

# Predator diversity and environmental change modify the strengths of trophic and nontrophic interactions

ARNAUD SENTIS<sup>1,2,3</sup>, CHARLÈNE GÉMARD<sup>1</sup>, BAPTISTE JAUGEON<sup>1</sup> and DAVID S. BOUKAL<sup>1,2</sup>

<sup>1</sup>Department of Ecosystem Biology, Faculty of Science, University of South Bohemia, 370 05 České Budějovice, Czech Republic,

<sup>2</sup>Laboratory of Aquatic Insects and Relict Ecosystems, Biology Centre CAS, Institute of Entomology, 370 05 České Budějovice,

Czech Republic, <sup>3</sup>Unité Mixte de Recherche 5174 'Evolution et Diversité Biologique', Centre National de la Recherche Scientifique, Université de Toulouse, Institut de Recherche pour le Développement, École Nationale de Formation Agronomique, BP 22687, 31326 Castanet-Tolosan, France

## Abstract

Understanding the dependence of species interaction strengths on environmental factors and species diversity is crucial to predict community dynamics and persistence in a rapidly changing world. Nontrophic (e.g. predator interference) and trophic components together determine species interaction strengths, but the effects of environmental factors on these two components remain largely unknown. This impedes our ability to fully understand the links between environmental drivers and species interactions. Here, we used a dynamical modelling framework based on measured predator functional responses to investigate the effects of predator diversity, prey density, and temperature on trophic and nontrophic interaction strengths within a freshwater food web. We found that (i) species interaction strengths cannot be predicted from trophic interactions alone, (ii) nontrophic interaction strengths vary strongly among predator assemblages, (iii) temperature has opposite effects on trophic and nontrophic interaction strengths, and (iv) trophic interaction strengths decrease with prey density, whereas the dependence of nontrophic interaction strengths on prey density is concave up. Interestingly, the qualitative impacts of temperature and prey density on the strengths of trophic and nontrophic interactions were independent of predator identity, suggesting a general pattern. Our results indicate that taking multiple environmental factors and the nonlinearity of density-dependent species interactions into account is an important step towards a better understanding of the effects of environmental variations on complex ecological communities. The functional response approach used in this study opens new avenues for (i) the quantification of the relative importance of the trophic and nontrophic components in species interactions and (ii) a better understanding how environmental factors affect these interactions and the dynamics of ecological communities.

**Keywords:** biodiversity–ecosystem functioning, facilitation, functional response, global change, interference, multiple-predator effects, prey density, temperature

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## Introduction

Determining the factors and mechanisms influencing the distribution and strengths of species interactions is crucial to understand and predict the dynamics and stability of ecological communities (McCann *et al.*, 1998; Berlow, 1999; Bascompte *et al.*, 2006; Tang *et al.*, 2014; Vázquez *et al.*, 2015). In particular, a mechanistic understanding of how environmental drivers affect species interaction strength will provide critically important information about global change impacts on populations and communities (Gilbert *et al.*, 2014; Sentis *et al.*, 2014). However, developing such mechanistic framework is challenging because species are linked by a multitude of interactions that can be affected

differentially by global change drivers (Tylianakis *et al.*, 2008). Moreover, until recently, most studies were limited to the effects of a single driver on one type of interaction (e.g. predation, competition, or mutualism) in simple experiments typically involving only 1–3 species (Tylianakis *et al.*, 2008). This leaves open three fundamental questions about the dependence of species interaction strengths on (i) species diversity, (ii) multiple types of interactions, and (iii) interacting environmental drivers.

While food-web studies traditionally focus on trophic interactions and their strength is most often measured in single-predator experiments, an increasing number of studies has highlighted the importance of nontrophic interactions among multiple predators and their prey to fully understand and predict species interaction strengths in ecological communities (Okuyama & Bolker, 2007, 2012; McCoy *et al.*, 2012; Davenport &

Correspondence: Arnaud Sentis, tel. +33 5 61 75 32 32, fax +33 5 61 75 03 09, e-mail: arnaud.sentis@gmail.com

Chalcraft, 2013). Indeed, pairwise interaction strengths are often not independent from each other and nontrophic interactions among predators and their prey (also referred to as nonconsumptive predator effects, trait-mediated indirect interactions, or emergent multiple-predator effects) can significantly affect the strength of species interactions and the dynamics of ecological communities (Sih *et al.*, 1998; Vonesh & Osenberg, 2003; Werner & Peacor, 2003). Nevertheless, the effects of key environmental drivers on the strength of nontrophic interactions in multipredator systems remain largely unknown. We argue that the currently lacking understanding of these effects is crucial to forecast the impact of environmental change on the dynamics and functioning of ecological communities.

Changing temperature is one of the key environmental drivers with far-reaching consequences. Temperature influences nearly all metabolic and biological rates, species interactions, population dynamics, and ecosystem functioning (Petchey *et al.*, 1999; Brown *et al.*, 2004; Tylianakis *et al.*, 2008). Recent studies (O'Connor, 2009; O'Connor *et al.*, 2011; Gilbert *et al.*, 2014) have addressed how climate change might impact consumer–resource interaction strength but did not address multiple-predator interactions. In simple ectotherm predator–prey systems, warming typically increases short-term trophic interaction strength (i.e. feeding rate) because predators are more efficient at searching and handling prey (Rall *et al.*, 2012; Sentis *et al.*, 2012; Dell *et al.*, 2013). In multiple predator–prey systems, the effects of temperature on the relationship between predator diversity and species interaction strengths remain little explored. Barton & Schmitz (2009) and Sentis *et al.* (2014) showed that warming strengthens intraguild predation (i.e. predation among predators sharing the same prey). However, to our knowledge, no studies have investigated thermal dependence of nontrophic interactions among predator species. This leaves an important gap in our understanding of how climate change alters species interactions in predator-rich communities.

A challenge in assessing the relative importance of trophic and nontrophic interactions is to account for the nonlinearity and time dependence of species interactions (McCoy *et al.*, 2012; Okuyama & Bolker, 2012). Indeed, the feeding rate of most consumers is characterized by density-dependent functions such as Holling type II functional response that saturates with prey density (Jeschke *et al.*, 2004). However, most studies focusing on nontrophic interactions among predators are conducted using additive and substitutive experimental designs that assume linear interaction strengths and do not account for prey depletion (Vance-Chalcraft & Soluk, 2005; Griffen, 2006; Schmitz, 2007; Griffen & Williamson, 2008). These limitations bias results at high

prey densities or when prey depletion is substantial (McCoy *et al.*, 2012; Sentis *et al.*, 2013a). To overcome these biases, recent studies have stressed the importance of using functional relationships based on mechanistic models and experimental designs that incorporate a gradient of the key ecological variable (e.g. prey density) rather than simple factorial designs that do not account for time dependence and nonlinear interaction strength (Denny & Benedetti-Cecchi, 2012; McCoy *et al.*, 2012; Okuyama & Bolker, 2012). In particular, characterizing the predator functional response and considering predator–prey dynamics have been identified as particularly important to (i) adequately assess the strengths of trophic and nontrophic interactions, (ii) identify the mechanisms driving changes in species interactions, and (iii) extrapolate the results to larger temporal and spatial scales (McCoy *et al.*, 2012; Okuyama & Bolker, 2012).

In this study, we used a functional response approach to investigate the effects of predator diversity, prey density, and temperature on the strengths of trophic and nontrophic interactions within a freshwater food web. The combination of modelling and experimental approaches enabled the quantification of the relative importance of trophic and nontrophic interactions and the identification of the mechanisms by which temperature and resource density affect these two interaction types. Our study emphasizes the importance of considering multiple environmental factors together with different interaction types to better understand and predict relationships between species diversity and interaction strengths in a rapidly changing world.

## Materials and methods

### Biological system

The experimental system consisted of larvae of three-predatory aquatic insects: *Sympetrum vulgatum* (Odonata: Libellulidae), *Libellula quadrimaculata* (Odonata: Libellulidae), and *Chaoborus obscuripes* (Diptera: Chaoboridae). The prey was *Daphnia magna* (Cladocera: Daphniidae). The predators represent three widespread and common European genera, whose species are important predators in small standing waters and readily prey on *Daphnia* (Klecka & Boukal, 2012). These predators have essentially the same hunting mode representing a sit-and-wait strategy but occupy different habitat domains (i.e. the spatial extent to which a microhabitat is used by a species; Schmitz, 2007): *C. obscuripes* is a pelagic predator foraging exclusively within the water column, *L. quadrimaculata* is a benthic predator foraging exclusively from the benthic zone, and *S. vulgatum* is a predominately benthic predator that can access prey in the water column (Klecka & Boukal, 2014; Sentis A., personal observation). The prey, *D. magna*, is an active filter feeder with a broad habitat domain spanning the entire

water column including the layer just above the benthic zone (see Method S1 for the capture, rearing, and standardisation of predators and prey).

We expected that (i) predators with overlapping habitat domains (i.e. any conspecifics or the two dragonfly species) interact negatively by intraguild predation and intraguild predator avoidance or by direct physical interference among predators (i.e. interference competition) and (ii) coexistence of predators with nonoverlapping habitat domains (*C. obscuripes* with *L. quadrimaculata* or with *S. vulgatum*) may lead to facilitation when prey escape behaviour from one predator increases the encounter probability with the other predator. Predators and prey were standardized to avoid confounding intraspecific effects of body size and developmental stage (see Methods S1 for details).

### Experiments

Our aim was to investigate the effects of predator diversity, prey density, and temperature on the strengths of trophic and nontrophic interactions within the freshwater food web described above. As recently suggested (McCoy *et al.*, 2012; Okuyama & Bolker, 2012), we used a functional response approach to estimate the density and thermal dependencies of trophic and nontrophic interactions. Our approach consists in (i) investigating the effects of abiotic factor (i.e. temperature) on the functional response of each predator species in single-predator experiments; (ii) using the functional response estimates to predict trophic interactions strength along thermal and prey density gradients for any predator combinations; (iii) empirically measuring the effects of temperature, predator diversity/density, and prey density on species interaction strength; and (iv) comparing the predicted and observed species interaction strengths to deduce nontrophic interaction strength and its dependence on predator diversity, temperature, and prey density.

#### Experiment 1. Effects of temperature on single-predator trophic functional responses

To quantify the *per capita* functional response of each predator species, we used a full factorial design with nine prey densities: 5, 15, 30, 50, 75, 110, 150, 200 and 280 prey L<sup>-1</sup> (for *C. obscuripes*, the highest density was not assayed because, during a pilot experiment, maximum predation rate was already achieved at 200 prey L<sup>-1</sup>) and two temperatures: 16 ± 0.5 and 20 ± 0.5 °C (based on continuous measurements using Ebro© EBI 20-TE1 units). The regime of 16 °C approximated water temperature at the locality when predators were collected and 20 °C matched the increase of 4 °C predicted for 2100 (IPCC, 2013). Experimental arenas consisted of plastic jars (length 12.5 cm, width 8 cm, height 19 cm) filled with 1 L of aged tap water and lined with a 1–2 cm layer of fine crystalline sand (diameter <1 mm). Prey were introduced in the arenas and allowed to acclimate to experimental temperature for 2 h before the start of the experiment. One predator larva was then introduced in each arena and allowed to feed under continuous light conditions for 6 h. Surviving prey were counted

to establish prey mortality. Natural mortality of *D. magna* was assessed in control treatments without predators. Eight replicates per each treatment were performed.

#### Experiment 2. Effects of temperature, prey density, and predator diversity on species interaction strength

To examine the effects of predator density and diversity, prey density, and temperature on species interaction strengths, we used a factorial design with two temperatures (16 and 20°C), three prey densities (15, 75, and 150 prey L<sup>-1</sup>) and eight predator assemblages: no predator (controls), two conspecific predators (all three species were tested), two heterospecific predators (all three combinations were tested), and three heterospecific predators. Inclusion of further treatments with three-predator individuals was logistically impossible due to limited space and number of available animals. Ten replicates per each treatment were performed. Prey and predator standardization, experimental set-up and duration, and data recording were the same as in Experiment 1. Both experiments were conducted simultaneously to avoid temporal effects. During Experiment 2, we observed intraguild predation of *S. vulgatum* on *C. obscuripes*. However, intraguild predation was too rare (12 of 120 replicates involving the two species) to perform meaningful analysis and, as this study focuses on nontrophic interactions, these data were excluded from subsequent analyses.

### Data analyses

*Experiment 1. Effects of temperature on single-predator trophic functional response.* Standard procedure (Text S1) indicated a type II functional response for all three predators and both temperatures (Table S1). We therefore used type II Rogers' random predator equation (Rogers, 1972) to account for prey depletion during the time course of the experiment:

$$N_e = N_0(1 - \exp(-a(t - hN_e))), \quad (1)$$

where  $N_e$  is the number of prey eaten,  $N_0$  is the initial prey density (prey L<sup>-1</sup>),  $t$  is the duration of the experiment (day),  $h$  is the prey handling time (day prey<sup>-1</sup>), and  $a$  is the predator search rate (L day<sup>-1</sup>). Before fitting the model to the data, we used the Lambert  $W$  function to solve Eqn (1) for  $N_e$  (see Bolker 2008 for details):

$$N_e = N_0 - \frac{W(ahN_0e^{-a(t-hN_0)})}{ah} \quad (2)$$

For each predator species, we determined the effects of temperature on functional response parameters  $a$  and  $h$  by considering different functional response models covering all possible combinations of temperature dependence in each parameter and using temperature as a categorical variable: only  $a$  depends on temperature, only  $h$  depends on temperature, both  $a$  and  $h$  depend on temperature, or neither  $a$  nor  $h$  depends on temperature (Sentis *et al.*, 2015). This yielded a total of four candidate models that were fitted to the data using a maximum-likelihood method with a binomial distribution and with temperature dependence modelled as a parameter argument allowing  $a$  and/or  $h$  to have different

values for each temperature (package 'bbmle'; Bolker, 2008). We selected the best-fitting model by ranking the candidate models according to their AICc values (Burnham & Anderson, 2002). Data were not corrected for natural prey mortality in these analyses because mortality in the controls was negligible (overall mean  $\pm$  SE =  $3.04 \pm 0.51\%$ ).

*Experiment 2. Effects of temperature, prey density, and predator diversity on species interaction strength.* Many different measures and definition of interaction strengths (IS) have been used, ranging from the relative effect of a consumer on the resource population abundance to parameters of the functional response or population-dynamic models (Berlow *et al.*, 1999). Although these IS metrics integrate different aspects of consumer–resource interactions, they all strive to quantify the total or *per capita* fluxes of energy and materials among species within a community. We thus express IS(C,R) as the total flux from resource R to consumer(s) C measured as the relative change in resource density or biomass after the introduction of the consumer(s) (Berlow *et al.*, 1999):

$$\text{IS}(C, R) = 1 - \frac{N_{R|C}}{N_R}, \quad (3)$$

where  $N_R$  and  $N_{R|C}$  are observed densities of resource R in the absence and presence of consumers C, respectively.

For each experimental treatment, we calculated IS using Eqn (3), which gives the proportion of prey eaten in each trial. Effects of predator assemblage, temperature, prey density, and their interactions (three-two-way interactions and one-three-way interaction) on IS were then analysed using a binomial GLM with a logit link function. We detected model overdispersion and thereby corrected the standard errors using a quasibinomial GLM model (Zuur *et al.*, 2009). The most parsimonious model was determined by the sequential deletion of the least significant explanatory parameters (or interaction terms) from the saturated model (i.e. the model with all fixed effects and possible interaction terms). Parameter significance was assessed using *F*-tests from analysis of deviance. The final model contained only explanatory variables that had a significant effect ( $P < 0.05$ ) or were part of significant interaction terms. *Post hoc* Tukey's tests were then used to determine differences among treatment means.

To disentangle predator density and diversity effects, we next excluded the treatments with the three heterospecific predators and tested the effects of interaction type (interspecific or intraspecific for predator assemblages involving two heterospecific or conspecific predators, respectively), temperature, prey density, and their interactions (three-two-way interactions and one-three-way interaction) on IS using a quasibinomial GLM with a logit link function as described above.

*Estimating temperature and prey density dependence of trophic species interaction strengths in multiple-predator systems.* To apply the proposed framework to our experimental case study, we used empirical estimates of trophic functional response from Experiment 1 to parameterize a population-dynamic model and predict how prey density changes

in time as a function of initial prey density, predator identity and density (see also McCoy *et al.*, 2012), and temperature:

$$\frac{dN_D}{dt} = \frac{a_C(T)N_D N_C}{1 + a_C(T)h_C(T)N_D} - \frac{a_L(T)N_D N_L}{1 + a_L(T)h_L(T)N_D} - \frac{a_S(T)N_D N_S}{1 + a_S(T)h_S(T)N_D}, \quad (4)$$

where  $N$  is the predator or prey population density (prey  $L^{-1}$ ),  $h(T)$  is the prey handling time (day prey $^{-1}$ ) at temperature  $T$ ,  $a(T)$  is the predator search rate (L day $^{-1}$ ) at temperature  $T$ , and respective subscripts D, C, L, and S refer to densities of *D. magna*, *C. obscuripes*, *L. quadrimaculata*, and *S. vulgatum*.

We calculated expected prey survival in the absence of nontrophic interactions for all tested assemblages of the three-predator species (Experiment 2) by integrating Eqn (4) over time using the R package 'deSolve' (Soetaert *et al.*, 2010). Initial values of  $N_D$  were set at the experimental initial prey densities (i.e. 15, 75, and 150 prey  $L^{-1}$ ). To account for uncertainty in the functional response parameter estimate, we performed a global sensitivity analysis using the 95% confidence intervals (CIs) of each functional response parameter estimate to generate 100 random parameter sets using a Latin hypercube sampling algorithm (Soetaert & Petzoldt, 2010). For each parameter set, we then integrated Eqn (4) over time and calculated expected prey survival at each output interval using the 'sensRange' function in the R package 'FME' (Soetaert & Petzoldt, 2010). We then recorded the minimal and maximal expected prey survival values obtained for the 100 simulations and used these values as a confidence interval around the mean predicted value. We next calculated the expected assemblage-level trophic interaction strengths  $IS_T(C^*, D)$  for each predator assemblage  $C^*$  by dividing the numbers of surviving prey eaten calculated from Eqn (4) by the initial prey density. Note that we can distinguish  $IS_T$  from IS because  $IS_T$  values are predicted from Eqn (4) that does not include nontrophic interactions among predators and their prey.

*Effects of predator diversity, temperature, and prey density on nontrophic species interaction strength.* We first visually compared model predictions and empirical observations by assuming that the observed IS (from Experiment 2) and the expected  $IS_T$  values (from Eqn 4) do not differ when their CIs overlap. We next statistically compared the observed IS and the expected  $IS_T$  values using a quasibinomial GLM with  $IS_T$  as an offset (i.e. predictor variable). A positive or negative significant intercept, respectively, indicates that  $IS_T$  values underestimate or overestimate IS.

We calculated the strength of nontrophic interactions  $IS_{NT}$  by deducting  $IS_T$  from IS and investigated the effects of temperature, prey density, predator diversity, and their interactions on  $IS_{NT}$  using an analysis of covariance (ANCOVA), with predator assemblage as categorical variable and temperature and prey density as continuous variables. Inspection of the residuals suggested a nonlinear prey density effect. We therefore included a quadratic term for prey density in the ANCOVA. This choice was supported by a lower AICc for the model including the quadratic term (AICc =  $-626.3$ ) as compared to the linear model (AICc =  $-614.9$ ). The process of model simplification

described above for the analysis of IS (Experiment 2) was repeated for  $IS_{NT}$ , and *post hoc* Tukey's tests were used to determine differences among means for the predator treatments.

We then excluded the treatment with the three heterospecific predators and tested the effects of interaction type (inter-specific and intraspecific), temperature, prey density, and their interactions on  $IS_{NT}$  using an ANCOVA and the process of model simplification as described above for species interactions strength IS. Statistical analyses were performed using R version 2.15.3 (R Development Core Team, 2013).

## Results

### Experiment 1. Effects of temperature on single-predator trophic functional responses

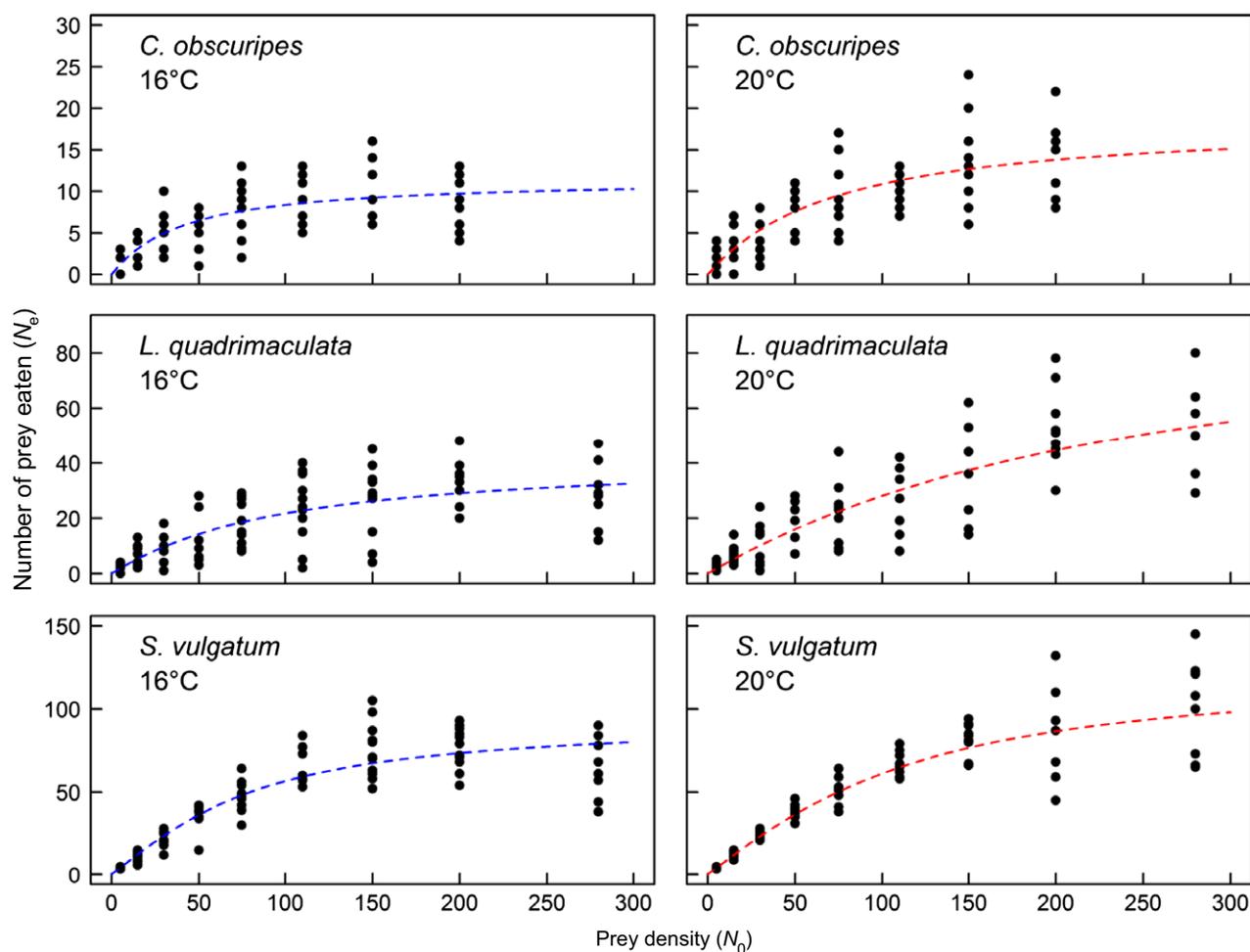
Functional response of each predator species depended on temperature (Fig. 1): the data were best described

by a model in which temperature significantly influenced handling time but not search rate (Table S2).

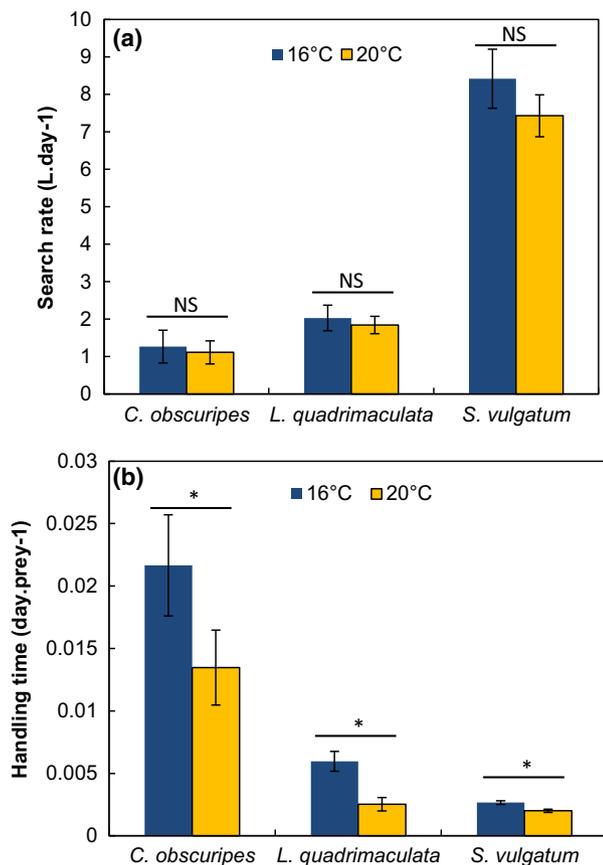
Handling time was significantly shorter at 20 °C than at 16 °C for all three predators, and this thermal effect was more pronounced for *C. obscuripes* and *L. quadrimaculata* than for *S. vulgatum* (Fig. 2). Among the three species, *C. obscuripes* had the longest handling time compared to the two dragonfly species, for which handling time values were of similar magnitude. *S. vulgatum* had the highest search rate followed by *L. quadrimaculata* and *C. obscuripes* (Fig. 2).

### Experiment 2. Effects of temperature, prey density, and predator diversity on species interaction strength

Model selection indicated that IS was not significantly influenced by the three-way interaction between



**Fig. 1** Functional responses of the three predators at 16 °C (left column, blue lines) and 20 °C (right column, red lines) estimated from Experiment 1. Points represent observed values, and dotted lines correspond to the fitted model. Predator and prey habitat domains: *Chaoborus obscuripes*, water column, *Libellula quadrimaculata* forages only within the benthic zone, and *Sympetrum vulgatum* is a predominantly benthic predator that can access prey in the water column; *Daphnia magna*, entire water column including the layer just above the benthic zone. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**Fig. 2** (a) Search rate  $a$  of (b) handling time  $h$  and the three predators at two temperatures (16 °C in blue and 20 °C in yellow) estimated from Experiment 1. Error bars indicate 95% confidence intervals. Within predator species, 'NS' stands for not significant and '\*' indicates significant differences ( $P < 0.05$ ) between the two temperature regimes. Search rate is not significantly influenced by temperature, whereas handling time decreases with temperature for the three-predator species. See Fig. 1 for predator and prey habitat domains. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

temperature, prey density, and predator assemblage and the two-way interaction between temperature and predator assemblage ( $P > 0.05$ ). The minimum adequate model indicated that IS increased with temperature ( $F_{1, 452} = 54.1$ ,  $P < 0.0001$ ), decreased with prey density ( $F_{1, 451} = 154.9$ ,  $P < 0.0001$ ), and was generally stronger in predator assemblages including *S. vulgatum* (predator assemblage,  $F_{6, 445} = 279.1$ ,  $P < 0.0001$ ; Fig. 3). Prey density effect also depended on predator assemblage (predator assemblage  $\times$  prey density,  $F_{6, 438} = 2.52$ ,  $P = 0.021$ ; Fig. 3) and was weaker, but still significant ( $P < 0.05$ ) for interactions involving two dragonflies (S\_S, L\_L, and S\_L) compared to the other interactions. Moreover, temperature effect depended on prey density (temperature  $\times$  prey density,  $F_{1, 444} = 7.8$ ,  $P = 0.006$ ). Subsequent analyses using a separate

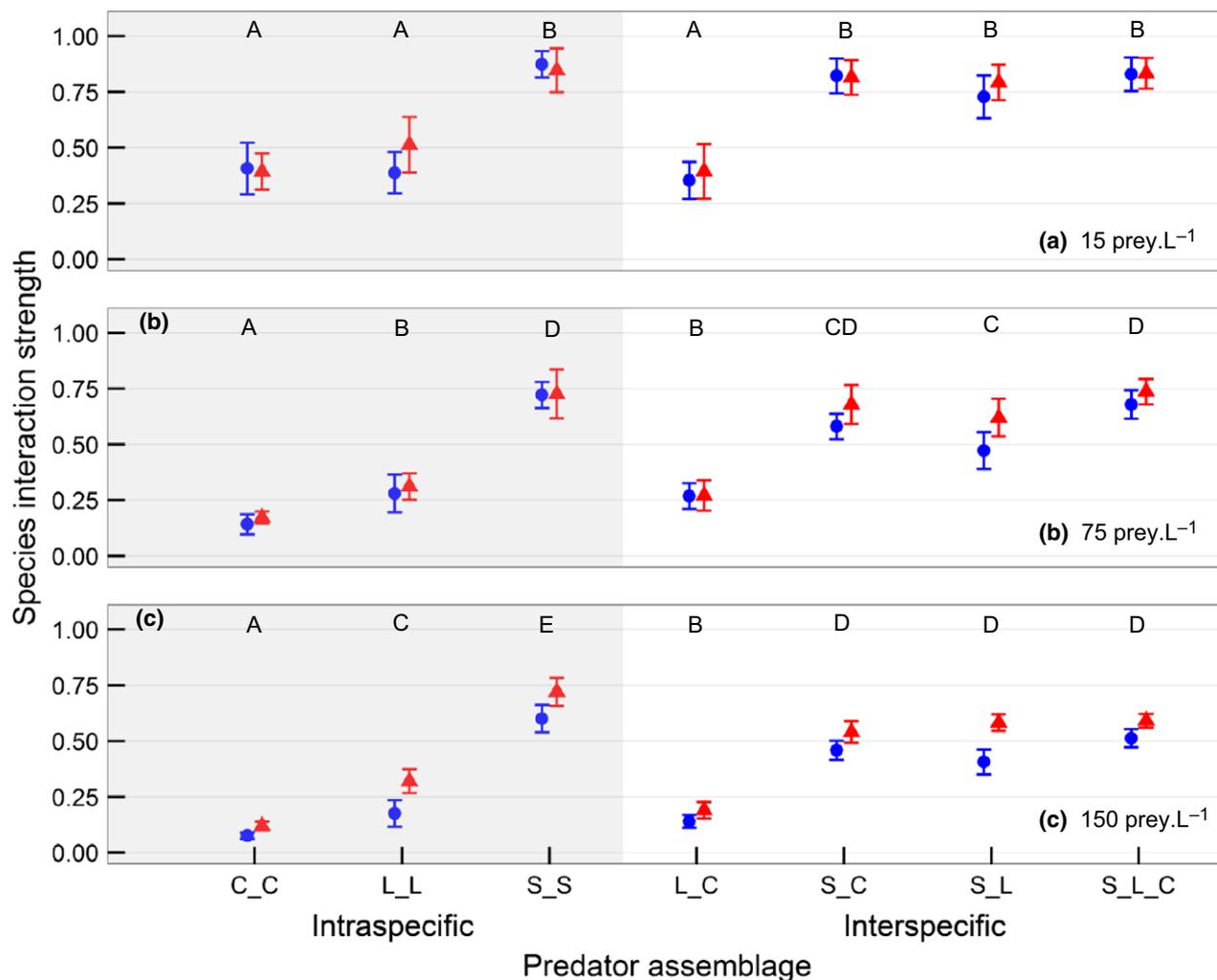
quasibinomial GLM for each prey density revealed that warming only increased IS at the two highest prey density levels (temperature effect at: 15 prey L<sup>-1</sup>:  $F_{1, 149} = 0.27$ ,  $P = 0.60$ ; 75 prey L<sup>-1</sup>:  $F_{1, 149} = 8.5$ ,  $P = 0.004$ ; 150 prey L<sup>-1</sup>:  $F_{1, 150} = 46.1$ ,  $P < 0.0001$ ).

Model selection indicated that IS was not significantly influenced by any interactions between temperature, interaction type (intra- and interspecific), and prey density ( $P > 0.05$ ). The minimum adequate model indicated that IS increased with temperature ( $F_{1, 370} = 10.6$ ,  $P = 0.001$ ), decreased with prey density ( $F_{1, 369} = 23.9$ ,  $P < 0.0001$ ), and was stronger in assemblages including two heterospecific predators than in those including two conspecific predators (interaction type,  $F_{1, 368} = 7.9$ ,  $P = 0.005$ ).

#### *Effects of predator diversity, temperature, and prey density on nontrophic interaction strength*

Visual comparison of the predicted IS<sub>T</sub> and the observed IS revealed that, in most cases, the predicted IS<sub>T</sub> overestimated the observed IS (Fig. S1), yielding negative values of IS<sub>NT</sub>. Moreover, the intercept of the quasibinomial GLM with predicted IS<sub>T</sub> as an offset (value  $\pm$ SE:  $-0.61 \pm 0.03$ ) was significantly different from zero ( $t$  value =  $-21.18$ ,  $df = 451$ ,  $P < 0.0001$ ) indicating that, on average, predictions overestimated observed IS by 32% (Fig. S1). Values of IS<sub>NT</sub> were not significantly influenced by the three-way interaction or any of the two-way interactions between temperature, prey density, and predator assemblage ( $P > 0.05$ ). The final model indicated that IS<sub>NT</sub> increases with temperature ( $F_{1, 444} = 4.9$ ,  $P = 0.027$ , slope =  $6.3 \times 10^{-3} \pm 2.8 \times 10^{-3}$ ; Fig. 4a), differs between predator assemblages ( $F_{6, 444} = 12.5$ ,  $P < 0.0001$ ), and has a unimodal dependence on prey density ( $F_{2, 444} = 7.0$ ,  $P = 0.001$ , linear coefficient:  $-1.74 \times 10^{-3} \pm 4.56 \times 10^{-3}$ , quadratic coefficient:  $9.7 \times 10^{-6} \pm 2.6 \times 10^{-6}$ ) with a minimum at the intermediate prey density (Fig. 4b). Nontrophic interactions were weakest in the conspecific *C. obscuripes* assemblage (C\_C; Fig. 4c), strongest in the two dragonfly assemblages (L\_L, S\_S, and S\_L), and intermediate for assemblages involving *C. obscuripes* and one or both dragonflies (L\_C, S\_C, and S\_L\_C; Fig. 4c).

Additional analysis with the three-predator treatment excluded yielded very similar results. Interaction type (intra- and interspecific) and its interactions with temperature and prey density did not significantly influence IS<sub>NT</sub> ( $P > 0.05$ ) and the minimum adequate model also indicated that IS<sub>NT</sub> increased with temperature ( $F_{1, 368} = 5.01$ ,  $P = 0.026$ , slope =  $7.9 \times 10^{-3} \pm 3.5 \times 10^{-3}$ ) and had a unimodal dependence on prey density ( $F_{2, 368} = 6.4$ ,  $P = 0.002$ , linear coefficient: -



**Fig. 3** Mean ( $\pm$ SE) species interaction strength (IS) for each predator assemblage at three prey densities (a: 15 prey L<sup>-1</sup>; b: 75 prey L<sup>-1</sup>; c: 150 prey L<sup>-1</sup>) and two temperatures (16 °C in blue circles and 20 °C in red triangles) estimated from Experiment 2. Within each prey density, different capital letters denote significant differences between predator assemblages. Predator abbreviations: S = *Sympetrum vulgatum*, L = *Libellula quadrimaculata*, C = *Chaoborus obscuripes*. See Fig. 1 for predator and prey habitat domains. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

$2.02 \times 10^{-3} \pm 0.56 \times 10^{-3}$ , quadratic coefficient:  $1.14 \times 10^{-5} \pm 0.33 \times 10^{-5}$ ) with a minimum at the intermediate prey density.

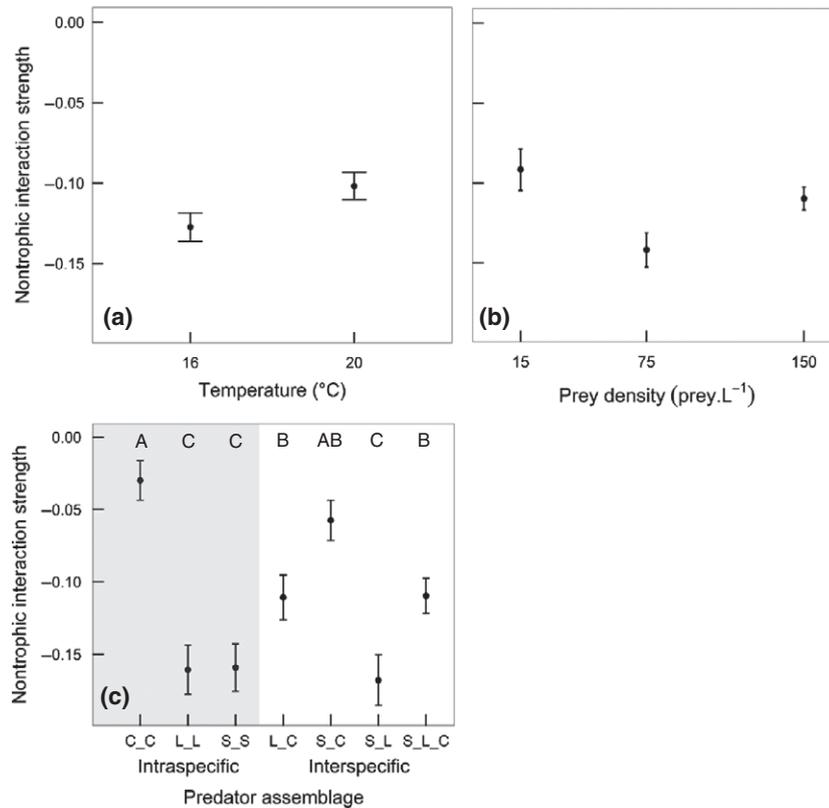
## Discussion

Revealing the links between environmental factors, species diversity, and species interaction strengths is crucial to understand the structure and dynamics of ecological communities (Duffy *et al.*, 2007; Sentis *et al.*, 2014). However, little is known about how predator diversity influences strengths of trophic and nontrophic interactions along environmental gradients. Here, we used a functional response approach combined with laboratory experiments to investigate the effects of temperature, prey density, and predator diversity on the

strength of trophic and nontrophic interactions in an aquatic food web. We showed, for the first time, that temperature can strongly influence nontrophic interactions among predators and their shared prey. Our results have several implications for the stability and persistence of food webs, and our functional response approach provides the potential for a broadly applicable framework of the environmental dependence of trophic and nontrophic interactions.

### *Species interaction strength and its trophic and nontrophic components*

We measured the trophic functional response of each predator species in single-predator experiments and used it to parameterize a model for multiple



**Fig. 4** Mean ( $\pm$ SE) nontrophic species interaction strength  $IS_{NT}$  for each temperature (a), prey density (b) and predator assemblage (c). Different capital letters denote significant differences between predator assemblages in panel (c). Predator abbreviations: S = *Sympetrum vulgatum*, L = *Libellula quadrimaculata*, C = *Chaoborus obscuripes*. See Fig. 1 for predator and prey habitat domains.

co-occurring predators. Most importantly, our model often overestimated species interaction strength measured in multiple-predator treatments, indicating that nontrophic interactions led to predation risk reduction for the prey. This occurs when negative interactions among predators or changes in prey behaviour or habitat domain in response to predators reduce prey mortality (Vance-Chalcraft & Soluk, 2005; Schmitz, 2007; Woodcock & Heard, 2011; Davenport & Chalcraft, 2013). Because we observed no clear-cut changes in prey behaviour or habitat use during the experiment (Sentis *et al.*, personal observations), we tentatively attribute the risk reduction to predator interference, which is ubiquitous in food webs (Vance-Chalcraft & Soluk, 2005; Griffen, 2006; Griffen & Williamson, 2008).

We hypothesized that differences in predator habitat domain (pelagic vs. benthic), body size, or functional traits such as foraging mode will underlie the observed differences in predation risk reduction among predator assemblages. Based on the habitat domain framework (Schmitz, 2007), we expected that risk reduction would be weak for predators with nonoverlapping habitat domains (*Chaoborus* and *Libellula*), whereas it would be

strong for predators with overlapping habitat domains (*Chaoborus* and *Sympetrum*, conspecific predators). However, we found that risk reduction was significantly weaker for the conspecific *Chaoborus* assemblage compared to all other assemblages involving any of the two dragonflies. *Chaoborus* is the smallest and has the lowest feeding rate of the three-predator species used in the experiment. For a given prey density, the level of intraspecific exploitative competition is thus probably lower for *Chaoborus* than for the two larger dragonfly predators, which likely explains why intra- and interspecific interference was lower for the assemblages involving *Chaoborus*. Intraguild predator avoidance by *Chaoborus* when in interaction with *Sympetrum* can also partly explain the more substantial risk reduction in that assemblage, although risk reduction was stronger in the *Chaoborus-Libellula* assemblage, in which intraguild predation did not occur. Whatever the exact process, we found that, on average, trophic interaction strength overestimated species interaction strength by 32% in our experiment, indicating that nontrophic interactions must be considered to predict the effects of multiple predators on species interaction strengths.

*Effects of environmental changes on interaction strengths*

Effects of prey density on species interaction strengths in complex food webs remain little explored. Previous studies reported that the strength of trophic interactions among predators (i.e. intraguild predation) generally decreases with increasing prey density (Lucas & Rosenheim, 2011; Sentis *et al.*, 2013a, 2014). In this study, we found that prey density systematically weakens both the predicted trophic interaction strengths and the measured species interaction strengths. This result likely arises from the nonlinear feeding rates (i.e. type II functional responses): when prey become abundant, handling or digestion of prey is limiting and decreases the proportion of prey eaten (Jeschke *et al.*, 2002; Sentis *et al.*, 2013a, b). This implies that nonlinear, density-dependent interactions drive temporal changes in interaction strength and highlights the importance of considering nonlinear functional responses to adequately interpret results from short-term experiments and to predict food-web dynamics along environmental gradients.

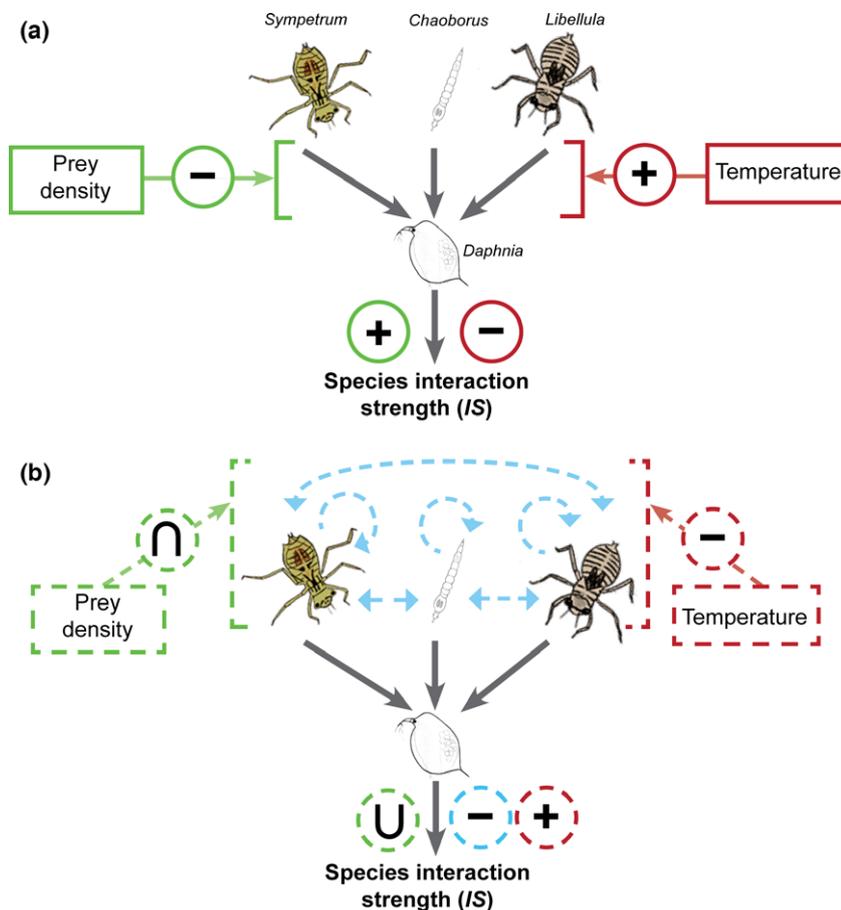
We also found that variation in feeding rates cannot fully explain prey density effects on species interaction strengths, because nontrophic interactions among predators also varied with prey density. The observed unimodal relationship likely arises from a balance between predation rates and satiation of competing predators. Nearly all prey are consumed at low resource densities, which hides any interference effects, while at high resource density, predators are not prey limited and thus mostly satiated, which decreases interference strength due to predator foraging behaviour. Interference should thus be strongest at intermediate resource levels as we observed for every predator assemblage. This unimodal relationship may also explain why previous studies had difficulties to characterize the effects of resource availability on multiple-predator effects (Soluk, 1993; Vance-Chalcraft & Soluk, 2005; Griffen, 2006; Tylianakis & Romo, 2010). Moreover, these studies were conducted using additive or substitutive experimental designs that assume density dependence and neglect resource dynamics which can bias the results (McCoy *et al.*, 2012) and thus invalidate conclusion about the density dependence of nontrophic interactions among predators and their prey. We thus argue that further studies using the functional response approach are needed to better characterize the dependence of multiple-predator effects on prey density and understand the underlying mechanisms. This is especially important in order to go beyond the simple detection of nontrophic interactions and to predict species interaction strengths across a full range of population densities to better understand and predict the dynamics of ecological communities.

Temperature affects trophic interaction strengths and hence alters population dynamics and community stability (Binzer *et al.*, 2012; Rall *et al.*, 2012; Dell *et al.*, 2013; Sentis *et al.*, 2014). Our model parameterized with empirical data (Eqn 4) predicted that warming increases trophic interaction strengths only at higher prey densities. Measured species interaction strengths were consistent with this prediction: warming increased them at intermediate and high prey densities but had no effect at the low prey density. This result is driven by temperature-dependent functional responses: at low prey densities, predation rate depends mainly on search rate, which did not change with temperature in our experiment in line with previous studies on sit-and-wait predators (Novich *et al.*, 2014; Seifert *et al.*, 2014; Sentis *et al.*, 2015). At higher prey densities, predation rate is limited by handling time, which decreases with temperature, resulting in stronger trophic interactions.

Our study is the first to consider the more subtle, so far overlooked role of temperature in nontrophic interactions among predators. We found that the temperature effect on nontrophic interactions was independent of prey density and opposite to its effect on trophic interactions: warming weakened intra- and interspecific interference in all predator assemblages. As proposed by Lang *et al.* (2012), we hypothesize that predators need to cover higher metabolic demands at higher temperatures and thereby search more for prey, which leaves less time for interference. Moreover, the temperature effect was independent of the predator assemblage in our experiment. This suggests that the thermal dependence of nontrophic interactions among predators may follow some general, but currently unknown, rules. We thus call for further studies characterizing the shape and scaling of this dependence, including its modifications caused by multiple prey interactions, phenotypic plasticity, local adaptations, additional predator and prey functional traits, and experimental conditions (e.g. arena size, habitat complexity, thermal regimes) that are known to modify trophic interactions (Boukal, 2014; Klecka & Boukal, 2014; Lawson *et al.*, 2015; Sentis *et al.*, 2015).

*Species interaction strengths and stability of ecological networks*

Global change impacts on ecological networks depend on consequential changes in the type and strength of interactions among organisms within the community (Petchey *et al.*, 1999; Suttle *et al.*, 2007; Tang *et al.*, 2014). Here, we found a systemic increase in trophic interaction strengths with warming (Fig. 5a) that is consistent with studies on simple predator-prey systems (Rall



**Fig. 5** (a) Summary of the effects of temperature (red circles and arrows) and prey density (green circles and arrows) on trophic interactions (black arrows) with cascading effects on species interaction strengths. (b) Effects of nontrophic interactions (dashed arrows) on species interaction strength (dashed blue circle) and effects of increased temperature (dashed red circles) and prey density (dashed green circles) on nontrophic interactions, resulting in indirect effects on species interaction strength. Plus sign = positive effect; minus sign = negative effect; humped curve = unimodal effect. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

*et al.*, 2012). Given that strong species interactions tend to destabilize food webs (McCann *et al.*, 1998; Neutel *et al.*, 2007), warming could decrease food-web persistence. On the other hand, we found that prey density systematically weakens trophic interactions in multiple-predator systems (Fig. 5a), which could increase food-web persistence and dampen the destabilizing effect of warming. Taken together, consequences of global change on food-web stability will depend on the relative contributions of prey density and temperature (Binzer *et al.*, 2012; Sentis *et al.*, 2014): food webs should be less stable if the prey density effect becomes stronger and more stable if the temperature effect predominates. However, as outlined above, species interaction strengths and thus community stability cannot be predicted from trophic interactions alone. In our study, nontrophic interference among predators significantly decreased trophic interaction strengths (Fig. 5b). According to theory (Brose *et al.*, 2006; Rall *et al.*, 2008),

this may dampen population oscillations and stabilize food webs. Moreover, the strength of interference depended on temperature and prey density in our experiment (Fig. 5b). Based on our results and previous food-web models, we predict that food-web stability should be maximized at lower temperatures and intermediate levels of prey density with weak trophic interactions and strong predator interference.

Nontrophic interactions affect the dynamics and stability of ecological networks (Rall *et al.*, 2008; Kéfi *et al.*, 2012; Vázquez *et al.*, 2015), but their dependence on temperature and resource density remains largely unexplored. The functional response approach used in this study opens new avenues for a mechanistic theory to (i) quantify the relative importance of trophic and nontrophic components in species interactions, (ii) provide a better understanding of how environmental factors affect these interactions, and (iii) enable the extrapolation of experimental results to longer

temporal scales and other environmental scenarios. By combining theory and experiments, we show that the strengths of trophic and nontrophic interactions depend on predator diversity, temperature, and prey density. Our study indicates that taking these multiple factors as well as the nonlinearity of density-dependent species interactions (i.e. the predator functional response) into account is an important step towards a better understanding of the effects of environmental variations on complex ecological communities in a rapidly changing world. Importantly, qualitative impact of temperature and prey density on the strengths of trophic and nontrophic interactions in our experiment was independent of predator identity, suggesting a general pattern. This finding, if confirmed by further studies, could facilitate the development of a general theory for the environmental dependence of trophic and nontrophic interactions.

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### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Method S1.** Capture, rearing, and standardisation of predators and prey.

**Text S1.** Statistical analyses (logistic regression) used to determine functional response type.

**Table S1.** Results (logistic regression coefficient estimate, standard error, and *P*-value) of the statistical analyses used to determine the functional response type.

**Table S2.** Values of  $\Delta\text{AICc}$  and respective Akaike weights (*w*) of the different models of temperature effect on functional response parameters.

**Figure S1.** Mean ( $\pm\text{CI}$ ) predicted and observed interaction strength as a function of prey density, temperature ( $^{\circ}\text{C}$ ) and predator assemblages.