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Research Focus

Two stressors are far deadlier than one

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Natural organisms often face a barrage of stressors, both natural and human induced. Two known stressors that impact amphibian populations are pesticides and predators. Recent work by Relyea and by Mills and Relyea reveals strikingly strong, synergistic negative effects of these two factors on amphibian larvae. Adding predation risk on top of supposedly sublethal concentrations of a common pesticide caused a massive increase in larval mortality. Interestingly, the increased mortality did not require exposure to actual predation. That is, simply the 'smell of danger' (predator chemical cues) caused 80–90% of larvae that were held in otherwise 'safe' levels of the pesticide to die. Notably, this effect occurred in some species, but not in others. These new studies highlight the need for further interdisciplinary work on the conditions under which combinations of stressors have particularly strong negative effects on natural organisms.

When the senior author of this report was a new graduate student several decades ago, one of his mentors took him aside and advised him to focus on pristine, natural systems. The goal, after all, was to understand Nature – with a capital N; in particular, to understand how biotic factors (e.g. predation or competition) and abiotic disturbances interact to influence organisms and communities under natural conditions. More recently, the focus has shifted toward an increased emphasis on investigating the effects of anthropogenic stressors on organisms. Given that these new, human-induced stressors play out in a background of natural factors, an obvious question is whether multiple stressors – both natural and human induced – interact with each other. In particular, do multiple stressors have synergistic negative impacts on natural systems? In other words, are two stressors much worse than either one alone? Recent work by Rick Relyea of the University of Pittsburgh [1] and Nathan Mills then at the University of Missouri [2] documented a worst-case scenario for the combined impacts of an anthropogenic stressor (a pesticide) and a natural one (predation risk) on amphibian larval mortality. Either predation risk alone (simply the 'smell of danger') [3] or 'sublethal' concentrations of the pesticide alone caused very little mortality. In some species, however, (but not others) the combination of these factors caused very heavy mortality. These studies suggest that we might be peeking at just the tip of the iceberg of the synergistic, negative impacts that multiple stressors can have on natural systems. They also underscore the need for more bridges between the fields of ecology and ecotoxicology.

Pesticides (and chemical pollutants, in general) are among the main causes of population decline of species of conservation concern [4,5]. To provide quantitative information about impacts of pesticides, classic mechanistic studies in ecotoxicology quantify LC50s and LD50s, the concentrations or doses of chemicals that cause 50% mortality in controlled, laboratory conditions. LC50s and other measures are then used to determine national water quality criteria for safe concentrations and for allowable release of pollutants (e.g. allowable total maximum daily loads, TMDLs). Methods for estimating LC50s are well standardized (e.g. [6]). However, these methods typically involve exposure to pesticides in the absence of other stressors – either biotic (e.g. poor food supply, competition, predation or disease) or abiotic (e.g. suboptimal temperatures, pH or oxygen levels). To extrapolate from laboratory results on mortality to possible effects on populations in the field, we need to know more about how effects of pollutants interact with natural stressors.

Conversely, although literature shows that predators often have major impacts on prey communities, populations and individual traits [7,8], controlled experimental studies are typically done under conditions with no chemical stress from pollutants. Given that many bodies of water are mildly, if not highly polluted, predator–prey ecologists would do well to learn more about how chemical stress might influence predator–prey interactions. An ecologist who only studies predator–prey interactions in clean water might drastically misestimate predator effects in real-world situations, where prey are exposed to low–moderate concentrations of pesticides and other chemical stressors. Again, the key question is: how strong and widespread are synergistic effects of these multiple stressors?

The need to study synergies among multiple stressors seems particularly compelling for amphibians. For over a decade, there has been significant concern over global amphibian decline [9]. Although habitat loss is clearly a major cause of this decline, other factors that appear to play a role include pesticides, ultraviolet radiation, predators, parasites and disease [5,9–14]. In fact, recent studies show a clear correlation between pesticide exposure and decline of some amphibian populations (10,11). However, pesticide concentrations in natural ponds and streams are usually considerably lower than the estimated non-lethal levels for most amphibians (based on standard ecotoxicological methods). Relyea and Mills' recent work might help to resolve this paradox, because it suggests that concentrations that might be 'safe'

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in the laboratory can be quite unsafe in nature, because even low concentrations can synergistically interact with a common natural stressor: predators.

Relyea and Mills (2001) exposed gray tree frog tadpoles *Hyla versicolor* to low concentrations (well below their LC50) of a commonly used pesticide, carbaryl, in the presence versus absence of chemical cues from predatory salamanders *Ambystoma maculatum*. They found a striking synergistic effect. Carbaryl was two to four times more deadly when combined with predator chemical cues. Based on this surprising result, Relyea then asked if this effect is unique to this prey species, or is it a general effect? A new paper [2] addresses this question by repeating the experiment on six North American tadpole species from three families (wood frogs, *Rana sylvatica*; leopard frogs, *R. pipiens*; green frogs, *R. clamitans*; bullfrogs, *R. catesbeiana*; American toads, *Bufo americanus*; and gray tree frogs). Previous work involving three to four day exposures in the absence of predator cues documented carbaryl LC50s for these species ranging from 2.5–18 mg l^{-1}. Here, Relyea examined effects of 16 days of exposure to six carbaryl concentrations (0–6.5 mg l^{-1}) in the presence versus absence of chemical cues from caged newts *Notophthalmus viridescens*, a predator that commonly co-occurs with, and consumes, these tadpoles.

Two of the tadpole species showed no synergies between carbaryl and predator cues (wood frogs and, surprisingly, gray tree frogs, the species used in the earlier experiment). Two other species (leopard frogs and toads) exhibited synergies early in the experiment that disappeared by its end. For example, for toads held at the highest concentration of carbaryl, midway through the experiment, very few survivors remained in tubs that were also exposed to predator cues, whereas over half were still alive in tubs without predator cues. However, by the end of the experiment, regardless of predator cue treatment, no toad larvae survived chronic exposure to the high carbaryl concentration.

The most striking effects occurred in the remaining two species, green frogs and bullfrogs. After 16 days of exposure to an intermediate concentration of carbaryl (1.6 mg l^{-1}, field surveys have measured concentrations up to 4.8 mg l^{-1}), green frogs suffered only 10% mortality in the absence of predator cues, but 80% mortality in the presence of predator chemicals. That is, at this concentration, predator cues made carbaryl eight times more lethal than carbaryl alone. This synergy was even more drastic for bullfrogs. In the absence of predator cues, 16 days of exposure to 1.6 mg l^{-1} of carbaryl caused only 2% mortality (indistinguishable from carbaryl-free controls). However, with newt cues present, this same concentration caused 92% mortality. Predator chemicals made carbaryl 46 times more deadly.

Relyea and Mills' studies did not address the mechanisms underlying these strong synergistic effects. Because actual predation was not a possibility, the mechanism presumably involved an aspect of the combined physiological stress. In nature, prey are often exposed to both pesticides and actual predators (not just the smell of predators). In that case, additional mechanisms of synergy could emerge involving effects of pesticides on predator–prey behavior. For example, recent studies show that typical anti-predator behaviors are compromised by exposure to pesticides [15,16]. Of course, pesticides can also have negative effects on predator foraging abilities [17]. Overall impacts of pesticides on prey might then depend on a mix of direct and indirect effects mediated by relative susceptibilities to chemical stress and multi-species interactions in a community context.

Relyea and Mills' work and other recent studies suggest several exciting directions for future research. First, it is notable that synergies emerged for some prey species, but not others. In addition, a comparison of Relyea's recent paper and that by Relyea and Mills suggests that there might be population variation in synergistic effects. Whereas the earlier study [1] found strong synergetic effects of carbaryl and predator chemicals on gray tree frogs, the new study [2] found no such effect on the same species. Although this difference could be attributed to the use of different predatory salamander species, one other notable difference between the two studies is that they used gray tree frogs from different locations (Missouri versus Pennsylvania). Other studies have documented variation among sibships, populations and species of frogs in response to carbaryl [18]. Although such variation might cause headaches for those attempting to identify generalities about the effects of contaminants on behavior, it also gives reason for hope because variation offers the potential for the evolution of resistance. Future work should focus on patterns of variation in the combined effects of multiple stressors, and on proximate and evolutionary mechanisms underlying this variation.

Finally, Relyea's laboratory experiments address only a small part of the complexity that exists in nature. Synergistic effects in nature might be weaker than in the laboratory if stress levels fluctuate more rapidly than the 4–16 day timescale of Relyea's experiments, or if prey can avoid areas with high pesticide levels or predation risk. Conversely, because predation risk and pesticides are only two of a suite of factors that could have non-additive impacts on sensitive prey, synergistic effects might be even more important in nature than in Relyea's experiments. Future studies could look at interactions between multiple chemicals, and any of several important biotic (e.g. predation, competition or disease [19,20]) and abiotic (e.g. temperature or pH) factors that limit natural populations. Real organisms under natural conditions face multiple stressors. Understanding mechanisms of interaction between these multiple stressors will be crucial for applying ecological knowledge to solving environmental problems.

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Sustainability in a nutshell

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Sustainable exploitation is widely advocated as a strategy for reconciling economic pressures upon natural habitats with nature conservation. Two recent papers examine different aspects of the sustainability of the nut harvest on wild populations of Brazil nut trees *Bertholletia excelsa* in Amazonia. Peres *et al.* find that many populations of the Brazil nut tree lack juvenile trees and are not regenerating. In a socioeconomic study, Escobal and Aldana find that nut-gathering provides insufficient income on its own to support nut-gatherers and that their other income-raising activities damage the forest. The existence of a market for rainforest products is, therefore, not sufficient on its own to prevent habitat destruction or the overexploitation of the resource and a more sophisticated approach to sustainability is required. Development of a market in ethically traded Brazil nuts might be one solution.

With economic forces driving deforestation in the Amazon basin at an accelerating rate [1], the strategy of 'use it or lose it' has been promoted as one of the few realistic ways in which large areas of rainforest might be protected. Since 1989, when a study published in *Nature* [2] showed that the monetary value of tropical forest timber was less than the potential value per unit area of other rainforest products, such as fruits, every plea and campaign for rainforest preservation has emphasized the opportunity cost of each hectare felled. The problem is how to turn notional, paper values of what would be lost when a forest is felled into a real income for local people. If forest dwellers can earn an income from sustainably harvesting forest products, so the theory goes, they will protect the source of their livelihood and, therefore, the rainforest will be preserved. Anything more than limited timber extraction would clearly be self-defeating to such an enterprise and so the emphasis has been on so-called 'non-timber forest products' (NTFPs), which include saleable plants, fruits and animals. Top of the list of these is the Brazil nut (Figure 1). However, two recent studies [3,4] suggest that their exploitation is not currently sustainable.

Sustainability of the Brazil nut harvest

Many familiar commodities, from avocados and chewing gum to rubber and vanilla, originated in tropical forest, but the Brazil nut (from the tree *Bertholletia excelsa*) is the only one that is widely traded and still harvested from the wild rather than from plantations. Brazil alone exports 45 000 tons of nuts a year that are worth (US$33 million. Because of the importance of the Brazil nut to the economy of Amazonia, the ecology and socioeconomics of the harvest have been reasonably well studied. Zuidema and Boot [5] reported matrix projection models parameterized for two populations of *B. excelsa* in the Bolivian Amazon. The models showed that the structure of the two populations was stable and, thus, that the nut harvest was not damaging the ability of the tree populations to renew

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