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1 **Title:** Macroinvertebrate trophic structure on waterfalls in Borneo

2

3 **Short title:** Macroinvertebrate trophic structure on Waterfalls

4

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Abstract

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

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Introduction

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

79 Waterfalls are defined by their bedrock substrate and fast flows (Newson and
80 Newson, 2000) and are unique compared to other biotopes because of their sheer
81 shallow flows and lack of connectivity to the hyporheic zones. In addition, these
82 biotopes are formed and maintained by erosive processes that occur over geologic
83 periods creating stable conditions, which are relatively uniform across a range of
84 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 whereas waterfalls are more stable (Clayton and Pearson, 2016).

87 Although waterfalls have stable substrate, resident taxa have to be specialized
88 to live in an environment with relatively fast velocities and no hyporheic zone (Clayton
89 and Pearson, 2016; Hart and Finelli, 1999). **Animals need morphological adaptations,**

90 including hooks, suckers and modified legs, to enable them to move around the rock
91 without being washed away or to fit into cracks and crevices (Clayton and Pearson,
92 2016). For example, some odonates in a Malaysian stream inhabited trailing plants and
93 accumulated debris irrespective of water velocity, assisted by their body spines, which
94 aided attachment (Furtado 1969). In addition to the high velocity areas of the waterfalls,
95 there are some benign zones including the waterfall spray, these patches are usually
96 colonised by snails.

97 Taxa adapted to living on waterfalls benefit from the absence of fish and shrimp,
98 which have been found to be important in determining insect assemblage structure
99 (March *et al.* 2002; March and Pringle, 2003; Ramirez and Hernandez-Cruz, 2004). In
100 streams, fish and shrimp typically impact insects via predation and competition for food
101 resources, breaking up and consuming leaf litter and grazing periphyton (Pringle and
102 Hamazaki, 1997; Crowl *et al.* 2001; Flecker and Taylor, 2004; Ramirez and Hernandez-
103 Cruz, 2004; Coughlan *et al.* 2010). In addition, fish and shrimp can occur in high
104 densities, creating competition for space with invertebrates. Waterfalls are therefore
105 unique biotopes for invertebrates as they can reside on the substrate with minimal top-
106 down ecological disturbance. It is therefore predicted that the difference in community
107 structure on waterfalls, will create a distinctive food web compared to other tropical
108 biotopes (e.g. Brito *et al.* 2006; Li and Dudgeon, 2008; Coat *et al.* 2009).

109 Food web studies using stable isotope analysis in tropical streams have focused
110 on pool and riffle biotopes dominated by shrimp and fish (for example see: Brito *et al.*
111 2006; Li and Dudgeon, 2008; Coat *et al.* 2009). Some of these studies have found
112 invertebrates in the tropics to depend more on algal based food compared to leaf litter
113 (Brito *et al.* 2006; Lau *et al.* 2009). Even in shaded reaches, some tropical food webs
114 studies (e.g. in the Neotropics) have been found to be mainly algae based (March and

115 Pringle, 2003; Brito *et al.* 2006). Gaps in the canopy often occur near to waterfall,
116 including upstream, below and along the waterfall, as a result of the high rates of
117 erosion below the waterfalls, which create wide plunge pools (Odland *et al.* 1991). This
118 canopy gap enables more light to reach the waterfall substrate promoting periphyton
119 growth. In contrast, standing stocks of benthic organic matter tend to be low on
120 waterfalls because of fast flows, channel gradient and the lack of retentive structure.
121 Although waterfalls are discrete units they are not separated from the river continuum
122 with resident filter feeders, such as Simuliidae and Hydropsychidae, dependent on
123 seston and organic matter from upstream. However, the strength of upstream-
124 downstream linkages have been debated with Huryn *et al.* (2002) suggesting that local
125 factors are still more of a determinant of trophic resources.

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

138

139

Methods

140 *Study Sites*

141 Research was conducted at the Kuala Belalong Field Studies Centre (KBFS
142 of UBD (Universiti Brunei Darussalam) in Ulu Temburong National Park in Brunei
143 Darussalam. The landscape of Ulu Temburong National Park (550 km²) changes over
144 a relatively short distance (< 35 km) from steep mountainous reaches (913 m a.s.l. at
145 Bukit Belalong) to placid lowlands (KBFS 30 m a.s.l.) (Dykes, 1994). This creates a
146 geomorphic template for many waterfalls and cascades, therefore a suitable region for
147 this study (Fig. 1).

148

149 Brunei is in the tropics and weakly influenced by the South East Asian
150 monsoon; however, weather in Ulu Temburong is highly variable as a result of localised
151 storms (Dykes, 1996). Sampling took place in June and July (2014) when rainfall and
152 stream discharges are generally low making access to the waterfalls safe (Cranbrook
153 and Edwards, 1994). Individual waterfalls on twelve tributaries of Sungai Temburong
154 or Sungai Belalong were investigated (Fig. 1). Waterfalls selected to be sampled were
155 the first upstream after the confluence with the main rivers.

156

157 *Geomorphic Measurements and Functional Habitats*

158 We measured width and length of the waterfalls, except where waterfalls were very
159 steep, in which case their length was estimated. The presence and distribution of
160 functional habitats (e.g. moss, leaf litter, wood debris) were recorded (for an
161 explanation of functional habitats see Harper *et al.* 1995). Descriptions of substrate
162 heterogeneity and photos of the waterfalls were taken to enable qualitative image
163 analysis of waterfall substrate complexity.

164

165 *Macroinvertebrate trophic structure*

166 The common and widely distributed taxa on the waterfalls, including Heptageniidae,
167 Blephariceridae, Simuliidae, Hydropsychidae, and Buccinidae, were picked directly off
168 the waterfall substrate, either by hand or with forceps. Sampling was conducted over
169 the length of the waterfall, except on the larger waterfalls where it was unsafe to sample
170 in the middle. Trophic structure was then evaluated using gut contents analysis (GCA)
171 and stable isotope analysis (SIA). Species richness within these families in streams
172 surrounding KBFSC is uncertain. However, to date, three genera of Heptageniidae
173 (*Componeuria*, *Thalerosphyrus* and *Afronurus*), one genus of Blephariceridae
174 (*Blepharicera*), six genera of Hydropsychidae (*Hydromanicus*, *Macrostemum*,
175 *Hydropsyche*, *Cheumatopsyche*, *Potamyia* and *Polymorphanisus*) and one genus of
176 Buccinidae (*Clea*) have been recorded (Baker et al., in press). To date *Clea* is the only
177 freshwater genus from the Buccinidae family. The level of diversity within Simuliidae
178 is unknown in the streams of Ulu Temburong National Park.

179
180 *Gut Contents Analysis:* Gut contents analysis (GCA) has been used frequently to
181 investigate benthic macroinvertebrate diet (Rosi-Marshall and Wallace, 2002; Li and
182 Dudgeon, 2008). Due to the remote location, only three individuals of each of the taxon
183 were collected for GCA. Macroinvertebrates were preserved in 70% ethanol. In the
184 laboratory, foreguts were removed from the individuals under a dissecting microscope
185 and the contents mounted in Euparal on a microscope slide (Layer *et al.* 2012). The
186 contents were identified at 400-1,000x magnification and separated into five categories:
187 coarse particulate organic matter (CPOM) including leaf litter and wood, algae
188 (diatoms, green alga), fungal hyphae and conidia, animal tissue and amorphous detritus

189 identified by the lack of any defined cellular structure; from Yule *et al.* (2010).
190 Percentage of gut contents was then estimated by eye.
191 *Stable Isotope Analysis:* Stable isotopes have been widely used to evaluate energy flow
192 and trophic structure in a range of food webs (Peterson and Fry, 1987). Stable carbon
193 and nitrogen isotope ratios are a useful tool to calculate what has been assimilated by
194 animals and to determine the relative importance of basal food resources in the food
195 web. Carbon isotope ratios are effective in distinguishing between autochthonous
196 (aquatic) and allochthonous (terrestrial) food sources, with the former having higher
197 $\delta^{13}\text{C}$ values (Fry, 1991; Hershey and Peterson, 1996). Nitrogen isotopes have been used
198 to provide information about trophic levels, with $\delta^{15}\text{N}$ increasing with each trophic
199 level.

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

210 Measurements were carried out at the NERC Life Sciences Mass Spectrometry
211 Facility (LSMSF) in East Kilbride, using an Elementar Pyrocube elemental analyser
212 coupled with a Thermo Fisher Scientific Delta Plus XP mass spectrometer. Laboratory
213 standards Fluka gelatin, Sigma alanine and Sigma glycine (Sigma-Aldrich Company

214 Ltd, Gillingham, UK) were repeated with every 10 samples and were used to correct
215 for linearity and instrument drift over a 22 hour analytical run. The isotope ratios for
216 the lab standards were determined relative to a range of International standards from
217 IAEA (Vienna, Austria) and USGS (Reston, VA, USA). Isotope ratios are expressed in
218 the δ (delta) notation in parts per million (‰): $\delta X = [(R_{\text{sample}}/R_{\text{standard}})-1]$ where X = ^{15}N
219 or ^{13}C and R = the ratio of $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$ isotopes in a given sample relative to AIR
220 for nitrogen and PDB for carbon. The analytical precision for carbon and nitrogen
221 isotope measurements was better than 0.3‰.

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

230

231

Results

232

Geomorphic Measurements and Functional Habitats

234 The twelve waterfalls had a range of average slope angles, from 16° (waterfall
235 11) to 53° (waterfall 1) and lengths of the waterfalls were from 4.5m (waterfall 11) to
236 20.4m (waterfall 8-Table 1). All submerged rock surfaces were covered in periphyton
237 with at least a few leaves present. Half of the waterfalls had wood debris, which varied
238 in size from twigs to large tree trunks. At waterfalls 1, 4 and 7, large tree trunks became

Commented [BK1]: that is not parts per million, it is parts per thousand (sometimes expressed as parts per mil, mil being short for the Latin word for thousand)

239 lodged in between the bedrock and the narrow bankful widths. Our observations in the
240 field and subsequent qualitative image analysis indicated that waterfalls varied in
241 substrate complexity. Some of the waterfalls consisted of smooth rock while others
242 were more complex with a mix of ridges (millimetres to centimetres), ledges and
243 undercutting.

244

245 *Macroinvertebrate trophic structure*

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

249

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

261

262 *Stable isotopic composition of food sources:* Periphyton had less depleted ^{13}C values
263 ($\delta^{13}\text{C}$ periphyton = -30.06 ± 2.54 ‰) compared to leaf litter ($\delta^{13}\text{C}$ leaf litter = $-32.97 \pm$

264 1.58 ‰), making it possible to distinguish between these basal food resources (one-
265 way ANOVA, $F_{1, 83}=37.7$, $p<0.01$; Table 2; Appendix 1). The $\delta^{15}\text{N}$ (‰) values for
266 periphyton (1.29 ± 0.78 ‰) and leaf litter (-0.91 ± 0.99 ‰) were also distinct (one-way
267 ANOVA, $F_{1, 83}=142.6$, $p<0.01$), with periphyton having significantly higher $\delta^{15}\text{N}$
268 values than leaf litter. The C: N values were higher for the leaf litter (49.27) than for
269 periphyton (5.37). The lower ratio indicates a higher proportion of nitrogen in the
270 sample suggesting higher protein digestibility and therefore higher nutritional value to
271 the consumers (Giller and Malmqvist, 1998; Table 2).

272 *Stable isotopic compositions of consumers:* The $\delta^{13}\text{C}$ values for Heptageniidae at the
273 12 waterfalls varied from -35.51 ‰ to -26.83 ‰, while Blephariceridae ranged from -
274 35.68 ‰ to -27.41 ‰ (Table 2; Fig. 3; Appendix 1). Simuliidae varied from -29.29 ‰
275 to -27.33 ‰ and Hydropsychidae $\delta^{13}\text{C}$ values ranged from -31.12 ‰ to -27.2 ‰.
276 Buccinidae ranged from -28.03 ‰ to -24.04 ‰ (Table 2; Fig. 3). There was a statistical
277 difference between the $\delta^{13}\text{C}$ values of all consumers (one-way ANOVA, $F_{4, 151}=55.5$,
278 $p<0.01$), but the Tukey post hoc test showed no difference among Heptageniidae and
279 Blephariceridae. Mean $\delta^{15}\text{N}$ values of Heptageniidae ranged from a -0.56 ‰ to 2.51 ‰,
280 Blephariceridae varied from 0.51 ‰ to 2.64 ‰ and Simuliidae varied from 1.65 ‰ to
281 3.04 ‰ (Table 2). Hydropsychidae ranged from 2.26 ‰ to 4.50 ‰ and Buccinidae
282 ranged from 4.12 ‰ to 6.69 ‰ (Table 2). There was a statistical difference between
283 the consumers in $\delta^{15}\text{N}$ values (one-way ANOVA, $F_{4, 151}=275.7$, $p<0.01$). However,
284 the Tukey post hoc test showed no difference in $\delta^{15}\text{N}$ values between Heptageniidae
285 and Blephariceridae ($p=0.34$), and between Simuliidae and Hydropsychidae ($p=0.8$).

286

287 *Potential food sources of consumers using GCA and SIA:* Most Heptageniidae had $\delta^{13}\text{C}$
288 values that corresponded to periphyton (Table 2; Fig. 3). However, at waterfall 7,

289 Heptageniidae had a $\delta^{13}\text{C}$ value of approximately -27 ‰ and at waterfall 10 the $\delta^{13}\text{C}$
290 values were very depleted at approximately -35 ‰ (Table 2; Fig. 3). GCA found
291 Heptageniidae consumed CPOM, algae and, fungal hyphae and conidia, the latter food
292 source may explain the 'unknown' $\delta^{13}\text{C}$ values. At most waterfalls Blephariceridae
293 matched periphyton and leaf litter; however, at waterfall 10 $\delta^{13}\text{C}$ values were depleted
294 with $\delta^{13}\text{C}$ values less than -35 ‰. Due to the small size of the Blephariceridae, the GCA
295 could not be used to show any further resolution, with gut material classed as
296 amorphous detritus. Generally Simuliidae $\delta^{13}\text{C}$ values matched periphyton, although,
297 there were some Simuliidae at waterfall 11 that had $\delta^{13}\text{C}$ value of approximately -27
298 ‰, values that are on the edge of the periphyton $\delta^{13}\text{C}$ (Fig. 3). The GCA did not provide
299 any further resolution (Fig. 2). Hydropsychidae $\delta^{13}\text{C}$ values were similar to periphyton,
300 along with another food source with $\delta^{13}\text{C}$ of approximately -27 ‰ at waterfall 8 and
301 11. GCA of Hydropsychidae contained animal tissue, fungal hyphae and conidia,
302 CPOM and algae. Buccinidae $\delta^{13}\text{C}$ values did not match periphyton or leaf litter apart
303 from at waterfall 9 where $\delta^{13}\text{C}$ values corresponded to periphyton. Buccinidae gut
304 contents was amorphous therefore not possible to differentiate food types.
305 Range of $\delta^{15}\text{N}$ values indicate that macroinvertebrates on the 12 waterfalls fed at
306 different trophic levels (Fig. 4). Heptageniidae had the most depleted ^{15}N with a value
307 of 1.06 ± 0.77 ‰ and Blephariceridae had a $\delta^{15}\text{N}$ value of 1.50 ± 0.68 ‰ (Table 2; Fig.
308 4). Simuliidae and Hydropsychidae $\delta^{15}\text{N}$ values were higher, Simuliidae had average
309 $\delta^{15}\text{N}$ value of 2.16 ± 0.36 ‰ and Hydropsychidae $\delta^{15}\text{N}$ value was 3.07 ± 0.46 ‰ (Table
310 2; Fig. 3). This indicates that some of their diet was composed of animal tissue. GCA
311 showed that animal tissue was present in the stomach contents of Simuliidae, although
312 only at waterfall 9. Hydropsychidae had animal tissue in gut contents for half of the
313 waterfalls including 2, 3, 7, 8, 9 and 12. Whilst Buccinidae was distinctly predacious,

314 having the most enriched ^{15}N with values of 5.48 ± 0.70 ‰ (Table 2; Fig. 3). These
315 results suggest that there were at least two trophic levels, with Heptageniidae and
316 Blephariceridae as primary consumers and Buccinidae as a secondary consumer.
317 Simuliidae and Hydropsychidae were in the middle, with some taxa primary consumers
318 and others secondary (Fig. 4).

319

320 Discussion

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

335 The isotopic values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of basal food sources were distinct for
336 periphyton and leaf litter (Table 2), making it possible to use them as indicators to
337 distinguish between autochthonous and allochthonous basal food resources. The $\delta^{13}\text{C}$
338 values for leaf litter averaged -32.97 ‰, lower compared to other values reported from

339 the tropics. For example, Huang *et al* (2007) recorded CPOM from streams in Taiwan
340 with an average of -30.3 ‰ and values from Hong Kong averaged -31.4 ‰ (Mantel *et*
341 *al.* 2004). This range in $\delta^{13}\text{C}$ values can be likely attributed to the high tree diversity in
342 tropical rainforests – for example in Ulu Temburong national park there are
343 approximately 250 species of trees (Cranbrook and Edwards 1994).

344 The average $\delta^{13}\text{C}$ value of periphyton was -30.06‰. This was more depleted
345 compared to other studies: -22.96‰ Puerto Rico (March and Pringle, 2003), -25.4‰
346 Hong Kong (Lau *et al.* 2009b) and -21‰ Guadeloupe (Coat *et al.* 2009). However,
347 depleted $\delta^{13}\text{C}$ values of filamentous algae have been reported, such as -28.3‰ in Hong
348 Kong (Mantel *et al.* 2004). The difference in periphyton $\delta^{13}\text{C}$ values reported in the
349 literature compared to periphyton in our study could be a result of samples being taken
350 from pools and riffles, biotopes with different environmental conditions compared to
351 waterfalls. The $\delta^{13}\text{C}$ values of periphyton can be affected by several factors including
352 the availability of dissolved inorganic carbon, taxonomic composition of the
353 periphyton, water velocity, growth rate, and periphyton biomass (Hill and Middleton,
354 2006). The average $\delta^{15}\text{N}$ for periphyton (-1.29 ‰) was higher than in leaf litter (-0.91
355 ‰), which is not surprising as periphyton scrubs contain a complex mix of bacteria,
356 algae, fungi, protozoa and can include animal tissue, thus increasing the $\delta^{15}\text{N}$ value
357 (Wetzel, 2001; March and Pringle, 2003).

358 This study was conducted during a dry season when there are high rates of leaf
359 litter fall (Cranbrook and Edwards, 1994; Pendry and Proctor, 1996). Leaves were
360 therefore expected on all waterfalls during this study. However, during the rest of the
361 year when there is less leaf litter fall and faster river flows, it would be predicted there
362 would likely be less leaf litter. This study showed the importance of periphyton as a
363 food source. Waterfalls are often situated under a canopy gap due to the wide plunge

Commented [BK2]: Reviewer comment: But does not leaf litter also include these extras??

The reviewer is right but periphyton is a biofilm that is made up of a mix of things, whereas leaf litter is mainly...leaf litter!

364 pools directly below the waterfall, which increase the channel width. Therefore, unlike
365 other headwater tropical biotopes, waterfalls are less likely to be light limited. In
366 addition to leaf litter and periphyton food sources, some macroinvertebrate guts
367 contained fungal hyphae and conidia. It has long been acknowledged that microbial
368 conditioning of leaf litter increases nutritional value and palatability for
369 macroinvertebrates (Petersen and Cummins, 1974). In the tropics bacteria and
370 fungi have been suggested to play a more important role in leaf litter decomposition
371 due to the higher lignin content (Irons *et al.* 1994; Wallace *et al.* 1997; Boyero *et al.*
372 2015, 2016) and in the streams of Ulu Temburong it is not uncommon to see leaf litter
373 covered in fungi.

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

381 In the literature Simuliidae and Hydropsychidae have been classed as filter
382 feeders (Cummins, 1973; Cummins and Klug 1979; Fig. 3), however, these functional
383 feeding group classifications are based on taxa living in temperate streams and there is
384 a growing number of studies that suggest tropical taxa are more dependent on
385 periphyton than leaf litter or detritus (March and Pringle, 2003; Brito *et al.* 2006; Lau *et al.*
386 2009a). For example, Brito *et al.* (2006) found Hydropsychidae in a Brazilian
387 stream with $\delta^{13}\text{C}$ values matching aquatic macrophytes and microalgae. The low
388 digestibility of tropical leaf litter as suggested by the C: N values in this study may

389 explain why periphyton is a more prevalent food source (Table 2; Benstead, 1996).
390 Wootton (1977) argues that Simuliidae have been known to eat algae and will essentially
391 ingest any particle from 0.5 μm to a maximum that depends on their mouth size (Burton,
392 1973). In addition, it is possible the water column contains many fragments of
393 periphyton due to the frequent scouring flows. In Ulu Temburong National Park
394 convective rainfall causes river and stream levels to fluctuate, this can occur daily in
395 the wet season, with the power of the water scouring out habitats including periphyton
396 (Sheldon, 2011). These consistent fast flows may have also influenced Simuliidae
397 feeding behaviour causing them to scrape.

398 The SIA results showed that Simuliidae and Hydropsychidae had higher $\delta^{15}\text{N}$
399 values compared to herbivorous grazers (Heptageniidae and Blephariceridae; Table 2).
400 The $\delta^{15}\text{N}$ values of consumers tend to be 2-5 ‰ higher than that of their diets (Hobson
401 and Clark, 1992; Bearhop *et al.* 2002), therefore our results suggest that Simuliidae and
402 Hydropsychidae on the waterfalls were omnivorous. Simuliidae have been known to
403 consume Chironomidae (Serra-Tosio, 1967) and some studies have regarded
404 Hydropsychidae as omnivorous and even predacious (Fuller and Mackay, 1980; Allan
405 and Castillo, 1995). The GCA support the SIA with animal tissue present in the guts of
406 both Simuliidae and Hydropsychidae. This study is the first known stable isotope
407 analysis on Buccinidae snail, with the literature mainly focusing on its taxonomy and
408 geographic distribution (Polgar *et al.* 2015). Our results show that the snail had little
409 overlap with $\delta^{13}\text{C}$ values for leaf litter or periphyton (Table 2) and the $\delta^{15}\text{N}$ values
410 confirm reports in the literature that Buccinidae is predacious (Coelho *et al.* 2013).

411 Some taxa showed high variability of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Table 2). This
412 variability could be due to the coarse level of identification to family level.
413 Identification to genus or species level may yield greater information, with species

414 potentially having different life histories and food preferences. However, this level of
415 resolution is difficult in Borneo with macroinvertebrates still mostly undescribed
416 (Baker *et al.* In press; Jacobsen *et al.* 2008; Yule and Yong, 2004). Variability in dietary
417 composition could also be a result of substrate complexity along with food availability
418 and quantity, which was not formally investigated. In our study the waterfalls'
419 geomorphic complexity varied from smooth bedrock to substrate covered in numerous
420 ridges and holes promoting the formation of a range of functional habitats defined by a
421 mix of leaf litter, wood debris, moss and periphyton. Although these functional habitats
422 were not quantitatively recorded in this study, visual observations found higher levels
423 of wood debris and leaf litter on some waterfalls, particularly ones
424 with complex substrate. Waterfall complexity may also influence abundance of
425 different taxa on the waterfall and therefore number of prey for the predators, for
426 example Buccinidae $\delta^{15}\text{N}$ value was lowest (4.35 ‰) at waterfall 9, which had smooth
427 substrate. While, the highest level of $\delta^{15}\text{N}$ (6.63 ‰) was at waterfall 1 which had
428 heterogeneous substrate. However, further studies are required to investigate if these
429 changes in waterfall complexity influence macroinvertebrate feeding behaviour and
430 trophic level.

431 While the food web is mostly resolved, it appears there are likely some unknown
432 food sources. The first was a source with more negative $\delta^{13}\text{C}$ values (less than -33‰)
433 that matched Blephariceridae and Heptageniidae at waterfall 10 (Fig. 3). Reviewing
434 other tropical isotopic studies (including March and Pringle, 2003; Brito *et al.* 2006;
435 Coat *et al.* 2009; Lau *et al.* 2009b) few food sources matched such low $\delta^{13}\text{C}$ values.
436 However, Huang *et al.* (2007) did sample red algae, which had depleted ^{13}C with values
437 around -37.4‰. Although this does not exactly match the $\delta^{13}\text{C}$ values of
438 Blephariceridae and Heptageniidae it could suggest that the waterfalls do harbor some

439 types of algae with very depleted $\delta^{13}\text{C}$ values. The second 'unknown' food source had
440 a $\delta^{13}\text{C}$ value of -27% and matched the $\delta^{13}\text{C}$ value for Heptageniidae at waterfall 7,
441 Simuliidae at waterfall 11 and Hydropsychidae at waterfall 8 and 11. These values
442 match values of dissolved organic matter and Fine Particulate organic matter (FPOM)
443 from other studies, such as 28.6% on the Island of Guadeloupe (Coat *et al.* 2009) and
444 in a Brazilian stream FPOM had a mean $\delta^{13}\text{C}$ value of $\sim 29\%$ (Brito *et al.* 2006).

445

446 **Conclusions**

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

460

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462

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470

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630
631 **Tables**

632
633 Table 1. Physical measurements of waterfalls including average slope angle and
634 length. Presence (1) vs. absence (0) of functional habitats (leaf litter, wood debris and
635 moss).

636

Waterfall	Slope angle (°)	Length (m)	Leaf litter	Wood Debris	Moss
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

637
638
639

640 Table 2. Average carbon and nitrogen isotopic values of basal food sources and
641 consumers (mean \pm SD).

642
643

	<i>n</i>	C:N	$\delta^{13}\text{C}$ (‰): Mean \pm SD	$\delta^{15}\text{N}$ (‰): Mean \pm SD
Basal food sources				
Leaf litter	36	49.27	-32.97 \pm 0.26	-0.91 \pm 0.16
Periphyton	57	5.37	-30.06 \pm 0.34	1.29 \pm 0.10
Consumers				
Heptageniidae	36	4.56	-29.47 \pm 0.33	1.06 \pm 0.13
Blephariceridae	30	4.50	-31.45 \pm 0.42	1.50 \pm 0.12
Simuliidae	36	4.30	-28.14 \pm 0.09	2.16 \pm 0.06
Hydropsychidae	36	4.53	-28.58 \pm 0.14	3.07 \pm 0.08
Buccinidae	33	3.92	-26.18 \pm 0.16	5.48 \pm 0.12

644

645 **Figure captions**

646

647

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

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

650 Temburong district, the eastern section of Brunei (B). The twelve waterfalls are

651 situated on tributaries of the two main rivers Sungai Belalong and Sungai Temburong

652 (C) near to Kuala Belalong Field Study Centre (KBFSC)

653

654 Fig. 2. Average percent contributions of stomach contents including amorphous

655 detritus, animal tissue, CPOM, algae and fungal hyphae and conidia in the five

656 macroinvertebrate families - Hydropsychidae, Heptageniidae, Blephariceridae and

657 Simuliidae.

658

659 Fig. 3. Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of basal resources leaf litter (LL) and

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

661 Temburong National Park. Macroinvertebrates include Buccinidae, Blephariceridae,

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

663

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

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

666 analysis. Line thickness represents contributions of the sources.

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