

# Water Quality and Lichen Coverage in Monteverde Streams

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## ABSTRACT

This study examined lichen abundance and water quality in six streams in Monteverde, Costa Rica. By estimating lichen coverage on rocks over a 90 m transect, mean lichen coverage per stream was measured. Water quality was estimated by measuring temperature, dissolved oxygen concentration, pH, turbidity, nitrogen and phosphorous levels at each stream. Mean lichen coverage varied between each site, showing that the Quebrada Sucia had the smallest lichen coverage and the Quebrada Máquina had the highest lichen coverage. A significant positive correlation was found between percent lichen coverage and total dissolved oxygen concentration. There are at least four possible explanations for these trends: water enhances lichen growth, competition with algae limits lichen growth, interactions between source and sink populations affect lichen coverage, and pollution inhibits lichen growth in streams.

## RESUMEN

Se examinó abundancia de líquenes y contaminación de agua en seis quebradas en Monteverde. Por calculando densidad de líquenes en piedras en un transecto de 90 m, las abundancias medias de una quebrada fueron medidas. Un censo de agua calidad fue tomada y temperatura, turbidez, concentración oxígeno disuelto, nitrógeno, fósforo y pH, se obtuvieron por todas las quebradas. Abundancia media cambió entre las quebradas; demostrando que la Quebrada Sucia tuvo la abundancia más pequeña que otras quebradas, y la Quebrada Máquina tuvo la abundancia más grande que otras quebradas. Se encontró una correlación significativa y positiva entre el porcentaje de líquenes y concentración oxígeno disuelto. Los resultados de relaciones sugieren cuatro tendencias: agua es un recurso importante en el crecimiento de líquenes, competición con alga pone un límite en crecimiento de líquenes, interacciones entre población de fuente y población de frezadero afectan los líquenes, y contaminación para el crecimiento de líquenes en quebradas.

## INTRODUCTION

Lichens are an association between a fungus and an alga or cyanobacterium and therefore demonstrate unique morphological and ecological characteristics (Umaña and Sipman, 2002). Because lichens are composed of two organisms, they cannot be classified as a true species. Yet, the pair comprises an obligate mutualism and is named as a unit. The alga provides the fungus with sugars from the photosynthesis and the fungus retains moisture for the alga and protects the alga from exposure to harsh environmental conditions. The symbiosis is especially important for the hydration of the alga because alga do not have mechanisms to obtain water from their substrate and must depend on rain, mist, and dew to collect in the fungi (Umaña and Sipman, 2002). When water falls on lichens some of it is drawn into grooves in the lichen body called chinks (Darbishire, 1914). Lichens are extremely efficient at absorbing nutrients and water efficiently from

their environment. It is this biological adaptation that makes lichens especially prone to death by atmospheric pollution.

Pollution in the atmosphere can affect the distribution and coverage of lichens in various ways. Lichens are effective biological indicators for air pollution because heavy metals in the atmosphere become trapped in the lichen body and can eventually kill the specimen (Scott and Hutchinson, 1993). For example, pollution may enhance or disrupt lichens natural adherence to a substrate (Umana and Sipman, 2002). Furthermore, Lawrey and Hale (1979) found that growth rates were suppressed in juvenile thalli when atmospheric lead was detected in the air. Studies conducted by Chatenet *et al.* (1999) have shown that lichens are capable of fixing micropollutants, such as copper, lead, and cadmium, in water and can be used as measures of dissolved metal concentrations. The growth of the lichens under polluted and non-polluted conditions were compared in two Venezuelan cloud forests (Gordon *et al.*, 1995). A study conducted by Gill (1990) showed that vegetative components of freshwater systems respond to direct organic carbon enrichment. It is believed that lichens may also respond to water quality, but this is not known.

Water pollutants are also likely to disrupt distribution and coverage of lichens on rocks or other substrates in rivers. Streams in Monteverde, Costa Rica, are polluted by the small industries, businesses, and farms in the region (Schultz, 1998). Anderson (1996) witnessed industries inputting large amounts of grey water and oil into the Quebrada Sucia several times a week. Other pollutants include pulses of acidic and caustic soda washes from cheese processing near the Fabrica de Quesos (Gill, 1990). The majority of the pollution in the region is from the farms because fertilizers, feces, and organic runoff contribute to higher levels of phosphates in water. If lichens are sensitive to water quality, then their communities may change along pollution gradients in the Monteverde region.

It is hypothesized that overall lichen coverage will decrease along streams that have high turbidity levels, relatively low dissolved oxygen concentrations, pH levels above 8 and below 4, warm temperatures, and high phosphorous and nitrogen counts. It is predicted that lichen coverage will decrease because of the inability of lichens to adhere to substrates in polluted environments. Thus, lichen coverage on rocks and the frequency of rocks with lichens should vary between sites on a pollution gradient. If lichen density is affected by water quality, then conservation efforts could focus on preserving lichen biodiversity through stream management, which would be especially influential in areas of high endemism such as the Monteverde cloud forest. The purpose of this paper is to examine lichens role as a bioindicator in Monteverde streams.

## METHODS

### Study Sites and Time

Lichen coverage assessment and stream monitoring took place between April 20<sup>th</sup> - May 10<sup>th</sup>, 2003, in Monteverde, Puntarenas, Costa Rica (W 85° N10 20') at elevations between 1300 m and 1500 m. Six river sites were compared in terms of stream quality and lichen coverage: the Quebrada Máquina, Quebrada Cuecha, Quebrada Berros, Quebrada Santa María, Quebrada Rodríguez, and Quebrada Sucia. Although currently most are in highly modified surrounding, the streams were originally in habitat classified as premontane wet

forest (Holdridge, 1967). All six rivers run near the main street in Monteverde and Santa Elena.

The climate in Monteverde has a distinct wet season and dry season. The dry season extends from February to April. It is important to note that this study was conducted in the dry season because water quality indicators such as dissolved oxygen concentration, pH, temperature of water, and turbidity are influenced by rainfall. Nitrogen and phosphorous levels vary seasonally because rain increases the potential of fertilizers to enter streams through runoff.

### Data Collection

River sites were chosen using a regional map of Santa Elena and Monteverde. Streams were chosen based on their predicted pollution amount. At each of the sites, rocks in the streams that were larger than 20 cm diameter were sampled and were examined for the presence or absence and quantity of lichens over a 90 m transect. The percent coverage of lichens on each rock was estimated with a grid. Water quality indicators such as dissolved oxygen concentration (DOC), nitrogen and phosphorus levels, pH, turbidity, and temperature were measured at each of the six sites using the Lamotte water testing kit (Renn, 1968). Nitrogen concentration was measured using the cadmium reduction method. Phosphorous concentration was measured using the stannous chloride chemical method. Jackson Turbidity Units were used when examining turbidity by comparing tap water to stream water. A standard buffer solution was used to measure the pH at each of the six sites. The dissolved oxygen concentration and temperature were obtained by using the Oakton 300 DO and temperature meter.

### Statistical Analysis

A Chi-squared test was used to test the prediction that lichen presence and absence varied between sites. A Spearman Rank correlation test was used to examine the relationship between the water quality variables and the average lichen percent coverage per rock across the six sites. An ANOVA test compared the average lichen coverage between the six sites.

## RESULTS

Rock censuses detected varying degrees of lichen coverage at the six sites (Table 1). In the six sites sampled, a total number of 556 rocks were examined for mean lichen cover. A Chi-Squared test was used to evaluate the frequency of occurrence of rocks with and without lichens ( $X^2 = 21.155$ ,  $p = 0.0008$ ,  $df = 5$ ).

The variation in mean area of lichen coverage per rock was compared to pH, temperature, dissolved oxygen concentration, turbidity, nitrogen and phosphorous concentration (Table 2). Spearman Rank tests showed that the mean lichen coverage per rock was positively correlated with an increase in dissolved oxygen concentration (Figure 1) ( $R^2 = 0.773$ ,  $p = 0.0209$ ,  $n = 556$ ; Figure 1). The Quebrada Máquina had the highest DOC value of 82.2%. The Quebrada Sucia had the lowest DOC value of 54%. The large Quebrada Cucha had a DOC of 60.2%. The Spearman Rank test also revealed that there

may be a negative correlation between mean area lichen coverage per rock and phosphorous concentration ( $p = 0.1671$ ,  $R^2 = 0.44$ ,  $n = 556$ ; Figure 2). The Quebrada Sucia has the highest phosphorous concentration of 1.0 ppm. No relationship was found between the mean lichen coverage per rock and pH, temperature, turbidity, or nitrogen count (Table 3). Quebrada Cuecha had the highest nitrogen concentration of 7.5 ppm.

One-Way ANOVA tests were conducted to examine whether there was a difference in the mean lichen concentration between sites. The One-Way ANOVA indicated that there were significant differences in percent of lichen coverage between sites ( $f = 4.679$ ,  $p = 0.0008$ ,  $df = 5$ ; Figure 5). The Quebrada Cuecha had the highest lichen coverage with a mean lichen density cover of 11.18%. The lichens in the Quebrada Sucia had the lowest with a 1.16% mean lichen coverage. The largest stream, the Quebrada Cuecha, had a 7.421% mean lichen coverage. The Fisher's PLSD test revealed that the Quebrada Máquina had a significantly higher mean area of lichen coverage than the Quebrada Berros ( $p = 0.0058$ ), the Quebrada Cuecha ( $p = 0.0279$ ), the Quebrada Santa Maria ( $p = 0.0024$ ) and the Quebrada Sucia ( $p < 0.0001$ ). The Fisher's PLSD test also showed that the Quebrada Sucia mean area of lichen cover was consistently lower than the Quebrada Berros ( $p = 0.0451$ ), the Quebrada Cuecha ( $p = 0.0043$ ), the Quebrada Máquina ( $p < 0.0001$ ), and the Quebrada Rodriguez ( $p = 0.0059$ ).

## DISCUSSION

This study reveals a positive correlation between mean lichen coverage and dissolved oxygen concentration in streams found in Monteverde. The Quebrada Sucia had the lowest mean lichen density and lowest DOC, the Quebrada Máquina had the highest mean lichen coverage and the highest DOC. There are at least four possible explanations for these trends: water enhances lichen growth, competition with algae limits lichen growth, interactions between source populations and sink populations affect lichen abundance, and pollution deteriorates lichen growth in streams.

The positive correlation observed between mean lichen coverage and dissolved oxygen concentration may be the result of indirect factors such as water availability. Dissolved oxygen concentration is a measure of the solubility of oxygen in a stream, but is influenced by watershed characteristics such as flow rate, stream size, and stream health (Allan, 1995). Therefore, when there is a swift current with many riffles, there is usually a high dissolved oxygen concentration. Fast streams with many riffles thrust more water into the atmosphere, through splashing, and can provide the lichens in the stream with an unlimited supply of water. Most of the water falling onto the lichens rebounds but some is drawn into the chinks where it will be absorbed (Darbishire, 1914). The unlimited supply of water may promote lichen growth and reproduction in streams.

Due to the usual association between dissolved oxygen concentration, flow rate and stream size, one might assume that the large and swift Quebrada Cuecha would have a high dissolved oxygen concentration as well. However, the Quebrada Cuecha only has a 60.2% dissolved oxygen concentration, a relatively low concentration for a large stream. Respiration processes occurring along the river explain this phenomenon. Even though the Quebrada Cuecha is large and fast moving, it has a great deal of bacteria slime located at the sample site after the Lechería. The bacteria populations flourish

downstream as a result of the constant supply of carbon dioxide supplied by the Lechería (Gill, 1996). Therefore, the bacteria use up the oxygen, and create anoxic conditions for the river.

The significant difference in the mean lichen coverage between the Quebrada Sucia, Quebrada Cuecha and the Quebrada Máquina may be due to competition for a substrate between algae and lichens. In the Quebrada Sucia and the Quebrada Cuecha algae was found in abundance. In contrast, a lesser abundance of algae was found in the Quebrada Máquina. The results here suggest that there may be a negative correlation between mean lichen coverage and an increase in phosphorous concentration (Figure 2). Phosphorous and nitrogen are commonly considered to be the inorganic elements that are most likely to limit primary production in algae (Steiman and Mulholland, 1996). The Quebrada Sucia contains a phosphate concentration of >1 ppm and the Quebrada Cuecha has a nitrogen level of 7.5 ppm and both streams had relatively high alga abundances in Monteverde. When sun, fresh air, and clean water are not limiting factors, higher plants and algae make their appearance and rapid algae growth ensues. Slower growing lichens have less of a chance to become established because they are out competed (Darbishire, 1914).

MacArthur and Wilson's Island Biogeography Theory (1967) may also explain trends observed in lichen coverage in streams. When a source population of lichens is large and located close to a riparian area it has the potential to support the island populations located on the rocks in a stream. By definition, a source population of lichens is continually growing and has the optimal conditions for growth and reproduction. The source population can disperse a large amount of propagules to close rock island populations, as long as there is a suitable substrate for lichens. If the source populations are near, then there will be a higher rate of lichen immigration than if the source populations are far. This occurs because spores will not have to travel large distances to reach the rock substrate in the streams and there is a better chance for establishment. If the river is larger and has numerous rocks throughout, then there is also larger target substrate for the incoming spores. According to this theory, the larger rivers with the most substrate located near source populations should have the highest lichen coverage on their rocks. This trend was observed in my study. The Quebrada Máquina is larger than the Quebrada Sucia and has a higher lichen coverage throughout. The Quebrada Máquina flows through a small private cloud forest reserve behind the Estación Biológica. This forest has the potential to act as a source lichen population for the stream. In contrast, the Quebrada Sucia site is located in the heart of downtown Santa Elena, far from forests. It is highly unlikely that there is a source lichen population near the sample site.

Another possible explanation for the significant difference in the mean lichen coverage between sites is air pollution, which causes lichen populations to decline. Although these factors were not measured directly, one can infer that air pollution was higher in the Quebrada Sucia because of its location. The Quebrada Sucia runs through an area with high traffic volumes, and has a mean lichen cover per rock of 1.16%, making it the stream with the lowest lichen diversity. Lichens are one of the most sensitive organisms to atmospheric pollution due to the fact that they have direct contact with the water droplets in mist and fog (Gatriona *et al*, 1995). Therefore, one can postulate that the streets in Santa Elena notably affected the lichen growth in streams near

the main road. Experiments showed that when lichens in cloud forests were transplanted to more polluted sites, there were significant increases in heavy metal contents in the lichen (Gordon *et al.*, 1995).

The results presented here suggest that mean lichen coverage may be influenced by water availability, competition with algae, the lichen metapopulations, and air and water pollution, among other factors. As is true with most studies conducted in the field, many factors contribute to the abundance of species found in a riparian community. Limitations in my study include a small sample size, which may influence the strength of the trends found. For example, the apparent trend between phosphorous concentration and lichen coverage may have been stronger had more streams been sampled. Further research should explore different trends in lichen species abundance in streams. Examining lichen communities on a species level may provide insight into trends unseen in this study. Perhaps different species of lichens in Monteverde have varying degrees of sensitivity to abiotic factors. Thus, in order to use lichens as stream quality indicator species, we must first obtain a better understanding of lichen abundance patterns.

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Table 1. Lichen coverage on six Monteverde streams.  $N = 556 \geq 20$  cm in diameter along an 80 m long transect of stream.

	Quebrada Rodríguez	Quebrada Berros	Quebrada Maquina	Quebrada Cuecha	Quebrada Santa Maria	Quebrada Sucia
X	8.4	5.9	11.2	7.4	4.9	1.2
N	59	97	120	155	75	56
SD	16.7	13.6	18.6	13.4	8.9	3.6
Range	5-100	1-90	1-80	1-80	2-50	5-20

Table 2. Water quality parameters for Monteverde streams, including as pH, °C, turbidity (JTU), N (ppm), DOC (O%), and P (ppm)

	Quebrada Rodríguez	Quebrada Berros	Quebrada Máquina	Quebrada Cuecha	Quebrada Santa María	Quebrada Sucia
pH	7.4	7.9	7.8	7.3	7.7	7.6
°C	18.2	18.1	20.7	18.8	18.1	19.0
DOC (‰)	76.1	77.9	82.2	60.2	70.3	54.0
Turbidity (JTU)	5	0	2.5	2.5	2.5	5.0
N (ppm)	1.25	1.25	3.75	7.50	1.50	0.00
P (ppm)	0.35	0.15	0.15	0.10	0.10	1.00

Table 3. Results table containing *p* values for Spearman rank tests between mean lichen density and stream quality indicators such as pH, °C, turbidity (JTU), N (ppm), DOC, and P (ppm)

	pH	°C	Turbidity	N	DOC	P
P-Value	0.6003	0.4011	0.4040	0.5516	0.0209	0.1671
R-value	0.08	0.18	.018	0.10	0.77	0.42
N	556	556	556	556	556	556



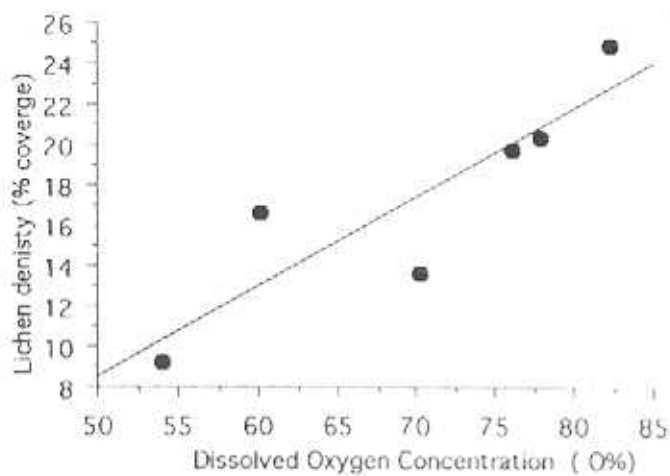


Figure 1. The relationship between the mean area of lichen coverage per rock sampled in six streams in Monteverde and the dissolved oxygen content of each stream ( $R^2 = 0.773$ ,  $p = 0.0209$ ).

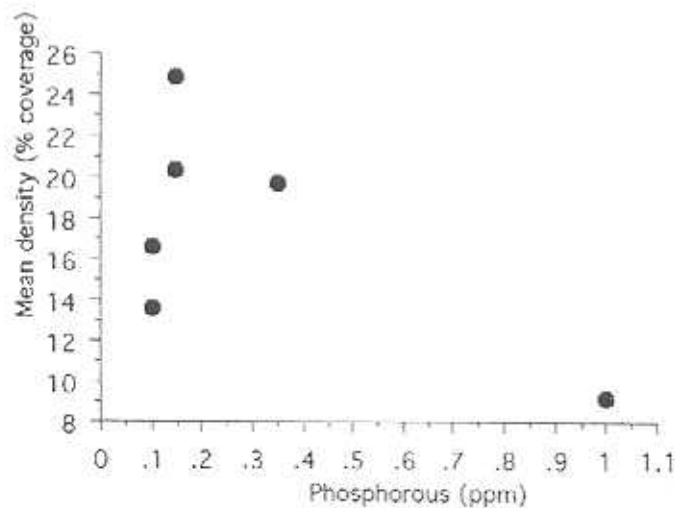


Figure 2. The relationship between the mean area of lichen coverage per rock sampled in six streams in Monteverde and phosphorous count per site. There is no significant correlation between the average area covered by lichens per rock and stream phosphorous content (ppm) (Spearman rank correlation,  $r=0.42$ ,  $p = 0.167$ ).

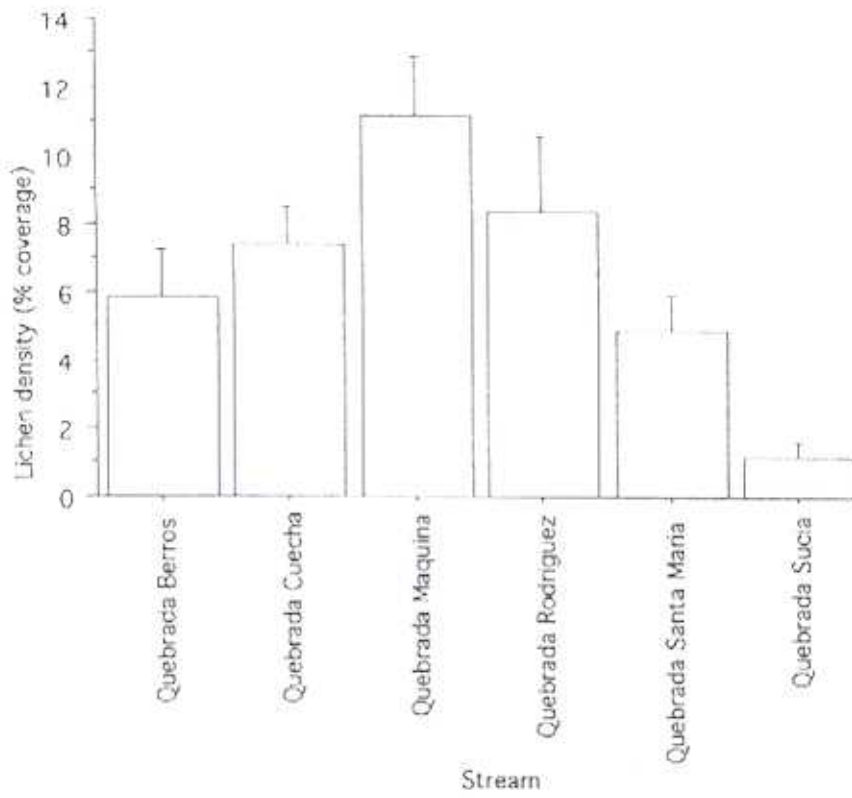


Figure 3. Mean lichen density (% coverage) on rocks in Monteverde varies significantly between sites ( $f = 4.679$ ,  $p = 0.0008$ ,  $df = 5$ ).