

**REVIEW AND
SYNTHESIS****Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems**

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Abstract

The field of ecotoxicology is experiencing a surge in attention among ecologists as we gain a deeper appreciation for how contaminants can impact natural ecosystems. This interest is particularly strong in aquatic systems where many non-target organisms experience pesticides. In this article, we assess how pesticides affect freshwater systems by applying the conceptual framework of density- and trait-mediated indirect effects from the field of basic ecology. We demonstrate the utility of this framework for understanding the conditions under which pesticides affect species interactions, communities and ecosystems. Through the integration of laboratory toxicity tests and this ecological framework, ecotoxicologists should be better able to identify the mechanisms through which pesticides affect communities and ecosystems. We also identify several areas of research that are in critical need of empirical attention including synergistic effects between pesticides and natural stressors, the importance of pesticides on community assembly via habitat preferences and oviposition effects, the timing and frequency of pesticide applications, pesticide effects on population dynamics, the evolution of pesticide resistance in non-target organisms and ecosystem recovery. With this knowledge, one can improve upon management decisions and help protect non-target species that are of conservation concern.

Keywords

Biodiversity, density-mediated indirect effects, ecosystem function, fungicide, herbicide, insecticide, trait-mediated indirect effects.

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INTRODUCTION

The fields of ecology and toxicology have largely evolved as separate disciplines over the past century with unique journals, unique tools and a distinct jargon that has reinforced an allopatric evolution of ideas. While ecologists have focused on how biotic and abiotic factors affect species distribution and species interactions, toxicologists have traditionally focused on single-species toxicity tests. There is, however, the growing field of ecotoxicology, a name that naturally implies a hybrid of ideas and approaches from ecology and toxicology. Given that the number of ecotoxicological studies has experienced tremendous growth in the past decade, it is an excellent time to evaluate what we have learned. In this essay, we evaluate the study of ecotoxicology in freshwater systems, an arena that has received a great deal of research focus. In doing so, we examine how one can use general ecological theory to

integrate ecology and ecotoxicology to better understand and conserve the ecology of aquatic systems.

TOXICOLOGY VS. ECOTOXICOLOGY

The rise of ecotoxicology (also termed ‘environmental toxicology’) is generally associated with the 1960s and the first formal definition came from Truhaut (1977) who considered ecotoxicology to be the branch of toxicology concerned with the effects of pollutants on the constituents of an ecosystem in an integrated context. A number of variants on this definition have appeared over the years (reviewed in Newman 1998), but all of them embrace much of Truhaut’s (1977) original concept. However, even with these definitions, there will always be some disagreement over what types of studies qualify as ecotoxicological; some researchers favour single-species toxicity tests (e.g. LC₅₀ tests to determine the lethal concentration of a pesticide that

is expected to kill 50% of a test population) that serve as definitive tests of cause and effect while others favour tests that include both single-species and multispecies contexts under more complex, natural conditions.

A number of excellent reviews of aquatic ecotoxicology have been conducted during the past decade (de Noyelles *et al.* 1994, Brock *et al.* 2000a,b, Fleeger *et al.* 2003; also see Special Features in Environmental Toxicology and Chemistry 1996 and Ecological Applications [1997]). These reviews have provided extensive information on the direct toxic effects of a wide range of contaminants and, in some cases, the indirect effects of pesticides that occur when a given species is eliminated or reduced in number. Our goal in this review and synthesis article is not to re-review this extensive literature, but to consider the effects of lethal and sublethal pesticide concentrations in systems containing multiple species and apply an emerging framework of food web theory (illustrated with a few key examples) to allow a more general conceptualization of how pesticides affect aquatic communities.

WHAT KIND OF RESEARCH HAS BEEN CONDUCTED?

To evaluate any field of study, it is often instructive to examine patterns in the literature to determine where we have and have not been placing our research efforts. With this goal in mind, we surveyed the literature for ecotoxicological studies of pesticides in freshwater systems to understand the types of studies that have been conducted. As noted by Fleeger *et al.* (2003), the vast majority (80%) of aquatic toxicology studies are freshwater systems. We searched the freshwater aquatic literature from the past 14 years (1992–2005) using the Web of Science bibliographic search engine and a number of key words [(pesticide or insecticide or fungicide or herbicide) and (community or pond or stream or mesocosm or tadpole or insect or snail or fish or zooplankton or algae)]. Our operational definition was that a study had to include at least two species that could potentially interact. Thus, we did not include single-species toxicology studies or studies that examined multiple species that were each raised separately. From a total of 319 studies that hit upon these keywords, 133 studies (42%) included at least two species that could potentially interact.

The studies exhibited a number of interesting patterns. For example, the number of ecotoxicological studies published annually has steadily increased from 1992 to 2003 with a large increase during the past 2 years (Fig. 1). Across all years, 71% of studies have been conducted in lentic systems (lakes, ponds and wetlands) while 29% have been conducted in lotic systems (streams and rivers). Among these studies, most (80%) have examined a single

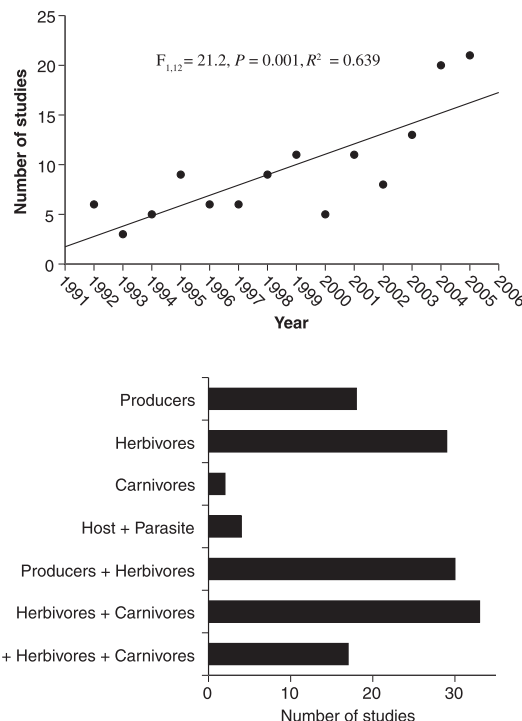


Figure 1 Upper panel: the number of ecotoxicological studies published from 1992 to 2005 in aquatic systems in which there were at least two species potentially interacting. Lower panel: the number of ecotoxicological studies by trophic group.

pesticide with considerably fewer studies that compared different pesticides applied separately (8%) or mixtures of pesticides (8%; an additional 4% were field surveys). Among the single-pesticide studies, the major research focus has been on insecticides (65%), with a moderate focus on herbicides (33%), and a minor focus on fungicides (2%). In contrast, data from real-world applications of pesticides (based on the mass of active ingredient; Donaldson *et al.* 2002) indicate that herbicides were actually the most commonly used pesticides (52%), followed by insecticides (35%) and fungicides (13%). Thus, the most commonly used category of pesticides has not received the majority of our research attention.

The types of studies were wide ranging including surveys of natural habitats after contamination (14%), surveys of natural habitats with experimental contamination (10%), field experiments using enclosures (16%) and mesocosm studies (60%). Among the mesocosm studies, most were conducted as pond mesocosms in large outdoor tanks (42%) or in laboratory tubs (35%), while others were conducted in artificial streams (19%) or small outdoor tubs (4%). The number of trophic levels examined in a study also varied, with most of our attention given to only herbivores or simple food webs composed of herbivores plus producers

or herbivores plus carnivores (Fig. 1). The taxa examined in these studies include macrophytes (24 cases), periphyton (32 cases), phytoplankton (37 cases), macroinvertebrates (65 cases), amphibians (20 cases), zooplankton (48 cases), fish (14 cases) and parasites (four cases).

PESTICIDE OCCURRENCE IN AQUATIC SYSTEMS

Pesticides have the potential to enter aquatic habitats from direct application, terrestrial runoff or wind-borne drift. Because there are thousands of different pesticides used around the world, data on aquatic contamination for any particular pesticide is usually quite limited. However, studies conducted in lentic and lotic systems have detected a variety of pesticides including the insecticides malathion, endosulfan and diazinon as well as the herbicides atrazine and glyphosate (LeNoir *et al.* 1999, Hayes *et al.* 2002; Kolpin *et al.* 2002, Thompson *et al.* 2004). Interestingly, many pesticides found in aquatic systems are not intended or legally registered for application to aquatic systems, but they still appear (e.g. Thompson *et al.* 2004). The concentrations found in surveys of natural habitats are often lower than the concentrations used in experimental tests, although these surveys are typically snapshots in time that are not always designed to detect peak concentrations (i.e. at the time of application). In most cases, we simply lack extensive data on natural pesticide concentrations to properly evaluate the validity of concentrations used in experiments. Given that pesticides find their way into aquatic systems, the relevant question is whether they affect the species in these systems.

HOW PESTICIDES CAN AFFECT SPECIES INTERACTIONS

To address the effects of pesticides on aquatic communities, we must first consider the ways in which pesticides might affect interactions between species. The magnitude of direct interactions (i.e. between two species) is a function of the density of each species and the per capita interaction strength between the species (which is a function of individual traits such as behaviour, morphology and physiology). Hence, the magnitude of a direct interaction can be altered anytime we change a species' density or traits. However, when there are more than two interacting species, there is the possibility of indirect effects in which one species affects the abundance of another species via a third, intermediate species. Such indirect effects include top-down or bottom-up trophic cascades, keystone predation, exploitative competition, apparent competition and indirect facilitation (Abrams *et al.* 1996).

The traditional focus in ecology has been on how changes in a species' density generate indirect effects (termed

'density-mediated indirect effects'), but there is a growing appreciation in basic ecology that changes in a species' traits can also generate indirect effects (termed 'trait-mediated indirect effects'; Abrams 1995). For example, predators can directly reduce herbivore density and thereby indirectly favour producers. However, predators can also induce herbivores to forage less which also indirectly favours producers (Fig. 2a; Werner & Peacor 2003; Peacor & Werner 2004; Schmitz *et al.* 2004). Importantly, these two processes occur simultaneously in nature as predators kill herbivores (thereby reducing the prey density) and, by killing the prey, induce herbivores to alter their traits (i.e. inducible defences; in aquatic systems, these trait changes are typically induced via water-borne chemical cues emitted by predators when they eat prey). Indirect effects caused by trait-mediated mechanisms can be of similar magnitude as indirect effects caused by density-mediated mechanisms (Peacor & Werner 2004; Schmitz *et al.* 2004; Preisser *et al.* 2005).

The above conceptual framework has become an extremely useful way to think about the structure and function of ecological communities. Because pesticides can also affect the densities and traits of organisms, we propose that this framework may also be effective for considering how pesticides cause indirect effects in ecological communities. For example, at relatively high concentrations, pesticides will be lethal and thereby cause density-mediated indirect effects (de Noyelles *et al.* 1994, Brock *et al.* 2000a,b; Fleeger *et al.* 2003). However, there is a growing appreciation that lower (i.e. sublethal) concentrations of pesticides can alter a wide range of individual traits including changes in neurotransmitters, hormones, immune response, reproduction, physiology, morphology and behaviour (including reduced foraging and changes in swimming ability, predator detection, learning and social interactions; reviewed in Weis *et al.* 2001). While these trait changes certainly affect the individual, they may also cause trait-mediated indirect effects in the communities in which the individuals are embedded. Unlike the traditional conceptualization of density- and trait-mediated effects in which an organism (e.g. a predator) is the initiator of density and trait changes, in our conceptualization the pesticide is the initiator of density and trait changes (Fig. 2).

There have been few attempts at modelling the combined density- and trait-mediated indirect effects of pesticides on ecological communities. The most recent models have examined simple two-species (consumer–resource) models to address how pesticides could affect resources by altering the density and behaviour of consumers (Preston and Snell 2001; Fleeger *et al.* 2003), but without making the more generalized link to density- and trait-mediated indirect effects. Interestingly, these modelling efforts have been paralleled by several researchers in basic ecology who have

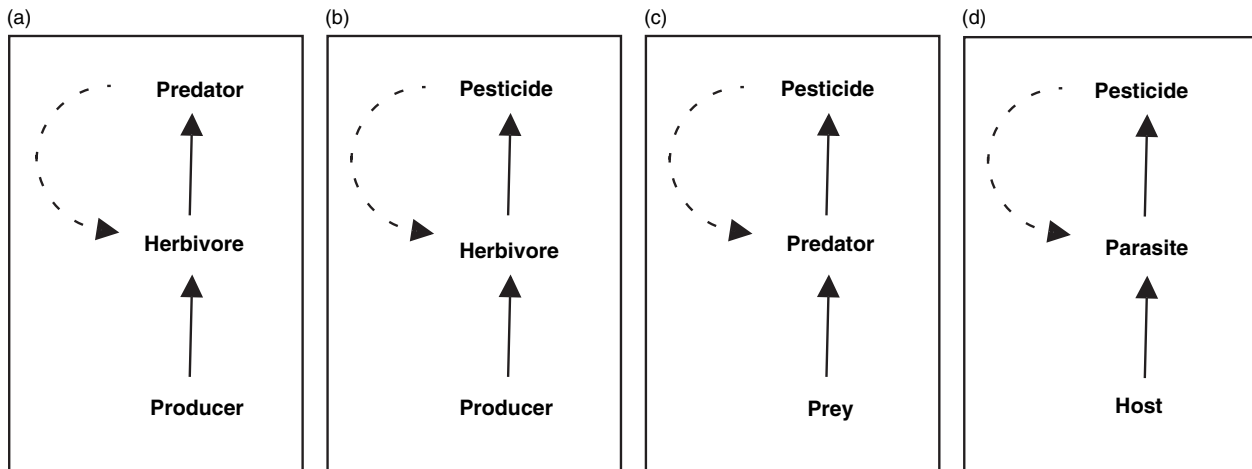


Figure 2 Density- and trait-mediated indirect effects in a food web that are initiated by either predators or pesticides. In panel (a), a predator initiates indirect effects on resources both by reducing the density of its prey (via consumption, solid lines) and by altering the foraging traits of its prey (via fear-inducing chemicals, dashed line). As demonstrated in panels (b–d), a pesticide can also serve as an initiator of indirect effects by reducing the density of a species (via direct toxicity) and inducing trait changes in a species (e.g. behaviour, growth, physiology or life history) such that the abundance of another species is indirectly affected.

explicitly modelled density- and trait-mediated indirect effects (e.g. Abrams 1995; Peacor & Werner 2004).

Conceptualizing the pesticide (rather than an organism) as the initiator of density- and trait-mediated indirect effects produces several interesting predictions. First, many organisms that are initiators (e.g. predators) cause simultaneous density- and trait-mediated indirect effects (although some trait-mediated indirect effects can be induced with minimal predation and, therefore, minimal density-mediated indirect effects). In contrast, depending upon the dose–response curves of a given pesticide on trait changes and lethality, pesticides may be capable of causing either primarily density-mediated indirect effects, primarily trait-mediated indirect effects or both processes simultaneously (Fig. 3). For example, consider a scenario in which we have an insecticide, an insect herbivore and a resource. Suppose that increasing concentrations of a pesticide initially reduce herbivore foraging, while further increases reduce herbivore survival (Fig. 3a,b). Using the models of Peacor & Werner (2004) for density- and trait-mediated indirect effects on resources at equilibrium, at low concentrations the addition of the insecticide makes the herbivore less effective at foraging for resources and this can have an indirect positive effect on the resource (i.e. a trait-mediated indirect effect). At high concentrations, the addition of the insecticide immediately kills the herbivore and this also has an indirect positive effect on the resource (i.e. a density-mediated indirect effect). Interestingly, at intermediate pesticide concentrations, only a portion of the herbivores will be killed and those that survive would exhibit reduced foraging ability. Thus, at intermediate concentrations, we should see the occurrence of both density- and trait-mediated indirect

mechanisms. These predictions are substantially different when the response curves for trait changes and mortality shift in relative position (Fig. 3c–e). Fortunately, for many pesticides and organisms we already have data from single species, laboratory tests on the range of concentrations that induce changes in traits and mortality. Most studies find that the concentrations that cause trait changes are considerably lower than the concentrations that cause mortality (Weis *et al.* 2001), suggesting that trait- and density-mediated indirect effects caused by pesticides may be a bit simpler to understand and predict than the simultaneous trait- and density-mediated indirect effects caused by organisms (i.e. predators, competitors and parasites).

Although trait-mediated indirect effects are likely widespread among different types of species interactions, the major current focus is on predator–prey interactions (Werner & Peacor 2003; Schmitz *et al.* 2004; Preisser *et al.* 2005). Thus, it could be valuable to directly compare the phenomena caused by predators and pesticides (Table 1). Both factors induce many of the same traits, but only the predator-induced responses are likely to be adaptive: predator-induced traits typically reduce the likelihood of being killed by predators whereas pesticide-induced traits probably do little to reduce the likelihood of being killed by the pesticide (although one might discover interesting exceptions to this latter generalization). Increased predator density should simultaneously cause increased prey death and larger anti-predator trait changes, thereby causing larger density- and trait-mediated indirect effects. In contrast, increased pesticide concentrations should initially cause trait changes and subsequently cause density changes via direct toxicity. The time scale of experiments may

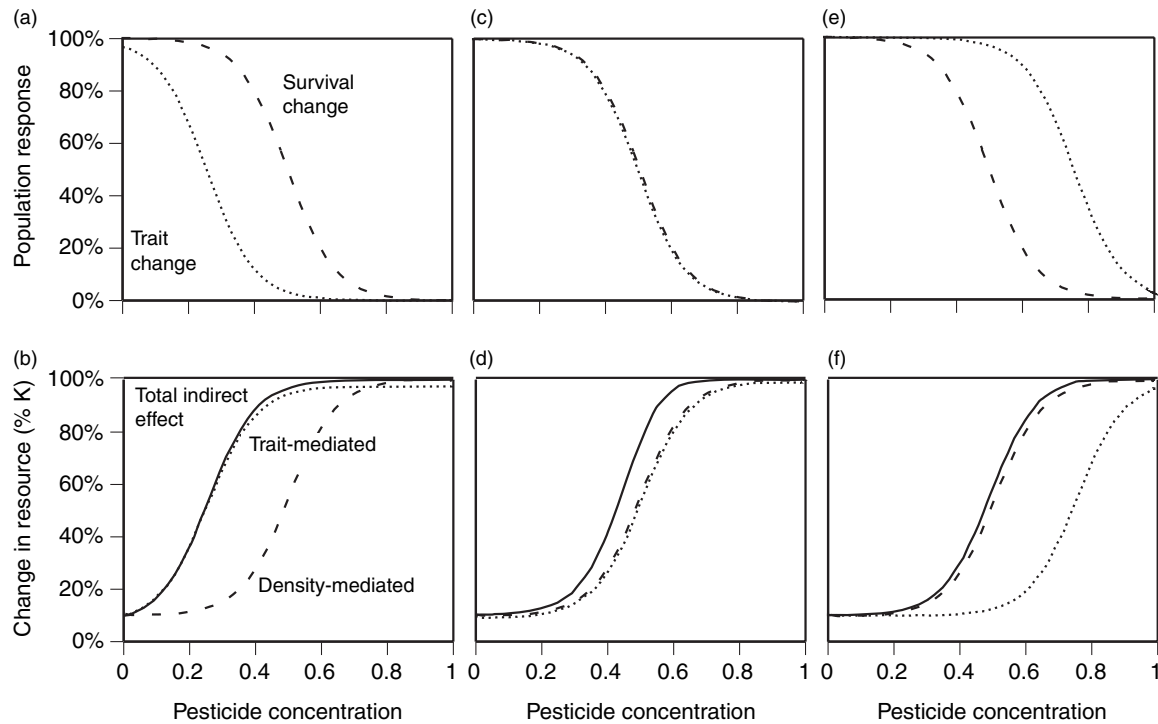


Figure 3 The relative importance of density- and trait-mediated indirect effects in a consumer resource system based on existing equilibrium models (Peacor & Werner 2004; assuming logistic functions of trait and mortality changes). In panel (a), increasing pesticide concentrations first induce trait changes (dotted line) and then cause mortality in the consumer (dashed line). As a result (panel b), trait-mediated indirect effects (dotted line) are important in determining the total indirect effect (solid line) at low concentrations but density-mediated indirect effects (dashed line) are not. At higher concentrations, both mechanisms are important. In panels (c, d), increasing pesticide concentrations cause trait changes and mortality at similar concentrations. As a result, both trait- and density-mediated indirect effects are important in determining the total indirect effect across all concentrations. In panels (e, f), increasing pesticide concentrations first cause mortality and then induce trait changes in the consumer. As a result, density-mediated indirect effects are important in determining the total indirect effect at low concentrations but trait-mediated indirect effects are not. At higher concentrations, both mechanisms are important in determining the total indirect effect.

also cause quite different effects. For example, in a simplified scenario, predation rates may remain stable over time (ignoring issues of gape limitation and prey size refuges) whereas pesticides can break down over time and switch from having lethal effects to having sublethal effects and, eventually, to having no effect (although there may be lasting developmental impacts). In reality, the comparative effect of time between predators and pesticides will likely be complex because trait- and density-mediated indirect effects over time critically depend upon predator dynamics (including predator emergence, colonization, reproduction and functional responses) and pesticide dynamics (including environment-specific breakdown rates and the impact of subsequent pesticide applications). Moreover, pesticides that are sublethal for short exposure durations can be lethal when exposed for longer exposure durations (i.e. chronic exposure). Thus, exposure duration and breakdown rates must be considered when we try to predict the impacts of pesticides using

basic ecological models that are focused on predators. Finally, recent reviews of predator-induced, trait-mediated indirect effects have concluded that these effects can be of similar magnitude as density-mediated indirect effects. Our earlier theoretical exploration suggests that the same should be true of pesticide-induced trait-mediated effects, but we currently have few data to arrive at any strong generalizations. With this conceptual framework as a background, below we review some of the more interesting examples of the two processes at work.

LETHAL PESTICIDE CONCENTRATIONS AND THE RESULTING DENSITY-MEDIATED INDIRECT EFFECTS

Herbivore–producer interactions

A prominent interaction in aquatic systems is between herbivores and producers. When herbicides are applied to

Table 1 A comparison of how predators and pesticides directly and indirectly affect other species in a freshwater community

Factor	Predators	Pesticides
Types of density changes	Reduced survival	Reduced survival
Types of trait changes	Behaviour, morphology, physiology, endocrinology and life history	Behaviour, morphology, physiology, endocrinology and life history
Adaptiveness of trait changes	Typically adaptive: trait changes offer a benefit of increased survival against predation but usually come at some fitness cost	Typically non-adaptive: trait changes offer no benefit to survival against the pesticide and usually come at some fitness cost
Effects of increasing predator number or pesticide concentration	Increased mortality and larger inducible defences of prey	First, there is an induction of trait changes, then an increase in mortality
Exposure duration	Longer exposure time should cause greater mortality (although prey defences can ameliorate predation risk)	Longer exposure time can make sublethal concentrations become lethal, but pesticides break down over time
Occurrence of trait-mediated indirect effects	Typically simultaneous with density-mediated indirect effects	Not necessarily simultaneous with density-mediated indirect effects; should dominate at low pesticide concentrations (generally unconfirmed)
Relative magnitude of trait-mediated indirect effects compared to density-mediated indirect effects	Similar	Generally unknown

lotic and lentic systems, the abundance of periphyton, phytoplankton and macrophytes will often decrease and, in turn, herbivore biomass decreases (Juttner *et al.* 1995; Brust *et al.* 2001). For example, the application of the herbicide atrazine to a lentic system resulted in lower periphyton abundance and, as a result, reduced herbivore biomass (Rohr & Crumrine 2005). Similarly, in plankton communities, the application of the herbicide Simetryn resulted in lower abundance of phytoplankton and, consequently, lower abundance of herbivorous zooplankton (Kasai & Hanazato 1995). However, bottom-up cascades are not always observed when herbicides are applied. In tadpoles, the addition of the herbicide Roundup caused a 75% decline in tadpole density and a concomitant 40% increase in the total amount of periphyton (i.e. a top-down cascade, Relyea 2005a). While it is possible that the periphyton community initially declined with Roundup (and may have altered the species composition of the periphyton), the large and immediate reduction in herbivore abundance drastically reduced grazing pressure during the recovery period. Thus, although herbicides are expected to negatively effect producers, they can also directly effect herbivores and influence the direction and magnitude of trophic cascades. In sum, these results suggest that ecotoxicologists should be able to use LC_{50} data to determine which species (i.e. producers vs. herbivores) are more sensitive to particular concentrations of herbicides and provide testable predic-

tions about the direction of indirect effects within a community.

We can also assess the effects of insecticides on the interactions between herbivores and producers. By targeting invertebrates, insecticides are expected to lead to top-down trophic cascades via reduced densities of invertebrate herbivores. For example, several studies of zooplankton have found that when insecticides are added, there can be large reductions in zooplankton abundance and, in turn, increases in the phytoplankton upon which zooplankton feed (Tidou *et al.* 1992; Barry & Logan 1998; Rand *et al.* 2000, 2001; Wendt-Rasch *et al.* 2003; Mills & Semlitsch 2004; van Wijngaarden *et al.* 2005). In addition to reduced grazing pressure, insecticides can also benefit producers via the release of nutrients from decaying animals. For example, after the application of the insecticide detamethrin, Knapp *et al.* (2005) attributed a phytoplankton bloom to the nutrients released from decaying herbivorous arthropods that were killed by the insecticide. Interestingly, the phytoplankton bloom was observed in open mesocosms where an adequate light supply was present, but not in shaded mesocosms, underscoring the importance of considering the interactive effects of abiotic conditions (see below, *Synergistic interactions between pesticides, abiotic factors and biotic stressors*). Collectively, the above studies make it evident that pesticides can have a variety of density-mediated indirect effects on herbivore–producer interactions.

Predator–prey interactions

A number of studies have shown that pesticides can reduce predator densities and lead to improved prey survival (Van den Brink *et al.* 1996; Woin 1998; Boone & Semlitsch 2003; Relyea *et al.* 2005). For example, Woin (1998) demonstrated that an oligochaete (*Stylaria lacustris*) benefited from the application of insecticides that eliminated insect predators. Moreover, after recolonization of insects into the mesocosms, oligochaete abundance decreased while the abundance of predator-resistant ostracods (*Herpetocypris reptans*) increased. Similarly, the insecticide malathion eliminated predatory beetle larvae (*Dytiscus* sp.) from pond mesocosms and positively affected the survival and growth of three tadpole species (Relyea *et al.* 2005). However, in mesocosms containing vertebrate predators (i.e. newts) that were not eliminated by the insecticide, tadpole survival was not improved by the addition of malathion. Thus, predator identity (e.g. invertebrate vs. vertebrate) plays an important role in the transmission of indirect effects in aquatic communities when pesticides are applied. In addition to predator identity, the relative sensitivity of predators and prey to insecticide concentrations can also affect the interaction. At low concentrations, prey may benefit from the addition of pesticides that target sensitive predators, but at higher concentrations both predators and prey may die. While we have a basic understanding of the indirect effects of pesticides on predator–prey interactions, future studies that incorporate real-world scenarios such as different predator species, different prey species, and different pesticide concentrations should be extremely insightful.

Interspecific competition

One of the most clear cases in which pesticides can affect interspecific competition can be found in examples of differential sensitivity to a pesticide that results in competitive release. For example, several studies have demonstrated that different groups of zooplankton, which compete for phytoplankton, have different sensitivities to insecticides (typically, cladocerans are more sensitive than copepods). Thus, when moderate concentrations of insecticides are added to communities containing both cladocerans and copepods, we see a dramatic decline of the more sensitive cladoceran species and a substantial increase in the abundance of copepods (Hanazato & Yasuno 1990; Hanazato 1991; Havens & Hanazato 1993; Havens 1994, 1995; Van den Brink *et al.* 2002; Relyea 2005a). Researchers also have examined the effects of insecticides on macroinvertebrate communities composed of several competing species (Van den Brink *et al.* 1996; Schulz & Liess 2001; Zrum & Hann 2002; Schulz *et al.* 2003). For example, because chironomids and crustaceans were more sensitive to

a mixture of lindane and chlorpyrifos than gastropods and oligochaetes, the latter increased in abundance in response to the pesticide addition (Cuppen *et al.* 2002). Differential sensitivity to herbicides has also been documented in producers. In a macrophyte–green algae system, the application of the herbicide linuron resulted in reduced macrophyte abundance but increased green algae abundance. While the herbicide had direct toxicity on the green algae, the nutrients released from decaying macrophytes benefited algal recovery (Slijkerman *et al.* 2005). From these studies, it is clear that differential sensitivity of competitors to pesticides can lead to competitive release.

SUBLETHAL PESTICIDE CONCENTRATIONS AND THE RESULTING TRAIT-MEDIATED INDIRECT EFFECTS

Herbivore–producer interactions

As noted above, sublethal pesticide concentrations that alter species' traits can also generate indirect effects in the absence of any changes in a species' density. For example, pesticides have the potential to alter herbivore–producer interactions by affecting the traits of the participants and thereby altering the interaction strengths between the species. Given that many pesticides (e.g. carbamates and organophosphates) inhibit animal foraging activity, one would expect this effect to cause a reduction in growth. Simple experiments using controlled food rations perhaps permit the strongest tests of this mechanism and the existing data suggests that pesticides do cause reduced consumption of food and slower growth (Relyea 2004a). If sublethal applications of pesticides lead to reduced grazing pressure, we might expect an increase in producer biomass (i.e. a trait-mediated indirect effect). From several non-pesticide-based studies, we know that trait-mediated indirect effects can be similar or larger in magnitude to density-mediated indirect effects (Werner & Peacor 2003; Schmitz *et al.* 2004). However, the role of pesticide-induced trait changes in affecting herbivore–producer interactions appears to have received little attention.

Predator–prey interactions

The effect of pesticides on predator–prey interactions has received particular attention. For example, pesticides can lead to non-adaptive behaviours in prey which result in lower survival. In stream mayflies (*Baetis*), organophosphate and pyrethroid insecticides cause mayfly larvae to spend more time on top of rocks and, as a result, become more visible to the fish and experience a 10 to 30 times higher rate of predation (Schulz & Dabrowski 2001). This finding suggests that the insecticide either interfered with the

mayfly's ability to detect the predator or with the mayfly's ability to adaptively respond to the predator by moving under rocks. Similarly, Dodson *et al.* (1995) found that high concentrations of carbaryl induced spinning behaviour in *Daphnia pulex* and individuals exhibiting this behaviour were preferentially killed by fish because they were more easily detected. A few other studies have demonstrated that sublethal concentrations of pesticides can increase predation rates, but the underlying mechanisms are unknown (Broomhall 2002, 2004).

Whereas sublethal effects from pesticides can have clear detrimental effects, positive trait-mediated effects can possibly offset this negative effect. For instance, acetylcholine esterase inhibitors (e.g. carbamates and organophosphates) induce prey to reduce their movement and foraging activity (Bridges 1997; Brewer *et al.* 2001; Glennemeier & Denver 2001; Punzo 2005). Because predation risk is often linked to time spent foraging (e.g. Relyea 2001), a pesticide-induced reduction in foraging should make prey less susceptible to predators. Moreover, these same insecticides should reduce the movement and coordinated neural function of predators, making any strikes on prey potentially much less effective. We have recently demonstrated this trait-mediated effect across a range of predator and prey species; the prey become less active, the capture efficiency of predators is reduced by 80%, and as a result, prey experience up to 73% better survival (R. A. Relyea and K. L. Edwards, unpublished data).

Interspecific competition

Competitive interactions may also be affected by the presence of pesticides via trait-mediated indirect effects. At sublethal concentrations, the effects of pesticides on competitive interactions will depend upon how the pesticide affects the abilities of each competitor to compete for the resources (trait-mediated indirect effect). One of the primary difficulties in understanding the relative importance of trait-mediated indirect effects in competitive scenarios (and thereby gaining predictive ability) is that very few studies involving pesticides and competition have quantified changes in the traits of each competitor. For example, a number of studies have shown changes in growth rates among species of larval anurans which compete (or potentially compete) for periphyton. However, rarely are there data on how the pesticides may have altered the foraging activity, foraging morphology or physiology of the competitors (Boone & Semlitsch 2001, 2002; Boone *et al.* 2004; but see Mills & Semlitsch 2004; Relyea 2005a). Only when we understand the mechanistic pathways in which pesticides can affect competitive outcomes will we begin to arrive at any general rules of thumb.

Parasite–host and pathogen–host interactions

A final type of interaction that can be affected by pesticides is that of parasites or pathogens and their hosts. We were unable to find any cases of density-mediated effects of pesticides on parasite–host interactions (although such effects likely occur when pesticides kill the host or the parasite). However, there are a few cases of trait-mediated effects of pesticides on parasite–host interactions. For example, a mixture of pesticides can reduce the proliferation of lymphocytes in amphibians, leading to a compromised immune system and an increase in the infection rate of lung parasites (*Rhabdias ranae*; Christin *et al.* 2003, 2004; Gendron *et al.* 2003). Similarly, Kiesecker (2002) observed that pesticides at low concentrations inhibited the immune systems of wood frog tadpoles (*Rana sylvatica*) which permitted an increase in the number of encysting trematodes. In an interesting alternative scenario, Ibrahim *et al.* (1992) discovered that an insecticide, which had no direct effect on the production of parasitic *Schistosoma mansoni* cercaria, was capable of completely preventing infected snails from shedding these cercaria (although the mechanism was unknown). In a host–pathogen system, Forson & Storfer (2006) found that low and moderate concentrations of atrazine reduced infectivity by a virus in larval salamanders (*Ambystoma macrodactylum*) and, in turn, reduced salamander mortality from the virus. The cause of this reduced infectivity was unknown, but the authors thought it might be related to compromised viral efficacy. While many of the existing studies on hosts and their parasites and pathogens are focused on amphibians (largely motivated by global amphibian declines), the observation that pesticides can have both positive and negative effects on parasite–host interactions likely applies to a wide variety of taxa. It is clear that this topic is in need of much further exploration.

The above studies demonstrate that sublethal concentrations of pesticides can cause a wide diversity of trait-mediated indirect effects. Because the mechanisms underlying these effects on interspecific interactions are intrinsic to any interspecific interaction and therefore potentially widespread, we need to incorporate trait-mediated indirect effects into our ecological studies of pesticides. When trait-mediated effects are operating, it is critical that we identify the traits that change and the mechanisms responsible so that we can develop general predictions about the direction and magnitude of pesticide-induced changes in predator–prey interactions. Therefore, future studies must move beyond just quantifying mortality rates and include observations of changes in behaviour, physiology and life history that are necessary to determine if sublethal doses of pesticides will affect the structure and function of communities.

PATTERNS IN DIVERSE COMMUNITIES

As we move from these simple linear food chains to more diverse communities, the expected outcomes become more complex and more interesting. For example, in more diverse communities, each trophic level or guild is represented by a number of species and each will have its own particular sensitivity to a given pesticide. As a result, the application of a pesticide might kill all individuals of one species, kill a portion of individuals of a second species, and kill none of a third species. For example, a recent community experiment by Relyea (2005a) found that the addition of insecticide carbaryl eliminated larval predatory beetles (*Dytiscus* sp.) but had little effect on the survival of larval dragonflies (*Anax junius*) and adult water bugs (*Belostoma flumineum*). At the same time, there was a 22–30% improvement in survival of wood frog and leopard tadpoles (*Rana sylvatica* and *Rana pipiens*; the prey of the insects). In this study and other studies like it (including experiments and surveys of contaminated natural systems), invariably the conclusion is that the improved survival occurred because the predator assemblage was reduced in number (i.e. a density-mediated indirect effect; Van den Brink *et al.* 1996; Woin 1998; Zrum & Hann 2002; Wendt-Rasch *et al.* 2003). However, we now know that while the reduced predator density does contribute to the indirect positive effect on the prey, we must also consider that the surviving predators likely had reduced capture efficiency and the prey likely were less active; both of these pesticide-induced trait changes would lead to improved prey survival. In short, just as we expect density- and trait-mediated effects to occur simultaneously on a given species at intermediate pesticide concentrations that kill some fraction of all individuals (Fig. 3), we also expect density- and trait-mediated effects to occur simultaneously in more diverse communities at intermediate concentrations that kill some fraction of the species from an assemblage (e.g. competitors and predators). Once again, if we know the lethal and sublethal effects of a pesticide from single-species laboratory experiments, we may be able to make testable predictions about the impacts of pesticides on diverse communities.

In more diverse communities, the effects will undoubtedly be more complex. For example, Preisser's *et al.* (2005) meta-analysis of indirect effects found that the density-mediated effects become weaker and trait-mediated effects become stronger as one moves from two to three trophic links. One might predict similar patterns in communities impacted by pesticides. However, in more complex communities we currently lack theory and sufficient empirical data even in basic ecology to draw any generalities about the effects of density- and trait-mediated indirect effects. The impacts of these two processes in communities contaminated by pesticides is currently

unknown and in need of both theoretical and experimental investigation.

EFFECTS OF PESTICIDES ON ECOSYSTEMS

When aquatic habitats receive pesticides, there can be substantial perturbation to the ecosystem. A major goal in many ecotoxicological studies has been to determine the perturbative effects of pesticides on ecosystem-level parameters including species diversity, primary productivity and abiotic conditions (e.g. pH and dissolved oxygen; for a comprehensive review, see Brock *et al.* 2000a,b). For example, the application of insecticides can reduce invertebrate diversity and abundance, decrease decomposition rates (via the loss of invertebrates) and increase primary productivity (as measured by chlorophyll *a*; Cuppen *et al.* 2002; Relyea 2005a). As many aquatic invertebrates have complex life cycles that include a terrestrial stage, insecticides can also impact the export of energy and nutrients to terrestrial ecosystems by reducing adult emergence (Fairchild & Eidt 1993) which may cause cross-ecosystem cascades (e.g. Knight *et al.* 2005). Ecosystems are also impacted by the application of herbicides. For example, reductions in producer biomass can lead to lower dissolved oxygen, reduced pH, increased alkalinity and increased conductivity (Juttner *et al.* 1995; Cuppen *et al.* 1997; Downing *et al.* 2004). Pesticides can also lead to changes in community structure as species that are more sensitive to the pesticide are eliminated and those that are more tolerant come to dominate the system (Fairchild & Eidt 1993; Relyea 2005a). Importantly, these changes in community structure are a function of the pesticide concentration applied to the ecosystem (Kreutzweiser *et al.* 2002). Once again, using data from laboratory experiments and an understanding of density- and trait-mediated effects among species in the ecosystem, ecotoxicologists can generate predictions about changes in ecosystem function (at least over the short term) across a range of pesticide concentrations.

Another way to examine the effects of pesticides on ecosystems is to assess resiliency or the ability of the ecosystem to return to its original state after perturbation. However, the focus of most pesticide research in aquatic systems has been to determine how pesticides alter the community after some pre-determined amount of time. Since natural communities continue through time, we need to determine whether and how communities recover from pesticide exposure. With all of our attention focused on what pesticides do to species and ecosystems, we need to step back and ask the longer-term question, what do species and ecosystems do once the pesticides are gone? Recovery time should depend on the species' ability to recolonize and generation time (Brock *et al.* 2000a,b), and on the half-life of the pesticide, which can range from days to years. Resiliency

can occur through the increased abundance of species still within the system or through migration of individuals from outside the system. For example, studies in lotic systems have found that macroinvertebrate diversity is resilient to the application of insecticides because individuals can colonize from upstream sites that are unaffected by the insecticide (Wallace *et al.* 1996). Similarly, insects can recolonize lentic systems after pesticide exposure, thereby contributing to the resiliency of the system (Van den Brink *et al.* 1996). This suggests that the regional species pool and metapopulation structure may play an important role for recovery of lentic systems.

There is some evidence that pesticides can have long-lasting effects on ecosystems. For example, Woin (1998) demonstrated that 2 years after the application of the insecticide fenvalerate an invertebrate pond community was significantly different in species diversity and abundance compared with control sites. Likewise, the application of herbicides can lead to reductions in producer biomass that persist due to species-specific recovery periods (Spawn *et al.* 1997). In sum, these studies show that ecosystem resiliency to pesticide exposure will depend on a number of parameters (beyond the lethal and sublethal effects on each member of the community) including pesticide breakdown rate, habitat type (lentic vs. lotic), migration rates, extinction and recolonization dynamics, species-specific sensitivity and species-specific recovery rates.

UNEXPLORED RESEARCH AREAS

The entrance of ecology into the field of toxicology is still relatively young and therefore we have only begun to address the diversity of possible questions that could be asked. In this section, we propose a number of research areas that have received little or no attention and yet are likely to produce interesting ecological insights.

Synergistic interactions between pesticides, abiotic factors and biotic stressors

Ecotoxicologists are beginning to appreciate that the effects of pesticides can change under different environmental contexts. For example, a variety of abiotic factors can alter the lethality of pesticides including changes in pH, temperature and ultraviolet radiation. In some cases, the interaction is an effect of altered breakdown rates of the pesticide which causes a change in the total exposure time to the organism (Wauchope & Haque 1973). However, in other cases, the mechanism responsible for higher lethality is unknown (Zaga *et al.* 1998; Edginton *et al.* 2004).

Synergistic effects between pesticides and stressors are not limited to abiotic factors. For example, Relyea & Mills (2001) discovered that the insecticide carbaryl (a carbamate

insecticide that inhibits acetylcholine esterase) became two to four times more lethal to grey tree frog tadpoles (*Hyla versicolor*) when predator cues were in the water than when predator cues were absent. Subsequent studies in other species of tadpoles demonstrated that predator cues were capable of making carbaryl up to 46 times more lethal (Relyea 2003). Subsequent studies have found that malathion (an organophosphate insecticide that also inhibits acetylcholine esterase) also can become more lethal in the presence of predatory stress (Relyea 2004b) as can the herbicide Roundup (containing glyphosate and a polyethoxylated tallowamine surfactant; Relyea 2005b). The mechanism underlying this phenomenon has yet to be identified and is an important area for future investigation. The phenomenon may not be restricted to predator-induced stress. For example, Hanazato & Hirokawa (2004) observed that the insecticide carbaryl has a much larger negative effect on *Daphnia* population size when they are experiencing the stress of limited food resources. This suggests that pesticides may interact with a range of different natural stressors.

The existence of this phenomenon also means that our current distinction between lethal and sublethal pesticide concentrations may in fact be a relatively blurry line. While traditional laboratory experiments may often correctly predict lethal and sublethal effects in a community, these experiments will not make correct predictions when the lethal effects change substantially with changing stressors. Consequently, synergies between pesticides and natural stressors will alter the importance of density- vs. trait-mediated indirect effects of pesticides in ecological communities. Because the existence of synergistic interactions poses serious concerns when extrapolating the results of standard (i.e. relatively stress-free) laboratory experiments to natural communities, it is imperative that we know how common such interactions are in nature and therefore whether they need to be incorporated into our predictions.

Pesticide effects on oviposition and habitat use

When pesticides contaminate aquatic systems, it is reasonable to expect that the contamination will be heterogeneous and that organisms might respond by altering their habitat use. Altered habitat use not only pertains to where an individual decides to spend its time, but also for many organisms with complex life cycles, where they decide to oviposit. For most aquatic communities, a major fraction of the biodiversity arrives via oviposition by terrestrial adults including insects and amphibians. Although pesticide impacts on habitat use and oviposition have been documented in terrestrial insect systems (e.g. Desneux *et al.* 2004), there appear to be no studies that have examined such effects in aquatic organisms. However, aquatic organisms are well known for avoiding habitats containing

predators, parasites and competitors which they likely detect via chemoreception (Resetarits & Wilbur 1989; Kiesecker & Skelly 2000; Murphy 2003). When we consider the impacts of predator, competitor and pesticide environments on habitat use and oviposition, we arrive at several intriguing possibilities. If organisms cannot detect pesticides (e.g. via chemoreception) but they can detect and avoid enemies (i.e. predators, competitors and parasites), they will unknowingly expose themselves or their progeny to a potentially harmful pesticide environment (depending upon pesticide concentration and breakdown rate). Alternatively, if organisms can detect and avoid pesticides (either due to adaptation or exaptation), they may concentrate themselves in pesticide-free habitats that contain enemies that reduce growth, development and survival. The above scenarios make it clear that we need to assess whether pesticides affect habitat use and oviposition decisions, how these decisions interact with decisions to avoid predators and competitors, and how these decisions affect the recovery of previously impacted systems.

Pesticide application time and frequency

When one compares the scenarios between experimental pesticide research and the actual application of pesticides in the world, one frequently observes a substantial disconnect. For example, in the real world, many pesticides are sprayed at particular times of the year (e.g. pre-emergent herbicides) while others are sprayed multiple times throughout a growing season (e.g. most insecticides). Thus, pesticides have specific application times and application frequencies that will often produce multiple pesticide pulses into a system. However, most laboratory experiments (and some mesocosm experiments) are 'renewal' experiments which maintain a constant pesticide concentration. In contrast, most mesocosm studies are single pesticide doses at the beginning of the experiment. It is unclear how these experimental traditions have developed, but it is clear that we need our experiments to be better connected to the reality of real-world applications. This timing matters because species have specific breeding seasons and particular periods in ontogeny that are sensitive to pesticides (e.g. Bridges 2000). Moreover, repeated applications of a pesticide may produce community effects that do not appear with a single application. Several years ago, Fleeger *et al.* (2003) reported finding no studies that explicitly examined the effect of exposure regime on indirect effects in freshwater systems and this still holds today.

Population dynamics

One of the most challenging issues to address is whether pesticide effects on individuals actually affect population

dynamics. For example, when a pesticide causes low to moderate mortality in a species or the feminization of males due to endocrine effects (e.g. Hayes *et al.* 2002), it is an open question whether these effects will affect population growth. Before we can make such an extrapolation, especially for taxa of conservation concern, we need to know whether any negative effects of pesticide exposure are additive or compensatory with other negative effects (e.g. predation) and whether the life stage examined is important to the growth of the population. Without long-term data on populations (including examining museum specimens; Reeder *et al.* 2005) or, alternatively, modelling the impacts of the mortality using life-table response experiments (Caswell 2001), extrapolation to future population-level impacts will remain conjecture. At the same time, it is important to appreciate that assessing the impacts of pesticides on population dynamics is not a trivial task and has thus far only been carried out (to our knowledge) on species with very short generation times (Sih *et al.* 2004). We need more work in this area, but the data will not come easily for most species.

The evolution of pesticide resistance

A common observation in single-species LC₅₀ experiments is that a moderate concentration kills many individuals of the test species, but not all of them. If heritable genetic variation for pesticide resistance exists, then populations may be able to evolve resistance. While such evolved resistance has been the bane of the pesticide industry when trying to eradicate undesirable pests, the same process may ensure the continued existence of many non-target species that we wish to conserve. Few studies have examined heritable genetic variation for pesticide resistance in non-target aquatic organisms (but see Kasai & Hanazato 1995; Semlitsch *et al.* 2000; Bridges & Semlitsch 2001) and, to our knowledge, no study has demonstrated natural patterns of more resistant populations of non-target organisms in more contaminated aquatic habitats. However, such patterns may be common in nature, despite strong density effects of pesticides that may lead to population bottlenecks that reduce genetic variation and thereby limit the ability of evolutionary responses.

Pesticide mixtures

While pesticides in nature frequently occur as mixtures of several pesticides, few studies have examined the impact of pesticide mixtures and even fewer have examined pesticide mixtures experimentally. The data from a limited number of studies suggest that pesticide mixtures can have both additive and synergistic effects, although in some studies one cannot separate the effects of combined pesticides from

the confounded increase in total pesticide concentration (Gendron *et al.* 2003; Christin *et al.* 2003; Relyea 2004a; Hayes *et al.* 2006). Thus far, mixture studies have largely focused on only one or two species at a time. We need studies that examine the effects of pesticide mixtures on whole communities. For example, mixtures of insecticides could have larger top-down effects than either insecticide alone whereas herbicides and insecticides would affect communities from both the top and bottom of the food chain.

CONCLUSIONS AND FUTURE DIRECTIONS

The field of ecotoxicology has experienced a surge of interest from ecologists and this has provided a great deal of insight into how anthropogenic chemicals can affect species, interactions, communities and ecosystems. The traditional approach of examining a few species of model organisms under highly controlled laboratory conditions is an efficient and necessary protocol for assessing the nearly 80 000 registered chemicals that exist. However, the outcomes of pesticide experiments under more natural conditions can generate very different outcomes due to both density- and trait-mediated indirect effects that cannot occur in single-species experiments. This is not to say that short term laboratory tests are without value. Indeed, such tests provide a tremendous amount of useful data to develop testable predictions about how pesticides might alter species' densities and traits and thereby alter community structure and ecosystem function. By integrating short term laboratory results with long-term community experiments, we can move from primarily phenomenological studies in which we add pesticides, quantify an outcome, and infer a mechanism to more mechanistic studies in which we determine the underlying causes of community and ecosystem changes. Only when we identify underlying mechanisms can we begin to develop a general understanding of how thousands of different pesticides impact aquatic ecosystems. Furthermore, while we have reviewed the research in freshwater aquatic systems, the phenomena discussed and the recommendation for a more mechanistic approach based on the density- and trait-mediated framework should be effective for a wide variety of systems (e.g. intertidal and terrestrial).

As we become more mechanistic in our approach, we are still faced with the daunting task of potentially evaluating thousands of pesticides. We propose that the best tactic would be for ecotoxicologists to prioritize this long list of potential candidates. For example, empirical work could focus on those pesticides that are the most widely applied and that occur in aquatic habitats based on survey data (LeNoir *et al.* 1999; Hayes *et al.* 2002; Kolpin *et al.* 2002; Thompson *et al.* 2004). Further, some pesticides can be grouped by mode of action (e.g. inhibitors of acetylcholine

esterase) which may allow us to develop generalities using a limited number of representative chemicals.

There are a large number of experimental questions that still need to be addressed. For example, we need to identify the general patterns of pesticide effects in aquatic systems to offer better predictive ability in the field of ecotoxicology, particularly when there are important management or conservation issues that rely on our knowledge and assessments of contaminated habitats. To achieve this goal, we need to examine the role of sublethal pesticide effects on individuals and communities to understand the conditions under which trait-mediated indirect effects are important to community structure and ecosystem function. Part of this effort includes further investigation of synergistic effects of pesticides, both when combined with other pesticides into a mixture and when combined with natural stressors. We also need increased attention on the recovery of contaminated ecosystems and how that recovery is shaped by the species that survive the contamination and the species that choose to colonize. We must better connect our experimental investigations to the real world of specific application times and application frequency. Finally, we should make an effort to look for heritable genetic variation and evolved resistance in natural population as well as attempt, where feasible, to determine how pesticides affect the population dynamics of aquatic organisms. Collectively, these challenges are certainly a tall order that will require a large number of investigators, a great deal of time and a substantial amount of resources. However, having this improved understanding provides a tremendous benefit to managing and conserving freshwater systems which provide important services to our society.

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