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1 Title: Macroinvertebrate trophic structure of	n <mark>waterfalls</mark>	in Borneo
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- 3 Short title: Macroinvertebrate trophic structure on Waterfalls

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39	Abstract
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41	Waterfalls have unique physical characteristics and harbour highly specialized
42	macroinvertebrate communities, but have been the subject of very few ecological
43	studies. There are no previous studies of trophic structure of waterfall assemblages. It
44	was hypothesized that due to the steep gradient of waterfalls and low retention of
45	terrestrial based resources, the abundant basal food resources would be periphyton. In
46	addition, due to the frequent scouring flood events, it was predicted that periphyton
47	would also be a significant source of food for filter feeders. Waterfalls in the Ulu
48	Temburong National Park in Brunei Darussalam (northern Borneo) were used as this
49	case study. Methods included stable isotope analysis (SIA; $\delta^{13}C$ and $\delta^{15}N$ of leaf litter
50	and periphyton) and gut contents analysis (GCA) of the most the abundant
51	macroinvertebrates. δ^{15} N values ranging from -1.9‰ to 5.5‰, literature suggests that
52	this indicates there were herbivores (Heptageniidae and Blephariceridae), omnivores
53	(Simuliidae and Hydropsychidae) and predators (Buccinidae) living on the waterfalls.
54	Apart from Buccinidae, all the taxa had δ^{13} C signatures ranging from -33‰ to -26‰,
55	with a high dependence on periphyton, which is similar to other tropical stream
56	biotopes. This study does suggest that despite the scouring velocities, waterfalls
57	support animals with a range of diets, based on grazing/scraping, filter feeding and
58	predation.
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Introduction

Waterfalls have received limited attention with very few published papers to 69 70 date (Rackemann et al. 2013; Clayton and Pearson, 2016). Research conducted on waterfalls in the tropics have generally focused on them acting as a longitudinal 71 barrier for fish movement, creating an upstream refuge for taxa including shrimp and 72 73 tadpoles (Baker et al. 2016; Covich et al. 2009; Hein and Crowl, 2010; Torrente-Vilara et al. 2011; Kano et al. 2012), with very little information on the waterfall 74 75 assemblages themselves (Clayton and Pearson, 2016; Rackemann *et al.* 2013 are 76 exceptions). This lack of research may be explained by several factors including waterfalls being perceived to be biological dead zones, too complex to sample 77 effectively and being inherently dangerous environments due to fast flows and sheer 78 79 drops (Rackemann et al. 2013).

Waterfalls are defined by their bedrock substrate and fast flows (Newson and 80 81 Newson, 2000) and are unique compared to other biotopes because of their sheer shallow flows and lack of connectivity to the hyporheic zones. In addition, these 82 83 biotopes are formed and maintained by erosive processes that occur over geologic 84 periods creating stable conditions, which are relatively uniform across a range of discharges (Clayton and Pearson, 2016). In comparison, other biotopes such as pools 85 and riffles change daily, frequently re-shaped by erosion and deposition events, 86 87 whereas waterfalls are more stable (Clayton and Pearson, 2016).

88 Although waterfalls have stable substrate, resident taxa have to be specialized 89 to live in an environment with relatively fast velocities and no hyporheic zone 90 (Clayton and Pearson, 2016; Hart and Finelli, 1999). Animals need morphological adaptations, including hooks, suckers and modified legs, to enable them to move 91 around the rock without being washed away or to fit into cracks and crevices (Clayton 92 and Pearson, 2016). For example, some odonates in a Malaysian stream inhabited 93 trailing plants and accumulated debris irrespective of water velocity, assisted by their 94 body spines, which aided attachment (Furtado 1969). In addition to the high velocity 95 areas of the waterfalls, there are some benign zones including the waterfall spray, 96 these patches are usually colonised by snails. 97

Taxa adapted to living on waterfalls benefit from the absence of fish and 98 shrimp, which have been found to be important in determining insect assemblage 99 100 structure (March et al. 2002; March and Pringle, 2003; Ramirez and Hernandez-Cruz, 101 2004). In streams, fish and shrimp typically impact insects via predation and 102 competition for food resources, breaking up and consuming leaf litter and grazing periphyton (Pringle and Hamazaki, 1997; Crowl et al. 2001; Flecker and Taylor, 103 104 2004; Ramirez and Hernandez-Cruz, 2004; Coughlan et al. 2010). In addition, fish and shrimp can occur in high densities, creating competition for space with 105 106 invertebrates. Waterfalls are therefore unique biotopes for invertebrates as they can reside on the substrate with minimal top-down ecological disturbance. It is therefore 107 predicted that the difference in community structure on waterfalls, will create a 108 distinctive food web compared to other tropical biotopes (e.g. Brito *et al.* 2006; Li and 109

110 Dudgeon, 2008; Coat *et al.* 2009).

Food web studies using stable isotope analysis in tropical streams have focused on pool and riffle biotopes dominated by shrimp and fish (for example see: Brito *et al.* 2006; Li and Dudgeon, 2008; Coat *et al.* 2009). Some of these studies have found invertebrates in the tropics to depend more on algal based food compared 115 to leaf litter (Brito et al. 2006; Lau et al. 2009). Even in shaded reaches, some tropical food webs studies (e.g. in the Neotropics) have been found to be mainly algae based 116 (March and Pringle, 2003; Brito et al. 2006). Gaps in the canopy often occur near to 117 waterfall, including upstream, below and along the waterfall, as a result of the high 118 rates of erosion below the waterfalls, which create wide plunge pools (Odland et al. 119 1991). This canopy gap enables more light to reach the waterfall substrate promoting 120 121 periphyton growth. In contrast, standing stocks of benthic organic matter tend to be low on waterfalls because of fast flows, channel gradient and the lack of retentive 122 123 structure. Although waterfalls are discrete units they are not separated from the river continuum with resident filter feeders, such as Simuliidae and Hydropsychidae, 124 dependent on seston and organic matter from upstream. However, the strength of 125 126 upstream- downstream linkages have been debated with Huryn et al. (2002) suggesting that local factors are still more of a determinant of trophic resources. 127

This study examined macroinvertebrate trophic structure on waterfalls in Ulu 128 129 Temburong Park, which has extensive pristine tropical rainforest with free flowing rivers (Sheldon, 2011). The study combines complementary techniques of gut 130 131 contents analysis (GCA) and stable isotope analysis (SIA), to enable a more complete characterization of waterfall trophic structure. SIA is useful for tracing energy flows 132 133 through food webs but gives poor taxonomic resolution (Fry, 2007; Grey et al. 2001). 134 In contrast, GCA can provide higher taxonomic resolution but provides only a snapshot of ingestion rather than assimilation (Layer et al. 2013). It was hypothesized 135 that due to the steep gradient of waterfalls and low retention of terrestrial based 136 137 resources, the predominant basal food resources of macroinvertebrates would be periphyton. In addition, due to the frequent scouring flood events, it was predicted 138 that periphyton would also be a significant source of food for filter feeders. 139

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141	Methods
142	Study Sites
143	Research was conducted at the Kuala Belalong Field Studies Centre (KBFSC)
144	of UBD (Universiti Brunei Darussalam) in Ulu Temburong National Park in Brunei
145	Darussalam. The landscape of Ulu Temburong National Park (550 km ²) changes over
146	a relatively short distance (< 35 km) from steep mountainous reaches (913 m a.s.l. at
147	Bukit Belalong) to placid lowlands (KBFSC 30 m a.s.l.) (Dykes, 1994). This creates a
148	geomorphic template for many waterfalls and cascades, therefore a suitable region for
149	this study (Fig. 1).
150	
151	Brunei is in the tropics and weakly influenced by the South East Asian
152	monsoon; however, weather in Ulu Temburong is highly variable as a result of
153	localised storms (Dykes, 1996). Sampling took place in June and July (2014) when
154	rainfall and stream discharges are generally low making access to the waterfalls safe
155	(Cranbrook and Edwards, 1994). Individual waterfalls on twelve tributaries of Sungai
156	Temburong or Sungai Belalong were investigated (Fig. 1). Waterfalls selected to be
157	sampled were the first upstream after the confluence with the main rivers.
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159	Geomorphic Measurements and Functional Habitats
160	We measured width and length of the waterfalls, except where waterfalls were very

steep, in which case their length was estimated. The presence and distribution of 161 functional habitats (e.g. moss, leaf litter, wood debris) were recorded (for an 162 explanation of functional habitats see Harper et al. 1995). Descriptions of substrate 163

- heterogeneity and photos of the waterfalls were taken to enable qualitative image
 analysis of waterfall substrate complexity.
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167 *Macroinvertebrate trophic structure*

- The common and widely distributed taxa on the waterfalls, including Heptageniidae, 168 Blephariceridae, Simuliidae, Hydropsychidae, and Buccinidae, were picked directly 169 off the waterfall substrate, either by hand or with forceps. Sampling was conducted 170 over the length of the waterfall, except on the larger waterfalls where it was unsafe to 171 sample in the middle. Trophic structure was then evaluated using gut contents 172 analysis (GCA) and stable isotope analysis (SIA). Species richness within these 173 174 families in streams surrounding KBFSC is uncertain. However, to date, three genera 175 of Heptageniidae (Compsoneuria, Thalerosphyrus and Afronurus), one genus of Blephariceridae (Blepharicera), six genera of Hydropsychidae (Hydromanicus, 176 Macrostemum, Hydrospyche, Cheumatopsyche, Potamyia and Polymorphanisus) and 177 178 one genus of Buccinidae (*Clea*) have been recorded (Baker et al., in press). To date Clea is the only freshwater genus from the Buccinidae family. The level of diversity 179 within Simuliidae is unknown in the streams of Ulu Temburong National Park. 180
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Gut Contents Analysis: Gut contents analysis (GCA) has been used frequently to investigate benthic macroinvertebrate diet (Rosi-Marshall and Wallace, 2002; Li and Dudgeon, 2008). Due to the remote location, only three individuals of each of the taxon were collected for GCA. Macroinvertebrates were preserved in 70% ethanol. In the laboratory, foreguts were removed from the individuals under a dissecting microscope and the contents mounted in Euparal on a microscope slide (Layer *et al.* 2012). The contents were identified at 400-1,000x magnification and separated into five categories: coarse particulate organic matter (CPOM) including leaf litter and wood, algae (diatoms, green alga), fungal hyphae and conidia, animal tissue and amorphous detritus identified by the lack of any defined cellular structure; from Yule *et al.* (2010). Percentage of gut contents was then estimated by eye.

Stable Isotope Analysis: Stable isotopes have been widely used to evaluate energy 193 flow and trophic structure in a range of food webs (Peterson and Fry, 1987). Stable 194 carbon and nitrogen isotope ratios are a useful tool to calculate what has been 195 assimilated by animals and to determine the relative importance of basal food 196 197 resources in the food web. Carbon isotope ratios are effective in distinguishing between autochthonous (aquatic) and allochthonous (terrestrial) food sources, with 198 the former having higher δ^{13} C values (Fry, 1991; Hershey and Peterson, 1996). 199 200 Nitrogen isotopes have been used to provide information about trophic levels, with δ^{15} N increasing with each trophic level. 201

Invertebrates were collected for stable isotope analyses using the same 202 203 methods as gut contents analysis (see above). From 3-50 individuals per taxon were selected to ensure enough sample material for analysis. All taxa were left in stream 204 205 water for 12 hours to promote gut clearance. Putative food sources (leaf litter and periphyton rock scrubs) were also taken at each waterfall. All samples were dried at 206 207 70 degrees Celsius for a 24hour period before being transported back to the UK. 208 Samples were weighed to approximately 0.5mg and loaded into 5x7mm tin capsules. Leaf litter was ground into a fine powder. Initial analysis found that three replicates 209 were sufficient for macroinvertebrates and leaf litter, and due to the increased 210 variability, five replicates were required for the periphyton. 211

212 Measurements were carried out at the NERC Life Sciences Mass 213 Spectrometry Facility (LSMSF) in East Kilbride, using an Elementar Pyrocube 214 elemental analyser coupled with a Thermo Fisher Scientific Delta Plus XP mass spectrometer. Laboratory standards Fluka gelatin, Sigma alanine and Sigma glycine 215 (Sigma-Aldrich Company Ltd, Gillingham, UK) were repeated with every 10 samples 216 217 and were used to correct for linearity and instrument drift over a 22 hour analytical run. The isotope ratios for the lab standards were determined relative to a range of 218 International standards from IAEA (Vienna, Austria) and USGS (Reston, VA, USA). 219 Isotope ratios are expressed in the δ (delta) notation in parts per thousand (‰): $\delta X =$ 220 $[(R_{sample}/R_{standard})-1]$ where $X = {}^{15}N$ or ${}^{13}C$ and R = the ratio of ${}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$ 221 222 isotopes in a given sample relative to AIR for nitrogen and PDB for carbon. The analytical precision for carbon and nitrogen isotope measurements was better than 223 224 0.3‰.

225 Data Analysis: Analysis of variance (ANOVA) was used to assess differences in stable isotope compositions for food sources and consumers. It was also used to 226 assess differences among all the waterfalls. Tukey Post Hoc test were used after the 227 228 ANOVA to examine specific differences (Thomas et al. 2013). The statistical computing environment R (R Core Team 2013) was used to conduct the ANOVAs. 229 The focus of the study was to examine allochthonous (leaf litter) and autochthonous 230 (periphyton) food sources on the waterfalls. As not all food sources were sampled 231 232 (e.g. moss, seston, fungi), a mixing model was not applied to the data.

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Results

236 Geomorphic Measurements and Functional Habitats

237 The twelve waterfalls had a range of average slope angles, from 16° (waterfall
238 11) to 53° (waterfall 1) and lengths of the waterfalls were from 4.5m (waterfall 11) to

239 20.4m (waterfall 8-Table 1). All submerged rock surfaces were covered in periphyton with at least a few leaves present. Half of the waterfalls had wood debris, 240 which varied in size from twigs to large tree trunks. At waterfalls 1, 4 and 7, large tree 241 trunks became lodged in between the bedrock and the narrow bankful widths. Our 242 observations in the field and subsequent qualitative image analysis indicated that 243 waterfalls varied in substrate complexity. Some of the waterfalls consisted of smooth 244 245 rock while others were more complex with a mix of ridges (millimetres to centimetres), ledges and undercutting. 246

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248 *Macroinvertebrate trophic structure*

The sampled macroinvertebrates included Simuliidae, Hydropsychidae,
Blephariceridae, Heptageniidae and Buccinidae. At waterfalls 8 and 11 there were no
Blephariceridae and no Buccinidae at waterfall 8.

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Gut Contents Analysis; Amorphous detritus was most abundant material in 253 macroinvertebrate guts, ranging from $\sim 45\%$ to $\sim 100\%$ (Fig. 2). CPOM was the next 254 most abundant, ranging from 0 to 30% while algae varied from 0 to ~20% (Fig. 2). 255 Animal tissue varied from 0 to ~10% and the least abundant matter in the 256 macroinvertebrate guts was fungal hyphae and conidia varying from 0 to 5% (Fig. 2). 257 258 Blephariceridae contained 100% amorphous detritus. Simuliidae contained a high percentage of amorphous detritus (~95%), but in addition included CPOM, algae, and 259 in one individual an insect head (Waterfall 9; Fig. 2). Heptageniidae contained a high 260 261 percentage of amorphous detritus, but also contained algae and fungal hyphae and conidia (Fig. 2). Hydropsychidae had a more even mix of all observed food types 262 (Fig. 2). 263

Stable isotopic composition of food sources: Periphyton had less depleted ¹³C values 265 $(\delta^{13}C \text{ periphyton} = -30.06 \pm 2.54 \text{ }\%)$ compared to leaf litter ($\delta^{13}C$ leaf litter = -32.97 ± 266 1.58 ‰), making it possible to distinguish between these basal food resources (one-267 way ANOVA, F_{1, 83}=37.7, p = <0.01; Table 2; Appendix 1). The $\delta^{15}N$ (‰) values for 268 periphyton $(1.29 \pm 0.78 \text{ }\%)$ and leaf litter (-0.91 $\pm 0.99 \text{ }\%)$ were also distinct (one-269 way ANOVA, $F_{1, 83}=142.6$, p=<0.01), with periphyton having significantly higher 270 δ^{15} N values than leaf litter. The C: N values were higher for the leaf litter (49.27) than 271 for periphyton (5.37). The lower ratio indicates a higher proportion of nitrogen in the 272 sample suggesting higher protein digestibility and therefore higher nutritional value to 273 274 the consumers (Giller and Malmqvist, 1998; Table 2).

Stable isotopic compositions of consumers: The δ^{13} C values for Heptageniidae at the 275 12 waterfalls varied from -35.51 % to -26.83 %, while Blephariceridae ranged from -276 35.68 ‰ to -27.41 ‰ (Table 2; Fig. 3; Appendix 1). Simuliidae varied from -29.29 ‰ 277 to -27.33 ‰ and Hydropsychidae δ^{13} C values ranged from -31.12 ‰ to -27.2‰. 278 Buccinidae ranged from -28.03 % to -24.04 % (Table 2; Fig. 3). There was a 279 statistical difference between the δ^{13} C values of all consumers (one-way ANOVA, F₄) 280 $_{151}=55.5$, p<0.01), but the Tukey post hoc test showed no difference among 281 Heptageniidae and Blephariceridae. Mean δ^{15} N values of Heptageniidae ranged from 282 a -0.56 ‰ to 2.51 ‰, Blephariceridae varied from 0.51 ‰ to 2.64 ‰ and Simuliidae 283 varied from 1.65 ‰ to 3.04 ‰ (Table 2). Hydropsychidae ranged from 2.26 ‰ to 284 4.50 ‰ and Buccinidae ranged from 4.12 ‰ to 6.69 ‰ (Table 2). There was a 285 statistical difference between the consumers in $\delta^{15}N$ values (one-way ANOVA, F₄ 286 $_{151}=275.7$, p=<0.01). However, the Tukey post hoc test showed no difference in $\delta^{15}N$ 287

values between Heptageniidae and Blephariceridae (p=0.34), and between Simuliidae
and Hydropsychidae (p=0.8).

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291 Potential food sources of consumers using GCA and SIA: Most Heptageniidae had δ^{13} C values that corresponded to periphyton (Table 2; Fig. 3). However, at waterfall 292 7. Heptageniidae had a δ^{13} C value of approximately -27 ‰ and at waterfall 10 the 293 δ^{13} C values were very depleted at approximately -35 ‰ (Table 2; Fig. 3). GCA found 294 Heptageniidae consumed CPOM, algae and, fungal hyphae and conidia, the latter 295 food source may explain the 'unknown' $\delta^{13}C$ values. At most waterfalls 296 Blephariceridae matched periphyton and leaf litter; however, at waterfall 10 δ^{13} C 297 values were depleted with δ^{13} C values less than -35 ‰. Due to the small size of the 298 Blephariceridae, the GCA could not be used to show any further resolution, with gut 299 material classed as amorphous detritus. Generally Simuliidae δ^{13} C values matched 300 periphyton, although, there were some Simuliidae at waterfall 11 that had δ^{13} C value 301 of approximately -27 ‰, values that are on the edge of the periphyton δ^{13} C (Fig. 3). 302 The GCA did not provide any further resolution (Fig. 2). Hydropsychidae δ^{13} C values 303 were similar to periphyton, along with another food source with $\delta^{13}C$ of 304 approximately -27 ‰ at waterfall 8 and 11. GCA of Hydropsychidae contained 305 animal tissue, fungal hyphae and conidia, CPOM and algae. Buccinidae δ^{13} C values 306 did not match periphyton or leaf litter apart from at waterfall 9 where δ^{13} C values 307 corresponded to periphyton. Buccinidae gut contents was amorphous therefore not 308 possible to differentiate food types. 309

Range of δ^{15} N values indicate that macroinvertebrates on the 12 waterfalls fed at different trophic levels (Fig. 4). Heptageniidae had the most depleted ¹⁵N with a value of 1.06 ± 0.77 ‰ and Blephariceridae had a δ^{15} N value of 1.50 ± 0.68 ‰ (Table 2;

Fig. 4). Simuliidae and Hydropsychidae $\delta^{15}N$ values were higher, Simuliidae had 313 average δ^{15} N value of 2.16 ± 0.36 ‰ and Hydropsychidae δ^{15} N value was 3.07 ± 314 0.46‰ (Table 2; Fig. 3). This indicates that some of their diet was composed of 315 animal tissue. GCA showed that animal tissue was present in the stomach contents of 316 Simuliidae, although only at waterfall 9. Hydropsychidae had animal tissue in gut 317 contents for half of the waterfalls including 2, 3, 7, 8, 9 and 12. Whilst Buccinidae 318 was distinctly predacious, having the most enriched ¹⁵N with values of 5.48 ± 0.70 ‰ 319 (Table 2; Fig. 3). These results suggest that there were at least two trophic levels, with 320 321 Heptageniidae and Blephariceridae as primary consumers and Buccinidae as a secondary consumer. Simuliidae and Hydropsychidae were in the middle, with some 322 323 taxa primary consumers and others secondary (Fig. 4).

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Discussion

There are few ecological studies of waterfalls (Rackemann et al. 2013; 326 327 Clayton and Pearson, 2016) and we are unaware of any published papers on waterfall food webs. Waterfalls are unique biotopes, lacking a hyporheic zone and a water 328 column of sufficient depth for most fish. Therefore the normally abundant 329 herbivorous fish and shrimp are absent, leaving highly specialized macroinvertebrates 330 to dominate the waterfalls (Clayton and Pearson, 2016; Hart and Finelli, 1999). This 331 study, using SIA and GCA, has established a trophic structure for waterfalls in 332 pristine catchments in northern Borneo showing there are at least two, and potentially 333 three, trophic levels -detritivore/herbivore (Heptageniidae and Blephariceridae), 334 335 omnivore (Simuliidae and Hydropsychidae) and predator (Buccinidae). There was some variation in carbon and nitrogen isotopic values of macroinvertebrates among 336 the waterfalls, which is likely linked to variations in functional habitats including both 337

quantity and quality. Further research relating functional habitats and waterfall
geomorphology to the trophic ecology of waterfall resident taxa would be required to
elucidate these patterns.

The isotopic values (δ^{13} C and δ^{15} N) of basal food sources were distinct for 341 periphyton and leaf litter (Table 2), making it possible to use them as indictors to 342 distinguish between autochthonous and allochthonous basal food resources. The $\delta^{13}C$ 343 values for leaf litter averaged -32.97 ‰, lower compared to other values reported 344 from the tropics. For example, Huang *et al* (2007) recorded CPOM from streams in 345 Taiwan with an average of -30.3 ‰ and values from Hong Kong averaged -31.4 ‰ 346 (Mantel *et al.* 2004). This range in δ^{13} C values can be likely attributed to the high tree 347 diversity in tropical rainforests - for example in Ulu Temburong national park there 348 are approximately 250 species of trees (Cranbrook and Edwards 1994). 349

The average δ^{13} C value of periphyton was -30.06‰. This was more depleted 350 compared to other studies: -22.96‰ Puerto Rico (March and Pringle, 2003), -25.4‰ 351 Hong Kong (Lau et al. 2009b) and -21‰ Guadeloupe (Coat et al. 2009). However, 352 depleted δ^{13} C values of filamentous algae have been reported, such as -28.3% in 353 Hong Kong (Mantel *et al.* 2004). The difference in periphyton δ^{13} C values reported in 354 the literature compared to periphyton in our study could be a result of samples being 355 taken from pools and riffles, biotopes with different environmental conditions 356 compared to waterfalls. The δ^{13} C values of periphyton can be affected by several 357 factors including the availability of dissolved inorganic carbon, taxonomic 358 composition of the periphyton, water velocity, growth rate, and periphyton biomass 359 (Hill and Middleton, 2006). The average $\delta^{15}N$ for periphyton (-1.29 ‰) was higher 360 than in leaf litter (-0.91 %), which is not surprising as periphyton scrubs contain a 361

362 complex mix of bacteria, algae, fungi, protozoa and can include animal tissue, thus 363 increasing the δ^{15} N value (Wetzel, 2001; March and Pringle, 2003).

This study was conducted during a dry season when there are high rates of leaf 364 litter fall (Cranbrook and Edwards, 1994; Pendry and Proctor, 1996). Leaves were 365 therefore expected on all waterfalls during this study. However, during the rest of the 366 year when there is less leaf litter fall and faster river flows, it would be predicted there 367 would likely be less leaf litter. This study showed the importance of periphyton as a 368 food source. Waterfalls are often situated under a canopy gap due to the wide plunge 369 370 pools directly below the waterfall, which increase the channel width. Therefore, unlike other headwater tropical biotopes, waterfalls are less likely to be light limited. 371 372 In addition to leaf litter and periphyton food sources, some macroinvertebrate guts 373 contained fungal hyphae and conidia. It has long been acknowledged that microbial conditioning of leaf litter increases nutritional value and palatability for 374 macroinvertebrates (Petersen and Cummins, 1974). In the tropics bacteria and 375 376 fungi have been suggested to play a more important role in leaf litter decomposition due to the higher lignin content (Irons et al. 1994; Wallace et al. 1997; Boyero et al. 377 378 2015, 2016) and in the streams of Ulu Temburong it is not uncommon to see leaf litter covered in fungi. 379

Both Heptageniidae and Blephariceridae are defined as herbivorous grazers in the literature (Tonnoir 1930; Alverson *et al.* 2001; Ghee, 2004). However, in this study Blephariceridae had δ^{13} C values matching both periphyton and leaf litter. It is possible for the Blephariceridae to graze the periphyton and fungi that have colonized the leaf surface and as a consequence consume the leaf litter (Petersen and Cummins, 1974). The δ^{15} N values of Heptageniidae and Blephariceridae generally stayed under 2.5 ‰ implying these taxa are herbivorous (Table 2), with the GCA supporting theseresults.

In the literature Simuliidae and Hydropsychidae have been classed as filter 388 389 feeders (Cummins, 1973; Cummins and Klug 1979; Fig. 3), however, these functional feeding group classifications are based on taxa living in temperate streams and there 390 is a growing number of studies that suggest tropical taxa are more dependent on 391 periphyton than leaf litter or detritus (March and Pringle, 2003; Brito et al. 2006; 392 Lau et al. 2009a). For example, Brito et al. (2006) found Hydropsychidae in a 393 Brazilian stream with δ^{13} C values matching aquatic macrophytes and microalgae. The 394 low digestibility of tropical leaf litter as suggested by the C: N values in this study 395 may explain why periphyton is a more prevalent food source (Table 2; Benstead, 396 397 1996). Wooton (1977) argues that Simuliidae have been known to eat algae and will essentially ingest any particle from 0.5 µm to a maximum that depends on their mouth 398 size (Burton, 1973). In addition, it is possible the water column contains many 399 400 fragments of periphyton due to the frequent scouring flows. In Ulu Temburong National Park convective rainfall causes river and stream levels to fluctuate, this can 401 occur daily in the wet season, with the power of the water scouring out habitats 402 including periphyton (Sheldon, 2011). These consistent fast flows may have also 403 influenced Simuliidae feeding behaviour causing them to scrape. 404

405 The SIA results showed that Simuliidae and Hydropsychidae had higher $\delta^{15}N$ values compared to herbivorous grazers (Heptageniidae and Blephariceridae; Table 406 2). The δ^{15} N values of consumers tend to be 2-5 % higher than that of their diets 407 408 (Hobson and Clark, 1992; Bearhop et al. 2002), therefore our results suggest that Simuliidae Hydropsychidae waterfalls 409 and on the were omnivorous. Simuliidae have been known to consume Chironomidae (Serra-Tosio, 1967) and some 410

411 studies have regarded Hydropsychidae as omnivorous and even predacious (Fuller and Mackay, 1980; Allan and Castillo, 1995). The GCA support the SIA with animal 412 tissue present in the guts of both Simuliidae and Hydropsychidae. This study is the 413 first known stable isotope analysis on Buccinidae snail, with the literature mainly 414 focusing on its taxonomy and geographic distribution (Polgar et al. 2015). Our results 415 show that the snail had little overlap with δ^{13} C values for leaf litter or periphyton 416 (Table 2) and the δ^{15} N values confirm reports in the literature that Buccinidae is 417 predacious (Coelho et al. 2013). 418

Some taxa showed high variability of δ^{13} C and δ^{15} N values (Table 2). This 419 variability could be due to the coarse level of identification to family level. 420 421 Identification to genus or species level may yield greater information, with species 422 potentially having different life histories and food preferences. However, this level of 423 resolution is difficult in Borneo with macroinvertebrates still mostly undescribed (Baker et al. In press; Jacobsen et al. 2008; Yule and Yong, 2004). Variability in 424 425 dietary composition could also be a result of substrate complexity along with food availability and quantity, which was not formally investigated. In our study the 426 waterfalls' geomorphic complexity varied from smooth bedrock to substrate covered 427 in numerous ridges and holes promoting the formation of a range of functional 428 habitats defined by a mix of leaf litter, wood debris, moss and periphyton. Although 429 430 these functional habitats were not quantitatively recorded in this study, visual observations found higher levels of wood debris and leaf litter on some waterfalls, 431 particularly ones with complex substrate. Waterfall complexity may also influence 432 abundance of different taxa on the waterfall and therefore number of prey for the 433 predators, for example Buccinidae δ^{15} N value was lowest (4.35 ‰) at waterfall 9, 434 which had smooth substrate. While, the highest level of $\delta^{15}N$ (6.63 ‰) was at 435

waterfall 1 which had heterogeneous substrate. However, further studies are required
to investigate if these changes in waterfall complexity influence macroinvertebrate
feeding behaviour and trophic level.

While the food web is mostly resolved, it appears there are likely some 439 unknown food sources. The first was a source with more negative δ^{13} C values (less 440 441 than -33‰) that matched Blephariceridae and Heptageniidae at waterfall 10 (Fig. 3). Reviewing other tropical isotopic studies (including March and Pringle, 2003; Brito et 442 al. 2006; Coat et al. 2009; Lau et al. 2009b) few food sources matched such low δ^{13} C 443 values. However, Huang et al. (2007) did sample red algae, which had depleted ¹³C 444 with values around -37.4‰. Although this does not exactly match the δ^{13} C values of 445 446 Blephariceridae and Heptageniidae it could suggest that the waterfalls do harbor some types of algae with very depleted δ^{13} C values. The second 'unknown' food source had 447 a δ^{13} C value of -27‰ and matched the δ^{13} C value for Heptageniidae at waterfall 7, 448 Simuliidae at waterfall 11 and Hydropsychidae at waterfall 8 and 11. These values 449 match values of dissolved organic matter and Fine Particulate organic matter (FPOM) 450 from other studies, such as 28.6% on the Island of Guadeloupe (Coat *et al.* 2009) and 451 in a Brazilian stream FPOM had a mean δ^{13} C value of ~29‰ (Brito *et al.* 2006). 452

453

454 Conclusions

455 Macroinvertebrate communities living on tropical waterfalls are significantly 456 under researched, with this study being the first paper to examine their trophic 457 structure. Results from both SIA and GCA show two, and potentially three, trophic 458 levels occurring including the herbivore (Heptageniidae and Blephariceridae), 459 omnivore (Simuliidae and Hydropsychidae) and predator (Buccinidae). In agreement 460 with some tropical trophic structure studies on pools and riffles, the basal food resource on the waterfalls were found to be predominantly algae based. However, this study does suggest that despite the scouring velocities, waterfalls can retain functional habitats, with leaf litter being assimilated. The degree of functional habitat retention appears to be dependent on substrate heterogeneity and complexity. Quantitative analysis of substrate complexity is necessary to understand if the macroinvertebrate isotopic variation is related to waterfall complexity. In addition, further research is required to identify the unknown food sources.

468

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638	
639	Tables
640	

Table 1. Physical measurements of waterfalls including average slope angle and

642 length. Presence (1) vs. absence (0) of functional habitats (leaf litter, wood debris and

643 moss).

644

Waterfall	Slope angle	Length (m)	Leaf litter	Wood Debris	Moss
	(°)	()		200115	
1	53	5.7	1	1	0
2	26	4.93	1	0	0
3	20	6.16	1	0	0
4	50	15	1	1	1
5	47	7.2	1	0	1
6	39	7	1	0	1
7	47	~6	1	1	1
8	35	20.4	1	0	0
9	27	~13	1	0	0
10	16	9.13	1	0	0
11	40	4.47	1	0	1
12	48	16.2	1	1	0

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647

Table 2. Average carbon and nitrogen isotopic values of basal food sources and

649 consumers (mean \pm SD).

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651

	п	C:N	δ13C (‰): Mean <u>+</u> SD	δ15N (‰): Mean <u>+</u> SD
Basal food sources				
Leaf litter	36	49.27	-32.97 + 0.26	-0.91 + 0.16
Periphyton	57	5.37	-30.06 + 0.34	1.29 + 0.10

Consumers				
Heptageniidae	36	4.56	-29.47 + 0.33	1.06 + 0.13
Blephariceridae	30	4.50	-31.45 + 0.42	1.50 + 0.12
Simuliidae	36	4.30	-28.14 + 0.09	2.16 + 0.06
Hydropsychidae	36	4.53	-28.58 + 0.14	3.07 + 0.08
Buccinidae	33	3.92	-26.18 + 0.16	5.48 + 0.12

652	Figure	captions
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653 654

Fig. 1. Map showing the location of Brunei Darussalam in northern Borneo (A).

Brunei Darussalam is split into two parts with this study being conducted in the

- 657 Temburong district, the eastern section of Brunei (B). The twelve waterfalls are
- situated on tributaries of the two main rivers Sungai Belalong and Sungai Temburong
- 659 (C) near to Kuala Belalong Field Study Centre (KBFSC)
- 660

Fig. 2. Average percent contributions of stomach contents including amorphous
detritus, animal tissue, CPOM, algae and fungal hyphae and conidia in the five
macroinvertebrate families - Hydropsychidae, Heptageniidae, Blephariceridae and
Simuliidae.

665

Fig. 3. Mean δ 13 C and δ 15 N values of basal resources leaf litter (LL) and

667 periphyton (P) for the abundant macroinvertebrates found on the waterfalls in Ulu

668 Temburong National Park. Macroinvertebrates include Buccinidae, Blephariceridae,

669 Heptageniidae, Hydropsychidae and Simuliidae. Error bars indicate ± 1 SD.

670

Fig. 4. Food web of the abundant macroinvertebrates present on waterfalls in Ulu

672 Temburong National Park. Based on stomach contents analysis and stable isotope

analysis. Line thickness represents contributions of the sources.