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ABSTRACT

Elfin forest tank bromeliads were shown to have lower pH when positioned below mats of epiphytic bryophyte than in open gaps. The effect of lower pH was studied to see if it influenced microorganism community abundance, species richness, evenness and diversity. It was found that increasing levels of pH over a range of 4.3 – 5.8, increased population sizes of most microorganisms. In fact abundance of individuals in the entire community spanned over an order of magnitude (82- 1936 individuals). Species richness also increased with pH but only as a function of increased abundance of individuals. One species richness was corrected for differences in abundance; the pattern of increasing species richness with pH was lost. It is possible that acidic environments limit productivity which would in turn reduce population sizes due to a limited resource base. Both diversity and evenness had no correlation with pH. Corrected species richness was also determined to be lower in those bromeliads with moss cover than those of open gaps. This could be attributed to moss bromeliads being more stable due to their protective location under the canopy, which results in a lower species richness by virtue of the Intermediate Disturbance Hypothesis.

RESUMEN

Bromelias de tanques en el bosque de Elfin mostraron pH más bajos cuando las coloque bajo esteras de briofitas epifita que las rajas abiertas. Se estudia el efecto de los pH bajos para ver si existe una influencia sobre las comunidades de microorganismos con respecto a la abundancia, el número de especies y la diversidad. Se encontro que al aumenta niveles de pH en una escala de 4.3 a 5.8, se aumenta las abundancias de la mayoria de las poblaciones. En realidad la abundancia de individuos en la comunidad entero se extiende sobre un orden de magnitud (82 – 1936 individuos). El número de especies aumentan también, pero solamente como una función de la abundancia de los individuos. Cuando el número de especies se corrigieron en las diferencias en abundancia, la tendencia de los numeros de especies creciente con el pH se perdió. Por eso, el aumento de especies fue una función del aumento la abundancia. Es posible que los ambientes ácidos limiten la productividad que posiblemente reduce los tamaños de poblaciones por el faltante de recursos. La diversidad y uniformidad no mostraron tener correlación con pH. Las especies corrigade determinaron ser más bajo en las bromelias con musgo como ellos en rajas abiertas. Eso puede ser distribuido a las bromelias con musgo ser mas equilibrado debido los localizaciones protegidos debajo de la cubierta, que resulta en un numero bajo de especies en virtud de la hipótesis del tumulto intermedio.

INTRODUCTION

Aquatic Ecosystems are impacted by altered habitat conditions such as changes in sediment load, organic debris levels, flow reduction, chemical additives and acidity (Allan 1995). Anthropogenic impacts have helped to create these changes through the building of dams, pollution and acid rain (Allan 1995). Acid rain is formed when nitrate and sulfate compounds from the burning of fossil fuels, dissipate into the atmosphere and mix into cloud systems (Grifo and Rosenthal 1997 in Calla 1999).

The harmful effects of increasing acidity on aquatic systems are well known to reduce the numbers of species and individuals. One survey looked at 34 invertebrate taxa from stream sites that varied in pH. It was determined that the number of taxa decreased in response to increases in acidity (Hildrew et al 1984 in Allan 1995). Another study that took place in the streams of Ashdown forest of Southern England saw a decrease in microarthropod species richness with decreasing pH. The pH was studied in the range of 4.0 – 7.0, and found a decrease from 25 taxa in the more neutral waters to only five taxa in the more acidic water (Rundle and Omerod 1991 in Allan 1995). There has also been increasing evidence that acidity reduces microbial populations important in organic matter decomposition. Hildrew et al. (1984) also found that acidic stream water had a considerable negative effect on decomposition rates. Since most protozoa rely on decaying organic debris or decompositional bacteria as food, it would be reasonable to expect that protozoan communities would also be affected by decreases in pH (Patterson 1996). These negative effects might work through the food chain to produce the differences in microinvertebrates found by Rundle, Omerod and Hildrew.

Tank bromeliads (Bromeliaceae) are epiphytic plants that have a central tank created by overlapping leaf bases on a shortened stem, to collect precipitation and nutrients (Utley and Burt-Utley 1984). The standing, nutrient-rich pool serves as a fertile environment for a number of aquatic organisms (Utley and Burt-Utley 1984). These organisms make up natural microcommunities, which can be used to test theories of community ecology such as Island Biography Theory (Martin 1998; Sandin 1993), species composition and productivity (Maltzman 1994), disturbance (Calla 1999) and many other attributes.

In general, it has been found in microorganism communities, that protist do not respond to differences in size or volume of the bromeliad tank (Sandin 1993). It was proposed that habitat sizes of tank bromeliads are many orders of magnitude outside the scale of protozoan communities. Another study mimicked bromeliad tanks using three different sized containers and looked at rates of colonization, diversity, species richness and abundance of protozoans in relation to Island Biogeography Theory (IBT). The biggest container was found to hold more species, and the smallest to hold the fewest. However, abundance of individuals was found to be inconsistent with the IBT in that the greater overall abundance of individuals was found in the smaller containers (Martin 1998). Both Martin and Sandin suggest that habitat size wasn’t the major determinant in protozoan diversity but rather debris density and food availability. Alicia Maltzman (1994) looked at species richness with increasing rosette diameter, assuming that the bigger rosettes would collect more detritus and productivity would be greater. She found that species richness did not appear to increase with increasing productivity or detritus in the bromeliad.

An additional important factor for tank bromeliad communities maybe the effect of pH. In the Elfin forest, where epiphytic moss is common, pH varies in bromeliads depending on their location. Calla (1999), looked at the pH of tank bromeliads in Elfin and pasture habitats, and found that tanks in the Elfin were lower in pH than those of the pasture. The increase in acidity was due to the interception of precipitation by epiphytic moss which filtered the moisture, resulting in a throughfall more acidic than the precipitation first reaching the canopy (Clark, et al. 2000). Other forms of pH variability in the absence of moss cover may be the result of filtering effects of the canopy vegetation or the varying pH found in precipitation. Bromeliads that were found under protective canopy in the absence of moss cover were found to have higher pHs than those that were found in unprotected areas. This buffering effect of the canopy vegetation may help buffer anthropogenic pH changes in bromeliad aquatic systems (Calla 1999).

pH in tanks has been studied for macroinvertebrates but not for protists. The range of pH studied was from 4.0 to 6.7. It was found that in the lower pH ranges (4.0 – 4.5) there was a trend of decreasing macroinvertebrates species richness. The greatest species richness was found to be at intermediate pH levels (about 5.0). This general trend of highest species richness at intermediate pH fits the description of the intermediate disturbance hypothesis which states that there may be smaller populations of more pollution-tolerant organisms coexisting with smaller populations of more pollution-sensitive species (Calla 1999). This is assuming that pH is limiting population sizes, directly or indirectly. It may be possible that similar trends can be found for protist communities.

This study will look at how tank bromeliad microorganism communities are affected by varying levels of pH and position. The acidity of tank bromeliads will be compared for those found under epiphytic bryophytes and those in gaps in order to determine if the Throughfall collecting in tanks is buffered by the moss. Then microorganism abundance, richness, evenness and diversity will be measured from tanks of varying pH to see if decreasing pH lowers these community measures as is the case for other systems. The influence of varying abiotic conditions, such as light and temperature extremes, in moss versus gap surroundings, on microorganism communities will also be considered.

MATERIALS AND METHODS

Study Sites

Tank bromeliads were studied within the Elfin forest (about 1770 – 1820m) of the Estación Biológica Monteverde, Costa Rica. The Elfin forest is designated as a lower montane wet forest, and the section studied was along the Pacific slope. Both eukaryotic microorganisms, henceforth referred to as microorganisms, and pH samples were taken from bromeliads of approximately the same width (200-500 mm leaftip to leaf tip) and tank volume (10-15ml) to avoid any possible species-area effects. Bromeliads were chosen based on their placement as it relates to throughfall (i.e. rain or mist) which collects in the tanks. Throughfall is the moisture that is first filtered by what is in the forest canopy before collecting in bromeliad tanks. Samples were classified as having either moss overhead (M), which included mats of epiphytic bryophytes hanging from branches or on the trunks of trees, or gap overhead (G), which included gaps where nothing was filtering the moisture collecting in the tanks. A total of 67 bromeliads were measured for pH. Fifty-one of these were under moss surroundings and 16 were under gap, reflecting the relative abundance of bromeliads in these microhabitats.

Community Composition

Thirteen tanks from both moss and gap surroundings were sampled for microorganisms. The tanks were chosen over a 4.0-6.0 range in pH. Each tank was initially mixed using a syringe to create a mixture of microorganisms and debris. A minimal amount of solution was collected to measure pH on site using a pH Test 2 electrode with a + 0.1 accuracy. The sample was then used to identify and count protozoan species. Three drops of the well-mixed sample were analyzed under 400 x magnifications. The number of drops was decided upon by exhaustively sampling two preliminary tank samples. After 3 drops, new species were rare or no longer found. The microorganisms of 13 bromeliads were counted and identified down to morphospecies based on appearance and mobility.

Statistics

A t-test was used to compare the differences of average pH between moss and gap surroundings. Simple linear regressions were run for abundance, species richness, evenness and species diversity of each bromeliad versus pH. Species richness was corrected for uneven sample size (i.e. unequal number of protists per bromeliad) using the Menhinick correction formula. Diversity was measured using Shannon-Weiner, Simpson's Index, and Fisher's alpha diversity indices. Shannon-Weiner was calculated as a standard measure of diversity. Simpson's index was calculated with a correction factor for uneven sample size, and the negative natural logarithm of the Simpson's value was used when running the regression. Finally, Fisher's alpha was calculated due to its independence of uneven sample sizes. Multiple regressions were used to measure differences in abundance, species richness, evenness and species diversity versus both pH and position (i.e. moss versus gap surroundings).

RESULTS

Study Sites

The distribution of the pH data was tested for normality (Komolgorov-Smirnov, DF = 2, $X^2 = 1.075$, $P > .9999$), and therefore parametric statistics were used. The average pH of tanks influenced by moss (pH = 4.8) was less than the average pH of tanks found in gaps (pH = 5.3. t-test, $p < .0001$, Figure 1). The pH range of moss bromeliads was 3.8 to 5.7. The pH range of gap bromeliads was from 4.3 to 6.1.

Community Composition

The total number of protozoan individuals increased significantly with increasing pH ($r^2 = 497$, $p = .0072$). Tanks at the lowest pH's (4.2 – 4.5, n = 5) had a mean abundance of 515 organisms, compared to those at the high end (> 5.5, n = 3) having a mean abundance of 1440. This trend resulted from a general increase across common species (Species A, $p = .0762$; Species B, $p = .1098$), except one bromeliad, where abundance was largely the result of one species increasing while the others stayed the same. The general increase across the majority of species can also be inferred from the lack of correlation between evenness and change in pH ($p = .7693$)

There was also a non-significant trend of species richness increasing with increasing pH (simple linear regression, $r^2 = .497$, $p = .0741$). The mean species richness at lower pH's (4.2 – 4.5) was 30 species, compared to those greater than 5.5 with a mean richness of 38 species. However, once species richness was corrected for unequal sample size using the Menhinick formula, this trend was lost ($p = .2847$). All three indices of diversity also showed no significant correlation with pH (p = .8542, .9891, .6666 respectively).

Multiple regressions were run with pH and position (M vs. G) versus species abundance, richness, evenness and diversity. Moss bromeliads were depressed in connected species richness with increasing pH as compared to gap bromeliads ($p = .0499$). The mean corrected species richness of moss bromeliads was 1.06 and the mean corrected species richness of gap bromeliads was 1.56. The corrected species richness values ranged from 0.5 to 3.0. However, there was not a difference between moss versus gap bromeliads in regards to abundance or evenness of protozoan communities, (p = .1235, . 1909 respectively). All three indices of diversity showed a trend of moss bromeliads having less diversity than those of gap bromeliads, although in no case was this statistically significant (Shannon-Weiner, $p = .1260$, Simpson's Index, $p = .0855$, Fisher's alpha, $p = .0634$).

DISCUSSION

Bromeliad tanks are rich in microorganism species that, in turn, may influence large organisms living in the tanks. This study showed that pH was an important factor regulating microorganism communities. At lower levels of pH, microorganism diversity and species richness was expected to decline. What was found was that population sizes increase with increasing pH, but species richness and diversity were not affected. It was also discovered that species richness was lower in bromeliads under moss cover than bromeliads found in gaps.

When pH was measured for bromeliads under epiphytic bryophytes and bromeliads in gaps, there were significantly lower levels of pH in those bromeliads affected by moss. When precipitation first passes through moss, the moss acidifies the throughfall to average levels of 4.8 (Jungens 1992). This is the precipitation that eventually collects in the tanks. Bromeliads that are found in gaps collect moisture that is not filtered by bryophyte mats, and for this reason the pH is determined directly by precipitation. Such water in the form of rain or mist in the elfin forest is typically of the pH 6.0 (Calla 1999). The average pH of tanks found in gaps during this study was of pH 53. The reduction in pH in gap bromeliads from that of the precipitation may have been due to organic debris collected in the tanks or the precipitation acidity at the time of data collection being slightly greater than normal.

When comparing bromeliads under moss to those in gaps, there was a trend that bromeliads under moss had fewer species. There were also slight, non-significant trends, that moss bromeliads had lower microorganism diversity than gap bromeliads. This may be due to lower light and temperature found in the forest as opposed to the gap, which could limit productivity. However, abundance versus pH suggests, if anything, moss bromeliads have a slightly higher abundance than those of gap bromeliads. If productivity was limiting in moss surroundings, you would expect abundance to be lower as well from the depressed resource base of food. Therefore, limited productivity in moss bromeliads is probably not the cause of lower richness and diversity compared to gap bromeliads. Another possible explanation could be that moss bromeliad communities are more stable. The light, temperature and pH extremes are less varied in the protected forest cover as opposed to the gaps. According to the intermediate disturbance hypothesis you would expect systems with more stability to have a lower species richness and diversity (Tilman 2000). This is because the more stable system is dominated by organisms that are competitively adept at those conditions, while systems with an intermediate level of disruption can support more coexisting populations of organisms that are optimally adapted to varying levels of light, temperature and pH.

The most important effect of pH on microorganism communities was that of abundance. As pH increased so did the total number of microorganisms in the tanks. Also, in bromeliads with higher pH, more species were encountered. However, once species richness was corrected for uneven sample size (i.e. unequal number of microorganisms) species richness no longer increased with increasing pH. Therefore, the increase in species richness was probably a function of the increase in abundance.

When evenness was measured against pH it showed no correlation. This suggests that at higher pH population sizes increase, accounting for the increase in abundance. Also, when the abundance of the most common protozoans was compared against pH, they all increased; therefore it is more than just one species accounting for the increase in abundance with pH. Higher levels of productivity may be able to explain the increase in abundance with increasing pH. High pH may be favorable for greater amounts of productivity. With a greater resource base a microorganism's population can increase, explaining the increase in abundance with increasing pH.

These responses to pH conflict with those on stream aquatic ecosystems in that species richness and diversity here do not decrease with increasing acidity. In stream ecosystems it has been shown that once pH drops below 5.0 species diversity and richness declines (Allan 1995). It is possible that this trend is not observed in the acidic ecosystems of my study, which has pH levels below 5.0, because the increasing acidity is a natural and chronic phenomenon. Microorganisms that live in these communities may have evolved to tolerate acidic conditions. In contrast, the studies done with aquatic ecosystems deal with changes in pH brought about by anthropogenic causes. The organisms affected in stream aquatic ecosystems probably have not adapted a tolerance to increases in acidity. For this reason species richness and diversity declined as pH changed.

Because protists serve as food resources for larger organisms, it would be expected that declines in protist abundance would result in population declines of their predators. In the forest where bromeliads have lower pH's and therefore lower abundances, one would expect competition for the limited resource base would decline macroinvertebrate abundance and possibly species richness of those living in the tank bromeliads. If one were to keep moving up the food chain, smaller communities of larger organisms would ultimately be found in the more acidic bromeliads due to the effects of acidity on lower trophic levels.

In future studies it would be interesting to compare tank bromeliads in more types of habitat, including forest cover without the presence of moss. It would also be interesting to look at productivity and decompositional rates within tank bromeliads with pH and microorganism diversity. This may be able to clarify the mechanisms responsible for increasing abundance with higher pH.

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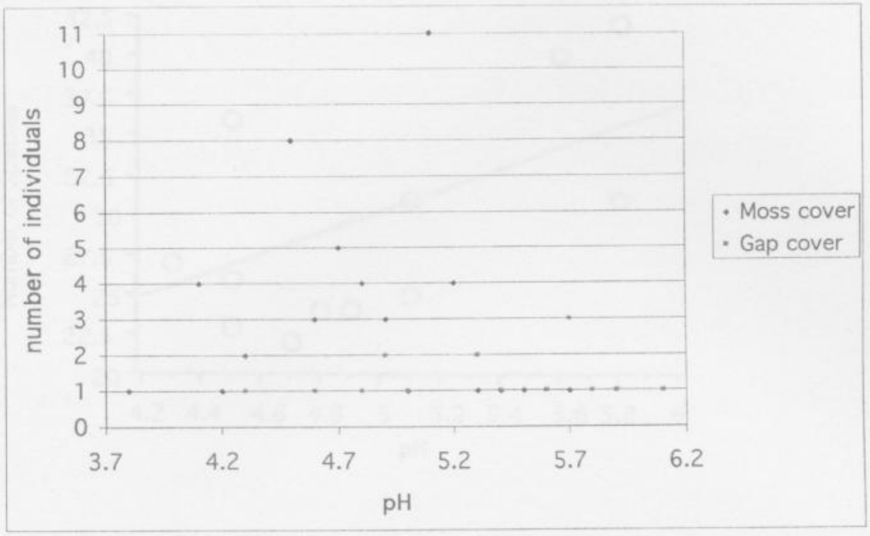

Figure 1. Frequency distribution of moss versus gap bromeliads with pH. The mean pH of moss bromeliads (4.8) is significantly less than the mean pH of gap bromeliads (5.3). (t-test, $p < .0001$).

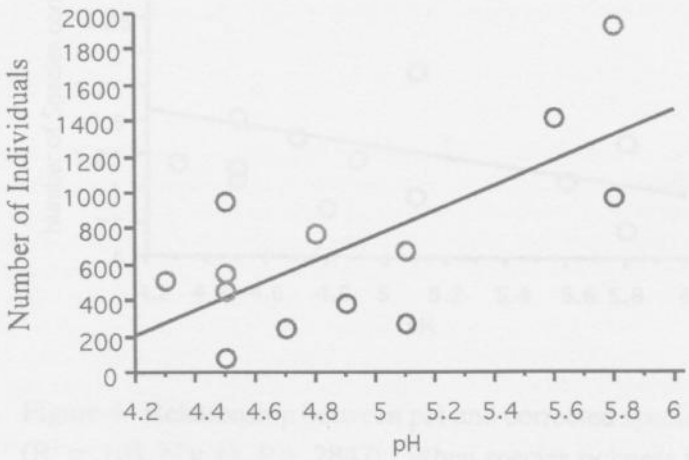

Figure 2. Relationship between pH and the total number of microrganisms found in each tank bromeliad. $R^2 = .497$, N = 13, **P = .0072**. The abundance of protists increases with increasing pH. Grey circles indicate moss cover, black indicate gap. (Y = -2725.453 + 697.362 * X; R^2 = .497).

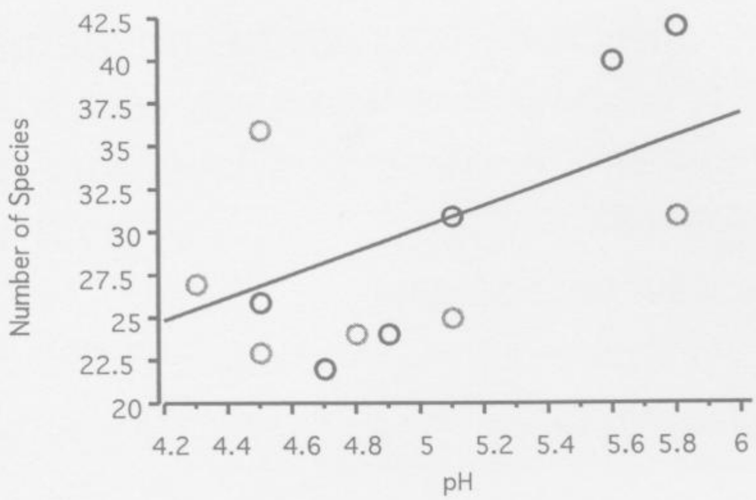

Figure 3. Relationship between pH versus Species richness uncorrected for differences in abundance. $R^2 = .262$, N = 13, **P = .0741**. As pH increases the number of species increases. Grey dots indicate moss cover, black indicates gap cover. (Y = -3.216 + 6.69 * X; R^2 = .262)

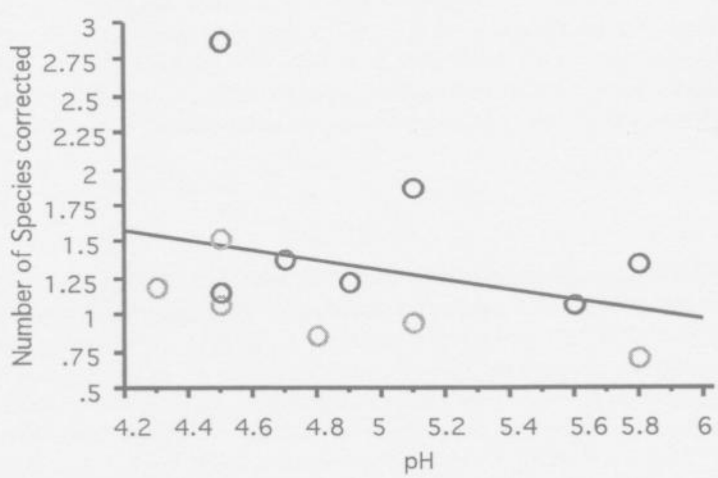

Figure 4. Relationship between pH and corrected species richness. ($R^2 = .103$, N = 13, P = .2847). When species richness is corrected for uneven sample size, the pattern for increasing species richness with pH is los. Grey dots indicate moss cover, black indicates gap. (Y = 3.012-.342 * X; $R^2 = .103$)