HANGING WITH MY PHAT BRO(MELIADS): THE NEGATIVE EFFECTS OF INCREASING ACIDITY ON AQUATIC INVERTEBRATE ABUNDANCE IN *GUZMANIA MONOSTACHYIA* (BROMELIACEAE)

ANKIT RASTOGI, SARAH C. ISBEY, HA LINH VU,
ZACHARY A. MAYER, AND SAMUEL J. HAYNOR

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Abstract: Acid rain has had profound effects on aquatic ecosystems worldwide. Decreases in rain pH have lowered the biomass of aquatic organisms in lower trophic levels that are unable to tolerate higher acidities. Invertebrate communities living in bromeliad tanks may be particularly susceptible to changes in rain pH because they obtain water by directly collecting precipitation, and therefore, they have no buffering capacity from surrounding geological substrates. We predicted that increasing water acidity would negatively affect invertebrate abundance in bromeliad tanks. We first measured the natural variation in pH and invertebrate abundance in nine bromeliads from the Estación Biologia Monteverde in Puntarenas, Costa Rica. We found that bromeliad pH naturally varied between 3 and 4.9, and natural invertebrate abundance was marginally negatively correlated with acidity. We then manipulated pH in 27 bromeliad tanks (three levels: pH = 2.4, 3.1, and 5.6) to experimentally test the effects of pH on invertebrate abundance. When pH was experimentally manipulated, invertebrate abundance was significantly lower in treatments with a pH of 2.4 compared to both 3.1 and 5.6. The pH levels of 5.6 and 3.1 did not significantly differ from each other. These data suggest that lower levels of acidity than naturally found in bromeliad tanks can lead to lower invertebrate abundance. Further studies are needed to fully understand the implications of increasing acidity of rain in neo-tropical environments for ecosystem processes relating to bromeliad tank communities.

Key Words: acid rain, phytotelmata, Monteverde, bromeliad

INTRODUCTION

Acid rain and its effects on biological systems are becoming increasing apparent as the pH of rain continues to drop. Rain was once believed to have a pH of 5.6, as this represents the pH of distilled water saturated with CO_2 (Schindler 1988). However, rain water commonly has a pH near 5.0 (Schindler 1988). Regions with high levels of human activity (e.g., areas of manufacturing density or high levels of emissions) have even lower rain pH values because of anthropogenic sulfur emissions (Schindler 1988). In addition, sulfur emissions can affect rain pH globally because atmospheric sulfur pollution can migrate between countries and across oceanic barriers (Schindler 1988; Raven et al. 1999).

Increases in acid rain during the last century have lowered the pH of many aquatic ecosystems in North

America and Northern Europe, resulting in the disappearance of some invertebrates in lower trophic levels (Schindler 1988). Acid rain can also lead to increases in aluminum concentrations through increased leaching, which negatively affects fish and other aquatic organisms, by changing the solubility of aluminum in water (Raven et al. 1999). Although organisms in higher trophic levels may be relatively resilient to pH values less than 5.5, organisms in lower levels may be less able to acclimate to these changes (Schindler 1988). The reasons for increased susceptibility to acid rain of organisms in lower trophic levels are unknown, but their declines in response to acidity are well documented (Schindler 1988). While the United States and Canada have reduced sulfur emissions in the last fifty years, acid rain is still a major problem in other North American countries, such as those in Central America (Schindler 1988; Pringle et al. 1993). Therefore, further increases in acid rain could have drastic effects on invertebrate communities worldwide.

Many species in the bromeliad family host complex aquatic invertebrate communities in their central tanks and surrounding leaves (Morales 2001). These communities are particularly vulnerable to changes in rain pH because they do not have the buffering capacity from the weathering of geological substrates that are present in freshwater communities (Schindler 1988). Additionally, epiphytic bromeliads are more susceptible to direct effects of rain pH changes because they obtain their moisture either by absorption from the air or direct collection of rainwater (Morales 2001).

In the Monteverde Cloud Forest (MCF), bromeliads are important sources of standing water, providing breeding habitats for many species of invertebrates (Weller and Cushman 2004). MCF is a diverse tropical cloud forest in the Puntarenas region of Costa Rica. Past studies on bromeliads in MCF have focused on the correlations of tank water volume, detritus level, and nutrient availability with invertebrate densities (Weller and Cushman, 2004; Matsuura et al., 2005; Treirweiler et al., 2005). However, no previous studies, to our knowledge, have investigated the repercussions of pH levels on invertebrates inhabiting bromeliad tanks.

To examine the effects of pH on this unique aquatic environment inside bromeliad tanks, we studied the effects of natural and experimental variation in tank water pH on invertebrate abundance. Specifically, we addressed three questions about bromeliad tank communities and acidity: (1) What is the natural pH variation in

bromeliad tanks? (2) How does pH correlate with invertebrate abundance? and (3) How does invertebrate survivorship vary with experimentally manipulated pH? We predicted that increasing acidity would negatively affect invertebrate abundance for both natural and experimental variation in tank pH.

METHODS

We conducted our study in a tropical cloud forest at La Estación Biologia de Monteverde, Costa Rica on 20-22 January, 2007. We collected bromeliads of the species *Guzmania monostachyia* (Bromeliaceae) along the Sendero Principal trail at varying elevations. We were only able to obtain bromeliads that were growing at less than 5 m up the trunk because higher bromeliads were inaccessible. Within the bromeliads, we sampled the diverse community of invertebrates, including adults in the orders Nematoda, Annelida and Copepoda, and larvae from the orders Diptera and Coleoptera.

We measured the natural variations in bromeliad tank pH on nine bromeliads in situ using a Milwaukee SM102 pH Meter, rinsing the probe in tap water between readings to eliminate contamination. We characterized the sizes of the bromeliads by measuring the length of the longest leaf on the outermost ring of leaves, diameter of the bromeliad, depth of the tank from the first separation of the innermost leaves, and diameter of the tank at the point where depth was determined. We took these measurements to see if bromeliad size covaried with invertebrate abundance.

We tested the relationship between the natural pH level of the bromeliads and the invertebrate communities within their tanks. We collected the organic material in 9 bromeliads to sample the aquatic invertebrate community. After removing and washing the detritus in each plant, we cleaned the plant with 15 half-second sprays of water and collected the contents in a cup. We examined the contents of each plant, counting and classifying any remaining live aquatic macroinvertebrates within each cup by order.

For the pH experiment, we collected 27 bromeliads and determined their starting pH, size, and invertebrate community, as described above, and placed them into individual holes in a patch of secondary forest 20 m east of the research station. We removed as much water as possible within each invertebrate sample and returned them to their respective plant. We used three pH treatments: 5.6, 3.1, and 2.4, and randomly assigned nine bromeliads to each. The 5.6 and 3.1 treatments reflect the extremes of natural pH variation of the bromeliads (see Results), and the 2.4

treatment reflects the conditions the bromeliad macroinvertebrates might experience in the event of acid rain. If bromeliads were to encounter acid rain with a pH of 2.4, as has been recorded in nature, their tanks would have the same pH due to their inability to buffer against pH fluctuations (Schindler 1988). To re-saturate the bromeliad tanks, we created three solutions with varying pH using lemon juice and tap water. To the first treatment, we added tap water with a pH of 5.6. To the second and third treatment group, we added lemon juice-water solutions with a pH of 3.1 and 2.4, respectively. Estimates of bromeliad size (as described above) did not differ among the pH treatments (MANOVA: $\lambda = 0.73$, $F_{8, 42} = 0.91$, $P = 0.52$).

After 24 hours, we recollected and analyzed the contents of the bromeliads to determine the change in the invertebrate community. We sampled invertebrates using the methods described above for initial invertebrate sampling. The total number of invertebrates before and after the pH experiment was used to determine the percent change in the number of aquatic invertebrates. We assumed that there was no recruitment of new invertebrate individuals to the bromeliads in our experiment.

Statistical analyses. We used correlation analyses to assess the relationship between the natural variation in pH and the number of invertebrates in the bromeliad tanks. We used a one-way ANOVA to test the effects pH treatments (pH=5.6, 3.1, and 2.4) on the percent change in invertebrates. We used a Tukey Highly Significant Difference multiple comparisons test to test which experimental pH treatments were significantly different from each other at alpha = 0.05. To normalize the data from our bromeliad pH experiment, we arcsine transformed the percent change in invertebrate abundance.

RESULTS

In our observational survey of nine bromeliads in the Monteverde cloud forest, the natural pH varied from 3.0 to 4.9, with a mean of 3.9 ± 0.1. As natural acidity of the water in the bromeliads increased, the number of aquatic invertebrates in the bromeliads decreased; however, this result was not statistically significant ($r = -0.62$, $n = 9$, $P = 0.076$; Figure 1). To have an 80% chance of finding a significant correlation between natural acidity and number of aquatic invertebrates at $\alpha = 0.05$, we would have needed to sample ten more bromeliads.

We also found a marginally significant positive correlation between the tank depth of each bromeliad and the abundance of invertebrates in the bromeliads ($r = 0.59$, $n = 9$, $P = 0.088$). However, tank

depth and acidity were not correlated (r = -0.10, n = 9, P = 0.79). To have an 80% chance of finding a significant correlation between tank depth of each bromeliad and number of aquatic invertebrates at alpha = 0.05, we would nave needed 22 more samples.

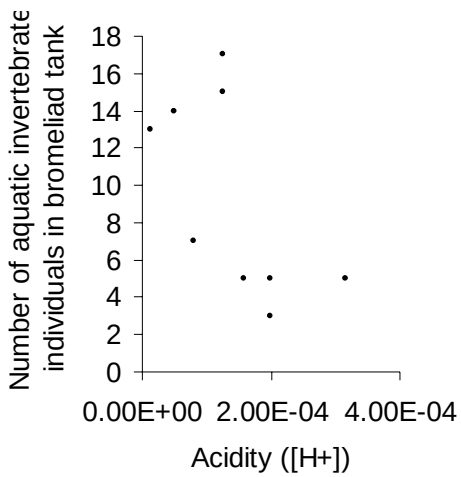

Figure 1: Number of aquatic invertebrate individuals in nine bromeliad tanks across their natural gradient of hydrogen ion concentrations. As the concentration of hydrogen ions increases, the number of invertebrates found in the bromeliads tends to decrease, with marginal significance.

In our pH manipulation, we found a significant difference among pH treatments in the percent change in aquatic invertebrate abundance after 24 hours (Figure 2). The pH=2.4 treatment was significantly different from the pH=3.1 and pH=5.6 treatments at α = 0.05, but the higher pH treatments were not significantly different from each other (Figure 2). In the pH=2.4 treatment, each bromeliad lost an average of 144% more invertebrate individuals than the pH=3.1 treatment, and an average of 234% more invertebrate individuals than the pH=5.6 treatment.

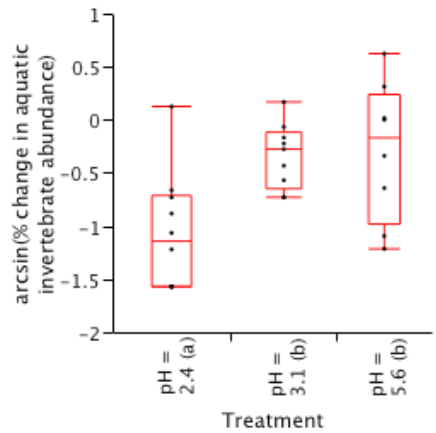

Figure 2: Percent change in aquatic invertebrate abundance in bromeliads across three pH treatments (pH 2.4, 3.1, 5.6). N = 9 plants per treatment. Treatments marked (a) are significantly different from treatments marked (b), which do not differ significantly from each other.

DISCUSSION

Our study suggests that there is a negative effect of acidity on bromeliad tank macroinvertebrate communities. While previous studies have focused on correlations between bromeliad physical characteristics, such as tank depth, with aquatic invertebrate abundance (Weller and Cushman 2004), we have shown that tank acidity is important as well. Additionally, we found that while tank depth and acidity were both correlated with aquatic invertebrate abundance, they were not correlated with each other,

indicating independence of tank depth and acidity effects. Because the two correlations of tank depth and number of invertebrates with acidity were only marginally significant, a future study should increase sample size of bromeliads to substantiate the two correlations described and investigate possible additive vs. non-additive effects of these two factors on number of invertebrates.

Our prediction that experimentally increasing acidity beyond natural levels would negatively affect aquatic invertebrate abundance was also supported. Both the tap water treatment and the lower threshold of the natural range acidity treatment had acidity levels that roughly spanned the natural range of bromeliad tank acidity, whereas the high acidity treatment was well below the natural range found in tanks. We suspect that there is a threshold between a pH of 3.1 and 2.4, below which the mortality of aquatic invertebrates significantly increases. To investigate this threshold prediction, further studies should examine mortality across a gradient of pHs. The percent change in aquatic invertebrate abundance in the tap water treatment indicates a tolerance for slightly higher pH levels than found in natural variation. Since rainfall in undisturbed habitats has a pH near 5.0 (Schindler 1988), invertebrate tolerance of water less acidic than the natural range of pH in bromeliads is important for survivorship.

While we were able to include a larger sample size in our study compared to previous bromeliad studies (e.g., Weller and Cushman 2004), our study is limited by its short time-span. In addition to the effects of acidity on invertebrate mortality, freshwater microhabitats can also experience negative sub-lethal effects (Stucliffe and Hildrew 1989). For example, acidic streams and lakes often affect invertebrate populations by containing less available nutrients or deterring larger predators, both of which alter species composition over time. To understand the sub-lethal effects of acidity on bromeliad communities, experimental studies should observe changes in the inhabitants over a longer time period than our 24 hour time period. Increasing acidity can also negatively affect invertebrate communities by decreasing food availability over longer time periods (Stucliffe and Hildrew 1989). It is possible that both acid treatments pH=3.0 and 2.4 would negatively affect invertebrate communities over a long time period due to time lag effects. Longer-term experimental studies should examine shifts in invertebrate species composition and abundance as a bromeliad tank acidifies over time.

Our study examined only bromeliads growing on the lower 5

m of tree trunks at the Monte Verde cloudforest. However, because invertebrate abundances and species composition in subcanopy bromeliads are similar to those in the upper canopy (Nadkarni and Longino, 1990), our study results may also be applicable to bromeliads growing higher in the canopy. Nonetheless, other tropical rainforests may have very different bromeliad communities and thus different responses to tank acidification. To better test the natural acidification on tropical forests in general, our study could be repeated in multiple different forests. In addition, to achieve natural responses to acidic solution additions, the bromeliads should be left attached to their host tree while the experiment is in progress. Leaving the bromeliads attached to the tree may also facilitate natural invertebrate migration in and out of acidic bromeliads.

Acid rain has the potential to greatly affect tropical habitats. With distinct wet and dry seasons, tropical habitats can experience 50% of their annual rainfall in as little as 15 days (Monte Verde Institute 2004). Shifts in the acidic content of this rain could have immediate detrimental effects on forest fauna. We found that in the acidic treatments, only the smallest aquatic invertebrates, such as nematodes and annelids were still alive. While Costa Rica is only responsible for 1% of Central America's industrial nitrogen and 2% of its sulfuric oxide emissions (Earth Trends 2003), acidic air pollution can travel distances far beyond Costa Rica's borders.

The significance of bromeliad acidification goes beyond acid rain alone. In highly productive tropical environments, sulfite leaching from high concentrations of detritus can acidify soils and habitats to levels equivalent to severe acid rain (Johnson et. al., 1979). Additionally, sulfuric emissions from volcanic activity can create acid rain in nearby microclimates (Pringle et. al., 1993). The acute effects of acidification on aquatic invertebrates have been demonstrated in many freshwater systems (Schindler, 1998), and are even thought to have caused decreases in the abundances of larger animals, such as amphibians (Blaustine and Wake, 1995). The effects of acid on aquatic invertebrates provide examples of the overall effects of acid on microhabitats. Acidification-related invertebrate mortality could rapidly cascade up the food-web, greatly altering all trophic levels in tropical rainforests. With longer-term research on the effects of acid inputs on bromeliad community response, we may be better able to understand how acidity can alter not only the inhabiting invertebrates, but also community food-web dynamics and ecosystem processes.

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