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Heat waves trigger swift changes in the diet and life-history of a freshwater snail --Manuscript Draft--

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Abstract:	<p>Extreme climatic events, such as heat waves, may induce changes in nutrient acquisition by omnivorous ectotherms. Likely modulated by the intensity, frequency and duration of these pulse events, dietary shifts may threaten the stability of freshwaters. We investigated the effects of heat wave duration on diet assimilation and life-history traits of the freshwater gastropod <i>Radix balthica</i>. We compared the magnitude of the effects of a short (one week) and a long heat wave (seven weeks) on the assimilation of animal and plant-based diets, measuring performance in terms of growth rate and reproduction. We hypothesized that heat waves should increase the proportion of plant material assimilated on the mixed diet and change the performance of snails on the animal and plant-based diets. Both heat waves increased the assimilation of plant material on the mixed diet and growth rate, with minor negative effects on reproduction. However, responses were disproportional to heat wave duration, as the short heat elicited swift and relatively stronger responses. Our findings showcase the role of phenotypic plasticity in aiding ectotherms to cope with increased</p>													

	thermal stress and acclimate. Temporarily changing the strength of trophic interactions, dietary shifts during heat waves may alter community dynamics in freshwater habitats.
Suggested Reviewers:	<p>Otto Seppälä otto.seppaelae@eawag.ch Co-author in publications by Katja Leicht on the effects of heat waves in a freshwater snail and very likely a reviewer of an earlier version of the manuscript submitted to Biology Letters. Katja Leicht has no online presence.</p>
	<p>Kwang Pum Lee Seoul National University College of Agriculture and Life Sciences kwanglee@snu.ac.kr Relevant publications on the topic of temperature and nutrient interactive effects on ectotherms.</p>
	<p>Peiyu Zhang p.zhang@nioo.knaw.nl Previous publications on temperature and nutrient interactive effects on freshwater snails.</p>

**Heat waves trigger swift changes in the diet and life-history of a
freshwater snail**

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ABSTRACT

Extreme climatic events, such as heat waves, may induce changes in nutrient acquisition by omnivorous ectotherms. Likely modulated by the intensity, frequency and duration of these pulse events, dietary shifts may threaten the stability of freshwaters. We investigated the effects of heat wave duration on diet assimilation and life-history traits of the freshwater gastropod *Radix balthica*. We compared the magnitude of the effects of a short (one week) and a long heat wave (seven weeks) on the assimilation of animal and plant-based diets, measuring performance in terms of growth rate and reproduction. We hypothesized that heat waves should increase the proportion of plant material assimilated on the mixed diet and change the performance of snails on the animal and plant-based diets. Both heat waves increased the assimilation of plant material on the mixed diet and growth rate, with minor negative effects on reproduction. However, responses were disproportional to heat wave duration, as the short heat elicited swift and relatively stronger responses. Our findings showcase the role of phenotypic plasticity in aiding ectotherms to cope with increased thermal stress and acclimate. Temporarily changing the strength of trophic interactions, dietary shifts during heat waves may alter community dynamics in freshwater habitats.

KEYWORDS

climate change, diet selection, extreme climatic event, *Radix balthica*, stable isotopes

INTRODUCTION

The climatic changes projected under the most recent scenarios demand a better understanding of the ecological responses to global warming and extreme climatic events, especially in organisms such as ectotherms (Stoks et al., 2017). Temperature governs many physiological and ecological processes (Gillooly et al., 2001; Angilletta, 2009), and there is a growing interest for its effects on nutrient acquisition. Changes in nutrient acquisition mediated by temperature may affect consumer interactions with both resources and other consumers, which may threaten the stability of ecosystems (Boukal et al., 2019). Freshwater communities are disproportionally at risk (Parmesan, 2006; Woodward et al., 2010), as they are mainly composed of ectotherms with a temperature-dependent metabolism (Parmesan & Yohe, 2003; Clusella-Trullas et al., 2011; Paaijmans et al., 2013). Studying temperature and nutrient interactions across organization levels will be a critical step to develop realistic predictions of the ecological responses to global changes (Cross et al., 2015; Kutz et al., 2019).

Two hypotheses link temperature, food quality (i.e. C:N:P nutrient ratios) and diet selection. The Growth Rate Hypothesis (Elser et al., 1996) predicts that higher temperatures increase phosphorous and nitrogen demand, needed to sustain increased growth (Persson et al., 2011) and protein turnover rates (Lemoine et al., 2013). In contrast, the Respiration Hypothesis predicts that higher temperatures increase the relative demand for carbon over nitrogen or phosphorus (Cross et al., 2015), as the stronger effect of temperature on catabolism than on anabolism promotes a greater increase in respiration than in growth (Karl & Fischer, 2008; Forster et al., 2011). Consequently, under higher temperatures ectotherms require extra carbon in their diet (Acheampong et al., 2014) and may select for increasingly carbon-rich foods (Anderson et al., 2017), which should better compensate for the energetic deficit induced by warming (Kingsolver & Woods, 1997;

Lemoine & Burkepile, 2012). Additionally, temperature may influence nutrient acquisition through different scaling effects on other metabolic processes. For instance, higher temperatures promote a greater increase in feeding and gut passage rates than in assimilation rates (Seifert et al., 2014), and this imbalance may increase the assimilation efficiency of carbohydrates (Croll & Watts, 2004).

Phenotypic plasticity is an ubiquitous response to different environmental conditions (Ghalambor et al., 2007), and dietary shifts may constitute an important aspect of the plastic responses to different temperatures. Supporting increased herbivory at higher temperatures, dietary shifts have been documented experimentally in several arthropod taxa – copepods (Boersma et al., 2016; Malzahn et al., 2016), decapods (Carreira et al., 2017) and insects (Lee & Roh, 2010; Clissold et al., 2013; Lee et al., 2015; Rho & Lee, 2017; Rendon et al., 2019); in anuran larvae (Carreira et al., 2016); and in fish (Emde et al., 2016; Vejříková et al., 2016). These findings agree with the higher prevalence of herbivory in fish communities of warmer waters (Floeter et al., 2005; Jeppesen et al., 2010; González-Bergonzoni et al., 2012) and with the seasonal increases in fish herbivory during summer (Guinan Jr et al., 2015; González-Bergonzoni et al., 2016). However, other studies have reported opposite effects showing a greater protein intake at higher temperatures (Lemoine et al., 2013; Schmitz et al., 2016), context-dependent temperature effects on nutritional requirements (Anderson et al., 2017), and no temperature effects on feeding preferences (Miller *et al.*, 2009; Zhang *et al.*, 2018a). As such, the potential for temperature to change nutritional requirements and for warming to increase herbivory is still under debate (Anderson et al., 2017; Carreira, 2017). More studies are required to understand how temperature mediates changes in nutrient acquisition in ectotherms and the extent to which this link may impact ecological communities (Rosenblatt & Schmitz, 2016; Rho & Lee, 2017).

Extreme climatic events such as heat waves constitute large, infrequent and stochastic perturbations, which can act as a major selective force shaping population dynamics and species interactions (Parmesan, 2006; Walther, 2010; Palmer et al., 2017). Recent work showed omnivorous ectotherms to increase the assimilation of plant diets during heat waves (Carreira et al., 2016, 2017), suggesting that these organisms optimized energy intake at higher temperatures by avoiding protein-rich diets. In natural systems, dietary shifts in response to heat waves may prove disruptive, temporarily changing the strength of trophic interactions and even the predominant impacts of invasive alien species (Carreira et al., 2017). The severity of heat wave effects on organisms and communities depends on their intensity, frequency and duration (Mironidis & Savopoulou-Soultani, 2010; Dowd et al., 2015). Likewise, these properties may also dictate the magnitude of the dietary shifts observed during heat waves and, in turn, the magnitude of their impacts on species functional roles and species interactions. Even though studies have assessed the effects of changes in the frequency and intensity of heat waves (Sentis et al., 2013, 2017; Ma et al., 2015), less is known about the effects of changes in their duration (Leicht et al., 2013).

Here, we studied the magnitude of heat wave effects on dietary choices and fitness related traits of *Radix balthica* (Linnaeus, 1758). Widespread throughout freshwater habitats in Europe and Asia, this hermaphroditic pulmonate gastropod is common in the Iberian Peninsula (Pfenninger et al., 2011), where it regularly experiences heat waves. We aimed to investigate both the effects of currently commonly occurring short heat waves, as well as the effects of uncommonly long heat waves, expected to become more frequent in the future (Meehl & Tebaldi, 2004; Diffenbaugh & Field, 2013). For this, snails were fed animal, plant or mixed diets and exposed to simulated heat waves for a short duration (one week) or a long duration (seven weeks). Performance was measured

in terms of growth rate and reproduction, and the proportion of animal and plant material assimilated by snails on the mixed diet was estimated using stable isotope mixing models. We predicted that heat waves effects should: 1) increase the assimilation of plant material by snails on the mixed diet; 2) change the relative performance of snails on the animal and plant diets; and 3) be proportional to heat wave duration.

METHODS

Heat wave characterization in the study area

The severity of heat waves has increased globally since 1950 (Perkins et al., 2012). Climatic models project an increase in their frequency of occurrence (up to 31%) and average duration (up to 35%), as well as greater positive anomalies (over 3°C) – especially in the areas currently most susceptible, like the Mediterranean basin (Easterling, 2000; Meehl & Tebaldi, 2004). Furthermore, there is a dramatic increase in the likelihood of statistically unlikely extreme events (Dasari et al., 2014), such as the one that afflicted Europe in 2003 (Christidis et al., 2015). Following global trends, the Iberian Peninsula (Western Europe) experienced an increase in mean temperature during the latter half of 20th century, especially marked in early summer (Dasari et al., 2014). During the 21st century, the mean annual temperature in this climate change hotspot is expected to increase up to 6°C (Giorgi, 2006; Jerez et al., 2013).

Heat waves are considered to occur when the daily maximum temperature exceeds the average maximum temperature by 5°C for more than five consecutive days, the reference period being 1961–1990 (Frich et al., 2002). In the southwest of Portugal, heat waves occur frequently during spring, when the larval and adult stages of many ectotherm species complete their life cycle in Mediterranean temporary ponds, before pond drying in the summer. Using a 10-year data set of air temperatures recorded during the period of

2002-2012, we characterized the most common spring heat waves occurring in southwestern Portugal. Typically, this region is afflicted by a heat wave in the end of April which lasts on average 7.8 ± 1.3 days and is often followed by another heat wave in mid-May lasting on average 7.5 ± 1.3 days (B. M. Carreira, *unpubl. data* 2002-2012). A five-year survey carried out in 42 temporary ponds located in southwestern Portugal during April and May revealed that at this time water temperatures ranged from 17 to 25°C with an average temperature of 20°C (R. Rebelo, *unpubl. data* 2010-2014).

Experimental setup and procedures

Young adult individuals of *Radix balthica* (10.5 ± 1.4 mm; N=200) were collected in southwestern Portugal (38°47'30.6''N, 9°25'9.0''W) on 18 April 2014. The snails were brought to the facilities at the University of Lisbon and kept in an experimental room at 20°C in 15L aquaria (ca. 25 snails per aquarium) under 12L:12D photoperiod. To ensure standardized isotopic signatures, snails were maintained in these conditions for a month, while being fed *ad libitum* commercial fish food (Pond Sticks, ©Tetra) every two days along with water change.

At the start of the experiment, snails were blotted dry in paper towel, weighed to nearest 0.1mg, and photographed for determination of initial body size (using ImageJ software). Snails were then transferred individually to 80mL mesh-covered cups and placed in water baths. We followed a fully factorial experimental design with diet (three levels) and temperature (three levels) as factors and assigned 18 randomly selected snails to each treatment combination. While subject to the experimental treatments, snails produced egg clutches that were removed along with feeding and water renewal every two days. The position of the snails in the water baths within a given treatment was randomized after each water change and feeding, and the number of eggs per clutch was

recorded. Water bath temperatures were checked daily and adjusted when necessary. The experiment ended after 49 days, when snails were blotted dry in paper towel, weighed, photographed and euthanized by rapid freezing at -18°C.

The snails were fed one of three experimental diets offered *ad libitum*: Animal diet (A) – defrosted Chironomidae larvae; Plant diet (P) – defrosted spinach leaves; Mixed diet (M) – both food items in diets A and P offered *ad libitum* in similar proportions of fresh weight. These food items were obtained commercially and selected based on previous experimental studies on *R. balthica* (Johansson et al., 2016). The food items complied with the basic assumptions that the animal diet was protein-rich and the plant diet protein-poor, and that both food items had distinct isotopic signatures and C:N ratios (Table 1).

Snails experienced one of three temperature treatments (Fig. 1): No Heat Wave (NHW) – constant temperature of 20°C for seven weeks; Short Heat Wave (SHW) – constant temperature of 20°C for six weeks followed by one week at 25°C; Long Heat Wave (LHW) – constant temperature of 25°C for seven weeks. The range of temperatures experienced by the snails in the experimental treatments was selected to match the water temperatures recorded in temporary ponds during spring. The duration of the short heat wave aimed to simulate current spring heat waves in the southwest of Portugal (ca. one week). Like in (Roth et al., 2010), the duration of the long heat wave aimed to simulate unusually long heat waves, such as the one that afflicted Europe in 2003 for nearly 50 days (Schär et al., 2004), which are expected to become more frequent in the future (Meehl & Tebaldi, 2004; Diffenbaugh & Field, 2013).

Stable isotope analysis

A subsample of 9 snails per treatment was randomly selected for stable isotope analysis. After shell removal, the muscle tissue of each snail was dried at 60°C for 24 h and ground to a fine powder with mortar and pestle. The samples were cleansed of storage lipids with chloroform-methanol (2:1; Dobush et al., 1985), and then re-dried at 60°C for 24h. Stable isotope ratios ($^{13}\text{C}/^{12}\text{C}$; $^{15}\text{N}/^{14}\text{N}$) and elemental analyses (C:N) were determined by continuous flow isotope mass spectrometry (CF-IRMS) (Preston & Owens, 1983), on a Hydra 20-22 (Sercon, UK) stable isotope ratio mass spectrometer, coupled to a EuroEA (EuroVector, Italy) elemental analyser for online sample preparation by Dumas-combustion. The standards used were IAEA-CH6 and IAEA-CH7 for carbon isotope ratio, and IAEA-N1 and USGS-35 for nitrogen isotope ratio; $\delta^{13}\text{C}$ results were referred to PeeDee Belemnite (PDB) and $\delta^{15}\text{N}$ to Air. Precision of the isotope ratio analysis was $\leq 0.2\text{‰}$ (6 to 9 replicates of laboratory standards in every batch).

Statistical analyses

Time-integrated estimates of the dietary choices of the snails maintained on the mixed diet were obtained with stable isotope mixing models. Using the Stable Isotope Analysis in R package (SIAR; Parnell *et al.*, 2013), we fit a Bayesian model to the proportions of animal and plant material contributing to the isotopic signature of the snails on the mixed diet. The resulting probability distribution functions (10.000 iterations) for the proportions of the two sources assimilated by these consumers incorporated variability in the sources, trophic enrichment factors and elemental composition (Parnell et al., 2010). Following standard procedures, we specified the isotopic signatures and C:N ratios of the food items, building a model for each temperature treatment. We accounted for the variability in the trophic enrichment factors across sources by using the isotopic signatures of the snails fed on the single diets (animal or plant) at each temperature. The

food items differed considerably in their C:N ratio and more than 2‰ in the isotopic signature of both isotopes (Table 1). Based on the data obtained by (Thomas & Crowther, 2015), we estimated the half-life for ^{13}C and ^{15}N turnover rates of the snails on the mixed diet (body weight: 167.6 ± 48.9 mg) as ca. 13 days.

Treatment effects on snail growth rate, calculated as $\ln$ of daily weight increase (mg), were tested with a general linear model in the software STATISTICA 12.6.255.0 (StatSoft 2012). Treatment effects on the total number of oviposited eggs were tested with a generalized linear model specifying a negative binomial distribution with a log link function in the software SPSS 24 (IBM). Initial shell length (10.5 ± 1.4 mm; defined as the distance between the apex and the edge of the outer shell lip) was included as a covariate in both models. Post hoc pairwise comparisons were corrected for multiple comparisons with Bonferroni test.

RESULTS

Regardless of the temperature treatment, plant material contributed two times more to the isotopic signature of *R. balthica* than animal material – the overall median proportion of plant material assimilated by snails on the mixed diet averaged 0.69. The median proportion of plant material assimilated increased from 0.59 in NHW (Bayesian credible interval (BCI) = 0.34-0.81) to 0.70 in SHW (BCI = 0.52-0.85) and to 0.78 in LHW (BCI = 0.63-0.89) (Fig. 2a). In other terms, relative to NHW, the median proportion of plant material assimilated by snails on the mixed diet increased by 0.11 at the end of SHW and by 0.19 at the end of LHW. These effects were non-linear and, relative to heat wave duration, the snail's response in SHW was four times greater than in LHW. In other terms, the increment in the proportion of plant material assimilated by snails at the end of SHW corresponded to 58% of that of snails at the end of LHW.

The growth rates of snails on the plant and mixed diets were similar, and two times greater than on the animal diet (Table 2; Fig. 2b). The growth rate in LHW was two times greater than in NHW ($P < 0.001$) and 24% greater than in SHW ($P < 0.001$), while the growth rates in NHW and SHW were not significantly different (Table 2; Fig. 2b). Effects on the growth rate of *R. balthica* were non-linear and, relative to heat wave duration, the response to SHW was four times greater than to LHW. Overall, the increase in snail growth rate triggered by the one-week heat wave (SHW) corresponded to 59% of that triggered by the seven-week heat wave (LHW). This strong disproportion in the snail's response to heat wave effects in SHW and LHW was also verified in the snails on the mixed diet and its magnitude was comparable (61%). We found no significant effects of diet $\times$ temperature interactions on snail growth (Table 2; Fig. 2b).

We found a positive relationship between reproduction (total number of eggs produced) and shell length at the end of the experiment ($r=0.387$; $P<0.01$). Reproduction on the plant diet was over two times greater than on the animal diet, but not significantly greater than on the mixed diet (Table 2; Fig. 2c). Temperature had no significant main factor effect on reproduction (Table 2; Fig. 2c), even though the mean number of eggs laid per individual decreased from 59 in NHW to 47 in SHW and to 30 eggs in LHW. Relative to heat wave duration, the reduction in reproduction observed in SHW was three times greater than in LHW, corresponding to 41% of the reduction observed in LHW. We found diet $\times$ temperature interactions indicating that: i) on the plant diet, reproduction in NHW and LHW was greater than in SHW ($P < 0.001$), unlike on the animal diet ($P = 0.531$) and on the mixed diet ($P = 0.633$); ii) in SHW, there was no diet effect on reproduction ($P = 0.113$), unlike in NHW ($P < 0.05$) and LHW ($P < 0.01$) (Table 2; Fig. 2c). These interactions were caused by a lower proportion of ovipositing snails on the plant diet in the SHW treatment ($X^2 = 9.186$; $P < 0.01$). While egg production on plant

diet by the animals that oviposited was similar in all temperature treatments, the proportion of snails that oviposited in SHW (46%) was half of the proportion of the snails that oviposited in NHW (91%) and LHW (100%).

DISCUSSION

Previous studies reported heat waves effects on the survival (Garrahou et al., 2009) and internal resource budgets of organisms, leading to changes in life history traits (Jiguet et al., 2007; Chiu et al., 2014; Fischer et al., 2014; Dinh et al., 2016; Arambourou et al., 2017). We investigated the expression of phenotypic plasticity in function of heat wave duration, comparing the magnitude of the effects of short- and long-lasting heat waves on the diet and life history traits of a freshwater gastropod. Although both types of heat wave increased the assimilation of plant material and the growth rate of *R. balthica*, with minor costs in reproductive output, the magnitude of the responses was disproportional to heat wave duration. Our results revealed a steep thermal reaction norm and suggest that organisms may be unable to sustain the magnitude of short-term responses over long periods. The strong responses deployed by *R. balthica* under the relatively short exposure to high temperatures showcase the major role of phenotypic plasticity as an important mechanism aiding ectotherms to cope with heat waves. Furthermore, presenting the first evidence for temperature-induced dietary shifts in gastropods, our findings add to those obtained in other taxa, supporting a general temperature effect on nutrient acquisition in omnivorous ectotherms and warming to increase herbivory.

Assimilation shifts

290 Snails on the mixed diet increased the assimilation of plant material under the heat
291 waves, in agreement with the findings of other studies in omnivorous ectotherms (Lee et
292 al., 2015; Boersma et al., 2016; Carreira et al., 2016, 2017). Supporting the Respiration
293 Hypothesis (Cross et al., 2015), *R. balthica* used trophic plasticity to increase the relative
294 assimilation of carbon at higher temperatures. The assimilation shifts in SHW (11%) and
295 LHW (19%) are similar to those reported in tadpoles (Carreira et al., 2016) and crayfish
296 (Carreira et al., 2017) under short heat waves (5-11%) and long heat waves (22%).
297 However, the magnitude of the responses displayed may be partially driven by the
298 difference in the C:N ratios of the food items available, as suggested by the findings of
299 Zhang *et al.* (2018b) showing a relationship between relative consumption rates and the
300 C:N and C:P ratios of animal and plant food items offered to amphipods and gastropods.
301 If the C:N ratios of the food items offered are relatively close, individuals may need to
302 perform larger dietary shifts to efficiently achieve nutritional balance. While the
303 magnitude of the assimilation shifts was similar, the net increase in the C:N ratio of the
304 snails' diet (SHW: 6%; LHW: 9%) was not as high as in the previous studies (10-90%).
305 The C:N ratios of the diets offered to the snails differed only by a factor of two (Table 1),
306 whereas in the two other studies there was a three to sevenfold difference in the C:N ratio
307 of the food items composing the animal and plant diets. The weaker response by *R.*
308 *balthica* may indicate a higher tolerance to heat waves than in tadpoles or crayfish,
309 supporting that these extreme events impact species differently and change freshwater
310 communities.

311 The increased assimilation of plant material at higher temperatures in our study is
312 typically interpreted as increased herbivory (Lee et al., 2015; Boersma et al., 2016;
313 Vejříková et al., 2016). However, the single study examining temperature-induced
314 changes in the feeding choices of a freshwater snail (*Lymnaea stagnalis*, Linnaeus 1758)

found no evidence for dietary shifts (Zhang *et al.*, 2018a). The seemingly contradictory findings in the two studies suggest that temperature effects on ectotherm feeding preferences could be species-specific or may simply reflect differences in the thermal sensitivity of the two model species. Additionally, the difference in the methodologies used may have also contributed to this contradiction. Owing to the impracticality of obtaining reliable consumption rates in a small-sized species, we used stable isotopes analysis to estimate assimilation rates. Mismatches between consumption and assimilation are known to occur (Schmidt *et al.*, 2017) and could be amplified or partially modulated by temperature, as the greater feeding and gut passage rates at higher temperatures may increase the relative assimilation efficiency of plant diets (Myrick & Cech, 2000; Seifert *et al.*, 2014). Although we cannot disentangle the contribution of the two pathways (consumption and assimilation), our results support temperature to modulate nutrient intake in *R. balthica* and a greater dependence on plant material at higher temperatures. Future studies should assess both feeding preferences and assimilation to determine their role in mediating temperature-induced changes in ectotherm nutrient intake.

Performance on animal and plant diets

The higher performance on the plant diet, in terms of growth and reproduction, supports the diet assimilation results. Despite the substantial contribution of animal material, *R. balthica* snails on the mixed diet were closer to the herbivory end of the omnivory gradient, with plant material contributing to ca. 70% of their isotopic signature. Previous studies found diet $\times$ temperature interactions supporting higher temperatures to decrease performance on animal diets and to increase performance on plant diets (Boersma *et al.*, 2016; Carreira *et al.*, 2016, 2017; Malzahn *et al.*, 2016). However, we

found no evidence for the performance of the snails on the single diets to change across the temperature regimes. Our experimental setup aimed to compare the effects of short and uncommonly long heat waves, replicating spring conditions. Thus, the thermal contrast provided (5°C) may have been insufficient to detect changes in the relative performance of the diets, especially for temperatures close the species preferred thermal range – 16-20°C in Icelandic populations (Johansson et al., 2016; Johansson & Laurila, 2017). The reduction in oviposition observed in the snails on the plant diet in SHW could be interpreted as a cost of the acclimation to the short heat wave. However, it resulted from fewer snails reproducing throughout the experiment, rather than from fewer oviposited eggs after snails experienced the short heat wave. Thus, we cannot link the short heat wave to any direct effect on the reproduction of snails on the plant diet. We used data from the snails alive at the end of the experiment and it is possible that random variation in the number of reproducing snails, together with a small sample size ($N=11$), may be responsible for the interaction found in SHW.

Heat wave effects on dietary shifts and life history traits

Generally, the two heat waves had similar effects, with the long heat wave prompting stronger responses in *R. balthica*. The increases in the assimilation of plant material on the mixed diet and in the overall snail growth rate were substantially larger in LHW than in SHW. Heat waves also caused an apparent reduction in the number of eggs produced (non-significant), in agreement with results obtained in northern European populations of *R. balthica*, indicating failure of reproduction at 24°C (Johansson et al., 2016). The increased growth rate in the heat waves, together with the apparent reduction in fecundity, could suggest a trade-off between these traits, not observed in other freshwater snails (Leicht et al., 2013). The positive relationship between size and

fecundity supports that allocating resources to growth, by temporarily reducing reproduction under heat waves, could potentially increase snail fecundity in the adult stage. Thus, close to the minimum reproductive size, *R. balthica* snails may have maximized fitness by postponing reproduction to a larger size during optimal growth conditions. However, such a trade-off could prove non-adaptive over prolonged periods.

Heat wave effects on fitness related traits of freshwater snails have been reported in *L. stagnalis* (Leicht et al., 2013, 2017). This species increased growth and reproduction under heat wave conditions, while their immune defences were reduced (Leicht et al., 2013, 2017). Phenotypic plasticity was also evident in *R. balthica*, with the relatively stronger effects of the short heat wave on plant material assimilation and growth rate suggesting a steep thermal reaction norm. Within a week under heat wave conditions, the snails deployed the equivalent to 60% of the plastic responses observed in the snails under heat wave conditions for seven weeks. Heat waves have been proposed to affect survival, behaviour and life history traits in non-linear ways (Chiu et al., 2014), but the responses to the short heat wave were particularly strong. Although snails in LHW should be close to isotopic equilibrium at the end of the experiment, snails in SHW are unlikely to have reached the half-life values for ^{13}C and ^{15}N turnover rates (13 days), even when accounting for weight gain during the short heat wave (9%). Likely underestimating the assimilation of plant material in SHW, our results suggest a larger assimilation shift in SHW than in LHW. As negative effects on performance get pronounced under prolonged heat waves (Leicht et al., 2013), snails may have been unable to sustain the strong assimilation shift implicit in SHW for a long period, reducing the assimilation of plant material as they acclimated to heat wave conditions in LHW.

Methodological issues

Logistic constraints prevented the implementation of cyclical dial variation in our experimental setup, which could have buffered the effects of high daytime temperatures and helped snails to recover from the thermal stress (Davis et al., 2006; Putnam et al., 2010). While our results may overestimate the strength of *R. balthica*'s responses in nature, this may be only partially true. We tested the effects of spring heat waves, characterized by relatively mild temperatures (25°C) and this species occurs more frequently in semi-permanent and permanent habitats, experiencing higher temperatures and more intense heat waves during summer. Although the narrow temperature range tested here may prevent generalizations, it showed strong responses to small changes in temperature that otherwise may have been masked or replaced by responses to extreme temperatures. For instance, acute heat stress may increase the demand for nitrogen used in the synthesis of heat shock proteins (Podrabsky & Somero, 2004; Lemoine et al., 2013).

CONCLUSIONS

Short-term climatic stress arising from extreme events, such as heat waves, poses organisms with a greater challenge than changes in mean climatic conditions (Easterling, 2000; Chapman et al., 2014; Vasseur et al., 2014). As climate change progresses and the severity of heat waves increases, phenotypic plasticity may prove determinant for immediate and effective responses. Although diet regulation and changes in life history traits may help omnivore ectotherms to cope with short-term thermal stress from heat waves, drastic changes may be unsustainable over long periods. The greater dependence on plants diets at higher temperatures underscores the potential for warming and heat waves to alter trophic interactions, threatening the stability of ectotherm-dominated ecosystems like freshwaters. While individuals may experience heat waves only briefly in the lifecycle, their responses are likely to have cascading effects, as feeding behavior

is key in shaping the structure and functioning of communities and food webs (Kortsch et al., 2015). Understanding how the multiple temperature effects on metabolism, growth and intake impact nutritional requirements under normal and extreme physiological conditions will be critical to assess the ecological effects of climate change on ectotherms.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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TABLES

Table 1. Isotopic signature and elemental ratio of the experimental diets (average $\pm$ standard deviation).

	Animal diet	Plant diet
$\delta^{13}\text{C}$	-24.61 ± 0.13	-27.79 ± 0.12
$\delta^{15}\text{N}$	5.09 ± 0.92	0.42 ± 1.01
C:N	3.79 ± 0.05	6.55 ± 0.71

Table 2. General linear model for growth rate and generalized linear model for reproduction (negative binomial distribution).

	Growth			Reproduction		
Factors	<i>df</i>	F	<i>P</i>	<i>df</i>	χ^2	<i>P</i>
Diet	2, 68	5.29	<0.01*	2, 68	11.71	<0.01*
Temperature	2, 68	3.94	<0.05*	2, 68	3.23	0.199
Diet $\times$ Temperature	4, 68	1.49	0.216	4, 68	10.97	<0.05*

*Denotes *P* values <0.05

FIGURE CAPTIONS

Fig. 1 Temperature variation over time in the experimental treatments

Fig. 2 Proportion of plant material assimilated (a), LN of growth rate in mg.day^{-1} (b) and total number of oviposited eggs (c) on animal (grey), plant (white) and mixed diets (black). Boxes show the median and the 25th – 75th percentiles and whiskers indicate the 2.5th – 97.5th percentiles (a) or the minimum and maximum (b,c). Significant contrasts are depicted with different letters

Figure 1.

Figure 2.