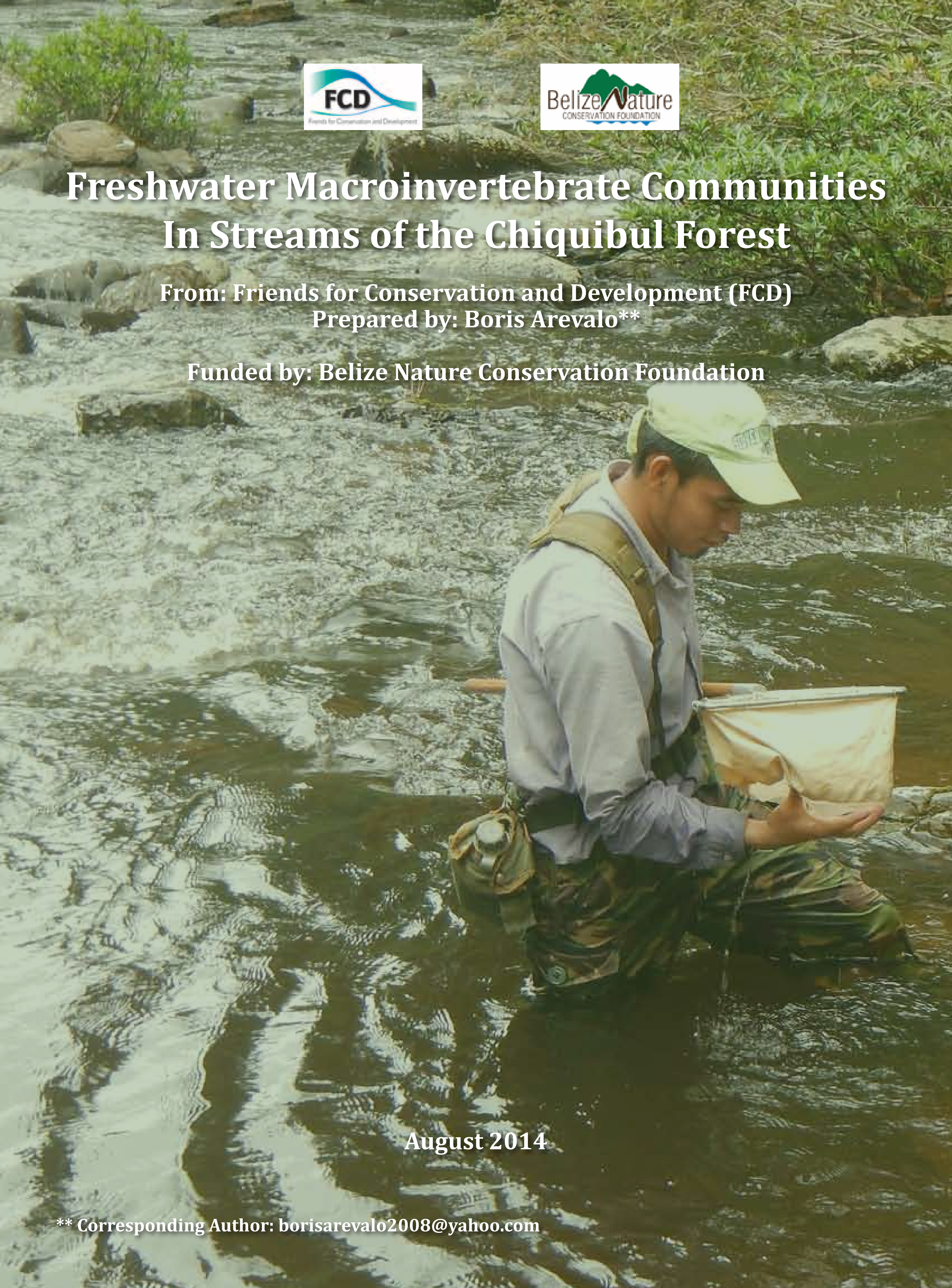
Freshwater Macroinvertebrate Communities

In Streams of the Chiquibul Forest



Freshwater Macroinvertebrate Communities In Streams of the Chiquibul Forest

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August 2014

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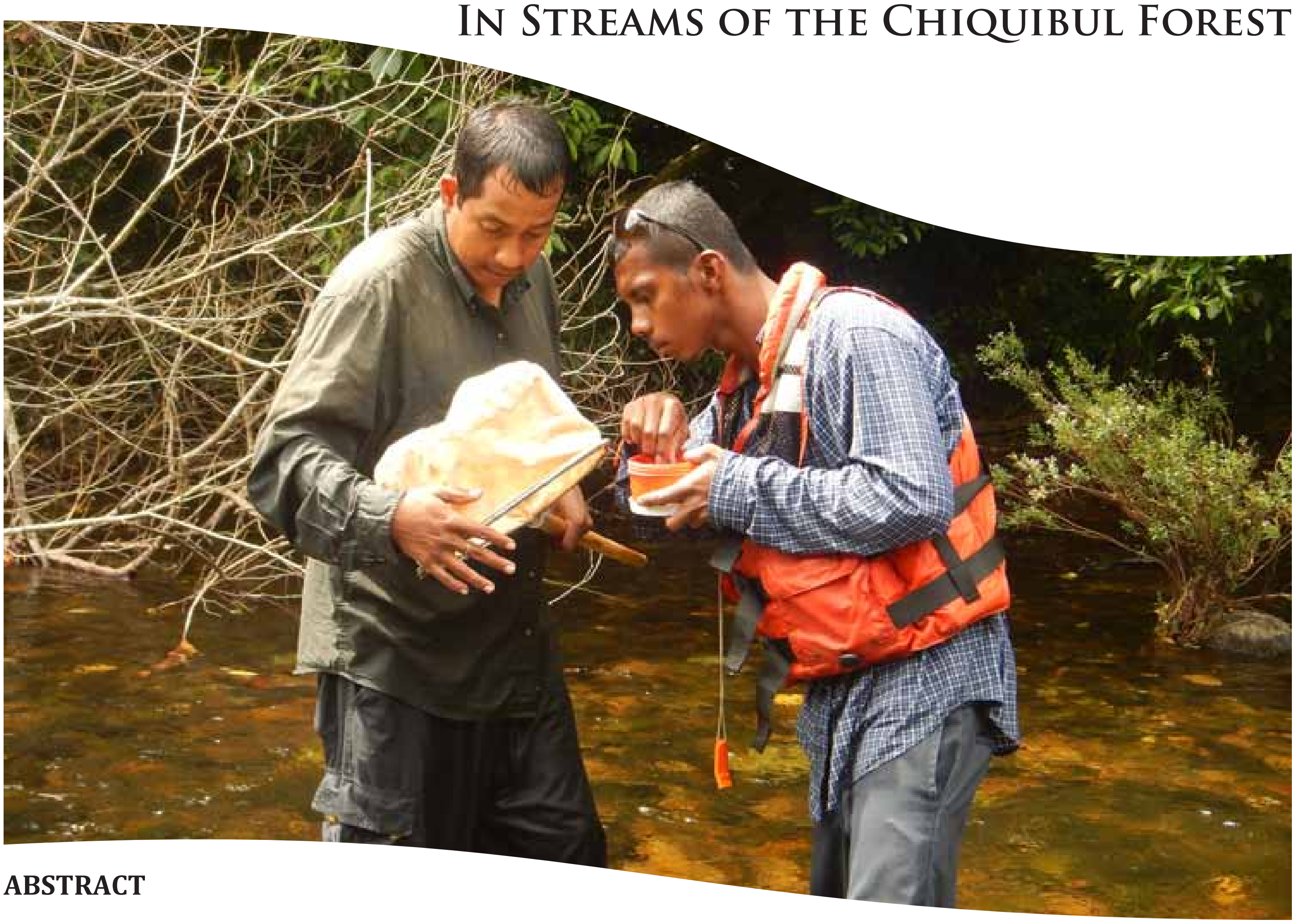


ABSTRACT

Freshwater macroinvertebrates are commonly used as biological indicators since they respond to water quality changes overtime; changes reflected in community composition and abundance. In this study 38 sampling sites were distributed covering four sub-basins in the Chiquibul Forest. A total of 7,785 individuals representing 62 families were recorded. From the 62 families recorded, Elmidae (Coleoptera) was most dominant but overall benthic community showed a much even distribution. In terms of Functional Feeding Groups (FFG), scrapers composed 53% of the recorded abundance followed by predators (18%); which also composed 45% of the benthic community diversity followed by scrapers (21%) and shredders (20%). The greatest community richness was recorded for the Southern Chiquibul and Raspaculo River. The abundance ($F = 3.09$; $p = 0.04$), richness ($F = 4.50$; $p = 0.0092$) and percentage contribution ($F = 5.27$; $p = 0.0043$) of pollution sensitive Ephermeroptera, Plecoptera and Trichoptera did show significant differences as did the abundance of the more pollution tolerant Chironomidae family ($F = 4.03$; $p = 0.0148$). SIGNAL 2 Site Scores were relatively high and showed no significant difference among sub-basins ($F = 1.26$, $p = 0.3022$) indicating relatively healthy headwaters for the Belize River Watershed. The “healthy” stream conditions was also supported by the high percentage abundance (75%) and diversity (66%) of macroinvertebrates expressing sensitivity to pollution based on the SIGNAL 2 Band Score but also indicated that 22% of the abundance and 24% of the richness was composed of organisms showing tolerance to organic pollution. Even though SIGNAL 2 Site Scores were high, the results indicated a significant difference in abundance of macroinvertebrates being very tolerant to pollution ($F = 3.59$, $p = 0.0235$). Higher abundance was recorded in the Southern Chiquibul River sub-basin, area being affected by illegal gold panning but it is important to note that this sub-basin registered greatest taxa richness as well. The indication of high abundance of pollution tolerant species may start to shed some light into the motion that illegal gold mining is impacting the water quality of this system; a trend that can only be detected if a systematic macroinvertebrate monitoring system is set in place.

INTRODUCTION

Freshwater macroinvertebrates are commonly used as biological indicators for aquatic ecosystems health, serving as indirect measure for water quality (Stark *et al*. 2001). Many authors argue that monitoring changes in macroinvertebrate community composition is important as they respond to water quality changes overtime and such changes are reflected in community composition and abundance (Resh and Jackson, 1993; Lenat, 1993; Barbour *et al*., 1995, 1996; Gerritsen, 1995; Fore *et al*., 1996; Wallace *et al*., 1996; Carlisle and Clements, 1999; Roldan 2003). On the contrary, chemical and physical water analysis provides a snap shot of the system only (Alba-Tercedor 1996). Freshwater macroinvertebrates serve as good stream health indicators because they: (i) have great diversity at both species level and at functional groups and abundance; (ii) are relatively sedentary allowing to draw conclusions based on what is happening at the place of capture; (iii) have a relative long life cycle of at least 6 months providing a good snap shot of the dominant physical and chemical conditions of the water body; and (iv) respond to stress (Boothroyd and Stark 2000; Mandaville 2002).

The Chiquibul Forest, an ecological unit, comprised of the Chiquibul National Park, the Chiquibul Forest Reserve and the Caracol Archeological Reserve, contain the headwaters of the Belize River Watershed. This is the most important watershed in Belize, providing water for a Belizean population of over 130,000, for agriculture irrigation along the Belize River Valley and for hydropower generation. Presently it is under stress due to anthropogenic activities such as gold panning, logging, non-timber forest products (legal and illegal) and agricultural encroachments. It is uncertain to what extent all these activities compromise the ecological integrity of the water systems. The objective of this assessment was to study the freshwater macroinvertebrate communities in the major sub-basins of the Chiquibul Forest in order to describe community composition and form a baseline for future monitoring efforts.

METHODOLOGY

Study Site

This study was carried out in the Chiquibul Forest (*Figure 1*), located within the Cayo District, covering an area of 176,999 ha (437,376 acres). It comprises of three protected areas being the Chiquibul National Park (106,838 ha), Chiquibul Forest Reserve (59,822 ha) and the Caracol Archeological Reserve (10,339 ha), with central UTM coordinates 1,878,200 – 1,871,800 North and 265,600 – 322,600 East. Meerman and Sabido (2001) identified 17 different ecosystems within the area, all being variants of Tropical Broadleaf Forests, except for a pine forest and a small non-mechanized agricultural category. The region has a subtropical climate with a marked dry season between February to June and a rainy season coinciding with the hurricane season starting from July to November (Salas and Meerman 2008). Cretaceous limestone forms the parent rocks found in the western half of the Chiquibul while Permian meta-sediments are dominant on the East (*Figure 1*) (Cornec 2003). On the extreme south of the Main Divide there are volcanic deposits. The soils are generally derived from limestone and are regarded fertile in comparison to other tropical areas but on the steeper limestone slopes, Wright *et al.*(1959) classifies the soils as skeletal where the bedrock tends to protrude out as a consequence of the soil layer being a few centimeters thick. Due to the calcareous bedrock of the Chiquibul Forest, many streams and some rivers are subterranean; it also leads to many first order streams drying out during the dry season.

Based on the underlying geology all streams in the Chiquibul Forest can be categorized as hard-bottom streams (meaning gravel, cobbles, boulders and bedrock substrate dominate more than 50% by area of the streambed).

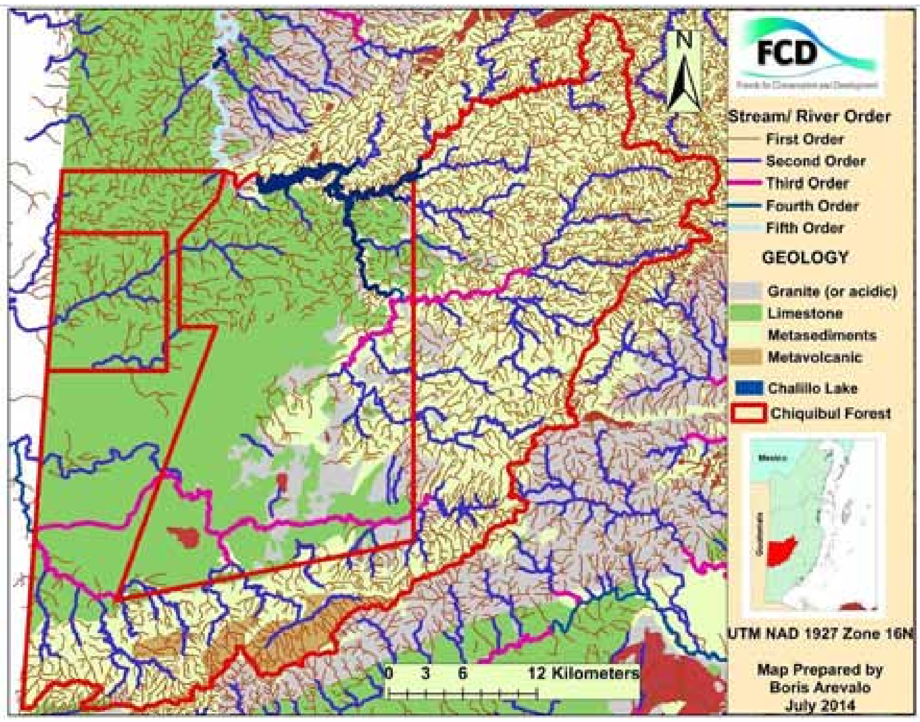


Figure 1*: Hydrology of the Chiquibul Forest.*

Stream channel characterization

Channel characteristics of stream reach often determine the abundance and distribution of benthic macro-invertebrates, so it is important to describe these attributes. The following stream channel characteristics were measured: water discharge, stream average depth and width, stream substrate, macro-habitat diversity, over-story density (using a densitometer), floodplain width and entrenchment depth.

Macroinvertebrate sampling and identification

Sampling was carried out in the headwaters of the Belize River Watershed (*Figure 2*). A total of 38 sample sites [stream reach] (first order: n = 2; second order: n = 20; third order: n = 16 [most to all first order streams dry up at the onset of the dry season]) were surveyed during the dry season (February to May) of 2014. At each sampling site a sequence of riffle-pool-riffle of at least 40 times greater than the width of the stream was surveyed (Klemm *et al*. 2002). At each stream reach micro-habitats representing ≥5% (percentage visually estimated) of the reach area were mapped and sampled. A total of 20 sub-samples were collected from each site but placed in two different containers (one for pool and one for riffle). Distribution of sub-samples was based on percentage of stream reach covered by each micro-habitat, where 1 sub-sample was allotted to every 5% covered by micro-habitat. Samples were collected using the kick and sweep method employing a D-net. This method is highly used in macroinvertebrate research and quite versatile (Resh and Jackson 1993; Carter and Resh 2001). All samples collected were fixed and preserved in 80% ethanol for later identification in the Laboratory. Collected samples were washed under running water, passing through metal sieves of mesh size 4000, 2000, 500 and 250 microns; then preserved in plastic vials with 80% ethanol until these were identified under a dissecting microscope. Identification of macroinvertebrates was done to family level following the Carrie *et al*. 2014 identification key.

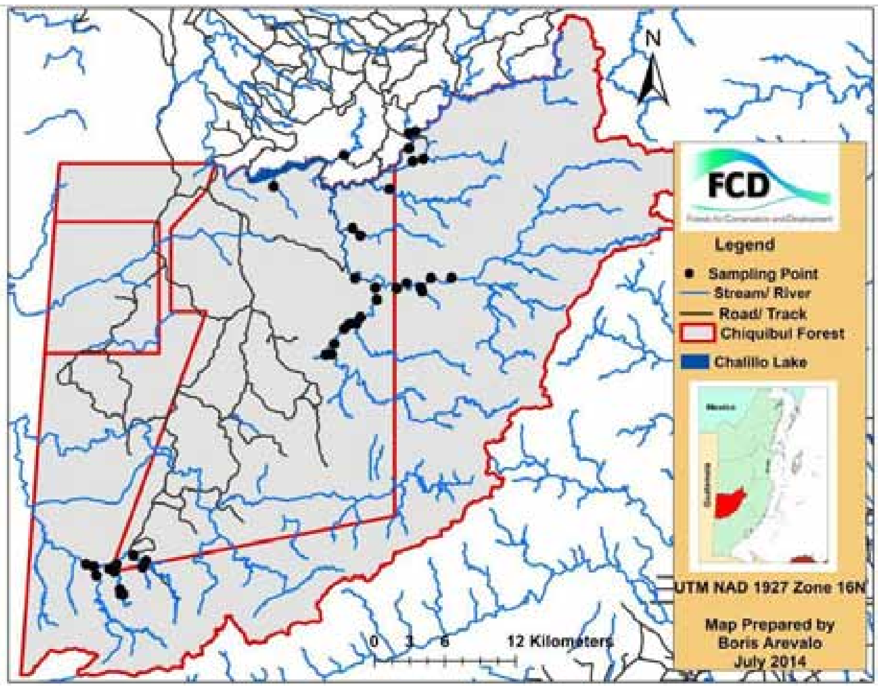


***Figure 2**: Spatial distribution of macroinvertebrate and fish sampling sites in the Chiquibul Forest.*

Drainage Systems

ANOVA tests were conducted using a sub-basin approach. The sub-basins used were Southern Chiquibul River (n = 12), Monkey Tail River (n = 9), Raspaculo River (n = 9) and Macal River (n = 8).

Macroinvertebrate metrics

Macroinvertebrate metrics recorded included richness and abundance by family, order; trophic level (herbivore, carnivore, detritivore, omnivore), Functional Feeding Groups (FFG) (scrappers, predator, filtering collector, gathering collector, shredder) and Stream Invertebrate Grade Number – Average Level 2 (SIGNAL 2), and Band Score. Each macroinvertebrate was assigned to one of four categories based on their respective SIGNAL 2 Grade Score. The categories included: Very sensitive to pollution, Sensitive to pollution, Tolerant to pollution and Very tolerant to pollution, based on Chessman (2003). Composition measures such as EPT (Ephemeroptera, Plecoptera, Trichoptera) richness [the sum of the different families belonging to the three orders], EPT% [percentage that the abundance of Ephemeroptera, Plecoptera and Trichoptera contributed to the total abundance of macroinvertebrates], Chironomidae abundance, EPT/ Chironomidae Ratio [ratio of the total number of Ephemeroptera, Plecoptera and Trichoptera to the total number of Chironomidae] and SIGNAL 2 Site Score were calculated.

SIGNAL 2 Site Scores were calculated following the Chessman (2003) methodology. SIGNAL is a simple biotic index for macroinvertebrates that make use of their organic pollution tolerance to create a site score and water quality rating of an aquatic system. It was especially designed for Australian freshwater systems but can be adapted to other areas but results can be less conclusive. Freshwater systems that have a high SIGNAL site score are likely to have high levels of dissolved oxygen with low levels of turbidity and adequate nutrient levels. Each macroinvertebrate family was assigned a grade number between 1 and 10. A low-grade number means that the organism is tolerant to a range of environmental conditions including forms of water pollution. A high number indicates that the macroinvertebrate is sensitive to most forms of pollution. To calculate the SIGNAL 2 Site Score all macroinvertebrate families were assigned a respective signal grade; then a weighted factor was calculated based on family abundance (1 – 2 individuals = 1; 3 – 5 = 2, 6 – 10 = 3; 11 – 20 = 4; > 20 = 5). The sum of all weight factors was calculated. Subsequently, the grade score was multiplied by the weight factor and a total sum of all products was calculated. To calculate the SIGNAL 2 Site Score the sum of all products from grade score and weight factor were divided by the sum of weight factors. SIGNAL 2 Site Score were then plotted using a biplot as a function of family diversity. A cutting line of 50% on both axis was used to divide biplot in 4 quadrants to determine stream health conditions following Chessman (2003).

Statistical analysis

The statistical package InfoStat (Di Reinzo *et al*. 2008) was used for data analysis. Summary metrics were calculated at stream reach level for both stream physical and environmental variables. Macroinvertebrate metrics were calculated by pooling all 20 sub-samples. Analysis of Variance (ANOVA) using LSD Fisher mean comparisons were performed using calculated macroinvertebrate metrics. Variables that did not meet the ANOVA assumptions were rank transformed. Quality assurance and quality control (QA/QC) procedures for processing (sorting, counting, and identification) of macroinvertebrate samples included recounting and re-identification of 10% (n = 4) of the macroinvertebrate samples (through a random process) and verification of difficult organisms by outside experts (Klemm *et al*. 1990; USEPA 1994, 2001; Barbour *et al*. 1999).

Fish Sampling and analysis

Direct observation of fish species was conducted while sampling for macroinvertebrates at sampling sites. Since the objective was to only conduct an inventory of fish species in the Chiquibul Forest no need for more detail sampling methodology was observed.

RESULTS

General stream characteristics

Over-story density, stream depth and stream width was significantly different among sub-basins (F = 3.27, p = 0.0329; F = 3.24, p = 0.0338; and F = 4.63, p = 0.0080 respectively). The Raspaculo River had the greater stream widths among sites sampled, while Southern Chiquibul River had the narrowest sites sampled. Over-story density was greater on the Macal and Raspaculo Rivers and least at Monkey Tail. The Southern Chiquibul River had the most shallow sampling sites, while Monkey Tail, Raspaculo and Macal Rivers had deeper stream channels but significantly different from each other. Stream water discharge was not significantly different among sub-basins (F= 2.19, p = 0.1076). *Table 1* shows a summary of the stream channel characteristics measured.

Table 1: *Summary of stream channel variables measured by sub-basin in the Chiquibul Forest.*

Drainage	Variable	Mean	Minimum	Maximum
Southern Chiquibul River	Over-story Density (%)	79.88	0	92.5
	Stream width (m)	8.05	2.55	18.85
	Stream depth (cm)	33.01	13.05	59.12
	Volume (m^3/Sec)	34.51	3.97	91.29
	Flood plain width (m)	46.47	8.2	245.2
	Flood prone area (m)	13.46	6.2	31
	Entrenchment depth (m)	1.62	1	4.14
Raspaculo River	Over-story Density (%)	85.81	78.15	92.71
	Stream width (m)	19.13	5.00	35.00
	Stream depth (cm)	53.00	21.92	90.32
	Volume (m^3/Sec)	125.39	10.19	326.7
	Flood plain width (m)	51.78	9.6	142
	Flood prone area (m)	22.31	6	42.6
	Entrenchment depth (m)	3.2	0.96	11.4
Macal River	Over-story Density (%)	87.64	76.82	93.66
	Stream width (m)	10.13	4.75	20.50
	Stream depth (cm)	40.86	13.79	61.99
	Volume (m^3/Sec)	73.48	12.36	296.07
	Flood plain width (m)	39.88	9.8	150
	Flood prone area (m)	11.36	4.4	21.5
	Entrenchment depth (m)	1.7	1	2.7
Monkey Tail River	Over-story Density (%)	64.94	31.66	83.9
	Stream width (m)	16.86	6.50	23.00
	Stream depth (cm)	54.85	32.00	95.00
	Volume (m^3/Sec)	106.93	7.72	264.85
	Flood plain width (m)	74.22	31	150
	Flood prone area (m)	36.78	23	50
	Entrenchment depth (m)	3.16	2	4.4

Global freshwater macroinvertebrate community structure

A total of 7,785 individuals representing 62 macroinvertebrate families were recorded. The highest number of individuals were collected from Sample Site FMP006 (n = 544), followed by FMP001 (n = 527); while FMP020 (n = 34) and FMP014 (n = 9) yielded the least number of individuals (*Figure 3*). Greatest family richness was recorded at site FMP003 (n = 29), followed by FMP001 and FMP034 (n= 27); while FMP020 (n = 11) and FMP014 (n = 7) recorded lowest number of families (*Figure 4*). Number of individuals collected and family richness was 1.72 and 1.4 times greater in riffles than at pools, respectively.

Fish communities

Fish communities in the streams of the Chiquibul Forest are not diverse and share similar composition across sub-basins. Appendix I indicates fish species recorded. A factor that may help explain low fish diversity in the Chiquibul Forest may be attributed to major rivers having complex systems of natural obstructions such as waterfalls, preventing the establishment of diverse fish assemblages (Greenfield & Thomerson 1997).

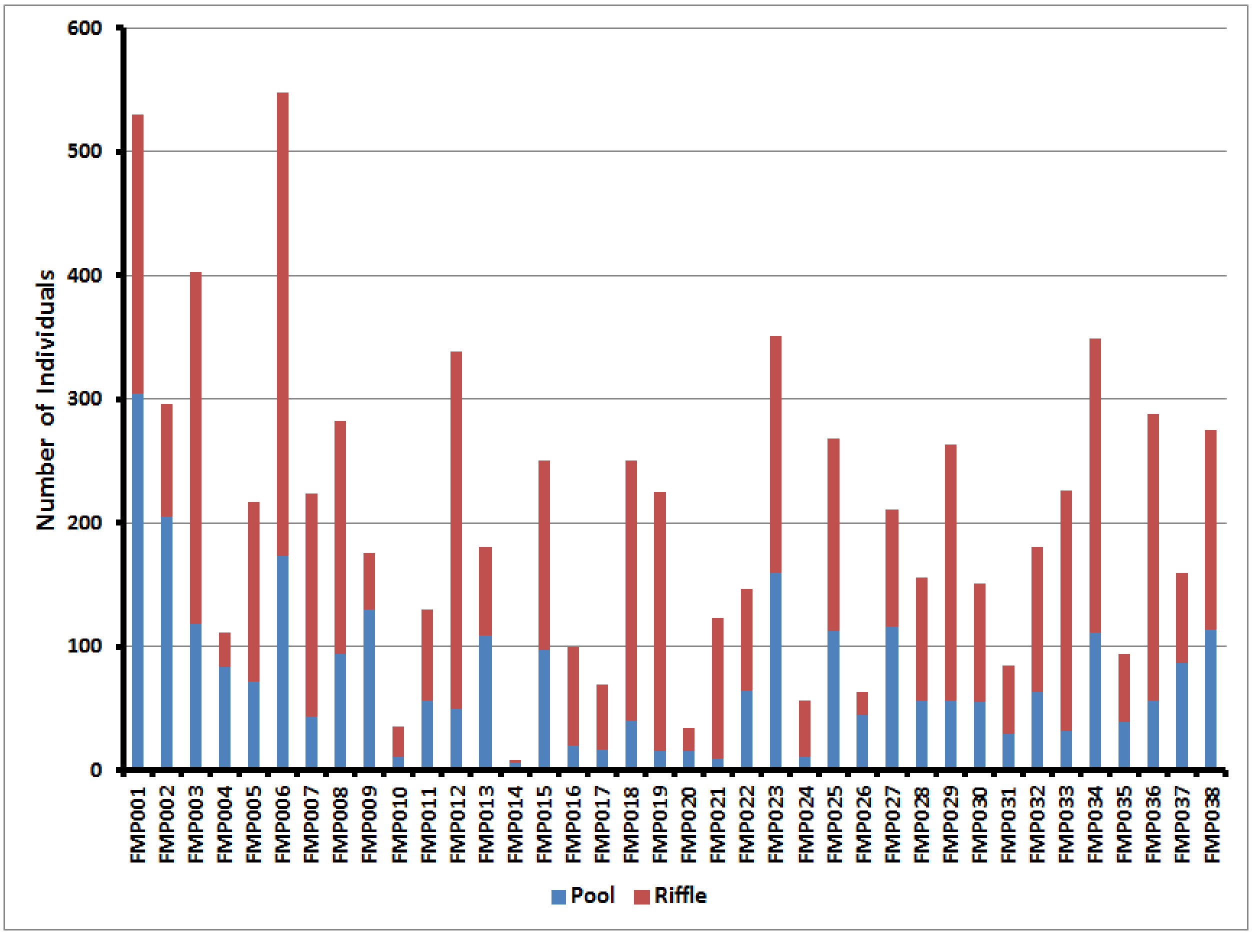


Figure 3: *Total individual macroinvertebrates collected per site in the Chiquibul Forest*

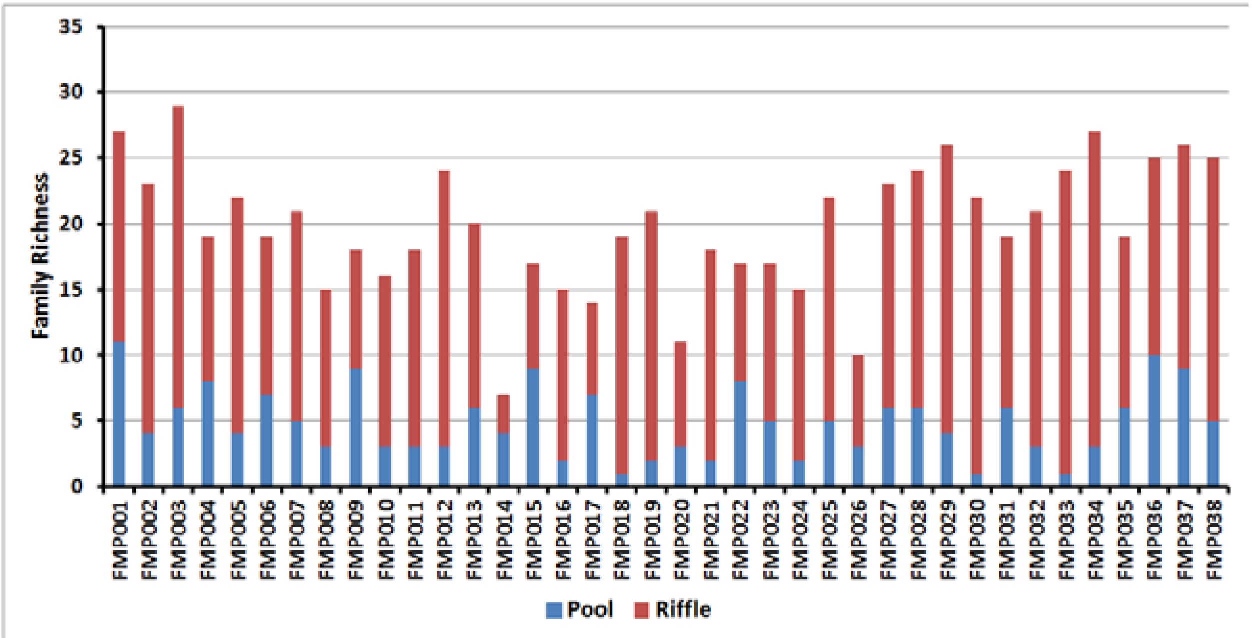


Figure 4: *Macroinvertebrate family richness by sampling site in the Chiquibul Forest*

Most abundant orders recorded were Ephemeroptera, Coleoptera, Diptera and Trichoptera while Decapoda and Oligochaeta were some of the least abundant (*Figure 5*).

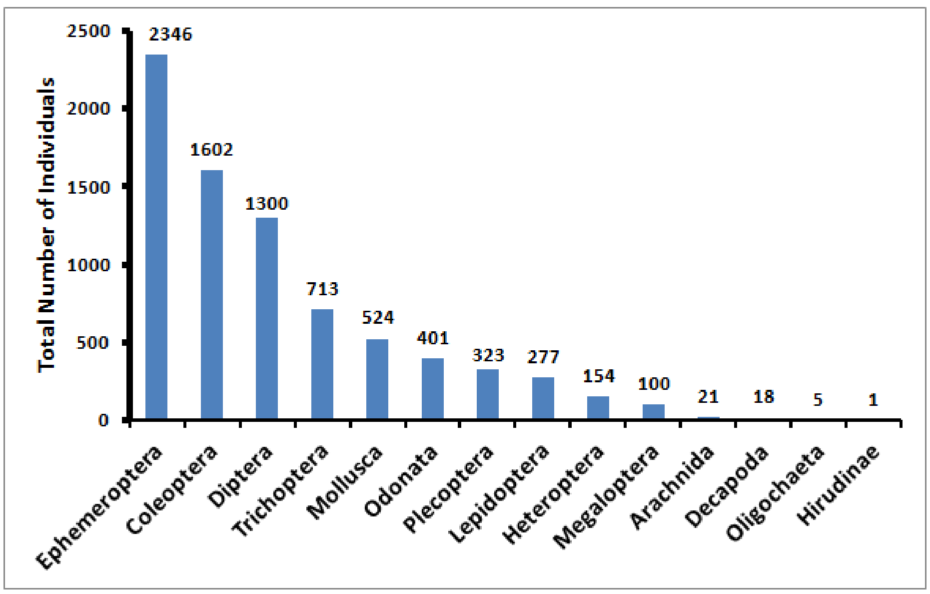


Figure 5*: Total number of individuals per order.*

The most abundant families recorded were Elmidae (n = 1220), Baetidae (n = 990), Leptophlebiidae (n = 736), Chironomidae (n = 512) and Pachychilidae (n = 498); while the least abundant were Belostomatidae, Ephydridae, Glossiphoniidae, Syrphidae and Tabanidae, each represented by a single individual. There was no noticeable dominance by a particular family as the most abundant family only showed a relative abundance of less than 0.15 (*Figure 6*).

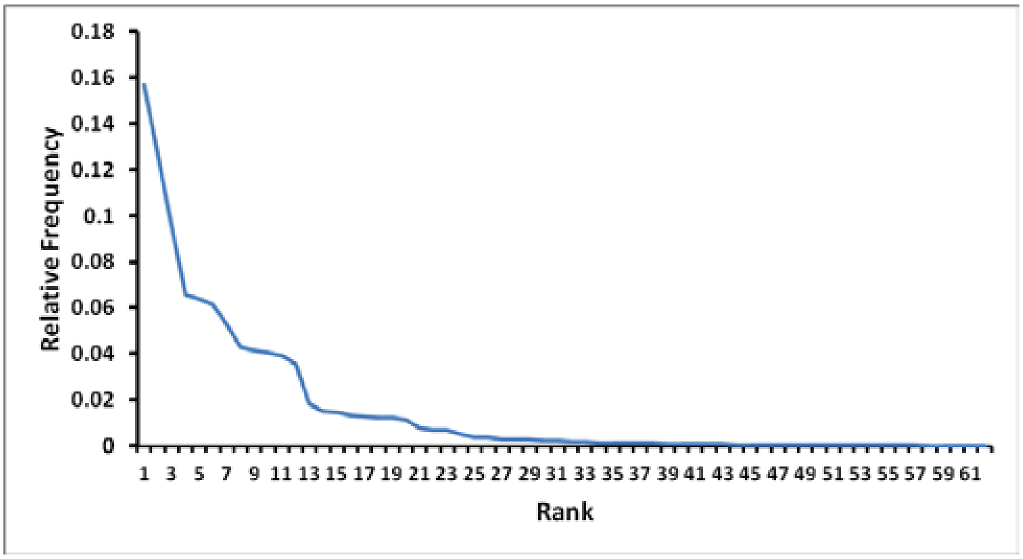
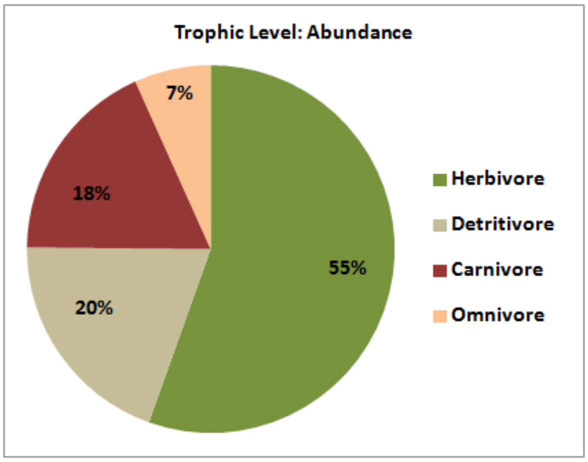


Figure 6: *Rank-Abundance Curve for freshwater macroinvertebrate families recorded in streams of the Chiquibul Forest.*

Based on trophic level, herbivores formed more than 50% of the total recorded individuals while omnivores represented only 7% (*Figure 7* A). Based on family richness, carnivores represented 45%, while herbivores and detritivores represented a similar percentage of richness (*Figure 7* B). Based on Functional Feeding Groups (FFG); Scrapers accounted for 53% of the total number of individual macroinvertebrates collected while Fileter Collectores and Shredders were the least abundant (*Figure 7* C). 45% of the richness were predators followed by scrapers (21%), while filtering collectors was the least diverse FFG (*Figure 7* D).



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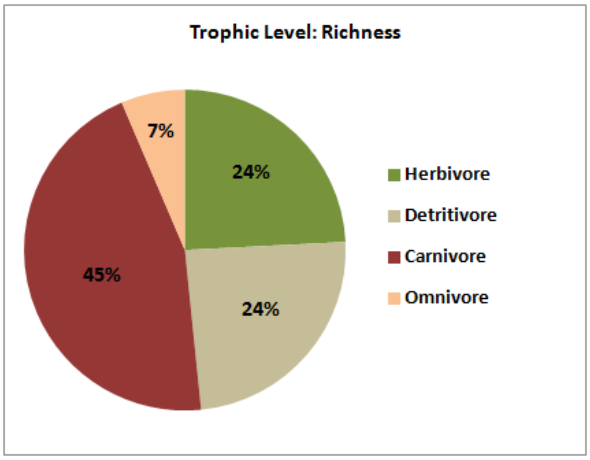

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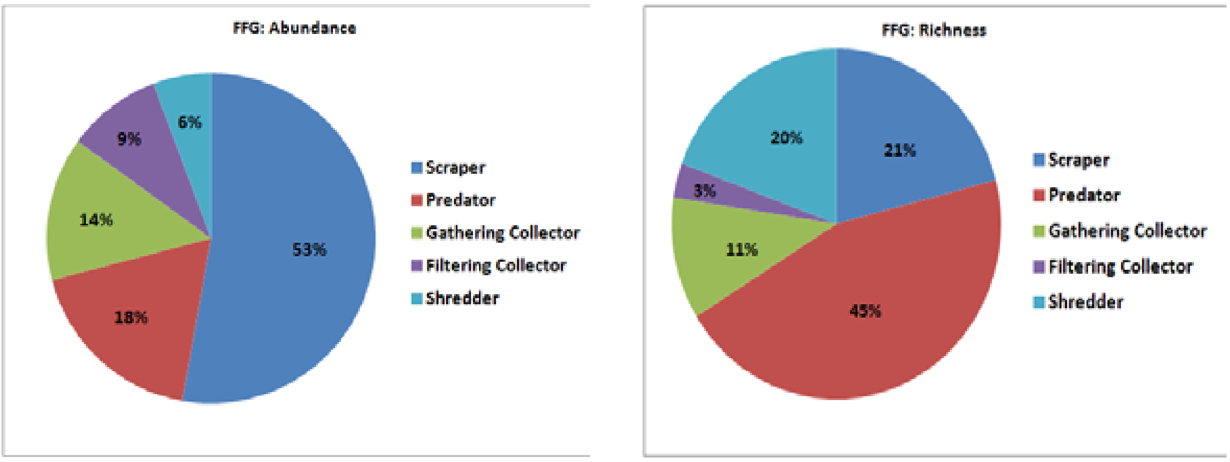


C **D**

Figure 7: *Total number of individuals and richness for trophic level and Functional Feeding Group for macroinvertebrate communities in the Chiquibul Forest.*

Macroinvertebrates expressing sensitivity to organic pollutants (based on the Stream Invertebrate Grade Number Average Level 2 Biotic Index [SIGNAL 2] Band) accounted for 75% and 66% of recorded benthic community abundance and richness, respectively (*Figure 8*). Organisms showing high tolerance to pollution accounted for 3% of the recorded benthic community abundance but represented 10% of the recorded richness (*Figure 8*).

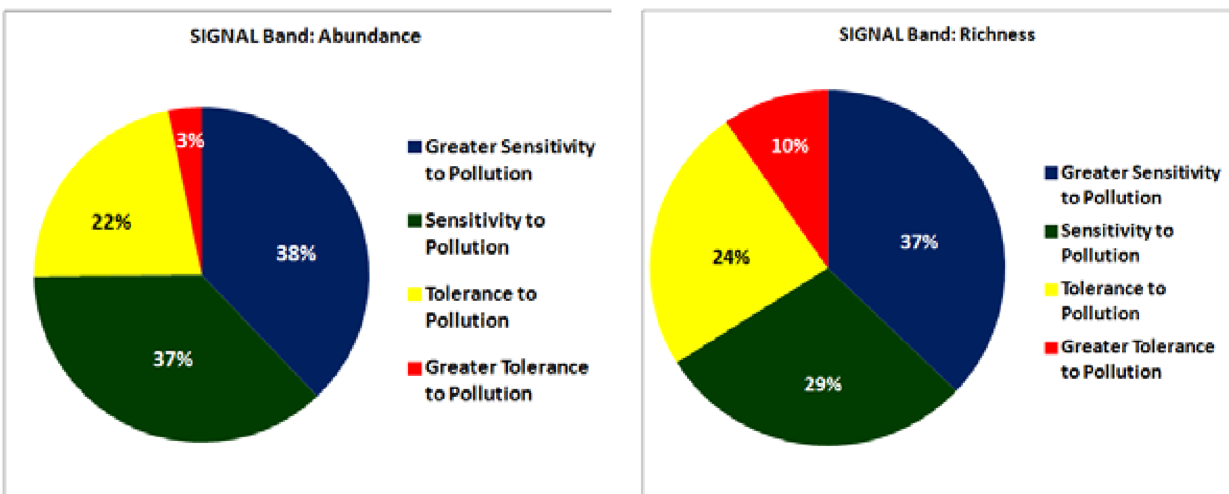


Figure 8: *Percentage abundance and richness of macroinvertebrates based on sensitivity and or tolerance to pollution (SIGNAL 2 Band Score).*

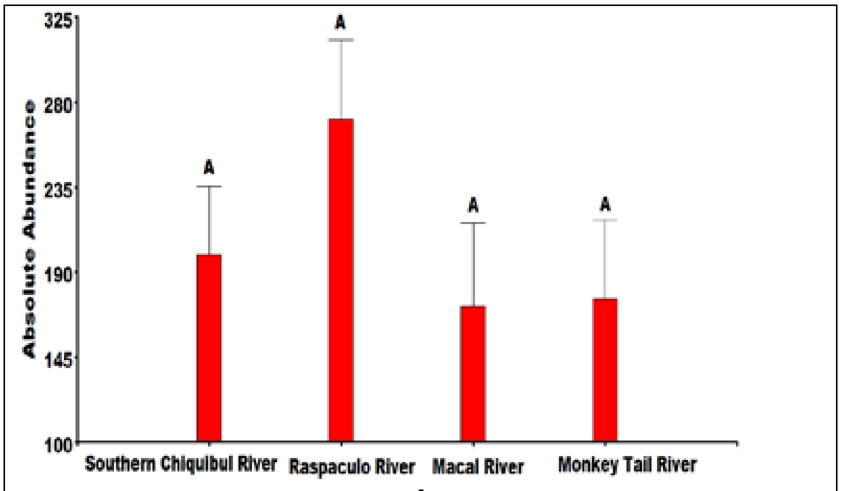
Macroinvertebrate community structure based on geology

Geology is believed to play an important role in determining freshwater macroinvertebrate communities. Results indicate that there was no significant difference based on geology for total number of individuals, richness, EPT total number of individuals, EPT richness, EPT %, EPT/ Chironomidae ration and SIGNAL 2 Site Score (*Table 2*).

***Table 2**: Mean comparisons for macroinvertebrate community composition metrics based on geology of the area. Limestone (n = 8), metasediments (n = 30). Mean with different letters are significantly different (p≤ 0.05)*

Variable	Geology		F-value	p-value
	limestone	metasediments		
Total Number of Individuals	174.50 ± 44.73	212.97 ± 23.10	0.58	0.4498
Richness	20.88 ± 1.76	19.60 ± 0.91	0.41	0.5239
EPT Total Number of Individuals	78.13 ± 27.48	91.90 ± 14.90	0.20	0.6587
EPT richness	7.13 ± 0.85	6.63 ± 0.44	0.26	0.6119
EPT %	41.15 ± 5.83	37.75 ± 3.01	0.27	0.6074
EPT/ Chironomidae ratio	8.04 ± 6.16	15.44 ± 3.18	1.14	0.2928
SIGNAL 2 Site Score	5.48 ± 0.13	5.70 ± 0.07	3.61	0.0655

Macroinvertebrate community structure at the sub-basin level



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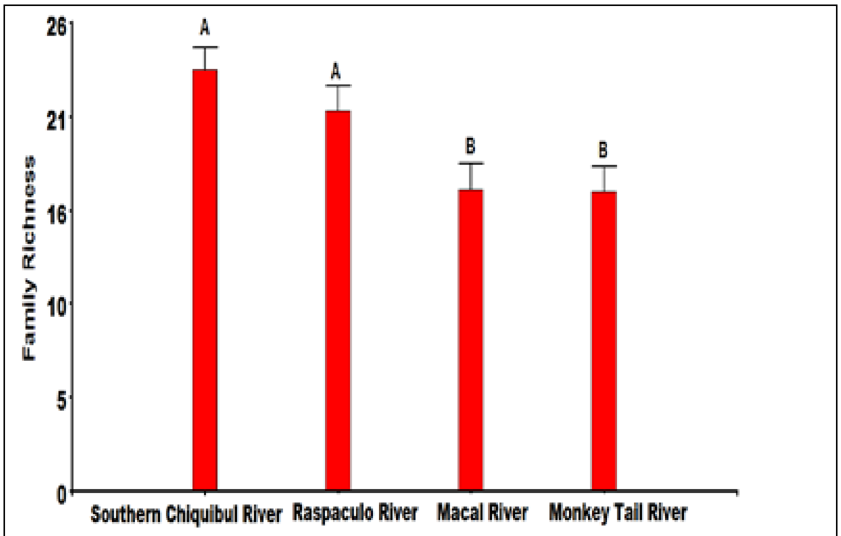

B

Figure 9: *Mean comparison of abundance (**A**) and Richness (**B**) of benthic communities recorded by sub-basin in the Chiquibul Forest.*

Total number of individuals showed no significant difference (F = 1.21; p = 0.3218) among sub-basins studies (*Figure 9* A) but family richness did (F = 6.69; p = 0.0011). The Southern Chiquibul and Raspaculo Rivers recorded greater family richness (*Figure 9* B), than that of the Macal and Monkey Tail Rivers. Both Shannon (F = 7.70, p = 0.0005) and Simpson (F = 3.20, p = 0.0356) Diversity Indexes showed significant statistical differences. With the Shannon Diversity Index, the Southern Chiquibul River showed significant greater diversity than the other three drainages; while with the Simpson Diversity Index, the Southern Chiquibul River recorded greater diversity than Macal and Monkey Tail River, and the Raspaculo River showed an intermediate diversity.

Mean trophic level abundance was not significantly different for herbivores, carnivores, detritivores and omnivores (*Table 3*). Mean trophic level richness was significantly different for carnivores and detritivores. Greater carnivore richness was recorded for the Southern Chiquibul River, while the other three sub-basins had less mean richness but similar to each other (*Table 3*). Highest detritivore richness were recorded for both Southern Chiquibul and Raspaculo Rivers.

Table 3: *Mean comparison for trophic level abundance and richness recorded in the sub-basins of the Chiquibul Forest. Mean with different letters are significantly different (p ≤ 0.05).*

Variable	Sub – basins				F-Value	p-Value
	Southern Chiquibul River	Raspaculo River	Macal River	Monkey Tail River		
Abundance						
Herbivore	85.83 ±23.16	172 ± 27.27	101.25 ± 28.92	103.33 ± 27.27	2.10	0.1187
Carnivore	47.25 ± 6.19	26.78 ± 7.14	32.13 ± 7.58	38.44 ± 7.14	1.75	0.1755
Detritivore	50.33 ± 12.72	63.56 ± 14.69	29.88 ± 15.58	13.00 ± 14.69	2.34	0.0905
Omnivore	15.83 ± 4.04	8.78 ± 4.67	8.38 ± 4.95	20.89 ± 4.67	1.64	0.1988

Richness						
Herbivore	6.75 ± 0.49	6.89 ± 0.57	5.38 ± 0.60	5.89 ± 0.57	1.61	0.2050
Carnivore	9.75 ± 0.68 **a**	7.00 ± 0.78 **b**	7.13 ± 0.83 **b**	6.89 ± 0.78 **b**	3.80	**0.0189**
Detritivore	5.17 ± 0.37 **a**	6.22 ± 0.43 **a**	3.25 ± 0.46 **b**	2.67 ± 0.43 **b**	14.89	**0.0001**
Omnivore	1.75 ± 0.24	1.0 ± 0.28	1.0 ± 0.30	1.22 ± 0.24	1.91	0.1467

Mean abundance for the Functional Feeding Group (FFG) Scrapers, Predators, Filter Collectors and Shredders was not significantly different among all sub-basins but was for Gathering Collectors (*Table 3*). The Southern Chiquibul River registered highest Gathering Collector abundance while the Monkey Tail River had the least. Results indicate significant mean richness difference for the FFG's Predators, Gathering Collectors and Shredders (*Table 4*). Highest mean predator richness was recorded for Southern Chiquibul River, while the other sub-basins had lower richness both similar to each other. Monkey Tail River showed lowest Gathering Collector richness, while Southern Chiquibul, Macal and Raspaculo River had greatest richness and similar to each other. Shredder mean richness was similar in the Southern Chiquibul and Raspaculo River but higher than that of the Macal and Monkey Tail River (*Table 4*).

Table 4: *Mean comparison for Functional Feeding Group (FFG) abundance and richness recorded in the sub-basins of the Chiquibul Forest. Mean with different letters are significantly different (p ≤ 0.05).*

Variable	Sub – basins				F-Value	p-Value
	Southern Chiquibul River	Raspaculo River	Macal River	Monkey Tail River		
Abundance						
Scrapers	80.25 ± 23.12	164.22 ± 26.69	99.50 ± 28.31	97.11 ± 26.69	2.04	0.1260
Predators*	24.38 ± 3.10	14.06 ± 3.58	16.69 ± 3.80	20.94 ± 3.58	1.83	0.1604
Gathering collectors*	26.21 ± 2.63 **a**	23.61 ± 3.04 **ab**	16.50 ± 3.22 **bc**	9.11 ± 3.04 **c**	6.96	**0.0009**
Filtering collectors*	21.46 ± 3.19	19.28 ± 3.68	15.63 ± 3.90	22.33 ± 3.68	1.08	0.3708
Shredders*	21.42 ± 3.00	26.22 ± 3.47	13.63 ± 3.68	15.44 ± 3.47	2.70	0.0612
Richness						
Scrapers	6.00 ± 0.44	6.22 ± 0.51	5.00 ± 0.54	5.00 ± 0.51	1.64	0.1980
Predators	9.75 ± 0.68 **a**	7.00 ± 0.78 **b**	7.13 ± 0.83 **b**	6.89 ± 0.78 **b**	3.80	**0.0189**
Gathering collectors	3.17 ± 0.22 **a**	3.11 ± 0.26 **a**	2.63 ± 0.27 **a**	1.67 ± 0.26 **b**	7.64	**0.0005**
Filtering collectors	1.42 ± 0.20	1.33 ± 0.23	0.75 ± 0.24	1.22 ± 0.23	1.66	0.1933
Shredders	3.08 ± 0.32 **a**	3.44 ± 0.37 **a**	1.25 ± 0.39 **b**	1.89 ± 0.37 **b**	7.63	**0.0005**

Abundance, richness and percentage of Ephemeroptera, Plecoptera and Trichoptera (EPT) was significantly different among drainages (*Table 4*). EPT abundance and percentage was highest at Raspaculo River, while lowest at Macal and Monkey Tail Rivers. Chironomidae abundance was significantly different, being highest as Raspaculo River while Monkey Tail River reported low Chironomidae abundance. EPT/ Chironomidae Ratio was not significantly different (*Table 4*).

Table 5: *Mean comparison for EPT metrics Chironomidae metrics measured in the sub-basins studied in the Chiquibul Forest. Mean with different letters are significantly different (p ≤ 0.05). * = variable was Rank Transformed.*

Variable	Sub – basins				F-Value	p-Value
	Southern Chiquibul River	Raspaculo River	Macal River	Monkey Tail River		
EPT Abundance	85.58 ± 20.53 **ab**	147.67 ± 23.70 **a**	49.50 ± 25.14 **b**	70.00 ± 23.70 **b**	3.09	**0.04**
EPT Richness	7.67 ± 0.61 **a**	8.11 ± 0.70 **a**	5.25 ± 0.75 **b**	5.44 ± 0.70 **b**	4.50	**0.0092**
EPT %	42.43 ± 4.07 **ab**	50.66 ± 4.69 **a**	26.06 ± 4.98 **c**	32.00 ± 4.69 **bc**	5.27	**0.0043**
Chironomidae Abundance*	21.75 ± 2.87 **ab**	27.33 ± 3.31**a**	14.94 ± 3.51 **bc**	12.72 ± 3.31 **c**	4.03	**0.0148**
EPT/ Chironomidae Ratio*	19.50 ± 3.21	15.56 ± 3.70	18.38 ± 3.93	24.44 ± 3.70	1.00	0.4051

Mean macroinvertebrate abundance being very sensitive to pollution (F= 0.09, p = 0.9662), sensitive to pollution (F = 1.28, 0.2970) and tolerant to pollution (F= 2.43, 0.0823) [based on SIGNAL 2 Band Score] showed no significant difference among sub-basins studied but there was significant difference for mean abundance of macroinvertebrates being very tolerant to pollution (F = 3.59, p = 0.0235) (*Figure 1* A). Southern Chiquibul Rivers reported greatest abundance of macroinvertebrates being very tolerant to pollution, while the Macal and Monkey Tail Rivers had the least. SIGNAL 2 Band Score for mean richness of very sensitive to pollution (F =1.80, p = 0.1659) and very tolerant to pollution (F = 2.20, p = 0.1062) macroinvertebrates were not significantly different among sub-basins in the Chiquibul Forest but significant difference was recorded for sensitive to pollution (F= 2.82, p = 0.0500) and tolerant to pollution (F = 9.72, p = 0.0001) macroinvertebrates (*Figure 10* B). Highest mean richness of sensitive to pollution and tolerant to pollution organisms was recorded for the Southern Chiquibul River, while the Monkey Tail River had the least mean richness (*Figure 10* B).

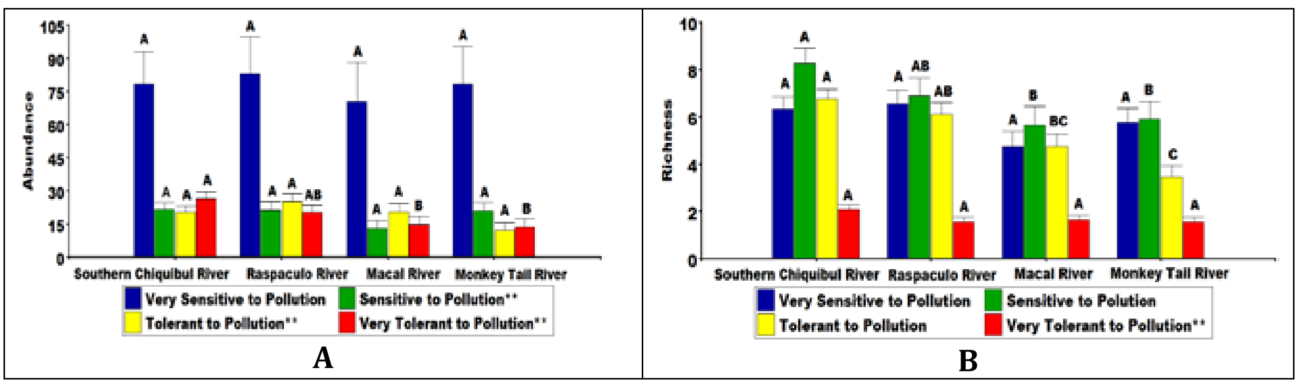

Figure 10*: Mean abundance and richness for SIGNAL 2 Band scores for macroinvertebrate communities studied in the Chiquibul Forest. different letters indicate significant mean difference p ≤ 0.05.*

Results indicated no significant SIGNAL 2 Site Score mean difference ($F = 1.26$, $p = 0.3022$). The biplot in *Figure 11* indicates that 93% of all sampling sites fell in Quadrant 1; indicating healthy streams with high macro-habitat diversity plus 98% of the sampled sites showed a high SIGNAL 2 Site Score ranging between 5 and 6.6.

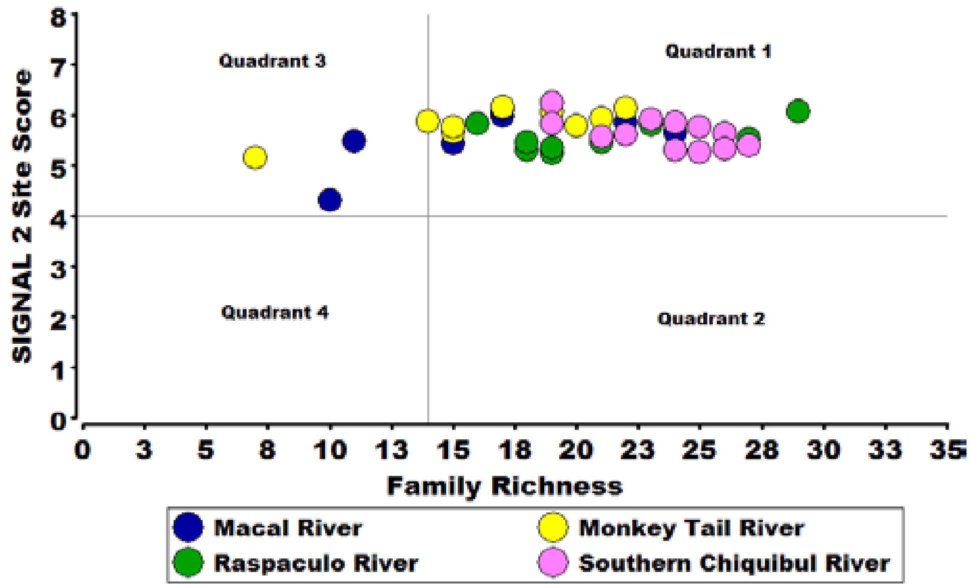

Figure 11: *SIGNAL 2 Site Scores for streams surveyed in the Chiquibul Forest.*

DISCUSSION

Stream channel characteristics and water discharge differ across flowing water ways in the Chiquibul Forest but no significant relationship was found with freshwater macroinvertebrate communities. There was no difference in abundance and richness of macroinvertebrates based on the geology of streams within the Chiquibul Forest, as has been noted by other researchers (Carrie pers. Com. 2014). Benthic communities were well represented in the four sub-basins of the Chiquibul Forest but the Southern Chiquibul River drainage revealed indications of high abundance of organic pollution tolerant species; an abundance significantly different from the Macal, Monkey Tail and Raspaculo Rivers. From the 62 families recorded, Elmidae (Coleoptera) was most dominant but in general terms there was much evenness in abundance of benthic community species; a typical characteristic of a relatively healthy ecosystem with diverse niches created by high microhabitats diversity in the sampled streams and rivers.

The trophic level analysis indicated a high abundance of herbivores (55%) followed by detritivores (20%) but this pattern was not seen in terms of diversity where a high percentage of carnivore species (45%) followed by 48% of the diversity being accounted by herbivores and detritivores was observed. Similar patterns were observed in terms of Functional Feeding Groups (FFG), where scrapers composed 53% of the abundance followed by predators; predators also composed 45% of the diversity followed by scrapers and shredders. The observed pattern does not follow much of the River Continuum Concept (RCC) (Vannote *et al.* 1980) as all sampling site were located in headwater stream (first order = 2 sampling sites, second order = 20 sampling sites and third order = 16 sampling sites). Based on the RCC, headwater streams should harbor high percentage of shredders (> 50%) (Vannote *et al.* 1980) followed by scrappers and predators but in the Chiquibul Forest streams, these guilds were not dominant.

Environmental factors that may have affected these patterns were heavy flooding events occurring during the later months of 2013 and January of 2014. These flooding events washed away course and fine particulate organic matter (CPOM and FPOM), causing a shift in the abundance of shredders and detritivores as these FFG may have been displaced by the strong flowing waters and displacements of respective micro-habitats. CPOM and FPOM on average represented less than 10% of the area sampled at each stream reach while bedrock, boulders, cobbles, gravel and sand accounted for more than 75% of the sampled substrate. The high abundance of the former microhabitats and over-story density of less than 80% may explain the high abundance and diversity of scrapers as these hard surface microhabitats plus adequate amounts of direct sunlight allow for the establishment of algae and other aquatic vegetation.

Family richness significantly differs among the sub-basins studied. The Southern Chiquibul and Raspaculo River recorded greatest diversity. The abundance, richness and percentage contribution of pollution sensitive Ephermeroptera, Plecoptera and Trichoptera did show significant differences as did the abundance of the more pollution tolerant Chironomidae family among sub-basins but did not segregated sub-basins from each other preventing preliminary conclusion as to which of these systems is more polluted relative to the others. The EPT/ Chironomidae Ratio was high for all sub-basins and was not significantly different, indicating that studied streams are in relative "healthy" conditions. The "healthy" stream conditions was also supported by the high percentage abundance (75%) and diversity (66%) of macro-invertebrates expressing sensitivity to pollution based on the SIGNAL 2 Band Score but also indicated that 22% of the abundance and 24% of the richness was composed of organisms showing tolerance to organic pollution.

The SIGNAL 2 Site Score also revealed the "healthy" state of all studied streams (no significant difference among sub-basins) having an average site score of 5.65 of a maximum of 10. Chessman 2003, states that most SIGNAL 2 Site Scores using the family as the lowest possible taxonomic level yields scores no greater than 7. The SIGNAL 2 Site Score as a function of macroinvertebrate family richness clearly indicated the "healthy" state of the streams as 93% of the sites were located in Quadrant 1. Chessman (2003), suggests that site in Quadrant 1 are typical of relatively undisturbed natural aquatic systems with good forest cover supporting high macroinvertebrate diversity and functional groups and stress factors such as toxic chemicals and other pollutants plus harsh physical conditions are absent. Sites falling in Quadrant 3, showed low macroinvertebrate richness but high Site Score values. This happens because of the presence of harsh physical conditions such as limited micro-habitat diversity and extreme water level dynamics. In this study the three sites falling within Quadrant 3 showed the stated limiting factors; where both of the Macal River sites were located in the Mountain Pine Ridge Forest Reserve with limited micro-habitat diversity (mostly solid granite bedrock) and open canopy with shallow waters. The Monkey Tail River site showed indications of transforming into standing pools of water during extended drought periods.

Even though SIGNAL 2 Site Score indicate healthy stream conditions, closer analysis of taxa sensitivity and tolerance to pollution indicated a significant difference in abundance of very tolerant to pollution macroinvertebrates. Higher abundance of these organisms was recorded in the Southern Chiquibul River that also registered highest richness of macroinvertebrates. Higher abundance of very tolerant to pollution organisms in the Southern Chiquibul Forest may be attributed to perturbations in the waterways caused by illegal gold extraction. Illegal gold panning has been adding unknown amounts of pollutants and sediments to the water column plus altering micro-habitats' spatial distribution and diversity, since all of the activity is concentrated to the streams' beds and immediate banks. Other studies have also attributed the occurrence of high counts of Total Coliform and *Escherichia coli* to illegal gold extractors since these individuals that can range over a hundred in the area have no sanitary facilities (Belize Environmental Technologies 2012). The Ceibo Chico and Ceibo Grande creeks, which are major tributaries to the Southern Chiquibul River have also been altered by legal alluvial or placer gold mining since 1999 but the mining company had halted all operations about a year before the assessment was carried out. Environmental perturbations reduce taxa richness creating niches for a few tolerant and generalist species (Couceiro *et al*. 2006) that can lead to changes in the ecological functioning of an ecosystem (Covich *et al*. 1999).

CONCLUSION AND RECOMMENDATIONS

Results indicate that lotic waters in the Chiquibul Forest are healthy systems supporting a diverse group of macroinvertebrates, where most of the benthic community composition is represented by species having high degree of sensitivity to pollution. High abundance and diversity of these organisms are indicators of good water quality coming from the headwaters of the greater Belize River Watershed, the most important watershed in the country of Belize.

The data suggests that macroinvertebrate communities in the Chiquibul Forest behaved different from that expected through the River Continuum Concept (Vannote *et al.* 1980) but can be attributed mainly to severe flooding events in the later months of 2013 and to the low occurrence of CPOM and FPOM preventing the establishment of the FFG Shredders but leading to the high occurrence of scrapers due to high percentage of sampling site area composed of more stable micro-habitats such as bedrock, boulders, cobbles and gravel.

SIGNAL 2 Site Scores were relatively high and showed no significant difference among sub-basins indicating relatively healthy headwaters for the Belize River Watershed. Even though Site Scores were high, the results indicated a significant difference in abundance of macro-invertebrates being very tolerant to pollution. Higher abundance was recorded in the Southern Chiquibul River sub-basin, the area being affected by illegal gold extraction but important to note that this sub-basin registered greatest taxa diversity. The indication of high abundance of pollution tolerant species may start to shed some light into the motion that illegal gold mining is impacting the water quality of this system; a trend that can only be detected if a systematic macroinvertebrate monitoring system is set in place.

Based on the results of this assessment, the following is recommended:

- Continue monitoring macroinvertebrate communities in the entire Chiquibul Forest to study community composition trends over time and correlate observable change with anthropogenic and or natural perturbations in the environment.
- Conduct chemical water analysis and relate these with macroinvertebrate communities to draw better conclusions and water quality in the Chiquibul Forest sub-basins.
- Develop a local biotic index score that reflects local macroinvertebrate composition and respective pollution tolerance levels that will provide a better picture of the water quality of the streams.
- Control illegal gold panning on the headwaters through coordinated reinforcements thereby preventing further impacts on the Greater Belize watershed.
- Attract more attention to the importance of the Greater Belize Watershed and engage key institutions nationally including Department of the Environment and the Climate Change Center in order to support the protection of this critical watershed.

ACKNOWLEDGEMENTS

This project was made possible through a generous fund from the Belize Nature Conservation Foundation (BNCF). Many thanks to the FCD field staff: Francisco Galicia, Roberto Tzib, Elroy Reyes, Demery Harris and Pedro Bol. Likewise gratitude goes to the FCD Ranger Team for providing assistance and logistical support. Gratitude also to the Forest Department for allowing the research team to conduct research across the Chiquibul Forest. To Rachael Carrie and the Ya'ache Conservation Trust staff, for providing training on macroinvertebrate collection and identification, thank you. Many thanks also to the Belize Defence Force and the Police Department for providing security during work in the Southern Chiquibul River. FCD is also grateful for the technical review made by Belizean limnologist, Dr. Ed Boles. To Las Cuevas Research Station for providing lodging, many thanks.

REFERENCES

Alba-Tercedor, J. 1996. Macroinvertebrados acuaticos y calidad de las aguas de los rıos. IV Simposio del agua en Andaluca (SIAGA). Almerıa 2: 203–231.

Barbour, MT; Gerritsen, J; Snyder, BD; Stribling, JB. 1999. Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers: Periphyton, Benthic Macroinvertebrates, and Fish, 2nd ed., EPA 841-B-99-002, U.S. Environmental Protection Agency, Washington, D.C.

Barbour, MT; Stribling, JB; Karr, JR. 1995. The Multimetric Approach for Establishing Biocriteria and Measuring Biological Conditions, *in* W. S. Davis and T. Simon (*eds.*), *Biological Assessment and Criteria, Tools for Water Resource Planning and Decision Making*,Lewis Publishers, Boca Raton, FL, pp. 63–77.

Belize Environmental Technologies. 2012. Environmental Impact Assessment for Bioton Minerals Limited to prospect and min e gold within the Chiquibul Forest. Belize.

Boothroyd, IKG; Stark, JD. 2000. Use of invertebrates in Monitoring In: New Zealand Stream Invertebrates: Ecology and implications for management. *In* K. Collier & MJ. Winterbourn. (*eds.*) New Zealand Limnological Society, Hamilton. pp. 344-373.

Carlisle, DM; Clements, WH. 1999. Sensitivity and variability of metrics used in biological assessments of running waters. Environmental Toxicology and Chemistry 18: 285–291.

Carter, JL; Resh, VH. 2001. After site selection and before data analysis: sampling, sorting and laboratory procedures used in stream benthic macroinvertebrate monitoring programs by USA state agencies. Journal of the North American Benthological Society 20(4): 658-682.

Chessman, B. 2003. SIGNAL 2 – A scoring system for macro-invertebrate ('water bugs') in Australian Rivers. Monitoring River Health Initiative Technical Report no. 31. Commonwealth of Australia, Canberra. p. 34.

Cornec, J. H. 2003. Geology Map of Belize.

Couceiro, SRM; Hamada, SLB; Bruce RF; Pimentel TP. 2006. Deforestation and sewage effects on aquatic macroinvertebrates in urban streams in Manaus Amazonas, Brazil. Hydrobiologia 119 -– 140.

Covich, AP; Palmer, MA; Crowl, TA. 1999. The role of benthic invertebrate species in freshwater ecosystems: Zoobenthic species influence energy flows and nutrient cycling. Bioscience 49: 119 – 140.

Di Reinzo JA; Casanoves , F; Balzarini, MG; Gonzalez, L; Tablada, M; Robledo, CW. 2008. InfoStat versión 2008. Grupo InfoStat. FCA. Universidad Nacional de Cordoba, Argentina.

Gerritsen, J. 1995. Additive biological indices for resource management. Journal of the North American Benthological Society 14:451–457.

Greenfield, DW; Thomerson, JE. 1997. Fishes of the continental waters of Belize. The University Press of Florida, Gainesville. USA.

Fore, LS; Karr, JR; Wisseman, RW. 1996. Assessing invertebrate responses to human activities: Evaluating alternative approaches. Journal of the North American Benthological Society 15: 212–231.

Klemm, DJ; Blockson, KA; Thoeny, W; Fulk, FA; Herlihy, AT; Kaufmann, PR; Cormier, SM. 2002. Methods development and use of macroinvertebrates as indicators of ecological conditions for streams in the Mid-Atlantic Highlands Region. Environmental Monitoring and Assessment 78:169-212.

Klemm, DJ; Lewis, PA; Fulk, F; Lazorchak, JM. 1990. Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters, EPA.600/4-90/030, Environmental Monitoring Systems Laboratory, Office of Modeling, Monitoring Systems, and Quality Assurance, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.

Lenat, DR. 1993. A biotic index for the southeastern United States: Derivation and list of tolerance values, with criteria for assigning water-quality ratings. Journal of the North American Benthological Society 12: 279–290.

Mandaville, SM. 2002. Benthic Macroinvertebrates in freshwater – Taxa Tolerance Values, Metrics, and Protocols. Soil and Water Conservation Society of Metro Halifax. P. 128.

Meerman and Sabido. 2001. Ecosystem Map of Belize.

Resh, VH; Jackson, J. 1993. Rapid Assessment Approaches to Biomonitoring Using Benthic Macroinvertebrates. *in* D. M. Rosenberg and V. H. Resh (eds), *Freshwater Biomonitoring and Benthic Macroinvertebrates*, Chapman & Hall, NY, pp. 195–233.

Roldan, G. 2003. Bioindicacion de la calidad del agua en Colombia: Propuesta para el uso del metodo BMWP/Col. In: Coleccion Ciencia y Tecnologıa. Editorial Universidad de Antioquia, Colombia, p. 170.

Salas, O; Meerman, JC. 2008. Chiquibul National Park Management Plan 2008-2013. p. 191.

Stark, JD; Boothroyd, IKG; Harding, JS; Maxted, JR; Scarsbrook, MR. 2001. Protocols for sampling macrioinvertebrates in wadeable streams. New Zealand Macroinvertebrate Working Group Report No. 1. Ministry of the Environment. Sustainable Management Fund Project No. 5103. p. 65.

USEPA. 1994, Environmental Monitoring and Assessment Program: Surface Waters and Region 3, Regional Environmental Monitoring and Assessment Program: 1994 Pilot Laboratory Methods Manual for Streams, EPA/640/R-94/003, U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory and National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC.

USEPA. 2001. Environmental Monitoring and Assessment Program: Surface Waters and Region 3, Regional Environmental Monitoring and Assessment Program Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory and National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC.

Vannote, RL; Minshall, GW; Cummins, KW; Sedell, JR; Cushing, CE. 1980. The River Continuum Concept. Canadian Journal for Fish and Aquatic Science 37: 130 – 137.

Wallace, JB; Grubaugh, JW; Whiles, MR. 1996. Biotic indices and stream ecosystem processes: Results from an experimental study. Ecological Applications 6: 140–151.

Wright, ACS; Romney, DH; Arbuckle, RH; Vial, VE. 1959. Land in British Honduras. Colonial Res. Publication No. 24.

APPENDIX I

Fish species recorded in the streams of the Chiquibul Forest

Family	Species	Sub-basin			
		Southern Chiquibul River	Raspaculo River	Macal River	Monkey Tail River
Anguillidae	*Anguilla rostrata*		✓	✓	
Pimelodidae	*Rhamdia sp.*	✓	✓	✓	✓
Poeciliidae	*Xiphophorus helleri*	✓	✓	✓	✓
Poeciliidae	*Poecilia teresae*	✓	✓	✓	✓
Poeciliidae	*Heterandria bimaculata*	✓	✓	✓	✓
Characidae	*Astyanax aeneus*	✓	✓	✓	✓
Cichlidae	*Cichlasoma spirurum*	✓	✓	✓	✓
Cichlidae	*Cichlasoma salvini*	✓	✓	✓	✓
Cichlidae	*Cichlasoma intermedium*	✓			

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