

How damaging are pesticide spills in freshwater ecosystems?

A review of the evidence in the literature

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Despite pesticides receiving increasing research interest for their effects on ecosystems, most freshwater studies focus on chronic, low-concentration application. This is reflective of contamination caused by leaching and run-off from agriculture but does not adequately represent the high-concentration, point-source entry that is typical of a pesticide spill. Pesticide spills are known to occur frequently in well monitored areas but there is great uncertainty in both severity and frequency globally. This review draws together the scattered knowledge of pesticide-spill effects and presents an overview of the ways that contaminated communities can be changed. Effect type and magnitude is highly dependent on four key categories of variables: the pesticide compound, original community structure, the type of freshwater body and the presence of other stresses. Indirect effects are shown to be as, if not more, important in determining the effect of a pesticide on an aquatic community. Limited evidence suggests that ecosystem processes appear to be less affected by pesticide spills and can recover to original states faster than the community structure and trophic interactions. This review calls for further research into spill effects by opportunistic observation of accidental spills and mesocosm studies for a wide range of habitat and pesticide types so that policy makers may more accurately assess the risk posed by pesticide spills.

Introduction

Why the question needs to be addressed

Freshwater aquatic ecosystems offer a wide and relatively little understood range of ecosystem functions (Postel, Carpenter and Daily, 1997; Wilson and Carpenter, 1999). The function of ecosystems are known to rely on complex interactions that depend on the community composition and structure for stable nutrient cycling and ecosystem service provision (Clements, 1936; Odum, 1969). There is a large body of evidence stating that disturbance and stress (such as hydrological and seasonal temperature variation) can be fundamental parts of a system rather than negative or damaging interruptions to a system's succession (Pickett, 1989). However, there is little or no precedent that suggests a system will recover adequately from the stress of severe pesticide spills. Studies conducted on other biome types have found that anthropogenic stresses that do not mimic 'natural' changes can be far more damaging (Bengtsson et al., 2000) than the regular, natural stresses on ecosystems such as drought and floods. Current policy on regulating pesticide use, storage and disposal is based on wildlife damage that is poorly understood and therefore impossible to value (Pimentel et al., 1992). This makes it important that we understand the ability of pesticide spills to damage or alter freshwater communities so that the benefits and costs of pesticide use can be more accurately balanced.

The scale of pesticide use and the associated risk of spill

Globally, around 236, 000 tonnes of pesticidal active ingredient are applied annually (based on 2000 and 2001 estimates) (Kiely, Donaldson and Grube, 2004). The increasing rate of pesticide use in developing nations has resulted in the growing use of older, more toxic chemicals being used with little consideration for their proper storage or health effects (Ecobichon, 2001).

The broader questions of terrestrial effects of this volume of application have already been addressed by other reviews (Somerville and Walker, 1990) in response to the terrestrial evidence of pesticide drift. However, chemical testing of many aquatic systems has shown low-level, chronic contamination to be almost ubiquitous in agricultural regions globally (Claver et al., 2006; Jasim et al., 2006; Leong et al., 2007). It could therefore be argued that low level pesticide effects are integral to some affected natural systems and consequently may even be covered by some present ecological understanding of those freshwater systems (although research is not usually conducted in the developed lower reaches of rivers). It is also worth noting that freshwater systems of all types should not be seen as isolated from surrounding terrestrial and riparian zones. The high level of interaction across these system boundaries is increasingly well documented (Gregory et al., 1991; Robinson, Tockner and Ward, 2002; Paetzold et al., 2005) and therefore the damage done by pesticide spills or contamination to these communities may well exacerbate the damage done to the freshwater system itself.

The exact frequency of pesticide spills is almost impossible to obtain for even the best equipped of environmental organisations. These point-source events are highly ephemeral, with some (particularly hydrophobic) pesticides being rapidly adsorbed by sediment particles, thereby not remaining detectable in the water column long enough for any feasible monitoring regime to identify. A study of rural ponds in Canada found that 16 of 168 (9.5%) ponds adjacent to treated agricultural land had been contaminated by accidental pesticide spills or containers that had been improperly disposed of (Frank et al., 1990).

In the United Kingdom, an Environment Agency report (1998) survey found that 20% of farmers routinely disposed of pesticides (such as organophosphate sheep-dip mixtures) by pouring it over land or directly into surface water and only 30% had received proper training on pesticide use and disposal. A 2006 report by the Belgian Science Policy and Department of Crop Protection Chemistry suggested that groundwater (and presumably also surface water) was subject to spills of pesticide by back-siphoning and rinsing of spraying equipment near to bore-holes and other water sources used to fill spraying tanks. Such findings suggest that there are a large number of unreported spills.

What would be considered damaging

In asking how damaging pesticide spills are to freshwater ecosystems, this review raises many further questions. Firstly there is a need to establish what would constitute damage and how this can be distinguished from stress alone. There is a trend in published literature to label an ecosystem or community as damaged when productivity or function is reduced (Jones and Schmitz, 2009) or even when there is any anthropogenic interference. Simply detecting interference would serve little purpose in this review as it seeks evidence of potential for damage in response to pesticide leaks and interference is already known to occur as a consequence of widespread, chronic pesticide contamination at low concentrations (Ritter, 1990).

Odum (1985) describes the many levels at which an ecosystem can exhibit signs of stress. If the conditions of duration and capacity for recovery are added, this provides a basis for damage indication. However, many of the observations Odum lists do not necessarily lead to or signify damage so they will be seen as possible contributors to damage rather than damaging effects themselves. System-level traits such as resource use efficiency may decrease, but this could easily occur due to hydrological stresses such as drought as well and does not necessarily have long-term consequences for the community. Many ecosystem traits such as biomass and respiration rate vary seasonally, and are affected by many other natural stressors that are hard to control for. In addition, stability of these traits could mask significant changes in the processes and structure controlling them. These will therefore not be considered adequate indicators of damage.

On a community level, any reduction in diversity could be argued to be a damaging effect. The beneficial effects of community diversity for both resilience and productivity are well documented (Lehman and Tilman, 2000; McCann, 2000). Unlike biomass, which can be restored a short time after a stress event (Steinman and McIntire, 1990), diversity changes can be more permanent when taxa or species are lost and can take longer to recover even if the eliminated species ever do recolonise (Wallace, Vogel and Cuffney, 1986; Harding et al., 1998).

As this review primarily aims to estimate damage at a community or system level. Damage or interference at smaller scales will be considered as evidence for higher level effects and the possible implications of them will be estimated when possible by comparison with similar documented cases or theories in the published literature. As this article draws from a wide array of experiments and reports that measure different traits in varying systems with many types of pesticide contamination no direct quantification is possible.

Complications of the question

The known effects and behaviours of pesticides can only be expected to hold true in the type of habitat in which they have originally been studied. The world's freshwater ecosystems are far from uniform. Table 1 demonstrates the diversity of the habitat types contained at a broad level. Within these are further subdivisions according to the biome type the riparian system runs through, and other variables that will be discussed in this review.

<i>Type</i>	<i>Sub-types</i>	<i>General qualities</i>
Lotic (Flowing)	Headwaters	Highly allochthonous nutrient supply. High oxygen concentration, low temperature (Vannote et al., 1980)
	Mid-Reaches	Substrate and lower turbidity allows for greater primary production but marginally lower oxygen concentration
	Lower Reaches	Often slower flow with high sedimentation. Higher temperature and low turbidity cause lower oxygen concentrations. Generally more contaminants present due to accumulation through system.
	(Estuarine)	High salinity. Marine system boundary.
Lentic (Still)	Wetlands	Qualities largely dependent on area, depth, climate and geology. Additionally divided into pelagic, benthic and (in deep lakes) profundal zones.
	Ponds/Pools	
	Lakes	

Table 1: A summary of systems considered for pesticide spill vulnerability

This heterogeneity complicates any damage prediction as a chemical will likely remain at high concentration in the water of a small, poorly drained pond far longer than it would a fast-flowing upland stream. A headwater community dominated by invertebrate breakdown of organic material from the riparian zone might respond differently to a herbicide spill than would a lower river system that depended more on affected primary producers for energy input (Vannote et al., 1980). Caldas et al. (2000) demonstrated that hydrophobic organochlorine pesticide remained in sediment and predatory organisms for a far greater duration than it did in the water itself. Therefore even within the sub-types of habitat listed in the table, there are many different habitats that will interact with contaminants differently.

Type of pesticide

There are a large number of different compounds in use as pesticides globally. The U.S. state of California, which maintains detailed data on the use of pesticides, publishes an annual report of the "Top 100 Pesticides Used Statewide". That 58 tonnes was used of the pesticide that ranked 100 in the report goes some way to suggest the variety (and volume) used and threatening contamination (California Department of Pesticide Regulation, 2011). Table 2 shows the broad categories that will be most referred to in this review.

<i>Pesticide class</i>	<i>Example compound(s)</i>	<i>Target</i>	<i>Selected reported effects</i>
Organochlorines	DDT, dicofol, dieldrin	Insecticide	Lethal and irreversible damage to acetylcholinesterase, an enzyme essential to neurotransmission in a wide range of organisms (Vivjerberg et al., 1982)
Organophosphates	diazenon, chlorpyrifos	Insecticide	Reversible inhibition of acetylcholinesterase (Casida, 1963)
Carbamates	aldicarb, carbofuran, carbaryl	Insecticides	Paralyse invertebrates and fish (Soderlund et al., 2002)
Pyrethroids	prallethrin, permethrin, esfenvalerate	Insecticides	Disrupt protein synthesis in broadleaf weeds and grasses (Chaleff and Mauvais, 1984)
Sulfonylureas	bensulfuron	Herbicide	Damage photosystem II of plants, algae and cyanobacteria (Tietjen et al., 1996)
Triazines	atrazine, terbutryn	Herbicide	Paralyses insects. Some toxicity in mammals (Fishel, 2005)
Neonicotinoids	imidacloprid, dinotefuran	Insecticide	

Table 2: A summary of pesticide classes used globally and selected reported effects.

How the damage is being assessed

The literature published to date on the damage of pesticides generally falls into one of four categories. The first (entitled 'simple' below) measure direct effects of a pesticide on a population of organisms in isolation. The second, generally referred to as mesocosm studies, looks at a model community that contains more than one species in a way that permits some degree of interaction that can be measured or observed (Odum, 1984). These allow indirect effects to be considered as well, but the direct effects are less valid as community processes may worsen or mitigate exposure damage (Relyea and Hoverman, 2006). Observations and investigations of contaminated 'natural' systems make up the third which cannot truly distinguish between direct and indirect effects as controls are generally not available.

The three categories and examples of such studies and findings are given below:

1: 'Simple' Ecotoxicity studies

These studies are highly numerous as a result of both the diversity of pesticide compounds and the requirement of toxicity values for licensing in most regions. They typically involve repeated exposures of one or more species to a range of pesticide concentrations in water and the calculation of an LC₅₀ (concentration resulting in 50% lethality) or an LCt₅₀ (with a time component).

Whilst these are useful for flagging certain pesticide compounds as threats and candidates for further investigation, these tests are of little use in predicting the total damage of a pesticide spill as they only typically target one representative species from each higher-level group. A comprehensive assessment of over one hundred pesticides by Helfrich et al (1996) specifies LC₅₀ values for each, but only for two species of fish. The value of such information would theoretically be in determining acceptable levels for freshwater management but in reality this is questionable as the aforementioned article showed a high level of discrepancy between the two species' response to many compounds that suggests a wider range of values across all unsampled fish species. The report makes no

mention of the ecological consequences of any tolerance discrepancy and preferential survival, even between the two species selected.

The vast majority of published ecotoxicity articles are more complex than exposure of one pesticide to one species. This complexity tends to come from using more than one pesticide or more than one species. However, the two species are rarely treated in combination, but separately in isolation. It is therefore rare that such publications pick out any effects above individual or population level. A review by Relyea and Hoverman in 2006 found that interaction studies were increasing but that the communities tested were limited in diversity. Fewer than 15% (17) of the studies incorporated representatives from producing, herbivorous and carnivorous trophic groups.

Another review of ecotoxicology (Köhler and Triebskorn, 2013) showed that all pesticide effect studies were biased heavily towards the investigation of damage to domestic and common laboratory model species. Whilst this makes studies easier to conduct, the lack of investigation of a more representative species spectrum (as in Helfrich et al., 1996) could mean that even the direct effects of a spill on an organism's physiology and behaviour could be inaccurately estimated by an inadequate surrogate sample. A study of invertebrates in the order Cladocerans (Hyasaka et al., 2011) found that the sensitivity of 5 species to neonicotinoid pesticide varied by two orders of magnitude. This could have very serious implications for a spill in the habitat of a threatened aquatic species or group.

Despite their limitations, the studies can and do highlight interesting direct effects that could not be predicted from previous tests and raise questions about what might occur in and after spill event. A good example of this is the observation that Atlantic salmon smolts exposed to atrazine in freshwater subsequently suffer lower survival levels when exposed to salinity relatively to those that have not been exposed (Waring and Moore, 2004). This might imply that future research may reveal atrazine to have indirect effects not only in freshwater systems but also in marine ecosystems.

Data from other 'simple' exposure experiments suggests that common pollutants in rivers used by Atlantic salmon can make individuals more vulnerable to predation, adding to any population damage caused by a pesticide spill (Scholz et al., 2000). Salmon olfactory capabilities can also be affected by pesticide exposure (Waring and Moore, 1996). As no work has as yet looked at interaction between these two specific reported effects of different pesticides, it is unknown whether the effects would be additively damaging or if there would be interactions that could lead to synergistic negative effects on salmon spawning and population survival. Other reported individual-level effects (in fish alone) include endocrine disruption, increased agonistic behaviour and reduced mobility/predator evasion (Scott and Sloman, 2004). That these articles dealt with regular run-off levels of pesticide (as could be expected to be found in a stream), and not spill concentrations, increases the uncertainty of the risk this article seeks to estimate.

2: Mesocosm-based studies

Mesocosm-based studies aim to reproduce communities of varied scale and complexity in artificial, controlled environments that bridge the gap between laboratory experiments and natural ecosystem observation (Odum, 1984). This permits the investigation of effects at population, community and (debatably) ecosystem levels. The range of scales in such study set-ups is also wide, varying from microcosms smaller than one square meter to semi-controlled ecosystems such as *in situ* bags and natural ponds. What these tell us about pesticide damage is therefore more applicable to risk assessment as they should more accurately mirror the way a contaminant would behave if spilled into a natural ecosystem.

That these studies allow indirect effects to be measured is a key advantage. A herbicide spill may not be expected to affect carnivore and herbivore biomass but it can be shown that both are reduced in a model community assemblage (Diana et al., 2000) as a result of trophic knock-on effects. However, as it is essentially impossible to distinguish the direct from indirect effects, it remains ambiguous in the article as to whether the decrease in herbivore biomass was due to the reduced mass of periphyton and phytoplankton or a direct effect of the high concentrations of atrazine herbicide. Despite this, the experiment showed clearly that there was a cost to the wider community which had a functioning food chain as found in a natural lentic environment.

The same issue is found with other mesocosm studies. Relyea (2005) observed several unexpected effects in a similarly diverse mesocosm with insecticide application such as unexpected survival of

species highly related to target species, altering zooplankton community composition and unpredicted (albeit minimal) population impacts on larger herbivores such as snails and tadpoles. The direct or indirect causation of these symptoms remains unresolved, but there are more general community-level observations for which the resolution between direct and indirect effects is made essentially redundant. The same study recorded reductions in community species richness (in zooplankton and carnivorous insects) and in using four different pesticides gave one of the broadest and most useful assessments yet published. This might make the uncertainty in causal mechanisms seem relatively unimportant, but Rohr and Crumrine's experiments in treated ponds (2005) led to the observation that the eventual outcome of pesticide treatment and the resulting community structure is at least partly determined by the composition of the community itself. This suggests that any mesocosm-derived results are only quantitatively valid for communities of highly similar composition and structure. Therefore, understanding mechanisms behind community level changes might yield understanding that is applicable to a wider range of spill circumstances than the overall effect alone would be.

The more complex assemblages and interactions of mesocosms have another benefit. The more dynamic nature of these communities mean that it is beneficial to increase the time scale of the studies as they must be allowed to 'settle' (Verdonschot and Ter Braak, 1994) to a relatively stable structure before any final conclusions can be drawn. This may mean that long-term effects that are missed in short ecotoxicity assays (typically four days or less) can take effect.

Mesocosm experiments can also introduce factors that are hard to categorise. Relyea and Mills (2001) discovered that a combination of predator stress and longer exposure time (relative to prior toxicity studies) significantly decreased the tolerance of amphibians (*Hyla versicolor* tadpoles) to a carbamate insecticide. As a refinement of a direct effect test but in combination with an indirect effect (predatory stress) this further undermines 'simple' ecotoxicity studies even as predictors of survival of an individual species in a pesticide spill.

Although the majority of mesocosm studies look at vertebrates and macro-invertebrates, Downing et al. (2003) took natural microorganism assemblages from water samples in Florida canals and subjected them to high concentrations of four pesticides *in vitro*. This supported the trends towards reduction in taxonomic diversity seen with larger organisms. The naturally sourced assemblages gave this study another advantage, they were able to test for resistance in samples taken from canal sections that harboured greater pesticide concentrations. The microorganisms investigated should in theory have had the capacity to adapt new stressors and evolve resistance at a rate greater than other members of the aquatic community due to the related traits of shorter generational time (Laird, McConaughy and McCarthy, 1969) and smaller body size (Martin and Palumbi, 1969). However, the organisms tested showed no significant resistance in any population. This may be because the canal concentrations were not great enough to exert a selective pressure, but the lack of adaptation means that there may not be development of resistance in other organisms. It is therefore possible that populations regularly stressed by pesticide contamination could be more vulnerable to spill effects, rather than having a greater tolerance of them which could make further study of this hypothesis a priority.

3: Contamination observation studies

These studies capitalise on accidental spills or high levels of contamination in natural ecosystems and monitor community characteristics over time. Observational studies have the advantage that long term effects and effects that would not necessarily be measured in mesocosm and ecotoxicity studies might be noticed when occurring in a natural system. These 'chance' observations include endocrine disruption resulting in gender bias in freshwater fish species (Tyler and Jobling, 2008), reduced egg clutch viability in alligators (Guillette et al. 1995) and the significance of storm run-off events that reduce macro-invertebrate abundance by increasing pesticide leaching into river systems (Leonard et al., 1997).

In terms of relevance to pesticide spill risk, these articles can offer the most realistic portrayal of effects. However, at present the literature is limited and few incidents have been documented in any detail. Lambert's account of the consequences of a large organochlorine leak near to a river tributary caused by bombing in Somaliland (1996) had potential for good insight as the event was a point-source spill that was noticed immediately after its occurrence. Unfortunately, the location, limited resources and lack of urgency in determining spill risk at the time meant that only reptile and amphibian populations were monitored as indicators of damage. As the article focussed (and

therefore tested for) the presence of dieldrin (an organochlorine that strongly adheres to sediment particles) much of the investigation was centred around the toxicity of contaminated soil to native species. The article did yield some important findings, such as support for the already established knowledge of the environmental persistence of organochlorines (residue levels remained consistently high up to 9km downstream) and the greater vulnerability of amphibians compared to lizard species to dieldrin. With greater monitoring effort and proper aquatic surveys across all trophic levels, analysis of the event may have been able to answer many of the questions still unanswered today.

In Colombia, a 1989 a spill of chlorpyrifos (see Table 1) from an agricultural centre reached an 82km² marine bay (Cowgill et al., 1991). 15 tonnes of dead fish were collected by fishermen employed by the responsible company and although the invertebrate kill was unrecorded, the LC₅₀ for fish and tested invertebrates is similar for chlorpyrifos and so it can be assumed to be considerable. The study began three days after the spill with live sampling of hook-caught fish, limited (as acknowledged by the authors) sampling for zooplankton and surface sediment sampling. Although the original amount that entered the bay is unknown, the authors were able to calculate degradation half-lives in different mediums (8 and 9 days in sediment and water respectively and 11 days in fish). The authors conclude that the bay had recovered 23 days after the spill, which might be seen as a good indication of future contamination durations but the currents and hydraulic processes of the bay are poorly understood and so the amount that was transferred to the surrounding ocean is not calculable. As with most spill-event follow-ups, there was no control measurement of diversity or any community descriptors taken before the spill, so any attempt to label full recovery is problematic. Unlike Lambert's (1996) account of the Somaliland spill, there was a recovery effort in Colombia that consisted on removal and safe storage of contaminated sediment (Cowgill et al., 1991). The effect this had on the recovery time was not estimated but it likely mitigated the damage.

Another chlorpyrifos spill occurred in the River Kennet in the UK in 2013. This incident followed two similar recent incidents in the River Wey in 2002 (Angling Trust, 2013) and the Ouse in 2001 (BBC, 2001). The chlorpyrifos is thought to have entered the river through a sewage treatment works and reportedly exterminated some invertebrate populations for 15km downstream (Thames Anglers' Conservancy, 2013). Surprisingly, given that Helfrich et al. (1996) describe chlorpyrifos as having a "super/extreme" toxicity, with Rainbow trout's 96hour LC₅₀ quoted as just 3 µg/L there appeared to be little damage to fish populations (Thompson, Personal Communication, 2014). It is therefore suspected that it was a small amount that entered the river (approximately 20ml). Were it not for an extensive network of volunteer river monitors (at 43 sites along the river) the spill may have gone unnoticed (Action for the River Kennet, 2013). As data for this project is still being collected, the effects are still uncertain, as is the state of recovery. The data (which includes fish and invertebrates as well as community function such as leaf litter decomposition) could be the most comprehensive study of lotic pesticide effects when published.

Present knowledge of damage

Direct effects and their predictability

The direct effect damage of a pesticide spill is arguably the most predictable. As there are LC₅₀ values available for all major pesticides used for at least laboratory model species, it is possible to predict the extent to which a spill will be lethal which is important for short-term assessment of the danger presented by a spill event. The uncertainty lies primarily in the breadth of the effect (across different taxa), the size of the effected area and the duration of the effect.

The variety of organisms affected by a pesticide should be dictated by the title of the pesticide class and its function (see Table 1). It could be assumed that a Triazine herbicide would directly affect producers and therefore reduce their abundance. However, even without indirect effect intervention, this can be unreliable. Triazines have been shown to be less damaging to small diatom producers than larger filamentous chlorophyta by Gurney and Robinson (1989). This unpredictable variance in tolerance will likely mean that certain species (or even populations within species) could be damaged or even eliminated in cases where chemical testing might deem concentrations 'safe' after a spill.

Indirect damage and damage to higher organisational levels

This section refers to effects that would not be experienced by a test population in isolation, but have been observed in more diverse assemblages. The first significant stage of a community effect would likely be the increased mortality of affected groups. The magnitude of this effect would depend on the concentration and type of pesticide used. Despite not being specifically targeted, vertebrates are affected to some extent by six of the seven pesticide classes listed in table 2 (sulfonylurea herbicides excluded as there is little relevant evidence)(Brucker-Davis, 1998; Tomizawa et al., 1991).

Macro-invertebrates would be affected by the same proportion (6/7) with direct lethality caused by the insecticide classes and reproductive success lowered by many triazines (Peters et al., 1991). The lethal and sublethal damage has been observed to alter invertebrate communities in model streams (Liess and Schulz, 1998), but the effects on natural aquatic plant and fungal communities is less examined than the animal populations. The action of herbicides on different target plant species appear as diverse as those of insecticides on invertebrates. Helfrich et al.'s toxicity report (1996) lists six sampled herbicides and four families of target producer. Of these, no herbicide acts on all groups and algal species are only affected by two of the six pesticides. This suggests that a spill would alter community structure as observed with invertebrates by selectively damaging just one type of macrophyte and allowing another to become more abundant in the community by a form of competitive release. This is supported in mesocosm studies that found functional redundancy in macrophytes to be important in preventing ecosystem function reduction that might have otherwise occurred from community alteration (Fairchild, La Point and Schwartz, 1994) The study does not comment on the future vulnerability of the altered community to sustained stress.

Microorganisms are often overlooked in favour of larger, more visibly affected freshwater life but are vital to healthy community function. Algae, bacteria and protozoa are crucial to the maintenance of stable phytoplankton populations and influence multiple trophic levels in freshwater communities (Pomeroy and Wiebe, 1988). Downing et al.(2003) showed that as well as reducing microbial diversity, some pesticides could change relative abundances between taxa and that different pesticides changed these in opposite ways (e.g. atrazine increased the relative abundance of diatoms and chlorothalonil reduced their relative abundance).

The common theme of pesticide effect variability is applied to the microbial community by DeLorenzo, Scott and Ross (2001) as well. The article confirms the assumption that herbicides will affect photosynthetic microbes more severely than others. This has implications for heterotrophs that would normally rely on nutrients provided by such producers as well as for competing autotrophs. Whilst herbicides might seem to be the most threatening pesticide class to photosynthesising algae, other pesticide types cannot be assumed to be safe as there is evidence that fungicides inhibit carbon uptake by algae (Peterson et al., 1994) and carbamate insecticides can be also be toxic to algae and cyanobacteria (Ma et al., 2006). This suggests that where one might expect microbial communities to take up part of the nutrient cycling and decomposition function of invertebrates after an insecticide spill, they may be unable to do so.

Trophic interactions are known to both be affected by pesticide presence, and to alter the pattern of pesticide damage in a system. The trophic effects of a stress are generally defined as top-down (whereby a consumer's change affects producers or lower level consumers) or the inverse, known as bottom-up. Trophic cascades in either direction can be caused in freshwater systems by behavioural, physiological or population attributes of a species (Brett and Goldman, 1996). It has been argued that indirect effects (such as those transmitted by trophic cascades) are more important than direct systems and so non-target organisms might make more sensitive system stress indicators than those actually targeted by the class of pesticide spilled (Lampert et al., 1989).

Top down changes effected by esfenvalerate (see table 2) have been identified in two outdoor mesocosm studies. One recorded that the resulting reduction in bluegill (*Lepomis macrochirus*, a sunfish) survival and abundance was associated with an increase in abundance of large zooplankton and phytoplankton, which would normally be consumed by the bluegill (Fairchild et al., 1993). Further cascade effects were not clear in causation and so could not be so clearly attributed to top-down modification. At higher concentrations (>0.2µg/L) the esfenvalerate eliminated most crustaceans, chironomids and juvenile bluegills but grazing gastropods and oligochaetes were found to increase in relative abundance (Lozano et al., 1992). Whether this is a top-down cascade effect (reduced bluegill predation) or a result of reduced competition from crustacean and chironomid decline remains unclear but Fairchild et al.'s (1993) findings would suggest that the trophic cascade is partly responsible. The effect difference between adult and juvenile bluegill could be physiological or it could be due to the ontogenetic niche shift that bluegill (and many other freshwater fish species) undergo, moving from feeding on invertebrates in littoral vegetation (as juveniles) to feeding on pelagic zooplankton in their adult stage (Mittelbach, Osenberg and Leibold, 1988). The fact that juvenile bluegill feed in the littoral zone to avoid predator stress rather than because of any nutritional preference (Werner and Hall, 1988) might suggest that the contaminant stress could have a similar effect to predator stress, adding to the positive change observed in (generally pelagic) zooplankton and phytoplankton abundance.

Chlorpyrifos has also been the focus of top-down trophic interaction studies. Macrozooplankton abundance reductions were strongly associated with increases in microzooplankton abundance, an effect that was suggested to be caused jointly by predatory and competitive (by the macrozooplankton) release (Papst and Boyer, 1980). It was also suggested that where algal blooms would normally occur after a pyrethroid treatment (Hughes et al., 1980) the increased grazing rates of the microzooplankton kept the algal abundance at a normal level for around two days before it began to increase as would have been expected with the consumption release (Hurlbert, Mulla and Willson, 1972). This could be evidence for a secondary cascade effect which, although likely to exist, is difficult to demonstrate experimentally with pesticide treatment. Carbamate insecticides appear to cause similar top-down cascades with the elimination of *Daphnia* and decline in zooplankton biomass resulting in increases in algal biomass (of which *Daphnia* are grazers)(Havens, 1995).

Bottom-up effects from pesticide contamination typically involve herbicides that damage producers and so atrazine, a common triazine, is frequently chosen for assessment. A clear demonstration of two bottom-up trophic cascades was found in two experimental pond studies by deNoyelles et al. (1989). The atrazine treatment damaged the submersed and emergent macrophytes which inflicted further indirect damage on the tadpoles, benthic insect grazers and macrophyte-feeding fish (bluegill and grass carp (*Ctenopharyngodon idella*)) that rely on macrophyte production. However, the producers that were more tolerant to the atrazine did not pass on indirect damage to their consumers. Phytoplankton biomass was not severely reduced by the treatment and therefore no significant treatment effects were observed in the crustacean zooplankton and filter-feeding fish *Dorosoma cepedianum* that fed upon it. These consequences of macrophyte production decrease were supported by observations in eight earlier pond mesocosms, with different assemblages to the deNoyelles et al. study (1989), which showed that herbivorous insect population sizes were greatly reduced with atrazine treatment whilst predatory insect populations were not as affected (Dewey, 1986).

The pond-based results were found not to differ greatly from those in artificial indoor streams where a herbicide directly reduced periphyton and thus indirectly reduced oligochaete population growth-rates (by 50% at just 6 µg/L) by reducing production in the periphyton that the oligochaetes consumed. The indirect effects on the oligochaetes occurred at a concentration three orders of magnitude lower than the LC₅₀ for the species (Brust et al. 2001).

The bottom-up trophic effects of insecticides are less studied than herbicides, but also show significant indirect damage. When chlorpyrifos was added to a series of littoral mesocosms contained in a natural pond the diet of the native fathead minnow (*Pimephales promelas*) larvae was forced to change by reduced invertebrate populations and their growth rates were significantly reduced (Brazner and Kline, 1990). Another article notes that in the same natural littoral mesocosms, the invertebrate responses to chlorpyrifos were more severe than would be predicted using laboratory derived LC₅₀ values resulting in a reduction of the larval growth-rate of native fish (Siefert et al., 1989). Therefore, even if fish mortality is not observed in a pyrethroid insecticide spill, there could be potential for damage in larval development some time after the event.

Fleeger, Carman and Nisbet (2003) highlight the difficulty in separating trophic cascade effects from those of competitive release within trophic levels despite the very different processes that cause them. The authors call for future research to carefully distinguish between such effects but this is difficult without compromising the complexity of an experimental system and diminishing the worth of a study in reflecting natural responses. It could be argued that, with the sporadic covering of present research, the target should remain accurately modelling natural responses rather than debating the relative strengths of the processes that cause them.

In marine systems, keystone species have been identified that are important in community function and composition and can indirectly affect many other species when faced with contaminant stress (Dumbauld, Brooks and Posey, 2001). In freshwater however, there is not a clear consensus on which species qualify as keystone and although some have been suggested most are labelled based on speculation and anecdotal evidence (Bond, 1994). Of those that have been suggested (and that are geographically widespread) amphibians (Kupferberg, 1997) and *Daphia* (Sarnelle, 2005) are amongst the strongest cases. Both have been found highly vulnerable directly and indirectly to pesticide concentrations well below those expected of a spill and so the topic of keystone species damage in freshwater communities is certainly one that warrants further investigation, both to identify species that are vital (for possible translocation when the pesticide concentration returns to habitable levels or monitoring) and what effect their removal has on a community and ecosystem.

Due to the influence of a freshwater body on its catchment area, the question of indirect effects of a pesticide spill on the surrounding riparian and terrestrial community should not be ignored. Adjacent riparian biomes are linked to the aquatic community by trophic interactions in both directions (Nakano, Miyasaka and Kuhara, 1999; Bastow et al. 2002; Paetzold, Schubert and Tockner, 2005) and it is feasible that damage could travel by trophic cascades to terrestrial ecosystems (Knight et al., 2005) or even microbial communities in local groundwater that are known to act as both a source and sink of organic matter for conventional freshwater bodies (i.e. Those featured in table 1) (Brunke and Gonser, 1997). Effects in other biomes may also be triggered by changes in populations of species that have complex life cycles with both terrestrial and aquatic stages, such as by emergence reduction of insects in bog ponds (Fairchild and Eidt, 1993).

The effect on terrestrial fauna could be worsened by biomagnification (Henny et al., 2002). In the freshwater system the hydrophobic behaviour of many pesticide groups (such as organochlorines) means that concentrations in sediment and organisms can be far higher than found in the water column. Concentrations were seen to increase with trophic level to over 140 times the ambient level in fish (Favari et al., 2002) in a reservoir polluted with organochlorine and organophosphate compounds. Ellegehausen et al. (1980) note that damage caused by the bioaccumulation of hydrophobic and lipophilic substances is often mitigated by the slow entry into the food web when the compounds adhere to sediment, suggesting that systems with less sediment (e.g. lotic headwaters) may exhibit greater bioaccumulation than lentic systems or sediment-filled lotic lower reaches.

Effects on ecosystem processes and conditions

Aside from community structure damage, some work has looked at the way pesticides interrupt ecosystem and community processes and conditions as a whole and the most significant findings are given below:

Oxygen concentration: Herbicides offer a clear cause for reduction of oxygen levels in destroying photosynthesising organisms and this is supported by mesocosm experiments (Jüttner et al., 1995) but Dewey reported no change in experiment ponds (1986). The literature is far from conclusive in deciding the effects of other pesticides as well. Organophosphate insecticides have been shown to

initially drop oxygen concentrations before a rise above original levels in mesocosms with macrophyte communities (Kersting and van der Brink, 1997). This is explained by an algal bloom several days after treatment by Butcher, Boyer and Fowle (1977).

Primary productivity: This measure is linked to oxygen concentration but excludes the effects of any oxygen depleting functions (i.e. respiration, decomposition) and is generally assessed by measures of phototropic carbon assimilation and chlorophyll α concentrations. Natural canal assemblages in microcosms were found to have both chlorophyll α and carbon assimilation reduced significantly by the strongest treatment of atrazine (Downing et al., 2004). However, as with oxygen concentration, there is also evidence for contrary effects. Schaffer and Sebetich (2004) tested two herbicides and one algicide on phytoplankton assemblages and found that the three compounds worked as stimulants to primary production in some concentrations and inhibitors (up to 45% relative to untreated controls) at others.

pH levels: Cuppen et al. (1997) report lower pH values after treatment, with the strength of the effect increasing with treatment concentration of a phenylurea herbicide. This effect has also been found with the herbicide atrazine (deNoyelles et al., 1989).

Habitat provision: Although this is difficult to measure experimentally, it is likely that destruction of macrophytes by a herbicide spill would also therefore affect littoral fauna populations, which rely on macrophytes for nutrients (direct consumption or consumption of habitat periphyton) and for cover from pelagic predators (Carpenter and Lodge, 1986).

Decomposition: Herbicides seem not to have great effect on decomposition rates (of particulate organic matter) in freshwater systems (Cuppen et al. 1997) although a field study of leaf-litter breakdown in streams found that pesticides (in combination from agricultural run-off and leaching) reduced breakdown rate significantly (Schäfer et al., 2012). In addition, the reduction in diversity by elimination of sensitive species in a spill would also impact degradation rates, possibly even in guilds thought to have high levels of redundancy (Jonsson and Malmqvist, 2000).

Analogous terrestrial effects

Whilst the habitat and organisms are different, the study of terrestrial effects of acute pesticide application may offer some insight into as yet unstudied aspects of freshwater aquatic responses. Barrett's (1968) early efforts at understanding terrestrial pesticide community effects in an enclosed grassland identified many of the now known trends in aquatic systems such as reduced decomposition rates and inequality in sensitivity between taxonomic groups and species. Recent discoveries of indirect pesticide effects on eusocial insect colonies (Gill et al., 2012) suggest that foraging and behavioural effects could be more widespread or complex in aquatic systems than currently known. Terrestrial effects are, however, more focussed on chronic exposure rather than acute point-sourced exposure (i.e. a spill event) as terrestrial spills tend to drain into groundwater and soils rather than effecting large fractions of an ecosystem as would occur in an aquatic setting .

Recovery and damage determinants

The duration and recovery time of a pesticide spill could be considered a good measure of the damage where full recovery is possible for the context of ecosystem service provision. Brock and Budde (1994) conclude that recovery from pesticide stress would depend four main factors (1-4). Three further criteria have been added (5-7):

1. *The persistence of the pesticide in the affected system-* Increased persistence has been shown to amplify community effects in zooplankton mesocosms (Hanazato and Masayuki, 1990a) and community recovery times are longer when traces of the stressor remain (Niemi et al. 1990). The length of persistence is impossible to generalise as it depends on many chemical attributes of the hundreds of pesticides available but varies from days to decades (Meharg et al. 2000; Raven and George, 1989). In addition the variety of freshwater environments and their characteristics makes case comparisons largely invalid (table 1). Fast flowing rivers have been shown to have better self-purification rates than slow flowing rivers (Warren et al., 2003) and rivers would self-purify by 'flushing' much faster than lentic bodies of similar size. The hydrophobicity of many organic pesticides can mean that even when communities appear to have recovered (no lethality observed) after a spill,

there are still pesticide traces in sediment and organism that can act as both sink and secondary source of contaminants over time (Long et al. 1998).

2. *Recovery time of the physio-chemical environment*- This largely depends on the previous (1) measure. Any changes in the chemical properties (e.g. pH, temperature, nutrient availability) are generally thought to occur as a result of biotic processes (i.e. increased decomposing matter, lower primary productivity)(Vanni, 2002) and so whether this does effect recovery rate itself is questionable. In cases where extreme spills (or compounds spilt in a pesticide application mixture) do change the chemical environment beyond the point at which the community can regulate it, the duration would depend on the abiotic degradation rate of the compound and the flow rate of the system.

3. *Generation time of affected organisms*- The mechanism for this criteria is clear (as long as a viable population remains) but there are also implications for biomonitoring. Many invertebrate species used to monitor freshwater system health have short generation times (e.g. Chironomidae) and so damage can be missed by monitors due to rapid population recovery that would more heavily impact species with longer generation times even within invertebrates (Takamura, Hatakeyama and Shiraishi, 1991).

4. *The ability of eliminated populations to recolonise the affected area*- This depends on several further sub-criteria such as the life-history of the affected organisms and the availability and access to nearby refugia (Yount and Niemi, 1990). The migratory mechanism has been demonstrated to be more important than reproductive capacity in spill-affected fish populations but this could vary by organism (Johnston and Cheverie, 1980).

5. *The downstream consequences*- This criteria accounts for the fact that lotic systems may recover rapidly by 'flushing' a pesticide spill and thus move the damage to estuarine or marine communities. A spill of a mixture of pesticides and other chemicals on the Rhine river in 1986 found that an organophosphate was detectable over 850km downriver from the point of the source, meaning that major spills in most rivers could easily reach estuarine or marine environments in high concentrations, especially given that the majority of agricultural development occurs around rivers' lower reaches (Capel et al., 1988).

6. *Interaction or additive effects of other stressors*- Whilst a pesticide spill would likely create the most intense stress that a community is facing, pesticide spills are not the only anthropogenic (or natural) stress that freshwater systems face. The stresses of nutrient loading, acidification, flow disruption and chemical pollution are widespread (Malmqvist and Rundle, 2000) as are the threats of invasive species and habitat destruction (Dudgeon et al., 2006). Stressed ecosystems are more vulnerable to invasion by non-native species (Sutherst, 2000). Climate change is predicted to exert stresses on freshwater ecosystems with both temperature changes and carbon dioxide driven changes in detritus input composition (Woodward, Perkins and Brown, 2010). Given the evidence that combined stresses often lead to greater damage than single stresses (Relyea and Mills, 2001; Janssens, 2013) there is potential for future pesticide spills to cause far greater damage than has previously been witnessed, regardless of whether these stresses operate additively or synergistically (Coors and Meester, 2008) (Darling and Côté, 2008).

7. *The timing of the spill*- The time of year at which a spill occurs is another determinant of the effects and recovery ability of a community. Plankton communities in pond mesocosms responded differently depending on the season and size of autumnal plankton blooms (Jüttner et al., 1995) and recovery of some organisms of a zooplankton community was observed to take longer with seasonal temperature drops (Hanazato and Masayuki, 1990b).

Research trends and gaps that need addressing

Relyea and Hoverman (2006) showed that current pesticide studies are generally increasing in number annually and that different levels of community complexity are all well represented. A more recent review showed that although the total number of pesticide effect publications is increasing, there is an increasing focus on organophosphates and organochlorines that leaves some groups (such as imidazoles) relatively neglected and a steadily increasing proportion of effects-based articles in pesticide publications (from 10% to 35% between 1988 and 2013) (Köhler and Triebskorn, 2013).

That research effort is increasing despite consistency in the global rate of pesticide use is encouraging. However, the vast number of variables discussed in this review means that for accurate prediction of spill-effects an unviable number (for effort and ethical reasons) of field or field-mesocosm studies would be required. For this reason, any accidental spills should be seen as valuable opportunities for further understanding. Modelling for pesticide effects is still relatively new as an application but recent studies have addressed chronic exposure in non-aquatic biomes (Engelman et al., 2012) and ecological effects in an agricultural context (Amano et al., 2011) and show promise for future freshwater application when combined with hydrological models that can describe a pollutant's behaviour in a river environment (Mossman, Schnoor and Stumm, 1988).

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