ASSESSMENT OF FUNCTIONAL FEEDING GROUPS OF AQUATIC INSECT COMMUNITIES IN THE MOHLAPITSI RIVER, SOUTH AFRICA

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(Received 25th Mar 2022; accepted 11th Jul 2022)

Abstract. The changes occurring in the catchment of the Mohlapitsi River in South Africa as a result of anthropogenic activities are affecting the integrity of the river and may subsequently alter the composition and functional structure of aquatic insect assemblages. The aim of this study was to assess aquatic insect composition and richness of the functional feeding groups at different sites along the river. The insect structural composition differed among sites and seasons. The number of taxa and the diversity of insects remained relatively high across the river, especially in the downstream. The highest abundance of aquatic insects was recorded at the downstream sites, S5 and S6. Taxa richness and abundance were higher during the dry season than during the wet season. Collector-gatherer was the dominant functional feeding group in abundance and the predator was the dominant group in taxa (family) richness. The spatial and temporal functional composition were related to the environmental variables in the river. These relationships suggest that the physicochemical variables have influence on the distribution, abundance, and diversity of functional groups. However, the low abundance and taxa richness in the midstream suggest that the activities along the river are gradually impacting the river. It is important to implement proper measures to reduce agricultural and domestic discharges into the river in order to maintain its integrity and conserve the aquatic biota.

Keywords: bioindicators, functional structure, land use changes, macroinvertebrates, water quality

Introduction

Many freshwater ecosystems are being polluted due to discharges from mining, industrial, agricultural, and domestic activities (Li et al., 2018; Chen et al., 2019). Furthermore, land use changes have caused destruction of riparian vegetation and loss of habitats, which have affected both the integrity of freshwater bodies and the aquatic biota. The community structure of many aquatic organisms, specifically aquatic insects, represents a high degree of spatial variation along rivers and therefore serves as good indicators of water quality (Keke et al., 2017; Sor et al., 2017; Addo-Bediako, 2021). The spatial variation in their composition may be influenced by physicochemical variables (Al-Shami et al., 2013; Cortes et al., 2013; Kumar and Khan, 2013) and trophic factors (Nicola et al., 2010; Cai et al., 2012).

Many metrics such as abundance, taxa richness and diversity of macroinvertebrates have been used in monitoring freshwater ecosystems. Recently, a functional approach based on macroinvertebrate functional feeding groups (FFG) is being used as indicator of ecosystem attributes and to assess the ecological health of rivers/streams (Merritt et al. 2005; Fierro et al., 2017). Functional feeding group is an important tool for establishing trophic relationships and community dynamics (Vannote et al., 1980; Cummins et al., 2005; Fu et al., 2016).

The classification of FFG considers the morphological and behavioural characteristics used in food acquisition (Ramirez and Gutiérrez-Fonseca, 2014). Macroinvertebrates can be classified into five groups based on consumption of diverse food resources and feeding

strategies: shredders, collector-gatherers, collector-filterers, predators and scrapers. Shredders feed on living or dead parts of plants and therefore, perform an important role in the transformation of coarse particulate organic matter (CPOM) to fine particulate organic matter (FPOM) in rivers; collector-gatherers feed on smaller particulate organic matter deposited on substrates; collector-filterers filter organic particles directly from the water column; predators feed on other live animals, whole or part of them; and scrapers feed on organic matter, algae and other associated organisms (periphyton), that usually form a matrix on the surface of substrates, such as rocks and submerged plant material. Thus, the food sources and availability influence significantly the distribution of FFGs (Allan and Castillo, 2007). Generally, shredders and scrapers are more sensitive to disturbances that might change the availability of certain food or habitat, while collectors (filterers and gatherers) are more tolerant, so they can potentially be used to assess aquatic ecosystem health (Bhawsar et al., 2015; Meira et al., 2021).

The Mohlapitsi River is an important tributary of the Olifants River Basin and it has long been known to provide the basin with water of good quality. However, due to the increasing agriculture, sand mining activities, together with human settlements in the catchment, the river is being degraded. Little is known about the ecological impact of the activities on the river and aquatic biota such as insects. There is a need therefore to study the spatial community structure of organisms and their relation to environmental factors in the river. The objective of the study was to assess the spatial and temporal differences in FFG of aquatic insects and relate these to differences in ecosystem attributes. It was hypothesized that there is a change in the FFG structural composition from upstream to downstream of the river in relation to changes in energy flow as predicted by the River Continuum Concept (RCC).

Materials and methods

Study area

The Mohlapitsi River in South Africa is an important tributary of the Olifants River, as it supplies the latter with water of good quality. The river takes its source in a protected Wolkberg Wilderness area, then flows downstream passing through various agricultural fields and human settlements before joining the Olifants River. The communities in the area depend on the river for domestic use, irrigation and livestock. Six sampling sites were selected along the river (Fig. 1); Site S1 (24.1650044S; 30.1043448E) was in the Wolkberg Wilderness area; it is surrounded by vegetation, comprising trees, shrubs and ferns, with little bank erosion. Site S2 (24.1738869S; 30.1027902E) was adjacent to a small human settlement, with a very little disturbance and there are big trees which provide shade to a greater part of the site. Site S3 (24.1806804S; 30.0975124E) was below a weir, with less vegetation around, though there are reeds and shrubs, with a few fig trees adjacent to the river. Site S4 (24.2367189S; 30.0778399E) was adjacent to a settlement (Ga Mafefe village), sand mining, washing of clothes occur at this site and also serves as source of drinking water for some of the communities and livestock. Site S5 (24.2370664S; 30.0785938E) was near a cattle grazing area, though it was in the downstream, the site is surrounded by trees, especially wild fig trees, which provide shade to a greater part of this section of the river. Site S6 (24.2371333S; 30.0781493E) was at the confluence of the Mohlapitsi River and the Olifants River. The area is mostly surrounded by shrubs and grasses. The sites were selected to cover upstream (S1 and S2), midstream (S3 and S4), and downstream (S5 and S6).

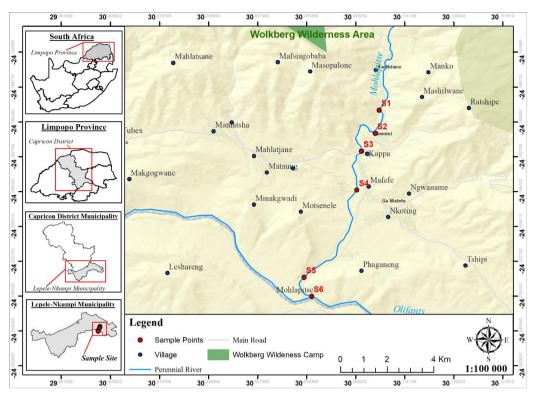


Figure 1. The selected sampling sites of the Mohlapitsi River of the Olifants River Basin

Water sampling

Water samples were collected at the six sites of the Mohlapitsi River in March (autumn), June (winter), October (spring) and December (summer), 2019. The water samples were collected in 1000 ml acid pre-treated polyethylene bottles. The water was stored at 4°C prior to chemical analysis. The pH, water temperature, dissolved oxygen, total dissolved solids (TDS), electrical conductivity and salinity were recorded *in situ* at sampling sites using the YSI Model 554TM Datalogger multiprobe (YSI Inc., Yellow Springs, Ohio). The water samples were analyzed for nutrients (nitrate, nitrite, total nitrogen, ammonia and phosphate), and turbidity in the laboratory (Waterlab) using a spectrophotometer (Spectroquant Pharo 100, Merck, Germany). Flow velocity was measured using a Flo-mate portable flowmeter Model 2000 (Marsh McBirney, Maryland, US). The width and water depth were measured using a measuring tape and graduated measuring rod, respectively.

Sampling of aquatic insects

Aquatic insects were collected at the selected sites using a standard net of 300 mm x 300 mm with the mesh size of approximately 500 μ m. The kick sampling method described by Dickens and Graham (2002) was used. The substrate was disturbed by kicking with the feet while sweeping the net in a zig-zag manner to free insects. At each site, approximately 6 min was spent sampling all aquatic habitats (i.e. riffles, pools and vegetated margins) and were combined to form one composite sample. The insect samples collected were identified to the family level in the field using an Invertebrate Field Guide Manual (Gerber and Gabriel 2002), with the aid of magnifying glass. However, those insects which could not be identified in the field were preserved in 70% ethanol in a litre polypropylene containers and transported to the laboratory for further

identification using a stereomicroscope (Leica EZ4). The insects were then classified into functional feeding groups (FFGs): Shredders (Sh), Collector-gatherers (CG), Collector-filterers (CF), Scrapers (Sc) and Predators (P), using the criteria of Merrit and Cummins (1996), Cummins et al. (2005) and Cummins (2016).

Data analysis

The mean and standard deviation of the physicochemical parameters were calculated. One-way ANOVA was used to compare means of physicochemical variables of water and insect distribution across the sites, after data was checked for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) and log transformations where necessary, using Statistica Version 10. The percentage contribution of each FFG to the different communities was determined to find out the relative contribution of each group. The influence of physicochemical variables on macroinvertebrate communities and functional feeding groups were determined by canonical correspondence analysis (CCA) using CANOCO version 5.1 software (Ter Braak and Smilauer, 2002).

Results

Physicochemical parameters

The measured physicochemical parameters are shown in *Table 1*. There were no significant differences in the physicochemical variables among the sampling sites (p < 0.05), however, DO and conductivity showed significant differences among the sites (F = 3.979, p = .013 and F = 2.822, p = 0.047 respectively). The upstream site, S1 had the lowest mean values for all parameters except DO. The highest mean water temperature and salinity levels were recorded at S3 and the highest pH, conductivity and TDS were recorded at S6. Generally, nutrient levels were very low at all sites. Ortho-phosphate was below detection level at all sites. The highest nitrate and total nitrogen concentrations were recorded at S5. The highest mean ammonium concentration was at S2 and the concentration of nitrite was the same at all sites except S2.

Physicochemical parameters	S1	S2	S 3	S4	85	S 6	Guideline values
Velocity (ms ⁻¹)	0.47 ± 0.2	0.66 ± 0.3	0.84 ± 0.7	0.80 ± 0.3	0.42 ± 0.1	0.50 ± 0.5	
Depth (m)	0.46 ± 0.1	0.31 ± 0.1	0.51 ± 0.2	0.57 ± 0.1	0.47 ± 0.2	0.64 ± 0.8	
Width (m)	6.12 ± 0.9	7.40 ± 1.0	6.80 ± 1.7	8.15 ± 0.8	10.2 ± 2.1	15.8 ± 0.3	
pH	7.6-7.9	7.7-8.0	7.9-8.4	7.9-8.4	7.8-8.2	7.9-8.5	$6.5.0-9.0^{1}$
Temperature (°C)	18.1 ± 2.5	19.5 ± 2.4	20.4 ± 2.0	20.2 ± 1.5	19.6 ± 1.7	20.0 ± 1.2	
Conductivity (µS/cm)	140.3 ± 32	231.6 ± 67	234.0 ± 67	234.5 ± 67	266.3 ± 61	275.1 ± 68	1700^{1}
TDS (mg/l)	104.6 ± 20	165.8 ± 45	166.7 ± 45	165.7 ± 45	194.7 ± 44	296.8 ± 224	1200 ¹
DO (mgl)	10.5 ± 1.7	9.7 ± 1.1	9.3 ± 0.8	8.7 ± 0.8	8.4 ± 0.6	7.6 ± 0.7	-
Salinity (ppt)	0.07 ± 0.02	0.11 ± 0.3	0.34 ± 0.4	0.12 ± 0.03	0.16 ± 0.02	0.2 ± 0.14	0.5^{1}
Nitrate (mg/l)	0.15 ± 0.06	0.2 ± 0.01	0.15 ± 0.1	0.15 ± 0.06	0.25 ± 0.17	0.2 ± 0.14	$< 11.0^{1}, 13^{2}$
Nitrite (mg/l)	0.05 ± 0.01	0.03 ± 0.01	0.05 ± 0.0	0.05 ± 0.00	0.05 ± 0.01	0.05 ± 0.1	$< 0.9^1, 0.6^2$
Ammonium (mg/l)	0.07 ± 0.6	0.63 ± 0.4	0.45 ± 0.4	0.45 ± 0.1	0.61 ± 0.4	0.6 ± 0.4	< 0.011
Total Nitrogen (mg/l)	0.31 ± 0.05	0.25 ± 0.03	0.2 ± 0.1	0.2 ± 0.06	0.39 ± 0.1	0.25 ± 0.13	< 0.5 (oligotrophic)
Ortho-Phosphate (mg/l)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	

Table 1. Mean water physicochemical variables (\pm standard deviation) recorded across thesix sites of the Mohlapitsi River

¹DWAF (1996)

²CCME (2012)

Insect community structure

The abundance of the insect taxa and functional feeding groups (FFG) assigned are shown in *Table 2*. A total of 6 386 individual insects belonging to 51 families and seven orders were recorded. Diptera had the highest taxa (11), followed by Odonata (10), Tricoptera (9), Hemipteran (8), Ephemeroptera (6), Coleoptera (6), and then Plecoptera (1). The most abundant family was Baetidae (2527), followed by Hydropsychidae (700) and then Gomphidae (587). The highest abundance of insects was recorded at S5, followed by S6, S1, S2, S4 and the least abundance at S3.

Order	Family		S1	S2	S 3	S4	S 5	S 6	Total	FFG
Plecoptera	Perlidae	Perl	17	1	2	0	0	4	24	Р
	Baetidae	Baet	541	477	91	158	658	602	2527	CG
	Caenidae	Caen	25	0	0	0	0	0	25	CG
E-b	Ephemeridae	Ephem	0	0	0	0	17	0	17	CG
Ephemeroptera	Heptageniidae	Hept	9	19	0	17	1	41	87	SC
	Oligoneuridae	OligoN	0	0	0	8	0	0	8	CF
	Tricorythidae	Tric	0	11	3	5	0	41	19	CG
	Calopterygidae	Calo	0	7	14	0	78	6	105	Р
	Chlorocyphidae	Chlo	1	0	0	0	33	6	40	Р
	Syntestidae	Synt	0	0	0	0	95	0	95	Р
	Coenagrionidae	Coen	9	8	5	7	57	6	92	Р
Odonata	Lestidae	Lest	3	0	0	18	0	12	33	Р
Odollata	Platycnemidae	Plat	0	0	12	0	0	0	12	Р
	Aeshnidae	Aesh	51	16	12	12	27	4	122	Р
	Corduliidae	Cord	0	0	7	12	16	0	35	Р
	Gomphidae	Gomp	101	143	57	64	114	108	587	Р
	Libelliludae	Libe	20	1	6	4	17	1	49	Р
	Nacauridae	Naca	0	9	4	3	5	0	21	Р
	Notonectidae	Noto	0	2	0	0	0	0	2	Р
	Belostomatidae	Belo	1	0	0	0	13	6	20	Р
Hemiptera	Gerridae	Gerr	3	1	16	0	0	0	20	Р
Heiniptera	Hydrometridae	HydroM	0	1	0	2	0	0	3	Р
	Corixidae	Cori	0	3	0	0	0	0	3	Р
	Veliidae	Veli	0	16	2	35	0	0	53	Р
	Nepidae	Nepi	6	8	1	0	27	0	42	Р
	Dipseudopsidae	Dips	74	0	0	0	0	0	74	CF
	Ecnomidae	Ecno	65	16	31	0	43	106	261	CF
	Hydropsychidae	HydroP	294	90	23	8	135	150	700	CF
	Philopotamidae	Philo	3	27	0	0	0	78	108	CF
Tricoptera	Polycentropodidae	PolyC	4	0	5	0	9	0	18	CF
	Psychomyiidae	Psyc	0	12	0	0	74	50	136	CG
	Hydroptilidae	Hydropt	0	0	0	5	6	10	21	CG
	Lepidostomatidae	LepiDM	1	9	0	6	16	11	43	SH
	Leptoceridae	LepTC	0	9	0	0	11	0	20	SH
	Dystiscidae	Dyst	2	0	0	0	0	0	2	Р
	Gyrinidae	Gyri	0	2	0	4	0	0	6	Р
Coleoptera	Elmidae	Elmi	3	8	22	13	43	19	108	SC
Coleoptera	Helodidae	Helo	0	0	0	0	8	0	8	SH
	Psephenidae	Psep	10	10	27	87	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	41	260	SC
	Hydrophilidae	Hydroph	0	0	0	0	0	59	59	CG

Table 2. Functional feeding groups of aquatic insects recorded at different sites of theMohlapitsi River

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	1			1		1	1		1	
	Athericidae	Ather	23	11	8	4	1	5	52	Р
	Ceratopogonidae	Cerat	28	35	61	31	32	0	187	Р
	Chironomidae	Chiro	3	0	0	11	11	0	25	CG
	Culicidae	Culi	1	0	0	3	0	3	7	CF
	Dixidae	Dix	0	0	1	0	0	6	7	CF
Diptera	Ephydridae	Ephy	0	0	4	0	0	0	4	SH
	Muscidae	Musc	0	0	0	0	5	4	9	Р
	Psychodidae	Psycho	5	2	23	15	15	0	60	CG
	Syrphidae	Syrp	0	0	4	15	0	0	19	CG
	Simulidae	Simu	0	2	0	3	5	16	26	CF
	Tabanidae	Taba	34	19	11	2	44	15	125	Р
Total			1337	975	452	552	1701	1369	6386	

Functional organization

In terms of the functional feeding group (FFG) abundance, collector-gatherer was the dominant group (45.5%), followed by predator (27.2%), collector-filterer (18.9%), scraper (7.1%), and then shredder (1.2%). There were differences in abundance and proportion of the functional feeding groups among sites (*Table 3*), but there were no significant differences (p > 0.05). The highest abundance of the collector-filterers was at S1, and collector-gatherers, predators, scrapers and shredders at S5 (*Fig. 2*). Predators, collector-gatherers, collector-filterers, shredders and scrapers comprised 25, 10, nine, four and three families respectively. In the CCA analysis, axis 1 was positively correlated with high DO and associated with S1, and the site was characterized by CF and CG. The second axis was associated with S3 and S4, and correlated with velocity, depth and NO₂, and the sites were characterized by scrapers. Sites S2 and S5 were characterized by shredders and were associated with TDS and width of the river (*Fig. 3*). The first three CCA axes accounted for 98.2% variation, with the first and second axes contributing 92.4% of the total variation (Monte Carlo test; P < 0.05) (*Table 4*).

Site	S1	S2	S 3	S4	S 5	S6
CF	441	135	60	22	192	359
CG	574	502	121	209	781	721
Р	299	283	218	198	564	177
Sc	22	37	49	117	129	101
Sh	1	18	4	6	35	11
Total	1337	975	452	552	1701	1369

Table 3. Abundance of functional feeding groups of the various sites in the Mohlapitsi River

Table 4. The CCA results which indicate correlation between physicochemical variables and the aquatic insects of the Mohlapitsi River

Axes	1	2	3	4	Total inertia
Eigenvalues	0.020	0.010	0.001	0.000	
Taxa-environment correlations	1.000	1.000	1.000	1.000	
% cumulative variance of taxa-environment	67.1	92.4	98.2	100	
Sum of eigenvalues					0.025

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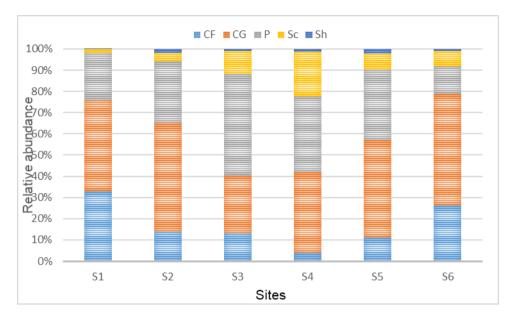


Figure 2. Relative abundance of the functional feeding groups at studied sites of the Mohlapitsi River (CF = collector filterers, CG = collector gatherers, P = predators, Sc = scrapers, Sh = shredders)

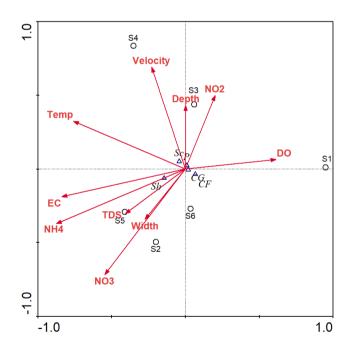


Figure 3. Canonical correspondence analysis (CCA) showing the relationship between environmental variables and macroinvertebrate functional feeding groups

Seasonally, the highest abundance was in autumn (2057), followed by winter (2051), spring (1607) and then summer (671). The highest number of taxa was during autumn (38), followed by winter (37), spring (35) and then summer (21) (*Table 5*). Collector-gatherers, collector-filterers were numerically dominant during autumn, predators and

shredders were dominant in winter (*Fig.* 4). However, there were no significant variations in terms of distribution of the FFGs among seasons (p > 0.05).

	Summer	Autumn	Winter	Spring	Total
CF	67	450	389	303	1209
CG	366	941	940	661	2908
Р	183	446	564	546	1739
Sc	51	202	112	90	455
Sh	4	18	46	7	75
Total	671	2057	2051	1607	6386

Table 5. Seasonal abundance of functional feeding groups in the Mohlapitsi River

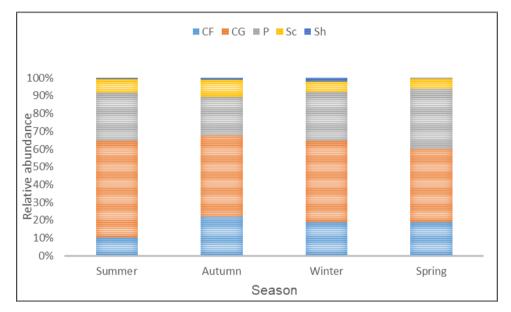


Figure 4. Relative abundances of the functional feeding groups of insects at different seasons ($CF = collector \ filterers, \ CG = collector \ gatherers, \ P = predators, \ Sc = scrapers, \ Sh = shredders$)

Discussion

Physicochemical parameters

Most of the physicochemical parameters and nutrients measured were within the standard guideline values. Thus, the human disturbances such as runoff, mainly from agricultural areas and the discharge of domestic waste waters have not significantly impacted the river. However, there was increasing concentration of TDS, electrical conductivity and turbidity from upstream to downstream. The nutrient levels were generally lower in the upstream but higher at the midstream and downstream sites due to increasing flow discharge and flooding from upstream and midstream of the river (Magoale et al., 2022). This is an indication that human activities are gradually having impact on the river. The most visible disturbances observed during this study were road construction near S3, which has resulted in the removal of most of the riparian vegetation and causing erosion of stream banks and sand mining at S4. These have

contributed to the sediment loads entering the river and may reduce available microhabitats for the biota (Addo-Bediako, 2021).

Insect community structure

The high abundance and taxa richness of the Mohlapitsi River could be attributed to the presence of riparian vegetation along most parts of the river which may provide food and breeding sites for many insect taxa. The high numbers of Ephemeroptera, Tricoptera and Odonata is attributed to clear water and a high level of dissolved oxygen in the river (Dobson et al., 2002). The high richness and abundance of insects at tsites, S5 and S6 might be attributed to the large width and depth of the river, these conditions promote different microhabitats and high nutrient and sediment load (Al-Shami et al., 2013). In addition, the high nutrient and high temperature recorded in the downstream of the river may provide ideal conditions for phytoplanktons (Vannote et al., 1980), and subsequently increase the abundance and richness of zooplanktons including some insect larvae and nymphs (Cai et al., 2012).

Functional organization

The collector-gatherers were the dominant group at all sites of the river, but the highest abundance was at the downstream sites, S5 and S6. The collector-filterers were also well represented at all the sites. The high abundance of the collectors could be due to the fact that they can feed on a broad range of food materials (Merritt et al., 2002; Addo-Bediako, 2021). The predators were well represented at all the sites. The high abundance and taxa richness of predators along the whole longitudinal gradient of the river may be due to availability of food (prey) and less competition (Ono et al., 2020). The predator distribution was almost similar at all sites, in support of the fact that they usually have similar proportion throughout the length of a stream channel, according to the river continuum concept (Vannote et al., 1980). The low number of shredders recorded is not unique to this river, such pattern has also been reported in many tropical and sub-tropical rivers and does not support the RCC model (e.g., Oliveira and Nessimian, 2010; Masese et al., 2014; Brasil et al., 2014; Doong et al., 2021, Makgoale et al. 2022). The significance of shredders in decomposition of organic material tends to decrease with low latitude (Gonçalves et al., 2006), instead there is a faster microbial decomposition due to the higher temperatures (Kaboré et al., 2016; Madomguia et al., 2016). Furthermore, the presence of secondary compounds in leaves of tropical trees reduce the palatability and nutrient content (Wantzen et al., 2002).

The CCA analysis indicated a significant relationship between the environmental factors and the functional groups in the axis 1 and axis 2 (p < 0.05). Shredders were mainly found at S2 and S5 and scrapers were mainly found at S4, where it is exposed to more sunlight, which promotes better growth of periphyton. However, collector-gatherers, collector-filterers and predators were well distributed throughout the sites, an indication that there was abundant food for these groups.

Seasonal influence was noted in the structural and functional organization of the aquatic insects due to seasonal differences in water quality and habitat characteristics. The abundance of most taxa was considerably lower in the hot and wet summer season than in the cold and dry seasons. This was also observed in other studies of tropical rivers, where abundance increased during the dry season (Tumwesigye et al., 2000; Arimoro et al., 2012). Flow reduction during the dry season contributes to seasonal

variability in physicochemical conditions that could influence aquatic insect community structure. In this study, the increase in the number of insects and taxa during winter and autumn (relatively dry seasons) was probably related to a better water quality and increased algal availability as a result of reduced turbidity. Thus, algal food sources for scrapers probably were limited during the wet season. Whiles the increase in shredder abundance during the dry seasons could be due to more food (fallen leaves from the riparian vegetation). However, in other studies of tropical rivers, abundance decreased during the dry season (Masese et al., 2009, 2014).

Conclusion

The abundance and relative proportion of the functional feeding group showed variation across sites. In general, collector-gathers and predators were the dominant groups at all sites. The variation in functional feeding group distribution across sites may have significant implications in understanding the spatial changes in aquatic insect community structure. The shredder and collector co-dominance in the headwaters was not observed as predicted by RCC. Though, the collector-gatherers dominated the downstream of the rivers, which supports the prediction of the RCC. The fact that changing environmental conditions influenced the FFG pattern confirms that FFG is an effective tool to assess ecological integrity of rivers. The spatial patterns of FFG insect community structure in the Mohlapitsi River were affected by resource availability, habitat heterogeneity and human alterations. Seasonal changes played a major role determining the distribution of aquatic insect taxa. The results suggest that policies governing changes in land use occurring in the Mohlapitsi River catchment should take into consideration the impact on biodiversity.

Acknowledgements. The author is grateful to the Flemish Inter-University Council (VLIR-UOS), Belgium for the financial support and postgraduate students in the water lab for their assistance in the data collection.

Data availability. Data will be made available on request.

Conflict of interests. The author reports no conflict of interests.

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DOI: http://dx.doi.org/10.15666/aeer/2005_42834294

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