturally-occurring intoxication, and after that, shortly discuss the role that human development is playing.

Examples of chemical use in nature

Many organisms have evolved chemicals to kill or repel their enemies. A broad variety of plants produce secondary metabolites to deter grazers. The perennial plant pyrethrum (*Chrysanthemum cinerariaefolium*) produces potent insecticides (pyrethrins) with neurotoxic activity (especially toxic to insects), which at lower doses seem to repel insects. The synthetic pyrethroid insecticides are derived from these naturally-produced chemicals. Many plant species produce tannins: bitter-tasting polyphenolic compounds. Consumption of large amounts of the tannin-rich acorns is known to be problematic for cattle. Water hemlock (*Cicuta sp.*) produces cicutoxin,[3] a highly toxic unsaturated aliphatic alcohol. This compound acts as a stimulant in the central nervous system, resulting in seizures. Livestock is especially at risk, leading to this plant's common name 'cowbane'.

Figure 1.1: The foxglove (*Digitalis purpurea*) contains potent cardiac glycosides, which can easily be fatal for humans. A purified component (digoxin) is used for the treatment of heart conditions. This drawing from "Köhlers Medizinal-Pflanzen", by Franz Eugen Köhler (1887).

[3] There is a strong tendency in biology and toxicology to relate the name of a toxicant to the species that produces it.

A bit closer to home, in fact, *at* home, many of our house and garden plants pack a powerful punch. For humans, the deadliest common houseplant is probably the oleander (*Nerium oleander*). Its sap contains a glycoside (oleandrin) that causes gastrointestinal and cardiac effects. The foxgloves (*Digitalis sp.*) contain similar glycosides, and are also extremely toxic. However, one of the purified glycosides from the foxglove (digoxin) is used for treatment of heart conditions.[4] Some more moderately toxic plants in and around the house are dumb cane (*Dieffenbachia sp.*, its English common name refers to the effects of needle-shaped calcium oxalate crystals on the throat when chewing on the leafs), hortensia (*Hydrangea sp.*), Swiss cheese plant (*Monstera deliciosa*, the second part of the scientific name probably refers to the tasty ripe fruit), and most spurges (family Euphorbiaceae). The yews (*Taxus sp.*) that are commonly planted in gardens and parks contain highly poisonous alkaloids (known as taxanes), which are also used in chemotherapy because they inhibit cell division. In fact, alkaloids are very popular defence chemicals among plants; it is estimated that 10-25% of the higher plants produces these compounds.

Not all of these poisons are effective in repelling curious mammals; the alkaloids include some of our favourite drugs such as caffeine, cocaine and nicotine, and pharmaceuticals such as morphine, codeine, ephedrine and quinine. Humans have also discovered that some alkaloids (commonly referred to as curare) can be used efficiently in hunting, to poison arrows and darts. A different use by humans of plant toxins is, oddly enough, in fishing. Several tropical and subtropical plant species (e.g., in the genus *Lonchocarpus* and *Derris*) produce rotenone, a respiratory inhibitor (interfering with the electron transport chain in mitochondria). Rotenone is highly toxic to insects and fish, but only mildly toxic for mammals. Various indigenous tribes use plant extracts in fishing; they collect the dead or dying fish that float to the surface for consumption.

The use of defensive chemicals is of course not restricted to plants. Many fungi produce potent toxins such as aflatoxin (by several *Aspergillus* species). The exact reason why these organisms do so is not so clear, although it may be to protect themselves from being eaten by nematodes, amoeba and springtails. Other fungi produce anti-insect toxins to protect the plants with which they live symbiotically [68]. Also animals use chemicals to protect themselves from being eaten. A famous example are the puffer fish (family Tetraodontidae), many of which produce the extremely potent neurotoxin tetrodotoxin, with no known antidote for humans. Despite (or perhaps thanks to) this toxicity, the well-prepared fugu is considered a delicacy in Japan. Another famous example are the poison dart frogs (family Dendrobatidae) that excrete alkaloid poisons from their skin. Alkaloids are also used by ladybirds (family Coccinellidae) to prevent being eaten. Toads from the genus Bufo apply, amongst other components, glycosides, similar to that of the oleander and foxgloves to protect themselves. Some animals are able to re-use the toxins from their food; as an example, the caterpillar of the cinnabar moth (*Tyria jacobaeae*) feeds on ragwort (*Jacobaea vulgaris*) and assimilates the bitter tasting alkaloids to become unpalatable itself. Chemical protection can also take on a more active form such as the apitoxin that honey bees (*Apis sp.*) use in their sting to protect their hive.

Some organisms use chemicals to gain a competitive advantage. When stressed, *Penicillium* fungi produce a compound that causes cell death in bacteria (which led

[4] Which underlines that the difference between a poison and a cure can just be in the dosing.

Figure 1.2: Ragwort (*Jacobaea vulgaris*) contains a range of toxic alkaloids that make it particularly dangerous for horses and cattle (especially dried, as the bitter taste is lost, but not the toxicity). The cinnabar moth (*Tyria jacobaeae*) assimilates the alkaloids to protect itself. Left plate: a reproduction of a painting by the Swedish botanist C. A. M. Lindman (1856 - 1928), taken from his book "Bilder ur Nordens Flora". Right plate: taken from John Curtis's "British Entomology" Volume 5 (1840s).

to the first 'modern' antibiotic treatment[5]). The black walnut (*Juglans nigra*) secretes a poison (juglone) from its roots that acts as a respiratory inhibitor to some other plant species, complicating gardening in its neighbourhood. Some endoparasites, such as trematodes in snails induce their host to grow to a much larger body size than usual (gigantism). This feat is probably accomplished by some chemical released by the parasite.

A range of predators employ toxic chemicals to kill or immobilise their prey, in which case the chemicals are called 'venom'. Snakes and spiders are probably the groups with the best-known examples of venomous predators. However, in marine invertebrates, venom also appears to be very popular, for example in the cone snails (*Conus sp.*), the greater blue-ringed octopus (*Hapalochlaena lunulata*, which applies

[5] As early as the Middle Ages, blue cheese (with *Penicillium* fungi) was presumably used to fight infections. It is however not clear to me if the medicinal value was due to penicillin or due to some other component of the cheese.

the same tetrodotoxin as the puffer fish for offence rather than defence), and almost the entire phylum *Cnidaria* (that contains jellyfish and sea anemones). The peripatus or velvet worm (*Euperipatoides kanangrensis*) applies a sticky secretion to catch its prey, a secretion which, surprisingly, contains the surfactant nonylphenol [32], an industrial pollutant of particular concern. The use of toxins in an offensive manner is not restricted to animals. The bacterium *Clostridium botulinum* produces extremely potent neurotoxins (often designated the 'most toxic compound known'), responsible for botulism. Apparently, the bacterium is using these toxins to kill larger organisms that are subsequently used as a source of nutrients. Interestingly, the same compounds are also used cosmetically as 'botox' injections to remove wrinkles by paralysing the facial muscles.

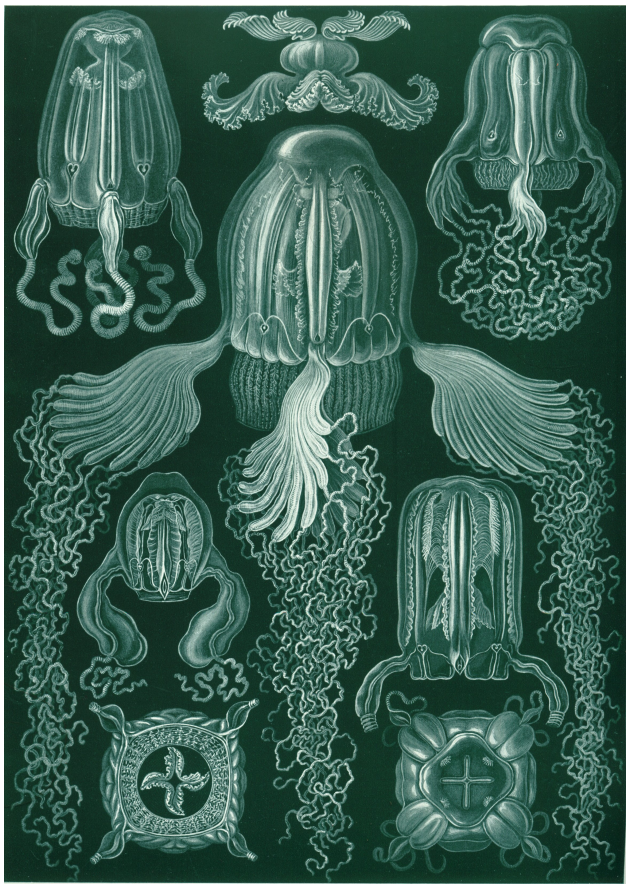

Figure 1.3: A collection of box jellyfish (class Cubozoa). Several species in this class produce a very potent venom, extremely painful, and sometimes even fatal for humans. This drawing from Ernst Haeckel's "Kunstformen der Natur" (1904).

Sometimes, organisms release chemicals as a by-product of their normal metabolism, which can be toxic to other species. One example is the oxygen produced by photosynthesising organisms, as we have seen above. Another extreme example are the cave-dwelling bacteria that form 'snottites', which produce sulphuric acid at a pH close to zero. Even a naturally occurring physiological process like ageing is generally assumed to relate to the toxic action of reactive oxygen species, produced as a by-product of aerobic metabolism (this will be treated in Section 5.5). The chemicals (inadvertently) excreted by a predatory organism, can be used by prey organisms as a cue to alter their

morphology and/or life-history traits (reviewed by [164]). For example, 'kairomones' excreted by fish have been found to shift the life history of water fleas to smaller body sizes and earlier reproduction. This makes evolutionary sense as fish tend to prefer larger water fleas as food. One species (*Daphnia cucullata*) is known to grow a helmet and spine as a response to the presence of predators. A general discussion, and more example of changes in morphology and life history can be found in [97].

The role of humans

With the short, and perhaps somewhat random, overview above, I hope to clarify that chemical stress is a natural phenomenon. Organisms are continuously involved in an intricate chemical warfare with attack and counter-attack measures. Therefore, organisms have evolved systems to cope with such stresses to some extent. However, one particular species of mammal, *Homo sapiens*, is currently testing these defence systems (including their own) in ways that the earth has never seen before. Not only did we distribute naturally occurring chemicals such as heavy metals in more available forms around the globe, we also developed an enormous array of synthetic chemicals to support our way of living. We developed pharmaceuticals to cure ailments, pesticides to protect our crops, surfactants to clean our clothes, flame retardants to protect us from fire, and the list continues. Chemical industry is big, very big, business. Total production value of 2020 was estimated at 3.5 trillion Euro (that is 35 followed by 11 zeros).[6]

Despite the obvious benefits of synthetic chemicals, and their substantial contribution to our economy and lifestyle, there are side effects. Inevitably, most of these chemicals (or their breakdown products) will enter the environment where they interact with organisms. Organisms are able to deal with chemical stress, and can develop defence mechanisms, as the rise of resistance towards antibiotics and pesticides has shown. However, the problem with synthetic chemicals is one of scale; the sheer number of chemicals with very different structures and properties, the spatial scales that are affected (for a number of chemicals that means the global scale), and the time scales that are involved. Evolution requires time, and generally plenty of it. So the evolution of defence against synthetic chemicals is only an option for organisms with very short generation times and flexibility in their physiological basis. And, there is no such thing as a free lunch; adaptations come with costs for the organism, such as a reduced tolerance against other stresses, or decreased reproductive output. Furthermore, we should realise that we are also exposing our own bodies to these chemicals, not only directly but also indirectly as these chemicals interact with organisms that we feed on (crops and livestock).

This is not a book about the dangers of the chemicals we humans have been, and are still, using. However, I certainly do not consider this to be a trivial issue: human activities are placing an excessive burden on the planet, and chemical pollution is an important part of that burden. This is a book about understanding and predicting how chemicals impact individual organisms. My hope is that such an understanding will help to evolve the (still quite juvenile) science of ecotoxicology, and that it can eventually also play a part in reducing the chemical pressure on ecosystems (although

[6] Information from Cefic, The European Chemical Industry Council, at https://cefic.org/a-pillar-of-the-european-economy/facts-and-figures-of-the-european-chemical-industry/, accessed 29 September 2022.