Diversity, abundance and habitat characteristics of mayflies (Insecta: Ephemeroptera) in Chambok, Kampong Speu Province, southwest Cambodia

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មូលន័យសង្ខេប

Mayflies (Ephemeroptera) គឺជាលំដាប់មួយនៃសត្វល្អិតទឹក។ ក្នុងដំណាក់កាលជាកូនញាស់ ជីវិតរបស់វាមានទំនាក់ទំនងជាមួយនឹងប្រភេទទីជម្រកផ្សេងៗក្នុងប្រព័ន្ធអេកូឡូស៊ីទឹកសាប។ យើងបានធ្វើការសិក្សាពីនានាភាពនៃចំនួនប្រភេទ ចំនួនឯកត្ត: និងទំនាក់ទំនងទីជម្រករបស់ Mayflies ក្នុងស្ទឹងមួយ ដែលមិនធ្លាប់មានការសិក្សាពីមុនមក គឺស្ទឹងនៃតំបន់ទេសចរណ៍ធម្មជាតិចំបក់ ក្នុងខេត្តកំពង់ស្ពឺ ភាគខាងត្បូងឈៀងខាងលិចនៃប្រទេសកម្ពុជា។ Mayflies ចំនួន១២ប្រភេទ ស្ថិតក្នុង៥អំបូរ ត្រូវបានរកឃើញ។ ចំនួនឯកត្ត:នៃអំបូរ Teloganodidae មានវត្តមានច្រើនជាងគេនៅក្នុងស្ទឹងចំបក់ ដែលមានចំនួន៥២ក្បាល។ បន្ទាប់មកគឺឯកត្ត:នៃអំបូរ Heptageniidae (៣៥ក្បាល), Baetidae (៣៥ក្បាល), Leptophlebiidae (១៨ក្បាល) និង Caenidae (៩ក្បាល)។ យើងរកឃើញថា ចំនួនប្រភេទ តម្លៃនានាភាព (Shannon-Wiener) និងចំនួនឯកត្ត: មានការកើនឡើងចាប់ពីផ្នែកខាងលើរហូតដល់ផ្នែកខាងក្រោមនៃខ្សែទឹក។ ផ្នែកលើការវិភាគតាមអនុគមន៍លីនេអ៊ែរ ចំនួននិងតម្លៃដែលបានរកឃើញនៅតាមទីតាំងសិក្សានីមួយៗមានទំនាក់ទំនងជាអវិជ្ជមានជាមួយនឹងកម្រិតកករក្នុងទឹក និងរយៈកម្ពស់ ប៉ុន្តែវាក៏មានទំនាក់ទំនងជាវិជ្ជមានផងដែរជាមួយនឹងបរិមាណគម្របព្រៃឬស្សី និងវត្តមានថ្មបាតស្ទឹងដែលមានទំហំមធ្យម (៦៤–២៥០ មីលីម៉ែត្រ)។ នៅតាមទីជម្រកក្នុងទីតាំងខ្សែទឹកខាងក្រោម លម្ហូរទឹកមានលក្ខណៈយឺតៗ កម្រិតកករក្នុងទឹកទាប និងមានគម្របព្រៃជុំវិញ ដែលជាលក្ខខណ្ឌសមប្រកប (ឧ. ជាទីជម្រក និងប្រភពអាហារដ៏ល្អ) សម្រាប់ Mayflies។ លទ្ធផលនៃការសិក្សានេះផ្តល់នូវមូលដ្ឋានគ្រឹះដ៏សំខាន់សម្រាប់ការស្រាវជ្រាវបន្ថែមពីនានាភាព និងរបាយណ៍របស់ Mayflies នៅក្នុងទីតាំងសិក្សា និងកន្លែងផ្សេងៗទៀតនៃប្រទេសកម្ពុជា។

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Abstract

Mayflies (Ephemeroptera) are an aquatic order of insects whose larval stages are generally associated with different habitat types in freshwater ecosystems. We investigated the species diversity, abundance and habitat associations of mayflies along a previously unstudied freshwater stream in the Chambok area of Kampong Speu Province, southwest Cambodia. Twelve mayfly taxa belonging to five families were recorded. Members of the Teloganodidae dominated the study stream with 52 individuals, followed by Heptageniidae (35 individuals), Baetidae (35), Leptophlebiidae (18) and Caenidae (9). We found that the taxonomic richness, Shannon-Wiener's diversity and abundance of mayflies increased from upstream to downstream sections of the stream. Based on linear regression models, these patterns were negatively associated with water turbidity and altitude, but positively associated with bamboo cover and availability of medium-sized stone substrates (64–250 mm) at sampling sites. Habitats at the downstream sampling sites were characterised by slow-flowing water, lower water turbidity and surrounding forest cover, all of which are typically considered to provide suitable conditions (e.g., good shelter and food sources) for mayflies. Our results provide a useful baseline for further investigations on the diversity and distribution of mayflies in the study site and elsewhere in Cambodia.

Keywords Ephemeroptera, Shannon-Wiener diversity, environmental parameter, habitat quality, water quality.

Introduction

Mayflies (Ephemeroptera) are a small order of insects which are characterised by their distinct nymph morphology: seven pairs of abdominal gills, three caudal filaments and mouthparts (Barber-James *et al*., 2008). The order is an ancient group of aquatic insects which is composed of approximately 440 genera belonging to 40 families with 3,330 described species (Sartori & Brittain, 2015). Mayflies are longer in the aquatic nymph stage compared to their winged stage (subimago & imago) and undergo a series of ecdysis as they grow (Sartori & Brittain, 2015). They commonly possess soft-bodies, which are small to medium-sized, and bear gills along the sides of the abdomen and two or three segmented caudal filaments (Borror *et al*., 1989). A variety of morphological adaptations also define their functional feeding behaviours and specific microhabitat preferences (Sartori & Brittain, 2015).

Mayfly nymphs inhabit lentic and lotic freshwater habitats (Khoo, 2004), although their diversity and abundance are usually higher in the latter (Barber-James *et al*., 2008). They commonly forage on particulate organic matter, macrophytes, algae, and periphyton on substrates (i.e. stones, silt and aquatic vegetation) (Sartori & Brittain, 2015) and their local species diversity depends greatly on habitat characteristics such as temperature, water quality, altitude, food availability and water flow velocity (Brittain & Sartori, 2009). For instance, high species richness has been found in shallow littoral stream habitats with slow current velocities and clean water (Sartori & Brittain, 2015; Vilenica *et al*., 2018). Conversely, the diversity of mayfly nymphs is lower in deeper streams with high sediment loads and water pollution (Extence *et al*., 2011; Sartori & Brittain, 2015). Due to their sensitivity, mayflies are regarded as useful bio-indicators of water quality, particularly good quality waters with high levels of dissolved oxygen (Bauernfeind & Moog, 2000). Partly as a result, many studies have investigated the relationship between environmental factors (e.g., physical and chemical variables) and mayfly species richness, abundance and assemblage composition (Minshall *et al*., 1985; Hawkins *et al*., 1997; Khoo, 2004; Finn & Poff, 2005; Gustafson, 2008; Ross *et al*., 2008; Arimoro & Muller, 2010).

Numerous studies have shown that mayfly diversity and abundance are threatened by anthropogenic activities including watercourse alterations (including small dams or weirs along mountain streams), habitat fragmentation, forest degradation and water pollution (Bauernfeind & Moog, 2000; Benstead & Pringle, 2004; Lange *et al*., 2018). However, understanding of their status in countries such as Cambodia is currently limited by a lack of studies. Because so few studies have been undertaken to date, the same knowledge gap also applies to many other aquatic insect groups in the country. Those that have include site-based assessments of the diversity of aquatic Polyphaga (Freitag *et al*., 2018), a national checklist for aquatic Hemiptera (Zettel *et al*., 2017), studies of relationships between water quality and aquatic insects in Phnom Penh city (Chhy *et al*., 2019) and the distribution patterns of macroinvertebrates in the Lower Mekong Basin, including Cambodia (Sor *et al*., 2017). As such, investigations of mayflies and their habitat associations are needed to improve basic understanding of the group in Cambodia. To this end, we investigated the species diversity, abundance and habitat associations of

mayflies along a previously unstudied freshwater stream in southwest Cambodia.

Methods

Study site

Our study was conducted on an elevational gradient along a freshwater mountain stream in the Chambok Community-Based Ecotourism Site, which is located in Chambok Commune, Phnom Sruoch District, Kampong Speu Province, southwest Cambodia. Sampling was undertaken in three undisturbed locations at higher elevations of the stream (site codes S01–S03), four locations at intermediate elevations (S04–S07), and three locations in lower elevation areas characterised by bamboo forest and patches of agriculture (S08–S10) (Table 1). One of the sampling sites (S04) included a waterfall and semi-natural pool which is regularly visited by tourists and used by local people for its clean water.

Field sampling

Sampling was undertaken on one occasion at each of the ten locations selected from 13–15 August 2018. Mayfly samples were collected in different areas of the stream at each location i.e. along both banks and in the mid-stream area. Each area was sampled for 30 minutes by sweeping hand-held nets with a 0.1 mm mesh size in the water and brushing the surfaces of microhabitats and substrates (e.g., stones, dead wood and leaves) into nets.

Table 1 Sampling locations, elevations and dates in Chambok, Kampong Speu Province, southwest Cambodia.

Site	Latitude, Longitude	Elevation (m)	Sampling Date
S01	11.21251°N, 104.06108°E	425	13 August
S02	11.21277°N, 104.06143°E	394	13 August
S03	11.21313°N, 104.06123°E	391	13 August
S04	11.21534°N, 104.06124°E	293	14 August
S05	11.2159°N, 104.06173°E	241	14 August
S06	11.22039°N, 104.06267°E	197	14 August
S07	11.22071°N, 104.06356°E	150	15 August
S08	11.2214°N, 104.06427°E	133	15 August
S09	11.22246°N, 104.06448°E	117	15 August
S10	11.22307°N, 104.06499°E	116	15 August

The contents of nets were frequently transferred into a white tray for sorting and to avoid overloading of nets. This material was then slowly rinsed and all mayflies present were gently removed by hand with forceps and preserved in labelled plastic vials containing 97% ethanol. These specimens were later sorted in the laboratory and identified to genus level using keys provided by MRC (2006), Khoo (2004) and Sartori *et al*. (2008).

Water temperature, conductivity and turbidity were measured at each sampling location using a HI 7609829 Multiparameter, Portable Water Quality Meter (Hanna Instruments Ltd., Bedfordshire, UK). Additional variables recorded included altitude, water depth, stream width and flow velocity. The percentage cover of different stream substrates in each sampling location was measured. Three categories of substrate were employed for this purpose, based on the size of stones present: 'large', stones >250 mm; 'medium', stones 64–250 mm; 'small', stones 2–64 mm. Land cover (i.e. forest, agriculture, residential areas, orchards, bamboo and shade) were measured using the AusRivAS (Parsons *et al*., 2002) and RHS protocols (Raven *et al*., 1998). The results of these measurements are provided in Table 2.

Statistical analyses

We used three measures to analyse the mayfly fauna of our study sites: taxonomic richness, Shannon-Wiener's diversity and abundance. Shannon-Wiener's diversity (H) was calculated using the *vegan* package of *R* (Oksanen et al., 2015). Multiple linear regression (MLR) models were first used to test for associations between environmental variables and mayfly diversity and abundance, after which stepwise selection was employed to retain the most important variables. Each response variable (taxonomic richness, abundance and diversity) was regressed (univariate regression) against each of the remaining significant environmental variables. The response of taxonimic richness, diversity and abundance to environmental factors was assessed using the standardized regression coefficient, whereas the performance of regression was evaluated using the coefficient of determination (adjusted R^2). Probability values of <0.05 were considered significant. All statistical analyses were performed in *R* software vers. 3.6.2 (R Core Team, 2019).

Results

We collected and identified a total of 149 mayfly nymphs belonging to 12 taxa, 10 genera and five families. Members of the Teloganodidae dominated the study stream with 52 individuals, followed by Heptageniidae (35 individuals), Baetidae (35), Leptophlebiidae (18) and Caenidae

Table 2 Environmental variables recorded at sampling sites in Chambok, Kampong Speu Province, southwest Cambodia.

Environmental variable	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
Forest (%)	70	100	80	80	60	70	60	20	20	10
Agriculture (%)	0	0	0	0	0	0	0	45	50	40
Bamboo (%)	30	0	20	20	40	30	40	35	30	50
Shade (%)	75	80	95	75	95	70	92	40	40	60
Dead wood (%)	1	2	2	1	2	2	2	2	1	1
Large stones (>250mm) (%)	60	50	45	65	45	48	55	65	55	35
Medium stones (64–250mm) (%)	15	25	15	25	15	20	20	10	25	25
Small stones (2–64mm) (%)	10	15	10	5	20	15	7	10	7	25
Sand (<2mm) (%)	10	8	10	3	10	10	5	10	3	10
Silt/clay (%)	5	2	20	2	10	7	13	5	10	5
Water depth (m)	0.37	0.47	0.24	0.44	0.42	0.40	0.45	0.46	0.29	0.24
Stream width (m)	6.6	5.6	10.7	20.5	9.8	13.5	8.8	6.2	6.6	8.0
Velocity (m/s)	1.27	1.50	2.45	2.10	1.95	1.90	2.36	1.03	3.90	2.75
Altitude (m)	425	394	391	293	241	197	150	133	117	116
Conductivity (EC.uS.cm)	14	14	14	12	13	13	11	12	13	14
Total dissolved solid (ppmTds)	7	7	7	6	7	7	6	6	6	7
Turbidity (FNU)	19.9	12.2	24.0	11.6	10.9	11.2	12.4	11.7	10.3	10.2
Temperature (°C)	23.71	23.77	23.70	23.69	24.07	24.23	24.42	24.42	24.24	24.19

(9). The twelve taxa identified comprised *Dudgeodes* sp. (52 individuals; Fig. 1), *Cinygmina* sp. 1 (6), *Cinygmina* sp. 2 (12), *Thalerosphyrus* sp. (15; Fig. 1), *Asionurus* sp. (2), *Liebebiella* sp. 1 (28), *Liebebiella* sp. 2 (3), *Labiobaetis* sp. (2), *Platybaetis* sp. (2), *Choroterpes* sp. (2), *Isca* sp. (16) and *Caenoculis* sp. (9).

Taxonomic richness, diversity & abundance

The distribution of taxonomic richness, diversity and abundance of mayflies across our sampling sites is shown in Fig. 2. In summary, all three values increased from upstream to downstream sections of the stream, although mayfly abundance was relatively low at two of the downstream sampling sites (S07 and S08).

Habitat associations

Four environmental variables were significantly associated with our response variables in the MLR models and stepwise selection process. Water turbidity and altitude were negatively associated with taxonomic rich-

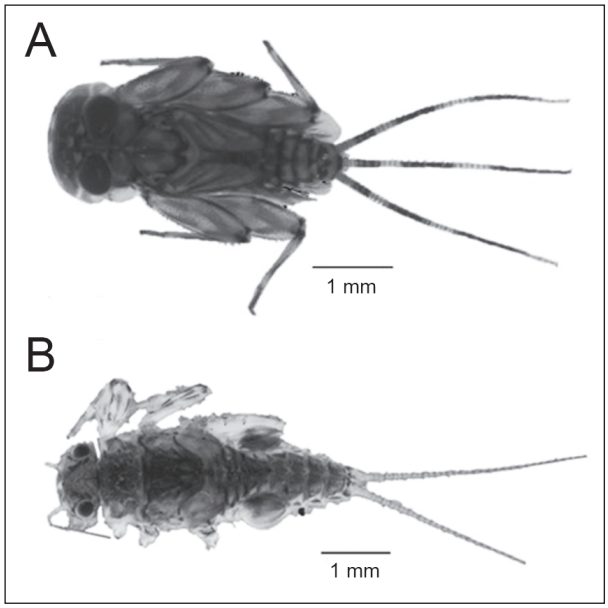

Fig. 1 Two mayfly taxa recorded during the study in Chambok, Kampong Speu Province, southwest Cambodia: A) *Thalerosphyrus* sp., B) *Dudgeodes* sp.

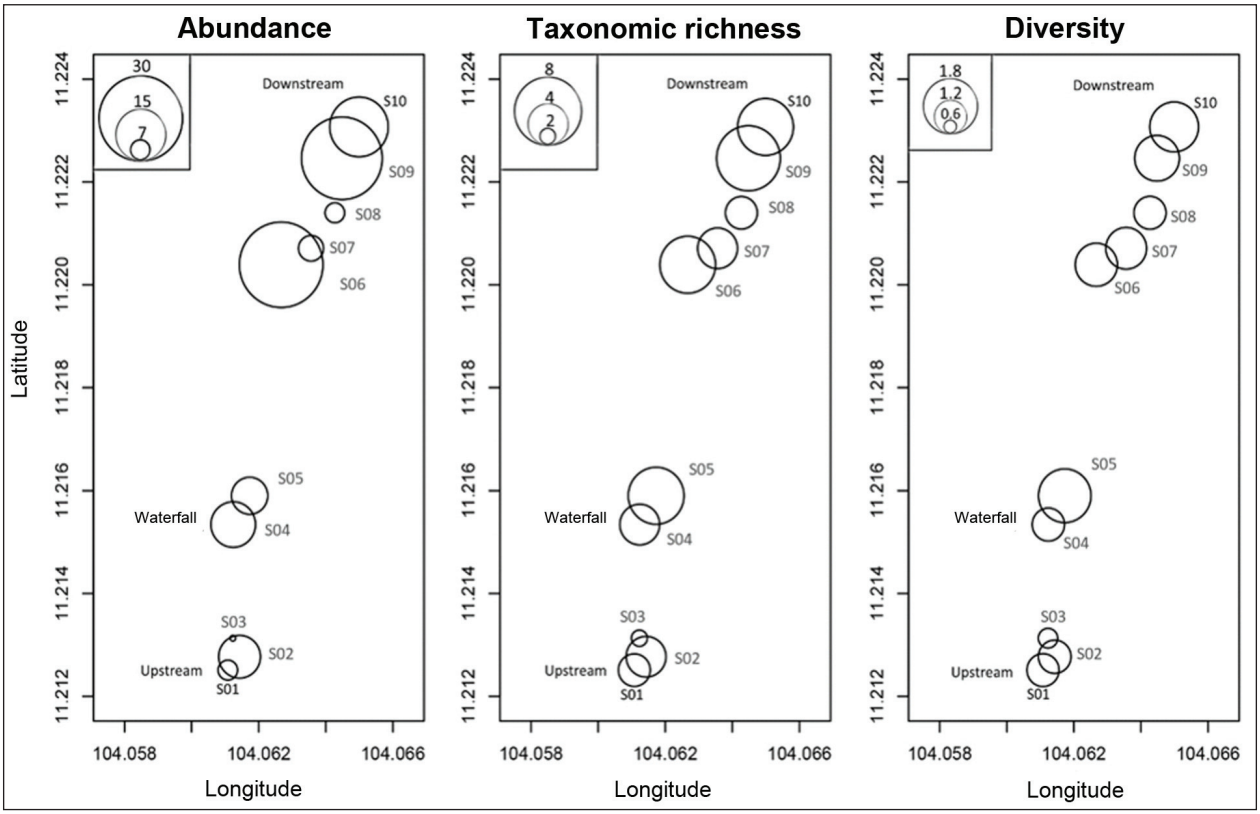

Fig. 2 Bubble plots showing the distribution of mayfly abundance, taxonomic richness and Shannon-Wiener's diversity along the study stream in Chambok, Kampong Speu Province, southwest Cambodia.

ness, diversity and abundance (Figs. 3A-B), whereas the percentage cover of bamboo and medium-sized stone substrates were positively associated with diversity and abundance (Figs. 3C-D).

Discussion

Ours is the first study of mayflies in the Chambok area of Cambodia, although we only recorded 12 taxa arranged in 10 genera and five families. These figures are relatively low compared to other studies of aquatic insects, water quality and habitat characteristics (Bauernfeind & Moog, 2000; Siegloch *et al*., 2008; Sor *et al*., 2017; Chhy *et al*., 2019). *Dudgeodes* sp. (Teloganodidae) was the dominant taxon in our study, being abundant at all of our sample sites. This may be due to the fact that it can apparently survive low and high turbidity environments, being found in waters at Chambok with turbidity values of 10.2 FNU and 24.0 FNU, respectively. Members of the genus also occur in relatively undisturbed habitats surrounded by secondary forest and highly disturbed areas including settlements (Garces *et al*., 2020). *Dudgeodes* species have also been found in fast-flowing waters with sand or gravel substrates (Sartori *et al*., 2008) and studies outside the region have found that Teloganodidae are the dominant family (Harrison & Agnew, 1962). In contrast, *Caenoculis* sp. (Caenidae) was the least common taxon in our study and only occurred at five sampling sites characterised by high vegetation cover and medium water flows. Members of the genus may be indicative of good quality or slightly polluted waters (Alhejoj *et al*., 2014).

Habitat associations

We found that the taxonomic richness, Shannon-Wiener's diversity and abundance of mayflies increased from upstream to downstream sections of our study stream. Previous studies have shown that water flow rates, turbidity and forest cover are important drivers of mayfly distributions (Ogbogu & Akinya, 2001; Rueda *et al*., 2002; Buss & Salles, 2007) and that slow-flowing and less turbid waters, with medium-sized stone substrates and surrounding forest cover may provide more suitable micro-habitats for mayflies (Siegloch *et al*., 2008; Sartori & Brittain, 2015; Vilenica *et al*., 2018). All of these factors likely reflect our finding of high mayfly diversity

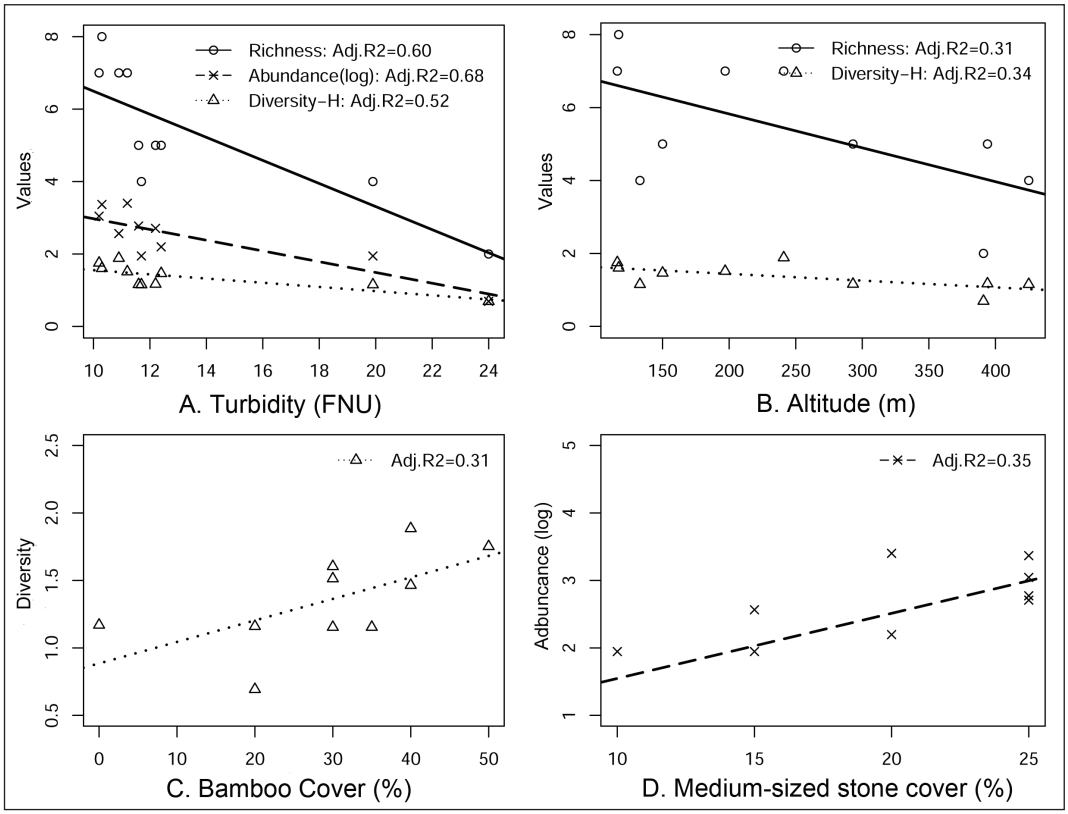

Fig. 3 Linear regression models of environmental variables significantly associated with the taxonomic richness, diversity and abundance of mayflies at the study site. Adj.R2 = Adjusted R-square (coefficient of determination).

in downstream sampling sites (S06–S10), where average water turbidity was 11.16 ±0.93 FNU, medium-sized stone cover was 20 ±6.12% and bamboo cover was 37 ±8.37%. This was supported by our regression models. Bamboo forest cover may provide good habitats and food sources for insects (Siegloch *et al*., 2008; Sor *et al*., 2017), whereas medium-sized stones might provide suitable ecological niches for foraging (Siegloch *et al*., 2008). However, one exception was found in the form of low mayfly abundance at S07 and S08, although their taxonomic richness and diversity were relatively comparable to other downstream sites. Further investigations are warranted to explore our observations at these sites.

The diversity of mayflies was somewhat lower in the upstream portions of our study stream where our sampling sites were characterised by large stone substrates, fast-flowing waters and high turbidity. High levels of turbidity resulting from greater sediment loads in fast-flowing waters can reduce the availability of certain food sources (e.g., macrophytes, algae and diatoms) (Sartori & Brittain, 2015) for mayflies and our regression models suggest that water turbidity is negatively associated with mayfly diversity. Upstream locations also naturally occur at higher altitudes where most adult female mayflies typically lay their eggs, which subsequently get washed downstream (Robinson, 2005). As such, this factor may have contributed to the higher mayfly diversity we observed in the downstream sections of the stream.

While our results provide useful insights into factors influencing the taxonomic richness, diversity and abundance of mayflies in Chambok, they are based on a single sampling event and so could primarily reflect the prevailing conditions at that time. As such, additional sampling with replication should be undertaken over time to assess whether the patterns we observed are indeed typical for the mayfly fauna of Chambok. It would also be interesting to explicitly test if our finding that the percentage cover of medium-sized stone substrates is positively associated with mayfly diversity holds true on a larger temporal and spatial scale. If found to be the case, this could have practical implications for restoration and management of degraded stream habitats. Taken as a whole, our study provides a useful baseline for further investigations of the diversity and distribution of mayflies in Chambok and elsewhere in Cambodia.

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