Proceedings

Dead Shells Bring to Life Baselines for Conservation, Revealing Invisible Biodiversity Loss †

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† Presented at the 1st International Electronic Conference on Biological Diversity, Ecology and Evolution, 15–31 March 2021; Available online: https://bdee2021.sciforum.net./

Abstract: We are living in a time of rapid biodiversity loss. Numerous studies have shown that modern extinction rates are higher than pre-human background rates. However, these biodiversity studies almost exclusively focus on large vertebrates: mammals, birds, fish and reptiles. We lack sufficient long-term records for many invertebrate taxa to track biodiversity loss. Aquatic, benthic, calcareous invertebrates, however, have the advantage of leaving a long-term record that can readily be sampled along with living communities. They leave easily-fossilized remains in the form of mineralized skeletons that accumulate in the very same sediments in which they live. These so called “death assemblages” contain an underutilized record for long-term monitoring. Here, we leverage calcareous micro- and macro-faunal remains from three aquatic environments spanning a two-dimensional gradient from freshwater to fully marine and polluted to remediated. We find death assemblages of lacustrine, calcareous microcrustaceans (Ostracoda) faithfully record human impacts, both pollution and remediation, across a fresh to hypersaline environmental gradient today. Death assemblages from calcareous marine, macrofauna (Bivalvia) also faithfully reconstruct temporal variation in human impact encompassing pristine, polluted, and successful remediation. We thus establish that death assemblages can act as useful gauges of changes in community assembly and population structures at local and regional scales which would be impossible with only contemporaneous monitoring of the living communities. These examples demonstrate that death assemblages from easily-fossilized taxa represent an effective tool for environmental managers to establish baselines for conservation targets and identify when community assembly approaches natural conditions in remediated ecosystems, rendering previously unrecognized biodiversity loss visible.

Keywords: biodiversity conservation; aquatic environments; molluscs; ostracods; conservation paleobiology

1. Introduction
We are currently facing many environmental catastrophes caused by human impacts on the natural environment. The rapid loss of biodiversity is one of those. Hundreds (potentially thousands) of species are being driven to extinction every year, leading to extinction rates that far exceed background rates [1,2]. This loss of biodiversity will have devastating effects on the Earth’s ecosystem function and services [3,4]. Although invertebrates comprise an estimated 80% of all animal species [5], and play important roles in regulating ecosystem health and function [5], estimates of biodiversity loss focus almost entirely on larger vertebrate species, e.g., [2,6–8]. Very little work has been done to document invertebrate biodiversity declines and less than 1% of invertebrate species have been assessed for threat by the IUCN [5,8]. The data that does exist for invertebrates suggests that they are experiencing declines in population size, extinction of species, and range contraction similar to those observed in vertebrates [8]. Freshwater ecosystems, and the invertebrates that inhabit them, are especially at risk [5,9].

Because they are fundamental contributors to functioning ecosystems, it is imperative that we effectively conserve invertebrate species. However, we lack sufficient long-term data for many invertebrate taxa and populations to effectively track declines in biodiversity and set conservation goals. Very few long-term surveys contain information on invertebrate species, and historical records that provide insight for conservation baselines for larger vertebrates, e.g., [10–12] do not exist for small invertebrates. The data gap can be filled by utilizing death assemblages, mineralized skeletal remains of organisms that accumulate in sediments. These not-quite-fossils can help us to track changes in the biodiversity of aquatic, benthic invertebrates with mineralized shells.

Death assemblages are gaining acceptance in the paleontological community as tools for recognizing ecosystems that are impacted by anthropogenic change and reconstructing pre-impact ecosystems, e.g., [13–18]. Discordance between surveys of living assemblages and the collected death assemblages arise when the living community responds to environmental perturbation. In contrast, death assemblages include a time averaged (temporally coarse accumulation of noncontemporaneous individuals) assemblage that may contain individuals that pre-date the anthropogenic impact. Of additional use to conservation, death assemblages have the ability to capture relative abundances (e.g., community structure) [19–22], and can be used for identifying species invasions/extirpations [22].

Here, we describe the use of live-dead comparisons for two benthic aquatic taxa, molluscs and ostracodes, in three case studies. These case studies span a range of environments (freshwater–marine) and habitat conditions (pristine–remediated) demonstrating the utility of death assemblages in multiple environments (see Table 1). We show that in all of these case studies, discordance between living and death assemblages occurs in areas of human impact.

<table>
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<th>Geographic Location</th>
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<th>Environment</th>
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<th>n, Habitats</th>
<th>Current Condition</th>
<th>Total Shells</th>
<th>Species Richness</th>
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2. Materials and Methods

2.1. Sampling Methods, Ostracodes

Living communities and associated time-averaged death assemblages were collected from Bahamas lakes during field expeditions in March 2009 and December 2013. Results from these Bahamian lakes were previously published in Michelson et al. [15]. Comparable living communities and death assemblages were sampled from lakes in the state of Wisconsin.
Wisconsin, USA in April 2015 and June 2016, results of which are reported here for the first time. In both The Bahamas and Wisconsin, eight samples of sediment were collected from each lake. At each point-sample, approximately 125 mL of sediment was extracted from the upper 2 cm of sediment. Each sediment sample contains both the remains of dead ostracodes in the form of mineralized calite shells and living benthic individuals that co-occur with time-averaged remains. To identify organisms collected alive (or recently dead), Rose Bengal was added to all sediment samples. Rose Bengal stains the chitinous hinge joining the two valves of the ostracode carapace bright pink [23]. In order to avoid false positives, only those ostracode valves with organic appendages visible (“soft parts”) and stained pink were counted as alive at the time of sampling. Because Rose Bengal will also stain partially-decayed organic material, some valves recorded as from live individuals may have come from recently-deceased organisms.

Sediment samples were stored in plastic containers before lab analysis. All sediment samples were subdivided into “coarse” (>125 μm) and “fine” (63–125 μm) fractions by wet sieving. The coarse fraction was picked for ostracode valves using a dissecting microscope. Because of convergent juvenile morphology and multiple molts per individual (between eight and nine), only valves from adult ostracodes were recorded. All valves were identified to species when possible following the higher taxonomic classifications of Brandão et al. [24].

2.2. Sampling Methods, Bivalves

Living communities for bivalves from Southern California were supplied by the Los Angeles Country Sanitation District (LACSD). LACSD performs annual surveys of the living communities in the summer along several transects off the Palos Verdes peninsula as part of a biomonitoring program. Samples are collected using a Van Veen grab, washed on a 1mm sieve, and picked, counted, and identified to species level using a standardized regional taxonomy [25]. The living bivalve assemblage used here is pooled from the 30 m and 61m stations of LACSD’s Line 10. This was done to approximate the living assemblages at the intermediate depth (50 m) where the death assemblages were collected.

Bivalve death assemblages were collected using a box corer (50 cm × 50 cm) along LACSD’s sampling transect Line 10 at 50 m depth in September of 2012. Each large box corer was subsampled using smaller plexiglass cores from which sediment was extruded in 2 cm increments. The bivalve death assemblages presented here came from 2 box cores, one with 3 subcores the other with 4 subcores. To parallel sampling of the ostracode death assemblages, we used bivalve shells only from the upper 2 cm of the sediment cores. After core increments were extruded, sediment was washed on a 1 mm mesh and the sieve residue (primarily calcareous shells) was dried. Bivalve shells that retained at least half of the hinge line, or the umbo, were counted as a single individual and identified to the lowest taxonomic unit possible (usually species). Data from the LACSD living surveys, as well as the bivalve death assemblages, have previously been published [16] where additional information about collection methods can be found.

2.3. Data Analysis

Match between the living community and associated time-averaged death assemblage in this paper is expressed in all cases using two metrics: Spearman’s rho and Jaccard-Chao agreement [26] following Kidwell [13,14,27]. Spearman’s rho (hereafter referred to simply as rho) measures the rank-abundance agreement (absolute abundances of species are converted to ranks) between dead-occurring species and live-occurring species; therefore, it is a measure of agreement in population size. A maximum value of +1 indicates the most abundant species alive is also the most abundant dead and the least abundant species alive is also the least abundant dead species. A minimum value of −1 indicates mismatch: the most abundant species alive is the least abundant in the death assemblages and vice versa. Jaccard-Chao measures the proportion of shared species between living
communities and death assemblages and thus ranges from 0 to +1. It is a modified version of the Jaccard measure of agreement corrected for “unseen shared species” owing to undersampling of the living community, death assemblage, or both [26]. It thus measures match in species composition between living communities and death assemblages. These two metrics have become standard practice in conservation paleobiological studies testing for live/dead mismatch associated with human impact, e.g., [28–30].

For ostracode live/dead comparisons, agreement metrics were calculated in two ways: at point-sample scale and at lake (habitat) scale. Point-samples reflect living communities and death assemblage at single location of sampling within a lake. All of the lakes sampled are relatively small, so little spatial variation in ecologically-meaningful factors exists in these lakes other than distance from shore [31]. To assess live/dead agreement at the lake scale, all individuals of the same species from all point samples within a lake were summed together, keeping separate live and dead individuals. In this way, an estimate of habitat-scale live/dead agreement was generated by pooling point-samples. Bivalve samples from the Southern California continental shelf are represented by a single point sample so the point sample is equivalent to the habitat scale.

T-tests were used to test whether there exist differences in means of both metrics of live/dead agreement in lakes experiencing different human impact in the same region: between impacted and “pristine” lakes in the Bahamas and between impacted and remediated lakes in Wisconsin.

3. Results

At point-scale, live/dead agreement is significantly lower in impacted lakes than in “pristine” lakes (Bahamas; Figures 1A and 2A,C) and remediated lakes (Wisconsin; Figures 1B and 2A,C) using both metrics of live/dead correspondence. This lower live/dead agreement persists when all point samples from the same environment are pooled to produce one estimate per habitat, but loses quantitative significance (Bahamas Figures 1C and 2B,D and Wisconsin Figures 1D and 2B,D). Our sample from Southern California displays high agreement in species composition, but not in population rank-abundance (Figures 1B and 2A,C).

While samples from impacted lakes do show significantly lower mean live/dead agreement in population rank-abundance and species composition, they are not uniformly so. Samples from impacted lakes show greater variation in both metrics of live/dead agreement as some point-scale samples show high live/dead agreement like un-impacted and remediated lakes (Figures 1A,B and 2A,C).

At the habitat scale, impacted lakes have lower live/dead agreement in both metrics (Bahamas Figure 1C; Wisconsin Figure 1D), but this reduced live/dead agreement is not statistically significant (α = 0.05) in either metric of agreement (population rank-abundance Figure 2B; species composition Figure 2D). Southern California is represented by one sample so the habitat-scale is equivalent to the point-scale.
Figure 1. Live/dead agreement at point- (A,B) and habitat-scales (C,D) in Bahamian (A,C), as well as Wisconsin and Southern California environments (B,D). Agreement in population rank-abundance is plotted on the x-axis. A value of +1 indicates the most abundant species in the living community is also the most abundant species in the death assemblage, while a value of −1 indicates the most abundant species in the living community is the least abundant in the death assemblage. Agreement in species composition is plotted on the y-axis as the proportion, corrected for undersampling [26], of species common to both the living community and the death assemblage. Symbols correspond to human impact by location: unfilled circles represent samples from impacted environments, filled circles samples from “pristine” Bahamian lakes, crosses samples from remediated Wisconsin lakes, and the asterisk represents the remediated sample from the Southern California continental shelf.
Figure 2. Bar plots of population rank-abundance agreement as measured by Spearman’s rho correlation coefficient (A,B) and species composition agreement as measured by Jaccard-Chao (C,D). Both represent mean live/dead agreement at point- (A,C) and pooled habitat scale (B,D). Colors correspond to human impact: black represent impacted environments, while grey represent “pristine” environments (Bahamas) and remediated environments (Wisconsin and Southern California). Error bars represent 95% confidence intervals. Southern California is represented by one sample so lacks error bars. Sample size of each category listed above. “*” indicates significantly difference in mean (p < 0.0001) live/dead agreement of samples from same location. Mean live/dead agreement at habitat scale is not significantly different (α = 0.05) in either metric in Bahamas or Wisconsin.

4. Discussion

The case studies presented here demonstrate the effectiveness of live/dead comparisons in identifying ecosystems that have been affected by human impacts. A single survey of the living assemblage compared to the death assemblage has the potential to demonstrate change in community composition and/or structure, thereby indicating human modification of the natural ecosystem. Throughout the analysis presented above, we see many instances of ecosystems classified as impacted falling into the low fidelity or very low fidelity quadrants defined by Kidwell [14,26], indicating that human activities have caused ecological response within the timespan over which death assemblages have accumulated. It is possible that a live-dead survey in an impacted environment may fall in the high fidelity quadrant (Figure 1 and 2). This does not mean the the community is not
at risk, but could indicate that the impact has not been prolonged or acute enough to cause ecological turnover, or, on the other hand, that impact has been so long-standing that the death assemblage reflects the human impact [22]. In cases where this is suspected, it may be wise to explore the possibility of collecting short sediment cores, e.g., [16] to provide a longer time scale for comparison. Determination of the degree of fidelity can also be used to determine if conservation or management is effective. In these case studies, we show that several live/dead collections from remediated ecosystems fall in the “high fidelity” quadrant. In our case studies from Wisconsin lakes and the southern Californian coastline, both areas that experienced remediation for eutrophication beginning in the 1970s, live/dead comparisons showing high fidelity could indicate success of remediation attempts (e.g., return to a pre-impact community preserved in the death assemblage).

Mismatch between the living and the death assemblage occur as species are gained or lost from a community, and as the relative abundances of species change in that community in response to anthropogenic impact. Because of this, individuals found in death assemblages, but not found in the living assemblages, could indicate local extirpations e.g., [18]. These “canaries” [15] can be used as indicators of environment and ecological deterioration. In addition, changes in the relative abundances of species (indicated by rho), can indicate changes in community structure. For example, in the southern California case study, the death assemblage contains a high proportion of the pollution tolerant and nutrient-loving bivalve *Parvilucina*, which was abundant during the 1970s at the height of eutrophication, but has since declined in abundance due to wastewater treatment (e.g., remediation) [16].

The time scale that is captured in the death assemblage can vary among habitats depending on the degree of time-averaging of the skeletal components. In continental shelf environments, studies have shown that bivalve assemblages can contain individual valves that are several millenia old in the upper 8 cms of the sediment column [32]; this may also be the case for molluscan assemblages in some lakes [33]. In cases where time-averaging is suspected to be this long, as in our Southern California case study, the death assemblage will contain individuals that span a wide gradient of human impact. Based on dating of the sediments in which they occur, we suspect that the time-averaging of ostracode assemblages is multi-decadal. Future work using direct dating of the ostracode shells will allow more precise estimates of the window of time-averaging of these death assemblages.

5. Conclusions

We strongly urge the use of death assemblages to supplement ongoing biodiversity surveys, especially where invertebrates with mineralized skeletal remains play an important role in the ecosystem. The collection of death assemblages is low-tech and low-cost, especially in terrestrial freshwater environments. Processing of death assemblages and the identification and quantification of taxa preserved in death assemblages can be time-consuming, however, this time cost can be minimized in cases where there are bio-indicators or key species of interest. Death assemblages have the ability long-term data (multi-decadal in many cases) that can prove key in the establishment of conservation baselines and can provide context to biodiversity changes to help bring to light the invisible biodiversity loss occurring in many invertebrate groups.

**Author Contributions:** Both J.L.-P. and A.V.M. contributed to writing the manuscript. J.L.-P. contributed the bivalve data from Southern California. A.V.M. contributed the ostracod data from The Bahamas and Wisconsin. A.V.M. performed statistical analyses. J.J.S., K.C.K.-L., S.F. and L.P.B. contributed to study design and ostracode data collection. All authors have read and agreed to the published version of the manuscript.

**Funding:**

**Institutional Review Board Statement:**

**Informed Consent Statement:**
Data Availability Statement:

Acknowledgments: Collection of Bahamian samples was enabled by National Science Foundation (U.S.) grant EAR-1249831 to AVM and EAR-0851847 to LPB and carried out under permits G252, G234, and G235 from the Bahamas Environment, Science & Technology Commission. Collection of Wisconsin samples was enabled by funding from the Keck Geology Consortium. Collection of samples from Southern California was enabled by NSF EAR 1124189 awarded to S. Kidwell and A. Tomasových. Living surveys from LACSD were made available by D. Cadien. S. Kidwell provided helpful, thoughtful, and constructive advice on the use and interpretation of live/dead agreement. We are grateful to J. Ash, V. Haley-Benjamin, L. Knowles, S. Bright, M. Dalman, E. Frank, E. Draher, S. Bua-Iam, A. Conaway, J. Dannehl, S. Garelick, T. Mooney, and A. Waheed for invaluable field assistance. K. Brady Shannon, A. Myrbo, and the National Lacustrine Core Facility provided indispensable logistical and scientific support. E. and T. Rothfus of the Gerace Research Centre enabled the collection of Bahamian samples by managing communicating with the Bahamian government. M. Davila-Baney, O. Diana, and M. Viteri provided valuable lab assistance.

Conflicts of Interest: The authors declare no conflict of interest.

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