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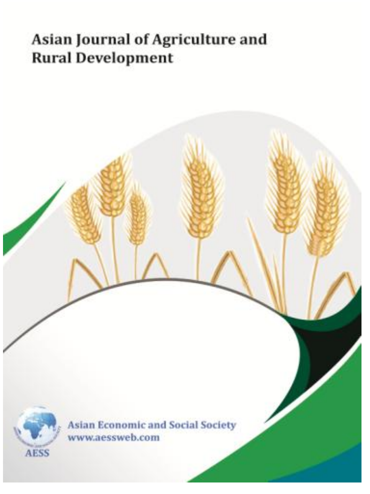

Diversity of Aquatic Insects in Keniam River, National Park, Pahang, Malaysia

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Abstract

The study on biodiversity of aquatic insects was carried out covering the area of Kuala Keniam to Kuala Perkai River, National Park, Pahang, Malaysia. The macro invertebrate community was found in the different types of micro-habitat and various flowing speed levels in good quality of water of Keniam Rivers consisted mainly of aquatic insects. There are large numbers and wide species of aquatic insects in aquatic habitats make them of great ecological importance. There are three divided strata with total of nine sampling location were carried out within several varieties of microhabitats such as sandy, cobble, gravel, leaf and the pool area. The aquatic insects were collected and sampled by using a D-framed aquatic kick net. There was a wide variety of aquatic insects belonging to at least 8 orders in the study area. The orders of insect were Odonata, Coleoptera, Diptera, Trichoptera, Thysanura, Orthopthera, Hemiptera and Ephemeroptera. Throughout the study period, there is range from total of 140 to 604 individuals of aquatic insect trapped monthly and collected in Keniam River from September 2009 to December 2010. Some group of aquatic insects were found significant (χ^2<0.05) different abundance between strata and sampling dates as well as habitat on the diversity of aquatic insects in Keniam River. The abundance and distribution of aquatic insects' species were varied and not constant from one month to another during the study period due to biotic and abiotic factors. Species diversity of aquatic insects varied in different strata of the Keniam River. This indicates the richness and diverse groups of aquatic insects in the study area. It adds to the fact that the undisturbed habitat quality is most suitable for insects to breed and multiply under the natural ecosystem with abundant food supply. Moving upstream from Kuala Perkai to lower stream to Kuala Keniam, one can observe various types of habitats for aquatic insects to live.

Keywords: Aquatic Insect, biodiversity, Habitat quality, Keniam River

Introduction

National Park Pahang is the largest protected area located in the central regions of the Peninsula Malaysia. The establishment of Taman Negara was initially to nurture and conserve the fauna and flora that are of interest to entomologist, geologist, archaeologist, historian, ethnology and others. It functions as protection of wild life, conservation of nature, conservation of the ecology system as well as recreation and ecotourism activities (Hayati et al., 2010). This study is one of the crucial parts to identify the species diversity and distribution of aquatic insects which given emphasizes on biotic and abiotic factors. National Park at Kuala Keniam, Pahang was chosen as a study area because it has the undisturbed rainforest which gave more chances to study on population and distribution of aquatic insects species. Aquatic insect is an important invertebrate organism both economically and ecologically. There are several orders of aquatic insects found in stream of Kuala Keniam, National Park, Pahang. Large numbers and wide variety of aquatic insects in the aquatic habitats make them of great ecological importance (Yule and Yong, 2004). Midges and other aquatic flies are of general importance to freshwater ecosystems, often being a major fish food and an important part of bottom-dwelling communities.

Aquatic insect can be found almost in every type of aquatic habitat throughout the world including lakes, torrential streams, highly saline pools, phytotelmata, coastal waters and estuaries, acid peat swamps, groundwater, hot springs and even pools of crude oil seeping from the ground (Yule and Yong, 2004). The warm, wet climate of the Malaysia region is ideal for the life cycles of many aquatic insects which are usually dependent on moist habitat. The presence of a wide variety of environments, particularly in tropical rainforest, provides an enormous number of ecological niches. Unfortunately habitat destruction is causing the extinction of many aquatic insects (Yule and Yong, 2004). Some of them, especially diptera adults are of medical importance, for example mosquitoes and house flies are the major vectors of a wide range of diseases of humans and other animals. Game fishes feed on many kinds of Diptera that live in and on the water (McCafferty, 1983). The extinction of aquatic insect may affect the entire population in the ecological system because they act as an important source of food for many invertebrate. For instance, if some species particularly the Diptera larvae become extinct, their absence will decrease the invertebrate animal, thus may decrease the fish population. Because of their position in food web, aquatic insects including the dipterans are excellent organism to sample and analyzed for the present of contaminants (Cushing and Allan, 2001). Some species of aquatic insects particularly Diptera larvae are saprophagous or scavengers on decaying or dead plants and animal matter (Peterson, 1960). Without them decaying organic materials such as dead plants and animal, the water will become more polluted, and consequently other animal will affect due to severe pollution. For instance, Diptera larvae namely Chironomus plumosus from family Chironomidae is important as fish food. Additionally, the larvae have a potential to be as farm fish diet (Bogut et al., 2007).

Some of Diptera may prefer highland and prefer lowland streams habitat such as Chironomidae (Polypedilum breviantennatum and Stictochironomus sp.) that prefer lowland streams (Tolkamp and Both, 1978). Example of Diptera larvae that prefer highland habitat and inhabitants of flowing water are Simuliidae and Blephariceridae. The larvae of Simuliidae are often found in the submerged rock because of their modified posterior prolegs anchored to silk secretion by the larva. Blephariceridae have an extreme morphological modification which made them adhere to smooth rock (Merritt and Cummins 1984). Some species of aquatic insects such as stonefly, mayfly, and caddis fly are very sensitive to pollution and live in high quality environment with good water quality. The absence of species that are sensitive to pollution and high number of dipteran Chironomidae that can tolerate with pollution is a good sign of pollution that happens at that place. The existences of certain organism in the water indicate the quality of the water and provide an overall view about physical and chemical properties of the water (Che Salmah, 2000). In this study further investigation of ecological importance of aquatic insects in Keniam River was initialized. The understanding of biology and ecological importance would contribute to future use of some orders of aquatic insect such as Diptera as a bioindicator. This is because some of them are well adapted to live in clean water. This study was carried out with the following objectives firstly to identify diversity of aquatic insects along Keniam River according to different strata, to assess the distribution and relationship of aquatic insect in relation to environmental parameters and water quality Keniam River. The information would be documented as a baseline data to help in the future study and conservation works.

Materials and Methods

a) Study area

The study was conducted along the Keniam River (Figure 1), located in the north-western portion (102º2813' N, 4º3142' E) from Kuala Tahan jetty of National Park, at Kuala Keniam, Pahang. Habitat and elevation might be influenced the distribution of aquatic insects at different elevation. National Park at Kuala Keniam, Pahang was chosen as a study area because it has the undisturbed rainforest which can give more chances to study on population and distribution of insects' species.

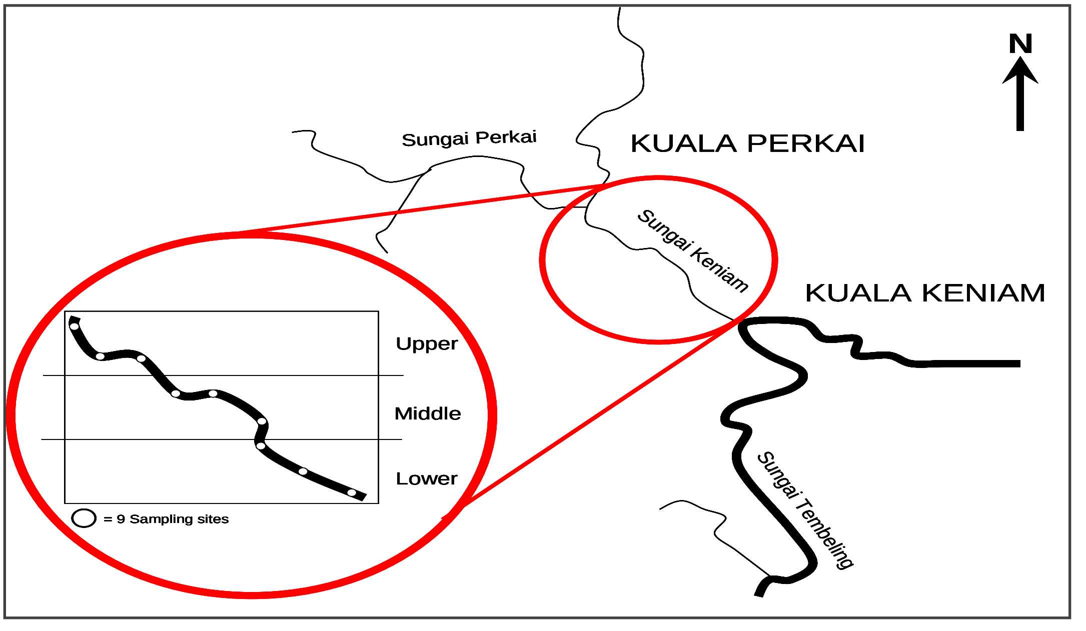

Figure 1: Sampling site of aquatic insects

b) Sampling Layout, Methods and Techniques

There are nine sampling sites at the sampling layout (Figure 1). Aquatic insects were collected according to different strata namely upper, middle and lower which each stratum had three sampling point. The distance measured of each sampling points was 50 meter interval between sampling point and range 800 meter to 1000 meter interval between strata using global positioning system (GPS). Habitat and elevation of strata might be influenced the distribution and abundance of aquatic insects.

Samplings of aquatic insects are the collection of the specimens. The main purposes of sampling are to provide specimen of aquatic insects, provide numerical estimates, detect environment change over space or time, and to determine relationship between variables (Nurulain 2008). The streams were monitored for 6 sampling dates (equivalent to 10 months duration period), from November 2009 until September 2010. Insects and water samples were collected in all three strata of Keniam River bimonthly. For distribution and abundance of aquatic insects, aquatic insects were catch using hand net and about three times for every sampling site were observed. All samples were brought back to the laboratory for counting and preservation purposes. The abundance of aquatic insects according to the orders was distinguished according to sampling sites. For sorting and classification of aquatic insects, specimens with common features were initially placed under different broad groups called order, further into sub-categories and placed in different families and genus, until it is ultimately identified up to species level. For systematic sampling according to different strata, aquatic insects were collected systematically according to different strata which lower, middle and upper strata. Each stratum had different habitats which each habitat had different species of aquatic insects.

c) Sampling of aquatic insects, measuring water and other parameters

The relative distribution of aquatic insects was collected along the Keniam River according to sampling point, water quality and abiotic factor such as temperature, water pH, water velocity and water depth. Aquatic insects were catch using hand net about three times for every sampling site was observed. The abundance of aquatic insects according to the orders was distinguished according to sampling sites. Aquatic insects were collected systematically according to different strata which lower, middle and upper strata. Each stratum had different habitats which each habitat had different species of aquatic insects. For sampling of aquatic insects, nine samples were collected from each stratum within three group of microhabitats namely S1 (sandy + leaf litter), S2 (under shed + pool area + gravel + cobble), and S3 (Open area + pool area + gravel + cobble). The aquatic insects were sampled by using a D-framed net which giving total of 27 samples every two months. The D-framed net was placed and dragged about a meter on river submerge vegetations. The sampling area was disturbed gently by rolling and rubbing the substratum surfaces approximately 1 meter in front of the net, and also by sweeping through sediment. The samples were placed into a labelled bottle containing small amount of water. The samples were brought back to the laboratory for sorting and identification. For water parameters, the water parameters were measured to evaluate their effect on diversity and abundance of aquatic insects. The measurement of water parameters were done and the sampling process simultaneously. The dissolved oxygen (DO) and water temperature were recorded using Dissolved Oxygen Probe to determine the concentration of oxygen in aqueous solutions in the field. The pH of water was measured by using pH meter (YSI PH100). The water velocity (ms^{-1}) was measured by using a Velocity Auto-flow watch (JDC Instrument). The depth of the streams was measured manually by using measuring tape. Water samples were also used to assess and correlate the quality of water from the streams with aquatic insect abundance.

d) Laboratory Analysis

In the laboratory, the samples were immediately sorted to avoid deterioration and death of organisms. The movement of living micro invertebrates was easier to spot and pick. Then the specimens were stored in a labelled universal bottle filled with 75% ethyl alcohol. This bottle is the permanent storage container that is sealed tightly to prevent evaporation of the alcohol. All the specimens were identified to Order and selected species generic level by using taxonomical key of Yule and Yong (2004) and Merritt and Cummins (1984). Two water samples were collected for chemical analysis. The biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonium-nitrogen (NH3-N) and total suspended solid (TSS) were all determined according to the standard method procedures.

e) Data Analysis

Data was analysed using appropriate statistical tools. Prior further analysis, the normality test of the distribution was carried out on the abundance of aquatic insect using Kolmogorov-Smirnov test. This test was carried out by using the SPSS software (version 14.0). The populations sampled of aquatic insects are not normally distributed (data not shown). Therefore, non-parametric Kruskal Wallis Test ($P<0.05$) was used to test the difference in the means of aquatic insects among the various levels of strata, locations and occasions. For ecological indices reflecting the population features were calculated by using Richness Indices to calculate number of species present in order to estimate the species richness. Diversity indices incorporate both species richness and evenness into a single value (Magurran, 2004). Margalef’s and Menhinick’s index were used to determine the richness of a genus or species in a community. For Diversity Index, the Shannon Index is the probability that second drawn at random from a population belongs to the same species. Shannon Index was to determine what species on individual drawn from a population will belong to. Pielou’s Eveness Index (1975, 1977) was used to observe the equal abundance of species present during the sampling period. All data analysis of the ecological indices using following formula:

Margalef's (1958) Richness Index $R_1 = s - 1 / \ln(N)$

Whereby R_1 = Margalef's Index; s = Total number of species sampled; N= Total number of individual

Menhinick's (1964) Richness Index $R_2 = s / \sqrt{N}$
Whereby R_2 = Menhinick's Index; s= Total number of species sampled; N= Total number of individuals observed

Simpsom's Diversity Index

$$D = 1 - \sum [n_i (n_i - 1)] / N(N-1)$$

Whereby D = Simpson's Index; n_i = Number of species; N = Total number of Individual
Shannon – Wiener Diversity Index

$$H' = - \sum [(n_i / N) \ln (n_i / N)]$$

$$H' = - \sum_{i=1}^{S} p_i \ln p_i$$

Whereby H' = Shannon's Index; n_i = Number of species; N = Total number of
S = species richness (total # of species present); Pi = proportion of total sample belonging to the ith species; Ln = natural log
Pielou's Evenness Index
E = H' / ln (s)

Whereby E = evenness index; H'= Shannon index; s = Total number of species

The effects and influences of water parameters and environmental parameters on the abundance of aquatic insects' Order and specifically Diptera larvae population were examined using the Pearson Correlation. R value more than 0.3 were considered as high enough to exert some influence in the population. Canonical Correlation Analysis Test (CCA Test) was carried out to determine how much the species is related to environmental parameters and water parameters. To calculate the Water Quality Indices (WQI) (Department of Environment, 2002) to classify them into classes, all the chemical parameters should be considered. They are pH, DO (mg/L), Ammonia-Nitrogen, COD (mg/L), BOD (mg/L), and TSS (mg/L).

WQI = 0.22 * SIDO + 0.19 * SIBOD + 0.16 * SICOD + 0.15 * SIAN + 0.16 * SISS + 0.12 * SIpH

Whereby, SI = Sub Index for each parameter; DO = Dissolved Oxygen; BOD = Biological Oxygen Demand; COD = Chemical Oxygen Demand; AN = Ammonia

Nitrogen; TSS = Total Suspended Solid; pH = Acidity/ Alkalinity

Results

Relationship of Abundance of Aquatic Insects on the types of habitat, Different Strata and Seasonal Impact

Significant differences were detected for several abundances of orders of aquatic insect at different strata of Keniam River as well as time of samplings. For Kruskal-wallis test on insects' Order of the aquatic insects, the calculated value of Kruskal-Wallis obtained is significant difference for order Coleoptera (χ^2 = 9.218, df = 2, $p < 0.05$), Diptera (χ^2 = 12.957, df = 2, $p < 0.05$), Tricoptera (χ^2 = 8.934, df = 2, $p < 0.05$) and Hymenoptera (χ^2 = 6.018, df = 2, $p < 0.05$) (Table 1) at different strata. In Table 2, the result shows that Kruskal-wallis test on sampling dates, the value obtained found that there were significant difference for Odonata (χ^2 = 40.367, df = 5, $p < 0.05$), Coleoptera (χ^2 = 18.033, df = 5, $p < 0.05$), Diptera (χ^2 = 17.735, df = 5, $p < 0.05$), Hemiptera (χ^2 = 39.391, df = 5, $p < 0.05$) and Lepidoptera (χ^2 = 11.262, df = 5, $p < 0.05$) at different sampling dates. In the aspect of habitat effect to abundances of orders of aquatic insects, three types of habitats were selected and defined in this study namely S1 (sandy area + leaf litter), S2 (undershed area + pool + gravel + cobble) and S3 (open area + pool + gravel + cobble) in the Kuala Keniam River. The Chi square-value order of aquatic insects was found that Coleoptera (χ^2 = 15.882, df = 2, $p < 0.05$), Diptera (χ^2 = 8.341, df = 2, $p < 0.05$), Hymenoptera (χ^2 = 12.561, df = 2, $p < 0.05$) and Lepidoptera (χ^2 = 13.636, df = 2, $p < 0.05$) were significant different in number at different habitat in Keniam River (Table 3). For reason, the aquatic insects probably varies and not constant in one habitat. Its depends on the location is either have a lot of food supply, no disruption from natural enemies and abiotic factors such as water depth, water speed, temperature and water pH.

Table 1: Non parametric (Kruskal Wallis) Test on Differences between Abundance of Orders of Aquatic Insects and Strata of the Keniam River

	Odonata	Coleoptera	Diptera	Hemiptera	Hymenoptera	Lepidoptera	Ephemeroptera	Tricoptera
Chi-Square	4.705	9.218	12.957	2.620	6.018	.760	1.734	8.934
df	2	2	2	2	2	2	2	2
Asymp. Sig.	.095	.010	.002	.270	.049	.684	.420	.011

a Kruskal Wallis Test; b Grouping Variable: River Strata

Table 2: Non parametric Test (Kruskal Wallis) on Differences between Abundance of Orders of Aquatic Insects and Sampling Dates along Keniam River

	Odonata	Coleoptera	Diptera	Hemiptera	Hymenoptera	Lepidoptera	Ephemeroptera	Tricoptera
Chi-Square	40.367	18.033	17.735	39.391	3.910	11.262	7.988	6.880
df	5	5	5	5	5	5	5	5
Asymp. Sig.	.000	.003	.003	.000	.562	.046	.157	.230

a Kruskal Wallis Test; b Grouping Variable: Sampling Date

Table 3: Non parametric (Kruskal Wallis) Test on Differences between Abundance of Orders of Aquatic Insects and Habitat Types along Keniam River

	Odonata	Coleoptera	Diptera	Hemiptera	Hymenoptera	Lepidoptera	Ephemeroptera	Tricoptera
Chi-Square	4.835	15.882	8.341	1.188	12.561	13.636	2.143	3.697
df	2	2	2	2	2	2	2	2
Asymp. Sig.	.089	.000	.015	.552	.002	.001	.343	.157

a Kruskal Wallis Test
b Grouping Variable: Types of Habitat

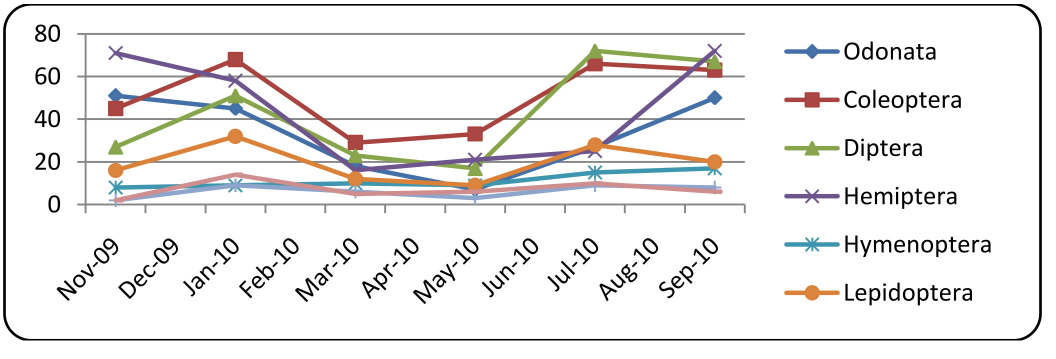

Figure 2: Population Dynamic of Aquatic Inscet (Order) in Keniam River from November 2009 to September 2010

Figure 2 shows that the population of insects order were fluctuated throughout the year. However, most of the orders of aquatic insects were found decreased in their numbers tremendously from March to May 2010. The highest population was recorded for Hemiptera (71) followed by Odonata and the lowest was tricoptera and ephemeroptera in November 2009. Subsequently, the population of Coleoptera increased to be the highest (68) and the least was recorded for Ephemeroptera and Tricoptera in January 2010. Inerestingly, Diptera was found to be the highest (72) Order found, followed by Coleoptera (66) in July 2010. The trend of population changes in the Keniam River due to several factors such as biotic and abiotic factors specifically respond to the environmental factor including availability of trophic resources, changes in their habitat, and to physical and chemical variation of water (Salman et al., 2011). Additionally, the abundance and population dynamic also influenced by current speed, water depth, light intensity and temperature regimes and water quality (Hellawell, 1986; Che Salmah et al., 1999).

Diversity of Aquatic Insect (Analysis using Ecological Indices)

The result of Ecological Indices (Simpson's (d) index, Margalef's (R_1) index, Menhenick's (R_2) index, and Pielou's index) were summarized and shown in Table 5. The table shows the Shannon's (H') index for three strata of Keniam River including Upper, Middle, and Lower stream were ranged between 1.26 and 1.85 for the entire sampling period. Species richness was higher in Middle stream River compare to other strata supported by Margalef's (R_1) index, with the highest 2.13 recorded (mean=1.59) and Menhinick's (R_2) index was the highest 1.37 (mean=0.89). Both the indices show that the species richness was highest in Middle stream River. Upper stratum of Keniam River has the highest value of diversity indices with Shannon's (H') value was 2.28 and Simpson's (d) value was 5.25 particularly in November 2009. Middle stratum of Keniam River has the lowest diversity index with Shannon's (H') value was ranged 1.16 to 1.85 and Simpson's (d) value was ranged between 2.30 and 5.68. Both indices show that species diversity varied in different strata of the Keniam River. The highest value for Evenness (J') index was recorded in Middle Stratum with 0.68. The lowest value was recorded in Middle Stratum with 0.46 in July 2010. This is due to high abundance of Coleoptera, Diptera and Hemiptera that was collected in this river compare to other Orders. The diversity of Orders of aquatic insects were found very high variation in H' values (Metcalfe, 1989). Based on this index, the correlation of aquatic insect and environmental parameters, it can describe the quality of the river (Metcalfe, 1989; Salman, et al., 2011) is very good.

Table 5: Mean of ecological indices for Aquatic Insects (Orders) in Three Strata of Keniam River from November 2009 to September 2010

Strata of Rivers	Month	Diversity Index		Richness Index		Evenness
		Shannon's	Simpson's	Margalef's	Menhinick's	Pielou's
Middle stream	Nov 2009	1.44	3.54	1.76	1.28	0.53
	Jan 2010	1.38	2.96	1.70	0.85	0.51
	March 2010	1.85	5.68	2.13	1.37	0.68
	May 2010	1.59	3.10	2.10	1.17	0.59
	July 2010	1.16	2.30	1.30	0.69	0.43
	Sept 2010	1.59	3.87	1.68	0.84	0.59
Upper stream	Nov 2009	2.28	5.25	1.54	1.51	0.47
	Jan 2010	1.26	2.38	1.27	0.66	0.47
	March 2010	1.56	4.45	1.13	0.65	0.58
	May 2010	1.70	4.58	1.68	0.99	0.63
	July 2010	1.29	2.65	1.31	0.70	0.48
	Sept 2010	1.68	4.69	1.65	0.96	0.62
Lower stream	Nov 2009	1.39	3.30	1.61	1.08	0.51
	Jan 2010	1.73	4.93	1.94	1.32	0.64
	March 2010	1.47	4.27	0.92	0.57	0.54
	May 2010	1.44	4.36	1.14	0.87	0.53
	July 2010	1.29	3.15	0.97	0.63	0.48
	Sept 2010	1.27	2.96	1.32	0.90	0.47

Aquatic Insects Composition According to Types of Habitats

The sampling process was done for six sampling dates which is from November 2009 until September 2010. Sampling could not be done as planned because of the bad weather during December 2009 and October 2010. Therefore, the aquatic insects cannot be sampled from the streams. During the 6 months sampling period, total of 1287 individuals of aquatic insect were sampled from three strata of Keniam River. They consist of eight orders which are Odonata, Coleoptera, Diptera, Trichoptera, Thysanura, Orthopthera, Hemiptera and Ephemeroptera (Table 6). For specific order of Diptera, there was several families were found such as Athericidae, Simuliidae, Chironomidae, Ceratopogonidae, Tipulidae, Tabanidae, Cullicidae and Blephariceridae and then were further identified (Table 7). In all three strata (upper, middle and lower strata), coleoptera was the dominant family. This followed by order Hemiptera, Odonata and Hemiptera. The highest percentage composition of aquatic inscets in Upper stream and Lower stream was the order Coleoptera and there are no Coleoptera in middle strata (Location 4, Location 5 and Location 6) of the Keniam River.

Table 6: Composition of Aquatic Insect from November 2009 to September 2010

Insect Order	Keniam River								
	Lower Stream			Middle Stream			Upper Stream		
	Sandy + Leaf litter (S1)	Under shed (S2)	Open area (S3)	Sandy+ leaf litter (S1)	Under shed (S2)	Open area (S3)	Sandy+ Leaf litter (S1)	Under shed (S2)	Open area(S3)
Odonata	11.00 (17.46 %)	15.67 (22.38)	14.67 (22.56%)	14.33 (18.53%)	5.33 (11.27 %)	12.00 (16.82 %)	10.00 (10.45 %)	6.67 (17.09 %)	10.33 (21.38%)
Coleoptera	13.67 (21.69 %)	21.00 (30.00 %)	10.33 (15.90%)	9.00 (11.64%)	13.00 (27.46 %)	2.33 (3.27%)	48.67 (50.87 %)	9.33 (23.93 %)	4.00 (8.28%)
Diptera	6.67 (10.58 %)	9.33 (13.33 %)	14.00 (21.54%)	11.00 (14.22%)	19.33 (40.85 %)	22.67 (31.78 %)	5.00 (5.23%)	3.33 (8.55%)	12.33 (25.52%)
Hemiptera	15.67 (24.87 %)	16.67 (23.81 %)	18.00 (27.69%)	23.00 (29.74%)	5.67 (11.97 %)	19.00 (26.64 %)	12.00 (12.54 %)	11.67 (29.91 %)	13.33 (27.59%)
Hymenoptera	1.67 (2.65 %)	0.67 (0.95 %)	2.67 (4.10%)	5.33 (6.90%)	0.67 (1.41%)	6.67 (9.35%)	6.00 (6.27%)	1.00 (2.56%)	3.33 (6.90%)
Lepidoptera	8.33 (13.23 %)	3.00 (4.29 %)	3.67 (5.64%)	13.00 (16.81%)	2.33 (4.93%)	4.67 (6.54%)	9.33 (9.76%)	3.67 (9.40%)	1.67 (3.45%)
Ephemeroptera	1.33 (2.12 %)	1.33 (1.90 %)	0.33 (0.51%)	0.67 (0.86%)	1.00 (2.11%)	3.67 (2.14%)	3.00 (3.14%)	0.33 (0.85%)	2.00 (4.14%)
Trichoptera	4.67 (7.41 %)	2.33 (3.33 %)	1.33 (2.05%)	1.00 (1.29%)	0.00 (20.00 %)	0.33 (0.47%)	1.67 (1.74%)	3.00 (7.69%)	1.33 (2.67%)
Total	63.00	70.00	65.00	77.33	47.33	71.33	95.67	39.00	48.33

Table 6 shows the lists of Aquatic Insect' Order (mean + percentage) that were identified according to the river strata and types of area. For overall total mean number of individual in every stratum, lower Stratum River consists of the highest number of aquatic insects with mean number 198 individuals per m^2. This followed by middle stratum with 195.99 individuals, and upper with only 182.987 individuals. Nevertheless, the area of S1 (sandy + leaf litter) at upper stratum were noted that Coleoptera to be the highest in mean number and the least at open area in middle stratum. Mean of aquatic insects collected between three selected strata (9 locations) of stream is different with another. Mean for the selected strata from November 2009 until September 2010 show a consistant value. Mean number of aquatic insect for Keniam was the lowest during March and May 2010 with 119 and 105 number of individuals per m^2 respectively. During July 2010, the mean of aquatic inscets starting to increase in all strata including upper, middle, and lower stratum of the river. However, mean in upper stream during November 2009 increase drastically to 46.0 number of individuals per m^2 and it was the highest mean recorded. Mean for the selected strata from November 2009 until September 2010 show a consistant value. Mean for upper stream was the highest in total mean number of aquatic insects for sandy + leaf litter during November with 46 number of individuals per m^2 and the least was found in May 2010. Similarly, during Novembert 2009, the mean of aquatic inscets highest for undershed area in the middle stratum. However, mean in lower stream during July increased drastically to 14 number of individuals per m^2 for sandy + leaf litter area. Meanwhile, Table 7 shows the composition of species of Diptera at different strata throughout the sampling dates. The highest number of species in Order of Diptera is Simulium species which belong to family Simulidae with 183 individual at upper strata. However, the Hexatoma, Chironomus and Asuragina were more abundant in lower strata compare to others.

Table 7: Composition of Species of Diptera at Different Strata in Kuala Keniam River

Order	**Family**	**Genus**	**Keniam River**					
			Upper Strata		**Middle Strata**		**Lower strata**	
			Total	%	Total	%	Total	%
Diptera	Ceratopogonidae	Dasyhelea	37.00	6.32	21.00	4.21	6.00	1.28
Diptera	Blephariceridae	BlephEricera	2.00	0.34	5.00	1.00	3.00	0.64
Diptera	Simuliidae	Simulium	183.00	48.38	117.00	33.47	34.00	24.26
Diptera	Athericidae	Asuragina	55.00	9.40	74.00	14.83	82.00	17.45
		Atrichops	9.00	1.54	3.00	0.60	2.00	0.43
Diptera	Chironomidae	Polypedilum	11.00	3.59	4.00	0.80	9.00	1.91
		Clinotanypus	6.00	2.74	18.00	4.61	14.00	2.98
		Chironomus	4.00	5.81	15.00	13.03	78.00	16.60
Diptera	Tipulidae	Antocha	15.00	2.56	35.00	7.01	19.00	4.04
		Hexatoma	88.00	15.04	93.00	18.64	106.00	28.94
		Ormosia	9.00	1.54	2.00	0.40	3.00	0.64
		Pseudolimnophila	12.00	2.05	4.00	0.80	3.00	0.64
		Tipula	3.00	0.68	3.00	0.60	1.00	0.21
Diptera	Tabanidae	Tabanus	7.00	1.03	1.00	0.20	4.00	0.64
		Grand Total	**441.00**	**100**	**395.00**	**100**	**364.00**	**100**

Relationship of Aquatic Insects with Environmental, Abiotic (Rainfall) and Water Quality Parameters

The values of environmental parameters are depth, current of stream (water speed) and rainfall that were recorded during the sampling period. The depth during six sampling periods range from 0.50 m to 1.41 m in November 2009, 0.43 m to 1.42 m in January 2010, 0.40 m to 1.25 m in March 2010, 0.33 m to 1.5 m in May, 0.50 m to 1.30 m in July, and 0.7 m to 1.7 m in September 2010. The water speed of Keniam River during sampling is range from 0.62 m/s to 2.50 m/s November 2009, 0.4 m/s to 1.23 cm in January 2010, 0.5 m/s to 2.0 m/s in March 2010, 0.45 m/s to 2.1 m/s in May 2010, 0.45 m/s to 3.0 m/s in July 2010, and 0.8 m/s to 4.0 m/s. Rainfall volume recorded for each sampling dates is different with sampling dates in September 2010 has the highest number with 302 mm, followed by May 2010 with 227.5 mm, and the least was recorded in March 2010 with only 92.5 mm. Based on the figure, some of orders have closed relationship with rainfall such as Coleoptera and Hemiptera.

Table 8 shows the output of correlation test between the abundance of aquatic insects and the parameters. Only the Order of the insect that has correlation with the parameters was shown in the table. From this correlation test, Odonata was strongly related to the parameters such as depth of water, velocity of water, pH, salinity, COD, and Ammonia. It is also strongly correlated inversely with salinity and BOD. The results of correlation test between the density of Diptera larvae and the parameters. Strong positive correlations were observed between the Diptera larvae density and depth of River (P = 0.337). It indicates that Diptera larvae density will increase when the depth of river increase. Additionally, Diptera larvae density were negatively influenced by water velocity (P = -0.498), COD and Ammonia. Table 9 shows that some negative correlation were observed between the Diptera larvae density and Biological oxygen demand (BOD) of the water (P = -0.285), salinity (P= -0.306) and Total suspended solid (TSS) (P = -0.341) in Lower Stratum of the river. It indicates that Diptera larvae density will increase when the BOD, salinity and TSS of river decrease. In middle stream, Diptera larvae density were more negatively influenced by depth of water (P = -0.332) and salinity (P = -0.332). But strong positive correlations were observed between the Diptera larvae density and velocity (P = 0.318) and highly correlated with DO (0.183). It indicates that certain Diptera larvae can adapt to current speed of the river due to their preference to live and hide on the cobble and gravel. In Upper Stratum of the river, Diptera larvae density were strong positively influenced by biological oxygen demand (P = 0.420). Negative correlations were observed between the Diptera larvae density and Ammonia (P = -0.276) and depth of water (P = -2.72) of Keniam River. It indicates that Diptera larvae density will increase when the BOD of river increase. Table 10 shows that the order of aquatic insects are positively correlated with total suspended solid (TSS) with (r = 0.6444) and biochemical oxygen demand (BOD) with (r = 0.3285). They are negatively correlated with dissolved oxygen (DO) with (r = -0.2766), ammonia (r = -0.2718), depth (r = -0.3416), temperature (r = -0.0092), conductivity (r = -0.0646), velocity (r = -0.2462) and pH (r = -0.1952). The orders environment correlation values are 0.619, 0.521, 0.397 and 0.385. Sum of all eigenvalues is 1.348 and sum of all canonical eigenvalues is 0.291. Test of significant of all canonical axes is 0.291 with F-ratio = 3.027 and $p<0.01$. The Water Quality Index (WQI) was categorized into class based on the National Quality Standards as shown in Table 11. The value for WQI calculated for Keniam River is shown in a Table 12. Its mean that Middle stream River, Upper stream, Lower stream River and Middle strata of Keniam River have good water quality.

Table 8: Correlations of Odonata, Diptera and Lepidotera with Water Quality and for the Whole Sampling Periods

	Odonata	Diptera	Lepidoptera
Depth	0.211(**)0.007	0.337(**)0	-.081.312
Velocity	0.174(*)0.011	-0.498(**)0.010	.157(*).046
Temperature	-0.0310.651	0.150(*)0.05	.064416
pH	0.357(**)0	.042.594	-.146.063
DO	0.1150.089	.106.181	-.181(*).021
Salinity	-0.149(*)0.027	.212(**).007	-.254(**).001
Conductivity	.132.095	-.092.245	.262(**).001
COD	0.396(**)0.008	-0.177(**)0.009	-.082.301
Ammonia	-.177(*).024	-0.160(*)0.017	-0.1290.057
BOD	-0.360(**)0.015	.120.129	.155(*).049
TSS	.069.340	-.139.077	.261(**).001

Table 9: Relationship of the Abundance of Diptera with Environmental and Water Parameters According to Strata of the Keniam River

	Abundance Lower Stratum	Abundance Middle Stratum	Abundance Upper Stratum
Temperature	.042 (.763)	.202 (.142)	.048 (.733)
DO	.040.772	0.183(**)0.007	-.211.126
Conductivity	-.146.293	-.034.809	.184.183
Velocity	.109.431	0.318(*).019	.156.259
Depth	-.018.903	-.332(*).014	-.272(*).047
pH	.193.162	.233.089	-.033.812
Ammonia	.302(*).026	-.093.505	-.276(*).043
Salinity	-.306(*).024	-.332(*).014	.123.376
BOD	-.285(*).037	-.010.941	0.420(**)0.012
COD	.148.284	-.103.459	-.228.097
TSS	-.341(*).012	.002.989	.124.373

Table 10: Output of CCA Test

Salinity	-0.2766	1.000
DO	-0.5824	0.6154
pH	-0.1952	-0.2561
COD	-0.1565	0.1953
BOD	0.3285	-0.4841
Ammonia	-0.2718	0.3658
Total Suspended Solid (TSS)	0.6444	-0.6205
Temperature	-0.0092	-0.1008
Conductivity	-0.0646	0.0846
Velocity (speed)	-0.2462	-0.0521
Width	-0.4513	0.3303
Depth	-0.3416	0.2304

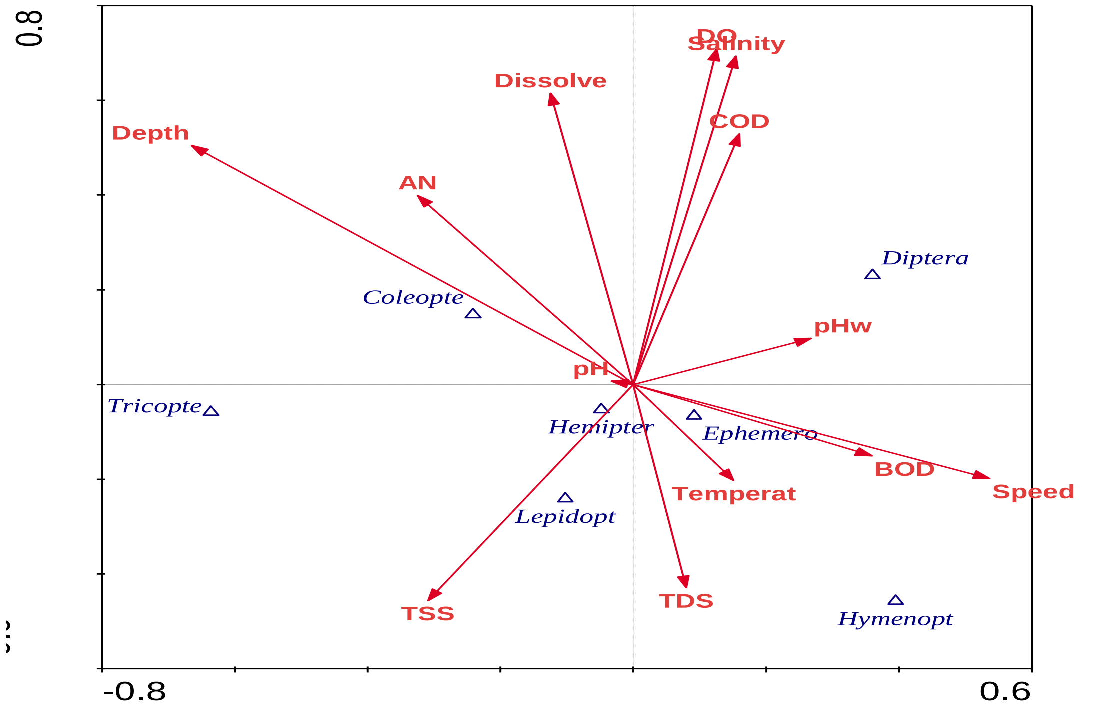

Figure 3: The ordination diagram (CCA) for the first two canonical axes of the aquatic insects (orders) and physical and water quality parameters in the Keniam River

Table 11: National Water Quality Standards (Department of Environment, 2002)

Water chemical Parameter	**Class**				
	I	**II**	**III**	**IV**	**V**
	Very Good	Good	Moderate	Bad	Worst
pH (mg/L)	>7	6-7	5-6	<5	>5
DO (mg/L)	>7	5-7	3-5	1-3	<1
Ammonia (mg/L)	<0.1	0.1-0.3	0.3-0.9	0.9-2.7	>2.7
COD (mg/L)	<10	10-25	25-50	50-100	>100
BOD (mg/L)	<1	1-3	3-6	6-12	>12
TSS (mg/L)	<25	25-50	50-150	150-300	>300
WQI	>92.5	76.5-92.7	51.9-76.5	31.0-51.9	<31.0

Table 12: Water Quality Analysis (In-Situ Reading)

Parameter/ Location	**N 04^{O} 32.534' E 102^{O} 27.135'**	**N 04^{O} 32.051' E 102^{O} 27.963'**	**N 04^{O} 31.125' E 102^{O} 27.135'**
Temperature (^{O}C)	26.241	26.63	27.03
Conductivity (µs/cm)	52	42	45
Total dissolve solid	0.033	0.027	0.028
Salinity	0.02	0.02	0.02
Dissolve oxygen (%)	97.0	106.4	100.9
Dissolve oxygen (mg/L)	7.84	8.43	7.95

pH	9.38	8.84	8.29
Biological Oxygen Demand (mg/L)	2.30	3.50	2.80
Chemical Oxygen Demand (mg/L)	1.33	3.00	5.00
Ammonia-Nitrogen (mg/L)	0.199	0.178	0.187
Total Suspended Solid (mg/L)	97.35	97.29	97.29
Water quality index	I	I	I

Discussion

Distribution, Abundance and Diversity of Aquatic Insects in Keniam River, National Park, Pahang

There are three sampling sites selected at each of stratum of Keniam River namely S1 (S1=Sandy area + Leaf Litter), (S2=under shed area + pool area + gravel + marvel) and (S3=Open area + pool area + gravel + marvel) respectively. The result showed that there was high diversity of insects-orders in the Keniam River at every stratum of the Keniam River. However, the diversity varied and changed over the time (bimonthly sampling) at every stratum of the Keniam River. This study demonstrated that diversity was much relies to environmental parameters and water quality that changed over the time. At Upper stratum result showed that the greater diversity of aquatic insects was found at S1 area of Keniam River. It is because, S1 (S1=Sandy area + Leaf Litter) have leaf litter that act as a habitat to numerous aquatic insects compared at the S2 (S2=under shed area + pool area + gravel + marvel) and S3 areas (S3=Open area + pool area + gravel + marvel). In the middle stratum of Keniam River, results showed that the greater diversity of aquatic insects was found at S1 area at Keniam River. It is probably due to microhabitat's effect which influences the diversity at middle stratum such as water depth and the presence of several aquatic plants in this area. Plants act as habitat to numerous aquatic insects that live along Keniam River. Results showed that the greater diversity of aquatic insects was also found at S2 area of Keniam River. It is because, S2 area has a lot of plants that probably act as a habitat and favourable to numerous aquatic insects compared to S1 and S3 areas in this stratum. Besides the natural changes, several environmental parameters' factors obviously lead to the abundance of aquatic insects such as water quality (Che Salmah, 2000), water depth, current (water speed) of stream, temperature, water pH, and types of habitat.

Significant differences were detected for several abundances of orders of aquatic insect at different strata of Keniam River as well as time of samplings. Shannon and Simpson index were higher in Lower stream River. Coleoptera, Diptera, Tricoptera and Hymenoptera were found that significantly different among three selected strata of Keniam River. This indicated that these orders are very selective to their habitats. Each habitat is unique and has their own chances for exploitation and the environmental parameters the stability of water depth and speed, temperature, light, and water quality (Hellawell, 1986; Che Salmah, 2000). Basically, the diversity and evenness indices were higher at upper stream and decreased at downstream (Salman et al, 2011) followed the same trend as that of the river water quality. All strata of Keniam River have greater aquatic insect diversity compared to other. The reason could be due to environment and suitability of the habitat that they live in. In this study, the difference between diversity indices among the stratum probably due to the sampling site in a short distance did not give significant in diversity and insect species but it significantly difference in term of time which due to the abiotic especially rainfall. Rainfall will influence the water depth and speed as well as water quality to the insect abundance and diversity in the extreme conditions.

Middle stream River has the highest species richness with higher value of Margalef and Menhinick index particularly during March 2010. However, the lowest species richness indicated from Margalef and Menhinick index was middle stratum of Keniam River during July 2010. For Evenness index, middle stratum of Keniam River has the highest value in March 2010 and the lowest during July 2010. This is because the orders of insect collected in this stratum are mostly equal in number compared to other strata which has higher number.

The density of Orders of aquatic insects for each River has few different for each sampling dates. However, the density of insect collected in each stratum of the river is nearly same and not much different throughout the year except dry season. This may be due to the environmental parameters of Keniam River which are not easily affected by weather or other parameters. Differences in insect-density only happen between dry season (March 2010 to May 2010) and wet season (September to January 2010). That is the reason why sampling cannot be done during December 2009. When raining, the water flowing will swept the most of the insect particularly larvae stages away from its habitat. That is why less number of Diptera larvae has been collected in this study. This is not a surprising, as according to Hernandez et al., (2009), Diptera especially family Simuliidae is often extremely abundant with approximately 2072 described species worldwide and can play an important role in the diet of other aquatic organisms such as fish. This show that Keniam River has very clean water and are not polluted because Simuliidae cannot tolerate with polluted water. Another family was Tipulidae as second dominant family in this river. According to Stehr 1991, Tipulidae inhabit extremely wide geographic habitat and distribution of the family. Other family of Diptera larvae that was very important is Chironomidae. The genus identified was the Polypedilum, Clinotanypus and Chironomus. The other families of Diptera larvae which can be categorized as 'minor' family present in the river. This is due to the number of individuals collected in the selected rivers. Some Diptera species was not detected in the study due to rapid current and cool water are unfavourable for the development of Diptera larvae (Medvedev and Panyukova, 2005).

Distribution of Aquatic Insects in relation to environmental parameters, water parameters and water quality.

In streams, biological condition is strongly influenced by water chemistry and habitat quality. The combination of water analysis, diversity indices and water quality indices were satisfactorily applied to investigate the river health (Salman et al., 2011) particularly in Keniam River. Low dissolved oxygen, high bacterial, nitrate or phosphorous concentrations, and low pH can cause reduced water quality. Good habitat quality is generally characterized by a heterogeneous habitat with both slow and fast moving water, woody debris, substrate variety, and well-vegetated, stable banks. Impairment of habitat and water chemistry can lead to reduce the diversity of aquatic macro invertebrates (Resh and Betts, 2007). Pearson correlation was carried out to investigate the effects of environmental parameters and water parameters towards Orders of aquatic insects' density. The test also carried out to know the relationship of the aquatic insects such as Diptera larvae with environmental and water parameters.

Based on calculation of Water Quality Index, the value of WQI for Lower stream River is the highest. This indicates that water quality in Lower stream River is cleaner compared to other rivers. Consequently, the species of aquatic insects such as Ischnura elegans from order Odonata and Diptera that act as bioindicator to the vicinity was identified. Based on the analysis, several species of Odonata such as Ischnura elegans, Diptera such as chironomid were detected in Keniam River. This species is exhibited a strong ability on accumulation of cadmium in water (Zhou et al., 2008). The diversity of Orders of aquatic insects were found very high variation in H' values (Metcalfe, 1989). Based on this index, the correlation of aquatic insect and environmental parameters, it can describe the quality of the river (Metcalfe, 1989; Salman, et al., 2011) is very good. Overall, the result demonstrated that even though the diversity of aquatic insects in Keniam River were varied on the strata and time, the undisturbed habitat quality in this river is most likely suitable for insects to breed and multiply under the natural ecosystem which is abundant food supply for the insects.

Conclusion

From our results throughout the study period, there are total of 1287 individuals of aquatic insects collected in all nine locations from February 2009 to January 2010. There is no significant different between strata and but different in term of habitat on the diversity of aquatic insects in Keniam River, National Park, Pahang. Various types of habitat for aquatic insects live starting from upstream from Kuala Perkai to lower stream at Kuala Keniam of Keniam River Species diversity of aquatic insects varied in different strata of the Keniam River. There were 8 orders identified. For selected order specifically Diptera, there was 7 families and 15 genus were identified. Simuliidae was the dominant family and followed by Tipulidae. Since the most abundance Diptera larvae found in most of the stratum Keniam River is Simulium, the river is considered very well because Simulium cannot tolerate to polluted water. This led them as a good indicator of clean water. The abundance and distribution of aquatic insects species varies and not constant from one to another month during the study period. Their distribution was not constant from one sampling date (bimonthly) to another. The abundance of the aquatic insect recorded as the highest number during July and September 2010 in Keniam River. This indicates the richness and diverse group of aquatic insects in the study area. It adds to the fact that the habitat quality is most suitable for insects to breed and multiply under the natural ecosystem. There is abundance food supply for the insects too. Our results can conclude that aquatic insects were diverse and some species from Diptera are good bioindicator to know the quality of water. The findings of this study also demonstrated that some species of aquatic insects (order) are able to adapt to their habitat and variety of environmental parameters. This is indicated and proven by the different correlation value for different type of habitats although there are same orders of aquatic insect. The pollution tolerant family such as Chironomidae present very low in number in all three strata of the river, but it is not a dominant family. Further study in Keniam River must be carried out because some species did not only important as bioindicator insects, but it also have it role in pollination, food sources (food chain), medical importance and also to stabilize the streams ecosystem. The information gathered from this study will hopefully help for further study by researchers, students and officers who have given responsibility in conservation of flora and fauna, especially the aquatic insects in Keniam River of National Park at Kuala Keniam, Pahang.

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