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Simultaneous exposure to a pulsed and a prolonged anthropogenic stressor can alter consumer multifunctionality

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Ecosystems face multiple anthropogenic threats globally, and the effects of these environmental stressors range from individual-level organismal responses to altered system functioning. Understanding the combined effects of stressors on process rates mediated by individuals in ecosystems would greatly improve our ability to predict organismal multifunctionality (e.g. multiple consumer-mediated functions). We conducted a laboratory experiment to test direct and indirect, as well as immediate and delayed effects of a heat wave (pulsed stress) and micropollutants (MPs) (prolonged stress) on individual consumers (the great pond snail *Lymnaea stagnalis*) and their multifunctionality (i.e. consumption of basal resources, growth, reproduction, nutrient excretion and organic-matter cycling). We found that stressful conditions increased the process rates of multiple functions mediated by individual consumers. Specifically, the artificial heat wave increased process rates in the majority of the quantified functions (either directly or indirectly), whereas exposure to MPs increased consumption of basal resources which led to increases in the release of nutrients and fine particulate organic matter. Moreover, snails exposed to a heat wave showed decreased reproduction and nutrient excretion after the heat-wave, indicating the potential for ecologically relevant delayed effects. Our study indicates that the immediate and delayed effects of stressors on individual organisms may directly and indirectly impact multiple ecosystem functions. In particular, delayed effects of environmental stress on individual consumers may cumulatively impede recovery due to decreased functioning following a perturbation. Reconciling these results with studies incorporating responses at higher levels of biological complexity will enhance our ability to forecast how individual responses upscale to ecosystem multifunctionality.

Keywords: ecosystem processes, environmental stress, grazer, heat wave, micropollutants

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Introduction

Ongoing global environmental change is dramatically altering the Earth's ecosystems (Sala et al. 2000, Boyd et al. 2014). Anthropogenic perturbations, such as global change and chemical pollution, can, for example, disrupt trophic links (Winder and Schindler 2004, Visser and Both 2005) and food-web structure, thereby modifying energy transfer rates and efficiencies (O'Connor 2009, Svensson et al. 2017, Zarco-Perello et al. 2017, Nordström and Bonsdorff 2017). Predicting the occurrence and magnitude of such ecosystem-level affects is, however, difficult. This is because understanding the mechanistic basis of these effects requires linking the impacts of anthropogenic perturbations across multiple levels of biological organisation: in other words, scaling from individual organisms to complete ecosystems (Woodward and Perkins 2015). At the individual level, exposure to stress commonly leads to alteration of the organismal phenotype and function, such as behaviour and growth (Maltby 1999, Ghalambor et al. 2007, Sulmon et al. 2015, Salo et al. 2017) – which may be directly linked to processes such as resource consumption, productivity and release of nutrients at ecosystem level. Therefore, examining the magnitude and (co-)variation in individual responses to environmental stress, and linking them to the functions these organisms mediate in natural ecosystems, can help estimating how different taxa and/or types of organisms contribute to higher-level effects of environmental stress (Smith et al. 2009, Moran et al. 2016, Galic et al. 2018).

Examining individual-level phenotypic responses to environmental stress is especially relevant for keystone species that disproportionately influence community or ecosystem properties (Hendry 2016, Felton and Smith 2017). Primary consumers are an important link across trophic levels in food webs and can control primary production and species composition through consumption (Sheldon 1987, Chaneton and Bonsall 2000) and nutrient excretion (Reusch et al. 1994). Furthermore, environmental conditions can alter the strength of consumer–resource interactions. In aquatic ecosystems, for example, warmer temperatures can increase the impact of herbivore communities on resource consumption (O'Connor 2009, O'Connor et al. 2009, Svensson et al. 2017). However, the mechanistic basis for individual organism contribution to such effects remains to be investigated.

Thermal and chemical stresses, mediated through heat waves (i.e. short-term extreme high temperature events; Meehl and Tebaldi 2004) and micropollutants (MPs; low concentrations of pharmaceuticals, pesticides and herbicides in complex mixtures; Schwarzenbach et al. 2006), respectively, are common in aquatic habitats. Heat stress is particularly relevant as aquatic ecosystems are dominated by ectotherms, whose metabolic demands (Vannote and Sweeney 1980, Salo et al. 2017), growth (Leicht et al. 2013) and reproductive output (Salo et al. 2017) is largely determined by environmental temperature (Angilletta 2009).

At the same time, chemical pollution may further alter organismal performance, such as resource requirements (Dinh et al. 2016) and reproduction (Salo et al. 2017). Maintaining organismal function at higher temperatures, while simultaneously needing more resources for detoxification of chemical toxicants (Broomhall 2002), can cause energetic conflicts. Thus, combined exposure to heat and chemical stress could cause additive or interactive changes on organismal functionality, consequently altering consumer-mediated ecosystem processes. Furthermore, while immediate stress effects are often the main endpoint in stress studies, some effects of single and combined stressors may be delayed (e.g. become visible only after the stress event has ended) – such as reduced recovery of damselflies (Dinh et al. 2016) or adjusted brood size of snails (Salo et al. 2017) following an experimental heat wave. Such potential for temporal delays in stress effects contributes to the challenges in estimating effects of stress on consumer multifunctionality. Using an aquatic keystone consumer, the great pond snail *Lymnaea stagnalis*, we previously found both immediate individual, additive and synergistic effects of experimental heat waves (pulsed stress) and MPs (prolonged stress) on individual level physiological responses (Salo et al. 2017). Shortly, exposure to heat led to a metabolic imbalance that caused tradeoffs between immune defence and reproductive output, while exposure to MPs reduced fecundity and the combination of both stressors further intensified the metabolic imbalance (Salo et al. 2017). Here, we broaden the scope by investigating how such sublethal organismal effects may affect consumer multifunctionality (i.e. multiple consumer-mediated functions). We conducted a laboratory experiment to explicitly quantify (at the individual level) how thermal and chemical stress affect parameters relevant for ecosystem processes and multifunctionality (e.g. how stress affects consumption of different basal resources differing in nutritional quality and how this relates to nutrient cycling). Moreover, we quantified both immediate and delayed effects of the heat wave. As both temperature and pollutants increase process rates due to higher maintenance costs (Vannote and Sweeney 1980, Leicht et al. 2013, Dinh et al. 2016, Salo et al. 2017), we expected a positive rate change in the quantified processes (e.g. consumption of basal resources, nutrient excretion) under stress. Based on our earlier results, we expected mainly additive effects of the two stressors. Furthermore, as many biological processes are causally linked, we expected stress to affect some processes directly (i.e. respiration, reproduction, consumption of basal resources), while only indirect effects were expected for other processes (e.g. nutrient excretion and production of particulate organic matter, i.e. rates that were expected to be modified for example by resource consumption; see Supplementary material Appendix 1 Fig. A1 for the predicted causality of different processes). Due to increased metabolic demands during stress, we expected to see higher rate change for consumption of basal resources of higher nutritional quality compared to resources of lower nutritional quality.

Material and methods

Study system

Lymnaea stagnalis is a hermaphroditic pulmonate gastropod with a wide distribution in the northern hemisphere. It lives in stagnant or slowly flowing water bodies, such as lakes and ponds, and may reach very high population densities in these habitats, with hundreds of adult individuals in a few m^2 area, dominating secondary biomass (Salo et al. unpubl.). It is a generalist consumer with a diverse diet ranging from phytoplankton to macrophytes and plant detritus, but may also feed on deceased conspecifics (Elger et al. 2004, Doi et al. 2010, Zhang et al. 2018). As an important prey and host source for predators and parasites (Faltýnková et al. 2007), it forms an important link between trophic levels.

The snails used in this study originated from a laboratory stock population that was started from 88 individuals collected from a shallow pond in Zürich, Switzerland (47°23′N, 8°33′E; see Salo et al. 2017 for details), in 2014. The laboratory population was maintained in a large (800 l) tank with slowly flowing water at ca 18°C and fed three times a week with fresh lettuce and pulverized *Spirulina*.

Experimental design

The experimental design involved individual-level exposure of snails to heat and MP stress in the laboratory. An individual level design was chosen to enable highly precise quantification of individual phenotypes/performance and individual level estimates of parameters related to consumer multifunctionality. The design was a fully randomized factorial design consisting of a combination of two temperature (heat wave and stable benign temperature) and three chemical treatments (MPs, technical control and control). Each of the six treatment combinations was sampled in two separate phases: at the end of an eight-day heat wave (immediate effects of heat wave) and eight days after the heat wave had ended (delayed effects of heat wave). Due to the number of quantified variables, the experiment was conducted in two blocks initiated two days apart. Each treatment combination was replicated 10 times in each experimental phase and block (in total n = 20 for each treatment combination per sampling phase). In total, 240 adult snails of similar size (29.0 ± 1.9 mm; average ± SD) were haphazardly chosen from the laboratory population and assigned to the different treatments, blocks and sampling phases. As the sampling was destructive, measurements on each snail (and ecosystem parameter) were only quantified once per individual. Thus, snails sampled at the end of the heat wave phase were removed from the experiment at this point and snails sampled at the end of the post-heat wave phase were not measured at earlier time points. The size of the individuals at the beginning of the study did not differ between any of the chemical and temperature treatment combinations, nor between blocks (separate two-way univariate permutational analysis, p > 0.05 for both chemical and temperature treatments, the interaction and the block).

The experiment was conducted in two separate walk-in climate chambers at the laboratory facilities of Eawag, Dübendorf, Switzerland. These chambers are mirror images of each other, connected via ventilation, and have identical cooling and lighting systems, which reduced confounding chamber effects between the experimental treatments. The snails were kept individually in 200 ml glass containers in 14/10 h light/dark cycle. The order of these containers was randomized across all factors. Initially, all snails were kept in one walk-in climate chamber (14.0 ± 1.5°C) for eight days. One third of the snails (n = 80) were kept in aged non-chlorinated tap water spiked with MPs (MP treatment, Salo et al. 2017) and one third (n = 80) in aged tap water mixed with low concentration of MeOH (technical control, TC). The TC was needed to control for the effects of MeOH that was used to solubilize chemicals in the MP treatment. The MeOH concentration in the TC was same as in the MP treatment (i.e. 8.71 mg l^{-1}). In addition, one third of the snails (n = 80) were assigned to a second control (C) consisting of aged tap water without any added chemicals. However, as the responses in the two control treatments were largely similar in quantity and direction (see also Salo et al. 2017), results are presented only for the MP and TC treatments (n = 160). The snails were maintained in their respective chemical treatment (i.e. MP, TC or C) throughout the experiment.

As MPs typically occur as a complex mixture in aquatic environments (Schwarzenbach et al. 2006), the MP treatment consisted of spiking with a standardized mixture of 17 chemicals. This consisted of low concentrations of pharmaceuticals, a hormone, a herbicide, a fungicide, a pesticide, an insecticide, a corrosion inhibitor and a sweetener (Supplementary material Appendix 1 Table A1) similar to the mix described in Stamm et al. (2016) and Salo et al. (2017). These chemicals and concentrations were chosen because they are commonly observed in freshwater ecosystems in central Europe (Wittmer et al. 2010, Munz et al. 2017) and known to cause organismal level effects in snails (Salo et al. 2017). To ensure constant exposure to MPs and sufficient water quality, water was changed every second day in all experimental units. To analyse whether MP concentrations were affected by the temperature treatments or by the presence of snails, water samples from haphazardly selected experimental units and control units without snails were collected on two occasions during the heat wave both after and before the water change (see Salo et al. 2017 for detailed information on the MP concentration analyses). The concentrations of eight chemicals decreased slightly in the high temperature treatment, while the concentration of two chemicals decreased more rapidly in the absence of snails (Supplementary material Appendix 1). However, due to the frequent water change, these slight differences in end concentrations are not expected to have had major implications on the experienced stress levels in different treatments.

After eight days at 14°C, half of the snails within each chemical treatment were exposed to an experimental heat wave (24 ± 2°C). In our study region, summer water temperatures in ponds can rapidly increase from relatively low (<16°C) to high (20–30°C) and last high for weeks (Supplementary material Appendix 1 Table A2). However, as the average duration of heat waves in western Europe is 8.4 days (Meehl and Tebaldi 2004), the experimental heat wave was adjusted to eight days. In the heat wave treatments, the containers were moved on day 9 to the second climate chamber (24°C) to allow a slow (ca 1°C h^{-1}) increase in water temperature until the target temperature was reached. The experimental units in the 24°C chamber were placed in randomized order in similar positions in relation to lighting, cooling and ventilation system as the experimental units kept in the 14°C chamber. Ten haphazardly selected individuals (or less in case of mortality) from each temperature and chemical treatment combination in each block (total n = 20) were sampled at the end of the heat wave (day 16) to assess single and combined effects of the heat wave and MP exposure on different ecosystem processes. For the remaining snails (i.e. 10 per treatment combination and block), water temperature was slowly decreased back to 14°C during ca 10 h and the snails were allowed to recover from the heat wave for eight days. These snails were then sampled in a similar manner at the end of the post-heat wave phase (day 24).

Comparing treatment effects between non-replicated experimental systems (here: two climate chambers set to different temperatures) increases the risk for confounding factors affecting the results. Accordingly, we cannot fully exclude the possibility that the effects of the temperature treatment were confounded by climate chamber effects. However, specific reasons make it likely that such confounding effects were minor in our study. Firstly, we have no reason to expect a major confounding effect because the conditions in the two climate chambers were highly controlled and therefore closely similar apart from the temperature difference (14°C versus 24°C). Secondly, our results are in line with typical temperature effects on organismal performance (e.g. growth and reproduction). Temperature is one of the most influential factors determining performance of ectotherms (Angilletta 2009) as well as ecosystem processes (Brown et al. 2004), thus it is likely this overrode any possible confounding factors by an order of magnitude.

The snails were fed ad libitum with a mix of freshly collected leaves of *Potamogeton natans* (henceforth *Potamogeton*) and dried *Alnus glutinosa* (henceforth *Alnus*). *Potamogeton* and *Alnus* were used as a basal resource to test the autochthonous and allochthonous food web channels, respectively. These plant species were chosen because a pilot study with six different types of plant material commonly observed in ponds indicated that these were the two preferred food sources by the snails (Salo unpubl.). Clean *Potamogeton* plants without visible epiphytes were collected from a slowly flowing shallow stream in the Swiss lowlands (47°45′ N, 8°43′E) in August 2016 three days prior to the start of the experiment. The

plants were cleaned from any associated fauna and kept in a 800 l tank with slowly flowing water at 18°C until used in the experiment. *Alnus*-leaves were collected in fall 2015 (47°4'N, 8°59'E) shortly after they were shed from the trees, air dried and stored in cool, dry and dark environment. Prior to feeding to the snails, the leaves were conditioned in aged tap water for six days in 14°C to increase palatability. To minimise the potential effects of higher temperature on palatability of the plant material, the food items were replaced every second day.

Survival of all the snails was assessed every second day, but as only five individuals died (2% of total; with no obvious patterns over temperature or chemical treatments), mortality was not analysed and data collected from these individuals were excluded from any analyses. Thus, we concentrated on how sublethal effects of stress may affect individual level responses and multifunctionality. This design ignores intra- and interspecific interactions (e.g. competition and facilitation) and does not allow to directly infer effects on population or ecosystem-level processes, but was chosen as the first step towards standardized estimates of individual level ecosystem parameters.

Response variables

To quantify whether the studied stressors have any immediate or delayed effects on ecosystem processes mediated by the consumers (here: snails) and to explore links between these processes, we quantified the following parameters: consumption of basal resources (autochthonous and allochthonous resources), nutrient excretion, production of fine particulate organic matter (henceforth FPOM), metabolic rates (respiration measured as mg O$_2$ h^{-1}), snail growth, reproduction and fat content. Many of these parameters can be considered both as ecological and physiological responses (e.g. respiration/metabolic rate).

Consumption of basal resources
Consumption of basal resources (i.e. realized feeding rates) may be used as an indicator for metabolic uptake of an individual, but are also essential ecosystem functions controlling prey biomass in the system. Consumption of autochthonous plant resources directly controls the biomass of plants and algae. Allochtonous resources (e.g. tree leaves) can be the major source of energy in many temperate-zone aquatic ecosystems. The consumption of *Potamogeton* and *Alnus* leaves was assessed for each experimental snail during 30 h, five days after the start of the heat wave or post-heat wave period. For this assessment, snails were moved from their experimental container to a clean container filled with water from the respective chemical treatment and offered an approximately equal amount of fresh *Potamogeton* leaves (ca 0.2 g wet weight, WW) and soaked *Alnus* leaves (i.e. detritus; ca 0.19 g WW). Consumption (g WW) was then calculated as the change in weight of each leaf type separately during the 30 h trial period, whereafter snails were returned to ad

libitum feeding in the experiment. To account for any autogenic changes in the plant material (e.g. due to photosynthesis or microbial decomposition), the initial and final weight of three replicates of each leaf type, submerged in water in separate containers without snails, were measured within each chemical and temperature treatment. In each replicate with a snail, the starting WW was corrected by the change in the no-snail treatments according to Eq. 1,

$$WW_{corrected} = \frac{WW_{initial} \times WW_{CF}}{WW_{CI}} \qquad (1)$$

where $WW_{initial}$ is the initial WW of the leaf offered to the snail and WW_{CI} and WW_{CF} are the initial and final weight of the control leaf, respectively (following Salo et al. 2017). Final food consumption was then calculated by subtracting the final WW from the corrected start WW and dividing it by the feeding time in hours.

Nutrient excretion and FPOM production
Invertebrates often play an important role in processing coarse particulate organic matter and converting it to fine particulate organic matter (FPOM) which, in turn, can be a key food resource for other invertebrate groups (e.g. collector-filterers and gatherers). Additionally, organisms excrete some of the nutrients they consume. Nutrient concentrations ($mg\ l^{-1}$) and FPOM production ($mg\ h^{-1}$) were measured during the same 30 h period as the consumption of basal resources. After 30 h, the focal snail was moved to another container and the water filtered with pre-weighed glass microfiber filters. The volume of water was measured and the water was stored in 8°C in closed containers until analysed for dissolved inorganic N (NO_3^-, NO_2^-, NH_4^+), organic-N and PO_4-P. The Supplementary material Appendix 1 describes the specific methods for each nutrient. The filters were dried (48 h, 60°C) and weighed. The difference between the final and initial weight of the same filter was used as an estimate for FPOM production.

Respiration
Respiration is a key measure of metabolism reflecting organismal maintenance rates (i.e. energy required by an organism), and increase in respiration may indicate stress or higher metabolic activity (Brown et al. 2004). At the same time, dissolved inorganic carbon (i.e. CO_2) is needed for primary production. Respiration rate of each individual ($mg\ O_2\ h^{-1}$, directly proportional to the release rate of CO_2) was assessed on the second last day of the respective experimental phase. Snails were placed in separate 185 ml plastic containers filled with water from their respective chemical treatment and incubated at temperatures corresponding to their experimental treatment at the time of the measurement (14°C or 24°C). Magnetic stirrers were deployed in each container to ensure constant mixing of the water. Snails were allowed to acclimatize in the incubation container for 10 min prior to any measurements. Respiration was measured using a system with optical oxygen sensors. Oxygen concentration

($g\ O_2$) was recorded at least 25 times during a 15 min period. Respiration rates ($mg\ O_2\ h^{-1}$) were then calculated from periods with linearly decreasing ($R^2 > 0.85$) oxygen concentration. In total 15 (of 157) measurements with lower linear fit were excluded from the results.

Growth, reproduction and fat content
Growth and reproductive rates of an individual contribute to secondary production and help transfer energy to different trophic levels (i.e. when individuals die and decompose or are preyed on). The reproduction was assessed as the total number of eggs that each individual oviposited during the entire experimental phase. For this, all egg clutches produced during a given experimental phase were removed from the rearing container at the end of that phase and photographed with a digital camera. The number of eggs in each digital image was then counted manually with the help of Cell Counter-plugin in Image J software (ImageJ 1.49k). Growth of individuals was quantified both in terms of changes in length and weight. As snail growth (in length) typically follows a power function (Seppälä et al. 2013), specific growth rate of each snail was assessed according to Eq. 2,

$$specific\ growth\ rate = \frac{(\ln S_2 - \ln S_1)}{\Delta t} \qquad (2)$$

where S_2 and S_1 are shell length (precision: 0.1 mm) at the end of and before the experimental phase, respectively, and t is the time between the measurements in days. In addition to length based growth rate, biomass based growth rate for each individual was assessed as the proportional change in weight (WW, precision: 0.01 g) during the experimental phase.

The nutritional quality of an organism (e.g. fat content or the stoichiometry of biomass) may affect the phenotype of predators (Malzahn et al. 2010), such as predatory insects or fish that typically feed on freshwater snails. The effect of environmental conditions on the nutritional quality of snails was quantified as percentage fat (lipids) in the snail soft-tissue. The snail was removed from the shell at the end of its respective experimental phase and stored at −20°C until the analyses were conducted. The analyses were conducted according to Reznick (1983) with modified drying times. Shortly, the snail tissue was dried for 48 h in 60°C, weighed (to get initial dry weight) and subsequently soaked in diethyl ether for 72 h. Thereafter the snail was dried again for 48 h in 60°C and finally weighed to get lipid free dry weight. The lipid content was then calculated as the proportional difference in the dry weight before and after the diethyl ether treatment. Nine samples (3.8 %) could not be measured because of human errors.

Data analysis

The data was analysed in two phases. Due to the strong temperature effects during the heat wave phase prohibited teasing apart the effects of experimental phase and temperature in a model incorporating the temporal scale of the

experiment. Thus, all analyses were conducted separately for the heat wave phase and the post-heat wave phase. First, we analysed the data with multivariate permutational analyses (PERMANOVA+ ver. 1.0.3 package in PRIMER ver. 6.1.13) to quantify whether the heat wave, MPs or both interactively affected the overall rates of ecosystem processes. In these multivariate analyses, data included all of the response variables (i.e. production of eggs, growth in length and mass, fat content, consumption of autochthonous and allochthonous resources, FPOM production, nutrient release and respiration). To decrease the number of variables with high correlations in the model, a principal component analysis (PCA) was conducted with the nutrient data separately for the heat wave and post-heat wave phases. The first principal component axis (PCA1) for nutrients (DIN, organic-N, PO_4^{3-}) was then used in the model (instead of separate nutrient variables). PCA1 explained 60.8% and 49.4% for the heat wave and post-heat wave phases, respectively. The predictors included in the multivariate analysis were temperature, chemicals and their interaction, as well as block (but interactions of block with other factors were excluded). As many of the quantified processes are size-dependent, logarithm of the geometric mean of body size (mm) of each individual was included as a covariate in the analyses. Prior to analyses, the data were normalized and the resemblance matrix were based on Euclidean distance. The data were cube root–transformed when necessary to account for data dispersion. All analyses were run 9999 times using type I SS. Dispersion of the data were inspected in PERMDISP analyses and MDS plots (Anderson et al. 2008). Effect sizes for the variables included in the PERMANOVA (Nilsson et al. 1976) are presented in the Supplementary material Appendix 1 Table A3.

Second, to assess the direct and indirect impacts of heat wave and MPs on different ecosystem processes, as well as the causality of the processes, the data was further analysed using Structural equation modelling (SEM) in AMOS (ver. 22.0.0). In these SEM analyses, we only included factors that showed significant effects in the multivariate PERMANOVAs as exogenous variables (i.e. any non-significant main factors were excluded from the SEM models). The original models were created based on our best biological knowledge and estimates of the causality of processes (Supplementary material Appendix 1 Fig. A1). The models were optimized based on model selection procedure. The model fit estimates were based on χ^2, root mean square of approximation (RMSEA), comparative fit index (CFI) and Akaike information criteria (AIC) values (Grace 2006). When no large differences between two compared models were observed (AIC < 2), the model with lower number of paths was always selected for according to the principle of parsimony. In some cases this led to removal of endogenous variables from the model. As many of the exogenous variables had non-normal distributions, the model fit was further estimated by Bollen–Stine bootstrapping. Standardized regression weights were calculated for each path. Squared multiple correlations (R^2) were calculated for each endogenous variable to summarize both direct and indirect treatment effects on the different processes.

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.4q7813g> (Salo et al. 2018).

Results

The temperature (but not chemical) treatment had a significant impact on the multivariate response during the heat wave phase (Table 1) and explained 17.5% of the observed variation in the measured parameters. The effect of the heat wave declined over time, however, and the temperature treatment explained only 4.2% of the multivariate response after the heat wave phase. The impacts of the chemical treatment were only detectable after an extended exposure (i.e. during the post-heat wave phase) and explained 9.7% of the observed variation. No significant interactive impacts of temperature and chemical treatment were found (Table 1).

Immediate effects of the heat wave

The heat wave had strong immediate direct and indirect effects on individual level estimates of ecosystem process rates (Fig. 1a). Specifically, direct effects of the heat wave were seen as increased rates of consumption (both autochthonous and allochthonous resources), FPOM production, nutrient excretion and reproduction (Fig. 1a). Indirect positive effects of

Table 1. Results from multivariate permutational analyses for (a) a heat wave phase and (b) a post-heat wave phase from an experiment testing the effects of heat wave, micropollutants and their interactions on the combined ecosystem processes in *Lymnaea stagnalis*. η^2 indicates the proportion of variance explained by each factor. Bold values indicate significant results at $\alpha = 0.05$. Covariate is based on the geometric mean of body size.

Source	Heat wave					Post-heat wave				
	df	MS	Pseudo-F	η^2	p(perm)	df	MS	Pseudo-F	η^2	p(perm)
Covariate	1	31.92	5.17	6.33	**0.0001**	1	12.86	0.95	2.51	0.46
Temperature	1	88.25	14.39	17.51	**0.0001**	1	21.57	3.16	4.21	**0.0023**
Chemical	1	7.83	1.26	1.55	0.26	1	49.38	7.50	9.65	**0.0001**
Block	1	8.83	1.44	1.75	0.18	1	36.36	5.58	7.10	**0.0001**
Temp × Chem	1	11.34	1.85	2.25	0.07	1	7.16	1.10	1.40	0.36
Residual	58	6.14		70.60		59	6.52		75.13	

(a)

Heat wave

0.42 → Reproduction ($R^2 = 0.29$)
0.37
0.42 → Cons. autochton. ($R^2 = 0.18$)
0.40
0.38 → Cons. allochton. ($R^2 = 0.14$)

0.21 (Cons. autochton. → Reproduction)

0.25
0.41 → Nutrients ($R^2 = 0.66$)
0.44
0.41 → FPOM ($R^2 = 0.42$)
-0.23 → Growth ($R^2 = 0.05$)

(b)

Previously experienced heat wave | Micropollutants

-0.34 → Reproduction ($R^2 = 0.11$)
-0.22
0.64 → Cons. autochton. ($R^2 = 0.40$)
0.25 → Cons. allochton. ($R^2 = 0.06$)
0.26 → Respiration ($R^2 = 0.07$)

0.39 → Nutrients ($R^2 = 0.20$)
0.36 → FPOM ($R^2 = 0.13$)
0.27 → Growth ($R^2 = 0.07$)

Figure 1. Structural equation model for the ecosystem processes mediated by the snails (a) during and (b) after an experimental heat wave. Effects of MPs were only detected after the heat wave, while heat wave effects were visible during both experimental phases. No interactive effects were observed. Black solid arrows indicate significant positive effects, black dashed arrows indicate significant negative effects, and the grey arrow indicates a non-significant positive effect. These represent standardized estimates and the thickness of the arrow, and the value next to it, indicate the strength of the correlation. R^2 indicates the amount of variation that is explained by the applied stressor(s) in the model. The models are significant (Bollen–Stine bootstrap p > 0.05).

the heat wave were seen as increases in nutrient excretion and FPOM production. The former was due to increased reproduction and consumption of autochthonous material, the latter due to an increased consumption of autochthonous and allochthonous material. An indirect negative effect of the heat wave was seen as reduced growth (i.e. body length), caused by increased consumption of allochthonous material (Fig. 1a). Bootstrapping suggested that the obtained model for the heat wave phase was correct (Bollen–Stine bootstrap, p = 0.114). In addition to the R^2-values indicating the amount of variation for each variable explained by the heat wave (Fig. 1a), the summarized direct and indirect effects of the heat wave for the variables included in the final SEM are presented in Fig. 2.

Delayed effects of the heat wave and MPs

The previously experienced heat wave decreased reproduction and nutrient excretion (Fig. 1b). In contrast, exposure to MPs strongly increased consumption of autochthonous resources after the heat wave phase, which increased both nutrient excretion and FPOM production. Further, exposure to MPs directly enhanced consumption of allochthonous resources and respiration (reflecting metabolic demand), thereby indirectly increasing growth (in length; Fig. 1b). Bootstrapping indicated that the model was correct (Bollen–Stine bootstrap,

p = 0.433). In addition to the R^2-values indicating the amount of variation for each variable explained by the heat wave and exposure to MPs (Fig. 1b), the summarized direct and indirect effects of these factors for the variables included in the final SEM are presented in Fig. 3.

Discussion

We investigated how the effects of two important anthropogenic stressors (high temperature and chemical stress) on individuals translate to parameters reflecting consumer multifunctionality. We used a laboratory experiment to quantify how a short-term heat wave and constant exposure to MPs independently and jointly may alter multiple ecosystem processes mediated by an aquatic consumer *L. stagnalis*. We found a transient increase in rates of several consumer-mediated processes as a result of our experimental heat wave. Specifically, consumption rates and reproduction increased, which subsequently increased nutrient excretion and production of particulate organic matter. In contrast, delayed effects of the experimental heat wave (i.e. those seen during the post-heat wave phase) on consumer-mediated processes appeared to be negative, evident as lower levels of reproduction and nutrient excretion. Our results further indicate that extended exposure of individuals to sublethal concentrations
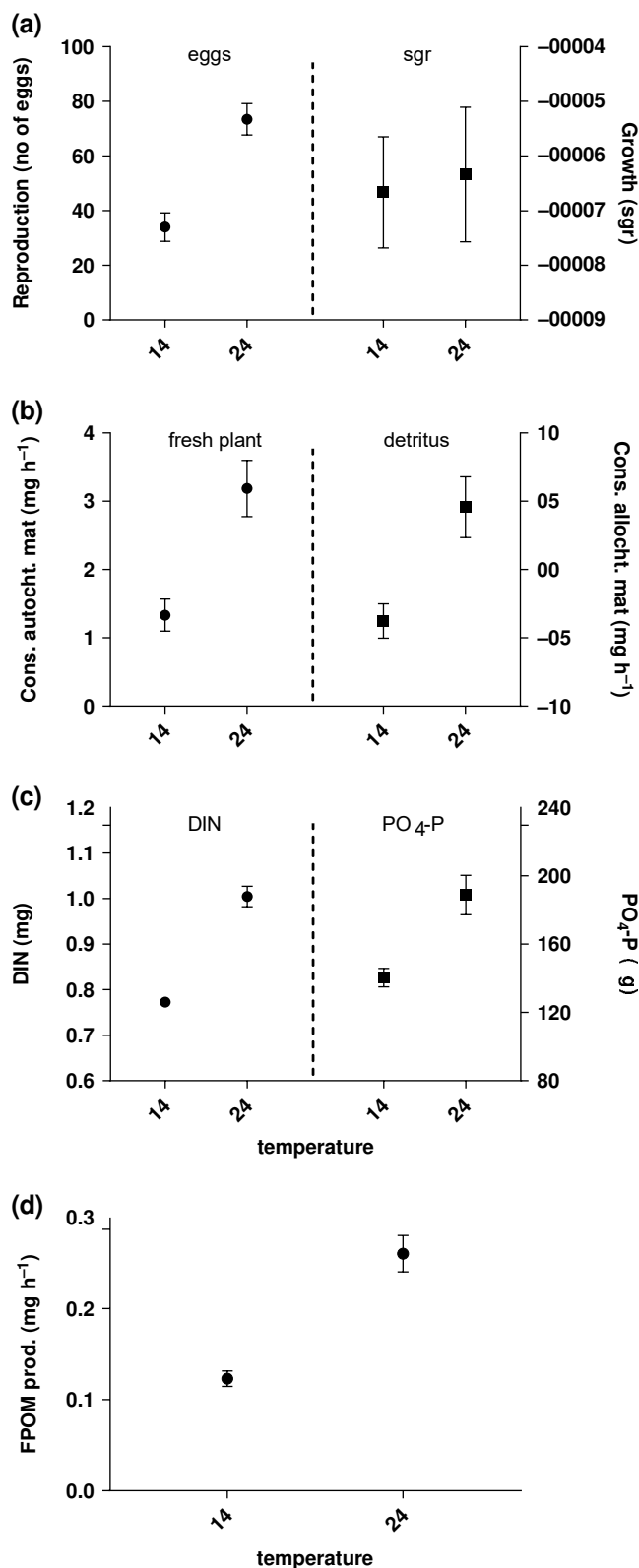
Figure 2. The summarized direct and indirect effects of the two temperature treatments (constant temperature = 14°C, heat wave = 24°C) on (a) reproduction (number of eggs produced) and individual growth (sgr, in mm), (b) consumption of autochtonous (fresh plant) and allochtounous (detritus) material, (c) nutrient excretion (DIN and PO_4-P) and (d) FPOM production during the heat wave phase. Data are means ± SE.

of chemical pollutants may have trophic consequences: MPs strongly enhanced consumption of an autochtonous resource, increasing nutrient excretion and FPOM production.

Because our experiment was conducted at the individual (rather than community or ecosystem) level, our results provide mechanistic evidence for how organismal responses to environmental stress may strengthen 'top–down' control of ecosystem processes (O'Connor 2009, O'Connor et al. 2009). For instance, consumers (i.e. snails) likely increased their consumption of basal resources when exposed to stressful conditions (i.e. heat wave or MPs) to maintain organismal function due to higher energetic demands. This, in turn, increased nutrient excretion. While an increase in available resources (e.g. in nutrient concentrations) can enhance primary production (Reusch et al. 1994), selectively increased consumption of (autochtonous) plant material can also alter plant species richness and composition (Sheldon 1987) or modify interspecific plant interactions (Chaneton and Bonsall 2000). Consequently, the biomass of suitable food items for a specific grazer may change with altered grazing pressure – resulting in an ecological feedback from organismal exposure to environmental stress. Accordingly, several simulations suggest that increased primary consumption is more likely to lead to overexploitation of plant biomass raising the risk for local extinctions for both plants (prey) and grazers (predator) (Binzer et al. 2012, Fussmann et al. 2014). However, this problem may be less relevant in aquatic ecosystems receiving detrital subsidies from the adjacent terrestrial environment, which can help stabilize trophic dynamics (Takimoto et al. 2002, Rooney et al. 2006).

In addition to resource consumption rate, environmental change may alter diet preference of organisms (Lee et al. 2015, Schmitz et al. 2016). For instance, detritus has lower N:C and P:C ratios compared to live plant material. In our experiment, we saw a higher relative consumption of autochtonous plant material (fresh *Potamogeton* leaves) compared to allochtonous detritus (dried *Alnus* leaves), likely reflecting a preference for a diet with higher energy content and palatability. Interestingly, however, despite the continuous access to both dietary options ad libitum, the snails continued to consume the detritus source throughout the experiment and even increased their consumption rate to some degree when exposed to stress. While detritus consumption resulted in higher quantity of released FPOM – indicating transfer into the decomposer food chain - the negative correlation of detritus processing with growth indicates that overconsuming such an energy-sparse resource comes at a cost to the individual organism. Consumption of lower quality food (e.g. detritus) may, however, enhance the stability of a system as foraging of high quality food may yield stronger trophic cascades during stress (Hall et al. 2007). Given high variability in quality of available resources in nature, how impacts of anthropogenic stress on individuals are mediated to ecosystem processes may depend on resource availability. Accordingly, resource quality may also influence the sign and magnitude of the stress responses. Evidence for this comes from the discrepancy in the findings of our current study and a previous study where the same population of *L. stagnalis* was exposed to the same

stress combinations – but provided with a different food source (Salo et al. 2017). Both food consumption, respiration and growth rates showed a less dramatic stress response in the previous experiment. This may have been because snails had access to a resource with higher palatability (i.e. lettuce; Salo et al. 2017) than in the current experiment were they received the less palatable *Potamogeton* and *Alnus* leaves, more typical resources available in nature. Thus, not only resource quantity, but also resource quality, are important determinants of how stress translates from physiological to ecological responses, and potentially across hierarchical levels of biological complexity (Galic et al. 2018).

Natural communities are complicated systems with complex feedback loops between species (Fussmann et al. 2007) as well as between species and environment factors (Kéfi et al. 2016). As the variability in ecological responses is likely to decrease when upscaling to higher levels of biological organization (Felton and Smith 2017) and the changes in systems
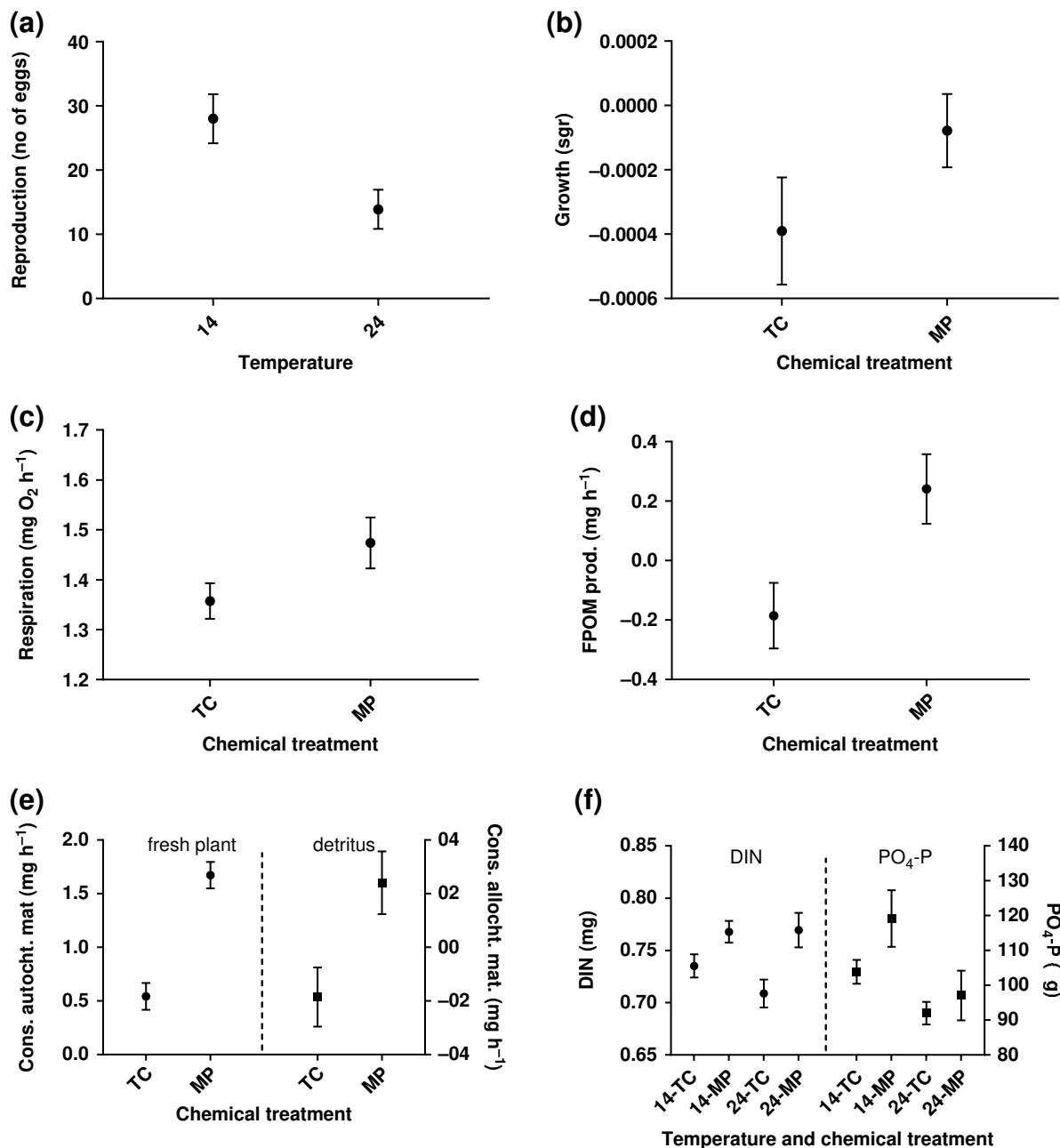

Figure 3. The summarized direct and indirect effects of the two temperature treatments (constant temperature = 14°C, heat wave = 24°C), chemical treatments (technical control = TC, micropollutants = MP) or both on (a) reproduction, (b) specific growth rate, (c) respiration, (d) FPOM production, (e) consumption of autochtonous (fresh plant) and allochtonous (detritus) material and (f) nutrient excretion after the heat wave phase. Data are mean ± SE.

may be non-linear (Smith et al. 2009), predicting community or ecosystem level changes based on individual is challenging, and may overestimate the impact on the system due to asymmetric responses and functional redundancies. However, key species in a community may have a disproportionate impact on the resilience of the ecosystem and strong individual level responses have the potential to change interactions at population or community level (Felton and Smith 2017). Further, as the processes on community and ecosystem level take longer time to be modified compared to the individual level responses (Smith et al. 2009) individual-level responses may help estimating the direction of the change in process rates during and after stress. However, as especially multiple stressor effects may increase with level of biological organization, direct extrapolations may underestimate the severity of stress on populations and ecosystems (Galic et al. 2018).

Additionally, with intraspecific variation in environmental responses, the covariation of traits can be useful when predicting population and eco-evolutionary dynamics (Hendry 2016, Moran et al. 2016). In our experiment, we minimized the effect of biotic interactions on the measured ecosystem parameters to mechanistically quantify the underlying causes of how stress may affect an important node, primary consumers, in benthic food webs. Our study indicates that the stress sensitivity of reproduction and growth (fluctuations during and after stress) may increase fluctuations in and, hence, reduce predictability of resource availability for predators. However, exclusion of intra- and interspecific interactions may have caused us to under- or overestimate the impacts of stress on the individuals and the studied ecosystem processes. In particular, resource limitation, parasitism and predation continuously affect individuals and populations in natural ecosystems, and thus may also affect the rate of ecosystem processes. For example, higher than optimum temperatures tend to decrease plant productivity (Salo and Pedersen 2014), while herbicides may reduce photosynthesis and lead to reduced primary production (Lichtenthaler 1996), thereby limiting resource availability for grazers. The presence of higher trophic levels may reduce the foraging of individuals, and thus also the rates for different consumer-mediated processes (Schmitz et al. 2008). Further, food processing can increase exposure to parasites (Seppälä et al. 2011, Seppälä and Leicht 2015) altering infection risk under environmental stress.

To conclude, we took a strictly ecological perspective in our assessment of how individual-level stress responses may transfer to multiple ecosystem properties mediated by snails. We found that these effects may be either positive or negative depending on the function. We showed that environmental stress affects resource consumption, reproduction and growth, demonstrating the potential effects that different anthropogenic stressors may have on consumer-mediated functions. While we still lack information about how individual-level responses couple with community and ecosystem level responses (Smith et al. 2009, Galic et al. 2018), future work should aim to combine individual-level responses with responses at different levels along the hierarchy of biological organization for more realistic estimates on how stress may alter ecosystem functioning over larger temporal scales. Similarly, due to the intimate interactions between ecological and evolutionary processes (i.e. eco-evolutionary feedbacks, Hairston et al. 2005, Matthews et al. 2014, Hendry 2016), studies on phenotypic and genetic stress responses would elucidate the ecosystem-level consequences of evolution in changing environments.

References

Anderson, M. J. et al. 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. – PRIMER-E.

Angilletta, M. J. 2009. Thermal adaptation – a theoretical and empirical synthesis. – Oxford Univ. Press.

Binzer, A. et al. 2012. The dynamics of food chains under climate change and nutrient enrichment. – Phil. Trans. R. Soc. B 367: 2935–2944.

Boyd, P. W. et al. 2014. Biological ramifications of climate-change-mediated oceanic multi-stressors. – Nat. Clim. Change 5: 71–79.

Broomhall, S. 2002. The effects of endosulfan and variable water temperature on survivorship and subsequent vulnerability to predation in *Litoria citropa* tadpoles. – Aquatic Toxicology 61: 243–250.

Brown, J. H. et al. 2004. Toward a metabolic theory of ecology. – Ecology 85: 1771–1789.

Chaneton, E. J. and Bonsall, M. B. 2000. Enemy-mediated apparent competition: empirical patterns and the evidence. – Oikos 88: 380–394.

Dinh, K. V. et al. 2016. Exposure to a heat wave under food limitation makes an agricultural insecticide lethal: a mechanistic laboratory experiment. – Global Change Biol. 22: 3361–3372.

Doi, H. et al. 2010. Stable isotopes indicate individual level trophic diversity in the freshwater gastropod *Lymnaea stagnalis*. – J. Moll. Stud. 76: 384–388.

Elger, A. et al. 2004. Disturbances as a structuring factor of plant palatability in aquatic communities. – Ecology 85: 304–311.

Faltýnková, A. et al. 2007. Larval trematodes (Digenea) of the great pond snail, *Lymnaea stagnalis* (L.), (Gastropoda, Pulmonata) in central Europe: a survey of species and key to their identification. – Parasite 14: 39–51.

Felton, A. J. and Smith, M. D. 2017. Integrating plant ecological responses to climate extremes from individual to ecosystem levels. – Phil. Trans. R. Soc. B 372: 2016142.

Fussmann, G. F. et al. 2007. Eco-evolutionary dynamics of communities and ecosystems. – Funct. Ecol. 21: 465–477.

Fussmann, K. E. et al. 2014. Ecological stability in response to warming. – Nature 4: 206–210.

Galic, N. et al. 2018. When things don't add up: quantifying impacts of multiple stressors from individual metabolism to ecosystem processing. – Ecol. Lett. 21: 568–577.

Ghalambor, C. K. et al. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. – Funct. Ecol. 21: 394–407.

Grace, J. B. 2006. Structural equation modeling and natural systems. – Cambridge Univ. Press.

Hairston, N. G. Jr. et al. 2005. Rapid evolution and the convergence of ecological and evolutionary time. – Ecol. Lett. 8: 1114–1127.

Hall, S. R. et al. 2007. Food quality, nutrient limitation of secondary production, and the strength of trophic cascades. – Oikos 116: 1128–1143.

Hendry, A. P. 2016. Eco-evolutionary dynamics. – Princeton Univ. Press.

Kéfi, S. et al. 2016. When can positive interactions cause alternative stable states in ecosystems? – Funct. Ecol. 30: 88–97.

Lee, K. P. et al. 2015. Macronutrient balance modulates the temperature-size rule in an ectotherm. – Am. Nat. 186: 212–222.

Leicht, K. et al. 2013. An experimental heat wave changes immune defense and life history traits in a freshwater snail. – Ecol. Evol. 3: 4861–4871.

Lichtenthaler, H. K. 1996. Vegetation stress: an introduction to the stress concept in plants. – Plant Physiol. 148: 4–14.

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

Malzahn, A. M. et al. 2010. Differential effects of nutrient-limited primary production on primary, secondary or tertiary consumers. – Oecologia 162: 35–48.

Matthews, B. et al. 2014. Under niche construction: an operational bridge between ecology, evolution and ecosystem science. – Ecol. Monogr. 84: 245–263.

Meehl, G. A. and Tebaldi, C. 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. – Science 305: 994–997.

Moran, E. V. et al. 2016. Intraspecific trait variation across scales: implications for understanding global change responses. – Global Change Biol. 22: 137–150.

Munz, N. A. et al. 2017. Pesticides drive risk of micropollutants in wastewater-impacted streams during low flow conditions. – Water Res. 110: 366–377.

Nordström, M. C. and Bonsdorff, E. 2017. Organic enrichment simplifies marine benthic food web structure. – Limnol. Oceanogr. 62: 2179–2188.

O'Connor, M. I. 2009. Warming strengthens an herbivore: plant interaction. – Ecology 90: 388–398.

O'Connor, M. I. et al. 2009. Warming and resource availability shift food web structure and metabolism. – PloS Biol. 7: 3–8.

Reusch, T. B. H. et al. 1994. Blue mussels *Mytilus edulis* do not interfere with eelgrass *Zostera marina* but fertilize shoot growth through biodeposition. – Mar. Ecol. Prog. Ser. 108: 265–282.

Reznick, D. 1983. The structure of guppy life histories: the tradeoff between growth and reproduction. – Ecology 64: 862–873.

Rooney, N. et al. 2006. Structural asymmetry and the stability of diverse food webs. – Nature 442: 265–269.

Sala, O. E. et al. 2000. Global biodiversity scenarios for the year 2100. – Science 287: 1770–1774.

Salo, T. and Pedersen, M. F. 2014. Synergistic effects of altered salinity and temperature on estuarine eelgrass (*Zostera marina*) seedlings and clonal shoots. – J. Exp. Mar. Bio. Ecol. 457: 143–150.

Salo, T. et al. 2017. Resilience to heat waves in the aquatic snail *Lymnaea stagnalis*: additive and interactive effects with micropollutants. – Freshwater Biol. 62: 1831–1846.

Salo, T. et al. 2018. Data from: Simultaneous exposure to a pulsed and a prolonged anthropogenic stressor can alter consumer multifunctionality. – Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.4q7813g>.

Schmitz, O. J. et al. 2008. From individuals to ecosystem function: toward an integration of evolutionary and ecosystem ecology. – Ecology 89: 2436–2445.

Schmitz, O. J. et al. 2016. Temperature dependence of predation stress and the nutritional ecology of a generalist herbivore. – Ecology 97: 3119–3130.

Schwarzenbach, R. et al. 2006. The challenge of micropollutants in aquatic systems. – Science 313: 1072–1077.

Seppälä, O. and Leicht, K. 2015. Quality attracts parasites: host condition-dependent chemo-orientation of trematode larvae. – Funt. Ecol. 29: 791–795.

Seppälä, O. et al. 2011. Food makes you a target: disentangling genetic, physiological and behavioral effects determining susceptibility to infection. – Evolution 65: 1367–1375.

Seppälä, O. et al. 2013. Are sick individuals weak competitors? Competitive ability of snails parasitized by a gigantism-inducing trematode. – PloS One 8: 1–6.

Sheldon, S. P. 1987. The effects of herbivorous snails on submerged macrophyte communities in Minnesota lakes. – Ecology 68: 1920–1931.

Smith, M. D. et al. 2009. A framework for assessing ecosystem dynamics in response to chronic resource alterations induced by global change. – Ecology 90: 3279–3289.

Stamm, C. et al. 2016. Unravelling the impacts of micropollutants in aquatic ecosystems: interdisciplinary studies at the interface of large-scale ecology. – Adv. Ecol. Res. 55: 183–223.

Sulmon, C. et al. 2015. Abiotic stressors and stress responses: what commonalities appear between species across biological organization levels? – Environ. Pollut. 202: 66–77.

Svensson, F. et al. 2017. In situ warming strengthens trophic cascades in a coastal food web. – Oikos 126: 1150–1161.

Takimoto, G. et al. 2002. Seasonal subsidy stabilizes food web dynamics: balance in a heterogeneous landscape. – Ecol. Res. 17: 433–439.

Vannote, R. and Sweeney, B. 1980. Geographic analysis of thermal equilibria: a conceptual model for evaluating the effect of natural and modified thermal regimes on aquatic insect communities. – Am. Nat. 115: 667–695.

Visser, M. E. and Both, C. 2005. Shifts in phenology due to global climate change : the need for a yardstick shifts in phenology due to global climate change : the need for a yardstick. – Proc. R. Soc. B 272: 2561–2569.

Winder, M. and Schindler, D. E. 2004. Climate change uncouples trophic interactions in an aquatic ecosystem. – Ecology 85: 2100–2106.

Wittmer, I. K. et al. 2010. Significance of urban and agricultural land use for biocide and pesticide dynamics in surface waters. – Water Res. 44: 2850–2862.

Woodward, G. and Perkins, D. M. 2015. Chapter 6. Biodiversity, ecosystem functioning, and services in fresh waters: ecological and evolutionary implications of climate change. – In: Belgrano, A. et al. (eds), Aquatic functional biodiversity. Academic Press, pp. 127–155.

Zarco-Perello, S. et al. 2017. Tropicalization strengthens consumer pressure on habitat-forming seaweeds. – Sci. Rep. 7: 820.

Zhang, P. et al. 2018. The effect of temperature on herbivory by the omnivorous ectotherm snail *Lymnaea stagnalis*. – Hydrobiology 812: 147–155.