How to Link Field Observations with Causality? Field and Experimental Approaches Linking Chemical Pollution with Ecological Alterations

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Abstract This chapter summarizes field and laboratory investigations dealing with metals and pesticides (90) and emerging compounds' (10) effects on fluvial communities. The Arkansas River case study is a good example showing how field observations, together with long-term natural experiments and microcosm experiments, provide consistent evidence of metals effects on macroinvertebrate communities. In the case of biofilms, microcosm and mesocosm experiments confirm that metals and pesticides are responsible for the loss of sensitive species in the community, and that this influence is modulated by several biological and environmental factors. Information about the effects of emerging pollutants is very scarce, highlighting the existence of a missing gap requiring future investigations. The examples provided and the recommendations given are proposed as a general guide for studies aiming to link chemical pollution with ecological alterations.

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Contents

1 General Introduction

Among the long list of compounds included in the Priority Pollutants (PP) list (European Water Framework Directive 2000/60/EC [1]) and those not included but considered also of environmental concern, few studies demonstrate their real impact on fluvial ecosystems. Discriminating the influence of environmental variability from chemical pressure on the biota is a challenging issue. It is necessary to detect joint and specific effects of simultaneously co-occurring stressors. This difficult task requires an interdisciplinary approach including environmental chemistry, toxicology, ecotoxicology, ecology, etc., and also a multiscale perspective from field investigations to laboratory experiments, microcosm, mesocosm studies, and back to the field in order to establish cause-and-effect relationships.

The change of scale is easily achieved working with fluvial communities. Exposure might be done under controlled experimental conditions in microcosms and mesocosms or in the field where the response can be evaluated under real exposure conditions. It allows defining non-effect concentrations, and also assessing the effects of different types of stress alone or in conjunction (multiple stressors). In contrast to single-species tests, ecotoxicological studies with communities involve a higher degree of ecological realism, allowing the simultaneous exposure of many species. Furthermore, it is possible to investigate community responses after both acute and chronic exposure, including the evaluation of direct and indirect toxic effects on entire communities.

In particular, fluvial biofilm and benthic macroinvertebrate communities fulfill these conditions and have been used to investigate single- and multiple-stress situations at different spatial and temporal scales.

Fluvial biofilms (also known as phytobenthos or periphyton) are attached communities consisting of cyanobacteria, algae, bacteria, protozoa, and fungi embedded within a polysaccharide matrix [2]. In rivers, these communities are the first to interact with dissolved substances such as nutrients, organic matter, and toxicants. Biofilms can actively influence the sorption, desorption, and decomposition of pollutants. Fluvial biofilms are relatively simple and easy to investigate compared to other communities (i.e., macroinvertebrate or fish communities).

Biological monitoring programs employed by state and federal agencies to assess effects of contaminants have routinely focused on benthic macroinvertebrate communities. Benthic macroinvertebrates are exposed to contaminants in water, sediment, and biofilm, providing a direct pathway to higher trophic levels. Because of considerable variation in sensitivity among species, community composition and the distribution and abundance of benthic macroinvertebrates are useful measures of ecological integrity.

The main aim of this chapter is to present key studies dealing with priority and emerging pollutants that provided clues to link field observations with causality following a community ecotoxicology approach.

While not being an exhaustive review, 100 different investigations have been analyzed, including field and laboratory investigations, most of them dealing with metals and pesticides (90), but several investigations focused on emerging compounds (10) are also provided (Fig. 1).

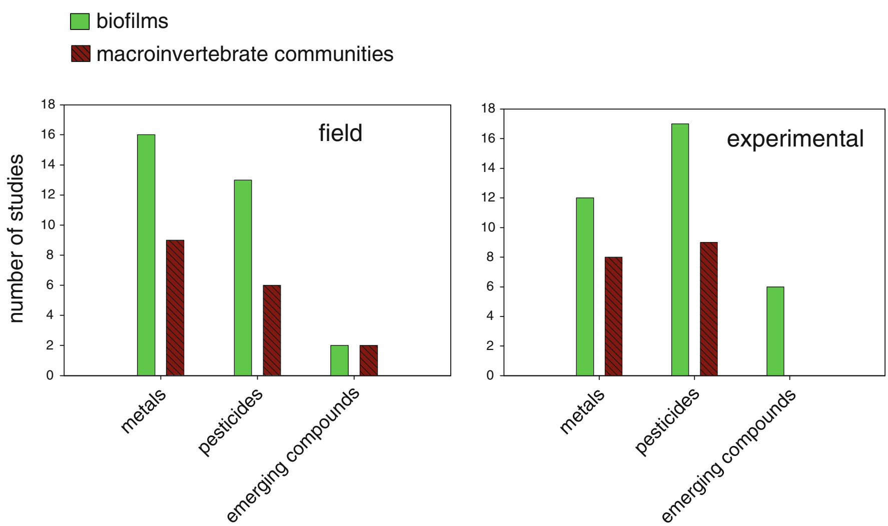

Fig. 1 Summary of field and experimental investigations addressing the effect of toxic exposure on biofilm and macroinvertebrate communities. Studies are grouped on the basis of the type of chemical investigated: metals, pesticides, and emerging compounds

2 Field Investigations Used to Generate Hypotheses

Field investigations, although being the basis of ecotoxicological research, have a high uncertainty linked to environmental variability. Fluvial ecosystems are very dynamic and complex systems, requiring the compilation and statistical analysis of extensive sets of samples and environmental data to characterize the exposure and response of their living organisms. This holistic approach, commonly used in ecology, is less extended in ecotoxicology (see [3] for more details).

Uncertainty might be reduced depending on the scale, type of pollution, and set of response variables investigated (Fig. 2). Large-scale studies are influenced by regional scale variability that may conceal the effects of human impacts. Therefore, human impacts may be better detected in small-scale studies, including reference and impacted situations, within a restricted range of environmental variability [124]. The presence of a specific or dominating type of pollution may also contribute to link causes and effects. The effects of toxic exposure, although being present, are more difficult to detect under multiple-stress situations with many chemical compounds occurring at low concentration and environmental stress factors affecting the biota. Choosing an appropriate set of response variables may allow identifying different types of stress and their ecological consequences (Fig. 2).

The link between chemical pollution and ecosystem damage has been addressed in mining areas (e.g., [4, 5]). Metal pollution in other environments is less

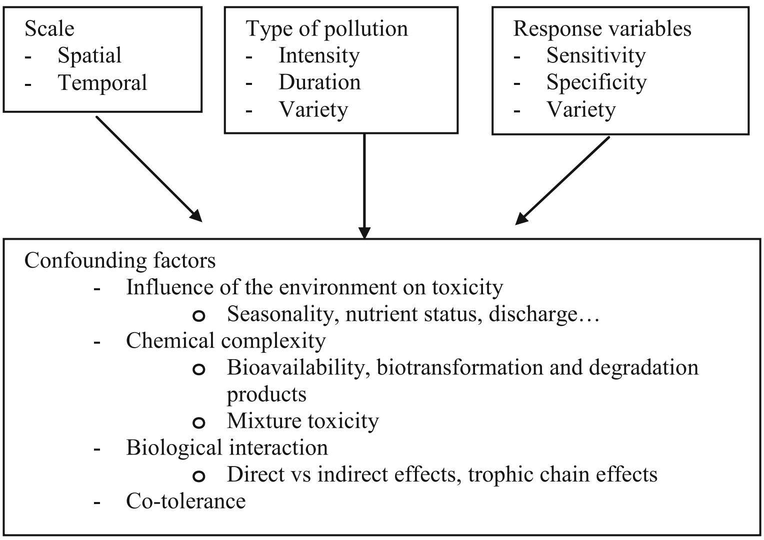

Fig. 2 Scheme showing the main factors influencing the degree of uncertainty in field studies aiming to assess the impact of chemical pollution on natural communities: scale, type of pollution, and set of response variables investigated. Reducing uncertainty will allow to distinguish biological effects caused by toxic exposure from other confounding factors caused by environmental variability, chemical complexity, biological interactions, or co-tolerance

documented. Influences of pesticide pollution or other contaminants received less attention. Emerging pollutants (a group where pharmaceuticals, nanomaterials, the family of perfluorinated compounds, hormones, endocrine disrupting compounds, pesticide degradation products, and newly synthesized pesticides and others are included) are unregulated pollutants, but may be candidates for future regulation depending on research on their potential health effects and monitoring data regarding their occurrence in ecosystems [6]. Their occurrence in aquatic ecosystems has been recently detected and/or their effects on biota recently investigated [7–9].

Many field investigations on metal, pesticides, herbicides, and emerging compounds's toxicity were focused on biofilms, the basis of the food chain, and consumers, mainly macroinvertebrate communities.

2.1 Biofilms

Different types of field studies have been performed to assess the effects of metal and organic contamination on fluvial biofilms (Table 1) such as seasonal monitoring [10], biofilm translocation experiments [11, 12], sampling before and after impact [13, 14], and the study of tolerance induction [15–20].

A key aspect in many ecotoxicological studies is to link water concentration with real exposure conditions due to the variety of factors influencing bioavailabilty and/or the stability of a compound. In the case of biofilms, the analysis of the chemical concentration within the biofilm matrix may contribute to partially overcome this constrain. Evidence of the link between metal exposure (water concentration) and metal contents in biofilms is provided in Behra et al. [21]; Meylan et al. [22]; Le Faucher et al. [23], and also between sediment and biofilm metal concentrations [24] (Table 1), highlighting their possible effects through the trophic chain [25]. Internal concentrations are more difficult to assess and less reported for organic toxic compounds [26].

The link between metal pollution and community changes has been shown in many field studies (see [27] for more details). In particular, the effects of metals on diatom communities have been well documented in mining areas. Hill et al. [13] evaluated the effects of elevated concentrations of metals (Cd, Cu, and Zn) on stream periphyton in the Eagle River, which is a mining impacted river in central Colorado. They found differences in diatom taxa richness, community similarity, biomass, chlorophyll *a* (chl-*a*), and the autotrophic index (AI) that were able to separate metal-contaminated sites from reference or less impacted sites. Sabater [14] evaluated the impact of a mine tailings spill on the diatom communities of the Guadiamar River (SW Spain) showing a clear decrease of diversity (Shannon–Wiener index) and water quality diatom indices. Other variables such as the size of individuals were also attributed to metal pollution in the Riou-Mort [5, 28].

Biomass reduction due to metal toxicity was suggested by Hill et al. [13]; however, these effects were not confirmed in other highly metal-polluted sites

Table 1 Summary of field studies approaching the effects of chemical pollution on fluvial biofilms

Site/sites	Metal/s in water (μg/L) or [a]sediments (μg/g)	Effects	Remarks	Reference
Metals				
High				
Dommel (Belgium)	Zn:6,675; Cd:765 (max)	Bacterial communities in metal-polluted sites more tolerant to Zn Tolerance induction in phototrophs less clear	Zn bioavailability expected to be low due to Fe hydroxide and organic matter	Admiraal et al. [15]
The Coeur d'Alene (Idaho, USA)	[a]As:149; Cd:884; Cu:1,175; Hg:3	Metal contents in polluted sites 10–100× greater than in reference sites Metal concentration of food-web components ranks: biofilm and sediments >invertebrates >whole fish	Bioaccumulated metals may cause physiological effects in indigenous fish	Farag et al. [110]
Boulder River Watershed, Montana (USA)	[a]As:17; Cd:9; Cu:69; Zn:1,800 (max)	Metal concentration of food-web components ranks: biofilm>macroinvertebrates≥sediment>fish tissues>water and colloids, due to entrapment of colloid-bound metals by the biofilm	Metal concentrations higher in low-flow condition	Farag et al. [111]
Guadiamar (after mine tailing spill)	Cd:37 /31[a] Cu:224/1,470[a] Zn:1,080/12,000[a] Pb: 30/1,910[a] As: 1,530/200[a] (max)	Changes in diatom species composition and marked reduction of diversity and diatom indices	Slight signs of recovery 14 months after the spill	Sabater [14]
Dommel and Eindergatloop streams (Belgium)	Cd: 330, Zn: 2,864 in Dommel Cd: 282, Zn: 3,147 in Eindergatloop (max)	Changes in diatom species after translocation to metal-polluted sites	Metal tolerance was not the only selective factor	Ivorra et al. [16]
Churnet and Manifold Rivers (England)	Cu: 3,030[a]; Zn: 4,705[a]; Cd: 53[a]; Pb: 3,236[a]	Biofilm metal accumulation more sensitive than water contents (Cd) Marked seasonal variability but identification of metal-tolerant diatom species	Specific diatoms species highlighted metal-tolerant indicator diatom	Holding et al. [24]
Fifteen rivers across Europe	Zn: 2,263 (max)	Regional difference in Zn tolerance in the biofilm (physical and chemical differences)	Large-scale patterns did not follow PICT prediction	Blanck et al. [30]
Riou-Mort River, France	Zn: 2,305, Cd: 27 (max)	The size distribution of diatoms changing from larger to smaller individuals, changes in the taxonomic composition of the assemblages, higher amounts of abnormal frustules Cd in biofilms: 250–1,200 μg/g	No effects of metal pollution on algal biomass due to high nutrients and organic pollution Metal accumulation and diatoms different between seasons	Morin et al. [28], Duong et al. [5]

(continued)

Table 1 (continued)

Metals

Site/sites	Metal/s in water (μg/L) or [a]sediments (μg/g)	Effects	Remarks	Reference
Moderate				
Eagle River, Colorado	Zn: 800, Cu: 18, Fe: 600	Diatom species composition different in metal-polluted sites; increase of valve deformities; lower chl-*a* and AI	Results in agreement with macroinvertebrate studies	Hill et al. [13]
Catalan (NE Spain) $N = 25$	Cd: 0.8, Cr: 6.6, Cu: 50, Pb: 10, Ni: 80, Zn: 63, As: 10, Hg: 4 (max)	CCU (cumulative Criterion Unit) indicated potential toxicity in many sites. Poor relation between concentration and diatom species composition	Scale effects masked metals effects	Guasch et al. [31]
Low				
Birs and Thur River (Switzerland)	Cu: 0.4–10, Zn: 0.7–37 (ref and metal polluted)	Increases in water Cu and Zn reflected by increased metal contents in biofilm and sediments Biofilm contents 35–173× higher in polluted than i n reference sites	Influence of speciation on adsorption on bioavailability	Behra et al. [21]
Furtbach stream (Switzerland)	Cu: 2.5–7.5 (8.95[a], 16[b] max), Zn: 2.9–9.6 (17[a], 25[b] max).	Dissolved and adsorbed metal concentrations increased steeply during rain events	Rain events influenced the metal speciation, and subsequently the metal accumulation	Meylan et al. [22]; Le Faucher et al. [23]
East Fork Poplar Creek (Oak Ridge, Tennessee)	Cu: 3.1, Cd: 0.25, Zn 25 (max)	In comparison to reference sites: Cu, Zn, and Cd biofilm metal contents were 4, 15, and 50× greater (respectively) in 1995. Increase in chl-*a* over time attributed to metal toxicity reduction	Metal reduction downstream attributed to biofilms retention	Hill et al. [25]
Fluvia River (NE Spain)	Cu: 4.2, Cd: 0.08, Zn: 80, Pb: 0.75 (max)	Water and biofilm metal contents in human-impacted sites 3–15× greater than in reference sites. Biofilm metal contents explained a small part of diatom species composition variance	Biofilm metal contents more sensitive than water contents	Guasch et al. [31]

Pesticides

Site/sites	Toxicants in water (μg/L)	Effects	Remarks	Reference
Morcille River (France)	Diuron and its metabolites (DCPMU and 3,4-DCA)	Individual and combined effects of diuron and DCPMU had both short-term effects and long-term effects on phototrophic biofilms, whereas environmental concentrations of 3,4-DCA did not affect biofilm photosynthetic activity	Diuron was more toxic for algae than its metabolites	Pesce et al. [36]
Ozane River (France)	77 pesticides (atrazine, isorpoturon)	Chronic exposure induced community tolerance to atrazine and isoproturon	PICT was confirmed	Dorigo and Leboulanger et al. (2001) [33]
	Diuron		PICT was confirmed	Pesce et al. [35]

(continued)

Table 1 (continued)

Pesticides

Site/sites	Toxicants in water (μg/L)	Effects	Remarks	Reference
Morcille River (France)		Diuron exposure during biofilm colonization caused an increase in community tolerance with little effects of other environmental variables (nutrients, conductivity, and temperature)		
Avencó River (Spain)	Reference site	Atrazine toxicity on biofilms was influenced by biofilm maturity and light history	Highlights the influence of the environment on toxicity	Guasch et al. [18]
20 rivers across Europe	Pesticides (atrazine) and nutrients	Diatom taxa indicators of different degrees of pollution	Difficulty to separate effects of nutrients from atrazine toxicity	Guasch et al. [19]
7 rivers (Catalunya area, Spain)	Atrazine	Light influenced atrazine toxicity. Biofilms from open-canopy sites were more sensitive to atrazine than biofilms from shaded sites. Differences in the proportion of algal groups and pigment composition were also observed	The influence of environmental light conditions on atrazine toxicity was confirmed	Guasch and Sabater [32]
Ter River (Spain)	Atrazine, Cu	Atrazine and Cu toxicity on biofilms were related to algal abundance and species composition. Seasonal differences in sensitivity were observed	Difficulty to elucidate the causes of the observed temporal and spatial variability in community tolerance	Navarro et al. [17]
Llobregat River (NE Spain)	22 pesticides (mixture)	Organophosphates and phenylureas related with functional and structural changes in biofilms No relationship with the macroinvertebrate community	Biofilms were more sensitive than invertebrates to contamination. Difficult to separate effects of nutrients from phenylureas toxicity [10, 78]. The use of taxonomic distinctness indices recommended for toxicity assessment on algae and benthic invertebrates (Ricciardi et al. 2010)	Ricart et al. [10, 78] Ricciardi et al. (2009)

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Table 1 (continued)

Pesticides

Site/sites	Toxicants in water (μg/L)	Effects	Remarks	Reference
Morcille River (France)	Pesticides	The upstream–downstream gradient of chemical and nutrients pollution caused changes in diatoms species composition and an increase in biofilm biomass. Biofilm from impacted sites had high capacity to recover when they were translocated to non-impacted sites	Shows biofilm capacity to recover their structure if the impact disappears Difficulty to elucidate the causes of the observed changes	Morin et al. [12]
Don Carlos stream, Argentina[a]	Pb: 13, Cu: 23, phthalate: 20	Changes in community composition, increase of abnormal diatom frustules, drop in Net Primary Production and increase of bacterial density	Difficulty to elucidate specific effects of different toxicants	Victoria and Gómez [38]
Elbe river, (Germany)	Prometryn	Prometryn tolerance increased when biofilms were translocated from reference-R to polluted-P sites and decreased when the translocation was opposite (from P to R)	The exposure history of communities defined the time-response of recovery and adaptation	Rotter et al. [11]

Emerging pollutants

Site/sites	Toxicants in water (μg/L) or sediments[a] (μg/g)	Effects	Remarks	Reference
Llobregat River (NE Spain)	Pharmaceutical products: 0.01–18.7	Pharmaceutical products influenced the distribution of the invertebrate community. No effects on diatom community. High concentrations of anti-inflammatories and β-blockers and high temperatures were related to greater abundance and biomass of *Chironomus* spp. and *Tubifex tubifex*	Multivariate analyses revealed a potential causal relation between stressors and effects along gradients and allowed the generation of hypothesis to be tested in laboratory studies	Muñoz et al. [59]
Don Carlos stream (Argentine Pampean plain)	Phthalates (plastiziers) (max: *n*-butyl phthalate: 20[a])	Shift in the composition of the translocated biofilms, presence of abnormal frustules on diatoms, drop in net primary production	Taxonomic and metabolic variables responded differently to translocation	Victoria and Gómez [38]

Water concentration in μg/L

[a]Indicate sediment conc in μg/g

such as the Riou-Mort where metal pollution co-occurs with high levels of nutrients and organic matter (Table 1).

Some investigations reported the induction of metal tolerance after chronic metal exposure [15] using the Pollution Induced Community Tolerance (PICT) approach. Based on the PICT concept, natural communities colonizing different river sites can be used to investigate their exposure history by comparing their physiological responses to a sudden exposure (short-term toxicity tests referred to as PICT tests in this paper) to high doses of the toxicant investigated [29].

Other publications [16] highlight the presence of many selective factors in addition to metal exposure. It may explain the lack of relationship between metal exposure and community tolerance in large-scale studies [30].

Obtaining clear causal relations is difficult in situations of low but chronic metal pollution due to the co-occurrence of many stress factors (Table 1). A low portion of the diatom species composition variance was explained by biofilm metal contents in a small-scale study [31]. Le Faucher et al. [23] found phytochelatines' (PC) production as a response to low metal exposure, but could not identify the specific metal responsible for that.

Most field studies evaluating the effects of pesticides on biofilms were focused on herbicides (atrazine and its residues, diuron and its residues, prometryn or isoproturon) targeting phototrophic organisms (Table 1). Some investigations (Table 1) evaluated the influence of environmental variability (e.g., light conditions, nutrients, maturity, seasonality, temperature, and hydrology) on biofilm's tolerance to pesticides (e.g., [17–20, 32–36]). Other studies investigated the capacity of biofilms to be adapted to sites presenting pesticides' contamination or their capacity to recover from chemical exposure [11, 12]. Monitoring studies aiming to discriminate the effects of pesticides from other stressors are also reported [10, 19]. In the Llobregat River, organophosphates and phenylureas were related with a significant but low (6%) fraction of variance of several biofilm metrics including algal biomass and the photosynthetic efficiency of the community [10]. In the same case study, a significant correlation between the taxonomic distinctness index of diatoms and diuron concentration was obtained [37]. In many other field studies (Table 1), discerning the specific effects of pesticides toxicity on biofilms was complicated by the co-occurrence of pesticide pollution with other types of pollution such as nutrients, organic pollution, or metals (e.g., [12, 19, 38]). On the other hand, the sensitivity of biofilms to pesticides has also been shown to be influenced by biofilm's age [11, 18] or light conditions [32].

In the case of organic pesticides acting as PSII inhibitors, the PICT approach has been demonstrated to be a valuable tool to assess the effects of chronic exposure [29, 39]. For example, Dorigo et al. [34] showed that the response of algal communities is likely to reflect past selection pressures and suggest that the function and structure of a community could be modified by the persistent or repeated presence of atrazine and isoproturon in the natural environment.

Other pesticides or PPs and emerging contaminants have been poorly investigated (Table 1), probably due to their occurrence at low dose in complex mixtures and the lack of sensitive methods applicable to complex field samples [40].

Overall most ecotoxicological field studies have been focused on structural changes, clearly demonstrated under high pollution conditions. Effects on functional attributes (e.g., photosynthesis) are less clear, probably due to functional redundancy between non-impacted communities and those adapted to chemical exposure (see [41] for more details). Besides, the link between moderate toxic exposure and biological damage is less documented. In these cases, a clear increase of chemical concentration in the biofilm matrix is commonly described, mainly in cases of metal pollution; however, the link with persistent effects on the community is, in most cases, less evident. Moreover, almost all the field studies reported point out the influence of environmental variables on biofilm toxicity. In particular biofilm age, nutrients' availability, and light conditions have been shown to influence biofilms' tolerance to various contaminants. This observation highlights the importance of an extensive monitoring, including a multidisciplinary approach to better assess the effects of pollution in complex fluvial ecosystems.

2.2 Macroinvertebrate Communities

Biomonitoring studies conducted with macroinvertebrate communities have been performed to assess effects of metals and organic contaminants on streams (Table 2). Some of these studies have been conducted at relatively large spatial scales [42, 43], allowing investigators to quantify effects of landscape-level characteristics on responses to stressors. These investigators have also developed new approaches based on the biotic ligand model (BLM) to quantify metal bioavailability and effects in the field [43, 44]. Other studies have employed a more traditional longitudinal study design, in which upstream reference sites are compared to downstream contaminated sites [45–47]. Although these single watershed studies are more restricted spatially, they often include investigations that provide a long-term perspective. For example, Clements et al. [47] documented changes in macroinvertebrates and water quality over a 17-year period and related changes in community composition to long-term improvements in water quality.

One of the most consistent observations from these descriptive studies of metals and organic pollution is that certain groups of macroinvertebrates, especially mayflies (Ephemeroptera), are highly sensitive to chemical stressors [42, 45, 48–50]. Interspecific variation in sensitivity to organic and inorganic contaminants forms the basis for the development of new community-level measures to quantify toxicological effects, such as the species at risk (SPEAR) model developed for pesticides and other organics [46, 51]; see also [3]. Recent studies conducted with macroinvertebrates have identified specific morphological and physiological characteristics that are likely responsible for interspecific variation in sensitivity to toxic chemicals [52].

The study of the effects of organic pollutants such as PCBs (polychlorinated biphenyls) and PAHs (polycyclic aromatic hydrocarbons) on macroinvertebrate communities is scarce in the literature, as has been reviewed by Heiskanen and

Table 2 Summary of field studies approaching the effects of chemical pollution on macroinvertebrates

Metals				
Site/sites	Metal/s in water (μg/L)	Effects	Remarks	Reference
Basento River, Italy	As 10–17, Cd 0.3–8, Cr 17–56, Cu 15–156, Pb 10–1,680, Zn 58–1,048	Benthic communities strongly influenced by metals; metal uptake varied significantly among taxa	Uptake of metals from sediment was more important than bioconcentration from water	Sontoro et al. (2009) [125]
Numerous (>150) streams in Colorado, USA	Cd 0.01–8, Cu 0.15–935, Zn 0.25–1,940	Negative effects were observed at concentrations below the chronic toxicity threshold	A new toxic unit model based on a modification of the biotic ligand model was developed to quantify effects	Schmidt et al. [43]
Numerous (412) streams from England, Japan, Scotland, and USA	Numerous metal cations ranging from bd to >100 × toxic concentrations	Threshold relationships established between metal mixtures and species richness of mayflies, stoneflies, and caddisflies	Related concentrations of cationic metallic species and protons to field effects	Stockdale et al. [44]
Numerous (95) sites in Colorado, USA	Cd, Cu, Zn	Heavy metal concentration was the most important predictor of benthic communities across 78 randomly selected sites	Ability to detect effects dependent on the taxonomic resolution; total abundance of mayflies and abundance of heptageniids were better indicators than abundance of dominant mayfly taxa	Clements et al. [42]
River Avoca, Ireland	Cd, Cu, Fe, Pb, Zn (concentrations not reported)	Acid mine drainage (AMD) sites characterized by lower abundance of EPT taxa and increased abundance of chironomids	Considerable variation in sensitivity among macroinvertebrate metrics	Gray and Delaney [45]
Several tributaries to the Hasama River, Japan	Cd 0.003–4.9, Cu 0.12–5.2, Pb 0.1–19, Zn 5–812	Ephemeroptera abundance and diversity were highly sensitive to metals; orthoclad chironomids were positively associated with metals	Biomass of important prey items for drift-feeding fishes was reduced, demonstrating potential indirect effects on predators	Iwasaki et al. [48]
	Cd, Cu, Zn			

Arkansas River, Colorado, USA		Spatial, seasonal, and annual variation of macroinvertebrates associated with metal concentrations	Macroinvertebrates quickly responded to improvements in water quality after remediation	Clements et al. [47]
Ten streams in northern Idaho, USA	As bd-228, Cd bd-3.2, Pb bd-419, Zn 12–523	Elevated levels of Cd and Zn were correlated with lower macroinvertebrate diversity	Community-level effects persisted 75 years after mining cessation	Lefcort et al. (2010) [126]
Several Peruvian streams	Al 13,000, As 3,490, Mn 19,650, Pb 876, Zn 16,080 (8–3,500 × greater at contaminated sites than at reference sites)	Polluted sites dominated by dipterans and coleopterans; reference sites dominated by crustaceans, ephemeropterans, plecopterans, and trichopterans	Unique challenges associated with high altitude streams; despite elevated metals, diverse communities were observed at polluted sites	Loayza-Muro et al. [50]
Pesticides				
Site/sites	Toxicants in water (µg/L)	Effects	Remarks	Reference
Lourens River, South Africa	Organophosphates (azinphos-methyl and chlorpyrifos): bd (below detection)−0.038 total OP (suspended particles)	Particle-associated OPs affected community structure; greatest effects on mayflies and caddisflies	Study linked population dynamics of sensitive taxa to changes in community structure	Bollmohr and Schulz [49]
29 streams from Finland and France	Mixture of pesticides from agriculture activity in adjacent crop fields	Test the hypothesis that community structure and function (leaf-litter breakdown) can be impaired by pesticides	Reduction of sensitive species in the community due to the presence of pesticides and correlation between elimination of sensitive species and diminution of leaf-litter breakdown	Schäfer et al. [112]
19 sites in the Ob River Basin, southwestern Siberia	Mixture of synthetic surfactants, petrochemicals, and nutrients	Test the hypothesis that a community measure of sensitivity to organic contamination ($SPEAR_{organic}$) was independent of natural variation	($SPEAR_{organic}$) was independent of natural longitudinal variation and strongly dependent on organics	Beketov and Liess [46]

(continued)

Pesticides

Site/sites	Toxicants in water (μg/L)	Effects	Remarks	Reference
Several small streams in Finland, France, and Germany	Mixtures of pesticides expressed using a toxic unit approach	Compare the use of species- and family-level data to show pesticide effects using a species at risk ($SPEAR_{pesticide}$) model	Family-level model had adequate explanatory power to quantify pesticide effects across a broad geographical region	Beketov et al. [51]
150 medium-sized mountain rivers in France	PAH, PCB, and metals in sediments	Some species traits are sensitive to the presence of those pollutants	Species traits is a good tool to predict pollution impacts	Archaimbault et al. [54]
Llobregat River, Spain	Pesticides	Abundance and biomass distribution of macroinvertebrates were not explained by the presence of pesticides but by environmental conditions	Different responses of biofilms and invertebrates communities to pesticides	Ricart et al. [10, 78]

Emerging pollutants

Site/sites	Toxicants in water (μg/L)	Effects	Remarks	Reference
Llobregat River, Spain	Several therapeutic families in water	Potential causal association between the concentration of some anti-inflammatories and β-blockers and the abundance and biomass of several benthic invertebrates	Statistical methodologies were useful tools in primary approaches of stressor's effects. Concentrations of pharmaceuticals in rivers are susceptible of generate changes in communities	Muñoz et al [59]
Llobregat River, Spain	Pharmaceuticals	Calculation of hazard quotients for a mixture of these emerging pollutants. Contrast with macroinvertebrate diversity	A clear negative relationship between diversity indices and higher pharmaceutical concentrations	Ginebreda et al. [113]

Solimini [53]. Archaimbault et al. [54] used invertebrates' species traits in order to relate the structure of the community to the presence of PCBs, PAHs, and metals and predict impacted sites by these compounds. As in the case of the SPEAR approach the use of species traits was also useful in order to predict impacted zones. Beasley and Kneale [55] studied the effects of PAHs and heavy metals by multivariate statistical analysis and they found a clear response of the community to the presence of these pollutants with a decrease in diversity.

The effects of pharmaceuticals on aquatic biota have been recently taken into account and have generated concern [56–58]. Despite the existence of experimental evidence of their effects on aquatic invertebrates, the potential effects of these substances on invertebrates' communities have been poorly studied in the field. Muñoz et al. [59] indicated possible relationships between the presence of some families of pharmaceuticals and the shift in the abundance and biomass of different macroinvertebrate species in freshwater communities.

2.3 Summary of the Hypotheses Generated

Differing in the degree of uncertainty, several hypotheses can be derived from the set of community ecotoxicology field investigations reviewed (Table 3).

3 Experimental Studies Searching for Causality

Although biomonitoring studies and other field approaches provide important insights into the effects of contaminants on aquatic ecosystems, these descriptive studies are limited because of their inability to demonstrate cause-and-effect

Table 3 List of hypotheses derived from field community ecotoxicology investigations

Since chemical pollution is first observed as an increased concentration in the biota, further effects on biological integrity are expected
Chemical exposure is expected to cause morphological and physiological alterations such as:
Photosynthesis reduction if PSII inhibitors are present
Morphological and /or physiological characteristics linked to species sensitivity (e.g., macroinvertebrate life traits)
Chemical exposure is expected to affect the community structure in terms of:
Loss in abundance (biomass)
Loss of sensitive species
Loss in species richness
Community adaptation
Environmental and biological factors expected to influence toxicity effects on biofilms are:
Light conditions during growth
Nutrient status
Age of the community (biofilm's thickness)
Emerging pollutant and especially pharmaceuticals (molecules designed to be biologically actives) present in very low concentrations in rivers are expected to provoke sublethal effects on individuals and community changes after long-term exposure

relationships between stressors and ecological responses. Establishing cause-and-effect relationships between stressors and responses is widely regarded as one of the most challenging problems in applied ecology [60]. Because biological assessments of water quality rely almost exclusively on observational data, causal inferences are necessarily weak [61]. In addition, most descriptive studies are unable to identify underlying mechanisms responsible for changes in aquatic communities. Because contaminants often exert complex, indirect effects on aquatic communities, an understanding of underlying mechanisms is often critical. In general, descriptive approaches such as biomonitoring studies provide support for hypotheses rather than direct tests of hypotheses. Equivocal results of biomonitoring studies result from the lack of adequate controls, nonrandom assignment of treatments, and inadequate replication [62].

Various approaches have been developed to address the lack of causal evidence in ecotoxicological investigations. Several investigators have provided useful advice on how to strengthen causal relationships in descriptive studies [63–65]. Weight of evidence approaches, such as the sediment quality triad [66], combines chemical analyses with laboratory toxicity tests and field assessments. These integrated approaches can provide support for the hypothesis that sediment contaminants are responsible for alterations in community structure. Recent approaches developed by the U.S. Environmental Protection Agency (U.S. EPA) employ formal methods of stressor identification, analogous to those used in human epidemiological studies [63], to determine causes of biological impairment in aquatic systems (http://cfpub.epa.gov/caddis/). Hill's [63] nine criteria and modifications of these guidelines [64, 65, 67] have been employed to strengthen causal relationships between stressors and ecological responses:

- Strength of the association between stressors and responses
- Consistency of the association between stressors and responses
- Specificity of responses to contaminants
- Temporal association between stressors and responses
- Plausibility of an underlying mechanistic explanation
- Coherence with our fundamental understanding of stressor characteristics
- Analogous responses are observed to similar classes of stressors
- A gradient of ecological responses are observed as stressor levels increase
- Experimentation support for the relationship between stressor and responses

Perhaps the most important of these nine criteria is the availability of experimental data to support a relationship between stressors and responses. Regardless of the established strength, consistency, specificity, coherence, plausibility, etc., of the relationship between stressors and responses, there is no substitute for experimentation to demonstrate causality. Experimental approaches provide an opportunity to demonstrate causal relationships between stressors and ecologically relevant responses across levels of biological organization. These experiments are a practical alternative to single-species toxicity tests and address the statistical problems associated with field biomonitoring studies. The maturity of a science such as ecotoxicology is often defined by the transition from purely descriptive to manipulative approaches. The ability to test hypotheses with ecologically realistic experiments represents a major shift in the quality of the questions that can be addressed [68].

Microcosm and mesocosm experiments are often designed to manipulate single or multiple environmental variables, providing opportunities to quantify stressor interactions and identify underlying mechanisms. It is the ability of an investigator to manipulate and isolate individual factors that makes the application of microcosm and mesocosm experiments particularly powerful in ecotoxicological research. Microcosm and mesocosm experiments address two of the key limitations associated with environmental assessment of contaminant effects: the lack of ecological realism associated with traditional laboratory experiments and the inability to demonstrate causation using biomonitoring. In addition, these experimental approaches provide the opportunity to investigate potential interactions among stressors.

Because of the limited spatiotemporal scale, measuring certain responses in microcosm and mesocosm experiments presents significant challenges. The duration of microcosm and mesocosm experiments is of critical importance when assessing effects of contaminants. An important consideration in the development of microcosm and mesocosm approaches is whether greater statistical power and ability to demonstrate causation outweigh their limited spatiotemporal scale. Thus, combining these experimental approaches with results of field studies conducted at a larger spatiotemporal scale is the most reliable way to demonstrate causation.

3.1 Experimental Studies Addressing Cause-and-Effect Relations Between Toxicant Exposure and Biofilm Communities Responses

Different experimental designs have been used to investigate, under controlled conditions, the effects of toxic substances on biofilm communities (Table 4). The scale of exposure ranges from hours to weeks, either continuous (the most common experimental design) or pulsed. After controlled toxic exposure, the PICT approach is often used to assess differences in sensitivity at community level with the same procedure applied to natural biofilms (e.g., see Sect. 2.1).

Many authors have investigated metal and pesticides' effects in microcosms and mesocosms by adding toxicants to natural water (Table 4). This experimental approach was used to investigate the single effect of a toxicant, the effect of mixtures, the bioaccumulation (only for metals), the tolerance and co-tolerance induction (using the PICT approach), and also the interaction between toxicity and environmental and biological factors such as nutrients, light conditions (including UV radiation), or grazing (Table 4). Effects of the most commonly reported metals in the environment: Cu, Zn, Ni, Ag, Cu, Pb, and Cd were investigated at environmental realistic concentrations. As in the field studies reported above, most of the

Table 4 Experimental studies addressing cause–effect relations between toxic exposure and their effects on biofilms

Metals

Hypothesis	Experimental design			Conclusions and remarks	References
	Conditions	Toxicant (µg/L)	Duration		
Bioaccumulation (bioac) driven by speciation and the presence of ligands	Growth in lab, river water (Glatt River)	Cu:16, Zn: 33 (max)	24 h	Zn bioac controlled by Zn^{2+}. Cu-bioac controlled by weakly complexed Cu due to very low free Cu^{2+} in natural environments	Meylan et al. [81]
Chronic Cu exposure increases community tolerance to Cu and co-tolerance to other metals	Outdoor glass aquaria system	Cu: 317 (max)	16 weeks	Hypothesis confirmed with changes in the community composition (taxonomic changes) and co-tolerance to Zn, Ni, and Ag	Soldo and Behra [85]
Biofilm maturity influences their sensitivity to metals	Growth in river and exposure in indoor channels	Cd: 100	4 and 6 weeks	Cd exposure affected the whole biofilm and diatom assemblages' structure. Mature biofilm less affected	Duong et al. [83]
The biofilm structure offers protection to the communities which live embedded	Laboratory-grown monospecific biofilms[a]. Growth on artificial subtrata in the Meuse river[a] and in indoor aquaria[b]	Cu: 0 < 445 < 953 < 1,905 and 572[b] (max)	7 and 24 h[a] Chronic[b]	Physical structure of the biofilm and not the species composition influenced Cu toxicity during short- and long-term exposures. Cu reduced P uptake	Barranguet et al [114[a], 115[b]]
EPS production as metal tolerance mechanisms in metal-adapted communities	Aliquota from Valencia mine (Mexico) used to colonize artificial substrata in the lab	Cu: 636–6,350, Zn: 654–65,270	1 and 5 days	Metal exposure increased the EPS production and the immobilization of metals. Metals' effects on biomass and the relative abundance of the dominant taxa	Garcia-Meza et al. [88]
Zn (400 µg/L) causes structural and functional damage	Indoor microcosms. Biofilms exposed to Zn and Zn+Cd	Zn: 400, Cd: 20	5 weeks	The effects of the metal mixture could be attributed to Zn toxicity. Photosynthesis was inhibited during the first hours of exposure, and algal growth, mainly diatoms, decreased after 2 weeks of exposure	Corcoll et al. [116]
Zn exposure reduces P-availability	Growth in Gota Alv river	Zn: from 3 to 2,000	4 weeks	Low levels of Zn affected biomass production in biofilms by inducing phosphorus depletion	Paulsson et al. [82]
Phosphate reduces metal toxicity (Zn and Cd)	Growth in the field and exposure in indoor aquaria	Zn: 1,000, Cd: 64, P: 284	3 weeks	Hypothesis confirmed for each metal alone but not for the combination. Sensitivity also influenced by the species composition and their autoecology	Ivorra et al. [84]

Nutrient (P) concentration reduces Cu toxicity	Growth in river and exposure in indoor channels[ab]. Growth in aquaria using algal inocula from the river[c]	Cu: 15[a], 4.2[b] (max in the river), 30[c]	12 days[ab] 3 weeks series[c]	Hypothesis confirmed. The effect of P on Cu toxicity changed depending on the parameter[c]. Biofilms' results confirmed with algal monocultures[b]. Highlights the importance of using a range of parameters that target all biofilm components	Guasch et al. [117] Serra et al. [89], Tlili et al. [39]
UV radiation influences Cd toxicity	Indoor microcosms	Cd: 225–6,800	Long term	UVR: biomass reduction, change in species composition, and an increase in the ratio of UVR-absorbing compounds/physical structure of the biofilm and not the species composition *a*. Induced community tolerance to high-UVR and co-tolerance to Cd	Navarro et al. [90]

Pesticides

Hypothesis	Experimental design			Conclusions and remarks	Reference
	Conditions	Toxicant (µg/L)	Duration		
Isoproturon produces alterations on the diatom species composition	Indoor microcosm system based on large water tanks containing: sediment, macrophytes, mollusks, and periphyton	Isoproturon:5 and 20 Isoproturon in sediment: 100 and 400 µg/kg	34–71 days	Isoproturon produced a reduction of diatoms density. After 71 days, periphyton was adapted to isoproturon exposure by dominant heterotrophic organisms and smaller diatoms species	Pérès et al [75]
Oxyfluorfen exposure produces oxidative stress in biofilms and reduce photosynthetic efficiency, chla	Growth in artificial indoor streams	Oxyfluorfen: 0 < 3<7.5< 15<30<75<150	40 days	Hypotheses not confirmed. At the end of exposure, biofilms pre-exposed to high concentrations of oxyfluorfen presented a higher capacity to answer to oxidative stress (catalase) linked with changes in algal community (RNA 18S).	Bonnineau PhD
Linuron causes direct and indirect effects in a simple freshwater trophic chain	Outdoor microcosms containing water, phytoplankton, biofilms, zooplankton	Linuron: 15–500	2–8 weeks	Linuron affected the community composition causing a decrease in the amount of algal species digestive for zooplankton altering the energy transfer in the studied trophic chain	Daam et al. [118]

(continued)

Pesticides

Hypothesis	Experimental design			Conclusions and remarks	Reference
	Conditions	Toxicant (μg/L)	Duration		
Diuron produces direct and indirect effects on biofilms.	Growth and exposure in artificial streams	Diuron: 0.07–7	29 days	Hypothesis confirmed. Diuron produced direct inhibitory effects on algae (photosynthesis inhibition, reduction of biovolume diatoms, and increase of chl-*a*) but also indirect effects on bacteria	Ricart et al. [73]
Long-term exposure to isoproturon, atrazine and prometryn increases community tolerance (PICT	Indoor aquaria	Isoproturon: 2.4–3,120 Atrazine: 7.5–2,000 Prometryn: 2.5–320	10–129 days PICT tests: 1 h	Hypothesis confirmed but the sensitivity of biofilms decreased with increasing age and biomass, respectively	Schmitt-Jansen and Altenburger [87, 119–121]
Long-term diuron exposure increases community tolerance (PICT)	Growth in microcosms from aliquota from Mulde river (Germany)[a] or from Morcille river (France)[b]	Diuron: 0.4–100[a] Chronic: 1[b] Pulses: 7 and 14[b]	3–12 weeks[a] 28 days[b] PICT tests: 1h[a], 3h[b]	PICT confirmed[ab]. The tolerance increased by a factor of 2–3 (based on EC_{50} values)[a]. PICT enhanced after pulse exposure[b]	McClellan et al. [86] Tlili et al [69]
Phosphate influences the diuron-induced community tolerance (PICT)	Growth in aquaria from an aliquota from the Morcille river (France)	Diuron: 10	3 weeks PICT tests: 2–3 h	PICT confirmed but the phosphate gradient affected the response of biofilm to diuron. For PICT approaches it is necessary to dissociate the real impact of toxicants from environmental factors	Tlili et al. [39]
Roundup exposure has an effect on biofilm colonization in "clear" and "turbid" waters	Outdoor mesocosms: "clear" (aquatic macrophytes/ metaphyton) "turbid" (phytoplankton/suspended inorganic matter)	Glyphosate (Roundup®): 8,000	42 days	Hypothesis confirmed. Roundup exposure provoked a delay in biofilm colonization, an increase in eutrophication (due to Roundup degradation), and a shift from"clear" to "turbid" mescosms	Vera et al. (2010) [127]
The exposure duration influences herbicides toxicity towards biofilms and their recovery potential	Growth in Esrum Mollea strem (Denmark). Exposure in 10 mL vials	Isoproturon, metribuzin: $0 < 0.4 < 2 < 10 < 50 < 250 < 1{,}250$ Pendimethalin, hexazinone: $0 < 0.4 < 2 < 10 < 50$	1 h and 24 h for all 2, 6, 18, 23, and 48 h for metribuzin	Hypothesis confirmed for isoproturon, metribuzin, and pendimethalin. Isoproturon and metribuzin toxicity increased throughout exposure time. Pendimethalin hysteresis effect only visible after 1–2 h of exposure. No effect of exposure duration on biofilm recovery from metribuzin exposure	Gustavson et al. [122]

Hypothesis	Conditions	Toxicant (µg/L)	Duration	Conclusions and remarks	Reference
Grazing enhances atrazine toxicity	Growth in mesocosms	Atrazine: 14 µg/L	18 days	Grazing increased atrazine toxicity on biofilms and affected the metabolism and structure of the algal community	Muñoz et al. [71]
Grazing enhances diuron toxicity to biofilms	Growth in artificial channels	Diuron: 2	29 days	Hypothesis not confirmed. Bacterial survival and photosynthetic activity were affected by diuron but no interaction between toxicant exposure and grazers	López-Doval et al. [74]
Variation in light intensity, in comparison to constant light influences the sensitivity of phototrophic biofilms to isoproturon	Growth in Sensée river (France). Exposure in laboratory under constant or dynamic light conditions	Isoproturon: 0–2,000 (constant light) 2.6–20 (dynamic light)	7 h	A dynamic light regime increased biofilm sensitivity to isoproturon by challenging its photoprotective mechanisms such as the xanthophyll cycle because both stressors (isoproturon and light) affected the photosynthetic activity	Laviale et al. [70]
Light history modulates biofilm response to herbicides exposure	Growth in artificial indoor streams under different light conditions Exposure in 10 mL vials	Glyphosate: 10–1,000,000 AMPA: 10–500,000 Oxyfluorfen: 1.5–1,000 Cu: 20–2,000	6 h	Hypothesis confirmed for glyphosate. Co-tolerance between high-light intensity and glyphosate in terms of photosynthetic efficiency. No effect of light history on oxyfluorfen (increase in ascorbate peroxidase activity) and Cu (decrease in protein content) toxicity	Bonnineau PhD

Emerging pollutants

Hypothesis	Experimental design			Conclusions and remarks	Reference
	Conditions	Toxicant (µg/L)	Duration		
Impacts of pharmaceuticals and personal care products depend on their pre-exposure history	Growth of natural biofilms upstream and downstream of a WWTP. Exposure in 20-mL glass screw-capped test tubes	Triclosan: 0.012–1.2 Ciprofloxacin: 0.015–1.5 Tergitol NP10: 0.005–0.50	13 days	Hypothesis not confirmed. All three compounds caused marked shifts in the community structure at both the upstream and downstream sites and a decline in algal genus richness (at high concentration)	Wilson et al. [79]
Pharmaceuticals at environmental relevant concentrations alter the structure and activity of fluvial biofilms	Growth and exposure in rotating annular bioreactors	Ibuprofen, carbamazepine, furosemide, caffeine: 10	8 weeks	Hypothesis confirmed for the algal and bacterial component of the biofilm. Pharmaceuticals exhibited both nutrient-like and toxic effects on fluvial biofilms	Lawrence et al. [77]

(continued)

Emerging pollutants

Hypothesis	Experimental design			Conclusions and remarks	Reference
	Conditions	Toxicant (μg/L)	Duration		
Triclosan (TCS) bioavailability is lower in biofilm-associated cell than in suspended ones	Growth on glass discs. Exposure in standard test vessels	TCS: 270–17,300	24 h	Biofilm-associated diatoms were less affected (in terms of photosynthetic efficiency) than suspended cells. Differences in sensitivity could not be attributed to the life form, suggesting that TCS may address multiple target sites	Franz et al. [76]
TCS and triclocarban (TCC) cause effects on the structure and function of river biofilms	Growth in rotating annular bioreactors	TCS, TCC: 10	8 weeks	TCS and TCC affected community structure, architecture, and functioning of the biofilm communities. TCS also produced changes in bacterial community	Lawrence et al. [123]
TCS directly affects the bacterial community (due to the mode of action), while effects on algae are indirect	Growth and exposure in a flow-through system of indoor channels	TCS: 0.05–500	48 h	TCS toxicity was higher for bacteria than algae. The increase in bacterial mortality can be attributed to a direct mode of action of triclosan. Direct effects on algae cannot be disregarded	Ricart et al. [78]
3 β-blockers have toxic effects on fluvial biofilms	Growth from river inocula in crystallizing dishes	Propranolol: 0–531 Metoprolol: 0–522 Atenolol: 0–707,000	24 h	Hypothesis confirmed. Toxicity: Propanolol (photosynthesis inhibition) > metroprolol (bacterial mortality) > atenolol (affected both the algal and bacterial components)	Bonnineau et al. [80]

pesticides studied were herbicides (atrazine, diuron and its residues, isoproturon, prometryn) targeting photosynthetic processes and hence the sensitivity of the phototrophic component of biofilms to these compounds (Table 4).

Investigating pesticides' effects at different temporal scales from hours, days [69, 70], to weeks [39, 71–74] or months [75] allowed to assess the physiological and structural biofilm responses. Herbicides' indirect effects on invertebrates [71, 74] or bacterial communities [73] were also evaluated. Among the long list of the so-called emerging pollutants that are commonly found in environmental samples, few pharmaceuticals and personal care products were selected to investigate their potential effects on biofilms (Table 4), the bactericide triclosan (TCS) being the most investigated compound [76–79]. These studies addressed the protective role of biofilms and the effects of TCS on structural and functional attributes of their heterotrophic and autotrophic component. Short-term effects of three similarly acting β-blockers were investigated by Bonnineau et al [80] using a multibiomarker approach (Table 4).

Overall, the experiments reported tested an important number of hypotheses on toxicant availability, their effects on biofilm communities, and the potential modulating factors (Fig. 3). It was shown that metal bioaccumulation was driven by speciation and the presence of ligands [81]. Negative effects on growth were also reported (Table 3) as a reduction in biomass production (i.e., [82]), diatoms density [75, 83], and in diatoms biovolume [73]. It was also shown that prolonged exposure to a toxic compound alters the community composition causing a selection of tolerant species in most cases (e.g., [83, 84]), an increase in the tolerance of the community in many cases (e.g., [85–87]), and the production of EPS in some occasions [88]. Other investigations also demonstrated that these community responses were modulated by different environmental and biological factors (Fig. 3).

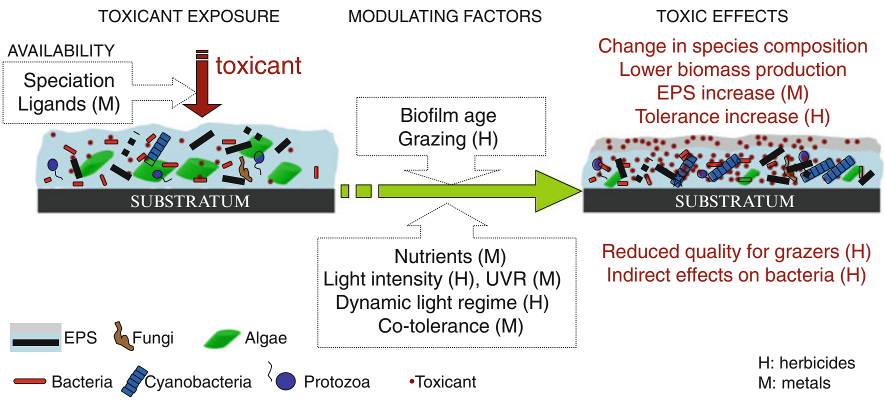

Fig. 3 Figure summarizing experimentally tested hypotheses on (1) toxicants' availability; (2) the biological and environmental factors modulating toxicity; and (3) the toxic effects after prolonged exposure of biofilm communities to different types of toxicants. M indicates hypotheses mainly tested with toxic metals; H for those tested with herbicides. No label for those tested for at least a metal, an herbicide, and an emerging compound

That biofilm's maturity and/or thickness may reduce toxicity is a general rule demonstrated for metals (i.e., [83]), herbicides (e.g., [87]), and emerging compounds such as TCS [76]. On the other hand, increased herbicide toxicity was demonstrated under grazing pressure [71], linked with the observation that grazing was responsible for maintaining the biofilm younger (Fig. 3).

Nutrient concentration may also influence the effects of toxic exposure (Fig. 3). It was shown for toxic compounds like Zn or Cu directly affecting nutrients availability (e.g., [89]).

Light history as well as light conditions during toxicant exposure affected toxicity. It was shown by the increase in sensitivity to Cd observed in biofilms adapted to UVR [90] or the increase in toxicity of isoproturon under a dynamic light regime [70]. In the case of Cu, co-tolerance to other metals was also demonstrated [85].

The toxicity of three similarly acting β-blockers was confirmed, but at very high concentration. In this study, differences in toxicity between the three compounds highlight the need to increase ecological realisms in toxicity testing used to derive environmental quality standards [80].

3.2 Experimental Studies Addressing Cause-and-Effect Relations Between Toxicant Exposure and Macroinvertebrates

Experimental studies have been conducted in aquatic microcosms and mesocosms to examine the effects of heavy metals, pesticides, and other organic chemicals on the structure and function of macroinvertebrate communities (Table 5). Although the duration of most studies was relatively short (1–4 weeks), the duration of several mesocosm and field experiments was considerable longer [91–93]. Endpoints measured in these studies included responses across several levels of organization, from physiological effects to alterations in community structure and ecosystem function. Some of these studies were conducted to provide additional support for field studies, thereby strengthening arguments for causation [60], while others were conducted to verify results of laboratory toxicity tests [46, 94]. Several studies were conducted to identify direct sources of toxicological effects in systems receiving multiple stressors [95, 96] or to quantify the influence of natural habitat characteristics on contaminant effects [97, 98]. Also experiments to examine competition/predation interactions between populations after exposure to pollutants have been developed [99]. A common goal of many experiments was to examine interactions among stressors [91] or to quantify the potential cost of tolerance associated with contaminant acclimation or adaptation [100–102]. A consistent justification for the application of these ecologically realistic approaches in risk assessment was to integrate responses across multiple levels of biological organization [93, 103].

Table 5 Experimental studies addressing cause–effect relations between toxic exposure and their effects on macroinvertebrate communities

Metals					
Hypothesis	Experimental design			Conclusions and remarks	Reference
	Conditions	Toxicant	Duration		
Field experiments to assess the influence of water quality and substratum quality on benthic macroinvertebrate communities	Colonization of clean and metal-contaminated substrate		30 days	Water was acutely toxic to most taxa; however, responses to metals in contaminated biofilms were species-specific	Courtney and Clements [95]
Microcosm experiments conducted to support results of field studies	Communities transferred from field to microcosms	Zn alone, Zn + Cd, and Zn + Cu + Cd	10 days	Experiments confirmed predicted "safe" concentrations of metals; some evidence of synergistic effects due to metal mixtures	Clements [60]
Seasonal variation in metal effects	Communities transferred from field to microcosms	Cd, Cu, Zn	7 days	Effects of metals greater in summer because populations dominated by smaller, early instars	Clark and Clements (2006) [128]
Cost associated with increased tolerance to metals	Communities transferred from reference and metal-contaminated sites to microcosms	Cd, Cu, Zn	10 days	Communities from metal-contaminated sites were tolerant of metals but more susceptible to other stressors (predation, acidification, UV-B radiation)	Clements [100], Courtney and Clements [101], Kashian et al. [102]
Heavy metals and UV-B radiation interact to structure communities	Field experiment excluded UV-B; microcosm experiment quantified UV-B effects on reference and contaminated communities	UV-B + metals (Cd, Cu, Zn)	Field: 60 days microcosm: 7 days	Effects of UV-B treatments are consistently greater in metal-contaminated streams compared to reference streams	Zuellig et al. [91]
Separate relative effects of acidity and metals in acid mine drainage (AMD)	Measured colonization of AMD-contaminated substrate in the field	Acidity and metals	5 weeks	Most toxicity associated with acidity leaching from sediment rather than metals	Dsa et al. [96]

Pesticides					
Hypothesis	Experimental design			Conclusions and remarks	Reference
	Conditions	Toxicant (μg/L)	Duration		
Determine no observed effect concentrations for several structural and functional measures	Small, synthetic microcosms seeded with zooplankton, phytoplankton, and snails from a pond	Chlorpyrifos, carbendazim, and linuron	2–3 weeks	Responses and effect similar to those observed in larger systems; absence of sediment and macrophytes accounted for greater persistence of pesticides in these systems	Daam and Van den Brink [97]

(continued)

Pesticides

Hypothesis	Experimental design			Conclusions and remarks	Reference
	Conditions	Toxicant (μg/L)	Duration		
Quantify the drift responses of benthic invertebrates	Mayflies, blackflies and amphipods exposed to pesticides in 1.2 m glass channels	11 different pesticides	48 h	Drift initiated by 6 of 11 pesticides at levels 7–22 times lower than LC_{50} values; greatest effects from neurotoxic insecticides	Beketov and Liess [46]
Quantify effects of a single pulse of insecticide	Mesocosm study designed to compare community LOEC with LC_{50} for individual organism	Thiacloprid	7 months	Long-term community LOEC comparable to LC_{50} for sensitive species; rate of recovery more dependent on life history characteristics than pesticide concentrations	Beketov et al. [92]
Demonstrate causal relationship between pesticide and responses	Field experiment conducted in tributary of the River Arrow, UK	Cypermerthrin, chlorpyrifos	20 months	Effects of pesticides mitigated by no-spray buffer zones; individual-level effects occurred at low concentrations, but were not translated to populations or communities	Maltby and Hills [93]
Compared structural and functional measures	Outdoor stream channels with 4 species of benthic invertebrates	Pyrethroid	10 d	No effects on macroinvertebrate density; greater algal biomass and reduced decomposition in treated mesocosms	Rasmussen et al. (2008) [129]
	Field deployed stream mesocosms containing natural benthic communities	Imidacloprid	20 days	Significant effects on macroinvertebrate abundance and diversity; demonstrated the importance of integrating studies of individual species with model mesocosm studies	Pestana et al. [103]
Measure effects of a mixture of insecticide and herbicide	Indoor microcosms exposed to a range of concentrations	Atrazine and lindane	14 weeks	Direct and indirect effects observed; macroinvertebrate community was affected at all but the lowest levels (0.01 TU)	Van den Brink et al. [94]

Test direct and indirect effects on community structure	Outdoor microcosms treated after 6 weeks of initial colonization	Carbendazim	8 weeks	Both direct toxic effects and indirect effects due to reduced competition and altered food chain	Daam et al. (2010) [130]
A mixture of pesticide produces effects on the ecology of aquatic microcosms	Indoor freshwater plankton-dominated microcosms	Atrazine: 55 Lindane: 28	28 days	Lindane seriously affected macroinvertebrate community. Atrazine produced fewer effects than expected, probably due to decreased grazer stress on the algae as a result of the lindane application	Van den Brink et al. [94]
Test changes in species composition and in competence/predation relations	Experimental pond	Atrazine and endosulfan	4 weeks	The pesticides changed the intensity in the competitive or predatory interactions	Rohr and Crumrine [99]
Test if refuge zones are important in the recuperation of communities from short-term exposure to insecticide	Experimental ditches	Lufenuron	161 days	Existence of refuge zones and species' vital traits are important factors enhancing recovery	Brock et al. [98]

3.3 Integrating Descriptive and Experimental Approaches to Demonstrate Causation in Stream Bioassessment Studies: Case Study of a Metal-Polluted Stream

To demonstrate how descriptive and experimental approaches can be integrated to demonstrate causation, we present results of a case study conducted in a metal-polluted stream in Colorado, USA. A long-term monitoring program of water quality and benthic macroinvertebrates was initiated in the Arkansas River in 1989 [47]. Concentrations of heavy metals (Cd, Cu, and Zn) are greatly elevated downstream, and often exceed acutely toxic levels. Over the past 17 years, we measured physicochemical characteristics, habitat quality, heavy metal concentrations, and macroinvertebrate community structure seasonally (spring and fall) at locations upstream and downstream from several sources of metal contamination. Three years after we began this research, a large-scale restoration program was initiated to reduce metal concentrations and reestablish trout populations in the upper Arkansas River basin. Because these data were collected before and after remediation, this long-term research provided a unique opportunity to quantify ecological responses to improvements in water quality. For the purposes of this case study, we present macroinvertebrate and metals data collected from one upstream and one downstream station collected from 1989 to 2006.

To support this descriptive study, microcosm experiments were conducted to develop concentration–response relationships between heavy metals and measures of macroinvertebrate community structure. Benthic macroinvertebrate communities for these experiments were obtained from an uncontaminated reference stream with no history of metal contamination using a technique previously described [104]. Communities were transferred to stream microcosms and exposed to combinations of Cu and Zn at concentrations that bracketed those measured at metal-contaminated sites in the field. Because these experiments involved a mixture of heavy metals, an additive measure of toxicity was used to express metal concentrations relative to the U.S. EPA chronic criterion values. The cumulative criterion unit (CCU) was defined as the ratio of the measured metal concentration to the hardness-adjusted criterion value and summed for each metal.

Significant concentration–response relationships were developed for total abundance and species richness in stream microcosms (Fig. 4). Total macroinvertebrate abundance was more sensitive to metals than species richness, a finding previously reported from stream microcosm experiments [105]. The LC_{20} concentrations for macroinvertebrate abundance and species richness, defined as the CCU levels that caused a 20% reduction, were approximately 2.3 and 9.0, respectively. These experimental data support the hypothesis that macroinvertebrate communities were highly sensitive to heavy metals and that relatively low concentrations resulted in significant alterations in community composition.

Heavy metal concentrations in the Arkansas River were seasonally variable, but decreased significantly after the remediation program was initiated (Fig. 5). Metal

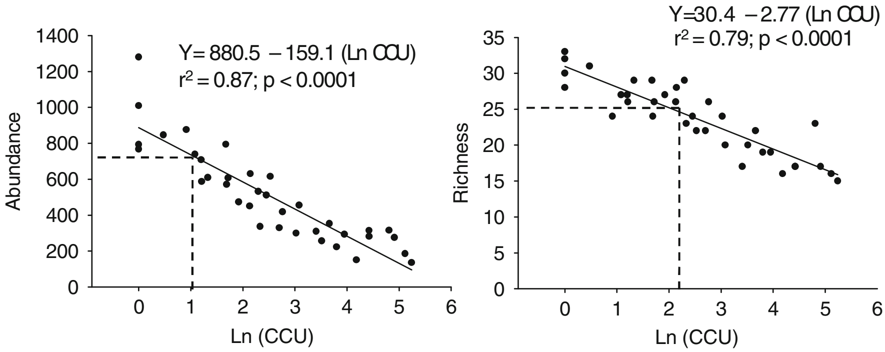

Fig. 4 Results of microcosm experiments showing concentration–response relationships between metal concentration (CCU), total macroinvertebrate abundance, and species richness. *Dashed lines* show the estimated LC_{20} concentrations for each metric

concentrations at station EF5 rapidly decreased below the estimated EC_{20} values in 1992. In contrast, metal concentrations at station AR3 remained elevated until about 1999, fluctuated between the estimated EC_{20} values for several years, and then decreased below these levels in 2003.

Macroinvertebrate communities in the Arkansas River responded to these improvements in water quality after remediation (Fig. 5). Macroinvertebrate communities at station EF5 quickly recovered after metal levels were reduced below the EC_{20} values. Abundance and species richness recovered more slowly at station AR3, consistent with expectations based on long-term changes in metal concentrations and estimated EC_{20} values for these metrics. Total macroinvertebrate abundance recovered in approximately 2003, whereas species richness recovered in 2000.

Although the biomonitoring results from the Arkansas River were consistent with the hypothesis that heavy metals are responsible for reduced abundance and richness, these data are not sufficient to demonstrate causation. Long-term improvements in water quality and the associated increases in abundance and species richness after remediation represent a natural experiment that allowed a more rigorous test of this hypothesis. Finally, highly significant concentration–response relationships between macroinvertebrate community metrics and heavy metal concentration from stream microcosm experiments provided estimates of metal levels that likely impact benthic communities. The consistency of these LC_{20} estimates with concentrations measured in the field where abundance and richness recovered greatly strengthened the argument that heavy metals were responsible for alterations in benthic communities. These results demonstrate the importance of employing ecologically realistic experimental techniques to support descriptive studies for developing causal arguments.

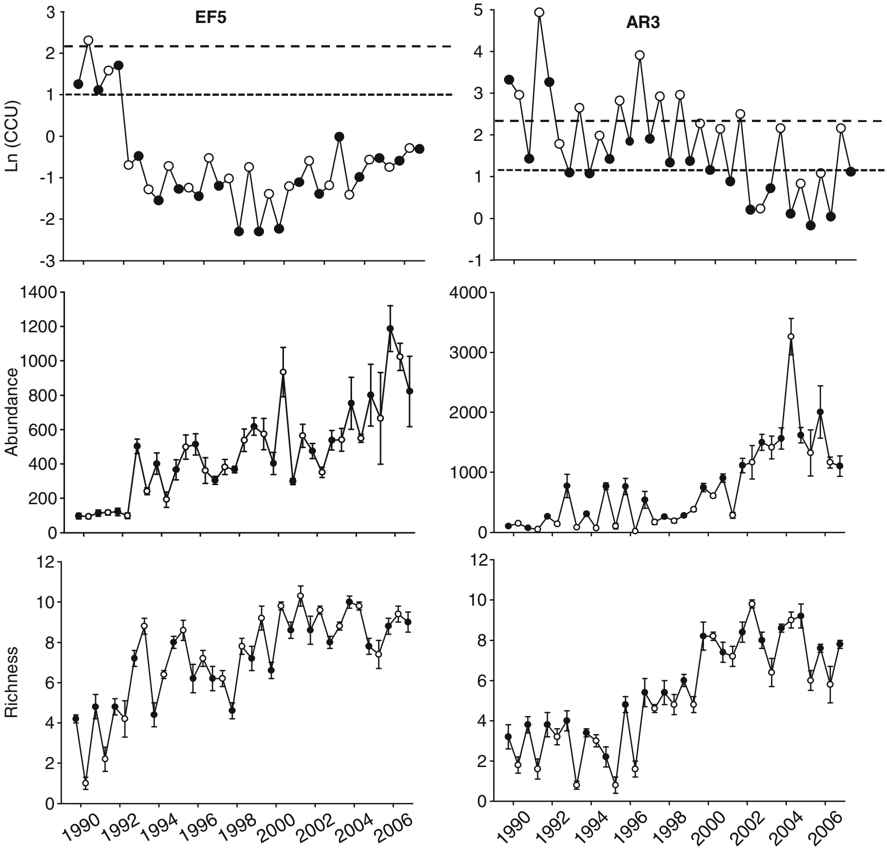

Fig. 5 Long-term changes in metal concentration (CCU), abundance, and species richness at Arkansas River stations EF5 and AR3. *Horizontal lines* correspond to the EC20 values for species richness (*dashed*) and abundance (*dotted*) estimated from microcosm experiments. Alternating *solid* and *open* symbols refer to samples collected in spring and fall, respectively. Macroinvertebrate data are means ±s.e.

4 General Discussion and Prospects

The papers found in the literature show how field and laboratory investigations evolved from the end of the 1990s, when community ecotoxicology studies focused on metals and pesticides began, to present days, when the first emerging compounds investigations have appeared (Fig. 1). To date, quite a lot of information has been generated about the effects of emerging pollutants (mixtures or simple compounds) on single organisms but community approaches are very scarce, highlighting the existence of a missing gap requiring future investigations.

The set of studies presented in this chapter illustrate how field and laboratory investigations complement to provide causality between toxicant exposure in running waters and benthic communities' responses. As a general scheme (Fig. 6), hypotheses based on field observations cannot be confirmed without experimentation and thus needed to provide causal evidence. If the formulated hypothesis is not confirmed, new field observations may be required and the results should be analyzed again in order to evaluate the role of different environmental factors (confounding factors) influencing the biological responses observed. The newly generated hypothesis should also be experimentally tested including possible interactions by simulating multiple-stress situations (Fig. 6).

Many of the hypotheses derived from field investigations have been validated following this general scheme. The Arkansas River case study is a good example showing how field observations, together with long-term natural experiments and microcosm experiments, provide consistent arguments and evidence of metals' effects on the biota. More precisely, metal pollution levels were responsible for reduced abundance and taxa richness of the macroinvertebrate community. Long-term monitoring studies following temporal trends in both chemical pollution and the biological responses are very informative, but unfortunately very scarce. In the case of biofilms, microcosm and mesocosm experiments confirmed that metals and pesticides are responsible for the loss of sensitive species in the community, and that this influence is modulated by several biological and environmental factors including the successional stage (biofilms age), the trophic status of the river or stream (nutrient concentration), and the light regime (whether is it an open canopy or shaded reach). These concluding findings should be included, in the future, in

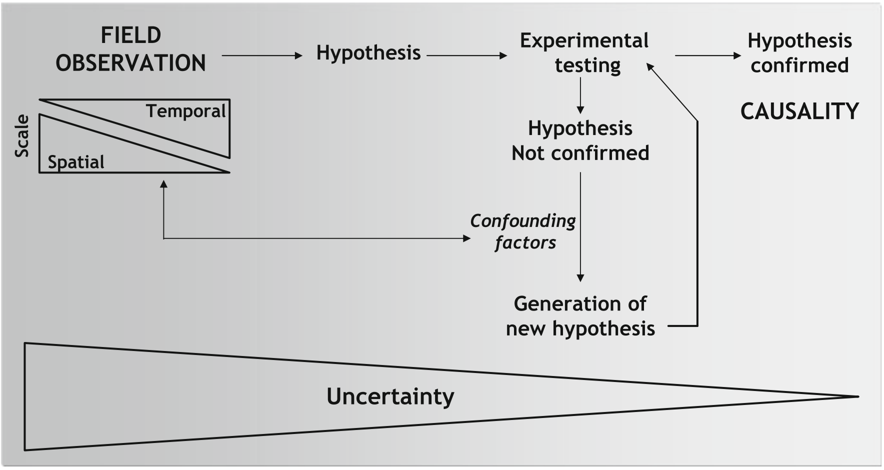

Fig. 6 Graph illustrating the steps required to derive causality. Hypothesis formulated on the basis of field observations should be experimentally tested for confirmation. If the formulated hypothesis is not confirmed, new field observations may be required and the results analyzed again in order to evaluate the role of different environmental factors (confounding factors) influencing the biological responses observed. The newly generated hypothesis should also be experimentally tested including possible interactions simulating multiple-stress situations

risk assessment models in order to account for the influence that the environment exerts on the effects of chemicals on the biota.

In contrast to the results obtained with metals and pesticides already included in the list of priority pollutants, emerging pollutants and especially pharmaceuticals (molecules designed to be biologically actives) are not expected to cause changes on natural communities easily to detect. Given that the levels reported in rivers for these substances in waters and sediments are generally low, no lethal effects on the species are expected at concentrations found in the environment [56, 106]; hence, new approaches should be used in order to know the effects of these substances on natural communities. Experimental investigations on communities are difficult because of the long-term studies required and because of the inconspicuous endpoints that need to be studied, whereas in field studies the difficulties in predicting the effects of emerging pollutants and changes in community arise from the nature of the effects caused by these substances, such as feminization or changes in behavior or emergence time, not studied enough in invertebrates in wildlife. Effects in communities can be detected by examining other mechanisms than the direct effect in species' density (like in the case of pesticides) such as sublethal or long-term effects on physiology, on reproductive traits or hormone-mediated processes (Endocrine Disrupting Compounds as explained in Lagadic et al. [107] and Soin and Smagghe [108]), or on other less obvious traits such as behavior [109].

Overall, the examples provided in this chapter, together with the recommendations given, are proposed as a general guide for studies aiming to link chemical pollution with ecological alterations. The proposed approach, although being complex and probably expensive in terms of dedication, is strongly recommended for investigative monitoring, situations where routine monitoring may fail in the detection of the causes accounting for a poor ecological status.

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