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Parasites and Ecotoxicology: Fish and Amphibians

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Article Outline

Glossary

Acanthocephalan Phylum Acanthocephala. Thorny-headed worms. Intestinal parasites of vertebrates, with an arthropod intermediate host. Characterized by spiny proboscis and lacking a digestive system. Sexes separate.

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Arthropod Phylum Arthropoda. Includes insects, crustaceans, and others. Body segmented with exoskeleton.

Bacteria Single-celled organisms lacking a nucleus and organelles.

Biodiversity The genetic diversity within species, the diversity of species within ecosystems and the diversity of ecosystems.

Cestode Class Cestoidea, phylum Platyhelminthes. Tapeworms. Intestinal parasites of vertebrates, with 1–2 intermediate host, the first usually an arthropod. Scolex (holdfast) with suckers, sometimes hooks, and body segmented with each segment containing male and female reproductive organs. Lacking a digestive system.

Community The assemblage of species that occurs in a particular habitat.

Complex life cycle The occurrence of more than one host in the life cycle, each of which is required for development or reproduction.

Crustacean Class Crustacea. Arthropods with 2 pairs of antennae, 1 pair of jaws, and 2 pairs of maxillae on the head.

Definitive host The host in a parasite's life cycle where sexual reproduction occurs.

Direct life cycle A life cycle without metamorphosis. In parasitology, a life cycle with only 1 host.

Ecosystem A complex of interacting organisms and their environment.

Ectoparasite A parasite that lives on the external surface. On fish, they are found on the fins, skin, and gills.

Endoparasite A parasite that lives in/on internal organs or tissues.

Food web The network formed by consumer interactions among organisms.

Fungi Nonphotosynthetic multicellular organisms with cytoplasm enclosed in rigid tubes.

Guild Functionally similar species within a community.

Host A living organism that is the habitat for a parasite.

Intermediate host A host that is required by a parasite for development and/or growth.

Macroparasite Relatively large parasites that do not proliferate directly on the host.

Microparasite Relatively small parasites that reproduce and increase in numbers on a single host.

Monogenean Class Monogenea, phylum Platyhelminthes. Generally ectoparasitic on fish and amphibians, with a direct life cycle. Body with oral sucker and posterior holdfast organ (opishaptor).

Nematode Class Nematoda, phylum Nemathelminthes. Roundworms. Free-living and parasitic forms. With cuticle and complete digestive system. Separate sexes.

Protist Single-celled organisms with nucleus and organelles. Also called protozoans.

Trematode Class Trematoda, phylum Platyhelminthes. Flukes. The most common are Digenea, which are parasites of vertebrates, with complex life cycles, the first intermediate host is usually a mollusc. Oral and ventral suckers, hermaphroditic, incomplete digestive system.

Trophic interaction Consumer relationship between species. One species feeding on another.

Virus Among the smallest life forms, with a single kind of nucleic acid, and limited enzymatic capacity. Depends on host cell for metabolic activity and reproduction.

Definition

An organism that lives in or on another that is physiologically dependent on its host and causes some degree of harm to its host.

Parasitism is a lifestyle, and parasitic organisms of animals can be found in most phyla. Broadly described, they typically include microparasites (bacteria, viruses, fungi, protists) and macroparasites (helminths, arthropods). Parasites are ubiquitous; virtually, all species are hosts for parasites, and parasitism may be the most common lifestyle among organisms. By definition, parasites exert some degree of harm on their hosts.

Historical Background

Research in environmental parasitology has taken two basic directions. In the first, researchers have explored the notion that parasite populations and communities in fish can be used as indicators of pollution. In the second, studies have been conducted examining the interaction between contaminants and parasites in individual organisms, particularly fish and invertebrates. These research directions date back more than 20 years, but both have seen significant progress in the last two decades.

Characteristics of Parasites

Parasites are natural stressors that can be found in all ecosystems. Virtually, all species are host to parasites at one time or another. Thus, it may be both useful, because of their ubiquity, and important, because of their potential effects, to consider parasites in ecotoxicology. Marcogliese and Price (1997) present a brief primer on the biology of parasitism that is reviewed below.

Life cycles may be direct or complex. Parasites with direct life cycles infect only one host during their lifetime. In contrast, parasites with complex life cycles infect two or more hosts during their lifetime. The definitive host is defined as that in which sexual reproduction occurs, while those required for growth and development are termed intermediate hosts. Many parasites also have free-living infective stages that are released into the environment to infect the next host in the life cycle.

Parasites are typically divided for functional reasons into microparasites and macroparasites, based on their size and life cycle. Microparasites consist of viruses, bacteria, fungi, protists, and some monogeneans. These parasites have a direct life cycle. They reproduce and proliferate directly on the host. Macroparasites typically consist of the helminths (monogeneans, trematodes, cestodes, nematodes, acanthocephalans) and arthropods. Most, but not all of the macroparasites, have complex life cycles.

Parasites can also be characterized by their site of infection on the host. Ectoparasites are located on the fins, body surface, and gills. Endoparasites are located internally. Any tissue may be infected, although adult endoparasites often inhabit the gastrointestinal tract.

Applications: Effects on Biomarkers

As stated above, parasites are naturally occurring stressors. Numerous studies demonstrate that fish infected with certain parasites have lower survival than uninfected conspecifics when exposed to various contaminants (Boyce and Yamada 1977; Pascoe and Cram 1977; Moles 1980; Gheorgiu et al. 2006; Marcogliese and Pietrock 2011). More recently, combined effects of parasites and pollutants have been shown to have enhanced sublethal effects on their fish and frog hosts compared to either stressor alone (Sakanari et al. 1984; Christin et al. 2003; Jacobson et al. 2003; Marcogliese et al. 2005, 2009, 2010; Thilakaratne et al. 2007; Marcogliese and Pietrock 2011). Indeed, in some cases, the parasite in question may not have a detectable effect in control or reference conditions, but become pathogenic in polluted waters (Marcogliese et al. 2005, 2010; Thilakaratne et al. 2007; Marcogliese and Pietrock 2011).

Parasites not only can cause stress, but they can modulate biomarker responses in organisms, including some that are routinely employed in ecotoxicological studies, such as metallothionein, cytochrome P450, oxidative stress enzymes, and heat shock proteins (Morley et al. 2006; Sures 2007, 2008a, 2008b; Marcogliese et al. 2010). In cases such as these, parasites can interfere with the natural protective mechanisms of the host organism, thus enhancing the effects of exposure to contaminants (Sures 2008b). Selected examples of biomarkers in fish and frogs that are are positively or negatively modulated by parasites are shown in Table 1.

Effects of parasites and pollution may also be antagonistic, for example, as measured by plasma cortisol in European eels (Sures 2004, 2007, 2008a; Sures et al. 2006). In such cases, infection with parasites would lead to false-negative results when investigating pollution effects in wild populations (Sures 2004, 2007).

Parasites may function as natural endocrine disruptors. Indeed, certain parasites are known to feminize their male hosts (Jobling and Tyler 2003; Morley 2006; Sures 2006). One of the best-known examples of modified reproductive function caused by parasitism is via infection with larval cestodes (*Ligula intestinalis*), which castrate their fish intermediate hosts. Gonadal development is arrested in both male and female fish, with infection causing lower levels of sex steroids, follicle-stimulating hormone, and luteinizing hormone (Carter et al. 2005; Trubiroha et al. 2009, 2010). Plasma vitellogenin (VTG) was lower in female bream (*Abramis brama*) infected with *L. intestinalis* (Hecker and Karbe 2005). Consequently, infections with this parasite may lead to false-negative VTG results in studies of endocrine disruption (Schabuss et al. 2005).

Furthermore, it cannot be overemphasized that parasites should be correctly identified to the lowest possible taxon. Not all parasites are equal, nor do they have the same pathological effects under the same conditions (Marcogliese and Pietrock 2011).

Applications: Bioindicators of Pollution

The use of parasites as indicators of pollution has been extensively reviewed (Khan and Thulin 1991; Poulin 1992; Overstreet 1993; MacKenzie et al. 1995; Lafferty 1997; Marcogliese 2004, 2005; Blanar et al. 2009; Vidal-Martínez et al. 2010). Typically, in ecotoxicology, parasitism and disease are expected to increase when animals are under stress. However, this is not necessarily the case. Species richness of endoparasites that have complex life cycles typically decreases in polluted conditions (Mackenzie 1999; Marcogliese 2005). This is because free-living infective stages of parasites may be directly affected by exposure to toxic chemicals (Morley et al. 2003; Pietrock and Marcogliese 2003) or because the populations of their intermediate or definitive hosts are negatively impacted. In these cases, parasite transmission would be reduced. In addition, parasitized hosts may be more susceptible to contaminants than nonparasitized organisms (Sures 2004). Parasites with complex life cycles often depend on trophic interactions for transmission, and the community of parasites in a host can be an indicator of ecosystem stress, food web structure, and biodiversity. Essentially, any changes in the food web caused by pollution will affect the transmission of parasites among the hosts that participate in that food web (Marcogliese 2004, 2005).

Parasites with direct life cycles often increase in polluted waters, because the host's immune response is compromised, allowing the parasites to proliferate on that host (MacKenzie et al. 1995; MacKenzie 1999; Marcogliese 2005). These parasites typically include protists and certain monogeneans, as well as microbial

Parasites and Ecotoxicology: Fish and Amphibians, Table 1 Selected biomarkers of fish and amphibian health that have been subsequently shown to be modulated either positively or negatively by parasites

Biomarker	Host	Parasite	Direction	References
Hematocrit	Striped bass (*Morone saxatilis*)	*Anisakis* sp. (nematode)	Decreased	Sakanari et al. (1984)
Cortisol	European eel (*Anguilla anguilla*)	*Anguillicoloides* (=*Anguillicola*) *crassus* (nematode)	Increased	Sures et al. (2006)
Condition factor	Spottail shiner (*Notropis hudsonius*)	*Echinorhynchus rutili* (acanthocephalan)	Decreased	Thilakaratne et al. (2007)
Lipid peroxidation	Yellow perch (*Perca flavescens*)	*Apophallus brevis* (trematode)	Increased	Marcogliese et al. (2005)
Catalase, glutathione *S*-transferase, glutathione reductase, and glutathione peroxidase activities in liver and/or kidney	Carp (*Cyprinus carpio*)	*Ptychobothrium* sp. (cestode)	Increased	Dautremepuits et al. (2003)
Lipid peroxidation	South American catfish (*Rhamdia quelen*)	*Clinostomum detruncatum* (trematode)	Increased	Belló et al. (2000)
Serum lysozyme activity	Rainbow trout (*Oncorhynchus mykiss*)	*Lepeophtheirus salmonis* (crustacean)	Increased	Ruane et al. (2000)
Leukocyte oxygen radical production	Rainbow trout (*Oncorhynchus mykiss*)	*Lepeophtheirus salmonis* (crustacean)	Decreased	Ruane et al. (2000)
Plaque-forming cell assay	Chinook salmon (*Oncorhynchus tshawytscha*)	*Nanophyetus salmonicola* (trematode)	Decreased	Jacobson et al. (2003)
% leukocytes % granulocytes Acetylcholinesterase activity	Bullfrog (*Lithobates* [= *Rana*] *catesbeianus*)	*Haematoloechus* sp. (trematode)	Increased	Marcogliese et al. (2009)
Dehydroretinol	Bullfrog (*Lithobates* [= *Rana*] *catesbeianus*)	Strigeid metacercariae (trematode)	Increased	Marcogliese et al. (2009)
Dopaminergic activity in hypothalamus	California killifish (*Fundulus parvipinnis*)	*Euhaplorchis californiensis* (trematode)	Increased	Shaw et al. (2009)
Serotonergic activity in hippocampus	California killifish (*Fundulus parvipinnis*)	*Euhaplorchis californiensis* (trematode)	Decreased	Shaw et al. (2009)

Parasites and Ecotoxicology: Fish and Amphibians, Table 2 Guidelines and criteria for the selection of parasites and hosts to be used as environmental indicators (Adapted and modified from MacKenzie et al. (1995), Overstreet (1997), and Williams and MacKenzie (2003))

1.	The area of concern ideally should be well studied for a long period
2.	Host species should be local and nonmigratory
3.	The ecology of the host species should be well understood
4.	Hosts of smaller body size should be preferred over larger individuals
5.	Preferred hosts are those in which the parasite attains high levels of infection
6.	Preferred hosts are those that are infected by a relatively large number of parasite species, or at least a variety of different types of parasites with differing life cycles
7.	Both hosts and parasites should be readily available and consistently present in the ecosystem
8.	Parasites should be easily seen in the host and easily identifiable to the lowest taxonomic level possible
9.	The life cycles of the parasites should be known
10.	If the parasite has a wide host spectrum, those hosts that are dead ends and do not participate in the continuation of the parasite's life cycle should be identified
11.	The distribution of the parasites should be known, as those near the edge of their range may be more sensitive to environmental changes
12.	Ectoparasites and parasites of the gastrointestinal tract may serve as good indicators because they are in direct contact with contaminants
13.	Highly site-specific parasites may be sensitive to pollution-induced changes in host tissues
14.	Where possible, contaminants should be identified and quantified

pathogens. While these parasites often numerically increase on fish exposed to contaminants, total parasite species richness and diversity still tend to decrease.

Different taxonomic levels or categories of parasites can be used as indicators. Parasite communities can be used, much in the same way that researchers use them to discriminate among fish stocks in fisheries (Marcogliese 2005). However, sometimes, populations of individual species may be sufficient as indicators of pollution. In other cases, it may be more pertinent to focus on particular taxa, guilds, or suites of parasites, based on characteristics such as their shared life history patterns (Marcogliese 2005). For example, parasites that use molluscs as intermediate hosts (e.g., trematodes) have been shown to be useful indicators of environmental damage from acid precipitation and subsequent recovery (Cone et al. 1993; Marcogliese and Cone 1996, 1997).

Other advantages of using parasites for environmental research are listed in Marcogliese and Cone (1997). Criteria and guidelines for good candidate species are presented in MacKenzie et al. (1995), Overstreet (1997), and Williams and MacKenzie (2003). These are summarized in Table 2. The use of parasites as environmental indicators is not completely free of problems or controversy. Limitations and caveats in their use are discussed by Lafferty (1997), Kennedy (1997), Overstreet (1997), and Marcogliese (2005).

Applications: Accumulation Indicators

A number of intestinal parasites have been shown to bioaccumulate toxic contaminants, especially heavy metals. Cestodes and particularly acanthocephalans in fish have the capacity to take up and accumulate heavy metals hundreds of times higher than the tissues of their host fish and tens of thousands of times more than the surrounding water (Sures 2004, 2007, 2008b; Sures et al. 1999). Thus, Sures and colleagues have suggested that intestinal parasites can be used as sensitive bioindicators of heavy metal contamination in the environment (Sures 2004, 2008b; Sures et al. 1999).

Infection with parasites can also moderate contaminant load in host organisms (Sures 2006, 2007, 2008a, 2008b). Fish infected with intestinal acanthocephalans actually accumulate less metals in their tissues than do uninfected fish (Sures and Siddall 1999), thus potentially reducing their impact on fish health.

Conclusions

Because parasites have complex life cycles and rely on predator-prey relationships for transmission, they integrate together information on the ecological effects of pollution and environmental stress on their host, as well as on other organisms that participate in their life cycles. In addition, free-living stages may be directly sensitive to pollutants. Gastrointestinal parasites which bioaccumulate environmental toxins within a host can also be used as sensitive indicators of exposure to certain contaminants. Thus, parasite communities, populations, or other categories of assemblages can be used as environmental indicators at several different hierarchical levels of biological organization. Parasites also are important because they can become more pathogenic in polluted conditions, or affect the expression of physiological biomarkers in their hosts. In either case, knowledge of alteration of physiological responses and ecotoxicological measurements is essential to correctly interpret results from field studies, in addition to extrapolating laboratory studies on parasite-free organisms to natural conditions.

Multidisciplinary approaches are required to best understand the impacts of contaminants on species and ecosystems (Marcogliese 2005, 2008; Hayes et al. 2010). Good examples incorporating both ecotoxicology and parasitology can be found in Landsberg et al. (1998), Broeg et al. (1999), Diamant et al. (1999), and Vidal-Martínez et al. (2003, 2006).

There are a number of ways forward to incorporate parasitology into ecotoxicological sciences that can improve our understanding of the environmental effects of contaminants. Parasitologists and ecotoxicologists should not only be encouraged to work together, but they should be encouraged to work on the same individual organisms, to increase our understanding of potential interactions (Marcogliese

2008). The use of experimental mesocosms for experimental exposures to contaminants may be a useful approach to blend ecotoxicology and parasitology into a more controlled context (Marcogliese and Pietrock 2011). This methodological approach allows for more realistic food web interactions and the detection of indirect effects, while at the same time providing suitable replication for more robust statistical analyses of effects and their interactions.

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Cross-References

► Amphibian Ecotoxicology
► Aquatic Biomarkers
► Aquatic Immunotoxicity
► Aquatic Mesocosms in Ecotoxicology
► Parasites and Ecotoxicology: Molluscs and Other Invertebrates

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Parasites and Ecotoxicology: Molluscs and Other Invertebrates

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Article Outline

Definition

Parasites are typically small-sized organisms exploiting their host both as a food resource and as habitat (Loreau et al. 2005) often with demonstrable negative effects on the host.

This form of animal association has been defined by Crofton (1971) who also characterized its main features:

1. Ecological relationship between two different organisms, one designated the parasite, the other the host.
2. The parasite is physiologically or metabolically dependent upon its host.
3. Heavily infected hosts will be killed by their parasites.
4. The reproductive potential of the parasite exceeds that of their hosts.
5. There is an overdispersed frequency distribution of parasites within the host population. That is, the parasite population is not evenly distributed among the host population nor is it randomly distributed but clumped, so some hosts have a lot of parasites, but most have very few.

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Characteristics of Parasites

Host–parasite interactions are ubiquitous in nature and are important in shaping the life history strategies of both hosts and their parasites (Kuo et al. 2008). Based on life history traits such as size and mode of replication, parasites are separated into two categories. The microparasites, including viruses, bacteria, and protozoans, are usually associated with pathology. Transmission may be direct or involve vectors. The macroparasites include metazoans such as helminths (monogeneans, cestodes, nematodes, trematodes, acanthocephalans) and arthropods (crustaceans, insects, acarids). These parasites can live and reproduce (1) on the external body surface of the host (ectoparasites), (2) in internal cavities such as digestive tract or lung, and (3) inside blood vessels or cells (endoparasites). The macroparasites may have direct or complex life cycles with one or several intermediate hosts.

Transmission is accomplished through diverse means, including ingestion and penetration. Moreover, some parasite species can also manipulate their host behavior to facilitate transmission (Bandi et al. 2001; Rigaud et al. 2005; Morand and Deter 2007).

Many parasites have diverse life history strategies involving more than one host, thereby providing information about the presence of other organisms participating in their life cycles and their trophic interactions in that ecosystem (Marcogliese and Cone 1997; Marcogliese 2005). Moreover, parasites may play an important role in structuring ecosystems (Poulin 1999; Lafferty et al. 2006). Considering that virtually all organisms are hosts for parasite species and that both contaminants and parasites may be considered as stressors (Marcogliese and Pietrock 2011), the interaction between contamination and parasitism could have serious implications for environmental risk assessment.

Historical Background

Considering the literature published in the framework of environmental parasitology during the last 15 years (for reviews see Lafferty 1997; Marcogliese 2005; Blanar et al. 2009; Vidal-Martínez et al. 2010), studies dealing with interaction between parasites and environmental pollution focused mainly on three aspects: (1) parasites as indicators of pollution; (2) parasites modifying biomarker responses of their hosts and more generally their physiology, thus interfering with ecotoxicological applications; and (3) parasites themselves being useful as accumulation indicators (Sures 2004).

Historically parasitism has been primarily studied in organisms of commercial interest, such as fishes and to a lesser extent some marine invertebrates. Among invertebrates, molluscs have been the most thoroughly studied with little attention paid to other organisms.

Molluscs are common in aquatic ecosystems and ecologically and commercially important on a global scale (Morley 2010). Many species are considered useful bioindicators for aquatic environmental monitoring and at the same time fulfill the main criteria for the selection of parasites and hosts to be used as environmental indicators (for more information, see the entry on "▶ Parasites and Ecotoxicology: Fish and Amphibians"). Molluscs, due to their medical, veterinary, and economic importance, have been the subject of numerous investigations in recent years (see reviews of Morley et al. 2003, 2006; Morley 2010).

Applications: Effects on Physiology and Biomarkers

During the last two decades, the need to detect and assess the impact of pollution on environmental quality has promoted the development of biological markers (i.e., biomarkers) in several vertebrate and invertebrate species. However, many biotic and abiotic environmental factors other than pollution (e.g., stage of development, reproduction, food availability, season) can influence biomarker responses and cause difficulties in interpreting results (Moore et al. 2004).

Parasites can induce physiological changes and pathology in their hosts, affecting metabolism, immune response, growth, development, and fecundity (Marcogliese 2004). In natural environments and also in experimental investigations, parasitism can represent a confounding factor interacting with other stressors (Sures 2004; Minguez et al. 2009; Marcogliese and Pietrock 2011).

Table 1 gives an overview of the effects of pathogens and parasites on both physiological and biological responses in selected species of bivalves, gastropods, crustaceans, and insects. Clearly, a diverse array of pathogens and parasites can affect a variety of organismal responses and physiological processes which are often used as ecotoxicological biomarkers and bioindicators. Laboratory experiments show that the freshwater bivalve, *Pisidium amnicum*, parasitized by trematodes was more tolerant of contaminants such as polychlorobiphenylates (Heinonen et al. 2001). In contrast, other infected freshwater invertebrate species, including the amphipod *Gammarus pulex* and the clam *Cerastoderma edule*, were more susceptible to environmental perturbations such as aluminum or hypoxia, compared to uninfected animals (McCahon and Poulton 1991; Wegeberg and Jensen 1999; Prenter et al. 2004; for a review see Marcogliese and Pietrock 2011).

Other physiological functions such as cellular defenses can be influenced by parasites. Effects on antioxidant activity (e.g., superoxide dismutase) vary according to the species. Its activity increased in the oyster *Crassostrea gigas* infected by the trematode *Polydora* sp. (Chambon et al. 2007) or decreased in the decapod *Palaemonetes argentinus* infected by the isopod *Probopyrus ringueleti* (Neves et al. 2000). The lysosomal system was reduced when the zebra mussel, *Dreissena polymorpha*, was infected by the ciliate, *Ophryoglena* spp., or by the

Parasites and Ecotoxicology: Molluscs and Other Invertebrates, Table 1 Overview of the effects of parasites and pathogens on both physiology and biological responses in selected species of bivalves, gastropods, crustaceans and insects

Host	Stressor	Biomarker/bioindicator	Direction of response	References
Bivalves				
Anodonta piscinalis	*Rhipidocotyle campanula* or *R. fennica* (trematode, 1st host) under anoxia or starvation conditions	Mortality	↗	Jokela et al. (2005)
Anodonta piscinalis	*Rhipidocotyle fennica* (trematode, 1st host)	Growth	↙	Taskinen (1998)
Cerastoderma edule	*Himasthla elongata* (trematode, 2nd host) or *Labratrema minimus* (trematode, 1st host)	Metallothionein synthesis	↗	Baudrimont et al. (2006), Desclaux-Marchand et al. (2007)
Cerastoderma edule	*Himasthla elongata* (trematode, 2nd host)	Cd accumulation	0 (Except after 14 days)	Paul Pont (2010)
	Himasthla elongata and *Vibrio tapetis (bacterium)*	Metallothionein synthesis	↗	
	Himasthla elongata	Mitochondrial activity	↗	
	Himasthla elongata and/or *Vibrio tapetis*	Phagocytosis	0	
	Himasthla elongata or *Vibrio tapetis*	Production of reactive oxygen species	↗	
	Himasthla elongata and/or *Vibrio tapetis*	Hemocyte number	↗	
Cerastoderma edule	*Himasthla elongata*	Survival after 30 h of hypoxia	↙	Wegeberg and Jensen (1999)
Crassostrea gigas	*Polydora* sp. (polychaete)	Condition	↙ (Weight)	Chambon et al. (2007)
			↗ (Partial pressure in O2)	
		SOD (expression)	↗	

Crassostrea gigas	*Perkinsus marinus* (dinoflagellate)	Apoptosis	~	Hughes et al. (2010)
Crassostrea virginica	*Perkinsus marinus*	Mortality	↗ (+ TBT exposure)	Fisher et al. (1999)
Crassostrea virginica	*Perkinsus marinus*	pH of hemolymph	↙	Paynter (1996)
Dreissena polymorpha	*Phyllodistomum macrocotyle* (trematode, 1st host)	Weight	↙	Kraak and Davids (1991)
		Metal accumulation (Cu, Zn, Cd, Pb)	↗	
Dreissena polymorpha	*Ophryoglena* spp. (ciliates) or Rickettsiales-like organisms (bacteria)	Digestive lysosomal system	↙	Minguez et al. (2009)
	coinfection (*Ophryoglena* and RLOs)		↗	
	Ophryoglena spp. or Rickettsiales-like organisms	Digestive neutral lipid content	0	
	coinfection (*Ophryoglena* and RLOs)		↙	
Mytilus edulis, M. galloprovincialis, Pecten maximus and *Ostrea edulis*	*Prosorhynchus squamatus* (trematode, 1st host)	Gonad mitosis	(−) (In vitro)	Cousteau et al. 1991, 1993 in Morley (2006)
Mytilus galloprovincialis	*Marteilia refringens* (Haplosporidium)	Hemocyte number	↗	Carballal et al. (1998)
	Mytilicola intestinalis (copepod)		0	
	Urastoma cyprinae (Turbellarian)		↗	
Mytilus galloprovincialis	*Pseudomyicola spinosus* (copepod)	Condition index	↙	Olivas-Valdez and Cáceres-Martínez (2002)
Perna perna	*Bucephalus* sp. (trematode, 1st host)	THC (total hemocyte count)	↙	da Silva et al. (2002)
		DHC (differential hemocyte count)	↙	
		Plasmatic protein	0	
Pisidium amnicum	Trematode	Sensibility to 2,4,5-trichlorophenol and BaP	↗	Heinonen et al. (2000)
Pisidium amnicum	Trematode	Sensibility to pentachlorophenol	↙	Heinonen et al. (2001)

(*continued*)

Parasites and Ecotoxicology: Molluscs and Other Invertebrates, Table 1 (continued)

Host	Stressor	Biomarker/bioindicator	Direction of response	References
Ruditapes philippinarum	*Perkinsus marinus* (dinoflagellate)	Hemocyte size	↗ (+ Toxic algae exposure)	da Silva et al. (2008)
Ruditapes philippinarum	*Perkinsus olseni* (dinoflagellate)	Hemocyte number	↗ (+ Toxic algae exposure)	Hégaret et al. (2007)
		Phagocytosis	↙	
Ruditapes philippinarum	*Himasthla elongata* (trematode, 2nd host)	Cd accumulation	0	Paul Pont (2010)
		Hemocyte number	0 or ↙ (Dependent on bivalve population and Cd exposure)	
		Hemocyte viability	0 or ↙ (Dependent on bivalve population and Cd exposure)	
		Phagocytosis	0	
	Himasthla elongata and Vibrio tapetis (bacterium)	Metallothionein synthesis	↗	
	Himasthla elongata and/or *Vibrio tapetis*	Phagocytosis, ROS production, hemocyte number, hyalinocyte concentration	0	
	Himasthla elongata	Hemocyte viability	0	
	Vibrio tapetis	Hemocyte viability	↙	
Gastropods				
Biomphalaria alexandrina	*Schistosoma mansoni* (trematode, 1st host)	Growth	↗But after 6 weeks ↙↙	Ibrahim (2006)
		Reproduction	↙	
		Survival	After 4 weeks postinfection ↙	
Biomphalaria alexandrina	*Schistosoma mansoni*	Calcium concentration in shell	↙	Mostafa (2007)

Biomphalaria glabrata	*Echinostoma caproni* (trematode, 1st and 2nd host)	Lutein and β-carotene concentrations	0	Evans et al. (2004)
Biomphalaria glabrata	*Schistosoma mansoni*	Glucose and maltose contents	↙	Jarusiewicz et al. 2006
Biomphalaria glabrata	*Schistosoma mansoni*	Sensitivity to high temperatures	↗	Lee and Cheng (1971)
Biomphalaria glabrata	*Schistosoma mansoni*	Serotonin and dopamine concentrations	↙	Manger et al. (1996) in Morley (2006)
Bulinus truncatus	*Schistosoma haematobium* (trematode, 1st host)	Calcium concentration in shell	↙	Mostafa (2007)
Cerithidea californica	*Euhaplorchis californiensis* (trematode, 1st host)	Metallic ion accumulation	Mg : ↙	Kaufer et al. (2002)
			Fe, Cu, Zn, Na, K : 0	
			Ca : ↗	
Helisoma trivolvis	*Echinostoma trivolvis* (trematode, 1st host)	Lutein and β-carotene concentrations	0	Evans et al. (2004)
Ilyanassa obsoleta	*Zoogonus lasius* (trematode, 1st host)	Spermatogenesis	(−) (In vitro)	Pearson and Cheng 1985 in Morley (2006)
Littorina littorea	*Himasthla elongata* (trematode, 2nd host) *and Cryptocotyle lingua* (trematode, 1st host)	Phagocytosis	(−)	Iakovleva et al. (2006)
Littorina littorea	Trematode larvae	Metal accumulation (Cu, Fe, Ni)	↙	Evans et al. (2001)
		Lead accumulation	↗	
Littorina saxatilis	*Microphallus* sp. (trematode, 1st host)	Fatty acids composition	~	Arakelova et al. (2003, 2007)
		Respiration	↙(Young organisms castrated by the parasite)	
		Glycogen	↙	
Lymnaea palustris	*Metaleptocephalus* sp. (trematode, 1st host)	Phagocytosis, ROS production, hemocyte number	0	Russo and Lagadic (2000)
		Lectin	↗	

(*continued*)

Parasites and Ecotoxicology: Molluscs and Other Invertebrates, Table 1 (continued)

Host	Stressor	Biomarker/bioindicator	Direction of response	References
Lymnaea truncatula	*Fasciola hepatica* (trematode, 1st host)	Succinate and lactate dehydrogenase activities	20 days postinfection: ↗	Humiczewska (2004)
			60 days postinfection: ↙	
Lymnaea truncatula	*Fasciola hepatica*	Lipid contents	↙	Humiczewska and Rajski (2005)
Potamopyrgus antipodarum	*Microphallus sp.* or *Notocotylus gippyensis* (trematodes, 1st host) under starvation	Survival	↙	Jokela et al. (1999)
Semisulcospira libertina	Trematode larvae	Food intake and respiration	↗	Shinagawa et al. (2001)
Crustaceans				
Corophium volutator	*Maritrema subdolum* (trematode, 1st host)	Mortality	↗ (+ enhanced temperatures)	Mouritsen and Jensen (1997)
Gammarus pulex	*Pomphorhynchus laevis* (acanthocephalan, 1st host)	Cadmium sensibility	↗	Brown and Pascoe (1989)
Gammarus pulex	*Pomphorhynchus laevis*	Mortality	↗	McCahon et al. (1988, 1989)
		Food intake	↙	
Gammarus pulex	*Pomphorhynchus laevis*	Aluminum or ammonium sensitivity	↗	McCahon and Poulton (1991)

Gammarus pulex	*Pomphorhynchus laevis*	Lipids	↙ (gravid females)	Plaistow et al. (2001)
		Glycogen	↗	
Gammarus pulex	*Echinorhynchus truttae* (acanthocephalan, 1st host)	Ammonium tolerance	↙	Prenter et al. (2004)
Gammarus roeseli	*Polymorphus minutus* (acanthocephalan, 1st host)	Salinity tolerance	↗	Piscart et al. (2007)
Gammarus roeseli	*Polymorphus minutus* + palladium exposure	HSP expression	(−)	Sures and Radszuweit (2007)
Cyathura carinata	Microphallidae gen. sp. (trematode, 1st host)	Mortality	↗	Ferreira et al. (2005)
		Growth	↙	
		Fecundity	↙	
Palaemonetes argentinus	*Probopyrus ringueleti* (isopod)	SOD activity and respiration	↙	Neves et al. (2000)
Palaemonetes argentinus	*Probopyrus ringueleti*	Glycogen	↗	Neves et al. (2004)
		Lipids	↙	
Palaemonetes pugio	*Probopyrus pandalicola* (isopod)	Hg accumulation	↙	Bergey et al. (2002)
Daphnia magna	*Pasteuria ramosa* (bacterium) or *Flabelliforma magnivora* (microsporidian)	Mortality	↗ (+ Carbaryl exposure)	Coors et al. (2008)
Insects				
Aedes aegypti	*Vavraia culicis* (Microsporidian) or *Ascogragarina culicis* (Apicomplexa)	Adult survival	↙	Fellous and Koella (2010)

Rickettsiales-like bacteria but was enhanced in the case of mixed infection (Minguez et al. 2009). The cockles, *C. edule* and *Ruditapes philippinarum*, when infected by trematodes and *Vibrio tapetis*, showed an increase in metallothionein synthesis, involved in the homeostasis and detoxification of metals (Baudrimont et al. 2006; Desclaux-Marchand et al. 2007; Paul Pont 2010). Heat shock protein response was modulated in the amphipod *Gammarus roeseli* naturally infected with the acanthocephalan *Polymorphus minutus*, preventing increase of this defense protein after exposure to palladium (Sures and Radszuweit 2007). The trematodes *Bunodera luciopercae* and *Schistosoma mansoni* influence the function of the immune and endocrine systems of their hosts (i.e., *Pisidium amnicum* and *Biomphalaria glabrata*, respectively) (Heinonen et al. 2001; Morley 2006), thus potentially distorting the results of endocrine disruptor studies of invertebrate populations exposed to contaminants.

Applications: Invertebrate Parasites – Could They Be Used as Bioindicators of Pollution?

Table 2 reports selected studies dealing with effects of pollution and other stressors on parasites and pathogens in invertebrates. It is apparent that interactions of both parameters in invertebrate populations are complex and vary with host, parasite, and contaminant. As such, elucidating general trends in interactive processes remains difficult.

Major trends have been reviewed by Sures (2004) and Marcogliese (2005). Pollution can increase parasitism if host defense mechanisms are negatively affected, thereby increasing host susceptibility or simply increasing the population densities of suitable intermediate and/or final hosts. On the other hand, pollution can decrease parasitism if (1) infected hosts suffer more from environmental exposure than do uninfected hosts, (2) parasites are more susceptible to the particular pollutant than their host, or (3) pollution kills the intermediate or final hosts. Furthermore, effects of pollution can vary not only among parasite species but also developmental stages within species because larval and adult parasites can be affected in different ways. Usually, parasites with complex life cycles requiring multiple hosts tend to decrease because environmental perturbations may affect a host or transmission at any point in the life cycle (Huspeni et al. 2005).

In contrast to fishes, the potential use of parasites as indicators of pollution in invertebrate species is rather poorly studied. In this case, studies focus mainly on particular parasite species rather than broader indices of parasite species richness and diversity, which are commonly used in vertebrate populations. The lack of interest in some invertebrate species with no commercial value and technical limitations in collecting and identifying parasites may partially explain this situation. Only few studies testing the use of parasite populations to assess environment quality have been reported in the literature.

Parasites and Ecotoxicology: Molluscs and Other Invertebrates, Table 2 Effects of pollution and other stressors on infection in selected species of bivalves, gastropods, crustaceans, and insects

Host	Parasite	Stress	Infection	References
Bivalves				
Bathymodiolus sp.	*Bucephalus* sp. (trematode, 1st host)	PAHs	↙	Powell et al. (1999)
Crassostrea gigas	*Vibrio splendidus* (bacterium)	Mechanical stress	↗	Lacoste et al. (2001)
Crassostrea virginica	*Perkinsus marinus* (dinoflagellate)	PAHs	↗	Chu and Hale (1994), Chu et al. (2002)
Crassostrea virginica	*Perkinsus marinus*	TBT	↗	Fisher et al. (1999)
Crassostrea virginica	*Perkinsus marinus*	Herbicide Weed-B-Gone (concentrations higher than those recommended by the manufacturer)	↗	Bushek et al. (2007)
		Ammonium, nitrate, phosphate, fluoranthene, phenanthrene	0	
Crassostrea spp.	Cestodes	Cd	↙	Kim et al. (1998)
	Perkinsus marinus	Cd	↙	
	Nematodes	PAHs, PCBs	↙	
	Nematopsis sp. (Apicomplexa)	HAPs	↗	
	Cestodes	Hg	↗	
	Rickettsia sp. (bacterium)	Ni, Cd	↗	
	Perkinsus marinus	PAHs, Hg	↗	
Crassostrea spp.	Cestodes	Metals and pesticides	↙	Kim et al. (2008)
	Gregarines	Pesticides	↙	
Mytilus trossulus	Trematode (metacercaria)	Untreated effluents	↗	Moles and Hale (2003)
	Ciliates in gills		0	
Mussels (*Mytilus edulis*, *Mytilus californianus*, *Dreissena polymorpha*)	Total parasites	Cu	↙	Kim et al. (1998)
	Ciliates in gills	Ni	↙	
	Total parasites	Se	↗	

(*continued*)

Parasites and Ecotoxicology: Molluscs and Other Invertebrates, Table 2 (continued)

Host	Parasite	Stress	Infection	References
	Bucephalus sp. (trematode, 1st host)	PCBs	↗	
	Ciliates in gills	Cd, Se	↗	
Dreissena polymorpha	Rickettsiales-like organisms (bacteria)	Metals (Cr, Ni, Cu, Pb, Zn)	↗	Minguez et al. (2011)
	Bucephalus polymorphus (trematode, 1st host)	Metals, PAHs	↙	
Gastropods				
Cerithidea californica	Larval trematodes	Environmental impacts (e.g., habitat destruction)	↙	Huspeni and Lafferty (2004), Huspeni et al. (2005)
Lymnaea peregra or *Physa fontinalis*	*Echinoparyphium recurvatum* (trematode, 1st and 2nd host)	TBTO	↙	Morley et al. (2004)
Physella columbiana and *Lymnaea palustris*	Trematodes, ciliates, nematodes, oligochaete, etc.	Metals	↙	Lefcort et al. (2002)
Stagnicola elodes	Trematode	Cd	↙	Pietrock et al. (2008)
Amphipods				
Corophium volutator	*Maritrema subdolum* (trematode, 1st host)	Higher temperatures	↗	Mouritsen and Jensen (1997)
Monoporeia affinis	Microsporidia	PFOS	↗	Jacobson et al. (2010)
Decapods				
Farfantepenaeus duorarum	Helminths	Metals, PAHs, PCBs, pesticides, . . .	0	Vidal-Martínez et al. (2006)
	Symbionts	Pesticides, unresolved complex mixture, PCBs	↙	
Pandalus borealis	Microsporidia	PAHs	↗	Moles (1999)

Lafferty and collaborators (Huspeni and Lafferty 2004; Huspeni et al. 2005; Hechinger et al. 2006) underlined that trematodes in the California horn snail, *Cerithidea californica*, can potentially be developed as indicators of benthic diversity in coastal ecosystems in California. However, while some studies revealed that trematodes in gastropods can be used as good indicators of bird communities, it is still unclear as to whether they are useful as indicators of benthos and fishes. Although they found some positive associations between species richness and abundance of trematodes in snail populations and surrounding benthic communities, the results remained inconsistent. In another study, Siddall et al. (1993) suggested that trematode communities in the snail *Buccinum undatum* may be used as indicators of trace metal pollution in marine environments.

A variety of molluscan species are used as bioindicators to monitor the status and trends of coastal water quality in the US Mussel Watch Program. However, Kim and coworkers (Kim et al. 1998, 2008) observed that the distribution of parasites/pathologies within taxa and along coasts is sufficiently complex such that a universal pattern associated with contaminants may be an unachievable goal but that relationships may be present at smaller spatial scales within taxa. Helminths and symbionts of the pink shrimp, *Farfantepenaeus duorarum*, were also examined in an environmental context (Vidal-Martínez et al. 2006). The authors found no significant associations between pollutants and helminths but negative associations of pesticides, PCBs, and UCM (unresolved complex mixture) with symbiont numbers after controlling for shrimp size and spatial autocorrelation. They concluded that shrimps and their symbionts appear to be promising bioindicators of organic chemical pollution. In this study, the authors examined contaminants and symbionts in the same organisms by cleverly combining histological and chemical analyses. More recently, Minguez et al. (2011) showed that sites of different environmental qualities (i.e., chemical contamination) exhibited different parasite communities characterized by different trematode species and parasite associations in the freshwater mussel, *D. polymorpha*. In addition, significant correlations between metallic and organic contamination and prevalence rates of Rickettsiales-like bacteria (positive) and the trematode, *Bucephalus polymorphus* (negative), reinforced the discrimination between sites.

Conclusions

It is clear from studies cited in this entry that parasites can strongly influence molluscan response to contaminant exposure. In particular the effects they have on the host's physiological function could distort results of ecotoxicological studies, providing false-negative or false-positive results, if studies do not assess infectious disease occurrence in individual molluscs (Morley 2010) or other invertebrate species.

While promising, the use of parasites/pathologies as bioindicators of pollution in biomonitoring still remains unclear in some cases. Previous studies recommend further investigations in order to (1) examine larger-scale patterns to confirm observed correlations, (2) assess the normal seasonal variations of epidemiological parameters, (3) evaluate the influences of environmental stressors, and (4) quantify the costs and benefits of different sampling techniques. The traditional ecological indices used to study fish–parasite systems cannot always be applied to mollusc–parasite interactions, but other methods, such as clustering and nonmetric analyses, could be useful in characterizing parasite assemblages and may represent complementary and/or alternative methods in biological assessment of water quality (Baudrimont et al. 2006; Minguez et al. 2011). Therefore, the significant role infectious diseases play in structuring the population dynamics of aquatic communities needs to be more widely recognized by ecotoxicologists (Morley 2010). Ecotoxicologists should share their expertise with parasitologists, and inversely, so as to develop multidisciplinary research programs in this area (see Marcogliese 2005, 2008; Hayes et al. 2010).

Cross-References

► Aquatic Biomarkers
► Aquatic Immunotoxicity
► Bivalves in Ecotoxicology
► Parasites and Ecotoxicology: Fish and Amphibians

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Phototoxicity in Ice

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Article Outline

Synonyms

Photo-activated toxicity; Photoactivation; Photo-induced toxicity

Glossary

Cryosphere The term cryosphere describes the portions of the Earth's surface where water is in solid form (sea ice, lake ice, river ice, snow cover, glaciers, ice caps and ice sheets, frozen ground which includes permafrost).

Nucleophile A nucleophile is a chemical reagent that forms a chemical bond to its reaction partner (the electrophile) by donating both bonding electrons. All molecules or ions with a free pair of electrons (electron-rich chemical species) can act as nucleophiles being attracted to a full or partial positive charge.

Photochemistry Photochemistry is a subdiscipline of chemistry, which studies interactions between light and atoms or molecules and describes chemical reactions that proceed with the absorption of light by a chemical species.

Phototoxicity Photo-induced toxicity, Phototoxicity is caused by the transfer of light energy to the chemical molecule (photoexcited state) leading to reactions that form new chemical species with higher toxicity than the parent compound.

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Often, photoexcited chemical transfers the energy to an oxygen molecule or water molecule, creating highly reactive molecules or radicals that damage biomolecules in living organism.

Polycrystalline Polycrystalline materials are solids that are composed of many crystallites of variable size and orientation. Almost all common metals, many ceramics, and also solid or snow are polycrystalline.

Trophosphere The troposphere is the lowest part of Earth's atmosphere containing approximately 75% of the atmosphere's mass and 99% of water vapors and aerosols. The average depth of the troposphere is 17 km in the middle latitudes being deeper in the tropical regions (up to 20 km) and shallower near the poles (7 km).

UV (ultraviolet) light Electromagnetic radiation with higher energies and shorter wavelength than that of visible light (range 10–400 nm). It is so named because the frequencies of the waves are higher than those that humans identify as the violet color.

Definition

Upon irradiation with UV or visible light, chemicals trapped in solid matrices such as ice or snow undergo unique chemical processes, which are very distinct from their fate in aqueous media. For example, new toxic compounds can be formed in ice or snow (such as hydroxylated chlorinated biphenyls formed during irradiation of chlorophenols). Thawing of ice and the release of the newly formed compounds from the ice may serve as a new unexpected secondary source of contamination.

Photo-induced toxicity (i.e., increased toxic effects of organic contaminants after irradiation) has been known for a long time. Most experimental and field studies have documented aquatic phototoxicity of contaminants such as polycyclic aromatic hydrocarbons (PAHs) and their derivatives, pesticides, and others (Pelletier et al. 2006; Lin et al. 2008). In spite of extensive research of photo-induced toxicity (phototoxicity) in water, new aspects of organic photochemistry have recently been discovered in solid water matrices (Grannas et al. 2007a).

In solid ice or snow, compounds undergo unique reactions of environmental concern since solute molecules, such as organic hydrophobic compounds, accumulate in the unfrozen solution surrounding the crystal walls of the polycrystalline state when their aqueous solutions freeze, and this phase transition radically modifies the reaction environment. The solute concentration at the grain boundaries increases, migrations and conformational motions of molecules are suppressed, and these conditions may lead to formation of new unexpected (and possibly highly toxic) products.

Historical Background

The cryosphere forms a substantial proportion of the Earth's surface with seasonal maxima around 40% of land and several percent of the oceans covered by snow or ice. In the past, ice and snow have been considered a “cap” preventing emissions from the surfaces below and acting as a sink of atmospheric chemical species. However, recent studies showed that the polar cryosphere may have a major influence on the overlying atmosphere by being highly photochemically active (Grannas et al. 2007a).

Another important part of the global cryosphere is the ice present in the upper parts of the atmosphere inside the tropospheric ice clouds. Although far from the Earth’s surface and less understood, they are important for global climate as they cover about 20% of the globe, they reflect more UV, and they absorb more infrared radiation than water clouds (Stephens and Kummerow 2007). A large surface of the small ice particles in the troposphere may contain various anthropogenic contaminants, which could undergo unique processes described in detail below.

In the past decade, laboratory research results have provided evidence that many organic compounds can undergo light-induced chemical transformations in the ice matrix (Klan et al. 2000a,b, 2001, 2003; Klan and Holoubek 2002). The presence of free water in the liquid matrix usually leads to the hydrolysis of original molecules upon irradiation, and it has been well described in several reviews (e.g., Malato et al. 2009). Moreover, formation of new and unexpected toxic compounds has been recorded in the solid ice matrix. This may have large consequences for the environment since many secondary photoprocesses may occur in natural ice or snow with photoproducts completely different from those obtained in liquid solutions or gas phase.

Results and Case Studies

It is now well recognized that ice and snow in polar areas comprise a highly (photochemically) active matrix contributing to global cycling of chemicals including anthropogenic contaminants. Results of some experimental studies that demonstrated the importance of ice and snow in the context of phototoxicity are given below.

- **New toxic compounds are formed in ice.** A series of studies with widespread organic industrial chemicals, chlorophenols and halobenzenes, demonstrated that phototransformations within the polycrystalline ice include coupling reactions leading to formation of highly toxic compounds such as polychlorinated biphenyls (PCBs) or even polychlorinated dioxins (PCDD/Fs; Klan et al. 2000a, b, 2001, 2003); Klan and Holoubek 2002). Detailed toxicological investigations with 2- and 4-chlorophenols showed formation of new coupling photoproducts (hydroxylated chlorobiphenyls; Klanova et al. 2003) that were found to have high acute toxicity in a bacterial luminescence test and induced dioxin-like effects in vitro by modulating the aryl hydrocarbon receptor, AhR (Bláha et al. 2004). These studies provided evidence that solar radiation can trigger formation of new types of organic pollutants in polar areas or tropospheric ice cloud particles.

- **Gases and small molecule chemical species are formed in ice.** Irradiated snow or ice is known to play an important role in the processing of atmospheric chemicals, including photochemical production of NO_X, HONO, molecular halogens, alkyl halides, and carbonyl compounds, among others (Sumner and Shepson 1999; Honrath et al. 1999). A detailed study of Grannas et al. (2007b) demonstrated highly enhanced rates of photochemical nucleophilic substitutions of a model system containing p-nitroanisole with pyridine. Reaction rates were enhanced by a factor of up to 40 when frozen at temperatures between 236 and 272 K. Thus, the direct extrapolations of liquid-phase kinetics to reactions occurring in frozen water systems may not be valid for certain reactions.
- **Ice is a source of bioavailable iron.** Laboratory as well as outdoor experiments in Ny-Alesund (Svalbard, 78° 55'N) showed that ice (snow)-covered surfaces and ice-cloud particles containing iron-rich mineral dusts provide a source of bioavailable iron when they thaw (Kim et al. 2010). While the photoreductive dissolution of insoluble iron oxides proceeded slowly in water, it was shown to be significantly accelerated in polycrystalline ice upon both UV and visible light irradiation (Kim et al. 2010). Subsequently, thawing of ice and snow leads to release of substantial amounts of bioavailable ferrous iron, which is a known water "fertilizer" stimulating growth of algae and other microorganisms.
- **Photodegradation in ice may be faster and more efficient than in water.** Weber et al. (2009) demonstrated that the organophosphorus pesticides methylparathion and fenitrothion are more efficiently photolyzed in ice than in aqueous solutions. Authors also demonstrated the presence of oxons in ice following irradiation of pesticides, providing an additional formation mechanism of these toxicologically relevant compounds in cold environments. Photochemistry of pesticides in ice thus might be an environmentally important sink of toxic chemical species in cold environments.
- **Photoreductive dehalogenation in ice.** Studies with representatives of persistent organic pollutants (polychlorinated biphenyls PCB-7 and PCB-153) trapped in snow at environmentally relevant concentrations showed that the main photodegradation pathway occurs via reductive dehalogenation (Matykiewiczova et al. 2007). The authors also demonstrated that water from ice was not involved in the reactions, but traces of small compounds trapped in ice (such as ethanol or hydrogen peroxide) acted as photo-reactants. Based on their results, it was estimated that the average lifetime of PCBs in surface snow, connected exclusively to the photoreductive dechlorination process, is 1–2 orders of magnitude longer than that in surface waters when subjected to the equivalent solar radiation. However, should the concentration of hydrogen peroxide in natural snow be sufficient, the photo-induced oxidation process could be more pronounced than the most commonly occurring photoreductive dechlorination (Matykiewiczova et al. 2007).

Impact on Biota

The impact of the processes described above on biota has not been studied in detail yet. Nevertheless, there are several lines of evidence indicating exposures of organisms to the products of the ice photo-transformation. Melting of ice, in particular, which is common under current global change, may lead to mobilization of the photoproducts and their release into the environment in bioavailable (dissolved) forms.

The major expected exposure routes for organisms are:

1. Whole-body exposure and direct contact of aquatic biota with water, which contains newly formed (and potentially toxic) photoproducts released from melted ice.
2. Respiratory exposures of terrestrial organisms to newly formed gases and small reactive molecules released after photochemical reactions in the ice. The environmental importance of the gases is rather in their role in relation to atmosphere physics, but some known photoproducts may be directly toxic including nitrogen oxides, sulfur oxides, or small volatile organic compounds (VOCs) such as formaldehyde (Sumner and Shepson 1999).

Impact may be either negative (potential toxicity of new photoproducts as outlined in the previous paragraph) or positive, for example, growth stimulation of photosynthetic organisms after fertilization of water by bioavailable iron (Kim et al. 2010). However, uncontrolled eutrophication of waters is another known environmental problem. Stimulations of phytoplankton growth lead, for example, to the formations of toxic cyanobacterial water blooms with tremendous indirect impacts on the whole aquatic ecosystem (Pearl and Huisman 2009).

Conclusions

Solid ice and snow are important environmental matrices, but their role in the fate of anthropogenic contaminants has not been fully explored. Recent studies document unique photochemical processes in ice that contribute to global cycling and impacts of various chemicals such as (1) unexpected formation of new highly toxic compounds, (2) photochemical release of volatile molecules and gases, or (3) enhanced photochemical reactions in ice in comparison with water (e.g., formation of bioavailable iron or photodegradation of pesticides). Several lines of evidence thus indicate that solar radiation can trigger formation of new pollutants in polar areas or tropospheric ice-cloud particles that may have direct or indirect impacts on biota in the aquatic environment (Chapman and Riddle 2005).

Cross-References

► Environmental Transformation of Organic Substances in the Context of Aquatic Ecotoxicology

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Phytoremediation in Ecotoxicology

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Article Outline

Synonyms

Phytoremediation toxicity studies; Wastewater treatment plant remediation

Glossary

Constructed wetland Artificial marsh or swamp, created for anthropogenic discharge with the aim that the wetland will act as a biofilter and induce sedimentation and decrease of pollutant concentrations.

Chlorophyll *a* fluorescence When green plants are illuminated they fluoresce. At room temperature, the light energy absorbed by chlorophyll associated with photosystem II is mainly responsible for this phenomenon. This endpoint is often used to assess the health status of plants.

Definition

Phytoremediation is the use of plants and their associated microorganisms to remove, neutralize, or biodegrade contaminants, as in polluted soil, water, or air. For aquatic applications (mainly constructed wetland), plants are mostly involved in heavy metal and organic contaminants (pesticide, pharmaceutical, etc.) removal.

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In the majority of cases, laboratory experiments have shown a positive relationship between plant tolerance and removal rate. Therefore, the use of toxicity tests in order to determine a plant's tolerance to contaminants can be considered as a first step in plant selection for phytoremediation purposes.

Moreover, toxicity testing can give information on which parts of plants are in contact with a pollutant and on its translocation inside the plant.

Historical Background

Ecotoxicology essentially began in the 1960s (see entry on "▶ Ecotoxicology: Historical Overview and Perspectives"). From the 1980s onward, phytoremediation experiments were well in progress, and their use accelerated from 2000 onward especially after links between toxicity testing and phytoremediation became apparent. Intrinsically, finding relations between ecotoxicology and phytoremediation is an important means of selecting plant species best adapted for remediation processes as such investigations can also give information on contaminant translocation in different plant parts.

Applications and Case Study Examples

From a phytoremediation perspective, plants to be employed should mainly display high uptake rates of contaminants. In the majority of cases, high uptake rate by plants was associated with a high level of tolerance, regardless of their terrestrial (Baker et al. 2000; Sulmon et al. 2007) or aquatic origins (Dosnon-Olette et al. 2009, 2010a). In this last study (Dosnon-Olette et al. 2010a), results showed that *Lemna minor* was less sensitive than *Spirodela polyrhiza* to dimethomorph for tested parameters (growth rate and chlorophyll *a* fluorescence emission) but was the most efficient for the removal of this fungicide. In 1992, Roy et al. (1992) demonstrated a correlation between peroxidase activity (i.e., an anti-oxidative stress and/or detoxification enzyme) and plant tolerance to pollutants.

A hypothesis advanced to explain the positive relationship between plant tolerance and removal rate suggests that a plant with adequate tolerance and high uptake capacity will likely have an efficient detoxification metabolism. Therefore, toxicity studies exploring chlorophyll *a* fluorescence emission, growth rate, or anti-oxidative stress enzyme activities as toxicity endpoints appear useful in plant species selection for phytoremediation purposes.

However, the relationship between plant sensitivity and uptake capacity has not always been clearly demonstrated in the scientific literature, especially when algae are involved in the experiments (Dosnon-Olette et al. 2010b). Indeed, even negative relationships have been found in some studies (Tang et al. 1998; Weiner et al. 2004).

In others situations (e.g., semiaquatic plants), monitoring of toxicological biomarkers can give evidence on how and where contaminants can enter plants (Dordio et al., 2009) and therefore can help to understand phytoremediation mechanisms involved in the cleaning process.

Toxicity is clearly not the only parameter involved in plant selection for phytoremediation, as accumulation, survival, transport, storage, metabolism, climatic adaptation, etc., can also be important endpoints.

Conclusions

Ecotoxicological research with respect to phytoremediation is expected to increase in future in order to support plant species selection for clean-up processes. Relationships between plant tolerance, uptake capacity, and detoxification are still unclear and need to be investigated more fully.

Cross-References

▶ Aquatic Macrophytes in Ecotoxicology
▶ Environmental Transformation of Organic Substances in the Context of Aquatic Ecotoxicology
▶ Microbial Bioremediation of Aquatic Environments
▶ Monitoring of Oil-Degrading Bacteria by Stable Isotope Probing

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Suggested Resources

Cost Action 837: Plant biotechnology for the removal of organic pollutants and toxic metals from wastewaters and contaminated sites. http://lbewww.epfl.ch/cost837/

Cost Action 859: Phytotechnologies to promote sustainable land use and improve food safety. http://w3.gre.ac.uk/cost859/publications.html

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Phytotoxicology: Contaminant Effects on Markers of Photosynthesis

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Article Outline

Glossary

Nanomaterials Materials with a size smaller than 100 nm in at least one dimension.

PPCPs (Pharmaceuticals and Personal Care Products) Refers, in general, to any product used by individuals for personal health or cosmetic reasons or used by agribusiness to enhance growth or health of livestock. PPCPs comprise a diverse collection of thousands of chemical substances, including prescription and over-the-counter therapeutic drugs, veterinary drugs, fragrances, and cosmetics.

Definition

The toxicity of chemical compounds that affect the photosynthesis process.

Photosynthesis is arguably the most important biological process on Earth. It is the process by which plants, algae, cyanobacteria, and photosynthetic bacteria convert carbon dioxide into organic compounds, especially sugars, using the energy

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from sunlight. Nearly all life either depends on it directly as a source of energy or indirectly as the ultimate source of the energy in their food.

Numerous researchers, motivated by the importance of photosynthesis for life and by its central position within plant biosynthesis, are presently trying to determine the toxicity of contaminants on the physical and chemical processes involved in the photosynthetic mechanism. Contaminants generally have negative impacts on plants, although the impacts are highly variable and often require field studies to predict accurately. Because photosynthesis provides an interactive link between the internal metabolism of a plant and the external environment, many initial symptoms of environmental stresses are manifested by reductions in the rate of photosynthesis. Therefore today, some markers of photosynthesis are used to identify the effects of contaminant of various origins and to assess the health or integrity of the plant in a specific environment.

Overview

Plants play a vital role in the Earth's ecosystem by converting light energy, water, and carbon dioxide into organic compounds and oxygen. These byproducts fuel the food chain on which all life depends. Consequently, the study of the capacity of contaminants (such as pesticides or heavy metals) to cause temporary or long-lasting damage to plants, i.e., phytotoxicology, has become an important subject of applied biological research especially in the field of ecotoxicology. Phytotoxic effects can range from slight browning of leaves to death of the plant. Damage symptoms vary with the type of contaminants at play, their concentrations, and the type of plant that has been affected. Markers are often used to assess the effects (toxicity) of these chemical compounds on plant health. A biological marker (biomarker) is a molecular, biochemical, cellular, or physiological response on either exposure to or effects of xenobiotic chemicals (after Huggett et al. 1992).

Herein, emphasis is on markers directly related to the photosynthetic process for visualizing, diagnosing, and quantifying plant stresses that are available in ecotoxicological studies and risk assessment.

Dry Matter as a Marker of Photosynthetic Activity

Historically, reported markers of measuring photosynthetic activity were originally estimated based on the accumulation of dry matter from a plant from the start of contamination until the end of exposure (Millan-Almaraz et al. 2009). Salt stress (i.e., NaCl salinity) was shown to affect dry matter accumulation in rice or bean plants (Sultana et al. 1999; Alves Pinheiro et al. 2008) as well as heavy metals such as chromium (Shanker et al. 2005).

O_2 Evolution

Variation of aqueous and/or gaseous concentrations of O_2 in an analyzed sample has been used extensively as a measure of photosynthetic activity (Delieu and Walker 1972; Hunt 2003) and is a reliable indicator of pollutant toxicity (Wang and Freemark 1995). Heavy metals, such as Hg, Cd, and Cu, and herbicides (e.g., atrazine) influenced photosynthetic oxygen emission, whereas the insecticide, Gusathion, had no effect (Van Der Heever and Grobbelaar 1997). Herbicides such as flumioxazin at a concentration of 3 μg/L can significantly decrease oxygen emission of *Lemna minor* (53%) and *Scenedesmus obliquus* (34%) after a 6-h exposure. After a 48-h exposure, this inhibition reached, respectively, 92% and 62% for duckweed and algae (Geoffroy et al. 2004). More recent studies on the toxicity of nanomaterials are emerging, however, and some results have evidenced that nano-TiO_2 or engineered nanoparticle (e.g., dendrimers) treatments induced an increase in oxygen evolution. To explain effects, authors have surmised that nanoparticles might enter the chloroplast, and its oxidation-reduction reactions might accelerate electron transport and consequently oxygen evolution (Hong et al. 2005; Petit et al. 2010). This marker is also used in photosynthetic-based biosensors providing valuable information about biological effects of pollutants on algal cell suspensions (*Chlorella, Scenedesmus, Pseudokirchneriella*) (Pandard et al. 1993).

CO_2 and O_2 Gas Exchange

Since plants fix carbon dioxide (CO_2) and release oxygen (O_2) during the photosynthetic process, gas exchange (CO_2 and O_2) by leaves or algae constitutes one of the most commonly utilized techniques for photosynthesis measurement (Schulze 1972; Takahashi et al. 2001).

It has been shown that Cd^{2+} application ($5.4\ 10^{-5}$ to 100 μM) on barley or *Arabidopsis thaliana* L. induced a decrease in CO_2 assimilation (Vassilev et al. 1997; Perfus-Barbeoch et al. 2002). Exposure to the herbicide glyphosate caused a slowing of carbon assimilation. Gas exchange measurements revealed that disruption of chloroplast carbon metabolism was an early and important factor in mediating glyphosate effects, perhaps by slowing the rate of ribulose bisphosphate regeneration (Geiger et al. 1987). Acid rain and iron can also affect gas exchange in *Eugenia uniflora* (Rust Neves et al. 2009). Gas exchange is also negatively affected by heavy metal or salt stress (Cambrollé et al. 2010; Kalaji et al. 2010).

Thermoluminescence

Contaminant effects on photosynthesis can also be determined by measuring thermoluminescence (TL) as discovered in the 1950s via the detection of thermally stimulated light emission from pre-illuminated photosynthetic material (Arnold and Sherwood 1957). Several additional investigations have firmly established the participation of various oxidation states of the water-oxidizing complex, the redox-active tyrosine residues, and the quinone electron acceptors of photosystem II (PS II) in the generation of photosynthetic glow curves (for a review, see Vass and Govindjee 1996). Since TL characteristics are very sensitive to subtle changes in the redox properties of the involved electron transport components, the TL method has become a powerful marker in studying the damaging mechanisms of environmental contaminants (Vass 2003). The mode and site of action of heavy metals, including Cu^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+}, have been studied using TL (Mohanty et al. 1989a, b; Horváth et al. 1998).

Photoacoustic Signal

Light absorption by plant leaves accounts for the release of heat, light (i.e., fluorescence), and oxygen, generating a photoacoustic signal that has its amplitude and phase serving as a marker of photosynthetic activity (Mesquita et al. 2006). The works of Malkin and Cahen (1979) with Poulet et al. (1983) marked the onset of photoacoustics as a method of observing photosynthetic activity. Since then, photoacoustic used for the study of photosynthesis has been well established, even if not very often used, and additional information can be found in comprehensive review articles (Fork and Herbert 1993; Malkin and Puchenkov 1997). The advantage of this marker is in its ease of interpretation since the signal obtained by a hydrophone is proportional to the fraction of light energy that is dissipated as heat. More heat means less photochemically stored energy when the photosynthetic apparatus is damaged.

Cells of *Synechococcus leopoliensis* (cyanobacteria) grown in medium containing lead showed a decrease in their photosynthetic energy storage efficiency with time and with lead concentration. Reduction of photosynthesis progressed with time and increased with lead concentration, reaching up to 80% at the highest lead concentration (200 ppm) after 7 days (Pinchasov et al. 2006).

Simultaneous fluorescence and photoacoustic measurements have been used to study the effects of metal ions (copper, lead, and mercury) during dark incubation of thylakoid membranes (Boucher and Carpentier 1999). It was observed that photosynthetic energy storage measured by photoacoustic spectroscopy also decreased, but a large portion of energy storage remained unaffected even at the highest metal ion concentrations used (attributed to the possible recurrence of cyclic electron transport around PS II). A maximal inhibition of photosynthetic energy storage of 80% and 50% was obtained with Hg^{2+}- and Cu^{2+}-treated thylakoids, respectively, while energy storage was insensitive to Pb^{2+} (Boucher and Carpentier 1999). Similar effects were observed in entire plants exposed to Hg^{2+}, Cu^{2+}, and Ni^{2+} (Murthy and Mohanty 1995; Ouzounidou 1996). Photoacoustic spectroscopy was also used to monitor herbicide effects on plants and helped to determine inhibition of photosynthesis after 2 days of atrazine treatment at a concentration of 200 μM in *Solanum nigrum* L. (Fuks et al. 1992).

Chlorophyll Fluorescence

In recent years, measurement of chlorophyll fluorescence has become ubiquitous in plant ecophysiology studies. The principle underlying chlorophyll fluorescence analysis is relatively straightforward. Light energy absorbed by chlorophyll molecules in a leaf can undergo one of three fates: it can be used to drive photosynthesis (photochemistry), excess energy can be dissipated as heat, or it can be reemitted as light—chlorophyll fluorescence (only 1% or 2% of total light absorbed) (Maxwell and Johnson 2000). The photochemical reaction of photosynthesis and emission of fluorescence competes for the same absorbed quanta (i.e., more demand on photochemistry results in less fluorescence). Because of this complementarity, chlorophyll fluorescence is a built-in probe (fluorescence fingerprint) of photosynthesis, and indeed, this biomarker is a popular method in phytotoxicology employed to investigate electron transport and CO_2 assimilation in leaves and algae (Tyystjärvi et al. 1999; Keränen et al. 2003). The first significant findings on the relationship between primary reactions of photosynthesis and Chl-*a* fluorescence came from Kautsky and Hirsch (1931) and MacAllister and Myers (1940). For reviews on chlorophyll-*a* fluorescence, readers may consult, among others, Bi Fai et al. (2007) and Baker (2008).

Since these first reports, the advancement in knowledge and instrumentation, as well as the number of publications on this topic, has rapidly increased. In 1987, Schreiber and coworkers (Neubauer and Schreiber 1987; Schreiber and Neubauer 1987) started to use short saturating pulses of light of particularly high intensities for induction of fluorescence transients, leading to the subsequent development of a modulated fluorometer allowing for routine, nondestructive estimation of photochemical and non-photochemical quenching components in leaves (Schreiber et al. 1986).

At high excitation irradiance, dark-adapted leaves show characteristic polyphasic fluorescence kinetics that can be affected by environmental conditions, reflecting the response of plants to various stresses and hence relate to their health status (Bussotti et al. 2007; Küster et al. 2007; Eullaffroy et al. 2009). The most frequently used parameter is the maximum quantum efficiency of primary photochemistry (F_V/F_M or Φ_{PSII}), but other parameters such as the operational plant capacity to convert light energy into chemical energy (Φs_{PSII}), photochemical quenching (Q_P), and non-photochemical quenching (Q_N) are also very useful to assess the health status of plants (Juneau et al. 2002; Frankart et al. 2003; Strasser et al. 2004). Many ecotoxicological studies have used F_V/F_M to evaluate the health status of a plant. However, some studies have shown that this ratio is not always the most suitable parameter to display contaminant toxicity or plant sensitivity (Force et al. 2003; Christen et al. 2007). Based on OJIP transients, Strasser and his team have developed a test called the "JIP test" (Strasser and Strasser 1995; Strasser et al. 2000) that quantifies in vivo energy fluxes passing through the photosystems and evaluates plant photosynthetic performance.

Phytotoxicity of a multitude of contaminants affecting the photosynthetic process can be detected, thanks to the chlorophyll fluorescence marker. After 1 min of exposure to several herbicides, *Scenedesmus obliquus* showed a drastic increase in the magnitude of the fluorescence ratio, F684/F735, which reflects photosystem II and photosystem I photochemistry (Eullaffroy and Vernet 2003). After a 1-h exposure, in *Zostera marina*, stress due to an infection by a pathogen can be quantified (Ralph and Short 2002). Sensitivity of different plant species to heavy metals or herbicides can be estimated (Lahive et al. 2010). Impact of polycyclic aromatic hydrocarbon, nanomaterials, or PPCP (Pharmaceutical and Personal Care Products) is also easily assessed (Pan et al. 2008; Petit et al. 2010; Shao et al. 2010). It can be noticed that analysis of photosynthetic pigment concentration generally confirms results obtained by chlorophyll fluorescence measurements (Ferrat et al. 2003).

Fluorescence emission is also a marker of photosynthesis used to highlight stress due to a combination of different environmental factors (Eullaffroy et al. 2007; Pokora and Tukaj 2010) since contaminants rarely occur alone in the environment. This marker revealed different patterns of response, i.e., synergy, additivity, or antagonism. It is worthy of mention to recall that many researchers have already established correlations between chlorophyll fluorescence and photoacoustic signal (Snel et al. 1990), CO_2 assimilation (Seaton and Walker 1990), and photosynthetic O_2 evolution (Pospišil and Dau 2000). Finally, several fluorescence-based bioassays have been described as short-term and sensible tools (with detection limits as low as 0.1 μg/L of contaminant) to allow rapid chemical effect screening for large numbers of samples (Schreiber et al. 2002, 2007; Küster et al. 2007).

Conclusion

Photosynthesis is a key process since it governs all life on Earth. It is then an obvious necessity to assess the toxicity of chemical contaminants that affect this process.

Markers of photosynthesis can be used for the early detection of alterations in environmental quality. These markers reliably assess the effects of contaminants on the photosynthetic process and can be used as rapid screens of environmental samples. The combination of multiple markers of photosynthesis could however allow achieving a better estimate of toxicity and in detecting effects that are not captured when only using a single marker.

Cross-References

► Aquatic Macrophytes in Ecotoxicology
► Diatoms in Ecotoxicology
► Phytotoxicity of Engineered Nanomaterials (ENMs)

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Phytotoxicity of Engineered Nanomaterials (ENMs)

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Article Outline

Glossary

Engineered nanomaterial Any form of material that is deliberately created such that it is composed of discrete functional and structural parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less.

Abbreviations

ENMs	Engineered nanomaterials
MWCNTs	Multiwalled carbon nanotubes
QDs	Quantum dots
ROS	Reactive oxygen species
SWCNTs	Single-walled carbon nanotubes

Definition

The ecotoxicity of emerging contaminants, namely, engineered nanomaterials (ENMs), on higher plants and algae.

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ENMs are manufactured materials with nanoscale dimensions, homogenous composition, and specific structure or surface properties. Their unique physicochemical properties and reactivities have led to an increasingly widespread use of ENMs in many consumer products. The rapid developments in nanotechnology raise concerns about adverse effects of ENMs on the environment since increased release of ENMs in the environment is likely.

Recently, some studies have evaluated the ecotoxicity of ENMs on higher plants and algae which are the primary producers of terrestrial and aquatic ecosystems. Considering their novel and changing properties, adequate physiochemical characterizations of ENMs are prerequisite for assessing their phytotoxicity.

Historical Background

During the last two decades, ENMs have attracted a lot of attention and concern due to their rapidly increasing applications in a variety of domestic and industrial products such as cosmetics, electronic components, cleaning products, and drug carriers. Currently, ENMs can be found in more than 800 consumer products (Woodrow Wilson International Centre for Scholars, http://nanotechproject.org). Engineered nanomaterials (ENMs) are generally defined as materials with sizes smaller than 100 nm in at least one dimension. ENMs can be grouped into three categories: (1) carbon-based materials usually including fullerenes, single-walled carbon nanotubes (SWCNTs), and multiwalled carbon nanotubes (MWCNTs); (2) metal-based nanoparticles including element metals (such as nano-Zn and nano-Al) and metal oxides (such as TiO_2, ZnO, and Al_2O_3); and (3) organic composites and hybrids which combine nanoparticles with other nanoparticles or with larger, bulk-type materials (e.g., quantum dots or dendrimers) (Colvin 2003; Maynard et al. 2006; Dang et al. 2010). The increase in the production, manufacture, and use of ENMs has enhanced their potential to reach the environment either intentionally or unintentionally. Higher plants and algae, essential base components of all ecosystems, are expected to be affected following exposure to ENMs since they interact strongly with their immediate environment. As a result, increasing numbers of publications concerning the toxicity of ENMs toward higher plants and algae have emerged in the past few years.

Beneficial Interactions of ENMs with Higher Plants

First, the study of Lu et al. (2002) showed beneficial effects of a mixture of SiO_2 and TiO_2 nanoparticles at low concentrations on nitrate reductase activity, ability to absorb water and fertilizer, and the antioxidant system of soybean. SiO_2 nanoparticles also promoted the growth of Changbai larch and enhancement increased with concentrations up to 500 mg/L (Lin et al. 2004). Other studies revealed beneficial effects of nano-sized TiO_2 on spinach. Zheng et al. (2005) showed that TiO_2 can increase the germination of aged spinach seeds and the growth of seedlings. Moreover, Hong et al. (2005a, b) showed that TiO_2 nanoparticles could increase light absorbance, accelerate transport and transformation of light energy, protect chloroplasts from ageing, and prolong photosynthetic time of chloroplasts. Finally, Gao et al. (2006, 2008) found that the amount and the activity of RuBisCo activase were increased by TiO_2 treatment, leading to a high rate of photosynthesis. In addition to these effects on photosynthesis, TiO_2 nanoparticles were shown to improve nitrogen-fixation capability in roots of spinach (Yang et al. 2006, 2007). More recently, Khodakovskaya et al. (2009) demonstrated that the exposure to MWCNTs enhanced seed germination and growth on tomato plants. The authors hypothesized that this stimulation might be related to the capability of carbon nanotubes to penetrate the thick seed coat and thus support water uptake inside seeds.

Characteristics of ENMs Influencing Their Phytotoxicity

Despite these seemingly beneficial effects of ENMs on higher plants, most studies have indicated a certain degree of phytotoxicity owing to ENMs. In addition, these studies have highlighted the importance of adequate physiochemical characterizations of ENMs. Indeed, at the nanoscale, physical and chemical properties of ENMs differ substantially from their bulk and molecular counterparts. A greater portion of atoms or molecules are orientated on the surface rather than within the interior of the material, hence allowing adjacent atoms and substances to interact more readily. The large surface area to mass ratio and size-dependent properties (classical versus quantum mechanics) of ENMs allow them to perform exceptional feats of conductivity, reactivity, and optical sensitivity that can be exploited to provide products with enhanced applications (Borm et al. 2006; Maynard et al. 2006; Nel et al. 2006; Elder et al. 2009). The exceptional properties of ENMs, however, may result in different environmental fate and behavior outcomes when compared with their bulk counterparts and lead to unexpected health or environmental hazards (Maynard et al. 2006; Wiesner et al. 2006; Nowack and Bucheli 2007; Boczkowski and Hoet 2010; Ma et al. 2010). Several physical and chemical characteristics of ENMs including dissolution ratio, particle size, surface properties, and state of aggregation can influence their phytotoxicity (Gagné et al. 2007; Ma et al. 2010).

Examples of Phytotoxicity Induced by ENMs

Determination of phytotoxicity of metallic nanoparticles due to the dissolution of metallic ions has been investigated. A first report in 2005 demonstrated that ENMs can exert negative effects at relatively low concentration (Yang and Watts 2005).

The authors showed that Al_2O_3 nanoparticles inhibited root elongation of corn, cucumber, soybean, cabbage, and carrot. It was commented that the toxic effect observed might be due to the release of dissolved aluminum (Murashov 2006). Another metallic oxide nanoparticle, ZnO, significantly inhibited seed germination and root elongation of ryegrass, radish, and rape (Lin and Xing 2007). Further studies on ZnO nanoparticles showed that they caused biomass reduction and root cap deformity in ryegrass (Lin and Xing 2008). This study indicated that ZnO nanoparticle toxicity was not solely explained by the release of dissolved ZnO in the rhizosphere. This was also observed for *Arabidopsis* exposed to ZnO nanoparticles (Lee et al. 2010). A study on the phytotoxicity of five ENMs (MWCNTs, Ag, Cu, ZnO, Si) on zucchini concluded that ENM dissolution only partially explains the observed phytotoxicity in higher plants (Stampoulis et al. 2009). In contrast, Franklin et al. (2007) showed that toxicity of ZnO nanoparticles to the green microalga *Pseudokirchneriella subcapitata* was essentially due to dissolved Zn. This result was confirmed by Aruoja et al. (2009) on the same alga with both ZnO and CuO nanoparticles. Similarly, studies on the phytotoxicity of Ag nanoparticles to a microalga (*Chlamydomonas reinhardtii*) and to a marine diatom (*Thalassiosira weissflogii*) revealed that photosynthesis inhibition was mainly due to the release of silver ions (Miao et al. 2009; Navarro et al. 2008a, b).

Several other recent studies have also indicated that ENM particle size can incur phytotoxicity. Hund-Rinke and Simon (2006) reported that smaller particles of TiO_2 (d = 25 nm) caused a stronger effect than larger particles (d = 100 nm) in green algae *Desmodesmus subspicatus*. Similarly, Van Hoecke et al. (2008) determined that the ecotoxic effects of SiO_2 nanoparticles in *P. subcapitata* were related to the size and surface area of the particle and not to its mass. Particle chemical nature has been also shown to play a role in ENM toxicity. Petit et al. (2010) established that cationic PAMAM dendrimers decreased viability of *C. reinhardtii* but stimulated the photosynthetic process which could be explained by the cationic nature of these dendrimers. Additional to the chemical nature of ENMs, another study indicated that their surface properties may also determine their toxicity. Indeed, Saison et al. (2010) demonstrated that copper oxide nanoparticles were toxic to *C. reinhardtii* only if they were contained within an organic polymeric layer. After exposure to SWCNTs, root elongation was affected in tomato and lettuce, while it was enhanced in onion and cucumber (Canas et al. 2008). The authors showed that nonfunctionalized carbon nanotubes were generally more toxic than functionalized nanotubes. This work highlighted the importance of surface properties of carbon nanotubes in studies of phytotoxicity.

Again, another study affirmed that, in addition to the chemical-based phytotoxicity of ENMs, physical interactions between ENMs and plants can be responsible for their toxicity. Indeed, Asli and Neumann (2009) showed that colloidal suspensions of clay or TiO_2 nanoparticles in the root media of maize seedlings can reduce the hydraulic conductivity of primary roots and induce symptoms of water stress, namely, reduced transpiration and leaf growth.

The role of ENM aggregation in their toxicity to microalgae has also been investigated. Indeed, TiO_2 nanoparticles were found to form characteristic aggregates entrapping algal cells which may have played a major role in their toxicity to *P. subcapitata* (Aruoja et al. 2009). This study also claimed that the shading of light by TiO_2 nanoparticles was not contributing to the overall toxic effect, as observed in *D. subspicatus* (Hund-Rinke and Simon 2006). However, the encapsulation of cells by particles may cause a "direct" shading effect. In addition, particle adhesion may also lead to physical effects (such as disruption of cell membrane) or reducing cellular uptake of nutrients (Hartmann and Baun 2010). Van Hoecke et al. (2008) also showed that SiO_2 nanoparticles adsorbed to the cell wall of *P. subcapitata*. Another study demonstrated that CeO_2 nanoparticles clustered around *P. subcapitata* cells and could incur toxicity (Van Hoecke et al. 2009).

Finally, studies demonstrated that ENMs are able to generate ROS by interacting with plant cells, thereby inducing oxidative stress and thus phytotoxicity (Navarro et al. 2008a). After exposure of *C. reinhardtii* to TiO_2 or quantum dots (QDs), growth inhibition was observed, associated with lipid peroxidation, indicating oxidative stress in algal cells (Wang et al. 2008). Saison et al. (2010) showed that toxicity of core-shell copper oxide nanoparticles to *C. reinhardtii* was due to their photocatalytic activities causing ROS formation.

Conclusions and Prospects

Recent studies on phytotoxicity of ENMs have shown that these emerging contaminants can be potentially harmful to both higher plants and algae due to their unique physicochemical properties. While relatively high concentrations are needed to induce observable toxicity on higher plants (Ma et al. 2010), algae appeared to be much more sensitive to ENMs (Blaise et al. 2008; Griffitt et al. 2008). The apparent lower toxic potential in higher plants could be related to the ability of ENMs to penetrate into plant cells. In addition, phytotoxicity of ENMs is dependent on their composition, concentration, size, as well as other important physical and chemical properties. Therefore, characterizing the state of nanomaterials (e.g., size, surface charge, and degree of agglomeration) is an imperative prerequisite prior to conducting phytotoxicity investigations.

Cross-References

► Aquatic Macrophytes in Ecotoxicology
► Diatoms in Ecotoxicology
► Emerging Issues in Ecotoxicology: Characterization of (Metallic) Nanoparticles in Aqueous Media

► Nanomaterials in the Environment
► Phytotoxicology: Contaminant Effects on Markers of Photosynthesis

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POCIS Passive Samplers in Combination with Bioassay-Directed Chemical Analyses

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Article Outline

Abbreviations

DGT	Diffusive gradients in thin films
EDA	Effect-directed analysis
EPA	Environmental Protection Agency
HLB	Hydrophilic-lipophilic balanced
HPLC	High-performance liquid chromatography
PES	Polyethersulfone
POCIS	Polar organic chemical integrative sampler
SPMD	Semipermeable membrane device
TIE	Toxicity identification evaluation
TWA	Time-weighted average

Definition

An integrated effect-directed analysis (EDA) scheme combining passive sampling techniques and bio-analytical approaches to characterize and identify toxicants causing effects in the aquatic environment.

Integrative passive sampling devices ensure continuous diffusion of pollutants from water to the sampler receiving phase in order to sample and to concentrate

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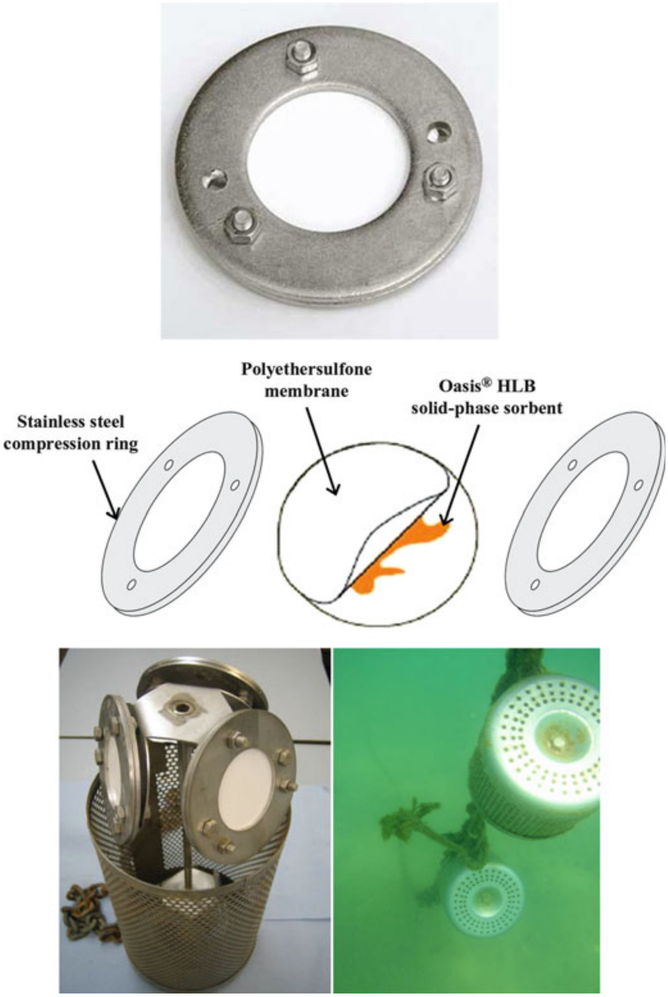
trace levels of dissolved waterborne pollutants during the sampler deployment period (up to several weeks). In particular, the polar organic chemical integrative sampler (POCIS) is designed for sampling of hydrophilic organic pollutants (classically with Log Kow ranging from 1 to 3), such as pesticides, hormones, pharmaceutical, and personal-care products (Fig. 1). In comparison with spot sampling techniques, passive samplers provide a more representative picture of water quality.

The effect-directed analysis (EDA), combining biotesting, toxicity-based fractionation, and chemical analysis, is an integrated bio-analytical approach that aims at identifying chemical stressors in the environment without targeting specific compounds. In addition, combining EDA with passive sampling (e.g., POCIS) can in the near future provide useful information concerning the relative toxicological significance of waterborne contaminants.

Historical Background

Passive samplers have been used in environmental monitoring since the beginning of the 1970s. The early designs were used to measure concentrations of gaseous pollutants in air. Later on, passive water samplers were developed for monitoring pollutants in aquatic environments from a range of chemical classes including metals (e.g., DGT in 1994), nonpolar organics (e.g., SPMD in 1990), volatile organics (e.g., diffusion bags in 1997), and polar organics (e.g., POCIS in 1999; Chemcatcher in 2000). Huckins et al. (1990) presented one of the first studies on passive sampling of nonpolar organic contaminants in water using semipermeable membrane devices (SPMDs). The POCIS was developed in 1999 to monitor a wide array of polar organic contaminants (Alvarez et al. 2004). Up to now, most research on organic passive water samplers has focused on SPMDs or similar nonpolar organic sampling devices, while less numerous studies have reported data on the use of polar organic passive samplers in water (Söderström et al. 2009).

Moreover, linking biological effects to exposure to specific active agents is often problematic due to the large numbers of compounds present in the environment. Bioassays likely constitute a solution to analyze toxic effects in samples, but cannot identify compounds. Again, current chemical-analytical techniques provide excellent sensitivity in the analysis of known compounds, but they cannot give information on potency and will easily miss compounds that were not included in the specific quantification method (Houtman 2007). In order to draw causal links between effects observed in the environment and to assess the results of chemical analysis, an increasing number of research groups have started to combine biological (mostly bioassays) and chemical techniques. Different types of combined studies can be distinguished (Streck 2009). The first type includes surveys based on target analysis of preselected compounds and correlation of results with findings from biological analysis. In this approach, individual compounds are preselected, so



POCIS Passive Samplers in Combination with Bioassay-Directed Chemical Analyses, Fig. 1 Picture (*top*) and sectional view (*middle*) of a POCIS (pharmaceutical configuration) and POCIS in their cage (*left bottom*) deployed in the field (*right bottom*)

that optimized and validated methods for chemical analysis can be used. However, a quantitative agreement between chemically derived effect estimations and measured effects as a crucial basis for reliable conclusions is rarely achievable (i.e., mass balance agreement). This approach does not provide the possibility of identifying unknown causes of effects. The most promising approach to solve this problem is the second type of study, which follows schemes such as Toxicity Identification Evaluation (TIE) and effect-directed analysis (EDA). Such studies aim at identifying chemical stressors without targeting specific compounds. The commonalities and differences between TIE and EDA are discussed comprehensively elsewhere (Brack et al. 2008). Briefly, TIE, originating from effluent control in a regulatory context in the USA, is based on guidelines by the US EPA (1989 and 1991) using in vivo testing to identify the cause(s) of acute toxicity in effluents, while EDA is a more scientific approach applied to both in vivo and in vitro tests in order to detect active fractions and potentially hazardous compounds in various environmental or technical matrices, even if the concentrations present will not necessarily cause acute effects (Brack et al. 2008).

Characteristics of Passive Sampling

Passive sampling can be defined in its broadest sense as any sampling technique based on free flow of analyte molecules from the sampled medium to a receiving phase in a sampling device as a result of a difference between the chemical potentials of the analyte in the two media. The net flow of analyte molecules from one medium to the other continues until equilibrium is established in the system or until the sampling period is stopped (Gorecki and Namiesnik 2002).

Passive integrative samplers present numerous advantages compared with conventional spot sampling of waters. First of all, they enable estimation of (1) the time-weighted average (TWA) water concentrations and (2) the biologically available fraction of pollutants over long periods of time, with just one sample collection. This TWA assessment is critical for an improved understanding of the consequences of prolonged exposure to environmental contaminant mixtures. Another advantage is that the masses of substances accumulated during deployment can ensure that analytes fall within the range of the detection limit requirements of common instrumental techniques. Hence, the detection of compounds present in water at concentrations lower than analytical detection (or quantification) limits, such as metabolites of some pollutants or steroid hormones capable of exhibiting toxicity at such low levels, can be envisaged. Passive samplers also have the potential to replace the use of living organisms in assessing bioavailability since they offer a number of advantages including lower cost, greater repeatability, and smaller variability. However, although they can be used as simple biomimetic tools, they cannot mimic metabolization and active uptake by organisms. In addition, they are logistically simpler in application, thereby enabling to increase both the frequency and spatial distribution of measurements (e.g., lowering cost, permitting in situ extraction of compounds with a minimal disturbance of speciation, no power and maintenance requirements), to more easily detect episodic events, and also to yield more stable samples. Last but not least, in addition to instrumental analysis of pollutants, sampler extracts can be subjected to toxicity testing using bioassays that give information on toxic and ecotoxic risks associated with the sampled substances (regardless of whether substances are identified or not) (see section “Applications” below).

However, the drawbacks of passive integrative samplers are their requirements of pre-calibrated sampling rates for the target analytes and calibration of, and adjustment for, the site-specific effects of environmental conditions on the uptake of a target analyte. The latter drawback can be corrected by using the performance reference compound (PRC) approach, where the in situ release of an analytical noninterfering compound, added to the sampler phase prior to field deployment, is assumed to be related to the uptake rate of the analogue natural compound sampled. This approach, successfully applied for SPMDs (Huckins et al. 2002), is still under development for POCIS (Mazzella et al. 2007).

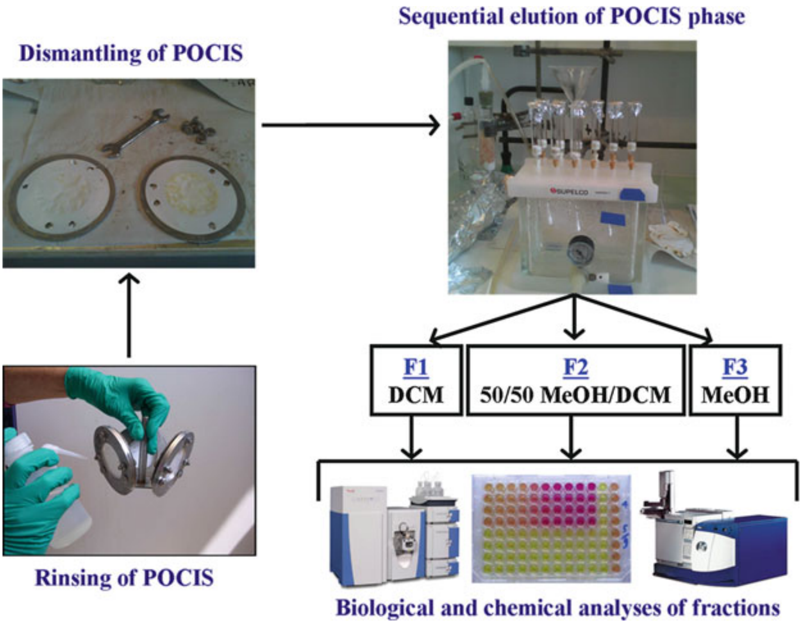
Characteristics of EDA

One of the major requirements in the characterization of complex mixtures found in the environment is the identification of those compounds causing effects. It is crucial to reduce the complexity of the mixture to a limited number of candidate compounds and finally to individual toxicants. EDA, combining biotesting, toxicity-based fractionation, and chemical analysis, is the most innovating and promising approach to meet this requirement (Brack 2003). This approach enables to detect and to identify both nontarget known and unknown toxicants (e.g., degradation products) based on their effects on the environment (see entry on "▶ Effect-Directed Analysis in Ecotoxicology" for a comprehensive description of this approach).

EDA involves stepwise fractionation procedures that systematically reduce the complexity of the sample by isolating groups of toxicants into individual fractions. At each fractionation step, bioassays identify active fractions, so that non-active fractions can be excluded from further processing. The manipulations are directed by bioassays until it is possible to identify the compounds responsible by chemical analysis. Then, advanced chemical identification techniques (based on mass spectrometry detection and increasingly on high-resolution systems allowing accurate mass measurements, e.g., Hogenboom et al. 2009) reveal compounds responsible for the adverse effects quantified by biological analysis. Finally, confirmation steps validate the findings (Brack et al. 2008).

Illustration of a Procedure Combining EDA with POCIS

A schematic view of a POCIS is illustrated in Fig.1. This device consists of a 200-mg solid receiving phase sorbent (sequestration medium) sandwiched between two microporous polyethersulfone (PES) membranes held in place by two stainless steel compression rings. The type of sorbent material used can be changed to specifically target certain chemical classes. Two configurations are commercially available: a "generic" configuration contains a triphasic admixture (Isolute ENV + hydroxylated polystyrene divinylbenzene resin and Ambersorb 1,500 carbonaceous sorbent



POCIS Passive Samplers in Combination with Bioassay-Directed Chemical Analyses, Fig. 2 Example of a simple procedure combining POCIS and an EDA approach. *MeOH* methanol, *DCM* dichloromethane

dispersed on S-X3 Bio-Beads styrene divinylbenzene copolymer) and is used to monitor most pesticides, hormones, and other water-soluble organic chemicals. The "pharmaceutical" configuration contains an Oasis™ HLB (poly[divinylbenzene]-co-*N*-vinylpyrrolidone; HLB: hydrophilic-lipophilic balanced) solid-phase sorbent and is designed for drug residues (Alvarez et al. 2004). However, it should be noted that the "pharmaceutical" configuration appears to be the most popular to sample a wide range of chemical compounds, such as pesticides, hormones, and pharmaceuticals (Mazzella et al. 2007; Zhang et al. 2008). POCIS samplers are deployed in the field up to several weeks mounted inside a protective perforated stainless steel canister.

Figure 2 then illustrates an example of how a simple procedure combining POCIS and an EDA approach can be carried out. After exposure, each POCIS sampler is rinsed with ultra-pure water to remove any material present on the outer surface of the membranes (particles and biofilms). The metal disks are disassembled, and the membranes are detached from the disk. The sorbent is carefully transferred into an empty glass solid-phase extraction (SPE) tube by rinsing it with ultra-pure water. The sorbent is dried by applying vacuum. Organic compounds are then successively eluted in separated fractions by appropriate solvents or solvent mixtures. The fractions are evaporated near to dryness and finally dissolved in a solvent suitable for either chemical analyses or bioassays. Each fraction is assessed by chemical analyses and bioassays.

However, a mass balance agreement between chemically derived effect estimations and measured effects is not often achievable at this stage. An additional fractionation step using HPLC (HPLC hyperfractionation) of the positive POCIS fractions can also be performed in order to more finely isolate chemical compounds and allow the identification of toxic compounds using high-resolution mass spectrometric techniques.

Applications

There are several applications in which POCIS samplers can be used. The four major applications are (1) water quality monitoring including screening for presence/absence and monitoring of spatial distribution and TWA concentrations in various aquatic environments like sewage water, rivers, and lakes; (2) detection of specific sources of contamination (like hospital effluents, waste water from drug manufacturing plants and livestock farms, illegal dumping); (3) estimation of the worst case exposure scenario for aquatic organisms; and (4) collecting time-integrative extracts in situ for toxicity assessment. POCIS is the sampler type which has been used in most field deployments aiming to assess environmental problems (e.g., Vermeirssen et al. 2005; Matthiessen et al. 2006; Togola and Budzinski 2007). Sampling strategy using POCIS has gained general approval and is starting to be considered as a standard in water quality monitoring of emerging polar organic compounds.

In addition to instrumental analysis of pollutants, the pre-concentrated extracts obtained from elution of the sampler receiving phases can be subjected to toxicity testing using a variety of bioassays that give information on toxic and ecotoxic risks associated with the sampled substances (those identified or not). In some in vitro bioassays used to assess the health of an ecosystem, problems can occur due to the difficulty of obtaining suitable water samples for testing. For example, most organic contaminants are only present in the aquatic environment at trace levels. The extraction of several liters of water would be required to yield sufficient amounts of analyte for subsequent bioassay. The use of "bio-mimetically" separated extracts from passive samplers can overcome this problem. Passive samplers have been used in combination with toxicity assays to determine total toxicity of pollutants in a water body, using Chemcatcher (Escher et al. 2006; Muller et al. 2007; Shaw et al. 2009; Vermeirssen et al. 2009) or POCIS (Vermeirssen et al. 2005; Matthiessen et al. 2006; Alvarez et al. 2008). Acute effects of POCIS extracts have also been tested on natural phototrophic biofilm communities (Pesce et al. 2011).

Moreover, EDA can be combined with the use of passive samplers to form an integrated EDA scheme for the detection and identification of readily bioavailable toxicants in waters (Dévier et al. 2011). EDA has proved to be a powerful tool for identifying unknown toxicants and linking effects observed in bioassays to individual compounds (Brack 2003). However, very few studies have used passive samplers in combination with an EDA approach to identify the toxic fractions among the many compounds accumulated during deployment. In fact, this approach has only been applied using SPMDs to detect substances with estrogenic activity in a number of rivers in Germany and the United Kingdom (Rastall et al. 2006) or to identify potential environmental hazards from compounds accumulated in POCIS samplers deployed in a French river by using several in vitro bioassays that detect endocrine-like and dioxin-like compounds (Creusot et al. 2010; Tapie et al. 2011). However, the toxicants responsible for observed effects have still to be identified. Furthermore, HPLC hyperfractionation of the positive POCIS fractions can also be performed in order to more finely isolate chemical compounds, thereby allowing the identification of toxic compounds using high-resolution mass spectrometric techniques.

In addition, new homemade POCIS sampling designs (e.g., nylon membranes, mixed solid-phase sorbents, miniaturized samplers) are now being developed in the laboratory in order to sample a wider range of organic pollutants (in particular, the highly polar compounds) and to prove applicable to aquatic media that are difficult to access (e.g., piezometer well).

Conclusions

One of the key challenges in environmental chemistry and ecotoxicology is to characterize and identify toxicant-causing effects in the aquatic environment. However, many of the current bottlenecks in the assessment of organic contaminants in our environment concern the difficulty to evaluate diverse chemical classes and biological effects within complex mixtures. To tackle these analytical challenges, integrated biological and chemical-analytical approaches developed recently provide an important step toward an estimation of the portion of an effect that can be explained by the analyzed chemicals, but they do not provide the possibility to identify unknown causes of effects. The most promising approach to solve this problem is EDA that has gained increased interest in ecotoxicological studies and environmental risk assessment. The EDA approach can be applied to elucidate unknown causative agents and their combined effects.

In addition, combining EDA with passive sampling instead of undertaking classical spot water sampling could also help to improve environmental risk assessment. By their combined ability to provide TWA concentrations and to concentrate bioavailable waterborne pollutants, these integrative passive devices allow increasing representativeness and reliability of data obtained. In particular, the applications of POCIS samplers show strong potential, especially their time-integrative and in situ collection of extracts for performing assays in a wide range of biological screening procedures. This new strategy should in the very near future be able to provide useful breakthroughs in knowledge concerning the relative toxicological significance of waterborne contaminants.

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Cross-References

► Active Biomonitoring
► Aquatic Mesocosms in Ecotoxicology
► Artificial Mussels
► Bioavailability of Contaminants
► In Situ Bioassays in Ecotoxicology
► Rapid Tests for Community-Level Risk Assessments in Ecotoxicology

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Pollution Acclimation, Adaptation, Resistance, and Tolerance in Ecotoxicology

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Article Outline

Definition

Acclimation as well as adaptation, resistance, or tolerance can be defined as the ability of organisms to cope with stress, either natural such as temperature changes, salinity variations, oxygen level fluctuations, and plant toxins or chemicals depending on anthropogenic inputs of many different classes of contaminants into the environment.

Herein we define **acclimation** or physiological **adaptation** as the second phase of stress. Several authors working on biomarkers in ecotoxicology use the term **adaptation**. However, numerous papers define **adaptation** as synonymous of **resistance** (Booth and Biro 2008; Demmig-Adams et al. 2008), and **resistance** is frequently used in the scientific literature as a synonym for **tolerance** (Forbes and Forbes 1994). Several authors have tried to clarify these terms. The definitions proposed by different authors may be different, and none is currently generally adopted (Lotts and Stewart 1995; Morgan et al. 2007). Today, most authors use the term **tolerance** in acceptance of the general first definition above. The use of the term **resistance** is generally preferred by those interested in the genetic basis of an organism's ability to survive in a contaminated environment (Amiard-Triquet et al. 2010).

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Historical Overview

The capacity of physiological adaptation or acclimation toward a stressor is related to the stress syndrome. The term stress indicates organism responses induced by a stressor at any level of biological organization. Selye (1956) defined the original concept of stress which is “the sum of all the physiological responses by which an animal tries to maintain or reestablish a normal metabolism in face of a physical or chemical force.” According to Wedemeyer and Goodyear (1984), this definition has evolved into the concept that stress is the biological effect of any force that challenges homeostatic or stabilizing processes and extends them beyond their normal limits at any level of biological organization: individual, population, or ecosystem. In brief, during physiological adaptation/acclimation which requires energy, the stress response will succeed in maintaining health by establishing a new equilibrium between the organism and the altered environment, thereby increasing the probability of survival. In contrast, if failure to achieve acclimation occurs, exhaustion appears with a number of deleterious events which can lead to death.

Moriarty (1988) reviewed the first recorded effects of pollution on population genetics. A first example of metalliferous plants tolerant to copper was described by Prát (1934) who found specimens of *Melandrium silvestre* growing on copper mine wastes in Czechoslovakia, and in 1971, Antonovics et al. (1971) reported that a considerable number of plant species were able to produce genotypes resistant to one or more metals. Again according to Moriarty (1988), resistance to pesticides in the field was first detected in 1908 when a population of an insect pest of fruit trees was found to be resistant to lime sulfur (Melander 1914), but in the second half of the twentieth century, the use of new synthetic insecticides accelerated the development of resistance. In 2003, some 520 insect and mite species, a total of nearly 150 plant pathogen species, and about 273 weeds species were resistant to pesticides (Pimentel 2005). For aquatic organisms, first reports dealing with tolerance were published for algae exposed to metals in the 1950s and for bacteria in the 1970s (quoted in Klerks and Weis 1987).

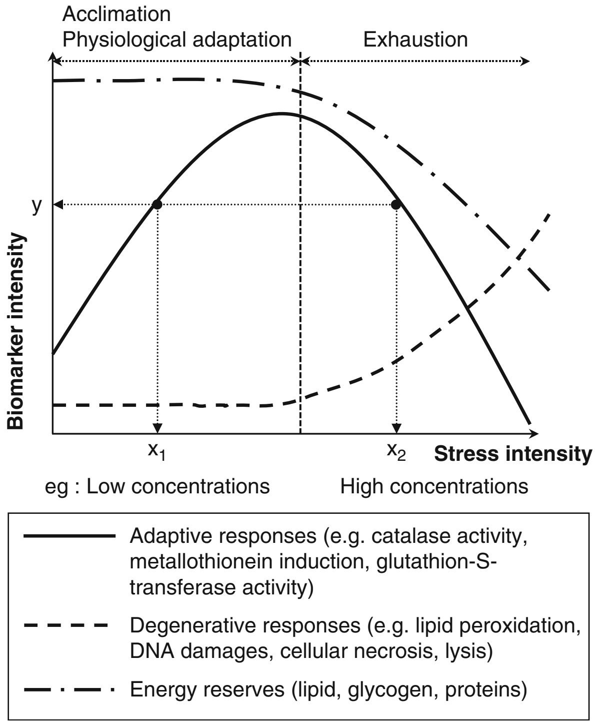
An important step forward in knowledge came from work published by Klerks and Weis (1987) dealing with genetic adaptation to heavy metals in aquatic organisms which has been cited 226 times till now (2010). These authors clearly distinguished the two reasons explaining why organisms may be tolerant (resistant) to a pollutant, namely, physiological acclimation that has no genetic basis and is not transferable to the progeny and/or genetic adaptation through the action of natural selection in response to the pressure of environmental stress. More recently, a number of papers have established the existence of tolerance to organic chemicals in many different taxa (Amiard-Triquet et al. 2010). Klerks and Weis (1987) also underlined that evolution of resistance may have important implications for interpreting the results of bioassays and biomonitoring programs and for making decisions regarding safe ambient toxicant levels. In 1991, Calow conceptualized the physiological costs of combating chemical toxicants and evoked the ecological implications of this cost of tolerance. Calow's work has been quoted in numerous papers as an explanation for many observations indicating increased metabolic rates in organisms exposed to a number of different stresses with subsequent induction of metallothioneins, heat-shock proteins, biotransformation enzymes, and anti-oxidative defenses. In addition, many reports indicate that organisms which have developed tolerance toward a given stressor can be penalized when exposed to a new stress (Amiard-Triquet et al. 2008). Another key paper is that by Heugens et al. (2001) showing that tolerance to chemicals dropped when organisms were submitted to stressful conditions such as unusual temperatures or decreased food availability in addition to toxicants. It contradicted the intuitive feeling that organisms tolerant to harsh natural conditions (e.g., estuarine organisms) were able to cope with any other stress including chemical stress. Tolerance also has implications in ecological and human health since, depending on the mechanisms involved (storage of contaminants under detoxified forms or increased elimination), tolerant species can represent highly contaminated links in food webs, including our own species (Amiard-Triquet et al. 2008).

Physiological Responses to Chemical Stress (Biomarkers)

Biological responses to contaminant stressors can range from changes at the molecular level (e.g., genetic integrity, biochemical responses), organ and physiological levels (e.g., histopathological perturbations, immunotoxicological changes), to population and even community levels (e.g., dynamics, structure).

It is generally accepted that effects of environmental stressors at any of the higher levels of biological organization begin with effects (biomarkers) at lower (subindividual, individual) levels. Sublethal stressor effects are common, and because the resulting stress can be both indirect and delayed, cause-and-effect relations are difficult to recognize. Thus, tests were (and still are) undertaken to determine these cause/effect relationships of pollution on organisms in controlled laboratory exposures. A large portion of research concerns adaptive mechanisms induced to counteract toxic impact of chemical contaminants at the individual level. When organisms are submitted to stressors, release of stress hormones in the blood (catecholamine, corticosteroid) initiates metabolic changes.

In individuals exposed to chemical compounds (e.g., heavy metals, organic pollutants), marked increases in response of biochemical biomarkers (e.g., metallothionein induction, metabolism of biotransformation, oxidative stress) can occur. Conversely when acute or chronic chemical stress exceeds compensation limits, biomarker responses are often depleted and can even decrease below control values (Fig. 1, Biagianti-Risbourg 1990; Mosleh et al. 2006). Thus, biochemical approaches have the advantage of a rapid response time. However, the limitation of uncertain biological and toxicological relevance owing to a similar level of biomarker intensity (Fig. 1) may be related to very contrasted stress conditions and



Pollution Acclimation, Adaptation, Resistance, and Tolerance in Ecotoxicology, Fig. 1 Response profiles of biomarkers related to increased stress syndrome (Modified from Paris-Palacios et al. 2000; Dagnino et al. 2007)

to individual responses reflecting states of physiological adaptation or exhaustion (x1–x2 Fig. 1, Paris-Palacios 1999). During exhaustion, depletion of energy reserves and degenerative events occur (e.g., lipid peroxidation, DNA damage, necrosis, cellular lysis). A multidisciplinary analysis using a panel of varied biomarkers (including anatomic-morphological changes, histo-cytopathology, immunotoxicology) is indispensable to characterize the functional state of organs and thus the probable health status of individuals (Klaunig et al. 1979; Biagianti-Risbourg 1990; Paris-Palacios et al. 2000).

Ecological and Ecophysiological Aspects of Tolerance

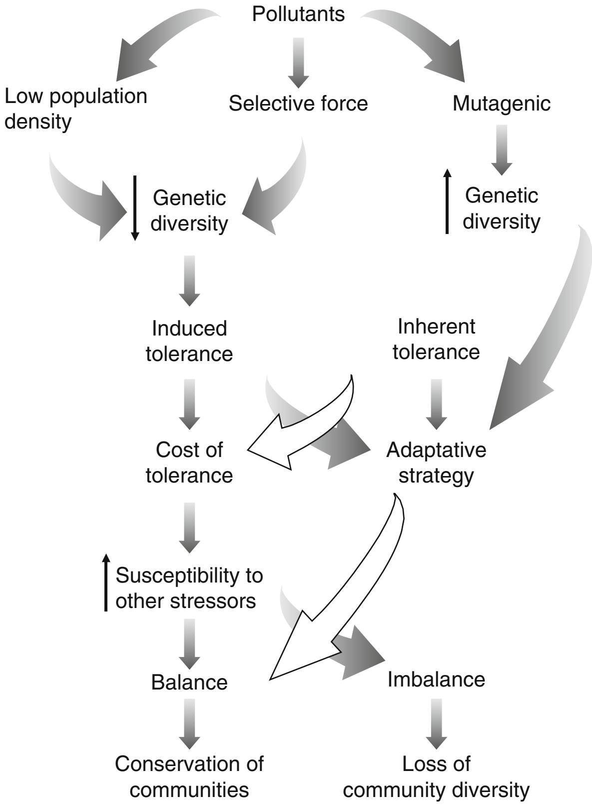
Tolerance and the Conservation of Biodiversity

If one considers stress effects in organisms chronically exposed to chemicals in their environment (to long-term exposure for several generations in the laboratory), no simple or general responses can be described. Tolerance can be due either to physiological acclimation, based on the same mechanisms of defense as those described previously, nontransferable to the progeny or to genetic adaptation. Depending on species and, within the same species, on population life history, responses can be highly variable according also to the type of stressor. In the worst case, chemicals can have harsh effects (avoidance/escape, death) leading to a drop of population densities. If organisms survive, exposure to chemicals can exert a selective pressure leading to the presence of resistant genotypes in impacted areas. In such areas, an increased frequency of resistant genotypes favors maintenance of DNA integrity. However, exposure to chemicals can lead to different types of DNA damage (Fig. 1) such as DNA adduct formation, chromosomal aberrations, oxidative DNA damage, and mutations. Mutations frequently have noxious effects on reproductive ability, viability and survival, and carcinogenesis. Again, in some cases, mutations can confer a selective advantage leading to the selection of resistant genotypes. Depending on the type of response, genetic diversity can be either increased or decreased.

It is intuitively accepted that when reduction in genetic diversity occurs in polluted populations, the prevailing genotypes are associated with increased tolerance. However, in a recent review, Weis (2010) regrets that only few studies have concurrently examined genetic diversity and tolerance. Either acquired or inherent, tolerance to chemicals represents an adaptive strategy, enabling tolerant populations/species to persist in a contaminated environment, but in return, costs of tolerance are well documented (Mouneyrac et al. 2010). Depending on the balance or imbalance of benefits and risks of being tolerant, consequences at higher levels of biological organization can be contrasted, contributing to the conservation of communities or to a loss of community diversity (Fig. 2). In a biological community exposed to toxicants in a contaminated environment, the most sensitive organisms are lost as a consequence of pollutant pressure, whereas tolerant organisms are maintained. Consequently, the new community as a whole is more tolerant to the toxicant responsible for selection than another community, initially identical, but which has never been exposed to this toxicant. Such a pollution-induced community tolerance (PICT, first described by Blanck et al. 1988) is well documented in microbial communities (in Amiard-Triquet et al. 2010), nematodes (Millward and Grant 1995, 2000), and the same philosophy is behind the use of lichen communities in the monitoring of air pollution (Hawksworth and Rose 1976, quoted in Millward and Grant 1995).

The Cost of Tolerance

Physiologically, the ability to tolerate increased levels of a toxic substance can be expensive in terms of energy or other resources (Sibly and Calow 1989). Energy costs are due to processes that protect organisms against stressors (e.g., avoidance or escape reactions, mucous barriers, synthesis of heat-shock proteins) or contribute to rid the body of a stressor (e.g., metabolizing/excreting contaminants). According to the metabolic cost hypothesis (Calow 1991), a decrease of energy reserves (carbohydrates, lipids, proteins) is usually observed in different species following laboratory or field exposure to various types of contaminants. However, intensity of impact of the stressor on energy expenditure varies greatly among species and types



Pollution Acclimation, Adaptation, Resistance, and Tolerance in Ecotoxicology, Fig. 2 Exploration of the links between chemical contamination, genetic variability, tolerance, and consequences at the community level (from Weis in Amiard-Triquet et al. 2010, with permission)

of stressors (Mouneyrac et al. 2010) because of the effect these stimuli have on different biological traits (Calow 1991).

Physiological acclimation to toxicant conditions also depletes energy reserve levels (Fig. 1). For example, in *Daphnia magna*, organisms preexposed to zinc (and having acquired a tolerance toward this metal) did not mobilize their energy reserves further following a laboratory exposure to zinc (0.1 and 1.0 μM) compared with nonexposed animals (Canli 2005).

Increased investment in the tolerance mechanism can initiate a trade-off between benefits of adaptation and costs exerted by a decreased expenditure into other energy-demanding processes, such as growth and reproduction, thereby reducing individual fitness. These significant trade-off costs have been identified in several multigenerational acclimation experiments, when animals established tolerance to the target toxicant (Mouneyrac et al. 2010). For instance, the Cd-tolerant population of the killifish *Heterandria formosa*, although threefold more tolerant to Cd than the control population, showed a significant reduction in brood size, delay in reproduction, and shorter female life span (Xie and Klerks 2004).

The adaptive benefit of tolerance can have other negative counterparts associated with the potential cost of tolerance leading to a systemic deficiency to respond to additional stressors. For example, marine aquatic organisms living in polluted environments that experience high standard metabolic rates due to defense responses will have reduced aerobic capacities for additional stress responses (Lannig et al. 2006). Long-term exposure to metals can result in a metal-tolerant community, which in turn is more sensitive to UV-B than similar communities without a history of metal contamination (Kashian et al. 2007), suggesting a potential fitness cost associated with increased heavy metal tolerance (Wilson 1988). Recently Vogt et al. (2010) showed in a multigeneration approach with the midge *Chironomus riparius* that their preexposure to a higher TBT concentration resulted in a significantly higher susceptibility to subsequent Cd stress. Thus, the compensatory ability of an organism in a given environment is dependent on its evolutionary history and the reserve energy that can be allocated to offset effects of a new stressor.

Conclusions and Operational Consequences

The literature provides a number of reports which do not mention acclimation or adaptation in organisms chronically exposed to environmental contaminants. Because publication of negative results is usually less easily achieved than publication of positive results, it can be suspected that tolerance is not a universal phenomenon. However, tolerance to chemicals has been frequently observed in unicellular organisms, invertebrates, and vertebrates exposed to a large range of different contaminants both organic and inorganic. Thus, it is necessary to evaluate the consequences of this phenomenon.

Many mechanisms of defense described above are currently used as biomarkers of exposure in biomonitoring programs. Keeping in mind Fig. 1, it is clear that misinterpretations are possible because identical levels of a given biomarker (e.g., catalase, EROD, MT) can correspond to different degrees of contamination depending as to whether individuals are in an acclimation or exhaustion phase or whether organisms are tolerant or not. Natural organisms and populations are subjected to multiple stressors, unfavorable or fluctuating physical and chemical conditions (e.g., temperature, salinity, dissolved oxygen, varying degrees of pollution, parasites). Considering the complexity of environmental conditions, integrative multidisciplinary approaches allow improved analysis of adaptive or degenerative processes set up by organisms prior to the onset of environmental pressures (Paris-Palacios et al. 2000; Falfushynska et al. 2009).

Depending on the pollutant-handling strategies involved (i.e., uptake limitation, increased elimination, accumulation of detoxified forms), tolerant organisms can represent highly contaminated links in food chains, even those leading to our own species. If the tolerance mechanism of a prey/food species is based on storage detoxification, there is a real risk of increased trophic transfer of contaminants (Amiard-Triquet et al. 2010).

The concentration of a chemical below which no deleterious effects will occur for many species in a field situation is determined from bioassays. A large range of biological models is proposed for this purpose, including many different taxa which were all shown as having a potential to acquire tolerance in contaminated environments. In addition, from a practical point of view, these species are generally tolerant in order to survive easily under laboratory conditions. Determination of threshold concentrations then calls for the use of uncertainty or security factors. Extrapolation factors take into account inter- and intraspecific variabilities in order that the threshold thus obtained can efficiently protect both sensitive and tolerant organisms. At each level, the use of a factor of 10 is generally recommended. If differences in tolerance ratios are relatively small, then they can be encompassed by the conventional safety factors used to establish protective guidelines. However, some investigations suggest that safety margins may not be adequate for all contaminant classes. A very striking example is provided in an expanded study by Nacci et al. (2009) who reported that the LC_{20} (lethal concentration for 20% of the individuals tested) to PCB126 in killifish embryos from 24 estuarine sites in the USA from Maine to Virginia ranged over three orders of magnitude, and sensitivities of the response reflected sediment total PCB concentrations.

Cross-References

► Aquatic Biomarkers
► Emerging Issues in Ecotoxicology: Persistent Organic Pollutants (POPs)
► Environmental Research Needs (in Ecotoxicology) in Relation to Public Policies
► Evolutionary Toxicology
► Impacts of Land Contaminants on Aquatic Ecosystems

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Polychaetes in Ecotoxicology

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Article Outline

Glossary

Biotic coefficients Coefficients based upon the percentages of abundance of five ecological groups (group I: very sensitive species; group II: indifferent species; group III: tolerant species; group IV: second-order opportunistic species; group V: first-order opportunistic species), according to their sensitivity to an increasing pollution gradient (Borja et al. 2000).

Mussel watch A biomonitoring program based on the determination of contaminant concentrations in bivalves.

Abbreviations

ASTM	American Society for Testing and Materials
BO2A	Benthic Opportunistic Annelida Amphipod index
ECOMAN	Ecosystem management bioindicators
ICES	International Council for the Exploration of the Sea

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I2EC *I*ndice d'*É*valuation de l'*E*ndofaune *C*ôtière (Coastal endofauna assessment index)
US EPA United States Environmental Protection Agency

Definition

Polychaete worms form a taxonomic group that is very important in the structure and functioning of aquatic ecosystems, being represented by several species exhibiting high densities and biomasses, particularly in estuarine and marine sediments.

Polychaetes as a whole taxon, or as particular species, may be used as benthic indicators able to reveal different kinds of stress such as organic matter enrichment or the impact of oil spills. Many polychaetes are endobenthic species, living in close contact with sediments which are the final sink for many chemical contaminants, both organic and inorganic, introduced into the environment as a consequence of anthropogenic pressure. Thus, they can be used as bioaccumulator species, useful when assessing the bioavailability of sediment-bound compounds. In addition, they can be employed as biological models for the determination of a number of biomarkers at different levels of biological organization, such as enzyme activities, energy reserves, and behavioral disturbances (Dean 2008). In the ECOMAN project, an approach designed to define sustainable ecosystem function, worm species were selected as sentinels (Galloway et al. 2006). They have also been proposed as biological test methods for the ecotoxicological evaluation of dredged material and sediments.

Historical Overview

Polychaetes were first used in ecotoxicology as benthic indicators to determine the extent of alterations resulting from large municipal discharges (Reish 1959; Bellan 1967, both quoted in Reish and Bellan 1995). The catastrophic oil spills which have occurred since the 1960s have been widely studied and shown to affect soft-bottom communities (Gómez Gesteira and Dauvin 2000). In general, there was a very low impact of the spills on polychaetes, but a high one on amphipod crustaceans. Thus, a polychaete/amphipod ratio was proposed to reflect temporal changes (degradation/recovery) in soft-bottom faunal communities. The implementation of the European Water Framework Directive (WFD) in marine coastal waters has triggered a large debate and many proposals from scientists around Europe (Dauvin et al. 2009). Suggested biotic indices include the use of many different species of polychaetes which might be sensitive to contaminants to different degrees or opportunistic benefiters of an environmental change.

In a second phase of research, marked attention has been devoted to the use of biological indicators of metal contamination in aquatic environments. Biomonitoring programs based on bioaccumulators were mainly developed based on filter feeders in the water column (Mussel Watch). However, polychaetes – as endobenthic species, living in close contact with sediments which are the final sink for many chemical contaminants, both organic and inorganic, introduced in the environment as a consequence of anthropogenic pressure – can be used as bioaccumulator species, notably when assessing the bioavailability of sediment-bound compounds. As early as the 1980s (Bryan et al. 1980), the polychaete *Nereis diversicolor* from many different estuaries in SW Britain proved to be a suitable indicator for local bioavailabilities of silver, cadmium, copper, and mercury (with the exception of zinc which it regulates) (Poirier et al. 2006 and literature quoted therein). Applying *N. diversicolor* for the biomonitoring of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) has also been documented (Ruus et al. 2005; Amiard et al. 2009), and uptake of such organic chemicals was also shown in different species.

However, chemical measurements of the different classes of chemicals which can enter into organisms do not provide sufficient information on the real impact of pollutants. In the 1990s, biomarker methods were developed to correct this situation. The following definition of a biomarker was given by Depledge (1994): “A biochemical, cellular, physiological or behavioral variation that can be measured in tissue or body fluid samples or at the level of whole organisms that provides evidence of exposure to and/or effects of, one or more chemical pollutants (and/or radiations).” Again because of their close contact with sediments, polychaetes were widely employed for the determination of numerous biomarkers including biochemical, physiological, and behavioral responses. Many examples are provided in the literature, for instance, by Amiard et al. (2006) for metallothioneins (MTs), Moreira et al. (2006) for feeding behavior and key physiological functions, Sandrini et al. (2008) for biomarkers involved in antioxidant defenses, Jørgensen et al. (2008) for phase I and phase II enzymes, and Mouneyrac et al. (2010) for burrowing behavior and fitness.

At the dawn of ecotoxicological studies, the toxicities of a large range of different chemicals were tested using many different species, among which polychaetes were frequently chosen as test species (see monographs by D. Taylor on metals and metalloids As, Cd, Cu, Cr, Hg, Pb, Ni, and Zn, published by Imperial Chemical Industries, Brixham Laboratory). With the improvement of knowledge and methodologies, polychaetes are still unquestionably recommended for bioassays, particularly for the evaluation of dredged material and sediments (Nendza 2002 and literature quoted therein). In this respect, there is notable interest for species which can be cultured in the laboratory (e.g., *Neanthes arenaceodentata*, California State University; *Nereis virens*, Shoreline Polychaete Farms, Northumberland, UK).

Because of their recognized tolerance to contaminants and bioaccumulation potential, ecotoxicological studies with polychaetes have focused on their role regarding pollutant transfer into food webs. Sediment-dwelling organisms, as important prey items of several bottom-dwelling fish species and wading birds (Ruus et al. 2002), may therefore contribute to the transport of contaminants to higher levels in the food chain and ultimately cause adverse effects in predators (Rainbow et al. 2006; Boyle et al. 2008).

Polychaetes as Benthic Indicators

Our present approach to environmental monitoring was pioneered by Reish in 1959 (see Reish and Bellan 1995) describing the ecological effects of pollution in Los Angeles-Long Beach Harbors. Others followed in his footsteps in various geographical areas in Europe and North America (Reish and Bellan 1995). These authors described concentric areas around an effluent discharge: (1) a maximum pollution zone deprived of macroscopic life; (2) a polluted zone characterized by few species, one of which is invariably the polychaete *Capitella capitata* (one or more of its "sibling species"); (3) a subnormal zone showing species enrichment but still exhibiting a dominance of polychaetes; and (4) a normal zone unaffected by effluents. However, it soon became evident that pollution indicators were not totally confined to polluted situations, and species initially proposed as indicators were incorporated into much longer lists of benthic organisms responsive to organic enrichment but in many cases to other disturbances (Wilson and Jeffrey 1994).

The distribution of benthic invertebrates according to organic pollution in coastal areas was at the basis of the definition of five ecological groups outlined by Glémarec and Hily (1981): I, species sensitive to organic enrichment; II, species indifferent to enrichment; III, species tolerant to excess organic matter enrichment; IV, second-order opportunistic species (slight to pronounced unbalanced situations); and V, first-order opportunistic species (pronounced unbalanced situations). This classification has been widely used by later scientists, for instance, in the *AZTI* Marine Biotic Index (AMBI) developed by Borja et al. (2000). More than 800 taxa representative of the most important soft-bottom communities present in European estuarine and coastal systems have been assigned to these ecological groups, among which nearly 40% are polychaetes. Only 16% of sensitive species belonging to group I are polychaetes, mainly deposit-feeding tubicolous polychaetes, whereas in group II, polychaetes are overrepresented (71%). Polychaete representation is also slightly unbalanced in group III, in which tubicolous spionids and nereids represent a consistent part of the 53% of polychaetes in this group. In agreement with the tolerance to organic enrichment generally recognized in polychaetes, these worms represent 95% of group IV (small-sized polychaetes: subsurface deposit feeders, such as cirratulids). Only a few species were assigned to group V ($N = 11$) among which were several capitellid polychaetes, *Malacoceros fuliginosus* and *Scolelepis* *fuliginosa*. Even though these ecological groups were initially described in relation to organic matter enrichment, Borja et al. (2000) explored the relationships between the AMBI and the concentrations of xenobiotics in sediments. Except for arsenic and mercury, sediment concentrations of all other metals were positively correlated with biotic coefficients (BC). For organic chemicals, the only significant correlation was found between BC and PCB concentrations. This methodology involving defined ecological groups has been recognized as efficient for assessing the environmental quality of harbor sediments and the potential impact of dredging (I2EC, standing for "Indice d'Évaluation de l'Endofaune Côtière" by Grall and Glémarec 2003; Grall et al. 2003 in Alzieu 2003). Gómez Gesteira and Dauvin (2000) studied the effects of oil spills on infralittoral muddy-sand macrobenthic communities. They observed the disappearance of amphipods, with a very low, but progressive, recovery rate during the four post-spill years that this study lasted. In contrast, polychaetes generally appeared to be resistant to high levels of hydrocarbons, with few changes in the sites where hydrocarbons dominated. These authors proposed an opportunistic polychaete/amphipod ratio which varied from ≤ 1, given a relative absence of pollution, to >1 in stations subjected to high levels of pollution, where amphipods disappeared completely. This ratio was reexamined and modified so that it could be used to assign estuarine and coastal communities to the EcoQ (Ecological Quality Status) classes suggested by the WFD BOPA (*B*enthic *O*pportunistic *P*olychaete *A*mphipod) index (Dauvin and Ruellet 2007). In estuaries, the main problem appears to be that all indices applicable for determining anthropogenic stress consider the abundances of stress-tolerant species, which may also be tolerant of natural stressors such as salinity, temperature, and hypoxia. To establish estuarine biological conditions, the US EPA's Environmental Monitoring and Assessment Program (EMAP) developed a benthic index that incorporates changes in diversity, structure, and abundance of selected estuarine benthic species (e.g., polychaetes, molluscs, crustaceans) (McDonald et al. 2004). For use in the freshwater zones of transitional waters (i.e., up to the upper limit of tidal range), Dauvin and Ruellet (2009) proposed an adaptation of the BOPA index, the Benthic Opportunistic Annelida Amphipod index (abbreviated BO2A), by adding clitellate annelids (Oligochaeta and Hirudinea) which are very common in muddy sediments of the tidal freshwater part of estuaries.

Tolerance in Polychaetes

The fact that polychaetes can survive in areas strongly affected by pollution inputs leads to the hypothesis that these organisms have been able to develop mechanisms of defense and to cope with the presence of a number of contaminants. This hypothesis was first confirmed in populations of the intra-sedimentary polychaete *Nereis diversicolor* living in a zone highly contaminated by metals (Restronguet Creek, UK) which had acquired tolerance to such contaminants (Bryan and

Hummerstone 1971, 1973). Hateley et al. (1989) observed that this tolerance was inheritable, demonstrating its genetic basis. Tolerance to metals has been shown even in less contaminated areas ever since, and the biological mechanisms responsible for metal tolerance have been described (Berthet et al. 2003; Mouneyrac et al. 2003). Tolerance of polychaetes to PAHs has also been demonstrated (Chandler et al. 1997; Bach et al. 2005; Lewis and Galloway 2008), and in sibling species of *Capitella capitata*, it was attributed to the ability of different species to biotransform these compounds into less hydrophobic metabolites that are more easily excreted.

Polychaetes as Bioaccumulator Species

Their Use in Biomonitoring

In a recent book devoted to analytical measurements in aquatic environments, Coelho et al. (2010) report that polychaetes, which are generally deposit-feeding detritivores, are a group frequently considered for sediment biomonitoring purposes, particularly *Nereis (Hediste) diversicolor*. In agreement with Bryan et al. (1980), they underline that some caution is necessary in their use, since these worms are known to regulate their tissue concentrations of several trace elements. The suitability of selected polychaetes from the German Wadden Sea for biomonitoring (*N. diversicolor, Nephtys hombergii, Nephtys cirrosa, Scolelepis squamata, Arenicola marina*) was demonstrated by toxicokinetic experiments with the elements Pb, Cd, Cu, and Zn (Bernds et al. 1998). The absence of additional bioaccumulation of Cd, Cu, and Zn was observed at some sites with very high metal concentrations in sediments. Thus, Saiz-Salinas and Francès-Zubillaga (1997) concluded that *N. diversicolor* was an unreliable biomonitor of metal contamination, but Berthet et al. (2003) showed that the lack of enhancement of metal concentrations in worms was associated with the absence of detectable release of the three metals from sediments at different pHs. Thus, these worms are able to reveal the bioavailable concentrations of metals which are those with the higher toxic potential. Bioaccumulators are also of great interest for those metals which can be present in the environment as organometallics. In the case of mercury, methylmercury MeHg – the organic form which is more bioaccumulated and more toxic than inorganic mercury – accounted for an average of 0.7% of the total Hg in sediments from the Scheld estuary (Belgium) and 18% of the total Hg in *N. diversicolor* (Muhaya et al. 1997).

Sediment-bound hydrophobic organic contaminants (tetrachlorobiphenyl TCBP, hexachlorobenzene HCB, and benzo[a]pyrene BaP) were absorbed with assimilation efficiencies of 55–92% in *Nereis succinea* (Ahrens et al. 2001). Bioaccumulated concentrations of PCBs in the marine polychaete *Neanthes arenaceodentata* are mainly derived from sediment with the aqueous phase accounting for less than 3% of total uptake. Since activated carbon amendment reduced

PCB uptake by 95% in laboratory experiments, bioaccumulated concentrations seem representative of bioavailable PCBs (Janssen et al. 2010), and even if in the field, confounding factors make data interpretation more delicate (Janssen et al. 2011).

In the spionid polychaete *Streblospio benedicti* exposed to sediment-associated polynuclear aromatic hydrocarbons, bioaccumulation of the most abundant sediment-associated PAH, fluoranthene (FL), was very high (9.5-13.7X FL sediment concentrations) after 28-day exposures (Chandler et al. 1997). Polychaetes (*Nereis virens, N. diversicolor*) also seem able to reveal the bioavailable concentrations of PAHs in sediments (Vinturella et al. 2004; Cornelissen et al. 2006).

However, because polychaetes are well equipped with biotransformation enzymes, they are able to excrete PAHs efficiently, thus resulting in relatively low concentrations in their tissues compared to sediments. In contrast, they are far from being quite so efficient in PCB biotransformation and, in this case, tissue concentrations may be much higher than sediment concentrations (Amiard et al. 2009).

The Risk of Contaminant Transfer into the Food Web

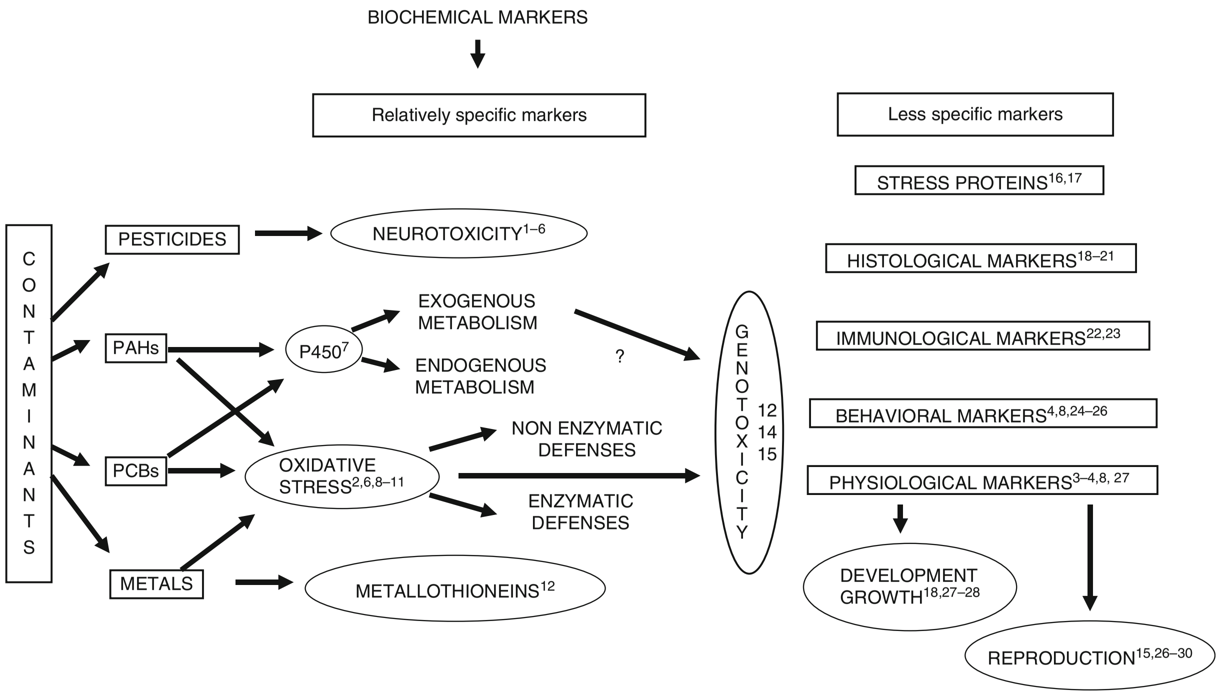
Determining the physicochemical forms of storage of chemicals is crucial in order to predict their fate and effects in organisms at a higher level in the food web (Amiard-Triquet and Rainbow 2011). Bioaccumulators with high concentrations of metals or organic chemicals in their tissues can be responsible for trophic transfer, provided that their body content can be assimilated by their predators. Some contaminants accumulated in prey species as very stable compounds may be eliminated without any measurable bioaccumulation in the predator. On the contrary, other bioaccumulated fractions are easily bioaccessible to the predator as is the case for mercury, particularly MeHg. This is why several studies deal with the role of benthic invertebrates, particularly polychaetes, in the trophic transfer of contaminants, such as copper (Rainbow et al. 2004), mercury (Coelho et al. 2008), and organic xenobiotics (Rice et al. 2000; Palmqvist et al. 2006).

In some cases, this trophic transfer has been found responsible for biological impairments in predators. Specifically, Boyle et al. (2008) provided evidence that As accumulated in fish fed a diet consisting of *N. diversicolor* from a highly contaminated area in Southwest England (Restronguet Creek) can affect reproduction, highlighting the ecotoxicological significance of As in the creek. Metal-rich invertebrates (*N. diversicolor*) that have accumulated metals from the sediments of particular SW England estuaries can transfer these metals to their predators (*N. virens* and *Palaemonetes varians*). This trophic transfer may be significant enough to have ecotoxicological effects as shown particularly in the decapod crustacean *P. varians* (Rainbow et al. 2006). Polychaetes (*Armandia brevis*) exposed to clean sediments supplemented with benzo(a)pyrene (PAH), pp'DDE (a metabolite of DDT), Aroclor 1254 (PCBs), or field sediments collected from two sites contaminated predominantly with PAHs or chlorinated compounds were fed to juvenile English sole (*Pleuronectes vetulus*). Fish growth was lower than in reference fish in all but one of eight groups fed contaminant-exposed polychaetes, and juvenile sole exposed to BaP-exposed worms showed clear evidence of hepatic DNA adducts revealing genotoxicity (Rice et al. 2000).

Biomarkers in Polychaetes

The major categories of biomarkers corresponding to different classes of contaminants are shown in Fig. 1. Neurotoxicity is often revealed by the inhibition of acetylcholinesterase (AChE), a response which was initially considered to be specific of carbamate and organophosphate pesticides. More recently, it has been recognized that AChE is able to respond to different chemical stress involving metals, detergents, and algal toxins (Leiniö and Lehtonen 2005). After AChE was characterized biochemically in the polychaete annelid *Nereis diversicolor* (Scaps and Borot 2000), it was widely used as a biomarker of damage in this species (Fig. 1). Biotransformation of organic compounds in marine polychaetes proceeds in a two-phased process similar to those well studied in vertebrates; phase I enzymes belonging to the cytochrome P450 (CYP) enzyme family, along with a few phase II enzymes, have been identified in marine polychaetes (review by Jørgensen et al. 2008). Among phase II enzymes, glutathione *S*-transferase seems very responsive in polychaetes including *Neanthes succinea* (Rhee et al. 2007) and *N. diversicolor* (Ait Alla et al. 2006; Durou et al. 2007; Kalman et al. 2009; Bouraoui et al. 2010). Biomarkers able to display oxidative stress were observed in *N. diversicolor* (Ait Alla et al. 2006; Moreira et al. 2006; Sun and Zhou 2008; Bouraoui et al. 2010) and other species (*Laeonereis acuta*: Sandrini et al. 2008; *Perinereis aibuhitensis*: Sun et al. 2009). Metallothioneins, a family of metalloproteins involved in both detoxification and homeostasis of metals, were present in different species (review by Amiard et al. 2006), but in *N. diversicolor* and *P. aibuhitensis*, their concentrations were not increased in the presence of metals whereas their turnover was. Thus, despite MTs being able to fulfill their biological role (Ng et al. 2008), these species are not a useful matrix for the determination of MT concentrations as biomarkers of contaminant metal exposure (Poirier et al. 2006). Genotoxicity was demonstrated in different species including *Capitella* sp. (Palmqvist et al. 2003), *Arenicola marina*, and *Nereis virens* (Lewis and Galloway 2008, 2009). Among the less specific biomarkers, stress proteins were shown to be induced by heat shock in *N. diversicolor* and in two sibling species of the spionid genus *Marenzelleria* as well as by cadmium in the former (Ruffin et al. 1994; Blank et al. 2006).

Toxico-genomic approaches using the tools of molecular biology provide an understanding of a new aspect of the impacts of chemical contaminants on living organisms. When no data are available on the nature of pollutants or on the genes involved in the responses to stress, different genomic tools allow exploring the patterns of gene expression over several thousand genes. The potential of genetic markers as diagnostic tools for a better knowledge of the impact of contaminants has



Polychaetes in Ecotoxicology, Fig. 1 Major categories of biomarkers corresponding to different classes of contaminants (Modified after Narbonne and Michel 1992) and studies in which they were determined in polychaetes. [1]Scaps and Borot 2000, [2]Ait Alla et al. 2006, [3]Durou et al. 2007, [4]Kalman et al. 2009, [5]Solé et al. 2009, [6]Bouraoui et al. 2010, [7]Review by Jørgensen et al. 2008, [8]Moreira et al. 2006, [9]Sandrini et al. 2008, [10]Sun and Zhou 2008, [11]Sun et al. 2009, [12]Review by Amiard et al. 2006, [13]Palmqvist et al. 2003, [14,15]Lewis and Galloway 2008, 2009, [16]Ruffin et al. 1994, [17]Blank et al. 2006, [18]Hutchinson et al. 1998, [19]Mouneyrac et al. 2003, [20]Geracitano et al. 2004, [21]Poirier et al. 2006, [22]Review by Galloway and Depledge 2001, [23]Review by Fournier et al. 2005, [24]Bonnard et al. 2009, [25]Rosen and Miller 2010, [26]Mouneyrac et al. 2010, [27]Pook et al. 2009, [28]Durou et al. 2008, [29]Mouneyrac et al. 2006, [30]Lewis et al. 2008 already been used on bivalve and fish populations. Recently, the transcripts obtained in the polychaete *Perinereis nuntia* included a number of stress- and cell defense-related genes (e.g., heat shock protein family, antioxidant-related genes, cytochrome P450 genes) that are potentially useful for sediment monitoring at the molecular level (Rhee et al. 2012).

Up to date there are studies dealing with both histological damages (e.g., effect of municipal sewage effluents in *Platynereis dumerilii*, Hutchinson et al. 1998; effect of copper in *Laeonereis acuta*, Geracitano et al. 2004) and defense responses (e.g., detoxification of metals, Mouneyrac et al. 2003; Poirier et al. 2006) in polychaete species. Many environmental chemicals have been shown to affect the immune system in vertebrates, and reviews by Galloway and Depledge (2001) and Fournier et al. (2005) indicate in vitro and in vivo evidence of immunotoxicity in invertebrates, including annelids. Among annelids, the most studied species belonged to oligochaetes, but immunotoxicities of copper to the polychaete *Eurythoe complanata* and of PCBs to *Glycera dibranchiata* were also reported (Galloway and Depledge 2001). The normal functioning of physiological mechanisms may be affected in several ways by xenobiotics, and special attention must be devoted to the processes of energy acquisition and allocation. Determination of digestive enzyme activities has been considered as an indicator tool for assessing the physiological status of organisms. Toxicant-induced inhibition of digestive activity was demonstrated in *N. diversicolor* (Kalman et al. 2009). Depression of assimilation efficiency, associated with impairments of feeding rate, and the energy cost of defense mechanisms (Pook et al. 2009) can result in metabolic disruption such as changes in the levels of energy reserves (Durou et al. 2007) and increased rate of an organism's anaerobic metabolism, as evidenced by an enhancement of lactate dehydrogenase activity (Moreira et al. 2006). Consequently, direct effects on population parameters such as growth and reproduction may be expected. Condition indices have been determined to reveal the physiological status of polychaetes in multi-polluted environments, such as the relationship between length of the first three segments (L3) and wet weight of *N. diversicolor* in estuaries (Durou et al. 2008). Since sexual products are freely suspended in the body cavity, thus easily accessible, polychaetes are remarkable models for studies dealing with parameters involved in reproduction success, such as fecundity (Durou et al. 2008; Pook et al. 2009; Mouneyrac et al. 2010), fertilization success, and postfertilization development rates (Lewis et al. 2008; Lewis and Galloway 2009). Reproductive disorders at the level of sexual hormones can also be shown in polychaetes (Mouneyrac et al. 2006). In order to expand the ecotoxicological toolbox, Lewis and Watson (2012) recommend the inclusion of polychaete reproductive endpoints.

Besides physiological biomarkers, promising behavioral biomarkers (e.g., feeding and burrowing behavior) have received particular attention in *N. diversicolor* and *Neanthes arenaceodentata* exposed to contaminants either in the laboratory or more realistically in the field (Fig. 1). The family Nereidae appears to be the most commonly used taxon of polychaetes for the determination of biomarkers.

Comparing biomarker responses in a bivalve mollusc and the ragworm *N. diversicolor* from a littoral enclosure in the SW Spain, Solé et al. (2009) concluded that the latter was the potentially most robust sentinel in this ecosystem. This opinion was confirmed by Kalman et al. (2010), since several biomarkers are generally more sensitive to pollution factors than to natural factors (e.g., salinity of the studied area, size of the ragworms), thereby avoiding most problems owing to so-called confounding factors.

Biological Testing with Polychaetes

As mentioned above in the historical overview, polychaetes were widely used in toxicity tests for individual compounds, and this strategy is still applied (Méndez et al. 2009). An inventory of marine biotest methods for the evaluation of dredged material and sediments was compiled by Nendza (2002) on behalf of the Federal Environmental Agency of Germany. For assessing acute toxicity, several studies using *Arenicola marina*, *Nereis/Neanthes* sp., and *Streblospio benedicti* were carried out according to ASTM and ICES guidelines. The effect of a 10-day exposure in the laboratory to whole sediment was assessed considering different endpoints: survival, casting rate, and growth. For assessing long-term toxicity, survival, growth, and reproduction were determined in *A. marina* and *Nereis/Neanthes* sp. after a 28-day exposure. However, in agreement with field data concerning the relative sensitivity of amphipods and polychaetes to xenobiotics, Anderson et al. (1998) showed that 78% of sediment samples collected from bays and harbors in California inhibited survival of the amphipod *Rhepoxynius abronius*, whereas 2% and 26% inhibited *Nereis/Neanthes arenaceodentata* survival and biomass, respectively.

Conclusion and Operational Consequences

Polychaetes are crucial for ensuring the structure and functioning of estuarine and coastal systems, representing nearly 40% of taxa constituting the most important soft-bottom communities in Europe, whereas their presence and ecological significance are not as important in freshwater systems. Consequently they are commonly incorporated in biotic indices especially relative to the Benthic Opportunistic Polychaete Amphipod index (BOPA). Living in close contact with sediments which are the final sink for most xenobiotics entering aquatic environments, they are very relevant as sentinels in many ecotoxicological studies: (1) as bioaccumulators not only able to reveal the presence and quantities of contaminants in their medium but also to give an insight into their bioavailability and (2) as models for the determination of many biomarkers of interest, including classical biomarkers and ecologically relevant biomarkers (see the entry on "► Biomarkers of Ecological Relevance in Ecotoxicology" in this encyclopedia). Most species commonly employed in these ecotoxicological studies have been assigned to ecological group III consisting of "tolerant species" by ecologists interested in biotic indices. On the one hand, this means that they will be available for use in most sites which need to be monitored for their health status and, on the other hand, that they will not be very sensitive in bioassays such as those included in environmental risk assessment procedures (e.g., evaluation of dredged material and sediments). Moreover, because tolerance may be the consequence of chronic exposure in the field, it will be necessary to collect test organisms from so-called "pristine" environments, in fact from environments that are "as clean as possible" and, when possible, to obtain them from aquaculture facilities. Lastly, the presence of tolerant polychaetes in highly contaminated areas, associated with their ability to accumulate pollutants and their importance as prey species, will require assessing their importance in food chain transfer events and perhaps even in the biomagnification processes involving xenobiotics.

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Cross-References

► Aquatic Biomarkers
► Benthic Community Ecotoxicology
► Biomarkers of Ecological Relevance in Ecotoxicology
► Contaminated Sediment Core Profiling
► In Situ Bioassays in Ecotoxicology
► Sediment Ecotoxicity
► Sediment Quality Guidelines
► Sediment Toxicity Identification Evaluation

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Protozoans in Ecotoxicology

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Article Outline

Synonyms

Ciliata in ecotoxicology; Protozoa in ecotoxicology

Glossary

Axenic culture A culture of protozoans under bacteria-free conditions
Deformation Change in size and/or shape of the cell caused by a stressor

Definition

The use of protozoans to measure the toxicity of pure compounds and/or environmental samples.

Several species of protozoans mainly from the group of ciliates have been used in ecotoxicological investigations: *Tetrahymena pyriformis*, *T. thermophila*, *Paramecium caudatum*, *P. bursaria*, *Spirostomum ambiguum*, *S. teres*, and *Colpidium campylum*.

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The assays include chronic toxicity tests with proliferation rate and population density measurements and acute tests with different endpoints: nonspecific mortality, morphological malformations and chemosensory behavior, and specific biochemical changes.

Historical Background

Protozoans have been used as bioindicators for the saprobic states of freshwaters ever since the beginning of the twentieth century (Pauli et al. 2001). The species from four genera, namely, *Tetrahymena*, *Colpidium*, *Paramecium*, and *Spirostomum*, have been favorite models in cell biology for over 70 years (Nałęcz-Jawecki 2004). Since the 1980s, several ecotoxicological assays with ciliates were developed as screening tools to assess contaminant toxicity. Both short-term and chronic tests were employed as alternatives to fish or mammalian assays.

The term “protozoan” has historically referred to unicellular, animal-like protists. According to the International Society of Protistologists (Adl et al. 2005), the term protozoan is no longer valid and should not be used in the scientific literature. However, according to ITIS (Integrated Taxonomic Information System) classification, protozoa make up one of the five biological kingdoms.

Biology and Features of Ciliated Protozoans

Many features have contributed to making protozoans, especially ciliates, good test bionts (Gilron and Lynn 1998; Pauli et al. 2001). Protozoans are the simplest eukaryotes, containing in a single cell all features necessary for independent life. Ciliates are the most complex group of Protista characterized by the presence of hair-like organelles called cilia. Protozoans play an important role in aquatic ecosystems as decomposers and primary consumers and comprise a key component of water and sewage purification systems. They are a link between a highly productive pico- and nanoplankton and the metazoans of the classical food web (Pauli et al. 2001). They feed on bacteria, small phytoplankton, and detritus and are responsible for the transfer of energy from the microbial food web to metazoan zooplankton (Gilron and Lynn 1998).

Freshwater ciliates vary in size ranging from 50 μm (*Tetrahymena*) to 3 mm (*Spirostomum ambiguum*). Asexual reproduction occurs by binary fission, and the growth rate depends on the size of the ciliate with doubling time ranging from 4–5 h to 72 h for *T. termophila* and *S. ambiguum*, respectively.

Freshwater ciliates are easily cultured in a laboratory. Culturing methods can be found in references cited in Table 1. *Tetrahymena* can be grown axenically (Schultz 1997), which could explain its extensive use in cytotoxicology

Protozoans in Ecotoxicology, Table 1 Characteristics of some toxicity tests with ciliated protozoans

Toxicity test / species	Medium	Test format	Assessment endpoint	Measurement endpoint	References
Chronic toxicity tests					
Tetratox *Tetrahymena pyriformis*	Proteose peptone-based medium	Erlenmeyer flask	Growth inhibition with optical density measurement of protozoan population at 540 nm	40 h-IGC_{50}	Schultz (1997)
Protoxkit F™ *Tetrahymena thermophila*	Food suspension in mineral medium	Disposable polystyrene spectrophotometric cells (4 ml)	Growth inhibition with optical density measurement of food suspension at 440 nm	24 h-EC_{50}	Pauli and Berger (2000)
Colpidium campylum	Bacterial suspension (*E. coli*) in mineral medium	Disposable polystyrene vials (30 ml)	Growth inhibition with cell density counted electronically	24 h-EC_{50}	Dive et al. (1989)
Acute toxicity tests					
Spirotox *Spirostomum ambiguum*	Mineral medium (diluted Tyrod solution)	Disposable 24-well microplate (6 × 4 wells)	Mortality, morphological deformations observed under dissection microscope	24 h-LC_{50}, 24 h-EC_{50}	Nałęcz-Jawecki (2005)
12 species of freshwater ciliates tested individually	Natural water	Disposable 24-well microplate (6 × 4 wells)	Mortality	24 h-LC_{50}	Madoni (2000)
Tetrahymena thermophila	Osterhout's mineral medium	Microcentrifuge tubes, multiwell filter plate	Viability assessed with fluorescent dyes	24 h-EC_{50}	Dayeh et al. (2005)
Rapid toxicity tests					
Tetrahymena pyriformis	Osterhout's mineral medium	Special test chambers with oxygen probe	Oxygen uptake rate measurements	5 min-LOEC	Slabbert and Morgan (1982)
Chemosensory test, *Tetrahymena thermophila*	Tris buffer, pH = 7.4	Special test chambers: outer portion containing organisms and inner portion functioning as a trap	Number of cells in the inner chamber determined electronically	90 min-EC_{10}	Pauli et al. (1994)

(*continued*)

Protozoans in Ecotoxicology, Table 1 (continued)

Toxicity test / species	Medium	Test format	Assessment endpoint	Measurement endpoint	References
Chemosensory test, *Tetrahymena pyriformis*	MOPS-buffered saline solution	Special test chambers: glass trough with capillary	Number of cells migrating in the capillary	1 h or 5 h-EC_{50}	Berk and Roberts (1998)

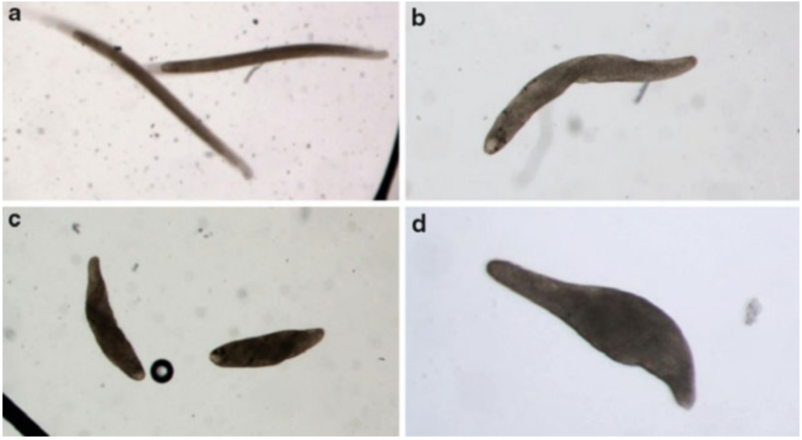
IGC_{50} 50% impairment growth concentration

(Nilsson 1989). The biochemistry and physiology of ciliated protozoans, especially for *Tetrahymena*, are well known, and thus, several test endpoints can be assessed including chemosensory behavior, ingestion rate, and enzyme activity measurements (Berk and Roberts 1998; Dias and Lima 2002; Nilsson 1989).

Types of Tests with Protozoans and Their Applications

A number of parameters can be used as test endpoints in the evaluation of protozoan toxicity. Table 1 describes several toxicity tests with protozoans. They are divided into three groups. In the population growth impairment tests, small rapidly growing species are used. Exposure duration can vary from 1 to 4 days, which include 4–10 generations. Growth rate can be evaluated directly by measurement of cell density with a microscope and counting chamber or an electronic counter. Axenic culture conditions guarantee high reproducibility of these assays. They have been used for comparing the sensitivity of protozoans with other organisms and QSAR studies of organic chemicals (Nezteva and Schultz 2005; Enoch et al. 2008). These tests are well standardized, but the addition of organic medium may cause the decrease of bioavailability of some toxicants, e.g., metals (Nilsson 1989). In the Protoxkit F™ specially designed for environmental samples, *T. thermophila* is fed with a food suspension, such that a much lower level of dissolved organic carbon is applied resulting in lower sorption of toxicants (Pauli and Berger 2000). In the Protoxkit F™ population growth rate is evaluated indirectly by measuring the turbidity of the remaining food suspension.

Acute toxicity assays evaluate survival, a common endpoint in many standard bioassays. The viability of small ciliates is measured with fluorescent dyes, while larger protozoans are examined with the aid of a dissection microscope. Besides mortality, sublethal effects can be observed, e.g., morphological deformations and changes in locomotion (Nałęcz-Jawecki 2005). Exposure time can be as long as 24 h. In contrast with chronic toxicity assays, a mineral (inorganic) medium is used in acute tests. This approach mimics the conditions of surface water and increases the sensitivity of test organisms to metals and other compounds, which are readily adsorbed to organic material.



Protozoans in Ecotoxicology, Fig. 1 Ciliated protozoan *Spirostomum ambiguum*. (**a**) Healthy cell, (**b–d**) morphological deformations of *S. ambiguum*; (**b**) bending of the cell; (**c**) shortening of the cell; (**d**) “club” shape

Due to their high metabolic ratio, small cell volume, and relatively high surface contact with their environment, ciliates can respond very rapidly to chemical stress. A marked advantage of the respiration test is that the incubation time is very short – only 5 min (Slabbert and Morgan 1982). The movement of ciliates toward or away from chemicals, i.e., chemosensory behavior, has been applied in several toxicological studies (Berk and Roberts 1998; Pauli et al. 1994). The exposure time for the chemoattraction assays is quite short, ranging between 15 and 30 min. Chemosensory behavior is relatively simple to implement and has a broad range of applications (Gilron and Lynn 1998).

Assays with ciliates have been applied to measure the toxicity of pure chemicals, including heavy metals, industrial organics, and pharmaceuticals (Berk and Roberts 1998; Madoni 2000; Nałęcz-Jawecki 2004). In addition, several investigations have dealt with the assessment of industrial effluents and leachates (Dayeh et al. 2005). More recently, ciliates have been used as an important component of a battery of bioassays as they represent a neglected trophic level (Mankiewicz-Boczek et al. 2008).

Illustration of Endpoints in the Spirotox Test

A healthy cell of *S. ambiguum* is shown in Fig. 1a. Some sublethal morphological deformations of *S. ambiguum* are illustrated in Fig. 1b, c, d. Such endpoints can be easily observed under a dissection microscope at an 8 x magnification.

Advantages of Conducting Tests with Ciliated Protozoans

Bioassays with protozoans allow the examination of a large population of organisms in a short time period. Ciliates belong to eukaryotes, but they can be cultured both easily and economically like bacteria. They can be used as screening and alternative tests prior to conducting whole organism assays at higher levels of biological complexity.

Conclusions and Prospects

Biotests with protozoans are an essential component of a battery of microbiotests to assess toxicity of both chemicals and environmental samples. Their application should markedly expand in the future, as different parameters can be used as test endpoints.

Cross-References

► Biological Test Methods in Ecotoxicology
► Microbiotests in Ecotoxicology
► Test Batteries in Ecotoxicology

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Pulse Exposure in Ecotoxicology

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Article Outline

Synonyms

Fluctuating exposure; Intermittent exposure; Peak exposure; Time-varying exposure

Glossary

Acute quality criterion (plural: criteria) Maximum acceptable concentration in an environmental compartment preventing severe effects, that is, short-term (acute) effects, on the ecosystem.

Chronic quality criterion (plural: criteria) Maximum acceptable concentration in an environmental compartment preventing long-term (chronic) effects on the ecosystem.

PCBs Polychlorinated biphenyls, comprising a class of organic compounds (with 1 to 10 chlorine atoms attached to a biphenyl structure, which is a molecule

J.-F. Férard, C. Blaise (eds.), *Encyclopedia of Aquatic Ecotoxicology*,
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composed of two benzene rings). PCBs were used as cooling and insulating fluids in industrial transformers, for example.

Pesticide A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest preventing the growth of crops.

Recovery The potential of an organism, a population, or a community to recover its full capacity after a stress.

Runoff Runoff is a term used to describe the water from rain, snowmelt, or irrigation that flows over land surface and is not absorbed into the ground. Runoff water flows into streams or other surface waters.

Standardized tests Testing methods, which describe and define the type and condition of organisms used for testing, exposure conditions, and reporting requirements. Protocols are validated via laboratory intercalibration to ensure test reproducibility and are recognized internationally. Examples of standardizing agencies are OECD, ISO, and Environment Canada.

Time-dependent effect An effect dependent on duration of exposure. At a specific concentration, an increase in effect is generally observed with increasing exposure duration.

Toxicodynamic Chemical, biological, and physiological effects occurring after adsorption of a chemical by an organism.

Toxicokinetic Kinetic of adsoption and excretion of a toxicant in an organism.

Veterinary substance A substance with a pharmacological action administered to treat animal pathologies from households and farmyards.

Definition

Nonconstant exposure of organisms to a chemical (or a mixture of chemicals) characterized by exposures of short duration (minutes to hours) that can happen repeatedly.

Pulse exposures are the consequence of intermittent discharge of chemicals in the environment. These can be accidental but are mainly observed in streams during rain events in either agricultural or urban regions. Monitoring indicates that pulses are mostly observed in small- and medium-size rivers during high flows.

Pulse exposure events generally integrate effects during the pulse exposure, as well as those following exposure. When pulses occur repeatedly, cumulative effects may be observed.

Historical Background

Traditionally, ecotoxicology focused on continuous exposure of organisms to one or a mixture of compounds. However, the environmental exposure of organisms to chemicals is rarely constant. This is especially true for the freshwater compartment. The first debate on the difficulty of assessing effects of pulse exposures essentially began in the early 1990s (Seager and Malby 1989; Handy 1994). More emphasis was given to this issue at the beginning of this century, with the growing need of defining water quality criteria for pesticides (Reinert et al. 2002). Advances have been made in both the modeling of effects during pulse exposure (Ashauer et al. 2006) and in laboratory testing methods (Vallotton et al. 2008).

Sources of Pulses in the Aquatic Environment

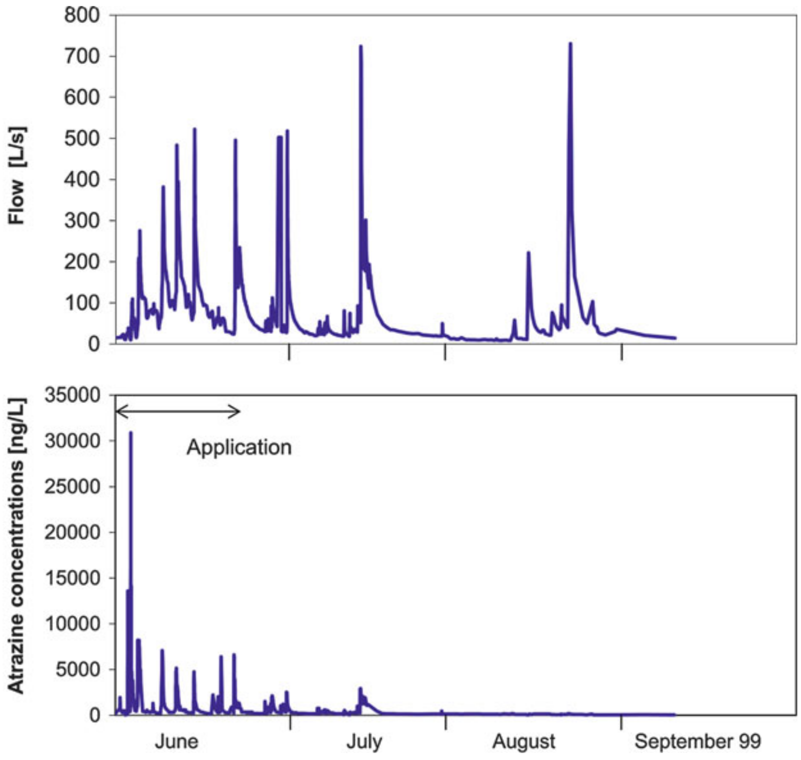
Apart from accidental events, the main source of intermittent chemical discharges in the environment is linked to rainfall events. Surface runoff occurring during such events leaches agriculture and urban surfaces and transport soluble substances as well as particles into receiving waters. In agricultural catchments, transported substances are typically pesticides and veterinary substances (Reinert et al. 2002). In urban catchments, the most commonly detected substances are of diverse sources and nature and can include biocides (Plagellat et al. 2004), heavy metals (Chèvre et al. 2011), PCBs (Rossi et al. 2004), and others.

Types of Pulses in the Aquatic Environment

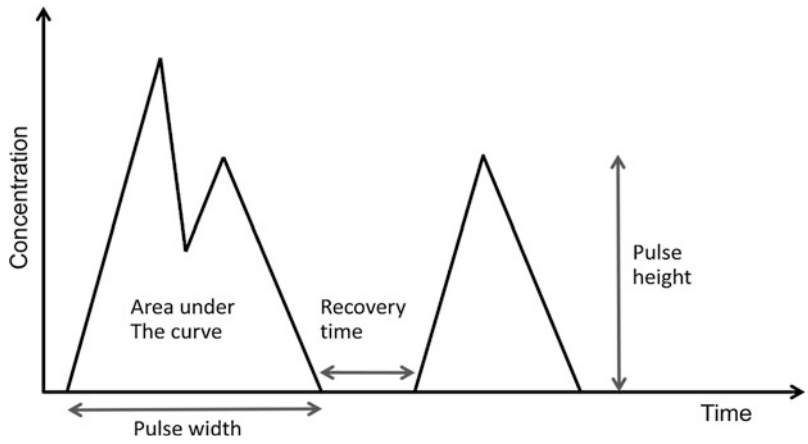
Figure 1 displays typical pulse exposures of an herbicide, as monitored in the river of agriculture catchments. In agricultural regions, pesticide concentrations reach their maximum level during rainfall events occurring during the field application period. In urban areas, the highest exposures are also observed during rainfall events; however, there is no specific seasonal occurrence. Each pulse can be characterized by its width, its height, or alternatively an integration of both parameters with the area under the curve, while recovery time between pulses is a critical parameter to consider in the assessment of sequential exposures (Fig. 2).

New Laboratory Testing for Pulses

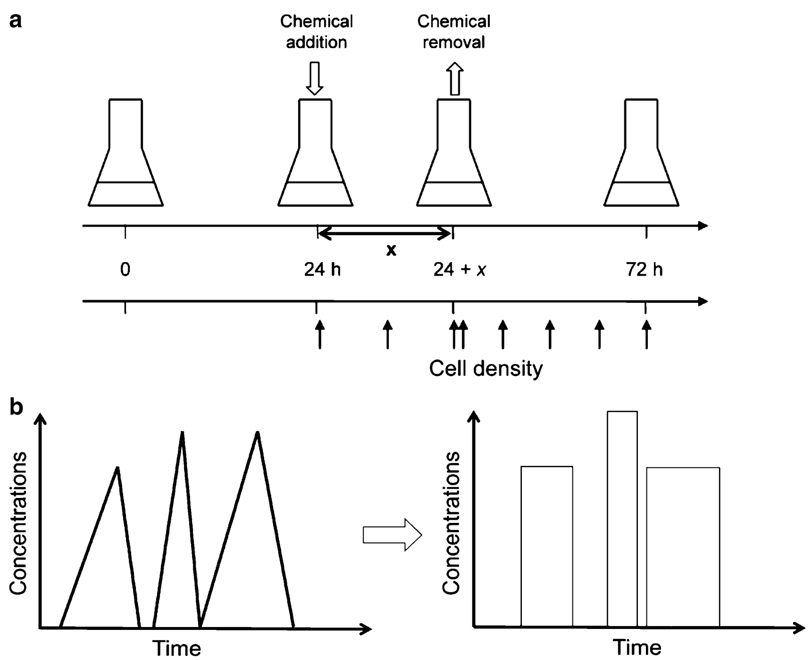
Laboratory testing of pulse exposure differs from standardized testing, as the exposure should be nonconstant and include the observation of postexposure effects, such as delayed effects following the pulse exposure. One of the main difficulties in assessing effects of a single pulse exposure in the laboratory is to reproduce a realistic exposure. Flow-through systems allow a close simulation of the rapid increase and decrease in concentration, as observed during a pulse. However, most of the time and for practical reasons, pulse exposures are assessed with simplified exposure scenarios either by adding and removing the chemical from experimental



Pulse Exposure in Ecotoxicology, Fig. 1 Fluctuating concentrations of atrazine in a small river. Concentrations reach a maximum during a rainfall event and during the application period (Adapted from Chèvre et al. (2004) and Leu (2003)). In this example, peak concentrations are at least 20-fold higher than the background concentration



Pulse Exposure in Ecotoxicology, Fig. 2 Main features considered to describe pulses (Adapted from Reinert et al. (2002))



Pulse Exposure in Ecotoxicology, Fig. 3 (**a**) Exposure scenarios to assess time-dependent effects on growth rate of *S. vacuolatus* during pulse exposures (duration x) and subsequent recovery (from Vallotton et al. 2008a). A simplification of the exposure scenario often undertaken is illustrated in (**b**)

vessels (Vallotton et al. 2008a) or by transferring organisms to vessels containing the test concentration for a defined duration (Tlili et al. 2008). Figure 3a and b portrays an experimental setup to assess effects of a single pulse on algae.

Effect assessment during fluctuating exposures needs to consider the infinite variety of exposure scenarios observed in the environment, which constitutes a second difficulty. Indeed, fluctuating exposures are a combination of single pulses of different width, height, and varying time between pulses. Sequential pulses can be simulated by repeated short constant exposure (Fig. 3b). However, establishing representative scenarios to be tested in a laboratory is not an easy task. Several scenarios have been proposed for agriculture (http://focus.jrc.ec.europa.eu/) and urban wet-weather discharges (Rossi et al. 2009a). These are mostly based on modeling of wet-weather discharge, which aims to describe the behavior of chemicals in the environment.

Effects of Pulses

Research on the effects of pulse exposure has focused to a large extent on toxic effects of insecticides to fish and macroinvertebrates. In general, pulse insecticide exposures cause less toxicity than long exposure at the same concentration

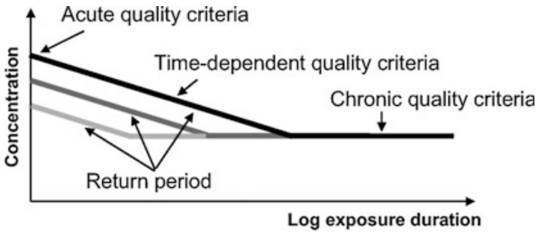
(Handy 1994; Van der Hoeven and Gerristen 1997; Peterson et al. 2001; Reynaldi and Liess 2005), although a few studies have shown that short exposure could cause higher toxicity (Buhl et al. 1993; Schulz and Liess 2000). The level of damage during pulse exposure usually depends both on the concentration and duration of the exposure (Cold and Forbes 2004; Duquesne et al. 2006). Furthermore, short pulses can induce latent effects in the recovery period (Van der Hoeven and Gerristen 1997) and influence the response of organisms during a second pulse, especially if the recovery is incomplete. The degree and rapidity of recovery could be related to the mode of action of the pesticide. Toxicokinetic and toxicodynamic models have been developed to describe the effects of pulse exposure in a given organism (fish, microcrustacea, etc.; for a review, see Ashauer and Brown 2008). Their application is dependent on certain assumptions such as the speed of recovery or the existence of a toxicodynamic threshold

In contrast to insecticides, little research has focused on the effect assessment of fluctuating exposure of herbicides (Klaine et al. 1997; Vallotton 2007). Recently, Tlili et al. (2008) have shown that exposure to environmentally realistic fluctuating concentrations of a common herbicide, diuron, can have measurable effects on freshwater periphyton communities and especially on algal communities. The toxic effects of pulse exposure to several herbicides have also been observed in algal cultures. In a recent study, Vallotton et al. (2008a, b) observed (1) differences in the time-dependent effects to herbicides and (2) the speed of recovery. They also observed that sequential exposures to a specific herbicide, isoproturon, induced cumulative effects over time, despite rapid recovery between exposures (Vallotton et al. 2009).

Ecological Risk Assessment of Pulses

Standard risk assessment in surface water evaluates the risk of a substance by comparing its predicted (PEC) or measured (MEC) concentration to its water quality criterion derived from, for example, the Predicted No Effect Concentration (PNEC). This PNEC should protect organisms from long-term exposure to a chemical (see entry on "► Toxic Units (TU) Indicators" in this encyclopedia). Some guidelines also recommend deriving acute quality criteria, which should never be exceeded to protect organisms. Predicting the risk of repeated pulse exposures in the environment is challenging, as risk cannot solely relate to exposure concentration, but needs to include additional parameters specific to pulses such as exposure and recovery duration and frequency of exposures.

Rossi et al. (2009b) have suggested a concept to establish water quality criteria to address the risk of pulse exposure. The measured pulse described in terms of concentration and exposure duration is compared to time-dependent quality criteria, which are illustrated in black in Fig. 4. In addition, the criterion should be more stringent for sequential pulses, especially when the return period is short.



Pulse Exposure in Ecotoxicology, Fig. 4 Concept for time-dependent water quality criteria. Allowable concentration in receiving waters depends on exposure duration and return period, that is, the frequency of the pulses. The maximum concentration during a single pulse should be compared to the *dark line* corresponding to the time-dependent quality criteria for a single event; the maximum concentration during sequential pulses should be compared to the *lightest lines* depending on the frequency of pulses, thus including the notion of "return period." Risk is considered as non-negligible when the maximum concentration of a single peak exceeds the criteria (from Rossi et al. 2009b)

Several questions nevertheless remain open as to how to consider additional uncertainties in the definition of water quality criteria for fluctuating exposure. For example, safety factors applied to extrapolate effects observed in the laboratory or model predictions to the field should be established. Furthermore, additional factors such as postexposure effects and adaptation of organisms to sequential pulses should be considered in risk evaluation.

Conclusions and Prospects

Wet-weather events induce the transport of chemicals in both agriculture and urban environments, resulting in exposure to fluctuating concentration of chemicals. Laboratory methods have and are being refined to assess effects of pulse and sequential exposures, with the goal of better predicting environmental risk related to substances present in runoff waters. Ongoing research focuses on improving the evaluation of pulse effects of chemicals with different modes of action to several aquatic species and communities. In parallel, key exposure, toxicokinetic, toxicodynamic, and response parameters are being refined with the goal of improving computational predictions. These should in turn allow predicting effects and related risk of the infinite variety of fluctuation exposure scenarios.

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Cross-References

- ▶ Benthic Community Ecotoxicology
- ▶ Biological Test Methods in Ecotoxicology
- ▶ Emerging Issues in Ecotoxicology: Persistent Organic Pollutants (POPs)
- ▶ Macroinvertebrate Ecotoxicity Testing (MET)

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