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Stream site selection of Hyalinobatrachium fleischmanni (Centrolenidae)

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Abstract

Census work and habitat analysis for the glass frog species *Hyalinobatrachium fleischmanni* was performed on four streams in Cañitas and Monteverde, Costa Rica. At each of six sites, the habitat occupied by *H. fleischmanni* was contrasted with a nearby, paired site where individuals were absent. Seven habitat parameters were investigated: water quality based on dissolved oxygen and phosphate content; water speed; discharge; distance from human development; extent of canopy cover; and presence or absence of common monocots (Zingiberaceae: *Hedychium coronarium*, Costaceae: *Costus spp*., Musaceae: *Musa acuminata*). No statistically significant differences were found between Occupied and Unoccupied locations though patterns suggested a preference for sites with faster water speed, greater discharge, and higher levels of dissolved oxygen. No gradients in group size based on habitat characteristics were observed. Population densities found in this study compared to densities reported by other sources and past studies showed an increase in density. This is especially significant in light of drastic frog population declines in past years in the Monteverde area.

Resumen

Un censo y un análisis del hábitat de la especie de rana de vidrio, *Hyalinobatrachium fleischmanni*, se llevó a cabo en seis lugares de cuatro quebradas en el area de Cañitas y Monteverde, Costa Rica. En cada sitio, el hábitat ocupado por *H. Fleischmanni* se comparó con un lugar similar donde *H. fleischmanni* estaba ausente. Siete parámetros del hábitat fueron investigados: la calidad del agua basada en pruebas de oxígeno disuelto y fosfato; la velocidad del agua, la descarga, la distancia al desarollo humano, la extensión de cubierta del dosel y la presencia o ausencia de monocotiledóneas comunes (Zingiberaceae: *Hedychium coronarium*, Costaceae: *Costus spp*., Musaceae: *Musa acuminata*). No se encontraron diferencias significativas entre las localidades ocupadas y desocupadas, aunque los patrones sugirieron una preferencias por sitios con agua más rápida, más descarga y niveles altos de oxígeno disuelto. No hubo ningun gradiente en el tamaño de los grupos basado en las caracteristicas del hábitat. En comparación a otras investigaciones, la población de ranas encontrada en esta investigación mostró un aumento de densidad. Este resultado es importante debido al declive de poblaciones de los años pasados en Monteverde.

Introduction

Hyalinobatrachium fleischmanni is a small, nocturnal frog found on vegetation overhanging streams (Savage, 2002). Rather than being evenly spread out along the length of the stream, the males are usually heard calling from groups in patchy distributions (Mark Wainwright, personal communication). It is possible that these male aggregations form to attract females and that a site is selected for its conduciveness to signal propagation. More open habitat and elevated perches are shown to enhance acoustic effects of calls (Gerhardt, 1994). Calling leks would be more conspicuous to females and would provide them more mating options (Villegas and Wainwright, in press). It is also possible that a site is selected due to physiological requirements, and the biotic and abiotic habitat parameters at that location are preferable to those at other spots. Anurans are sensitive to environmental quality since their skin is a breathing surface, and *H. fleischmanni* lays eggs on vegetation over streams so tadpoles can fall into the water (Savage, 2002). This aspect of life history relies on vegetation and water conditions being favorable for these stages of development.

Previous studies have made many speculations as to the preferred habitat of *H. fleischmanni groups*. A study on one stream in Monteverde observed *H. fleischmanni* to be very adaptable to disturbed areas with a partiality for altered forest riparian canopy versus pristine forest riparian canopy. It was also noted that the groups seemed to prefer fast-moving streams to slow ones and gingerlily (Zingiberaceae: *Hedychium coronarium*) vegetation above other types of vegetation (Hayes, 1979). The author made these observations as side notes to the experiment taking place and suggested that further research would be needed to make any authoritative conclusions on these points. Other sources report findings that this species is tolerant of high levels of pollution (http://www.redlist.org, November 21, 2004). A detailed study specifically exploring factors of canopy cover, stream quality, vegetation types, and human impact could reveal if there are stream site selection preferences for *H. fleischmanni* groups.

Hyalinobatrachium fleischmanni is one of the more commonly found anuran species in the Monteverde area and, as a result, has been studied to a fair extent. While listed under the category of Least Concern on the IUCN Red List (http://www.redlist.org, November 22, 2004), the situation in Monteverde provides a special case. Since research done by Hayes (1979) and others was conducted in Monteverde, drastic frog population declines have been reported here in the years 1987, 1994, and 1998 (Pounds et al., 1999). Data from past studies showed capture rates of *H. fleischmanni* ranging from 27 individuals in 18 hours (1.8 per hour; Jacobson and Werman, 1981), to 22 individuals in four hours (5.5 per hour; Buskirk et al., 1981), both on the Río Guacimal below the Cheese Factory. By 1990, the population on that same stretch of stream had disappeared and that section still remains unoccupied (Mark Wainwright, personal communication). In the greater Monteverde area, another source details that populations have declined from one male per meter of stream to a few males over several hundred meters (http://www.globalamphibians.org, November 22, 2004). Causes of decline have been speculated to be anything from global warming to chytridiomycosis or some combination of effects (http://www.redlist.org, November 22, 2004).

The goal of this investigation was to census populations of *H. fleischmanni* on streams in the Monteverde area and observe biotic and abiotic factors of the habitat where groups were located in to see if any patterns exist. Seven specific habitat parameters were analyzed: dissolved oxygen content, phosphate content, flow rate, discharge, proximity to human development, extent of canopy cover, and presence or absence of common monocots (Zingiberaceae: *Hedychium coronarium*, Costaceae: *Costus spp*., Musaceae: *Musa acuminata*). It was predicted that *H. fleischmanni* groups would prefer faster-moving streams with a greater volume of discharge. Less canopy cover was expected to be preferred, as well as the presence of *H. coronarium* or similarly broad-leafed vegetation. It was not known whether human development or water quality would have a negative impact on the groups. The population sizes found will be valuable to compare to previous data from before the large-scale declines and to determine habitat preferences to inform future conservation decisions.

Materials and Methods

Study Sites

Five groups of *H. fleischmanni* in Cañitas and one group in Monteverde were investigated. The study sites in Cañitas were on the Quebrada Santamaría, Quebrada Berros, and the stream parallel to the road to Las Nubes. The occupied locations will be referred to as Quebrada Berros 1 (accessed from the farm of Segundo Santamaría), Quebrada Berros 2 (accessed from the highway to Abangares), Quebrada Santamaría 1 (on the same side of the street as the Miramontes Hotel, accessed from the highway to Abangares), the Quebrada Santamaría 2 (on the opposite side of the street as the Miramontes Hotel, accessed from the highway to Abangares), and Calle a Las Nubes. The stream used in Monteverde was behind Tramonti's restaurant and the El Bosque Hotel, and the occupied location will be referred to as Tramonti's. For each Occupied location studied, there was a corresponding Unoccupied location used on the same stream, selected by measuring a distance of at least 20 meters from the boundary of the Occupied location, upstream or downstream depending on which was least obstructed. The Unoccupied location was of approximately equal size as its paired Occupied location. All sites were in the range of 1280 to 1370 meters of elevation within the Premontane Wet Forest Holdridge Life Zone (Haber, 2000).

Censusing

Fieldwork was done between October 28 and November 7, 2004. In order to census the populations of *H. fleischmanni*, groups were located on streams prior to the censusing period, and then maps were made of the area to facilitate counting. The censuses were performed between 1900 hours and 2130 hours and weather conditions including rain, wind, and moon, were noted. Observations were made by walking in the streambed or closely alongside it. Calling individuals were marked on the site map, and the perch location in the vegetation was flagged with as much precision as possible to avoid recounting. Uncertainties in the number of individuals in a specific location where calls were dense were noted.

Habitat Analysis

Fieldwork was done between October 28 and November 20, 2004. Data were collected on each stream during daylight hours after the censusing of the group was completed. The length of stream occupied by the group was measured and boundaries were marked with flagging tape. An Unoccupied location was established based on the criteria discussed previously. One to three canopy density measurements were taken using a canopy densitometer (Spherical Densiometer Model – C) depending on whether the canopy cover in the site (Occupied or Unoccupied) varied greatly. Water quality was assessed according to two indicators: dissolved oxygen and phosphate. Two water samples were taken at each Occupied and Unoccupied location and the dissolved oxygen and phosphate content were measured in parts per million (ppm). Water speed was determined for each location by the amount of time it took a lemon to float three separate distances within that location and averaging the speeds. The discharge (the product of speed by width by depth) was calculated by measuring the width of the stream and average depth (based on three readings) at each spot the water speed was taken at and averaging the three values. Distance from human development was measured starting at the bank of stream and measuring to the closest human construction. If the distance was greater than 100 meters, it was recorded as greater than 100 meters instead of an exact distance under the assumption that the distance was too great to have any differing effect on the frogs.

Statistical Analysis

Six of the seven factors (canopy density, dissolved oxygen content, phosphate content, average water speed, average discharge, and distance from development) for which data were collected for Occupied and Unoccupied locations were analyzed using the Wilcoxon Paired Samples Test. Common monocots were analyzed on a presence or absence basis and did not require statistical analysis.

Results

Hyalinobatrachium fleischmanni groups at Quebrada Berros 1 and 2, and Quebrada Santamaría 1 and 2 were censused and found to contain group sizes of 15, 16, 36, and 45 males, respectively. Censusing lasted from one hour to one hour and 45 minutes with discovery rates between 8.57 individuals per hour to 30 individuals per hour. The average rate was 18.8 individuals per hour. The average density of individuals on these two streams was 0.79 individuals per meter. Males of *H. fleischmanni* were heard calling in specific locations on both the Calle a Las Nubes stream and Tramonti's stream, but these two sites were not censused due to weather conditions that caused the frogs to stop calling before a census could be completed. For Tramonti's stream, the population size was estimated by local naturalist Mark Wainwright, who had extensive knowledge of the site. Based on the time of year, length of stretch of the stream used, and number of hours spent listening, the population was estimated to be ten individuals, assuming good weather conditions. No estimate was made for the Calle a Las Nubes stream. There was no apparent pattern between any of the habitat parameters and the resulting size of the group present in those conditions. The average water speed, distance from development, and average discharge were the three parameters that showed variation among the groups (Figures 1 – 3). The extent of canopy cover, dissolved oxygen content, and phosphate content remained at nearly the same level among all groups.

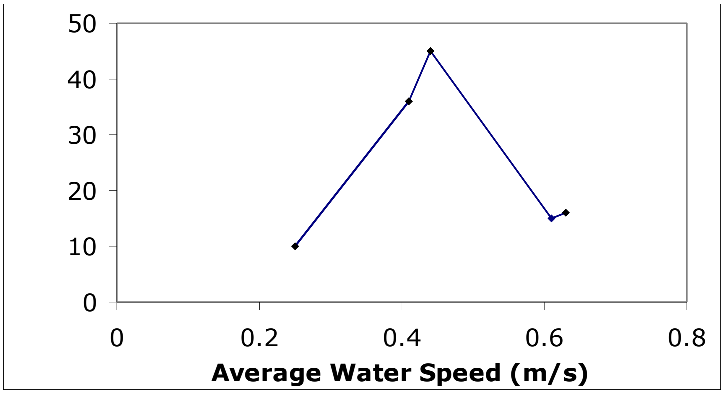

Figure 1. The number of individuals of H. fleischmanni *males at each Occupied location as a function of average water speed.*

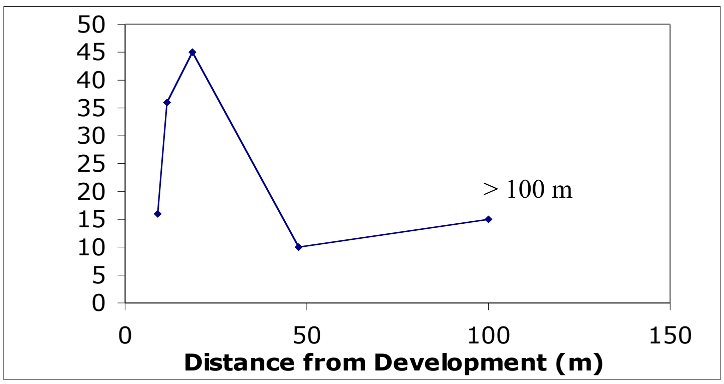

Figure 2. The number of individuals of H. fleischmanni *males at each Occupied location as a function of distance from human development.*

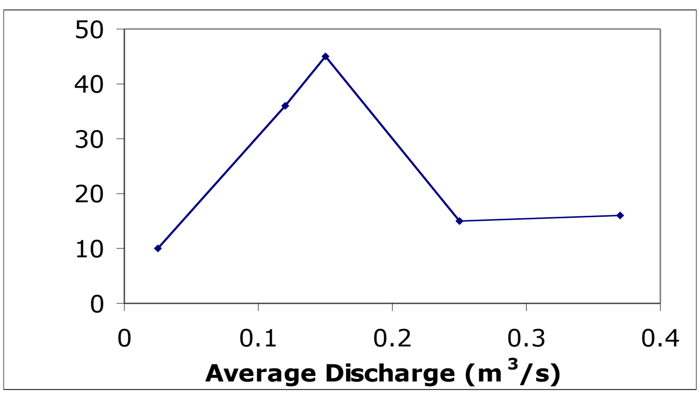

Figure 3. The number of individuals of H. fleischmanni *males at each Occupied location as a function of average discharge.*

The Wilcoxon Paired Samples Test showed no significant differences between Occupied and Unoccupied paired locations for any of the habitat parameters. The test for dissolved oxygen content was inconclusive due to frequency of tied values. However, there is a trend that Occupied locations contained greater or equal dissolved oxygen content to their corresponding Unoccupied locations (Figure 4).

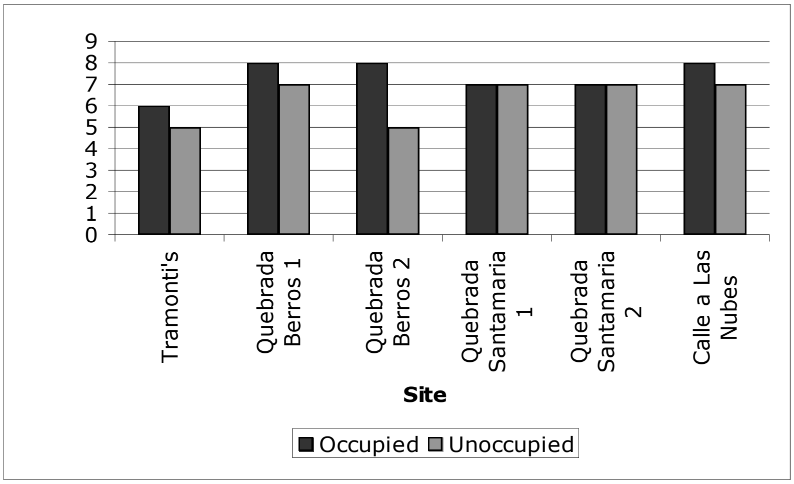

Figure 4. Comparison of dissolved oxygen levels between Occupied locations of H. fleischmanni *and Unoccupied locations at six sites. Occupied values are greater or equal to Unoccupied values.*

Average water speed of paired Occupied and Unoccupied locations did not differ significantly (Wilcoxon Paired Test, $T_- = 1.5$, $T_+ = 19.5$, $T_\alpha = 0$; Figure 5), nor did the average discharge of paired locations (Wilcoxon Paired Test, $T_- = 3.5$, $T_+ = 17.5$, $T_\alpha = 0$; Figure 6). These factors showed Occupied locations having higher average water speed and discharge than Unoccupied locations in all cases except one of the locations on the Quebrada Santamaría. The difference between the Occupied and Unoccupied locations that varied from the trend was particularly small for the average water speed.

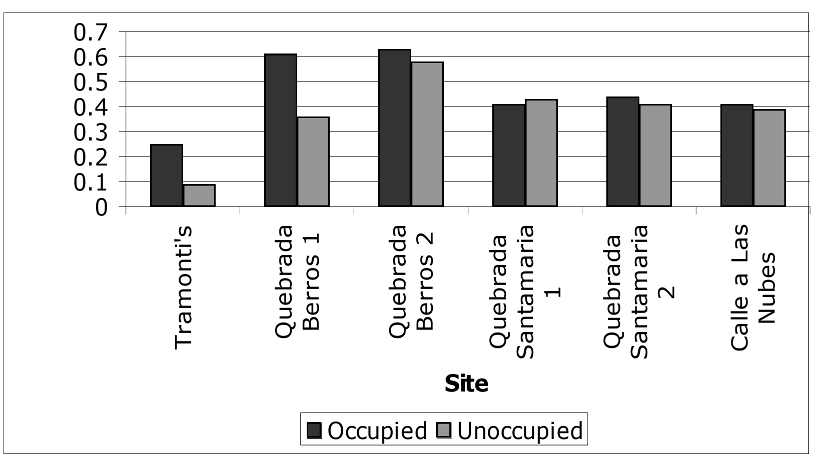

Figure 5. Comparison of average water speed between Occupied locations of H. fleischmanni *and Unoccupied locations at six sites. Quebrada Santamaría 1 is the only site where the Unoccupied location had a higher reported value though the difference between Occupied and Unoccupied bars is small.*

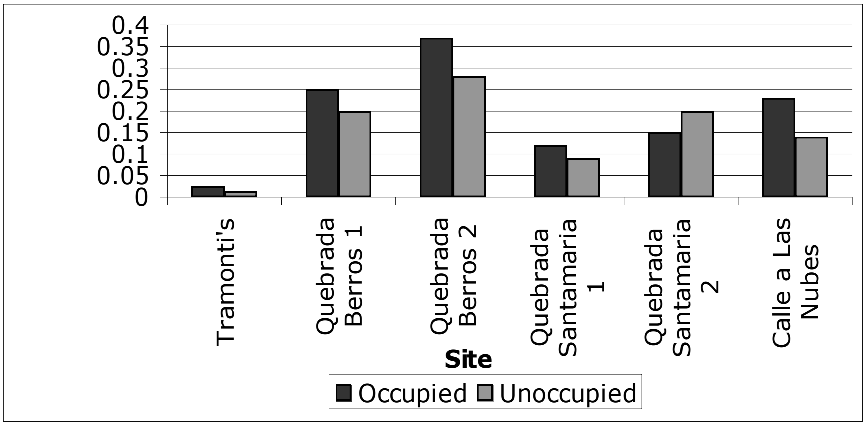

Figure 6. Comparison of average discharge between Occupied locations of H. fleischmanni *and Unoccupied locations at six sites. Quebrada Santamaría 2 is the only site where the Unoccupied location had a higher reported value.*

Differences in distance from human development were also inconclusive due to tied values between Occupied and Unoccupied locations at Quebrada Berros 1 (Figure 7).

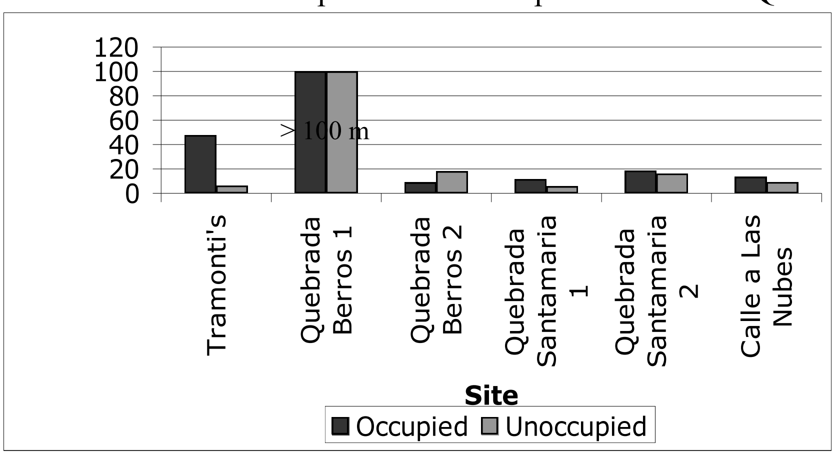

Figure 7. Comparison of distance from development between Occupied locations of H. fleischmanni *and Unoccupied locations at six sites.*

Phosphate content analysis also provided inconclusive results due to tied values (Figure 8).

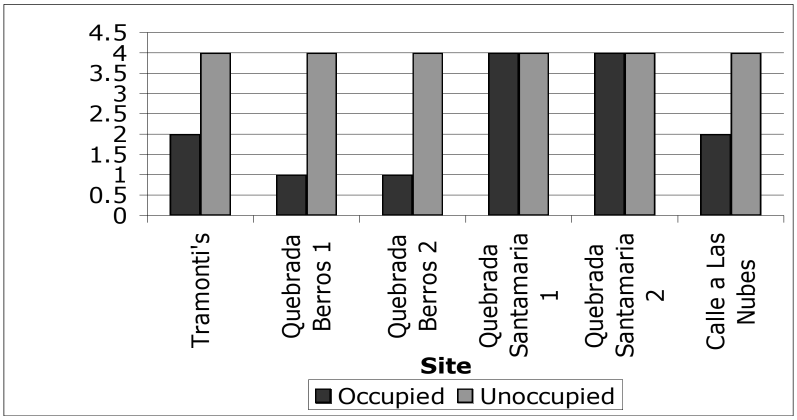

Figure 8. Comparison of phosphate content between Occupied locations of H. fleischmanni *and Unoccupied locations at six sites.*

The extent of canopy cover showed no difference between Occupied and Unoccupied paired locations (Wilcoxon Paired Test, $T_- = 12$, $T_+ = 9$, $T_\alpha = 0$; Figure 9). Cover remained within a 20% range for all locations.

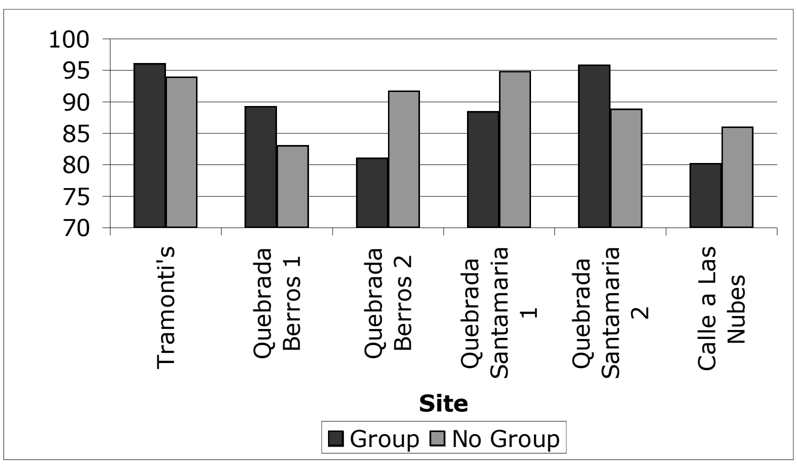

Figure 9. Comparison of percent canopy cover between Occupied locations of H. fleischmanni *and Unoccupied locations at six sites.*

The presence or absence of common monocots in each site was not analyzed statistically. While *H. coronarium*, *Costus spp.*, or *M. acuminata* were found at all sites except Tramonti's, they were found equally at the Unoccupied locations as the Occupied (Appendix 1). In only one site, Calle a Las Nubes, was one of these monocots the dominant vegetation. *H. coronarium* was present in large quantities at the Occupied location of Calle a Las Nubes but was also present in the Unoccupied. In all other sites, the main vegetation was made up of larger trees forming a high canopy. There did not appear to be a relationship between the presence of these monocots and the Occupied locations.

Discussion

Although analysis of the seven habitat parameters produced no statistically significant results, some interesting information can still be extrapolated. The results from the average water speed, average discharge, and dissolved oxygen show trends that can be related to the physiological requirements of the frogs and their life history. Tadpoles of *H. fleischmanni* develop in the stream water at the locations their parents select (Leenders, 2001), so qualities of the stream at Occupied locations could be chosen by the parents to give their offspring a better chance of survival.

Faster streams with greater discharge are more turbulent, which contrary to intuition, is actually better for the tadpoles. *H. fleischmanni* females lay their eggs on vegetation over the stream and when the tadpoles hatch, they drop from the leaves into the water. More turbulent water makes falling tadpoles less noticeable to stream predators waiting below, such as fish. Rain during hatching can also enhance this effect (McDiarmid, 1983). In most sites, the average water speed and average discharge were greater in the Occupied locations than the paired Unoccupied locations. This pattern is supported by previous findings from Hayes (1979) that faster flowing streams were preferred by *H. fleischmanni*. Selection of locations with faster water speed and greater discharge by adults could be a result of the survival needs of tadpoles.

High dissolved oxygen content in the stream water could also be indicative of such parental behavior. Streams with elevated dissolved oxygen levels are healthier for tadpoles to develop in. Even though the results were statistically inconclusive, the trend showed that the Occupied locations had either greater or equal dissolved oxygen content to that at the paired Unoccupied locations (Figure 4). It is possible that adults choose locations by streams with greater dissolved oxygen content to lay their eggs. Further research might support this observation. There are limitations to the reliability of the water test results due to the level of accuracy provided by the test kit used. It is possible that with a more accurate kit, the data from the water quality analysis would have shown statistically significant results. Content in ppm was determined by matching the color of the water sample after a tablet was dissolved in it to a color scale on a laminated card. Variability in light levels during readings could possibly have affected the content reported. Previous studies on Quebrada Berros and Quebrada Santamaría measured levels of dissolved oxygen in percent saturation and found content to be relatively high (Buckman, 2003; Payne, 2003). While percent saturation is not directly comparable with content in ppm, both scales agree that dissolved oxygen levels in these streams are elevated, lending support to the trend observed in this study.

Results from the phosphate test showed neither a preference for high or low phosphate levels. The presence of phosphate in Occupied locations does show that tadpoles and adults are most likely tolerant of this pollution, as other sources have pointed out (http://www.redlist.org, November 21, 2004). Phosphate levels reported in this investigation varied little or not at all among samples, but other studies on both Quebrada Berros and Quebrada Santamaría have shown levels of phosphate present

(Buckman, 2003; Payne, 2003). Failure to detect differences could be due to the coarseness of the detection scale and the same limitations as the dissolved oxygen test.

Though distance from development, canopy cover, and vegetation type did not show clear trends like the last three parameters assessed, the lack of trend still provides information about *H. fleischmanni*'s life history patterns. When male groupings are choosing the location along the stream in which to group, it seems that they might want to avoid human development due to the level of disturbance that comes with it, for example, increased noise, presence of domesticated animals, and light pollution. The effect of distance from development in this study was shown to be inconclusive in its relation to Occupied and Unoccupied sites. Quebrada Berros 1 was greater than 100 meters away from the nearest human development at both its Occupied and Unoccupied locations, and so was assumed to be unaffected. For the remaining sites, all but Quebrada Berros 2 had Occupied locations farther from development than the paired Unoccupied locations. In other Monteverde sites not included in the study it was observed that individuals were within three meters of human structures. This lack of a clear pattern in preference for distance from development corresponds with other sources that point to *H. fleischmanni* being tolerant of human development (http://www.redlist.org, November 22, 2004).

Vegetation and canopy cover could be very important to both adults and tadpoles in their physiological requirements. Adult males spend most of their time during mating season perched on vegetation directly over the water (Leenders, 2001) and females select males to mate with based in part on their perch site (Villegas and Wainwright, in press). Tadpoles hatch out of their eggs on the leaf surface and from there must make it into the water. While previous studies suggest altered canopy and *H. coronarium* are preferred by *H. fleischmanni* (Hayes, 1979), the results of this investigation show an opposite trend where all Occupied locations had high canopy densities and low occurrence of monocots. The extent of canopy cover in all Occupied and Unoccupied locations ranged from approximately 80% to 96%. Although there appeared to be a pattern in presence of broad-leafed monocots in this study, this vegetation type was not the most common type at any of the sites with the exception of the Calle a Las Nubes stream. There was no significant difference between Occupied and Unoccupied locations for either canopy cover or monocot vegetation, and therefore these parameters fail to explain the selection of one location over another. It is clear that altered, less dense canopy and broad-leafed monocots in the vegetation are not a necessity for *H. fleischmanni* groups since Occupied locations were found without them.

The fact that none of these parameters showed statistically significant results could mean that other theories for the location of a male group provide a better explanation. One of the theories previously mentioned was that males simply group out of leking behavior. Habitat parameters at multiple locations on the stream might be equally acceptable, but males group in one location in order to make themselves more available to females. Another possibility is that certain locations enhance signal propagation. Calling entails high energetic costs to an individual male, so locating habitat that augments acoustic effects would be preferable. Some factors that affect sound propagation include open areas, certain plant leaves, atmospheric turbulence via wind or warm air pockets, high available perches, and interfering objects (Gerhardt, 1994). Groups may form in locations where calls are more enhanced and therefore less energy is expended. Neither the leking nor the acoustic theories exclude habitat selection for physiological needs, and it is very likely that there is a combination of these factors determining site selection.

Valuable information was gained in this study outside of analysis of habitat parameters. Censusing of four groups on two streams, Quebrada Santamaría and Quebrada Berros, produced higher discovery rates than the capture rates reported from studies by Jacobson (1981) and Buskirk (1981) pre-dating the population declines of *H. fleischmanni* in Monteverde. This can be expected since the census in this study only involved counting individuals by call and not physically locating each one. Other sources reporting on declines in Monteverde described population sizes as a few males over several hundred meters (http://www.globalamphibians.org, November 22, 2004). Censusing done for this study has shown that on at least two streams in the Monteverde area, population sizes have reached greater densities. The groups found on the remaining streams used in this study were also observed to contain good-sized populations though exact counts were not taken. Both the Quebrada Berros and the stream by the Calle a Las Nubes contained other groups of *H. fleischmanni* that were not studied. Other streams in Monteverde not analyzed in this investigation were also found to contain groups of *H. fleischmanni,* including the stream behind Johnny's Pizzeria and the drainage ditch next to Stella's Bakery. Limits on time due to a change in weather prevented censusing of these additional streams and groups located within the study area. Calling rate of the males drops significantly or ceases altogether when there are dry weather conditions, heavy rain, strong wind, or bright moonlight (Leenders, 2001; Villegas and Wainwright, in press). During the last week of the study period, winds reached a great enough force that no individuals were calling on any of the streams in the study area. In addition, there was a three-day dry period that substantially decreased air humidity. The combined effects of wind and drought made it impossible to census the remaining two sites analyzed in the study or any additional streams. Continued work on these streams and other streams in the area might support the finding that *H. fleischmanni* has been able to successfully recolonize areas where its numbers were previously low. The Río Guacimal was not analyzed, but future studies might investigate why the stretch below the Cheese Factory remains uninhabited by this glass frog species.

In summary, this study showed some patterns in habitat preference of *H. fleischmanni*, though none were statistically significant. With more research, average water speed of streams and average discharge could be shown to be preferred by groups of *H. fleischmanni*. Water quality tests performed at finer scales might show a preference for high dissolved oxygen levels and a tolerance for phosphate levels. It is unlikely that monocots are a prerequisite for occupied habitat, though detailed analysis of vegetation types could produce some patterns in preferred tree species and canopy cover. Distance from human development seems unlikely to be an influence on habitat choice based on this study as well as past studies. Detailed information on group sizes at four locations in the Monteverde area and additional information for sites not censused was presented in this study. Much speculation exists as to the causes of population declines of *H. fleischmanni* in the past and to the recolonization in recent years. It is heartening to see that populations of *H. fleischmanni* in at least some areas of Monteverde are rebuilding. More knowledge of how to protect these frogs and their habitats is needed if the population is to continue its upward trend in Monteverde.

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Appendix

FROG GROUPS STREAM	ELEVATION	CENSUS	LENGTH OF SECTION	GINGER PRESENT
TRAMONTI'S	1370	10	13.7 m	NO
QUEBRADA BERROS FINCA	1335	15	20.1 m	NO (Costus)
QUEBRADA BERROS CALLE	1280	16	25.6 m	NO (Banana)
QUEBRADA SANTAMARÍA SAME SIDE	1350	36	42.7 m	NO (Costus)
QUEBRADA SANTAMARÍA OPPOSITE SIDE	1350	45	52.7 m	NO (Costus)
CALLE A LAS NUBES	1350	N/A	17.2 m	YES

NO FROG GROUPS STREAM	ELEVATION	DISTANCE AWAY FROM GROUP	LENGTH OF SECTION	GINGER PRESENT
TRAMONTI'S	1370	> 20 m UPSTREAM	N/A	NO
QUEBRADA BERROS FINCA	1335	> 20 m (28.8) UPSTREAM	20.2 m	NO (Costus)
QUEBRADA BERROS CALLE	1280	20 m DOWNSTREAM	23.2 m	NO (Banana)
QUEBRADA SANTAMARÍA SAME SIDE	1350	20 m UPSTREAM	39.2 m	NO (Costus)
QUEBRADA SANTAMARÍA OPPOSITE SIDE	1350	20 m DOWNSTREAM	52.3 m	NO (Costus)
CALLE A LAS NUBES	1350	20 m DOWNSTREAM	6.1 m	YES