An Assessment of Freshwater Macroinvertebrate Communities in Streams of the Chiquibul Forest

Study No. 3

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ABSTRACT

Freshwater macroinvertebrates are used as biological indicators of water quality. The changes are reflected in community composition and abundance. In this study 38 sampling sites were distributed across the Chiquibul Forest. Overall mean abundance and richness was significantly greater during 2017 compared to both 2014 and 2020 surveys. There was significant difference on mean abundance and family richness of freshwater macroinvertebrate based on their sensitivity and/ or tolerance to organic pollution (SIGNAL 2 Band Score) where greater means was reported during 2017 for "Blue", "Green", "Yellow", and "Red" categories except for mean richness on class "Yellow". Overall, the stream of the Chiquibul Forest have good water quality; where most sampling sites were classified in Quadrant 1 (high SIGNAL 2 Site Scores and macroinvertebrate diversity) and both abundance and richness of macroinvertebrates tolerant to organic pollution were significantly less irrespective of survey period. The observed patterns can be used to generate hypothesis about factors which may be affecting macroinvertebrate assemblage and be tested in future studies. Future studies can incorporate the potential effects of stream substrate, physical and chemical water parameters to investigate variations in macroinvertebrate assemblages.

INTRODUCTION

Freshwater macroinvertebrates are used as biological indicators of water quality (Stark *et al*. 2001). Authors argue that monitoring changes in freshwater macroinvertebrate community composition and abundance is important because of their response to water quality over time (Resh & Jackson, 1993; Lenat, 1993; Barbour *et al*., 1995, 1996; Gerritsen, 1995; Fore *et al*., 1996; Wallace *et al*., 1996; Carlisle & Clements, 1999; Roldan 2003) compared to chemical and physical water analysis which only provide a snap shot of the system (Alba-Tercedor 1996). Freshwater macroinvertebrates are ideal biological indicators because organisms: (i) are abundant and diverse both at species and functional groups level; (ii) are relatively sedentary and have a long life cycle of at least 6 months providing a good snap shot of the dominant physical and chemical conditions and anthropogenic disturbances of the water body at a spatial and temporal scale; and (iii) respond to environmental stress (Boothroyd & Stark 2000; Mandaville 2002).

The Chiquibul Forest, comprised of public protected lands is the headwaters of the largest watershed in Belize, the Greater Belize River watershed (GBRW). The GBRW has a surface area of approximately 10,500 km^2 and is the most populated watershed in Belize, accounting for 44% (125,098) of the national population. Belize City accounts for 48% of the population, followed by San Ignacio and Santa Elena (13%). In addition, the agricultural belt of the Central Belize River Valley is completely dependent on the GBRW resource for irrigation. Within the Chiquibul Forest are the Chalillo, Mollejon, and Vaca hydroelectric facilities, generating more than 30% of the country's electric energy supply.

With an increase in population and present investment initiatives in agriculture, tourism, reduced impact selective logging, gold mining, and road infrastructure; water demand will only increase, and pollution is certain. In addition, within the Chiquibul National Park and Caracol Archaeological Reserve, illegal gold panning and agricultural encroachments are eminent threats. It becomes important to monitor the water quality of the head waters to guide management and conservation of this vital resource. Water management and conservation are an integral part of the Chiquibul National Park and Cave System management plan and forms part of the Chiquibul Forest Biodiversity Research Monitoring and Inventory Framework. The ecological damage of these disturbances is uncertain. The goal of this assessment was to compare freshwater macroinvertebrate composition and abundance with previous assessments (2014 and 2017) to evaluate any changes and where possible correlate how ecological disturbances within the Chiquibul Forest are impacting water quality.

METHODOLOGY

Freshwater macroinvertebrates were collected at 38 sampling sites [stream reach] between September-March of 2013-2014 (2014 Census), 2016-2017 (2017 Census), and 2019-2020 (2020 Census), and distributed across four major sub-basins (Macal River: n = 8; Raspa River: n = 9; Monkey Tail River: n = 9; and Southern Chiquibul River: n = 12) in the Chiquibul Forest (CF;**Error! Reference source not found.**), Belize. The CF, Belize, which comprises 176,999 ha of protected lands is dominated by tropical broadleaf forests (Meerman & Sabido 2001). Riparian habitats are described as deciduous broadleaf lowland riparian shrubland on hills ranging from 400 to 1000-m elevation (Meerman & Sabido 2001; Penn et al 2004). Annual rainfall varies between 2 and 3 m, and flood events are frequent during the rainy season. 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 karstic nature of the areas give rise to subterranean streams and rivers. The underlying geology of the Chiquibul Forest give rise to hard-bottom streams (gravel, cobbles, boulders, and bedrock substrate dominate more than 50% by area of the stream bed).

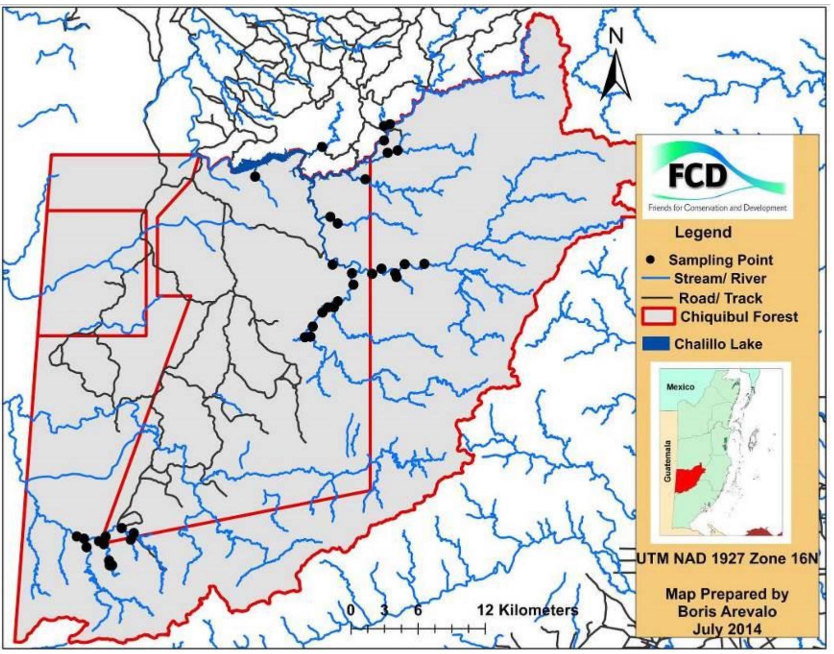


Figure 1: *Spatial distribution of macroinvertebrate sampling sites in the Chiquibul Forest*

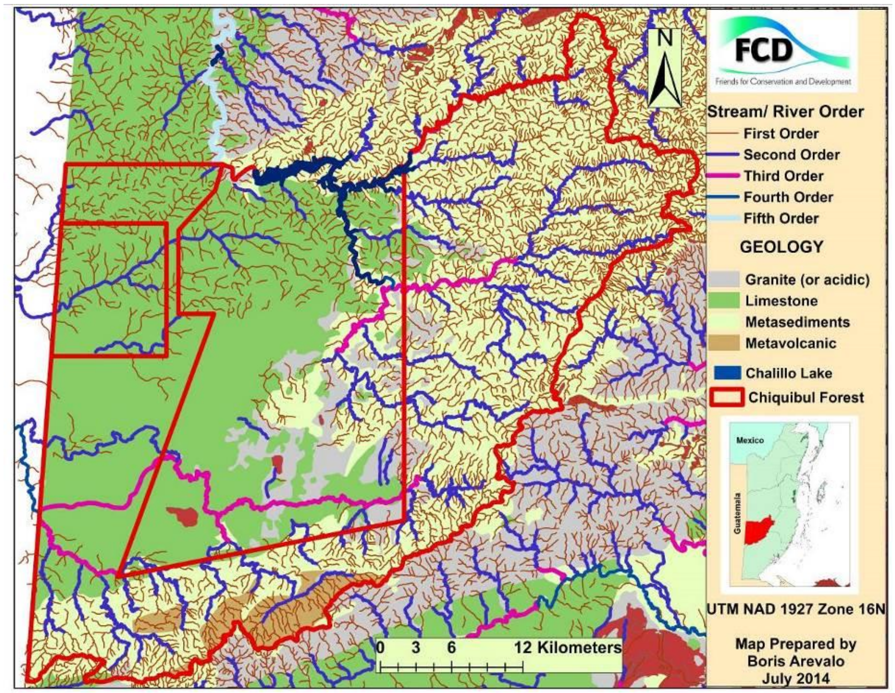


Figure 1: *Hydrology of the Chiquibul Forest.*

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 and 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 frequently used in freshwater macroinvertebrate research and quite versatile (Resh and Jackson 1993; Carter & Resh 2001). All samples collected were fixed and preserved in 70% by volume isopropyl alcohol for later identification. Collected samples were washed under running water, passing through metal sieves of mesh size 4000, 2000, 500 and 250 microns for sorting from sample debris, then freshwater macroinvertebrates were placed in plastic vials containing 70% by volume isopropyl alcohol for later identification. Freshwater macroinvertebrates were identified to family level following the Carrie *et al.* 2014 identification key.

All identified freshwater macroinvertebrate were classified into their respective Functional Feeding Groups (FFG) (scrappers, predator, filtering collector, gathering collector, shredder) and Stream Invertebrate Grade Number Band Score Level 2 (SIGNAL 2). Each freshwater macroinvertebrate was assigned to one of the four SIGNAL 2 Grade Score categories [Very sensitive to pollution (Blue), Sensitive to pollution (Green), Tolerant to pollution (Yellow), and Very tolerant to pollution (Red), based on Chessman (2003)].

SIGNAL 2 Site Scores were calculated for each sampling site by survey following Chessman (2003) methodology. SIGNAL is a simple biotic index for freshwater macroinvertebrates based on their tolerance to organic pollution yielding a site score and a water quality rating of an aquatic system. It was designed for Australian freshwater systems but can be adapted to other areas; however, results can be less conclusive. High SIGNAL site score (above 5 since most sites yield a score of no more than 7) are indicative of high dissolved oxygen with low turbidity and adequate nutrient levels. Each freshwater macroinvertebrate family was assigned a SIGNAL 2 grade number between 1 and 10. A low-grade number means that the organism is very tolerant to water pollution. A high number indicates that the freshwater macroinvertebrate is sensitive to most forms of pollution. A weighting factor based on each family abundance was calculated (1–2 individuals = 1; 3-5 = 2, 6-10 = 3; 11-20 = 4; > 20 = 5). The sum of all weight factor and the product of each family SIGNAL 2 Band Score by weighting factor was calculated for each sampling site by survey. To calculate the SIGNAL 2 Site Score the sum of all products of each family grade score by weight factor were divided by the sum of weighting factors for each sample site per survey. SIGNAL 2 Site Score were then plotted using a quadrant plot (biplot) as a function of family richness. The biplot was divided into 4 quadrants by using a cutting line of 50% on both axis and stream health following Chessman (2003).

A one-way ANOVA was performed to compare mean abundance and richness for functional feeding group and SIGNAL Band by census. A post-hoc Tukey Honest Significant Difference (HSD) mean comparison was done. HSD was used to control for the Type I error rate across multiple comparisons. The assumptions of normality and homogeneity of variance were evaluated using the Shapiro-Wilk and the Levene Test, respectively. If assumptions were not met, variables were squared root transformed for ANOVA and HSD but means and confidence intervals were calculated using untransformed variables. All statistical analysis was carried out using R 3.6.1 (R Core Team 2019). Unidentified organisms and Diptera pupae were excluded from analysis.

RESULTS

Greatest total abundance of freshwater macroinvertebrates was recorded for 2017 (n = 24,350 individuals), followed by 2020 (n = 13,291 individuals), while the least abundance was recorded for the 2014 survey (n = 7,785; Figure 2A) but total family richness was relatively similar across survey (62, 64, and 59 species during 2014, 2017, and 2020 respectively; Figure 2B). The family Elmidae was dominant, representing 15.67%, 27.23%, and 23.12% of recorded abundance during 2014, 2017, 2020 surveys, respectively. The dominance of Elmidae was reflected in the Rank-Abundance Curves and overall trends in freshwater macroinvertebrate family evenness was similar for the three different survey periods (Figure 3).

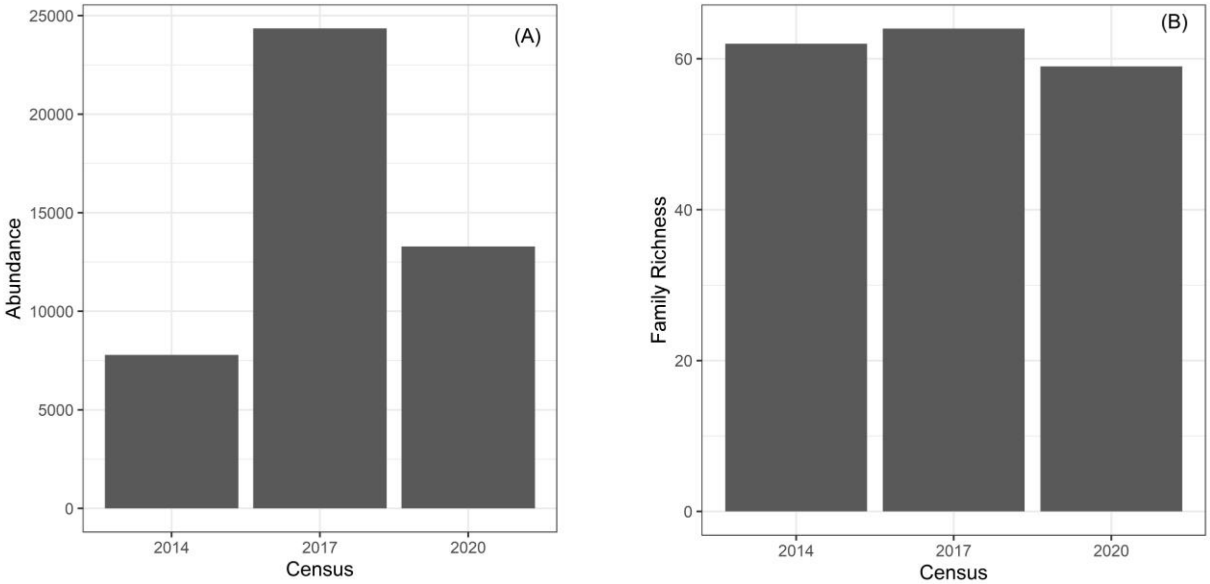


Figure 2: *Total recorded abundance (A) and total family richness (B) of freshwater macroinvertebrates recorded by survey in stream of the Chiquibul Forest. Census 2014 = 2013-2014 survey, Census 2017 = 2016-2017 survey, Census 2020 = 2019-2020 survey.*

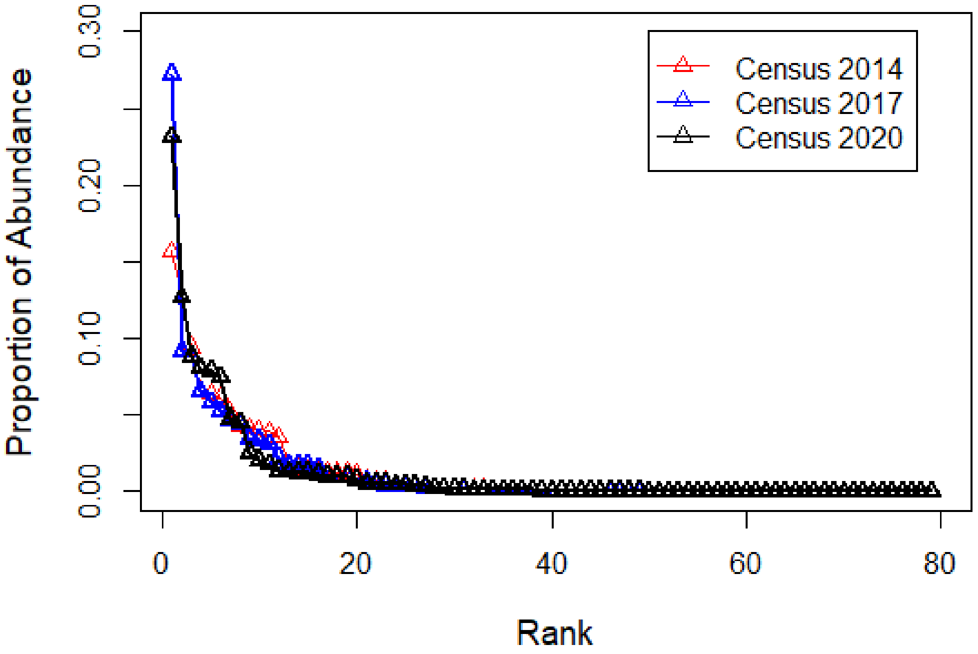
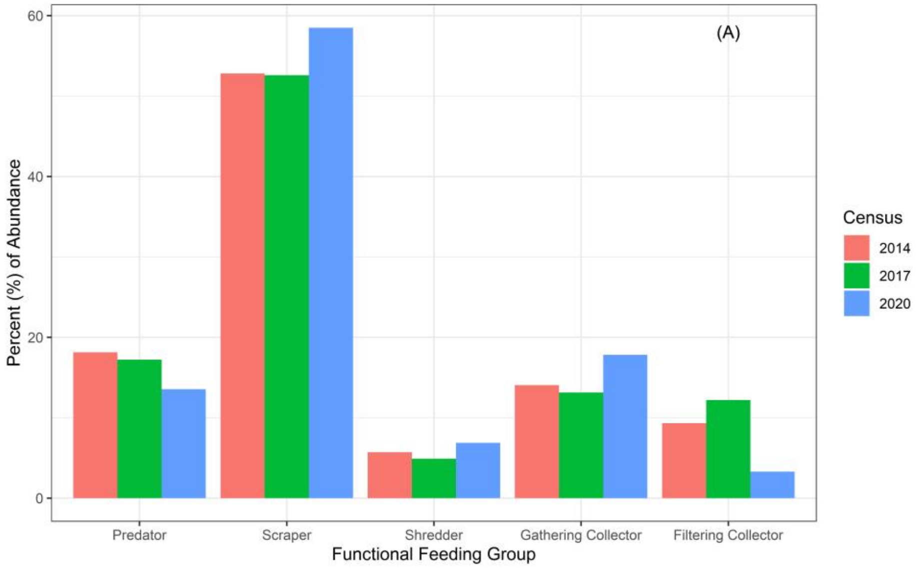


Figure 3: *Rank-Abundance Curve for freshwater macroinvertebrate abundance by family recorded in streams of the Chiquibul Forest during the 2014, 2017, and 2020 surveys.*

Trends in the percentage of total abundance by functional feeding group was similar during the three-survey period, where Scrapers accounted for the highest percentage of abundance during all survey periods while Shredders were represented the least (Figure 4A). Predators accounted for the highest percentage of macroinvertebrate richness during the three survey periods, followed by scrapers, while Filtering Collectors accounted for the least richness and this general trend was maintained for the three survey periods (Figure 4B).



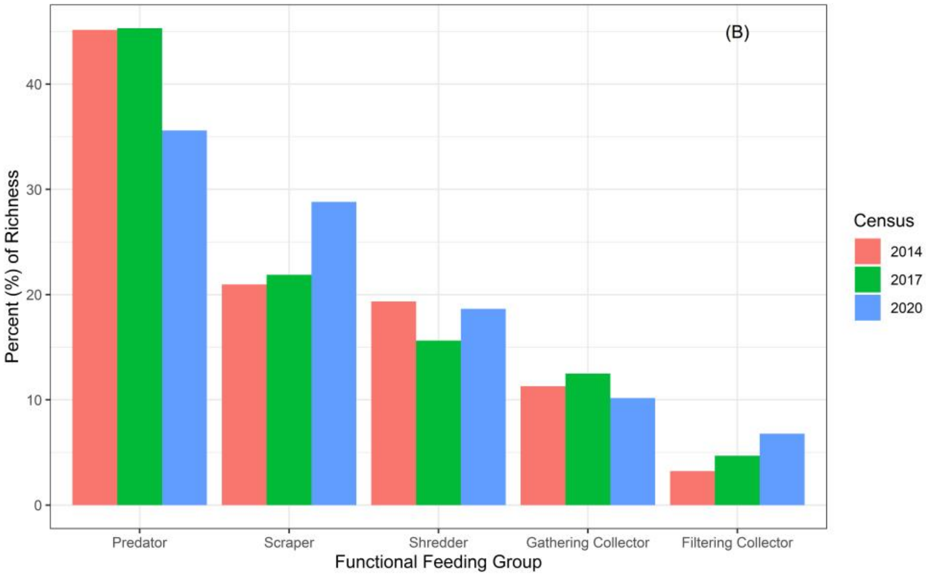


Figure 4: *Percentage of total abundance (A) and family richness (B) by function feeding group of freshwater macroinvertebrates recorded in streams of the Chiquibul forest. Census 2014 = 2013-2014 survey, Census 2017 = 2016-2017 survey, Census 2020 = 2019-2020 survey.*

Freshwater macroinvertebrates categorized as very sensitive and sensitive to organic pollutants (Blue and Green, respectively) were most abundant while very tolerant to organic pollutants were the least abundant (Red). A slight increasing trend in percent abundance of class “Red” (macroinvertebrates very tolerant to organic pollutants) was observed from 2014 to 2020 survey as was for class “Blue” (very sensitive to organic pollution) but a decreasing trend for class “Green” (Figure 5A). The recorded richness base on SIGNAL 2 Band Scores showed a general even distribution across the “Blue”, “Green”, and “Yellow” categories for the three surveys (Figure 5B). Similar to abundance, family richness of freshwater macroinvertebrates “very tolerant to organic pollutants” were recorded the least but showed a slight increase in richness from 2014 to 2020 surveys (Figure 5B).

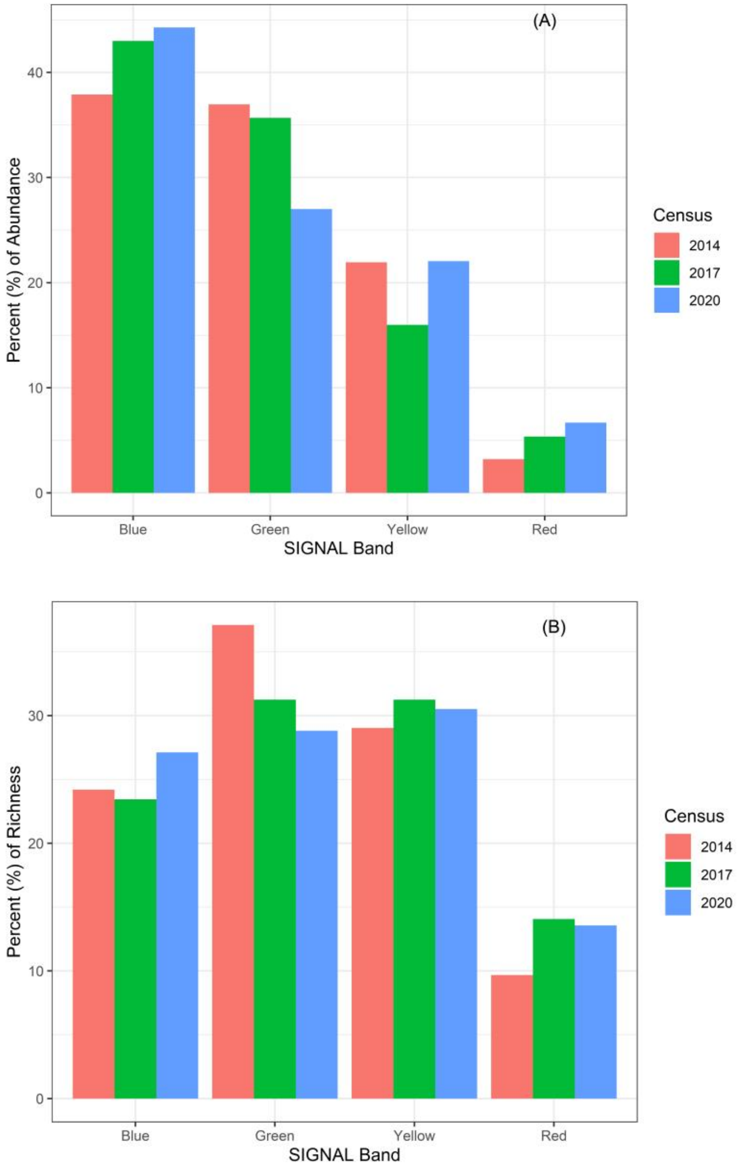


Figure 5: *Percentage of total abundance (A) and total richness (B) by survey base on SIGNAL 2 Band Score for freshwater macroinvertebrate communities surveyed in streams of the Chiquibul Forest. Blue = Very sensitivity to pollution, Green = Sensitive to pollution, Yellow = Tolerant to pollution, and Red = very tolerant to pollution. Census 2014 = 2013-2014 survey, Census 2017 = 2016-2017 survey, Census 2020 = 2019-2020 survey.*

All sampling site during the 2017 survey fell in Quadrant 1, while 34.21 % and 23.7% of site during 2014 and 2020 respectively fell into Quadrant 3 (Figure 6). Irrespective of surveys, no sampling sites fell into Quadrant 2 or Quadrant 4; indicating good water quality of the streams within the Chiquibul Forest. Mean SIGNAL 2 Site Score during 2014 survey was 5.643 (min = 4.294; max = 6.235), 5.506 during 2017 (min = 4.912; max = 5.884), and 5.156 (min = 3.688; max = 5.967) during 2020.

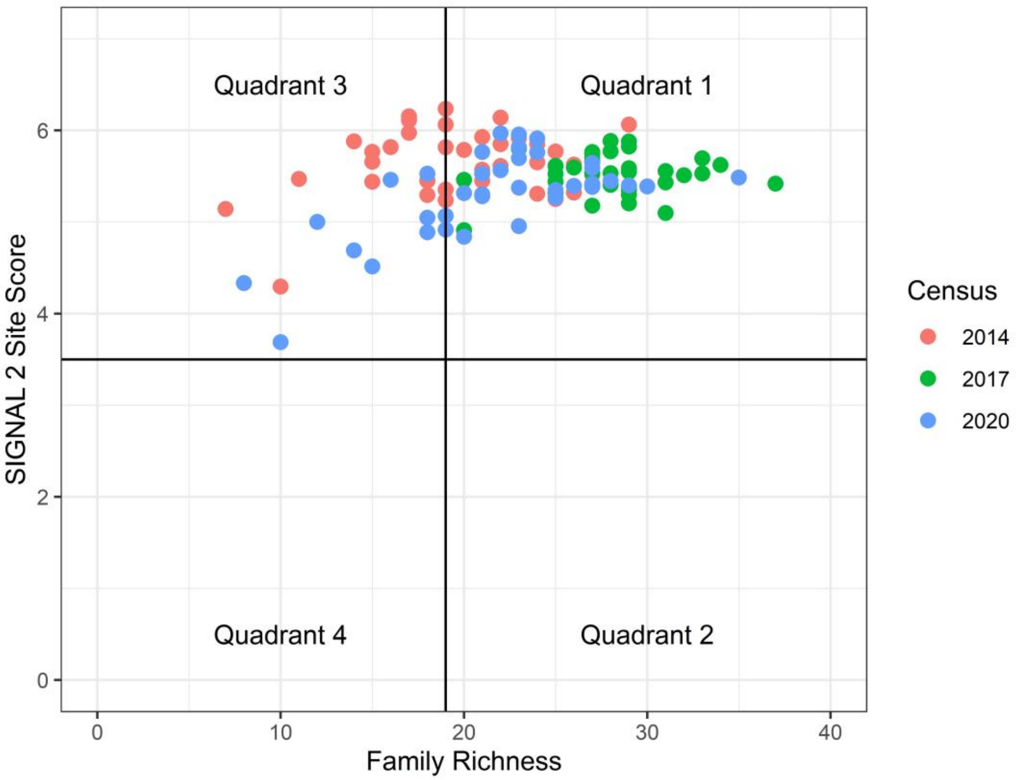


Figure 6: *The quadrant diagram for stream reaches surveyed during three time periods in the Chiquibul Forest, where SIGNAL 2 Site Scores are plotted as a function of freshwater macroinvertebrate family richness. Census 2014 = 2013-2014 survey, Census 2017 = 2016-2017 survey, Census 2020 = 2019-2020 survey.*

Mean abundance and richness of freshwater macroinvertebrate

Mean abundance was significantly different by survey (F-value = 35.2, p-value <0.0001) as was mean family richness (F-value = 18.48, p-value <0.0001). Mean abundance was significantly lower during the 2014 survey compared to both 2017 and 2020 (adjusted p-value <0.0001, and 0.0130, respectively), and mean abundance was lower during census 2020 compared to 2017 (adjusted p-value <0.0001; Figure 7A). Mean family richness was significantly greater during the 2017 survey compared to 2020 (Adjusted p-value = 0.0001) and 2014 (Adjusted p-value = 0.0006) but there was greater variation in the data during the 2017 survey as shown in Figure 7B. There was no significant difference in mean richness between 2014 and 2020 (Adjusted p-value = 0.88).

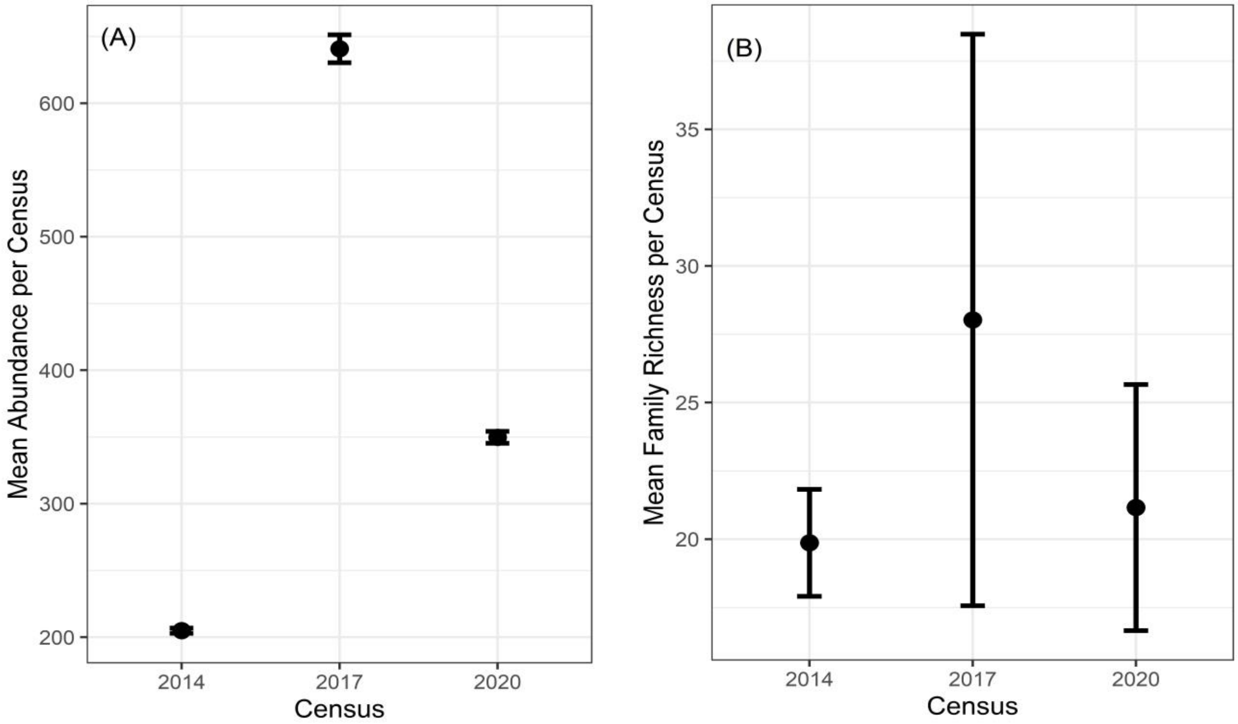


Figure 7: *Mean abundance (A) and richness (B) of freshwater macroinvertebrates by survey recorded in streams of the Chiquibul Forest. Census 2014 = 2013-2014 survey, Census 2017 = 2016-2017 survey, Census 2020 = 2019-2020 survey.*

There was significant difference in the mean abundance of freshwater macroinvertebrates categorized as very sensitive to pollution (Blue) by survey period (F-value = 24.84, p-value = 1.21e-09) as was for Green (F-value = 20.7; p-value = 2.3e-08), Yellow (F-value = 11.05; p-value = 4.2e-05), and Red (F-value = 31.29, p-value = 1.68e-11; Figure 8A). During 2017, greater mean abundance of freshwater macroinvertebrates that are very sensitive to pollution (Blue) compared to both 2014 and 2020 surveys (adjusted p-value of < 0.0001 and 0.0001, respectively) was reported. Mean abundance of macroinvertebrates sensitive to pollutants (Green) was significantly greater during 2017, compared to 2014 (Adjusted p-value = 0.00001) and 2020 (Adjusted p-value = 0.00006) while no difference in mean abundance between 2014 and 2020 (Adjusted p-value = 0.5769) was recorded. During the 2014 survey, lower mean abundance of macroinvertebrates tolerant to pollution (Yellow) were recorded compared to 2017 (Adjusted p-value = 0.00002) and 2020 (Adjusted p-value = 0.0333), but mean abundance was similar during 2017 and 2020 (Adjusted p-value = 0.0829). There was lower mean abundance of very tolerant to pollution (Red) macroinvertebrates during the 2014 survey compared to the 2017 (Adjusted p-value <0.0001) and 2020 (Adjusted p-value = 0.0001), while mean was not significantly different during the 2017 and 2020 census (Adjusted p-value = 0.1016).

Mean family richness was significantly different for freshwater macroinvertebrates categorized as very sensitive to pollution (Blue) during the different census (F-value = 8.429, p-value 0.0004) as was for Green (F-value = 18.82; p-value = 9.15e-08), Yellow (F-value = 20.64; p-value = 2.39e-08), and Red (F-value = 8.192, p-value = 0.00048; Figure 8B). Significantly greater Blue family richness was recorded during 2017 compared to 2014 (Adjusted p-value = 0.0013) and 2020

(Adjusted p-value = 0.0019), while no significant difference in mean blue richness between 2014 and 2020 Census (Adjusted p-value = 0.99). Green mean richness was greater during 2017 compared to 2014 (Adjusted p-value = 0.00001) and 2020 (Adjusted p-value = 0.00001), while no difference between 2014 and 2020 (Adjusted p-value = 0.9692). Yellow richness during 2017 was greater than during 2014 (Adjusted p-value = 0.00001), and mean richness was significantly greater during 2020, than 2017 (Adjusted p-value = 0.000006), while mean richness was not significantly different between 2014 and 2020 (Adjusted p-value = 0.59169). Mean richness of Red was significantly lower during 2014 compared to both 2017 (Adjusted p-value= 0.00098) and 2020 (Adjusted p-value = 0.004), while no difference between 2017 and 2020 census (0.90453).

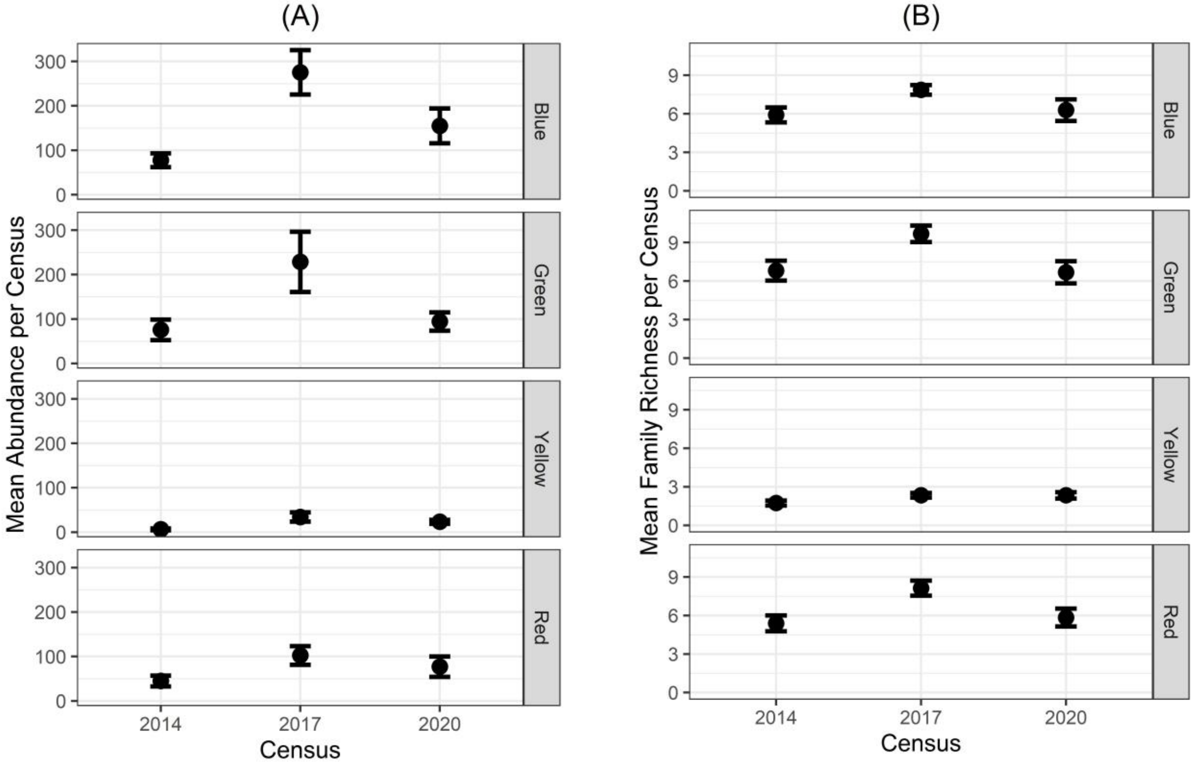


Figure 8: *Mean abundance (A) and richness (B) for freshwater macroinvertebrates classified by sensitivity to water pollution in streams of the Chiquibul Forest. Blue = Very sensitivity to pollution, Green = Sensitive to pollution, Yellow = Tolerant to pollution, and Red = very tolerant to pollution. Census 2014 = 2013-2014 survey, Census 2017 = 2016-2017 survey, Census 2020 = 2019-2020 survey.*

DISCUSSION

The freshwater macroinvertebrate abundance recorded in streams and rivers of the Chiquibul Forest indicates good water quality but significantly different between surveys. The observed trend in abundance and richness was indicative of systems with heterogeneous micro-habitats which have allowed the establishment of a diverse assemblage of organisms. The similarity in recorded macroinvertebrate richness among surveys may result because “family” was used as the lowest taxonomic level of analysis. If organisms were identified to genus or species level, results may differ. The Rank-Abundance Curve had a steep gradient indicative of low evenness as the high-ranking species had greater abundance, compared to a shallow gradient indicating evenness where abundance by species is similar. Elmidae (Riffle Beetles) was dominant during all surveys. Elmidae are specialists to fast flowing, shallow and narrow streams where they feed on algae and detrital biofilms (Brown 1991); these habitat conditions are present in streams of the CF.

Scrapers dominated macroinvertebrate abundance was followed closely by predators and Gathering Collectors whereas family richness was dominated by Predators and Scrapers. The observed pattern in functional feeding group abundance and richness does not follow the River Continuum Concept (RCC; describes the entire river system as a continuously integrating series of physical gradients and associated biotic adjustments as the river flows from headwater to mouth; Vannote *et al.* 1980). Based on the RCC, headwater streams support high percentage of shredders and collectors (> 75%; Vannote *et al.* 1980) followed by scrappers and predators, but in the CF streams, Shredders were the least abundant and diverse group. Environmental factors that may have affected these patterns were heavy flooding events occurring during the later months of 2013 and January of 2014 and during the passage of Hurricane Earl in August 2016, followed by prolonged dry seasons in 2018 and 2019. Flooding events washed away course and fine particulate organic matter (CPOM and FPOM) whereas droughts prevent the transport of food matter or increase tannins in the water column, which may have caused a shift in the abundance of shredders and detritivores. CPOM and FPOM on average represented less than 10% of the area sampled at each stream reach while bedrock, boulders, cobbles, and gravel, were the dominant substrates. The high abundance of the former microhabitats, fast flowing and shallow waters which allow stream substrate to cover with algae and biofilm may explain the high abundance and diversity of scrapers which in turn attract predators.

Overall, the quadrant plot of SIGNAL 2 Site Score as a function of freshwater macroinvertebrate family richness indicates “healthy” streams within the Chiquibul Forest. Sample from the 2016-2017 survey had slightly better site scores and all fell in Quadrant 1 while the 2013-2014 and 2019-2020 surveys site scores showed greater variability both in family richness and SIGNAL 2 Site Scores. Using the family as the lowest taxonomic level of analysis results in SIGNAL 2 Site Scores no greater than 7 (Chessman 2003). Chessman (2003), suggests that site in Quadrant 1 are typical of relatively undisturbed natural streams with good forest cover and heterogeneous micro-habitats supporting high macroinvertebrate diversity and stress factors such as toxic chemicals and organic pollutants plus harsh physical conditions are absent. Sites falling in Quadrant 2 indicate lower SIGNAL 2 site scores and a high diversity of macroinvertebrate but are within streams with higher turbidity, nutrients, and salinity levels than those sites in Quadrant 1. The high macroinvertebrate diversity suggests that physical conditions are still favorable and toxic chemicals are minimally present. Site falling in Quadrant 3 represents high values of SIGNAL 2 Site Scores but low in macroinvertebrate diversity. Sites with toxic pollution, low pH, and high concentrations of heavy metals (such as site below dams and mines) usually fall within Quadrant 3 or 4. This classification is possible because macroinvertebrate families respond different to diverse pollutants. For example, mollusks (snails) are organic pollution tolerant but sensitive to heavy metals while Corydalidae (alderfly) and Leptoceridae (caddisfly) are very sensitive to organic and other pollutants but sensitive to heavy metals. Harsh physical conditions such as flooding and homogeneous habitats can also result in sites falling in quadrant 3, even if water quality is suitable. Poor sampling technique or inadequate sampling effort can also result in a site falling in quadrant 3, because few macroinvertebrates are collected even though many are present. Quadrant 4 represents sites both with low SIGNAL 2 site scores and macroinvertebrate richness. Most sites falling into this quadrant experience high levels of organic pollution and anthropogenic disturbances indicative of low water quality.

SIGNAL 2 metrics respond to the most common forms of water quality variation, such as organic and nutrient enrichment and salinity. Sites with unusual forms of pollution may still have high SIGNAL scores. Linking SIGNAL 2 assessments to other types of information will increase the weight of evidence and lead to more confident conclusions. Such information might include physical and chemical water quality monitoring, physical habitat assessments and assessments of other life forms, such as vegetation. Following Chessman (2003) classification of macroinvertebrate family response to organic pollutants, macroinvertebrate families that were associate (positive or negative) to either elevation or canopy were all taxon categorized as being sensitive to organic pollutants, except for the Planorbidae family which is very tolerant to organic pollutants. Similarly, the most abundant families recorded are sensitive to organic pollutants, giving an indication of good water quality of the sampled streams.

The observed greater significant difference in overall mean abundance, family richness, and for freshwater macroinvertebrates classified by sensitivity to water pollution (SIGNAL 2 Band Score) during 2017 survey compared to 2014 and 2020 is very difficult to ascertain because no physical and chemical water quality parameters were measured. The frequent flooding events during the later months of 2013 and January of 2014 may have “washed out” macroinvertebrate and reduced microhabitat heterogeneity which may have contributed to the low recorded diversity. Stream reaches with greater habitat heterogeneity generally support more taxa than structurally simple streams (Hubert et al. 1996) but landscape characteristics also affect macroinvertebrate distributions and abundance (Miserendino 2001). Forest cover is homogeneous throughout the CF but variations in soils and geology occur which may alter water chemistry. Alluvial gold mining and illegal panning has been occurring disturbing stream bed and bank stability but restricted to the Southern Chiquibul Rivers. Environmental perturbations reduce taxa richness creating niches for a few tolerant and generalist species (Couceiro *et al.* 2006) which can lead to changes in the ecological functionality of an ecosystem (Covich *et al.* 1999).

CONCLUSION

The objective of this assessment was to briefly describe and quantify macroinvertebrate assemblages in the Chiquibul Forest and compare results with previous assessments. Such approach is limited in detecting or interpreting more subtle environmental and physical-chemical factors that lead to changes in assemblages (abundance, richness, and composition) as was the case with this study. However, these freshwater macroinvertebrate assessments serve as a critical baseline of the present macroinvertebrate assemblages in the CF. The observed patterns can be used to generate hypothesis about factors which may be affecting assemblage. Future studies can incorporate the potential effects of stream substrate, physical and chemical water parameters to investigate variations in macroinvertebrate assemblages.

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.

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.

Brown, H. 1991. Elmidae (Dryopoidea) Pp. 404-407 in F. Stehr, ed. Immature Insects, Vol. 2. Dubuque, Iowa, USA.

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.

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

Hubert, W. A., J. Lavoie, and L. D. DeBray. 1996. Densities and substrate associations of macroinvertebrates in riffles of a small High Plains Stream. Journal of Freshwater Ecology 11:21–26.

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.

Miserendino, M. L. 2001. Macroinvertebrate assemblages in Andean Patagonia rivers and streams: Environmental relationships. Hydrobiologia 444:147-158.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

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 macroinvertebrates in wadeable streams. New Zealand Macroinvertebrate Working Group Report No. 1. Ministry of the Environment. Sustainable Management Fund Project No. 5103. p. 65.

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.