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Impact of food type on respiration, fractionation and turnover of carbon and nitrogen stable isotopes in the marine amphipod *Gammarus aequicauda* (Martynov, 1931)

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ABSTRACT

This study experimentally determined the impact of food source type on turnover rate and trophic enrichment factors (TEFs or Δ) of $\delta^{13}C$ and $\delta^{15}N$, as well as on respiration rate, in captive populations of the marine amphipod *Gammarus aequicauda*. *Gammarus aequicauda* (318 individuals) were fed ad libitum with three food sources animal, algae, and dead *Posidonia oceanica* leaves (also called "litter"), varying in palatability, digestibility, nutritional qualities and isotopic compositions, for between four and six weeks in a controlled feeding experiment. The resulting death rate was lower for the amphipods fed with animal treatment (30.9%) than for individuals fed with algal (65.9%) or litter treatment (64.4%), indicating a better fitness of the individuals fed with the animal food source. Respiration rates also differed highly among the treatments. Animal treatment showed higher respiration rates than algal and litter treatments, potentially due to the toxicity of the algae and the very low nutritional quality of the litter. Amphipods fed with these treatments might have entered in a "low activity state" to cope with these unsuitable food sources, inducing low respiration rates. Due to the very low assimilation and toxicity of the algae source, turnover rate for $\delta^{13}C$ was impossible to determine. Turnover rate for $\delta^{13}C$ was much faster (half-life = 12.55 days) for amphipods fed with the animal food source than for amphipods fed with litter (half-life = 51.62 days), showing the faster assimilation of the most nutritionally optimal food sources by *G. aequicauda*. Turnover for $\delta^{15}N$ was impossible to determine because the amphipods were already at isotopic equilibrium at the beginning of the experiment. Despite the detritus feeder status of *Gammarus aequicauda*, TEFs for the animal treatments were in accordance with values generally found for carnivorous organisms ($\Delta^{13}C = 0.9 \pm 0.7‰$; $\Delta^{15}N = 2.9 \pm 0.6‰$). TEFs for the litter treatment were in accordance with values generally corresponding to detritivorous organisms ($\Delta^{13}C = 1.2‰$; $\Delta^{15}N = 1.0 \pm 0.4‰$). SIAR mixing model outputs obtained with these new TEF values were more constrained and coherent than outputs obtained with general literature TEFs. This study thus demonstrated the non-negligible impact of the food source on *Gammarus aequicauda* physiological status, fitness and turnover rates, but also on TEFs—highlighting the importance of TEF experimental calculations for every potential food source of a given organism to ensure more robust isotopic data interpretation.

1. Introduction

Stable carbon (C) and nitrogen (N) isotope analysis is nowadays a common and efficient method for studying diet, energy flow and food web structure in ecology (Fry, 2006). Dietary inferences based on naturally occurring isotopes, such as those of N and C, are possible because these isotopes are transferred from food source to consumer and are reflected in the consumer's tissues (DeNiro and Epstein, 1978; Fry, 2006). Moreover the stable isotope ratio of a consumer reflects its diet over some period of time, not just the most recently eaten food. The stable isotope ratio, SIR ($^{13}C/^{12}C$ expressed as $\delta^{13}C$ or $^{15}N/^{14}N$ expressed as $\delta^{15}N$), of a consumer will differ slightly from that of its food source and a shift, referred to as trophic fractionation or trophic enrichment factor (TEF or Δ), occurs because isotopes of a given element, as a result of their different atomic mass, react slightly differently during all biochemical reactions (e.g., photosynthesis, respiration, organic matter incorporation) (Fry, 2006). The overall result of combined isotopic effects associated with trophic processes is generally a net enrichment of the consumer's tissue in ^{13}C compared to the food source, by TEF range of 0–1‰ (DeNiro and Epstein, 1978; Caut et al., 2008; Michel et al., 2015). TEF for ^{15}N is typically higher, ranging from 2 to 5‰ (Rau et al., 1983; Hobson et al., 1994; Vander Zanden and Rasmussen, 2001; Post, 2002; Vanderklift and Ponsard, 2003). TEF values can vary dramatically depending on the tissue being sampled and the species in question (Vanderklift and Ponsard, 2003; Suring and Wing, 2009; Caut et al., 2009).

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TEF values for marine detritivorous invertebrates are rare in literature (Vanderklift and Ponsard, 2003; Kaufman et al., 2008; Mancinelli, 2012; Michel et al., 2015) and this might be an important issue for isotopic data interpretation (Bond and Diamond, 2011). The use of Bayesian mixing models is common nowadays to assess diets with many possible food sources and uncertainty on TEF values, isotopic values of the food sources and isotopic values of the consumers (Cherel, 2008; Browning et al., 2014; Michel et al., 2015), but these models all significantly depend on the number of replicates, on the number of food sources but also on the accuracy of the TEFs to give robust and reliable results (Bond and Diamond, 2011; Phillips et al., 2014). Thus, it has been suggested there is a need to undertake experiments under controlled feeding conditions to establish TEF values and turnover rates at a species specific level (Gannes et al., 1997). Some such experiments have been conducted in recent years (e.g., Logan et al., 2006; Kaufman et al., 2008; Suring and Wing, 2009; Caut et al., 2010; deVries et al., 2015) but to our current knowledge, this is the first study testing the impact of different food sources on TEFs, isotopic turnover and respiration of a Mediterranean detritus-feeder Gammaridean amphipod.

This study focused on the marine amphipod *Gammarus aequicauda* (Martynov, 1931), which is known to be the most important consumer of dead *Posidonia oceanica* (or Neptune grass) leaves found in detritus accumulations (Lepoint et al., 2006; Michel et al., 2015). These exported macrophytodetritus accumulations (hereafter called: "EMAs") composed of dead and alive Neptune grass leaves, rhizomes, macro-algae and microorganisms (hereafter called: "litter") are colonized by meiofauna (Mascart et al., 2015) and form an abundant and diverse vagile macrofauna community (Gallmetzer et al., 2005; Dimech et al., 2006). Due to the relatively low consumption of the living leaves of the Neptune grass (Pergent et al., 1994; Moore et al., 2004; Heck and Valentine, 2006; Cardona et al., 2007), the vagile fauna, largely dominated by detritivorous amphipods, plays a key role in the transfer of organic matter from the *P. oceanica* meadow to the upper components of the coastal food web (Lepoint et al., 2006; Sturaro et al., 2010; Michel et al., 2015).

Gammaridean amphipods are known to show low TEF for $\delta^{15}N$ (Michel et al., 2015; Mancinelli, 2012), much lower than TEFs generally admitted in literature for other animals (Post, 2002; McCutchan et al., 2003) and what influences TEFs remains unknown for *G. aequicauda*. For example, food elemental content may impact assimilation rate of a food source (Gergs and Rothhaupt, 2008) and therefore TEFs (Adams and Sterner, 2000; Caut et al., 2009). Ecological stoichiometry, formally defined as "the conceptual framework that considers the relative balance of key elements (e.g., C and N) in trophic interactions" (Brown et al., 2004; Cross et al., 2003), could therefore play a role in food assimilation, isotopic fractionation (and thus on TEFs) and isotopic turnover. Indeed, a consumer facing inappropriate dietary elemental ratios may modify ingestion, assimilation and/or respiration rates (Cross et al., 2005; Frost et al., 2002; Darchambeau et al., 2003), which could also influence TEFs. Metabolism impacts of an elementally non-optimal food source may include: growth and reproduction rate decrease, increased storage of the deficient element, or increased excretion of the element in excess (Sterner & Hessen, 1994; Hessen et al., 2004).

This study aimed to: (1) determine the impact of food elemental composition and stoichiometry on *Gammarus aequicauda* respiration rates; (2) determine the impact of food stoichiometry on isotopic turnover rate of C and N; (3) determine the impact of food type and elemental composition on TEFs; and (4) highlight the importance of precise species specific and food source specific TEFs on SIAR (Parnell et al., 2008) Bayesian Mixing Model results.

2. Materials and methods

2.1. Collection of G. aequicauda

Macrofauna inhabiting *P. oceanica* litter accumulation was manually sampled by scuba-diving, between 7 and 10 m depth, with 50 L plastic bags in November 2013 near the STARESO oceanographic station in the Bay of Calvi (Corsica, 8°45′E 42°35′N). Samples were brought back alive to Liège and put into two 500 L containment tanks along with fresh *P. oceanica* litter, its natural food source. In March 2014, after 5 months of tank rearing, 318 *Gammarus aequicauda*[1] (Martynov, 1931) (Fig.1) that had reproduced and lived freely in the aquarium were isolated from the remaining macrofauna.

Fig. 1. Male specimen of *Gammarus aequicauda*.

2.2. Experimental food sources

Three different feeding treatments were developed for this experiment: an animal treatment, freshwater *Gammarus* spp.; an algae treatment, *Flabellia petiolata* (Turra) (Nizamuddin, 1987); and a litter treatment, dead *P. oceanica* leaves; hereafter respectively "**AnT**", "**AlT**" and "**LiT**". These food sources were not randomly chosen. **AnT** was chosen because many *Gammarus* species are known to be cannibals or predators of other amphipods. A food source composed of amphipods of the same genus with close C:N ratio was thus considered appropriate. **AlT** was chosen because *Flabellia petiolata* is a common green algae found on *P. oceanica* rhizomes and commonly found in EMAs. **LiT** was obviously chosen because dead *P. oceanica* leaves are the main components of the EMAs and because preliminary studies indicate a non-negligible assimilation of dead leaf organic matter by *Gammarus aequicauda* inhabiting EMAs (Lepoint et al., 2006). The vegetal material for **AlT** and **LiT** were sampled near STARESO in November 2013, and the freshwater *Gammarus* spp. intended for **AnT** were sampled in a pristine headwater stream (Liège, Sart-Tilman) in February 2014. All the treatments were freeze-dried for 48 h using a Christ™ Alpha 1–4 Ldplus freeze-dryer and then manually ground and sieved to obtain 1–2 mm particles, a size compatible to the *Gammarus* mouth.

2.3. Feeding experiment design

The experiment lasted 30 days for **AlT** and **LiT,** due to mortality, and 43 days for **AnT**. These durations are compatible with the life span of *Gammarus aequicauda* (3–4 months) (Prato et al., 2006) and assumed to be sufficient for reaching isotopic equilibrium for a small amphipod (Crawley et al., 2007). From the 318 individuals, 30 were randomly sampled at the beginning of the experiment (Day 0) for evaluating initial conditions and 288 individuals were randomly placed in 24 microcosms (8 replicated microcosms per treatment with 12 individuals in each microcosm). The microcosms were made of a 450 mL plastic

[1] It must be pointed out that *Gammarus aequicauda* is a species morphologically extremely close to *Gammarus insensibilis* (Stock, 1966). Even if *Gammarus aequicauda* is much more frequent than *Gammarus insensibilis* in *P. oceanica* exported litter accumulations, and if identification was performed with all due care, rare cases of confusion cannot be excluded.