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Variation in environmental conditions influences diversity and abundance of Ephemeroptera in forest streams of northern Peninsular Malaysia

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Abstract: Effects of variations in stream environmental conditions on a nymphal ephemeropteran community were investigated in two forested catchments in Peninsular Malaysia; Gunung Jerai (GJ) and Royal Belum State Park (RBSP). Ephemeropteran nymphs were collected monthly from both catchments using an aquatic net over a period of a year (2008-2009). Ecological indices were calculated to compare community features and diversity of the ephemeropterans in the two catchments. Ephemeropteran taxa interactions with environmental parameters and their community structures were investigated using multivariate analysis (detrended canonical analysis and canonical correspondence analysis) and Bray Curtis similarity measure, respectively. From the two catchments, 22 ephemeropteran taxa were recorded and taxonomic richness was higher in undisturbed RBSP (20 taxa) than in slightly impacted GJ (12 taxa) streams. Environmental parameters were more important than geographical location in separating ephemeropteran communities in both catchments but similarities among communities in streams in the same catchment were detectable. Water temperature and pH were strong descriptors of ephemeropteran community structure in GJ. High water temperature was favourable to *Isonychia* sp., *Crinitella* sp., *Habrophlebiodes* sp. and *Tricorythus* sp. In RBSP, pH, water velocity, and canopy cover were important in shaping ephemeropteran communities. *Heptagenia* sp., *Campsoneuria* sp., and *Habrophlebiodes* sp. were negatively associated with elevating pH and fast running water. This study provides useful information for preservations of suitable habitats for the ephemeropterans in undisturbed and potentially disturbed forest streams in Malaysia.

Key words: Ephemeroptera, environmental conditions, forest streams, Malaysian tropical forest.

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Introduction

Ephemeroptera is one of the most abundant orders of aquatic insects found in tropical lotic and lentic habitats (Sites *et al.* 2001). In tropical areas, it constitutes up to 80 % of the aquatic invertebrate community (Dudgeon 2006). Among the three strictly aquatic orders, Ephemeroptera, Plecoptera and Trichoptera (EPT), Ephemeroptera (mayflies) shows the highest abundance in at least six tropical regions; Hong Kong, Sulawesi, Papua New Guinea, Ecuador Pacific, Ecuador Amazon, and

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Bolivia (Dudgeon 2008). Mayflies become an important component of the aquatic food web particularly as a food source for fish and other aquatic vertebrates (Parkyn *et al.* 2001).

Colonization of mayflies in headwater streams depends on several abiotic and biotic factors (Perbiche-Neves *et al.* 2012; Power *et al.* 1988). Active anthropogenic activities in the surrounding riparian areas especially in the developing countries such as Malaysia, which include deforestation, agricultural and urban land use, infrastructure development, and road construction may change the physical and chemical characteristics of the stream water and streambeds through erosion and sedimentation (De Billy *et al.* 2000; Kreutzweiser *et al.* 2005; Pond 2010). In many instances, when changes are severe, they have an adverse effect on abundance and diversity of mayflies in tropical (Che Salmah *et al.* 2012; Douglas *et al.* 1993; Dudgeon 2000; Iwata *et al.* 2003; Sodhi & Brook 2006) and temperate (Franken *et al.* 2001; Hicham & Lotfi 2007) streams.

There is growing interest among ecologists in the determinant environmental conditions shaping community structure of aquatic insects, particularly mayflies, in tropical streams. For example, Al-Shami *et al.* (2013) concluded that biochemical oxygen demand (BOD), velocity of the river, ammonia content, and dissolved oxygen (DO) were the determinant factors affecting diversity and abundance of EPT taxa at large spatial scale. Despite their importance in tropical streams, Ephemeroptera have not been well studied outside of a few geographical regions (e.g., Hong Kong, Australia, New Zealand and Africa) (Boyero *et al.* 2009). These geographical restrictions often lead to incomplete understanding of stream ecology in other tropical regions (e.g., South East Asia including Malaysia). In addition, biogeography and ecology of tropical Ephemeroptera are poorly known and the available data on their diversity are sparse and disconnected at local and regional scales (Boyero *et al.* 2009; Dudgeon 2000, 2006).

In Malaysia, little specific biological and ecological information is available on Ephemeroptera because most aquatic ecologists have studied mayflies only as a part of larger studies on aquatic macroinvertebrates (Al-Shami *et al.* 2011; Azrina *et al.* 2006; Che Salmah *et al.* 2013; Wahizatul *et al.* 2011) and EPT communities (Che Salmah *et al.* 2001, 2007; Suhaila *et al.* 2011, 2012). This study, aiming to address the limited information on biology and ecology of Ephemeroptera, was carried out in several streams of two catchments in peninsular Malaysia [Royal Belum State Park (RBSP) and Gunung Jerai (GJ)]. RBSP rivers are located in a preserved area and are expected to be minimally disturbed, whereas GJ may encounter slight human disturbance due to occasional recreational activities by the locals. The objective of the study was to (1) compare diversity and abundance of the mayflies between streams of the two catchments, and (2) explore the relationships between mayfly taxonomic composition and selected physical and chemical factors in each catchment. We hypothesized that the diversity and abundance of mayflies would show different patterns due to variations in physical and chemical conditions owing to human disturbance or natural occurrences. We also postulated that different mayfly taxa would respond differently to the physical and chemical factors of their environments.

Methodology

Study Areas

This study was carried out in two catchments in the northern peninsular Malaysia; Royal Belum State Park (RBSP) and Gunung Jerai Forest Reserve (GJ). The Royal Belum State Park is located between 05° 36’ 18.7” N and 101° 25’ 15.9” E) at approximately 295 m asl to 900 m asl. It has a very large catchment of 117,500 ha of impenetrable jungle in the state of Perak, stretches into the Thai-Malaysian border. The RBSP retains 90 % virgin forest while the rest is logged-over or secondary forest. The forest is covered with Dipterocarpaceae which constitutes over 85 % of the forested area concentrating at the lowland region below 1200 m asl. *Shorea platyclados* (locally known as meranti bukit) is abundant on hills and slopes of upper hill (> 700 m asl), *Shorea leprosula* (meranti tembaga) grows in meandering slopes (< 700 m asl), while *Dipterocarpus* (keruing) and *Intsia palembanica* (merbau) trees are abundantly found in wetter and warmer areas at the lower altitudes. RBSP forest is managed by the Royal Belum State Park of the Perak state.

Gunung Jerai Forest Reserve (GJ, located between 05° 47’44” N and 100° 26’4” E) is a small catchment of 8676.36 ha in Kedah State which is only about 7 % of the total area of RBSP. It begins from its peak at approximately 1214 m asl down to its base at 200 m asl (Fig. 1). This dipterocarp forest has high dominance of *I. palembanica* (merbau), *Koompasia malaccensis* (kempas), *Shorea platyclados* (meranti bukit) and *Droba-*

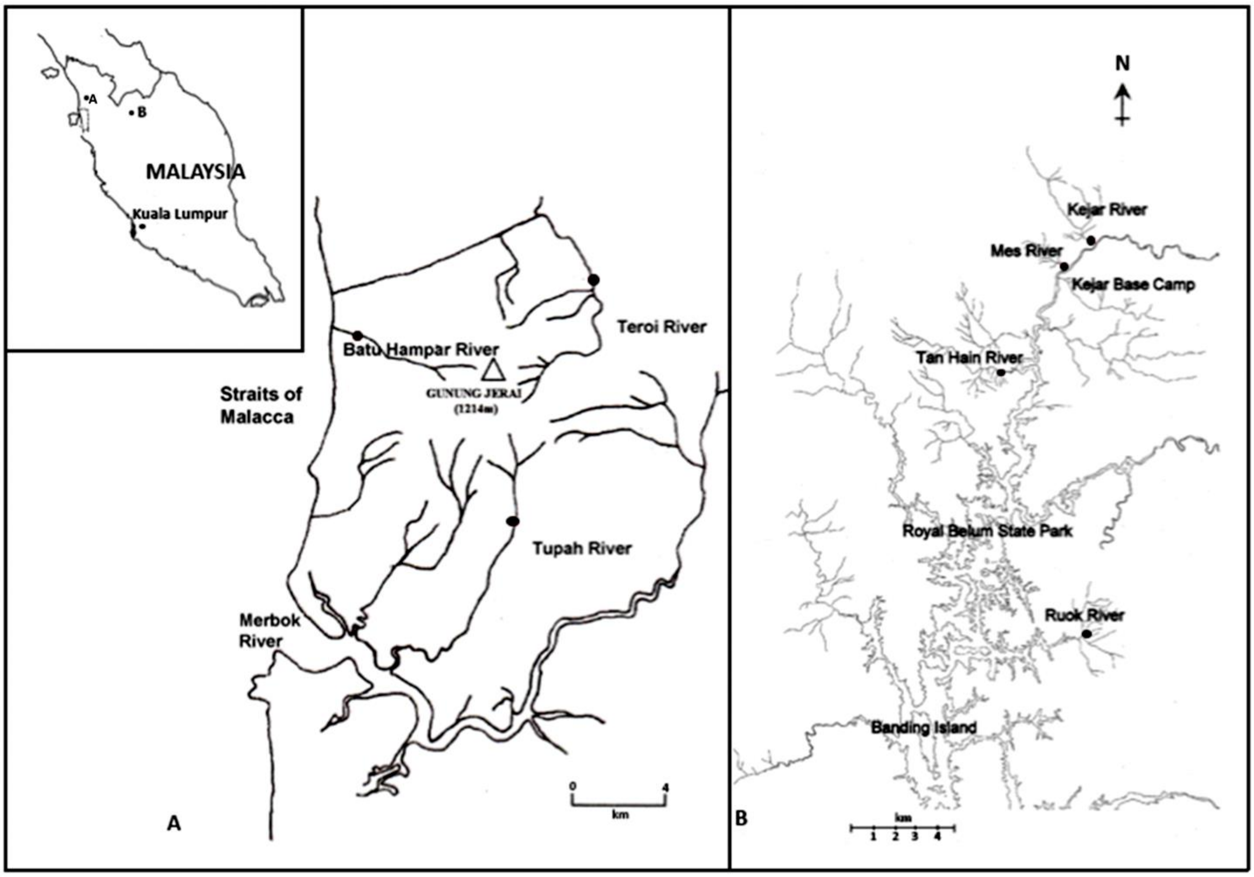

Fig. 1. Location of the northern Peninsular Malaysia study sites. A: Gunung Jerai (GJ) catchment in Kedah State. B: Royal Belum State Park (RBSP) catchment in Perak State. Inset is the map of Peninsular Malaysia showing the approximate locations of the sampling areas.

lanops aromatica (kapur). The GJ forest is under the direction of the Kedah State Forestry Department. Respective authorities in both forests had granted permission for this study to be conducted in their areas.

After preliminary sampling in the two catchments, four streams were selected in the RBSP (Kejar River, Mes River, Tan Hain River, and Ruok River) and three streams were chosen in the smaller GJ (Teroi River, Tupah River, and Batu Hampar River) (Fig. 1), on the basis of their accessibility and availability of suitable habitats for ephemeropterans. In RBSP, Kejar River is located at the northern part and Mes River is approximately 1 km to the south of Kejar River. Tan Hain River lies in the middle span of the park and Ruok River flows down a hill into Belum Lake near to the park entrance. All RBSP streams have pristine environment. Substrates in the four rivers vary from big boulders in Tan Hain and Kejar to small boulders and cobbles in Mes and gravel and course sands in Ruok. In GJ, Tupah and Batu Hampar streams are popular recreational sites at the foot of the hill (200 - 300 m asl). Teroi River is an upland recreational site, flowing down from the peak of the hill to lowland area. Although the sampling site at Teroi River was situated at a much higher altitude (about 1200 m asl), its environmental parameters did not vary much from other streams except for slightly higher water velocity (channel gradient approximately 17.5 %) and shallower water. Water temperature varied within a small range in all streams (Fig. 2). Stream substrates were comprised of boulders, cobbles, and gravels, except in Teroi River where the substrate was mainly bedrock. Several riffles and runs were observed in all streams in both catchments, but pools were relatively uncommon. The water was generally clean and clear except during occasional disturbances by visitors in GJ streams. Streams in GJ and RBSP were uphill streams in the second to third orders (Strahler

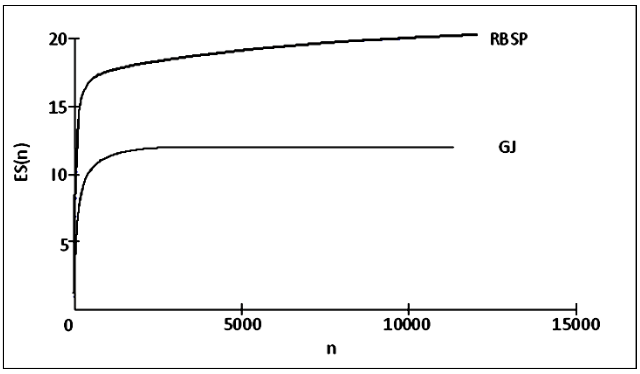

Fig. 2. Rarerified richness of ephemeropteran communities inhabiting RSBP and GJ streams.

1957). All investigated streams in both catchments were almost similar to each other in terms of annual rainfall (2000 - 3000 mm), humidity (70 % to 98 %), water temperature (20.8 °C - 24.4 °C), and water pH (5.25 - 6.02). In Teroi River (GJ), however, the pH was slightly more acidic because it was surrounded by relatively thick growth of riparian trees and decomposition of their fallen leaves contributed to lower pH of the water.

Physical and chemical parameters

Water temperature, DO, pH, depth, stream width, and canopy cover were simultaneously measured *in situ* during each ephemeropteran sampling occasion. Water temperature and dissolved oxygen (DO) were measured with a YSI 1550 (YSI Inc. USA) DO meter, and water pH was measured using a HACH SensIon 1 (HACH Company, USA) portable pH meter. A velocity meter (Global Water Velocity Meter, Model FP111) measured water velocity at various intervals across the streams and their averages were recorded. Width and depth of the streams were measured using a measuring tape and a metal ruler, respectively. The amount of shaded water surface was estimated with a Spherical Densiometer (Model A Convex, Forestry Suppliers, Inc.). Five 500 mL water samples were randomly collected from each stream and transported to the laboratory under cool conditions. Biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia-nitrogen (ammonia-N), and total suspended solid (TSS) were measured using a HACH digestion and calorimeter DRB200 and DR/890 following standard protocols. All water parameter measurements were recorded in triplicates.

Sampling of Ephemeroptera

Ephemeropteran nymphs were sampled monthly from both catchments, over twelve months from February 2008 to January 2009 using a D-frame aquatic net with a 38 cm opening and equipped with a meter long pole. A cone-shaped net of 0.3 mm mesh size was fixed to the frame. In each stream, sandy substrates or pool areas were sampled by dragging the net for one meter against the water current over the stream substrate or aquatic plants. Stony substrates in fast flow were sampled using the kick sampling technique and an area of 1 m^2 in front of the net was disturbed for 2 minutes (Merritt *et al.* 2008). Ephemeropteran nymphs collected in the net were transferred into labeled plastic bags filled with small amount of water. In the laboratory, the nymphs were sorted and preserved in 75 % ethanol. The Ephemeroptera nymphs were identified to the lowest practical taxonomic level, using keys of Dudgeon (1999), Morse *et al.* (1994), Yule & Yong (2004) and Webb & McCafferty (2008). In this study, most of the specimens were identified to the generic level due to lack of species-based taxonomic keys for Malaysian stream macroinvertebrates. It has been established that genus-level identification is considered useful in biodiversity studies (Heino & Soininen 2007).

Data analysis

Normality assumptions of the ephemeropteran data were tested using the Kolmogorov-Smirnov test. Then differences in mean mayfly abundance between catchments were examined using the t-test (SPSS software package, version 11.5). Calculations of various diversity measures provided information on diversity of Ephemeroptera in habitats at varying spatial scales. Alpha diversity measures the compacting of taxa within a habitat which reflects division of ecological resources. Beta diversity indicates the amount of turnover in species composition along environmental gradient (habitat specialization), while gamma diversity differentiates provinciality or endemicity of taxa in a large area (Sepkowski 1988). Margalef's, Shannon-Wiener, and Pielou indices allow both number of taxa and their relative importance (abundance) to be evaluated simultaneously. These indices were useful for comparison of diversity, richness, and distribution of the ephemeropterans among streams. Ephemeroptera taxa data in RBSP and GJ were rarified for unbiased compa-

Table 1. Physico-chemical parameters (mean ± SE) of Gunung Jerai (GJ) in Kedah and Royal Belum State Park (RBSP) streams.

	Gunung Jerai			RBSP			
	Teroi	Batu Hampar	Tupah	Kejar	Mes	Tan Hain	Ruok
pH	4.9 ± 0.23	6.0 ± 0.11	6.0 ± 0.13	5.3 ± 0.49	5.5 ± 0.51	5.6 ± 0.52	5.7 ± 0.53
Temp (°C)	20.8 ± 0.24	24.2 ± 0.18	24.4 ± 0.31	21.0 ± 1.91	21.5 ± 1.97	21.6 ± 1.98	21.9 ± 2.00
DO (mg L^{-1})	7.5 ± 0.32	7.1 ± 0.26	7.6 ± 0.23	7.1 ± 0.69	7.0 ± 0.67	6.9 ± 0.75	6.7 ± 0.66
BOD	0.8 ± 0.11	0.9 ± 0.10	1.9 ± 0.87	0.9 ± 0.15	0.8 ± 0.17	0.8 ± 0.21	0.8 ± 0.21
COD*	19.5 ± 3.48	10.3 ± 2.01	10.7 ± 2.06	3.6 ± 2.48	3.3 ± 2.43	4.3 ± 2.65	4.3 ± 2.74
TSS (mg L^{-1})	5.3 ± 0.36	1.4 ± 0.15	3.1 ± 0.34	2.4 ± 0.81	3.3 ± 0.91	1.9 ± 0.67	3.5 ± 0.66
Ammonia-N (mg L^{-1})	0.04	0.03 ± 0.01	0.02	0.02 ± 0.01	0.05 ± 0.02	0.05 ± 0.02	0.04 ± 0.02
Canopy (%)*	50.3 ± 0.25	70.1 ± 0.15	20.3 ± 0.13	30.5 ± 8.29	32.3 ± 8.62	20.8 ± 7.65	47.1 ± 9.03
Velocity (m s^{-1})*	1.3 ± 0.13	0.7 ± 0.13	0.6 ± 0.17	0.5 ± 0.05	0.4 ± 0.05	0.5 ± 0.08	0.5 ± 0.10
Width (m)*	4.0 ± 0.79	4.6 ± 0.37	4.1 ± 0.29	8.7 ± 0.85	4.8 ± 0.51	12.2 ± 1.19	4.6 ± 0.54
Depth (cm)*	0.3 ± 0.07	0.3 ± 0.06	0.3 ± 0.05	33.9 ± 3.54	21.8 ± 2.87	32.1 ± 4.44	20.7 ± 2.61

*****Parameter significantly different at $P < 0.05$ (Kruskal-Wallis test).

rison of their diversities due to unequal sample size collected from the two catchments. All analyses were performed using the SDR Program (Version 4.1.2; Seaby & Henderson 2006). Homogeneity assumption of sampling sites was examined using detrended correspondence analysis (DCA). Bray-Curtis similarity analysis was employed to investigate ephemeropteran distribution in various streams and the Bray-Curtis distance plot was drawn using BioDiversity Pro® software. The canonical correspondence analysis (CCA) estimated associations of ephemeropteran taxa with various stream physico-chemical parameters in the two catchments. The significance of the canonical axes generated was tested using Monte Carlo test ($P < 0.05$) with 500 iterations. DCA and CCA were performed using CANOCO (version 4.5) and their ordination biplots were drawn with CanoDraw for Windows 4.1.

Results

Physico-Chemical Parameters of Streams

Stream physico-chemical parameters, namely COD, canopy, velocity, and stream width and depth, varied significantly in the two catchments (Table 1). Except for water depth and stream width, most of the physical and chemical parameters were higher in GJ streams (Teroi River, Tupah River, and Batu Hampar River) than in RBSP streams (Kejar River, Mes River, Tan Hain River, and Ruok River). The water in Teroi River was slightly more acidic than in other streams and higher temperatures (± 24 °C) were recorded in Tupah and Batu Hampar. Ammonia-N was generally low in all streams and its lowest values were recorded in Tupah and Kejar streams (0.2 mg L^{-1} of each of the rivers). Kejar was the deepest stream while Tan Hain stream was the widest. Some streams were mostly shaded as observed in Batu Hampar as well as Teroi rivers, but Tan Hain and Tupah streams were only slightly shaded.

Composition of Ephemeroptera in different catchments

Higher mayfly diversity was recorded from the four RBSP streams compared to the three GJ streams and the highest (18 taxa) was recorded in two streams in RBSP (Table 2). Rarefaction curves produced using abundance data of ephemeropterans in the two catchments (Fig. 2) substantiated higher ephemeropteran diversity in RBSP. *Baetis* spp. dominated the streams in both catchments but their density was approximately eight times higher in Teroi River in GJ than in Tan Hain River, the stream that sustained the most *Baetis* spp. in RBSP. All taxa recaded from the three GJ rivers were collected from the four RBSP streams except for *Teloganodes* sp. and

Table 2. Density (individual m-2) of mayfly taxa in streams of Gunung Jerai (GJ) and Royal Belum State Park (RBSP).

Family	Genus	RBSP				GJ		
		Kejar	Mes	Tan Hain	Ruok	Batu Hampar	Tupah	Teroi
Baetidae	*Baetis*	111.3	216.7	219.6	61.7	182.1	384.7	1926.6
	Platybaetis	2.0	6.3	4.2	0.3	17.9	60.5	156.6
	Centroptilum	-	-	-	-	0.78	5.8	10.8
Heptageniidae	*Thalerosphyrus*	28.2	41.8	33.3	20.7	42.1	47.1	161.3
	Campsoneuria	12.6	4.7	13.7	8.6	6.1	4.0	26.32
	Heptagenia	6.2	4.1	12.9	6.1	-	-	-
	Epeorus	0.3	2.2	0.3	6.3	3.2	8.7	7.6
Potamanthidae	*Cinygmula*	-	-	-	0.4	-	-	-
	Rheonanthus	8.2	-	0.7	5.4	-	-	-
	Potamanthus	0.4	-	12.6	0.2	-	-	-
Leptophlebiidae	*Habrophlebiodes*	61.9	25.5	55.3	32.5	18.2	13.7	-
	Thraulus	1.2	0.9	0.5	0.1	-	-	-
	Choroterpes	3.4	5.5	0.9	-	-	-	-
Ephemerellidae	*Crinitella*	1.3	1.5	13.3	0.1	0.00	3.9	0.5
	Drunella	0.1	-	-	-	-	-	-
Oligoneuridae	*Isonychia*	3.0	0.5	3.3	6.8	4.2	-	-
Neoephemeridae	*Neoephemeropsis*	5.3	0.7	5.1	15.3	-	-	-
Caenidae	*Caenis*	40.5	21.7	17.5	14.9	13.2	19.2	-
Ephemeridae	*Ephemera*	3.1	7.5	0.9	3.9	-	-	-
Tricorythidae	*Tricorythus*	7.6	4.5	10.1	7.9	16.6	12.9	-
	Teloganella	-	-	-	0.3	-	-	-
	Teloganodes	-	-	-	-	-	0.8	2.9

Table 3. Diversity scores of mayfly communities in Gunung Jerai (GJ) and Royal Belum State Park streams (RBSP).

	RBSP				GJ		
	Kejar	Mes	Tan Hain	Ruok	Tupah	Batu Hampar	Teroi
Alpha$_{min}$ (α_{min})	9	5	11	9	5	4	4
Alpha$_{max}$ (α_{max})	14	12	14	14	11	9	7
Alpha$_{average}$ ($\alpha_{average}$)	11.75	8.67	12.00	11.17	7.83	6.25	5.17
Gamma (γ)	18	15	17	18	11	10	8
Gamma$_{estimated}$ ($\gamma_{estimated}$)	27.37	21.77	26.68	26.83	17.33	14.95	12.11
Beta (β)	1.53	1.73	1.42	1.61	1.40	1.60	1.55
Margalef's (R1)	2.19	1.74	1.98	2.32	1.30	1.28	0.77
Shannon-Wiener (H')	2.26	2.26	2.48	2.33	2.16	1.95	2.37
Pielou (J')	0.91	0.91	0.97	0.94	0.87	0.78	0.96

Centroptilum sp. Meanwhile, *Heptagenia* sp., *Cinygmula* sp., *Rheonanthus* sp., *Potamanthus* sp., *Thraulus* sp., *Choroterpes* sp., *Drunella* sp., *Neoephemeropsis* sp., *Ephemera* sp., and *Teloganella* sp. were exclusively found in the RBSP streams.

Higher ephemeropteran taxa richness in the four RBSP streams corresponded to relatively high values for most of the diversity indices calculated for the RBSP streams (Table 3). Specifically, the local (i.e., measured as $alpha_{average}$) and regional (i.e., gamma) diversities (measures of taxa richness) were the highest in RBSP streams especially in Kejar and Ruok. Other indices [Margalef's Richness (R1), Shannon-Weiner Diversity (H'), Pilou Evenness (J')] were also higher in RBSP in comparison to GJ. Margalef's index (R1) was the highest in Kejar River while H' and J' were the highest in Tan Hain River, both streams in the RBSP catchment. Among the RBSP streams, Mes River had higher turnover due to more variations of habitats (beta-diversity = 1.73). However, Ruok River (in RBSP) had similar production with Batu Hampar River in GJ (beta-diversity 1.61 and 1.60, respectively). In GJ, Teroi River had the highest diversity (H') and more evenly distributed ephemeropteran taxa although more genera were collected from Tupah River.

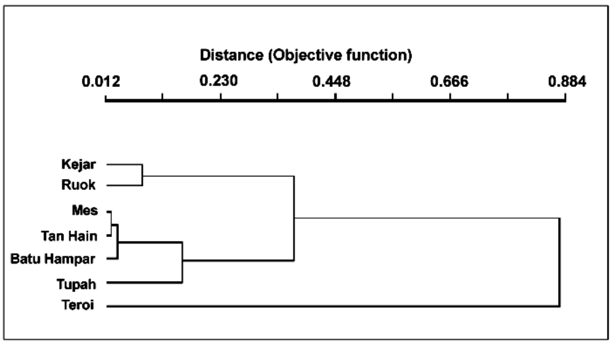

Fig. 3. A dendogram (Bray-Curtis similarity measure) separating streams in the two catchments based on mayfly taxonomic composition (RBSP: Kejar, Mes, Tan Hain, Ruok, GJ: Tupah, Teroi and Batu Hampar).

Distribution of ephemeropteran taxa richness in all streams separated mayfly compositions in GJ and RBSP into three major groups (Fig. 3). The ephemeropteran community in Teroi River was very different from the composition in other streams and hence formed a single group by itself. Some similarities of ephemeropteran assemblage were observed in streams of RBSP and GJ catchments. For instance, the ephemeropteran community in Mes and Tan Hain rivers (RBSP catchment) were comparable with that of Batu Hampar in the GJ catchment. Within the same catchment, Kejar and Ruok formed a single group while Tan Hain and Mes paired with each other. Batu Hampar and Tupah also formed a single group although with slightly greater variation.

Ephemeropteran assemblages in various streams were further substantiated by the results of DCA biplot produced in Fig. 4. In this analysis, streams from both catchments were grouped according to their similarities in selected environmental parameters which also justified homogeneity of habitats in the two catchments. Strong water velocity of Teroi River separated this stream from other streams which corresponded to different assemblages of the ephemeropterans. Water temperatures in Tupah and Batu Hampar fell within a similar range while Mes, Tan Hain and Ruok were grouped together based on similarity in ammonia content in the water. Mes River was almost as wide as Tan Hain River and Tan Hain, Tupah, and Ruok shared similar levels of TSS in the water. In general, variations in distribution of ephemeropterans in the two catchments closely followed their stream physico-chemical properties.

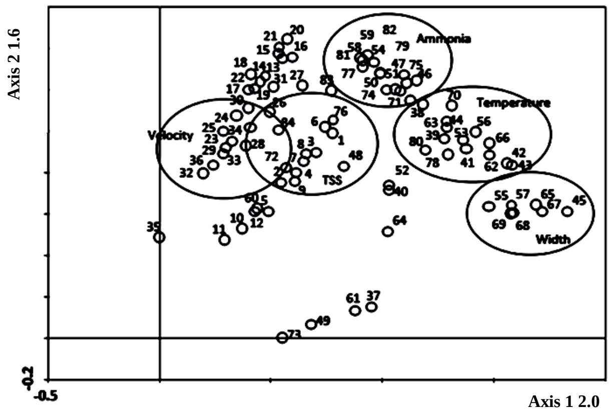

Fig. 4. A detrended correspondence analysis (DCA) showing grouping of habitats in streams in the two catchments mainly based on physico-chemical parameters (GJ: Tupah River 1 - 12, Batu Hampar River 13 - 24, Teroi River 25 - 36, RBSP: Kejar River 37 - 48, Mes River 49 - 60, Tan Hain River 61 - 72, Ruok 73 - 84).

In view of some dissimilarity among mayfly assemblages in streams in similar catchments, the multivariate CCA was applied to simplify the complex relationships between abundance of mayfly taxa and environmental conditions in each catchment. In the GJ streams, the canonical correlation model explained nearly half the variation in taxonomic composition, and the first two canonical

Table 4. Features of Canonical Correspondence Analysis (CCA) of measured enviromental parameters on ephemeropteran richness in RBSP and GJ streams at 500 iterations. Monte Carlo test $P < 0.05$.

		RBSP				GJ			
		Axis 1	Axis 2	Axis 3	Axis 4	Axis 1	Axis 2	Axis 3	Axis 4
Constrained correlation coefficients for all variables	pH	-0.3643	0.2065	-0.0067	0.3983	0.3846	0.5434	0.0717	0.0186
	Temperature	-0.0569	0.1513	-0.0926	0.2712	0.5413	-0.2298	0.1185	-0.0482
	DO	-0.5172	-0.2169	-0.2928	-0.0698	-0.3315	-0.2353	0.4258	0.0738
	BOD	0.1583	-0.0895	-0.0081	-0.2189	0.145	0.2032	0.2579	-0.0797
	COD	-0.1491	-0.0627	0.0029	0.0447	-0.0842	-0.2977	-0.0237	0.0147
	TSS	-0.2343	-0.1961	0.3789	-0.0391	-0.4397	0.0042	0.0828	-0.0724
	Ammonia-N	-0.0658	0.0098	0.0073	0.2018	-0.2427	-0.0322	-0.2405	0.1794
	Canopy	-0.0427	-0.4105	-0.0328	0.0935	-0.0138	-0.0538	-0.2402	0.3224
	Velocity	-0.3093	0.1611	0.1609	-0.3446	-0.4499	0.1113	-0.2773	0.0323
	Width	0.1138	0.7023	-0.0893	-0.0639	-0.0162	-0.0257	0.038	0.1222
	Depth	-0.1686	0.1925	-0.0565	-0.138	-0.1157	0.0176	0.2255	0.1123
Eigenvalues		0.14	0.065	0.037	0.031	0.225	0.109	0.06	0.018
Cumulative percentage variance of species-environment relation		41	59.9	70.8	79.9	52.1	77.5	91.3	95.5
Total Variance Explained (TVE) (%)		36.46				46.79			

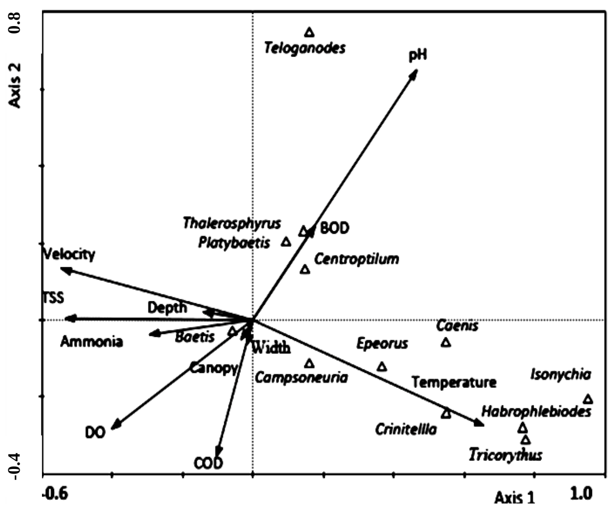

Fig. 5. The interaction between mayfly and water parameters in GJ is shown by the the ordination plot (CCA) for the first two canonical axes of the mayfly taxa and physicochemical parameters in the investigated streams of Gunung Jerai (GJ). Total inertia of the model was 0.92 with total variance explained (TVE) of 46.8 %.

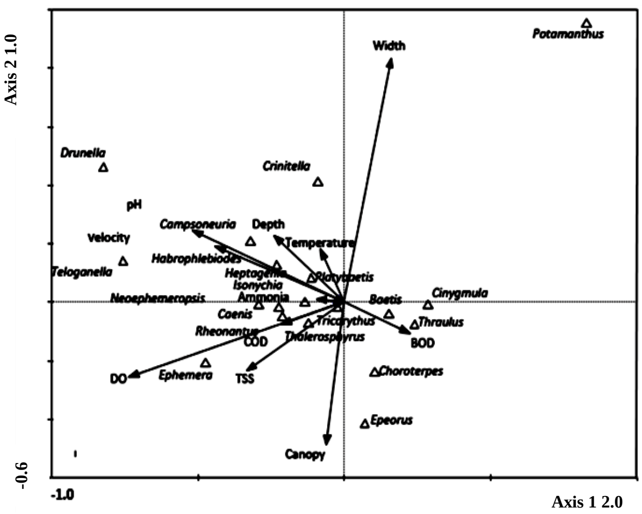

Fig. 6. The interaction between mayfly and water parameters in RBSP is shown by the ordination plot (CCA) for the first two canonical axes of taxa and physicochemical parameters in the investi-gated streams of Royal Belum State Park (RBSP). Total inertia of the model was 0.938 with total variance explained (TVE) of 36.5 %.

axes explained more than 36 % of the variation (Fig. 5 and Table 4). Elevated pH and high water temperature in GJ were associated with taxa such as *Campsoneuria* sp., *Epeorus* sp., *Caenis* sp., *Crinitella* sp., *Habrophlebiodes* sp., *Isonychia* sp., and *Tricorythus* sp., and BOD was associated with *Thalerosphyrus* sp., *Platybaetis* sp., and *Centroptilum* sp. The widespread and highly abundant *Baetis* sp. was not associated with any of the measured parameters.

In RBSP, the total extent of variation was high (TI = 0.938) (Fig. 6 and Table 4) and the canonical correlation model explained about 22 % of the variation in taxonomic composition. High DO, pH, velocity, and TSS were negatively associated with abundance of most ephemeropteran taxa, except *Baetis* sp., *Cinygmula* sp., *Thraulus* sp. and *Choroterpes* sp., while *Epeorus* sp. showed preference for habitats with high BOD.

Discussion

Diversity of Ephemeroptera in different catchments

Considerable variation in diversity of ephemeropteran nymphs in the streams of RBSP and GJ was observed in this study. A more diverse ephemeropteran community inhabited the cleaner streams of RBSP catchment compared to the slightly impacted streams of the GJ catchment. GJ streams supported an almost four times higher abundance of mayflies compared to RBSP streams, primarily due to enormous collections of *Baetis* sp. (76 % of total ephemeropterans), especially from Teroi River. Species of *Baetis* are collector-gatherers of FPOM (fine particulate organic matter) deposited on stream substrates and scraping algae from the surfaces of rocks (McShaffrey & McCafferty 1986). Fast water flow in Teroi River carried fine food particles from allocthonous organic matter that had been broken down by various EPT shredders and entrapped them in bedrock crevices (Suhaila & Che Salmah 2011). The lack of this type of substrate in RBSP streams was likely the cause of lower abundance of *Baetis* sp. there. Nevertheless, relatively big boulders were present in Kejar and Tan Hain streams and *Baetis* sp. was more abundant than other ephemeropterans collected from RBSP.

From the two catchments, we recorded 22 mayfly taxa that represented 12 families. Based on this record, the diversity of the ephemeropterans in GJ and RBSP was markedly lower than those recorded from streams in hilly dipterocarp forest of Borneo (Sartori *et al.* 2003). However, this assemblage is more diverse than the record of Sites *et al.* (2001) from Thailand and some other pristine highland streams in the tropics. For instance, only 15 taxa and 5 families were recorded from Brazil (Bispo *et al.* 2006) and a poorer community (9 taxa and 5 families) was reported from India (Dinakaran & Anbalagan 2007).

Influence of environmental parameters on Ephemeroptera in different catchments

Ephemeroptera are ubiquitous in streams, from lowland to mountainous altitudes living in variable environmental conditions (Heino & Peckarsky 2014). Usually upland streams provide physically heterogenous habitats to support more diverse macroinvertebrate assemblages (Che Salmah *et al.* 2013; Md Rawi *et al.* 2014). The presence of certain ephemeropteran taxa, such as heptageniids (e.g. *Campsoneuria* sp. and *Epeorus* sp.) reflects a relatively clean stream environment (Al Shami *et al*. 2013; Azrina *et al.* 2006; Merritt *et al.* 2008), although high abundances of widespread and tolerant taxa such as *Baetis* are often associated with moderate levels of organic pollution (Armitage *et al*. 1983). In this study, the values of all water parameters of all streams fell within the limits of an unpolluted environment. Except for slightly more acidic water in Teroi River, the pH in other streams ranged between 5.25 and 6.02, and was suitable for most aquatic organisms. Besides the difference in water pH, TSS and COD were higher in Teroi River. In this stream, a minor amount of nutrient enrichment, possibly introduced during the decomposition of fallen leaves from trees along the stream, contributed to extremely high abundance of *Baetis* sp., a moderately tolerant aquatic insect (Azrina *et al.* 2006). Consequently, *Baetis* spp. was 11 to more than 3000 times more abundant than other genera (Table 2) collected from the stream. For this matter, it was evident that water enrichment coupled with bedrock habitat enhanced the preponderance of this genus in Teroi River.

In this study, although we found that some water quality (e.g. COD) and habitat indicators (e.g. stream width and depth, canopy cover, water velocity) were significantly different between the two catchments, the ephemeropterans in both catchments were not separated clearly according to the catchment they occurred in, but more on the variations of water quality and habitat suitability in both catchments. This suggests that variations in water parameters and habitat quality among rivers in both catchments were more important in structuring mayfly richness (Kamsia *et al.* 2008) in the two areas. Nevertheless some similarities were detectable among streams in similar catchment.

The present study revealed that most mayfly taxa were sensitive to subtle changes in their habitat as indicated by the CCA ordination plots (Bere 2014). The range of DO, COD, and water velocity in both RBSP and GJ streams varied within small ranges which represented well-oxygenated streams paralleled with relatively low water temperatures (in the tropics). In GJ, the mean water temperature varied 3.62 °C among streams and we found that temperature was a fairly strong descriptor of change in the abundance of several mayfly taxa as shown in Fig. 5. This may reflect the importance of temperature variation, even small fluctuations, to community structure of mayflies. Sweeney (1995) suggests that small changes in temperature (2 - 5 °C) would result in obvious changes in the aquatic invertebrate composition and the species associated either with high or low water temperature are taxonomically very heterogenous (Burgmer *et al.* 2007). Some mayfly species in these catchments, such as *Isonychia* sp., *Habrophlebiodes* sp., and *Tricorythus* sp., were positively associated with water temperature. Such association among the ephemeropterans was similarly reported by Dobrin & Giberson (2003). Meanwhile, Haidekker & Hering (2008) found that increase in water temperature only increased mayfly abundance, but not the taxonomic richness.

In RBSP streams, responses of ephemeropteran taxa towards water parameters were almost similar to those observed in GJ streams. Water temperature was not a strong descriptor in this catchment, but ephemeropterans were also associated positively with this parameter. Other parameters were either negatively associated with taxa abundances or can be considered negligible.

The water flow in GJ streams was much faster than in RBSP streams and ephemeropteran abundance (specifically heptageniids) was markedly higher in GJ (particularly in Teroi River) than in RBSP streams. In the CCA analysis however, most of ephemeropteran genera in both catchments were negatively associated with elevated water current. In this situation, heptageniids and other families highly adapted to living in fast water may have a certain inherent threshold for water current (Hynes 1970). In very fast water, the current is fastest just below the water surface and slowest along the bottom (Leopold 1953) while a boundary layer is formed along the middle water column (Payne 1986). Usually, *Thalerosphyrus* (and other heptageniids) remains attached to rocky substrate in the area of slower current below the boundary layer (Voshell & Reese 2002). When the current rose to a level above its threshold limit, *Thalerosphyrus* was negatively affected by the main thrust of the strong current. Furthermore, many mayfly taxa can survive in relatively lower-oxygen environments in slow flowing water (Kamsia *et al.* 2008) as they are equipped with external gills.

The water pH of streams in both catchments was slightly acidic and different associations of the ephemeropterans with pH were observed in different catchments. In GJ streams, the ephemeropterans were positively influenced by elevated water pH but a negative association was observed in RBSP streams. This different response of ephemeropterans was likely related to the variations of pH in all streams. In GJ streams, the pH ranged from 4.98 to 6.02 while in RBSP it varied very little (5.25 - 5.68) suggesting that the most suitable pH for ephemeropteran taxa in these two catchments was slightly above 5.0. Teroi River in particular was the most acidic and we strongly suspected that natural acidification had taken place in this River. The water in this stream was brown in colour due to resin (containing tannin) introduced by dense growth of *Agathis alba* (Dipterocarpaceae) and associated humic acid produced during litter decomposition had made the water slightly more acidic (Nimptsch & Pflugmacher 2008). Among GJ streams, *Baetis* spp., was exceptionally plenteous in this stream but taxa richness was the lowest (8 genera) partially implying less suitable environmental condition for some taxa compared to other streams. According to Dangles & Guerold (2000) and Gerhard *et al.* (2005), some ephemeropterans survive well in acidic environments and Dangles *et al.* (2004) observed no difference in taxonomic richness of aquatic invertebrates between a naturally acidified stream and circum-neutral controlled streams. On the contrary, Guerold *et al.* (2000) reported that mayflies were totally absent in some acidic (< pH 4.0) streams.

Conclusions

We conclude that the ephemeropteran community structures in GJ and RBSP catchments were more influenced by physical and chemical conditions of stream environments than catchment characteristics. However, higher diversity of ephemeropterans was recorded in undisturbed RBSP that provided better habitats for mayfly community. Different responses towards stream physico-chemical parameters were observed among various ephemeropteran taxa in the two catchments. This study provides the information on the responses of mayfly communities in GJ and RBSP streams towards water physico-chemical parameters which is useful for preservation of suitable habitats in undisturbed and potentially disturbed forest streams and for biomonitoring of the ephemeropterans.

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