



# Salmon & Trout Conservation

## Beyond lethal toxicity: Sublethal effects of chemical inputs on the ecology of freshwater systems

### LITERATURE REVIEW

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## I. Introduction

The diversity and industrial production of chemicals has reached an extent that even the most remote areas on the earth's surface are affected (Gessner and Tlili, 2016). More than 300,000 inventoried/regulated chemicals are currently used in industry, household and agriculture (Acuña *et al.*, 2015). Statistics published by EUROSTAT in 2013 revealed that, between 2002 and 2011, over 50% of the total production of chemicals was represented by environmentally harmful compounds, with over 70% of them having significant environmental impacts (Gavrilescu *et al.*, 2015).

Rivers are net receivers of chemical stressors from anthropogenic origins. The composition of chemical inputs differs according to prevailing land uses around river catchments. Extensive agriculture, industrial activities and human conurbations all contribute different chemical mixtures to watercourses (Sabater *et al.*, 2016). The two main categories of chemical contaminants problematic for watercourses are:

**Pesticides:** Mainly originate from agricultural activities and enter aquatic environments through diffuse sources via runoff (Proia *et al.*, 2013). These chemicals can cause serious problems for freshwater biota as they are designed with the sole intention to kill organisms and are released into the natural environment intentionally (Hanazato, 2001). Often pesticides are non-specific, so harm other organisms as well as the intended target.

**Pharmaceuticals:** Point discharges from wastewater treatment plants are recognised as the dominant route by which pharmaceuticals enter the aquatic environment (Kay *et al.*, 2017). Concentrations of pharmaceuticals in rivers are usually lower than those of pesticides. However, their relevance is related to the chronic character of their input (Proia *et al.*, 2013)

Chemical pollution is one of the main causes of degradation and biodiversity loss in aquatic ecosystems (Vörösmarty *et al.*, 2010). Exposure can have adverse biological consequences on river biota; interactions may be lethal, where exposure causes direct mortality, or sublethal, where physiological pathways and natural behaviours are disrupted. Such interactions can have broader impacts on river systems as a whole, by altering food-web structure and ecosystem functioning (Thompson *et al.*, 2016). To properly understand and effectively manage chemicals in the environment, it is essential to consider these ecosystem-level consequences, looking beyond traditional, species-based lethal effects. However, evaluating impacts of sublethal exposure concentrations at these levels is complex and confounded by other environmental pressures (Hamilton *et al.*, 2016)

This review will explore the following topics:

- Sublethal effects of chemical inputs on invertebrates and fish and the broader ecosystem consequences as a result of these effects.
- The future of management and monitoring around chemicals in watercourses, given the importance of considering sublethal and additive chemical effects.

## II. Sublethal effects on invertebrates

Aquatic invertebrates are particularly susceptible to chemical perturbations, and peak pesticide concentrations can affect species communities on sublethal endpoints such as growth, emergence times, feeding and drift behaviour which ultimately may translate into altered community structure and function (Cedergreen and Rasmussen, 2017). Organisms that are slow to develop, like mayfly species, may be at higher risk than more rapidly developing groups due to chemical exposure from multiple pulses over the course of their development (Alexander, Heard and Culp, 2007)

### Drift

Downstream drift is a common response of lotic macroinvertebrates to various stress factors, including chemical disturbances. Most likely, aquatic organisms perform downstream drift behavior during chemical exposure in order to avoid high concentrations and to reach areas with lower exposure levels (Schulz and Liess, 1999). However, there is debate as to whether drift is an active behaviour reflecting increased activity or a consequence of direct toxicity-induced immobility (Lauridsen and Friberg, 2005). Increased rates of drift can cause significant changes in lotic community structure, as resident invertebrates are lost downstream. Knowledge about which toxicants can initiate drift, and at what concentrations, is limited, and the ecotoxicological consequences of drift are only known to a minor extent.

In the natural environment, chemical pulses are typically associated with sudden increases in flow velocity, water turbidity, and changes in water temperature. As these confounding factors have drift-inducing potential themselves, they must be disentangled from the pesticide effect to exactly quantify chemical impact (Berghahn *et al.*, 2012). Drift reactions of stream invertebrates can also vary significantly depending on the type of chemical, time of exposure and even taxonomic group. Drift initiating behaviour has been demonstrated in response to a wide range of chemicals, including but not limited to the following:

**Pyrethroids:** (Muirhead-Thomson, 1978b) exposed various invertebrate species to 30 minute pulses of permethrin at 0.5 and 5 µg/l. *Gammarus*, *Baetis* and *Simulium* showed a considerable degree of activation and detachment leading to downstream drift during the 30 minutes, whereas *Hydropsyche* and *Brachycentrus* showed little sign of drift induction. In contrast, (Breneman and Pontasch, 1994) found *Hydropsyche*, along with *Baetis* were the insects most frequently entering drift after exposure to 0.01 and 0.1 µg/l treatments of fenvalerate. However, this exposure was continuous and not pulsed like the previous study, meaning the results represent worst-case conditions. Another pulse experiment was conducted by (Lauridsen and Friberg, 2005), using one hour pulses of lambda-cyhalothrin at concentrations of 0.001, 0.01, 0.1, and 1.0 µg/l. *Gammarus pulex*, *Baetis rhodani* and *Leuctra fusca/digitata* all responded with catastrophic drift. For *G. pulex* this was significant at 0.001 µg/l, but for *B. rhodani* and *L. fusca/digitata* the drift response threshold was 0.01 µg/l.

**Neonicotinoids:** (Beketov and Liess, 2008) reported increased downstream drift of *G. pulex*, *Simulium latigonium* and *B. rhodani* in a stream microcosm within 2-4 hours of exposure to thiacloprid, imidacloprid and acetamiprid.

However, (Berghahn *et al.*, 2012) found that after 12 hour 12 g/L pulses of imidacloprid, drift occurred in *Gammarus roeselii* at concentrations 2.5-times lower than the lowest concentration tested for *G. pulex* by (Beketov and Liess, 2008).

**Organochlorine and organophosphate insecticides:** (Muirhead-Thomson, 1978a) found that short, sublethal exposures to the organophosphorus insecticides temephos and chlorpyrifos methyl readily activated drift in *G. pulex* and to a lesser extent *B. rhodani*. For invertebrates exposed to doses of endosulfan prebound to river sediment, (Hose *et al.*, 2002) found the lowest observed effect concentration for macroinvertebrate drift was 6.14 µg/l. Abundances of the mayfly *Jappa kutera* were significantly greater in drift samples at this treatment concentration. As *J. kutera* is a burrowing mayfly, it has a high degree of contact with sediment so may have had greater exposure to the contaminant than other species.

#### Feeding, growth & emergence

Sublethal toxicant levels also alter the energy budget of exposed organisms, diverting energy from growth and development. For mayflies, this can result in earlier emergence, at smaller body sizes. This is a result of a trade-off between suboptimal reproduction (e.g. fewer eggs) and survival as adults (Peckarsky *et al.*, 2001). (Alexander, Heard and Culp, 2007) found that 12 hour pulses of imidacloprid as low as 0.1 µg/l reduced head length in *Baetis* and thorax length in *Epeorus*. All impacts on survivorship and body size were found in males, which has negative implications for reproductive success in these species. Smaller head size can mean reduced eye size, which has been shown to reduce territory size and mate capture success in other insect orders (Rutowski, 2000). (Jarvis, Bernot and Bernot, 2014) also found a reduction in body size in nymphs of the Heptagenid mayfly *Stenonema* after exposure to the antiepileptic drug carbamazepine. Exposure to 2,000 ng/l of carbamazepine resulted in decreased mass in adult males and decreased length in adult females. Additionally, exposed individuals were found to experience an altered molt cycle, which hindered their development.

Pulses of imidacloprid greater than 0.5 µg/l over 24 hours were also shown to impair mayfly feeding, with feeding rates remaining low even four days after exposure (Alexander *et al.*, 2007). Because mayflies only feed as larvae, reduced consumption of foodstuffs can result in hampered larval development, reduced emergence, and smaller adult imagoes (Hatakeyama, Shiraishi and Uno, 1997). However, (Beketov and Liess, 2005) found evidence indicating that impacts on fecundity caused by sublethal exposure to chemicals could be compensated for by a greater food resource. Contamination with esfenvalerate at 0.1 and 0.01 µg/l, combined with low food availability resulted in significantly less egg production by *Cloeon dipterum*. But, in medium and high food availability no significant effect on egg production was observed. (Palmquist, Jepson and Jenkins, 2008) also demonstrated reduced fecundity (a significant decrease in egg weight as a percentage of total body weight) from 0.05, 0.1 and 0.2 µg/l pulse exposures of the same chemical to the caddisfly *Brachycentrus americanus*.

There is a wide range of evidence that shows shredding macroinvertebrates may reduce their consumption of litter following continuous exposure to different toxicants. Macroinvertebrate mediated leaf litter decomposition is the basis of heterotrophic food webs, where shredder species make energy that is bound in allochthonous organic matter available to local and downstream communities (Zubrod *et al.*, 2014). Reduced detrital processing can have ecosystem-level consequences through a decline in organic matter decomposition and thus food availability.

Changes in feeding rate may also change community structure by altering inter-species competition between shredder species (Whitehurst, 1991). Reduced activity, which may indicate reduced feeding performance, was demonstrated in *G. pulex* exposed to low concentrations (10–100 ng/l) of the pharmaceuticals fluoxetine and ibuprofen (De Lange *et al.*, 2006). Similar effects on locomotion and feeding were also found by (Castro-CatalÃ *et al.*, 2017) after 100 ng/l exposure of *G. pulex* to fluoxetine. Conversely, (Jonsson *et al.*, 2015) found that the antihistamine fexofenadine had no effect on leaf litter decomposition via impacts on larvae of the stonefly *Protonemura meyeri*. Despite no effect being observed, high levels of bioconcentration in the nymphs were identified, reinforcing that in the environment surrounding water concentrations are not always reflective of the chemical burden organisms may be experiencing.

For pesticides, (Flores *et al.*, 2014) showed a reduction in shredding performance of *Echinogammarus berilloni* following 0.5 µg/l exposure to the organophosphate diazinon. (Wieczorek *et al.*, 2018) also found reduced shredding in *Asellus aquaticus* following exposure to the pyrethroid etofenprox (0.04 µg/l) - a 44% decrease in feeding rate was observed. However, for pulse exposures, which is typically how aquatic organisms are exposed to pesticides in the environment, information regarding the effects on shredding invertebrates is scarce. (Nyman *et al.*, 2013) demonstrated that feeding of *G. pulex* was heavily inhibited by imidacloprid under a low constant treatment (15 mg/l) but feeding was not affected by repeated pulses of exposure.

### III. Sublethal effects on fish

Freshwater fishes are among the most threatened group of vertebrates worldwide (Hamilton *et al.*, 2016). Fish are exposed to chemical contaminants from the surrounding water, sediments, food and/or via maternal transfer. Consumption of contaminated prey items may also potentially be an important route of exposure to wild fish (Brodin *et al.*, 2014).

Most of the current understanding surrounding chemical impacts on fish is based on laboratory experiments, with fish kept in optimal conditions. These studies provide much needed insight into chemical effects, but are not completely representative of the wild environment. Chemicals may exert effects on fish that are difficult to measure in the laboratory, yet could be important for wild fish already subjected to other stressors such as habitat degradation (eutrophication, acidification and sedimentation), altered hydrology (dams, flow regulation and abstraction), introduction of non-native species and transfer of diseases and pathogens (Cowx and Portocarrero-Aya, 2011).

#### Reproduction & Development

Sublethal chemical effects on reproductive biology are numerous and varied, impacts can include: Intersex, testicular and ovarian histological damage, delayed gonad maturation and impairment of the vitellogenesis process (Mondal, Karmakar and Haque, 2015). Chronic effects from the presence of endocrine disrupting pharmaceuticals in the aquatic environment have been widely studied. Steroids have been the presumed causative agent in many studies that have documented effects on fish reproduction and gonadal development, since natural (17β-estradiol) or synthetic (17α-ethynylestradiol - EE2) estrogens are commonly present in wastewater effluents (Galus *et al.*,

2013). Male roach (*Rutilus rutilus*) living downstream of major UK wastewater treatment plants showed high occurrences (16–100%) of intersexuality. Additionally, elevated blood levels of the female egg yolk protein, vitellogenin, were observed in male roach, at levels significantly higher than those of males from upstream and control sites (Jobling *et al.*, 1998). Increased plasma vitellogenin was also recorded in male wild brown trout (*Salmo trutta*) downstream of a major wastewater treatment works in Ireland (Tarrant *et al.*, 2009). (Ankley *et al.*, 2003) demonstrated a sublethal impact of 17- $\beta$ -trenbolone (0.027  $\mu\text{g/l}$ , a concentration measured in run-off), a metabolite of a potent androgen used to increase muscle growth in cattle, on the fecundity of fathead minnows (*Pimephales promelas*). Female vitellogenin and plasma steroid concentrations were significantly reduced. Masculinisation of females also occurred, females produced dorsal (nuptial) tubercles, structures normally present only on the heads of mature males. (Parrott and Blunt, 2005) found a similar impact in the same species; exposure to EE2 at concentrations less than 1 ng/l decreased male sex characteristics and reduced egg fertilisation success.

A variety of studies also indicate impacts on reproductive biology, that are not likely to be mediated by steroid receptors. *D. rerio* exposed to a combination of chlorpyrifos and cypermethrin exhibited minimal to mild multifocal follicular atresia in the ovaries (Rajini, Revathy and Selvam, 2015). (Galus *et al.*, 2013) exposed *D. rerio* to dilutions of treated wastewater and a pharmaceutical mixture of acetaminophen, carbamazepine, gemfibrozil and venlafaxine. Both treatments showed a significant decline in embryo production and elevated oocyte atresia in the ovaries of females. Follicular atresia during the normal course of reproduction can potentially limit the number of eggs supported for vitellogenesis, as well as hindering maturation and ovulation in female fish. Ovaries of adult female common carp (*Cyprinus carpio*) also exhibited tissue damage when exposed to sublethal levels of dichlorvos (Mir *et al.*, 2012).

Reduced or retarded growth is a common result of pesticide exposure in fish (Heath *et al.*, 1997). Smaller fish can experience reduced competitive ability, increased rates of predation and decreased mating success. (Gormley and Teather, 2003) found that Japanese medaka (*Oryzias latipes*) eggs exposed to sublethal concentrations of endosulfan produced smaller fry than controls. (David and Pancharatna, 2009) exposed *D. rerio* embryos to paracetamol, which also resulted in larvae with lower body masses and length.

Juvenile anadromous fish undergo a transition from parr to smolt during their downstream migrations. This transition, known as 'smoltification', is orchestrated by hormones and prepares juvenile salmonids for entry into seawater (Russell *et al.*, 2012). Exposure to sublethal concentrations of chemicals in freshwater can have adverse biological consequences on smolt quality and readiness for life at sea (Moore *et al.*, 2003). Gill Na<sup>+</sup>/K<sup>+</sup> ATPase activity increase is one of the major physiological processes that allows salmon to adapt to saltwater environments. Activity of the enzyme increases two to five-fold during smoltification and signifies an increase in the ability of the fish to osmoregulate in saltwater. Disruption to this pathway has been demonstrated for both pharmaceuticals and pesticides. Exposure to the natural oestrogen 17 $\beta$ -estradiol and the oestrogen-mimicking compound 4-nonylphenol were found to cause a significant decline in salinity tolerance in Atlantic salmon (*S. salar*) smolts (Madsen, Mathiesen and Korsgaard, 1997; Madsen *et al.*, 2004). These findings were corroborated by (McCormick *et al.*, 2005) who also found lowered salinity tolerance in smolts. (Waring and Moore, 2004) found that exposures of 2, 5 and 10  $\mu\text{g/l}$  atrazine caused a significant reduction in Na<sup>+</sup>/K<sup>+</sup> ATPase activity. However, (Moore *et al.*, 2008) determined that exposure to a concentration of atrazine as little as 0.1  $\mu\text{g/l}$  over a 72-h period significantly reduced

gill Na<sup>+</sup>/K<sup>+</sup> ATPase activity. Contrastingly, (Matsumoto, Hosmer and Van Der Kraak, 2010) did not detect a significant effect on gill Na<sup>+</sup>/K<sup>+</sup> ATPase activity in salmon smolts after short term exposure of up to 100 µg atrazine/l.

Contaminants can also cause physiological deformities that hinder swimming ability. *C. carpio* exposed to sublethal levels of chlorpyrifos exhibited caudal bending, which greatly retarded the normal swimming pattern (Halappa and Muniswamy, 2009). Caudal bending was also identified as the prime morphological malformation in *Labeo rohita* exposed to another organophosphate pesticide (malathion) by (Patil and Muniswamy, 2010).

### Behaviour

At the level of the individual, sublethal toxicant effects are manifested in several ways, but among the most sensitive indicators of pollution stress are behavioural alterations (Patil and Muniswamy, 2010). Many pharmaceuticals are designed specifically to alter behaviour. These are particularly concerning as modifications of fish behaviour can influence aquatic community compositions and, consequently, the functioning of aquatic systems over time (Brodin *et al.*, 2013). Naturally, fish are active with well coordinated movements and respond instantly to the slightest disturbance. However, in a chemical contaminated environment these responses can be suppressed or changed (Mondal, Karmakar and Haque, 2015).

Depression in appetite is a common response of fish to stress and intermittence of feeding for longer periods can have a detrimental impact on growth and reproduction. Larger fish are more likely to avoid predation and are more fecund than smaller individuals, indicating that inhibition of growth through reduced feeding would likely have important population-level consequences (Floyd, Geist and Werner, 2008). (Floyd, Geist and Werner, 2008) observed inhibited feeding activity in *P. promelas* larvae after 1 day of exposure to sublethal concentrations of esfenvalerate. Conversely, wild European perch (*Perca fluviatilis*) exposed to the pharmaceutical benzodiazepine, at an environmentally relevant concentration (1.8 µg/l) found in waters receiving sewage effluents, had a higher feeding rate (Brodin *et al.*, 2013). Increased boldness and activity were also observed, which may explain why enhanced feeding took place.

Swimming behaviour of fish is frequently assessed as a response in toxicity investigations because altered locomotor activity can indicate effects to the nervous system (Brewer *et al.*, 2001). Changes in swimming behaviour as a result of exposure to sublethal pollutants can affect the ability of fish to feed, to flee from predators or even to reproduce (Barbieri, 2007). This is particularly important in the context of salmonids, as reduced swimming and feeding can cause a reduction in somatic growth of juvenile salmon, which is a critical determinant of freshwater and marine survival (Beamish, Mahnken and Neville, 2004). Pesticides can cause acute toxicity to neural transmission primarily through cholinesterase inhibition, which in turn can cause increased acetylcholine in synapses and potentially result in downregulation of muscarinic cholinergic receptors. This can impair multiple functions, including behaviour (Pavlov, Chuiko and Gerasimov, 1992). Changes in swimming speed and distance were significantly correlated with changes in cholinesterase activity in larval rainbow trout (*Oncorhynchus mykiss*), following exposures to sublethal concentrations of two organophosphate pesticides (Beauvais *et al.*, 2000). Research by (Sandahl *et al.*, 2005) found a close relationship between brain acetylcholinesterase inhibition and behavioural impairment in juvenile coho salmon (*Oncorhynchus kisutch*) exposed to chlorpyrifos. Brain

acetylcholinesterase inhibition and reductions in spontaneous swimming were significantly correlated. Similarly, chlorpyrifos has been shown to significantly inhibit acetylcholinesterase in the nervous system and muscle of juvenile steelhead trout (Sandahl and Jenkins, 2002).

Chemicals can also interfere with migratory behaviour, through previously mentioned changes in swimming speeds or changes in the abilities of fish to detect environmental cues. Chemoreception is an important contributor allowing fish to respond to their environment, and olfaction is believed to be the predominant chemical sense in fish (Scott and Sloman, 2004). Work by (Sandahl *et al.*, 2004) indicated sublethal excitotoxicity (where nerve cells are damaged or killed by excessive stimulation by neurotransmitters) to juvenile coho salmon central networks after exposure to 0.2 µg/L of the pyrethroid esfenvalerate. Although a loss in sensory function in response to natural odorants did not occur, the natural odorant L-Serine triggered bursts of postsynaptic activity in the olfactory bulb. Interference with olfactory function has the potential to inhibit olfactory-mediated behaviours crucial for the survival and migration of salmonids.

Inadequate detection and response to predators during early life-stages can compromise growth to reproductive age, in turn, eliminating reproductive potential (McGee *et al.*, 2009). (Painter *et al.*, 2009) exposed both embryonic and larval stages of fathead minnows (*P. promelas*) to environmentally relevant (ng/l) concentrations of several common antidepressants (fluoxetine, sertraline, venlafaxine, and bupropion) individually and in combination. The mixture of all four antidepressants was found to slow predator avoidance behaviours in larval fathead minnows regardless of the exposure window. Adverse effects on escape responses were also found by (McGee *et al.*, 2009), where minnows exposed to a sublethal mixture of estrone, 17β-estradiol, and 17α-ethinylestradiol showed a reduced ability to perform innate C-start escape behaviour, which is essential to rapidly move away from an approaching threat.

## VI. Recommendations

Current monitoring of priority substance-based chemical status according to the Water Framework Directive (WFD) covers only a tiny fraction of toxic risks, extensively ignores mixture effects and lacks incentives and guidance for abatement. While European water bodies are contaminated with complex mixtures of ten thousands of chemicals, chemical status is defined on the basis of 45 substances with little insight into links between chemicals and ecosystem effects (Brack *et al.*, 2018). Currently, assessments of chemical status are based on concentrations of a selection of priority pollutants, sampled at discrete points in time, and their comparison to concentrations considered safe for the environment (environmental quality standards, EQS) (Tlili *et al.*, 2016). Comprehensive toxicological data for most chemicals, especially for chronic exposures, are missing, even for chemicals with high usage rates that are ubiquitous in aquatic environments (Hamilton *et al.*, 2016). For pharmaceuticals there is a striking contrast between the number of pharmaceuticals produced and those that have an environmental quality standard (EQS) (Acuña *et al.*, 2015). This is a concerning gap that should be addressed.

Where concentration boundaries are set, chronic effects are often not taken into account due to a lack of available data on sublethal impacts on an appropriate range of species. For some chemicals the standard toxicity testing



organism *D. magna* is remarkably insensitive, so may not be the best indicator of sublethal/chronic effects (Beketov and Liess, 2008). It is widely accepted that riverfly and *Gammarus* species are particularly sensitive to water quality disturbances, therefore using these organisms more in sublethal toxicity testing seems logical (Gerhardt, Bloor and Lloyd Mills, 2011; Firmiano *et al.*, 2017). The link between new scientific research and regulation is also relatively disjointed. Many EQS values are not reflective of the most recent scientific findings and when new data on sublethal impacts are obtained, intuitive mechanisms for it to be fed into the EQS system are not in place. Currently, there is a clear 'reactive' rather than 'proactive' behaviour when it comes to managing chemical impacts in the environment which needs to change (Acuña *et al.*, 2015).

So far, most risk-assessment studies have focused on uptake of pharmaceuticals in organisms as a function of water concentrations, where additional uptake via consumption of exposed prey that, in themselves, bioconcentrate pharmaceutical substances is overlooked. (Brodin *et al.*, 2014) demonstrated that nearly 50% of a psychiatric drug in ingested prey accumulated in a predatory fish (*Perca fluviatilis*). This indicates that investigations of exposure through bioconcentration, where trophic interactions and subsequent bioaccumulation of exposed individuals are ignored, may be underestimating exposure. There is also potential for pharmaceuticals which increase feeding rates to trigger a positive feedback loop between behavioural change and bioaccumulation, as individuals exhibiting higher feeding rates are exposed to increasing levels of the pharmaceutical. Understanding bioaccumulative pathways of chemical exposure is essential to truly quantify the contaminant burden aquatic wildlife are being subjected to and manage chemicals accordingly.

Freshwater organisms experiencing chemical stress are also likely to be experiencing additional stresses such as habitat degradation, nutrient pollution and flow stress at the same time. Currently, these stressors are evaluated separately, and monitoring for chemicals and other water quality parameters is not integrated. This separation is detrimental to management as environmental stressors are often strongly interlinked. For example, flow stress is particularly important to consider as concentrations of chemicals in watercourses are related to dilution capacities of the receiving river. Rivers with low dilution capacity, such as those subjected to water abstraction pressures, will be under greater chemical pressure than high dilution capacity rivers receiving an equivalent chemical discharge (Petrovic *et al.*, 2011). Habitat quality is also a crucial element that can buffer or magnify the toxicity of contaminants; (Liess and Von Der Ohe, 2005) demonstrated that biological impairment observed at sites with high concentrations of pesticides and good habitat quality (uncontaminated upstream sections) was similar to those at sites where pesticide concentrations were low but habitat quality was poor. To grasp impacts of toxicants at ecosystem level, it is fundamental that better connections are made between physical, chemical and biological sampling regimes.

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