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Impacts and management of sublethal chemical exposure on freshwater invertebrates and fish

LITERATURE REVIEW

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 ecosystem-level consequences, looking beyond traditional, species-based lethal effects.

Evaluating impacts of sublethal exposure concentrations at these levels is complex and confounded by other environmental pressures (Hamilton *et al.*, 2016). The presence of chemicals in the aquatic environment as mixtures adds to this complexity. It is possible for chemical compounds to act independently (where toxicity is best estimated by addition of organism response), synergistically (toxicity has a greater-than-additive effect) or antagonistically (toxicity has a less-than-additive effect) on nontarget aquatic biota (Raby *et al.*, 2019). These mixture impacts are poorly understood and not addressed within the current chemical regulatory framework.

This review will cover the following topics:

- Sublethal effects of chemical inputs on invertebrates and fish and the broader ecosystem consequences as a result of these effects.
- Issues with, and recommendations for, the management and monitoring of chemicals in watercourses.

II. Sublethal effects on invertebrates

Aquatic invertebrates are particularly susceptible to chemical perturbations. Exposure can affect a variety of physiological and behavioural processes 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). This section provides examples from the scientific literature of how aquatic invertebrates are affected by sublethal chemical exposures. Issues with basing current chemical regulations on these toxicity studies are also highlighted.

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. Knowing which toxicants 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.

In the natural environment, aquatic organisms are more likely to be exposed to chemicals in the environment as pulses, rather than constant concentrations. Despite this, a large proportion of toxicity studies are based on lab experiments where a chosen organism receives continual exposure of a chemical at a designated concentration. For example, (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. Although low and potentially environmentally relevant concentrations were used, exposure like this is often representative of worst-case conditions and not necessarily a suitable proxy for impact in nature. Currently many environmental safety thresholds are based solely on the responses of single organisms in lab studies like this.

Pulse experiments are a much closer representation of how aquatic invertebrates experience chemical exposure. (Muirhead-Thomson, 1978) 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. (Lauridsen and Friberg, 2005) also conducted a pulse experiment, 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.

Field studies are the closest scientists can get to recreating a true to life response. However, there are associated implications with introducing chemicals into the environment for study. Unlike a laboratory experiment, containment of introduced chemicals is extremely difficult and can result in unintended ecological deterioration to the system. A possible option to overcome this is to use microcosm experiments. (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. Another option is to choose experimental sites where chemical inputs are known. (Wurzel *et al.*, 2020) examined invertebrate drift in response to incidental incursions of permethrin, from spraying adjacent to a watercourse. Despite in-river concentrations of permethrin remaining below the detection limit (<0.25 µg/l), an 11 fold increase in the density of drifting aquatic insects immediately after application was observed. An increase in drifting aquatic invertebrates was also found as far as 2.25 km downstream from the treatment.

Mode of exposure is also important to consider. For invertebrates that mainly inhabit the hyporheic zone, exposure to chemicals bound to sediment may be more relevant than concentrations in the water column. 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. Chemical legacies in sediment are currently not considered in the existing regulatory framework.

Reproduction & development

Sublethal toxicant levels can alter the energy budget of exposed organisms, diverting energy from growth and development (Muller, Nisbet and Berkley, 2010).

For some invertebrate species, sublethal chemical exposure has been demonstrated to result in emergence and body size alterations. (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) observed a similar response in *Stenonema* nymphs 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. (Barmentlo *et al.*, 2019) observed emergence reductions of up to 39%-65% in damselfly (*Ischnura elegans*) nymphs exposed to environmentally relevant concentrations (0.1 µg/l - 10 µg/l) of the neonicotinoid thiacloprid. This experiment was conducted both in a lab and in naturally colonised experimental ditches. The effect was more substantial in the natural population than the laboratory set up. This is further support for the inappropriateness of using solely lab tests to set species toxicity thresholds and base regulatory chemical standards.

After exposing the caddisfly *Brachycentrus americanus* to 0.05, 0.1 and 0.2 µg/l pulse exposures of esfenvalerate, (Palmquist, Jepson and Jenkins, 2008) demonstrated a significant decrease in egg weight as a percentage of total body weight. (Beketov and Liess, 2005) found that for *Cloeon dipterum,* this effect could be mitigated with an increased food resource. Contamination with esfenvalerate at 0.1 and 0.01 µg/l, combined with low food availability resulted in significantly less egg production. But, in medium and high food availability no significant effect on egg production was observed. When setting toxicity thresholds, it is crucial to consider the role that additional biotic factors, like food availability, play in governing toxicity in the natural environment. This again highlights how relying on lab-based toxicity thresholds alone are not fit for purpose.

Despite the importance and vulnerability of the egg stage in the life cycles of aquatic invertebrates, little knowledge exists regarding the effects of exposure duration on toxicity to different embryonic stages. Chemicals have various modes of action, which means they can interact with each life stage in a different way. It is not fully understood when the eggs of non-target aquatic invertebrates become sensitive or insensitive to chemicals, and whether there are specific embryonic stages that are vulnerable to chemicals with different modes of action. (Yokoyama, 2019) found that for eggs of the caddisfly *Cheumatopsyche brevilineata*, toxicity of the insecticide etofenprox was more dependent on the embryonic stage at which exposure occurred, rather than the duration. Species toxicity thresholds used for management rarely consider multiple life stages, which is problematic given the potential of a chemical to be harmful for only a short window of an organism's lifespan.

Feeding

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 food consumption has consequences on an individual level, with impacts such as hampered larval development, reduced emergence, and smaller adult imagoes (Hatakeyama, Shiraishi and Uno, 1997). The ecosystem as a whole is also affected, through declines in organic matter availability and alterations to inter-species competition (Whitehurst, 1991).

There is a wide range of evidence that shows macroinvertebrates may reduce their consumption of litter following continuous exposure to different toxicants. (Flores *et al.*, 2014) showed a reduction in shredding performance of *Echinogammarus berilloni* following exposure to 0.5 µg/l concentrations of the organophosphate diazinon. (Wieczorek *et al.*, 2018) also found reduced shredding by *Asellus aquaticus* following continuous exposure to the pyrethroid etofenprox (0.04 µg/l) - a 44% decrease in feeding rate was observed. 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. As previously mentioned, continuous exposure experiments are typically not an accurate representation of exposure in nature. (Nyman *et al.*, 2013) conducted a continuous and pulse experiment in parallel to compare feeding in *G. pulex* following exposure to the neonicotinoid imidacloprid. Feeding was heavily inhibited under the low constant treatment (15 mg/l) but feeding was not affected by repeated pulses of exposure. (Alexander *et al.*, 2007) found the opposite in mayflies, where pulses of imidacloprid greater than 0.5 µg/l over 24 hours impaired feeding, with feeding rates remaining low even four days after exposure.

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*. However, despite no effect being observed, high levels of bioconcentration in the nymphs were identified. This highlights that surrounding water concentrations are not always reflective of the chemical burden organisms may be experiencing. As chemicals in the environment are monitored and regulated on a singular basis, the existing chemical loads present in organisms are overlooked.

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).

Similarly to invertebrates, most of the current understanding surrounding sublethal chemical impacts on freshwater 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). This section explores the evidence surrounding sublethal chemical exposures to freshwater fish, again linking to some of the issues with current chemical management.

Reproduction

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. Although municipal wastewater treatments have been designed to efficiently remove major elements such as carbon, nitrogen and phosphorus, other chemical contaminants such as micropollutants, hormones and pharmaceutical residues are poorly removed (Schmitz *et al.*, 2018).

(Jobling *et al.*, 1998) found high occurrences (16–100%) of intersexuality in male roach (*Rutilus rutilus*) living downstream of major UK wastewater treatment plants. These fish had elevated blood levels of the female egg yolk protein, vitellogenin, at levels significantly higher than those of males from upstream and control sites. (Tarrant *et al.*, 2009) also recorded increased plasma vitellogenin in male wild brown trout (*Salmo trutta*) downstream of a major wastewater treatment works in Ireland. Currently, UK water companies are required to regularly monitor their final effluent before discharging to rivers, to ensure chemical concentrations are within legal limits. However, this monitoring does not take into account chemicals already present at discharge sites. Some chemicals can bind to river sediments and may build up as a result of frequent effluent discharges over time. To determine the actual chemical concentrations aquatic communities are being exposed to, regular monitoring of wastewater discharge points and not just the effluent being released would be beneficial. A monitoring approach that takes legacy effects into consideration is also essential.

Individually, pharmaceutical residues may fall under their classified environmentally safe concentration limits, but when combined they can form more potent mixtures. (Brian *et al.*, 2007) demonstrated that 5 oestrogenic chemicals (estradiol, ethynylestradiol, nonylphenol, octylphenol and bisphenol A) did not induce a significant response in fathead minnows (*Pimephales promelas*) individually; but the mixture had additive effects on reproductive performance at environmentally relevant concentrations (µg/l range). Following exposure to dilutions of treated wastewater and a pharmaceutical mixture of acetaminophen, carbamazepine, gemfibrozil and venlafaxine, (Galus *et al.*, 2013) found a significant decline in embryo production and elevated oocyte atresia in the ovaries of zebrafish (*Danio rerio*). 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. (Schmitz *et al.*, 2018) also found that a nonsteroidal pharmaceutical mixture exposure (of carbamazepine, irbesartan, paracetamol, naproxen and diclofenac) influenced reproductive traits. Significant overexpression of key genes involved in the maintenance of the ovaries of rainbow trout (*Oncorhynchus mykiss*) occurred after mixture exposure. Current management does not take into account the ecological risk associated with mixtures of chemicals being released. It is crucial that synergistic effects of mixtures on aquatic organisms are taken into account in the monitoring and regulation of chemicals.

Pharmaceuticals and pesticides can also enter watercourses through run-off from agricultural land. Treatments sprayed on crops, and veterinary medicines excreted from livestock treatments, can all be washed from land into adjacent rivers. (Ankley *et al.*, 2003) demonstrated a sublethal impact of 17-β-trenbolone (0.027 µg/l, an environmentally relevant concentration measured in run-off), a metabolite of a potent androgen used to increase muscle growth in cattle, on the fecundity of of fathead minnows (*P. promelas*). Female vitellogenin and plasma steroid concentrations were significantly reduced. Masculinisation of females also occurred, where females produced dorsal (nuptial) tubercles, normally only present on the heads of mature males. Assigning blame for diffuse pollution is challenging and obtaining evidence to link chemical degradation to a specific landowner is complicated and resource demanding. The existing regulations to protect watercourses from agricultural chemical pollution are sufficient to protect aquatic communities. However, education and enforcement of these rules is lacking. Additional resources are needed to deliver advice and incentives for farmers, in tandem with an enforcement regime that contains a realistic threat of detection and penalty to ensure compliance.

Development

Fish can experience reduced growth and physiological deformities as a result of chemical exposure (Heath *et al.*, 1997). Fish with deformities or a smaller body size have reduced competitive ability, greater susceptibility to 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. Common carp (*Cyprinus 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 rohu (*Labeo rohita*) exposed to the organophosphate pesticide malathion by (Patil and Muniswamy, 2010).

Juvenile anadromous fish undergo a transition from parr to smolt during their downstream migrations. This development phase, known as ‘smoltification’, is orchestrated by hormones and prepares salmonids for entry into seawater (Russell *et al.*, 2012). Gill Na+/K+ 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. (Waring and Moore, 2004) found that exposures of 2, 5 and 10 µg/l atrazine caused a significant reduction in Na+/K+ ATPase activity in Atlantic salmon (*Salmo salar*). Further work later determined that exposure to concentrations of atrazine as low as 0.1 µg/l over a 72-h period had the same detrimental impact ((Moore *et al.*, 2008). Conversely, (Matsumoto, Hosmer and Van Der Kraak, 2010) found that short term exposures of up to 100 µg atrazine/l did not significantly affect gill Na+/K+ ATPase activity in salmon smolts. Although these studies provide valuable insight into the adverse biological consequences of sublethal chemical exposure, the testing was conducted entirely on fish obtained from captive facilities. Even when hatchery-reared smolts are from the same strains as the wild smolts, they differ in physical condition and physiological status. The accelerated growth regime typically employed in hatcheries favours large body size and high fat content, which may affect life history characteristics (Thorstad *et al.*, 2012). These fish may exhibit biological and behavioural responses to chemical exposure different to that of wild smolts, so it is difficult to confidently determine harmful concentration thresholds based on them.

Swimming and migration behaviour

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.

Changes in swimming speed and distance were significantly correlated with changes in cholinesterase activity in larval rainbow trout (*O. 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). Locomotor activity of fish is the most frequently assessed sublethal endpoint in determining a behavioral change in response to a contaminant in toxicity testing (Makaras *et al.*, 2020). However, behavioural alterations in fish may be not noticed during short-term toxicity tests and response patterns may be overlooked due to inappropriate selection of behavioral endpoints for testing. This can be overcome by widening the range of endpoints examined, in combination with reasonable exposure duration and observation intervals.

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.

Predator avoidance

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). 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. As previously mentioned, synergistic effects of mixtures on aquatic organisms are not taken into account in the current regulatory framework, meaning freshwater organisms are not adequately protected from negative impacts like these.

Feeding activity

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.

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 (Brack *et al.*, 2018). Current assessment is based on sampling at discrete points in time, testing for chemicals on the priority list, and seeing if concentrations match the levels considered safe for the environment (environmental quality standards, EQS) (Tlili *et al.*, 2016). Although this provides a simple scheme for environmental protection and assessment, it is insufficient for comprehensive understanding of mixture impacts to aquatic ecosystems in a realistic, multiple-stress context (Posthuma, de Zwart and Dyer, 2019)

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 some 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).

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.

Looking at chemicals using a one by one approach has its merits for reducing lethal concentrations of specific chemicals in waterbodies. However, it does not take into account enhanced detrimental consequences resulting from mixture effects. (Cleuvers, 2003) stated that for a range of stream organisms, acute (lethal) effects of single substances in the aquatic environment were very unlikely, but considerable combination effects could occur. To better grasp the impact of mixture burdens, implementing a suitable chemical monitoring regime alone would likely be demanding and continuous measuring unrealistic. Incorporating biological monitoring could overcome this. As invertebrate communities experience continuous exposure to the water, they represent a much longer time period than water samples. Effect-based biological indicators are a powerful tool for identifying ecological pressure from contaminants. The invertebrate-based indicator SPEAR (SPEcies At Risk) uses trait information to identify chemical pressure and ecological effects in rivers (Knillmann *et al.*, 2018). SPEAR validation has been undertaken by scientists in a variety of countries already, including France and Finland (Schäfer *et al.*, 2007). Recommendations have been made by the Environment Agency to validate the SPEAR approach in the UK through field investigations. To grasp impacts of toxicants at ecosystem level, it is essential that better connections are made between chemical and biological sampling regimes. Currently in the UK no overlap exists between water quality and invertebrate monitoring. In order to achieve more informative and more intuitive monitoring, biological and chemical sampling should be completed together at the same locations.

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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V. References

Acuña, V. et al. (2015) “Balancing the health benefits and environmental risks of pharmaceuticals: Diclofenac as an example.,” Environment international, 85, pp. 327–333.

Alexander, A. C. et al. (2007) “Effects of insecticide exposure on feeding inhibition in mayflies and oligochaetes,” Environmental Toxicology and Chemistry, 26(8).

Alexander, A. C., Heard, K. S. and Culp, J. M. (2007) “Emergent body size of mayfly survivors,” Freshwater Biology, 53(1).

Ankley, G. T. et al. (2003) “Effects of the androgenic growth promoter 17-β-trenbolone on fecundity and reproductive endocrinology of the fathead minnow,” Environmental Toxicology and Chemistry, 22(6).

Barbieri, E. (2007) “Use of metabolism and swimming activity to evaluate the sublethal toxicity of surfactant (LAS-C12) on Mugil platanus,” Brazilian archives of biology and technology = (Archivos de biologia e tecnologia), 50.

Barmentlo, S. H. et al. (2019) “Environmental levels of neonicotinoids reduce prey consumption, mobility and emergence of the damselfly Ischnura elegans,” Journal of Applied Ecology, 56(8), pp. 2034–2044.

Beamish, R. J., Mahnken, C. and Neville, C. (2004) “Evidence that reduced early marine growth is associated with lower marine survival of coho salmon,” Transactions of the American Fisheries Society, 133(1), pp. 26–33.

Beauvais, S. L. et al. (2000) “Physiological measures of neurotoxicity of diazinon and malathion to larval rainbow trout (Oncorhynchus mykiss) and their correlation with behavioral measures,” Environmental Toxicology and Chemistry, 19(7), pp. 1875–1880.

Beketov, M. A. and Liess, M. (2005) “Acute contamination with esfenvalerate and food limitation: chronic effects on the mayfly, Cloeon dipterum.,” Environmental toxicology and chemistry, 24(5), pp. 1281–1286.

Beketov, M. A. and Liess, M. (2008) “Potential of 11 pesticides to initiate downstream drift of stream macroinvertebrates.,” Archives of environmental contamination and toxicology, 55(2), pp. 247–253.

Berghahn, R. et al. (2012) “Effects of repeated insecticide pulses on macroinvertebrate drift in indoor stream mesocosms.,” Aquatic toxicology (Amsterdam, Netherlands), 122–123, pp. 56–66.

Brack, W. et al. (2018) “Towards a holistic and solution-oriented monitoring of chemical status of European water bodies: how to support the EU strategy for a non-toxic environment?,” Environmental Sciences Europe, 30(33).

Breneman, D. H. and Pontasch, K. W. (1994) “Stream microcosm toxicity tests: Predicting the effects of fenvalerate on riffle insect communities,” Environmental Toxicology and Chemistry, 13(3).

Brian, J. V. et al. (2007) “Evidence of estrogenic mixture effects on the reproductive performance of fish.,” Environmental science & technology, 41(1), pp. 337–344.

Brodin, T. et al. (2013) “Dilute concentrations of a psychiatric drug alter behavior of fish from natural populations,” Science, 339(6121), pp. 814–815.

Brodin, T. et al. (2014) “Ecological effects of pharmaceuticals in aquatic systems - Impacts through behavioural alterations,” Philosophical Transactions of The Royal Society B Biological Sciences, 369(20130580).

Castro-CatalÃ, N. D. et al. (2017) “Evidence of low dose effects of the antidepressant fluoxetine and the fungicide prochloraz on the behavior of the keystone freshwater invertebrate Gammarus pulex,” Environmental Pollution, 231(Pt 1), pp. 406–414.

Cedergreen, N. and Rasmussen, J. J. (2017) “Low Dose Effects of Pesticides in the Aquatic Environment,” in Pesticide Dose: Effects on the Environment and Target and Non-Target Organisms. American Chemical Society, pp. 167–187.

Cleuvers, M. (2003) “Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects,” Toxicology letters, 142(3), pp. 185–194.

Cowx, I. G. and Portocarrero-Aya, M. (2011) “Paradigm shifts in fish conservation: moving to the ecosystem services concept,” Journal of fish biology, 79, pp. 1663–1680.

David, A. and Pancharatna, K. (2009) “Effects of acetaminophen (paracetamol) in the embryonic development of zebrafish, Danio rerio.,” Journal of applied toxicology: JAT, 29(7), pp. 597–602.

De Lange, H. et al. (2006) “Behavioural responses of Gammarus pulex (Crustacea, Amphipoda) to low concentrations of pharmaceuticals,” Aquatic Toxicology, 78(3), pp. 209–216.

Firmiano, K. R. et al. (2017) “Mayfly bioindicator thresholds for several anthropogenic disturbances in neotropical savanna streams,” Ecological indicators, 74, pp. 276–284.

Flores, L. et al. (2014) “Effects of a fungicide (imazalil) and an insecticide (diazinon) on stream fungi and invertebrates associated with litter breakdown,” Science of The Total Environment, 476–477, pp. 532–541.

Floyd, E. Y., Geist, J. P. and Werner, I. (2008) “Acute, sublethal exposure to a pyrethroid insecticide alters behavior, growth, and predation risk in larvae of the fathead minnow (Pimephales promelas),” Environmental Toxicology and Chemistry, 27(8), pp. 1780–1787.

Galus, M. et al. (2013) “Chronic effects of exposure to a pharmaceutical mixture and municipal wastewater in zebrafish,” Aquatic Toxicology, 123–133, pp. 212–222.

Gavrilescu, M. et al. (2015) “Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation.,” New biotechnology, 32(1), pp. 147–156.

Gerhardt, A., Bloor, M. and Lloyd Mills, C. (2011) “Gammarus: important taxon in freshwater and marine changing environments,” International Journal of Zoology, 2011(524276).

Gessner, M. O. and Tlili, A. (2016) “Fostering integration of freshwater ecology with ecotoxicology,” Freshwater Biology, 61(12).

Gormley, K. L. and Teather, K. (2003) “Developmental, behavioral, and reproductive effects experienced by Japanese medaka (Oryzias latipes) in response to short-term exposure to endosulfan,” Ecotoxicology and environmental safety, 54(3), pp. 330–338.

Halappa, R. and Muniswamy, D. (2009) “Behavioral Responses of the Freshwater Fish, Cyprinus carpio(Linnaeus) Following Sublethal Exposure to Chlorpyrifos,” Turkish Journal of Fisheries and Aquatic Sciences, 9, pp. 233–238.

Hamilton, P. B. et al. (2016) “Population-level consequences for wild fish exposed to sublethal concentrations of chemicals - a critical review,” Fish and Fisheries, 17(3).

Hanazato, T. (2001) “Pesticide effects on freshwater zooplankton: an ecological perspective.,” Environmental pollution (Barking, Essex: 1987), 112(1), pp. 1–10.

Hatakeyama, S., Shiraishi, H. and Uno, S. (1997) “Overall pesticide effects on growth and emergence of two species of Ephemeroptera in a model stream carrying pesticide- polluted river water,” Ecotoxicology. Kluwer Academic Publishers, 6(3), pp. 167–180.

Heath, A. G. et al. (1997) “Physiological responses of fathead minnow larvae to rice pesticides,” Ecotoxicology and environmental safety, 37, pp. 280–288.

Hose, G. C. et al. (2002) “A pulse of endosulfan-contaminated sediment affects macroinvertebrates in artificial streams.,” Ecotoxicology and environmental safety, 51(1), pp. 44–52.

Jarvis, A. L., Bernot, M. J. and Bernot, R. (2014) “The effects of the pharmaceutical carbamazepine on life history characteristics of flat-headed mayflies (Heptageniidae) and aquatic resource interactions,” Ecotoxicology, 23(9), pp. 1701–1712.

Jobling, S. et al. (1998) “Widespread sexual disruption in wild fish,” Environmental Toxicology and Chemistry, 17, pp. 2498–2506.

Jonsson, M. et al. (2015) “Effects of an antihistamine on carbon and nutrient recycling in streams,” Science of the Total Environment, 538, pp. 240–245.

Kay, P. et al. (2017) “Widespread, routine occurrence of pharmaceuticals in sewage effluent, combined sewer overflows and receiving waters.,” Environmental pollution (Barking, Essex: 1987), 220(Pt B), pp. 1447–1455.

Knillmann, S. et al. (2018) “Indication of pesticide effects and recolonization in streams,” 630, pp. 1619–1627.

Lauridsen, R. B. and Friberg, N. (2005) “Stream macroinvertebrate drift response to pulsed exposure of the synthetic pyrethroid lambda-cyhalothrin.,” Environmental toxicology, 20(5), pp. 513–521.

Liess, M. and Von Der Ohe, P. (2005) “Analyzing effects of pesticides on invertebrate communities in streams,” Environmental Toxicology and Chemistry, 24(4), pp. 954–965.

Makaras, T. et al. (2020) “Juvenile fish responses to sublethal leachate concentrations: comparison of sensitivity of different behavioral endpoints,” Environmental science and pollution research, (27), pp. 4876–4890.

Matsumoto, J., Hosmer, A. J. and Van Der Kraak, G. (2010) “Survival and iono-regulatory performance in Atlantic salmon smolts is not affected by atrazine exposure,” Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology, 152(3), pp. 379–384.

McGee, M. R. et al. (2009) “Predator avoidance performance of larval fathead minnows (Pimephales promelas) following short-term exposure to estrogen mixtures,” Aquatic Toxicology, 91(4), pp. 355–361.

Mondal, K., Karmakar, B. and Haque, S. (2015) “A review on effects of pyrethroids pesticides on fresh water fish behaviour and fish reproduction,” Journal of global biosciences, 4, pp. 2594–2598.

Moore, A. et al. (2008) “The impact of a pesticide on the physiology and behaviour of hatcheryâ reared Atlantic salmon, Salmo salar, smolts during the transition from fresh water to the marine environment,” Fisheries Management and Ecology, 15(5–6).

Muirhead-Thomson, R. C. (1978) “Lethal and behavioral impact of permethrin (NRDC 143) on selected stream macroinvertebrates.,” Mosquito News.

Muller, E. B., Nisbet, R. M. and Berkley, H. A. (2010) “Sublethal toxicant effects with dynamic energy budget theory: model formulation,” Ecotoxicology, 19(1), pp. 48–60.

Nyman, A. M. et al. (2013) “The insecticide imidacloprid causes mortality of the freshwater amphipod Gammarus pulex by interfering with feeding behavior,” PLoS ONE, 8(5), p. e62472.

Painter, M. M. et al. (2009) “Antidepressants at environmentally relevant concentrations affect predator avoidance behavior of larval fathead minnows (Pimephales promelas),” Environmental Toxicology and Chemistry, 28(12), pp. 2677–2684.

Palmquist, K. R., Jepson, P. C. and Jenkins, J. J. (2008) “Impact of aquatic insect life stage and emergence strategy on sensitivity to esfenvalerate exposure,” Environmental toxicology and chemistry, 27(8), pp. 1728–1734.

Patil, V. K. and Muniswamy, D. (2010) “Behavioral and morphological endpoints: as an early response to sublethal malathion intoxication in the freshwater fish, Labeo rohita,” Drug and chemical toxicology, 33(2), pp. 160–165.

Petrovic, M. et al. (2011) “Combined scenarios of chemical and ecological quality under water scarcity in Mediterranean rivers,” TrAC Trends in Analytical Chemistry, 30(8).

Posthuma, L., de Zwart, D. and Dyer, S. D. (2019) “Chemical mixtures affect freshwater species assemblages: from problems to solutions,” Current opinion in environmental science & health, 11, pp. 78–89.

Proia, L. et al. (2013) “Effects of pesticides and pharmaceuticals on biofilms in a highly impacted river.,” Environmental pollution (Barking, Essex: 1987), 178, pp. 220–228.

Raby, M. et al. (2019) “Acute Effects of Binary Mixtures of Imidacloprid and Tebuconazole on 4 Freshwater Invertebrates.,” Environmental toxicology and chemistry, 38(5), pp. 1093–1103.

Russell, I. C. et al. (2012) “The influence of the freshwater environment and the biological characteristics of Atlantic salmon smolts on their subsequent marine survival,” ICES Journal of Marine Science, 69(9), pp. 1563–1573.

Rutowski, R. L. (2000) “Variation of eye size in butterflies: inter- and intraspecific patterns,” Journal of Zoology, 252(2).

Sabater, S. et al. (2016) “Shared effects of organic microcontaminants and environmental stressors on biofilms and invertebrates in impaired rivers.,” Environmental pollution (Barking, Essex: 1987), 210, pp. 303–314.

Sandahl, J. F. et al. (2004) “Odor-evoked field potentials as indicators of sublethal neurotoxicity in juvenile coho salmon (Oncorhynchus kisutch) exposed to copper, chlorpyrifos, or esfenvalerate,” Canadian Journal of Fisheries and Aquatic Sciences, 61(3), pp. 404–413.

Sandahl, J. F. et al. (2005) “Comparative thresholds for acetylcholinesterase inhibition and behavioral impairment in coho salmon exposed to chlorpyrifos,” Environmental Toxicology and Chemistry, 24(1), pp. 136–145.

Sandahl, J. F. and Jenkins, J. (2002) “Pacific steelhead (Oncorhynchus mykiss) exposed to chlorpyrifos: benchmark concentration estimates for acetylcholinesterase inhibition,” Environmental Toxicology and Chemistry, 21(11), pp. 2452–2458.

Schäfer, R. et al. (2007) “Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe,” Science of The Total Environment, 382(2–3), pp. 272–285.

Schmitz, M. et al. (2018) “Environmentally-relevant mixture of pharmaceutical drugs stimulates sex-steroid hormone production and modulates the expression of candidate genes in the ovary of juvenile female rainbow trout.,” Aquatic Toxicology, 205, pp. 89–99.

Schulz, R. and Liess, M. (1999) “Validity and ecological relevance of an active in situ bioassay using gammarus pulex and limnephilus lunatus.,” Environmental toxicology and chemistry, 18(10), pp. 2243–2250.

Scott, G. R. and Sloman, K. (2004) “The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity,” Aquatic Toxicology, 68, pp. 369–392.

Tarrant, H. et al. (2009) “Raised plasma vitellogenin in male wild brown trout (Salmo trutta) near a wastewater treatment plant in Ireland,” Environmental Toxicology and Chemistry, 27(8).

Thompson, M. S. A. et al. (2016) “Gene-to-ecosystem impacts of a catastrophic pesticide spill: testing a multilevel bioassessment approach in a river ecosystem,” Freshwater Biology, 61(12).

Thorstad, E. B. et al. (2012) “A critical life stage of the Atlantic salmon Salmo salar: behaviour and survival during the smolt and initial post-smolt migration,” Journal of fish biology, (81), pp. 500–542.

Tlili, A. et al. (2016) “Pollution-induced community tolerance (PICT): towards an ecologically relevant risk assessment of chemicals in aquatic systems,” Freshwater Biology, 61(12).

Vörösmarty, C. J. et al. (2010) “Global threats to human water security and river biodiversity.,” Nature, 467(7315), pp. 555–561.

Waring, C. and Moore, A. (2004) “The effect of atrazine on Atlantic salmon (Salmo salar) smolts in fresh water and after sea water transfer,” Aquatic Toxicology, 66(1), pp. 93–104.

Whitehurst, I. (1991) “The Gammarus: Asellus ratio as an index of organic pollution,” Water research, 25(3), pp. 333–339.

Wieczorek, M. V. et al. (2018) “Structural and functional effects of a short-term pyrethroid pulse exposure on invertebrates in outdoor stream mesocosms,” Science of The Total Environment, 610–611, pp. 810–819.

Wurzel, S. et al. (2020) “Evaluating the impact of Permethrin on non-target invertebrates in an urban stream,” Hydrobiologia, (847), pp. 91–104.

Yokoyama, A. (2019) “Assessing Impacts of Insecticides on Different Embryonic Stages of the Nontarget Aquatic Insect Cheumatopsyche brevilineata (Trichoptera: Hydropsychidae).,” Environmental toxicology and chemistry, 38(7), pp. 1434–1445.

Zubrod, J. P. et al. (2014) “Effects of current-use fungicides and their mixtures on the feeding and survival of the key shredder Gammarus fossarum,” Aquatic Toxicology, 150, pp. 133–143.