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Wildlife Ecotoxicology of Pesticides: Can We Track Effects to the Population Level and Beyond?

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During the last 50 years the human population has more than doubled and global agricultural production has similarly risen. The productive arable area has increased by just 10%, thus increased use of pesticides has been a consequence of the demands of human population growth and its impact has reached global significance. Although we often know a pesticide´s mode of action in the target species, we still largely do not understand the full impact of unintended side effects on wildlife, particularly at higher levels of biological organization: populations, communities, and ecosystems. In these times of regional and global species declines, we are challenged with the task of causally linking knowledge of molecular pesticide action to possible interference with biological processes in order to develop reliable predictions about the consequences of pesticide use, and misuse, in a rapidly changing world.

Wildlife ecotoxicology has its roots in acute poisoning events in the late 19th century, however, public concern over the undesirable environmental effects of chemicals arose in the early 1960s with the publication of Rachel Carson´s 'Silent Spring', which publically broached the issue of environmental risks of pesticide use for the first time. Shortly thereafter, DDT and its metabolites were found to be responsible for population-level effects in raptorial birds and, with the realization of the global nature of organochlorine pesticide contamination, long-range studies on wildlife exposure, mainly on the basis of environmental analytical chemistry, were launched (*1*). At that time, in industrialized countries, attention was focused on acute mortality effects in wildlife following pesticide use, abuse, or misuse, mostly involving birds or fish. Currently, pesticide use is widespread in agriculture all over the world but, still, only very few countries have established wildlife poisoning surveillance programs (*2*). As a result, many data on pesticides remain scattered and/or not publicly available (*3*). Even fifteen years ago incident registration was already considered an insufficient approach for understanding the side effects of pesticide use in agriculture (*4*). Further shortcomings inevitably associated with research on incidents are the difficulties discriminating between poisoning and other causes of death and limitations of the analytical detection of pesticides in carcasses (*2*).

Consequently, in the last 25 years, research interest has shifted from documenting incidents, and exclusively quantifying chemical exposure, to effect studies aimed at linking laboratory, mesocosm, and field experiments. Since the early 1990s, the proportion of effect-related publications has continuously increased, even though a high number of mechanistically-oriented studies have been conducted on laboratory or domestic species, particularly mammals. In terms of sheer numbers of publications, most research on wildlife ecotoxicology deals with fish, insects, and, to a lesser extent, birds, amphibians, and arachnids (Fig. 1a). Effect-related research, which has addressed insecticides, herbicides, and fungicides in a rather constant proportion of published papers for more than 20 years, does not reflect the proportions of active ingredients applied in the U.S. or Europe but, rather, overemphasizes the effects of insecticides (Fig. 1c,d). Within the literature on pesticide effects, increasing numbers of publications have been recorded for some distinct insecticide classes in recent years indicative of the importance of these currently dominating active ingredients (Fig 1b). In this context, the last five years have revealed particular progression of interest in the effects of

organophosphates, pyrethroids, and the rather 'new' class of neonicotinoids. However, there remains ongoing interest in first generation organochlorine pesticides, like DDT, which is still in use in many developing countries (*5*). Even though the banning of highly persistent organochlorines in developed countries has shifted pesticide use towards a vast diversity of readily biodegradable ingredients, the explosiveness of organochlorines on a global scale cannot be ignored. The FAO estimates that half a million tons of 'old', obsolete pesticides have been scattered throughout developing regions in Asia, Latin America, and Africa.

Regulatory programs have considerably changed the array of pesticides used in agriculture. Since 1993 both the U.S. and the EU have implemented programs to update risk assessment for pesticides in use which made manufacturers to pull highly acutely toxic organophosphate and carbamate insecticides from these markets voluntarily. Current-use pesticides are mainly designed on the basis of their desired mode of action, which is aimed at displaying optimal efficiency in target, and minimum side effects in non-target, organisms. Due to the frequently close phylogenetic relationships of beneficial and pest species, however, it is ambitious to both target and protect. One of the major challenges in wildlife ecotoxicology, therefore, is to trace the effects and side-effects of chemicals from their cellular targets through levels of increasing complexity to communities of species and the function of ecosystems. Here, we provide an integrated view of the existing knowledge regarding pesticides of the past and present. This includes synthetic chemicals and biological compounds (spinosyns, azadirachtin, Bt δ-endotoxin) applied in agriculture, but excludes non-agricultural biocides used as antifouling or fracking compounds, parasiticides, or antibiotics.

Individuals and Populations

As events of acute poisoning in wildlife have decreased in number during recent decades, at least in developed countries, the problem of chronic pesticide toxicity has moved into the focus of scientific interest. Wherever pesticide application is spatially restricted and buffer zones (e.g. riparian buffers) are respected, wildlife vertebrates currently are considered unlikely to be exposed to pesticide levels that are acutely toxic, with the exception of some examples of exceedances of acute toxicity values in aquatic systems (*6*, *7*) and anticholinesterase poisoning of birds (*8*), Chronic toxicity, however, hast to be taken into account for all pesticides that are applied at regular intervals, particularly those which are highly persistent, such as, e.g., organochlorines. In addition to their acute toxicity, which has occasionally led to mass deaths in the past, this group of insecticides, including DDT and its metabolite DDE, an androgen receptor antagonist, is known to chronically act as endocrine disruptors (*9*) exerting estrogenic and/or androgenic effects in rats, birds, and fish (*10*). DDT itself is carcinogenic (*9*). To date, more than 120 endocrine disruptive pesticides are known, covering numerous chemical classes (*11*). Organochlorines, organophosphates, carbamates, pyrethroids, thiocarbamates, triazines, and triazoles furthermore exhibit thyroid disruption properties in rodents, birds, amphibians, and fish (*10*). Immunotoxicity, which is primarily caused by inhibition of serine hydrolases or esterases, oxidative damage, and modulation of signal transduction pathways has been reported for organophosphates (*12*). The organochlorine chlordane, carbamates, the phenoxy herbicide 2,4-D, and atrazine were found to interact with the immune system of vertebrates (*13*). Organophosphates and carbamates impair metabolic functions such as thermoregulation, water/food intake, and behavior (activity, foraging time, learning ability) in vertebrates. Further consequences are weight loss, impaired development, reduced reproduction and hatching success (*14*). Particularly in aquatic biota, a plethora of studies have revealed a broad range of pesticides representing a variety of chemical classes to induce embryotoxicity and teratogenicity in non-target fish, amphibia, and invertebrates, which result in organ malformations, delayed hatching, growth suppression and embryonic mortality (*15*). Some of these pesticide effects at the sub-individual or individual levels have been causally or plausibly linked to their consequences on populations (Fig. 2).

In general, information on the hazard of pesticides to wildlife is based on the knowledge of their environmental fate, persistence, application rate, and toxicity (*14*); the latter have been largely

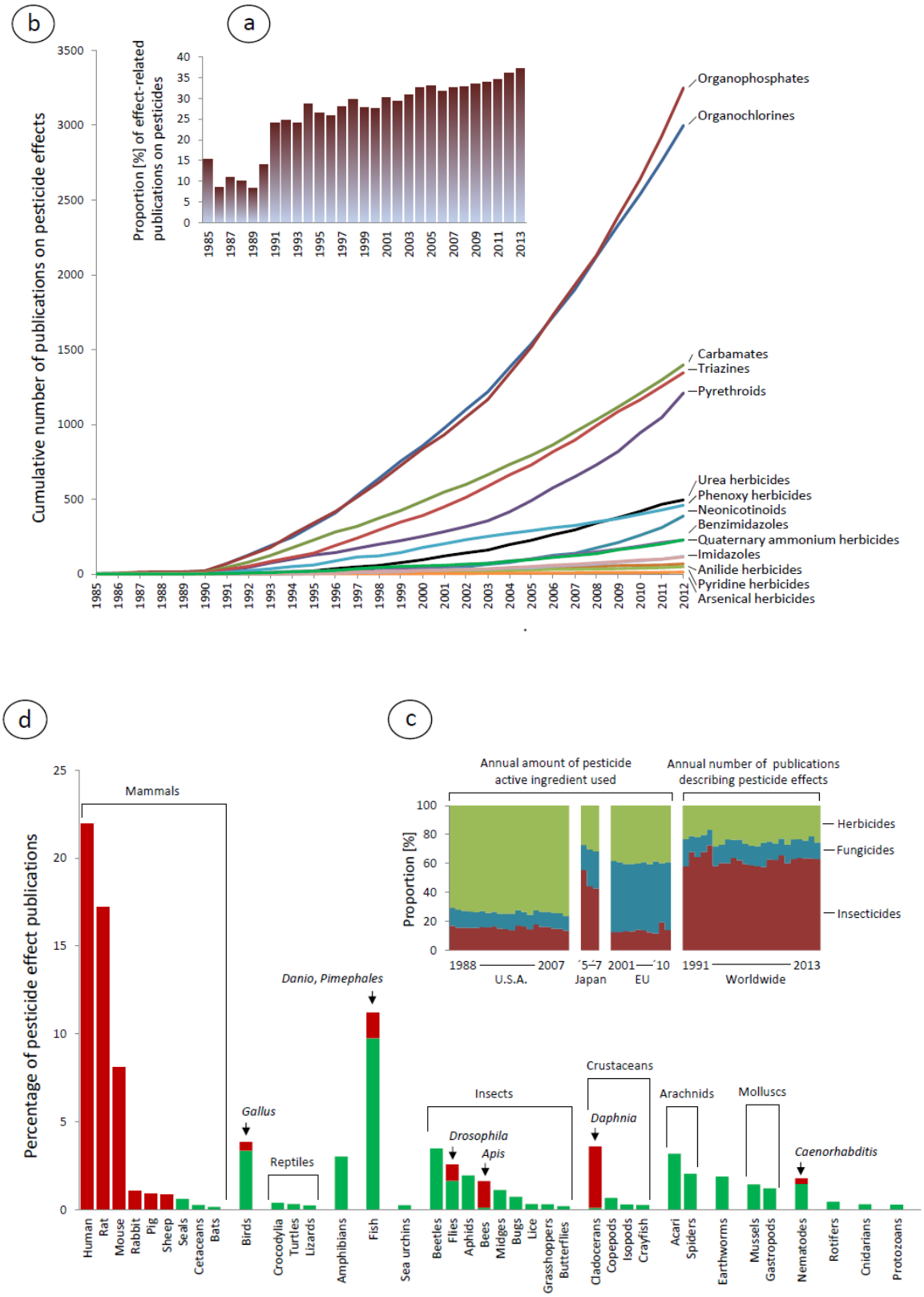

Fig. 1. Trends in research on pesticide effects and pesticide use. (**a**) Steadily increasing proportion of effect-related research among publications on pesticides in the last 28 years. (**b**) Journal publication numbers on effects related to pesticide classes. During the last years, the most substantial increase in the rate of publication was recorded for organophosphates, pyrethroids, and neonicotinoids. (**c**) The proportions of effect-related publications on herbicides, fungicides, and insecticides remained rather constant throughout the last 23 years, but did not reflect proportions of these pesticide classes used in the US and Europe. (**d**) Effect-related research shows a bias towards domestic and lab model species (in red, species names given respectively, including human cell lines) in relation to wildlife animals (in green). [Calculated from data obtained from Web of Science (March 2013), the US EPA, the European Crop Protection Association, and (*88*)].

gained from laboratory experiments predominantly conducted on vertebrates, including mammalian model organisms. While modern insecticides such as neonicotinoids previously were expected to exert only low toxicity on mammals, birds and fish, since these compounds have a low affinity for vertebrates relative to insect nicotinic receptors (*16*), current research has provided evidence for respiratory, cardiovascular, neurological, and immunological toxicity in rats and man (*17*, *18*). However, information for many endangered mammalian species, particularly for arctic marine biota, are scarce and limited to measurements of compounds and a few selected biomarkers, e.g. CyPIA1 activity (*19*). Effects indicative of endocrine disruption were reported for river otters, bears, seals, sea lions, and Beluga whales from organochlorine-polluted environments, but it was impossible to separate DDT effects from sympatrically present non-pesticide organochlorines (*20*).

In birds, population effects of pesticides have been linked to neurotoxicity and endocrine disruption. While acute mortality could be attributed to AChE inhibition exerted by organochlorines, organophosphates, and carbamates (*8*, *21*), chronic exposure via oral uptake to organochlorines and organophosphates in particular, but also carbamates and a variety of herbicides and fungicides, resulted in disturbances of the endocrine and reproductive system. DDT and its metabolite, DDE, had a devastating effect on many Laurentian Great Lakes bird species due to a reduction of eggshell thickness of up to 90% and, consequently, cracking, and even affected migrating eagles that had consumed fish from the Great Lakes two years previously (*5*, *22*). Similar effects of organochlorines were detected in ducks and herons from the Ebro delta, Spain (*21*). It is commonly accepted that these endocrine effects have caused the observed population declines. However, behavioral effects including impaired incubation and chick rearing behavior (*23*), which have been detected in captive birds after chronic exposure to all neurotoxic pesticide classes, have, as yet, not been linked to population declines (*24*).

The spill of highly persistent organochlorines (DDT and metabolites, diclofol, dieldrin, toxaphene) in Lake Apopka, Florida in 1980 is well known as the only example linking the endocrine effects of pesticides to juvenile population densities and unexpected adult mortality in wildlife reptiles (*25*). Population parameters of American alligators were impaired by disrupted steroidogenesis, reduced testosterone levels and penis lengths in males, and elevated 17β-estradiol levels in juvenile females (*5*). Worldwide, amphibians have also been suffering alarming population declines. Signs of endocrine disruption, like gonadal abnormalities and feminization of males (*5*, *26*, *27*), interference with metamorphosis (*28*), changing behavior (*5*, *28*), and retarded development (*26*), have been frequently found in wildlife frogs and toads, but it has been difficult to relate these pesticide effects directly to population parameters, gene frequencies, or sex ratios (*28*). A recent meta-analysis revealed overall environmental pollution to have large effects on abnormality frequencies but only medium effects on survival and no effects on time of development (*29*). A key to mechanistically link pesticide impact and population declines in amphibians may lie in an impaired immune function and, consequently, in increased infection rates (*28*). Whether high acute mortality recently observed in European common frogs following direct dermal application of recommended rates of four fungicides, two herbicides, and the insecticide dimethoate (*30*) is field-relevant remains to be investigated.

Fish ecotoxicology faces similar challenges. While literature on laboratory studies provides rich detail for sub-individual pesticide effects, attempts to link these to fish populations are rare. Apart from obvious relations in cases where pesticide runoff from orchards reached streams and caused fish kills (*31*), the difficulty in separating pesticide action from potentially interacting parameters in freshwater ecosystems within industrialized regions has hampered causality analysis. There is compelling global evidence that exposure to endocrine disruptive chemicals is compromising the physiology and sexual behavior of fish, including effecting permanent alterations of sexual differentiation and impairment of fertility; however, it is thus far impossible to quantify the specific contribution of pesticides to these impairments (*20*). Whereas pesticide-induced neurological, endocrine, and olfactory dysfunction following cholinesterase inhibition have been correlated with fish behavior (*32*), effects at the population level associated with exposure to mixtures of pesticides and other chemicals, have at most been plausibly linked to sub-individual effects by application of

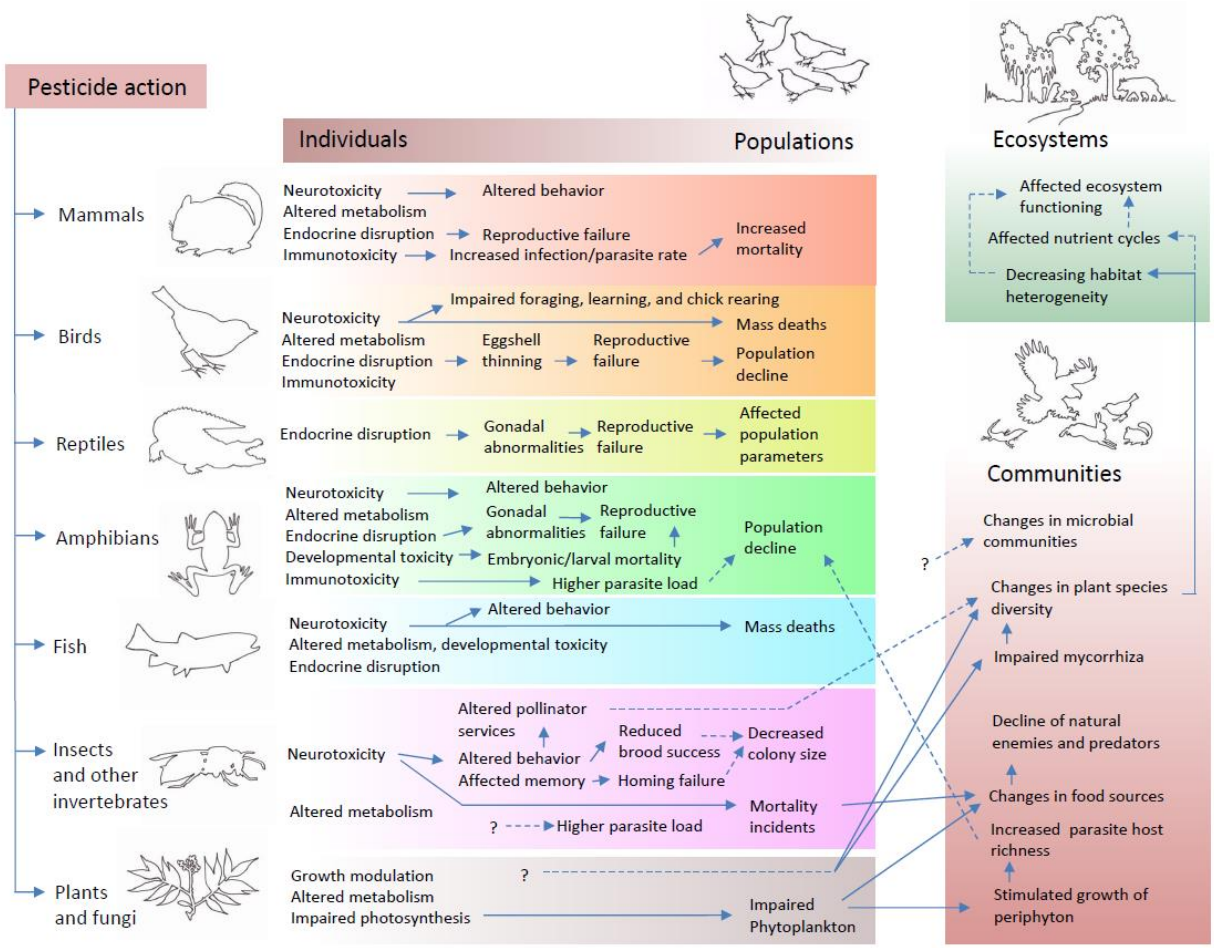

Fig. 2. Documented pesticide effects on wildlife at different levels of biological organization and known (solid arrows) or rather evidence-supported, anticipated (dashed arrows) interrelations among them. Research remains to be conducted wherever plausibly interrelated effects are not connected by arrows. Most of the sub-individual data for mammals are derived from non-wildlife studies.

Bradford-Hill´s criteria of causation (*33*, *34*). Generally, single-chemical risk assessment likely will underestimate actual risks of pesticide mixtures to fish, as combinations of organophosphates and carbamates were shown to exert synergistic neurotoxicity and unpredicted mortality in Pacific salmon (*35*).

Research on interrelations between individual and population effects of pesticides on invertebrates is dominated by studies on insects, particularly bees. Honey bee poisoning incidents in developed countries such as the UK or Germany declined from the mid 1990s onwards, in parallel to a decline in organophosphate incidents (*36*). The phenomenon of colony collapse disorder (CCD) and the suspicion that neonicotinoids and formamidines could be involved (*37*), however, has stimulated much recent research. There is evidence that neonicotinoid pesticides disrupt biogenic amine signaling and cause subsequent olfactory dysfunction, as well as affecting foraging behavior, learning and memory abilities (3, *37*, *38*), but it is still unclear whether bee societies can buffer individual effects at field-realistic dosages (*3*, *39*). Two recent studies found that bumblebees exposed to field-realistic concentrations of imidacloprid suffer from impaired foraging, brood development, and colony success in terms of growth rate and new queen production, particularly in combinatorial exposure to the pyrethroid λ-cyhalothrin (*39*, *40*). In honey bees, thiamethoxam caused high worker mortality due to homing failure (*41*), but possible risks for colony collapse remain controversial (*41*, *42*). Alternative approaches designed to reduce impact on beneficial insects, such as bees, favor compounds of microbial origin such as spinosyns or the Bt δ-endotoxin *Cry*. Spinosyns, however, affect various

physiological and behavioral traits of beneficial arthropods, particularly hymenopterans (*43*), whereas transgenic crops expressing *Cry* were shown to cause negative effects on the abundance of some insect taxa, predominantly on susceptible lepidopteran herbivores as well as their predators and parasitoids (*44-46*). So, despite all efforts to increase the specificity of insecticides, there is, as yet, no compound both targeting insect pests and leaving non-target insects unaffected.

Across the Levels of Biological Organization

For the most part, pesticide research remains a scattered assemblage of data recorded at the molecular, cellular, physiological, or individual levels for different species on the one hand, and records of population declines or altered community structure in areas with high pesticide input or persistence on the other hand. Evidence for causal links across the levels is still scarce and restricted to the mentioned examples. At present, two strategies are favored to move from one level of biological organization to the next, more complex one. Firstly, a multi-tiered approach combining controlled lab experiments, mesocosms, and field studies is needed to provide the basis for the application of Hill´s criteria of causation (*33*, *47*). Secondly, computational methods either relating observed population effects to underlying parameters (top-down strategy (*20*)) or translating toxicity data derived from individuals to the level of wild populations and beyond (bottom-up strategy) are increasingly being developed and refined (*48*). Refinement includes criteria quantifying the 'best' model selection (*49*) and the adoption of population dynamics and food web modeling from ecology, accepting that a sophisticated understanding of species interactions is essential to detect and explain indirect pesticide effects (*50*). New approaches in population modeling include population-level measures of toxicant effects (e.g. on population growth rate, age structure) and different sensitivity of life history traits, and aim to determine the probability of extirpation or recovery of populations after pesticide exposure (*51-53*). Despite recent promising achievements (*54*, *55*), however, population modeling is still considered a relatively new subdiscipline in ecotoxicology (*48*) and not yet developed well enough to fully assess pesticide impacts on endangered species (*56*).

Biotic Interactions and Communities – Indirect Effects

The current scarcity of incidents in developed countries, the shift from long-lived to mostly less persistent compounds (except for sulfonylurea herbicides and neonicotinoids), and the awareness of long-term sublethal effects of pesticides turned the attention of scientists and administrators towards the indirect consequences of pesticide use, which address changes in biotic interactions. Here, three main aspects have moved into focus: parasite-host interactions, predator-prey relationships, and pollination.

A number of pesticide compounds were proven to affect immune parameters, and some cases of immunosuppression (exerted by organochlorine pesticides, organophosphates, carbamates, atrazine, and 2,4-D) were correlated to higher susceptibility of organisms to infection and parasite-caused diseases. For example, oysters exposed to DDT, toxaphene, and parathion were shown to be susceptible to fungal infection and earthworms from triazine-treated orchards became infected with monocystid gregarines (*13*, *57*). In mammals, the use of anticholinesterase agents in agriculture can pose a threat of infections, disease outbreaks, and higher mortality, e.g. by tularemia in hares (*58*). Work on seals showed organochlorine pollutants, including pesticides, have immunotoxic properties, impairing resistance to phocine distemper virus (*59*). Particularly in view of the global loss of amphibian populations, which has resulted in nearly one-third of the world's species being threatened, this subject seems to be crucial. Laboratory exposure experiments and field studies have shown an association between atrazine, malathion, esfenvalerate, or glyphosate exposure and increased infection of tadpoles with trematodes (*60*, *61*). A field survey of the northern leopard frog, *Rana pipiens*, revealed atrazine pollution and inorganic phosphate to account for 74% of the variation in the abundance of trematodes (*62*). Further mesocosm studies in ponds showed that atrazine killed the

phytoplankton, thus allowing light to penetrate the water column and periphyton to assimilate the nutrients, including inorganic phosphate, released from the plankton. Presumably, periphyton growth provided more food to grazers and thus increased the richness of snails which act as trematode intermediate hosts (*62*).

Other prominent indirect pesticide effects act on food webs and species competition through removal of prey or competing species. Herbicides, which reduce plant cover of soil and change plant species diversity, were found to be responsible for reduced food availability and thus adverse secondary effects on soil invertebrates and butterflies (*63*). The fungicide benomyl, which suppresses arbuscular mycorrhizal fungi, altered the patch-level floral display and resulted, after three years of fungal repression, in a two-thirds reduction of the total number of floral visits and in a shift in the community of floral visitors from large-bodied bees to small-bodied bees and flies (*64*). Indirect herbicide effects have also been reported for many vertebrate species, as weed and many non-crop plants form important components of their diet. Pesticide-induced diet shifts decreased species abundance and diversity in small mammals (*63*), reduced survival and reproductive rates in seed-eating or carnivorous birds (*65, 66*), and resulted in declines of bird populations and species diversity (*63*). However, declining bird species are not found to be associated with particular plants but rather with reductions in overall diversity and the abundance of food plants in intensely managed arable land. Concomitantly, in these areas, a loss of insects and spiders, important sources of food for chicks of a wide range of bird species, was observed (*67*). Accompanying the trend towards monoculture on a large scale in the U.S. and parts of the developing world, herbicide use – particularly in combination with the cultivation of herbicide-tolerant crops – frequently has contributed to an overall reduction in habitat heterogeneity within agricultural landscapes and degraded their suitability as habitat for wildlife, including pollinators (*63*). Also the biological pesticide spinosad has a wide variety of sublethal effects on natural enemies of pests and can drastically affect demographic traits in parasitoids and predators (*43*). Bt-transgenic crops, as an alternative to conventional insecticide use, did not impair the function and abundance of natural pest enemies in a 6 year-study, but secondary effects by sublethally poisoned prey, and diminished food quality to predators cannot be excluded for this kind of pest control (*45*). In aquatic systems, the most detrimental effects of herbicides address the reduction of the complexity and structure of the plankton and the submerged vegetation, including periphyton, all acting as food source and refuge for phytophagous species like waterbirds and amphibian tadpoles (*21*, *68*). In this regard, structural alterations in the planktonic community can result from direct herbicide effects on microalgae, from indirect consequences of pesticides on filter feeders (*69*), or from changes in competitive interactions (e.g. small zooplankton (rotifers) was found to increase after larger zooplankton (cladocerans) was selectively decimated (*70*)). Species of higher trophic levels, like salmon, are most likely to be affected in population growth and productivity by indirect pesticide effects (*71*). Fleeger and co-workers list 56 cases of indirect pesticide effects on competition or predation in aquatic biota, identified in studies across trophic levels (*50*). It has to be concluded that, at least in aquatic systems, pesticides exert strong selection on invertebrates. Freshwater habitats are best studied in this respect, whereas marine and estuarine systems are underrepresented. Furthermore, it is noteworthy that not only modulations in the population structure of prey or predator species, but also pesticide effects on interspecific behavior, may change predator-prey interactions, as shown for glyphosate in tiger salamanders (*72*) or imidacloprid in zebrafish (*73*) and their respective prey.

Probably the most meaningful example of indirect pesticide effects, however, does not address the aquatic environment, but insect pollination. In bumblebee (*Bombus terrestris*) workers chronically exposed to realistic concentrations of imidacloprid and λ-cyhalothrin, pesticide-altered behavior was found to be associated with a declined pollen collecting efficiency (*39*). For these insecticides, as well as for spinosad impact on bees, it is likely that diminished foraging efficiency affects overall pollination services (*43*).

Can Microevolution Catch up?

The selection of resistant phenotypes following multi-generation exposure can be a problem in pest control, and, perhaps, a chance for non-target species with high reproductive output and short generation time. As a matter of principle, the elucidation of long-term pesticide effects in communities of animals and plants is often hampered by the long generation times of the species involved and, thus, the inevitable inertia of these systems. In contrast, microbial communities display microevolutionary responses within a rather short time period. Transient effects of herbicides including diuron and simazine, the dithiocarbamate fungicide mancozeb, and DDT on microbial populations and communities and their function in ecosystems as, for example, nitrification are regularly measurable, but studies congruently revealed their high capacity to recover and to develop tolerance to these pesticides (*68*). Quite often, these tolerant bacteria benefit from pesticide application and use the compound itself as a carbon source (*74*). Similarly, insect and pathogen pests were found to benefit from elevated protein levels in 2,4-D-treated corn plants (*75*) whose yields on a per-ha basis may thus equal those from organically managed corn (*76*). Studies also report at least partial restoration of community functions despite structural changes after pesticide treatment for communities of freshwater microalgae (*77*). It is, however, unclear to what extent the selection of resistance traits and/or a functional resilience of the community can be generalized throughout ecosystems, since studies on metazoans are rare. Recent field experiments revealed λ-cyhalodrin treatment to select ten-fold higher resistance against this pyrethroid in lady beetles *Eriopsis connexa* after 55 generations (*78*). Furthermore, the selection of resistance against deltamethrin was reported for the common green lacewing, *Chrysoperla carnea* (*79*) but, up to now, there is no indication for pesticide-tolerant bees, probably since the queens are not directly exposed to the toxin (*38*). However, the scarcity of information for non-target species does not allow the degree to which resistance contributes to the regeneration of populations to be judged. Independent from evolutionary processes however, ecological networks often allow for restoration by means of recruitment from the filial generation or immigrating individuals. Microcrustacean populations in stagnant waters, for example, usually recover from pesticide effects within a few weeks, provided that the compound is not persistent, the physicochemical environment remains intact, generation times are short and immigration from the residual population is possible (*80*). There is also evidence that reversal of intense pesticide use in arable systems can result in the rapid recovery of food sources for birds (*67*). In a review of the ecological consequences of insecticide use, Devine and Furlong listed a variety of cases in which terrestrial and aquatic insect, crustacean, lumbricid, and fish populations recovered within months when the pesticide treatment stopped (*81*). In this context, multilevel modeling allows situations in which reduced pesticide application will have the most benefit on restoring biodiversity to be detected (*54*).

Future Challenges in a Changing World

It is to be assumed that the global changes we are going to experience during the coming decades pose larger questions regarding pesticide impact on wildlife than we have been accustomed to. We cannot predict the consequences of a possible release of the bulk of obsolete pesticides that remain in developing countries. Shifts in the use of ‘old’ and highly persistent pesticides to modern compounds may surely improve the situation in many countries of the world but, as outlined, they are also far from being unproblematic. As far as we know, even the latest generation of biopesticides poses problems to wildlife – perhaps not directly by receptor interaction in non-target species, but at least indirectly via the impairment of species interactions.

Climate change will surely interact with the spatial distribution and effects of pesticides in nature (Fig. 3). Currently, it is possible to identify reasonable points of expected interactions, even though the magnitude of interference remains unclear. Elevated water temperatures may change the metabolite pattern of pesticides via alterations in biotransformation processes, and changes in

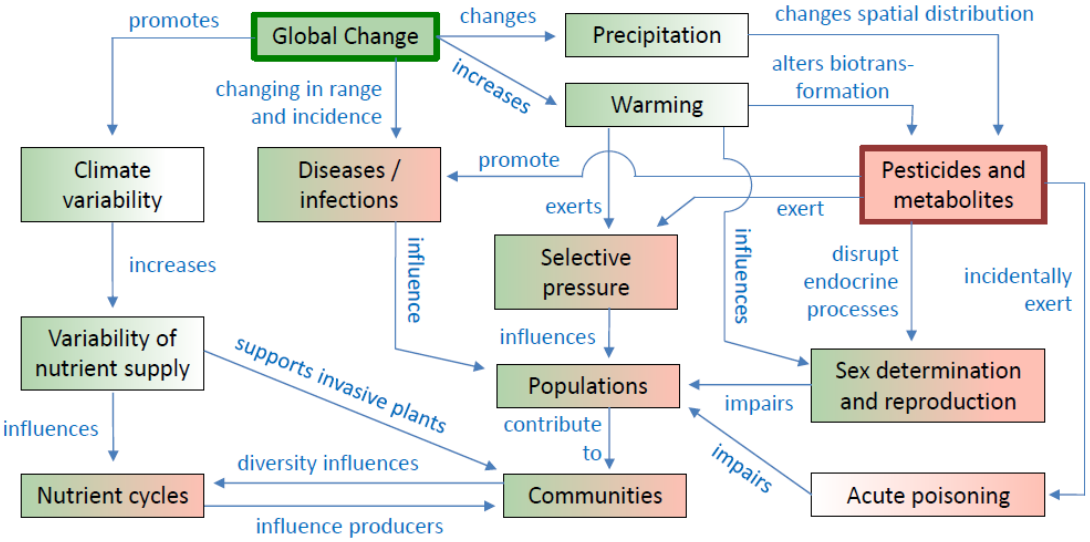

Fig. 3. Anticipated interactions of global change and pesticide effects on the physiology and ecology of wildlife species. Presumed impact of pesticides is depicted in red, presumed global climate change impact in green.

precipitation may result in changes in volatilization and deposition (*82*). Global warming is decisively expected to impact the ecotoxicological potency of pesticides, since 83% of ecotoxicological studies on combined effects of elevated temperature and pesticide exposure have revealed the synergistic action of these factors (*83*). Experimental evidence for this expectation has been provided by a study on the fungicide pyrimethanil applied under thermally realistic global change summer conditions simulated for Central Europe. In comparison to current temperatures, response to the conditions in this study predict increased mortality, a declining population growth rate, and considerably reduced genetic diversity in the midge *Chironomus riparius* (*84*). Pesticide interactions with global warming will likely influence the direction in which selection acts upon biota, a factor which will be particularly problematic for populations or species living at the edge of their physiological tolerance (*82*). Further problems in a warming world may result from temperature interactions with metabolic rates of heterothermic organisms and, with respect to endocrine disruptive compounds, with physiological processes involved in temperature-dependent sex determination, as is known for reptile species (*25*). In addition, changes in the geographic range and incidence of many infectious diseases which may be fostered by pesticide-exerted immunotoxicity have been predicted (*60*). Higher level pesticide effects, such as changes in plant communities, will likely interfere with the consequences of global change on biodiversity and thus affect ecosystem function. Increased heterogeneity of nutrient supply associated with global change was shown to strongly promote plant invasion and thus to alter plant communities (85). In turn, plant diversity is known to influence biomass production (*86*) and nitrogen cycling (*87*).

In the coming years, there will be a paramount need to causally link both direct and indirect pesticide effects across levels of increasing biological complexity. Specifically, it will be essential to detect and quantify confounding factors that act synergistically with pesticide exposure, and to identify processes of particular vulnerability to interactions of pesticide impact and climate change.

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