



Trophic discrimination factor and the significance of mangrove litter to benthic detritivorous gastropod, *Ellobium aurisjudae* (Linnaeus)

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ABSTRACT

In stable isotope analysis, the estimation of proportional contribution of carbon and nitrogen from mangrove to benthic invertebrates requires knowledge of the food-consumer trophic discrimination factor ($\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$). This study tested the hypothesis that the mangrove gastropod *Ellobium aurisjudae* can assimilate low quality refractory mangrove litter and aimed to determine the trophic discrimination values (TDV) of C and N isotopes between gastropod and the mangrove producer. The mean $\Delta\delta^{13}\text{C}$ for gastropods fed senescent leaves of the mangrove *Bruguiera parviflora* (Roxb) Wight & Arn and decomposing mangrove (unknown species from the same site) wood were estimated at $5.3 \pm 0.3\text{‰}$ and $3.2 \pm 0.5\text{‰}$ respectively, whereas for $\Delta\delta^{15}\text{N}$, these values were $4.2 \pm 0.2\text{‰}$ and $6.0 \pm 0.2\text{‰}$ respectively. The gastropod assimilated refractory carbon from mangrove leaf and wood litter with 49% and 18% efficiency respectively. Rearing experiment of gastropods ($n = 25$) fed only mangrove wood litter over 5months in the laboratory, showed mean weight increments of 14.8–74.4% depending on the initial animal weight. Significant deviation of the TDVs for *E. aurisjudae* from the generalized discrimination values for herbivory underscores the need to use specific TDV for the detritivory link.

1. Introduction

Mangrove forests and adjacent estuaries are among the most productive coastal habitats, fuelled by at least three primary producers; mangrove plants, microphytobenthos and phytoplankton all support these coastal food webs (Alongi, 2009; Chong et al., 2012a; Chai et al., 2012). The stable isotope approach based on the isotopic signatures of carbon ($^{13}\text{C}/^{12}\text{C}$ ratio) and nitrogen ($^{15}\text{N}/^{14}\text{N}$ ratio) of aquatic producers and consumers allows researchers to trace the carbon or organic matter flow from the primary source to the consumers (Minagawa and Wada, 1984; Rodelli et al., 1984; Peterson et al., 1985; Peterson and Howarth, 1987). This powerful technique supports detailed investigations into the mangrove detritus paradigm (Odum and Heald, 1975) thus resolving some of the long entrenched ideas of mangrove contribution to coastal food webs (Lee, 2005; Alongi, 2009). Most studies using this technique have concluded that despite the high production of mangrove litter (Gong and Ong, 1990), mangrove detritus is of limited nutritional quality, or its carbon is largely refractory to the nutrition of aquatic consumers especially those in offshore waters (Rodelli et al., 1984; Dittel et al., 1997; Hatcher et al., 1989; Bouillon et al., 2002). More recent studies, however, suggest that certain benthic invertebrates

such as mangrove grapsid crabs, hermit crabs, sergestid and mysids may assimilate mangrove carbon (Niiyama et al., 2012; Herbon and Nordhaus, 2013; Bui and Lee, 2014; Ramarn et al., 2014; Teoh and Chong, 2015).

Mangrove litter consumed by benthic fauna undergoes both physical and chemical changes due to the animal digestive processes (Thongtham and Kristensen, 2005; Florin et al., 2011). C isotopic ratio of the animal changes slightly after assimilation through a process, called trophic fractionation or discrimination, that results from preferential loss of ^{12}C from tissues during respiration (Rounick and Winterbourn, 1986). Two factors apparently regulate the degree of change in the trophic discrimination value (TDV) for N; first, the quality and quantity of the food protein, where the proportion of the most limiting amino acid affects the changes in the TDV (Florin et al., 2011); and secondly, the selective assimilation of amino acids, carbohydrates and lipids by the organism (DeNiro and Epstein, 1978; Herbon and Nordhaus, 2013). Thus, accurately tracing the C and N contributions of mangrove and other primary food sources to the benthic fauna requires knowledge of the TDV. The TDV may differ among species and at different trophic levels (Vander Zanden and Rasmussen, 2001). The dependence of many marine organisms on multiple food sources adds

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further complication (Phillips and Gregg, 2003; Alongi, 2009; Ramarn et al., 2014; Teoh and Chong, 2015). In nature, benthic grazers of mangrove litter may also supplement their diet through ingestion of dead animal tissues, bacteria, phytoplankton and benthic diatoms to maintain their growth (Kurata et al., 2001; Thongtham and Kristensen, 2005).

Previous studies such as by Vander Zanden and Rasmussen (2001) and McCutchan Jr et al. (2003) provided average TDV for the isotopic ratios of carbon and nitrogen (annotated as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) based on trophic groupings of various fauna (e.g. carnivores and herbivores). Such trophic grouping values are useful given the lack of species-specific TDVs. However, recent workers challenge the validity of applying such average values to many trophic ecology studies. For example, the TDV for $\delta^{13}\text{C}$ of mangrove grapsid crabs that ranged from 4.1‰ to 5.5‰ (Herbon and Nordhaus, 2013; Bui and Lee, 2014) was significantly higher than the group average of 0.5‰ recommended by McCutchan Jr et al. (2003). Bui and Lee (2014) further cautioned against the use of such average TDVs compiled from multiple proxy species rather than from the studied species.

The litter consuming pulmonate gastropod, *Ellobium aurisjudae*, represents one of most common species of benthic macroinvertebrates that inhabit the upper mangrove forest of Selangor River estuary (western coast of peninsular Malaysia) where it grazes on the leaves and wood litter that carpet the forest floor. Dormant individuals occur most often within the fissures and gaps of the decomposing mangrove wood, sometime in groups of two to five individuals. Little is known about this species in term of their biology and ecology despite their ubiquitous presence in the high shore mangroves. However, their consumption of mangrove litter (personal observation) suggests a potential mediating role of this gastropod in channelling trophic energy from mangrove-derived organic matter to higher consumers and in facilitating the nutrient recycling process. Despite the wealth of knowledge on the diversity of flora and fauna in the Selangor mangrove forest and its adjacent coastal waters (see Sasekumar and Chong, 2012), the evidence for mangrove detritus as a primary source supporting the estuarine or marine food web remains ambiguous. One reason for this may be the incorrect approximation of the isotopic discrimination values for detritivory leading to the underestimation of mangrove contribution (Herbon and Nordhaus, 2013; Bui and Lee, 2014).

This study thus tests the hypothesis that *E. aurisjudae* can assimilate low quality refractory mangrove litter, and aimed to determine the TDV of both C and N isotopes for this presumed mangrove detritivore. We test our hypothesis and address the study's objective by feeding captured gastropods from the mangrove forest only mangrove detritus (leaf and wood litter) for 5 months in the laboratory. Stable isotope values of both C and N ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) of mangrove and animal tissue were then followed during the feeding experiment.

2. Materials and methods

2.1. Field samplings

About 50 live *Ellobium aurisjudae* with shell lengths of 44–50 mm were collected during low tide on 9th July 2014, from the high shore mangrove forest that fringes the Selangor River estuary (N3°19'47.74"; E101°14'15.65"). We also collected samples of faecal matter of *E. aurisjudae*, leaf litter (*Bruguiera parviflora*), and fragments of decomposing mangrove wood litter (unrecognizable mangrove species) from separate fallen logs inhabited by the gastropods. The density of *E. aurisjudae* in the high shore zone of Kuala Selangor mangrove forest was estimated by random sampling of animals distributed within a 50-m strip located approximately 200 m inwards from the estuarine bank. The animals were sampled within a 3 m × 3 m quadrat laid 10 times. We counted the number of *E. aurisjudae* including individuals residing inside the mangrove wood litter, and measured their shell length on-site using Vernier calipers to an accuracy of 0.05 mm.

2.2. Feeding experiment

The mangrove leaves and wood used for feeding the gastropods were soaked in artificial seawater of salinity 25, which is within the salinity range of the area (Chong et al., 2012b) for 24 h to remove other fauna such as termites. Ten individuals of *E. aurisjudae* were placed inside each of the four 10-litre (24 cm × 33 cm × 16 cm) tanks containing about 0.5 cm deep artificial seawater (salinity of 25–28). All gastropods were allowed to acclimate to the new environment for 5 days and to evacuate their guts, after which individuals of *E. aurisjudae* in two of the tanks were fed yellow senescent mangrove leaves (Treatment 1) whereas individuals in another two tanks were fed mangrove wood litter (Treatment 2). The feeding experiments were continued for a period of 120 days. Animals were offered both feeds ad libitum. Two petri dishes (10 cm diameter) were placed upside down in each tank to allow the gastropods to emerge from the water. The tanks were covered with semi-transparent black plastic throughout the experimental period to mimic the dim environment of the mangrove forest floor. The tanks were cleaned and the water replaced weekly. Three replicate individuals from each of the treatment tanks were harvested at day-40, day-80, and day-120 and muscle tissues were taken. Faeces were sampled at day-120 from each tank. All samples were dried in the oven at 60 °C for 48 h before storage in a desiccator prior to stable isotope analysis.

2.3. Stable isotope analysis

In the laboratory, the sampled faeces, leaves, and wood for stable isotope analysis were dried in the oven at 60 °C for 24 h before storage in a desiccator. Three individuals of *E. aurisjudae* for initial (day-0) stable isotope readings were killed by freezing them at –20 °C for about 6 h. Samples of their muscle tissue were then taken and dried for 48 h before storage in a desiccator.

All samples from the feeding experiment were sent to the Marine Biological Laboratory (MBL) in Wood Hole, USA for determination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. At MBL, the samples were pulverised into powder form and weighed to $\pm 0.001 \mu\text{g}$. The isotopic compositions of the samples were determined using a Europa ANCA-SL elemental analyser attached to a continuous-flow Europa 20–20 mass spectrometer. The results were expressed as standard δ notation determined based on the following equation with Vienna Pee Dee Belemnite (VPDB) and N_2 in air (AIR) as standard reference materials for carbon and nitrogen respectively:

$$\delta^{13}\text{C}, \text{‰} = [({}^{13}\text{C}/{}^{12}\text{C})_{\text{sample}}/({}^{13}\text{C}/{}^{12}\text{C})_{\text{standard,VPDB}} - 1] \times 1000$$

$$\delta^{15}\text{N}, \text{‰} = [({}^{15}\text{N}/{}^{14}\text{N})_{\text{sample}}/({}^{15}\text{N}/{}^{14}\text{N})_{\text{standard,AIR}} - 1] \times 1000$$

Changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ readings over time (day-0 to day-120) were tested using one-way ANOVA at the 5% significance level. The TDV for carbon ($\Delta\delta^{13}\text{C}$) and nitrogen ($\Delta\delta^{15}\text{N}$) from mangrove leaves or wood litter to *E. aurisjudae* was determined by subtracting the $\delta^{13}\text{C}$ ($\delta^{15}\text{N}$) value of leaves or wood from the value of the gastropod tissue at day-120. This calculation was based on the assumption that the change in isotopic signature is consistent and predictable upon assimilation (Bouillon et al., 2008). Assimilation efficiency (A) of *E. aurisjudae* was determined based on the following equation with proportion of C in feed (F) and faeces (E) as input data (Bui and Lee, 2014):

$$A = \frac{F - E}{(1 - E)F}$$

2.4. Growth experiment

A total of 25 individuals of *E. aurisjudae* (shell lengths of 11–47 mm) collected from the same location were measured for shell length (SL) and total body weight including shell (W), using Vernier calipers (Mitutoyo 500-196-30, Advanced Onsite Sensor, accuracy of 0.01 mm)

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