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Hormesis in ecotoxicological studies: a critical evolutionary perspective

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Abstract

Many studies report negative associations between exposure to large concentrations of contaminants and life-history traits of animals. As a consequence, they argue to derive linear models that can be applied to predict effects in sub-threshold or low-dose exposure scenarios. However, several recent studies found that exposure to low doses of a contaminant may induce a hormetic response, i.e. stimulatory or beneficial effects at low concentrations, but harmful effects at higher concentrations. Hormesis might be a novel fundamental pillar in the field of ecotoxicology, as it may promote the evolution of adaptive coping mechanisms in rapidly changing and challenging environments. However, because hormesis occurs in the low-dose zone of the dose-response, it has been often neglected. In this review, we have discussed how low-dose contaminant exposure may adaptively modify molecular and physiological mechanisms that may lead to organisms better adapted to cope with challenging environments, how such responses may be transgenerational, and how thus this issue can no longer be ignored in ecotoxicological studies. In so doing, we have also identified some of the main limitations of ecotoxicological studies that, by masking potential hormetic responses of animals to chemicals, makes the integration of hormesis complicated to achieve.

Introduction

Ecotoxicological studies provide a fundamental contribution to our understanding of the effects of chemicals on terrestrial, freshwater, and marine ecosystems. It is increasingly recognised that the release of these chemicals into the environment is a major threat for the conservation of wildlife and ecosystem functioning, as chemicals have numerous effects on organisms. As a discipline, ecotoxicology was thus intended to deliver fundamental knowledge on the ecological effects of contaminants, providing grounds for the development of international guidelines to protect the environment and the species that inhabit it. Importantly, research within the ecotoxicology field has been growing at an exponential pace as shown by a fivefold increase in the published literature over the last fifteen years (Figure 1). Most studies report negative associations between exposure to environmental contaminants and markers of health status of exposed individuals [2-4]. Such effects are often described as a linear relationship between the environmental contaminant and a targeted marker of health (i.e. linear no-threshold models), or a relationship that changes after a given concentration is exceeded, the so-called threshold models [5, 6].

In this contribution, we make the point that low-dose contaminant exposure may lead to organisms better adapted to cope with challenging environment, a fundamental concept that is too often neglected in ecotoxicological studies. We highlight that the majority of studies are carried out on captive animals or *in vitro* (e.g. by testing the effect of contaminants on cells), and are often based on unrealistic concentrations that do not occur in nature [7]. Current environmental risk assessments and the formulation of laws is based on high-dose effects. Such approach, however, completely ignores that once released into the environment, chemicals can degrade or accumulate, thus occurring at concentrations that are often not comparable to those used in risk assessment on captive animals [8]. Importantly, these risk assessment models also ignore that (i) organisms (even if highly phylogenetically related) may differ in their sensitivity

to a single or a cocktail of chemical/s and (ii) organisms are equipped with endogenous cellular mechanisms able to detoxify, at least to a certain extent, potential harmful effects induced by exposure to toxic chemicals [8]. Furthermore, such models still ignore that chemicals can even trigger stimulatory or beneficial responses, especially when exposure occurs at low doses, while they are inhibitory at high concentrations (hormetic response; [8]).

Overview of hormesis in ecotoxicology

Recent work has highlighted that hormetic responses are more widespread than originally thought [9, 10], even for chemicals classified as highly toxic [11]. For example, methylmercury is considered a highly toxic chemical due to the well-known bioaccumulation and biomagnification properties [12]. But during *in ovo* development in mallards (*Anas platyrhynchos*), eggs injected with the lowest dose of methylmercury actually showed enhanced hatching success [13]. Similarly, Blévin et al. [14], found positive associations between persistent organic contaminants and metabolic rate in black-legged kittiwakes (*Rissa tridactyla*), suggesting potential stimulatory responses analogous to hormesis. Other examples are represented by the trace element cadmium and the insecticide imidacloprid, which had toxic effects at high concentrations, while at lower concentrations they stimulated the growth of (*Solanum melongena*) plants [15] or the reproduction of peach aphids (*Myzus persicae*) [16], respectively.

Hormesis is also used in relation to the conditioning effect (i.e. conditioning hormesis, [17]), where exposure to mild/moderate environmental stressors, including contaminants, results in organisms with better stress-coping abilities and resilience if they are subjected to a subsequent exposure with the same or a related stressor later in life [17, 18]. Beneficial effects of conditioning hormesis can emerge at any time throughout the individual's life, but are thought to be more robust when animals experience mild environmental challenges during early life [18, 19]. Soybean (*Glycine max*) plants pre-treated with low doses of the herbicide glyphosate preconditioned the soybean seedlings to have greater growth stimulation by a later glyphosate treatment than plants with no preconditioning glyphosate exposure [20]. Similarly, peach aphids exposed to sub-lethal doses of imidacloprid showed increased survival when exposed to a subsequent environmental stressor compared to control aphids that did not receive the treatment [21]. Such responses that might facilitate phenotypic/genotypic adaptation have been observed in many organisms studied so far, from plants to vertebrates [18, 22-24]. Conversely to physiological acclimation, exposure to low-magnitude stressors including environmental contamination, may induce long-lasting stimulatory transcriptional and post-transcriptional mechanisms that modify gene expression and enable organisms to adjust their phenotype to the prevailing environmental conditions [25].

Recent studies showed that hormetic responses have even more far-reaching implications. Such stimulatory effects at low doses may be transferred across multiple generations (i.e. transgenerational hormesis) and are generalizable among different organisms and sources of stress [26, 27]. Specifically related to environmental contaminants, studies investigating contaminant-induced transgenerational effects on plants found that cadmium enhanced biomass production compared to parental tomato (*Solanum lycopersicum*) plants [28], and that the offspring of low-dose glyphosate-treated barley (*Hordeum vulgare*) plants were more tolerant and faster growing than the offspring of control plants [29]. Similarly, Ullah et al. [30] found stimulatory effects when they evaluated the effect of low or sublethal concentrations of thiamethoxam (a neonicotinoid insecticide) on biological traits and genes expression in aphids (*Aphis gossypii*). The low dose treatment had transgenerational stimulatory effects as aphids in progeny generation showed increased expression of vitellogenin and ecdysone receptors genes [30]. Such studies suggest that the induction of transgenerational hormetic responses might be one mechanism by which parents might tailor the phenotype of their offspring for the adverse conditions they have to face [31].

Both types of hormetic responses - i.e. i) low-dose stimulation and ii) better copying ability at subsequent exposure - are fundamental phenomena in disciplines based on the dose-response mechanisms

as traditional medicine and ecotoxicology [32, 33]. Incorporating hormesis in ecotoxicological studies would therefore provide a step forward to understand how exposed animals adaptively respond to environmental cues on contaminants. Yet ecotoxicological studies are still carried out in a way that masks or neglects hormetic responses. In the following paragraph, we discuss key points that should be considered in ecotoxicological research to refine our inferences on the impact of contaminants on wildlife and environmental health.

Major flaws of ecotoxicological studies

High concentrations

One of the major factors that reduce the possibility of observing hormetic responses is represented by the excessively high concentrations used in most laboratory studies, reducing our ability to observe sub-lethal effects. As early as the 80's, Taylor [34] described how the median lethal concentration LD₅₀ of cadmium for the eastern oyster (*Crassostrea virginica*) is about 100 times higher than the concentrations known to induce several sub-lethal effects. Thus, both linear no-threshold and threshold models based on high and environmentally unrealistic exposure levels in animal model studies do not enable to make robust inferences [32]. Many laboratory studies rely on exposure to very high contaminant concentrations that free-living animals are unlikely to ever experience. It is therefore urgently needed to consider sub-threshold effects in regulatory risk assessment of chemicals that are not being extrapolated from linear dose-response models. We emphasise the importance of assessing effects of contamination within the environmentally relevant concentrations (i.e. that occur in natural environments), which may induce nonlinear or hormetic effects [35, 36]. This approach is particularly important for those contaminants, like pesticides, that easily degrade after being discharged into the environment, exposing animals to concentrations several orders of magnitude lower than those originally released. Thus, dose-response studies should be based on a detailed knowledge of the contaminant concentrations that occur in a given environment, and on how they vary across space and time.

Confounding factors

Free-living animals are naturally exposed to a cocktail of contaminants, so that it is hard to clarify which contaminant is associated with the visible effect. This is of fundamental importance because diverse chemicals may either enhance or reduce toxicity by antagonistic, additive, or synergic effects (mixture effect; [37]). But the current legislation remains mostly based on risk modelling extrapolated from single contaminants and does not account for a potential mixture effect [37, 38]. Contrary to the synergic or additive effects (which are generally easily recognised), antagonistic effects of chemicals are difficult to observe and may actually hide hormesis. For instance, the hormetic response to a given chemical for a specific physiological or molecular trait/pathway may be completely modified or obscured if the individual is simultaneously exposed to another chemical with an antagonistic effect on the same trait. Confounding factors may become of greater importance in conditions of low sample size. When sample size is low, the high among-individual variation in contaminant load (as usually observed in free-living animals) may prevent analytical power to detect a harmful effect at a high concentration, or a stimulatory effect at a lower concentration as described in Figure 2. Given the modest magnitude of the hormetic response (ca. 60-70% of control), rigorous and well-directed experiments are needed to minimise the effects of confounding factors on the interpretation of the results.

Simplification of the study

Laboratory studies are highly simplified and may not be entirely representative of the chemical, physical, and biological parameters of natural environments. In the natural environment, different sources of stress may exacerbate the sensitivity of individuals and populations to chemicals. For instance, the quality of an individual could be crucial in understanding the direction of an effect as high- and poor-quality individuals

may respond differently to a stressor [39]. This may be the case for studies in which higher quality individuals have access to food of higher nutritional value (potentially leading to improved health) and higher trophic level (leading to higher accumulation of some contaminants). If the contaminant induces a hormetic effect at low concentrations, the stimulatory response may only occur in those individuals in poorer nutritional status. The same physiological response may be thus enhanced in individuals in poor nutritional status (due to hormesis) and reduced in those individuals in good nutritional status (due to the higher exposure to the chemical). Such scenario would result in a lack of effect because the effect of the contaminant is simply masked by a covariate (i.e. the nutritional status of the individuals). Several studies also make assumptions by extrapolating data on threshold of effects that have been determined for other species, but this practice is full of uncertainties [8]. Not all individuals, species, or communities are equally vulnerable to changing environments and exposure to chemicals. Even closely related species from the same geographic area may respond differently when naturally exposed to similar concentration of environmental contaminants [40]. A given concentration may cause disruption in one species but elicit hormesis in another species. Thus, species-specific studies should be prioritized and extrapolation of data from other studies should be avoided or be carefully validated with appropriate pilot studies to prevent hiding potential hormetic responses.

Concluding remarks

In this article, we have highlighted the need to refine the use of simple linear dose response models for risk assessment because they are based on weak assumptions. There is mounting evidence for significant sub-threshold responses for a plethora of pollutants and in all sorts of organisms, thus this issue can no longer be ignored even in ecotoxicological studies. We stress that rigorous experimental studies are necessary to appropriately identify hormetic responses in animals, and that extensive research is required to better understand the ecotoxicological implications of low-dose exposure.

We also point out that ecotoxicological studies should look into more detail the transgenerational effects, where hormesis might favour adaptability of organisms to single or even cocktails of contaminants [31]. Further studies should focus on the molecular and physiological mechanisms through which transgenerational hormesis operates. How parents equip their offspring with protective physiological tools against changing environments remain largely unexplored areas of research in wild organisms. As of today, there are no studies investigating transgenerational hormesis in vertebrates.

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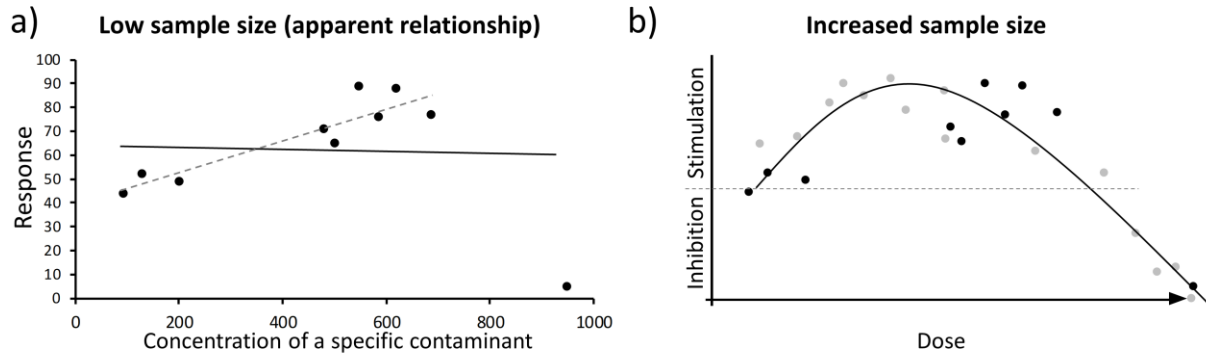


Figure 2: How a low sample size hides the hormetic response. In panel 2a, a typical ecotoxicology study with low sample size is shown. When all data are included, there is no relationship (black line). However, in ecotoxicological studies it is very common to find data point at the extreme of the distribution. Sometimes they are treated as outliers, and their removal modifies completely the relationship (grey dotted line). However, these points treated as outliers may be the most representative of the sample. In panel 2b, an increased sample size shows that more points are found at the extremes of the distribution, modifying it into an inverted U-shaped curve typical of an hormetic response.

Conflict of interest

The authors declare no conflict of interest

Credit author statement

Manrico Sebastiano: Writing- Original draft preparation, Visualization; **Simone Messina:** Writing- Reviewing and Editing, Visualization; **Valeria Marasco:** Writing- Reviewing and Editing; **David Costantini:** Writing- Reviewing and Editing, Conceptualization.

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