The dirt on snails: An experimental test of the effects of fine sediments on snail mortality

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Introduction

Lake Tanganyika's many endemic species are under threat by impacts from human development and land use changes. Anthropogenic sediment inputs into the lake have increased, with a suite of negative effects on the benthic fauna. Sedimentation reduces habitat heterogeneity, alters cycling of nutrients, interferes with visibility, and interferes with photosynthesis. For fauna of the rocky benthos, settled sediments may directly affect movement, digestion, and respiration. Experimental tests and field studies are needed to determine the species most at risk and the effects most damaging from excess sedimentation.

In a laboratory test of several distinct grain sizes of sediment on the endemic gastropod *Reymondia horei,* Donohue & Irvine (2003) showed that all sediments were lethal, and the finest grained sediments had the fastest effect on gastropods. However, several aspects of their experimental design may have significantly compromised the applicability of their results to natural settings. They used only one species, *R. horei*, which is a snail found primarily on the underside of rocks, thus would seldom encounter fine sediments in its natural habitat. By subjecting *R. horei* to an environment where only the upper surfaces of rocks were available, and then inundating that surface with sediment, Donoghue & Irvine created a situation where mortality seems a probable outcome. Futhermore, the sediments themselves were unrealistic for testing a lacustrine effect. The sediments were derived from terrestrial sources, thus would lack any of the normal complement of bacteria found on suspended and deposited aquatic particles. Their experimental treatments sieved and sterilized all sediments, which, while providing uniformity, established treatments that are far removed from natural variables and decreased relevance to the problem in the field. These authors ensured that sediments were a single particle size, not the mix of grain sizes found in natural sediments, which would decrease interstitial space for bacterial growth. Finally, sterilization of fine sediments by combustion oven, the replacement of treatment sediments every two days with new, sterilized, sieved terrestrial sediment, and the replacement of filtered lake water every two days all made starvation and suffocation a probable cause of mortality for these snails in a way that would not be the case in natural settings with similar sediment loads. This obscured the possible real effects of sediment on snail mortality. Sediments may interfere with digestive physiology, but natural sediments would not be totally devoid of organic matter, nor would natural lake water. Also, though sedimentation rates may be rapid, gastropods are not exposed to terrestrial sediments in nearly the quantities described in Donohue & Irvine (2003), which completely covered all available hard substrate. I aimed to repeat this study with a more relevant, realistic suite of variables.

Some of these aspects were addressed by a previous study. Mutatina (2003) conducted an experiment based on Donohue & Irvine (2003) that differed in several ways. A variety of gastropod species were used and several aspects of the sediment used in the experiment departed from the methods described by Donohue & Irvine (2003). However, the results of Mutatina's work were compromised by a shortage of aeration for replicate chambers, obscuring her findings. We solved these logistical challenges and expanded the study.

My experiment sought to build on the work of Mutatina by examining three of the assumptions of Donohue & Irvine (2003). First, I used three species, in addition to *R. horei,* which are common residents on rock surfaces and thus the most likely species to be subjected to sedimentation in the field. All species were residents, and generally unique to, unsedimented sites. Second, natural materials such as rocks, sediment, and lake water were not completely sterilized. This means that algal and microfaunal growth could theoretically occur. Therefore, if starvation were to cause snail mortality, it would be because sediments had prevented access to nutrients, not because the nutrients were never there. Third, experimental treatments of terrestrial sediments (as used by Donohue) were compared to treatments of aquatic sediments. This was done to examine the effects of inundating snails with sediments foreign to the lake, compare with sediments that were derived from the lake.

Methods

Our experiment consisted of two treatments and a control. One experimental treatment exposed snails to terrestrial sediments while the other exposed them to similar amounts of sediments derived from an aquatic source. There were eight replicates of each treatment, making a total of 24 experimental units.

The eight replicates were arranged in a randomized block design. We used plastic two-liter containers as aquaria. A rock was placed in each aquarium. In the experimental treatments, enough sediment was added to cover all but the top surface of the rock, with some fine sediment layered on the rock's surface. This approximates the situation observed at sites in the field that have been sediment impacted. The approximate amount of sediment added was recorded to control for experimental variation. In each aquarium, lake water was added to the two liter line, making the total volume of rocks, sediment, and water two liters for each aquarium. Once the sediments had settled, two snails from each of four species (*Lavigeria coronata, L. grandis, L.* species 'J', and *Reymondia horei*) were added to each aquarium for a total of eight snails per aquarium. Each aquarium was aerated using a stone bubbler.

Rocks and snails were both collected by snorkelers from a rocky substrate site known for its pristine condition, Kalalangabo South (KLG-S, site information in Huntington, this volume) at approximately 3-5m depth. Rocks were chosen for relatively flat upward facing aspect, selected to be similar in size and shape, transported to the lab in a bucket of water, measured, any macroinvertebrates removed, and placed directly in the experimental chambers to preserve natural algal condition on the surface. The surface area of each rock was quantified by cutting out an outline of the rock in aluminum foil, and weighing the cut out.

Sediments were obtained from a single site (Nondwa, the first sedimented area to the south of KLG-S) where sediments are naturally deposited in the water. Aquatic sediments were obtained by snorkelers at a depth of about 5 meters. Terrestrial sediments were obtained about 2 meters from shore at the same site. All sediments were sieved to remove macro-invertebrates and to ensure that the grain size for all treatments was less than 1 mm. The grain size distributions of both terrestrial and aquatic sediments were quantified using sieves.

Half the water was changed every other day in all aquaria to avoid buildup of toxic metabolites in these closed containers. We siphoned out old water, then slowly added fresh, aerated, lake water over a baffle which prevented disturbance to the snails and sediment.

The aquaria were examined every 24 hours at the same time of day to describe snail behavior and record mortality. Snail location was recorded as one of the following for each individual: on the rock, on the wall of the aquarium, on the substrate (sediment in experimental aquaria, bottom of aquarium in control aquaria), on the bubbler, or on another snail. If a snail was on another snail, the species of that other snail was recorded. If a snail was upside-down on the substrate, this was recorded as well. Snails that were not obviously alive (i.e. attached to the aquarium wall) were tested for reactions with a probe. Dead snails were removed, recorded, measured (total length), and preserved in alcohol. Newborn snails were removed from the chambers. Individual snails of the same species within an aquarium were not distinguished from each other.

Survival data for only the first ten days of the experiment were analyzed using JMP IN 5.1. One-way ANOVA's were performed on both combined species survival response and individual species survival response to the sediment treatment (aquatic, terrestrial, or control), followed by the Tukey-Kramer HSD (honestly significant difference) post-test for multiple comparisons. Data collection continued for several weeks after that reported here, and will be presented in a more detailed analysis of the full set of results (in prep.).

Results

An ANOVA revealed a significant effect of treatment, for all snail species combined (Fig. 1 A, $F_{2, 93}$= 4.84, P=0.01). A Tukey-Kramer HSD revealed that both the aquatic and terrestrial treatments had a significantly different response than the control treatments, though not from each other.

The ANOVAs by species (Table 1 and Fig 1 B) revealed that *R. horei* had a highly significant response to the treatments, whereas the three *Lavigeria* species did not.

Conclusions

This initial analysis of the experiment (after running for only ten days) generates two interesting findings. The first is that there are no significant differences between aquatic and terrestrial derived sediments in terms of their effect on snail mortality. This suggests that the experimental design assumption made by Donohue and Irvine (2003) end up being safe assumptions to make. There appears to be no consequence of using terrestrial sediment for the purposes of this experiment.

The second major finding is that *R. horei* was the only snail whose survival was significantly affected by terrestrial sediments. This has significant implications for the findings of Donohue and Irvine (2003), since *R. horei* was their only study species. As was mentioned in the introduction, the habitat preferences of *R. horei* suggests that it would rarely be exposed to sediments in natural settings and would therefore be behaviorally and physiologically maladapted to settings like those encountered in this laboratory experiment.

This hypothesis is stroingly supported in spades by the initial analysis of the data from this experiment. *R. horei* was the only species to be significantly affected by the addition of large quantities of fine sediment. All other species exposed to either terrestrial or aquatic sediments had a final survivorship statistically indistinguishable from the control treatment. This again is in agreement with knowledge of the natural histories of the study species. *R. horei* is the only snail in this study that lives primarily on the undersides of rocks. All other species are probably exposed to some fine sediments in their natural habitats on rock surfaces even in pristine settings, and thus have evolved coping strategies.

What these data suggest is that the findings of Donohue and Irvine are limited to the species they chose: *R. horei.* The relevance of their results to the real world then is quite limited. Since *R. horei* lives on the undersides of rocks, these snails are unlikely to experience sediment impacts until the whole of their habitat has been inundated – long after the surfaces of the rocks and the rest of the malacofauna has been affected. Only then would significant quantities of *R. horei* habitat be removed and only then would *R. horei* be forced to deal with fine sediments. Though sedimentation is currently happening in Lake Tanganyika at an alarming rate, it falls well short of the rates needed to make the above scenario a reality. It seems wise to focus future research attention on the taxa most likely to be affected by sedimentation in the real world.

Further studies

The complete experiment was carried out over 37 days, however only the first 10 days were analyzed for this report – I plan more complete analyses of the full data set as part of an ongoing study. For example, the data were presented as a percent survivorship, compared to Donohue and Irvine's method of comparing the rates of mortality over many days. Behavioral data is likely to add further insight into the responses of each species to sediment input.

These preliminary analyses also point to new experiments that can be done. Probably the most valuable follow-up study would be an *in-situ* experiment where the response of *R. horei* is compared to the other species in a natural setting exposed to increased sediment.

Acknowledgements

I would like to thank the Nyanza project and the NSF for giving me the opportunity to complete this study. Dr. Ellinor Michel, Dr. Jon Todd, and Pete McIntyre were invaluable resources that aided me in numerous aspects of experimental design. George Kazumbe recorded data in my absence, thus extending the life of this experiment from 10 days to 37 days, which is phenomenal. Christine Gans, Ranjan Muthukrishnan, Jessica Corman, and Winston Wheeler all helped me record data, each sacrificing a half hour of their time to save me a total of ten or more. Kevin Robinson watched me create randomized blocks using Excel's random number generator and tried to act as impressed as I was with myself.

References:

Donoghue, I. & Irvine, K. (2003) Effects of sediment particle size composition on survivorship of benthic invertebrates from Lake Tanganyika, Africa. Archiv fuer Hydrobiologie.

Mutatina, Alieth. (2003) An experimental test of the effects of sedimentation on Lake Tanganyika rocky shore gastropod survivorship . *The Nyanza Project 2003 Report.*

Species	df	F ratio	P-value
L. coronata	2, 21	1.152	0.335
L. grandis	2, 21	1	0.385
L. species J	2, 21	0.528	0.597
R. horei	**2, 21**	**5.914**	**0.009**

Table 1: ANOVAs of species-specific response to treatments (**bold** indicates significance)

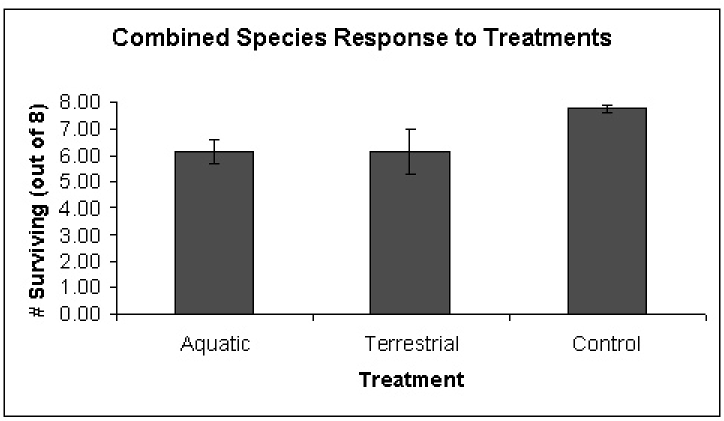

Figure 1A

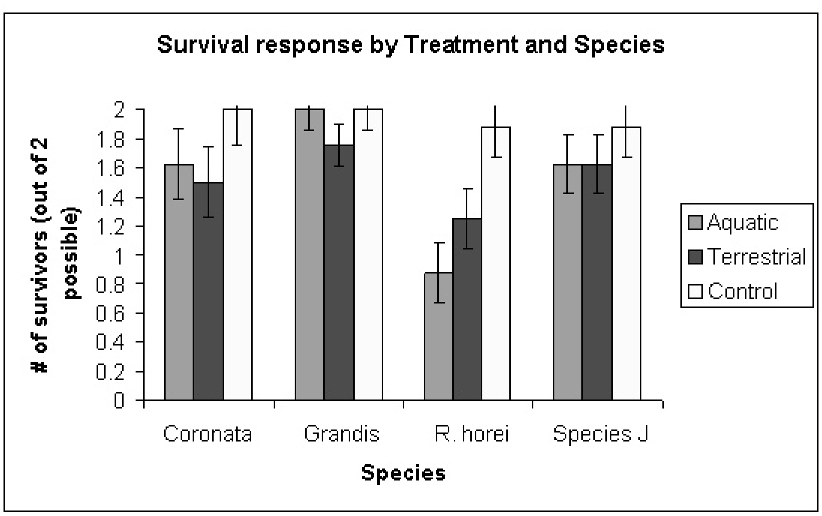

Figure 1B