RELATIONSHIP BETWEEN WATER QUALITY & BLACK FLIES (DIPTERA: SIMULIIDAE) ABUNDANCE IN TAMBUNAN DISTRICT, SABAH.

Nur Ashiqin Abdul Hamid[1*], Maria Lourdes T. Lardizabal[1], Hiroyuki Takaoka[2], Estherpeni Stephen[1] & Maznah Mahali

[1]Faculty of Science and Natural Resources, University Malaysia Sabah, Jln. UMS, 88400 Kota Kinabalu, Sabah, Malaysia
[2]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur
Corresponding author: Nur Ashiqin Abudul Hamid
Email: ashiqinhamid@gmail.com

ABSTRACT. *A study to investigate the relationship between black flies (Simuliidae) pupa abundance and physio-chemical parameters such as velocity, water temperature, pH, dissolved oxygen, conductivity and total dissolved solid was conducted at Tambunan district, Sabah. A total of six rivers were selected as sampling stations. Five sampling points located at a distance of 5-10 meters were established in each sampling station. Sampling was conducted every fortnight for a duration of six months from October 2015 until March 2017. Samples of black flies larvae and pupae were manually collected from substrates consisting of grasses, plant roots and plastics which were found stuck in between the rocks with running water area. Water quality parameters that were measured during every sampling, include water temperature, velocity, pH, dissolved oxygen, conductivity and total dissolve solids (TDS). Results from this study showed that The Principal Component Analysis (PCA) revealed two PC's which had eigenvalues >1.0 and together accounted for 78% total variability of the physio-chemical parameters. PC-1 which accounted 56% of variability defined a normal temperature (23-25^{0}C), high water velocity, high dissolved oxygen, low conductivity and low total dissolved solid. While PC-2 explained 22% of the variability was related to water pH. Pearson's correlation result shows that only velocity had a significant relationship with the abundance of black flies ($r=0.512$, $p<0.01$), while other parameters did not show any significant relationship with its abundance. In conclusion, results from this study revealed that only water velocity had a significant relationship with the abundance of black flies.*

KEYWORDS: Black flies, physio-chemical parameters, Tambunan.

INTRODUCTION

Black flies (Diptera: Simuliidae) are among the best-known aquatic insect in resource turnover and have been extensively studied worldwide. Black flies belong to the Order diptera and family Simuliidae (Zhang *et al.*, 1998). Their populations are distributed widely in Africa, America and some Asian countries such as India, Japan and South-East Asia (Srisuka *et al.*, 2015; Adler, 2005; Butler *et al.*, 1998). Immature black flies (larvae and pupa) prefer running water habitat which contains high oxygenated concentration in

waterways such as rivers, streams and waterfalls (Craig, 2003). Such habit makes the black flies important to the lotic ecosystem either for their integral role in organic matter processing in streams (Hart, 1986) or as a prey for food web dynamic in the lotic ecosystems (Cummins, 1988). According to Vincent & John (1975), *Simulium* is the first insect that appears in the recovery zone, which makes this insect group one of suitable bio-indicators for water quality. Some species are often found only in clean water, while some species can adapt to polluted water and these species are distributed in a wide range of ecological tolerances (Hamada & Grillet, 2001; Vincent & John, 1975).

On the contrary, some species of blackflies have been reported as disease vectors of river blindness' and skin problems in some countries such as in North America and Africa (Adler *et al*., 2004). This is caused by the adult females of these insects which are serious blood feeders that can cause the disease (Creadie *et al.*, 2011; Catherine *et al.*, 2010).

Black flies are less known in Malaysia and ecological research about this aquatic insect is still inadequate especially in the Borneo region (Takaoka, 1996; Takaoka, 2008). Most of the earlier studies on black flies in Sabah were related to taxonomy. Studies on the ecology and role of black flies as bio-indicator had so far not been well reported yet. Therefore, the aim of this study was to determine whether there is any relationship between black flies (Simuliidae) pupa abundance and the water quality.

MATERIALS AND METHODS

Study area: This study was conducted from October 2015 until March 2016. Black flies pupae and larvae were sampled once every fortnight which resulted 12 sampling efforts throughout the six months duration. Sampling stations were located in six selected rivers within the Tambunan district, Sabah. Habitat characterization and location of the rivers were as listed in Table 1.

Sampling procedure : At each sampling station, larvae and pupae were manually collected from all types of substrates that includes leaf litter, rocks, twigs and the artificial substrates such as plastics that are usually found stuck in between rocks in running water area at depths less than 100 cm (Figure 1). The river was chosen based on methods adapted from Zubaidah *et al.* (2016). Characteristics for choosing the streams would include the convenience of accessibility for sampling, the presence of substrates and continuous water flow. Five sampling points along the stream was set up at each site. The distance between sampling points ranged between 5-10 meters from each point (Figure 2). Sampling of pupae and larvae was conducted for about thirty minutes at each station. Physio-chemical parameters were also recorded during the sampling at each station. *In-situ m*easurement of the physio-chemical water quality was taken by using multi-parameter probe EUTECH PCD-650 for dissolve oxygen (DO), pH, conductivity, temperature and total dissolve solid (TDS).

Samples of black flies larvae and pupae were removed from the substrate and stored in eppendorf tubes containing 80% ethanol for preservation and identification. At the laboratory, mature pupae of black flies were sorted and placed into vials until the adult black flies emerged. Emerged adult black flies along with its pupae skin were preserved in eppendorf vials which contain 80% alcohol for further identification. Specimens of black flies were identified based on taxonomic key references (Takaoka 2001; Takaoka *et al.*, 2012).

Table 1: Habitat characterization of study areas.

Stations	GPS Location	Depth (m)	Width (m)	Habitat Description
Sg. Kerokot	N05°49'33.1"E116°29'39.5"	0.4	6.33	Fast flowing water, rocky bottom. Open canopy
Sg Lumondou	N05°42'54.7" E116°24'08.8"	0.45	7.67	Fast flowing water, rocky bottom. Open canopy.
Sg. Pegalan	N05°42'47.1" E116°24'28.2"	0.60	0.62	Fast flowing water, rocky bottom. Open canopy.
Sg. Tambunan	N05°41'42.6" E116°22'57.4"	0.15	1.33	Moderate water current, rocky and sandy bottom. Open canopy.
Sg. Malungung	N05°37'35.2" E116°17'12.7"	0.27	2.75	Fast flowing water, rocky bottom. Open canopy.
Sg. Kinabaan	N05°43'46.9" E116°23'27.5"	0.22	3.42	Moderate current, gravelly and rocky bottom. Open canopy

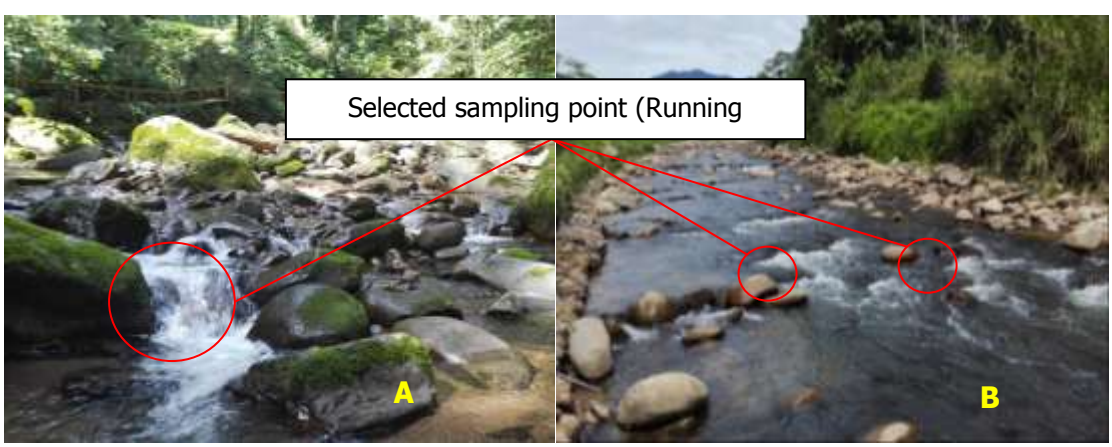


Figure 1: Running water area for black flies to breed. A) Sg Mahua, B) Sg Lumondou.

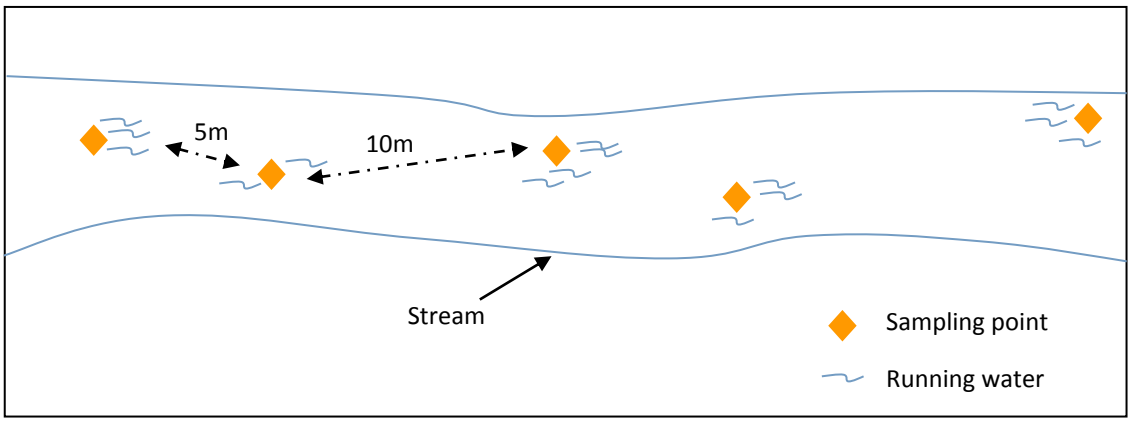
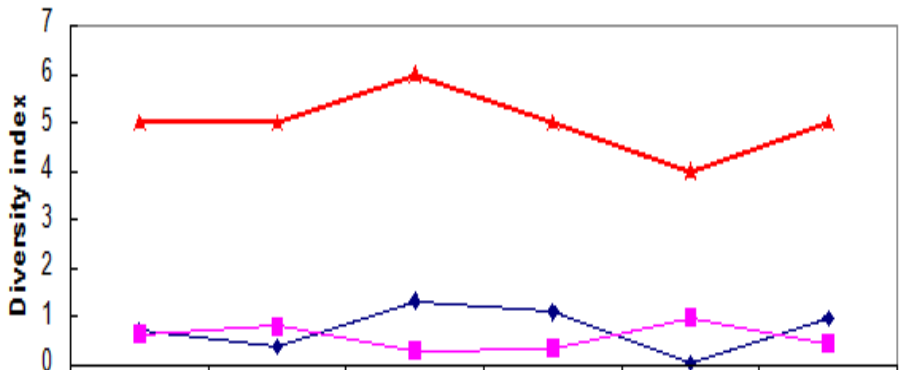


Figure 2: Distances of sampling points were set up at running water area.

Data Analysis : Diversity indices such as Shannon Weiner (H), Dominance (D) and species richness was analyzed with the aid of PAST version3 software (Srisuka *et al.*, 2015). Principal Component Analysis (PCA) was used to determine which combination of physio-chemical parameters were more predictive in describing each parameters which are velocity, water temperature, water pH, dissolved oxygen, total dissolve oxygen and conductivity (Scheiber & Debandi, 2008). The PCA was also used to reduce the number of parameters into groups of independent components (Zubaidah *et al.*, 2016). Spearman Correlation was used to determine the relationship between the principal component (PC) and the abundance of black flies (Zubaidah *et al.*, 2016). Pearson's correlation coefficient (r) was used to determine the interdependence of the physio-chemical parameters, whether the parameters (velocity, water temperature, water pH, dissolved oxygen, total dissolve oxygen and conductivity) were correlated each other and with the abundance of black flies (Popoola & Otalekor, 2011). All tests were considered significant at $p<0.01$. Water quality classification was referred based on the Water Quality Index Classification by Department of Environmental (DOE, 2006).

RESULTS AND DISCUSSIONS

A total of 8107 individual (pupa) of black flies were collected from October 2015 until March 2016. Throughout the sampling period, a total of seven black flies species were recorded. Figure 3 showed the diversity index for the six sampling stations in Tambunan. Diversity index values ranged between 0.04 and 1.3 which indicated a low diversity of black flies in the sampling stations. The highest Shannon Weiner (H) diversity index value was 1.3 that was recorded at Sg Tambunan. There were seven species of black flies recorded in Sg Tambunan, namely, *S. sabahense, S.beludense, S. keningauense, S. parahiyangum, S. sp, S. sheilae* and *S. aureohirtum*. Meanwhile, the lowest index value was 0.04 at Sg Lumondou with four species of black flies recorded which are *S. sabahense, S.beludense, S. keningauense,* and *S. parahiyangum,*. The highest dominance index was recorded in Sg Lumondou with the index value of 0.98, indicating the presence of a dominant species. The dominant species that recorded at this site was *S. beludense.*



	Sg Kinabaan	Sg Kerokot	Sg Tambunan	Sg Malungung	Sg Lumondou	Sg Pegalan
Shannon_H	0.7268	0.3874	1.321	1.097	0.04397	0.9658
Dominance_D	0.6553	0.8154	0.3154	0.3513	0.9877	0.4507
Species Richness	5	5	6	5	4	5

Figure 3: Diversity index value for black flies at selected streams in Tambunan.

Sg. Lumondou recorded the highest abundance of black flies pupae (2918 individual; 36% total abundance), while Sg. Tambunan recorded the lowest pupae abundance with 191 individuals (2.35% total abundance) of pupae (Table 2). Among the seven species recorded, *Simulium sabahense* and *Simulium beludense* (Figure 3) were recorded as a common species found at all the sites (Table 3). For this study, immature black flies from the species *S. sabahense, S.beludense, S. keningauense, S. parahiyangum* and *S. aureohirtum* was highly abundant in running water area with the water current velocity between 0.3 to 0.5 m/sec and range of river width of 3-6 m. While, *S. Sheilae* was found only at Sg Tambunan which has slow-flowing water velocity of 0.1-0.2 m/sec, and a similar finding was also reported by Takaoka (2001).

Table 2: Abundance of individual black flies and mean water quality parameter

Stations	N (Total pupa)	Abundance (%)	Velocity (m/sec)	DO (%)	pH	TDS (ppm)	Conductivity (µS/cm)	Temp (°C)	River Classification (DOE,2006)
S1	191	2.35	0.2±0.15	82.7±3.32	6.83±0.24	125.3±9.59	127.5±10.1	24.2±1.47	Class I (Notes : Class I – Conservation of natural environment; Water Supply I – practically no treatment (only boiling needed) Fishery I – very sensitive (aquatic)
S2	347	4.28	0.2±0.15	88.7±1.05	6.87±0.32	75.85±8.39	81.9±8.92	24±1.21	
S3	610	7.25	0.44±0.19	85.6±3.21	6.6±0.32	52.15±10.4	55.61±10.9	21.1±0.3	
S4	1275	15.73	0.2±0.55	85.8±2.61	6.7±0.26	40.9±15.01	35.2±15.03	22.2±0.82	
S5	2766	34.12	0.29±0.18	84.1±3.25	6.76±0.36	101.8±15.0	112.4±18.4	24±0.76	
S6	2918	35.99	0.36±0.28	82.8±3.90	6.68±0.33	63.87±9.22	61.94±8.79	23.6±1.5	

S1: Sg Tambunan; S2: Sg. Kinabaan; S3: Sg. Kerokot; S4: Sg. Pegalan; S5: Sg.Malungung; S6: Sg.Lumondou

Table 3: Composition and distribution of black flies at the sampling stations in Tambunan District, Sabah

Subgenus/ Species	Sg.Kerokot	Sg. Lumondou	Sg. Pegalan	Sg. Malungung	Sg. Tambunan	Sg.Kinabaan
Subgenus : *Simulium*						
S.sabahense	+	+	+	±	+	+
S.keningauense	+	+	+	+	+	+
S.aureohirtum	-	-	-	-	+	+
Gomphostilbia						
S.beludense	±	±	+	±	+	±
S.parahiyangum	+	+	+	+	+	+
S.sheilae	+	-	+	+	+	-

+: Present; ±: Common; -: absent

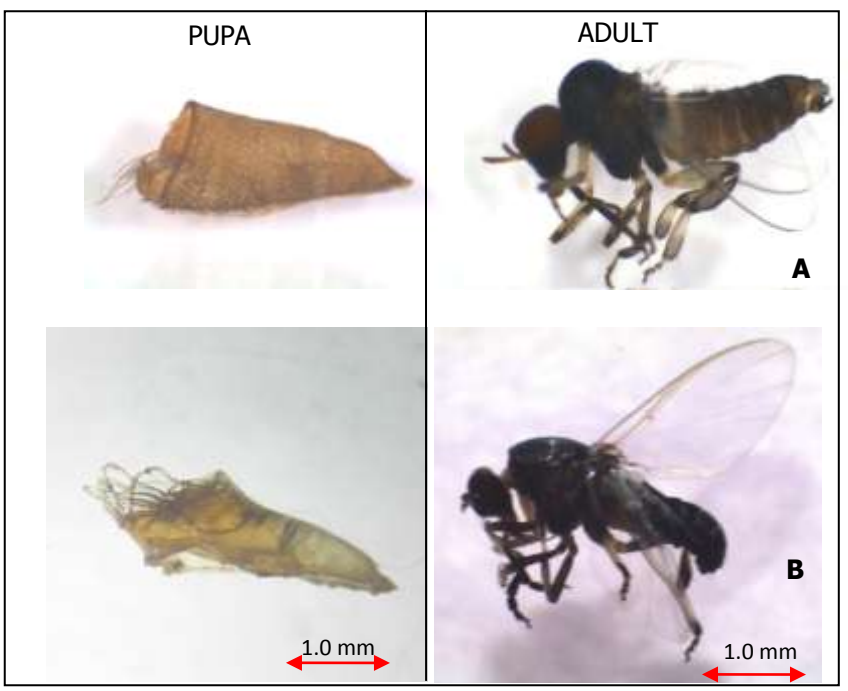


Figure 4: The common black flies species recorded A) *S. sabahense*, B) *S.beludense.*

Physiochemical parameter

Principal Component Analysis (PCA) of physio-chemical properties revealed two PC's (Figure 5), which have eigenvalue >1.0 with 78% total variance of the physio-chemical parameters (Table 4). PC-1 accounted 56% of variability, which was derived mostly from velocity, temperature, dissolved oxygen, conductivity and TDS. While PC-2 explained 22% of the variability was related to water pH. The sites with higher PC-1 were at normal temperature (23-25^{0}C), high water velocities, high dissolved oxygen, low conductivity and low total dissolved solid.

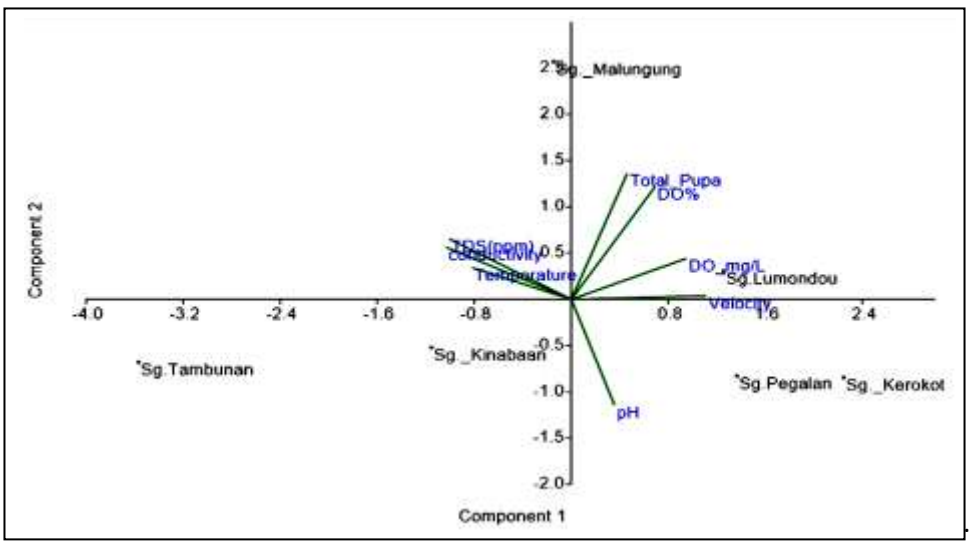


Figure 5: Biplot of sampled sites and physio chemical parameters resulting from PCA.

Result from PC-1 shows that velocity, temperature, dissolved oxygen, conductivity and TDS were associated with the black flies abundance. Previous findings showed the importance of water velocity for black flies distribution (Zubaidah *et al.*, 2016; Srisuka *et al.*, 2015). This is because the running water usually provides a good aeration, which also influenced the dissolved oxygen. According to Doisy *et al.* (1986), black flies larvae require at least 78% saturation of dissolve oxygen, while some species require between 92 to 98% of saturation.

Table 4: PCA and Spearman's rank coefficient of physio-chemical parameter in Tambunan.

Parameters	**Min - Max**	**Mean ± STD**	**PC1**	**PC2**
Velocity (ms^{-1})	0.3 - 0.7	0.62 ± 0.14	**0.988***	0.021
Temperature (^oC)	21 - 26	24.47 ± 1.76	**-0.729***	0.190
pH	7.21 - 7.29	7.25 ± 0.03	0.316	**-0.636****
Conductivity (μs^{-1})	63 - 119	96.92 ± 30.74	**-0.918****	0.313
Total Dissolved Solid (ppm)	58.4 - 134	91.39 ± 29.56	**-0.896****	0.363
Dissolved Oxygen (mg/L)	6.6 - 7.3	6.94 ± 0.29	**0.843***	0.245
Eigenvalues			4.5	1.7
% Proportion			56.39	22.05
Cumulative			56.39	78.44

*$p<0.01$, **$p<0.001$

Water temperature was also reported as one of the most important parameter that influenced the aquatic insect distribution (Scheibler & Debandi, 2008). The range of water temperature for this study was from 21^{o}C-26 ^{o}C. TDS and conductivity also influenced the abundance of black flies pupa, this is because there was a change of weather during the sampling period. The changes of weather may affect the condition of water against the total dissolve solids and conductivity (Laurince & Philippe, 2013). In this study the value of conductivity and TDS fluctuated due to the weather changed, and there was flood during the sampling. The higher value of TDS and conductivity was due to soil erosion caused by heavy rain. PC-2 showed pH as the only parameter which had negative relationship to the black flies distribution. For this study, the range of pH value was from 7.21 to 7.29.

Pearson's correlation coefficient (r) showed the relationship between the physio-chemical parameters and total pupa recorded at sampling sites (Table 5). The result showed that only velocity had a significant relationship with the total pupa ($r=0.52$, $p<0.01$), while other parameters did not show a significant relationship. The analysis showed that water velocity had a significant relationship with dissolved oxygen ($r=0.512$, $p=0.004$) and water pH ($r=-0.58$, $p=0.007$). The water velocity had moderate and positive correlation with dissolved oxygen, which meant that the higher the water velocity, the higher would be the saturation of dissolved oxygen. While other parameters such as pH, temperature, conductivity and TDS had inverse correlation with velocity. The temperature value recorded during the sampling period ranged between 21^{o}C to 24^{o}C, which were in the optimal range of tropical fresh waters (Popoola & Otalekor, 2011).

Pearson's correlation showed that water temperature had a strong relationship with dissolved oxygen (r=-0.84, p=0.04). Water velocity also was correlated negatively with conductivity and TDS. Pearson's correlation analysis showed that conductivity had a strong and positive correlation with TDS (r=0.93, p=0.003). Both conductivity and TDS indicated the presence of ions concentration that determined the quality of water (Tariq *et al*., 2006). Therefore, high water velocity may also decrease the source of dissolve ions in the water as in Siddaramu & Puttaiah (2013).

Table 5: Pearson's correlation (r) values between the physio-chemical parameters.

Parameters	Total Pupa	Velocity (ms^{-1})	pH	Temperature (^0C)	Conductivity (μs^{-1})	TDS (ppm)	Dissolve Oxygen (%)
Total Pupa	**1**						
Velocity	0.524*	**1**					
pH	-0.286	-0.581*	**1**				
Temperature	0.522	-0.502	0.227	**1**			
Conductivity	0.065	-0.560	0.065	-0.062	**1**		
TDS(ppm)	-0.084	-0.607	-0.098	0.060	0.929*	**1**	
DO%	0.516	0.512*	0.057	-0.844*	-0.313	-0.417	**1**

*$p<0.01$

From this study, all six selected rivers were classified as Class 1 river according to Water Quality Index Classification (DOE, 2006). From this study, all six selected rivers were classified as Class 1 rivers according to the Classification of Water Quality Index (DOE, 2006). Characteristics of a Class 1 river has a conserved natural ecosystem, practically no water treatment is required for water supply and sensitive aquatic species that exist in the river.

Limitations of this study had included the unpredictable change of weather that affected the sampling activities especially during rainy seasons. Heavy rain that caused flooding and high water current velocity washed away the substrates, thus leaving very little available substrates to sample. In addition, there were more potential stations around Tambunan, but some rivers were being restricted due to the "tagal" system in Sabah which thus makes the river inaccessible without the permission of the village chief.

CONCLUSIONS

As a conclusion, there were seven species of black flies recorded in this study. The dominant species at all study sites were *S. sabahense* and *S. beludense* , and results from the diversity index that ranged between 0.04 and 1.3 indicated that the diversity of black flies in Tambunan was still low. Findings from this study also showed that only water velocity had a significant relationship ($r=0.52$, $p<0.01$) with the black flies abundance. It is thus recommended that in-depth study on black flies habitat preferences, vertical and horizontal distribution of black flies should be done in the future to deepen the knowledge on ecology and biology of the different black flies species.

ACKNOWLEDGEMENT

This study was funded by SGPUMS Research Grant (UMS/SBK107-STWN-2013). We would also like to express our gratitude to the Ministry of Education for granting MyBrain15 (MyMaster). Special thanks also to the expertise from University Malaya (UM), Prof Hiroyuki Takaoka for helping in the identification of black flies species and also to Prof Dato'. Dr Mohd. Sofian bin Azirun and Dr. Chen Chee Dang for ideas and advises. Last but not least, our special appreciation to the Sabah Parks management for granting us the permission and research permit to conduct this study within the Kinabalu Park and Crocker Range Park area. Not forgetting also all staff of Kinabalu Parks who had rendered their guidance during our field sampling in the park areas.

REFERENCES

Adler, P.H., Currie, D.C., & Wood, D.M. (2004). The Black flies (Simuliidae) of North America. ROM Publication in Sciences, New York, NY

Adler, P. H. (2005). Black flies, the Simuliidae. In W. C. Marquardt (ed.).Biology of DiseaseVectors, 2nd edition. *Elsevier Academic Press*, San Diego. 127-140 pp.

Butler, J.F & Hogsette, J A. (1998). Blackflies. Simulium spp.(Insecta:Diptera:Simuliidae). University of Florida, IFAS Extension,EENY-030. Retrieved 23th November 2015.(http://entomolpgy.ifas.ufl.edu).

Catherine A.H, Jessica P & John F. (2010). Black Flies : Biology and Public Health Risk Department of Entomology. Purdue University, E-251-W.

Cummins, K.W. (1988). The functional role of black flies in streamecosystems,pp.1-10. In Kim, K.C. & Merrit, R.W (eds) Black flies : *Ecology, Population Management, and Annotated World List*. Pennslvania State University, University Park, P.A.

Craig, D.A. (2003). Geomorphology, Development of Running Water Habitats, and Evolutiuon of Black flies on Polynesian Islands. *BioScience*. **53** (11):1079-1093 pp.

Creadie, J.W., Adler, P.H & Beard, C.E. (2011). Ecology of Symbiotes of Larval Blackflies (Diptera: Simuliidae): Distribution, Diversity and Scale. EntomologicalSociety of America. *Environmental Entomology.* **40** (2): 259-302 pp.

Department of Environment. (2006). *Malaysia Environmental Quality Report*. Ministry of Natural Resources and Environment Malaysia, Kuala Lumpur. 86 pp.

Doisy, K.E, Hall, R.D. & Fischer, F.J. (1986). The Black flies (Diptera: Simuliidae) of an Ozark Stream in South Missouri and Associated Water Quality Measurement. *Journal of The Kansas Entomology Society*. **59** (1): 133-142 pp.

Hamada, N., & Grillet, M. E. (2001). Black flies (Diptera :Simuliidae) of the Gran Sabana keys for larvae and pupae, **16** (1):29-49 pp.

Hart, D.D. (1986). The Adaptive Significant of Territoriality in Filter-Feeding Larval Blackflies (Diptera:Simuliidae).*Oikos,* Vol. **46**, (1): 88-92 pp.

Laurince, M. Y., Celestin, B. A & Philippe, K. (2013). Composition, abundance and diversity of aquatic insects in fishponds of southern Ivory Coast, West Africa. *FaunisticEntomology.* **66**:123-133 pp.

Popoola, K. O. K & Otalekor, A. 2011. Analysis of Aquatic Insect's Community of Awba Reservoir and its Physio-chemical Properties. *Journal of Environmental and Earth Science.* **3**(4):422-428 pp. Scheibler, E. E., & Debandi, G. O. 2008. Spatial and Temporal patterns in the aquatic community of high altitude Andean stream (Mendoza, Argentina). *Aquatic Insect.Taylor and Francis Group*. **30** (2): 145-161

Siddaramu, D & Puttaiah, E.T. (2013). Physiochemical characteristic of Balagala Kere and Purali Kere of Shimoga District, Karnataka, India. *International Journal of Advanced Research*. **1** (8):313-321pp.

Srisuka, W. Takaoka. H, Yatsushi, O. Masako. F, Sorawat. T, Kristsana. Y, Wej. C, Atiporn, S. 2015.Seasonal biodiversity of black flies (Diptera: Simuliidae) and evaluation of ecological factor influencing species distribution at Doi Pha Hom Pok National Park, Thailand. Elsevier. 149: 212-219.

Takaoka, H. (2001). Two new and three newly recorded species of Black flies(Diptera: Simuliidae) in Sabah, Malaysia.*Japanese Journal of Tropical Medicine and Hygiene*. **29:** 111 114 pp. Takaoka, H. (2008). Taxonomic revision of *tuberosum* species-group of Simulium (Simulium) in Sabah and Sarawak, Malaysia (Diptera:Simuliidae). *Med Entomology and Zoology*. **59** (2):55-80 pp.

Takaoka, H., Sofian, M.A. Rosli, H. Yasushi, O. Daicus, M.B. & Tan, P.E. (2012). Relationships of Black fly species of the *Simulium tuberosum* species group (Diptera: Simuliidae) in Peninsular Malaysia, with keys to ten Malaysia Species. *The Raffles Bulletin of Zoology.* **60** (2): 533-538 pp.

Takaoka, H. (1996). Description of a new species of *Simulium (Simulium)* from Sabah, Malaysia (Diptera :Simuliidae). *Journal of Japan Medical Zoology.* **24** (3). 157-161 pp.

Tariq, M. , Ali, M & Shah, Z. (2006). Characteristic of industrial effluents and their possible impacts on quality of underground water. *Soil & Environment*. **25** (1) :64-49pp.

Vincent, H., & John, D. 1975. Water Quality Monitoring and Aquatic Organism: The Importance of Species Identification. Vol. (47).No 1, pp.9-19

Zhang, Y., Malmqvist, B. & Englund, G. (1998). Ecological processes affecting community structure of black fly larvae in regulated and unregulated rivers. *Journal of Applied Ecology*, 35: 678-686 pp.

Zubaidah, Y. Takaoka, H. Pramual, P. Low, V.L. & Sofian, A.M. 2016. Breeding habitat preference of preimaginal black flies (Diptera: Simuiidae) in Peninular Malaysia. *Acta Tropica, Elsevier.* 153: 57-63.