Paleo-ecotoxicology: what can lake sediments tell us about ecosystem responses to environmental pollutants?

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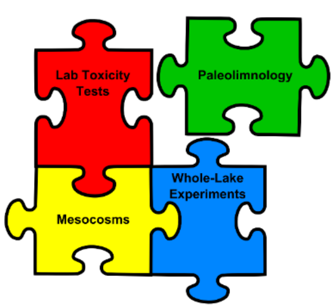
Abstract

The development of effective risk reduction strategies for aquatic pollutants requires a comprehensive understanding of toxic impacts on ecosystems. Classical toxicological studies are effective for characterizing pollutant impacts on biota in a controlled, simplified environment. Nonetheless, it is well-acknowledged that predictions based on the results of these studies must be tested over the long-term in a natural ecosystem setting, to account for increased complexity and multiple stressors. Paleolimnology (the study of lake sediment cores to reconstruct environmental change) can address many key knowledge gaps. When used as part of a weight-of-evidence framework with more traditional approaches in ecotoxicology, it can facilitate rapid advances in our understanding of the chronic effects of pollutants on ecosystems in an environmentally realistic, multi-stressor context. Paleolimnology played a central role in the Acid Rain debates, as it was instrumental in demonstrating industrial emissions caused acidification of lakes and associated ecosystem-wide impacts. “Resurrection Ecology” ” (hatching dormant resting eggs deposited in the past) records evolutionary responses of populations to chronic pollutant exposure. With recent technological advances (e.g. geochemistry, genomic approaches), combined with an emerging paleo-ecotoxicological framework that leverages strengths across multiple disciplines, paleolimnology will continue to provide valuable insights into the most pressing questions in ecotoxicology.



Introduction

Water and sediment quality guidelines for several common pollutants have been established for many regulatory frameworks, with the goal of balancing economic gains from industrial activities against the potential for ecological harm in freshwater ecosystems. Such guidelines are intended to provide a scientific basis for setting appropriate targets for the protection of aquatic life and ecosystem services, and are typically reported in environmental assessments. However, water and sediment quality guidelines are based largely on laboratory bioassays using a limited number of taxa as model organisms [1- 2]. Emerging –omics approaches (e.g. proteomics, metabolomics) provide further insights on the mode of toxic action and sublethal effects of contaminants on metabolic pathways, gene expression, endocrine functioning [3-5]. Syntheses of these studies are then used to estimate the concentrations above which we would expect a pollutant to have deleterious ecological effects [6].

It is well acknowledged, however, that classical lab toxicity tests do not necessarily reflect what is likely to occur in a natural ecosystem setting. For example, lab bioassays often use standardized aqueous media, which does not capture the full range of variability in water chemistry conditions likely to be encountered in the environment. The bioavailability and toxicity of pollutants in aquatic ecosystems can be highly dependent on physicochemical parameters like pH, alkalinity, and dissolved organic carbon [7-9]. In addition, only a small number of species are used routinely for laboratory toxicity testing, to allow for ease of comparison among studies. Care must be taken when extrapolating results based on a few species to whole communities and ecosystems, as sometimes even closely related species may have very different ecological tolerances [10-11]. There is also increased complexity in a natural ecosystem compared to a controlled laboratory setting due to ecological interactions (e.g.

competition, food web interactions) [12] and confounding stressors (e.g. climate change) [13]. This may result in organisms being more sensitive to pollutants in a natural ecosystem setting than would otherwise be expected based purely on lab bioassays [14]. Finally, lab toxicity testing is usually done on short timescales, as even chronic toxicity tests typically run for less than a month [15-16]. Sublethal effects [17] that can cause ecological harm to a population over the long-term may not be adequately captured over these short timescales, nor are complex ecological interactions readily observable in lab experiments. For example, a multi-year whole-ecosystem acidification experiment conducted at the Experimental Lakes Area in northwestern Ontario, Canada, showed that trout stopped reproducing at pH values higher than was anticipated based on lab toxicity experiments, which was the result of starvation following the loss of acid-sensitive prey species [18-19].

Controlled laboratory bioassays are clearly needed to establish the direct toxicity of pollutants on aquatic biota in the absence of confounding factors but, for the reasons stated above, the conclusions drawn from these studies need to be rigorously tested in a natural ecosystem setting. Mesocosm experiments conducted *in-situ* in a lake or stream ameliorate some of the limitations of lab experiments by simulating (albeit in a simplified way) the basic elements of an ecosystem [20-21]. Field surveys that characterize aquatic communities and biodiversity across broad environmental gradients can also be used to make inferences about the concentrations of pollutants required to cause deleterious ecological effects. Whole-ecosystem manipulation experiments, although logistically intensive and expensive, more fully capture the complexity of ecosystem responses to controlled inputs of a pollutant [22-23]. Each of the above-described approaches in aquatic ecotoxicology has strengths and limitations that, when used in a weight-of-evidence approach, can provide a more holistic picture of the potential for ecological harm from pollutant exposure. However, each of these approaches are still conducted on relatively short timescales (hours to a few years) due to logistical constraints, when a longer-term perspective is critical for characterizing the full range of natural variability needed to contextualize complex and nuanced responses of ecosystems to pollutants [24]. We have argued for the development of a new research framework that better integrates the principles of ecotoxicology with the use of lake or other sediment cores as natural archives of environmental change (the field of paleolimnology), providing the critical long-term perspective that is largely missing in ecotoxicology [25]. The purpose of this review is to document specific examples where paleolimnology has already contributed to advancing aquatic ecotoxicology, and discuss new opportunities to utilize lake sediment core records to directly test conclusions drawn from lab/mesocosm experiments in natural ecosystems, over long timescales. We also provide recommendations for future advances in the emerging sub-discipline of "paleo-ecotoxicology."

Contributions of paleolimnology towards our understanding of the movement and fate of contaminants in the environment

Lake sediments are partial sinks for many metals and persistent organic pollutants, and the use of paleolimnological approaches to study the movement of contaminants in the environment is well established [26]. Key questions such as "what is the doubling rate of chemicals in the environment," and "how long do they persist in the environment after bans or restrictions on production are enacted" could not have been adequately answered without the use of paleolimnological techniques [27-29]. For contaminants that have both natural and industrial sources, such as metals and polycyclic aromatic compounds (PACs), paleolimnology often provides the only means available to establish whether elevated contaminant burdens in a lake are due to industrial activities or are naturally occurring [30-31]. Paleolimnological studies have also been instrumental for understanding the movement of contaminants at a global scale, for example by providing evidence for the influence of the global fractionation hypothesis on latitudinal trends in contaminant histories [32-33], as well as the role biovectors like migrating seabirds and anadromous salmon play in concentrating pollutants in lakes [34-35]. With recent advances in methodology for studying post-depositional processes and source discrimination, paleolimnological research continues to generate new knowledge on the fate of contaminants in the environment [26].

Paleolimnology is not only useful for tracking contaminant deposition histories, but can also provide new information on the ecological consequences of long-term exposure to contaminants ("paleo-ecotoxicology"). Well-developed techniques are available for inferring both historic contaminant inputs (the stressor record) and concomitant changes in biological communities over time (the response variable) for several trophic levels across multiple habitats (benthic, littoral, pelagic), including many of the common model organisms in ecotoxicology (Figure 1).

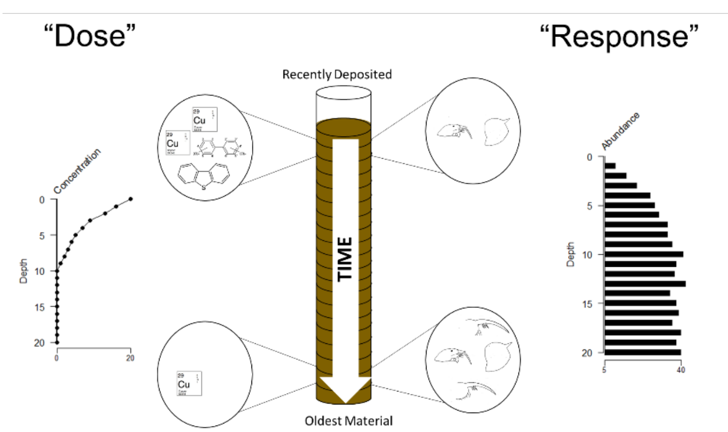


Figure 1. Lake sediment cores are routinely used to infer historic depositional trends for many environmental contaminants of interest, including metals and persistent organic pollutants (the "dose" or "stressor" record). Lake sediment cores also preserve the fossil remains of many different groups of biological organisms (the "response" record), including taxa routinely used as model organisms in ecotoxicology, such as Daphnia and Bosmina (shown as drawings of commonly recovered fossil remains).

Considerable advances have been made over the last few decades on our ability to recover high-resolution sediment cores that can be sectioned and dated (e.g. ^{210}Pb geochronology) to provide reconstructions of past pollution and ecosystem trajectories within a multiple-stressor context [36]. Importantly, sediment cores represent material that is temporally integrated (typically 2-10 years per 1-cm interval), reducing environmental noise from seasonal and inter-annual variability, and thus provide a clear indication of when significant ecological thresholds are crossed in response to a stressor. Merging paleolimnology with ecotoxicology can make significant, novel contributions to our understanding of the legacy of industrial chemicals in the environment and their effects on our ecosystems. With the exception of a few notable examples (which we will discuss), this is not commonly done, and represents a fruitful area for future research.

Biological organisms that can be studied from sediment cores

Many different groups of biological organisms leave some form of identifiable subfossil remain preserved in the sediment record [37-38]. Here, we focus on the most common biological proxies used in paleolimnological studies, with an emphasis on those that are especially relevant to ecotoxicologists.

Diatoms

Siliceous diatom frustules (cell walls) are well preserved in lake sediments. Historically, they are the most commonly used biological proxy in paleolimnological studies, used to quantitatively reconstruct changes in lakewater pH [39] and nutrients [40-41], as well as qualitatively assess changes in other limnological properties like dissolved organic carbon

(DOC) [42], and lake thermal and ice-cover regimes in response to climate warming [43]. Several studies have used diatoms in lake sediment cores to track metal contamination in mining-impacted regions, which in most cases was accompanied by acidification [44-46]. Abnormal (teratological) diatoms, which deviate from their usual morphological symmetry, have also been used as bioindicators of exposure to toxic substances (usually metals) in both ecotoxicological [47-48] and paleolimnological studies [49-50].

A few species of diatoms (e.g. *Navicula libonensis*) are used routinely to assess toxicity of chemicals for use in risk assessments [51]. Benthic diatoms are common pollution indicators in field assessments and mesocosm experiments to assess river pollution [52-53]. Various biotic indices, including species diversity, autoecological metrics, and abundance of select indicator taxa are used in diatom-based bioassessments of pollution [52]. These indices can also be applied in diatom-based paleolimnological studies, although inferences of diversity from sediment cores can be problematic, as changes in sedimentation rates that are unaccounted for can artificially alter diversity inferences [54]. Still, these challenges can be overcome, and paleolimnological approaches have much to offer the study of aquatic biodiversity [55], including the impacts of toxic substances.

To date, most of the diatom-based paleolimnological assessments of pollution have focused on acidification, eutrophication, and metals [49, 56-58], while an increasing number of ecotoxicological studies also focus on diatom responses to persistent organic pollutants such as pesticides and hydrocarbons [59-60]. For example, Bayona et al. [53] exposed diatom communities in biofilms to thiram and a hydrocarbon emulsion in outdoor flow-through mesocosms, and measured several structural and ecological traits to determine which measures were most sensitive. They found that ecological guilds were more sensitive to these chemicals than traditional bioindicator indices used for water quality assessments (e.g. for eutrophication). For example, motile diatom species were more tolerant of persistent organic pollutants than non-motile species [53, 59, 61]. Increased incidences of teratology have also been observed for benthic diatoms exposed to the genotoxic herbicide, maleic hydrazide [62]. Paleolimnological approaches have yet to be widely applied to the study of temporal changes in lake ecosystems occurring in response to pesticides, hydrocarbons, or other persistent organic pollutants, with some exceptions [63-64].

Chironomids

Non-biting midge larvae (Diptera, Chironomidae) are benthic macroinvertebrates that leave subfossil remains in lake sediments in the form of chitinized head capsules that can often be identified to the species-level [65-66]. Generally considered to be tolerant of pollution relative to other macroinvertebrates, chironomid communities have been an integral component of field and lab-based bioassessments of toxic substances [67-68], and several species in the *Chironomus* genus are common model organisms in ecotoxicology [69]. In paleolimnology, chironomids are commonly used in paleoclimate assessments [70], and are also used frequently to infer eutrophication and reconstruct changes in deepwater oxygen concentrations [71-73].

Chironomids typically live associated with the sediments, and therefore direct relationships between sediment quality (including concentrations of sediment-associated contaminants like pesticides) and the abundance and species assemblages of chironomid subfossil remains can be assessed through time in sediment cores. For example, chironomid subfossil remains were examined in tandem with reconstructions of DDT and its metabolites in a sediment core collected from a lake downstream of the St. Lawrence River at Montreal, Quebec

(Canada), where more than 16,000 kg of DDT were applied between 1965 and 1967, to investigate downstream ecological impacts of pesticide application [63]. Concentrations of DDT and its metabolites (∑DDT) exceeded sediment quality guidelines. Subfossil diatom assemblages showed little detectable response to ∑DDT, but chironomid taxa that live directly associated with sediments where ∑DDT partitions exhibited notable changes in abundance and species assemblage, as the absolute and relative abundances of *Paratanytarsus* and *Psectrocladius* declined markedly while Tanypodinae and *Tanytarsus* more than doubled following the application of DDT in the 1960s.

Similar to teratological diatoms, chironomid mouthpart deformities have been reported in higher percentages when exposed to various metals and other toxic substances [74-75]. However, deformities can also be natural occurrences [76]. A recent meta-analysis of studies on chironomid mouthpart deformities emphasized the importance of understanding background deformity rates in a community or population before asserting causal linkages to pollutant exposure [77]. Since one of the main advantages of a paleolimnological approach is the characterization of background conditions typically over decadal to centennial (and even millennial) timescales, and chironomid mouthparts are well preserved in sediments, paleolimnology may represent a promising tool for refining the use of chironomid mouthpart deformities as an early warning bioindicator of pollutant exposure [74].

Cladocera

Cladocera (Crustacea, Branchiopoda) are the main group of zooplankton that leave abundant and readily identifiable remains preserved in sediments cores, and provide several opportunities for the development of paleo-ecotoxicology. Many laboratory and mesocosm experiments have been conducted for *Daphnia* and other cladocerans to establish dose-response relationships with a wide variety of metals and industrial chemicals [78-80]. In addition to an ever-growing library of sensitivity to toxic substances, *Daphnia* also have a fully sequenced genome and a well-understood life history and ecology, and are considered a keystone species in lakes [81].

A primary challenge that has historically hindered the use of Cladocera in paleolimnological studies is the differential preservation of taxa, and only a few groups can be identified to the species-level [82, 83]. For *Daphnia*, only the post-abdominal claws and resting stages (ephippia) typically preserve in lake sediments, although headshields can preserve in rare cases [84-86]. Post-abdominal claws and ephippia cannot be identified to the species-level, but instead are assigned into one of two (sometimes three) species complexes, each containing several different species [87]. Different species within these complexes may display widely different ecological tolerances. For example, *Daphnia* generally have higher calcium optima compared to other zooplankton taxa, and declines in *Daphnia* have been observed in response to lakewater calcium decline (a legacy of acidification) in North America [88]. However, there are two species of *Daphnia* (*D. catawba* and *D. ambigua*) that are able to tolerate low ambient calcium concentrations [10]. These taxa cannot be resolved from other taxa within the broader species complexes based on morphology of their subfossil remains [87]. Instead, molecular genetics are required to distinguish *Daphnia* to the species-level, and well-developed techniques exist for extracting DNA from the resting egg bank [89-90]. Dormant resting eggs remain viable for decades, and can be hatched from different sediment intervals to examine evolutionary change in populations throughout different points in a lake's history in response to environmental pressures [91-92]. This application for subfossil cladocerans has made many important contributions to ecotoxicological research, and consequently is discussed in greater detail below, in the section titled “Resurrection Ecology.”

Because shells and headshields do not typically preserve, *Daphnia* cyclomorphosis in response to different predators cannot be tracked from sediments. This is unfortunate, as changes in water chemistry can limit their ability to produce morphological defenses, increasing *Daphnia* vulnerability to predation [93]. Thus, a common and important sub-lethal effect of *Daphnia* exposure to pollutants cannot be documented from lake sediments. Similarly, *Ceriodaphnia*, *Simocephalus*, *Scapholeberis*, and *Diaphanosoma* leave only a few small body parts preserved in the sediments [83], and are rarely reported in paleolimnological studies. In contrast, bosminids and chydorids are well preserved in sediments, with headshields, shells, ephippia, and postabdomens and/or postabdominal claws readily identifiable [83, 94]. Similar to *Daphnia*, *Bosmina* morphology is influenced by its dominant predators, and exposure to carbaryl has been linked to inhibition of anti-predator morphological defenses against copepod predators [95]. Since bosminid remains are well preserved, changes in size structure through time can be tracked from sediment cores [96], and carefully designed paleolimnological approaches have some potential use for testing hypotheses about the effects of low-level pollutant exposure on zooplankton predator-prey dynamics.

Paleolimnological approaches focusing on bosminids and chydorids can also be used to test the effects of pollutant exposure on life history traits, for example pollutant effects on sexual versus asexual reproduction in zooplankton [97-98]. Cladocerans and several species of rotifers, reproduce parthenogenically, where females produce clonal females asexually throughout most of the growing season, and switch to sexual reproduction during stressful conditions (e.g. overcrowding, limited food availability). Because the carapaces of parthenogenic females can be distinguished from ephippial females in bosminids and chydorids, the ratio of parthenogenic to ephippial females can be tracked through time from sediment cores to investigate changes in reproductive strategies in response to environmental stressors [99].

An important consideration for the use of cladoceran subfossils in ecotoxicological applications is that, for most species, exposure to pollutants is in the water column. Therefore, unlike benthic chironomids, where assemblage/morphologies can be more directly linked to pollutant concentrations in the sediments, the concentration of pollutants in sediment intervals do not necessarily reflect the historical exposure of cladocerans to pollutants in the water column. For example, pesticides and other polychlorinated biphenyls are hydrophobic, and bind readily to sediments. Several metal(loid)s, including arsenic, are redox sensitive, and can be remobilized from the sediments back into the water column during periods of anoxia. Metals (e.g. cadmium, chromium) and certain pesticides are known to bioaccumulate in *Daphnia* ephippia, and measurements of contaminants in subfossil ephippial remains have potential use to track historical exposure to contaminants in the water column [100-101]. To use this tool most effectively, further study is needed to understand the biological and chemical processes by which certain contaminants are sequestered in *Daphnia* ephippia. However, sediments contaminated by pesticides and fire retardants have also been shown to have significant negative effects on the hatching success of *Daphnia* from dormant eggs [102-103], and so the concentrations of pollutants in sediments do have some direct toxicological relevance to Cladocera.

While cladocerans are the best represented group of zooplankton in the sediment record, other taxa leave some identifiable fossil remains that have utility for a paleo-ecotoxicological approach. In Lake Orta, Italy, the abundance and diversity of rotifer (*Brachionus*) resting eggs was examined in a paleo-ecotoxicological application to assess the ecological effects of severe acidification and copper pollution [104]. The authors noted a persistence of viable rotifer and hatched egg cases in sediment intervals deposited during a period of severe acidification and copper contamination, and suggested that the production of resting eggs may have contributed to rotifer survival while other zooplankton taxa were extirpated. The above described study demonstrates the considerable potential that exists to develop a paleo-ecotoxicological approach to answer questions regarding the direct effects of sediment pollutants on zooplankton recruitment from resting eggs, an important source of colonizers for zooplankton communities in lakes.

Other Bioindicators

Other notable groups of biota that can be identified in sediment cores from their subfossil remains are the chrysophytes (algae), *Chaoborus* (phantom midges, macroinvertebrate predators), ostracods (crustaceans), and arcellaceans (testate amoeba). Although used less frequently than diatoms, chironomids, and cladocerans, other groups have considerable potential for integration into a developing paleo-ecotoxicological framework. For example, *Chaoborus* are traditionally used to indicate fish presence/absence, as certain species of *Chaoborus* cannot coexist with fish [105]. They were a crucial component in early studies on the limnological impacts of acidification (see “Acid Rain” below). They are also important predators on *Daphnia* and other cladocerans [93], and may be analyzed alongside cladoceran subfossil remains to infer the impacts of pollutant exposure on predator-prey dynamics [106]. The ostracods represent another useful bioindicator group for sediment toxicity testing [107-109], and could complement chironomid-based paleo-ecotoxicological studies in benthic habitats. Similarly, arcellaceans (testate amoeba) are benthic organisms that have been used as paleolimnological indicators of pH, oxygen, eutrophication, road salt, and metal pollution [110-112]. Macrofossils of aquatic macrophytes can also be identified in lake sediments [113], and have been used previously as part of a multi-proxy paleolimnological study to demonstrate that widespread application of tributyltin, an anti-fouling paint applied to boats, resulted in a collapse of aquatic macrophyte communities [64].

Case studies in paleo-ecotoxicology

Acid rain

Applied paleolimnology as a discipline gained prominence during the so-called "acid rain debates," as it was recognized that the unique long-term perspective provided by lake sediment cores held the answers to some of the most pressing questions of the time, which could not be answered through any other means. Most notably, while it was evident that many waterbodies on acid sensitive (poorly buffered) bedrock downwind of industrial centres were acidic, the argument was made by mainly vested industrial interest groups that these lakes were naturally acidic, since no pH measurements were available from the pre-industrial period. Diatom and chrysophyte species have well-defined, specific pH tolerances and optima, and thus it was possible to develop transfer functions using newly emerging, state-of-the-art statistical tools to quantitatively reconstruct changes in lakewater pH through time [114]. Using this approach, research groups from both Europe and North America were able to show that these lakes were not naturally acidic, but instead had undergone acidification consistent with the timing of industrial development [39, 115]. Another key contribution of paleolimnology to our understanding of the ecological impacts of acidification was to show that, in many cases, acidification led to the extirpation of fish [116]. Certain species of *Chaoborus* (e.g. *C.*

americanus) are heavily preyed upon by fish because they are large-bodied and do not undertake diel vertical migration as a behavioural adaptation for predator avoidance [117]. As such, these species do not typically co-exist with fish, and their presence in lake sediment cores is a strong indicator of fishless conditions in North American waters [105]. Observations of the appearance of *C. americanus* and other fishless indicators in dated sediment profiles, occurring coincident with diatom-inferred changes in lakewater pH, provided strong evidence that lake acidification can lead to the extirpation of fish. [116, 118-119].

Paleolimnological approaches continue to make important contributions to our understanding of the processes of chemical and biological recovery following acidification, by providing necessary information on pre-acidification conditions and the range of natural variability [49, 120]. For example, Jeziorski et al. [88] used a paleolimnological approach, interpreted in the context of lab bioassays and long-term monitoring [25], to demonstrate that lakewater calcium decline, a legacy of decades of acid rain, negatively impacts *Daphnia* populations and can inhibit recovery of zooplankton from acidification. Labaj et al. [121] compared multi-proxy palaeolimnological records from Sudbury (Canada) and nearby Killarney Provincial Park to tease apart the influence of metal contamination on biological recovery from acidification. The authors inferred that biological recovery in Sudbury lakes is likely inhibited by metals, which still exceed provincial water quality guidelines. These barriers to recovery are not readily apparent when only examining short-term mesocosm or lab studies.

The integration of paleolimnology into an extensive, interdisciplinary network of researchers studying acidification provides a useful roadmap for conceptualizing how paleolimnological approaches can be more effectively synthesized into ecotoxicological research moving forward. Mainly, key research questions were identified from the laboratory and field ecotoxicological research (are acidic lakes natural, did acidification lead to the extirpation of fish), and an innovative paleolimnological approach was devised (quantitative pH reconstruction, *Chaoborus*-based inferences of fish presence/absence) to leverage the unique perspective provided from the study of lake sediment cores to answer those questions.

Resurrection Ecology

Daphnia and several other species of zooplankton leave dormant resting stages in the sediments. These resting stages can be isolated from dated sediment cores and hatched to resurrect populations that existed at different time periods within a lake's history (termed "resurrection ecology") [92, 122-123]. Resting eggs potentially as old as 700 years have been hatched successfully in the lab [124]. Resurrection ecology provides the most direct example of how paleolimnology can be integrated with classical toxicity testing. Resurrected zooplankton populations from different time periods within a lake's history can be used in laboratory testing to determine if they differ in terms of their tolerances to a stressor, and to establish the genotypic and phenotypic basis for any observed differences [91, 124-125].

Zooplankton life history involves a sexual reproductive stage and short generation times, and thus potential exists for populations to rapidly evolve tolerances to a contaminant or other stressor. One of the fundamental questions that can be answered with resurrection ecology is if *Daphnia* (or other zooplankton taxa) become more tolerate of a contaminant with long-term exposure. If this is the case, then lab bioassays that are conducted on timescales of hours to weeks may actually over-estimate the sensitivity of natural populations to a contaminant, where sufficient time exists for evolutionary processes to ameliorate the harmful effects. This approach has been used to investigate evolutionary responses of *Daphnia* and other zooplankton taxa to acidification [126], metals [127-128], and pesticides [129-130]. For example, ancient genotypes of *Daphnia pulicaria* have been reported to be more sensitive to chlorpyrifos (an organophosphate insecticide) than younger genotypes, determined through acute toxicity tests [130]. In contrast, Rogalski [131] observed the opposite: *Daphnia ambigua* clones hatched from sediment intervals corresponding to a historical period of peak copper and cadmium contamination were in fact more sensitive than ancient clones hatched from the pre-impact period, apparent evidence of maladaptation to metal contamination. Future studies such as these, which pair resurrection ecology with paleolimnological reconstructions of contaminant deposition histories, can provide further insights into the trajectories of population evolutionary responses to long-term contaminant exposure.

The viability of dormant resting eggs can decrease over time, which may be an important confounding factor when interpreting differences in clonal tolerances to environmental conditions hatched from different time periods [132]. Recently, Rogalski [128] conducted a study to examine the combined influence of sediment age and metal contamination on both the hatching rate of *Daphnia* diapausing eggs, and juvenile mortality of hatched *Daphnia* in four Connecticut (USA) lakes over the past 40-100 years. The results showed that both sediment age and metal contamination were negatively correlated to hatching rate of diapausing eggs, but that metal contamination alone was positively, significantly associated with juvenile mortality of hatched eggs. This study demonstrates that it is not only sediment age which may influence hatching success of diapausing eggs, but also the environmental conditions in which the diapausing eggs were produced. Rogalski [128] provides several interesting explanations for possible mechanisms by which exposure to metals may influence hatching success of *Daphnia*, including potential fitness costs of bioaccumulation of metals, maternal investment, and genotoxic effects.

In summary, resurrection ecology provides unique insights into both evolutionary responses to long-term exposure to pollutants, and the effects of contaminant exposure on the functioning of sediment resting egg banks, both of which play a crucial role in long-term zooplankton population and community dynamics.

Recent studies on arsenic exposure

The toxic effects of long-term exposure of aquatic biota to arsenic has received relatively little attention (compared to copper, for example), despite being a prominent contaminant globally. Within the last year, two independent studies [133-134] were published that each examined the toxic effects of arsenic on multiple trophic levels under different scenarios and geographic settings. Chen et al. [133] collected sediment cores from two lakes in southwest China with documented histories of arsenic contamination from industrial tailings. Sediments clearly recorded arsenic enrichment consistent with the known history of arsenic contamination. Cladocera and diatoms were examined as ecological indicators of the potential toxic effects of arsenic. Both daphniid and bosminid fluxes (an estimate of abundance) decreased abruptly with arsenic contamination, a dramatic reversal of an increasing trend that was occurring with ongoing eutrophication. *Achnanthidium minutissimum*, a benthic diatom taxon known to be tolerant of metals, increased following arsenic contamination, while *Fragilaria contruens* and *F. crotenensis* decreased or were presumably extirpated. For both diatoms and cladocerans, critical ecological transitions occurred consistent with the increase in arsenic concentrations in water above 100 $\mu g\ L^{-1}$. No biological recovery was evident, despite recent decreases in arsenic.

Chen et al. [133] provided some of the first evidence of the long-term ecological consequences of arsenic contamination in freshwater ecosystems at the community level, and was rapidly followed by a second study also showing dramatic ecological impacts of arsenic exposure. Thienpont et al. [134] examined diatoms, Cladocera, chironomids, and *Chaoborus* in a naturally fishless, subarctic Canadian lake (Pocket Lake, Yellowknife, Northwest Territories) historically impacted by gold mining activities that released toxic arsenic trioxide dust as a byproduct. In contrast to the preceding example, Pocket Lake received arsenic contamination solely from atmospheric sources, rather than tailings. At the height of mining, arsenic concentrations in Pocket Lake were enriched by 1400%, and clear enrichment, albeit of a smaller magnitude, was also evident for antimony, mercury, and lead. At the height of mining activities, arsenic concentrations in the sediments were almost 4% arsenic by dry mass. Planktonic diatoms were lost from the sediment record, while benthic diatom taxa like *A. minutissimum* increased, consistent with Chen et al. [133]. In contrast, at the onset of arsenic pollution, *Daphnia* appeared for the first time in the sediment record and increased in abundance until the height of pollution, after which *Daphnia* and all cladocerans were functionally extirpated. *Daphnia* are known from lab toxicity tests to be relatively tolerant of arsenic [135]. The findings from Pocket Lake support this, suggesting that more sensitive, resident zooplankton populations were lost as a result of arsenic contamination (perhaps copepods which do not preserve well in the sediments, and receive less attention in ecotoxicological research; 136), leaving a niche available for *Daphnia* to exploit. This suggests that *Daphnia* may not be the most sensitive model organism for assessing the toxic effects of arsenic on waterbodies. However, arsenic contamination quickly exceeded the tolerances of *Daphnia*, leading to their eventual extirpation [134]. No recovery was evident for diatoms or Cladocera. Assemblage shifts in chironomids were observed consistent with the history of arsenic pollution, but no taxa were extirpated and some recovery was evident. No evidence of an impact of arsenic on *Chaoborus* was observed.

A third study [137], published within the same timeframe, contained similar themes to those in Thienpont et al. [134] and Chen et al. [133], although arsenic was not the primary contaminant of concern, but only one among many metal(loid)s (mainly Cd, Zn, and Cu). Ross Lake in Flin Flon, Manitoba (Canada) received metal-contaminated sewage effluent, and like Pocket Lake and the two lakes in Southwest China, an increase in metal(loid) concentrations was followed by a period of recovery following improved tailings management. Metal concentrations were consistent with the known pollution history, and the biodiversity of diatoms was reduced, as was the abundance of Cladocera (particularly *Bosmina*) and *Chaoborus* [137]. Assemblage changes in chironomids also occurred consistent with metal pollution. As was the case in the previous two examples, minimal signs of recovery were evident despite decreases in metal concentrations. An important finding of this study was that pre-industrial levels for several metal(loid)s also exceeded Canadian Council of Ministers of the Environment (CCME) probable effects guidelines for the protection of aquatic life, suggesting they are naturally elevated in this system. The authors stressed the need for appropriate knowledge of baseline conditions to assess toxic effects or remediation criteria. All three of these examples demonstrate how the dramatic ecological impacts of pollutants are clearly visible when long timescales are considered, and noise from inter-annual variability is reduced.

Recommendations for a new paleo-ecotoxicological framework

Paleolimnology has made significant contributions to our understanding of the movement of contaminants in the environment, and their impacts on lake ecosystems. With regards to the latter, however, our view is that the potential of paleolimnology to answer key questions related to toxic effects on aquatic biota has not been realized, due, in part, to limited communication amongst paleolimnologists and ecotoxicologists. Here, we provide recommendations for a new paleo-ecotoxicological framework (Figure 2), with the aim of stimulating development of new paleo-ecotoxicological approaches that use a long-term perspective to answer pressing questions in ecotoxicology, moving beyond simply providing an assessment of natural variability and baseline conditions, valuable though this information is.

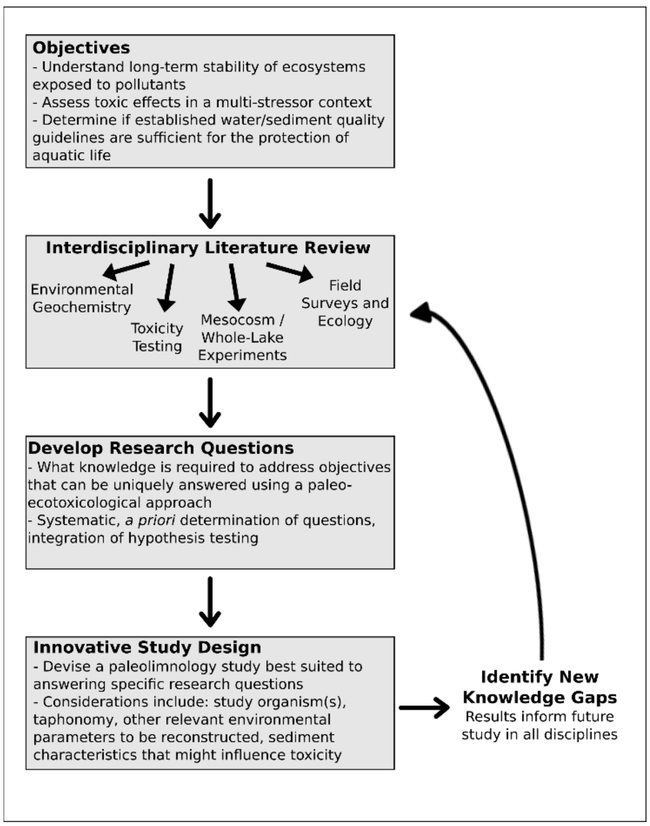


Figure 2. Our recommendation for what a new paleo-ecotoxicological framework could look like. Central objectives related to long-term ecological impacts of pollutant exposure in a multi-stressor context and evaluating regulatory guidelines are well suited to a paleoecotoxicological approach. A critical review of interdisciplinary literature can identify important knowledge gaps that can be addressed effectively using lake sediment records. A systematic study design, targeted to answering clearly defined a priori research questions, can take advantage of the wealth of information preserved in lake sediments. In turn, results of paleo-ecotoxicological studies can identify new knowledge gaps that can be addressed through studies using multiple approaches (e.g., toxicity testing, geochemistry). In this way, paleolimnology can be seamlessly integrated into an interdisciplinary context to provide novel insights into the toxic effects of pollutants on aquatic ecosystems.

A paleolimnological approach is well suited for investigations into the long-term stability of ecosystems exposed to toxicants, with well-developed techniques available for inferring both historical trends in key contaminants of interest, and ecological changes in key groups of aquatic biota. Importantly, it is feasible to analyze lake sediment cores from a suite of lakes in a region of interest, providing opportunities to compare ecotoxicological effects in lakes subjected to different environmental stressors, across wide limnological gradients, and with different pre-disturbance ecological communities. This, in combination with a long-term perspective over centennial to millennial timescales, also makes a paleolimnological approach well-suited for assessing toxic effects in a multi-stressor context across a full range of environmental conditions. Quasi-experimental designs that analyze sediment cores from strategically selected lakes [e.g. 34, 138] can be set up to further aid in the disentangling of multiple stressors, and influences of different water chemistry conditions (e.g. alkalinity) on ecosystem toxicity. Ultimately, a central objective of an emerging paleo-ecotoxicological framework should be to provide a means to evaluate the applicability and effectiveness of sediment and water quality guidelines for the protection of aquatic life. These general objectives can be applied across broad geographical scales, to address the ecological impacts of many potential contaminants, or to assess the impacts of industrial activities on specific regions of interest.

Paleolimnology is interdisciplinary by nature, and a paleo-ecotoxicological study requires a strong grasp of the available knowledge on environmental geochemistry (how a chemical of interest behaves in the environment), the mode of toxic action of that chemical, and its known ecological effects across different spatial and temporal scales (i.e. integrating lab toxicity testing, field surveys, ecosystem experiments). A thoughtful evaluation of the literature, including a critical review of the strengths and limitations of different study designs, is useful for identifying where critical knowledge gaps exist that can be addressed using a paleo-ecotoxicological approach. Systematic study design, with clearly defined *a priori* research questions (incorporating hypothesis testing where appropriate), should be developed with the overlying objectives in mind. An adaptable paleolimnological study can then be designed to most effectively answer the *a priori* established questions. For example, which biological proxies are best suited to address the questions of interest? Is a multi-indicator assessment most appropriate [139], or should the focus be on evaluating specific organisms (i.e. *Daphnia*) used in lab toxicity testing in a real-ecosystem setting? Is the main route of toxic exposure the sediments or in the water column (i.e. are we evaluating the efficacy of sediment or water quality guidelines), and which indicator(s) will best facilitate evaluation of this exposure route? Paleolimnology is a rapidly growing discipline, and as the ability to reconstruct past environmental conditions using lake sediment records continues to improve with time, so will the ability to ask increasingly nuanced questions about the toxic effects of pollutants. In particular, rapid advances in environmental DNA (eDNA) and DNA sequencing technology represents a powerful tool for detecting organisms that do not leave identifiable subfossil remains in sediment cores [140]. An interesting example of this emerging paleo-ecotoxicological tool can be found in Poulain et al. [141], where DNA extracted from a dated sediment core revealed an association between the phylogeny of the microbial mercuric reductase gene (merA) and the onset of the industrial revolution, evidence of a rapid evolutionary response by microbial communities to anthropogenic mercury deposition.

There are many opportunities available to directly apply tools used in ecotoxicology and environmental chemistry to evaluate the bioavailability and toxicity of the sediment matrix through time using lake sediment cores. For example, Xiao and colleagues [142] characterized the sediment matrix at varying intervals in a sediment core, including organic carbon content, grain size, and the chemical speciation of metals, and related this information to sediment risk assessments in order to evaluate changes in ecological risk through time in Lianhuan Lake, eastern China. This approach could be further expanded by examining the subfossil remains of benthic organisms that live associated with the sediments, in the same sediment intervals, in order to directly infer impacts on biotic assemblages. For example, we can characterize changes in the chemical make-up of sediment intervals and directly relate this to chironomid abundances and species assemblage, or the prevalence of chironomid mouthpart deformities, to determine at what concentration toxic effects are observable. It is more challenging to link sediment core proxies to historic changes in contaminant exposure in the water column, as the concentrations of chemicals measured in the sediments does not necessarily reflect what organisms were exposed to. It is possible, however, to provide indirect information on historic contaminant exposure in the water column. The potential also exists to use knowledge of chemical kinetics to estimate water column concentrations from sediment values (e.g. fugacity modeling), however this is not a straightforward process. This does not negate the effectiveness of a paleo-ecotoxicological approach, but does highlight the importance of an understanding of biogeochemistry and taphonomy (the process of fossilization/preservation in sediments) when designing and drawing conclusions from paleo-ecotoxicological studies. In cases where the sediment core record can be compared against limnological monitoring records (e.g. Chen et al [133]), it may still be possible to use the sediment core record to establish what concentrations of a pollutant cause ecological effects. Some pollutants can also be measured directly in subfossil remains of pelagic organisms (e.g. *Daphnia* ephippia [100]) to reconstruct historical exposure, though an understanding of contaminant partitioning and the related physiological processes that influence the concentration of pollutants in subfossil remains is beneficial for proper interpretation. The geochemical characterization of subfossil remains could also be examined for other relevant ecotoxicological questions, such as a record of changes in carbon cycling and food web structure (e.g. carbon and nitrogen stable isotopes) [143-144], in addition to providing a record of exposure to pollutants, to provide further insights into the chronic effects of pollutants on aquatic ecosystems.

Paleo-ecotoxicology as a sub-discipline has begun to gain prominence, especially within the last several years [31, 104, 127, 134 137]. It is increasingly recognized that lake sediment cores can provide novel information on the responses of aquatic ecosystems to many different pollutants, and is highly complementary to other ecotoxicological approaches. Like whole-ecosystem experiments, paleo-ecotoxicology allows for investigations into ecosystem-wide responses to pollutants, although mechanistic links are more challenging to tease out, and there is less temporal resolution, but longer timescales can be examined. However, paleolimnological studies can be replicated more easily than expensive and logistically-intensive whole-ecosystem experiments, both within a lake and on a regional scale, to span relevant limnological gradients. As part of an integrated framework, with better interdisciplinary collaboration and integration within ecotoxicology, paleo-ecotoxicology can leverage strengths and limitations across disciplines. Toxicity testing (lab and mesocosms), reductionist by design, provide the mechanistic, cause-effect basis for designing and interpreting biotic changes reported in paleo-ecotoxicological studies. Mesocosms, field surveys, whole-ecosystem experiments, and other ecological approaches can be used to infer the role of indirect, ecological effects of toxic exposures. With a solid grounding in the breadth of interdisciplinary literature available, the paleo-ecotoxicologist can develop targeted study designs that address key knowledge gaps, contributing to a more holistic picture of the toxic effects of pollutants. Knowledge which is necessary for establishing effective regulatory guidelines to protect aquatic life.

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