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RESEARCH ARTICLE

Consumption and performance responses of the amphipod *Echinogammarus berilloni* change during laboratory incubation

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Abstract – Microcosms try to mimic the behaviour of natural ecosystems; however, differences in experimental conditions reduce comparability among studies. A standard incubation duration may increase comparability between experiments without losing realism in the response. Some response variables can respond fast, acting as early warning signals of other, more important oncoming changes. In this experiment, we test the effects of resource quality and incubation duration on the performance of the amphipod *Echinogammarus berilloni*. Individuals were fed on five leaf species of contrasting quality and we measured their consumption rate, death rate, RNA:body mass (RNA:BM), growth rate, mass body condition and lipid body condition over time. We predicted that (i) resource quality would affect the response variables, (ii) consumption rate and RNA:BM ratio would act as early warning signals of oncoming changes in growth or death rates, and (iii) the inter-individual variation would gradually decrease with time. Resource quality was positively related to consumption rates, although it did not correlate to nutrient concentration and toughness of the materials. Amphipod body mass condition changed with diet, animals feeding on oak and beech showing the lowest values. Death rate, growth rate, RNA:BM, and lipid mass condition did not change with food resources. Consumption, growth rate and mass body condition changed with time. Moreover, consumption, mass body condition and RNA:BM significantly interacted with incubation duration. Variability among individuals in consumption and growth rate decreased with time, as predicted. Our results pointed that special care should be taken when comparing microcosm experiments with different incubation duration.

Keywords: Microcosm experiment / incubation duration / food resource quality / consumption / amphipod performance

1 Introduction

Freshwater ecosystems are affected by multiple stressors derived from global environmental change, including nutrients and pollutants, land use changes and geomorphological disturbances (Sabater *et al.*, 2018). Establishing the effects of those stressors is a major scientific challenge that has been approached from diverse perspectives. On one hand, observational field studies (*e.g.*, Ponsatí *et al.*, 2016) can offer information on the effects of these stressors on real conditions, but are strongly limited in the attribution of causality. Manipulative field experiments, especially those following a BACI design (*e.g.*, Arroita *et al.*, 2016), overcome some of the problems of observational studies, but can face the idiosyncratic nature of the study sites. On the other extreme, standardized assays, such as those common in ecotoxicology, use standard incubation water or commercially available organisms. Those assays are highly replicable and allow attributing causality as well as understanding the mechanisms of stress and resistance. Procedural details, though, can strongly affect the outcome of toxicological assays, and thus, authors strive for a standardization of their procedures. For instance, the "OECD Guidelines for the testing of chemicals" is a collection of internationally agreed testing methods for the selection and ranking of candidate chemicals in terms of toxicity and effects on natural systems (OECD, 2019). Following these guidelines, a variety of studies focus on understanding how each impact affects specific taxa and on how different stressors are ranked (*e.g.*, Dahl *et al.*, 2006; Lebrun *et al.*, 2017). However, incubation conditions in these standardized assays are usually far from real ecosystems and thus, responses can often be hardly extrapolated to the natural world. The model ecosystem approach (Landner *et al.*, 1989)

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stays in between standardized artificial assays and field experiments and can be referred to as microcosm or mesocosm studies. The aim of the model ecosystem approach is to mimic the target ecosystem (*e.g.*, using water, food and organisms collected in the target system) in order to observe its response to a potential stress. Nevertheless, even if the number of published microcosm assays based on model ecosystem approach is growing fast, the comparability among them is small, as experimental conditions often differ (see Rasmussen *et al.*, 2012; Zubrod *et al.*, 2015).

It has already been shown that temperature (Menéndez *et al.*, 2003), food quality (LeRoy *et al.*, 2007; Lecerf and Chauvet, 2008; Casas *et al.*, 2013), shredder taxa (Taylor and Chauvet, 2014) and mesocosm size (Uiterwaal and Delong, 2018) affect the responses and therefore, the comparability between those studies is not straightforward. The selection of response variables is also critical in microcosm experiments, as variables differ in the time needed to find an effect. Some variables respond fast to changes in their environment and thus, can act as early warning signals of oncoming biological effects at higher levels of biological organization. In stream ecosystems, leaf litter quality traits determine the breakdown rates (Lecerf and Chauvet, 2008; Hladyz *et al.*, 2009; Schindler and Gessner, 2009) and the quality and quantity of ingested resource will have direct consequences on consumer performance. In recent years, it is becoming increasingly common to assess individual metabolic status by using RNA-based measurements. The rationale for these measurements is the fact that the RNA content of whole organisms is primarily ribosomal RNA. Consequently, concentration of RNA is directly related to the protein synthesis (Elser *et al.*, 2000). DNA, on the other hand, is accepted as an surrogate of number of cells (Buckley *et al.*, 1999). Consequently, RNA:DNA, or RNA:biomass (RNA;BM) ratio is a proxy of growth and metabolic status and has been shown to be a good and very responsive indicator of nutritional condition (Stuck *et al.*, 1996; Wagner *et al.*, 1998; Kainz *et al.*, 2010). Individual growth and body condition also respond to food resource quality (Larrañaga *et al.*, 2014) but the time required to detect a response is supposed to be longer than for RNA-based ratios. It is then expected that each variable will need more or less time to respond to an environmental condition, and thus, will provide meaningful information only after different microcosm incubation times.

Our objective was to test the effects of leaf litter quality and incubation duration on the performance of the freshwater shredder invertebrate *Echinogammarus berilloni* (Catta.). The amphipod was fed with five leaf species of contrasting quality and analysed the responses (consumption rate, death rate, RNA:BM, growth rate mass body condition and lipid body condition) over time. Instead of aiming at explaining the mechanisms for the differences in the response variables with the resources offered, we wanted to test the consistency of the patterns among the various sampling days in an experiment lasting one month. We predicted that, (i) food resource quality would affect amphipod performance, (ii) consumption rates and RNA:BM ratios would act as early warning signals and precede changes of amphipod growth rate and body condition, which would respond later in time, and (iii) inter-individual variation would gradually decrease with time due to constant microcosm conditions and, as a consequence, the power to find differences among treatments would increase with time.

2 Materials and methods

2.1 Leaf litter and consumer

We used leaf litter of five common riparian tree species in European streams, chosen because they differ on traits such as nutrient content and toughness (Boyero *et al.*, 2017): *Alnus glutinosa* ((L.) Gaertn), *Corylus avellana* (L.), *Fraxinus excelsior* (L.), *Fagus sylvatica* (L.) and *Quercus robur* (L.). Leaves were collected near Bilbao (North Iberian Peninsula 43°15′47″N 2°56′06″W) after abscission in October 2015. Disks (12 mm diameter) were punched using a cork borer, air dried and stored in 24-well culture plates in a dark dry place until further use. Leaf litter elemental analyses were performed in five replicates per species after grinding. Nitrogen and carbon content were analysed with Eurovector EA 3000 CNH analyser (Eurovector, Milan Italy). Phosphorus was analysed after acid digestion (0.5 g dry mass in 15 mL of HNO_3 69%) in an optical emission spectrophotometer with inductively coupled plasma (ICP-OES, Horiba Jobin Yvon, Activa). Leaf toughness was measured in 15 replicates per species using a penetrometer with a 0.49 mm^2 area steel rod (Boyero *et al.*, 2011).

As consumer macroinvertebrate we chose the amphipod *Echinogammarus berilloni* (Catta.) as it is one of the most abundant species in the streams of the region (Larrañaga *et al.*, 2009a). Specimens were kick-sampled (500 μm mesh size) in Guriezo (Adino Stream, 43°20′27″ N 3°20′20″ W), individually enclosed in 30 mL containers with pores to allow water exchange, and carried to the laboratory immersed in transport boxes filled with stream water. Animals with a first thoracic segment length between 0.24 and 1.26 mm were selected (4.59 and 17.08 mm in total body length), but breeding females were discarded as they show a clearly distinct biochemical composition (Larrañaga *et al.*, 2009b). The animals were acclimatized in a controlled temperature room (15 °C) without food for 3 d prior to the experiment.

2.2 Experimental setup

We prepared microcosms, consisting of test tubes (2 cm diameter and 20 cm long) with 40 mL of filtered (100 μm nylon mesh) water from the same stream where the amphipods were collected and five leaf disks of one species. The treatments were named according to the leaf disks in the microcosm (*i.e.*, *Corylus*, *Alnus*, *Fraxinus*, *Quercus* and *Fagus*). Despite the small dimensions of the test tubes, the animals were able to move freely, we did not observe accumulation of leachates, and all tubes were aerated by means of direct bubbling. Prior to the experiment, the groups of five leaf disks were conditioned in stream water at the laboratory microcosms for 15 days at 15 °C, 12:12 light photoperiod, under constant aeration, and the water renewed at day 7. The conditioning intended to reduce the amount of leachable compounds and promote microbial colonization. Water was renewed before adding an individual of *E. berilloni* to each microcosm (20 replicates × 5 leaf species × 5 sampling times), and the rest of experimental conditions were kept as during conditioning. The experiment

lasted for 32 days; water was renewed at days 8, 16 and 24 to avoid accumulation of deleterious substances and shortage of nutrients for microbes.

Water samples were collected at each renewal (one sample taken from the container with new water), filtered through pre-combusted glass-fibre filters (Whatman GF/F, 0.7 μm pore size) and stored at −20 °C until analysis. The concentration of soluble reactive phosphorus (SRP) and ammonium in water was determined colorimetrically on a UV-1800 UV-Vis Spectrophotometer (Shimadzu Corporation, Kyoto, Japan) with the molybdate method and the salicylate method (APHA, 1998), respectively. The concentrations of chloride (Cl), sulfate (SO_4) and nitrate (NO_3) anions were determined with capillary ion electrophoresis (Agilent CE) (Environmental Protection Agency, 2007). Twenty replicates per treatment were destructively sampled at days 2, 4, 8, 16 and 32.

2.3 Response variables

We measured six response variables, leaf litter consumption (related to ecosystem functioning) and death rate, growth rate, mass body condition, lipid condition and RNA:Body mass ratio (related to the performance of the amphipod). Death rate was expressed as the natural logarithm (ln) of the difference between initial and final number of individuals alive per treatment and sampling day, standardized with incubation duration. Dead animals were not analysed, so reported values are always of individuals alive when they were collected.

2.3.1 Consumption rates

The five air-dried leaf disks of each microcosm were weighed before conditioning (air dry mass). At each sampling day, disks were retrieved from the microcosms, oven dried (70 °C, 72 h) and ashed (500 °C, 4 h) to calculate the ash free dry mass (AFDM). Mass loss during conditioning was measured and used to estimate the initial AFDM. Consumption rates were calculated from the difference between estimated initial dry mass and final weighed dry mass of the five disks in each microcosm, and standardized by the geometric average body mass of the individual and total incubation time (mg AFDM mg BM^{-1} d^{-1}). An additional set of nine microcosms per leaf species were incubated without amphipods to estimate mass loss due to microbial decomposition rates and therefore correct consumption rates.

2.3.2 Growth rate

Amphipods were photographed at the beginning and end of the experiment through a binocular microscope (Leica M165FC, Wetzlar, Germany). The dorsal length of the first thoracic segment (*DL*) was measured from photographs using the "Leica Application suite V4" program (LAS V4.1). Initial and final total body lengths (*BL*) were calculated using equation (1) (Flores *et al.*, 2014a). Instantaneous growth rate (*IGR*) for each individual was calculated using equation (2) where t is the incubation duration, BL_t is the body length at time t and BL_0 is the initial body length (Flores *et al.*, 2014a).

$$BL = 14.458 \cdot DL - 0.110; \text{ (mm)} \quad (1)$$

$$IGR = (\ln(BL_t) - \ln(BL_0)) / t; \text{ (mm d}^{-1}\text{)} \quad (2)$$

2.3.3 Body condition: mass and lipids

By means of the equation (3) (Flores *et al.*, 2014a) the body mass (BM) was estimated at the end of the experiment, and this value was used as the descriptor for each invertebrate dry mass in the experiment.

$$BM = 0.8213 \cdot BL - 4.3025; \text{ (mg)} \quad (3)$$

Half of the sampled amphipods (10 replicates per leaf species and sampling time) were freeze dried and weighed. We compared the dry mass obtained this way with the expected body mass calculated from equation (3) to be used as a proxy of mass body condition. The mass body condition was logaritmized (log10) before statistical analyses; a value of 0 indicates that the weighed mass exactly matches what is expected from the body length, while positive and negative values show individuals heavier or lighter than expected, respectively. Total individual lipid content was measured as a proxy of energy reserves stored. Lipid amount was quantified in freeze-dried entire individuals. Extraction was performed by incubating individuals in diethyl ether at 4 °C for 2 d and sonicated twice for 15 min (Bandelin Sonorex; Bandelin Electronic GmbH and Co., Berlin, Germany). Extraction was followed by digestion with H_2SO_4 (200 °C) and quantification by spectrophotometer. Cholesterol was used as standard. Lipid body condition was expressed as μg lipid mg BM^{-1}.

2.3.4 Nucleic acid content

The rest of the individuals (10 replicates per leaf species and sampling time) were stored in RNAlater solution (Ambion) until further nucleic acid analysis. Microplate fluorometric high-range assay with Ribo-Green was performed to quantify RNA after extraction with *N*-laurylsarcosine followed by RNase digestion as described in detail by Gorokhova and Kyle (2002). Fluorescence measurements were performed using a Tecan GENios microplate reader (Cavro Scientific Instruments, Sunnyvale, filters: 485 nm excitation and 520 nm emission). Measurements were performed in black solid flat-bottom microplates, scanned during 0.2 s $well^{-1}$, with 10 measurements per well at constant temperature (37 °C). Measured RNA concentrations were expressed as μg RNA mg BM^{-1}.

2.4 Data analysis

The critical alpha value for all analyses was 0.05 and all statistical analyses were conducted using R statistical software (version 3.1.2, R Core Team, 2018). A high degree of correlation was observed among leaf traits (minimum R^2 in pairwise Pearson correlation: 0.67; Supplementary material, Table S1). Thus, we integrated all variables in an index of leaf litter quality, calculated by means of equation (4), where T is the average of the measured value for the trait (C:N, C:P and toughness) and n the number of measured traits.

$$\text{Quality index} = 1 - ((T_1/(n \cdot T_{1(\max)})) + \ldots + (T_n/(n \cdot T_{n(\max)}))) \quad (4)$$

We fitted Gaussian models (Madsen and Thyregod, 2010; Zuur and Ieno, 2010) to test for the effect of leaf species (1st prediction) and incubation duration on the response variables (consumption, death rate, RNA:BM, growth rate, mass body condition and lipid body condition). For parameter estimation,

Table 1. Nitrogen and phosphorus molecular elemental ratios and leaf toughness (kPa) of the leaf species used in the experiment (mean values ± standard error). Different superscript letters indicate significant differences among leaf species. Significant *p*-values are in bold.

Leaf litter sp.	*Corylus*	*Alnus*	*Fraxinus*	*Fagus*	*Quercus*	*p*-value
C:N (n = 5)	19.92 ± 0.38^{a}	14.05 ± 0.07^{b}	22.76 ± 0.66^{c}	52.70 ± 0.11^{d}	37.39 ± 0.40^{e}	<**0.001**
C:P (n = 5)	838.69 ± 11.20^{a}	1141.87 ± 20.65^{b}	1128.87 ± 31.03^{b}	2073.13 ± 69.22^{c}	3259.20 ± 84.56^{d}	<**0.001**
Toughness (n = 15)	57.81 ± 3.09^{a}	70.47 ± 4.96^{ab}	81.52 ± 6.51^{b}	107.43 ± 5.04^{c}	123.48 ± 8.53^{c}	<**0.001**
Leaf quality index	0.632	0.604	0.520	0.165	0.097	

Table 2. Results of the ANOVA test for the effect of leaf species, experiment duration, and their interaction on the performance of the amphipod. Significant *p*-values are in bold.

		Consumption		Death rate		RNA:BM		Growth rate		Mass body condition		Lipid body condition	
Source of variation	DF	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
Species	4	62.76	<**0.001**	1.42	0.242	1.15	0.335	1.52	0.195	3.57	**0.007**	2.12	0.079
Day	4	8.46	<**0.001**	0.48	0.491	1.12	0.349	3.42	**0.009**	4.24	**0.002**	0.55	0.698
SpeciesxDay	16	1.83	**0.024**	0.57	0.682	1.72	**0.044**	0.67	0.820	1.99	**0.015**	1.01	0.442

generalized least squares (Pinheiro and Bates, 2000) were used, via the gls() function of the package nlme (Pinheiro *et al.*, 2018). The fixed structure of the model included leaf species (fitted as a discrete explanatory variable, with five levels, *Corylus*, *Alnus*, *Fraxinus*, *Quercus*, *Fagus*), incubation duration (fitted also as a discrete explanatory variable, with five levels: day 2, 4, 8, 16 and 32) and the interaction between both sources of variation. AIC comparisons revealed the need to add a variance structure to the models to deal with heteroscedascity, which allowed different variances per day (varIdent(form = ~1|Day)). No heteroscedascity was observed among leaf species. As both consumption and growth rates presented leptokurtic distributions, data were normalized via the gaussianize() function of the package LambertW (Goerg, 2011). To check how each response variable responded over incubation duration (2nd prediction) analyses of amphipod consumption and performance were carried out first using data from all sampling days and later with data from each sampling day separately. When using data from all sampling days, generalized least squares gls() were used where the fixed structure of the model consisted on leaf species (fitted as explanatory variables) and incubation duration included as a block factor. We added a variance structure to the models to deal with heteroscedascity, which allowed different variances per day (varIdent(form = ~1|Day)). No heteroscedascity was observed among the different leaf species. When using separate data from each sampling day, linear models lm() were used where the fixed structure of the model consisted on leaf species (fixed as explanatory variable). As both consumption and growth rates presented leptokurtic distributions, data were normalized as explained above.

Variance reduction over time was tested with linear models lm() from package nlme (Pinheiro *et al.*, 2018) for each response variable (3rd prediction). For the different response variables, we computed first the standard error for the variables of interest for each leaf species and day, and then we created models with the standard error as response variable, incubation duration fitted as a discrete explanatory variable and species as random factor.

3 Results

Stream water used over the experiment ranged in phosphorus concentration from 4.7 $\mu g\,L^{-1}$ to 28.2 $\mu g\,L^{-1}$ and in ammonia concentration from 35.6 $\mu g\,L^{-1}$ to 106.7 $\mu g\,L^{-1}$. The mean values of Cl, SO_4 and NO_3 were 11.4 ± 1.2 (SE) mg L^{-1}, 7.9 ± 1.0 mg L^{-1} and 2.4 ± 0.3 mg L^{-1} respectively. No temporal pattern was observed for water chemistry.

The five leaf litter species significantly differed in stoichiometry (molar C:N, C:P, C:K ratios), in toughness and, consequently, in their overall leaf quality index (Tab. 1). *Corylus* showed the highest quality index, with the highest concentration of phosphorus per carbon and lowest toughness. *Alnus* and *Fraxinus* only differed in nitrogen content, higher in the former species. The leaf species with the worst quality was *Quercus*, with the lowest concentrations of phosphorus per carbon and the highest toughness (Tab. 1).

Consumption rates were affected by leaf species and incubation duration ($p < 0.001$, Tab. 2, Fig. 1). Consumption rates increased significantly with leaf quality (Supplementary material, Table S2; $p < 0.001$). *Fraxinus* was the most consumed leaf species, with 0.594 mg AFDM mg BM^{-1} d^{-1} consumption at day 2 and 0.235 mg AFDM mg BM^{-1} d^{-1} at day 32. The less consumed leaf species were *Quercus* at day 2 and *Fagus* at day 32, with mean consumptions of 0.084 and 0.079 mg AFDM mg BM^{-1} d^{-1} respectively. Overall, the consumption rate decreased over time for all leaf species. Most importantly, there was a significant interaction between leaf

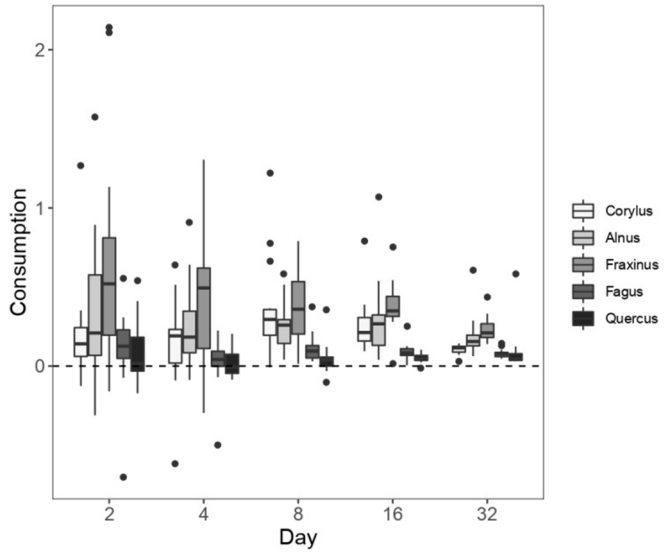

Fig. 1. Consumption rate (mg AFDM mg BM^{-1} d^{-1}) of the five leaf species along time (median and interquartile range, IQR ± 1.5*IQR; the dots represent outliers). Leaf species are ordered by leaf quality from light to dark.

species and incubation duration ($p = 0.024$, Tab. 2, Fig. 1), showing that the pattern of consumption of different leaf species was altered along the experiment.

Fourteen percent of the individuals died during the experiment, but neither the leaf species nor the incubation duration affected death rate (Tab. 2; Supplementary material, Table S3). The RNA:BM ratio was affected by the interaction between leaf species and incubation time ($p = 0.044$, Tab. 2, Fig. 2) showing different patterns along time for individuals fed with different resources. Leaf species did not significantly affect growth but incubation duration did ($p = 0.009$, Tab. 2, Fig. 2). The overall amphipod mass body condition was affected by leaf species, incubation duration and the interaction between both factors ($p = 0.015$; Day, Tab. 2, Fig. 2), meaning that mass body condition of individuals fed with different leaf species ranked differently over the duration of the incubation. Individuals fed with *Fagus* presented the best mass body condition at the second sampling day and the worst at day 16. However, amphipod lipid body condition was neither affected by leaf species nor incubation duration, and the interaction was also non-significant (Tab. 2, Fig. 2).

Consumption rate was significantly affected by resource quality from the second day on and it showed a very consistent pattern, as significantly varied with leaf species either when considering all the incubation times or when taking into account the different sampling days separately (Tab. 3). However, RNA:BM did not respond to the resource quality at any of the sampling days. Regarding amphipod body condition, the mass body condition was significantly affected by leaf species when all the data was gathered, however, this significance was only maintained at day 16 when analysing sampling days separately. Lipid body condition and growth rates were not affected by leaf species neither analysing all data together nor at separate sampling days. Results at sampling day 16 were similar to those using all the sampling days (Tab. 3).

The standard error decreased over time for growth rate and consumption (Growth rate, $p < 0.001$; Consumption, $p = 0.003$, Fig. 3). The standard error of growth rate significantly decreased from Day 2 (0.019) to Day 4 (0.009), Day 8 (0.004), and Day 32 (0.001). This reduction was statistically significant from day 2 to 16 for consumption, which decreased from 0.072 to 0.025 and then levelled constant until day 32 (Fig. 3). The rest of the measured variables did not show any reduction of the standard error.

4 Discussion

Organic matter consumption is widely measured in the scientific literature due to its importance to support terrestrial and aquatic food webs and the significant contribution to total carbon fluxes (Battin *et al.*, 2009). Our results, as many others before, showed that resource quality affects consumption rates. However, *Fraxinus* was the most consumed leaf species despite not being identified as the highest quality resource by our quality index. Similarly, in the literature, the correlation between food quality and consumption rates is not always straightforward (Huang *et al.*, 2006), which may suggest that consumption responds to non-measured variables, like the concentrations of tannins. Contrarily, consumers can also feed more on poorer materials, thus compensating for the lack of nutrients, which might have happened in our study (Flores *et al.*, 2014b). The differences in leaf quality, and in consumption rates, were expected to significantly affect amphipod performance however, most of the measured variables were not significantly affected. For example, it has been demonstrated that nutrient content can limit consumer growth (Mulder and Elser, 2009; Kendrick and Benstead, 2013). However, in the present study the effect of resource quality on gammarid growth rate was weak, as it was only significant when using all data together. Other previous, slightly longer, studies with contrasting resources have reported changes in growth rates of gammarids that can be attributed to resource quality (Graça *et al.*, 2001, Larrañaga *et al.*, 2014), which can highlight the need to perform longer experiments to be able to see a response of this variable, at least when dealing with relatively long living species like gammarids. The increase in RNA concentration has been interpreted as a proxy of growth rate (Dahl *et al.*, 2006) and also as a sign of activation of the defence system and repair processes (Maltby, 1999). However, contrary to expected, in the present experiment the RNA:BM ratio did not respond to the leaf species consumed. Mass body condition, which is directly linked to survival rates (Peig and Green, 2009) and correlates with body protein content (Larrañaga *et al.*, 2010), has been also described to respond to resource quality (Larrañaga *et al.*, 2014). In the present study, leaf species significantly affected the mass body condition, individuals fed with high resource quality (*Corylus*, *Alnus* and *Fraxinus*) presenting overall higher mass body condition. Similarly, resource availability and quality have been reported to affect total lipid body condition (Øie and Olsen, 1997) and, therefore, have consequences on growth and reproduction (Glazier, 2000); nonetheless, lipid body condition in our amphipods was not significantly affected by the resource gradient. The lack of the response could be related to the low percentage and the

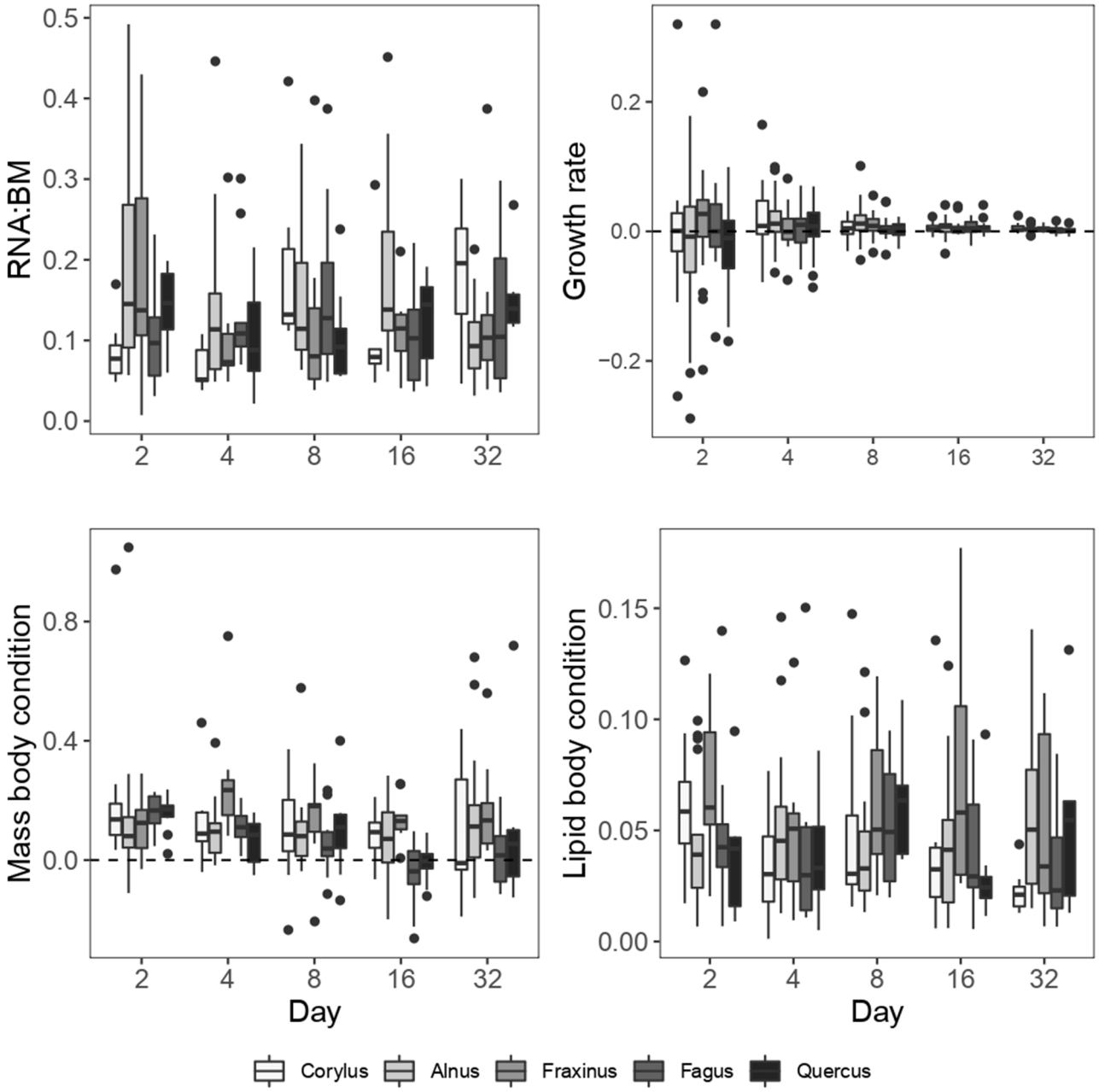

Fig. 2. RNA:BM (μg RNA mg BM^{-1}), growth rate (mm d^{-1}), mass body condition and lipid body condition (μg lipid mg BM^{-1}) of consumers feeding on the five leaf species along time (median and interquartile range, IQR ± 1.5*IQR; the dots represent outliers). Leaf species ordered by leaf quality from light to dark.

high inter-individual variability of the total lipid mass. In line with this, Larrañaga *et al.* (2014) did not found a significant response to resource quality for the amount of lipids after 3 and 6 weeks of incubation with resources varying in quality as much as in our study. Overall, the much larger differences for consumption rates than for the performance of the consumer supports that the amphipod was using compensatory mechanisms when facing materials of different quality.

We predicted that consumption rate and RNA:BM ratio would act as early warning signals and would precede responses occurring later in time, such as changes in growth or body condition. In our experiment consumption rates significantly responded to the resource quality by the second sampling day and the response was consistent over the experiment. However, differences tend to increase with the duration of the experiment (Haber, 1924), which is also true for experiments that compared consumers feeding on resources of contrasting quality (Canhoto and Graça, 1996). The use of consumption rate as early warning signal when testing the effect of resource quality can be quite straightforward because individual feeding preferences/decisions can partially affect their performance. However, when testing other stressors, such

Table 3. ANOVA results for leaf species and day as block factor (only when analysing all sampling days) for consumption and amphipod performance. The effect of leaf species is shown for all sampling days and for separate sampling days. Significant *p*-values are in bold.

	All samplings				Day 2		Day 4		Day 8		Day 16		Day 32	
	Day		Species											
	F-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
Consumption	8.03	<**0.001**	60.86	<**0.001**	7.92	<**0.001**	10.67	<**0.001**	9.53	<**0.001**	21.89	<**0.001**	16.85	<**0.001**
RNA:BM	1.10	0.358	1.37	0.246	2.28	0.075	1.78	0.150	0.84	0.509	2.16	0.090	2.45	0.062
Growth rate	3.45	**0.008**	1.53	0.193	0.88	0.480	0.61	0.655	1.69	0.159	0.08	0.989	1.18	0.326
Mass body condition	3.72	**0.006**	3.62	**0.007**	1.95	0.115	2.48	0.055	0.44	0.776	5.14	**0.001**	0.71	0.589
Lipid body condition	0.55	0.697	2.14	0.076	1.31	0.278	0.69	0.604	0.16	0.957	1.15	0.345	1.85	0.138

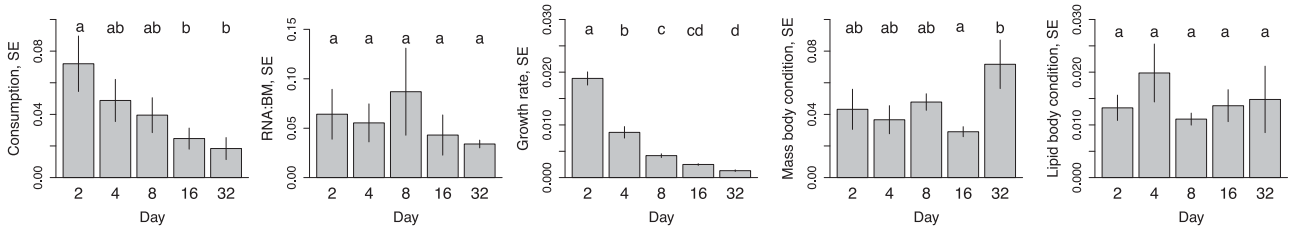

Fig. 3. Consumption, RNA:DM, growth rate, body mass condition and lipid body condition standard errors taking into account the five leaf species together along time (mean ± SE). Different superscript letters indicate significant differences among incubation durations.

as exposure to contaminants, other physiological variables, as RNA:BM ratio, might respond earlier or at the same time that consumption rate (*e.g.* Vrede *et al.*, 2002 and Pietrzak and Bednarska, 2010). The expected changes in RNA:BM ratio within the first incubation days were not observed in our individuals. Therefore, the RNA:BM cannot be used as early detection tool in this particular case, which can partially be a consequence of the larger lifespan of *Echinogammarus* (over 6 months, Maranhão and Marques, 2003) compared to the species that other researchers have used as sentinels (*e.g. Daphnia* and *Monoporeia*). Finally, we expected consumption and RNA:BM to precede later responses in growth rate and body condition that would occur as a consequence of different quality resource consumption. However, growth rate and lipid condition did not respond when taking into account separate sampling days or analysing all data together. Individual growth rates were calculated from measurements of the first thoracic segment length at the beginning and the end of the experiment. This measurement has a rather large error, which together with the relatively small sample size ($n = 20$ individuals per leaf species and sampling day), could have prevented significant responses even if there were clear non-significant patterns.

We observed incubation time to be an important factor affecting the response. As the materials were only offered at the beginning of the experiment, they were surely conditioned by fungi along the incubation, making them more palatable with time. Counter intuitively, consumption rates decreased with time, an observation that has been previously attributed to reduced energy requirements under laboratory conditions (Hessen *et al.*, 2013). Additionally, although the main nerves of the leaves were avoided when punching the discs, it is possible that individuals first ate the highest quality parts, thus reducing the overall quality and decreasing consumption of the leftovers. Most importantly, for consumption, RNA:BM and mass body condition, incubation time significantly interacted with leaf type. The significant interaction between leaf species and incubation time in our experiment indicates that responses changed with time, which other authors have attributed to the changes of the chemical properties of the resources over time (Aßmann *et al.*, 2011). These changes can be partly explained by the disparate colonization rates that microorganisms exhibit when facing leaf litter with contrasting traits (Gessner and Chauvet, 1994). The interaction between microbial colonisation and the intrinsic quality of the leaf litter might have regulated the changes in quality of the remaining materials, and thus, its consumption. In highly consumed leaf litter, as alder or common ash, microbial colonization is fast (Gessner and Chauvet, 1994), and as a consequence, the quality difference between different parts of the leaf litter larger than in less preferred materials. The remaining mass in these highly consumed materials might have become worse faster, which can explain the reduction of their consumption with time. For the low quality materials, as oak or beech, the microbial colonization is much slower (Gessner and Chauvet, 1994), and thus, the differences between the different patches of the same leaf smaller. The more constant consumption rate of these materials by the amphipod supports this idea. Regarding amphipod physiological responses, the interaction between leaf species and incubation duration significantly affected mass body condition and RNA:BM ratio. Those variations in the

physiological status and nutritional condition have been also reported for other crustaceans (Wu and Dong, 2002; Comoglio *et al.*, 2005), and might reflect switches in the use of energy from some type of reserves to another. The observed significant interactions, due to shifts in requirements or any other natural alteration of the animals along the experiment, showed the intrinsic difficulty when comparing studies performed with different incubation duration and highlight the importance of time-standardized experiments.

The high variability on the responses observed at the beginning of experiments could be a consequence of the elevated intraspecific variability present in natural populations. The main food source for stream communities, allochthonous organic matter, is distributed in patches in the field (Webster *et al.*, 2001) and microhabitats may differ greatly in the quality of the material retained (Flores *et al.*, 2013). For our study, the amphipods were kick-sampled at different microhabitats along the reach. As a consequence, collected individuals may have presented very diverse physiological status. Additionally, individual life stage also affects macroinvertebrate body condition and nutritional status (McCahon and Pascoe, 1988), which can partly explain the large intra-specific variability in stoichiometry that can be observed within a species (Small and Pringle, 2010). We narrowed down the size of the animals used in the experiment, but still the largest individuals in our study were 3 times longer than the smallest ones, which should have contributed to the large variability recorded. Thus, the high variability present in the life stage and the physiological status of the collected amphipods would lead to large variability in their response at the beginning of the experiment, which highlights the caution needed when interpreting very short experiments.

5 Conclusions

Stress signals on the physiology of biota facing a perturbation are expected to be followed by changes at the levels of the community and ecosystem functioning. Our results show that both resource quality and incubation duration are relevant factors affecting *E. berilloni* consumption rates, but not individual performance variables, which showed subtle responses. The interaction between resource quality and incubation time was significant for some of the measured variables, which shows the difficulty when comparing studies performed at different incubation times and highlights the need for time-standardized experiments. The RNA:BM ratio was no useful as an early warning signal for *E. berilloni*, as it did not show significant differences among the individuals feeding resources of different quality. Although consumption showed a fast and significant response to resource quality, we could not validate its use as early warning signal because it was not followed by changes in amphipod performance during the studied time span.

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Supplementary Material

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References

APHA. 1998. Standard Methods for the Examination of Water and Wastewater. Washington: American Public Health Association.

Arroita M, Flores L, Larrañaga A, *et al*. 2016. Water abstraction impacts stream ecosystem functioning via wetted-channel contraction. *Freshw Biol* 62: 243–257.

Aßmann C, Rinke K, Nechwatal J, von Elert E. 2011. Consequences of the colonisation of leaves by fungi and oomycetes for leaf consumption by a gammarid shredder. *Freshw Biol* 56: 839–852.

Battin TJ, Luyssaert S, Kaplan LA, Aufdenkampe AK, Richter A, Tranvik LJ. 2009. The boundless carbon cycle. *Nat Geosci* 2: 598–600.

Boyero L, Graça MAS, Tonin AM, *et al*. 2017. Riparian plant litter quality increases with latitude. *Sci Rep* 7: 1–10.

Boyero L, Pearson RG, Dudgeon D, *et al*. 2011. Global distribution of a key trophic guild contrasts with common latitudinal diversity patterns. *Ecology* 92: 1839–1848.

Buckley L, Caldarone E, Ong T. 1999. RNA–DNA ratio and other nucleic acid-based indicators for growth and condition of marine fishes. *Hydrobiologia* 401: 265–277.

Canhoto C, Graça MAS. 1996. Decomposition of Eucalyptus globulus leaves and three native leaf species (*Alnus glutinosa*, *Castanea sativa* and *Quercus faginea*) in a Portuguese low order stream. *Hydrobiologia* 333: 79–85.

Casas JJ, Larrañaga A, Menéndez M, *et al*. 2013. Leaf litter decomposition of native and introduced tree species of contrasting quality in headwater streams: how does the regional setting matter? *Sci Total Environ* 458–460: 197–208.

Comoglio L, Smolko L, Amin O. 2005. Effects of starvation on oxygen consumption, ammonia excretion and biochemical composition of the hepatopancreas on adult males of the False Southern King crab Paralomis granulosa (Crustacea, Decapoda). *Comp Biochem Physiol B Biochem Mol Biol* 140: 411–416.

Dahl U, Gorokhova E, Breitholtz M. 2006. Application of growth-related sublethal endpoints in ecotoxicological assessments using a harpacticoid copepod. *Aquat Toxicol* 77: 433–438.

Elser JJ, Sterner RW, Gorokhova E, *et al*. 2000. Biological stoichiometry from genes to ecosystems. *Ecol Lett* 3: 540–550.

Environmental Protection Agency. 2007. Method 6500. Dissolved Inorganic Anions in Aqueous Matrices by Capillary Electrophoresis.

Flores L, Díez JR, Larrañaga A, Pascoal C, Elosegi A. 2013. Effects of retention site on breakdown of organic matter in a mountain stream. *Freshw Biol* 58: 1267–1278.

Flores L, Banjac Z, Farré M, *et al*. 2014a. Effects of a fungicide (imazalil) and an insecticide (diazinon) on stream fungi and invertebrates associated with litter breakdown. *Sci Total Environ* 476–477: 532–541.

Flores L, Larrañaga A, Elosegi A. 2014b. Compensatory feeding of a stream detritivore alleviates the effects of poor food quality when enough food is supplied. *Freshw Sci* 33: 134–141.

Gessner MO, Chauvet E. 1994. Importance of stream microfungi in controlling breakdown rates of leaf litter. *Ecology* 75: 1807–1817.

Glazier DS. 2000. Is fatter fitter? Body storage and reproduction in ten populations of the freshwater amphipod Gammarus minus. *Oecologia* 122: 335–345.

Goerg GM. 2011. A nonparametric frequency domain EM algorithm for time series classification with applications to spike sorting and macro-economics. *Stat Anal Data Min* 4: 590–603.

Gorokhova E, Kyle M. 2002. Analysis of nucleic acids in Daphnia: development of methods and ontogenetic variations in RNA-DNA content. *J Plankton Res* 24: 511–522.

Graça MAS, Cressa C, Gessner MO, *et al*. 2001. Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. *Freshw Biol* 46: 947–957.

Haber F. 1924. Zur geschichte des gaskrieges. In Fünf Vorträge aus den jahren. Berlin, Heidelb.: Springer, 1920–1923, 76–92.

Hessen DO, Elser JJ, Sterner RW, Urabe J. 2013. Ecological stoichiometry: An elementary approach using basic principles. *Limnol Oceanogr* 58: 2219–2236.

Hladyz S, Gessner MO, Giller PS, Pozo J, Woodward G. 2009. Resource quality and stoichiometric constraints on stream ecosystem functioning. *Freshw Biol* 54: 957–970.

Huang YM, Mcclintock JB, Amsler CD, Peters KJ, Baker BJ. 2006. Feeding rates of common Antarctic gammarid amphipods on ecologically important sympatric macroalgae. *J Exp Mar Bio Ecol* 329: 55–65.

Kainz MJ, Johannsson OE, Arts MT. 2010. Diet effects on lipid composition, somatic growth potential, and survival of the benthic amphipod Diporeia spp. *J Great Lakes Res* 36: 351–356.

Kendrick MR, Benstead JP. 2013. Temperature and nutrient availability interact to mediate growth and body stoichiometry in a detritivorous stream insect. *Freshw Biol* 58: 1820–1830.

Landner L, Blanck H, Heyman U, *et al*. 1989. Community Testing, Microcosm and Mesocosm Experiments: Ecotoxicological Tools with High Ecological Realism. In: Chemicals in the Aquatic Environment. Springer Series on Environmental Management. Berlin, Heidelberg: Springer.

Larrañaga A, Basaguren A, Elosegi A, Pozo J. 2009a. Impacts of Eucalyptus globulus plantations on Atlantic streams: changes in invertebrate density and shredder traits. *Fundam Appl Limnol/Arch für Hydrobiol* 175: 151–160.

Larrañaga A, Basaguren A, Pozo J. 2009b. Impacts of eucalyptus globulus plantations on physiology and population densities of invertebrates inhabiting iberian atlantic streams. *Int Rev Hydrobiol* 94: 497–511.

Larrañaga A, Basaguren A, Pozo J. 2014. Resource quality controls detritivore consumption, growth, survival and body condition recovery of reproducing females. *Mar Freshw Res* 65: 910–917.

Larrañaga A, Layer K, Basaguren A, Pozo J, Woodward G. 2010. Consumer body composition and community structure in a stream is altered by pH. *Freshw Biol* 55: 670–680.

Lebrun JD, Uher E, Fechner LC. 2017. Behavioural and biochemical responses to metals tested alone or in mixture (Cd-Cu-Ni-Pb-Zn) in Gammarus fossarum: From a multi-biomarker approach to modelling metal mixture toxicity. *Aquat Toxicol* 193: 160–167.

Lecerf A, Chauvet E. 2008. Intraspecific variability in leaf traits strongly affects alder leaf decomposition in a stream. *Basic Appl Ecol* 9: 598–605.

LeRoy CJ, Whitham TG, Wooley SC, Marks JC. 2007. Within-species variation in foliar chemistry influences leaf-litter decomposition in a Utah river. *J North Am Benthol Soc* 26: 426–438.

Madsen H, Thyregod P. 2010. Introduction to General and Generalized Linear Models. *CRP Press.*

Maltby L. 1999. Studying stress: The importance of organism-level responses. *Ecol Appl* 9: 431–440.

Maranhão P, Marques JC. 2003. The influence of temperature and salinity on the duration of embryonic development, fecundity and growth of the amphipod *Echinogammarus marinus* Leach (Gammaridae). *Acta Oecologica* 24: 5–13.

McCahon CP, Pascoe D. 1988. Use of *Gammarus pulex* (L.) in safety evaluation tests: culture and selection of a sensitive life stage. *Ecotoxicol Environ Saf* 245–252.

Menéndez M, Hernández O, Comín FA. 2003. Seasonal comparisons of leaf processing rates in two Mediterranean rivers with different nutrient availability. *Hydrobiologia* 495: 159–169.

Mulder C, Elser JJ. 2009. Soil acidity, ecological stoichiometry and allometric scaling in grassland food webs. *Glob Chang Biol* 15: 2730–2738.

Øie G, Olsen Y. 1997. Live Food in Aquaculture. *Hydrobiologia* 358: 251–258.

Peig J, Green AJ. 2009. New perspectives for estimating body condition from mass/length data: The scaled mass index as an alternative method. *Oikos* 118: 1883–1891.

Pietrzak B, Bednarska A. 2010. Longevity of Daphnia magna males and females. *Hydrobiologia* 643: 71–75.

Pinheiro J, Bates D. 2000. Mixed-Effects Models in S and S-PLUS.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2018. nlme: Linear and Nonlinear Mixed Effects Models. P package version 3. 1–137.

Ponsatí L, Corcoll N, Petrović M, *et al*. 2016. Multiple-stressor effects on river biofilms under different hydrological conditions. *Freshw Biol* 61: 2102–2115.

R Core Team. 2018. R: A language and environment for statistical computing.

Rasmussen JJ, Monberg RJ, Baattrup-Pedersen A, *et al*. 2012. Effects of a triazole fungicide and a pyrethroid insecticide on the decomposition of leaves in the presence or absence of macroinvertebrate shredders. *Aquat Toxicol* 118–119: 54–61.

Sabater S, Elosegi A, Ludwig R. 2018. Multiple Stressors in River Ecosystems: Status, Impacts and Prospects for the Future. Elsevier.

Schindler MH, Gessner MO. 2009. Functional leaf traits and biodiversity effects on litter decomposition in a stream. *Ecology* 90: 1641–1649.

Small GE, Pringle CM. 2010. Deviation from strict homeostasis across multiple trophic levels in an invertebrate consumer assemblage exposed to high chronic phosphorus enrichment in a neotropical stream. *Oecologia* 162: 581–590.

Stuck KC, Watts SA, Wang SY. 1996. Biochemical responses during starvation and subsequent recovery in postlarval Pacific white shrimp, Penaeus vannamei. *Mar Biol* 125: 33–45.

Taylor BR, Chauvet EE. 2014. Relative influence of shredders and fungi on leaf litter decomposition along a river altitudinal gradient. *Hydrobiologia* 721: 239–250.

Uiterwaal SF, Delong JP. 2018. Multiple factors, including arena size, shape the functional responses of ladybird beetles, 2429–2438.

Vrede T, Persson J, Aronsen G. 2002. The influence of food quality (P: C ratio) on RNA: DNA ratio and somatic growth rate of Daphnia. *Limnol Oceanogr* 47: 487–494.

Wagner M, Durbin E, Buckley L. 1998. RNA: DNA ratios as indicators of nutritional condition in the copepod Calanus finmarchicus. *Mar Ecol Prog Ser* 162: 173–181.

Webster JR, Benfield EF, Hutchens JJ, Tank JL, Golladay SW, Adams JC. 2001. Do leaf breakdown rates actually measure leaf disappearance from streams? *Int Rev Hydrobiol* 86: 417–427.

Wu LX, Dong SL. 2002. Compensatory growth responses in juvenile Chinese shrimp, Fenneropenaeus chinensis, at different temperatures. *J Crustac Biol* 22: 511–520.

Zubrod JP, Englert D, Wolfram J, *et al*. 2015. Waterborne toxicity and diet-related effects of fungicides in the key leaf shredder *Gammarus fossarum* (Crustacea: Amphipoda). *Aquat Toxicol* 169: 105–112.

Zuur AF, Ieno EN. 2010. Mixed Effects Models and Extensions in Ecology with R. New York: Spinger.

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