Building Rural Adaptive Capacity in the Maya Golden landscape Freshwater Biomonitoring Component 2016-2017

End of Project Report

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For Ya'axché Conservation Trust

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Abbreviations

BMWP-CR	Biological Monitoring Working Party-Costa Rica
ASPT-CR	Average Score Per Taxon-Costa Rica
MMMC	Maya Mountain Marine Corridor
SVAP	Stream Visual Assessment Protocol
EPT families	Ephemeroptera, Plecoptera and Trichoptera Families

1. Introduction and Rationale

Macroinvertebrates are globally use as indicators to assess the condition of the stream ecological health. The use of macroinvertebrates in bio-assessments for Small Island Developing States (SIDS) like Belize provides several advantages that includes: macroinvertebrates being taxonomically and structurally diverse, their low-cost and relative ease of sampling, and their sensitivity to a wide range of environmental conditions (Resh, 2007; Resh and Rosenburg, 1993). Bio-assessment within freshwater ecosystems has become Belize's most active area of interest for conservation and management strategies (MMMC-CAS 2008, MGL ILM Strategy 2010). Freshwater rivers has become the most threaten systems by the intensive and increasing human activities along these systems. This has even led to the incorporation of freshwater bio-assessment in the National Biodiversity Monitoring Programme for Belize (UB-ERI, 2016).

Macroinvertebrate-based bio-assessment is at its youngest stage of becoming a full monitoring programme within the Maya Mountain Marine Corridor (MMMC). It is a conservation landscape comprising of several protected zones that covers an area of 3360 km² of land and sea. The Golden Stream Corridor Preserve (GSCP) and Bladen Nature Reserve (BNR) are two of the protected zones within the MMMC managed by The Ya'axché Conservation Trust. The two protected areas comprise of the four key watershed: Rio Grande, Golden Stream, Deep River and Monkey River that lies within ~2600 km² semi-deciduous upland and coastal-plain broadleaf rainforest and pine savanna draining into the Mesoamerican Barrier Reef System (MBRS). The watershed contains 70% of forest cover and 10% to deforestation (Cherrington et al., 2010). In the downstream of the catchments lies rural communities, whose livelihoods depend on the rivers.

The objectives of the freshwater bio-assessment component of this project within the MMMC aims to (1) build local capacity and knowledge for freshwater bio-assessment in the communities. (2) It aims to develop baseline data and potential station sites for continuous monitoring in order to assess long term trends of the stream condition. Moreover, (3) it is to test the potential biotic metrics robustness inter-annually and seasonally over two years in the MMMC.

2. Capacity Building

Ya'axché freshwater team developed and trained by Dr. Carrie continued to develop their capacity and expertise in freshwater bio-assessment and macroinvertebrate taxonomy. The long term purpose of the team is to continue monitoring of the streams within the MMMC and assist in building the capacity of local community watershed groups on implementing freshwater bio-assessment within their communities.

The freshwater team consisted of two para-biologist rangers from The Ya'axché Conservation Trust and a local graduate student of the area. Anignacio Makin (ranger), Octavio Cal (ranger)

and Devina Bol (graduate student) were trained in stream mapping of macroinvertebrates habitat, sorting and identification of macroinvertebrates to family level, taking measurements of basic physio-chemical parameters and visually evaluating the stream's physical condition, using the Stream Visual Assessment Protocol (SVAP). For the period of the project, the team carried out the four sampling periods at 12 sites that is distributed across the four key watersheds within the MMMC.

The lead freshwater member (graduate student) attended the freshwater identification training course at the Freshwater Biological Association (FBA) in Cumbria, Windermere, UK in November, 2015. The member took the course examination passing with a mark of 98% and obtained a certificate of competence in identification of freshwater invertebrates to family-level: IF01. More training on the identification of macroinvertebrates with the inclusion to genus level, would further continue to build her taxonomical skills of identifying macroinvertebrates.

3. Freshwater Biomonitoring

Four sampling periods was carried out over the two year duration of the project. Sampling was done twice per year within the dry season. Once at the beginning of the dry season that is referred as the wet season (November-December) and at the end of the dry season (April-May).

3.1. Planning and justification of the sampling sites

Biomonitoring was limited to the dry season for several reasons:

- Previous work indicated higher abundance and richness of intolerant macroinvertebrates families to organic pollution in the dry season as compared to the wet season (CLP report, 2013).
- During the actual wet season (June to November), sites are inaccessible due to high water level and at times flooding.
- For reasons of efficiency, streams with high water level results in turbid waters creating an inability to map macroinvertebrate habitats competently.

Overall reason of the selected sites was based on:

- The objectives of the organization (Ya'axché) and the Maya Mountain Marine Corridor Conservation Action Strategy (MMMC CAS, 2008) to develop knowledge and tools required to implement macroinvertebrate based bioassessment of stream condition in the four key southern watershed of Belize.
- Establishing baseline data and fixed station sites for the development of a long term programme in tracking trends in the stream conditions of the southern watersheds, specifically for the area Ya'axché manages: Golden Stream Corridor Preserve and Bladen Nature Reserve.
- Increasing the knowledge and awareness in the rural communities through the involvement of local community watershed groups on basic stream condition assessment and strategies to maintain freshwater quality through watershed protection.

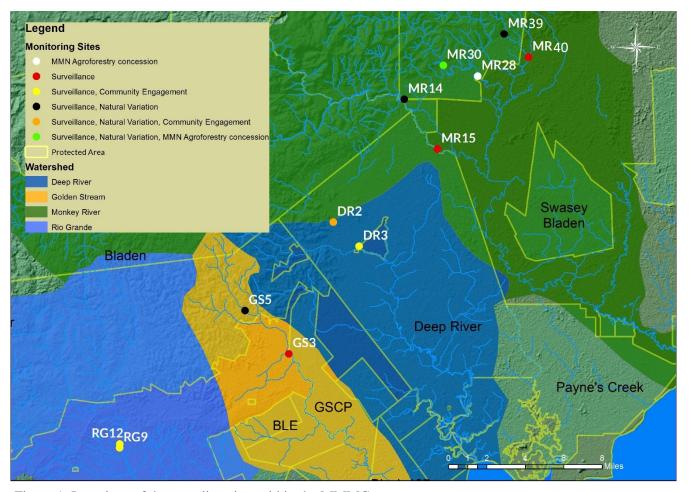


Figure 1: Locations of the sampling sites within the MMMC.

3.2. Sampling sites

The sampling sites are justified under four purposes. Some of the sites are found under more than one justification. Figure 1 illustrates the location of the sampling sites within the four key watersheds of the MMMC.

3.2.1. Natural Variation

Five of the best available forested sites (GS5, DR2, MR14, MR30, and MR39) are selected in the upstream of the watersheds, where minimal human impact (e.g. fishing) occurs. The sites are within the protected zones, thus selected to be potential reference condition sites within the watersheds of the MMMC. Two sites (GS5 and DR2) are within the calcareous tributaries and three sites (MR14, MR30, and MR39) are within siliceous tributaries. The site GS5 is located within the Columbia River Forest Reserve, site MR14 is located within the Bladen Nature Reserve and site MR30 is located within the Maya Mountain North Forest Reserve. The remaining two sites DR2 in the Deep River watershed and MR39 within the Monkey River watershed are located in the headwaters ~ 7 km from the nearest community Medina Bank village and San Pablo village respectively.

3.2.2. Maya Mountain North Forest Reserve Agroforestry Concession

Two sites (MR30, MR38) are selected on the Trio Branch River in the Monkey River watershed for the main purpose of assessing the impacts of newly developed agroforestry concession in the Maya Mountain North Forest Reserve on the stream water quality. The agroforestry concession is a project in the Trio Village that grants access and usage to a 165 acres along the reserve for local farmers. Of the two sites, MR30 is a selected natural forested site, and MR38 site is located below the agroforestry concession that has a road passing through the river for use during the dry season.

3.2.3. Community Engagement

Four sites (RG 9, RG 12, DR 3, and DR 2) on the Rio Grande and Deep River watershed are located near rural communities. The communities depend on the rivers daily for domestic use that includes: washing laundry, bathing, fishing, and agriculture. In both communities there are local watershed groups, who are keen to learn and develop their knowledge about macroinvertebrate-based bio-assessment, in order to create awareness and stewardship of monitoring the rivers in their community. Thus, the four sites were selected on the community interest to be informed on the condition of their rivers. San Miguel community is particularly interested to know the impacts of the small hydro-dam built in the upper reaches of the stream.

3.2.4. Surveillance

Eleven sites (RG12, RG9, GS5, GS3, DR2, DR3, MR14, MR15, MR30, MR 39, MR 40) are monitored for observation of changes or trends in the metrics and macroinvertebrate assemblage that can provide an evaluation of the condition of the stream water quality within the two year duration of the project. Some of the sites have other justification besides surveillance, as mention above. The sites GS3, MR15, and MR40 are downstream sites of their upstream natural sites. The GS3 site is downstream of the community Golden Stream that utilizes the stream for domestic purpose. It also borders the Golden Stream Corridor Preserve which is of interest to The Ya'axché Conservation Trust. The sites MR15 and MR40 are below small farms and acts as road access for the community Bladen and San Pablo villages, respectively.

3.3. Data

Biological and environmental data was collected at each of the sampling sites. The biological data only comprised of the collection of macroinvertebrates. Basic physiochemical parameters and a visual assessment of the physical stream condition are the environmental data collected at each sampling site.

3.3.1. Biological

Macroinvertebrates sampling was done following the AQEM project method (AQEM Consortium, 2002). At each site, twenty microhabitats was manually mapped in a sampling reach that was twice the average wetted width of the stream. The microhabitats was define by a combination of various substrate summarized into two flow types: riffle and pool. The macroinvertebrates were collected manually using a standard D-frame net (30-x25-cm, mesh

size: 500µm) and placed in Nalgene pots. The samples were preserved in 70% ethanol and the macroinvertebrates were sorted in the laboratory at The Ya'axché Conservation Trust field station in Golden Stream. Identification of the macroinvertebrates were also completed in the laboratory and identified to family level using the available taxonomic keys from Costa Rica (Springer et al., 2010) and a draft macroinvertebrate identification guide for bioassessment in Southern Belize (GIZ CATS, 2013).

Table 1: The sites are selected and justified under four categories based on the objectives of The Ya'axché Conservation Trust and the interest of the communities. The reason/s for sampling the selected sites are marked with an X.

Site Det	ails			Monit	oring Jus	tificatio	n
				Surveillance	Natural Variation	MMN Agroforestry concession	Community Engagement
Code	Stream	Site Name	UTM				
	nde Watershed	1		T	1		
RG 12	San Miguel Branch	Below hydro-dam	0293706 1802285	х			х
RG9	San Miguel Branch	Downstream of village	0293692 1801963	Х			Х
Golden	Stream Waters	hed			•		•
GS5	Indian Creek	Transect 1	0304194 1813446	х	х		
GS3	Golden Stream	Staff Gauge	0307869 1809830	х			
Deep Ri	ver Watershed	L	1		1		
DR2	Deep River	Forest	0311575 1820853	х	х		х
DR3	Deep River	0313749 1818837	х			х	
Monkey	River Watersh	ied					
MR14	Bladen Branch	BFREE Crossing	0317533 1831132	х	x		
MR15	Bladen Branch	Charley road	0320308 1826969	х			
MR30	Trio River	Old Man Lizard Forest	0320801 1833987	х	х	х	
MR 38	Trio River	Concession Crossing	0323677 1833072			х	
MR 39	Governor Creek	San Pablo upstream	0325892 1836608	х	х		
MR 40	Governor Creek	San Pablo downstream	0327933 1834665	х			

3.3.2. Environmental

The basic physio-chemical variables were collected at the same time of macroinvertebrate collection using hand-held portable meters. Triplicate readings of dissolve oxygen, temperature, pH, electrical conductivity and turbidity were all recorded at each site at three different points across the stream width. This was done to receive an average reading of the measured parameter for each site. After the macroinvertebrate and physio-chemical data was collected at each site, the freshwater team conducted a visual assessment of the stream's physical condition using the Natural Resource Conservation Service (1998) Stream Visual Assessment Protocol (SVAP) adapted for Belizean streams (Esselman, 2001). The SVAP is a qualitative scoring criteria of the different physical attributes of a stream, and ranks the stream physical condition from a 1 (worst) to 10 (best) condition. These attributes includes channel condition, riparian zone, shade (canopy), bank stability, hydrologic alteration, pools, water appearance, nutrient enrichment, sedimentation, insect/invertebrate habitat, in-stream fish cover, barriers to fish movement, fishing pressure, presence of garbage and manure presence. The scores of each attributes are average to result in an overall site score.

These data serve to enhance the evaluation process of the stream condition. It also assist to monitor sites over time to define any changes or trends that will assist in monitoring priorities and strategies.

3.3.3. Biotic Metrics

The potential metrics chosen for assessment in this project include: Biological Monitoring Working Party- Costa Rica (BMWP-CR) index, Average score per taxon- Costa Rica (ASPT-CR) index, relative abundance of Plecoptera, relative abundance of Tricoptera and the relative abundance of Predators. The BMWP and ASPT metrics are officially recognized by the government of Costa Rica and incorporated into the 'Law for the Evaluation and Classification of the Health of shallow bodies of warm water' (Wright, 2010). The BMWP-CR and ASPT-CR metrics are used to assess the streams in Belize, since Costa Rica and Belize have similar macroinvertebrates taxa. In addition, research has indicated they can distinguish environmental variation in Belizean streams (Carrie, 2017).

The metrics are tools that can be used to show a categorical scale of stream condition in streams and rivers. The BMWP function by assigning a sensitivity value from 1-10 to each macroinvertebrate family collected at the streams. The very pollutant tolerant families are assign a value of 1; while a very sensitive intolerant families are assign a value of 10. The sensitivity value from each family collected are sum to result in a final score which is called the BMWP score. A categorical scale is then used to determine the classification of the water quality at each site (Table 2). The ASPT-CR score is derived by dividing the BMWP score by the number of scoring taxa collected from each sample. The higher the ASPT score indicates the stream to be of high ecological status, while the lower the score indicates a stream of degraded ecological status (Table 2). An ASPT value greater than > 6 indicates high ecological status of the stream. The biotic metrics data was arc-sine transformed on proportion data to approximate normality of the data.

3.4. Sampling

The first sampling period began at the start of the dry season from December 29, 2015 to January 8, 2016. During this period, physiochemical data was not recorded as the dissolve oxygen and pH meter malfunctioned after sampling the first three sites (RG9, RG12, and GS5).

Macroinvertebrates were collected for all the sites. The second period was delayed waiting for the two meters to arrive. Which began June 2016 at the start of the rainy season, resulting in one site (RG 9) in the Rio Grande watershed not to be completed. This was due to the river level increasing quickly causing the site to be very turbid and unable to map the macroinvertebrates habitat. In contrast, site MR30 on the Trio River along the agroforestry concession could not be completed as the site was completely dry, even though the rains had started.

The third sampling period began at the start of the dry season (November 2016). The only sites that were not completed were RG9 and 12 on the San Miguel River, due to the previous heavy rains causing high water level and increase turbidity, resulted in difficulty to map macroinvertebrates habitat. The last sampling period was at the end of the dry season (April, 2016). This period was successfully completed with both physiochemical and macroinvertebrates data collected. The sorting of the macroinvertebrates for all four sampling were completed immediately after field collection and preserved in 70% ethanol by the ranger/para-biologist. The identification of the macroinvertebrates to family level was completed by the graduate student who also carried out the analysis and reporting.

Table 2: The Biological Monitoring Working Party and Average Score per Taxon - Costa Rica Index, a categorical scale for the evaluation of water quality.

Water Quality	BMWP-CR	Related Colour	ASPT Score		
Waters with excellent quality	>120	Blue			
Waters with good quality, no	101-120	Blue	>6	High	
contaminations or obvious					
distortions					
Waters with regular quality,	61-100	Green	4-5	Good	
eutrophic, medium contamination					
Waters with bad quality,	36-60	Yellow	3-4	Moderate	
contaminated					
Waters with bad quality, very	16-35	Orange	2-3	Poor	
contaminated					
Waters with very bad quality,	<15	Red	1-2	Bad	
extremely contaminated					

Source: Costa Rica Decree 33903 of 2007

4. Results

4.1. Physiochemical variables

The water temperature for the sites on the Rio Grande, Golden Stream and Deep River watershed ranges from 23°C - 26°C for all the three samplings in both wet and dry season. The water temperature of the sites on the Monkey River watershed for the wet season ranges from $23^{\circ}\text{C} - 26^{\circ}\text{C}$ and slightly increase in the dry season from 27°C - 32°C (figure 2). The dissolve oxygen at all sites in the Southern catchments and Monkey River catchments for the second and fourth sample (dry season) ranges from 5.4 - 8.5 mg/L. The only site in the fourth sample that gave an extremely high dissolve oxygen was site GS3 with $49.4 ~(\pm 0.1)$ mg/L (figure 2). Dissolve Oxygen in sample three for all the sites range from 3.2 - 8.8 mg/L. The sites with

lower dissolve oxygen in sample 3 were GS5, DR2 and DR3 with average readings of 3.23, 4.37 and 4.6 mg/L, respectively. The reading are below the 5 mg/L Mexico standard for aquatic life in warm surface waters. The pH level for all sites in all three samplings are neutral within a range of 7-8.7 (figure 2). Electrical conductivity of the sites on Rio Grande, Golden Stream and Deep River watershed ranges from 337-428.3 µS/cm for all three samplings (figure 2). The electrical conductivity for the sites on the Monkey River watershed illustrates three separate ranges (Figure 2). The first EC range is from 266-245 µS/cm for the sites on the Bladen River Branch for all three samplings, which includes the sites MR14 and MR15. The second EC range is from 107.4-122.4 µS/cm for the sites on the Trio River Branch for all three samplings, the sites includes MR30 and MR38. The third EC range is from $31.9 - 69.8 \,\mu\text{S/cm}$ for the sites on the Governor Creek Branch for all three samplings, the sites include MR39 and MR40. Significant test shows the three sub-catchments are different from each other: Bladen River vs. Trio River (PERMANOVA, t = 1.682, p < 0.018), Bladen River vs. Governor Creek (PERMANOVA, t = 1.8734, p < 0.012), and Trio River vs. Governor Creek (PERMANOVA, t = 1.5697, p < 0.041). Turbidity for all sites in all three samplings ranges from 0.1-10.2 NTU, which are within Mexico (<10NTU) and Costa Rica (<25NTU) standards for water quality.

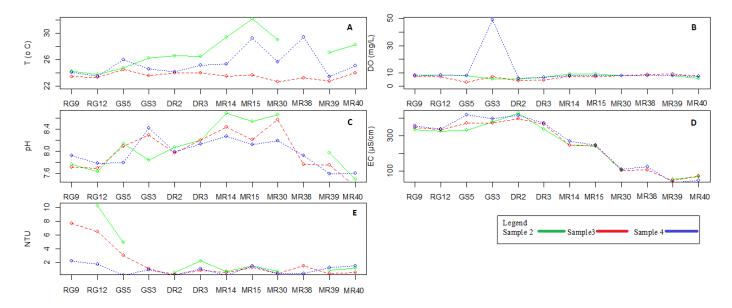


Figure 2: The physiochemical variables collected at all 12 sites for all three samplings within the MMMC. The Figure illustrates (A) temperature, (B) dissolve oxygen, (C) pH, (D) electrical conductivity [EC] and (E) turbidity [NTU]. The first sampling for the wet season was omitted due to several missing values for majority of the sites. Sample 2 and 4 are data collected for the dry season (end of the dry season) and sample 3 is data collected for the wet season (collected at the start of the dry season).

4.2. Macroinvertebrates Metrics

4.2.1. BMWP and ASPT

The BMWP-CR scores for the sites RG9, GS5, GS3, DR2, DR3, MR14, MR30, MR39 and MR40 gave very high scores ranging from 103 to 216 for all the four samples (Table 3). The sites are rated as good to excellent water quality according to the BMWP score of Costa Rica. The sites RG12 and MR15 scored slightly lower in the first sample with scores of 91 and 99, and in the second sample with scores of 84 and 61 respectively. Following the BMWP score for Costa Rica the sites are rated as regular water quality with medium contamination. The sites

MR15 and MR38 in the fourth sample gave very low scores of 50 and 37, which rates as bad water quality that is contaminated. However, the scores of RG12, MR15 and MR38 in the other samples scored within the range of 117 - 147. The ASPT score for all sites in all four samples scored within the range of 4.1 - 5.8, which rates according to the ASPT score of Costa Rica to be of good ecological status (Table 3).

4.2.2. Percentage Trichoptera

In the Monkey River watershed, site MR14 gave the highest percentage of Tricoptera families in both sample 3 and sample 4 with 16.4 and 17.2, respectively. This was followed by site MR15 with 11.1% in sample 2, MR39 (sample 4) and MR40 (sample 3) with 10%, and MR30 with 9.8% in sample 4. The lowest percentage were found in MR15 sample 1 and 3 with 1.1%. The site MR30 (sample2), MR39 (sample 1&3), MR40 (sample 2&4), and MR38 (sample 3&4) gave a range of 6.3-8.5%. Samples 1 (MR30, MR38, MR40, MR14), 2 (MR39, MR14), 3 (MR30) and 4 (MR15) gave a percentage within the range of 2.5 – 5.5%. In the southern catchment, site RG12 gave the highest percentage of 14.1 in sample 4, but a low percent in sample 1 and 2 with 2.0 and 1.4. RG9 site downstream of RG12 gave a percent of 7 and 3.1 in sample 1 and 4, respectively. No data was collected for sample 2 and 3 for RG9 site. GS5 site gave the lowest percentage for all 4 samples within the range of 1.2 - 4.8%. Site GS3 also gave a low 2.7-3.4% in sample 1, 2, 3, but sample 4 gave a high 9%. Site DR2 and DR3 gave high and low percentage of 9.6-11.3 in samples 1 (DR2), 3 (DR2 & DR3), and 4 (DR3). Low percentage of 2.9 -5.4 in samples 1 (DR3), 2 (DR2 &DR3) and 4 (DR2).

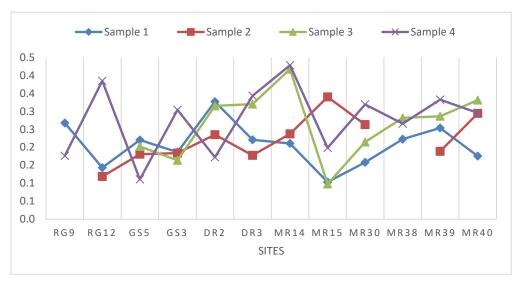


Figure 3: Percentage of Tricoptera

Site Details				BMWP			ASPT			SVAP				Overall Class Rate	
Code	Stream	Site Name	Sampling 1	Sampling 2	Sampling 3	Sampling 4	Sampling 1	Sampling 2	Sampling 3	Sampling 4	Sampling 1	Sampling 2	Sampling 3	Sampling 4	
Rio Gra	ande Watershed														
RG12	San Miguel Branch	Below hydro-dam	91	84	ND	117	4.6	4.2	ND	5.6	8.1	8.5	8.4	8.5	Good
RG9	San Miguel Branch	Downstream of village	160	ND	ND	150	5.2	ND	ND	5.6	7.9	8	7.5	8.2	Good
Golden	Golden Stream Watershed														
GS5	Indian Creek	Transect 1	168	184	119	142	5.4	5.4	5.2	4.9	9.6	9.8	8.3	8	Excellent
GS3	Golden Stream	Staff Gauge	204	103	131	161	5.2	4.5	5.2	5.4	7.9	7.8	9.6	9.4	Excellent
Deep Ri	iver Watershed														
DR2	Deep River	Forest	139	187	143	136	5.1	5.5	5.7	5.2	9.8	9.2	9.6	9	Excellent
DR3	Deep River	Medina Bank village	216	136	194	190	5.5	5.0	5.4	5.3	8.8	8.6	8.1	8.7	Excellent
Monkey	River Watershed														
MR14	Bladen Branch	BFREE Crossing	167	176	179	137	5.4	5.3	5.4	5.3	8.9	8.3	8.5	8.1	Excellent
MR15	Bladen Branch	Charley road	99	61	125	50	5.2	4.1	5	5	8.9	7.5	8.7	7.5	Good
MR30	Trio 1	Old Man Lizard Forest	160	167	156	153	5.7	5.6	5.2	5.5	9.4	8.9	9.1	8.6	Excellent
MR38	Trio 2	Concession Crossing	141	ND	147	37	5.6	ND	5.7	4.1	7.2	ND	8.3	8.1	Good
MR39	Governor Creek 2	San Pablo upstream	186	151	187	174	5.6	5.4	5.8	5.4	8.9	8.7	8.7	9.5	Excellent
MR40	Governor Creek 3	San Pablo downstream	141	167	202	111	5.6	5.2	5.6	5.6	9.3	9.1	9.1	9	Excellent

Table 3: Summary of the biomonitoring results of 2016-2017 in the MMMC. The BMWP, ASPT and SVAP score of all 12 sites over the four sampling periods (Sampling 1 and 3 are the wet season and Sample 2 and 4 are the dry season). The meaning of the colour code and scores of the BMWP and ASPT can be found in Table 2. The SVAP scores: 6.1-7.4 rates fair, 7.5-8.9 rates good and >9.0 is excellent physical condition of the stream. The overall class rate was define by the average of all samples from the BMWP, ASPT and SVAP scores separately. The final average score is rate against each scale for an overall condition of the water quality and physical stream condition.

4.2.3. Percentage of Plecoptera

In the southern catchment, site DR2 gave the highest percentage of 3.5 % in sample 4. The sites RG9 (sample 4), GS5 (sample 3), GS3 (sample 3), and DR2 (sample 1) gave a percentage ranging within 1.0-1.7 %. The sites RG12 (sample 1, 2, &4), GS5 (sample 1), GS3 (sample 2) and DR3 (sample 2 & 3) gave the lowest percentage of Plecoptera within the range of 0.0 – 0.2 %. The remaining samples of the sites RG9 (sample 1), GS5 (sample 2 &4), GS3 (sample 4 &1), DR2 (sample 2&3) and DR3 (sample 1&4) gave percentages within the range of 0.3-0.6%. The upstream sites in the Monkey River watershed gave a high percentage of Plecoptera that range from 0.3-1.4%, this was seen in all 4 samples of MR14, samples 4&3 of MR30 and samples 1, 3, &4 of MR 39. The downstream sites gave the lowest percentage that range from 0.0-0.2%. This was seen in all 4 samples of MR15 and MR40, samples 3&4 of MR38, samples 1&2 of MR30, and sample 2 of MR39. MR38 was the only downstream site to give a percentage of 0.4% in sample 1.

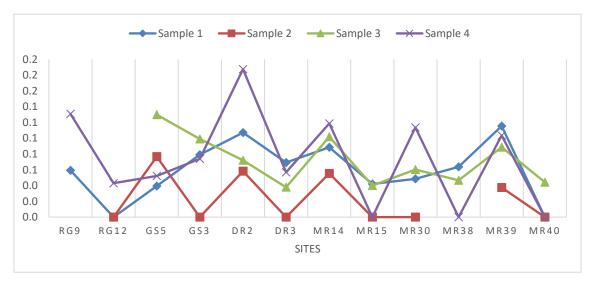


Figure 4: Percentage of Plecoptera

4.2.4. Percentage of Predators

The upstream sites DR2 and GS5 has a higher percentage of predators ranging from 6.8 – 17.4% in all four samples than their downstream sites. Site DR2 gave the highest percentage (17.4%) for the southern catchments, followed by GS5 with 13.7% in sample 3 and DR2 in sample 2 with 11.2%. The downstream sites DR3 and GS3 gave low percentage of predators ranging within 1.7-5.4% in all four samples. RG9 (9.6 & 9.1%) and RG12 (12.8 & 7.8%) gave high percentage in both sample 1 and 4. However, RG12 sample 2 gave a low percentage of 3.4. The sites in the Monkey River watershed all have high percentage of predators starting at 6.4%. The only two sample with the lowest percentage were from MR15 with 5.9% (sample 4) and 4.4% (sample 1). The highest percentage of predators were found in MR38 sample 4 (17.8%), MR39 sample 2, 3, and 4 (18, 17%) and MR40 sample 1, 2, 3 and 4 (12.4, 10.2, 18.3 &18.4%). The percentage in MR14 site range from 6.4-10.2% in all four samples.

The SVAP scores for sites RG9, RG12, GS3, GS5, DR3, MR14, MR15, MR30, MR38, and MR39 scored within the range of 7.5 – 8.9, indicating good physical condition of the sites

(Table 3). Four sites (GS3, GS5, MR30 and MR39) gave scores ranging within 9.1 -9.8 in one or two of the samples. MR38 gave one fair score of 7.2 in the first sample, but gave higher scores for the remaining three samples. The site DR2 and MR40 gave high scores of 9-9.8 through all four samples indicating excellent physical stream condition



Figure 5: Percentage Predator

5. Discussion

The BMWP and ASPT scores shows some consistencies over the two years and seasons within the four sampling period. Eight of the twelve sites in accordance to the BMWP score of Costa Rica would rate as excellent condition, while only four sites would rate as good condition. Of the four sites, two sites had low score of 50 and 37 in the fourth sampling, which may have been due to a low number of Ephemeroptera, Plecoptera and Tricoptera (EPT) families found in MR15 and MR38 sites. The EPT contains families with high BMWP scores, so the low number of these families found in a site would cause the BMWP score to be low. The SVAP scores were also consistent over the two years at all 12 sites and complements the BMWP and ASPT scores of each sites.

The good condition of the streams is further indicated by the water quality parameters recorded. The physicochemical parameters meet the Mexico and Costa Rica water quality standard for the ecological integrity of aquatic life in warm surface water. The EC for the streams within the Monkey River Watershed is of a lower range, than the Southern catchments sites. The geology of the streams in the Monkey River watershed are siliceous, while the southern catchments sites are calcareous that contributes to the different levels of EC within these two distinct geological streams (Carrie, 2015). The three different ranges found in the subcatchments of the Monkey River may be related to the significance difference of the three streams and substratum that characterize these stream (Neff and Jackson, 2011). Turbidity remains very low for all streams across the four monitoring. The only streams that quickly get turbid after light rains are the RG 9 and RG 12 sites on the San Miguel River in the Rio Grande Watershed, thus the reason macroinvertebrate mapping at times cannot be done.

5.1. Natural Variation

The five forested sites (GS5, DR2, MR14, MR30 and MR 39 rated excellent according to the BMWP-CR score (Table 3). The physical condition (SVAP) of the forested sites rated good, especially for the two attributes riparian zone and canopy that scored a 10. The good condition of these sites are also indicative by the number of EPT families found within them. The number of EPT families' ranges from 10-14 different families with those of high BMWP scores, thus resulting in the overall of each sites to be rated as an excellent condition.

The highest percentage of Tricoptera were found in site MR 14 on the Bladen River during the last two monitoring, which contain high BMWP scoring families such as *Odontoceridae* (9), *Glossosomatidae* (8), *Philopotamidae* (7), *Polycentropodidae* (6) and *Hydrobiosidae* (9). The other three forested sites (MR39, MR30 and DR2) also gave a high percentage of Tricoptera. The only forested site that had a low percentage of Tricoptera is GS5 in all four monitoring. However, the percentage of Plecoptera were the highest for all forested sites, especially for site DR2 in the Deep River Watershed. The order Plecoptera has only one family (*Perlidae*) identified in Belizean streams, which carries a high BMWP score (9). These high scoring families are good indicators of healthy stream as they require high oxygen, diverse habitat and riparian vegetation that feed the stream with organic matter. The relative abundance of predators is the only functional metric analysed over the four samples. It is the highest in the forested sites MR30, MR39, DR2 and GS5, indicating diversity and health of the ecology of the stream, as they play important roles of balance by controlling other species population.

5.2. Maya Mountain North Agroforestry Concession

The overall rate of the water quality for MR30 site score excellent and good for MR38 site, even though MR38 is below an agroforestry concession. It can be assume the concession does not have adverse impacts to the water quality since the site rated as good. Although the last sample for site MR38 gave a low score of 37. The low score for site MR38 may be attributed to single Tricoptera family collected at the site for that particular sampling period. Although, it was one family (*Philopotamidae*), it carried a high BMWP score (7) giving a fair score for the site. It also had a low percentage of Plecoptera probably contributing to the low score. However, the site had a higher percentage of predators compared to its upstream site MR30 in sample 4 (end of dry season). It is also important to note the number of families and EPT families are higher at the beginning of the dry season than at the end of dry season. This could be a factor of certain taxa life cycle to change into adults as a life strategy to avoid the low water levels in the dry season (Johnson *et al*, 2012), thus the low number of EPT families found at the site. However the high percentage of predators are mostly from the Order Odonata that develops biological traits or migrate when stream desiccates (Munné and Pratt, 2011), thus might be the reason there is a high percentage of predators found at site MR38.

5.3. Community Engagement

Rural communities rely heavily on the stream for domestic use such as laundry, bathing, and agriculture. They are very interested and supportive to studies relating to the condition of the streams. The sites on the Deep River watershed resulted in an excellent condition of the water quality according to the BMWP-CR, SVAP and ASPT-CR scores. Even the site DR3 in the community scored excellent regardless of the daily use (washing, bathing) that occurs in the stream. Plausible reason might be the physical condition (riparian zone and canopy) and diverse habitats found at each site. In addition, DR2 had a higher percentage of predator and Plecoptera that contributed to the high BMWP scores. The relative abundance of Tricoptera was alike

between both sites, thus contributing to the score of the site. The sites on the Rio Grande River resulted in a good condition, although it has moderate human impacts such a small hydro dam at the upstream and domestic use by the community of San Miguel. Although data was missing for the second and third samples for site RG9, due to turbid water and high water level, it rated as good water quality only from the two sample taken. This is probably due to the high number of scoring taxa found at the sites. For instance, the relative abundance of Tricoptera (14.1%) and predators (12.8%) were high in RG12. The relative abundance of Plecoptera were high in RG9 (9.6%) allowing for the BMWP score to increase.

5.4. Surveillance

The results for eight of the eleven sites selected for surveillance have already been discussed in the sections above. The remaining three sites to be discuss are the downstream sites GS3, MR15 and MR40. The sites GS3 and MR40 have rated as excellent water quality condition according to the BMWP-CR score. This can be attributed to high number of EPT families, relative abundance of Tricoptera and Plecoptera found for all three sites. Site MR15 has rated fairly in the fourth sample, probably due to the low number of families and EPT families found at the site. All the sites selected for surveillance during the two year period have resulted in a good to excellent condition according to the BMWP-CR score. Majority of sites have shown high number of families, especially for the EPT families and percentage of predators in one or more of the sampling periods.

6. Summary and Recommendations

The four sampling period over the two year period indicates a positive ecological trend in the stream condition of the twelve sites selected. This was shown by the consistent BMWP score over the four samples for all the 12 sites. The downstream and upstream sites in the Rio Grande, Golden Stream and Deep River were of similar class condition. The same can be said for the condition of the streams in the Monkey River watershed. The biotic metrics BMWP-CR, ASPT-CR, relative abundance of Plecoptera, relative abundance of Tricoptera and relative abundance of Predators from the two year study indicate they are sensitive to the changes of the season, and can be assumed that they are robust to be use in bio-assessment in the MMMC, without confounding with natural factors. The environmental variables: temperature and electrical conductivity were not influence by season, but by the environmental characteristics of the catchments.

Recommendations:

- 1. A continuous monitoring over a five year period or more would provide a more sufficient data to concretely conclude the robustness of the metrics yearly and seasonally.
- 2. A continued monitoring of the sites MR15, MR38 and RG12, especially within the dry season is required in order to evaluate how the water quality condition of the sites develops over time.
- 3. Sampling of all sites should be done within the same time frame (month) each year for both season.
- 4. Further research on the ecology aspect (environmental characteristics of each stream) that may influence the abundance or presence of certain families found along the stream.

7. Reference

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