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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.

beaker mounted at the bottom with a 500-μm mesh nylon net. The box was embedded into another seawater-filled, 750-mL plastic box. All boxes were placed in a common thermostatic bath. This design prevented the ingestion of feces and molts. Constant temperature (15 ± 0.8 °C), pH (8.1) and salinity (30 ± 1.4) of water was maintained for the duration of the experiment. Salinity of 30 was considered appropriate because it was easy to maintain constant and because *Gammarus aequicauda* is known to be a euryhaline species (Mancinelli and Rossi, 2002). Food sources were provided ad libitum in each mesocosm for each treatment, every 2 days to ensure that a sufficient amount of fresh food was provided but also to avoid food decay and water contamination. Every week from Week 1 to 6, surviving amphipods were counted and, for each treatment, between 10 and 16 individuals were sampled randomly (i.e., a maximum of 2 individuals from each microcosm). From the 16 individuals collected per treatment (per sampling time), 6 were immediately freeze-dried for later analysis and 10 were used for individual respiration measurements. Due to mortality, only 10 individuals were sampled for treatment **AlT** and 12 for treatment **LiT** at Week 4.

2.4. Respiration measurements

Ten out of the 16 collected individuals were randomly selected for respiration rate measurements. The basal metabolic respiration rate of *Gammarus aequicauda* was determined by measuring oxygen consumption by the "closed bottle method" (Lampert, 1984), using the MRCh System (Unisense A/S, Århus, Denmark). Amphipods were individually placed in a 1.5 mL glass respiration chamber in a thermostatically controlled water bath (15 ± 0.1 °C). The chambers contained 0.2 μm-filtered seawater. Dissolved oxygen content was measured with a calibrated Clark-type oxygen microsensor (model OX-MRCh; Unisense A/S). Amphipods were allowed to acclimate for 10 min, after which oxygen consumption rates were taken as the linear slope of the O_2 concentration plotted against time for each 5-min interval during the next 30–45 min. The lowest 5 min consumption rate observed was considered as the basal metabolic rate, reducing the impact of active movement respiration on measurements. It should be noted, however, that what was measured may not be the true basal metabolic respiration rate. Indeed, Specific Dynamic Action, SDA, can also impact respiration rate, sometimes leading to slight overestimation and variability of measures. SDA corresponds to the increase of oxygen consumption just after food ingestion and is very species specific (Whiteley et al., 2001; Secor, 2009). We thus postulate that our measurements are basal metabolic respiration plus a variable amount of SDA that could not be precisely evaluated. After measuring respiration, the amphipods were removed from the respiration chamber and frozen (−18 °C) for later analysis. The volume of water in the microchamber was then measured for assessing consumption rate to individual respiration.

2.5. Sample processing and C, N elemental and stable isotope analysis

After thawing, all individuals were pictured using a MOC-510 Mueller-Optronic 5 megapixel CMOS camera associated to a Zeiss Stemi 2000-C binocular, sexed and measured using Tucsen-TS View 7 software. Ovigerous females were counted and the eggs removed from the body prior to any analysis. All samples were freeze-dried for 48 h. Their digestive tracts were then manually removed to limit the bias of the gut content isotopic composition on the analysis. Samples were acidified under HCl 30% vapor for 15 h to limit the bias of carbonates on tissue isotopic composition. After freeze-drying, dry samples were weighted, ground to homogenous powder and put in tin cups prior to elemental and stable isotope analysis. The stable isotope ratio, SIR, of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), and the elemental composition was determined for each individual using an isotopic ratio mass spectrometer (Isoprime 100™, Isoprime, UK) interfaced in continuous flow with an elemental analyzer (vario MICRO cube™, Elementar). C and N elemental composition was reported in% of the DW of the sample. Isotope ratios for C and N were reported conventionally in per mil (‰) using standard delta (δ) notation relative to their respective international standards, Vienna-Pee Dee Belemnite (V-PDB) and atmospheric N_2:

$$\delta X = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right) \times 10^3 (‰) \quad (1)$$

where $X = {}^{13}C$ or ${}^{15}N$, $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$, and standard = Vienna-Pee Dee Belemnite (V-PDB) and atmospheric N_2 respectively for carbon and nitrogen. Pure gases of CO_2 and N_2 were used and calibrated against certified reference materials, i.e., sucrose (IAEA-C6; $\delta^{13}C = -10.8 \pm 0.3‰$) and ammonium sulfate (IAEA-N2; $\delta^{15}N = 20.3 \pm 0.3‰$), obtained from the International Atomic Energy Agency (IAEA, Vienna, Austria). The analytical precision was assessed by procedural blanks, internal replicates (i.e., glycine and in-house crustacean and seagrass reference material) and isotopic certified material (i.e., IAEA-C6 and IAEA-N2). Elemental data are expressed relative to dry mass (%DM) and C:N ratios are weight-based ratios. Standard deviations on replicated measurements presented hereafter were 0.4% for N elemental composition, 0.7% for C elemental composition, 0.1‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$. Neither chemical lipid extractions nor a posteriori lipid corrections were performed due to the C:N ratios values of *G. aequicauda* being equal or close to the 3.5 trigger recommended (Post et al., 2007), and also due to the often poor usefulness of a posteriori corrections for aquatic invertebrates containing high proportions of chitin in addition to lipids and proteins (Logan et al., 2008).

2.6. Turnover rate and trophic enrichment factors (TEF) calculation

The turnover rate was calculated using the classical one-phase exponential decay curve (Tieszen et al., 1983; Hobson & Clark, 1992):

$$\delta_{(t)} = \delta_f + (\delta_0 - \delta_f) e^{-ct} \quad (2)$$

where $\delta_{(t)} = \delta^{13}C$ or $\delta^{15}N$ (‰) at time t, $\delta_f = \delta^{13}C$ or $\delta^{15}N$ value (‰) of the amphipods at the end of the experiment approached asymptotically, $\delta_0 = \delta^{13}C$ or $\delta^{15}N$ value (‰) of the amphipods at the beginning (T0) of the experiment, c = fractional turnover of the respective isotopes (day^{-1}) and t = time (day) since diet modification. c and δ_f parameters were estimated for each food quality treatment by fitting the one-phase exponential decay model to the time-course of the experimental data.

The half-life (HL) of C and N was calculated as:

$$HL = \frac{\ln 0.5}{c} \quad (3)$$

where c = fractional turnover of the respective isotopes (day^{-1}). HL refers to the time required for the stable isotope composition of one consumer to reach a midpoint value between the equilibrium value of the consumer before the diet modification and the expected calculated equilibrium (Bosley et al., 2002) value of the consumer on the new diet.

The isotopic discrimination factors, or trophic enrichment factors (TEFs or Δ) were calculated as (Fry, 2006):

$$\Delta = \delta_c - \delta_{f \cdot s} \quad (4)$$

where δ_c is the isotopic composition ($\delta^{13}C$ or $\delta^{15}N$) of the consumer at equilibrium, $\delta_{f \cdot s}$ is the isotopic composition ($\delta^{13}C$ or $\delta^{15}N$) of the food source at equilibrium, and Δ refers to the global fractionation (‰) between a food source isotopic composition and the consumer composition at equilibrium for a given isotope. δ_c was individual values at isotopic when possible. When individual values did not approach asymptote value, δ_c was the theoretical asymptote value, leading to two slightly different methods of calculation. When possible, the TEF

Table 1
Summary table of the TEFs (‰) used for the different food sources in the three different scenarios (A, B and C). The right-hand column indicates where the chosen TEFs for the different scenarios come from in the literature.

		TEF values		
	Food source	$\Delta^{13}C$ Mean ± SD (‰)	$\Delta^{15}N$ Mean ± SD (‰)	
Scenario A	Dead *P. oceanica* leaves	0.50 ± 0.10	2.30 ± 0.20	McCutchan et al., 2003
	Drift photphilous macroalgaeEpiphvtes	0.50 ± 0.10	2.30 ± 0.20	McCutchan et al., 2003
	Drift sciaphilous macroalgae	0.50 ± 0.10	2.30 ± 0.20	McCutchan et al., 2003
	Pool of harpacticoid copepods	0.50 ± 0.10	2.30 ± 0.20	McCutchan et al., 2003
Scenario B	Dead *P. oceanica* leaves	0.20 ± 0.60	1.20 ± 0.50	Michel et al., 2015
	Drift photphilous macroalgaeEpiphvtes	0.20 ± 0.60	1.20 ± 0.50	Michel et al., 2015
	Drift sciaphilous macroalgae	0.20 ± 0.60	1.20 ± 0.50	Michel et al., 2015
	Pool of harpacticoid copepods	0.20 ± 0.60	1.20 ± 0.50	Michel et al., 2015
Scenario C	Dead *P. oceanica* leaves	1.00 ± 0.40	1.20	from this study
	Drift photphilous macroalgaeEpiphvtes	0.20 ± 0.60	1.20 ± 0.50	Michel et al., 2015
	Drift sciaphilous macroalgae	0.20 ± 0.60	1.20 ± 0.50	Michel et al., 2015
	Pool of harpacticoid copepods	0.90 ± 0.70	2.90 ± 0.60	from this study

calculation method with individual values was preferred because standard deviations of TEFs are important parameters for mixing model runs.

2.7. SIAR mixing model runs

The Bayesian Mixing Model from the package SIAR for R (Parnell et al., 2008, 2010) was used to demonstrate the importance of accurate TEF parameters for robust mixing results interpretation. Different TEF parameters were tested on isotopic data from *G. aequicauda* individuals (N = 40) sampled in 2011–2012 near the STARESO oceanographic station in the Bay of Calvi (Corsica, 8°45′E 42°35′N). The potential food sources entered into the model, identified from observations of individuals' gut contents, were: (1) dead litter; (2) photophilous algae; (3) sciaphilous algae; and (4) copepods. The "photophilous algae" source is composed of drift photophilous macroalgae and leaf epiphytes, sampled in 2011–2012 at the same time and locations, undistinguishable by $\delta^{13}C$ or $\delta^{15}N$ compositions. The "copepod" source is based on unpublished data collected for the four most abundant species of copepods sampled inside the exported litter accumulations in 2011-2012 (Mascart, 2015) at the same time and locations. The "dead litter" and "sciaphilous algae" sources were also sampled in 2011–2012 at the same time and locations. Three different modelling scenarios were applied: (A) SIAR run with identical TEFs from literature for all the food sources (McCutchan et al., 2003); (B) SIAR run with identical custom TEFs for all the food sources, calculated for *Gammarus aequicauda* fed with epiphytes (Michel et al., 2015); and (C) SIAR run with different TEFs for all the food sources, calculated in this study and from Michel et al. (2015). The TEF values used for our three different scenarios were: (A) general values of 2.3 ± 0.2‰ for ^{15}N and 0.5 ± 0.1‰ for ^{13}C (found in McCutchan et al., 2003) used for all food sources (Table 1); (B) values of 1.2 ± 0.5‰ for ^{15}N and 0.2 ± 0.6‰ for ^{13}C (found in Michel et al., 2015 for *Gammarus aequicauda*) used for all food sources (Table 1); and (C) values determined experimentally in this study for *Gammarus aequicauda* fed with different food sources (Table 1), along with values found in Michel et al. (2015) for food sources "sciaphilous algae" and "photophilous algae". The presentation of SIAR model proportion outputs consists of a Kernel density graphical representation based on the first 5000 raw SIAR outputs of modelled proportions of each source. This graphical representation is an alternative to the classical probability densities and credibility intervals plot generated by the SIAR software.

2.8. Statistical analysis

A Log Rank (Mantel-Cox) test on survival data, Spearman correlation, Non-Linear Regression (and subsequent AICc and Extra sum-of-squares F-test), and One-Phase-Decay curve fit performed on $\delta^{13}C$ and $\delta^{15}N$ data were performed using GraphPad Prism version 6.01 for Windows, GraphPad Software, San Diego California USA ($P < 0.05$).

F-statistics tested the significance of the regression model applied on $\delta^{13}C$ and $\delta^{15}N$ data ($P < 0.05$) using Welch approximation when heteroscedasticity was detected (Welch, 1951). F-statistics or Welch approximation was performed using STATISTICA 12 (StatSoft Inc.).

Other statistical analyses (2-way ANOVAs and post hoc HSD Tukey tests) were performed using statistical R Programming Environment (V3.2.1; R Development Core Team, 2015). Normality and variance homogeneity of length, dry mass, C:N ratios, $\delta^{13}C$, $\delta^{15}N$ and respiration data were tested with Shapiro-Wilk and Levene tests ($P < 0.05$). A few groups (weekly samples) did not meet the assumptions for parametric analysis, but as these assumptions are also required for non-parametric tests (Kruskal-Wallis rank test) along with symmetry of distribution, an ANOVA-test was considered more robust to these slight assumption violations than non-parametric equivalent analysis (Underwood, 1997). Thus, 2-way ANOVAS and post hoc HSD Tukey tests (when global significance was highlighted by ANOVAs) were performed on length, dry mass, C:N ratios, $\delta^{13}C$, $\delta^{15}N$ and respiration data, with significance level of $P < 0.01$ to limit erroneous interpretations.

3. Results

3.1. Death rate

Food type significantly affected survival rates of *G. aequicauda* (Fig. 2). At the end of the experiment, death rates were 30.9%, 65.9% and 64.4% for **AnT**, **AlT** and **LiT,** respectively. The **AnT** curve differed significantly ($P < 0.001$, Log-rank test) from **AlT** and **LiT** curves which were

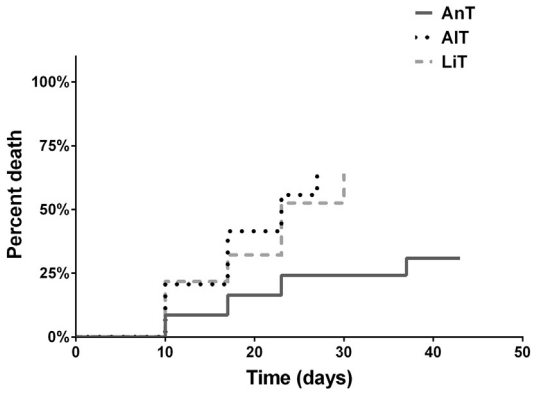

Fig. 2. Evolution in death rate of individual *Gammarus aequicauda* for the three different food sources during the experimentation. Plain grey line represents animal treatment (AnT); dotted black line represents algal treatment (AlT); dotted grey line represents litter treatment (LiT).

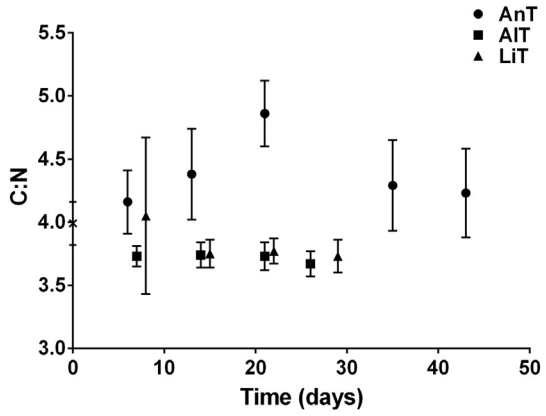

Fig. 3. Evolution of C:N ratio (mean ± s.d.) of *Gammarus aequicauda* for the three different food sources during the experiment. Circles represent animal treatment (AnT), squares represent algal treatment (AlT), triangles represent litter treatment (LiT) and the cross represents T0.

not significantly different from each other. **AnT** was responsible for a large part of these significant differences of length and dry mass, showing a significant increase (HSD Tukey, $P < 0.001$) through time, and becoming significantly different (HSD Tukey, $P < 0.001$) from **AlT** and **LiT** from Weeks 3 and 4, for length and mass respectively.

3.2. Other variables

a. Length and dry mass

Specimen length ranged from 5.9 to 15.6 mm and dry mass ranged from 0.57 to 8.43 mg (Table 1). Time, treatment and interaction all significantly affected (2-way ANOVA, $P < 0.001$) length and dry mass of *Gammarus aequicauda* during the experiment (Table 3). **AnT** was responsible for a large part of these significant differences of length and dry mass, showing a significant increase through time, and becoming significantly different from **AlT** and **LiT** from weeks 3 and 4, for length and mass respectively (HSD Tukey test, $P < 0.001$).

b. C:N ratios

C:N ratios of *Gammarus aequicauda* ranged from 3.7 ± 0.1 to 4.9 ± 0.3. Values for **AlT** and **LiT** did not vary much during the experiment, unlike **AnT** which went from a mean value of 4.0 ± 0.2 at the beginning of the feeding experiment to a mean value of 4.9 ± 0.3 at Week 3. After Week 3, C:N of *Gammarus aequicauda* in treatment **AnT** decreased slightly to 4.2 ± 0.4 at week 6. Time has no significant impact on measured C:N, but treatment and interaction between time and treatment showed a significant impact (2-way ANOVA, Table 3). Except for week 1, **AnT** was responsible for these significant effects, being significantly different from **AlT** and **LiT** from week 2 to week 4 (HSD Tukey test, $P < 0.001$). C:N values in **AlT** and **LiT** were not significantly different from each other throughout the duration of the experiment (Fig. 3, Table 3).

c. Respiration measurements:

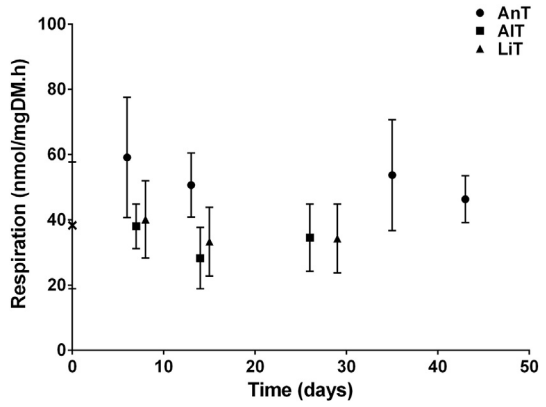

Fig. 4. Evolution of respiration rates (mean ± s.d.) of *Gammarus aequicauda* for the three different food sources during the experiment. Circles represent animal treatment (AnT), squares represent algal treatment (AlT), triangles represent litter treatment (LiT) and the cross represents T0.

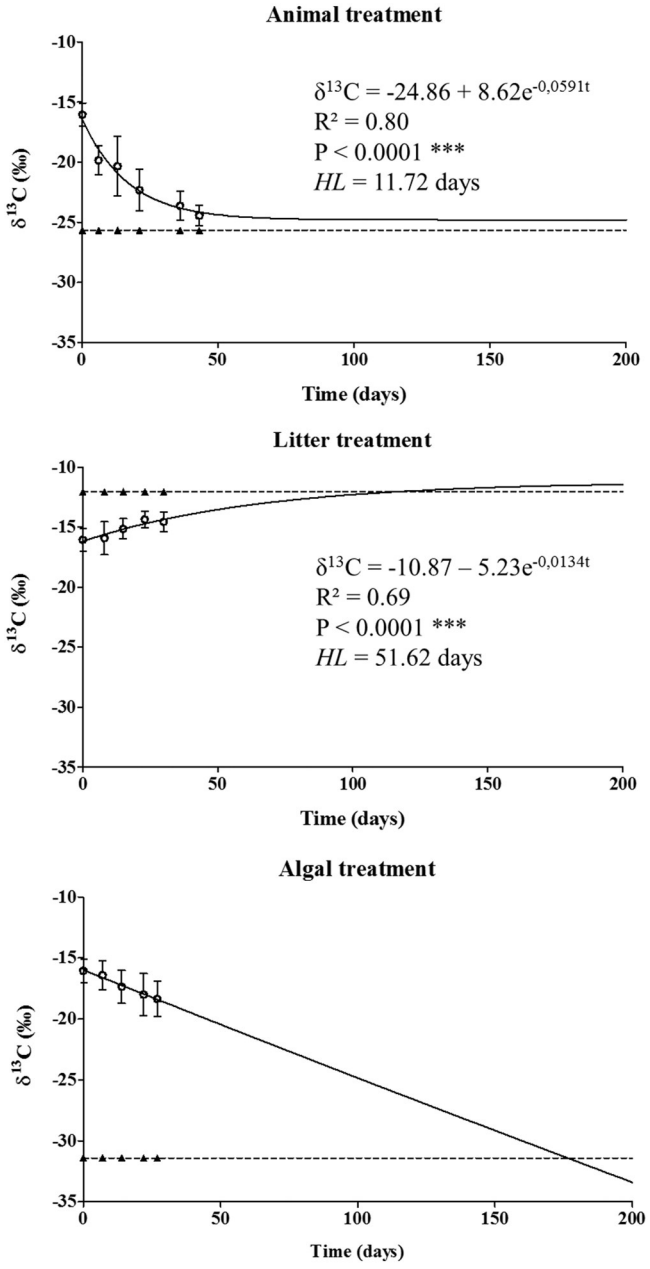

Fig. 5. Evolution of $\delta^{13}C$ values (mean ± s.d.) of *Gammarus aequicauda* for the animal, algal and litter treatments during the experiment. Experimental measures (empty circles) are plotted with error bars representing standard deviation. Black triangles and dotted black lines represent isotopic compositions of food sources. Black lines represent the theoretical one-phase exponential decay curves fitted to the data. Corresponding equations, associated regression coefficients, half-lives and P-values of dedicated F-statistics are also indicated for animal and litter treatment. These values were not possible to calculate for algal treatment.

Table 2
Summary table of every measurement taken on *Gammarus aequicauda* during the whole experiment for the three treatments. Values expressed in mean ± standard deviation.

Summary table (mean ± SD)									
Time	Treatment	Length (mm)	Sex ratio	Ovigerous females (%)	Dry mass (mg)	Respiration (nmol/mgDW/h)	C/N	δC (‰)	δN (‰)
Start of experiment	–	9.94 ± 1.64	0.27	80	2.44 ± 1.14	38.31 ± 19.41	3.99 ± 0.17	−16.06 ± 0.95	4.31 ± 0.61
Week 1	Animal	8.37 ± 1.64	1	100	2.15 ± 1.25	59.13 ± 18.42	4.16 ± 0.25	−19.85 ± 1.15	3.8 ± 0.5
	Algal	9.08 ± 1.72	0.27	62.5	2.02 ± 1.18	38 ± 6.84	3.73 ± 0.08	−16.37 ± 1.16	4.15 ± 0.84
	Litter	8.21 ± 1.38	0.18	75	1.49 ± 0.85	40.11 ± 11.85	4.05 ± 0.62	−16.39 ± 1.73	3.94 ± 0.77
Week 2	Animal	10.41 ± 1.96	0.83	40	2.93 ± 1.64	50.67 ± 9.82	4.38 ± 0.36	−20.98 ± 1.93	3.59 ± 0.56
	Algal	11.46 ± 1.99	2	0	3.42 ± 1.39	28.24 ± 9.4	3.74 ± 0.1	−17.04 ± 1.26	3.67 ± 0.58
	Litter	9.33 ± 1	0.71	0	2.14 ± 0.66	33.29 ± 10.55	3.75 ± 0.11	−15.15 ± 0.8	4.06 ± 0.53
Week 3	Animal	11.43 ± 1.58	0.57	28	4.16 ± 1.57	− ± −	4.86 ± 0.26	−22.34 ± 1.64	3.38 ± 1.28
	Algal	10.74 ± 1.66	1	0	2.96 ± 1.21	− ± −	3,73 ± 0,11	−18 ± 1.62	3.6 ± 1.32
	Litter	10.58 ± 1.13	1	0	3.03 ± 0.68	− ± −	3.77 ± 0.1	−14.37 ± 0.64	4.12 ± 0.44
Week 4	Animal	13.4 ± 1.34	0.71	100	4.92 ± 1.3	53.73 ± 17	4.29 ± 0.36	−23.62 ± 1.16	4.19 ± 0.6
	Algal	9.14 ± 1.52	0.8	0	1.74 ± 0.61	34.53 ± 10.29	3.67 ± 0.1	−18.35 ± 1.34	4.11 ± 0.41
	Litter	8.98 ± 1	0.33	0	2.07 ± 0.64	34.27 ± 10.57	3.73 ± 0.13	−14.57 ± 0.7	4.4 ± 0.4
Week 6	Animal	11.51 ± 1.59	0.72	90	4.86 ± 1.81	46.32 ± 7.16	4.23 ± 0.35	−24.71 ± 0.66	3.85 ± 1.15

Respiration rate measurements presented high individual variability. Respiration ranged from 16.72 to 93.98 nmol $O_2 \cdot mgDW^{-1} \cdot h^{-1}$. Respiration rate was significantly affected by treatment but neither by time nor interaction (Fig. 4, Table 3). *Gammarus aequicauda* feeding on **AlT** and **LiT** always presented lower respiration rates than those feeding on **AnT**. Further analysis showed that **AnT** was significantly different (HSD Tukey test, $P < 0.001$) from **AlT** and **LiT**, and that **AlT** and **LiT** were not different from each other (HSD Tukey test, $P = 0.77$).

3.3. Isotopic composition and turnover rate

All the treatments had very different isotopic compositions from the usual isotopic composition of wild *Gammarus aequicauda* in natural conditions, and were very different from one another in terms of stoichiometry (C:N ratio) and of isotopic composition for ^{13}C and ^{15}N (Table 4).

C and N isotopic compositions of the food sources of the three treatments were constant for the duration of the whole experiment (Table 4). Initial (T0) *Gammarus aequicauda* isotopic values for C and N were −16.1 ± 0.9‰ and 4.3 ± 0.6‰ respectively, reflecting their isotopic composition in the tank. $\delta^{15}N$ values of *Gammarus aequicauda* ranged from 2.3 to 5.7‰ during the experiment and no temporal trend occurred regardless of the treatment. $\delta^{13}C$ values ranged from −13.1 to −25.3‰ and showed important variations depending on treatment and time (Fig. 5). Time, treatment and interaction between the two had a significant influence on $\delta^{13}C$ but not on $\delta^{15}N$ (Factorial 2-way ANOVA; Table 2). $\delta^{13}C$ values in **AnT** differed significantly from both **AlT** and **LiT** from week 1 to week 4 (HSD Tukey, $P < 0.001$). $\delta^{13}C$ values in **AlT** and **Lit** were not significantly different from each other until week 2, but showed a significant difference from week 3 to week 4 (HSD Tukey, $P < 0.001$). (See Fig. 6.)

C isotopic compositions at equilibrium were estimated equal to −25.13‰ and −10.86‰ for amphipods feeding respectively on **AnT** and **LiT** (Fig. 5). *Gammarus aequicauda* feeding on **AnT** were close to the equilibrium value after 6 weeks while those feeding on **LiT** were still far from this value at the end of the 4-week feeding period. It was not possible to fit any one-phase exponential decay curve on **AlT data** (data not shown).

$\delta^{13}C$ turnover rate for **AnT** (0.06 ± 0.01‰·day^{-1}) was higher than **LiT** (0.01 ± 0.04‰·day^{-1}). C half-lives (*HL*), calculated for **AnT** and **LiT** were 12.55 days and 51.62 days respectively.

3.4. Isotopic discrimination factors, TEFs

TEFs were calculated according to the two methods described above (based on true equilibrium values or theoretical asymptote value), for carbon and nitrogen for **AnT** and **LiT**, but not for **AlT** as assimilation of this food source appeared to be far too low for robust TEF calculation (i.e., extremely low/no assimilation of carbon). **AnT** treatment showed

Table 3
Summary table of results of factorial 2-way ANOVAs on length, dry mass, C/N ratio, $\delta^{13}C$, $\delta^{15}N$ and respiration rates measured individually each week for each dietary teatment. MS = mean square between groups.*

	Length				Dry mass				C/N			
Factors and interaction	MS	F	df	P	MS	F	df	P	MS	F	df	P
Time	43.20	12.82	3	***	15.54	9.97	3	***	026	2.96	3	ns
Treatment	22.80	6.77	2	**	20.17	13.77	2	***	6.10	68.80	2	***
Time × treatment	22.57	6.70	6	***	826	5.32	6	***	0.52	5.86	6	***
Residuals			142				142				130	

	$\delta^{13}C$				$\delta^{15}N$				Respiration			
Factors and interaction	MS	F	df	P	MS	F	df	P	MS	F	df	P
Time	19.00	9.57	3	***	125	3.13	3	ns	457.00	2.76	2	ns
Treatment	457.50	230.56	2	***	1.00	2.49	2	ns	3779.00	22.83	2	***
Time × treatment	20.00	10.09	6	***	029	0.71	6	ns	17.00	0.1	4	ns
Residuals			122				126				76	

ns = not significant.
* = 0.01 < P < 0.001 = significant.
** = 0.001 < P < 0.0001 = highly significant.
*** = P < 0.0001 = very highly significant.

Table 4
Summary table of C and N elemental and isotopic (δ13C and δ15N) compositions of the three food sources throughout the experiment and of wild *Gammarus aequicauda*.

Treatment	$\delta^{13}C$ Mean ± SD (‰)	$\delta^{15}N$ Mean ± SD (‰)	C/N Mean ± SD (‰)
Animal	−25.7 ± 0.4	1.2 ± 0.2	4.1 ± 0.3
Algal	−31.4 ± 0.8	3.4 ± 0.9	11.5 ± 0.8
Litter	−12.1 ± 0.1	3.6 ± 0.9	63.3 ± 0.6
Wild *Gammarus aequicauda*	−15.3 ± 1.5	−2.0 ± 0.4	4.1 ± 0.3

a higher ^{15}N TEF value than **LiT** treatment (Table 5). TEFs calculated for ^{13}C for **AnT** and **LiT** were low, around 1‰. No significant correlations were found between $\Delta^{15}N$ or $\Delta^{13}C$ and sex, length, respiration or dry mass.

Non-negligible differences were observed (Fig. 7, A, B and C) when running the SIAR Bayesian Mixing Model on natural *G. aequicauda* data, depending on the different TEF values chosen in our three scenarios. Although the three different scenarios indicated a high proportion of assimilated litter, SIAR run with usual literature TEF values (Scenario A, Fig. 7, A) showed a surprisingly high proportion (95 confidence interval) of copepods in the diet (0–39%) and particularly wide ranges of proportion for all sources. With custom values for *G. aequicauda* calculated for epiphytes feeding experiment (Scenario B, Fig. 7, B). SIAR results showed a lower but still high proportion of copepods in the diet (0–22%). With our new TEFs (Scenario C, Fig. 7, C), SIAR results showed more constrained diet, with high proportions of litter (43–70%) and photophilous algae (28–64%), and very low proportions of copepods (0–3%) and sciaphilous algae (0–7%). Diagnostic matrix plots for all SIAR runs showed lower correlations between sources with the TEF values calculated in this study.

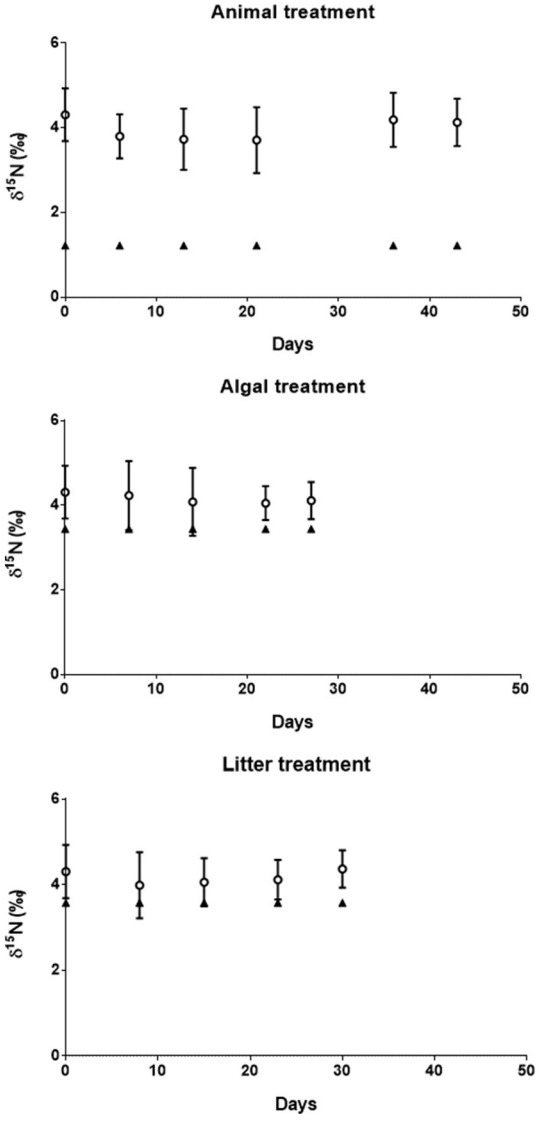

Fig. 6. Evolution of $\delta^{15}N$ values (mean ± s.d.) of *Gammarus aequicauda* for the animal, algal and litter treatments during the experiment. Experimental measures (empty circles) are plotted with error bars representing standard deviation. Black triangles represent isotopic compositions of food sources.

Table 5
Summary table of TEFs calculated for the three different food source treatments to which *Gammarus aequicauda* were subjected, according to the two available methods: (1) theoretical asymptote values; (2) equilibrium values. nc = not calculable.

		Animal food source	Algal food source	Litter food source
From asymptote value	$\Delta\delta^{13}C$ Mean ± SD (‰)	0.6	nc	1.2
	$\Delta\delta^{15}N$ Mean ± SD (‰)	nc	nc	nc
From "equilibrium" individual values	$\Delta\delta^{13}C$ Mean ± SD (‰)	0.9 ± 0.7	nc	nc
	$\Delta\delta^{15}N$ Mean ± SD (‰)	2.9 ± 0.6	nc	1.0 ± 0.4

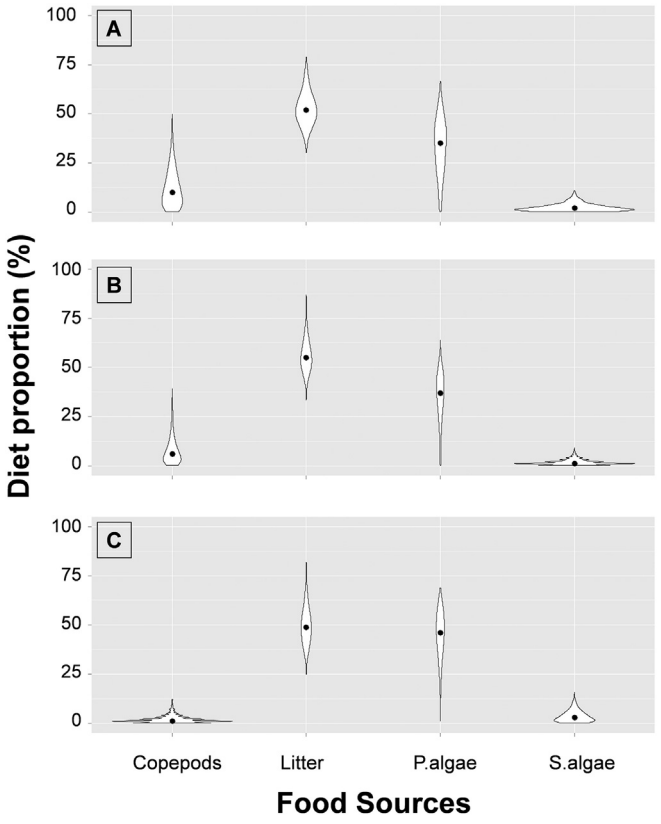

Fig. 7. Violin plot of Kernel density estimates of diet proportions for every potential food source of *Gammarus aequicauda*, based on the first 5000 raw SIAR outputs from the model. Black dots represent median values. A,B,C = SIAR runs with different TEF parameter scenarios. A: SIAR run with identical TEFs from literature for all the food sources (McCutchan et al., 2003); B: SIAR run with identical custom TEFs for all the food sources, calculated for *Gammarus aequicauda* fed with epiphytes (Michel et al., 2015); C: SIAR run with different TEFs for all the food sources, calculated in this study and from Michel et al. (2015). Potential food sources codes: "Copepods" = a pool of harpacticoid copepods representing the meiofauna; "Litter" = dead *P. oceanica* leaves; "P.algae" = a pool of photophilous brown and green algae; "S.algae" = a pool of sciaphilous red algae.

4. Discussion

This study is, to the best of our knowledge, the first to study the effect of food quality on the marine detritivorous amphipod *G. aequicauda*, a dominant species of coastal vegetated ecosystems and macrophytodetritic accumulations. It is also the first to present an experimental determination of isotopic turnover rates and trophic enrichment factors for different food quality.

Death rate and body growth were very variable between treatments. Algal and litter treatment showed a very high death rate (~65% after 3–4 weeks) compared to animal treatment (~30% after 5 weeks) showing that all food sources were not equal in their quality for our Gammaridae.

Respiration rate and C/N of all individuals was measured individually as it may constitute a good proxy of metabolic and physiologic state of a consumer (Cross et al., 2005). Respiration rate measurements may be difficult to interpret due to physiological state and physiological pathways implied (Lampert, 1984; McCue et al., 2005; McCue, 2006). A higher respiration rate for low food quality (e.g., high C:N) was expected (Darchambeau et al., 2003; Hessen et al., 2004). However, in our experiment, metabolic respiration rates were very different from this hypothesis and were significantly higher for *G. aequicauda* fed with the animal treatment. We hypothesized the vegetal treatments did not match at all the nutritive requirements of *G. aequicauda*, leading to the absence of growth, very low respiration and potential lipid reserve consumption. As will be discussed in detail below, amphipods fed with the two vegetal food sources potentially entered into a "standby state" to cope with this un-adapted food.

Respiration rates did not show any clear time pattern in any treatment and presented high individual variability. Food ingestion was impossible to control during this experiment, and as Specific Dynamic Action, SDA, can last for 48 to 72 h depending on the species, it can be assumed that measurements were performed on individuals at really every stage of the SDA cycle (McGaw and Curtis, 2013), explaining a part of the high variability observed. Aquatic invertebrates present a much higher stoichiometry homeostasis than other organisms (Persson et al., 2010), however, our *G. aequicauda* fed with the animal treatment were able to grow and store C reserves as shown by C:N ratio increases during the experiment.

Algal treatment was the only one where C turnover rate was too slow to fit the one-phase-decay curve. This may have been due to a particularly low C assimilation during this experiment. This observation is in contradiction with some studies that observed that marine *G. aequicauda* ingest and assimilate an important amount of algae in natural conditions (Lepoint et al., 2006; Michel et al., 2015). The green alga chosen for this experiment, *F. petiolata*, is very abundant on rhizomes of *P. oceanica* and rocky substrates in coastal Mediterranean ecosystems. As its natural isotopic composition is very different from the composition of wild *Gammarus aequicauda*, it makes it an apparently perfect choice for isotopic turnover experiments. Yet, after experimentation, it was acknowledged that *F. petiolata* can contain udoteal and petiodial, some terpenoid compounds playing a potential role as antimicrobial cytotoxic substances and deterrents for herbivores (Fattorusso et al., 1983; Fenical and Paul, 1984). When present, these compounds are known to drastically impact the consumption of algae by marine fishes (Targett et al., 1986; Paul, 1987). High mortality was also observed during the experimental feeding of gastropods *Lobatus costatus* with an udoteal-enriched food source (Paul and Fenical, 1986). The data presented here show a similar pattern of very low respiration, turnover rate, and high mortality for individuals fed with *F. petiolata*. The *Gammarus aequicauda* fed with that food source presented a low fitness and have potentially entered in a "low activity state", possibly explaining the low respiration rates and growth we observed. Terpenoids could also decrease amount of bacteria present in *G.aequicauda* digestive tracts and thus impact their ability to digest vegetal, poorly digestible compounds (Targett et al., 1986) and, consequently, drastically limit the efficiency of assimilation of this food source.

Litter treatment also appeared below optimal nutritional requirement for our Gammaridae. The reason for this is unlikely to be toxicity of the litter treatment, as it is known that dead leaves constitute a large part of wild *G. aequicauda* diets (Lepoint et al., 2006). Litter was assimilated more slowly than animal treatment ($\delta^{13}C$ turnover rate = $0.013 \pm 0.05‰ \cdot day^{-1}$, $HL = 51.62$ days). C isotopic composition shift took >150 days (Fig. 5) to reach asymptotic value, which might be considered long for organisms living approximately 140 days in experimental conditions (Prato et al., 2006). This treatment was considered in our initial experimental design as the one providing the lowest food quality (in terms of nutritional quality, i.e., C:N ratios) and, therefore, potentially inducing the highest respiration rate to cope with the excess of C (Darchambeau et al., 2003). Nevertheless respiration rates were the same in this treatment than for amphipods fed by *Gammarus* spp. and the expected higher respiration rate pattern was not observed. The *G. aequicauda* fed with litter have also potentially entered in a "low activity state", possibly explaining the low respiration rates we observed. Food elemental composition and stoichiometry of carbon, nitrogen or phosphorus mismatch between diet and consumer can induce lower growth/assimilation rates by the consumer even when food quantity is not a limiting parameter (Söderström, 1988; Frost et al., 2002; Frost and Elser, 2002). It is thus possible that a diet composed only of *P. oceanica* litter is not appropriate for *Gammarus aequicauda*, even if dead leaves constitute a major part (>50%) of its assimilated carbon in the wild (Lepoint et al., 2006; Michel et al., 2015). It can thus be hypothesized that for *G. aequicauda*, as for other amphipod species, a mixed diet is important for its survival (Cruz-Rivera and Hay, 2000) and that it cannot cope with a diet exclusively composed of a poorly digestible and unpalatable food source such as *P. oceanica* dead leaves. However it can also be hypothesized that *P. oceanica* litter can be consumed in quite large amounts if mixed along with various other vegetal, or even occasionally animal food sources, and still achieve nutritional balance (Bernays et al., 1994; Cruz-Rivera and Hay, 2000; Senior et al., 2015). Another hypothesis could be that our experimental protocol eliminated epiphytes, a very important and attractive constituent of litter (Kitting et al., 1984; Lepoint et al., 2006). Indeed, epiphytes (i.e., all organisms living fixed on a plant substrate) may represent a significant source of food for seagrass-inhabiting invertebrates (Alcoverro et al., 1997; Lepoint et al., 2000), and our freeze-drying-grinding protocol might have removed most of the epiphytes, influencing stoichiometry of this treatment and impacting drastically the fitness of the individuals fed with litter treatment. One last hypothesis concerning the low assimilation of litter treatment lies in the experimental protocol itself: coprophagy was avoided throughout the experiment. Coprophagy is a very common strategy in marine invertebrates to cope with poorly nutritive and digestible food (Zimmer and Topp, 2002) and it has been proved that if it is given the choice, *Gammarus roeselii* preferred ingesting fecal biodeposited material rather than the original food source (Gergs and Rothhaupt, 2008; Basen et al., 2013). This coprophagy strategy is also a means of "gardening" bacterial flora on the feces, allowing bacterial development and enzyme action before re-ingestion, increasing food quality and digestibility (Zimmer and Topp, 2002; Córdova-Murueta et al., 2003). As coprophagy was prevented to avoid the bias of feces re-ingestion by a mesh bottom in all microcosms, *Gammarus aequicauda* was not able to re-ingest fecal material and this might be a cause of the extremely low assimilation pattern of litter treatment stated above and the low fitness of individuals fed with this treatment.

The food source with the richest N content (i.e., lowest C:N ratio) in this study, the animal treatment, was assimilated faster than the litter treatment (Turnover rate = $0.059 \pm 0.02‰ \cdot day^{-1}$, $HL = 11.72$ days), potentially due to higher palatability. The turnover and HL values are in the same range as those already published for some amphipod species fed in adapted stoichiometry conditions (Kaufman et al., 2008;

Gergs and Rothhaupt, 2008). The positive response of *Gammarus aequicauda* to the animal treatment is confirmed by the better survival rate and body growth of individuals fed with this treatment compared to the twoother diets. The low ($+0.85 \pm 0.27$) but significant increase of body C:N ratios of gammarids observed during the first three weeks under a carnivorous regime might result from the accumulation of C reserves in response to the increased food quality compared to the diet prior to the experiment (Eriksson-Wiklund, 2002; Fagan et al., 2002; Prato and Biandolino, 2009). The decrease after the third week could be explained by the high proportion of ovigerous females observed at week 4 and week 5 (100% and 90% respectively). Indeed, eggs are very rich in C-rich lipids (Clarke et al., 1985). As eggs were removed before elemental and isotopic analyses, this could explain the decrease of C/N observed. It could also be explained by the excretion of excess N, a physiological process often associated with carnivore diets, and to an increase of TEF (see below) (Vanderklift & Ponsard, 2003; Edwards et al., 2010) These results highlight the very strong response of *Gammarus aequicauda* to a high quality food source, resulting in increased fitness.

The other major result of this study concerns TEF calculations. TEFs for C do not seem to vary very much with food source type and quality, accordingly to previous results (Vanderklift and Ponsard, 2003; Mancinelli, 2012). In contrast, values obtained for TEFs for N varied as a function of diet ($\Delta^{15}N = 1.0 \pm 0.4‰$ for litter treatment and $\Delta^{15}N = 2.9 \pm 0.6‰$ for animal treatment). This confirms the observation in the wild since *G. aequicauda* is considered to be a detritivorous amphipod (Lepoint et al., 2006; Michel et al., 2015), potentially displaying very low TEF values for N (McCutchan et al., 2003; Vanderklift and Ponsard, 2003). The value obtained for litter treatment was much lower than the value obtained for animal treatment, and also lower than 3.4‰ or 2.30‰, the values often applied for isotopic trophic level calculation in literature (Post, 2002; McCutchan et al., 2003). This drastic food-dependent variation highlighted the difficulty of using a unique TEF value based on $\delta^{15}N$ for absolute or even relative trophic level estimation. As TEFs of *G. aequicauda* for ^{15}N vary with food source type, showing typical carnivore TEF when fed with animal treatment and detritivore TEF when fed with litter treatment (Vanderklift and Ponsard, 2003; Michel, 2011; Mancinelli, 2012), it can be hypothesized that TEFs are more dependent on food source isotopic composition and C/N ratio, resulting metabolic rate and isotopic routing patterns rather than the general metabolism of a species (Del Rio et al., 2009; Caut et al., 2010; Remien, 2015).

SIAR model runs with different TEF parameters from literature and from this study showed that TEFs are of major importance when analyzing stable isotope data for delineating diets of detritivores. Diet proportions given by SIAR run with TEFs from this study are in accordance with previous studies focusing on gut content analysis and isotopic studies of *G. aequicauda* (Lepoint et al., 2006; Michel et al., 2015). Indeed, TEFs calculated here prevent the overestimation of a hypothetical copepod food source, one never found in the gut contents of any individual, showing the importance of precise species and food-source specific TEFs to reject improbable food sources. The SIAR run with TEFs calculated after this study also showed that SIAR performed better than with literature TEFs. Indeed "diagnostic matrix plots" (model robustness evaluation tool) showed lower correlations between sources when using our TEFs than when using literature TEFs. This showed that the SIAR mixing model discriminated much more efficiently the different food sources using these new TEFs. Using global TEFs widely found in the literature can induce biased diet estimations and poor result robustness.

5. Conclusions

This study thus showed that for *Gammarus aequicauda*, TEFs for N experienced a drastic food-dependent variation. This confirmed the need to calculate experimentally TEFs for every potential food source of an organism before trophic level estimation, and also before the use of mixing models and robust interpretation of trophic data. It also proved the major influence of food elemental composition and stoichiometry on fitness, growth, reproduction, respiration rate and isotopic turnover in marine *G. aequicauda*. A food source of low C/N, better quality and adapted stoichiometry is more efficiently assimilated, resulting in higher fitness and faster growth and isotopic turnover rate for individuals. On the contrary, low quality food sources lead to much slower assimilation, growth and isotopic turnover rates, resulting in lower fitness. Nevertheless, under natural conditions, *G. aequicauda* found in the *P. oceanica* exported litter accumulations are able to cope with this low palatability food source, probably by mixing it with other algal and sometimes animal material.

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