Responses of benthic macroinvertebrate community structure to anthropogenic environmental alterations

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McGill University Panama Field Study Semester

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Time Allocation

Number of full days in the field: 7
Number of full days in Panama City: 35
* 1 workday considered to be 8 hours. Hours do not include travel time required to get to and from field sites (See Appendix I for time chronogram).

Host Information

Created in 2002, INDICASAT AIP was initiated to promote development of science in Panama (INDICASAT Times). Our host institution conducts research with two main objectives. The first objective is to have a major impact on the generation of science-based knowledge and the second, to play an important role in the formation of national scientists (INDICASAT Times). Recently, INDICASAT has expanded its areas of research to include an ecology unit within the biodiversity and drug discovery center.

Our supervisor, Dr. Luis Fernando de León is an evolutionary biologist at INDICASAT in the ecology unit. He received his Bachelors of Science at the University of Panama, after which he went to McGill University to complete a PhD. His research focused on the ecology of adaptive radiation in Darwin's Finches. More specifically he investigated how ecological differences can initiate and maintain divergence and subsequent speciation. Furthermore he examined how anthropogenic influences may hinder this speciation process and thereby alter the processes that generate biodiversity. He continued on at McGill conducting Post Doctoral research on Darwin's Finches although switching emphasis away from how ecological dynamics influence evolution towards how evolution may in turn influence the environment's ecology. Such research has been called eco-evolutionary dynamics. Following his postdoctoral work he returned to Panama where he took on his current position. He now continues work on eco-evolutionary dynamics with Darwin's finches. Furthermore, his research interests have expanded towards an investigation of diversity within Panama, particularly within freshwater environments. One main focus has been directed towards another study organism, the electric fish that may be evolving by mechanisms that parallel what has been seen in the finches. The goals of these projects, as was seen with the finches, is to shed more light as to the processes that drive diversity within this group of neotropical fish. In addition he has been interested in examining other components of biodiversity within these environments and particularly implications of human influences. From the formation of the Panama Canal to extensive land use changes all may have played a role in structuring the current pattern of diversity in the country through the modification of both ecological and evolutionary processes. Thus within here, our project comes into play.

Project Background

Freshwater habitats are of fundamental importance as they support essential resources, provide a suite of essential ecosystem services, and contribute significantly to global biodiversity (Strayer & Dugeon, 2010). However at the current, these vital habitats are becoming increasingly threatened due to a wide range of anthropogenic stressors such as overexploitation, water pollution, land use change, habitat degradation, species invasions, flow modification, and climate change (Dudgeon et al., 2006; Ormerod et al., 2010). In freshwater streams, of particular concern is deforestation of riparian and surrounding catchment habitats as these terrestrial environments are tightly linked to aquatic processes (Wallace et al., 1997; Naiman & Décamps, 1997). Deforestation has been shown to cause many changes in the physical and chemical properties of stream habitats such as increased temperature, increased sediment input, higher insolation, elevated nutrient loading, and changes in proportions of different basal food resources (Benstead et al., 2003; Likens et al., 1970).

Recent work is now focusing on how these changes in habitat attributes in turn impact the biological integrity of stream ecosystems. Biological integrity refers to the "capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitat of the region" (Karr & Dudley, 1988). In essence, integrity describes the state and health of natural communities with respect to what is expected in the absence of disturbance. The use of aquatic bioindicators has shown to be very useful to detect such short and long-term effects of land conversion. Particularly, benthic macroinvertebrates have been widely used as bioindicators for several reasons. First they have varying sensitivities and respond rapidly to changes in water and habitat quality (Kasagaki et al., 2008). Community composition and diversity has been shown to be strongly dependent on the suite of physical parameters found within the habitat (Mesa, 2010; Buss et al., 2004; Lorion & Kennedy, 2009). They are also easily collected and identified. Moreover, macroinvertebrates are one of the most diverse and abundant groups within aquatic communities (Couceiro et al., 2007). Therefore the status of macroinvertebrate communities can be used to reflect overall ecosystem health and to what extent it may have been altered relative to what may be expected under unimpacted conditions.

Macroinvertebrates present further interest as they can be categorized into several distinct functional groups that perform differential ecosystem services such as sediment mixing, nutrient cycling and energy flow within foodwebs (Covich et al., 1999). As such, macroinvertebrates not only possess high taxonomic diversity but also high functional diversity within stream environments and are central to the integrity of stream functioning. Habitat conditions such as temperature and flow regimes, resource availability, and biotic interactions all influence the functional community structure (Covich et al., 1999). Different groups are found in different proportions based on the suite of habitat traits. For example, grazers are prominent in sites with high algae production rates (Covich et al., 1999). Therefore if human disturbances alter these habitat attributes, it may in turn affect the functional composition of macroinvertebrate communities. Several studies have supported this notion (Couciero et al., 2011; Helson & Williams, 2013; Tomanova et al., 2008). For example, Benstead et al. (2003) found that deforested sites were dominantly composed of generalist collector-gatherer species in contrast to forested sites that contained collector-filterer and detritivorous shredders in addition to collector-gatherers. Functional structure is a key component influencing biological integrity in addition to taxonomic composition however the two may be differentially impacted by disturbances (Gessner & Chauvet, 2002). For these reasons, in order to thoroughly examine human disturbance impacts on ecological communities, it is of great interest to see if these changes in community structure are occurring in these habitats as well.

Work on this subject in tropical streams is limited to date. However, the findings thus far indicate that deforestation has major implications on macroinvertebrate communities. It has been shown to reduce overall macroinvertebrate diversity and severely simplify and alter the taxonomic community composition (Benstead et al., 2003; Couceiro et al., 2007; Bojsen & Jacobsen, 2003; Iwata et al., 2003). As such, it is evident that loss of forests surrounding stream habitats has the potential to strongly alter biological communities, which could lead to local extirpations of different taxonomic groups or even species extinction.

Panama, our area of study, has been subject to substantial deforestation due to a variety of causes such as road construction, logging, mining, and colonization (Rompré et al., 2008; Suman, 2002). One particular cause of the extensive deforestation in Panama however has been the progression of agriculture. Between 2006 and 2011, forested land in Panama decreased at a rate of 0.37% annually with an increase in permanent cropland of 2.25% annually (FAOSTAT, 2014). Furthermore, deforestation due to agriculture is prevalent near stream environments due to the pattern in which this land use change occurs. Thomas Croat (1972), whose work was conducted primarily in Panama, described the movement of people as inextricably linked with waterways. Humans migrate along waterways, spread laterally and remove forest cover during the voyage (Croat, 1972). This land is then used for agricultural practices, which inevitably has lead to deforestation occurring within watersheds across the country (Croat, 1972; see Box 1, Figure 1 in Appendix II). In addition to the consequences of deforestation, conversion of land use for agriculture may have other consequences such as pesticide and fertilizer application (Harrison, 2011). Pesticide and fertilizer drift leaches into waterways, which in turn has consequences for aquatic ecosystems either through acting as toxins to ecosystem health or as nutrients in supplementing growth (Harrison, 2011; Egler et al., 2012). While Justus et al. (2010) suggested that herbivorous macroinvertebrates and algal density are linked with increased nutrients provided by runoff fertilizers; other studies have demonstrated the negative consequences of pesticide use on macroinvertebrate communities (Egler et al., 2012; Maltby & Hills, 2008). Despite the body of work that has already investigated the impacts of deforestation and agriculturally induced deforestation on macroinvertebrate communities, it is difficult to extrapolate findings from one region to another. As there is little information regarding how this land use change impacts stream communities in Panama specifically, the consequences have yet to be fully uncovered.

The aims of this study are thus threefold. First is to gain a greater understanding of freshwater communities in the Neotropics and how they respond to environmental anthropogenic disturbances. Here the focal disturbance is deforestation from agricultural land use alterations. Secondly, to provide insight as to the health and status of some of the freshwater environments in Panama. Finally this gained knowledge will hopefully allow us to provide insight as to what would be an effective management and mitigation strategy to conserve biodiversity in this region. From the above review, we hypothesized to see an overall reduction in diversity. We also hypothesized to see changes in community composition and functional structure. As there is a lack of consensus in the literature as to the pattern of change in both these components with respect to disturbance, we did not formulate specific predictions beyond this. Furthermore, we hypothesized that macroinvertebrate community composition would be dependent on a suite of habitat traits. We hypothesized that disturbed and undisturbed sites would differ with respect to these various physical characteristics and thus present the mechanism behind these biotic differences.

In order to investigate these questions, streams were studied within the Panama Canal watershed in both undisturbed locations with intact forests in the surrounding catchment areas and in disturbed streams that were found in regions heavily impacted by deforestation due to agriculture.

Methods

STUDY AREAS

The study was conducted at a total of six sites within the Panama Canal Watershed during the dry season, between January and April 2014. This is a moist tropical region (latitude 9°) experiencing average precipitation of 2921mm/y as well as relatively constant yearly temperatures between 23-27°C.

Three streams, Quebrada Juan Grande, Río Frijoles, and Río Frijolito located along Pipeline Road in Parque Nacional Soberania were studied as representatives of undisturbed sites. The national park has been undisturbed since the early 1900’s (Angermeier & Karr, 1983). The disturbed streams were all found within areas in which agriculture was the dominant surrounding land use. The first was an unnamed stream located on the property of a local farmer and thus will be referred to as Disturbed 1. The other two streams sampled were Quebrada Trinidad and Quebrada Grande (see Figure 2 in Appendix II).

DATA COLLECTION

Benthic macroinvertebrates were collected at the six sites. At each stream, two 100 m stretches were assessed, taking one sample within each 20 m segment to give a total of 5 samples per stretch. At each site within each river either a pool or riffle habitat was sampled. Where possible, equal numbers of pools and riffles were sampled within each river. Macroinvertebrates were collected using D-frame dipnets to cover 1 m^2-sampling quadrats. To do this sediment was disturbed by kicking while macroinvertebrates were collected in the net. Manual sampling was also conducted at sites containing stony bottoms. Material was then sorted on a tray; macroinvertebrates were removed and placed into vials of 95% ethanol for later identification. Physical characteristics of sampling sites were measured including pH, oxygen (mg/L), temperature (˚C), flow velocity (m/s), and canopy cover (%). The pH was measured using ExStik pH Meter that also performs temperature readings. The oxygen was measured using a Handy Polaris oxygen probe, which also measures the temperature. To determine flow velocity of the stream sites, a Model 3000 Current Velocity Meter was used. A spherical densiometer was used to determine canopy cover. Habitat integrity was assessed through the use of the Habitat Integrity Index (HII) created by Nessimian et al (2008). The index is a modified version of the Riparian, Channel and Environmental (RCE) Inventory created by Petersen (1992). The RCE was developed in temperate landscapes and as such, it was adjusted in the HII to be more applicable to tropical environments. Sites were visually inspected for 12 stream environment characteristics that may be related to human impacts (See Appendix IV). Subsequent calculation of the HII follow the procedure of Nessimian et al (2008). An HII value was determined at each site within each river then subsequently averaged to give a mean HII value for each river. HII values range from 0 indicating severely impacted to 1 indicating pristine un-impacted environments.

IDENTIFICATION

Due to limitations in resources to identify all macroinvertebrate groups, only those of the class Insecta were included in the study. Samples were identified to taxonomic family where possible by use of taxonomic keys (Merritt et al., 2005) and then grouped into morpho-species. Morpho-species were assigned to functional groups based on Merritt and Cummins (1996) and their abundance was determined. Merritt and Cummins (1996) classify macroinvertebrates into functional groups based on modes of feeding leaving six categories. Scrapers, who adapt to graze and scrape materials from substrates, shredders, invertebrates that take coarse particular organic matter (CPOM) and break it down into fine particulate organic matter (FPOM), collector gatherers, who feed on fine particulate organic matter, collector filterers, who remove FPOM from passing water using silken nets or filtering fans, collector gatherers, those which acquire FPOM from interstices in bottom of sediment and predators that capture and consume prey. After identification, samples will be stored at -20 ˚C for subsequent DNA analysis.

DATA ANALYSIS

t-tests were conducted for each environmental parameter to detect for differences between disturbed and undisturbed streams. The value of each environmental variable for each stream used in the test was taken as the mean of the values across all of the sites in each river.

To measure diversity within each river, richness, the Shannon Index (*H'*) and Simpson's Index (*D*) were calculated at the taxonomic order level and at the family level where applicable. All abundance data was summed across all sites of each river to give one value of each diversity index for each river. *t*-tests were used to test for differences in each diversity index between disturbed and undisturbed sites.

Spearman rank correlation was conducted to investigate a potential relationship between species richness and total number of insects sampled at each river.

Spearman rank correlations were conducted between HII and each diversity index at both the order and family level. *t*-tests were used to compare physical parameters and habitat integrity index between disturbed and undisturbed sites.

The relationship between environmental parameters and abundance of different orders was investigated through the use of spearman rank correlations. The analysis was conducted at the level of each individual site of each river. For every environmental parameter, the absolute abundance of each order within each site was paired with the value of the parameter at that site for a total of 60 points.

Canonical correspondence analysis was used to investigate the relationship between sampled sites, environmental variables, and community composition. This analysis allows variation in communities to be directly related to variation in the environment (Ter Braak 1986). A permutation test was conducted to test significance of the relationships described in the CCA.

Relative abundances for each of the macroinvertebrate orders and families were calculated for each one of the six sampled rivers. To test for differences between disturbed and undisturbed sites in terms of percent abundances, one-way ANOVA's tests (habitat as a factor) were conducted for each order and family. Tests were conducted on arcsine transformed relative abundance data.

To compare community composition between all streams, a one way ANOVA was conducted on arcsine transformed percent abundance data for each order. This was followed by tukey tests to detect specific differences.

For analysis of functional structure, individuals whose functional group could not be determined were eliminated. Total number of individuals in each functional group was summed across all sites in each river and divided by the total number of insects within the river to give percent abundance of each functional group. In order to detect differences in functional structure between disturbed and undisturbed sites, a one way ANOVA was conducted. Percent abundance data was arcsine transformed. Absolute abundance of each functional group was also compared between disturbed and undisturbed sites by use of one way ANOVAs.

All results were considered significant at the $p<0.05$ level.

This study was carried out following the Code of Ethics of McGill University.

Results

See Appendix V for raw data for rivers sampled.

PHYSICAL CHARACTERISTICS

(a) Environmental Variables

Temperature was significantly different between disturbed and undisturbed sites with a greater mean temperature in disturbed sites (*t*-test, $P<0.001$). Percent canopy cover was also significantly different between disturbed and undisturbed sites with lower mean cover in disturbed sites (*t*-test, $P<0.05$). Oxygen, flow velocity, pH, width, and depth had no significant difference between disturbed and undisturbed sites (*t*-test, all with $P>0.05$) (Table 1).

(b) Habitat Integrity Index

Habitat integrity index was significantly different between disturbed and undisturbed sites (*t*-test, $P<0.01$). All undisturbed sites had higher HII values than disturbed sites (Figure 2). Habitat integrity index was not significantly correlated to any diversity index at the order level (Shannon $R= -0.0286$, $P=1$; Simpson's $R = 0.3714$, P=

0.4972; Richness *R*=0.7590, *P*=0.08). It was also not significantly correlated at the family level (Shannon R=-0.2571 *P*=0.6583; Simpson's *R*= -0.1429, *P*=0.8028; Richness *R*=0.0304, *P*=0.9545) (Figure 3, Table 2).

RELATIONSHIPS BETWEEN ENVIRONMENTAL VARIABLES AND MACROINVERTEBRATE COMMUNITY COMPOSITION

There were significant correlations found between some environmental parameters and abundances of some orders (Table 3). Temperature was negatively correlated to Odonata ($p<0.05$). Oxygen was positively correlated to Coleoptera, Ephemeroptera, Hemiptera, Plecoptera, and Trichoptera ($p<0.05$). Diptera and oxygen were negatively correlated ($p<0.05$). Flow velocity was positively to Coleoptera, Hemiptera, Plecoptera, and Trichoptera ($p<0.001$). Diptera and pH were negatively correlated ($p<0.01$). pH was positively correlated to Ephemeroptera, Hemiptera, and Trichoptera ($p<0.01$). Width was positively correlated to Coleoptera, Ephemeroptera, Hemiptera, and Trichoptera ($p<0.05$). Depth was negatively correlated to Coleoptera, Ephemeroptera, Hemiptera, Odonata, Plecoptera, and Trichoptera ($p<0.01$). Canopy cover was the only environmental parameter that did not significantly correlate with any order abundance. Collembola and Megaloptera were the only orders that did not significantly correlated to any of the environmental parameters. A summary of the significant positive and negative correlations associated with each order are given in Table 4.

50.1% of the variance in community composition is accounted by the CCA. The cumulative variation of the taxa-environment relationship in the CCA accounted by the first two axes was 45.3%. Eigenvalues for each axis indicate the amount of variation seen along it or its strength in influencing community composition. Eigenvalues for axes 1 and 2 were 0.3939 and 0.04841 respectively. As the eigenvalue for axis 2 was quite low, it indicates a weak gradient and only the first axis was considered in further analysis.

Axes define gradients of a set of environmental parameters. The intraset correlations are the correlation coefficients between the environmental parameters and the axes. By looking at the magnitude of the intraset correlations it can be inferred which parameters are the most important variables influencing community assemblages. Here, the first axis was most strongly associated with oxygen, flow velocity, pH, and depth (Table 5).

Points for each order show the center of its distribution along a particular environmental gradient. The distance between this point and environmental vectors describes the relative importance of the environmental variable in explaining variation in the group's abundance; the closer to the vector, the more important it is. Areas around the perpendicular of a vector represent regions of no change in the variable. Therefore if a taxonomic point lies in that area, it can be inferred the perpendicular variable does not explain variation in that taxa. Table 4 summarizes environmental gradients associated with each order and their relative position along them.

Sites 1 to 10 correspond to Disturbed 1, 11 to 20 for Quebrada Grande, 21 to 30 for Quebrada Juan Grande, 31- 40 for Rio Frijoles, 41-50 for Rio Frijolito, and 51-60 for Rio Trinidad. Each site point lies at the centroid of all the points of species that are found in that site. Its position in relation to the axes defines where along the environmental gradient it falls. Figure 4 shows that sites from disturbed 1, Rio Frijoles, and Rio Frijolito generally fall on the left of the origin on axis one. Thus it can be inferred that these sites are associated with shallow, low oxygen and flow velocity conditions. Sites from Quebrada Grande, Rio Trinidad, and Quebrada Juan Grande are dominantly found on the right of the origin thus associated with deeper, higher oxygen and flow velocity conditions.

Permutation test for CCA indicate the relationship between environmental parameters and community composition were not due to chance ($p<0.01$).

COMMUNITY STRUCTURE

(a) Diversity

The Shannon or Simpson Index revealed no consistent trend between disturbed and undisturbed sites in changing diversity at either the order or family level (Table 6). Disturbed 1 always had the lowest value across all diversity indices at both the family and order level although the effect was more pronounced at the order level (Figure 5). At the order level, Quebrada Juan Grande always had the highest value across all diversity indices. At the family level, Rio Trinidad had the highest value. With the exception of Simpson's at the family level, undisturbed had greater diversity values than disturbed however this difference was not significant (t-test, $p>0.05$ for all indices) (Table 7).

Richness at both the level of order and family were not correlated to total number of insects sampled ($R=0.213$, $P>0.05$; $R= 0.455$, $P>0.05$).

(b) Macroinvertebrate Abundance

A total of 3626 insects were collected across all 6 rivers. 44 families were found across 9 orders.

Total insect abundance was greater for the disturbed streams (2,077 individuals vs. 1549 individuals found in undisturbed rivers), but this difference was not statistically significant (F value=0.372, p value=0.575, ANOVA test).

Diptera was the predominant order found in Disturbed 1(93.80% of the total insect abundance), and also in Río Frijoles and Río Frijolito (39.07% and 34.34% respectively). Ephemeroptera was the main order found in Quebrada Grande, Quebrada Juan Grande and Trinidad (50.90%, 40.89% and 56% of total insect abundance respectively) (Figure 7).

When relative abundances were compared, Collembola was the only insect order found to differ significantly ($p<0.05$) between disturbed and undisturbed streams, with a higher relative abundance in undisturbed sites (Figure 8). Odonates were more predominant in undisturbed sites as well (18.97% vs. 5.68% of the insect community in disturbed streams), and this difference was close to be significant ($p=0.061$) (Table 8).

At the family level, Staphilinidae (Coleoptera), Gomphidae and Protoneuridae (Odonata) were found to be significantly more abundant in undisturbed streams (Table 8). The relative abundance of Hydropsychidae (a Trichoptera tolerant species) did not significantly differ between disturbed and undisturbed rivers (F value=0.109, $p=0.758$, ANOVA test).

When relative abundances were compared across all 6 rivers, percent abundance of 7 orders were significantly different (Collembola F=0.0145, p<0.05, Diptera F=6.088, p<0.001, Ephemeroptera F=3.577, p<0.01, Hemiptera F=4.345, p<0.01, Megaloptera F=8.051, p<0.001, Odonata F=9.289, p<0.001, and Trichoptera F=5.209, p<0.001). The remaining 2 orders did not have significantly different percent abundances across rivers

(Coleoptera *F*=1.638, *p*>0.05; Plecoptera *F*=1.413, *p*>0.05). Tukey tests were unable to reveal which rivers differed (Table 9).

(c) Functional Structure

There was no significant difference in absolute or relative abundance of functional groups between disturbed and undisturbed sites (Figure 9, Table 10 and 11). The mean relative abundance in disturbed sites was greater for Collector Filterers, Predators, and Scrapers (ANOVA, *F*=0.645, P >0.05; *F*=0.4, P>0.05; *F*=1.21, P>0.05 respectively). The mean relative abundance in disturbed sites was lower than undisturbed for Collector Gatherers and Shredders (ANOVA, *F*=3.951, P >0.05; *F*=0.445, P>0.05 respectively). Absolute abundances showed the same pattern with greater mean values in disturbed sites for Collector Filterers, Predators, and Shredders (ANOVA *F*=0.107, P >0.05; *F*=0.836, P>0.05; *F*=0.726) and lower mean values in disturbed sites for Collector Gatherers and Shredders (ANOVA *F*=5.468, P >0.05; *F*=0.774, P>0.05).

Discussion

Macroinvertebrate community composition was found to be related to environmental parameters supporting one of our original hypotheses. However, disturbed and undisturbed sites were not found to differ significantly across the majority of environmental variables nor did they differ significantly in terms of diversity, abundances or structural community composition. These contradict the remaining two of our original hypotheses regarding the impacts of deforestation on stream environments.

PHYSICAL CHARACTERISTICS

(a) Habitat Integrity Index

The Habitat Integrity Index was indeed significantly lower across disturbed sites than undisturbed sites. This indicates that sites selected for each category did in fact differ in overall environmental health. Further, it can be inferred that human impact from agriculturally induced deforestation is altering freshwater environments across a range of environmental attributes.

(b) Environmental Variables

The only physical traits that differed between our sampled undisturbed and disturbed streams were canopy cover and temperature. Other studies have found similar trends. For example, Benstead et al. (2003) and Bojsen and Jacobsen (2003) also found an increase in mean temperature in deforested sites attributed to canopy loss.

On the other hand, we did not find a significant difference in the other physical traits (dissolved oxygen, flow velocity, pH and stream width/depth) between our undisturbed and disturbed sites. This is in contrast to several other studies that report differences such as in dissolved oxygen, pH and depth differences between streams of differing levels of deforestation (Couciero et al., 2007; Bojsen & Jacobsen, 2003).

There are a few key reasons why only temperature and canopy differences were detected between disturbed and undisturbed streams. In this study, we were limited to select sites solely based on surrounding land characteristics (i.e. “forested” vs. “deforested, agricultural” habitats). We were able to select streams all within the same watershed to reduce some levels of natural variation however we were not able to select sites consistent in other physical traits knwon to be very important in stream ecology such as stream order, elevation, upstream length, amount of water flow and surface geology (Helson & Williams, 2013). For example, one of our disturbed streams, Disturbed 1, was narrow and consisted only of pool habitats (i.e. very limited to no water flow) whereas our other chosen disturbed streams, Quebrada Grande and Río Trinidad, were wider (Quebrada Grande was more than 3 times Disturbed 1) with a combination of pool and riffle habitats, as well as a large water flow. As these variables were unable to be controlled for, there is the potential they may have confounded the ability to detect disturbance driven changes in other abiotic properties.

This is further emphasized by the fact that a similar study was conducted in the Panama Canal watershed that was able to more strictly control for these potential confounding variables (Helson & Williams, 2013). They did in fact see a difference between forested and deforested regions across a set of abiotic variables including lower dissolved oxygen in deforested sites.

Another potential explanation for lack of a relationship between disturbed and undisturbed sites is that there are other environmental parameters that were not measured in this study that may have differed between categories of disturbance. Other studies have detected differences in turbidity, conductivity, nitrogen and phosphorous, discharge, and sediment inputs (Couciero, 2007; Larsen et al., 2009; Mesa, 2010).

RELATIONSHIPS BETWEEN ENVIRONMENTAL VARIABLES AND MACROINVERTEBRATE COMMUNITY COMPOSITION

Results from both the Spearman rank correlations and the CCA show that community composition is related to environmental parameters supporting the final original hypothesis. These results are consistent numerous other studies that have also found abiotic factors to significantly explain variation in macroinvertebrate communities (Bosjen & Jacobsen, 2003; Buss et al., 2003; Coucerio et al., 2007; Kasangaki et al., 2006; Kasangaki et al., 2008).

The Spearman rank correlations show that oxygen, stream width and depth, flow velocity, and pH were all found to correlate significantly with several orders. Only canopy cover and temperature appeared to have little relationship with order abundance.

Similarly, as 50.1% of the variance in community composition is accounted by the CCA, it can be inferred that the environmental gradient generated by measured variables explains a considerable amount of variation in taxonomic composition. However this still leaves half of the variation left unexplained and thus indicates that likely there are other factors not measured here explaining variation in composition as well. Our disturbed streams were found within regions impacted by deforestation due to agriculture. Other studies have found that in agriculturally impacted streams, other variables not measured here were important in explaining species compositions including nitrates, conductivity and land-use percentage such as was found by Mesa (2010) in a study comparing pristine forested areas with areas affected by agricultural land use. It would therefore be useful in future work to measure these variables to investigate whether they are responsible for the remaining variation in species composition and to see if sites impacted by agriculture here show similar patterns to elsewhere.

There was a high level of consistency between results of the correlations and CCA in terms of which environmental parameters are most important in explaining patterns in abundance of each order. Coleoptera, Hemiptera, Plecoptera, Ephemeroptera and Trichoptera all appear to be associated with wider, and shallower, faster flowing, higher pH, oxygenated waters. These parameters were found to explain large proportions of variation in taxonomic data in previous studies (Coucerio et al., 2007; Kasangaki et al., 2006; Kasangaki et al., 2008). Diptera appear to be associated with opposing environments that are deeper, narrower, slower flowing, lower oxygenated and intermediate depths, oxygen levels, and velocity. In both analyses, Collembola and Megaloptera were not found to be associated with any of the measured variables. There are three possible explanations for this. The first is that their distributions are not associated with abiotic conditions. The second is that there are other variables not measured in this study that are exerting greater influence over their abundance patterns. The latter is of greater likelihood given the number of variables that were unable to be measured and included and the high amount of unexplained variation in the CCA. Another key consideration is the rarity in which both of these orders were observed in the sites sampled. A pattern may be hard to discern with such small abundances.

Within each river, sites were scattered throughout the CCA ordination diagram however there was a general area within which most sites were found. Based on these dominant areas occupied by each river, relationships with other rivers may be assessed. Rivers within the same disturbance category were not necessarily found closely to one another in the CCA ordination diagram. Instead, an alternative grouping of rivers are suggested. Quebrada Grande, Quebrada Juan Grande, and Río Trinidad appear to be closely associated. Río Frijoles and Río Frijolito also appear grouped and Disturbed 1 appears to be distinct from all other 5 rivers but closest to Río Frijoles and Río Frijolito.

This indicates that rivers within the same disturbance category were not necessarily similar in terms of the set of environmental parameters found within each and suggests that it is not the most important grouping variable here. As such it lends strong support to the concern that rivers were not chosen with consistent sets of other stream parameters and thus there are confounding parameters preventing this categorization scheme from being effective. This further explains potentially why no significant differences were seen in physical traits between disturbed and undisturbed.

Furthermore, the fact that streams within the same disturbance categories were not necessarily grouped together indicates they are not similar in terms of community composition but rather, community composition is more similar between the alternative grouping scheme described above.

COMMUNITY STRUCTURE

(a) Diversity

Our results indicate that disturbance had no affect on macroinvertebrate diversity within stream communities in the sense of both taxonomic richness and evenness. This contrasts the findings of several other studies investigating the effects of deforestation and agriculture in tropical environments. Benstead et al. (2003) found a significant decrease in agriculture impacted streams compared to forested streams in Madagascar. Similarly, alpha diversity decreased in deforested sites of Ecuadorian streams (Bojsen & Jacobsen, 2003). Furthermore, a study in Costa Rica too showed reduced alpha diversity in pasture streams compared to forested, non-impacted streams (Lorion & Kennedy, 2009). Kasangaki et al. (2006) found higher values for Shannon diversity index in forested sites compared to sites surrounded by heavy logging and agriculture. In these studies, it appears that surrounding land use did have significant influence on macroinvertebrate diversity.

There are several reasons that could explain why we did not observe such patterns. First as was illustrated in physical trait comparisions and in the CCA ordination diagram, there are the physical differences among the streams we chose to group within the *disturbed* and *undisturbed* categories. Future studies should consider stream selection carefully, and aim at having as high as possible replicate similarity to test differences in diversity due to deforestation per se rather than due to other variables (obviously as much as this is possible). This relates to our second point: although we did measure different physical and chemical stream attributes, we were not able to consider some very important variables related to deforestation such as: sedimentation, nitrogen, phosphorous, organic matter content, turbidity and substrate type. Therefore, the macroinvertebrate community could have been responding to different factors that we were not able to quantify. In addition, there is the timing of our field sampling: we sampled our first three rivers (the undisturbed ones) at the end of January and beginnings of February (mid-dry season), whereas we sampled our disturbed streams during Mid-March and early April (ends of the dry season). Cummins et al. (2005) recommend that macroinvertebrate sampling should be done when the populations are at their latter stages of growth (either end of dry season or end of wet season) as this facilitates their sorting and identification as well as targets macroinvertebrates in their stage most closely linked to their feeding modes. This could also be confounding our results. Additionally, the fact that we did not identify any of our collected Ephemeroptera individuals at the family level represents a serious limitation of our study because this macroinvertebrate order constituted almost half of our total sample in terms of abundance (Kasangaki et al., 2008).

Furthermore, HII was not significantly correlated to any of the diversity indices. This indicates that environmental health is not related to macroinvertebrate diversity. Nessimian et al. (2008) found results consistent with this. They too found that the index grouped together sites that shared the same disturbance categorization yet did not find significant correlations with taxonomic richness. Few other studies have used the HII to look directly at its relationship with macroinvertebrate diversity. Silva et al. (2010) however studied diversity of Hymenoptera in human impacted and unimpacted streams in Brazil and did find a significant correlation between HII and species richness. The objective behind the habitat integrity index is to discern the biological condition of stream environments through an assessment of physical attributes of stream environments. This is based on the assumption that biological communities are coupled with the state of its physical environment (Petersen, 1992). The original Riparian, Channel, and Environmental (RCE) Inventory index was found to successfully meet this objective in that it was significantly correlated to Shannon Diversity Index in temperate streams. Furthermore, it has been applied in tropical environments and indicated similar patterns in that both taxonomic richness and Shannon index increased with increasing RCE scores (Buss et al., 2002). The results here and other studies reveal that it is not certain whether this modified index is an effective tool to assess the status of macroinvertebrate diversity within streams and requires further investigation. Yet it should be noted that this analysis too may have been influenced by the above impacts affecting comparison of diversity between streams explaining the lack of relationship.

(b) Macroinvertebrate Abundance

Similar to what Benstead et al. (2003) found, there was no difference in overall macroinvertebrate abundance between disturbed and undisturbed sites. Furthermore, we did not find significant differences in the relative abundance for most of the insect orders: we found that only the abundance of Collembola was greater in our undisturbed streams. This contrasts greatly what was hypothesized based on results of previous studies investigating the impacts of deforestation on macroinvertebrate community assemblages. Helson and Williams (2013) found that the relative abundances of Trichoptera, Plecoptera, Odonata and Coleoptera decreased with increased disturbance. Similarly, Benstead et al. (2003) found a negative correlation between deforestation and Trichocoptera, Plecoptera, and Diptera. In addition they found a positive correlation between Ephemeroptera abundance and deforestation. Kasangaki et al. (2008) in contrast observed a decrease in Ephemeroptera abundance between deforested and forested sites in Uganda while Trichoptera did not change.

At the level of family, again there was a lack of relationships seen between relative abundance in disturbed versus undisturbed sites with the exception of Staphilinidae, Gomphidae, Protoneuridae. Again this contrasts what was expected. Other studies have shown variation between in family abundances. For example, Kasangaki et al. (2008) found that forested and deforested sites differed in which families were found within the orders Trichoptera, Ephemeroptera, Plecoptera and Odonata.

There are a few potential explanations as to why no relationship was found in this study. It must be emphasized that we were unable to identify Ephemeroptera to the family level. As this represented almost half of the total abundance this could be greatly preventing any relationships in family compositions between disturbed and undisturbed sites to be seen. Some Diptera were also unable to be identified to the family level as well as all Collembola although because this order was extremely rare it is likely not of great importance. In addition, an important difficulty for studies of macroinvertebrates in the tropics is found in their classification: the majority of macroinvertebrate taxonomic guides are based on temperate regions. This made it difficult to increase the "resolution" of the study, for example getting down to genus and/or species level, and may have important repercussions in the conclusions being drawn. For example, Helson and Williams (2013) could not find a difference in richness between disturbed and undisturbed sites when they looked at Ephemeroptera families, but did find a decrease in richness with increased disturbance when Ephemeroptera were analyzed at the genus level.

However a final key explanation of a lack of relationship comes from the fact that macroinvertebrate assemblages were more similar between rivers of different disturbance categories than within each category itself as indicated above from the CCA. Abundance data appears to support this suggestion. Quebrada Juan Grande, despite being classified as an undisturbed river appeared to have similar relative abundances across all orders with two of the disturbed streams Quebrada Grande, and Río Trinidad with the exception of Megaloptera that was only found within Quebrada Juan Grande. Similarly, Disturbed 1 had relative abundances more similar to that of two undisturbed streams, Río Frijoles and Frijolito.

As described previously, no relationship may have been seen because the site selection did not control for confounding variables and thus they are exerting greater influence on community composition as opposed to disturbance. This was again supported by the ANOVAs that compared abundances of each order across all individual rivers. It showed that the factor of stream had a significant effect on abundances of all orders besides Coleoptera and Plecoptera. Therefore it can be suggested that disturbance category was not the main factor driving differences in community composition but rather other variables between streams.

Abundances of particular macroinvertebrate groups are often used to infer levels of disturbance as they have been classified as being more tolerant or intolerant to disturbances (Chakona et al., 2009). Trichoptera families are one such group. Here we see that disturbed streams contain Trichoptera of the families Hydropsychidae and Hydroptiliidae (classified as “moderate tolerant”), Philopotamidae (“low tolerant”) and Polycentropodidae (“high tolerance”) in similar proportions to undisturbed streams suggests the possibility that studied disturbed sites (Río Trinidad and Quebrada Grande) may be more resilient to disturbance.

(c) Functional Structure

Our results indicate that there were no significant differences in functional structure between disturbed and undisturbed communities.

There were two key limitations that need to be addressed. First, the order Ephemeroptera has families that belong to three different functional groups: scrapers, collector gatherers and collector-filterers; as we could not quantify this for our study we acknowledge that the conclusions we will be able to draw from our results will be influenced by this important fact.

Secondly, we classified macroinvertebrates into functional groups based on the family classification for temperate regions. This brings up two further concerns. First, recent work has suggested that tropical macroinvertebrates do not always share the same functional classification as their temperate counterparts (Tomanova et al., 2006). Second, macroinvertebrates in tropical regions have been found to show flexibility in their food acquisition modes; this is reasonable as we would expect generalist-feeding strategies to be beneficial in the unpredictable resource availability conditions found in the tropics (Tomanova et al., 2006). However, this “plasticity” complicates their classification into a single functional feeding group. For example, in their study in Bolivia, Tomanova et al. (2006) found that omnivory was common among most taxa, and that they could be classified into more than one functional group. They have suggested that functional group identification should combine both mouthpart and gut content analysis strategies to address this issue.

Despite these limitations, we were still able to observe certain patterns when streams were compared at the individual level.

Although we did not measure leaf litter directly, there is the potential that the decrease in canopy cover found in our disturbed streams caused a reduction in leaf litter, as other studies have observed (Bojsen & Jacobsen, 2003). This could explain why shredders decreased in Quebrada Grande and Río Trinidad, although not why they were so underrepresented in Río Frijoles and Frijolito regardless of a high canopy cover. Shredder macroinvertebrates in our samples were represented by individuals from the following groups: Coleoptera (mostly Elmidae), Trichoptera and Plecoptera. Trichoptera shredders are usually found in running waters (Bouchard, 2004) thus the pool habitats found in Río Frijoles and Frijolito (as well as in Disturbed 1) could be partially responsible for their absence despite high canopy cover. These results are consistent with other studies that have found a decrease in shredder abundance with increased disturbance (Helson & Williams, 2013). This has been explained in part because as more specialized feeders shredders become more sensitive to human alterations (Helson & Williams, 2013).

In addition to its impact on organic inputs into streams, deforestation around stream environments has been found to allow higher amounts of light penetration, which can enhance algae and perphyton growth (Bosjen & Jacobsen, 2003). This would benefit scraper individuals. Indeed, two disturbed streams, Quebrada Grande and Río Trinidad showed higher scraper abundance in comparison to undisturbed sites (although statistically insignificant). Other studies have shown a positive correlation between algae and grazers abundance due to increased light levels (Wallace & Webster, 1996), but algae can also increase due to the addition of nutrients from the run-off of agricultural sites. On the other hand, streams in deforested sites have been found to contain higher sediment quantities and greater water turbidity (Murphy et al., 2012), thus algae growth would not necessarily be as high as predicted from an increase in light and nutrients. For future studies, it would be useful to quantify periphyton abundance to further test the validity of these correlations in these habitats. Additionally, scrapers were totally absent from Disturbed 1. It has been suggested that their feeding specialization also makes them more sensitive to disturbance, and different studies have observed a decrease in scraper abundance following a human impact gradient (Helson & Williams, 2013; Tomanova et al., 2008).

Furthermore, the supply of nutrients into the stream system from agricultural sites (organic pollution) as well as a decrease in canopy cover can increase the abundance of collector individuals (both filterers and gatherers) although this will depend on the input ratio of organic matter to inorganic matter (Murphy et al., 2012). In our study, we found that collector gatherers had the highest relative abundances in Río Frijoles and Frijolito and were comparatively quite low within all other rivers. This might be related to the fact we did not include Ephemeroptera in our functional group classifications. In disturbed streams, we would expect taxa that can take advantage of in situ primary production to increase in abundance, like some Ephemeroptera generalist collector gatherers (families Baetidae and Leptohyphidae) and large omnivorous collectors like certain Trichoptera families (Benstead et al., 2003). However, we did observe that collector-filterers were mostly present in Quebrada Grande and Río Trinidad (although in Quebrada Juan Grande as well), therefore this is likely due to the fact these were streams that had riffles. Filter feeders remove FPOM from suspension and supply larger particles via their feces to deposit-feeders. They exploit habitats with high degrees of particle transport, therefore they have high abundances in running-water environments (Wallace & Webster, 1996).

Furthermore, disturbance has been shown to alter not just overall differences in functional structure of communities between disturbed and undisturbed environments but also to diversity within functional groups (Couceiro et al., 2011). Here Río Frijoles and Río Frijolito appear similar to Disturbed 1 in functional structure (i.e. containing mostly predators and collector gatherers). However, by looking at the taxonomic composition of the functional groups we can see that the two undisturbed rivers have a wider variety of taxa corresponding to the same functional group. For example, chironomids, odonates, coleopterans, plecopterans and tipulidaes may function as predators in the undisturbed sites, whereas Disturbed 1 predators are mainly represented by chaeoboridaes and chironomids. Therefore this study too suggests that disturbance may reduce functional diversity.

Stemming from the discussion of altered functional structure due to anthropogenic impacts comes a gap in the literature. Little work has investigated what further consequences these changes in communities may be having on the system. Because different groups of macroinvertebrate functional groups contribute differentially to ecosystem processes, changes in its composition have been suggested to influence the relative levels in which these different functions continue to occur which in turn could have trophic cascade effects throughout the system (Chapin III et al., 2000). In other words, human impacts may establish a chain reaction of ecological alterations: disturbances modify the environment which alters community structure leading to further changes in the environment that continue to affect other components of communities throughout the system. One study has demonstrated this within stream communities whereby terrestrial litter inputs were excluded from stream sites (Baer et al., 2001). This lead to changes in the relative abundance of different functional groups as well as an overall decline in macroinvertebrate levels. A reduction in secondary production, altered detrital processing, and composition of organic matter were observed which in turn affected subsequent macroinvertebrate colonization rates. Another study investigating the effects of disease driven amphibian declines in tropical stream ecological processing also shed light on this concept from the perspective of another group of organisms (Whiles et al., 2013). Tadpoles are grazers predominantly and by impinging an impact that reduced this functional group, strong changes within the environment were witnessed with great increases in algae and fine detritus biomass, and altered nitrogen cycling. Thus from this it is strongly illustrated how impinging impacts that alter functional structure can feedback and alter the environment further. Although this study did not show significant differences in community structure due to disturbance, the limitations of our analysis as well as the strong consensus from other referenced studies implies that such changes may in fact still be at play and as such it would be beneficial to follow up on this concept further in these environments in order to understand the full circle of impacts associated with human activities.

Finally, the discussion here was limited to relationships between functional groups and disturbance levels and not with that of environmental parameters. Although it has been shown that environmental parameters are important in the functional structuring communities (Couceiro et al., 2011; Benstead et al., 2004), and as with the taxonomic community composition may be more important in our study than disturbance category, we concluded the lack of data from Ephemeroptera would impair our ability to draw conclusions from our results.

IMPLICATIONS AND CONCLUSION

There did not appear to be a strong relationship between deforestation and both abiotic and biotic variables considered in this study within stream environments. From this it appears that other variables are more important in determining community composition than surrounding catchment land use. However, caution should be taken in concluding that human impacts are irrelevant in determining the state of these freshwater environments. This can be explained by returning to the concept of biological integrity.

The integrity of an environment is given in reference to what its state would be under undisturbed conditions. The undisturbed sites in this case however cannot be used as reference sites for this comparison because of the noted differences in other natural stream habitat parameters. Therefore we were unable to address one question of the study in that we were unable to fully assess the states of some freshwater habitats are within Panama, particularly those in heavily deforested regions. The HII however, was still able to provide information regarding this question. As significantly lower values for disturbed sites were found, it indicates that the health of these agriculturally impacted sites may be jeopardized. We were also unable to provide a response to another key question as to how freshwater stream communities respond to disturbances. Yet we were able to show that within these habitats, environmental variables are strong predictors of community composition and this can be put into great use in the future. If changes are seen within stream environments in the future from either human or other causes, it can be expected that a change in biotic communities will likely result and efforts should thus be put in place to mitigate these potential impacts. Furthermore we were able to elucidate some variables that were particularly important in determining community composition. Thus it may be suggested that these could be the focus of monitoring activities in order to detect changes before their impacts are able to take effect. In this regard, we were able to accomplish our third goal to some degree.

There is room for a great deal of future work to illuminate our questions here further. First, studies in the future should look at disturbance in comparison to reference sites more reflective of the disturbed sites natural unimpacted condition in order to investigate more rigorously the effect of human impacts on biological integrity.

Furthermore, only macroinvertebrates were investigated here however there are several other components of the system that would be of great interest to study in order to understand the full range of impacts disturbance may be having on communities. Finally, there remains several environmental variables not measured here. It would be valuable to measure a greater range of variables in the future to gain better understanding of how environments shape community compositions.

This study has contributed to a better understanding of freshwater community dynamics within Panama that can be expanded on in the future so that we may learn how best to promote the biological integrity of these essential habitats.

Limitations and Challenges

The first and most limiting aspect to our internship was time. Firstly, we were unable to sample all of the rivers we would have liked due to time constraints on what was feasible. We would have liked to sample an additional three streams in the Bayano region where the agricultural frontier is pushing eastward but were unable to do so as we did not have enough days consecutively earlier enough in the internship to be able to go on the trip. Furthermore, a lack of time rendered us unable to finish classifying groups of organisms (most notably Ephemeroptera and Diptera). Taxonomic classification was the most time consuming part of our internship and with access to only one microscope, there was not enough time to classify these taxonomically diverse groups to the level of Family. Our next greatest limitation was limited access to lab equipment. Limited time combined with limited access to analysis equipment (e.g. microscopes) made processing of samples move quite slowly. This meant the lab equipment we did have access to was continuously under use. Furthermore, we were limited in the physical parameters we were able to measure at the study sites due to a lack of equipment. Of the equipment we did have, the absorbance spectrophotometer stopped working halfway through our sampling and thus were unable to use turbidity as a proxy for sedimentation. Also, we were unable to obtain standards to calibrate our salinity measure from the beginning. This reduced the number of environmental parameters that we were able to measure in the field at our different sites. Additionally, the task of classifying organisms taxonomically based on morphology was quite a challenging task. We were unable to classify organisms further than the level of Family and in some cases Order due to limited knowledge regarding taxonomic identification.

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Appendix I: Chronogram of Activities

Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**	**Sunday**
		01-Jan	02-Jan	03-Jan	04-Jan	05-Jan
06-Jan	07-Jan	08-Jan	09-Jan Meet with Luis/Literature Review	10-Jan Literature Review	11-Jan	12-Jan
13-Jan	14-Jan	15-Jan	16-Jan Equipment Testing/Field Prep	17-Jan Quebrada Juan Grande Practice Sampling	18-Jan Literature Review	19-Jan Literature Review
20-Jan	21-Jan	22-Jan	23-Jan	24-Jan	25-Jan	26-Jan
27-Jan	28-Jan	29-Jan	30-Jan Practice Identification	31-Jan Practice Identification/Meeting with Celestino	01-Feb Quebrada Juan Grande Sampling	02-Feb Quebrada Juan Grande Sampling
03-Feb Quebrada Frijoles Sampling	04-Feb Quebrada Frijoles Sampling	05-Feb Quebrada Frijolito Sampling	06-Feb Quebrada Frijolito Sampling	07-Feb Order Identification	08-Feb Order Identification	09-Feb Order Identification
10-Feb Order Identification	11-Feb Order Identification	12-Feb	13-Feb	14-Feb	15-Feb	16-Feb Order Identification
17-Feb Order Identification	18-Feb Order Identification	19-Feb	20-Feb	21-Feb	22-Feb	23-Feb
24-Feb	25-Feb	26-Feb	27-Feb Informal Presentation	28-Feb Order Identification	01-Mar Order Identification	02-Mar
03-Mar	04-Mar	05-Mar	06-Mar	07-Mar	08-Mar	09-Mar
10-Mar	11-Mar	12-Mar	13-Mar	14-Mar	15-Mar	16-Mar
17-Mar	18-Mar	19-Mar	20-Mar Disturbed 1 Sampling	21-Mar Order Identification	22-Mar Order Identification	23-Mar Quebrada Trinidad Sampling
24-Mar	25-Mar	26-Mar	27-Mar Order Identification	28-Mar Order Identification	29-Mar Order Identification	30-Mar Order Identification
31-Mar Order Identification	01-Apr Family Identification	02-Apr Family Identification	03-Apr Family Identification	04-Apr Family Identification	05-Apr Family Identification	06-Apr Quebrada Grande Sampling
07-Apr Family Identification	08-Apr Family Identification	09-Apr Family Identification	10-Apr Family Identification	11-Apr Family Identification	12-Apr	13-Apr
14-Apr	15-Apr	16-Apr	17-Apr	18-Apr Data Analysis	19-Apr Data Analysis	20-Apr Final Report Write Up
21-Apr Final Report Write Up	22-Apr Final Report Write Up	23-Apr Final Report Write Up	24-Apr Final Report Write Up	25-Apr Internship Symposium		

Appendix II: Agriculture Concept Map

Box 1. Agriculture Concept Map

Agriculture as a driver initiates changes in the surrounding environment which impacts aquatic systems. Firstly, with agriculture, deforestation takes place to make space for crop production. Loss of tree cover in turn leads to increased soil erosion and thus increased sedimentation into water systems. With increased sedimentation, light penetration into water is decreased although a lack of tree does increase the light able to hit the water surface. As a result, ground temperature is also increased. Both light and temperature increase algal growth which allows scrapers to increase with increased food supply. With deforestation there is also a depletion in the allochthonous food supply to water systems. This decreases the shredders who are able to convert CPOM to FPOM which in turn affects the collector gatherers and collector filterers. Furthermore, with the use of pesticides and fertilizers, toxins and nutrients are both added to waterways through runoff. Nutrients can stimulate algal growth which in turn stimulates scrapers while toxins will negatively impact all function groups.

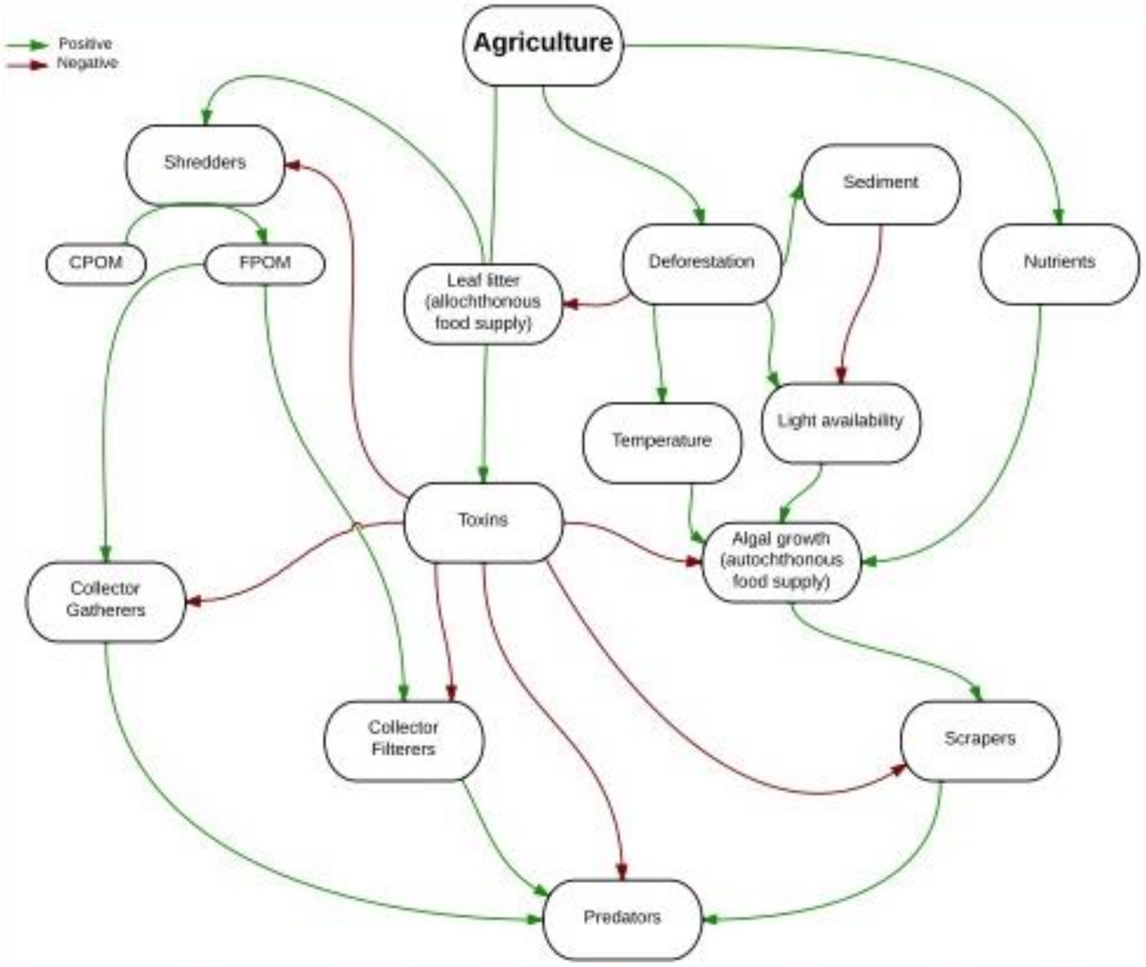
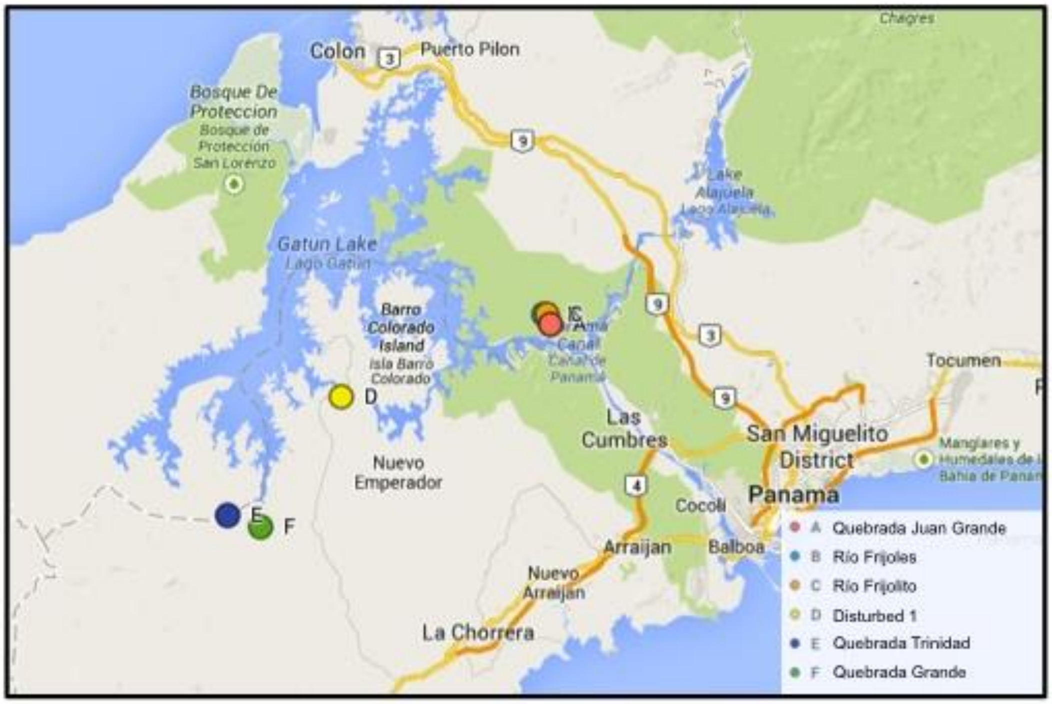


Figure 1. Concept map of agriculture as a driver and the associated changes it can have on aquatic ecosystem stocks.

Appendix III: Maps of Study Sites



Appendix IV: Habitat Integrity Index Table

Table 2 Habitat characteristics used in evaluation of sampling sites for HII calculations

	Characteristic	Condition	Score
F1	Land use pattern beyond the riparian zone	Primary continue forest/100 ha fragment/10 ha fragment	6
		Cecropia secondary forest/mixed secondary forest	5
		Vismia secondary forest	4
		Pasture	3
		Perennial crops	2
		Short-cycle crops/exposed soil	1
F2	Width of riparian forest	Continuous forest	6
		Forest width between 30 and 100 m	5
		Forest width between 5 and 30 m	4
		Forest width between 1 and 5 m	3
		Riparian forest absent, but some shrub species and pioneer trees	2
		Riparian forest and shrub vegetation absent	1
F3	Completeness of riparian forest	Riparian forest intact without breaks in vegetation	4
		Breaks occurring at intervals of >50 m	3
		Breaks frequent with gullies and scars at every 50 m	2
		Deeply scarred with gullies all along its length	1
F4	Vegetation of riparian zone within 10 m of channel	More than 90% plant density by non-pioneer trees or shrubs	4
		Mixed pioneer species and mature trees	3
		Mixed grasses and sparse pioneer trees and shrubs	2
		Grasses and few tree shrubs	1
F5	Retention devices	Channel with rocks and/or old logs firmly set in place	4
		Rocks and/or logs present but backfilled with sediment	3
		Retention devices loose, moving with floods	2
		Channel of loose sandy silt, few channel obstructions	1
F6	Channel sediments	Little or no channel enlargement resulting from sediment accumulation	4
		Some gravel bars of coarse stones and little silt	3
		Sediment bars of rocks, sand and silt common	2
		Channel divided into braids or stream channel corrected	1
F7	Bank structure	Banks inconspicuous	5
		Banks stable, with rock and soil held firmly by grasses, shrubs, or tree roots	4
		Banks firm but loosely held by grasses and shrubs	3
		Banks of loose soil held by a sparse layer of grass and shrubs	2
		Banks unstable, easily disturbed, with loose soil or sand	1
F8	Bank undercutting	Little, not evident or restricted to areas with tree root support	4
		Cutting only on curves and at constrictions	3
		Cutting frequent, undercutting of banks and roots	2
		Severe cutting along channel, banks falling in	1
F9	Stream bottom	Stone bottom of several sizes packed together, interstices obvious	4
		Stone bottom easily moved, with little silt	3
		Bottom of silt, gravel, and sand, stable in some places	2
		Uniform bottom of sand and silt loosely held together, stony substrate absent	1

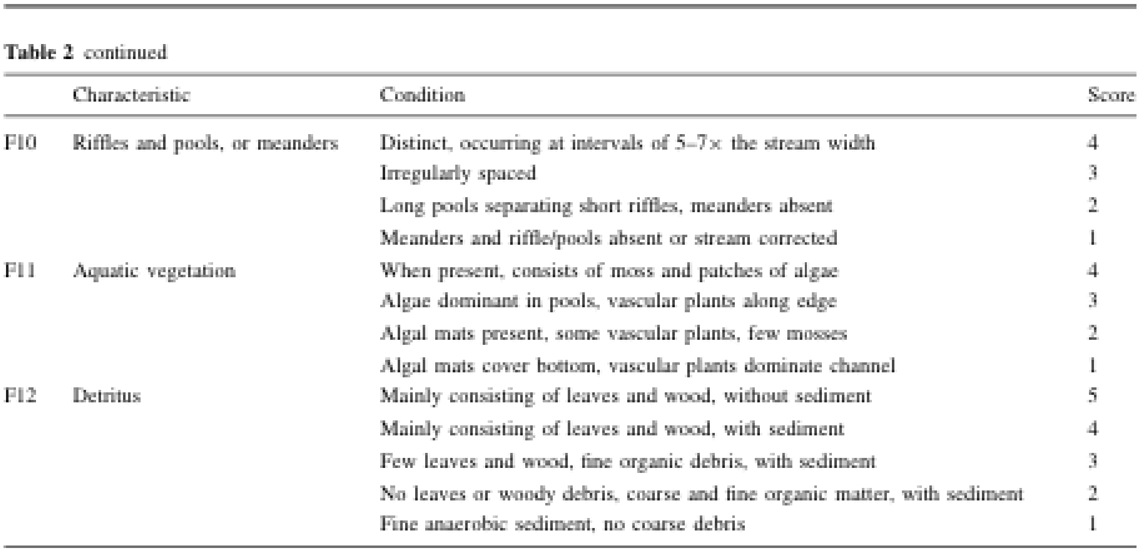


Table 2 continued

	Characteristic	Condition	Score
F10	Riffles and pools, or meanders	Distinct, occurring at intervals of 5–7× the stream width	4
		Irregularly spaced	3
		Long pools separating short riffles, meanders absent	2
		Meanders and riffle/pools absent or stream corrected	1
F11	Aquatic vegetation	When present, consists of moss and patches of algae	4
		Algae dominant in pools, vascular plants along edge	3
		Algal mats present, some vascular plants, few mosses	2
		Algal mats cover bottom, vascular plants dominate channel	1
F12	Detritus	Mainly consisting of leaves and wood, without sediment	5
		Mainly consisting of leaves and wood, with sediment	4
		Few leaves and wood, fine organic debris, with sediment	3
		No leaves or woody debris, coarse and fine organic matter, with sediment	2
		Fine anaerobic sediment, no coarse debris	1

Table taken from Nessimian et al. (2008)

Appendix V: Raw data for rivers sampled

Sum of Count		River						
Order	Family	Disturbed 1	Q. Grande	Q. Juan Grande	Río Frijoles	Río Frijolito	Río Trinidad	Grand Total
Coleoptera	Curculionidae					1		1
	Dytiscidae	6	1		5	4		16
	Elmidae		35	89	6	3	13	146
	Heteroceridae				1			1
	Hydrophilidae					1		1
	Hysteridae		1					1
	Psphenidae		11	2			39	52
	Ptiliidae						1	1
	Scirtidae	5			2	2		9
	Sphaeriusidae				2	1		3
	Staphylinidae			1	2	1		4
	Unknown				1	2		3
Coleoptera Total		11	48	92	19	15	53	238
Collembola	(blank)			6	1	2	1	10
Collembola Total				6	1	2	1	10
Diptera	Chaeboridae	261	8				2	271
	Chironomidae	83	48	27	113	125	67	463
	Culicidae	1		9		2		12
	Dolichopodidae					2		2
	Simuliidae					1		1
	Tipulidae		5	16		5	7	33
	Unknown	3	8	23	1	8	21	64
Diptera Total		348	69	75	114	143	97	846
Ephemeroptera	(blank)	6	283	348	98	130	645	1510
Ephemeroptera Total		6	283	348	98	130	645	1510
Hemiptera	Gelastocoridae		6	9	1		21	37
	Naucoridae	1	33	81	2	1	56	174
	Nepidae				1			1
	Scirtidae						1	1
Hemiptera Total		1	39	90	4	1	78	213
Megaloptera	Corydalidae			8				8
Megaloptera Total				8				8
Odonata	Aeshnidae			14				14
	Calopterygidae			3				3
	Coenagrionidae		26	2	19	3	35	85
	Corduliidae	3	6	1	12	2	3	27
	Gomphidae		1	45	12	33	7	98
	Lestidae				3	11		14
	Libellulidae	1	4	5	42	5	11	68
	Macromiidae		1					1

	Platystictidae		11	21			4	36
	Protoneuridae		1	10	3	9		23
	Unknown			1	1		20	22
Odonata Total		4	50	102	92	63	80	391
Plecoptera	Peltoperlidae		5					5
	Perlidae		3		2	1	28	34
	Pteronarcyidae			18				18
Plecoptera Total			8	18	2	1	28	57
Trichoptera	Calamoceratidae						1	1
	Calamocertidae		1					1
	Hydrophilidae						1	1
	Hydropsychidae		15	29	1	1	47	93
	Hydroptilidae		2	3			1	6
	Lepidostomatidae		3					3
	Leptoceridae		1	5			2	8
	Odontoceridae		2	1			1	4
	Philopotamidae		28	67	1	7	63	166
	Phryganeidae			3				3
	Polycentropodidae	1	3	4		3	8	19
	Psychomyidae		1				10	11
	Rhyacophilidae						1	1
	Unknown		3				33	36
Trichoptera Total		1	59	112	2	11	168	353
Grand Total		371	556	851	332	366	1150	3626

Appendix VI: Figures and Tables - Results

Physical Parameter	Disturbed	Undisturbed	*t*	p-value
Temperature (°C)	28.01	24.06	11.8472	0.0003*
Oxygen (mg/L)	5.69	3.54	0.9117	0.4135
Flow Velocity (m/s)	0.29	.08	1.3714	0.2422
% Canopy Cover	66.51	88.01	-3.829	0.0474*
pH	7.52	7.33	0.6675	0.541
Width (cm)	788.9	283.07	-1.312	0.2597
Depth (cm)	26.72	18.803	0.8639	0.4364
HII	0.7327315	0.9270833	7.1845	0.002*

Table 1. Comparison of disturbed and undisturbed mean physical parameter values. **p*-value significant at 0.05.

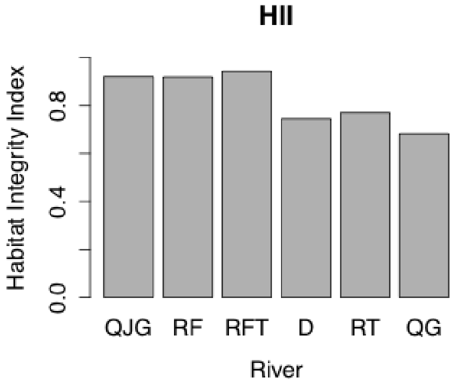


Figure 2. Habitat Integrity Index values for each of the six rivers sampled. River codes are as follows Quebrada Juan Grande = QJG, Rio Frijoles = RF, Rio Frijolito = RFT, Disturbed 1 = Disturbed, Rio Trinidad = RT, Quebrada Grande = QG

Taxonomic Level	Diversity Index	*R*
Order	Shannon	-0.0286
	Simpson’s	0.3714
	Richness	0.7590
Family	Shannon	-0.2571
	Simpson’s	-0.1429
	Richness	-0.0304

Table 2. Spearman rank correlation coefficients (*R*) between Habitat Integrity Index and diversity indices.

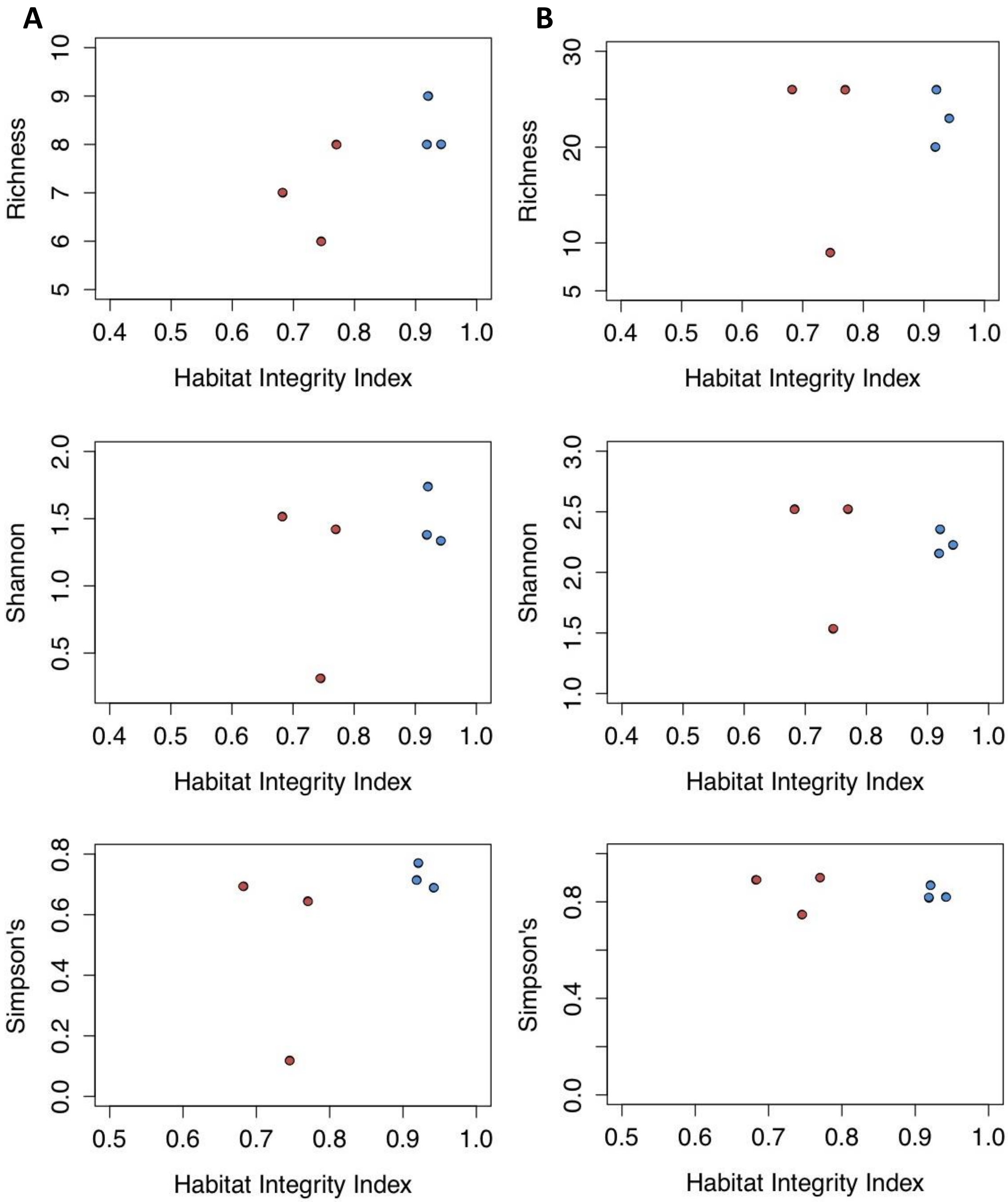
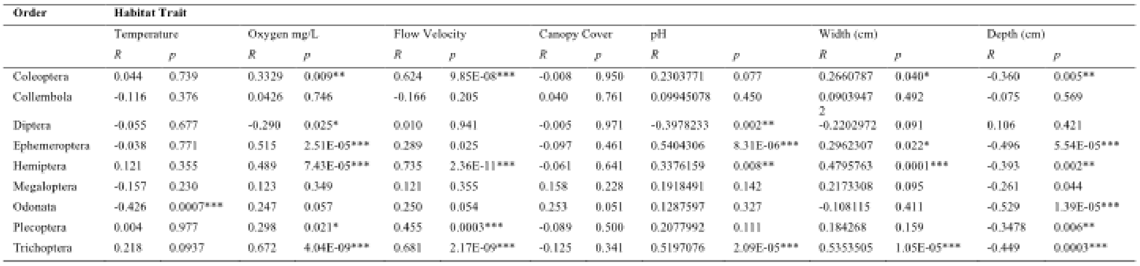


Figure 3. Relationship between habitat integrity index and diversity indices in the 6 sampled rivers. (A) at the level of order and (B) at the level of family. Red points indicate values for disturbed streams, blue points indicate values for undisturbed streams



Order	Habitat Trait													
	Temperature		Oxygen mg/L		Flow Velocity		Canopy Cover		pH		Width (cm)		Depth (cm)	
	R	*p*	*R*	*p*	*R*	*p*	*R*	*p*	*R*	*p*	*R*	*p*	*R*	*p*
Coleoptera	0.044	0.739	0.3329	0.009**	0.624	9.85E-08***	-0.008	0.950	0.2303771	0.077	0.2660787	0.040*	-0.360	0.005**
Collembola	-0.116	0.376	0.0426	0.746	-0.166	0.205	0.040	0.761	0.09945078	0.450	0.09039472	0.492	-0.075	0.569
Diptera	-0.055	0.677	-0.290	0.025*	0.010	0.941	-0.005	0.971	-0.3978233	0.002**	-0.2202972	0.091	0.106	0.421
Ephemeroptera	-0.038	0.771	0.515	2.51E-05***	0.289	0.025	-0.097	0.461	0.5404306	8.31E-06***	0.2962307	0.022*	-0.496	5.54E-05***
Hemiptera	0.121	0.355	0.489	7.43E-05***	0.735	2.36E-11***	-0.061	0.641	0.3376159	0.008**	0.4795763	0.0001***	-0.393	0.002**
Megaloptera	-0.157	0.230	0.123	0.349	0.121	0.355	0.158	0.228	0.1918491	0.142	0.2173308	0.095	-0.261	0.044
Odonata	-0.426	0.0007***	0.247	0.057	0.250	0.054	0.253	0.051	0.1287597	0.327	-0.108115	0.411	-0.529	1.39E-05***
Plecoptera	0.004	0.977	0.298	0.021*	0.455	0.0003***	-0.089	0.500	0.2077992	0.111	0.184268	0.159	-0.3478	0.006**
Trichoptera	0.218	0.0937	0.672	4.04E-09***	0.681	2.17E-09***	-0.125	0.341	0.5197076	2.09E-05***	0.5353505	1.05E-05***	-0.449	0.0003***

Table 3: Spearman rank correlation coefficients (R) between environmental parameters and abundance of each order at 60 sampled sites. *$p<0.05$, **$p<0.01$, ***$p<0.001$

Order	Spearman Rank Correlation		CCA	
	Positive	Negative	Positive	Negative
Coleoptera	Oxygen, Flow Velocity, Width	Depth	Width*, pH	
Collembola				
Diptera		Oxygen, pH	Depth*	Oxygen, Flow velocity, Width, pH
Ephemeroptera	Oxygen, pH, Width	Depth	Oxygen, Flow Velocity	Depth
Hemiptera	Oxygen, Flow Velocity, pH, Width	Depth	Oxygen, flow velocity, pH*, Width*	Depth
Megaloptera			Width	
Odonata		Depth	Width	Oxygen, Flow velocity
Plecoptera	Flow Velocity	Depth	Flow velocity Width*, pH*, Oxygen	Depth
Trichoptera	Oxygen, Flow Velocity, pH, Width	Depth	Oxygen, Flow Velocity, pH, Width*	Depth

Table 4: Summary of environmental parameters associated with each order as described by Spearman Rank Correlations and CCA ordination diagram. Reported variables from correlations were those found to be significant. Reported variables from the CCA are all those in which taxa points did not fall within the region perpendicular to them. * indicate the taxa point was located in close proximity to the vector.

	Intraset Correlation	
Environmental Parameter	Axis 1	Axis 2
Temperature	0.08017	0.388668
Oxygen	0.83762	0.205061
Flow.Velocity	0.72922	0.173518
Canopy.Cover	-0.2402	-0.773998
pH	0.66607	-0.321698
Width	0.41861	-0.49487
Depth	-0.66012	0.007322

Table 5: CCA results showing the intraset correlation of axis 1 and 2 to each environmental parameter

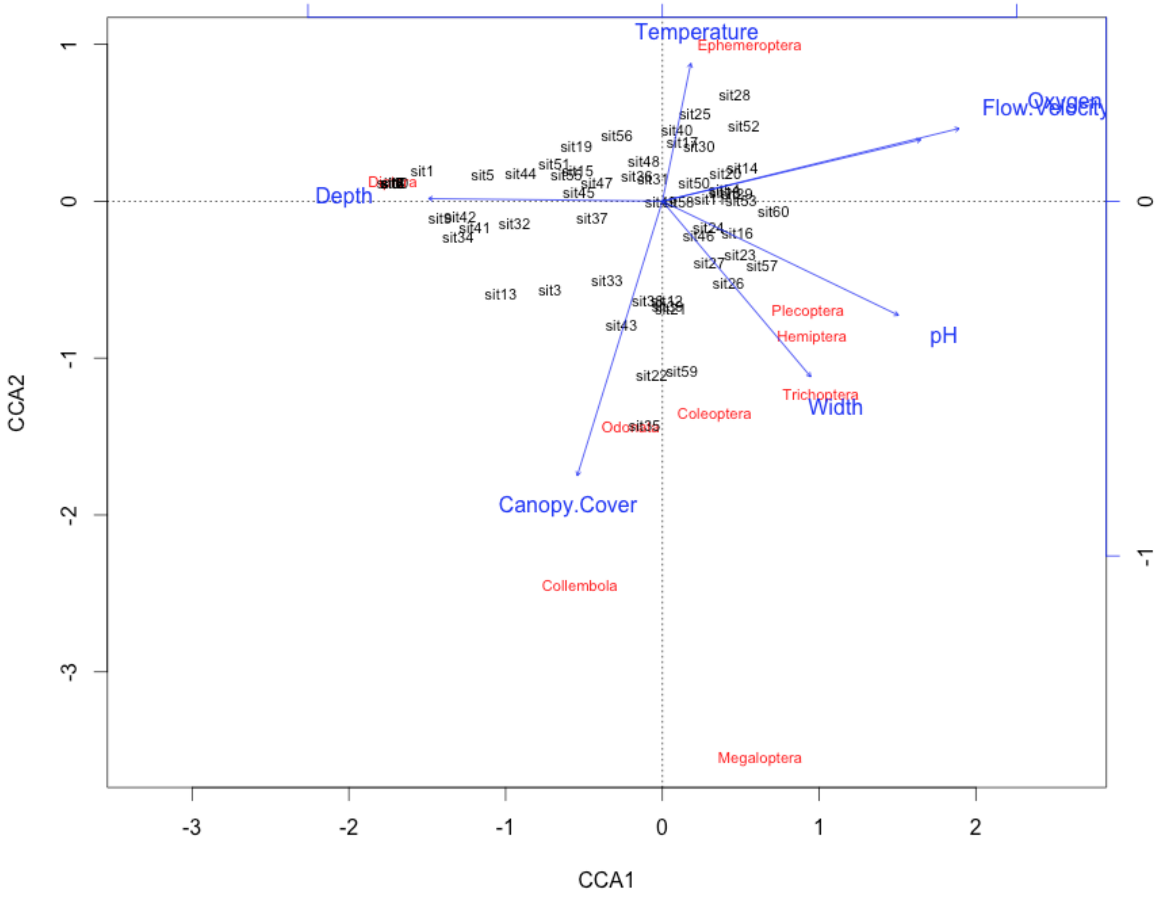


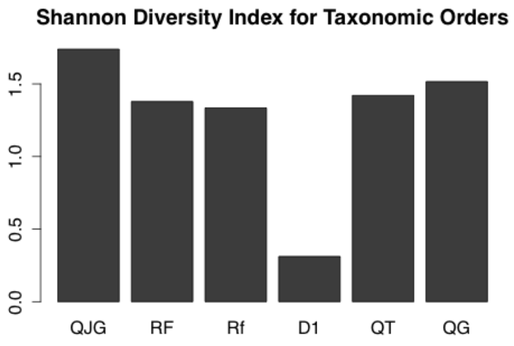
Figure 4: CCA ordination diagram with sites (black), insect orders (red), and environmental variables (blue arrows). Axis one is the horizontal and axis two is the vertical. Values of environmental variables increase in the direction of the arrow head and can be extrapolated back on the opposite side of the origin to represent a decreasing trend. The relative lengths of the arrows indicate their importance in explaining the variation in community composition shown in the diagram

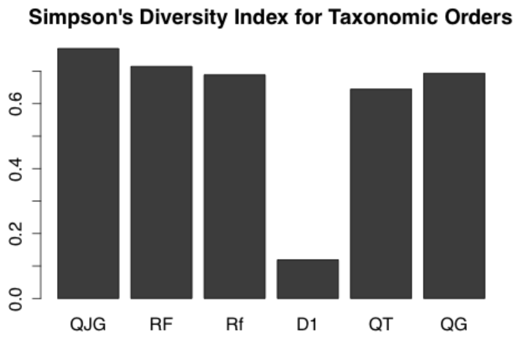
		Undisturbed			Disturbed		
		Juan Grande	Frijoles	Frijolito	Disturbed 1	Trinidad	Grande
Shannon	Order	1.7394	1.3789	1.3347	0.3118	1.4203	1.5162
	Family	2.3543	2.1563	2.2256	1.5336	2.5203	2.5224
	Odonata	1.5662	1.4432	1.4767	0.5623	1.2901	1.3644
	Plecoptera	0	0	0	0	0	0.6616
	Trichoptera	1.1302	0.6931	0.86	0	1.3273	1.4667
Simpson's	Order	0.7699	0.7147	0.6889	0.1189	0.6448	0.6936
	Family	0.8694	0.8161	0.8204	0.7474	0.9004	0.8912
	Odonata	0.7254	0.7064	0.7268	0.375	0.6436	0.6592
	Plecoptera	0	0	0	0	0	0.4688
	Trichoptera	0.5631	0.5	0.5124	0	0.6515	0.669
Richness	Order	9	8	8	6	8	7
	Family	26	20	23	9	26	26

Table 6. Diversity indices at the level of order and family as well as within Odonata, Plecoptera, and Trichoptera families at each stream as indicated by alpha diversity measures.

		Disturbed	Undisturbed	*t*	*p*-value
Shannon	Order	1.082767	1.484333	0.9862	0.3799
	Family	2.2454	2.1921	0.1594	0.8811
Simpson's	Order	0.7245000	0.4857667	1.2868	0.2676
	Family	0.8353000	0.8463333	-0.2105	0.8435
Richness	Order	8.333333	7.000000	2	0.1161
	Family	23.00000	20.33333	0.45	0.676

Table 7. Diversity and richness values for disturbed and undisturbed sites.





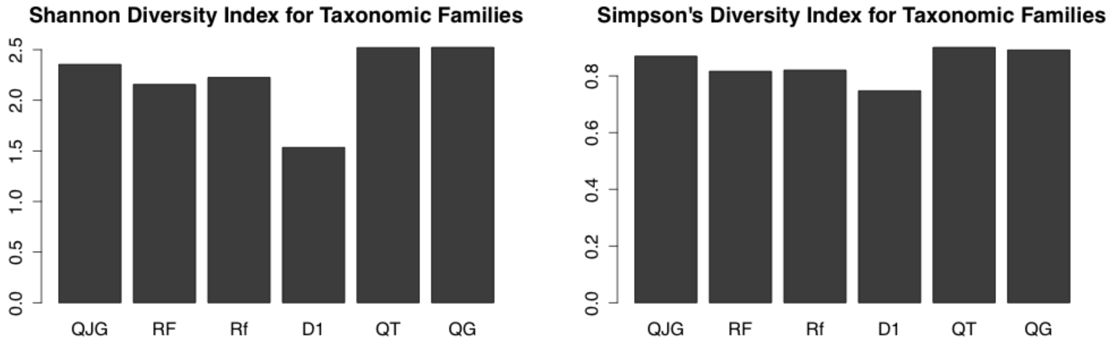


Figure 6. Values of diversity indices at the order and family level for each stream as indicated by alpha diversity measures. River codes are as follows Quebrada Juan Grande = QJG, Rio Frijoles = RF, Rio Frijolito = Rf, Disturbed 1 = D1, Rio Trinidad = QT, Quebrada Grande = QG

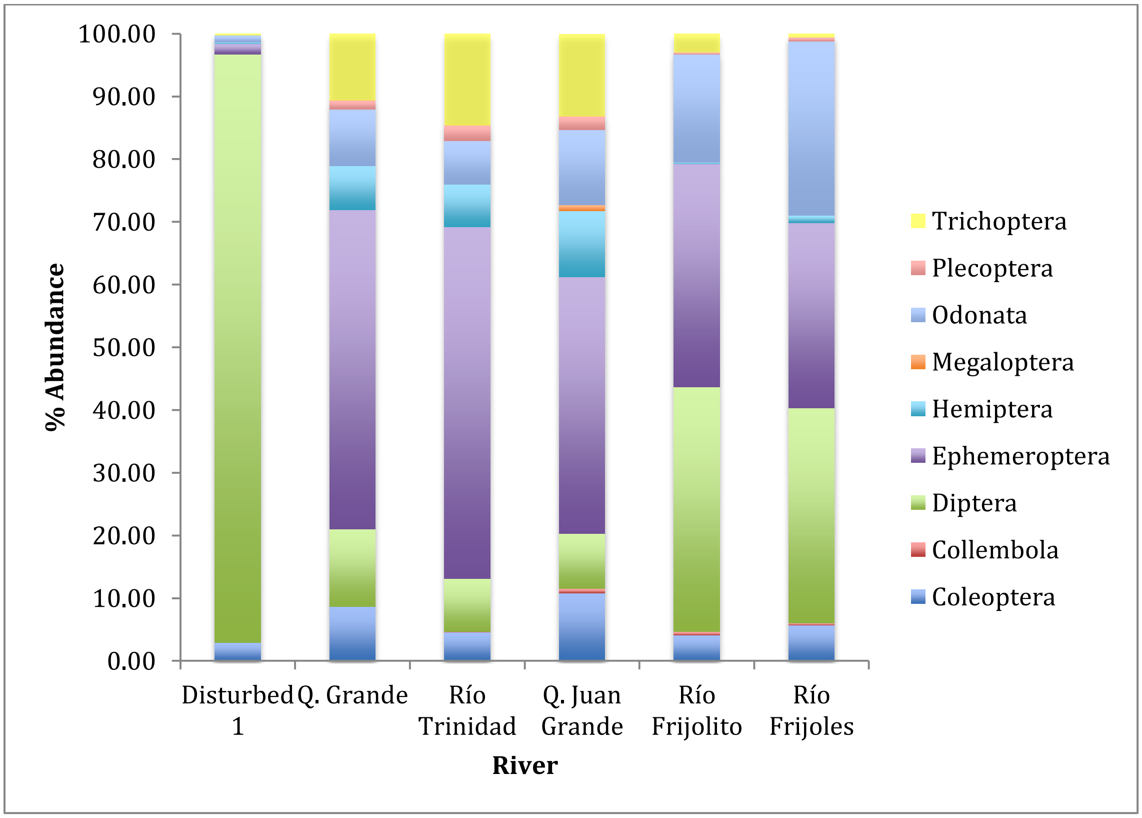


Figure 7. Relative abundances (%) of nine macroinvertebrate orders across our 6 sampled rivers.

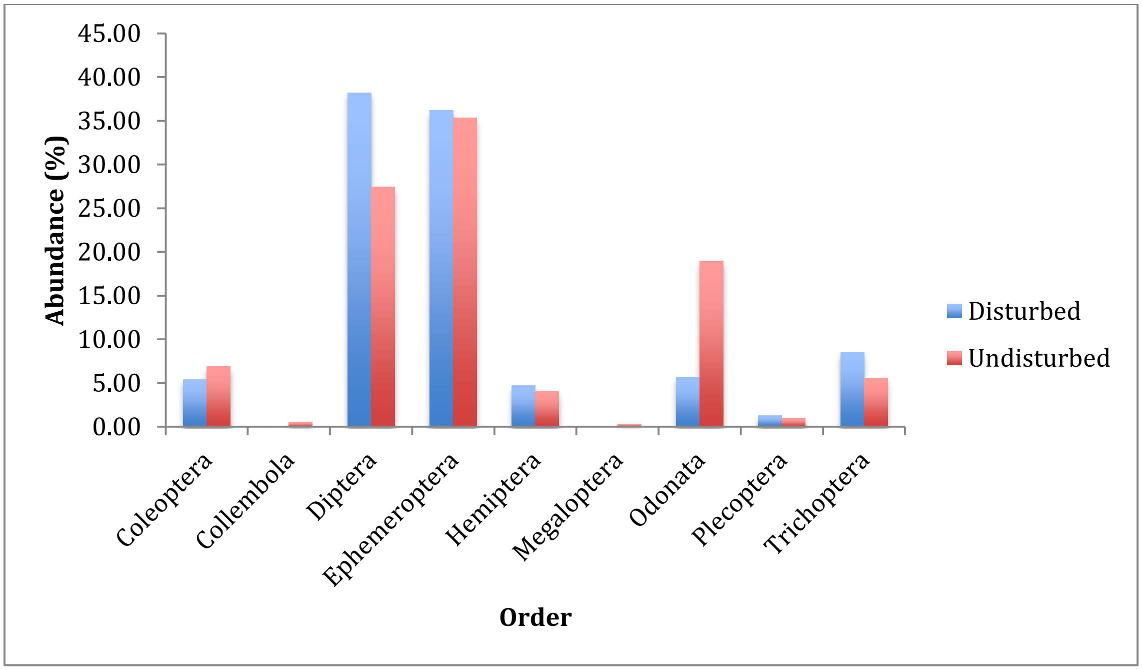


Figure 8. Mean Abundance (%) of nine insect orders found in disturbed vs. undisturbed streams.

		Mean Abundance (%)				
		Disturbed	**Undisturbed**	**Response to Disturbance**	**F Value**	**p Value**
Order	Odonata	5.68	18.970	Decrease	6.704	0.061
	Collembola	0.029	0.518	Decrease	22.04	**0.009
	Coleoptera	4.402	6.877	Decrease	0.334	0.594
	Diptera	38.215	27.407	Increase	0.124	0.743
	Ephemeroptera	36.201	35.310	Increase	0.039	0.853
	Hemiptera	4.689	4.018	Increase	0.073	0.800
	Megaloptera	0.000	0.313	Decrease	1	0.374
	Plecoptera	1.291	0.997	Increase	0	0.996
	Trichoptera	8.497	5.590	Increase	0.141	0.727
Family	Staphilinidae	0.000	0.331	Decrease	18.91	**0.012
	Gomphidae	0.263	5.973	Decrease	25.4	**0.007
	Protoneuridae	0.060	1.513	Decrease	20.25	**0.011

Table 8. Anova results from abundance (%) comparisons of insect taxa (Order and family) between disturbed and undisturbed streams. Only families with significant differences are shown. Percentages were arcsine transformed. ** Denotes significance ($p<0.05$).

Order	*F*	*P*-value
Coleoptera	1.638	0.166
Collembola	3.149	0.0145*
Diptera	6.088	0.000154***
Ephemeroptera	3.577	0.00725**
Hemiptera	4.345	0.00214**
Megaloptera	8.051	9.95E-06***
Odonata	9.289	1.99E-06***
Plecoptera	1.413	0.234
Trichoptera	5.209	0.000567***

Table 9. Results from ANOVA comparison of abundances (%) of each order between 6 rivers sampled. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

FFG	Disturbed	Undisturbed	Response to Disturbance	*F*
Collector-Filterer	15.84245474	8.378940855	Increase	0.645
Collector-Gatherer	22.99680567	43.80388423	Decrease	3.951
Predator	54.19099121	42.44165205	Increase	0.4
Scraper	6.132315522	2.413773814	Increase	1.21
Shredder	0.71020639	2.961749046	Decrease	0.445

Table 10. Mean % Abundance of each functional feeding group between disturbed and undisturbed streams and F-values from ANOVA.

FFG	Disturbed	Undisturbed	Response to Disturbance	*F*
Collector-Filterer	57.33333333	35.66666667	Increase	0.107
Collector-Gatherer	80.33333333	115	Decrease	5.468
Predator	195.6666667	135.6666667	Increase	0.836
Scraper	22.33333333	9.666666667	Increase	0.726
Shredder	2	13.66666667	Decrease	0.774

Table 11. Mean Absolute Abundance of each functional feeding group between disturbed and undisturbed streams and F-values from ANOVA.

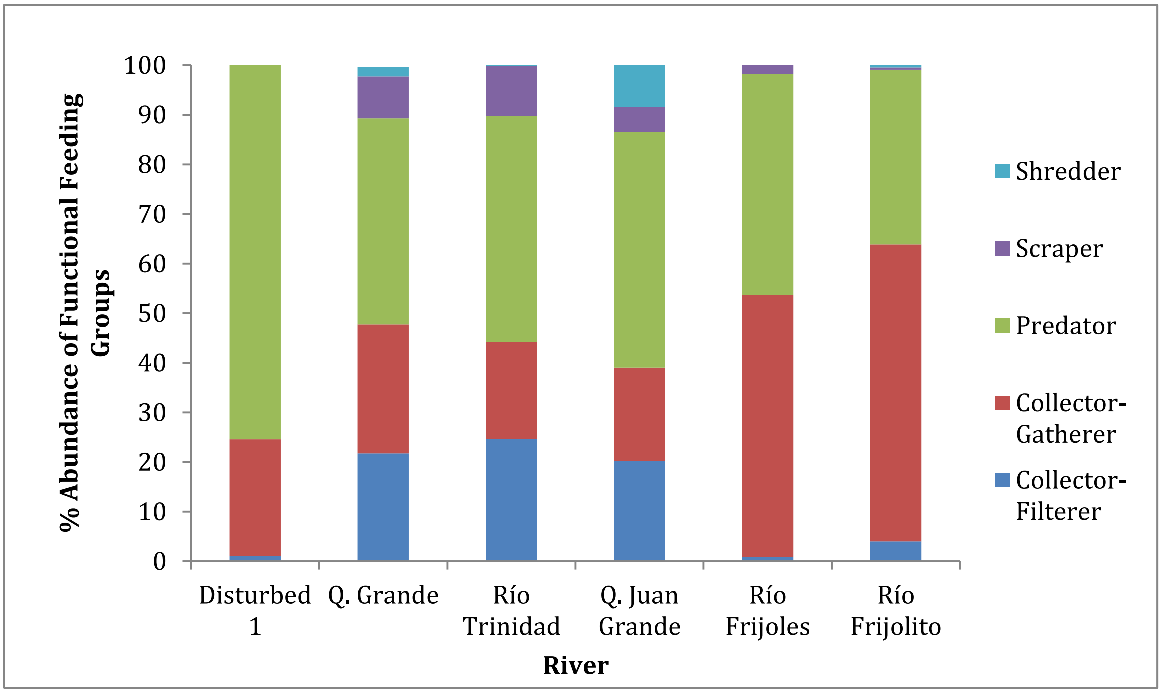


Figure 9. Relative abundance of each functional feeding group within each river.

Appendix VII: Final Product for Host

Responses of benthic macroinvertebrate community structure to anthropogenic environmental alterations

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Abstract: Deforestation is of great concern to the biological integrity of freshwater environments in the tropics. Despite the fact that Panama has been greatly transformed by this land use change, little research to date has investigated the specific implications of this on its freshwater habitats. Here we investigated the impact of deforestation caused by agriculture on stream communities. In particular, macroinvertebrates were investigated due to their known usefulness as bioindicators. Streams were sampled within the Panama Canal Watershed, 3 within undisturbed forested sites and 3 within sites surrounded by agriculture. Results indicate that deforested and forested sites did not differ significantly with respect to most abiotic variables nor did they differ in terms of their biotic variables including diversity indices, taxonomic and functional composition. However, measured environmental variables were found to significantly explain variation in community composition with oxygen, flow velocity, width, depth, and pH being the most important. Thus although conclusions cannot be made as to both the health of stream environments in Panama and the effects of deforestation on stream communities, these findings provide insight as to the dynamics of the biological components of these freshwater habitats within Panama. This can be expanded on in the future to better understand and protect the biological integrity of these essential habitats.

Resumen: La deforestación es una gran preocupación para la integridad biológica de los ambientes de agua dulce en las zonas tropicales. A pesar de que Panamá ha sido transformado por este tipo de cambios en el uso del suelo, pocos estudios han investigado las implicaciones específicas de esta alteración en los hábitats de agua dulce. En este estudio, investigamos el impacto de la deforestación causada por la agricultura en las comunidades de agua dulce a través del uso de macroinvertebrados, ya que éstos son reconocidos por su utilidad como fueron bioindicadores. Para llevar a cabo nuestro estudio, muestreamos 6 quebradas dentro de la cuenca del Canal de Panamá: 3 que se encontraban en sitios de bosques no perturbados y 3 quebradas ubicadas en sitios rodeados por la agricultura. Los resultados indican que los sitios deforestados y boscosos no difirieron significativamente con respecto a la mayoría de las variables abióticas ni tampoco en términos de sus variables bióticas incluyendo índices de diversidad taxonómica y composición funcional. Sin embargo, variables ambientales que medimos explicaron de manera significativa la variación en la estructura de la comunidad, siendo las más importantes: oxígeno, velocidad de flujo, el ancho del río, profundidad y pH. De esta manera, aunque no podemos formular conclusiones en cuanto a la salud tanto de los ríos en Panamá como sobre los efectos de la deforestación en éstas comunidades fluviales, nuestros resultados nos pueden ayudar a comprender mejor la dinámica de las comunidades biológicas en los hábitats de agua dulce en Panamá. Esperamos que este conocimiento sea ampliado en el futuro para comprender mejor y proteger la integridad biológica de estos hábitats tan esenciales.

Introduction

Freshwater habitats are of fundamental importance as they support essential resources, provide a suite of essential ecosystem services, and contribute significantly to global biodiversity (Strayer & Dugeon, 2010). However at the current, these vital habitats are becoming increasingly threatened due to a wide range of anthropogenic stressors such as overexploitation, water pollution, land use change, habitat degradation, species invasions, flow modification, and climate change (Dudgeon et al., 2006; Ormerod et al., 2010). In freshwater streams, of particular concern is deforestation of riparian and surrounding catchment habitats as these terrestrial environments are tightly linked to aquatic processes (Wallace et al., 1997; Naiman & Décamps, 1997). Deforestation has been shown to cause many changes in the physical and chemical properties of stream habitats such as increased temperature, increased sediment input, higher insolation, elevated nutrient loading, and changes in proportions of different basal food resources (Benstead et al., 2003; Likens et al., 1970).

Recent work is now focusing on how these changes in habitat attributes in turn impact the biological integrity of stream ecosystems. Biological integrity refers to the "capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitat of the region" (Karr & Dudley, 1988). In essence, integrity describes the state and health of natural communities with respect to what is expected in the absence of disturbance. The use of aquatic bioindicators has shown to be very useful to detect such short and long-term effects of land conversion. Particularly, benthic macroinvertebrates have been widely used as bioindicators for several reasons. First they have varying sensitivities and respond rapidly to changes in water and habitat quality (Kasagaki et al., 2008). Community composition and diversity has been shown to be strongly dependent on the suite of physical parameters found within the habitat (Mesa, 2010; Buss et al., 2004; Lorion & Kennedy, 2009). They are also easily collected and identified. Moreover, macroinvertebrates are one of the most diverse and abundant groups within aquatic communities (Couceiro et al., 2007). Therefore the status of macroinvertebrate communities can be used to reflect overall ecosystem health and to what extent it may have been altered relative to what may be expected under unimpacted conditions.

Macroinvertebrates present further interest as they can be categorized into several distinct functional groups that perform differential ecosystem services such as sediment mixing, nutrient cycling and energy flow within foodwebs (Covich et al., 1999). As such, macroinvertebrates not only possess high taxonomic diversity but also high functional diversity within stream environments and are central to the integrity of stream functioning. Habitat conditions such as temperature and flow regimes, resource availability, and biotic interactions all influence the functional community structure (Covich et al., 1999). Different groups are found in different proportions based on the suite of habitat traits. For example, grazers are prominent in sites with high algae production rates (Covich et al., 1999). Therefore if human disturbances alter these habitat attributes, it may in turn affect the functional composition of macroinvertebrate communities. Several studies have supported this notion (Couciero et al., 2011; Helson & Williams, 2013; Tomanova et al., 2008). For example, Benstead et al. (2003) found that deforested sites were dominantly composed of generalist collector-gatherer species in contrast to forested sites that contained collector-filterer and detritivorous shredders in addition to collector-gatherers. Functional structure is a key component influencing biological integrity in addition to taxonomic composition however the two may be differentially impacted by disturbances (Gessner & Chauvet, 2002). For these reasons, in order to thoroughly examine human disturbance impacts on ecological communities, it is of great interest to see if these changes in community structure are occurring in these habitats as well.

Work on this subject in tropical streams is limited to date. However, the findings thus far indicate that deforestation has major implications on macroinvertebrate communities. It has been shown to reduce overall macroinvertebrate diversity and severely simplify and alter the taxonomic community composition (Benstead et al., 2003; Couceiro et al., 2007; Bojsen & Jacobsen, 2003; Iwata et al., 2003). As such, it is evident that loss of forests surrounding stream habitats has the potential to strongly alter biological communities, which could lead to local extirpations of different taxonomic groups or even species extinction.

Panama, our area of study, has been subject to substantial deforestation due to a variety of causes such as road construction, logging, mining, and colonization (Rompré et al., 2008; Suman, 2002). One particular cause of the extensive deforestation in Panama however has been the progression of agriculture. Between 2006 and 2011, forested land in Panama decreased at a rate of 0.37% annually with an increase in permanent cropland of 2.25% annually (FAOSTAT, 2014). Furthermore, deforestation due to agriculture is prevalent near stream environments due to the pattern in which this land use change occurs. Thomas Croat (1972), whose work was conducted primarily in Panama, described the movement of people as inextricably linked with waterways. Humans migrate along waterways, spread laterally and remove forest cover during the voyage (Croat, 1972). This land is then used for agricultural practices, which inevitably has lead to deforestation occurring within watersheds across the country (Croat, 1972). In addition to the consequences of deforestation, conversion of land use for agriculture may have other consequences such as pesticide and fertilizer application (Harrison, 2011). Pesticide and fertilizer drift leaches into waterways, which in turn has consequences for aquatic ecosystems either through acting as toxins to ecosystem health or as nutrients in supplementing growth (Harrison, 2011; Egler et al., 2012). While Justus et al. (2010) suggested that herbivorous macroinvertebrates and algal density are linked with increased nutrients provided by runoff fertilizers; other studies have demonstrated the negative consequences of pesticide use on macroinvertebrate communities (Egler et al., 2012; Maltby & Hills, 2008). Despite the body of work that has already investigated the impacts of deforestation and agriculturally induced deforestation on macroinvertebrate communities, it is difficult to extrapolate findings from one region to another. As there is little information regarding how this land use change impacts stream communities in Panama specifically, the consequences have yet to be fully uncovered.

The aims of this study are thus threefold. First is to gain a greater understanding of freshwater communities in the Neotropics and how they respond to environmental anthropogenic disturbances. Here the focal disturbance is deforestation from agricultural land use alterations. Secondly, to provide insight as to the health and status of some of the freshwater environments in Panama. Finally this gained knowledge will hopefully allow us to provide insight as to what would be an effective management and mitigation strategy to conserve biodiversity in this region. From the above review, we hypothesized to see an overall reduction in diversity. We also hypothesized to see changes in community composition and functional structure. As there is a lack of consensus in the literature as to the pattern of change in both these components with respect to disturbance, we did not formulate specific predictions beyond this. Furthermore, we hypothesized that macroinvertebrate community composition would be dependent on a suite of habitat traits. We hypothesized that disturbed and undisturbed sites would differ with respect to these various physical characteristics and thus present the mechanism behind these biotic differences.

In order to investigate these questions, streams were studied within the Panama Canal watershed in both undisturbed locations with intact forests in the surrounding catchment areas and in disturbed streams that were found in regions heavily impacted by deforestation due to agriculture.

Methods

Study Areas

The study was conducted at a total of six sites within the Panama Canal Watershed during the dry season, between January and April 2014. This is a moist tropical region (latitude 9°) experiencing average precipitation of 2921mm/y as well as relatively constant yearly temperatures between 23-27°C.

Three streams, Quebrada Juan Grande, Río Frijoles, and Río Frijolito located along Pipeline Road in Parque Nacional Soberanía were studied as representatives of undisturbed sites. The national park has been undisturbed since the early 1900’s (Angermeier & Karr, 1983). The disturbed streams were all found within areas in which agriculture was the dominant surrounding land use. The first was an unnamed stream located on the property of a local farmer and thus will be referred to as Disturbed 1. The other two streams sampled were Quebrada Trinidad and Quebrada Grande (see Figure 1 in Appendix I).

Data collection

Benthic macroinvertebrates were collected at the six sites. At each stream, two 100 m stretches were assessed, taking one sample within each 20 m segment to give a total of 5 samples per stretch. At each site within each river either a pool or riffle habitat was sampled. Where possible, equal numbers of pools and riffles were sampled within each river. Macroinvertebrates were collected using D-frame dipnets to cover 1 m^2-sampling quadrats. To do this sediment was disturbed by kicking while macroinvertebrates were collected in the net. Manual sampling was also conducted at sites containing stony bottoms. Material was then sorted on a tray; macroinvertebrates were removed and placed into vials of 95% ethanol for later identification. Physical characteristics of sampling sites were measured including pH, oxygen (mg/L), temperature (˚C), flow velocity (m/s), and canopy cover (%). The pH was measured using ExStik pH Meter that also performs temperature readings. The oxygen was measured using a Handy Polaris oxygen probe, which also measures the temperature. To determine flow velocity of the stream sites, a Model 3000 Current Velocity Meter was used. A spherical densiometer was used to determine canopy cover. Habitat integrity was assessed through the use of the Habitat Integrity Index (HII) created by Nessimian et al (2008). The index is a modified version of the Riparian, Channel and Environmental (RCE) Inventory created by Petersen (1992). The RCE was developed in temperate landscapes and as such, it was adjusted in the HII to be more applicable to tropical environments. Sites were visually inspected for 12 stream environment characteristics that may be related to human impacts (Appendix II). Subsequent calculation of the HII follow the procedure of Nessimian et al (2008). An HII value was determined at each site within each river then subsequently averaged to give a mean HII value for each river. HII values range from 0 indicating severely impacted to 1 indicating pristine un-impacted environments.

Identification

Due to limitations in resources to identify all macroinvertebrate groups, only those of the class Insecta were included in the study. Samples were identified to taxonomic family where possible by use of taxonomic keys (Merrit et al., 2005) and then grouped into morpho-species. Morpho-species were assigned to functional groups based on Merrit and Cummins (1996) and their abundance was determined. Merrit and Cummins (1996) classify macroinvertebrates into functional groups based on modes of feeding leaving six categories. Scrapers, who adapt to graze and scrape materials from substrates, shredders, invertebrates that take coarse particular organic matter (CPOM) and break it down into fine particulate organic matter (FPOM), collector gatherers, who feed on fine particulate organic matter, collector filterers, who remove FPOM from passing water using silken nets or filtering fans, collector gatherers, those which acquire FPOM from interstices in bottom of sediment and predators that capture and consume prey. After identification, samples will be stored at -20 ˚C for subsequent DNA analysis.

Data Analysis

t-tests were conducted for each environmental parameter to detect for differences between disturbed and undisturbed streams. The value of each environmental variable for each stream used in the test was taken as the mean of the values across all of the sites in each river.

To measure diversity within each river, richness, the Shannon Index (*H'*) and Simpson's Index (*D*) were calculated at the taxonomic order level and at the family level where applicable. All abundance data was summed across all sites of each river to give one value of each diversity index for each river. *t*-tests were used to test for differences in each diversity index between disturbed and undisturbed sites.

Spearman rank correlation was conducted to investigate a potential relationship between species richness and total number of insects sampled at each river.

Relative abundances for each of the macroinvertebrate orders and families were calculated for each one of the six sampled rivers. To test for differences between disturbed and undisturbed sites in terms of percent abundances, one-way ANOVA's tests (habitat as a factor) were conducted for each order and family. Tests were conducted on arcsine transformed relative abundance data.

To compare community composition between all streams, a one-way ANOVA was conducted on arcsine transformed percent abundance data for each order. This was followed by Tukey tests to detect specific differences.

For analysis of functional structure, individuals whose functional group could not be determined were eliminated. Total number of individuals in each functional group was summed across all sites in each river and divided by the total number of insects within the river to give percent abundance of each functional group. In order to detect differences in functional structure between disturbed and undisturbed sites, a one- way ANOVA was conducted. Percent abundance data was arcsine transformed. Absolute abundance of each functional group was also compared between disturbed and undisturbed sites by use of one- way ANOVAs.

Spearman rank correlations were conducted between HII and each diversity index at both the order and family level. *t*-tests were used to compare physical parameters and habitat integrity index between disturbed and undisturbed sites.

The relationship between environmental parameters and abundance of different orders was investigated through the use of spearman rank correlations. The analysis was conducted at the level of each individual site of each river. For every environmental parameter, the absolute abundance of each order within each site was paired with the value of the parameter at that site for a total of 60 points.

Canonical correspondence analysis was used to investigate the relationship between sampled sites, environmental variables, and community composition. This analysis allows variation in communities to be directly related to variation in the environment (Ter Braak, 1986). A permutation test was conducted to test significance of the relationships described in the CCA.

All results were considered significant at the $p<0.05$ level.

This study was carried out following the Code of Ethics of McGill University.

Results

See Appendix III for the raw data for rivers sampled.

Physical Characteristics

(a) Environmental Variables

Temperature was significantly different between disturbed and undisturbed sites with a greater mean temperature in disturbed sites (*t*-test, $P<0.001$). Percent canopy cover was also significantly different between disturbed and undisturbed sites with lower mean cover in disturbed sites (*t*-test, $P<0.05$). Oxygen, flow velocity, pH, width, and depth had no significant difference between disturbed and undisturbed sites (*t*-test, all with $P>0.05$) (Table 1).

(b) Habitat Integrity Index

Habitat integrity index was significantly different between disturbed and undisturbed sites (*t*-test, $P<0.01$). All undisturbed sites had higher HII values than disturbed sites (Figure 2). Habitat integrity index was not significantly correlated to any diversity index at the order level (Shannon R= -0.0286, P=1; Simpson's R = 0.3714, P= 0.4972; Richness R=0.7590, P=0.08). It was also not significantly correlated at the family level (Shannon R=-0.2571 P=0.6583; Simpson's R= -0.1429, P=0.8028; Richness R=0.0304, P=0.9545) (Figure 3, Table 2).

Relationships between environmental variables and macroinvertebrate community composition

There were significant correlations found between some environmental parameters and abundances of some orders (Table 3). Temperature was negatively correlated to Odonata ($p<0.05$). Oxygen was positively correlated to Coleoptera, Ephemeroptera, Hemiptera, Plecoptera, and Trichoptera ($p<0.05$). Diptera and oxygen were negatively correlated ($p<0.05$). Flow velocity was positively to Coleoptera, Hemiptera, Plecoptera, and Trichoptera ($p<0.001$). Diptera and pH were negatively correlated ($p<0.01$). pH was positively correlated to Ephemeroptera, Hemiptera, and Trichoptera ($p<0.01$). Width was positively correlated to Coleoptera, Ephemeroptera, Hemiptera, and Trichoptera ($p<0.05$). Depth was negatively correlated to Coleoptera, Ephemeroptera, Hemiptera, Odonata, Plecoptera, and Trichoptera ($p<0.01$). Canopy cover was the only environmental parameter that did not significantly correlate with any order abundance. Collembola and Megaloptera were the only orders that did not significantly correlated to any of the environmental parameters. A summary of the significant positive and negative correlations associated with each order are given in Table 4.

50.1% of the variance in community composition is accounted by the CCA. The cumulative variation of the taxa-environment relationship in the CCA accounted by the first two axes was 45.3%. Eigenvalues for each axis indicate the amount of variation seen along it or its strength in influencing community composition. Eigenvalues for axes 1 and 2 were 0.3939 and 0.04841 respectively. As the eigenvalue for axis 1 was quite low, it indicates a weak gradient and only the first axis was considered in further analysis.

Axes define gradients of a set of environmental parameters. The intraset correlations are the correlation coefficients between the environmental parameters and the axes. By looking at the magnitude of the intraset correlations it can be inferred which parameters are the most important variables influencing community assemblages. Here, the first axis was most strongly associated with oxygen, flow velocity, pH, and depth (Table 5).

Points for each order show the center of its distribution along a particular environmental gradient. The distance between this point and environmental vectors describes the relative importance of the environmental variable in explaining variation in the group's abundance; the closer to the vector, the more important it is. Areas around the perpendicular of a vector represent regions of no change in the variable. Therefore if a taxonomic point lies in that area, it can be inferred the perpendicular variable does not explain variation in that taxa. Table 4 summarizes environmental gradients associated with each order and their relative position along them.

Sites 1 to 10 correspond to Disturbed 1, 11 to 20 for Quebrada Grande, 21 to 30 for Quebrada Juan Grande, 31- 40 for Rio Frijoles, 41-50 for Rio Frijolito, and 51-60 for Rio Trinidad. Each site point lies at the centroid of all the points of species that are found in that site. Its position in relation to the axes defines where along the environmental gradient it falls. Figure 4 shows that sites from disturbed 1, Rio Frijoles, and Rio Frijolito fall on the left of the origin on axis one. Thus it can be inferred that these sites are associated with shallow, low oxygen and flow velocity conditions. Sites from Quebrada Grande, Rio Trinidad, and Quebrada Juan Grande are dominantly found on the right of the origin thus associated with deeper, higher oxygen and flow velocity conditions.

Permutation test for CCA indicate the relationship between environmental parameters and community composition were not due to chance ($p<0.01$).

Community Structure

(a) Diversity

The Shannon or Simpson Index revealed no consistent trend between disturbed and undisturbed sites in changing diversity at either the order or family level (Table 6). Disturbed 1 always had the lowest value across all diversity indices at both the family and order level although the effect was more pronounced at the order level (Figure 5). At the order level, Quebrada Juan Grande always had the highest value across all diversity indices. At the family level, Rio Trinidad had the highest value. With the exception of Simpson's at the family level, undisturbed had greater diversity values than disturbed however this difference was not significant (*t*-test, $p>0.05$ for all indices) (Table 7).

(b) Macroinvertebrate Abundance

A total of 3626 insects were collected across all 6 rivers. 44 families were found across 9 orders. Richness at both the level of order and family were not correlated to total number of insects sampled ($R=0.213$, $P>0.05$; $R= 0.455$, $P>0.05$).

Total insect abundance was greater for the disturbed streams (2,077 individuals vs. 1549 individuals found in undisturbed rivers), but this difference was not statistically significant (F value=0.372, p value=0.575, ANOVA test).

Diptera was the predominant order found in Disturbed 1(93.80% of the total insect abundance), and also in Río Frijoles and Río Frijolito (39.07% and 34.34% respectively). Ephemeroptera was the main order found in Quebrada Grande, Quebrada Juan Grande and Trinidad (50.90%, 40.89% and 56% of total insect abundance respectively) (Figure 7).

When relative abundances were compared, Collembola was the only insect order found to differ significantly ($p<0.05$) between disturbed and undisturbed streams, with a higher relative abundance in undisturbed sites (Figure 8). Odonates were more predominant in undisturbed sites as well (18.97% vs. 5.68% of the insect community in disturbed streams), and this difference was close to be significant (p=0.061) (Table 8).

At the family level, Staphilinidae (Coleoptera), Gomphidae and Protoneuridae (Odonata) were found to be significantly more abundant in undisturbed streams (Table 8). The relative abundance of Hydropsychidae (a Trichoptera tolerant species) did not significantly differ between disturbed and undisturbed rivers (F value=0.109, p=0.758, ANOVA test).

When relative abundances were compared across all 6 rivers, percent abundance of 7 orders were significantly different (Collembola F=0.0145, p<0.05, Diptera F=6.088, p<0.001, Ephemeroptera F=3.577, p<0.01, Hemiptera F=4.345, p<0.01, Megaloptera F=8.051, p<0.001, Odonata F=9.289, p<0.001, and Trichoptera F=5.209, p<0.001). The remaining 2 orders did not have significantly different percent abundances across rivers (Coleoptera F=1.638, p>0.05; Plecoptera F=1.413, p>0.05). Tukey tests were unable to reveal which rivers differed (Table 9).

(c) Functional Structure

There was no significant difference in absolute or relative abundance of functional groups between disturbed and undisturbed sites (Figure 9, Table 10 and 11). The mean relative abundance in disturbed sites was greater for for CF, P, and S (ANOVA, F=0.645, P >0.05; F=0.4, P>0.05; F=1.21, P>0.05 respectively). The mean relative abundance in disturbed sites was lower than undisturbed for CG and SH (ANOVA, F=3.951, P >0.05; F=0.445, P>0.05 respectively). Absolute abundances showed the same pattern with greater mean values in disturbed sites for CF, P, and S (ANOVA F=0.107, P >0.05; F=0.836, P>0.05; F=0.726) and lower mean values in disturbed sites for CG and SH (ANOVA F=5.468, P >0.05; F=0.774, P>0.05).

Discussion

Macroinvertebrate community composition was found to be related to environmental parameters supporting one of our original hypotheses. However, disturbed and undisturbed sites were not found to differ significantly in environmental variables nor did they differ significantly in terms of diversity, taxonomic or structural community composition. These contradict the remaining two of our original hypotheses regarding the impacts of deforestation on stream environments.

Physical Characteristics

(a) Habitat Integrity Index

The Habitat Integrity Index was indeed significantly lower across disturbed sites than undisturbed sites. This indicates that sites selected for each category did in fact differ in overall environmental health.

(b) Environmental Variables

The only physical traits that differed between our sampled undisturbed and disturbed streams were canopy cover and temperature. Other studies have found similar trends. For example, Benstead et al. (2003) and Bojsen and Jacobsen (2003) also found an increase in mean temperature in deforested sites attributed to canopy loss.

On the other hand, we did not find a significant difference in the other physical traits (dissolved oxygen, flow velocity, pH and stream width/depth) between our undisturbed and disturbed sites. This is in contrast to several other studies that report differences such as in dissolved oxygen, pH and depth differences (Couciero et al., 2007; Bojsen & Jacobsen, 2003).

There are a few key reasons why only temperature and canopy differences were detected between disturbed and undisturbed streams. In this study, we were limited to select sites solely based on surrounding land characteristics (i.e. "forested" vs. "deforested, agricultural" habitats). We were able to select streams all within the same watershed to reduce some levels of natural variation however we were not able to select sites consistent in other physical traits shown to be very important in stream ecology such as stream order, elevation, upstream length, amount of water flow and surface geology (Helson & Williams, 2013). For example, one of our disturbed streams, Disturbed 1, was narrow and consisted only of pool habitats (i.e. very limited to no water flow) whereas our two other chosen disturbed streams, Quebrada Grande and Río Trinidad, were wider (Quebrada Grande was more than 3 times Disturbed 1) with a combination of pool and riffle habitats, as well as a large water flow. As these variables were unable to be controlled for, there is the potential they may have confounded the ability to detect disturbance driven changes in other abiotic properties.

This is further emphasized by the fact that a similar study was conducted in the Panama Canal watershed that was able to more strictly control for these potential confounding variables (Helson & Williams, 2013). They did in fact see a difference between forested and deforested regions across a set of abiotic variables including lower dissolved oxygen in deforested sites.

Another potential explanation for lack of a relationship between disturbed and undisturbed sites is that there are other environmental parameters that were not measured in this study that may have differed between categories of disturbance. Other studies have detected differences in turbidity, conductivity, nitrogen and phosphorous, discharge, and sediment inputs (Couciero, 2007; Larsen et al., 2009; Mesa, 2010)

Relationships between environmental variables and macroinvertebrate community composition

Results from both the Spearman rank correlations and the CCA show that community composition is related to environmental parameters confirming the final original hypothesis. These results are supported by numerous other studies that have also found abiotic factors to significantly explain variation in macroinvertebrate communities (Bojsen & Jacobsen, 2003; Buss et al., 2003; Coucerio et al., 2007; Kasangaki et al., 2006; Kasangaki et al., 2008).

The Spearman rank correlations show that oxygen, stream width and depth, flow velocity, and pH were all found to correlate significantly with several orders. Only canopy cover and temperature appeared to have little relationship with order abundance.

Similarly, as 50.1% of the variance in community composition is accounted by the CCA, it can be inferred that the environmental gradient generated by measured variables explains a considerable amount of variation in taxonomic composition. However this still leaves half of the variation left unexplained and thus indicates that likely there are other factors not measured here explaining variation in composition as well. Other such factors that could explain this variation include nitrates, conductivity and land-use percentage such as was found by Mesa (2010) in a study comparing pristine forested areas with areas affected by agricultural land use.

There was a high level of consistency between results of the correlations and CCA in terms of which environmental parameters are most important in explaining patterns in abundance of each order. Coleoptera, Hemiptera, Plecoptera, Ephemeroptera and Trichoptera all appear to be associated with wider, and shallower, faster flowing, higher pH, oxygenated waters. These parameters were found to explain large proportions of variation in taxonomic data in previous studies (Coucerio et al., 2007; Kasangaki et al., 2006; Kasangaki et al., 2008). Diptera appear to be associated with opposing environments that are deeper, narrower, slower flowing, lower oxygenated and intermediate depths, oxygen levels, and velocity. In both analyses, Collembola and Megaloptera were not found to be associated with any of the measured variables. There are three possible explanations for this. The first is that their distributions are not associated with abiotic conditions. The second is that there are other variables not measured in this study that are exerting greater influence over their abundance patterns. The latter is of greater likelihood given the number of variables that were unable to be measured and included and the high amount of unexplained variation in the CCA. Another key consideration is the rarity in which both of these orders were observed in the sites sampled. A pattern may be hard to discern with such small abundances.

Within each river, sites were scattered throughout the CCA ordination diagram indicating some differences in environmental parameters and species compositions however there was a general area within which most sites were found. Based on these dominant areas occupied by each river, relationships with other rivers may be assessed. Rivers within the same disturbance category were not necessarily found closely to one another in the CCA ordination diagram. Instead, an alternative grouping of rivers are suggested. Quebrada Grande, Quebrada Juan Grande, and Río Trinidad appear to be closely associated. Río Frijoles and Río Frijolito also appear grouped and Disturbed 1 appears to be distinct from all other 5 rivers but closest to Río Frijoles and Río Frijolito.

This indicates that rivers within the same disturbance category were not necessarily similar in terms of the set of environmental parameters found within each and suggests that it is not the most important grouping variable here. Thus it lends strong support to the concern that rivers were not chosen with consistent sets of other stream parameters and thus there are confounding parameters preventing this categorization scheme from being effective. This further explains potentially why no significant differences were seen in physical traits between disturbed and undisturbed.

Furthermore, the fact that streams within the same disturbance categories were not necessarily grouped together indicates they are not similar in terms of community composition. Streams in different disturbance categories were actually more similar that streams within the same disturbance category.

Community Structure

(a) Diversity

Our results indicate that disturbance had no effect on macroinvertebrate diversity within stream communities in the sense of both taxonomic richness and evenness. This contrasts the findings of several other studies investigating the effects of deforestation and agriculture in tropical environments. Benstead et al. (2003) found a significant decrease in agriculture impacted streams compared to forested streams in Madagascar. Similarly, alpha diversity decreased in deforested sites of Ecuadorian streams (Bojsen & Jacobsen, 2003). Furthermore, a study in Costa Rica too showed reduced alpha diversity in pasture streams compared to forested, non-impacted streams (Lorion & Kennedy, 2009). Kasangaki et al. (2006) found higher values for Shannon diversity index in forested sites compared to sites surrounded by heavy logging and agriculture. In these studies, it appears that surrounding land use did have significant influence on macroinvertebrate diversity.

There are several reasons that could explain why we did not observe such patterns. First, and as we already mentioned, there are the physical differences among the streams we chose to group under the *disturbed* and *undisturbed* categories (i.e. replicate selection). Future studies should consider stream selection carefully, and aim at having as high as possible replicate similarity to test differences in diversity due to deforestation per se rather than due to other variables (obviously as much as this is possible). This relates to our second point: although we did measure different physical and chemical stream attributes, we were not able to consider some very important variables related to deforestation such as: sedimentation, nitrogen, phosphorous, organic matter content, turbidity and substrate type. Therefore, the macroinvertebrate community could have been responding to different factors that we were not able to quantify. In addition, there is the timing of our field sampling: we sampled our first three rivers (the undisturbed ones) at the end of January and beginnings of February (mid-dry season), whereas we sampled our disturbed streams during Mid-March and early April (ends of the dry season). Cummins et al. (2005) recommend that macroinvertebrate sampling should be done when the populations are at their latter stages of growth (either end of dry season or end of wet season) as this facilitates their sorting and identification as well as targets macroinvertebrates in their stage most closely linked to their feeding modes. This could also be confounding our results. Additionally, the fact that we did not identify any of our collected Ephemeroptera individuals at the family level represents a serious limitation of our study because this macroinvertebrate order constituted almost half of our total sample in terms of abundance (Kasangaki et al., 2008).

Furthermore, HII was not significantly correlated to any of the diversity indices. Nessimian et al. (2008) found results consistent with this. They too found that the index grouped together sites that shared the same disturbance catagorization yet did not find significant correlations with taxonomic richness. Few other studies have used the HII to look directly at its relationship with macroinvertebrate diversity. Silva et al.

(2010) however studied diversity of Hymenoptera in human impacted and unimpacted streams in Brazil and did find a significant correlation between HII and species richness. The objective behind the habitat integrity index is to discern the biological condition of stream environments through an assessment of physical attributes of stream environments. This is based on the assumption that biological communities are coupled with the state of its physical environment (Petersen, 1992). The original Riparian, Channel, and Environmental (RCE) Inventory index was found to successfully meet this objective in that it was significantly correlated to Shannon Diversity Index in temperate streams. Furthermore, it has been applied in tropical environments and indicated similar patterns in that both taxonomic richness and Shannon index increased with increasing RCE scores (Buss et al., 2002). The results here and other studies reveal that it is not certain whether this modified index is an effective tool to assess the status of macroinvertebrate diversity within streams and requires further investigation. Yet it should be noted that this analysis too may have been influenced by the above impacts affecting comparison of diversity between streams explaining the lack of relationship.

(b)Macroinvertebrate Abundance

Similar to what Benstead et al. (2003) found, there was no difference in overall macroinvertebrate abundance between disturbed and undisturbed sites. Furthermore, we did not find significant differences in the relative abundance for most of the insect orders: we found that only the abundance of Collembola was greater in our undisturbed streams. This contrasts greatly what was hypothesized based on results of previous studies investigating the impacts of deforestation on macroinvertebrate community assemblages. Helson and Williams (2013) found that the relative abundances of Trichoptera, Plecoptera, Odonata and Coleoptera decreased with increased disturbance. Similarly, Benstead et al. (2003) found a negative correlation between deforestation and Trichocoptera, Plecoptera, and Diptera. In addition they found a positive correlation between Ephemeroptera abundance and deforestation. Kasangaki et al. (2008) in contrast observed a decrease in Ephemeroptera abundance between deforested and forested sites in Uganda while Trichoptera did not change.

At the level of family, again there was a lack of relationships seen between relative abundance in disturbed versus undisturbed sites with the exception of Staphilinidae, Gomphidae, Protoneuridae. Again this contrasts what was expected. Other studies have shown variation between in family abundances. For example, Kasangaki et al. (2008) found that forested and deforested sites differed in which families were found within the orders Trichoptera, Ephemeroptera, Plecoptera and Odonata.

There are a few potential explanations as to why no relationship was found in this study. It must be emphasized that we were unable to identify Ephemeroptera to the family level. As this represented almost half of the total abundance this could be greatly preventing any relationships in family compositions between disturbed and undisturbed sites to be seen. Some Diptera were also unable to be identified to the family level as well as all Collembola although because this order was extremely rare it is likely not of great importance. In addition, an important difficulty for studies of macroinvertebrates in the tropics is found in their classification: the majority of macroinvertebrate taxonomic guides are based on temperate regions. This made it difficult to increase the "resolution" of the study, for example getting down to genus and/or species level, and may have important repercussions in the conclusions being drawn. For example, Helson and Williams (2013) could not find a difference in richness between disturbed and undisturbed sites when they looked at Ephemeroptera families, but did find a decrease in richness with increased disturbance when Ephemeroptera were analysed at the genus level.

However a final key explanation of a lack of relationship comes from the fact that macroinvertebrate assemblages were more similar between rivers of different disturbance categories than within each category itself as indicated above from the CCA. Abundance data appears to support this suggestion. Quebrada Juan Grande, despite being classified as an undisturbed river was found to have similar relative abundances across all orders with two of the disturbed streams Quebrada Grande, and Río Trinidad with the exception of Megaloptera that was only found within Quebrada Juan Grande.

As described previously, no relationship may have been seen because the site selection did not control for confounding variables and thus they are exerting greater influence on community composition as opposed to disturbance. This was again supported by the ANOVAs that compared abundances of each order across all individual rivers. It showed that the factor of stream had a significant effect on abundances of all orders besides Coleoptera and Plecoptera. Therefore it can be suggested that disturbance category was not the main factor driving differences in community composition but rather other variables between streams.

Abundances of particular macroinvertebrate groups are often used to infer levels of disturbance as they have been classified as being more tolerant or intolerant to disturbances (Chakona et al., 2009). Trichoptera families are one such group. Here we see that disturbed streams contain Trichoptera of the families Hydropsychidae and Hydroptiliidae (classified as "moderate tolerant"), Philopotamidae ("low tolerant") and Polycentropodidae ("high tolerance") in similar proportions to undisturbed streams suggests the possibility that our studied disturbed sites (Río Trinidad and Quebrada Grande) may be more resilient to disturbance than we originally thought.

(c) Functional Structure

Our results indicate that there were no significant differences in functional structure between disturbed and undisturbed communities.

There were two key limitations that need to be addressed. First, the order Ephemeroptera has families that belong to three different functional groups: scrapers, collector gatherers and collector-filterers; as we could not quantify this for our study we acknowledge that the conclusions we will be able to draw from our results will be influenced by this important fact.

Secondly, we classified macroinvertebrates into functional groups based on the family classification for temperate regions. This brings up two concerns. First, recent work has suggested that tropical macroinvertebrates do not always share the same functional classification as their temperate counterparts (Tomanova et al., 2006). Second, macroinvertebrates in tropical regions have been found to show flexibility in their food acquisition modes; this is reasonable as we would expect generalist-feeding strategies to be beneficial in the unpredictable resource availability conditions found in the tropics (Tomanova et al., 2006). However, this “plasticity” complicates their classification into a single functional feeding group. For example, in their study in Bolivia, Tomanova et al. (2006) found that omnivory was common among most taxa, and that they could be classified into more than one functional group. They have suggested that functional group identification should combine both mouthpart and gut content analysis strategies to address this issue.

Despite these limitations, we were still able to observe certain patterns when streams were compared at the individual level.

Although we did not measure leaf litter directly, there is the potential that the decrease in canopy cover found in our disturbed streams caused a reduction in leaf litter, as other studies have observed (Bojsen & Jacobsen, 2003). This could explain why shredders decreased in Quebrada Grande and Río Trinidad, although not why they were so underrepresented in Río Frijoles and Frijolito regardless of a high canopy cover. Shredder macroinvertebrates in our samples were represented by individuals from the following groups: Coleoptera (mostly Elmidae), Trichoptera and Plecoptera. Trichoptera shredders are usually found in running waters (Bouchard, 2004) thus the pool habitats found in Río Frijoles and Frijolito (as well as in Disturbed 1) could be partially responsible for their absence despite high canopy cover. These results are consistent with other studies that have found a decrease in shredder abundance with increased disturbance (Helson & Williams, 2013). This has been explained in part because as more specialized feeders shredders become more sensitive to human alterations (Helson & Williams, 2013).

In addition to its impact on organic inputs into streams, deforestation around stream environments has been found to allow higher amounts of light penetration, which can enhance algae and perphyton growth (Bosjen & Jacobsen, 2003). This would benefit scraper individuals. Indeed, two disturbed streams, Quebrada Grande and Río Trinidad showed higher scraper abundance in comparison to undisturbed sites (although statistically insignificant). Other studies have shown a positive correlation between algae and grazers abundance due to increased light levels (Wallace & Webster, 1996), but algae can also increase due to the addition of nutrients from the run-off of agricultural sites. On the other hand, streams in deforested sites have been found to contain higher sediment quantities and greater water turbidity (Murphy et al., 2012), thus algae growth would not necessarily be as high as predicted from an increase in light and nutrients. For future studies, it would be useful to quantify periphyton abundance to further test the validity of these correlations in these habitats. Additionally, scrapers were totally absent from Disturbed 1. It has been suggested that their feeding specialization also makes them more sensitive to disturbance, and different studies have observed a decrease in scraper abundance following a human impact gradient (Helson & Williams, 2013; Tomanova et al., 2008).

Furthermore, the supply of nutrients into the stream system from agricultural sites (organic pollution) as well as a decrease in canopy cover can increase the abundance of collector individuals (both filterers and gatherers) although this will depend on the input ratio of organic matter to inorganic matter (Murphy et al., 2012). In our study, we found that collector gatherers had the highest relative abundances in Río Frijoles and Frijolito and were comparatively quite low within all other rivers. This might be related to the fact we did not include Ephemeroptera in our functional group classifications. In disturbed streams, we would expect taxa that can take advantage of in situ primary production to increase in abundance, like some Ephemeroptera generalist collector gatherers (families Baetidae and Leptohyphidae) and large omnivorous collectors like certain Trichoptera families (Benstead et al., 2003). However, we did observe that collector-filterers were mostly present in Quebrada Grande and Río Trinidad (although in Quebrada Juan Grande as well), therefore this is likely due to the fact these were streams that had riffles. Filter feeders remove FPOM from suspension and supply larger particles via their feces to deposit-feeders. They exploit habitats with high degrees of particle transport, therefore they have high abundances in running-water environments (Wallace & Webster, 1996).

Furthermore, disturbance has been shown to alter not just overall differences in functional structure of communities between disturbed and undisturbed environments but also to alter diversity within functional groups (Couceiro et al., 2011). Here Río Frijoles and Río Frijolito appear similar to Disturbed 1 in functional structure (i.e. containing mostly predators and collector gatherers). However, by looking at the taxonomic composition of the functional groups we can see that the two undisturbed rivers have a wider variety of taxa corresponding to the same functional group. For example, chironomids, odonates, coleopterans, plecopterans and tipulidaes may function as predators in the undisturbed sites, whereas Disturbed 1 predators are mainly represented by chaeoboridaes and chironomids. Therefore this study too suggests that disturbance may reduce functional diversity.

Stemming from the discussion of altered functional structure due to anthropogenic impacts comes a gap in the literature. Little work has investigated what further consequences these changes in communities may be having on the system. Because different groups of macroinvertebrate functional groups contribute differentially to ecosystem processes, changes in its composition have been suggested to influence the relative levels in which these different functions continue to occur which in turn could have trophic cascade effects throughout the system (Chapin III et al., 2000). In other words, human impacts may establish a chain reaction of ecological alterations: disturbances modify the environment which alters community structure leading to further changes in the environment that continue to affect other components of communities throughout the system. One study has demonstrated this within stream communities whereby terrestrial litter inputs were excluded from stream sites (Baer et al., 2001). This lead to changes in the relative abundance of different functional groups as well as an overall decline in macroinvertebrate levels. A reduction in secondary production, altered detrital processing, and composition of organic matter were observed which in turn affected subsequent macroinvertebrate colonization rates. Another study investigating the effects of disease driven amphibian declines in tropical stream ecological processing also shed light on this concept from the perspective of another group of organisms (Whiles et al., 2013). Tadpoles are grazers predominantly and by impinging an impact that reduced this functional group, strong changes within the environment were witnessed with great increases in algae and fine detritus biomass, and altered nitrogen cycling. Thus from this it is strongly illustrated how impinging impacts that alter functional structure can feedback and alter the environment further. Although this study did not show significant differences in community structure due to disturbance, the limitations of our analysis as well as the strong consensus from other referenced studies implies that such changes may in fact still be at play and as such it would be beneficial to follow up on this concept further in these environments in order to understand the full circle of impacts associated with human activities.

Finally, the discussion here was limited to relationships between functional groups and disturbance levels and not with that of environmental parameters. Although it has been shown that environmental parameters are important in the functional structuring communities (Couceiro et al., 2011; Benstead et al., 2004), and as with the taxonomic community composition may be more important in our study than disturbance category, we concluded the lack of data from Ephemeroptera would impair our ability to draw conclusions from our results.

Final Remarks

There did not appear to be a strong relationship between deforestation and both abiotic and biotic variables considered in this study within stream environments. From this it appears that other variables are more important in determining community composition than surrounding catchment land use. However, caution should be taken in concluding that human impacts are irrelevant in determining the state of these freshwater environments. This can be explained by returning to the concept of biological integrity. The integrity of an environment is given in reference to what its state would be under undisturbed conditions. The undisturbed sites in this case however cannot be used as reference sites for this comparison because of the noted differences in other natural stream habitat parameters. Therefore we were unable to address one question of the study in that we were unable to fully assess the states of some freshwater habitats are within Panama, particularly those in heavily deforested regions. We were also unable to provide a response to another key question as to how freshwater stream communities respond to disturbances. Yet we were able to show that within these habitats, environmental variables are strong predictors of community composition and this can be put into great use in the future. If changes are seen within stream environments in the future from either human or other causes, it can be expected that a change in biotic communities will likely result and efforts should thus be put in place to mitigate these potential impacts. Furthermore we were able to elucidate some variables that were particularly important in determining community composition. Thus it may be suggested that these could be the focus of monitoring activities in order to detect changes before their impacts are able to take effect. In this regard, we were able to accomplish our third goal to some degree.

There is room for a great deal of future work to illuminate our questions here further. First, studies in the future should look at disturbance in comparison to reference sites more reflective of the disturbed sites natural unimpacted condition in order to investigate more rigorously the effect of human impacts on biological integrity. Furthermore, only macroinvertebrates were investigated here however there are several other components of the system that would be of great interest to study in order to understand the full range of impacts disturbance may be having on communities. Finally, there remain several environmental variables not measured here. It would be valuable to measure a greater range of variables in the future to gain better understanding of how environments shape community compositions.

This study has contributed to a better understanding of freshwater community dynamics within Panama that can be expanded on in the future so that we may learn how best to promote the biological conservation of these essential habitats.

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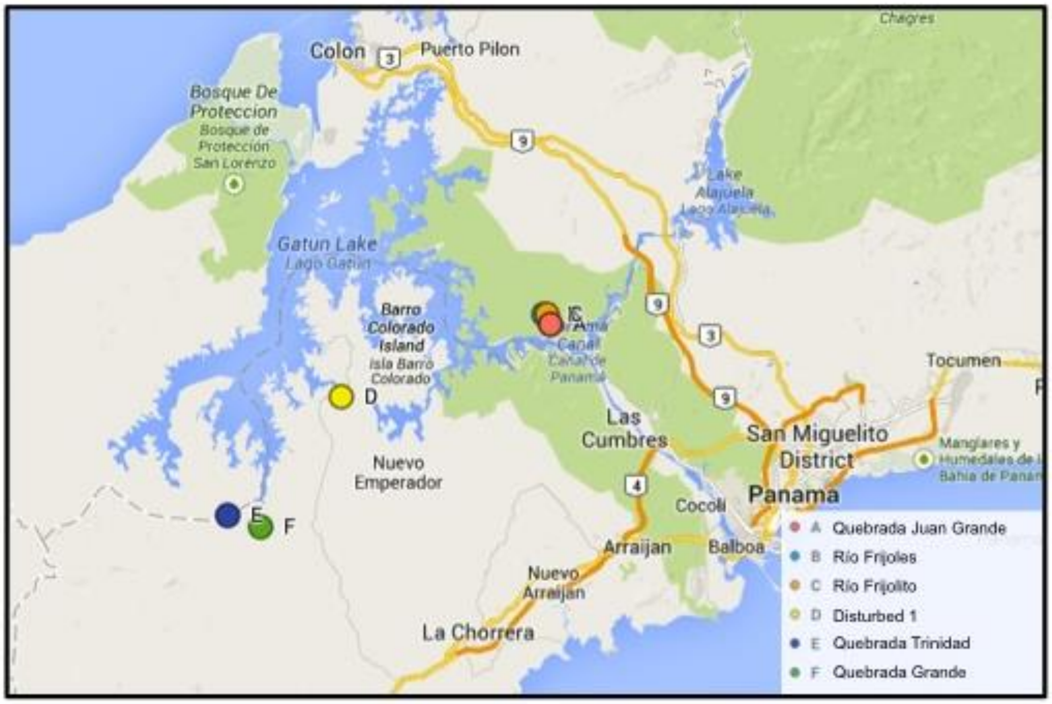
Appendix I: Maps of Study Sites



Appendix II: Habitat Integrity Index

Table 2 Habitat characteristics used in evaluation of sampling sites for HII calculations

	Characteristic	Condition	Score
F1	Land use pattern beyond the riparian zone	Primary continue forest/100 ha fragment/10 ha fragment	6
		Cecropia secondary forest/mixed secondary forest	5
		Vismia secondary forest	4
		Pasture	3
		Perennial crops	2
		Short-cycle crops/exposed soil	1
F2	Width of riparian forest	Continuous forest	6
		Forest width between 30 and 100 m	5
		Forest width between 5 and 30 m	4
		Forest width between 1 and 5 m	3
		Riparian forest absent, but some shrub species and pioneer trees	2
		Riparian forest and shrub vegetation absent	1
F3	Completeness of riparian forest	Riparian forest intact without breaks in vegetation	4
		Breaks occurring at intervals of >50 m	3
		Breaks frequent with gullies and scars at every 50 m	2
		Deeply scarred with gullies all along its length	1
F4	Vegetation of riparian zone within 10 m of channel	More than 90% plant density by non-pioneer trees or shrubs	4
		Mixed pioneer species and mature trees	3
		Mixed grasses and sparse pioneer trees and shrubs	2
		Grasses and few tree shrubs	1
F5	Retention devices	Channel with rocks and/or old logs firmly set in place	4
		Rocks and/or logs present but backfilled with sediment	3
		Retention devices loose, moving with floods	2
		Channel of loose sandy silt, few channel obstructions	1
F6	Channel sediments	Little or no channel enlargement resulting from sediment accumulation	4
		Some gravel bars of coarse stones and little silt	3
		Sediment bars of rocks, sand and silt common	2
		Channel divided into braids or stream channel corrected	1
F7	Bank structure	Banks inconspicuous	5
		Banks stable, with rock and soil held firmly by grasses, shrubs, or tree roots	4
		Banks firm but loosely held by grasses and shrubs	3
		Banks of loose soil held by a sparse layer of grass and shrubs	2
		Banks unstable, easily disturbed, with loose soil or sand	1
F8	Bank undercutting	Little, not evident or restricted to areas with tree root support	4
		Cutting only on curves and at constrictions	3
		Cutting frequent, undercutting of banks and roots	2
		Severe cutting along channel, banks falling in	1
F9	Stream bottom	Stone bottom of several sizes packed together, interstices obvious	4
		Stone bottom easily moved, with little silt	3
		Bottom of silt, gravel, and sand, stable in some places	2
		Uniform bottom of sand and silt loosely held together, stony substrate absent	1

Table 2 continued

	Characteristic	Condition	Score
F10	Riffles and pools, or meanders	Distinct, occurring at intervals of 5–7× the stream width	4
		Irregularly spaced	3
		Long pools separating short riffles, meanders absent	2
		Meanders and riffle/pools absent or stream corrected	1
F11	Aquatic vegetation	When present, consists of moss and patches of algae	4
		Algae dominant in pools, vascular plants along edge	3
		Algal mats present, some vascular plants, few mosses	2
		Algal mats cover bottom, vascular plants dominate channel	1
F12	Detritus	Mainly consisting of leaves and wood, without sediment	5
		Mainly consisting of leaves and wood, with sediment	4
		Few leaves and wood, fine organic debris, with sediment	3
		No leaves or woody debris, coarse and fine organic matter, with sediment	2
		Fine anaerobic sediment, no coarse debris	1

Table taken from Nessimian et al. (2008)

Appendix III: Raw data for rivers sampled

Sum of Count		River						
Order	Family	Disturbed 1	Q. Grande	Q. Juan Grande	Río Frijoles	Río Frijolito	Río Trinidad	Grand Total
Coleoptera	Curculionidae					1		1
	Dytiscidae	6	1		5	4		16
	Elmidae		35	89	6	3	13	146
	Heteroceridae				1			1
	Hydrophilidae					1		1
	Hysteridae		1					1
	Psphenidae		11	2			39	52
	Ptiliidae						1	1
	Scirtidae	5			2	2		9
	Sphaeriusidae				2	1		3
	Staphylinidae			1	2	1		4
	Unknown				1	2		3
Coleoptera Total		11	48	92	19	15	53	238
Collembola	(blank)			6	1	2	1	10
Collembola Total				6	1	2	1	10
Diptera	Chaeboridae	261	8				2	271
	Chironomidae	83	48	27	113	125	67	463
	Culicidae	1		9		2		12
	Dolichopodidae					2		2
	Simuliidae					1		1
	Tipulidae		5	16		5	7	33
	Unknown	3	8	23	1	8	21	64
Diptera Total		348	69	75	114	143	97	846

Ephemeroptera	(blank)	6	283	348	98	130	645	1510
Ephemeroptera Total		6	283	348	98	130	645	1510
Hemiptera	Gelastocoridae		6	9	1		21	37
	Naucoridae	1	33	81	2	1	56	174
	Nepidae				1			1
	Scirtidae						1	1
Hemiptera Total		1	39	90	4	1	78	213
Megaloptera	Corydalidae			8				8
Megaloptera Total				8				8
Odonata	Aeshnidae			14				14
	Calopterygidae			3				3
	Coenagrionidae		26	2	19	3	35	85
	Corduliidae	3	6	1	12	2	3	27
	Gomphidae		1	45	12	33	7	98
	Lestidae				3	11		14
	Libellulidae	1	4	5	42	5	11	68
	Macromiidae		1					1
	Platystictidae		11	21			4	36
	Protoneuridae		1	10	3	9		23
	Unknown			1	1		20	22
Odonata Total		4	50	102	92	63	80	391
Plecoptera	Peltoperlidae		5					5
	Perlidae		3		2	1	28	34
	Pteronarcyidae			18				18
Plecoptera Total			8	18	2	1	28	57
Trichoptera	Calamoceratidae						1	1
	Calamocertidae		1					1
	Hydrophilidae						1	1
	Hydropsychidae		15	29	1	1	47	93
	Hydroptilidae		2	3			1	6
	Lepidostomatidae		3					3
	Leptoceridae		1	5			2	8
	Odontoceridae		2	1			1	4
	Philopotamidae		28	67	1	7	63	166
	Phryganeidae			3				3
	Polycentropodidae	1	3	4		3	8	19
	Psychomyidae		1				10	11
	Rhyacophilidae						1	1
	Unknown		3				33	36
Trichoptera Total		1	59	112	2	11	168	353
Grand Total		371	556	851	332	366	1150	3626

Appendix IV: Figures and Tables: Results

Physical Parameter	Disturbed	Undisturbed	*t*	p-value
Temperature (°C)	28.01	24.06	11.8472	0.0003*
Oxygen (mg/L)	5.69	3.54	0.9117	0.4135
Flow Velocity (m/s)	0.29	.08	1.3714	0.2422
% Canopy Cover	66.51	88.01	-3.829	0.0474*
pH	7.52	7.33	0.6675	0.541
Width (cm)	788.9	283.07	-1.312	0.2597
Depth (cm)	26.72	18.803	0.8639	0.4364
HII	0.7327315	0.9270833	7.1845	0.002*

Table 1. Comparison of disturbed and undisturbed mean physical parameter values. **P*-value significant at 0.05.

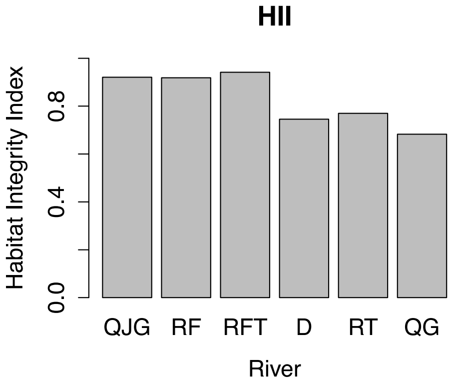


Figure 2. Habitat Integrity Index values for each of the six rivers sampled. River codes are as follows Quebrada Juan Grande = QJG, Rio Frijoles = RF, Rio Frijolito = RFT, Disturbed 1 = Disturbed, Rio Trinidad = RT, Quebrada Grande = QG

Taxonomic Level	Diversity Index	R
Order	Shannon	-0.0286
	Simpson’s	0.3714
	Richness	0.7590
Family	Shannon	-0.2571
	Simpson’s	-0.1429
	Richness	-0.0304

Table 2. Spearman rank correlation coefficients (R) between Habitat Integrity Index and diversity indices.

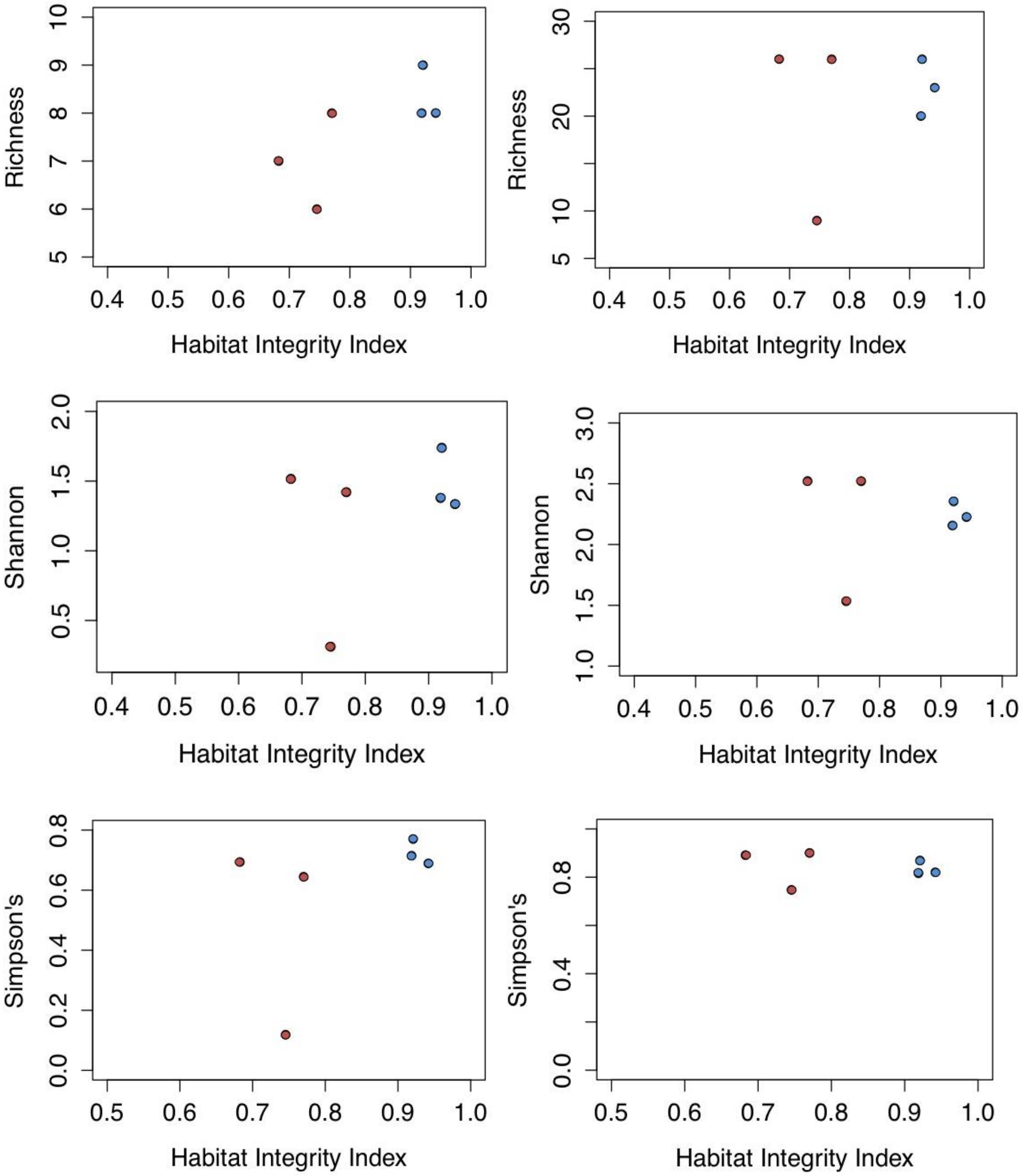


Figure 3. Relationship between habitat integrity index and diversity indices in the 6 sampled rivers. (A) at the level of order and (B) at the level of family. Red circles represent disturbed streams, blue circles represent undisturbed streams

Order	**Habitat Trait**													
	Temperature		Oxygen mg/L		Flow Velocity		Canopy Cover		pH		Width (cm)		Depth (cm)	
	R	*p*	*R*	*p*	*R*	*p*	*R*	*p*	*R*	*p*	*R*	*p*	*R*	*p*
Coleoptera	0.044	0.739	0.3329	0.009**	0.624	9.85E-08***	-0.008	0.950	0.2303771	0.077	0.2660787	0.040*	-0.360	0.005**
Collembola	-0.116	0.376	0.0426	0.746	-0.166	0.205	0.040	0.761	0.09945078	0.450	0.09039472	0.492	-0.075	0.569
Diptera	-0.055	0.677	-0.290	0.025*	0.010	0.941	-0.005	0.971	-0.3978233	0.002**	-0.2202972	0.091	0.106	0.421
Ephemeroptera	-0.038	0.771	0.515	2.51E-05***	0.289	0.025	-0.097	0.461	0.5404306	8.31E-06***	0.2962307	0.022*	-0.496	5.54E-05***
Hemiptera	0.121	0.355	0.489	7.43E-05***	0.735	2.36E-11***	-0.061	0.641	0.3376159	0.008**	0.4795763	0.0001***	-0.393	0.002**
Megaloptera	-0.157	0.230	0.123	0.349	0.121	0.355	0.158	0.228	0.1918491	0.142	0.2173308	0.095	-0.261	0.044
Odonata	-0.426	0.0007***	0.247	0.057	0.250	0.054	0.253	0.051	0.1287597	0.327	-0.108115	0.411	-0.529	1.39E-05***
Plecoptera	0.004	0.977	0.298	0.021*	0.455	0.0003***	-0.089	0.500	0.2077992	0.111	0.184268	0.159	-0.3478	0.006**
Trichoptera	0.218	0.0937	0.672	4.04E-09***	0.681	2.17E-09***	-0.125	0.341	0.5197076	2.09E-05***	0.5353505	1.05E-05***	-0.449	0.0003***

!

Table 3: Spearman rank correlation coefficients (R) between environmental parameters and abundance of each order at 60 sampled sites. *p<0.05, **p<0.01, ***p<0.001

Order	Spearman Rank Correlation		CCA	
	Positive	Negative	Positive	Negative
Coleoptera	Oxygen, Flow Velocity, Width	Depth	Width*, pH	
Collembola				
Diptera		Oxygen, pH	Depth*	Oxygen, Flow velocity, Width, pH
Ephemeroptera	Oxygen, pH, Width	Depth	Oxygen, Flow Velocity	Depth
Hemiptera	Oxygen, Flow Velocity, pH, Width	Depth	Oxygen, flow velocity, pH*, Width*	Depth
Megaloptera			Width	
Odonata		Depth	Width	Oxygen, Flow velocity
Plecoptera	Flow Velocity	Depth	Flow velocity Width*, pH*, Oxygen	Depth
Trichoptera	Oxygen, Flow Velocity, pH, Width	Depth	Oxygen, Flow Velocity, pH, Width*	Depth

Table 4: Summary of environmental parameters associated with each order as described by Spearman Rank Correlations and CCA ordination diagram. Reported variables from correlations were those found to be significant. Reported variables from the CCA are all those in which taxa points did not fall within the region perpendicular to them. * indicate the taxa point was located in close proximity to the vector.

Environmental Parameter	Intraset Correlation	
	Axis 1	Axis 2
Temperature	0.08017	0.388668
Oxygen	0.83762	0.205061
Flow.Velocity	0.72922	0.173518
Canopy.Cover	-0.2402	-0.773998
pH	0.66607	-0.321698
Width	0.41861	-0.49487
Depth	-0.66012	0.007322

Table 5: CCA results showing the intraset correlation of axis 1 and 2 to each environmental parameter

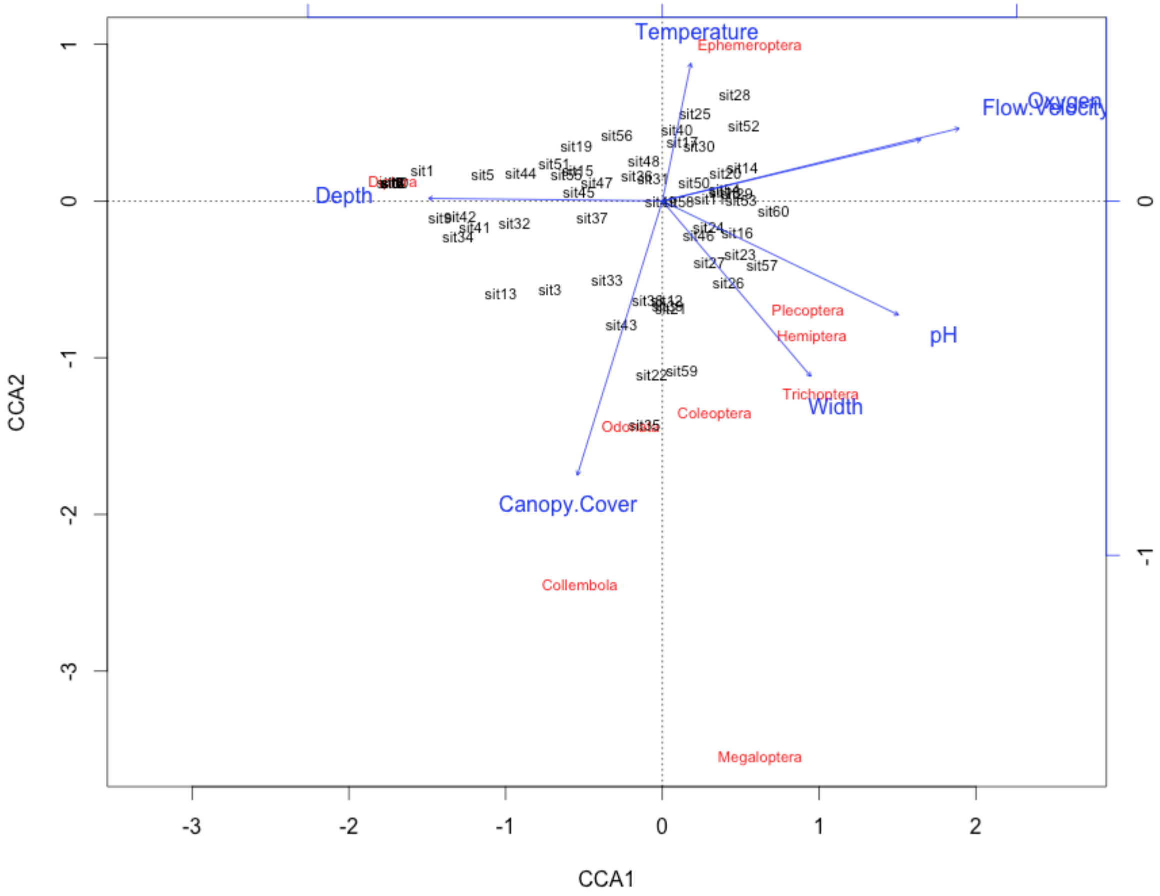


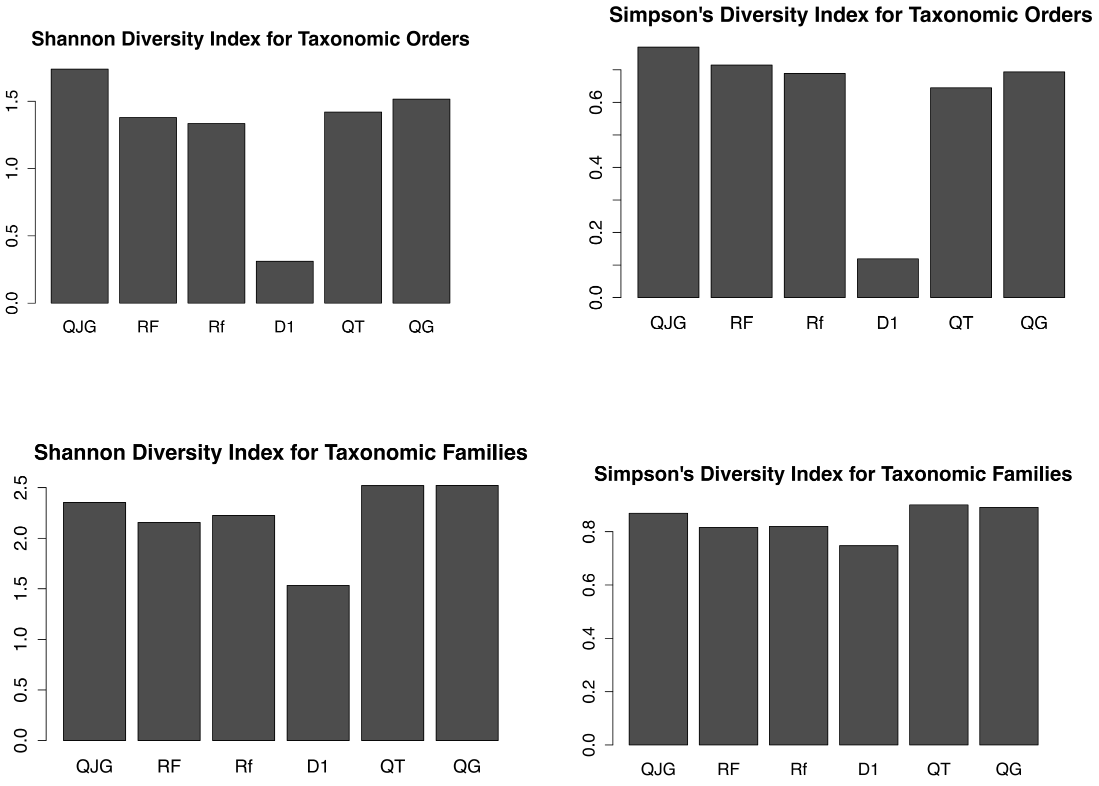
Figure 4: CCA ordination diagram with sites (black), insect orders (red), and environmental variables (blue arrows). Axis one is the horizontal and axis two is the vertical. Values of environmental variables increase in the direction of the arrow head and can be extrapolated back on the opposite side of the origin to represent a decreasing trend. The relative lengths of the arrows indicate their importance in explaining the variation in community composition shown in the diagram

		Undisturbed			Disturbed		
		Juan Grande	Frijoles	Frijolito	Disturbed 1	Trinidad	Grande
Shannon	Order	1.7394	1.3789	1.3347	0.3118	1.4203	1.5162
	Family	2.3543	2.1563	2.2256	1.5336	2.5203	2.5224
	Odonata	1.5662	1.4432	1.4767	0.5623	1.2901	1.3644
	Plecoptera	0	0	0	0	0	0.6616
	Trichoptera	1.1302	0.6931	0.86	0	1.3273	1.4667
Simpson's	Order	0.7699	0.7147	0.6889	0.1189	0.6448	0.6936
	Family	0.8694	0.8161	0.8204	0.7474	0.9004	0.8912
	Odonata	0.7254	0.7064	0.7268	0.375	0.6436	0.6592
	Plecoptera	0	0	0	0	0	0.4688
	Trichoptera	0.5631	0.5	0.5124	0	0.6515	0.669
Richness	Order	9	8	8	6	8	7
	Family	26	20	23	9	26	26

Table 6. Diversity indices at the level of order and family as well as within Odonata, Plecoptera, and Trichoptera families at each stream as indicated by alpha diversity measures.

		Disturbed	Undisturbed	*t*	*p*-value
Shannon	Order	1.082767	1.484333	0.9862	0.3799
	Family	2.2454	2.1921	0.1594	0.8811
Simpson's	Order	0.7245000	0.4857667	1.2868	0.2676
	Family	0.8353000	0.8463333	-0.2105	0.8435
Richness	Order	8.333333	7.000000	2	0.1161
	Family	23.00000	20.33333	0.45	0.676

Table 7. Diversity and richness values for disturbed and undisturbed sites.



Shannon Diversity Index for Taxonomic Orders

Simpson's Diversity Index for Taxonomic Orders

Shannon Diversity Index for Taxonomic Families

Simpson's Diversity Index for Taxonomic Families

Figure 6. Values of diversity indices at the order and family level for each stream as indicated by alpha diversity measures. River codes are as follows Quebrada Juan Grande = QJG, Rio Frijoles = RF, Rio Frijolito = Rf, Disturbed 1 = D1, Rio Trinidad = QT, Quebrada Grande = QG

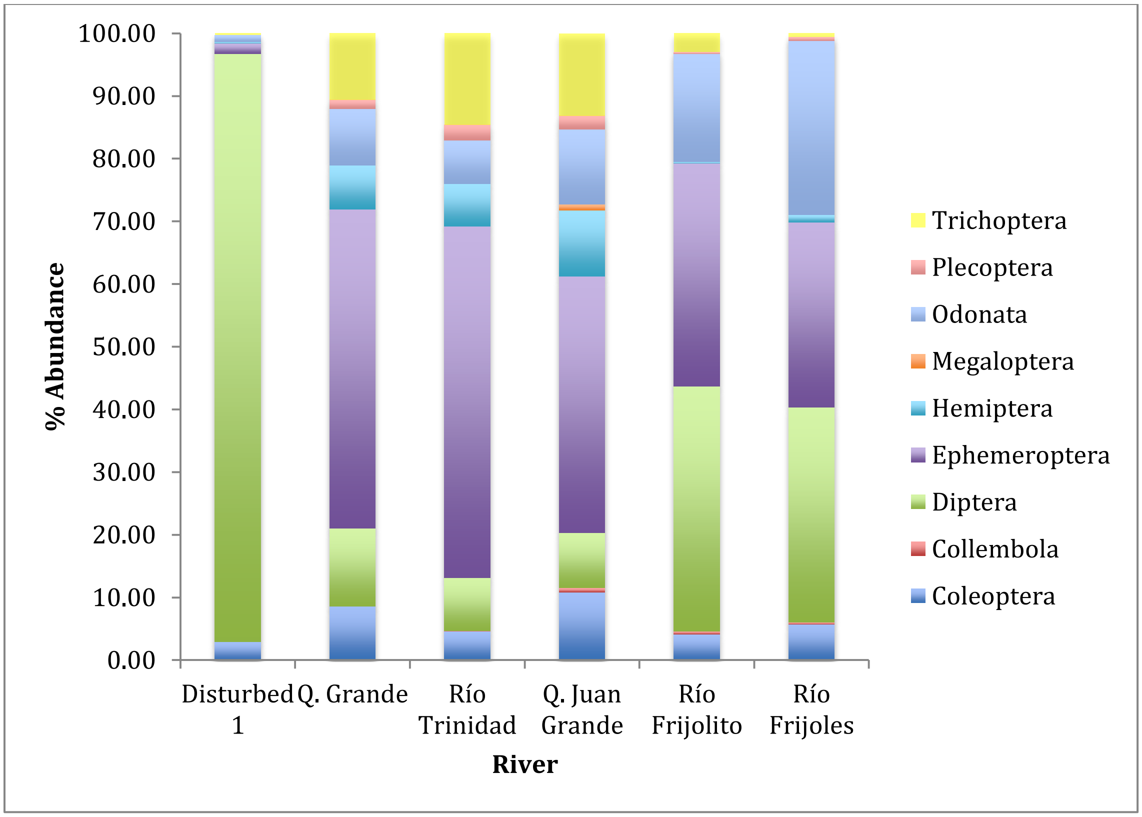


Figure 7. Relative abundances (%) of nine macroinvertebrate orders across our 6 sampled rivers.

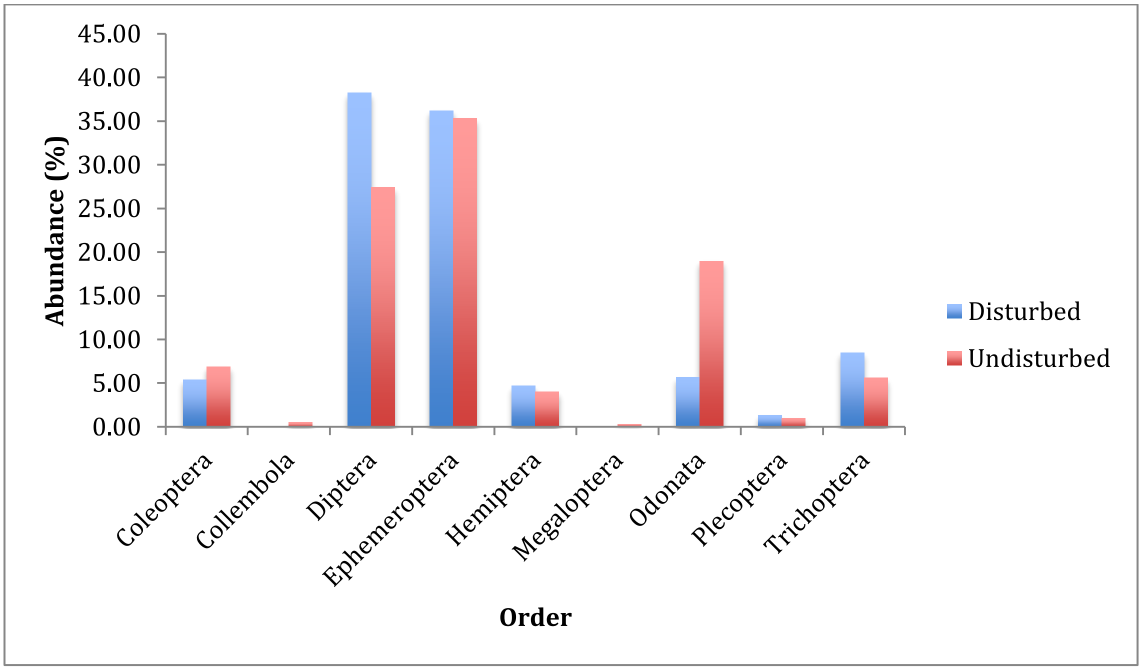


Figure 8. Mean Abundance (%) of nine insect orders found in disturbed vs. undisturbed streams.

		Mean Abundance (%)				
		Disturbed	Undisturbed	Response to Disturbance	F Value	p Value
Order	Odonata	5.68	18.970	Decrease	6.704	0.061
	Collembola	0.029	0.518	Decrease	22.04	**0.009
	Coleoptera	4.402	6.877	Decrease	0.334	0.594
	Diptera	38.215	27.407	Increase	0.124	0.743
	Ephemeroptera	36.201	35.310	Increase	0.039	0.853
	Hemiptera	4.689	4.018	Increase	0.073	0.800
	Megaloptera	0.000	0.313	Decrease	1	0.374
	Plecoptera	1.291	0.997	Increase	0	0.996
	Trichoptera	8.497	5.590	Increase	0.141	0.727
Family	Staphilinidae	0.000	0.331	Decrease	18.91	**0.012
	Gomphidae	0.263	5.973	Decrease	25.4	**0.007
	Protoneuridae	0.060	1.513	Decrease	20.25	**0.011

Table 8. Anova results from abundance (%) comparisons of insect taxa (Order and family) between disturbed and undisturbed streams. Only families with significant differences are shown. Percentages were arcsine transformed. ** Denotes significance (p<0.05).

Order	*F*	*P*-value
Coleoptera	1.638	0.166
Collembola	3.149	0.0145*
Diptera	6.088	0.000154***
Ephemeroptera	3.577	0.00725**
Hemiptera	4.345	0.00214**
Megaloptera	8.051	9.95E-06***
Odonata	9.289	1.99E-06***
Plecoptera	1.413	0.234
Trichoptera	5.209	0.000567***

Table 9. Results from ANOVA comparison of abundances (%) of each order between 6 rivers sampled. *p<0.05, **p<0.01, ***p<0.001.

FFG	Disturbed	Undisturbed	Response to Disturbance	*F*
Collector-Filterer	57.33333333	35.66666667	Increase	0.107
Collector-Gatherer	80.33333333	115	Decrease	5.468
Predator	195.6666667	135.6666667	Increase	0.836
Scraper	22.33333333	9.666666667	Increase	0.726
Shredder	2	13.66666667	Decrease	0.774

Table 10. Mean % Abundance of each functional feeding group between disturbed and undisturbed streams and F-values from ANOVA.

FFG	Disturbed	Undisturbed	Response to Disturbance	*F*
Collector-Filterer	15.84245474	8.378940855	Increase	0.645
Collector-Gatherer	22.99680567	43.80388423	Decrease	3.951
Predator	54.19099121	42.44165205	Increase	0.4
Scraper	6.132315522	2.413773814	Increase	1.21
Shredder	0.71020639	2.961749046	Decrease	0.445

Table 11. Mean Absolute Abundance of each functional feeding group between disturbed and undisturbed streams and F-values from ANOVA.

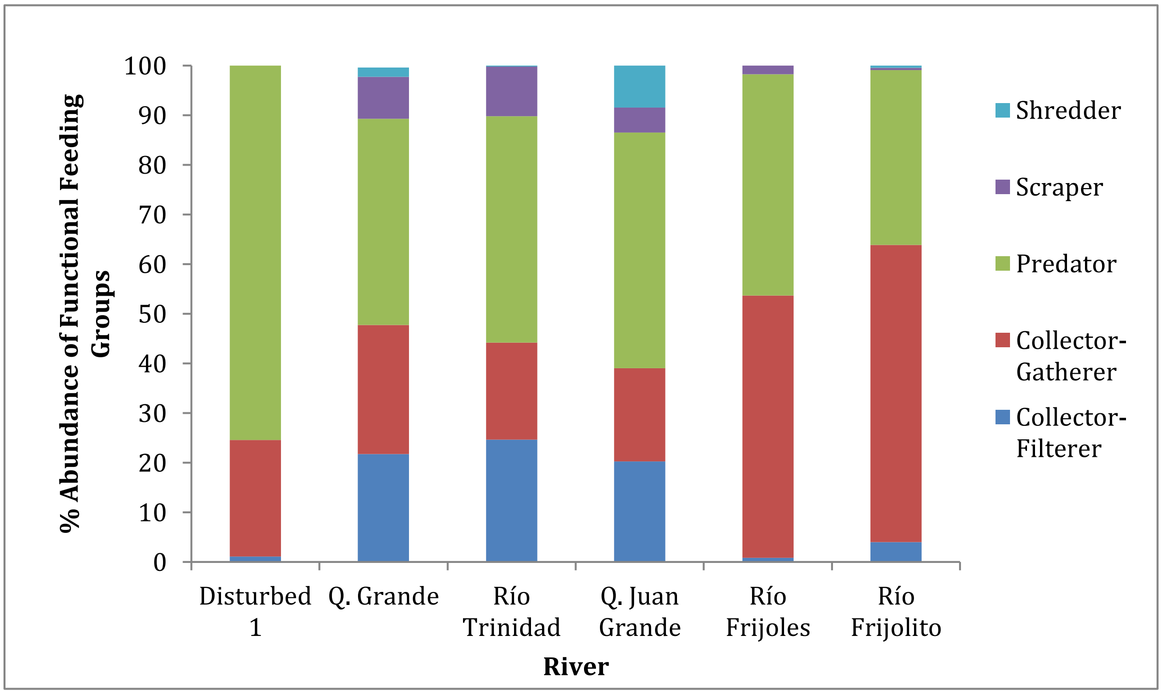


Figure 9. Relative abundance of each functional feeding group within each river.