

# **Influence of abiotic factors on the size of larval cases of the Order Trichoptera (Calamoceratidae) in Neotropical freshwater ecosystems**

**Carlos Andre Yanez-Schmidt & Cam Roy**

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**Host Organization: INDICASAT**

**Supervisor: Dr. Luis Fernando de León**

**Centro de Biodiversidad y Descubrimiento de Drogas INDICASAT- AIP**

**Edificio 219, Ciudad del Saber Clayton, Panamá**

**Tel. 517-0700**

**Fax. 517-0701**

**Email: [luis.deleonreyna@gmail.com](mailto:luis.deleonreyna@gmail.com)**



## Introduction

Despite covering less than one-thousandth of the global land surface, running water environments are complex, high-energy systems that support a plethora of life. Rivers are simply channels of freshwater flowing downslope – yet within these channels one can find a wide range of environments. Much of this heterogeneity is caused by the geomorphologic power of the stream itself, constantly transporting material and re-shaping its form. The characteristics of a stream's habitat are also a reflection of the land it passes through. Running water environments are dependent on their catchments' climate, vegetation, underlying geology, soil and land use, among other factors. In terms of the in-stream biota, physical heterogeneity is exceptionally important; particularly flow velocity and substrate composition (Wallace and Webster, 1996). In addition, the diversity of these micro-habitats is intensified by gradients of water properties, such as temperature, pH, dissolved oxygen, salinity and turbidity.

Ecological communities are always shaped by a broad range of abiotic (as mentioned above) and biotic factors with high spatial and temporal variation (Menge and Olson, 1990). In streams, particularly headwaters, a key factor in energy flow is allochthonous organic matter input (Gessner et al., 1999). Riparian canopy cover has been shown to be an important variable in terms of light availability and quantity of organic matter reaching the stream (Hill et al., 2001). In first and second order streams, the extent of the canopy cover can regulate biogeochemical processes, along with rates of detrital processing, organic matter input and benthic production (McKie and Cranston, 2001). Openings or gaps in riparian canopy strongly affect environmental variables such as water temperature and the quantity and quality of organic matter inputs (Franken et al., 2007). Canopy cover can also influence dissolved oxygen content,

as pulses of dropped leaves can deprive the underlying water of oxygen during the respiration process.

The evolution of life in running water is expressed through adaptations to the multifarious in-stream environment. The challenges faced by life in streams are quite different from those encountered on land, or even in lentic freshwater systems. Most of the adaptations used by stream biota involve withstanding the powerful force of the current. Body shape tends to be fusiform or flattened, often coupled with hooks, bristles, hairs or filaments (Hynes, 1970). Body size is also important for hydrodynamics and the ability to move in among bed sediments (Giller and Malmqvist, 1998), not to mention avoiding predation. Research has shown that in addition to morphology, the use of flow refuges is an important adaptation in streams (Stubbington, 2012). There are high gradients of flow velocity in all three dimensions in rivers (laterally, longitudinally and vertically), providing ample locations to avoid being dislodged and washed away. A common refuge, mostly for invertebrates, is the bed of the stream. This group comprises the benthos.

Benthic macro-invertebrates, of which 70-90% are insects (Hynes, 1970), occupy important functional roles in freshwater streams. In addition to being a food source for fish and other consumers, aquatic invertebrates can control the abundance of primary producers, accelerate detrital decomposition and release bound nutrients into solution by their feeding activities (Covich et al., 1999). They are thus key regulators of the biogeochemistry of lotic systems. Aquatic insects can be grouped into two distinct life cycles: *hemimetabolous* or *holometabolous* development (Cushing and Allan, 2001). Hemimetabolous insects, such as the Orders Ephemeroptera (mayflies) and Odonata (dragonflies), have three life stages: egg, nymph

and terrestrial adult. Holometabolous insects have four life stages: egg, larva, pupa and terrestrial adult, with a complete metamorphosis occurring between larva and pupa. Examples of holometabolous insects include the Orders Diptera (true flies) and Trichoptera (caddisflies).

The Trichoptera (caddisflies) are a diverse holometabolous insect order whose larvae occupy a wide range of freshwater environments. Their abundance, taxonomic diversity and variety of food sources make them important in terms of secondary production and energy transfer (Wiggins, 1996). Many families of Trichoptera have a distinctive adaptation to life in running water. The larvae build portable cases of organic or inorganic material, held together by silk. The silk, secreted through an opening at the tip of the caddis labrum, is composed of long unbranched polypeptide chains of fibroin (Giller and Malmqvist, 1998). The silk has exceptionally high tensile strength, and is being investigated for use as a new natural textile fibre (Michalak et al., 2005) and as biomedical tissue (Tszydel et al., 2015).

The function of the case is believed to be two-fold: firstly as protection from predation (Otto and Svensson, 1980) and secondly to improve the efficiency of respiration (Giller and Malmqvist, 1998). Case-building requires significant investment, with estimates that up to 12% of larval energy is used to spin the silk and gather the material necessary for a case (Otto, 1974). Adult feeding amongst caddisflies is minimal, thus resources diverted to larval case-building will be unavailable after free-flying emergence. Nitrogen has been identified as a critical nutrient as it is a major constituent of silk (Craig, 1997). In one study, Scottish researchers collected Trichoptera larvae from streams. The insects were removed from their cases and weighed. ‘Control’ insects were placed back in their cases while the experimental group was forced to rebuild their cases. The larvae were left to pupate into adults. Adults that had been induced to

expend more silk as larvae had lighter thoraces and smaller wings, although their abdomens did not differ in mass or N content. Extensive building thus has potential negative consequences for adult flight, which affects longevity and ultimately fitness (Stevens et al., 1999).

The family Calamoceratidae (Order: Trichoptera) have case-building larvae that are found in a range of riparian habitats throughout the Neotropics. They build distinctive “sleeping bag” cases out of highly-decomposed, black leaves. The cases are relatively large compared to the size of the larvae. Calamoceratidae cases are flattened and straight, comprising dorsal and ventral sections fastened together around the edges (Wiggins, 1997). They tend to choose less palatable leaves in order to deter other invertebrate leaf shredders (Graça and Cressa, 2001). Their cases are primarily a deterrent to fish predation, but also possibly linked to respiration. The larvae feed on filamentous algae and vascular plant tissue, most likely derived from detritus (Wiggins, 1997). Larvae can be found on both submerged leaves and rocks (Norwood and Stewart, 2002). They are common in headwater streams across Panama.

With case-building being a costly, yet potentially useful adaptation to running water environments, this raises the question: do larvae of case-building Trichoptera build different sized cases under different environmental conditions? More specifically: 1) can the morphology of larval cases be predicted from certain environmental variables? And 2) is there an effect of canopy cover on the morphology of benthic larval cases? To test these questions, we used Calamoceratidae of the genus *Phylloicus* across several streams in central Panama.

## **Hypothesis**

We hypothesized that we would find larger Calamoceratidae cases under extensive riparian canopy cover than in ‘open’ reaches of stream due to a combination of available case-building material and oxygen dynamics.

## **Methods & Materials**

### *Sampling Sites*

Ten distinct reaches were selected for sampling on Quebrada Juan Grande (QJG), a first order stream off of Pipeline Road in Soberanía National Park, Panama Province, Panama (9°08'14.9"N, 79°43'23.8"W). The QJG catchment is mature, secondary growth tropical dry forest. At the time of sampling flow was very low, and in fact, QJG dried up before reaching Lake Gatún. Seven distinct reaches were sampled in three streams in the Santa Fe region in Veraguas Province, Panama. Rio Santa Maria (RSM) was sampled at both its headwater stage (8°30'51.9"N 81°04'02.1"W) and further downstream where it is a higher-order meandering river (8°12'44.6"N 80°57'48.5"W). Upstream, its catchment is primarily cloud forest with some mixed agriculture and horticulture. Downstream, RSM winds through pastureland. Rio Bulaba (RB) is a mountain headwater stream that feeds RSM, with an almost exclusively forested catchment (8°31'53.1"N 81°08'58.1"W). Rio Gatú (RG) is a higher-order stream that feeds RSM at a lower elevation, near the town of San Francisco (8°18'02.4"N 81°00'34.6"W). Its catchment is primarily pastureland.

### *Site Selection and Initial Assessment*

Primarily, the choosing of sampling sites in Panama was guided by expected physicochemical characteristics and then narrowed down the selection by accessibility. The list of sites was then further constricted on arrival to the stream by doing initial assessments in the immediate vicinity. The tertiary, most specific decisions were guided by study design suitability through the expression of key environmental variations selected involved into the correlation. Specifically, we assessed and collected thirteen variables of which six are physical characteristics, six are probed aquatic properties of the water and the last one is time.

### *Physical Data Collection*

For the physical data we registered the local time of day at the start of collection together with the reach's cover type, the channel width, the flood width, the substrate character, GPS location and altitude. We used a team composed of two-to-three members as by commonly used field procedures . The optimized scheme for three members included one person tending to instruments and data taking. The other two members were tasked with manipulating the sampling gear.

First, the time, the position and altitude of the site was collected as UTC -5:00 time zone time and in the conventional WGS 1984 Datum respectively. The data was directly imported from a LG-852 mobile device connected to the Global Positioning System (GPS). Second, the stream section was classified as either a gap or a canopy covered portion. The methodology for selecting a site's specific portion to be sampled was purposefully biased to encourage the odds of detectable differences in the case morphology-related dependent variables. In other words, sites of ambiguous cover (i.e. not clearly a closed-canopy reach or a 'gap' reach) were excluded.

Third, the stream flow velocity was estimated with the Quick And Rough method . This method's advantage on material requirements is that they were easily found at the site. The method consists of measuring the time and the amount of distance traveled by a leaf deposited afloat on the water surface. Timing and walking 30 meters alongside the leaf's direction begins at the releasing of the leaf. In the recorded amount of time taken to travel the 30 meters, the leaf's distance is estimated in comparison. This yields a fairly correlated estimate ( $r=0.85$ ) of flow velocity in m/s (FAO, 2006). Then, one obtains the flow velocity by multiplying the leaf's approximated velocity by the depth x width (cross-sectional area) of the channel to give a water volume per units of time. Fourth, channel width at larger rivers was assessed by walking across the flow, counting the number of steps and estimating the distance by multiplying the average step distance (1 m) by the number of steps. The width for the larger-sized channels was determined as the average of two member's visual estimates. Bank-to-bank length was assessed with the same procedure for all sites. The accuracy given to this assessment is  $\pm 1$  m. Fifth and finally, for the substrate and channel character, we categorized the reach as either a sandy pool, sandy riffle, cobble pool or cobble riffle.

### *Instrument Calibration*

The digital physio-chemical data gathering involved setting up, calibrating, probing and saving the data. For turbidity no significant setup was required and the calibration (following the model's included user manual) of the Thermo Scientific AQUAfast AQ3010 was done at every site while for the YSI data the setup and calibration was done in advance at the INDICASAT laboratories following the YSI Professional Plus User Manual (March 2009, Revision D).



### *Aquatic Physio-chemistry Data Collection*

The previously mentioned six variables measured with the YSI probe include water temperature, pH, dissolved oxygen content, specific conductance (salinity adjusted for temperature), nitrate concentration and waterbody turbidity as summarized (*Table 1*).

Table 1: Details on physicochemical measurement

Variable	Units	Resolution	Accuracy / Uncertainty	Auto-stabilizing Variance Threshold (at 1/2 maximum sensitivity )
Temperature	°C	0.1	±0.2	0.01275 (1.275%)
Nitrates concentrations	mg/L	0.01	±0.01	Disabled
pH	pH Units	0.01	±0.2	0.015 (1.5%)
	mV	0.01	±0.2	
Conductivity	mS/cm	0.001	±0.001	0.07625 (0.7625% )
Dissolved Oxygen	mg/L	0.01	±0.02	0.01275 (1.275%)
	%	1%	±2%	
Turbidity	NTU	.01	±2%	N/A

Probe sampling of the water was done with a portable YSI Professional Plus multiparameter probe (see Appendix B for details). The number of data points collected varied based on the hydrologic variations found along the reach and along a cross-section of the river including deeper or shallower areas. Overall the number of data points never exceeded 5 per site. Collecting water and insects after rather than before probing allows for the most accurate capture of initial conditions of the site. The results then avoid effects on suspension of matter, nutrient

content, oxygen content and temperature gradients from the mixing resulting from walking into the stream. For turbidity measurements, the vials were handled by the cap, rinsed with 10 mL of the sample water, filled to the mark, wiped with lint-free cloth and placed into the reading well. Water sampled into a clean dry vial was done three times at each site to create an average reading.

### *Clearing, Washing & Rinsing*

Clearing, washing and rinsing samples has two functions. First, it enhances the detection rate of invertebrate subjects, and second, it ensures the risk of damaging samples during transport or storage is reduced<sup>1</sup>.

On that note, washing and rinsing the samples first involved removing the undesired contents including excessively large, decomposed or heavy materials that would jeopardize the detection or damage the samples (for instance seeds, large leaf remains or heavy rocks). Anything initially removed was first thoroughly inspected for latched subjects then returned to the ecosystem. Then, rinsing the samples was done with a sieve and a white plastic pan. Water is thoroughly added to thin a pan and sieved careful to avoid losing any material. The process was repeated until the mixture's clarity was sufficiently low to allow for efficient stereoscopy. This process was always done using the water from the sampling site, at the site itself. Additionally, the alcohol preservation often triggered the suspension of organic matter thereby reducing clarity in the sample after the *in-situ* rinsing therefore requiring a second washing and rinsing using the

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<sup>1</sup> Notes: In accordance to the principle on reducing the scientific footprint while sampling, the methodology involved extracting and returning on-site collected elements to the ecosystem. As such, large biotic and abiotic materials (e.g. wood chunks, sticks, rocks), animals (e.g. tadpoles, small fish) and especially egg-carrying subjects outside of this study's question and scope were intentionally returned.

laboratory sink's tap water. The light color of the pan increases the contrast of dark shapes and silhouettes rendering the task of detecting, recognizing and extracting invertebrates less demanding.

### *Macroinvertebrate Collection*

The extraction of Calamoceratidae from debris was done carefully by hand for larger individuals and with soft dissection kit tongs to avoid breaking the cases. The samples were then preserved in 95% ethanol as a killing and preserving agent. The fragile samples were put in lab-grade plastic vials and unprocessed (non-cleaned, unwashed or non-rinsed) samples were preserved similarly in alcohol inside site-labeled sealable plastic bags. Both sealable bags and vials were labelled on-site with the abbreviation of the site's name, the gap or canopy cover result and the number of the sample. Samples processed at the laboratories were screened both with the naked eye and a stationary stereoscope.

### *Data Processing*

To import data from the YSI probe, the Data Manager software was used while measuring case lengths was done through digital image capture done with a Dino-Lite digital microscope and a millimetric spreadsheet stationary setup. This allowed for a standardized image capture and measuring process across all individuals. The digital image capture and case lengths logging was done in the ImageJ software. Finally, Microsoft Excel software was used for assembling the collected data and the RStudio statistical processing software was used for graphing, plotting and correlational analysis.

## Results

A total of 138 Calamoceratidae larval cases were collected from twelve distinct sites in four streams in central Panama. The mean case length was 1.88 cm and the mean anterior case width (A) was 0.67 cm (*Table 2*). The cases were collected with contemporaneous physio-chemical sampling (*Table 3*). Photographic examples of Calamoceratidae cases are provided in Appendix A.

Table 2: Summary of Calamoceratidae case characteristics

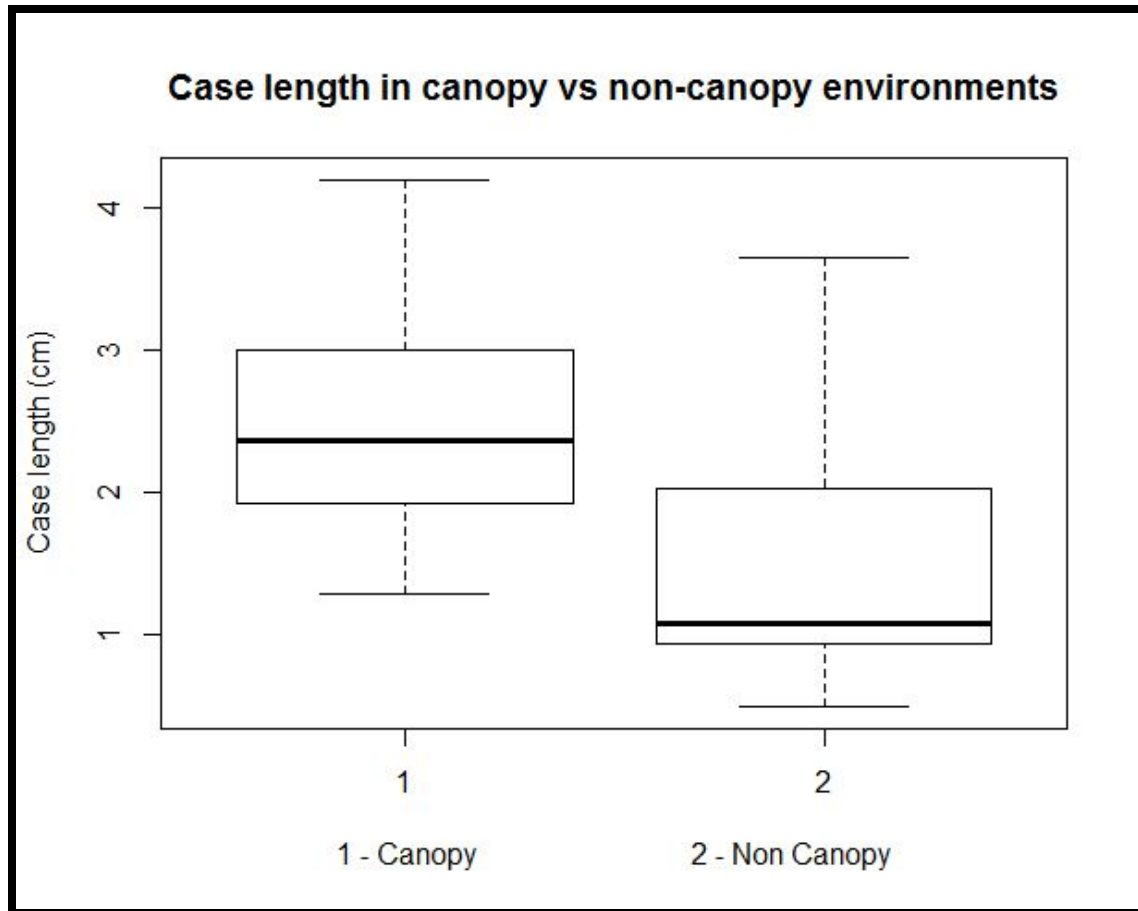
<i>n</i> =138	Case length (cm)	Case width A (cm)	Case width B (cm)	Length/Width A
<b>Mean</b>	1.88	0.67	0.61	2.93
<b>Standard deviation</b>	0.85	0.33	0.30	0.76
<b>Minimum</b>	0.50	0.21	0.10	1.39
<b>Maximum</b>	4.20	1.58	1.33	6.02

Table 3: Summary of physio-chemical data at sampling sites

<i>n</i> =12	Water temperature (°C)	Dissolved oxygen (mg/L)	pH	Specific conductivity (µS/cm)	Total dissolved solids (g/L)	NO <sub>3</sub> (mv)	Turbidity (NTU)
<b>Mean</b>	25.79	5.32	7.13	171.4	0.11	235.4	0.98
<b>Standard deviation</b>	1.67	2.24	0.79	98.9	0.06	20.7	1.40
<b>Minimum</b>	24.32	2.78	4.71	48.2	0.03	209.9	0.10
<b>Maximum</b>	29.28	8.73	7.91	283.3	0.18	272.5	4.54

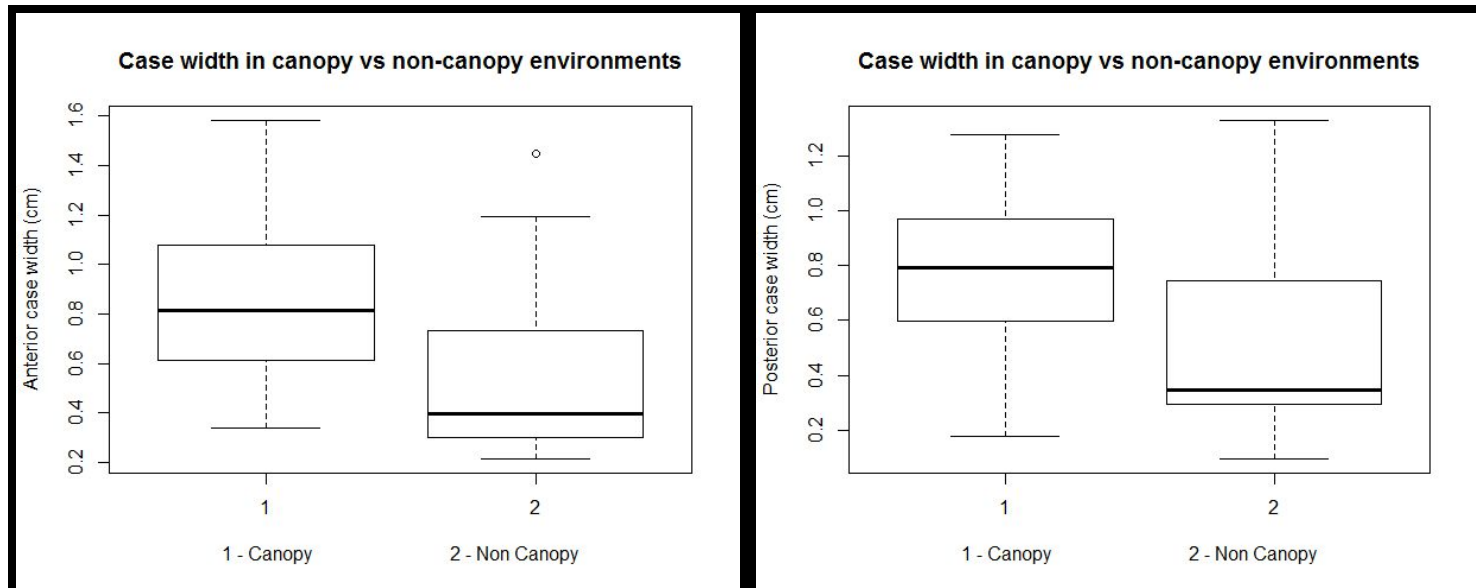
Larval cases were found to be significantly longer in canopy sites (mean=2.44 cm,  $n=63$ ) compared to non-canopy sites (mean=1.43 cm,  $n=75$ ) using a two-sample Welch's T-test ( $df=129.5$ ,  $p<0.001$ ) (*fig. 1*).

Fig. 1: Length of Calamoceratidae cases in different canopy environments



Anterior case widths (A) were also found to be significantly greater in canopy sites (mean=0.87 cm,  $n=63$ ) than in non-canopy sites (mean=0.52 cm,  $n=75$ ) ( $df=126.2$ ,  $p<0.001$ ) (*fig. 2*). The same relationship held with posterior widths (B), with canopy cases (mean=0.78 cm,  $n=63$ ) significantly greater than non-canopy cases (mean=0.48 cm,  $n=75$ ) ( $df=128.9$ ,  $p<0.001$ ) (*fig. 3*).

Fig. 2 & 3: Width (anterior and posterior) of Calamoceratidae cases in different environments



When looking only at QJG, which had the largest variation of canopy versus non-canopy sites, the relationship held. Canopy sites on QJG had significantly longer case lengths (mean=2.57 cm,  $n=51$ ) than non canopy sites on QJG (mean=2.18 cm,  $n=24$ ) ( $df=73$ ,  $p=0.04$ ). Across all streams, there was a significant positive correlation between percentage canopy cover and case length (*fig. 4*). The correlation was stronger when fit with a logarithmic trendline (*fig.5*).

Case length was significantly negatively correlated with dissolved oxygen concentration (*fig. 5*). There was also a negative correlation with  $\text{NO}_3$  concentration (*fig. 6*), although it must be noted that there is a strong positive correlation between  $\text{NO}_3$  levels and dissolved oxygen (*fig. 7*). Dissolved oxygen concentration (*fig. 8*) and water temperature (*fig. 9*) were weakly negatively correlated with percentage canopy cover.

Fig. 4: Calamoceratidae case length in relation to percentage canopy cover [case\_length (cm) =  $0.986 + 0.018(\% \text{canopy\_cover})$ ,  $R^2=0.38$ ,  $p<0.001$ ].

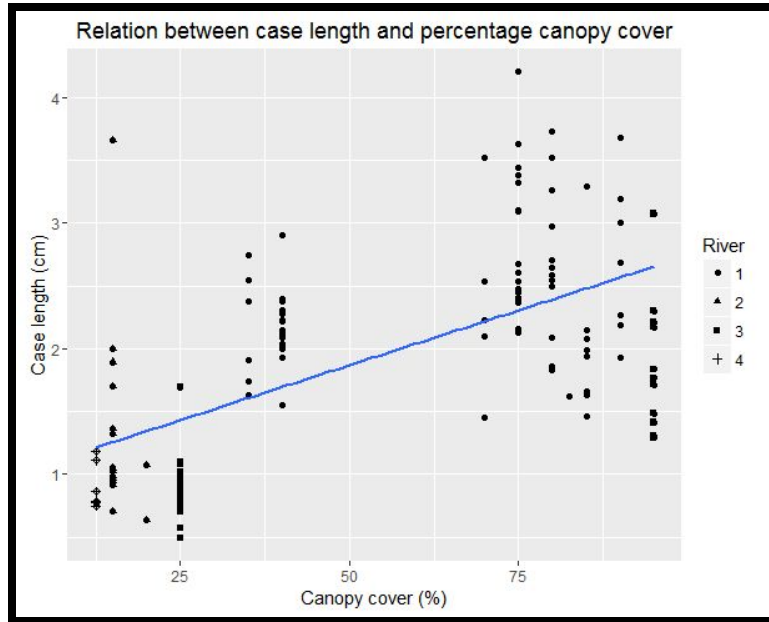


Fig. 5: Logarithmic relationship between case length and percentage canopy cover

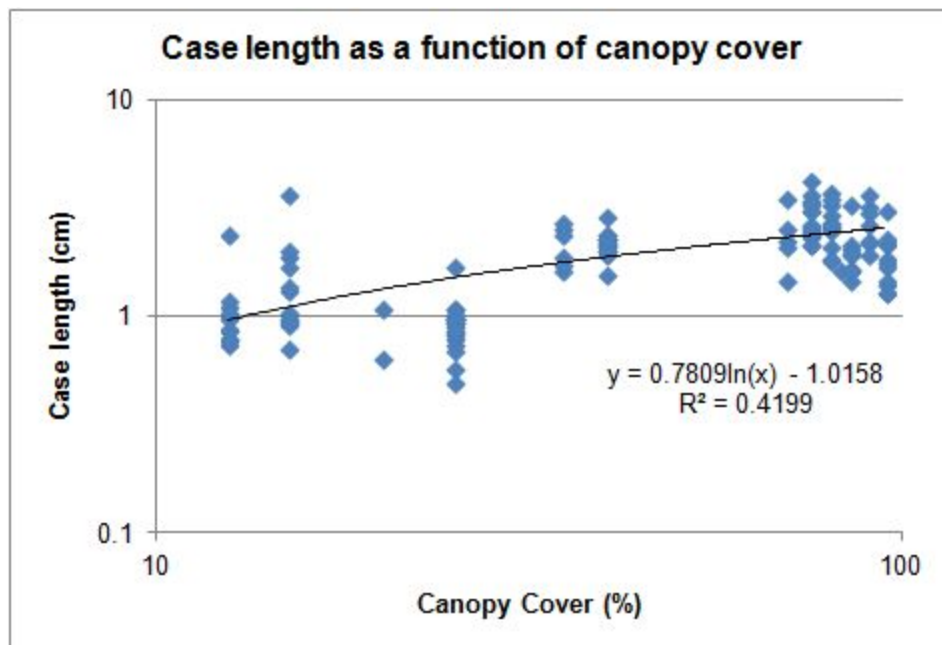


Fig. 5: Calamoceratidae case length in relation to dissolved oxygen concentration [ $\text{case\_length (cm)} = 8.425 - 1.685(\text{DO})$ ,  $R^2=0.47$ ,  $p<0.001$ ].

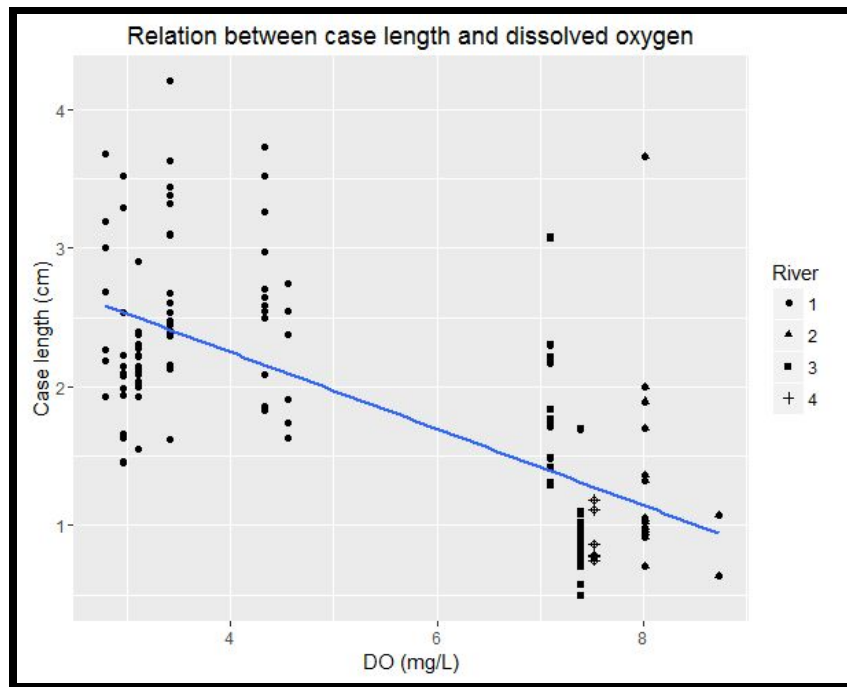


Fig. 6: Calamoceratidae case length in relation to  $\text{NO}_3$  [ $\text{case\_length (cm)} = 8.682 - 0.029(\text{NO}_3)$ ,  $R^2=0.44$ ,  $p<0.001$ ].

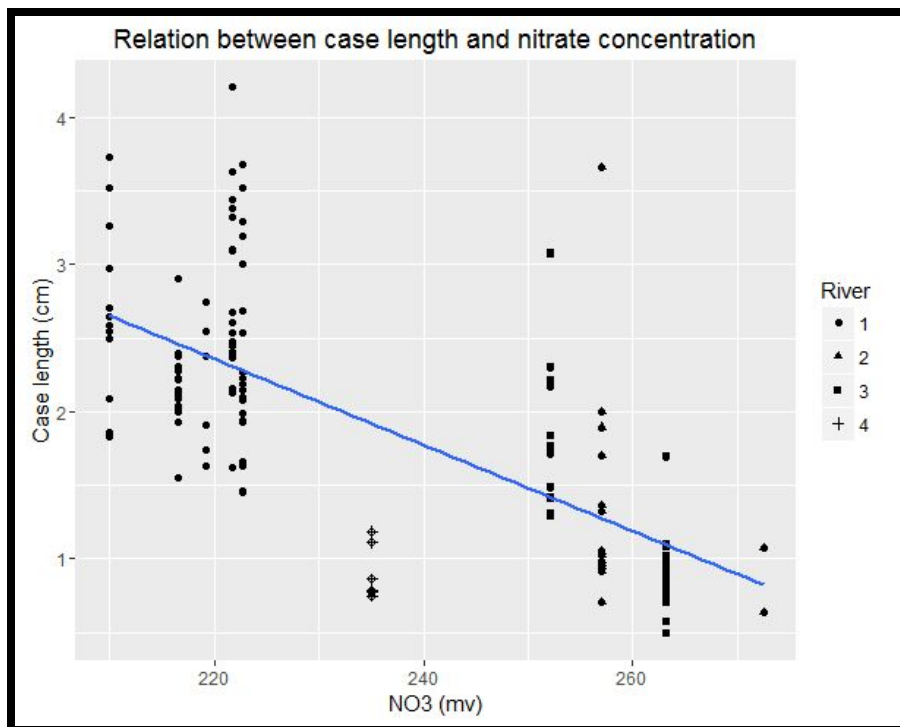




Fig. 7: Correlation of  $\text{NO}_3$  with dissolved oxygen concentration [ $\text{NO}_3$  (mv) =  $191.151 + 8.526(\text{DO})$ ],  $R^2=0.80$ ,  $p<0.001$ .

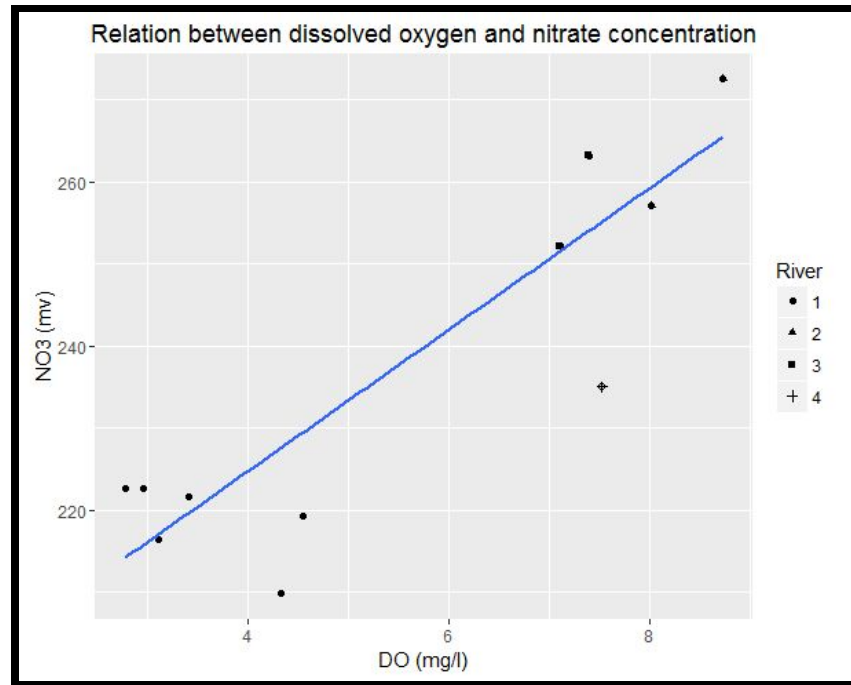


Fig. 8: Correlation of dissolved oxygen concentration with percentage canopy cover [ $\text{DO}$  (mg/L) =  $6.933 - 0.035(\text{canopy\_cover}\%)$ ],  $R^2=0.26$ ,  $p<0.001$ .

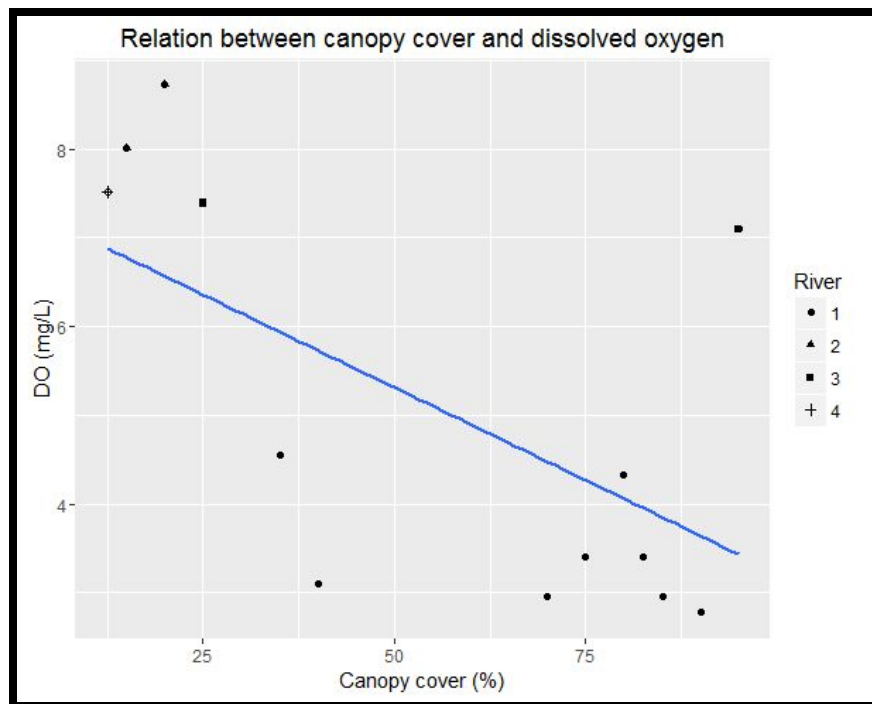
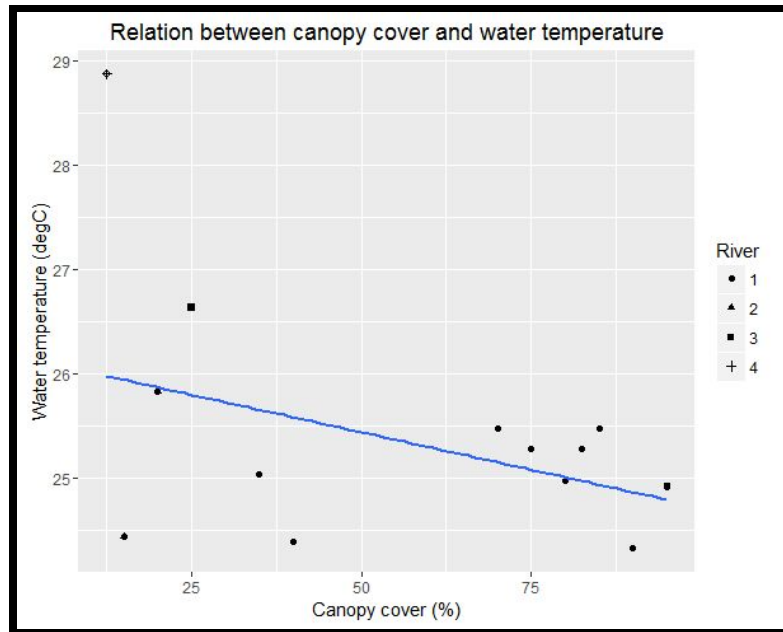
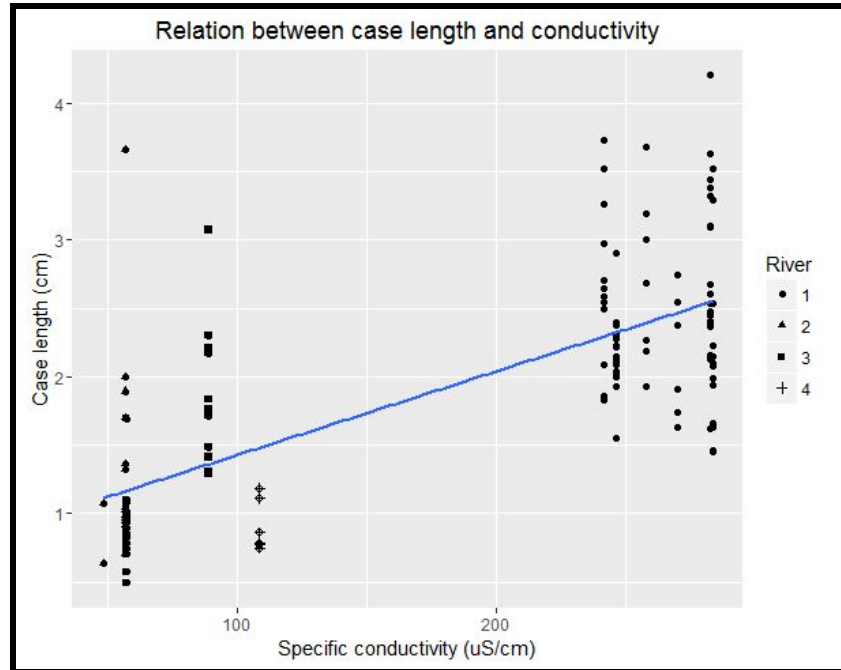


Fig. 9: Correlation of water temperature with percentage canopy cover [ $\text{water\_temperature } (^{\circ}\text{C}) = 26.691 - 0.022(\text{canopy\_cover}\%)$ ],  $R^2=0.23$ ,  $p<0.001$ .



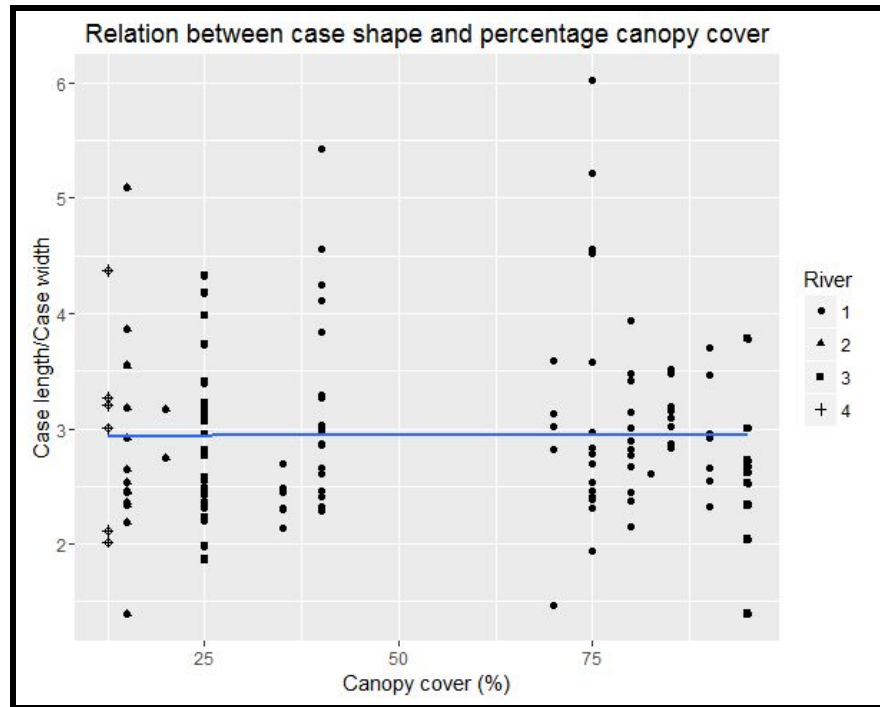
There were significant differences in case length and width of Calamoceratidae collected from reaches of stream with different primary substrate type. In stream reaches with beds primarily composed of sand, larval cases were larger (mean=2.40 cm,  $n=63$ ) than in cobble-based streams (mean=1.45 cm,  $n=75$ ) ( $df=136$ ,  $p<0.001$ ). Case length was negatively correlated with channel width ( $R^2 = 0.41$ ,  $p<0.001$ ), although channel width was also strongly negatively correlated with percentage canopy cover ( $R^2 = 0.55$ ,  $p<0.001$ ). Case length was positively correlated with specific conductivity (*fig. 10*).

Fig. 10: Calamoceratidae case length in relation to specific conductivity [case\_length (cm) =  $0.77 + 0.006(\text{sp\_conductivity } \mu\text{S/cm})$ ],  $R^2=0.51$ ,  $p<0.001$ .



There was no significant relationship between case morphology and either the abiotic variables of pH or turbidity. While case length and width were correlated with several variables, there was no difference in proportion amongst cases (length divided by width) across the measured environmental variables. For instance, the shape of Calamoceratidae cases showed no correlation with canopy cover (*fig. 11*).

Fig. 11: Calamoceratidae case shape (length divided by width) in relation to percentage canopy cover,  $R^2 < 0.01$ ,  $p = 0.78$ .



## Discussion and Conclusions

### *Implications of Results*

Our results highlight the importance of riparian canopy cover on stream ecology. The presence, or lack thereof, of canopy affects the stream directly through organic matter inputs and indirectly through its effects on in-stream factors, chiefly water temperature. It is hard to say which is more important in the context of this study.

With almost unlimited leaves available for case-building observed in closed-canopy reaches, it would appear that the quantity of leaf-fall is the major consequence of a closed riparian canopy. Our findings suggest that in open reaches *Phylloicus* larvae are limited in material that is appropriate for case-building. This theory is strongly backed by our findings at QJG, where there was little variation in abiotic factors between closed-canopy and open reaches. Yet one result from RB implies otherwise. One of the “closed canopy” reaches of the RB headwaters was in fact “closed” not by foliage but by a concrete bridge. Here, we found significantly larger *Phylloicus* cases than in open reaches on the same stretch of river. Under the bridge there was no observable difference in leaf-fall, suggesting that litter inputs were not controlling case size as hypothesized at QJG. Moreover, the negative correlation between water temperature and canopy cover (*fig. 9*) demonstrates the important indirect effects of shade. Physiological processes, such as respiration and digestion, are ultimately biochemical reactions - and biochemical rates are dependent on ambient temperature (Giller and Malmqvist, 1998). Water temperature is also negatively correlated with oxygen solubility. Ultimately, we cannot quantify how much of the observed difference in case size under varying canopy cover is due to litter inputs or alteration of other confounding abiotic factors. Previous studies have emphasized

both allochthonous litter inputs (Gilling et al., 2009) and the effects of shade (Cooper et al., 2015) as the more important drivers of invertebrate growth and assemblages.

At the time of sampling, Panama was in the midst of a historically strong dry season. In tropical dry forests, as at QJG, the dry season is the time when most trees drop their leaves (Wright, 1990). Overall, there were noticeable differences in the amount of leaf fall in closed-canopy reaches compared to open reaches, particularly in slow-moving QJG. There was abundant black, decaying leaves that Calamoceratidae use to construct their cases. It is reasonable to assume it would be easier for larvae to assemble materials to build their case in areas of plentiful leaf-fall, as was found in closed canopy reaches. Previous research has shown that it takes *Phylloicus* larvae about 24 hours to complete their case (Norwood and Stewart, 2002) when building material is abundant.

A secondary consequence of high leaf-fall is the depletion of dissolved oxygen in the underlying water. The decomposition of large quantities of organic matter removes oxygen from the water through microbial respiration. At QJG, many of the *Phylloicus* cases were collected from eddies that were not being replenished very quickly by more oxygenated water. Since we found a significant negative correlation between dissolved oxygen and case size, we expect oxygen depletion induced by the dry season leaf-fall to be playing a role in our results.

A possible explanation for the relationship between case size and dissolved oxygen involves the theory that the cases are built to facilitate respiration (Wiggins, 1997). The use of the cases for respiratory purposes has been extensively studied in other Trichoptera, particularly Leptoceridae and Rhyacophilidae, but the literature remains unclear on the extent to which Calamoceratidae use their case for purposes other than protection from predators. Our data

suggests that in oxygen-poor environments, Calamoceratidae might construct larger cases in order to maximize oxygen uptake. Further research into whether or not the case is used for respiration at all is needed before any conclusions can be drawn.

### *Cautions to Interpreting Results*

A major limitation of our study was our inability to resolve our cases to the species level. While we are reasonably certain that all 138 larval cases are from the genus *Phylloicus*, it is possible that cases collected from faster-moving streams in highland Veraguas Province were from a different species than those collected from lowland, sluggish QJG. As demonstrated by Norwood and Stewart (2002), case-building behaviour can differ between *Phylloicus* species, although Wiggins (1997) suggests that case architecture is characteristic at the genus level. In addition, if there are size differences between the larva themselves, that could result in different-sized cases. Differentiating between *Phylloicus* species is very difficult, which may dissuade future researchers from tackling this problem. On this note, this paper's methodology underlines the importance of investigating at higher taxonomic resolution the interactions between the broad Trichoptera order and the aquatic environments they are found in. This is especially true for the neotropics because of the high variations in habitat and adaptations observed.

This type of correlational analysis benefits from a broad spectrum of sites across different ecosystems. Due to time constraints, this study could not include an adequate range of fluvial sites. The trends displayed by the linear regressions included in this study should be accepted as 'reconnaissance' work that opens the door to more rigorous investigation. For instance, while there is a significant positive correlation between specific conductivity and case length, with a

relatively high  $R^2$  of 0.51 (fig. 10), we must be careful in interpreting this trend. The dataset is missing cases found in water with moderate conductivity that lies somewhere between the two extremes. More troubling, all the points with high conductivity were from QJG, so it is unclear whether case size increases with conductivity or some other factor related to the specific stream.

In addition, 'empirical' limnology as employed by this study should be coupled with experimental work that will elucidate the mechanisms behind what we find. With a sampling design such as ours, there is no way of truly understanding causal relationships. Even with canopy, we can only state that we expect to find larger Calamoceratidae cases under closed canopy, but we cannot say why. A possible experiment could be to collect Calamoceratidae cases with live larvae and bring them into the laboratory. There, one could manipulate variables such as leaf input, water temperature, light, dissolved oxygen or salinity. Larvae would be removed from their cases and forced to rebuild. Since they have been shown to build cases in one day (Norwood and Stewart, 2002), differences in case size could be observed on a reasonable time scale.

Two important additional unaccounted factor of influence are population density and predation intensity in a given habitat. It has been observed that under presence of predator fish, the leaf-processing behavior of *Phylloicus* is at its minimum, is lesser when in presence of other Trichoptera (predators or not) and at is at a maximum when in isolation (Rezende et al., 2015). This asserts a critical point that predator presence, and in fact any increase in density as well, affects the functional behavior of Calamoceratidae presents a potential impairment to its growth through impairing leaf and associated microfauna harvesting during leaf processing. Therefore, accounting for predation intensity and population presence is reasonably expected to yield a



contribution to case building, and by extension should be included in more extensive efforts to understand determinants of case morphology. Further evidence to encourage encompassing population density comes from Malaysian observations of higher biodiversity of aquatic tropical freshwater shredders both in terms of abundance and richness amongst sites located upstream compared to downstream sites (Yule et al., 2009). Assuming the findings of Rezende et al. (2009) to hold true, having more individuals in such sites could yield significant alterations to feeding behavior, growth and case morphology by extension. Evidently, population densities and predation are factors that correlational assessments should encompass get a better idea of the other variable's connection to case morphology differences.

Another variable playing into resource availability to individuals for growth is that of the match between known Calamoceratidae palatability and the qualities of leaf litter inputs composition. Specifically for tropical stream shredders, and for *Phylloicus* sp. in particular, there is ample evidence feeding preferences based on intrinsic leaf characteristics such as lignin, polyphenols, phosphorous or nitrogen content over plant leaf nativeness (Graca et al., 2001; Rincón & Martínez, 2006). Furthermore, *Phylloicus*'s growth in comparison to some temperate shredder counterparts is more noticeable and their survival more correlated to feeding on leaves “microbially colonized and partially degraded” (Graca et al., 2001). Again, this evidence points towards a worthwhile inclusion of leaf litter composition in a site as a contributing explanatory variable for growth and case morphology by extension once again.

#### *Technical limitations and observations*

Although the ocular estimation is a fast technique and requires only sight, its ability to provide accurate and precise readings is debatable. The lack of reproducible canopy results

affects the accuracy of the data correlated. This problem could be managed by having multiple individuals give simultaneous assessments and averaging the percentage results. Another way to improve the accuracy of estimates would be to use a have the procedure involve a fixed survey scale (perhaps Likert-type scale). It has been evaluated as a consistently underestimating method (Korhonen et al., 2006) therefore also affecting the precision of the estimate. Still using the ocular approximation for a lack of instrumentation, one way our design could have improved precision is by dividing the reach transect and make multiple readings to produce and average to be used. The researchers would have access to a densiometer were it not for a bunch of careless McGill students of yesteryear.

With regards to turbidity a perhaps unimportant limitation to obtaining accurate measurements is linked to vial preparation. There was omission to evenly apply an oil included in the kit which fills small scratches and diminish imperfection in the glass. This procedure was not applied throughout the sites thus it should be assumed that all sites were equally biased.

There is evidence of sieve mesh sizes  $>0.5$  or  $<0.5$  mm used in collection effectively biasing abundance and composition results for benthic invertebrate in various aquatic environments (Barba, Arrizabalaga, Otermin, del Campo, & Martínez, 2010; Mbaka, M'Erimba, Thiongo, & Mathooko, 2014; Schlacher & Wooldridge, 1996). This underlines the importance one needs to give to the collection materials and their role in determining results. This is especially important for smaller sized macroinvertebrates which will tend to be eliminated from the sampling as they are not retained through the rinsing and straining process. This paper however is not particularly affected by this bias for two main reasons. First, it does not focus on abundance nor on composition although it acknowledges that the size of the sample regulates the

statistical power of interpretations on the Calamoceratidae target population. Second, literature records of Calamoceratidae cases sizes range from 2 to 40mm (Thorp & Rogers, 2010; Wiggins, 1996). In light of this evidence, the mesh openings' size for the sieve and the kicknet were both 250 micrometers so as to retain any and all desired subjects.

One variable uncontrolled for is flow regime. Flow regime are accepted to be of critical importance to riparian ecology, including macroinvertebrate assemblages (Warfe et al., 2014). Although the data was collected allows for the incorporation of this variable into the scope, reasonable workload was restricted to collection of data for future reference or studies. Flow regime has the potential to be a design limitation in so far that a dry season diminishes access to Calamoceratidae niche areas on the dry banks so as to limit the selection of case leaves and nutrients accessible.

With regards to the chosen analysis, the focus of this study was to investigate case morphological changes and so, a standardized amount of time-effort at each site as performed in the pilot project was not necessary. Ensuring comparable amounts of individuals was irrelevant. Instead, the relevant concern or constraint was about collecting an adequate amount of individuals to pursue statistical analyses.

### *Conclusions*

The creation of closed-canopy reaches and 'gap' reaches are more often than not the result of natural processes. Just as in the tropical forest, light gaps are an important successional stage in tropical streams. However, the conversion of forest to pastureland is an on-going phenomenon in Panama, with subsequent reductions in riparian canopy cover. The findings of

this study further emphasize how a simple disturbance such as removing canopy cover from a stream can affect a slew of different organisms and interactions within the waterbody. It demonstrates how case-making Trichoptera larvae can be sensitive to slight changes in abiotic factors in their environment.

Leaf cases constitute critical adaptations for Calamoceratidae on the grounds of that they are most evidently a shelter and a tool for anchoring themselves, collecting food and regulate their oxygenation. In this way the stress of long-term changing conditions from climate change could prove to promote further functional evolutionary adaptations amongst Calamoceratidae. Since Calamoceratidae play a multitude of ecological roles, human activity-induced conditions in tropical freshwater systems is likely to create reverberating pressures on the food web, composition and structure of communities linked to the Calamoceratidae and similar Trichoptera families. Hence, to understand possible repercussions of human activity on biological diversity it is imperative to study the variables that accompany changes in case dimensions.

It is hoped that this research prompts further investigation into Calamoceratidae among Neotropical scientists, as well as into casebuilding Trichoptera in general. From an evolutionary standpoint, these are fascinating animals who are thought to have evolved outside of water and adapted first to life in standing water and then to running water (Hynes, 1970). They can tell us much about the interactions of biotic and abiotic factors and how they affect the expression of life.

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## Appendix A: Photographs of Calamoceratidae specimens

Fig. A1: Calamoceratidae larva in case collected from Quebrada Juan Grande



Fig A2: Empty Calamoceratidae case collected from Quebrada Juan Grande





Fig. A3: Calamoceratidae larva outside case, collected from Quebrada Juan Grande



## **Appendix B: Table of Multiparameter Probing Configuration**

<b>Serial Number</b>	<b>Item Description</b>
<b>6 050 000</b>	<b>Professional Plus Instrument</b>
<b>605 790</b>	<b>10-meter Field rugged cable ISE/ISE/DO/conductivity/temp</b>
<b>605 202 (5908)</b>	<b>Galvanic DO sensor</b>
<b>605 106</b>	<b>Freshwater Nitrate sensor (ISE)</b>
<b>605 203</b>	<b>Conductivity Four Electrode Cell sensor</b>
<b>605 101</b>	<b>Freshwater pH sensor (ISE)</b>