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Determination of food sources and trophic position in Malaysian tropical highland streams using carbon and nitrogen stable isotopes

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ABSTRACT

Stable isotope analysis has been extensively used as an effective tool in determination of trophic relationship in ecosystems. In freshwater ecosystem, aquatic invertebrates represent main component of a river food web. This study was carried out to determine potential food sources of freshwater organism together with pattern of trophic position along the river food web. In this study, rivers of Belum-Temengor Forest Complex (BTFC) has been selected as sampling site as it is a pristine area that contains high diversity and abundance of organisms and can be a benchmark for other rivers in Malaysia. Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were applied to estimate trophic position and food web paradigm. Analysis of stable isotopes based on organic material collected from the study area revealed that the highest $\delta^{13}C$ value was reported from filamentous algae ($-22.68 \pm 0.126^0/_{00}$) and the lowest $\delta^{13}C$ was in allocthonous leaf packs ($-31.58 \pm 0.187^0/_{00}$). Meanwhile the highest $\delta^{15}N$ value was in fish ($8.45 \pm 0.177^0/_{00}$) and the lowest value of $\delta^{15}N$ was in autochthonous aquatic macrophyte ($2.00 \pm 1.234^0/_{00}$). Based on the $\delta^{15}N$ results, there are three trophic levels in the study river and it is suggested that the trophic chain begins with organic matter followed by group of insects and ends with fish (organic matter < insects < fish).

1. Introduction

Tropical Asian countries such as Malaysia, Cambodia, Thailand and Indonesia have extensive freshwater resources that play important role in supplying the community with food and drinking water. Their forest rivers are suitable habitat for a diverse group of biota especially freshwater living organism and aquatic macroinvertebrates [25]. Malaysia with its diverse running water rivers has high diversity of aquatic invertebrates. Since the last decade, several studies aimed to investigate the ecological and biological aspects of tropical forested rivers in Malaysia (e.g., [4–7,17,18,40,74,86,90]).

Most of Malaysian rivers flow down from highlands to lowlands with disparate velocity depending on type of the river substrates [84]. The river substrate varies between upper river and downriver as it is changing from boulder, bedrock, cobbles and gravel at the upper river to be more sandy and muddy in the downriver [43]. Due to alteration in the hydrological characteristic between upper river and down river reaches, the water quality and availability of food sources also change [86]. Thus, upper reaches are usually rich with leaf litters and algal biofilms that attached to the stony substrates which were major food sources to the aquatic micro- and macroorganism of the river. However, slow flowing water in the downriver reaches is rich with aquatic macrophyte and phytoplankton that serve as food source to some aquatic macroinvertebrates [85].

The relationship between food utilization by organism and the pathway of energy transfer is called trophic structure [14]. The aquatic invertebrates are the main components for understanding the trophic structure in a river [21]. Therefore, the feeding habits of river invertebrates can influence all of their ecological and biological characteristics [83]. Feeding habits are typical traits reflecting the adaptation of species and they could group together across communities differing in taxonomic composition [73].

Aquatic and terrestrial habitats are both dependent on each other and have been described as intricate and complex continuum of linked habitats [78]. Thus, it is important to understand interaction among smaller habitats within a specific ecosystem. Furthermore, the interaction between these two habitats can be quantified by measuring the rates of energy exchange between trophic levels and proximate habitat using stable isotopes analysis [12]. Therefore, stable isotopes analysis is useful tool to determine the trophic base of food webs in the aquatic systems. Salas & Dudgeon [72] suggested isotope analysis to be the tool to determine the relative contribution of allochthonous and autochthonous sources to the production of consumer biomass.

Several stable isotopes ratios such as carbon, nitrogen and sulfur were widely used to trace the organic matter pathways among

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consumers in the aquatic ecosystem [50]. The application of isotopes has facilitated understanding the exchange of organic matter among aquatic organisms in the ecosystem from the primary sources to the predators [47,53,95]. In Malaysian rivers, studies highlighting the importance of the organic matter are restricted to investigating the diversity patterns of functional feeding groups (see [18]) or examining the decomposition rate leaf litter by aquatic insects [75].

The stable isotope values are usually reported in standard delta notation (δ) with unit of part per thousand ($^0/_{00}$) as for example, δ^{13}C and δ^{15}N [63]. Carbon isotope, ^{13}C has been used vigorously as a tracer of carbon flow pathways and also to differentiate among the organic matter sources [91] and usually show low variation among different trophic levels. The trophic enrichment of carbon isotopes in aquatic ecosystems usually ranges from 0 to $1^0/_{00}$ [39,81]. Carbon stable isotopes are also commonly used in mixing models functioning in quantifying the source distribution to a mixture [64]. A linear mixing model which involves two isotopic sources is commonly used to calculate fractional proportion of food sources in animal diets [65].

Nitrogen has been well established as a tool for trophic level determination as well as organic matter source information for benthic consumers. This is explained in the nitrogen metabolism which involved light isotope and heavy isotope. The light isotope (^{14}N) is concentrated in nitrogenous excretion products while the heavy isotope (^{15}N) is discriminated against and retained in body tissues [23]. Due to the importance of the nitrogen in metabolism, a progressive enrichment of trophic levels occurred by ~$+3.0^0/_{00}$ per trophic transfer. In addition, each particular organism can be assigned into specific trophic level by using the δ^{15}N values [1]. It has been proven that the δ^{15}N values increase constantly with the trophic levels. However, determination of organic nitrogen food sources is difficult due to trophic enrichment and various levels of δ^{15}N values among food sources cause difficulties in assigning the trophic levels. Due to all these intricate issues, most researchers prefer to use dual isotope simultaneously to determine both trophic level and food sources. For example, carbon and nitrogen dual-isotope approach is one of the most useful strategies that have been used intensively in analysis of aquatic food webs [63].

Although there are some available studies on stable isotopes in Malaysian ecosystems, they only focus on availability of nutrition for mangrove [19,60,69] and food preference of mudskipper [89]. Information on the aquatic food web structure in the forested tropical rivers of Malaysia using isotopes is still scarce. This study is considered as the first report of aquatic insects' food availability in forested rivers of Malaysia. Thus, this study aimed to investigate the structure of available food sources for aquatic insects in the forested rivers of BTFC, Perak. The output of this study can be an introductory knowledge on stable isotope analysis and trophic relationships among aquatic insects for further ecological studies in Malaysia.

2. Materials and methods

2.1. Study areas

Belum-Temengor Forest Complex (BTFC) is located in the most northerly region of Perak Darul Ridzuan (5°30′N, 101°20′E) covered approximately 300,000 ha which made it the second largest protected area in Peninsular Malaysia after Taman Negara National Park Pahang (Fig. 1). The dipterocarp rain forest located at a range of altitude from 130 to 1500 m above sea level (asl) was thickly covered with trees of various species of Dipterocarpaceae such as Keruing mempelas (*Dipterocarpus crinitus* Dyer), Mersawa (*Dipterocarpus costulatus* V.S1.), Jelutong (*Dyera costulata*, syn. *D. laxiflora*) and many other flora (bamboos and ferns) [2]. The forest complex is also rich with various fauna species, especially fishes. According to study done by Zakaria-Ismail & Lim [87], Temengor Lake supported a massive number population of freshwater fishes such as sebarau (*Hampala macrolepidota*), toman (*Channa micropeltes*) and Queen Danio (*Devario regina*). Meanwhile along the rivers, most encountered riparian vegetation and aquatic vegetation are weeds mainly the knobweed (*Hyptis capitata*: Lamiaceae), water straw grass (*Hymenachne amplexicaulis*: Poaceae) and Willdenow's spikemoss (*Selaginella willdenowii*: Selaginellaceae). A river from tributaries of the Temengor Forest (and associated lake) and two rivers from the Belum Forest were selected based on the highest availability of organic sources. The study sites have annual rainfall >2000 mm and the average temperature 24.51 °C.

2.2. Collecting samples for stable isotope analyses (plants, algae, aquatic macroinvertebrates and fish)

All samples were collected randomly once (in May 2013) from rivers of BTFC as representative of the entire complex for stable isotope analysis. Aquatic macrophytes, leaf litter and algae were selected as main sources of organic matter in this freshwater ecosystem [78]. Leaf litters that have been shed from the riparian canopy were collected from the river bed. Meanwhile, algae were scraped from the substrate and aquatic macrophytes were clipped above the roots. All samples were rinsed with distilled water to remove any organisms and sediment particles attached to them [48]. The samples were kept in plastic bags and stored in an ice chest (Coleman® chest).

A D-framed net was used to collect several potential aquatic insects using the 2 min kick-sampling technique. All samples were identified to their families using taxonomic keys of Morse et al. [57], Yule & Yong [85], and Orr [61]. Then, several families were assigned to represent the aquatic insects' community in BTFC such as Perlidae, Heptageniidae, Stenopsychidae, Hydropsychidae and Gomphidae. These families were chosen according to the literature of the insect functional feeding groups [52,86]. All samples were kept alive in small vials filled with clean water and covered with a 0.5 mm mesh cap for 1–2 days to clear the gut from ingested foods [72].

Fishing net was used to sample the most dominant fish from the study site which was identified to their species using identification keys by Ambak et al. [8] and Mohsin & Ambak [55]. The most dominant fish found in BTFC was the Queen Danio (*Devario regina*) from family Cyprinidae. The fish samples were kept alive in a small aquarium to clear the gut content. Then, the head, stomach, tail and scales were removed and the remaining tissue (fillet) was cut out using a scalpel and a probe under a dissecting microscope [41].

All the samples were pooled and processed for the isotope analysis. A total of 3 samples from the pooled samples were selected and were oven dried at 60 °C overnight and grounded into a fine, homogenous powder using a mortar and pestle. Prior to processing different samples, the mortar and pestle were cleaned with methanol and air dried. This precaution was taken to avoid any mixture of powder that can affect the result. Thereafter, samples in the form of powder were stored in a clean small glass vials and stored in a freezer until they were analysed [72].

2.3. Stable isotope analysis

The dry samples were brought to The Centre for Advanced Analytical Toxicology Services (CAATS) of Universiti Sains Malaysia (USM). Some procedures prior to analysis were necessary before all the organic samples were loaded into the elemental analyser-isotope ratio mass spectrometry (EA-IRMS). These procedures were carried out following standard practice for EA-IRMS [15]. A small amount of powder sample was carefully transferred into a tin capsule pressed light (weight 8 × 5 mm) using a laboratory spatula with a micro spoon and weighed using a microbalance (Mettler Toledo, 10/1 μg) to ~300 μg. Then, the tin capsule was folded into a small ball using a pair of forceps. While folding, all the air should be compressed out of the tin capsule to avoid error during analysis. Carbon and nitrogen stable isotopes were analysed together following the Urea-isotopic working standard (^{13}C,