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Effects of global warming on trophic interactions in ponds - an experimental approach

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Dissertation presented in partial fulfilment of the requirements for the degree of Doctor of Science (PhD): Biology

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Samenvatting

Hoe meerdere stressoren die gepaard gaan met klimaatsverandering met elkaar kunnen interageren bij het beïnvloeden van soorten en trofische interacties is van cruciaal belang geworden om onze voorspellingen voor biodiversiteit te verbeteren. Hoewel algemeen wordt erkend dat soorten en hun interacties kunnen reageren op stijgende gemiddelde temperaturen, hebben studies grotendeels de gecombineerde effecten met andere klimaatveranderingsstressoren, zoals bijvoorbeeld temperatuurschommelingen en eutrofiëring dat door algen geïnduceerde troebelheid veroorzaakt, genegeerd op soorten en vooral op trofische interacties in zoetwaterecosystemen. Bovendien hebben studies zich meestal toegespitst op thermische plasticiteit, terwijl de rol van thermische evolutie in het mediëren van de impact van klimaatsveranderingen op trofische interacties grotendeels onbekend is.

In deze thesis testte ik de ‘snelle evolutie’ in de thermische prestatiecurven (TPCs) van een belangrijke aquatische soort, de watervlo *Daphnia magna*, onder experimentele thermische selectie. Ik onderzocht de latitude-geassocieerde evolutie van thermische plasticiteit in predatiesnelheden van zijn predator, larven van de waterjuffer *Ischnura elegans*. Ik testte ook de ecologische en evolutionaire gevolgen van blootstelling aan opwarming (een +4 °C stijging van de gemiddelde temperatuur, een +10 °C stijging van de dagelijkse temperatuurschommelingen en een hittegolf van 32 °C) en troebelheid voor de predator-prooi interactie tussen de twee soorten, door het uitvoeren van een reeks gemeenschappelijke labo experimenten met predatoren en prooien afkomstig van verschillende breedtegraden. Om de onderliggende mechanismen te begrijpen, bestudeerde ik de effecten op de parameters van de ‘functionele response’ (zoeksnelheid en verwerkingstijd) en op andere parameters die bijdragen tot de interactiesterkte op lange termijn. Om de rol van thermische evolutie in de lange termijn interactie tussen predator en prooi onder klimaatsopwarming te onderzoeken, gebruikte ik waterjufferlarven (en in hoofdstuk IV ook watervlooien) van twee verschillende breedtegraden in Europa. De gekozen breedtegraden vertegenwoordigen een verschil in thermische regimes met een +4 °C hogere gemiddelde temperatuur, een +5 °C hogere maximale dagelijkse temperatuurschommeling, en frequentere hittegolven in ondiepe zoetwater vijvers tijdens zomers op de lage latitude. Dit komt overeen met de voorspelde toename van deze thermische factoren die volgens het IPCC RCP 8.5 scenario tegen 2100 op de hoge latitude zou optreden. Dit maakte het mogelijk een ‘ruimte-voor-tijd substitutie’ toe te passen om te testen of thermische evolutie in populaties van de hoge latitude de impact van de opgelegde temperatuurstressoren onder klimaatsopwarming kan matigen, en of de invloed van thermische

evolutie kan veranderen als de combinatie met een niet-temperatuurstressor (door algen geïnduceerde troebelheid) in aanmerking wordt genomen.

In het eerste hoofdstuk ontdekte ik in *D. magna* snelle thermische evolutie van de TPC van intrinsieke groeisnelheid, een belangrijke maat voor 'fitness', onder experimentele thermische selectie. De TPCs van de ingestie- en de metabolische snelheden daarentegen evolueerden niet, wat suggereert dat snelle thermische evolutie in de sleutelsoort niet noodzakelijk de 'top-down' impact van de consument op zijn hulpbron versterkt.

In het tweede hoofdstuk vond ik dat de breedtegraad-patronen in thermische plasticiteit en acclimatisatie van de predatiesnelheden van *I. elegans* larven die jagen op 'midden-latitude' *D. magna*, kritisch afhingen van het plasticiteitstype en de acclimatisatiemetriek, waardoor gemengde ondersteuning voor de klimatologische variabiliteitshypothese (KVH) werd gegenereerd. Hoewel larven van beide breedtegraden perfecte thermische compensatie vertoonden in predatiesnelheden tussen 20°C en 24°C, was de acclimatisatiecapaciteit die thermische acclimatisatie kwantificeert in verhouding tot de acute plasticiteit veel hoger bij larven van hoge latitude in vergelijking met die van lage latitude. Dit wijst op het grote acclimatisatiepotentieel van de hoge latitude larven als reactie op klimaatsopwarming.

In het derde hoofdstuk vond ik dat niet alleen stijgingen van de gemiddelde temperatuur, maar ook dagelijkse temperatuurschommelingen en hittegolven de interacties op lange termijn kunnen beïnvloeden in *I. elegans* larven die jagen op 'midden latitude' *D. magna*. Opwarming verzwakte de lange termijn interactiesterkte, behalve voor het hoge latitude trofische systeem bij dagelijkse temperatuurschommelingen en een hittegolf, waarbij plastische reacties daardoor het hoge latitude systeem mogelijks niet stabiliseren. Thermische evolutie van de predator kan de interactiesterkte op lange termijn van het hoge latitude trofische systeem verder verzwakken bij stijgingen van de gemiddelde temperatuur, zelfs bij dagelijkse temperatuurschommelingen en mogelijks ook bij hittegolven.

In het laatste hoofdstuk vond ik dat de troebelheid van het water de invloed van thermische plasticiteit en thermische evolutie van predator en prooi op de lange termijn interacties tussen lage- en hoge-latitude *I. elegans* en *D. magna* prooien onder klimaatsopwarming kritisch kan beïnvloeden. Mijn resultaten toonden aan dat thermische plasticiteit het hoge latitude trofische systeem onder klimaatsopwarming destabiliseert in helder water, maar niet in troebel water. Dit staat in contrast met mijn eerdere bevinding dat klimaatsopwarming de dynamiek van het trofische systeem zal stabiliseren. Eén van de functionele respons parameters (zoeksnelheid) droeg in grote mate bij tot de patronen van de interactiesterkten op lange termijn. Bovendien vond ik dat thermische evolutie van de predator (niet van de prooi) het hoge latitude systeem onder klimaatsopwarming stabiliseert in troebel water, maar niet in helder water.

De resultaten van mijn thesis benadrukken de noodzaak om zowel thermische plasticiteit als thermische evolutie in overweging te nemen bij het voorspellen van de effecten van meerdere klimaatsverandering gerelateerde stressoren op de sterkte van trofische interacties op lange termijn. Thermische evolutie zou het effect van klimaatsopwarming op de stabiliteit van voedselwebben kunnen verzwakken, maar dit hangt sterk af van andere klimaatsveranderings-gerelateerde stressoren en de breedtegraad.

Summary

How multiple global change stressors may interact with each other in affecting species and trophic interactions has become crucial to improve our forecast for biodiversity. While it is widely acknowledged that species and their interactions may respond to increasing mean temperatures, studies largely ignored the combined effects with other global change stressors, such as temperature variation and eutrophication causing algae-induced turbidity on species and especially on trophic interactions in freshwater ecosystems. Furthermore, studies have mostly focused on thermal plasticity, while the role of thermal evolution in mediating the global change impact on trophic interactions is mostly unknown.

In this thesis, I tested the rapid evolution in the thermal performance curves (TPCs) of a key aquatic species, the water flea *Daphnia magna*, under experimental thermal selection. I examined the latitude-associated evolution of thermal plasticity in predation rates of its predator, *Ischnura elegans* damselfly larvae. I also tested the ecological and evolutionary consequences of exposure to warming (a + 4 °C increase in mean temperature, a + 10 °C increase in daily temperature fluctuation, and a 32 °C heatwave) and turbidity for the predator-prey interaction strengths between the two species, by executing a series of common-garden experiments with predators and prey from different latitudes. To understand the underlying mechanisms, I studied effects on functional response parameters (search rate and handling time) and other parameters that contribute to long-term interaction strength. To investigate the role of thermal evolution in shaping long-term predator-prey interaction under global warming, I used damselfly larvae (and in chapter IV also water fleas) from two different latitudes in Europe. The chosen latitudes represent a difference in thermal regimes with a +4 °C higher mean summer water temperature, a +5 °C higher maximum summer daily temperature fluctuations, and more frequent heatwaves in the shallow freshwater ponds during summer at the low latitude. This matches the predicted increase in these thermal factors that would be imposed at the high latitude by 2100 under the IPCC RCP 8.5 scenario. This allowed for applying a space-for-time substitution to test if thermal evolution in high-latitude populations may mediate the impact of the imposed temperature stressors under global warming, and if the influence of thermal evolution may change if the combination with a non-temperature stressor (algae-induced turbidity) is considered.

In the first chapter, I detected rapid thermal evolution of *D. magna*’s TPC of intrinsic growth rate, an important fitness estimate, under experimental thermal selection. However, the

TPCs of ingestion rates and metabolic rates did not evolve, suggesting rapid thermal evolution in the key species may not necessary strengthen the top-down impact of the consumer on its resource.

In the second chapter, I found that the latitudinal patterns in thermal plasticity and acclimation of predation rates of *I. elegans* larvae preying on mid-latitude *D. magna* critically depended on the plasticity type and acclimation metric, thereby generating mixed support for the climatic variability hypothesis (CVH). Notably, while larvae from both latitudes showed perfect thermal compensation in predation rates between 20 °C and 24 °C, the acclimation capacity that quantifies thermal acclimation relative to the acute plasticity was much higher in high-latitude larvae compared to low-latitude ones. This highlights the large acclimation potential of high-latitude larvae in response to global warming.

In the third chapter, I found that not only increases in mean temperatures but also daily thermal fluctuations and heat waves can affect long-term interactions in *I. elegans* larvae preying on mid-latitude *D. magna*. Warming weakened the long-term interaction strength, except for the high-latitude trophic system at daily temperature fluctuations and a heat wave, where plastic responses therefore may not stabilize the high-latitude system. Thermal evolution of the predator may further weaken the long-term interaction strength of the high-latitude trophic system under increases in mean temperatures, even under daily temperature fluctuations and potentially also heat waves.

In the final chapter, I found that turbidity state can critically affect the influence of thermal plasticity and thermal evolution of predator and prey on long-term interactions between low- and high-latitude *I. elegans* larvae and *D. magna* prey under warming. My results revealed that thermal plasticity will destabilize the high-latitude trophic system under warming in clear water but not in turbid water. This contrasts with my previous finding that warming will stabilize trophic system dynamics. One of the functional response parameters (search rate) largely contributed to the patterns of long-term interaction strengths. Furthermore, I found that thermal evolution of the predator (not the prey) will stabilize the high-latitude system under warming in turbid water but not in clear water.

The results of my thesis highlight the necessity for considering both thermal plasticity and thermal evolution while predicting effects of multiple global change stressors in shaping long-term trophic interaction strength. Thermal evolution could ameliorate the impact of global warming on food web stability, but this critically depends on which other global change stressors and which latitude are taken into account.

List of abbreviations

ANOVA	analysis of variance
CT_{max}	critical thermal maximum
CVH	climatic variability hypothesis
CTI	consumer thermal impact
DTF	daily temperature fluctuation
FDR	false discovery rate
E_a	activation energy
HW	heatwave
IPCC	Intergovernmental Panel on Climate Change
T_{opt}	optimum temperature
TPC	thermal performance curve

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General introduction

Under heavy anthropogenic activities since industrialization, the Earth has been experiencing rapid global change (e.g. climate change, pollution, invasive species, habitat loss, overexploitation), whose negative impact has reached an unprecedented rate (McGill et al. 2015, Maxwell et al. 2016, Morris 2019). Whether and how species may respond to the multiple stressors under global change has become one of the most relevant research topics because this is closely associated with the diversity, function, stability, and resilience of ecosystems (reviewed Sage 2020, Wagner 2020, Halsch et al. 2021). Specifically, there is a rising awareness that some key stressors may interact with each other in affecting species (reviewed by Jackson et al. 2016, Côté et al. 2016, Orr et al. 2020), that species responses to these stressors may be plastic and/or genetic (reviewed by Merilä and Hendry 2014, Nadeau and Urban 2020), and that these responses may vary with temporal and/or spacial scale (reviewed by Jackson et al. 2021, Valladares et al. 2014). Moreover, global change stressors may indirectly affect species via modifying biotic interactions, such as trophic interactions (a collection of predation, herbivory and parasitism), mutualism, and competition (reviewed by Tylianakis et al. 2008, Urban et al. 2016, Stoks et al. 2017, Bartley et al. 2019). Therefore, to better understand and predict the impact of global change, we need to consider (i) multiple key stressors, (ii) both plastic and genetic responses, (iii) temporal/spatial variation, and (iv) biotic interactions.

Freshwater ecosystems are more vulnerable to global change compared to terrestrial and marine ecosystems (reviewed by Brönmark and Hansson 2002, Heino et al. 2009, Comte and Olden 2017, Bartley et al. 2019, Anton-Pardo et al. 2019). While hosting a larger proportion of biodiversity compared to terrestrial systems with ca. 1% surface area coverage and providing many essential ecosystem services (e.g. drinking supplies, irrigation and hydrological-climatic regulation), freshwater ecosystems receive the threats from multiple key global change stressors (Dudgeon et al. 2006, Jackson et al. 2016). Notably, species in freshwater ecosystems have relatively limited ability to disperse in response to stressors (Heino et al. 2009, Woodward et al. 2010), and the biodiversity of freshwater ecosystems has been reduced more severely than that of any other ecosystem (He et al. 2019). Therefore, the mechanistic understanding and prediction of the impact of global change on freshwater ecosystems require more investigation and multi-disciplinary integration under the urge of bio-conservation and sustainable management (Dodds et al. 2013, Perujo et al. 2021).

In this general introduction, I will introduce the conceptual framework of my thesis, including the general background of the investigated biotic interactions and global change stressors, the study species, and an overview of the research aims and outlines.

Investigated biotic interaction

Trophic interactions, defined as the feeding relationships between species, are perhaps the most common and fundamental biotic interactions in shaping community structure, because these determine the energy and element flow within ecosystems, affect the distribution and abundance of organisms, and mediate the effects of environmental stressors from individual to ecosystem levels (Holt and Loreau 2002). Notably, direct effects of environmental stressors may cascade into indirect effects via trophic interactions among species, which complicates the understanding and prediction of the impact of global change (including climate change and the dynamics of other anthropogenic factors) on the local persistence of species (Huey et al. 2012, Angert et al. 2013). Therefore, investigating effects on trophic interactions has become crucial to improve our forecast for biodiversity under global change (Urban et al. 2016).

A fundamental framework to mechanistically understand global change impact on trophic interactions is to quantify the trophic interaction strength across a set of global change stressors. A basic way to quantify the short-term interaction strength is to measure the per capita feeding rate (the number of prey consumed in a given amount of time by a single predator) at a single prey density (e.g De Block et al. 2013). However, predation rates vary with prey density, which relationship can be described as the functional response (Holling 1959). Therefore, a better way to assess effects on the short-term interaction strength is to measure the functional response. There are different types of functional responses, among which type II and type III are the most common in non-filter feeders (reviewed by Jeschke et al. 2004). These functional responses can be parameterized by the predator search rate (a) that determines the feeding rate at low prey densities, and the predator handling time (h) that determines the maximum predation rate at high prey densities (Holling 1959; Figure 1). In the type II function response the search rate remains constant across prey density (Figure 1a), while in the type III functional response the search rate increases with prey density (Figure 1b), which could be explained by learning and prey switching of the predator (Jeschke et al. 2002). A stronger short-term interaction strength is characterized by a faster a and shorter h.

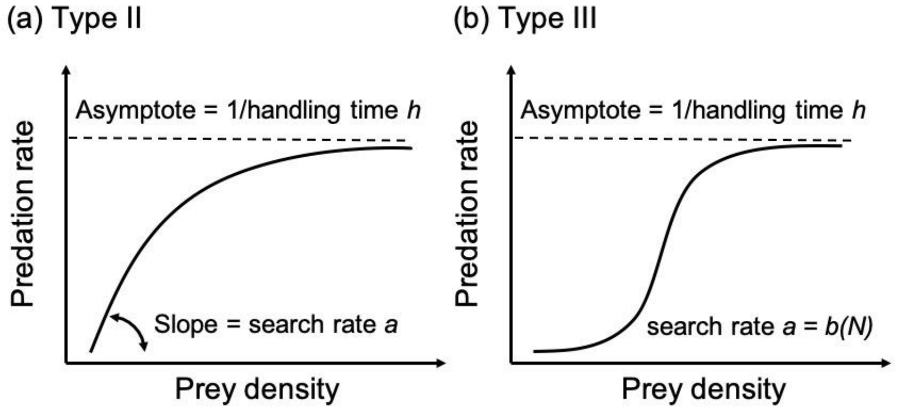


Figure 1. (a) Type II functional response curve. The slope at which predation rate initially increases with prey density is the search rate *a*. (b) Type III functional response curve, characterized by the increasing search rate with prey density. In both types the asymptote (i.e. maximal predation rate) is determined by the inverse of the handling time *h*.

The short-term trophic interaction strength estimated using a functional response can further be used to simulate trophic system dynamics (Archer et al. 2019) and estimate the long-term trophic interaction strength, i.e. the long-term numerical effect of the predator population on the prey population (Rall et al. 2010, Fussmann et al. 2014). Apart from functional response parameters, long-term interaction strength also considers other parameters of predator (e.g. metabolic rate and prey assimilation efficiency) and prey (e.g. intrinsic growth rate and carrying capacity). At higher long-term interaction strengths, there could be stronger population fluctuations under resource enrichment, thereby a higher risk of extinction due to population fluctuations (Rosenzweig 1971, Kratina et al. 2012). Hence, higher strengths indicate less stable systems. However, at lower long-term interaction strengths, species at the higher trophic level can be more easily starved to extinction if their metabolic lost cannot be compensated by the limited consumption regardless of food abundance (Vucic-Pestic et al. 2011). Note that there are other approaches to estimate long-term trophic interaction strength (e.g. Gilbert et al. 2014), and to indicate trophic system stability (e.g. Daugaard et al. 2019), which were not applied in this thesis.

Investigated global change stressors

Global warming

Global warming is the most studied component of global climate change. It is largely driven by the rapidly accumulating greenhouse gases (especially carbon dioxide, CO_2) and aerosols in the atmosphere as by-products of anthropogenic activities (Scheffers et al. 2016). According to the fifth assessment report (AR5) of Intergovernmental Panel on Climate Change (IPCC 2014), the average land surface temperature has already increased by 1.53 °C (ranging between 1.38 and 1.68 °C) from 1850-1900 to 2006-2015, while the global mean surface temperature has increased by 0.87 °C (ranging between 0.75 and 0.99 °C) during this period. A set of scenarios, the Representative Concentration Pathways (RCPs), are used to project future climate change based on the measures to control the accumulating atmospheric CO_2 concentration. It has been predicted that global mean surface temperatures will eventually increase on average with 4 °C (ranging between 3.7 and 4.8 °C) by 2100 if no measures will be taken (RCP 8.5) (Figure 2, IPCC 2014).

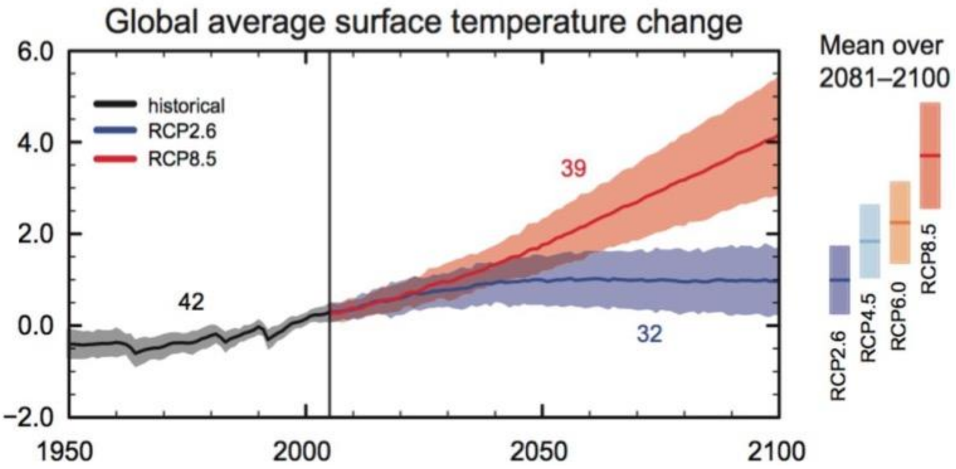


Figure 2. Increase in global mean surface temperatures relative to 1986–2005 (IPCC 2014). In the panel, the colored lines and shaded bands represent the predicted global mean surface temperature change and uncertainty range during the period 2006-2100 compared to the period 1986-2005 based on model simulations of scenarios RCP 2.6 (blue) and RCP 8.5 (red). The predicted means and uncertainties of multiple scenarios for the period of 2081-2100 are also given on the right side of the panel (adapted from IPCC 2014).

Apart from the increase in mean temperature, global warming is also accompanied by other thermal stressors, such as the increase of daily temperature fluctuations (DTFs, the temperature difference between the daily absolute maximum and minimum) (Colinet et al. 2015, Cattiaux et al. 2015, Vázquez et al. 2017), and the more frequent and severe heat waves (HW) (IPCC 2014, Beniston et al. 2017). In Western Europe, it has been demonstrated that daily temperature fluctuation could increase over 10% on average under scenario RCP 8.5 (Figure 3), and that the frequency of HW over 30 °C has mostly doubled during 2001-2015 compared to that during 1951-2965 (Figure 4).

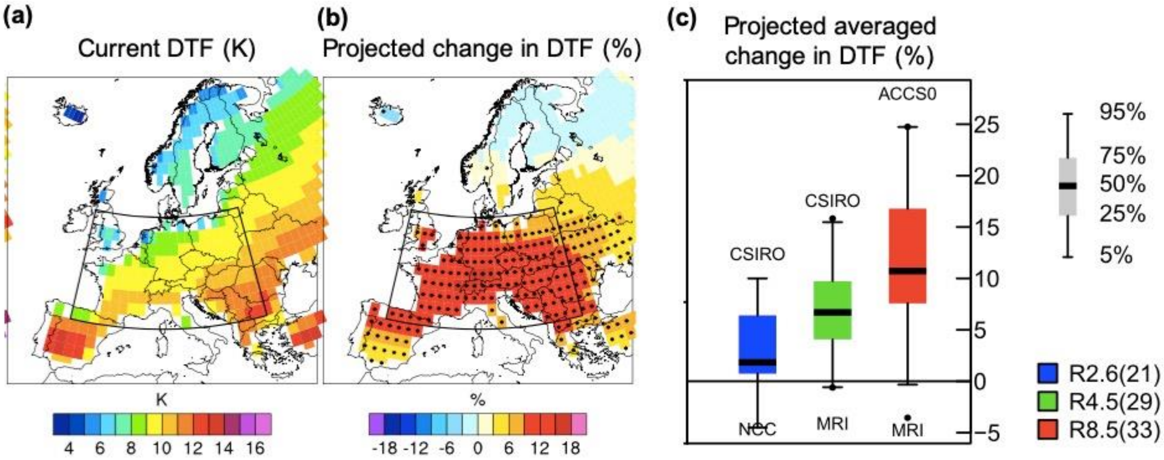


Figure 3. (a) Current (1979-2008) and (b) predicted change (%) of DTF in Europe during summer under scenario RCP 8.5. (c) Projection of averaged changes (%) in summer DTF under scenarios RCP 2.6 (blue), RCP 4.5 (green), and RCP 8.5 (red). Adapted after Cattiaux et al. (2015).

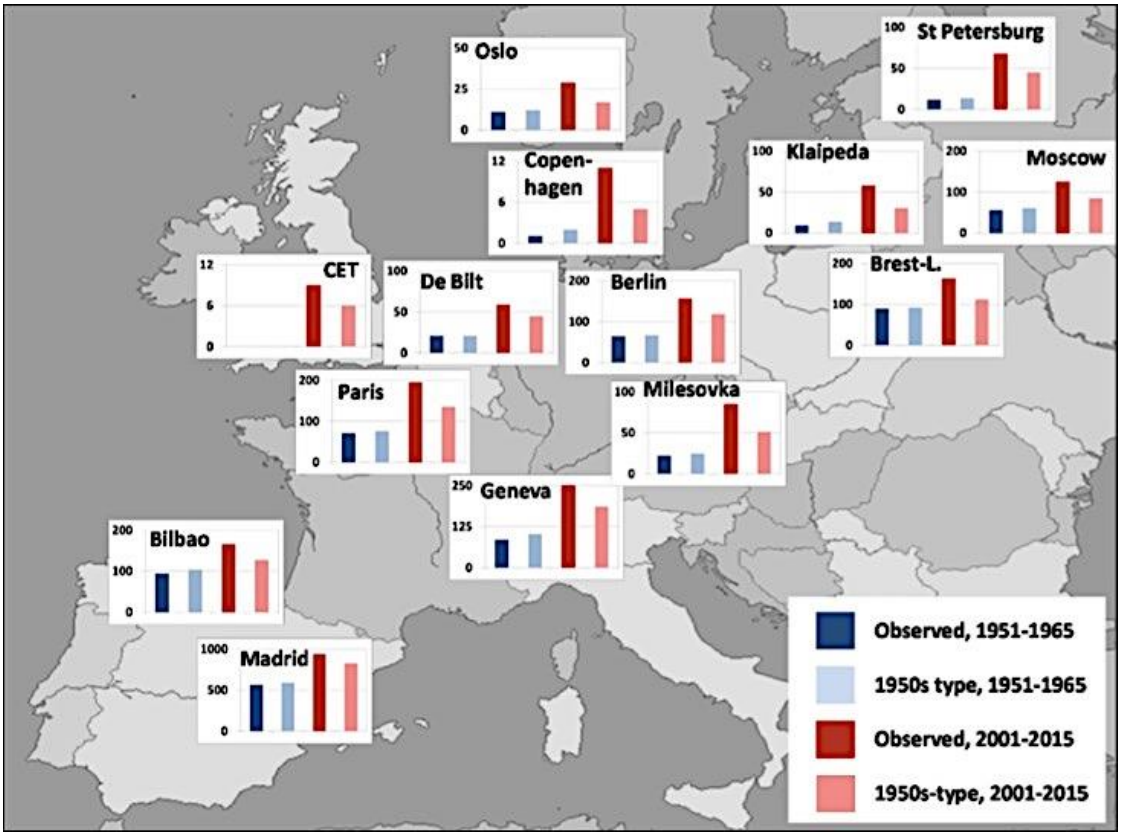


Figure 4. Frequency of hot days with a temperature above 30 °C during the period 1951-1965 (blue) and the period 2001-2015 (red) in Western Europe. Data based on observed values (dark) and de-trended values (light). Adapted after Beniston et al. (2017).

Temperature is probably the most important abiotic factor shaping species performance especially for ectotherms, because their body temperatures closely keep track of the environmental temperatures (Sinclair et al. 2016). The relationship between temperature and species performance is described as a thermal performance curve (TPC, Huey and Stevenson 1979, Angilletta Jr. 2009). The mechanisms behind temperature effects on ectotherm performance is closely associated with biochemical and physiological processes (Sinclair et al. 2016). The Metabolic Theory of Ecology (MTE), for example, suggests the thermal-dependent performances to be governed by the rate of organismal metabolism, which in turn is determined by body mass and temperature (Gilloly et al. 2001, Brown et al. 2004). According to this theory, performance y typically increases with temperature T (K), which can be described using an Arrhenius equation (Gillooly et al. 2001):

$$y\ (T) = y_0 e^{-E(\frac{1}{kT})} \text{ (Equation 1)}$$

where E is the activation energy (eV) of metabolic enzymes and k is the Boltzmann constant (8.62 × 10-5 eV K^{-1}). The E of metabolic rate and consumption rate can be used to estimate the consumer thermal impact (CTI), which determines the thermal dependence of the top-town impact of the consumer on its resource (further introduced in Chapter IV). When temperature increase above the optimum temperature (T_{opt}), the metabolic enzymes become inactivated, resulting in performance dropping with temperature toward zero (Englund et al. 2011, Sinclair et al. 2016). This performance relationship with temperature forms a unimodal TPC (Figure 5), which can be described using an Arrhenius-quadratic equation (Englund et al. 2011):

$$y\ (T) = y_0 e^{b\left(-\frac{1}{kT}\right)+q\left(-\frac{1}{kT}\right)^2} \text{ (Equation 2)}$$

with b and q as scaling coefficients. Because of the unimodal nature of TPC, an increase in temperature could lead to an increased, unchanged or decreased performance, depending on where the tested temperature interval is located on the TPC. This has also been demonstrated for performance related with trophic interactions, such as predation rate and the functional response parameters (reviewed by Englund et al. 2011). For example, warming below the optimum temperature may increase short-term interaction strength by increasing search rates and/or decreasing handling times (Thompson 1978, Sentis et al. 2015, Twardochleb et al., 2020), while it may decrease long-term trophic interaction strength by mechanisms other than functional response parameters (Rall et al. 2010, Sentis et al. 2015; further introduced in Chapter II and III). Therefore, it is important to consider temperature effects on both short- and long-term trophic interaction to determine the persistence and stability of the trophic system.

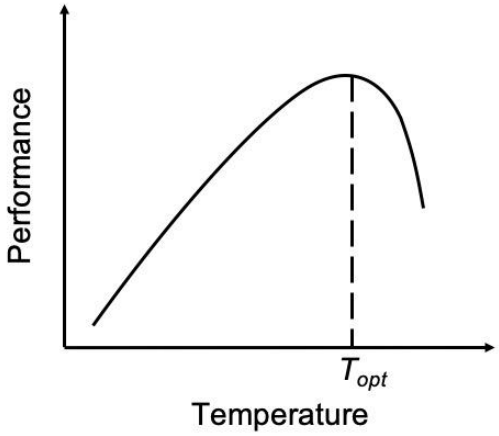


Figure 5. A unimodal thermal performance curve (TPC), with optimum temperature (T_{opt}) marked.

The increased daily temperature fluctuations and heatwave events add complexity to the prediction of species performance under global warming. For example, if the mean temperature is located near the start of the rising phase of the TPC, daily temperature fluctuations around the mean may result in higher average performance compared to the performance at constant mean temperature (Figure 6a). As the mean temperature shifts to or even above T_{opt}, temperature fluctuations around the mean may result in lower average performance compared to that at a constant mean temperature (Figure 6b-c) (Stoks et al. 2017). The property of such prediction based on a non-linear function is mathematically known as the Jensen's inequality, and applied in thermal ecology to project species responses to temperature variation (Ruel and Ayres 1999, Vázquez et al. 2017, Stoks et al. 2017). Despite recent studies suggesting the increase in temperature variation to pose greater risk to ectotherms than the increase in mean temperature (Paaijmans et al. 2013, Vasseur et al. 2014, Verheyen and Stoks 2019), little is known about the effects of daily temperature fluctuation and HW on trophic interactions (Stoks et al. 2017). Specifically, the single and combined effects of warming, DTF and HW have never been integrated to mechanistically investigate the response of freshwater trophic interactions to global change.

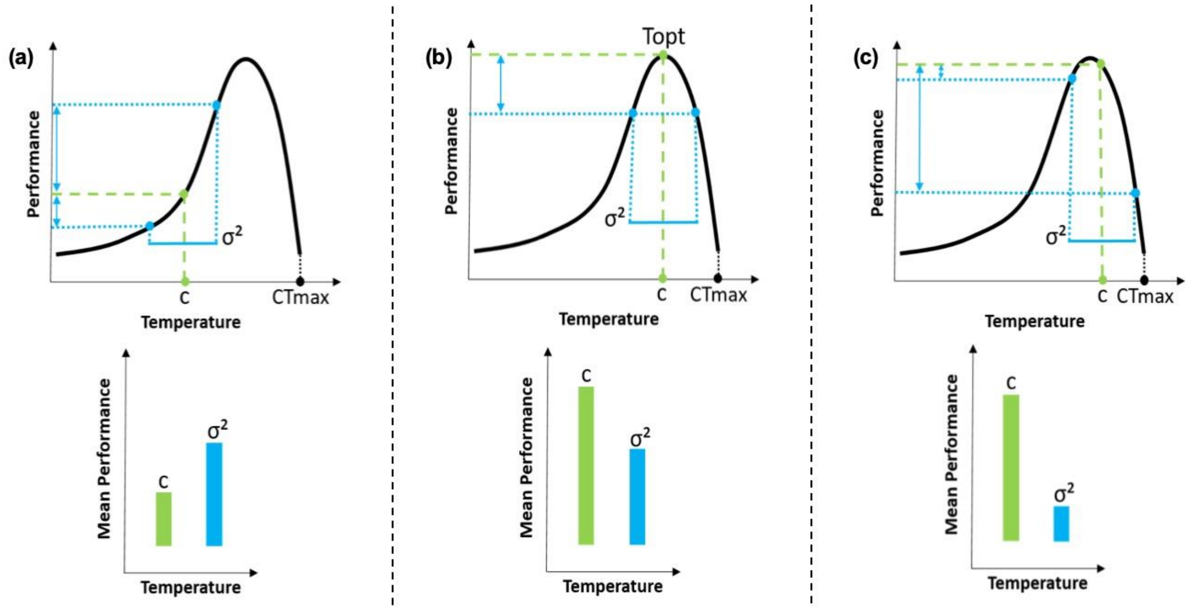


Figure 6. TPCs and comparisons between performance at a constant mean temperature c and fluctuating temperatures σ^2 when the mean temperature is (a) near the start of the rising phase of TPC, (b) at the T_{opt}, and (c) above the T_{opt}. (adapted from Stoks et al. 2017).

Another challenge of predicting the ectotherm performance under global change is to consider both plastic and evolutionary changes. While plastic changes refer to the changes of the phenotype of a given genotype, evolutionary changes refer to the changes in the genotype composition of a population (i.e. changes in allele frequencies). Thermal plasticity may evolve rapidly (Hoffmann and Sgrò 2011, Stoks et al. 2014, De Meester et al. 2018, Catulo et al. 2019) and vary within species (Liefting et al. 2009, Molina-Montenegro and Naya 2012, Verheyen and Stoks 2019). Such evolutionary changes in thermal plasticity may critically shape the predictions of warming impact of species under global warming (Valladares et al. 2014, Catulo et al. 2019). A powerful framework regarding intraspecific variation in thermal plasticity is the climatic variability hypothesis (CVH), which predicts thermal plasticity and acclimation capacity to increase with seasonal thermal heterogeneity of the environment, and thus to be higher at high-latitudes where the climate is more variable across the year than at low latitudes (Janzen 1967, Addo-Bediako et al. 2000, Ghalambor et al. 2006, Aguilar-Kirigin and Naya 2013; for a reversed latitudinal pattern of seasonality see Naya et al. 2011). This framework, however, has not been used to demonstrate the intraspecific variation of thermal plasticity in trophic interaction traits (applied in Chapter II). Two powerful approaches have been used to investigate the potential and outcome of thermal evolution (Stoks et al. 2014, Kelly 2019). One approach is experimental evolution, where experimental populations are exposed to well-known, controlled and projected thermal selection pressures (e.g. 4 °C warming) and tested after a certain number of generations (Garland and Rose 2009, Kawecki et al. 2012; applied in

Chapter I). Another approach is a space-for-time substitution, where the current phenotypes of low-latitude populations at their local thermal conditions are used as estimates for the phenotypes of high-latitude populations under projected warming after thermal evolution (De Frenne et al. 2013, Verheyen et al. 2019, applied in Chapters III and IV). Combined with common garden experiments, space-for-time substitutions and experimental thermal evolution trials have been proven useful in highlighting the importance of both plasticity and evolution of plasticity in shaping the observed phenotypic change under warming. Yet, these studies have never considered the functional response and long-term interaction strength.

Increase in algae induced turbidity

Algae-induced turbidity is mainly triggered by eutrophication, another well-known component of global change. Eutrophication is a progress of excessive nutrient (mainly as inorganic forms of N and P) enrichment in waterbodies, and is driven by anthropogenic factors, such as the increased fertilizer usage for agriculture, increased sewage production from industrial and household consumption, warming and related changes in hydrological patterns (Moss 2011, Sinha et al. 2017, Wurtsbaugh et al. 2019). Eutrophication has become one of the major global change-related stressors that can negatively affect freshwater ecosystems (Birk et al. 2020, Hering et al. 2015, Moss 2011, Woodward et al. 2010). According to the fifth Global Environmental Outlook (GEO-5) report, global river nutrient export has increased ca. 15%, and the algal and macrophyte gross productivity in lakes has increased 74% since 1970 (UNEP 2012). Eutrophication may trigger algal blooms (i.e. rapid increase of phytoplankton populations) which can affect aquatic ecosystems via toxin production, oxygen depletion due to decomposition of the overloading biomass, and increasing water turbidity (Alexander et al. 2017, Wurtsbaugh et al. 2019).

In shallow lake ecosystems, an increase in algae-induced turbidity marks the shift from a clear, submerged macrophyte dominated state to a turbid, algae dominated state. Such regime shifts can alter the structure of macroinvertebrate communities (Van de Meutter et al. 2005), and can be frequent and unstable (Bayley et al. 2007). As resource enrichment, algal blooms itself can intensify the oscillations of consumer-resource dynamics, leading to higher risk of oscillation-caused extinctions (Rosenzweig 1971, Kratina et al. 2012). As light absorbance and scattering factor, algae-induced turbidity can reduce available light underwater and alter trophic interactions (Ortega et al. 2020; further introduced in Chapter IV).

Study species

Damselflies (Odonata: Zygoptera) are important intermediate predators, while water fleas (Crustacea: Cladocera) are important algae grazers and prey for intermediate predators in freshwater ecosystems (Corbet 1999, Vanoverbeke 2008). During their semi-aquatic life cycle damselflies focus on growth and development in the aquatic larval stage, and on reproduction and dispersal in the terrestrial adult stage (Corbet 1999).

The cyclical parthenogenetic nature of water fleas allows them to alternate between parthenogenetic (asexual) reproduction during favorable conditions and sexual reproduction during stressful conditions (Ebert 2005; Figure 7). The genus *Daphnia* can be found in freshwater ecosystem around the world (Ebert 2005). The broad distribution, key role in food webs, small but manipulable body size, short generation time, and high sensitivity to environmental change make *Daphnia* an ideal experimental species in ecological, toxicological and evolutionary research (Lampert 2011, Miner et al. 2012).

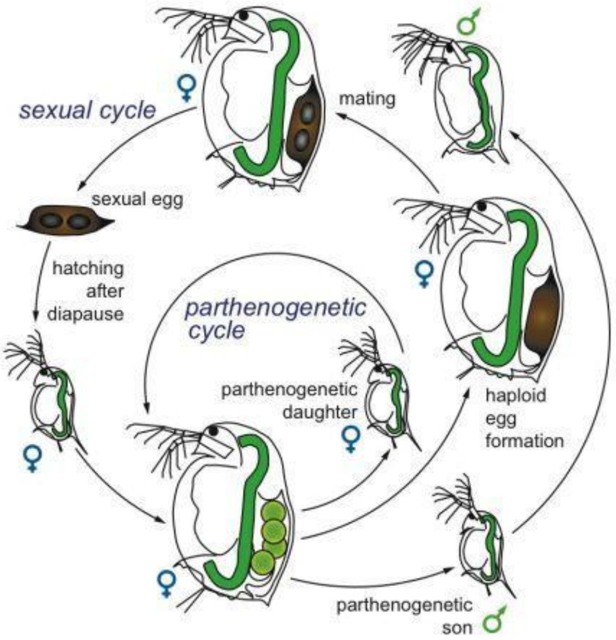


Figure 7. Life cycle of a cyclic parthenogenetic *Daphnia*. Adapted after Ebert (2005).

In this thesis, I studied the larvae of the Blue-tailed Damselfly, *Ischnura elegans* (Vander Linden, 1820) Coenagrionidae and the water flea, *Daphnia magna* (Straus 1820) Daphniidae, a well-studied predator-prey module in pond food webs (Begon et al. 2006) in which the predator typically demonstrates a type II functional response (Thompson 1978, Villalobos-Jiménez et al. 2017). As ectotherms that inhabit shallow waterbodies, they are vulnerable to thermal stressors (warming, daily temperature fluctuations and HW) and algae-induced turbidity (Scheffer and Van Nes 2007, Woodward et al. 2010). The broad distribution range and abundance of both species across latitudes (*I. elegans*: from southern Scandinavia in the north to the Mediterranean in the south, Dijkstra 2006, Gosden et al. 2011; *D. magna*: Holartic, De Gelas and De Meester 2005) makes them common predator and prey species that act as primary and secondary consumers in pond ecosystems in Europe; this makes them ecologically relevant species for eco-evolutionary research. The single and combined effects of stressors have been increasingly studied in both species. For example, warming makes *I. elegans* larvae more sensitive to pesticides (Verheyen and Stoks 2020), while it causes lower fitness of *D. magna* if combined with pesticides or food limitation (Cuenca Cambronero et al. 2018). With regard to trophic interactions, warming has been found to increase predation rates (De Block et al. 2013) and strengthen the functional response (i.e. increase search rates and decrease handling times) of *I. elegans* larvae preying on *D. magna* (Thompson 1978), while the combined effects of warming and other stressors are largely unknown.

The chosen high-latitude (Denmark and southern Sweden) and low-latitude (southern France) damselfly populations differ considerably in latitudinal distribution (55–57°N vs. 43°N) and annual temperature range (based on Addo-Bediako et al. 2000), making them suitable to test intraspecific variation of thermal responses and CVH in predation rates. In summer, the high-latitude populations experience a lower mean water temperature (20 °C), a lower maximal daily temperature fluctuation (5 °C, i.e. ± 2.5 °C around the mean) and a relatively low frequency of HW above 30 °C compared to the low-latitude populations which experience a mean water temperature of 24 °C, a maximal daily temperature fluctuation of 10 °C (i.e. ± 5 °C around the mean) and frequent HW of above 30 °C (De Block et al. 2013, Dinh Van et al. 2014, Verheyen and Stoks 2019, Van Dievel et al. 2019a). Notably, the difference in mean temperature between both latitudes matches the predicted 4 °C increase in mean temperature at the high latitude by 2100 under the IPCC scenario RCP 8.5 (IPCC 2014). Also the 10 °C daily temperature fluctuations and HW above 30 °C, that currently occur at the low latitude, can be expected under predicted warming at the high latitude (Beniston et al. 2017, González-Tokman et al. 2020). Previous studies have indicated latitude-associated thermal adaptation, i.e. adaptation to the thermal conditions across latitudes : (1) High-latitude *I. elegans* populations are semivoltine (have one generation every two years) while low-latitude populations are multivoltine (have three to four generations per year) (Corbet et al. 2006). (2)

High-latitude populations have evolved a slower growth rate, a slower development rate and a higher mass at emergence compared to low-latitude populations (De Block et al., 2013, Debecker and Stoks 2019, Dinh Van et al. 2014, Shama et al. 2011). Apart from temperature, other abiotic and abiotic characteristics (including algal turbidity) are similar between these latitudes (Debecker and Stoks 2019). This makes low- and high-latitude damselfly populations suitable to test the evolutionary change of short- and long-term trophic interaction un the high-latitude populations under climate change using the space-for-time substitution approach. Using this approach, previous studies have found that temperature variation makes the species more sensitive to increases in mean temperature unless thermal evolution occurs (Verheyen and Stoks 2019), and that temperature variation overrules the ability of thermal evolution to offset the increased pesticide toxicity under warming (Verheyen et al. 2019). In Chapters II and III, I chose a single clone of *D. magna* from an intermediate latitude (Belgium, 50°N) for the predation trials due to practical constraints. In Chapter IV, I included both *D. magna* and *I. elegans* from low- and high- latitudes to investigate the role of thermal evolution in both interacting species.

The short generation time and the production of clones by *D. magna* makes it also ideal for testing the evolution of TPCs using experimental thermal evolution. Therefore, in Chapter I, I used *D. magna* from control and heat (+4 °C)-selected mesososms, which were already established and kept as separate clones in the lab since 2009.

Research aims and outlines

My thesis is situated at the interface of global change biology, evolutionary biology and trophic ecology. I studied the rapid thermal evolution of TPCs in the prey *D. magna*, and the long-term latitude-associated evolution of predation rate and its thermal plasticity in the predator *I. elegans*. I also studied the single and combined effects of several global change stressors (increasing mean temperatures, increasing daily temperature fluctuations, presence of HW, and increasing algae induced-turbidity) on the short- and long-term trophic interaction strength between larvae of the damselfly *I. elegans* and the water flea *D. magna*. Thereby, I mainly focused on effects of the stressors on predator-prey interactions, the potential of evolution of the predator and/or the prey to mediate these effects, and their implications on food web stability. The general aims were to answer (1) if the TPC of prey can rapidly evolve under thermal selection, (2) if CVH is supported by predation rates across latitudes, (3) if thermal evolution in high-latitude predators can buffer the effects of increased DTF and the presence of HW on trophic system stability under global warming, and (4) if the thermal evolution of high-latitude predator and/or prey can influence the effects of warming on trophic system stability and whether this differs between clear and turbid water bodies.

In Chapter I, I focused on the experimental thermal evolution of the TPCs of *D. magna*. I therefore examined if the TPCs of several key performance traits related to fitness differ between the control and heat (+ 4 °C)-selected populations. I also indirectly evaluated if the thermal dependence of top-down impact the water fleas may have on their resource differed between the control and heat-selected populations by contrasting the activation energies of ingestion and metabolic rates.

In Chapter II, I quantified predation rates of low- and high-latitude *I. elegans* larvae on mid-latitude prey to test the CVH, using different types of thermal plasticity and thermal acclimation capacity metrics under projected mild warming (mean temperature increase of 4 °C, IPCC RCP 8.5 scenario) to examine if thermal plasticity and/or thermal acclimation capacity of predation rate is higher for high-latitude predators compared to low-latitude predators.

In Chapter III, I examined the effects of mild warming (mean temperature increase of 4 °C), a 10 °C DTF increase, and a 32 °C heatwave on the functional response and long-term interaction strength of high- and low-latitude *I. elegans* larvae preying on mid-latitude *D. magna*. I investigated if thermal evolution of the high-latitude predators (using a space-for-time substitution approach) can mediate these effects. I also investigated if the individual growth rate and functional response parameters show signs of latitude-associated thermal adaptation.

Since low-latitude predators experience more frequent HW, a higher DTF around a higher mean temperature compared to high-latitude predators, I applied a space-for-time substitution to assess the potential impact of thermal evolution on the long-term interaction strength in high-latitude predators.

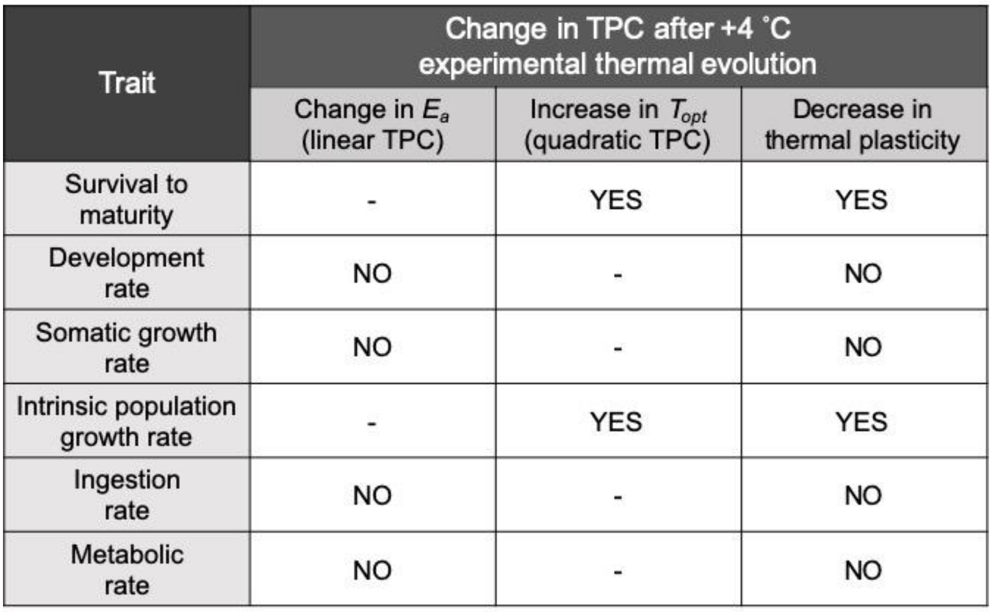
In Chapter IV, I exposed high- and low-latitude *I. elegans* larvae feeding on high- and low-latitude *D. magna* to 4 °C warming (a constant temperature of 20 °C and 24 °C), and algae-induced turbidity to investigate how both global change stressors shape the functional response and long-term interaction strength of the interacting species. I examined which components mainly contribute to the patterns of long-term interaction strength, and whether the thermal evolution of predator and/or prey can mediate warming effects, and if this differs between clear and turbid water bodies.

Chapter I

Rapid evolution of thermal performance curves but not of activation energies in *Daphnia magna*

Ying-Jie Wang, Nedim Tüzün, Luc De Meester, Arnaud Sentis and Robby Stoks

Manuscript under preparation.



Trait	Change in TPC after +4 ˚C experimental thermal evolution		
	Change in E_a (linear TPC)	Increase in T_{opt} (quadratic TPC)	Decrease in thermal plasticity
Survival to maturity	-	YES	YES
Development rate	NO	-	NO
Somatic growth rate	NO	-	NO
Intrinsic population growth rate	-	YES	YES
Ingestion rate	NO	-	NO
Metabolic rate	NO	-	NO

Abstract

Besides plastic responses, species may cope with warming through rapid evolutionary responses. Nevertheless, while thermal performance curves are considered powerful tools to understand the impact of warming on ectotherms, their rapid evolution is much less studied. We capitalized on a 2-year experimental evolution trial in outdoor mesocosms that were kept at ambient temperatures or heated 4 °C above ambient, by testing in a follow-up common garden experiment for rapid evolution of the thermal performance curves (TPCs) for multiple key performance traits of the water flea *Daphnia magna*. We found several cases of rapid evolution of the TPCs for life history traits. The heat-selected *Daphnia* showed evolutionary shifts of the quadratic TPCs for survival, fecundity of the first clutch and intrinsic population growth rate toward higher optimum temperatures and a less pronounced downward curvature indicating a better ability to keep fitness high across a range of temperatures. In contrast, we found no evolution of the linear TPCs for somatic growth and development rates, and for the traits related to energy gain (ingestion rate) and costs (metabolic rate). This adds evidence to the rare studies on this topic that TPCs of different traits may strongly differ in their evolutionary response, and matches theory that slopes of linear TPCs (activation energies) are less likely to evolve. As a result also the relative thermal slope of energy gain versus energy costs did not evolve. This suggests that despite thermal evolution of the intrinsic population growth rate of this consumer, its top-down impact on the resource may not evolve.

Introduction

Rapid climate change is among the key factors threatening persistence of ectotherm species, and therefore biodiversity and ecosystem functioning (Sánchez-Bayo and Wyckhuys 2019, Soroye et al. 2020, Halsch et al. 2021). The ability of species to respond through plastic and genetic changes to climate warming is crucial to understand and predict the impact of climate change (Sgrò et al. 2016, Stoks et al. 2014, Urban 2015, Nadeau and Urban 2019). Although genetic trait changes take longer time than plastic trait changes, these may be crucial as evolution may reverse maladaptive plastic responses and enhance adaptive plastic responses (Diamond and Martin 2016). Despite the increasing number of demonstrations of rapid thermal evolution (Catullo et al. 2019), studies on ectotherms have mainly focused on the ability to deal with extreme temperatures or measured traits at only two temperatures. This could lead to inaccurate predictions of the impact of climate change on ectotherms, because they can experience a range of temperatures, and their performance often does not follow a simple linear thermal reaction norm (Huey and Kingsolver 1993, Gillooly et al. 2001, Schulte et al. 2011).

A powerful tool to assess the impact of warming are the thermal performance curves (TPCs) that describe the response patterns of performance against a range of temperatures (Deutsch et al. 2008, Kingsolver 2009, Sinclair et al. 2016). Two important features that can be identified using TPCs are the activation energy (E_a) of biological reactions, being the slope of the increasing linear part of TPCs, and the optimum temperature (T_{opt}) at which the maximal performance is reached for unimodal TPCs (Gillooly et al. 2001, Brown et al. 2004, Angilletta Jr. 2009). Importantly, besides traits also their TPCs may rapidly evolve (reviewed by Sinclair et al. 2016), and this has been increasingly investigated in ectotherms (Santos et al. 2006, Higgins et al. 2014, Fragata et al. 2016, Carbonell and Stoks 2020, Mesas et al. 2021, Carbonell et al. 2021). Evolutionary changes of TPCs could in principle include changes in its two key features: E_a for the linear part of TPCs and T_{opt} for unimodal TPCs. While E_a values are assumed to be constant according to the metabolic theory of ecology (Gillooly et al. 2001), this has been highly debated (e.g. Kontopoulos et al. 2020) and evolution of E_a has been observed over long time scales associated with population differentiation along latitudinal gradients (Nilsson-Örtman et al. 2013; Kontopoulos et al. 2020). While rapid evolution of E_a has not been reported in ecotherms, it recently has been documented for photosynthesis in a phytoplankton species (Zhong et al. 2021). Instead, rapid evolution of TPCs toward a higher T_{opt} has been more often documented (in ectotherms: Santos et al. 2006, Carbonell and Stoks 2020, Mesas et al. 2021; in phytoplankton: O'Donnell et al. 2018, Schaum et al. 2017, Jin and Agustí 2018, Zhong et al. 2021). Evolutionary change of TPCs can involve trade-offs between the features of TPCs. Specifically, evolution of a higher T_{opt} and higher thermal tolerance range (i.e. less curvature of the TPC) have been associated with the evolution of a lower performance at T_{opt}, which is also known as the "specialist-generalist trade-off" (Huey and Hertz 1984, Sinclair et al. 2016, Jin and Agustí 2018, Zhong et al. 2021). So far, studies on TPC evolution mostly focused on single performance traits, yet the rare multi-trait studies showed this to be strongly trait-dependent (Santos et al. 2006, Fragata et al. 2016), asking for a multi-trait approach.

While the features of TPCs are typically used to predict the impact of rapid climate change at the population level (Sinclair et al. 2016), these may also inform about effects at the community level. Specifically, the net impact of warming on consumer-resource species couples is expected to depend on the relative thermal responses (activation energies, E_a) of energetic gains (ingestion of resources) and energetic costs (metabolism) of the consumer that mediate the flow of energy throughout a food web. If a consumer's ingestion rates increase more with temperature, hence have a higher E_a, than its metabolic rates, than the overall energetic efficiencies of the consumers will increase under warming, and therefore the consumer can be considered energetically warm-adapted (Vasseur and McCann 2005, Rall et al. 2010). At the community level, a higher E_a for ingestion than for metabolic rates in the consumer has been theoretically shown to result in an increasing top-down impact of the consumer on its resource under warming (Vasseur and McCann 2005). Despite its potential ecological importance, it remains untested whether rapid thermal evolution affects the relative scaling of the activation energies of energetic costs and gains.

We examined the rapid evolution of TPCs of multiple performance traits of a key aquatic species, the water flea *Daphnia magna* (Miner et al. 2012). We thereby capitalized on a powerful experimental evolution trial whereby *D. magna* were kept in outdoor mesocosms at ambient or heated (ambient +4 °C) conditions. It was shown before in this experiment that rapid evolution occurred in terms of an increased body size (Van Doorslaer et al. 2010), an increased competitive strength at higher temperatures (Van Doorslaer et al. 2009b), and a higher heat tolerance (CT_{max}) (Geerts et al. 2015). Here, we extend these studies by explicitly considering the evolution of TPCs (i.e. comparing two types of experimental populations selected by different thermal conditions), thereby reconstructing the thermal evolution of multiple traits across a range of temperatures. We expected that for traits with unimodal TPCs, the heat-selected *D. magna* to have evolved a higher T_{opt} (Santos et al. 2006, Carbonell and Stoks 2020, Mesas et al. 2021) and a larger thermal tolerance range (less curvature) accompanied with a lower performance at T_{opt} (Huey and Hertz 1984, Sinclair et al. 2016). For traits with a linear TPC we expected evolution of the activation energies to be less pronounced (Gillooly et al. 2001). Yet in case of evolution of activation energies, we expected the heat-selected *D. magna* to have evolved a higher scaling of the activation energies of ingestion rates versus metabolic rates, hence to become energetically warm-adapted (*sensu* Vasseur and McCann 2005, Rall et al. 2010).

Materials and Methods

Daphnia populations and rearing

We worked with *D. magna* clones obtained from a thermal selection experiment in outdoor mesocosms conducted in Ness Botanic gardens (53°16' N, 3°03' W, UK) in 2007 (Feuchtmayr et al. 2010, Van Doorslaer et al. 2009b, Van Doorslaer et al 2010). The source populations for this selection experiment were sampled as dormant eggs from the upper sediment layers from a shallow pond at Brown Moss, UK. Two thermal selection treatments were imposed in outdoor 3000 L mesocosms: ambient temperatures in non-heated mesocosms, and ambient +4 °C temperatures in heated mesocosms. The +4 °C warming was based on the expected mean temperature increase by 2100 under IPCC (2014) scenario RCP8.5. The mean summer water temperatures in the mesocosms were ~20 °C (non-heated) and ~24 °C (heated) (Van Doorslaer et al. 2010). Three previous studies have shown rapid thermal evolution in the *Daphnia* obtained from this experiment. After six months of thermal selection, a genetically based increased size at maturity was observed in the clones that underwent thermal selection (Van Doorslaer et al. 2010). Furthermore, heat-selected clones evolved after 1.5 years an increased competitive strength at higher temperatures against warm-adapted French clones (Van Doorslaer et al. 2009b), and after two years an increased heat tolerance (Geerts et al. 2015). The current experiment used the clones obtained from sexually produced dormant eggs after two years of selection that were studied by Geerts et al. (2015). These clonal lineages were kept through parthenogenetic reproduction at low densities in the laboratory for nine years under standardized conditions (20 °C, 14:10 L:D photoperiod) before use in current study. Under these conditions, mutations are unlikely to have affected the genotypic trait values of these clones (Goitom et al. 2018).

Fourteen *D. magna* clones were chosen for current study: seven clones from two non-heated, and seven clones from two heated mesocosms. Before the experiment, each clone was cultured for several generations (ca. 2 months) under standard conditions. For this, each clone was reared in a 500 mL glass jar with dechlorinated tap water, at a standard density (10-12 individuals jar^{-1}), temperature (20 °C) and photoperiod (14:10 L:D), and daily fed *ad libitum* green algae *Acutodesmus obliquus*. We only used 2nd-4th clutches to produce the next generation.

Life table experiment

To test for thermal evolution of the thermal performance curves for life history, behavioural and physiological traits, we set up a common-garden experiment where the clones from the two thermal selection groups (non-heated vs heated mesocosms) were exposed to a range of six test temperatures in the laboratory (12-16-20-24-28-30 °C). Note that during this experiment, clones were culture separately, hence no further evolution in terms of clonal sorting could occur. Temperatures between 12 °C and 24 °C are yearly experienced by clones in the UK, while 28 °C and especially 30 °C are only encountered during heat wave periods. Each clone was tested in two replicate jars per test temperature. This resulted in 2 thermal selection groups × 6 test temperatures × 7 clones × 2 replicates = 168 jars.

At the start of the experiment, 10-12 *D. magna* juveniles (<24-hr old) were transferred to 500 mL glass vials filled with dechlorinated tap water that were placed in water baths at one of the test temperatures at a 14:10 L:D photoperiod. *Daphnia* were daily fed *ad libitum* green algae *A. obliquus*. Temperature loggers within the jars showed that the experimental temperatures were within 0.2 °C from their target means. To minimize maternal effects, the *D. magna* clones were cultured at the six test temperatures for three generations before the actual life table experiment started. Throughout the pre-experimental and experimental rearing the medium was refreshed three times per week.

Response variables

We quantified four life-history variables (survival until maturity, development rate, somatic growth rate, and intrinsic population growth rate), a behavioural variable (ingestion rate) and a physiological trait (metabolic rate). These variables were chosen as they strongly associate with fitness (Angilletta et al. 2004, Van Doorslaer et al. 2009a), and show patterns of local thermal adaptation to different thermal habitats in *Daphnia* (Chopelet et al. 2008). We also quantified the relative scaling of the activation energies of ingestion rates and metabolic rates to assess the expected effects on the consumer's impact on its resource (Vasseur and McCann 2005, Iles 2014).

Survival until maturity was quantified as the proportion of individuals per jar that reached reproductive maturation. Development rate was estimated as the inverse of age of maturity, the moment when eggs were present in the pouch of one fourth of the adult *Daphnia* in a jar. somatic growth rate was quantified by dividing individual dry mass by age of maturity. Dry mass was obtained from one of the two replicate jars after the second clutch was released, by drying nine individuals at 60 °C for 24 h and weighing them to the nearest 0.01mg. Intrinsic population growth rate was measured based on survival and daily offspring production during the first two clutches (Zhang et al. 2018) using the Euler-Lotka equation (Lotka 1913):

$$1 = \int e^{-rx} \, l_x m_x dx \text{ (Equation 1)}$$

where l_x is the probability of survival to age x, and m_x is the number of offspring produced at day x. For the statistical analyses, we worked with one mean value per jar for survival until maturity, development rate, somatic growth rate and intrinsic population growth rate.

Ingestion rate and metabolic rate were measured in randomly sampled egg-free adult *Daphnia* (after the second clutch was released) from one of the two replicated jars (i.e. one for ingestion rate, the other for metabolic rate) per clone at each test temperature. This resulted in one value for ingestion rate and one value for metabolic rate per clone at each test temperature. For ingestion rate, six *Daphnia* were kept without food at the test temperature for >12 h to ensure gut clearance (Gillis et al. 2005). Thereafter, sets of two *Daphnia* were allocated to 60 mL glass vials with 50 mL dechlorinated water and 50 µL algae solution (10^5 cells/mL) at their respective test temperature. To prevent algal growth, the vials were placed in the dark (Mandal et al. 2018). Ingestion rate was measured as the algae depletion rate (per mg *Daphnia* dry mass) quantified by the reduction in algae concentration between the start and after 1 h of feeding using an AttuneTM acoustic focusing cytometer (Applied Biosystems®, Foster City, CA, USA). Two control vials containing no *Daphnia* were installed for every measurement session to quantify any background changes in algae concentration. For metabolic rate, nine *Daphnia* per clone at each temperature were randomly chosen and sets of three individuals were placed in 60 mL vials with dechlorinated tap water at their respective test temperature. Metabolic rate was measured as the oxygen depletion rate (per mg *Daphnia* dry mass) during 8 h, quantified by the reduction in oxygen concentration between the start and after 8 h using a non-invasive oxygen sensor spot on each vial with a fiber-optic cable connected to an oxygen meter (FIBOX 4, PreSens, Regensburg, Germany). Three control vials containing no *Daphnia* were installed for every measurement session to quantify the background oxygen depletion. Per clone we took at each test temperature the mean of the three values for each variable for further analyses, this resulted in one value for each variable per clone at each test temperature.

We estimated the consumer thermal impact (CTI) based on Iles (2014). CTI describes how the net impact of a consumer on its resources changes with temperature (Vasseur and McCann 2005). CTI was estimated following the equation:

$$CTI = E_I - E_J \text{ (Equation 2)}$$

where E_I and E_J are the activation energy (eV) for ingestion and metabolism, respectively. Positive (negative) CTI indicates that the top-down impact of a consumer on its resources increases (decreases) with temperature. Consumers with a positive (negative) CTI have a higher (lower) energetic efficiency, hence performance, at higher temperatures, and therefore can be considered warm- (cold-) adapted (Vasseur and McCann 2005, Iles 2014).

For some of the 14 *Daphnia* clones, all individuals died before maturation in both replicate jars at the highest temperatures (28 °C: two control clones from ambient mesocosms; 30 °C: five control clones and one heat-selected clone) or failed to produce juveniles (12 °C: one control clone and one heat-selected clone). We excluded the clones which died before maturation in further analyses for all response variables except for intrinsic population growth rate. For the clones which died before maturation or survived but failed to produce juveniles, we estimated the intrinsic population growth rate as zero (based on O'Neal and Juliano 2013).

Statistical analyses

Data were analyzed in R version 3.6.3 (R Core Team 2020). We estimated the temperature dependence of each response variable Y in each selection group fitting three TPC models following Twardochleb et al. (2020): an intercept only 'null' model, the Arrhenius model and the Arrhenius quadratic model. The intercept only model assumes no effect of temperature on the biological performance:

$$Y = c \text{ (Equation 3)}$$

where c is a normalization constant and model intercept. The Arrhenius model (Gillooly et al. 2001) describes the dependence of biological performance for temperatures below thermal optima (Englund et al. 2011):

$$Y = ce^{E_a\left(-\frac{1}{kT}\right)} \text{ (Equation 4)}$$

where c is a normalization constant, E_a is the activation energy (eV) describing the strength of the temperature response, k is the Boltzmann's constant (8.617 × 10−5 eV), and T is temperature (K). The Arrhenius-quadratic model describes the temperature response of biological performances for temperatures below and above thermal optima (Englund et al. 2011):

$$Y = ce^{b\left(-\frac{1}{kT}\right)+q\left(-\frac{1}{kT}\right)^2} \text{ (Equation 5)}$$

where c is a normalization constant, and b (eV) and q (eV^2) are scaling coefficients. Taking the natural logarithm of both sides of Equations 3, 4 and 5 results in the form of the multiple linear regression models used to estimate the normalization constant c, the first-order scaling coefficients E_a (Equation 4) and b (Equation 5) of temperature (-1/kT), and the second-order scaling coefficient q (Equation 5) of temperature $(-1/kT)^2$ (Iles 2014). We used Akaike's information criterion corrected (AICc) for small sample sizes to select the best-fitting model (see Table S1) for each response variable in each thermal selection group. When the difference between models in AICc is less than 2 we considered both models as having similar support (Burnham and Anderson 2002). When the Arrhenius equation had good support, indicating a linear TPC, we checked whether 84% confidence intervals for E_a (slope of the linear TPC) overlapped zero, and whether these overlapped between thermal selection groups. Overlap of 84% CIs matches a difference between two values based on z test at the $P = 0.05$ level (Payton et al. 2003, MacGregor-Fors and Payton 2013). Values with 84% CIs not covering zero were considered significantly different from zero, values with non-overlapping 84% CIs were considered as significantly different. When the Arrhenius-quadratic equation was selected, indicating a unimodal TPC, we checked whether 84% CIs for the thermal optimum T_{opt} overlapped between thermal selection groups. T_{opt} was calculated by the function *predict*, which uses the quadratic model to predict the best performance for a given set of temperatures. Its 84% CI was estimated as $T_{opt} \pm 1.406$ SE with SE based on 999 bootstrapped pseudovalues generated by fitting the bootstrapped data sets (obtained by randomly resampling the original data of each thermal selection group with replacement thereby keeping the sample sizes equal) to the Arrhenius model. We report the parameter values of the selected model for each response variable in Appendix S2.

Complementary to the TPC approach, we also fitted each ln-transformed response variable to a general linear model (GLM) to test the effects of temperature and the thermal selection group on the response variables (Appendix S3). Temperature, temperature^2 (both as continuous variables), and thermal selection group (control and heat-selected) were treated as fixed factors. Clone was originally added as a random factor nested in mesocosm (using package *lme4*, Bates et al. 2015), but it was never significant (all $P > 0.05$) and thus removed from the final models. Four outliers (> 1.5 interquartile above the upper or below the lower quartile, Ghasemi and Zahediasl 2012) out of 76 observations for ingestion rate and metabolic rate were removed to meet model assumptions.

To estimate the consumer thermal impact (CTI) for each thermal selection group, we followed the methodology of Iles (2014) after verifying the shape of TPC of metabolic rate and ingestion rates to be linear (fitting the Arrhenius model). We first obtained per temperature the per capita metabolic rates I (J/s) by multiplying the observed *Daphnia* metabolic rates expressed in µM O_2/s with the aerobic energy lost per unit oxygen consumption (0.44 J/µM O_2). Next, we estimated per temperature the per capita ingestion rates J (J/s) by multiplying the observed *Daphnia* ingestion rates expressed as algae dry mass/s with the algae biomass-energy conversion factor (7000 J/g, Peters 1986). We thereby converted algae cell counts into algae dry mass following the conversion factor 1.67×10^{-8} g/cell (Putman et al. 2015). Based on Iles (2014), we then fitted the I and J values to the universal temperature dependence (UTD) model which relates biological rates to body mass M (g) and environmental temperature T (K):

$$I = i_0 M^{b_I} e^{-E_I/kT} \text{ (Equation 6)}$$

$$J = j_0 M^{b_J} e^{-E_J/kT} \text{ (Equation 7)}$$

where i_0 and j_0 are normalization constants, b_I and b_J are allometric exponents, E_I and E_J are the activation energy (eV) for ingestion and metabolism based on the UTD model, and k is the Boltzmann's constant (details shown in Appendix S4). Finally, based on the estimated values for E_I and E_J we then calculated CTI for both control and heat-selected *Daphnia* using Equation 2. We calculated the 84% CIs of the CTI by propagating the standard errors associated with thermal selection group estimates of E_I and E_J based on the law of propagation of uncertainty.

Results

Thermal performance curves for life history

For survival until maturity, the Arrhenius-quadratic model provided the best fit in control *Daphnia* (ΔAICc > 9.8), while the intercept model provided similar support compared to the Arrhenius-quadratic model in heat-selected *Daphnia* (ΔAICc < 2, Table S1). Survival increased with temperature up to 18.52 °C (84% CI = 18.49 – 18.55) and then declined strongly in control *Daphnia*, while it slightly increased with temperature up to 21.01 °C (84% CI = 20.88 – 21.14) and then levelled off in heat-selected *Daphnia* (Figure 2a). The ~2.5 °C increase in T_{opt} of survival in the heat-selected *Daphnia* was significant based on the non-overlapping 84% CIs. This was supported by the GLM that showed both Temp × Selection group ($P = 0.004$) and Temp² × Selection group ($P = 0.06$) interactions (Table S3).

For development rate, the Arrhenius and Arrhenius-quadratic models had similar support in each selection group (ΔAICc < 2, Table S1). Development rates increased with temperature to 30 °C in a mainly linear way and only slightly levelled off; the E_a values being higher than zero but not differing between both selection groups (Figure 2b). This was also supported in the complementary GLM by significant effects of Temp ($P < 0.001$) and Temp² ($P = 0.018$) but no interactions with the selection group (Table S3).

For somatic growth rate, the Arrhenius model and the Arrhenius-quadratic model provided similar support in each selection group (ΔAICc < 2, Table S1). Moreover, in the heat-selected group the ‘intercept only’ model also had good support (ΔAICc < 2). The somatic growth rate slightly decreased in a mainly linear way with temperature to 30 °C, with the 84% confidence intervals of the negative E_a values overlapping between both selection groups, suggesting a shallower slope in the heat-selected *Daphnia* (Figure 2c). This was also supported by the complementary GLM showing significant effects of Temp ($P < 0.001$) and but no interactions with the selection group (Table S3).

For intrinsic population growth rate, the Arrhenius-quadratic model provided a better fit than the Arrhenius model in both selection groups (both ΔAICc > 2.4 compared to Arrhenius model, Table S1). The curvature of the TPC was much less in the heat-selected *Daphnia* whereby the ‘intercept only’ model also had good support (ΔAICc < 2). The intrinsic population growth rate increased with temperature up to 18.92 °C (84% CI = 18.88 – 18.96 °C) then strongly declined in control *Daphnia*, while it slightly increased with temperature up to 22.30 °C (84% CI = 22.17 – 22.43 °C) before levelling off in heat-selected *Daphnia* (Figure 2d). Based on the non-overlapping 84% CIs, the ~3.4 °C increase in T_{opt} of intrinsic population growth rate in heat-selected *Daphnia* was significant. These TPC differences between selection groups were supported by the GLM that showed both a significant Temp × Selection group interaction ($P = 0.003$) and a trend for a Temp² × Selection group interaction ($P = 0.073$) (Table S3).

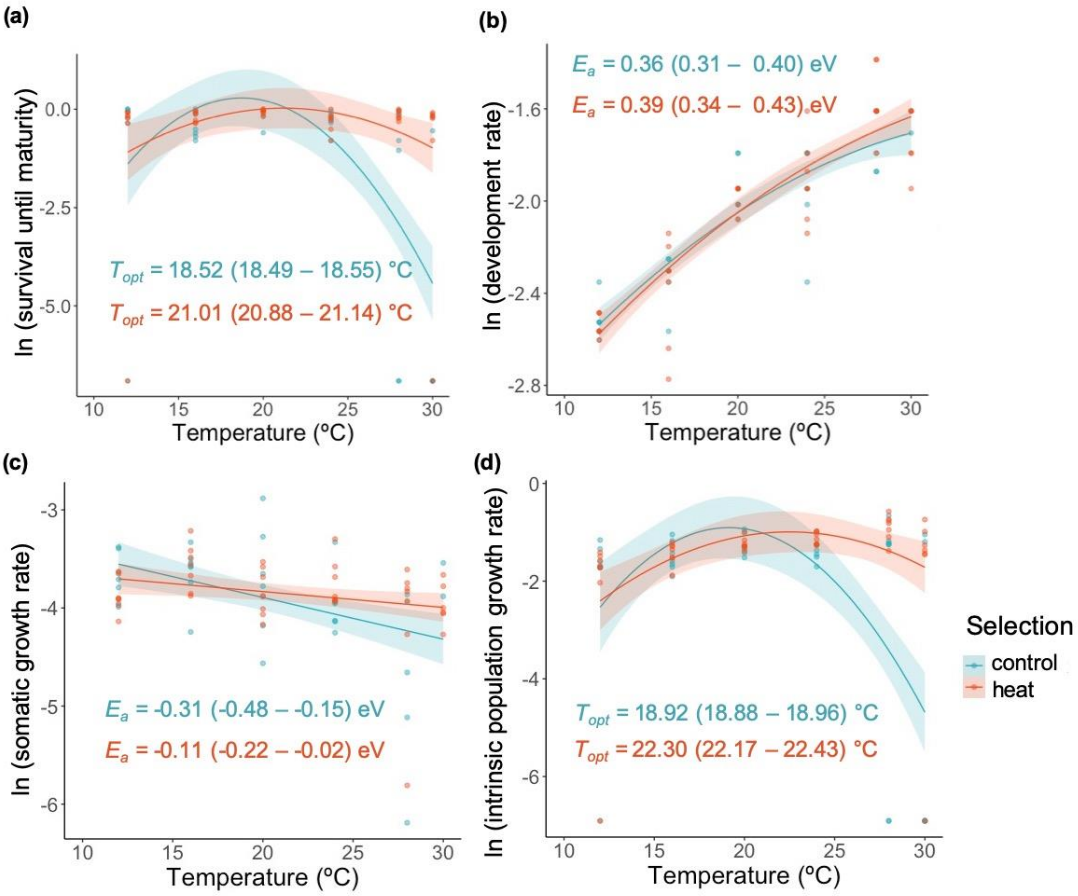


Figure 1. Thermal performance curves for life history traits of control (blue) and heat-selected (red) *Daphnia magna*. (a) Survival until maturity; (b) development rate; (c) somatic growth rate; (d) intrinsic population growth rate. For TPCs supported by the Arrhenius model we reported the activation energy, E_a (eV), and for TPCs supported by the quadratic Arrhenius model we reported the thermal optimum T_{opt} (°C), each time with 84% CI. Coloured areas represent 84% CI bands of the TPCs.

Thermal performance curves for food ingestion and metabolic rates

For ingestion rate, the Arrhenius model provided the best fit in each selection group (ΔAICc > 2, Table S1). The ingestion rate increased with temperature to 30 °C, with the positive E_a values not differing between both selection groups (Figure 2e). This was also supported in the GLM by significant effects of Temp ($P < 0.001$) without interactions with selection group (Table S3).

For metabolic rate, the Arrhenius model provided quite similar support compared to the Arrhenius-quadratic model in control *Daphnia* (ΔAICc < 2), and in heat-selected *Daphnia* (ΔAICc = 2.14, Table S1). The metabolic rate increased with temperature to 30 °C in a mainly linear way with the positive E_a values not differing between both selection groups (Figure 2f). This was also supported in the GLM by significant effects of Temp ($P < 0.001$) and a trend for Temp^2 ($P = 0.063$), but no interactions with selection group (Table S3).

The consumer thermal impact (CTI) of the control *Daphnia* was significantly lower than zero (zero not included in the 84% CI), while the CTI of the heat-selected *Daphnia* was not significantly different from zero (zero included in the 84% CI) (Figure 3). Nevertheless, the CTI values did not differ between both thermal selection groups (84% CIs overlapped).

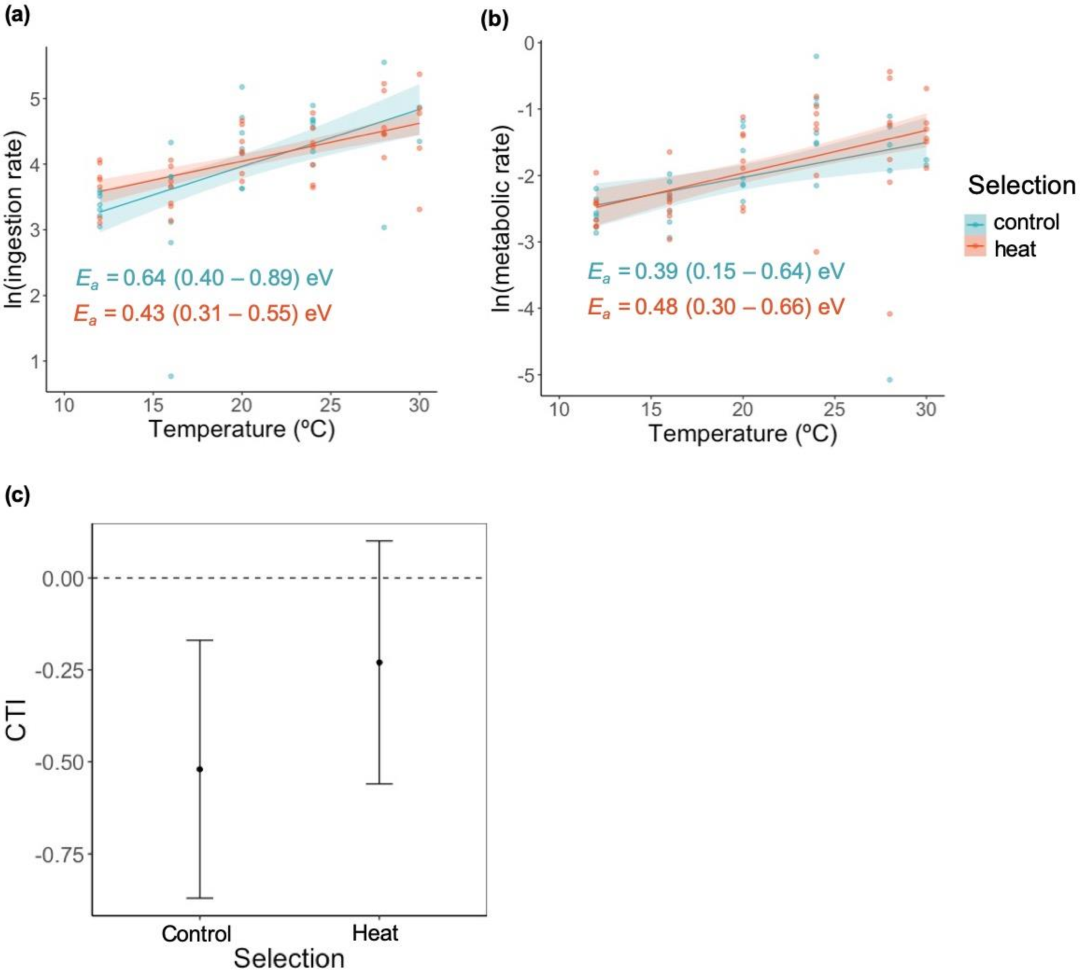


Figure 2. Thermal performance curves for (a) ingestion rate and (b) metabolic rate, and (c) the resulting patterns in the consumer thermal impact (CTI) for control and heat-selected *Daphnia*. For TPCs supported by the Arrhenius model we reported the activation energy, E_a (eV), each time with 84% CI. Colour areas represent 84% CI bands of the TPCs. For CTI, means are given with 84% CIs.

Discussion

As expected, we found several cases of rapid evolution of the TPCs for life history traits. We identified evolutionary shifts of quadratic TPCs for survival, fecundity at first clutch and intrinsic population growth rate toward higher optimum temperatures (T_{opt}) and less pronounced curvatures, yet without decreased performances at T_{opt}. In contrast, we found no evolution of the linear TPCs for somatic growth and development rates and for the traits related to energy gains and costs. This adds evidence to the rare studies on this topic in ectotherms that TPCs of different traits may strongly differ in evolutionary responses (Santos et al. 2006, Fragata et al. 2016).

After two years of thermal selection, the *Daphnia* from the outdoor mesocosms that were heated 4 °C above ambient temperatures evolved a higher thermal optimum for our fitness estimate, intrinsic population growth rate. This was driven by evolution of a higher T_{opt} of underlying life history traits that also showed a quadratic TPC: survival, and fecundity of the first clutch (Appendix S4). The shift of T_{opt} toward higher temperatures was greatest for intrinsic population growth rate (+3.38 °C), followed by survival (+ 2.49 °C), and fecundity of the first clutch (+1.15 °C). There was no perfect +4 °C shift in T_{opt}, matching the imposed warming scenario in the outdoor mesocosms. One reason may be that evolution near the optimum will go much slower and may take more than two years. The shifts of T_{opt} matches the pattern that the heat-selected *Daphnia* evolved a 3.6 °C higher heat tolerance (CT_{max} ~ 37 °C) compared to control *Daphnia* (CT_{max} ~ 33 °C) (Geerts et al. 2015). Note that these CT_{max} values were obtained by heating up the *Daphnia* at a high rate of 2.4 °C per min, hence reflect the upper temperatures that *Daphnia* can cope with during acute exposure for a couple of hours. As a result, these CT_{max} estimates result in much higher values than those that can be extrapolated from the quadratic TPCs of current study where *Daphnia* were chronically exposed to warming. It has even been demonstrated that acute and chronic thermal tolerance may trade off against each other across species (Rezende et al. 2014, Magozzi and Calosi 2015). Yet, this did not seem the case in current intraspecific study. The TPCs for intrinsic population growth rate, an important proxy for fitness, have been used to investigate the effects of climate warming on ectotherms (e.g., Deutsch et al. 2008, Tewksbury et al. 2008, Bernhardt et al. 2018), while other fitness-related traits were also studied in the context of thermal adaptation (e.g. somatic growth rate, Mitchell and Lampert 2001). Despite the general belief that it is crucial to consider rapid evolution when assessing the impact of climate change (Diamond and Martin 2016, Catullo et al. 2019), the thermal evolution of TPCs for intrinsic population growth rate has received little attention and only in the context of long-term evolution (e.g., at the among-species level: Frazier et al. 2006, across latitudes within species: Kontopoulos et al. 2020).

While a limited number of studies have demonstrated rapid thermal evolution of intrinsic population growth rate in multicellular ectotherms (Van Doorslaer et al. 2009b, Cavalheri et al. 2019), our study provided the first evidence of rapid evolution of its TPC (performance across a range of temperatures). Thermal evolution of the TPC of somatic growth, however, was not observed; nevertheless this TPC was previously found to be differentiated across latitudes (Mitchell and Lampert 2001). This may suggest that the somatic growth rate evolves less rapid than the intrinsic growth rate under warming.

Another key finding of our study was that thermal plasticity decreased in heat-selected *Daphnia* for traits with unimodal TPCs. Indeed, for survival, fecundity of the first clutch and intrinsic population growth rate both the quadratic Arrhenius model and the intercept null model had support in the heat-selected *Daphnia*. The decrease of curvature of unimodal TPCs indicates the *Daphnia* evolved a larger thermal tolerance range. In contrast with the idea of a trade-off (Huey and Hertz 1984, Sinclair et al. 2016), this was not accompanied by a decrease in performance at T_{opt} (based on the 84% CIs of TPCs), which is in line with a study by Nati et al. (2016). The here observed evolution of a less plastic response is beneficial as it resulted in a higher performance at temperatures around T_{opt} compared to the control *Daphnia*. A similar adaptive decrease in thermal plasticity beyond T_{opt} has also been found for photosynthesis in warm-adapted populations of a coral species (Gould et al. 2021).

While a set of life history traits (survival, intrinsic population growth rate, and fecundity of the first clutch) had a quadratic TPC, other life history traits (development rate and somatic growth rate) and the traits related to energy gain (ingestion rate) and costs (metabolic rate) had linear TPCs. This further illustrates that TPCs may strongly differ in shape among traits (David et al. 2005, Baker et al. 2016, Kellermann et al. 2019). Notably, while we observed thermal evolution of the TPCs of traits with a quadratic TPC, this was not observed for the traits with a linear TPC. This matches a central idea of the metabolic theory of ecology that activation energies (the slope of linear TPCs) are stable, as most of the variation in absolute biological rates being explained by variation in body mass (Gillooly et al. 2001, Allen and Gillooly 2007). Alternatively, the absence of evolution of the linear TPCs might be explained as a side-effect, whereby the here studied traits with linear TPCs may also be the traits showing low genetic variation and/or were constrained by genetic correlations, for example through life history trade-offs between traits (e.g. somatic growth vs. survival, Lancaster et al. 2017). The lack to detect evolution in resting metabolic rate may also be explained by thermal compensation (Seebacher et al. 2015). While the rapid evolution of intrinsic growth rate but not of resting metabolic rate may seem surprising, maximum metabolic rate may be more linked to intrinsic growth rate.

The absence of evolution in the slopes (E_a) of the linear TPCs for ingestion rate and metabolic rate also resulted in the absence of evolution of the difference between these two slopes, the so-called consumer thermal impact (CTI, Vasseur and McCann 2005). Nevertheless, there was some hint for an incipient differentiation as the CTI of control *Daphnia* was significantly lower than zero, while the one of heat-selected *Daphnia* was not, suggesting a tendency of increasing CTI, which would reflect an improved energetic warm-tolerance (Vasseur and McCann 2005, Iles 2014). The insignificant difference in CTI between control and heat-selected *Daphnia* suggests that the top-down impact of the *Daphnia* on its resource would remain stable, regardless of the rapid thermal evolution of the TPC for intrinsic population growth rate. To our knowledge, no other study has examined the long-term or rapid evolution of CTI.

Conclusion

Our insights add to the emerging insight that ectotherms may quickly adapt to climate warming not only through plasticity, but also through rapid evolution, and thereby highlighted several cases of rapid evolution of unimodal TPCs. Notably, we could demonstrate rapid evolution of intrinsic population growth rate, an important fitness estimate. In contrast, linear TPCs did not evolve, which is in line with the theoretically assumed constancy of activation energies (Gillooly et al. 2001). While rapid thermal evolution in consumers increased consumer fitness at higher temperatures, this was not accompanied by a change in the relative thermal scaling of ingestion rates and metabolic rates, suggesting this may not necessary strengthen the top-down impact of the consumer on its resource.

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Authors' contributions

RS developed the ideas, and together with YJW designed the experiments. LDM provided the *Daphnia* clones. YJW performed the experiments and analyzed the data with input from RS and NT. YJW and RS drafted the manuscript, after which all authors contributed and gave final approval for publication.

Appendix S1. Results of AICc model selection

Table S1. Results of AICc model selection for models testing relationships between temperature and response variables separately for the control and the heat-selected *Daphnia*. K is the number of model parameters. ΔAICc = AICc of the model - AICc model with lowest AICc . AICc weight is the probability that the model provides the best fit to the data.

Variable	Model	K	AICc	ΔAICc	ΔAICc weight	Log-likelihood
Survival until maturity						
Control	Intercept	2	204.32	16.28	0.00	0.00
	Arrhenius	3	197.92	9.87	0.01	0.01
	Arrhenius-quadratic	4	188.03	0.00	1.00	0.99
Heat-selected	Intercept	2	154.23	0.00	1.00	0.53
	Arrhenius	3	156.55	2.32	0.31	0.17
	Arrhenius-quadratic	4	155.28	1.05	0.59	0.31
Development rate						
Control	Intercept	2	22.82	53.61	0.00	0.00
	Arrhenius	3	-30.55	0.23	0.89	0.47
	Arrhenius-quadratic	4	-30.78	0.00	1.00	0.53
Heat-selected	Intercept	2	38.97	63.46	0.00	0.00
	Arrhenius	3	-24.49	0.00	1.00	0.56
	Arrhenius-quadratic	4	-24.04	0.44	0.80	0.45
Somatic growth rate						
Control	Intercept	2	65.17	4.66	0.10	0.06
	Arrhenius	3	60.51	0.00	1.00	0.64
	Arrhenius-quadratic	4	62.02	1.51	0.47	0.30
Heat-selected	Intercept	2	43.55	0.49	0.78	0.30

	Arrhenius	3	43.06	0.00	1.00	0.39
	Arrhenius-quadratic	4	43.52	0.46	0.80	0.31
Intrinsic population growth rate						
Control	Intercept	2	189.58	13.56	0.00	0.00
	Arrhenius	3	185.76	9.74	0.01	0.01
	Arrhenius-quadratic	4	176.03	0.00	1.00	0.99
Heat-selected	Intercept	2	141.65	1.49	0.48	0.27
	Arrhenius	3	142.59	2.42	0.30	0.17
	Arrhenius-quadratic	4	140.17	0.00	1.00	0.56
Ingestion rate						
Control	Intercept	2	89.05	10.29	0.01	0.00
	Arrhenius	3	78.75	0.00	1.00	0.76
	Arrhenius-quadratic	4	81.05	2.30	0.32	0.24
Heat-selected	Intercept	2	70.82	17.64	0.00	0.00
	Arrhenius	3	53.19	0.00	1.00	0.78
	Arrhenius-quadratic	4	55.67	2.48	0.29	0.22
Metabolic rate						
Metabolic rate$_c$	Intercept	2	90.17	3.74	0.15	0.08
	Arrhenius	3	87.22	0.80	0.67	0.37
	Arrhenius-quadratic	4	86.43	0.00	1.00	0.55
Metabolic rate$_h$	Intercept	2	100.37	10.75	0.01	0.00
	Arrhenius	3	89.62	0.00	1.00	0.74
	Arrhenius-quadratic	4	91.76	2.14	0.34	0.26

Appendix S2. Parameter estimates from selected models describing relationships between temperature and response variables

Table S2. Parameter estimates from selected models describing relationships between temperature and response variables separately for the control and heat-selected *Daphnia*. *P*-values less than 0.05 indicate that the value for that parameter differs from zero and affects the response variable. c is the model intercept. b is the activation energy E_a from the Arrhenius equation measured in electron volts (eV). q is the quadratic term of the Arrhenius-quadratic equation.

Response	Model	ln (c) ±(SE)	*P*	b*(eV) ±(SE)	*P*	q*(eV^2) ±(SE)	*P*	R^2
Survival until maturity								
control	Arrhenius-quadratic	-1.54 (0.33)	<0.01	-7.34 (2.11)	<0.01	-7.71 (2.11)	<0.01	0.36
heat-selected	Arrhenius-quadratic	-0.51 (0.22)	0.03	0.12 (1.43)	0.93	-2.72 (1.43)	0.07	0.04
Development rate								
control	Arrhenius	12.04 (1.24)	<0.01	0.36 (0.03)	<0.01			0.79
heat-selected	Arrhenius	13.33 (1.23)	<0.01	0.39 (0.03)	<0.01			0.79
Somatic growth rate								
control	Arrhenius	-16.29 (4.57)	<0.01	-0.31 (0.12)	0.01			0.16
heat-selected	Arrhenius	-8.54 (2.81)	<0.01	-0.12 (0.07)	0.10			0.04
Intrinsic population growth rate								
control	Arrhenius-quadratic	-2.41 (0.28)	<0.01	-5.25 (1.83)	<0.01	-6.63 (1.83)	<0.01	0.32
heat-selected	Arrhenius-quadratic	-1.52 (0.18)	<0.01	1.45 (1.20)	0.23	-2.62 (1.20)	0.03	0.09
Ingestion rate								
control	Arrhenius	29.50	<0.01	0.64	<0.01			0.30

		(6.70)		(0.17)		
heat-selected	Arrhenius	21.06 (3.41)	<0.01	0.43 (0.09)	<0.01	0.38
Metabolic rate						
control	Arrhenius	13.57 (6.69)	0.05	0.39 (0.17)	0.03	0.12
heat-selected	Arrhenius	17.12 (4.96)	<0.01	0.48 (0.13)	<0.01	0.25

Appendix S3. Results of the complementary GLMs testing for the effect of temperature and thermal selection group on *Daphnia* performance

Table S3. Effects of temperature (Temp, Temp^2) and thermal selection (Select) treatment on the response variables. Note that to avoid correlation between the linear and quadratic coefficients, we used orthogonal polynomial regression in R.

Variable	Factor	Estimate	SE	*t*	d.f.	*P*
Survival until maturity	Temp	-5.235	1.800	-2.908	78	**0.005**
	Temp^2	-7.354	1.800	-4.085	78	**0.001**
	Select	-0.515	0.196	-2.621	78	**0.011**
	Temp × Select	-5.338	1.800	-2.965	78	**0.004**
	Temp^2× Select	-3.504	1.800	-1.947	78	0.055
Development rate	Temp	2.666	0.161	16.530	70	<**0.0001**
	Temp^2	-0.387	0.160	-2.414	70	**0.018**
	Select	-0.005	0.018	-0.267	70	0.790
	Temp × Select	-0.165	0.161	-0.025	70	0.309
	Temp^2× Select	-0.014	0.160	-0.088	70	0.930
Somatic growth rate	Temp	-1.596	0.474	-3.368	70	<**0.001**
	Temp^2	-0.714	0.471	-1.517	70	0.134
	Select	-0.049	0.054	-0.920	70	0.361
	Temp × Select	-0.804	0.474	-1.698	70	0.094
	Temp^2× Select	-0.035	0.471	-0.074	70	0.941
Intrinsic population growth rate	Temp	-2.799	1.543	-1.815	78	0.073
	Temp^2	-6.564	1.543	-4.255	78	<**0.001**
	Select	-0.443	0.017	-2.631	78	**0.010**
	Temp × Select	-4.786	1.543	-3.103	78	**0.003**
	Temp^2× Select	-2.803	1.543	-1.817	78	0.073
Ingestion rate	Temp	3.624	0.630	5.753	66	<**0.001**
	Temp^2	-0.376	0.623	-0.603	66	0.548
	Select	-0.040	0.073	-0.543	66	0.589
	Temp × Select	0.674	0.630	1.070	66	0.289
	Temp^2× Select	-0.368	0.623	-0.591	66	0.557
Metabolic rate	Temp	2.968	0.743	3.994	66	<**0.001**

	Temp^2	-1.397	0.738	-1.892	66	0.063
	Select	-0.054	0.084	-0.648	66	0.519
	Temp × Select	-0.544	0.743	-0.731	66	0.467
	Temp^2× Select	-0.810	0.738	-1.097	66	0.276

Appendix S4. Estimation of the coefficients of the universal temperature dependence (UTD) model

To estimate the consumer thermal impact (CTI) for both control and heat-selected *D. magna*, we fitted the observations (y) of ingestion rate and metabolic rate to the universal temperature dependent (UTD) model. The UTD model is in the linear least square regression form $\ln(y) = b \ln(M) - E(1/kT) + \ln(i)$, where *M* is body mass (g), *T* is water temperature (K), and *k* is the Boltzmann's constant (8.62×10^{-5} eV K^{-1}). We obtained the robust regression coefficients (i.e. allometric exponent *b*, the activation energy *E*, and the normalization constant *i*) of the linear least square regression models by bootstrapping with 1000 replicates. We estimated 84% CIs for each coefficient.

Table S4. Robust linear regressions coefficients and statistics for the UTD models of the effect of body mass and temperature on metabolic and ingestion rates of control and heat-selected *D. magna*. Given are bootstrapped coefficients with the 84% CIs of the allometric exponent *b*, the activation energy *E* (eV), and the normalization constant *i*. The model statistics include the model *P* values ($\alpha < 0.05$ in bold), and the sample size *n*. A *P*-value < 0.05 indicates the linear regression model provides a better fit to the data than a model that contains no independent variable (i.e. an intercept model). Asterisks (*) and dots (·) denote significant ($P < 0.05$) and marginally significant ($p < 0.1$) effects.

Variable	Selection group	*b*	*E* (eV)	ln(*i*)	*P*	*n*
Ingestion rate	Control	0.35 (0.06 – 0.83)	0.27 (0.09 – 0.44)	7.78 (-0.35 – 16.62)	0.543	33
	Heat-selected	0.37 (-0.02 – 0.74)	0.15 (-0.08 – 0.33)	3.63 (-8.48 –13.46)	0.305	39
Metabolic rate	Control	1.02 (0.71 – 1.29)*	0.59 (0.38 – 0.85)*	20.07 (10.05 – 32.66)*	**0.0002**	33
	Heat-selected	0.85 (0.30 – 1.36)*	0.41 (0.13 – 0.65)·	11.64 (-3.47 – 24.85)	0.1211	39

Appendix S5. Thermal performance curves of age and fecundity at first and second clutches

To understand the mechanisms behind the differential thermal performance curves of intrinsic population growth rate of control and heat-selected *Daphnia*, we examined for each selection group the best fitted thermal dependence models among the three candidate models (intercept model, Arrhenius model, Arrhenius-quadratic model) for ln-transformed age and fecundity (offspring produced) at 1st and 2nd clutches. The activation energy, E_a (eV), was estimated for the Arrhenius model, while the optimum temperature, T_{opt} (°C), was estimated for the Arrhenius-quadratic model.We also analyzed effects of rearing temperature, temperature^2 and thermal selection group (control and heat-selected) on these variables using general linear models (GLMs). For the clones which died before maturation or survived but failed to produce juveniles, we estimated the fecundity as zero. To meet model assumption, 1 (> 1.5 interquartile above the upper or below the lower quartile) out of 65 observations for age at 1st and 2nd clutches, and 4 our of 78 observations for fecundity at 1st and 2nd clutches were removed.

For age at 1st clutch, the Arrhenius model provided similar support compared to the Arrhenius-quadratic model in each selection group (ΔAICc < 2). Age at 1st clutch decreased with temperature to 30 °C in a mainly linear way in both control and heat-selected *Daphnia*. The negative Ea values did not differ between both selection groups (Figure S1a). This was also supported in the complementary GLM by significant effects of temperature (t = -2.197, df = 65, P < 0.001) and temperature^2 (t = 0.035, df = 65, P = 0.035), but no interactions with selection group (all P > 0.20).

For age at 2nd clutch, the Arrhenius-quadratic model provided the best fit in control *Daphnia* (ΔAICc = 46.06 and 9.01), while it provided similar support compared to the Arrhenius model in heat-selected *Daphnia* (ΔAICc < 2). The age at 2nd clutch decreased with temperature to 27.63 °C (84% CI = 27.54 – 27.72) then levelled off in control *Daphnia*, while it continuously decreased with temperature to 30 °C in a nearly linear way in heat-selected *Daphnia* (Figure S1b). Based on 84% CIs of the TPCs, we found a lower age at 2nd clutch in heat-selected *Daphnia* compared to control *Daphnia* at the highest temperature. This was supported by the GLM that showed a significant temperature × selection group interaction (t = 2.22, df = 65, P = 0.03).

For fecundity at 1st clutch, the Arrhenius-quadratic model provided the best fit in the control *Daphnia* (ΔAICc > 7.4), while it had similar support compared to the Arrhenius model in the heat-selected *Daphnia* (ΔAICc < 2). The fecundity at 1st clutch increased with temperature to 16.94 °C (84% CI = 16.90 – 16.98) then declined strongly in control *Daphnia*, while fecundity at 1st clutch increased with temperature to 18.09 °C (84% CI = 17.99 – 18.19) then slightly declined in heat-selected *Daphnia* (Figure S1c). The T_{opt} of fecundity at 1st clutch was significantly higher in heat-selected *Daphnia* compared to control *Daphnia*, based on the non-overlapping 84% CIs. This was supported by the GLM that showed both a significant temperature × selection group interaction ($t = -3.260$, df = 74, $P = 0.002$) and a trend for a temperature² × selection group interaction ($t = -1.673$, df = 74, $P = 0.09$).

For fecundity at 2nd clutch, the Arrhenius-quadratic model provided the best fit in control *Daphnia* (ΔAICc > 7.3), while it provided similar support compared to the Arrhenius model in heat-selected *Daphnia* (ΔAICc < 2). The fecundity at 2nd clutch slightly increased with temperature to 16.87 °C (84% CI = 16.83 – 16.91) then strongly declined in control *Daphnia*, while it slightly decreased with temperature in heat-selected *Daphnia* (Figure S1d). This difference in TPC between both selection groups was supported by the GLM that showed significant temperature × selection group ($t = -3.050$, df = 74, $P = 0.003$) and temperature^2 × selection group interactions ($t = -2.147$, df = 74, $P = 0.035$).

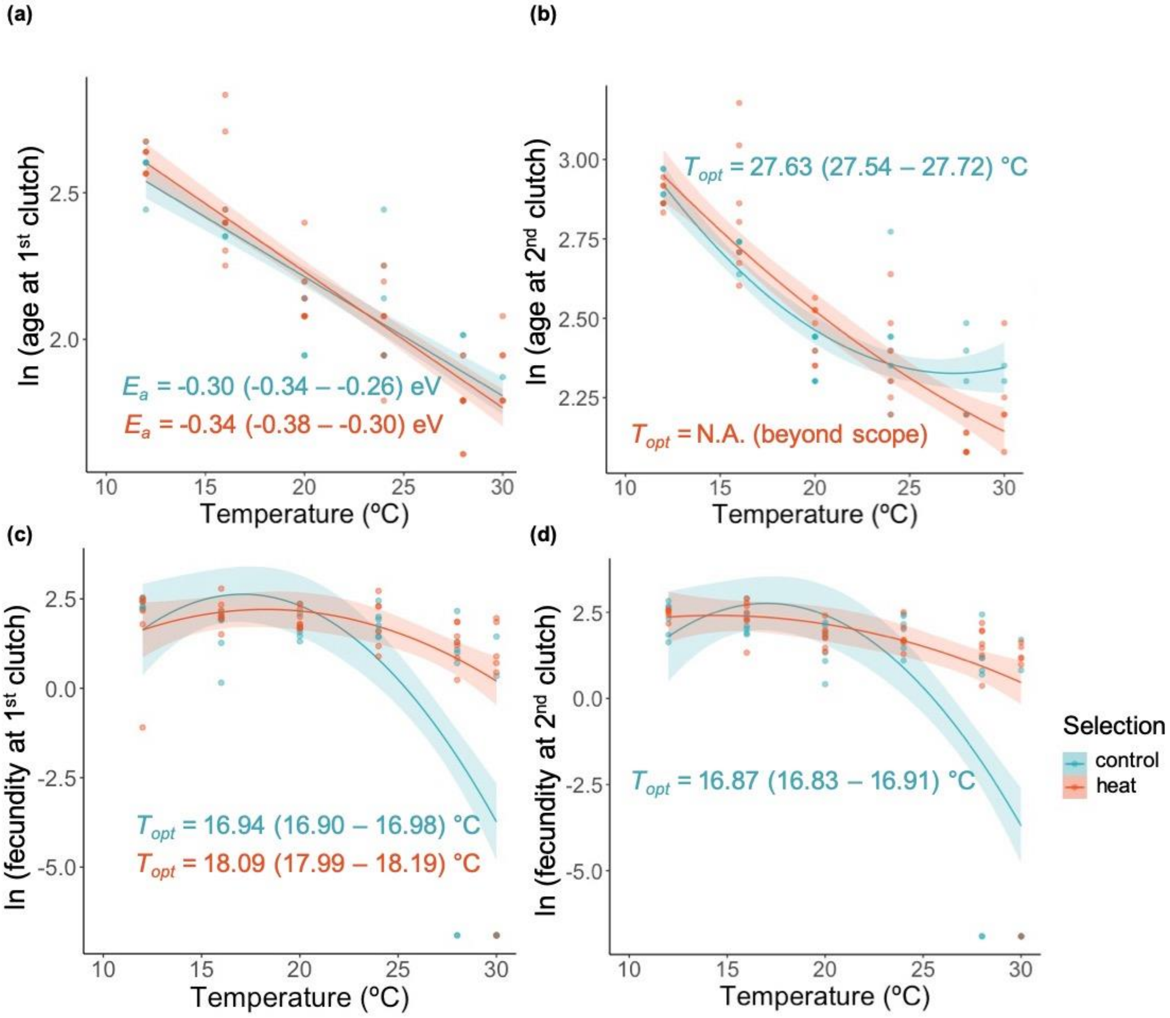

Figure S1. Thermal performance curves of control (blue) and heat-selected (red) *Daphnia magna*. (a) Age at 1st clutch; (b) age at 2nd clutch; (c) fecundity at 1st clutch; (d) fecundity at 2nd clutch. For TPCs supported by the Arrhenius model we reported the activation energy, E_a (eV), each time with 84% CI. Colour bands represent 84% CI bands of the TPCs.

Chapter II

Support for the climatic variability hypothesis depends on the type of thermal plasticity: lessons from predation rates

Ying-Jie Wang, Robby Stoks, Arnaud Sentis and Nedim Tüzün

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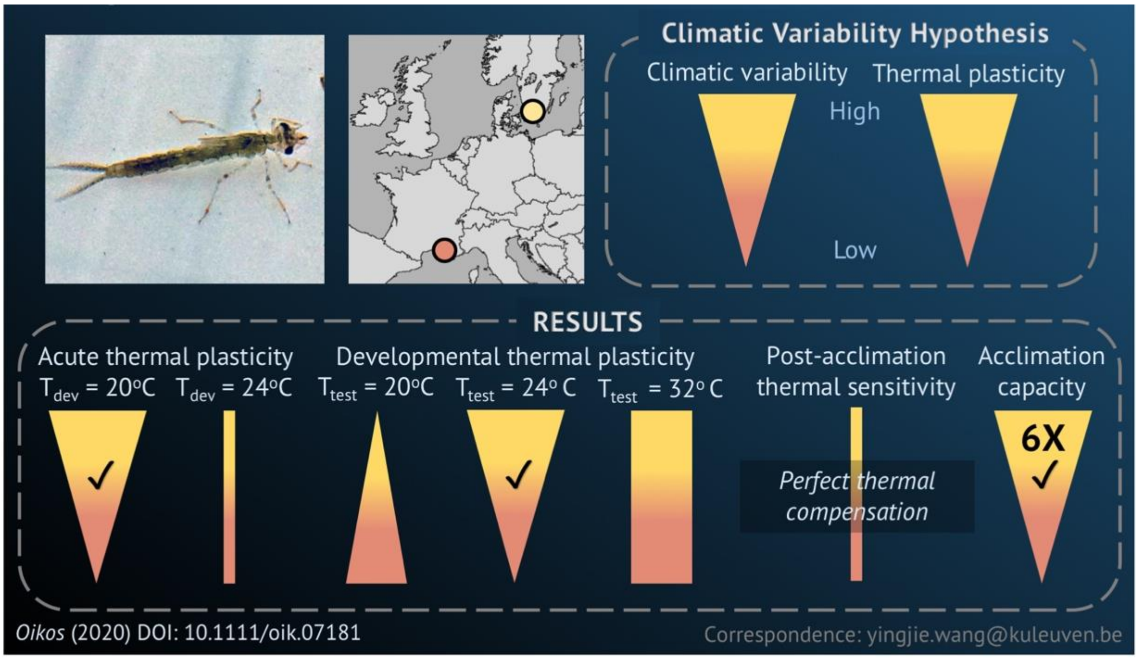


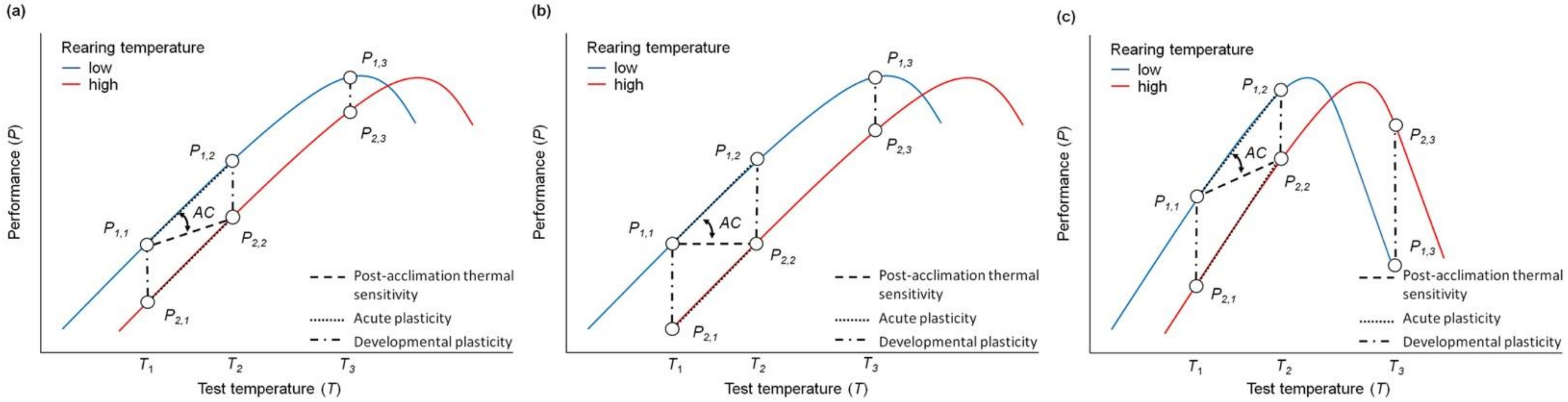
Abstract

Plastic and evolutionary changes in traits related to biotic interactions are crucial for the local persistence of populations under global warming. Yet, how acute and developmental thermal plasticity evolve and shape predation rates has been poorly studied, especially in the context of latitude-driven thermal evolution. A powerful predictive framework is given by the climatic variability hypothesis (CVH) stating that thermal plasticity and acclimation capacity evolve to be higher in high-latitude populations because these are exposed to higher thermal seasonal variability. We tested the CVH for predation rates and evaluated if the support for the CVH depended on the type of plasticity and acclimation metric. We examined effects of developmental temperature (20 and 24 °C) and acute changes in mean and extreme temperatures (20, 24 and 32 °C) on the predation rates of high- and low-latitude populations of a predatory aquatic insect, the damselfly *Ischnura elegans*. We documented opposing and interactive effects between developmental and acute temperatures, which urges caution when using thermal performance curves to forecast the impact of global warming on biotic interactions. Predation rates were higher in low-latitude than high-latitude predators, especially at the warmer developmental and test temperatures, suggesting thermal adaptation to the higher low-latitude temperatures. The latitudinal patterns in acute and developmental plasticities differed, providing mixed support for the CVH. Moreover, there was no latitudinal pattern in post-acclimation thermal sensitivity, indicative of perfect thermal compensation in predators from both latitudes. Strikingly, the acclimation capacity leading to perfect thermal compensation was ~6 times higher in high-latitude than in low-latitude predators. Our study provides new insights into the climatic variability hypothesis (CVH) by documenting that its support is critically dependent on the type of plasticity and acclimation metric used.

Introduction

Plastic and evolutionary changes in traits related to biotic interactions such as predation are crucial for the local persistence of populations under global warming (Urban et al. 2016). Important in this context is that thermal plasticity may evolve and vary within species across latitudinal gradients (Liefting et al. 2009, Molina-Montenegro and Naya 2012, Verheyen and Stoks 2019). Such intraspecific variation in thermal plasticity may critically shape the predictions of future distributions of species under global warming (Valladares et al. 2014). A powerful framework thereby is the climatic variability hypothesis (CVH), predicting thermal plasticity and acclimation capacity to increase with seasonal thermal heterogeneity of the environment, and thus to be higher at high-latitudes where the climate is more variable across the year than at low latitudes (Janzen 1967, Addo-Bediako et al. 2000, Ghalambor et al. 2006, Aguilar-Kirigin and Naya 2013; for a reversed latitudinal pattern of seasonality see Naya et al. 2011). Studies testing for the CVH provided mixed support (Liefting et al. 2009, Naya et al. 2011, Van Heerwaarden et al. 2014, Gunderson and Stillman 2015, Barria and Bacigalupe 2017). Despite its importance, surprisingly few studies tested for effects of thermal adaptation, thermal plasticity and thermal acclimation on predation rates (but see De Block et al. 2013, Sentis et al. 2015) and none did so across latitudes to test whether thermal patterns in predation rates follow the CVH. Such studies should also include extreme temperatures as these may differentially affect predation rates compared to mild increases in mean temperature (Englund et al. 2011, Gillespie et al. 2012, Sentis et al. 2013a, Dell et al. 2014).

Organisms can respond to temperature change with short- term (typically within hours) acute and/or longer-term (typically across many days to weeks) developmental plasticities (Gause 1942, Sgrò et al. 2016). These different plasticity types provide different information on how organisms can adapt to global warming (Stillman 2003, Calosi et al. 2008). Moreover, they may show different latitudinal patterns and thereby potentially underlie mixed support for the CVH across studies. How different types of thermal plasticity and thermal acclimation shape predation rates has been poorly studied (but see Sentis et al. 2015; see also Buxton et al. 1981 for a study on filtration rate), and never in the context of latitudinal differences. While there are numerous ways to quantify phenotypic plasticity (reviewed by Valladares et al. 2006), studies testing for the CVH typically estimate plasticity as the difference between performances at different temperatures (Liefting et al. 2009, Naya et al. 2011, Pereira et al. 2017, Barria et al. 2018, Kellermann and Sgrò 2018). Acute thermal plasticity is investigated by rearing organisms at a fixed developmental temperature and then testing them at a set of test temperatures (Van Heerwaarden et al. 2014, Frances and McCauley 2018). Acute thermal plasticity can then be estimated as the difference in performance between test temperatures (e.g. $P_{1,2}–P_{1,1}$, Figure. 1, dotted line). Acute plasticity can be used to evaluate and predict responses to short-term temperature variations and extremes (Kingsolver and Woods 2016). In contrast, developmental thermal plasticity is investigated by rearing organisms at two (or more) temperatures (T1 and T2) and testing them at a single temperature (Donelson et al. 2011, Sentis et al. 2015). Developmental plasticity can then be estimated as the difference in performance between both rearing temperatures (e.g. $P_{2,1}–P_{1,1}$, Figure. 1, stripped-dotted line). Developmental plasticity describes organismal responses as a function of their developmental temperature, and is relevant when predicting climate change impact considering both average thermal differences during development and short-term thermal variations or extremes. For example, it captures the differential effect of a heat wave between animals reared at a low versus a high temperature (Figure. 1d, contrast 5).



(d)

Thermal plasticity type	Developmental Temperature	Test Temperature		Developmental Temperature	Test Temperature	estimate	Contrast
Acute plasticity	low	T_1	vs.	low	T_2	$P_{1,2}$-$P_{1,1}$	1
	high	T_1	vs.	high	T_2	$P_{2,2}$-$P_{2,1}$	2
Developmental plasticity	low	T_1	vs.	high	T_1	$P_{2,1}$-$P_{1,1}$	3
	low	T_2	vs.	high	T_2	$P_{2,2}$-$P_{1,2}$	4
	low	T_3 (extreme)	vs.	high	T_3 (extreme)	$P_{2,3}$-$P_{1,3}$	5
Post-acclimation thermal sensitivity	low	T_1	vs.	high	T_2	$P_{2,2}$-$P_{1,1}$	6

Figure 1. Schematic presentation of the different thermal plasticity types (modified after Seebacher et al. 2015). In each plot two thermal performance curves (TPCs) are shown; one matching a low rearing temperature (T_1, blue) and one matching a high rearing temperature (T_2, red) that is moved horizontally toward higher temperatures. Horizontal shifts of TPCs are a common pattern of thermal acclimation to warmer temperatures. Although other patterns such as vertical shifts are also reported, we decided to represent only horizontal patterns for clarity. Furthermore, the identical shapes (hence also slopes) of the horizontally shifted TPCs are for illustrative purposes. The performance for an organism reared at developmental temperature i and tested at test temperature j is denoted as $P_{i,j}$. (a) Scenario with imperfect compensation so that after the long-term thermal exposure period, the post-acclimation thermal sensitivity will present, meaning that $P_{2,2} \neq P_{1,1}$. (b) Scenario with perfect compensation resulting in no post-acclimation thermal sensitivity. (c) Scenario with increased acute plasticity (steeper slope of the TPCs) resulting in a higher post-acclimation thermal sensitivity compared to (a). For each scenario we also indicated the associated acclimation capacity (AC) sensu Einum et al. (2019). AC was estimated as the absolute value of the difference between the post-acclimation thermal sensitivity and the mean acute plasticity across both developmental temperatures, both expressed as slope coefficients (Einum et al. 2019). (d) Overview of the different contrasts to statistically test for the plasticity types. Acute plasticity (dotted line, $P_{1,2} – P_{1,1}$ and $P_{2,2} – P_{2,1}$) is the difference in performance of animals reared at the same temperature (low or high) and tested at T_2 versus T_1. Developmental plasticity (stripped-dotted line, $P_{2,1} – P_{1,1}$, $P_{2,2} – P_{1,2}$, and $P_{2,3} – P_{1,3}$) is the difference in performance of animals reared at the high temperature versus the low temperature when tested at the same temperature (T_1, T_2, or T_3). Post-acclimation thermal sensitivity (stripped line, $P_{2,2} – P_{1,1}$) is indicated by the difference in performance of animals reared and tested at T_2 (high temperature) versus animals reared and tested at T_1 (low temperature).

Also relevant for predictions on the effects of global warming is the outcome of developmental plasticity: how sensitive organisms are to warming after thermal acclimation. This outcome has been called ‘post-acclimation thermal sensitivity’ and viewed as the capacity for acclimation (Seebacher et al. 2015). The post-acclimation thermal sensitivity can be estimated as the difference of performances between organisms reared and tested at temperature 1, and organisms reared and tested at temperature 2 (e.g. $P_{2,2}$ and $P_{1,1}$, Figure. 1). If thermal compensation is perfect, there will be no post-acclimation thermal sensitivity (Seebacher et al.

2015; Figure. 1, stripped line). It was recently pointed out that the level of acclimation needed for perfect compensation may depend on the magnitude of the acute plasticity (Einum et al. 2019). Therefore, ‘acclimation capacity’ was quantified as the absolute value of the difference between the post-acclimation thermal sensitivity and the mean of the acute plasticities across developmental temperatures (Figure. 1). The emerging view is that different plasticity types and acclimation metrics may show different patterns (Donelson et al. 2011, Schulte et al. 2011, Donelson and Munday 2012, Einum et al. 2019) and that the plasticity types may even interact (Fangue et al. 2009, Schulte et al. 2011, Grigaltchik et al. 2012, Sentis et al. 2015, Beaman et al. 2016; Figure. 1). Nevertheless, whether different plasticity types (acute and developmental) and the two acclimation metrics (post-acclimation thermal sensitivity and acclimation capacity) show consistent latitudinal patterns, hence consistently support the CVH remains poorly tested.

In this study, we examined the effects of developmental temperature and acute changes in mean and extreme temperatures on the predation rates of high- and low-latitude populations of a predatory aquatic insect, the damselfly *Ischnura elegans*. To address for the first time whether support for the CVH is dependent on the plasticity type and acclimation metric, we compared acute and developmental plasticities, and the resulting post-acclimation thermal sensitivity and thermal acclimation capacity between both latitudes. We have shown before that when *I. elegans* larvae were reared and tested at 20 °C and at 24 °C, predation rates were higher in low-latitude than in high-latitude larvae, and higher at the higher developmental temperature in both low- and high- latitude larvae (Stoks et al. 2012, De Block et al. 2013). To answer our current new and general research questions on the dependence of the CVH on the plasticity type, we here extend these studies by crossing developmental temperatures with acute (test) temperatures. We studied predation rates on the water flea *Daphnia magna*. This makes a well-studied text book example of a predator–prey module in pond food webs (Begon et al. 2006). To improve standardization across treatments and ease the interpretation of the predator thermal plasticities in driving the outcome of predation, we used a single clone of *D. magna* from an intermediate latitude and developmental temperature for all predation trials. We predicted predation rates to increase with test temperatures as well as with developmental temperatures as a consequence of increased energetic demands associated with higher metabolic rates (Brown et al. 2004). Based on Seebacher et al. (2015), and under the assumption of an acclimation-induced horizontal shift toward higher temperatures of the thermal performance curve (TPC, Figure. 1b), we predicted post-acclimation sensitivity to be weaker than acute and developmental plasticities (but see Einum et al. 2019). Finally, under the CVH (Janzen 1967, Addo-Bediako et al. 2000, Ghalambor et al. 2006, Naya et al. 2011), we predicted thermal plasticity and acclimation capacity to be consistently larger in high-latitude than in low-latitude populations. This is because the climatic variability, quantified as the difference between annual absolute maximum and the absolute minimum temperatures, is larger at the here studied high-latitude regions (55–57°N) compared to the low-latitude regions (43°N) (based on Figure. 4 in Addo-Bediako et al. 2000).

Materials and Methods

Damselfly populations and rearing

Three randomly chosen *Ischnura elegans* populations were sampled both at low-latitude (southern) and high-latitude (northern) sites. To assess consistency of patterns within a given latitude, we sampled three populations at each latitude. The three low-latitude populations were collected in France: Saint-Martin de Crau (43°37′57.8″N, 04°46′55.1″E), Bassin de Réaltor (43°28′11.1″N, 05°19′44.1″E) and La Durance (43°43′52.5″N, 05°44′53.0″E). The three high-latitude populations were collected in Denmark: Roskilde, (55°39′09.8″N, 12°08′01.7″E), and in Sweden: Hovgardsdammarna (57°14′24.3″N, 12°08′28.2″E) and Kalmar Dämme (56°40′04.6″N, 16°17′46.5″E). The water flea *Daphnia magna* co-occurs with *I. elegans* in both regions. In each population, ca 15 mated females were captured and kept individually in plastic vials to oviposit in wet filter paper. Egg clutches were transported to the laboratory and kept separately in plastic containers filled with aged tap water in incubators at 22 (± 0.2) °C and a 14:10 L:D photoperiod. Containers were daily checked for newly hatched larvae. Larvae were first kept in group per female to maximize survival (De Block and Stoks 2003). After 10 days, the larvae were placed individually in 100ml plastic cups filled with aged tap water and allocated to incubators at one of the temperature treatments. Throughout the rearing, larvae were fed daily ad libitum with *Artemia salina* nauplii.

Daphnia rearing

For the *D. magna* prey, a single clone was collected from the shallow lake ‘Oude Meren’ in Leuven, Belgium (50°51′49.0″N–4°43′24.4″E) that hosts a large population of *I. elegans* damselflies. The clone was kept in the laboratory for multiple generations before being used in the predation trials. Daphnia were cultured in 1 l glass vials in a water bath system at 22 (± 0.2) °C and a 14:10 L:D photoperiod. For logistic constraints, we did not rear the *Daphnia* at the two developmental temperatures of the damselfly larvae (as in Sentis et al. 2015). This precluded thermal acclimation of the prey, yet allowed for a more straightforward interpretation of the developmental plasticity in predation rates. We regularly renewed the medium, and daily added *Acutodesmus obliquus* green algae (1×10^5 cells ml^{-1}) as food for the *Daphnia*.

To obtain enough *Daphnia* for the predation trials, we transferred sets of 15 juveniles from the source culture to 1-l glass vials to start the grandmaternal lines. Next, sets of 15 juveniles (< 24 h-old) of the grandmaternal lines were transferred to 1-l glass vials to start the maternal lines. Only juveniles of the 2nd up to the 5th brood were used before a new maternal culture was started. Based on the number of individuals needed for the predation trials, we combined juveniles (< 24 h-old) from different maternal lines to assemble sets of 65 juveniles in 1-l glass vials as juveniles for the experiment. When the juveniles for the experiment were four days old, we assembled sets of 90 juveniles for the predation trials.

Experimental design

To examine the effects of predator latitude, predator developmental temperature and test temperature on predation rates, we conducted a full factorial laboratory experiment. Damselfly larvae of each latitude were kept at one of the two developmental temperatures (20 °C or 24 °C) and assayed at one of the three test temperatures (20, 24 or 32 °C). Temperature loggers showed that the experimental temperatures were within 0.2 °C from their target means. Throughout the experiment, larvae were kept at a 14:10 L:D photoperiod, matching the day length experienced by final instar I. elegans larvae in May in the natural populations. Damselfly larvae were kept at the developmental temperature from day 10 after hatching until 7 days after their molt into the final (F0) instar (> 8 weeks). This allowed time for thermal acclimation and possible thermal compensation. By scoring the predation rates of all larvae at the same day after molting into the final instar, we standardized the ‘physiological/developmental’ age across latitudes. The developmental temperatures correspond to the mean summer water temperatures of the shallow lakes inhabited by the species at the high-latitude (20 °C) and low- latitude (24 °C) sites (De Block et al. 2013, Dinh Van et al. 2014, Debecker and Stoks 2019). The 4 °C temperature dif- ference also represents the predicted temperature increase at the high-latitude sites by 2100 according to IPCC (2014) scenario RCP8.5. The test temperature of 32 °C mimics an extreme temperature that larvae experience yearly at the low-latitude sites (Arambourou and Stoks 2015). While this extreme temperature is not currently encountered at the high- latitude sites (Arambourou and Stoks 2015), this is expected under further warming (Nikulin et al. 2011).

We quantified the predation rates under each of the six temperature treatment combinations (Figure. 2). Each combination of latitude and temperature treatment was replicated 8–12 times, resulting in a total of 137 predation trials. For each latitude, we distributed the trials per temperature treatment as equally as possible over the three predator populations.

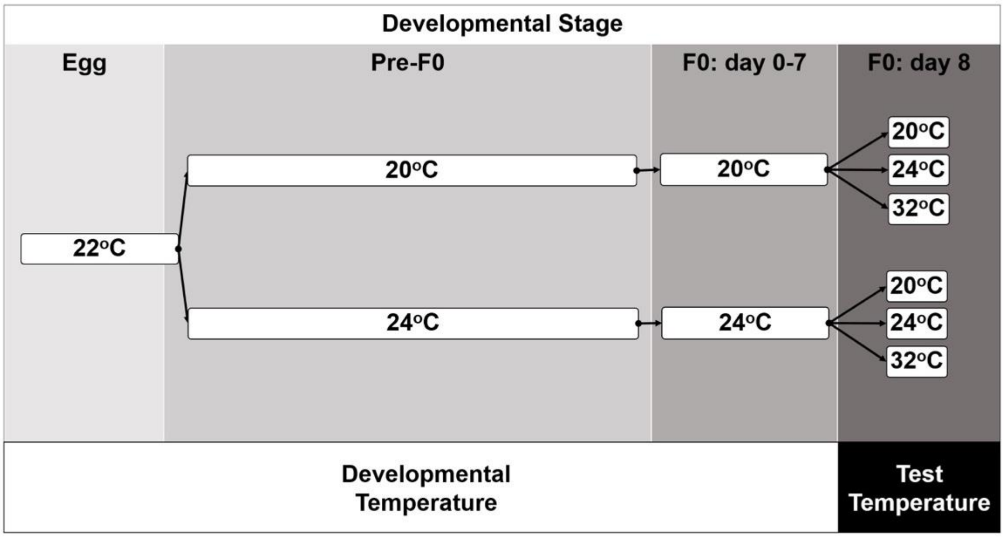


Figure 2. The experimental design showing the developmental and test temperature treatments imposed on the *Ischnura elegans* larvae. F0 is the final larval instar.

Before each trial, damselflies were gently blotted dry with tissue paper and weighed to the nearest 0.01mg, and both predators and preys were placed at the test temperature for 2h (based on Thompson 1978, Sentis et al. 2017). The trials were run in white 2-l (180 × 133 × 126 mm) arenas filled with 1 l aged tap water at the test temperature. At the start of each trial, one damselfly larva was released at the bottom of the arena together with 90 four-day-old *Daphnia* juveniles. All predation trials started between 11:00 and 13:00 and lasted 24 h. The relatively high initial number of *Daphnia* was chosen to avoid the full depletion of prey during the predation trials. Indeed, during preliminary experiments damselfly larvae never consumed more than 70 *Daphnia* per trial.

At the end of each trial, the damselfly larva was removed from the arena, and the remaining *Daphnia* individuals were counted to calculate predation rates. Natural mortality of *Daphnia* was assessed in eight replicates per temperature combination in the absence of damselfly predators; it was 0.65 ± 0.33% (Mean ± SE) and thus negligible. Predation rate was expressed as the number of *Daphnia* eaten per 24 h per mg damselfly wet mass.

Statistical Analyses

All statistical analyses were computed in R ver. 3.5.0 (<www.r- project.org>) using the function *lmer* in the package ‘*lme4*’ (Bates et al. 2015), and the function contrast in the package ‘*lsmeans*’ (Lenth 2016). To minimize the potential influence of nonlinear thermal performance curves between 20 °C and 24 °C, we consistently used ln-transformed values. We tested the main effects of predator source latitude, predator developmental temperature and test temperature, and all their interactions on predation rates using a linear mixed model (LMM). Damselfly population nested in latitude, and its interactions with developmental temperature and test temperature were included as random factors. None of these random factors reached significance (all $p \geq 0.10$), indicating consistent thermal response patterns between populations within a given latitude. We excluded non-significant random effects from the final model after testing for their significance using the function *ranova* in the package '*lmerTest*' (Kuznetsova et al. 2017). Finally, to evaluate significant interactions between fixed effects, we performed pairwise comparisons that were false discovery rate (FDR)-corrected to account for multiple comparisons. Within the LMM models, we explicitly tested for thermal plasticity of the predator on predation rates by constructing six contrasts based on combinations of the developmental and test temperatures (Figure. 1d). We also evaluated whether the effect of a given type of plasticity differed between latitudes by testing the interaction between source latitude and each of these contrasts. For acute thermal plasticity we contrasted larvae with the same developmental temperature but tested at a different test temperature: larvae reared at 20 °C and tested at 20 °C versus 24 °C (Contrast 1), and larvae reared at 24 °C and tested at 20 °C versus 24 °C (Contrast 2). For developmental plasticity, we contrasted larvae reared at a different developmental temperature but tested at the same test temperature: larvae reared at 20 °C versus 24 °C and tested at 20 °C (Contrast 3), at 24 °C (Contrast 4) and at 32 °C (Contrast 5). Finally, for post-acclimation thermal sensitivity the contrast tested differences between the predation rates of larvae reared and tested at 20 °C versus larvae reared and tested at 24 °C (Contrast 6). Note that the main effects and interactions of the LMM do not allow direct testing of the plasticity types. For example, the main effect of developmental temperature in the LMM does not directly test for developmental plasticity, as it compares the mean response at one developmental temperature (averaged across both test temperatures) with the mean response at the other developmental temperature (averaged across both test temperatures, Figure. 1).

In addition, we estimated the strength of thermal acclimation ('acclimation capacity') for both high- and low-latitude predators as the absolute value of the difference between the post-acclimation thermal sensitivity and the mean of the acute plasticities across both developmental temperatures (Einum et al. 2019, Figure. 1). Note that the formula suggested by Einum et al. (2019) expresses both plasticities as slopes, hence divides the changes in performance by the difference in test temperature (i.e. 4 °C). The resulting equation is:

$$Acclimation\ Capacity\ (AC) = \left|\frac{P_{2,2}-P_{1,1}}{T_2-T_1} - \frac{(P_{1,2}-P_{1,1})+(P_{2,2}-P_{2,1})}{2(T_2-T_1)}\right| \quad \text{(Equation 1)}$$

Using the law of propagation uncertainty (Rice 2007), we calculated the standard deviation of the estimates by propagating the standard errors associated with the estimates of performance parameters using the package *propagate* (Spiess 2014).

Results

General patterns in predation rates

Predation rates generally increased with test temperature (main effect Test temp), yet this further depended on the source latitude of the predator (Test temp × Latitude, Table 1, Figure 3). In low-latitude populations, predation rates were higher at 32 °C compared to 20 °C and 24 °C (both P < 0.001), but not different between 20°C and 24°C (P = 0.63, Figure 3). In high-latitude populations, predation rates were marginally higher at 32 °C compared to 24 °C (P = 0.083), yet lower at 20 °C (both P < 0.050). From the perspective of source latitude, low-latitude larvae had higher predation rates than high-latitude larvae (main effect Latitude), especially at test temperatures 20 °C (+ 74%, P = 0.001) and 32 °C (+54%, P = 0.001), but not significantly at 24 °C (+13%, P = 0.49) (Test temp × Latitude, Table 1, Figure 3).

The effect of test temperature depended also on the developmental temperature (Test temp × Dev temp, Table 1, Figure 3). Predation rates were overall lower at the higher developmental temperature of 24 °C (main effect Dev temp), but not when tested at 20 °C (P = 0.27) (Test temp × Dev temp, Table 1, Figure 3).

There was a significant interaction between source latitude and developmental temperature (Latitude × Dev temp, Table 1, Figure 3). Predation rates were higher in low-latitude larvae than in high-latitude larvae at the higher developmental temperature of 24 °C (P < 0.001), but not at the developmental temperature of 20 °C (P = 0.12). Vice versa, predation rates were overall lower at the higher developmental temperature of 24 °C than 20 °C for high-latitude larvae (P < 0.001), but not for low-latitude larvae (P = 0.53).

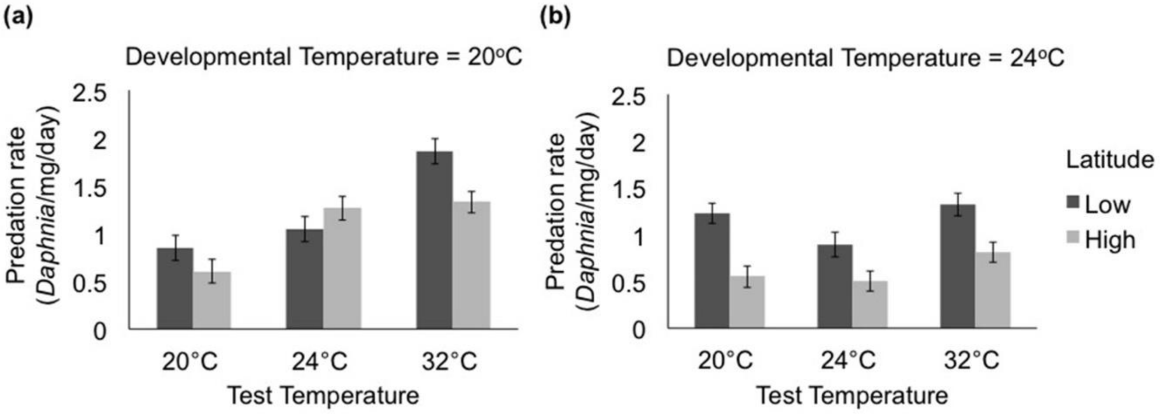


Figure 3. Effects of source latitude of the predator, developmental temperature of the predator and test temperature on the predation rates of *Ischnura elegans* larvae on *Daphnia magna* water fleas. Given are least-square means ± 1 SE of untransformed values.

Table 1. Results of the linear model testing for the effects of predator source latitude (Latitude), predator developmental temperature (Dev temp) and test temperature (Test temp) on the ln-transformed predation rates of *Ischnura elegans* larvae on *Daphnia magna* water fleas.

	Predation rate		
Effect	d.f.1, d.f.2	*F*	*P*
Latitude	1, 101	26.96	**< 0.001**
Dev temp	1, 101	14.51	**< 0.001**
Test temp	2, 101	19.91	**< 0.001**
Latitude × Dev temp	1, 101	8.30	**0.005**
Latitude × Test temp	2, 101	3.11	**0.049**
Dev temp × Test temp	2, 101	8.22	**< 0.001**
Latitude × Dev temp × Test temp	2, 101	1.54	0.220

Thermal plasticity and acclimation of predation rates

Acute plasticity at the developmental temperature of 20 °C showed a significant interaction with latitude (Table 2, Latitude × Contrast 1, P = 0.047): low-latitude populations did not show acute plasticity (P = 0.35) but high-latitude populations showed higher (+106%, P < 0.001) predation rates when tested at 24 °C compared to 20 °C (Figure 4a). Acute plasticity at the developmental temperature of 24 °C was not significant (Contrast 2, P = 0.094, Figure 4b).

Developmental plasticity differed between latitudes for test temperature 24 °C (Table 2, Latitude × Contrast 4: P = 0.006), and marginally for test temperature 20 °C (Latitude × Contrast 3: P = 0.084), but not for test temperature 32 °C (Latitude × Contrast 5: P = 0.69). When tested at 24 °C, low-latitude populations did not show developmental plasticity (P = 0.50), while high-latitude populations showed lower (–60%, P < 0.001) predation rates when developed at 24 °C compared to 20 °C (Figure 4d). When tested at 20 °C, low-latitude populations showed higher (+40%, P = 0.048) predation rates when developed at 24 °C compared to 20 °C, while high-latitude populations did not show developmental plasticity (P = 0.65, Figure 4c). When tested at 32°C, populations at both latitudes showed on average lower (-34%, P = 0.0002) predation rates when developed at 24°C compared to 20°C (Figure 4e).

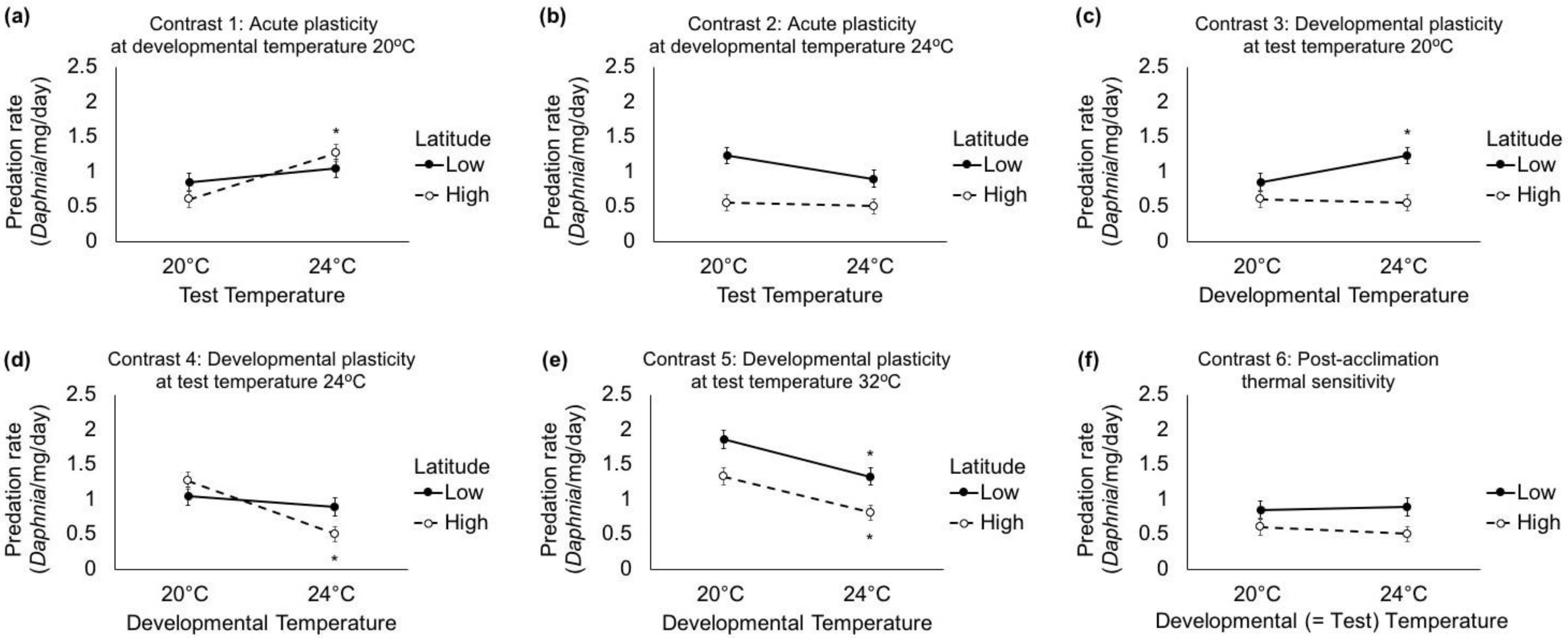


Figure 4. Contrast plots for patterns in thermal plasticity and acclimation. (a) Acute plasticity at developmental temperature 20 °C (Contrast 1), (b) acute plasticity at developmental temperature 24 °C (Contrast 2), (c) developmental plasticity at test temperature 20 °C (Contrast 3), (d) developmental plasticity at test temperature 24 °C (Contrast 4), (e) developmental plasticity at test temperature 32 °C (Contrast 5), and (f) post-acclimation thermal sensitivity (Contrast 6). Given are least-square means ± 1 SE of untransformed values. Significant ($P < 0.05$) differences between temperature treatments for a given latitude (based on ln-transformed values) are indicate by asterisks.

There was no significant post-acclimation thermal sensitivity, indicating perfect thermal compensation at both latitudes (Table 2, Latitude × Contrast 6, P = 0.61, Figure 4f). In other words, predation rates did not differ between damselfly larvae reared and tested at 20 °C vs. larvae reared and tested at 24 °C. The acclimation capacity leading to perfect thermal compensation was ~6 times higher in high-latitude larvae compared to low-latitude larvae (Figure 5).

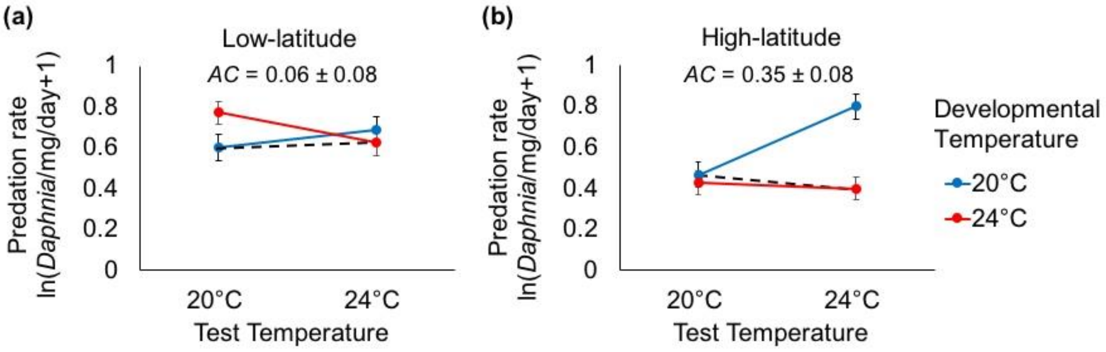


Figure 5. Thermal acclimation capacity (AC) of (a) low-latitude larvae and (b) high-latitude larvae, based on the estimation method of Einum et al. (2019). Given are means and standard deviations.

Table 2. Results of the contrast testing for the different types of thermal plasticity. The contrasts are further explained in Figure 1d. When the interaction between predator source latitude and the contrast was significant, contrasts are given separately per source latitude; otherwise the joint contrast for both source latitudes is given. Given are t-values (d.f. = 101) and P-values. Dev. T = developmental temperature; Test T = test temperature.

						Latitude × Contrast		*Joint contrast*		*Low latitude*		*High latitude*	
Contrast	Dev. T	Test T		Dev. T	Test T	*t*	*P*	*t*	*P*	*t*	*P*	*t*	*P*
1	20	20	vs.	20	24	2.007	0.047			0.933	0.353	3.916	**<0.001**
2	24	20	vs.	24	24	0.988	0.326	1.688	0.094				
3	20	20	vs.	24	20	1.743	0.084			2.005	**0.048**	0.451	0.653
4	20	24	vs.	24	24	2.808	**0.006**			0.671	0.504	4.948	**<0.001**
5	20	32	vs.	24	32	0.394	0.694	3.411	**<0.001**				
6	20	20	vs.	24	24	0.757	0.451	0.510	0.611				

Discussion

Our study provides new insights into the climatic variability hypothesis (CVH) by, for the first time, testing it for different plasticity types (acute and developmental) and for the two acclimation metrics (post-acclimation thermal sensitivity and acclimation capacity). A key result was that the latitudinal differences in the plasticity of predation rates strongly depended on the type of thermal plasticity and thermal acclimation metric used, thereby providing mixed support for the CVH. These latitudinal patterns in thermal plasticity likely translate into fitness patterns, as predation rates have been associated with growth and development rates in damselfly larvae (Stoks et al. 2005, Stoks et al. 2012, Pickup and Thompson 1990, McPeek et al. 2001).

Effects of test temperature

As expected, and reported for several other ectotherm species (Rall et al. 2012, Englund et al. 2011), acute warming increased predation rates of *I. elegans* larvae. Particularly, the acute extreme temperature of 32 °C led to higher predation rates than acute mild +4 °C warming (in low-latitude larvae). This matches previous findings showing that a heat wave may be beneficial for damselfly larvae as it can increase growth rates and immune components (Van Dievel et al. 2017). The extreme test temperature of 32 °C might have reduced *Daphnia*’s swimming ability, making them easier to catch for damselfly predators. Yet, Van Dievel et al. (2017) showed at 32 °C higher predation rates by *I. elegans* larvae on *Artemia* nauplii while these prey did not reduce their activity at 32 °C. Therefore, the higher predation rates at 32 °C are more likely explained by a thermal effect on the predator than on the prey. Note that, although a gradual temperature increase better matches a realistic field scenario, we observed in an additional experiment (Supplementary material Appendix S1) that the presence of such gradual temperature increase did not affect the predation rates at the extreme temperature.

General effects of the source latitude

In line with previous studies, low-latitude larvae had higher predation rates than high-latitude larvae (Stoks et al. 2012, Janssens et al. 2014, Debecker and Stoks 2019). This can be attributed to the faster lifestyle of low-latitude populations driven by their shorter generation time (Shama et al. 2011, Verheyen and Stoks 2019). This difference in predation rates was especially pronounced at the extreme temperature of 32 °C, indicating that low-latitude larvae were able to sustain a high feeding rate under extreme temperature which could compensate for the increased metabolic demand. It is thus plausible that the higher feeding rate of low-latitude larvae under extreme temperature reflects thermal adaptation to heat waves that are more frequent and intense at low latitudes (Nikulin et al. 2011).

Thermal plasticity and its latitudinal patterns

A key finding was the strong thermal acclimation leading to the absence of post-acclimation thermal sensitivity (sensu Seebacher et al. 2015) of predation rates in *Ischnura* larvae between the developmental temperatures 20 °C and 24 °C. This indicates that the thermal performance curve (TPC) for predation rates of *Ischnura* larvae shifted horizontally to perfectly compensate the higher developmental temperature (Figure 1b). Note that the non-parallel reactions norms of acute thermal responses (Figure 5, blue line vs. red line) may have been caused by changes in the TPC shape. Despite no post-acclimation thermal sensitivity, predation rates were in general lower at the developmental temperature of 24 °C in high-latitude larvae. This confirms that chronic exposure to 24 °C is suboptimal for high-latitude larvae that have lower growth rates when reared at 24 °C than at 20 °C (Debecker et al. 2017, Debecker and Stoks 2019). This matches the mean summer water temperature at the low latitude of 20 °C (De Block et al. 2013, Dinh Van et al. 2014, Debecker and Stoks 2019). This also resulted in developmental plasticity at the test temperature 24 °C only occurring in high-latitude larvae, where predation rates were ca. 60% lower when the developmental temperature was 24 °C as compared to 20 °C. Notably, acute thermal plasticity showed the opposite pattern: at the developmental temperature of 20 °C, only high-latitude larvae increased predation rates when tested at 24 °C as compared to 20 °C. This suggests that high-latitude larvae may exploit the infrequent short periods of warmer temperatures at their latitude.

The latitudinal patterns in the different plasticity types and in the two thermal acclimation metrics provided mixed support for the climatic variability hypothesis (CVH). The CVH predicts that high-latitude ectotherm populations, that experience a higher seasonal thermal variability (Addo-Bediako et al. 2000), should show a higher thermal plasticity and acclimation capacity (Janzen, 1967). This has been supported by several studies (e.g. Ghalambor et al. 2006, Aguilar-Kirigin and Naya 2013; for support under a reversed latitudinal pattern of seasonality see Naya et al. 2011). Notably, in the current study the hypothesis was not supported for post-acclimation thermal sensitivity (sensu Seebacher et al. 2015), but for the related and recently developed metric thermal acclimation capacity (sensu Einum et al., 2019). The reason for this discrepancy is that the absence of post-acclimation thermal sensitivity, hence the presence of perfect thermal compensation, was driven by a ~6 times higher acclimation capacity in high- than in low-latitude larvae. These contrasting patterns support the recently identified pitfall by Einum et al. (2019) which highlights the need to explicitly express acclimation capacity relative to acute plasticity (but see Rohr et al. 2019). Although our results of acclimation capacity sensu Einum et al. (2019) are as predicted by the CVH, they might be explained by mechanisms other than climatic variation. For example, the adaptation to a mean summer water temperature of 24 °C could make low-latitude larvae rely less on plasticity to acclimate between 20 °C and 24 °C. Furthermore, the CVH was only partially supported for acute plasticity and developmental plasticity. High-latitude larvae showed positive acute plasticity at the low developmental temperature (Figure 4a) and negative developmental plasticity at the higher two test temperatures (Figure 4d-e), while low-latitude larvae showed no acute plasticity but positive developmental plasticity at the lowest test temperatures (Figure 4c) and negative developmental plasticity at the highest test temperature (Figure 4e). Other studies have shown the predicted higher plasticity or acclimation capacity at higher latitudes to be absent (for acute plasticity: Van Heerwaarden et al. 2014, Barria and Bacigalupe 2017; for post-acclimation thermal sensitivity: Gunderson and Stillman 2015), or even reversed (for post-acclimation thermal sensitivity: Liefting et al. 2009, Seebacher et al. 2015). This suggests that mechanisms other than climatic variability may play a role. For example, daily temperature fluctuations may also shape thermal plasticity (Verheyen and Stoks 2019), yet show opposite latitudinal patterns than climatic variability in temperate regions by being stronger at lower latitudes (Wang and Dillon 2014).

While the current study followed the methodology of previous studies comparing thermal plasticity across latitudes by applying constant temperature and photoperiod conditions (e.g. fruit flies: Liefting et al. 2009; dandelions: Molina-Montenegro and Naya 2012; shrimps: Barria et al. 2018), these conditions might not represent well the natural seasonality, particularly for high-latitude animals which are exposed to a stronger seasonality. The constant photoperiod of 14:10 L:D used in our experiment matches the photoperiod experienced by final instar larvae in the field at both latitudes in May, hence provides similar seasonal information for high- and low-latitude larvae. In line with this, we have shown in previous studies that under these rearing conditions, damselfly larvae of the same low-latitude populations consistently show faster growth and development rates compared to high-latitude larvae thereby matching their higher number of generations per year in the field (e.g. Arambourou and Stoks 2015, Debecker and Stoks 2019; Verheyen and Stoks 2019). This may explain why we still observed the expected higher acute plasticity (when reared at 20 °C) and higher acclimation capacity in high-latitude compared to low-latitude larvae. Related to this, the CVH might be easier to detect when animals are exposed to their natural rearing temperature regime. Indeed, the acute plasticity of high-latitude larvae developed at 20 °C (Figure 4a, dashed line) was stronger compared to the acute plasticity of low-latitude ones developed at 24 °C (Figure 4b, solid line) (contrast test, P < 0.001), adding further support for the CVH.

The results of the higher acclimation capacity (sensu Einum et al. 2019) of high-latitude larvae seemed to be largely driven by the higher acute plasticity at their natural mean developmental temperature at 20 °C, rather than by a higher post-acclimation thermal sensitivity. The mechanism explaining why high-latitude larvae increased their predation rate in response to warming in the short term but not in the long term may be related to different acute versus chronic thermal responses of metabolism. Indeed, short-term exposure to higher temperatures typically increases resting metabolic rates, while longer exposure often leads to no increase or even a decrease (e.g. Donelson et al. 2011, Donelson et al. 2012). For the study species, short-term exposure to higher temperature increased metabolic rate (Debecker and Stoks 2019), while longer exposure resulted in perfect thermal compensation for metabolic rate (Van Dievel et al. 2019a). The increased metabolic rates under acute exposure to higher temperatures, imply higher energetic costs, and therefore ask for a higher food intake (hence predation rates). This need is relieved under long-term exposure to higher temperatures because of adjustment of the metabolic rates (Vucic-Pestic et al. 2011). Whatever the underlying mechanisms, our study extends the emerging view that intraspecific variation in thermal plasticity of fitness-related traits is important to better understand the effects of temperature changes (Seebacher et al. 2012, Valladares et al. 2014) towards traits related to the outcome of interspecific interactions.

Possible implications of thermal plasticity on the effects of global warming

Thermal plasticity is important for organisms to cope with global warming at a local scale (Deutsch et al. 2008, Seebacher et al. 2015, Sgrò et al. 2016). Our results for predation rates add to the insight that developmental temperature may shape the acute thermal response (Fangue et al. 2009, Grigaltchik et al. 2012, Sentis et al. 2015). Moreover, our results indicate that the effect of developmental temperature depends on test temperature and latitude. Indeed, developmental plasticity was positive (meaning higher predation rates when reared at 24 °C compared to 20 °C) for low-latitude larvae at test temperature 20°C, negative for high-latitude larvae at test temperature 24 °C, and negative for both low-latitude and high latitude larvae at test temperature 32 °C. If TPCs are solely based on developmental temperatures (e.g. Deutsch et al. 2008, Cohen et al. 2018) or test temperatures (e.g. Sitch et al. 2003), such interactions between developmental temperature and test temperatures may be problematic when using TPCs to predict the effects of global warming (Schulte et al. 2011, Sinclair et al. 2016). Our results highlight the importance of investigating the contribution of acute thermal plasticity across developmental temperatures to predict the impact of warming on organism performance and interactions (Magozzi and Calosi 2015, Padfield et al. 2016, Dillon et al. 2016).

The patterns of the plasticity types and acclimation metrics provide complementary information on the possible effects of global warming on predation rates in the absence of thermal evolution in the high-latitude populations that currently experience mean summer water temperatures of 20 °C (De Block et al. 2013, Dinh Van et al. 2014, Debecker and Stoks 2019). Our results of post-acclimation sensitivity and acclimation capacity suggest that in response to long-term exposure to the predicted 4 °C increase in temperature, the predation rates of high-latitude larvae at 24 °C may not change as compared to current predation rates at 20 °C because of a high thermal acclimation capacity. Our results of acute plasticity suggest that during short periods of 4 °C warming, high-latitude larvae are expected to increase their predation rates. Furthermore, our results of developmental thermal plasticity suggest that during a heat wave, high-latitude larvae will have lower predation rates when the mean summer water temperatures have increased to 24 °C compared to current 20 °C.

Conclusion

Our results added three important insights in thermal plasticity and thermal acclimation of predation rates and its intraspecific latitudinal variation that are directly relevant for understanding and predicting the impact of warming. First, developmental and acute temperatures had contrasting and interactive effects, which urges caution when using TPCs to forecast the impact of global warming and thermal extremes on biotic interactions (see also Schulte et al. 2011, Sinclair et al. 2016). Second, latitudinal patterns in thermal plasticity and acclimation across latitudes critically depended on the plasticity type and acclimation metric, thereby generating mixed support for the climatic variability hypothesis. Third, while both low- and high-latitude larvae showed perfect thermal compensation, this was driven by a much higher thermal acclimation capacity in high-latitude larvae. This supports the recent plea by Einum et al. (2019) to quantify thermal acclimation relative to the acute plasticity. We were able to isolate the predator's contribution in shaping predation rates by using a single prey clone, which allowed straightforward testing of the CHV for predator performance. Future studies should extend our study by also considering the latitude of the prey in shaping the outcome of predator-prey interactions. Taken together, our study underscores the importance for jointly considering acute and developmental plasticity between latitudes to obtain better insights in the role of thermal plasticity in shaping species interactions in a warming world.

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Authors’ contributions

YJW, AS and RS conceived the ideas and designed methodology. YJW collected and analysed the data. YJW and RS led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Appendix S1. Effect of a gradual temperature increase on the effect of the heat wave treatment

We conducted an extra heat wave treatment where gradual temperature increase was performed by exposing F0 animals at 24 °C for one day, 29 °C for one day, and 32 °C for five days before measuring predation rates. The analysis focusing on predation rates at the 32 °C extreme temperature showed that the presence of a gradual temperature increase had no significant effect on predation rates (Table S1, Figure S1). This extra analysis confirmed the effects of source latitude and developmental temperature. Low-latitude larvae had higher predation rates compared to high-latitude larvae, and larvae developed at 20 °C had higher predation rates compared to those developed at 24 °C (Table S1, Figure S1).

These results indicate that studies exposing animals more abruptly to their extreme temperature treatment (e.g. Seifert et al. 2015) may still generate meaningful results. Yet, it should be noted that larvae under the gradual temperature increase had been at 32 °C for several days before being tested, and this might have contributed to the results.

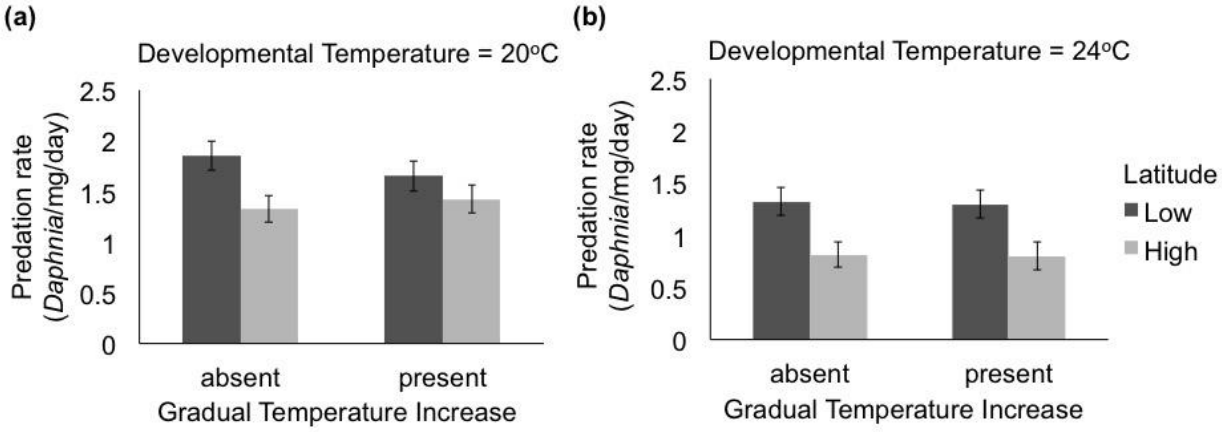

Figure S1. Effects of source latitude, developmental temperature and test temperature on the predation rates of Ischnura elegans larvae on Daphnia magna water fleas. Given are least-square means ± 1 SE.

Table S1. Results of the linear model testing for the effects of source latitude (Latitude), developmental temperature (Dev temp) and the presence of a gradual temperature increase (Gradual temp increase) on the predation rates of *Ischnura elegans* larvae at 32°C heat wave.

	Predation rate		
Effect	d.f.1, d.f.2	*F*	*P*
Latitude	1, 66	20.64	< **0.0001**
Dev temp	1, 66	27.30	< **0.0001**
Gradual temp increase	1, 66	0.14	0.7087
Latitude × Dev temp	1, 66	0.45	0.5069
Latitude × Gradual temp increase	1, 66	0.68	0.4118
Dev temp × Gradual temp increase	1, 66	0.04	0.8513
Latitude × Dev temp × Gradual temp increase	1, 66	0.55	0.4627

Chapter III

Thermal evolution ameliorates the long-term plastic effects of warming, temperature fluctuations and heat waves on predator-prey interaction strength

Ying-Jie Wang, Arnaud Sentis, Nedim Tüzün and Robby Stoks

Functional Ecology (2021), 00: 1–12.

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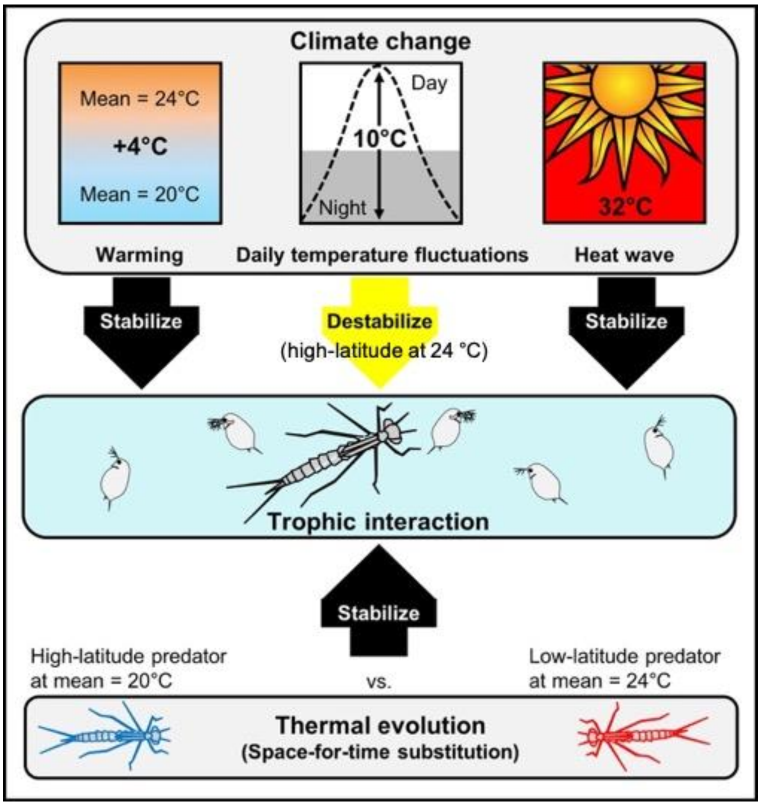

Abstract

How thermal evolution may affect trophic interactions and its implications for trophic system stability remains unstudied. To advance insights in how global warming shapes trophic interactions, we need to consider besides increases in mean temperatures, also daily thermal fluctuations (DTF) and heat waves (HW), and how their effects are modulated by thermal evolution. Using a common-garden approach we tested how each thermal factor affected predator metabolic rate and functional response parameters, and used these responses to predict long-term predator-prey interaction strength between larvae of the damselfly *Ischnura elegans* and the water flea *Daphnia magna*. By using high- and low-latitude predator populations with the latter being exposed to higher mean temperatures, higher DTF and more frequent HW, we assessed the potential impact of thermal evolution at the high latitude using a space-for-time substitution. In line with thermal adaptation, growth rates were faster and handling times shorter in low-latitude compared to high-latitude larvae at 24 °C, while the opposite was true at 20 °C. Warming weakened the long-term interaction strength, except for the high-latitude trophic system at DTF and HW where plastic responses therefore may not stabilize the high-latitude system. This extends the emerging insight that temperature variation may make ectotherms more vulnerable to warming. The contributions of metabolic rate, search rate and handling time in shaping thermal effects on interaction strength differed between latitudes. A key finding was that thermal evolution may further weaken the long-term interaction strength of the high-latitude trophic system under increases in mean temperatures, even at DTF and potentially also at HW. Our results underscore the importance of daily thermal fluctuations and heat waves in shaping predator-prey interactions, and may suggest an overall stabilizing contribution of predator thermal evolution ameliorating thermal plastic effects on food web stability.

Introduction

How global warming affects trophic interactions is crucial to forecast the local persistence of species (Urban et al. 2016). Most research on the biological effects of global warming focused on increases in temperature means, and much less on temperature extremes (Vasseur et al. 2014, Williams et al. 2016) and daily temperature fluctuations (Colinet et al. 2015, Stoks et al. 2017, Boukal et al. 2019). Nevertheless, these additional thermal factors may have a stronger impact and even reverse responses to increases in temperature means (Vasseur et al. 2014, Paaijmans et al. 2013). The few demonstrations of heat waves and daily temperature fluctuations shaping trophic interactions and resulting population dynamics support their importance (Stoks et al. 2017, Sentis et al. 2013a). Yet, these studies did not consider the underlying mechanisms determining the changes in trophic interaction strength, thereby precluding to model the long-term impact on predator and prey populations (as done for an increase in temperature means in Sentis et al. 2015).

The short-term interaction strength is typically studied using the functional response: the relationship between per capita predation rates and prey densities. It is parameterized by the predator search rate that determines feeding rate at low prey densities, and the predator handling time that determines the maximum predation rate at high prey densities (Holling 1959). Warming is expected to increase search rates and to shorten handling times, leading to higher predation rates at low and high prey densities, respectively (Thompson 1978, Sentis et al. 2012, Twardochleb et al. 2020). Yet, given that functional response parameters may show a hump-shaped relationship with environmental temperature (e.g. search rate, Englund et al. 2011), temperatures above an optimum may reverse these predictions. The thermal dependence of functional response parameters has been shown to be consistent across laboratory and field settings, supporting their value to predict warming effects on natural systems (Archer et al. 2019). In contrast to the predictions for short-term interaction, warming is expected to weaken long-term interaction strength; i.e. the long-term effect of the predator population on the prey population density. This is because warming may cause (i) for the prey a lower carrying capacity and a higher intrinsic growth rate thereby increasing the predator equilibrium density, and (ii) for the predator a higher metabolic rate thereby increasing the prey equilibrium density (Rall et al. 2010, Fussmann et al. 2014). Notably, by decreasing long-term interaction strength, warming is expected to increase trophic system stability by preventing predator and prey extinctions driven by population fluctuations (Sentis et al. 2015, Binzer et al. 2012, Kratina et al. 2012). Yet, if warming in the long run increases the energy loss (metabolic rate) more than the energy gain (i.e. predation rate), the predator population could suffer from a lower energetic efficiency and be at risk of extinction despite abundant resources (Vucic-Pestic et al. 2011). Importantly, the thermal dependence of metabolic rate and functional response parameters, and hence of short- and long-term trophic interaction strengths, may not always follow expectations (Rall et al. 2010, Fussmann et al. 2014, Uiterwaal and DeLong 2020). Two important reasons for this are that (i) most predictions about the thermal dependence of biological reaction rates assume the predators' experience temperatures below their thermal optimum, and overlook the decline in rates above thermal optimum (Englund et al. 2011), and that (ii) most predictions overlook the phenomenon of thermal compensation whereby 'passive' increases of costly rates such as metabolic rates are buffered in the long term (Seebacher et al. 2015, Havird et. al. 2020). In addition, increases in mean temperature can affect trophic system stability via shifting the functional response type (Daugaard et al. 2019). Taken together, to predict the impact of global warming on trophic interaction strengths and its implications for trophic system stability, we need empirical studies determining the responses of predator metabolic rate, the type and parameters of the functional response not only to increases in mean temperatures but also to daily temperature fluctuations and heat waves (Boukal et al. 2019).

Besides thermal plasticity (Sentis et al. 2015, Rosenblatt et al. 2016), thermal evolution may play an important role in shaping trophic interactions under global warming (Merilä and Hendry 2014, Stoks et al. 2014). Thermal evolution can be more important than thermal plasticity (Verheyen and Stoks 2019; González-Tokman et al. 2020), and even reverse predictions based on plasticity (Diamond and Martin, 2016). One way to study the impact of thermal evolution is a space-for-time substitution, where the current phenotypes of low-latitude populations at their local thermal conditions are used as estimates for the phenotypes of high-latitude populations under projected warming after thermal evolution (De Frenne et al. 2013, Verheyen et al. 2019). The few applications to trophic interaction studies were limited to single prey densities (De Block et al. 2013, O'Gorman et al. 2017). Therefore, how thermal evolution may affect trophic interactions and its implications for trophic system stability remains unstudied.

We here examined (i) how an increase in mean temperature, daily temperature fluctuations (DTF) and a heat wave (HW) influence the metabolic rate and the functional response of an aquatic insect predator, as well as the resulting short- and long-term interaction strengths, and (ii) how thermal evolution in the predator may modulate these effects. We studied larvae of the damselfly *Ischnura elegans* preying on the water flea *Daphnia magna*, a textbook example for the functional response in pond food webs (Begon et al. 2006). Both species inhabit shallow water bodies where DTFs and HWs can be pronounced. To apply a space-for-time substitution, we studied predators from low- and high-latitude populations in Europe. These populations inhabit water bodies with similar abiotic and biotic conditions (see Table S1 in Debecker and Stoks 2019) except for different thermal conditions between latitudes. Compared to high-latitude populations, low-latitude populations experience 4 °C higher mean summer water temperatures, higher daily temperature fluctuations reaching a maximum of 10 °C, and more frequent HW temperatures (Debecker and Stoks 2019, Verheyen and Stoks 2019, Van Dievel et al. 2019a). Given thermal adaptation (Verheyen and Stoks 2019, Debecker and Stoks 2019), we expected the plastic effects of the three thermal factors (increase in mean temperature, DTF and HW) to differ and potentially even reverse between latitudes. Depending on thermal plasticity being adaptive or maladaptive, we expected predator thermal evolution to magnify or oppose the impact of a higher mean temperature, DTF and HW on long-term interaction strength (Diamond and Martin 2016).

Materials and methods

We cultured *I. elegans* populations from three southern (low-latitude) and three northern (high-latitude) sites, and a single clone of *D. magna* from an intermediate latitude for all predation trials (Appendix S1). Due to practical reasons we used a single population of *D. magna* from an intermediate latitude (i.e. Belgium). Indeed, even current design resulted in an experiment with a very large number of predation trials (n = 755) which also required a large effort for the culturing and synchronization of both predators and prey. Using *Daphnia* from two latitudes would have doubled the number of predation trials. While this choice implies that any latitudinal patterns in the outcome of predation trials can be fully explained from the predator's perspective, this also has potential limitations which we specify in the Discussion.

Experimental design and general procedure

We conducted a full factorial experiment where damselfly larvae of each latitude were reared at one of six temperature combinations (Figure 1a): 2 mean rearing temperatures (20 °C or 24 °C) × 3 temperature variation levels [constant (CTE), 10°C daily temperature fluctuation (DTF), or a 32 °C heat wave (HW)]. Damselfly larvae were kept at the thermal treatments (except for the heat wave treatment) from day 10 after hatching until 7 days after their moult into the final (F0) instar when the predation trials were run. This period (> 8 weeks) allowed ample time for thermal acclimation. Given that heat waves are typically limited in time to a couple of days (Fischer and Schär 2010), we started the heat wave (HW) treatment one day after the larvae moulted into the final instar. The mean rearing temperatures correspond to the mean summer water temperatures of the shallow lakes inhabited by the populations at the northern (20 °C) and southern (24 °C) sites (De Block et al. 2013, Debecker and Stoks 2019). The 4 °C temperature difference also represents the predicted temperature increase at the northern sites by 2100 according to IPCC scenario RCP8.5 (IPCC 2014). This allows for a space-for-time substitution to examine the evolutionary response of the high-latitude predators to warming (Figure 1b) (De Frenne et al. 2013, Verheyen et al. 2019). The 10 °C daily temperature fluctuation (DTF) is the maximum daily variation at the low-latitude sites and the 32 °C is a heat wave temperature regularly experienced at the low-latitude sites based on both simulations with the 'FLake model' (a freshwater lake model for predicting thermal parameters) and direct observations with temperature loggers in the studied ponds (Verheyen and Stoks 2019, Van Dievel et al. 2019a). In the northern sites these thermal conditions can be expected under further warming (Beniston et al. 2017, González-Tokman et al. 2020). *Daphnia* were reared at a constant temperature of 22 °C, and were acclimated for 2 h at the respective test temperature before they were used in the predation trials (based on Thompson 1978; details in Appendix S1).

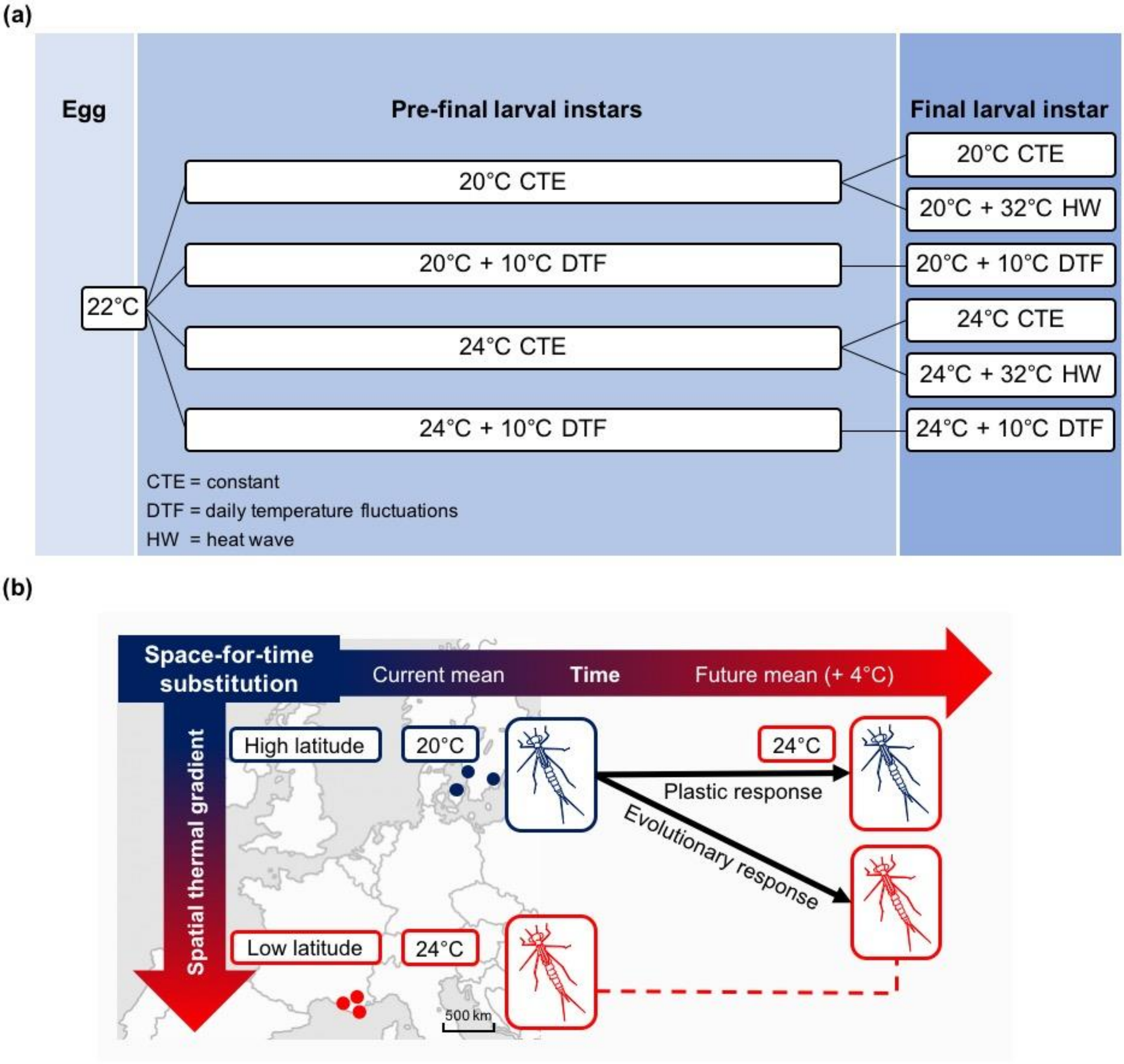

Figure 1. The experimental design showing (a) the six temperature treatment combinations of 2 mean rearing temperatures (20 °C and 24 °C) × 3 temperature variation levels (CTE = constant, DTF = daily temperature fluctuation of 10 °C, and HW = heat wave temperature of 32 °C), and (b) the study populations in Europe and the space-for-time substitution design (modified after Verheyen et al. 2019). Shown on the map are the sampled high-latitude (blue dots) and low-latitude (red dots) populations. The exposure of high-latitude populations (depicted as blue larvae) to the projected +4 °C warming (red box around blue larvae) allows testing thermal plasticity. The current phenotypes of low-latitude populations (depicted as red larvae) at their current mean temperature of 24 °C are used as estimates for the phenotypes of high-latitude populations under projected warming after thermal evolution.

To assess latitude-specific thermal adaptation of the predators, we first quantified their growth rate as an important fitness-related performance trait. We quantified growth rates as the increase in body mass between days 3 and 8 of the final instar. After 24-hour of starvation (to ensure empty guts), damselflies were weighed to the nearest 0.01 mg. We calculated growth rates as (ln W2 – ln W1) / 5 days, where W1 and W2 are the body masses measured at days 3 and 8. Results are shown in Appendix S2.

Directly after weighing at day 8, we reconstructed the functional response and estimated search rate a and handling time h for each of the 12 latitude × temperature treatment combinations. For each combination we conducted 8-12 predation trials at each of seven prey densities (5, 10, 15, 30, 50, 90 and 120 *Daphnia* per liter) (Thompson 1978). This resulted in 755 predation trials (predator and prey individuals were only used in one trial). Predation trials were conducted in white 2 L (18.0 × 13.3 × 12.6 cm) arenas filled with 1 L dechlorinated tap water. The test temperature matched the rearing temperature of the predator; 4-day old *Daphnia* prey were acclimatized to this temperature for 2 h (Thompson 1978). One damselfly was released at the bottom of an arena with one of the seven *Daphnia* densities. All predation trials started between 11:00 and 13:00 corresponding to the time period during which the temperatures of the DTF treatment matched the mean rearing temperatures, and lasted for 24 hours to cover an entire DTF cycle. At the end of each trial, the remaining Daphnia individuals were counted to calculate predation rates. Natural prey mortality was assessed in 8 replicates per temperature treatment using 90 *Daphnia* per liter in the absence of predators. Natural mortality was 0.65 ± 0.33% and thus negligible. Predation rates were expressed as the number of *Daphnia* consumed per damselfly per 24 h.

Twenty-four hours after the predation trials, we measured the metabolic rate on 6-8 randomly chosen damselfly larvae in each of the 12 treatment combinations (total of 88 larvae). Damselfly larvae were starved 24 hours and then placed in sealed 200 mL glass vials filled with aged tap water at their treatment temperature. The oxygen concentration was measured at the start and after 24 hours through a non-invasive method whereby oxygen sensor spots (placed inside each vial) were monitored with a fiber-optic cable connected to an oxygen meter (FIBOX 4, PreSens, Regensburg, Germany). Five control vials without damselfly larva were measured to determine background oxygen depletion in each thermal treatment group. Metabolic rate was expressed as the oxygen depletion rate per mg damselfly body mass (ppm O2 mg-1 day-1).

Statistical analyses and modelling

Statistical analyses were run in R v3.5.0 (R Core Team 2018). We determined the effects of predator latitude, mean rearing temperature, temperature variation (CTE, DTF and HW) and their interactions on growth and metabolic rates with linear mixed models. Damselfly population (nested in latitude) and its interactions with mean temperature and temperature variation, and mother identity (nested in population) were added as random factors. These random factors were never significant (likelihood ratio test; all $P > 0.05$) and were removed from the final models. To meet model assumptions, metabolic rate was ln-transformed. To evaluate significant interactions, we performed pairwise comparisons that were false discovery rate (FDR, Benjamini and Hochberg 1995)-corrected.

To analyze the functional response curves, we followed the procedure developed by Pritchard et al. (2018 package frair). Based on the statistical test to determine the functional response type (function *frair_test*, Appendix S3), we fitted the predation rates with the type II Rogers' random predator equation accounting for prey depletion (function *frair_fit*) to estimate the search rate a (L day-1) and handling time h (day):

$$N_e = N_0\left(1 - exp^{-a(t-N_e h)}\right) \text{ (Equation 1)}$$

where Ne is the number of prey eaten, N_0 is the initial prey density, and t is the total duration of the predation trial (in days). Due to an extremely high standard error of search rate in one of the 12 treatment groups, we removed 5 (out of 67) data points whose residuals were >2 interquartiles below the first or above the third quantiles (Villalobos-Jiménez et al. 2017). The fitted functional response curves were bootstrapped (n = 999) to visualize 95% confidence intervals (function *frair_boot*). We pairwise compared the search rate a and handling time h between treatment combinations using the "indicator variable" approach (function *frair_compare*) (e.g. Villalobos-Jiménez et al. 2017, Siepielski et al. 2020). We performed multiple comparisons within and between latitudes, and adjusted P-values with the FDR-method.

We estimated long-term interaction strength using a standard model of predator–prey dynamics (Smith 2008), following the procedure of Sentis et al. (2015) that is detailed in Appendix S4. Long-term interaction strength (I_L) was estimated as:

$$I_L = \left|\frac{\ln\left(N^+/N^-\right)}{P^+}\right| \text{ (Equation 2)}$$

where N^+ and P^+ are the prey and predator equilibrium densities when the predator and the prey coexist, and N^- is the prey equilibrium density in the absence of the predator (= carrying capacity

K).

We calculated the 84% confidence intervals (CIs) of the long-term interaction strength by propagating the standard errors associated with the group estimates of each experimental parameter (search rate, handling time and metabolic rate) based on the law of propagation of uncertainty. Unlike 95% CIs, a pattern of non-overlapping 84% CIs matches a difference between two values based on z test at the $P = 0.05$ level (Payton et al. 2003, MacGregor-Fors and Payton 2013). Treatment combinations with non-overlapping 84% CIs were therefore considered as significantly different (e.g. Harris et al. 2020, Drinkwater et al. 2020).

Results

Metabolic rates were not affected by 4 °C warming (Mean temperature: $F_{1,77} = 0.01$, $P = 0.93$) (Figure 2). Predator metabolic rate did not differ between the CTE and DTF treatments, but was higher under the HW treatment (Temperature variation: $F_{2,77} = 47.14$, $P < 0.001$), especially in the low-latitude predators (Latitude × Temperature variation: $F_{2,77} = 3.98$, $P = 0.023$) and at a mean of 20 °C (Mean temperature × Temperature variation: $F_{2,77} = 4.12$, $P = 0.020$). Low-latitude predators had a higher metabolic rate than high-latitude predators at a mean of 24 °C (Latitude × Mean temperature: $F_{2,77} = 4.64$, $P = 0.034$).

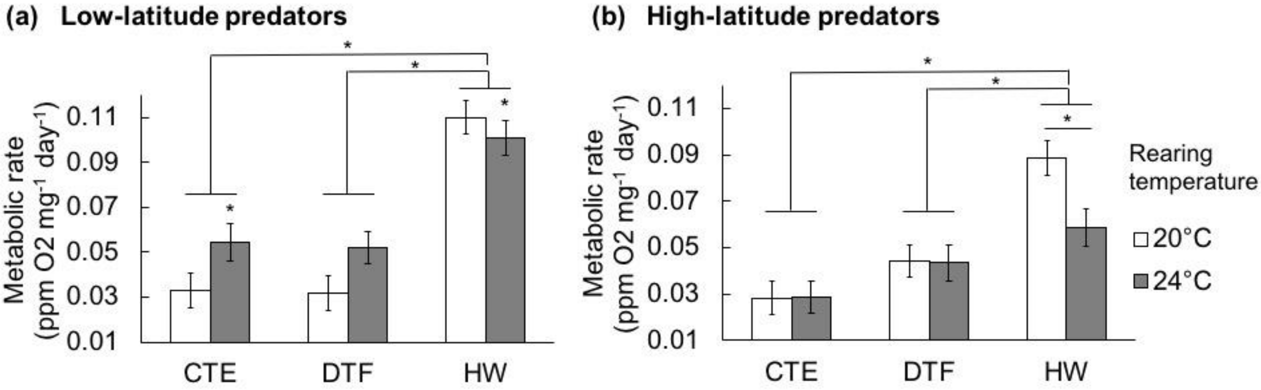

Figure 2. Effects of predator source latitude, mean rearing temperature and temperature variation (CTE = constant, DTF = daily temperature fluctuation of 10°C, HW = heat wave temperature of 32°C) on the ln-transformed metabolic rate of (a) low-latitude and (b) high latitude *Ischnura elegans* damselfly larvae. Given are least square means ± 1 SE. Asterisks associated with connecting lines denote significant (FDR-corrected $P < 0.05$) differences between groups. Asterisks on single bars denote significantly (FDR-corrected $P < 0.05$) higher values compared with the same thermal treatment combination of the other latitude.

Functional response parameters

For both low- and high-latitude populations, and across temperature treatments, prey consumption increased with prey density following a type II functional response (Figure 3).

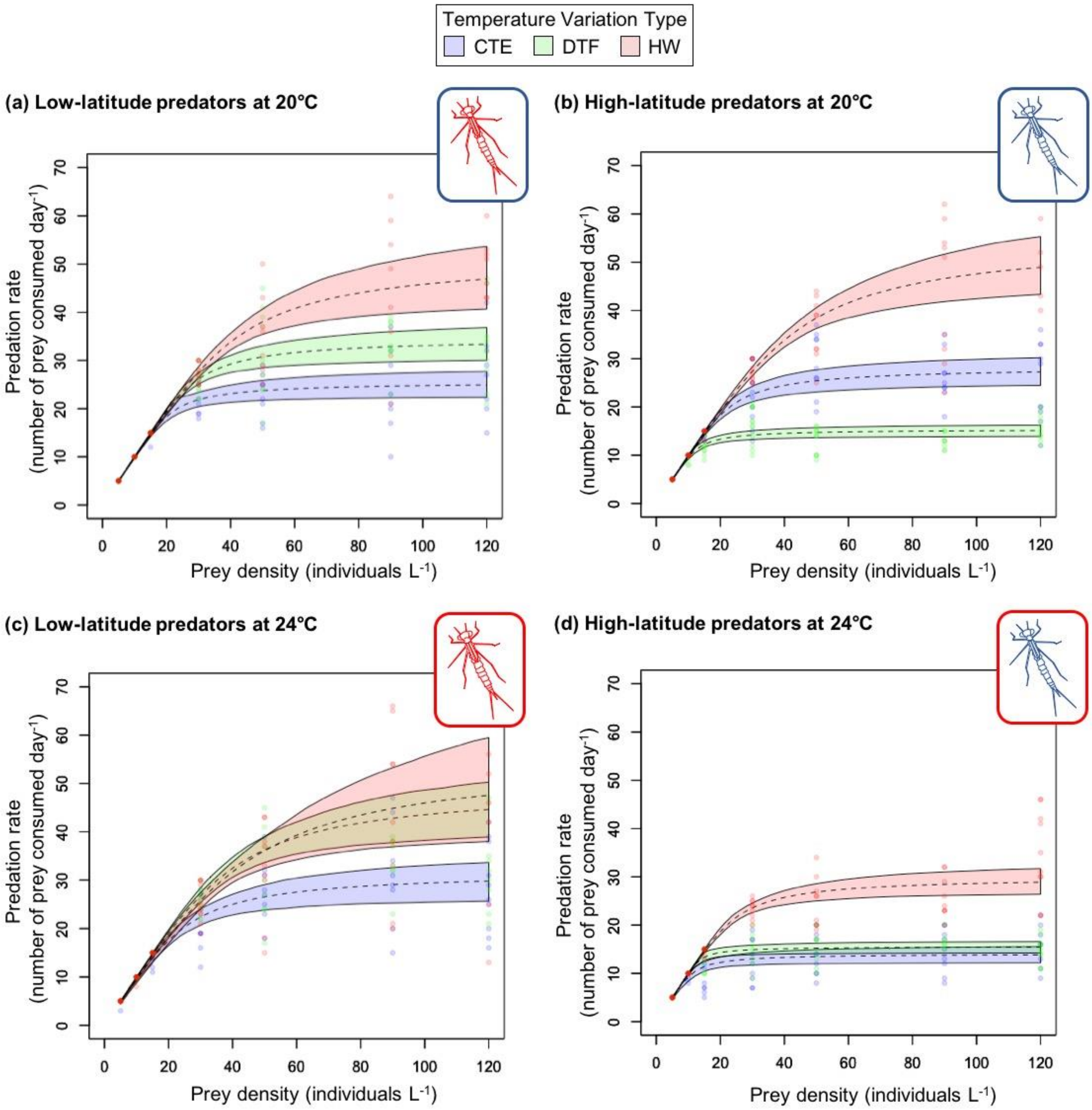

Figure 3. Type II functional response curves of (a,c) high-latitude and (b,d) low-latitude *Ischnura elegans* damselfly larvae preying on *Daphnia magna* at six thermal regimes: two mean rearing temperatures (20 and 24 °C) crossed with three levels of temperature variation (CTE = constant, DTF = daily temperature fluctuation of 10 °C, HW = heat wave temperature of 32 °C). Shaded areas represent 95% confidence intervals.

Search rates of low-latitude predators were -41% slower at 24 °C compared to 20 °C, while those of high-latitude predators did not differ between 20 °C and 24 °C (Figure 4a-b, Appendix S5). Search rates of low-latitude predators were not significantly affected by temperature variation, while those of high-latitude predators were 121% faster at DTF compared to HW at 24 °C. Low- and high-latitude predators had similar search rates at 20 °C, while high-latitude predators had faster search rates than low-latitude predators at 24 °C-DTF (+224%) and 24 °C-HW (+92%).

Handling times of low-latitude predators were shorter at 24 °C than 20 °C at CTE (-20%) and at DTF (-31%) but not at HW (Figure 4c-d, Appendix S5). Instead, handling times of high-latitude predators were longer at 24 °C than 20 °C at CTE (+102%) and at HW (+83%) but not at DTF. Handling times of low-latitude predators were overall shorter at DTF (-30%) and at HW (-24%) compared to CTE, especially at 20 °C. Handling times of high-latitude predators were at both mean temperatures shortest at HW, and longest at DTF (yet not different from CTE at 24°C). Low-latitude predators had generally shorter (-45%) handling times compared to high-latitude predators except at 20°C-CTE and 20°C-HW.

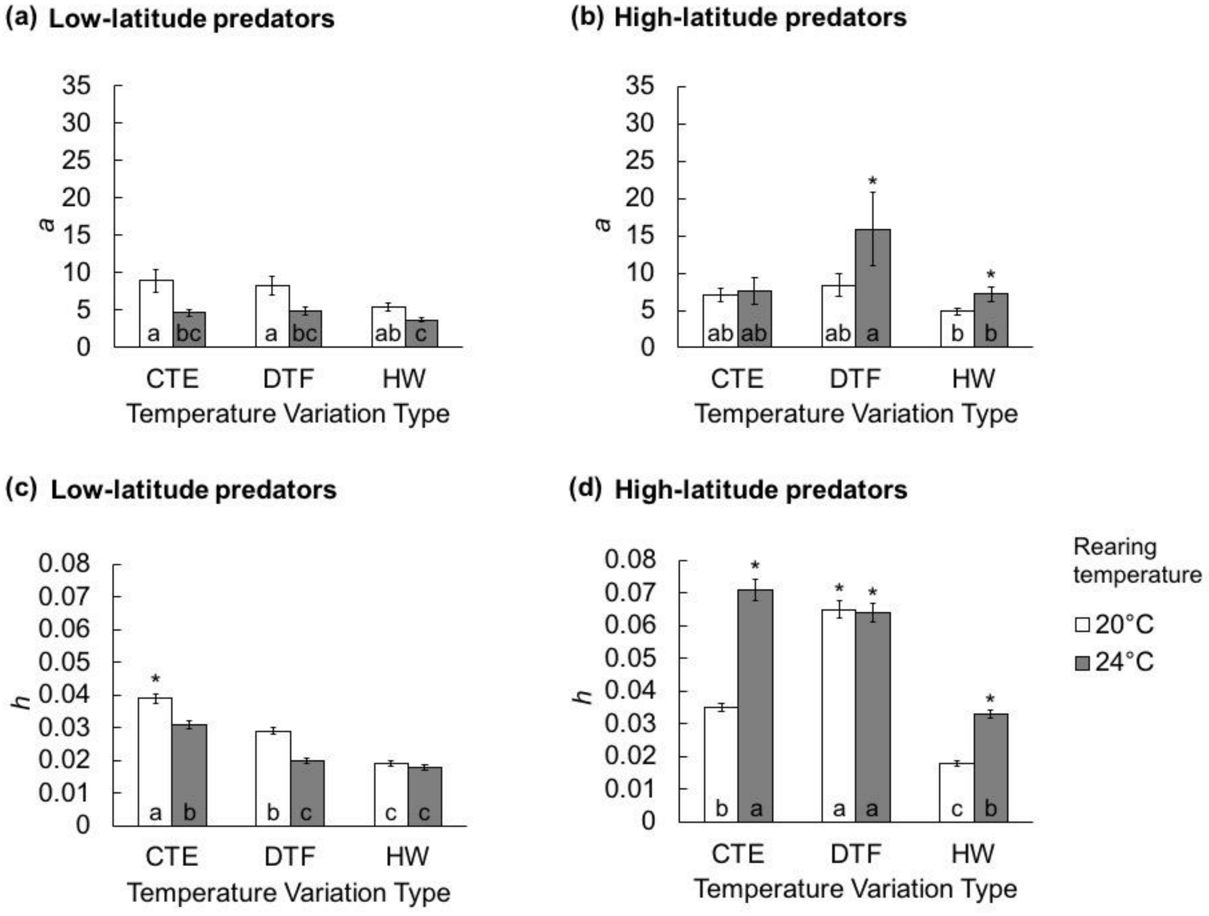

Figure 4. Effects of predator source latitude, mean rearing temperature and temperature variation (CTE = constant, DTF = daily temperature fluctuation of 10 °C, HW = heat wave temperature of 32 °C) on (a-b) the search rates *a* (L day-1) and (c-d) the handling times *h* (day) of *Ischnura elegans* damselfly larvae preying on *Daphnia magna*. Given are means ± 1 SE. Letters denote significant differences between groups within each latitude, and asterisks denote higher values compared to the other latitude based on FDR-corrected *P*-values < 0.05.

Predator–prey long-term interaction strength

Based on the 84% CIs, the long-term interaction strength generally was lower (-38%) at 24 °C compared to 20 °C except for the high-latitude system at DTF and HW (Figure 5). Compared to CTE, DTF increased the interaction strength (+117%) in the high-latitude system at 24 °C (Figure 5b), while the HW reduced it in both the low-latitude (-43%) and the high latitude (-30%) systems (Figure 5).

Focusing on the critical comparisons for the space-for-time substitution (illustrated in Figure 1b), the long-term interaction strength at CTE was reduced (-39%) through thermal plasticity when the high-latitude system experienced a 4 °C higher mean temperature, and was much lower (-64%) in the low-latitude system at 24 °C, suggesting thermal evolution may considerably lower the long-term interaction strength in the high-latitude system (Figure 5). Taking into account also DTF and HW, 4 °C warming did no longer cause a plastic reduction in interaction strength in the high-latitude system but thermal evolution would still do so (-62% at 24 °C-DTF and -11% at 24 °C-HW in the low-latitude system compared to the 20 °C-DTF and 20 °C-HW in the high-latitude system).

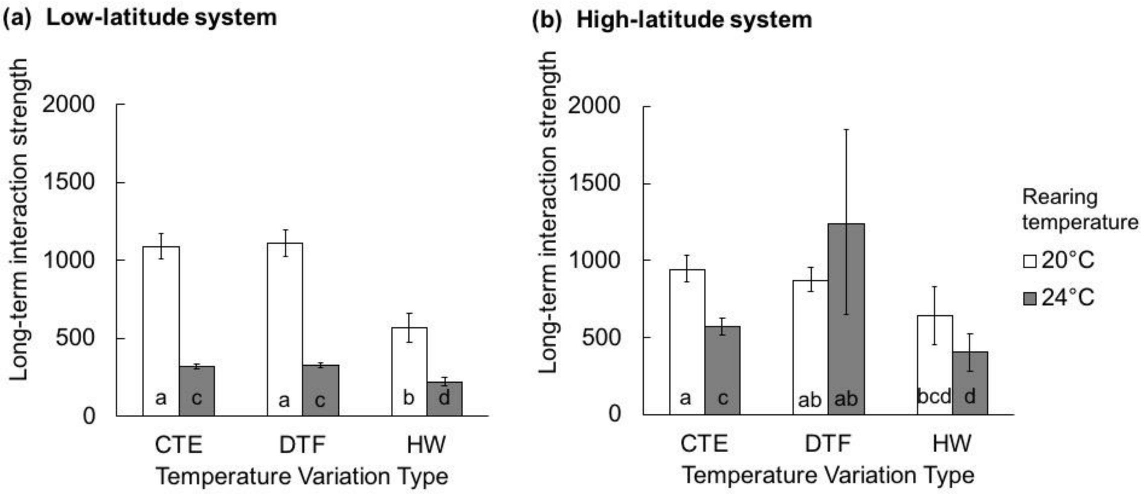

Figure 5. Effect of mean rearing temperature (20 and 24 °C) and temperature variation (CTE = constant, DTF = daily temperature fluctuation of 10 °C, HW = heat wave temperature of 32 °C) on the long-term interaction strength for (a) low-latitude and (b) high-latitude *Ischnura elegans* larvae feeding on *Daphnia magna*. Values given are estimated means with 84% CI. Letters denote significant differences between groups within each latitude.

Discussion

To improve predictions of the impact of global warming on natural systems, we need to consider (i) increases in mean temperature as well as daily temperature fluctuations (DTF) and heat waves (HW) (Vasseur et al. 2014, Colinet et al. 2015, Williams et al. 2016, Stoks et al. 2017, Boukal et al. 2019), (ii) mechanisms underlying trophic interaction strength (Rall et al. 2010, Fussmann et al. 2014, Sentis et al. 2015, Archer et al. 2019), and (iii) thermal evolution (Diamond and Martin, 2016, González-Tokman et al. 2020). By integrating these key aspects in a single study, we obtained several unique insights. First, while a 4 °C warming weakened the long-term interaction strength in the low-latitude system, this effect disappeared in the high-latitude system when warming was combined with DTF or HW. This extends the emerging insight that temperature variation may make ectotherms (in our case the high-latitude system) more vulnerable to warming (Paaijmans et al. 2013, Stoks et al. 2017). Second, the contributions of metabolic rate, search rate and handling time in shaping thermal effects on interaction strength often differed between latitudes. Third, predator thermal evolution, compared to thermal plasticity, could make the high-latitude trophic system more stable under warming even in the presence of DTF or HW.

Thermal patterns in metabolic rate

Metabolic rates did not increase under 4 °C warming, suggesting complete thermal compensation (Seebacher et al. 2015, Havird et al. 2020), thereby confirming the same pattern for cellular respiration in the study species (Van Dievel et al. 2019a). Complete compensation patterns where the ‘passive’ increase in energetically costly biological rates such as metabolic rates is countered can be considered adaptive (Seebacher et al. 2015). Based on the meta-analysis of Havird et al. (2020), complete thermal compensation of metabolic rate has been observed in several studies, albeit partial thermal compensation is more frequent. Our results suggest that, given ample time for acclimation (i.c. from day 10 in the larval stage), *I. elegans* larvae are capable to completely compensate in their final larval instar for increases in metabolic rate that can be expected due to passive thermal plasticity. However, at HW metabolic rates were higher, as also observed before in *I. elegans* (Van Dievel et al. 2017). This may reflect the upregulation of energetically costly defense mechanisms (such as heat shock proteins, Van Dievel et al. 2017). When reared at 24 °C, the increase in metabolic rate under the heat wave was smaller, suggesting that acclimation to warmer conditions reduced the energetic costs of exposure to the HW (Logan and Somero 2011).

Thermal patterns in the functional response parameters

As can be expected for ectotherm predators, the functional response parameters were affected by the thermal treatments (Thompson 1978, Sentis et al. 2012; Twardochleb et al. 2020). Notably, while 4°C warming did not influence the search rates of high-latitude larvae, it decreased those of low-latitude larvae. This contrasts with the pattern of latitude-associated thermal adaptation as we found for growth rate (Appendix B). Different relationships between search rate and temperature have been recorded: positive (Thompson 1978, Twardochleb et al. 2020, Petchey et al. 2010), negative (Grigaltchik et al. 2012), from positive to negative (Englund et al. 2011, Sentis et al. 2012), and no effect (Sentis et al. 2015). Our results may suggest that the thermal reaction curve of low-latitude predator search rates is in the decreasing phase of the quadratic Arrhenius model where biotic reaction rates decrease with temperature above the thermal optimum, a pattern detected in a meta-analysis on search rates by Englund et al. (2011). Search rates of predators may first increase with increasing temperatures when the metabolic rate is high enough to boost bioenergetic activities related with foraging, but then decrease with temperature when metabolic rates decrease by the stronger influence of catabolism (Sentis et al. 2012). A recent study found that the capture success of another damselfly decreased at temperatures above the environmental mean (Twardochleb et al. 2020). This may not have led to an increased foraging effort because metabolic demands did not increase under warming as suggested by the constant metabolic rates. Based on a previous study where capture success increased with aerobic scope (Preest and Pough 2003), we hypothesize that the high-latitude predators did not show decreased search rates at 24 °C because of their lower standard metabolic rate and thus the larger aerobic scope to maintain capture success high at 24 °C compared to low-latitude predators. An alternative hypothesis for decreasing search rates of low-latitude predators at the higher temperature could be an energy-saving strategy. At higher temperatures, the net energy return for energetically costly foraging at low prey densities may become too low, urging predators to lower search activities and allocate energy to prioritize functions such as rapid growth and development (based on Huey and Kingsolver 2019, Sokolova 2021). Such energy-saving strategy is expected to be more important in the low-latitude larvae which have a faster lifestyle (i.e. invest more energy in rapid growth and development) and a higher metabolic rate than high-latitude larvae at 24 °C (Debecker and Stoks 2019, this study).

In line with expectations (Thompson 1978, Sentis et al. 2012, Sentis et al. 2015, Twardochleb et al. 2020), also the handling time was strongly dependent on temperature.

Handling times are mainly determined by digestion time (hence physiology) and the actual time of handling when killing and eating a prey (Sentis et al. 2013b). The digestion time is longer than the actual handling time, and decreases exponentially with temperature (Sentis et al. 2013b). This may explain why handling times decreased at HW, and in low-latitude larvae at the higher mean temperature and at DTF. However, handling times of high-latitude larvae increased at the elevated mean temperature (except at DTF). This suggests high-latitude larvae suffered from the long-term exposure to higher temperatures resulting in increased digestion and actual handling times. In line with thermal adaptation, this resulted in low-latitude larvae having shorter handling times than high-latitude larvae at 24 °C, and high-latitude larvae having shorter handling times than low-latitude larvae at 20 °C CTE. As maximal predation rates are the inverse of the handling times, this reflects latitudinal thermal adaptation in food intake at high prey densities as shown for the predator species feeding on *ad libitum* levels of brine shrimp nauplii (Debecker and Stoks 2019). This may have contributed to the similar latitude-specific thermal adaptation pattern in growth rate (Appendix S2).

Most studies looked at effects of increased mean temperatures on the short-term trophic interaction strength (Thompson 1978, Sentis et al. 2012, Sentis et al. 2015, Twardochleb et al. 2020), while only few investigated the effects of the widespread daily temperature fluctuations (Barton and Schmitz 2018, Vangansbeke et al. 2015) and heat waves (Sentis et al. 2013a, Van Dievel et al. 2017). Notably, these few studies included only a single prey density, hence could not detect the here reported strong dependence of temperature variation effects on prey density. Moreover, our results demonstrate that temperature extremes and fluctuations differentially affect short-term trophic interaction strengths compared to mean temperature increases, and that this further depends on latitude. This adds to the insight that ignoring DTF and HW may result in less accurate estimates of the short-term interaction strengths, and emphasizes the importance to examine the impact of DTF and HW at both low and high prey densities.

Thermal patterns in long-term interaction strength

Consistent with Sentis et al. (2015), the long-term interaction strength decreased under 4 °C warming when assuming a constant temperature. In the simulation, we allowed prey carrying capacity (K) to decrease and prey intrinsic growth rate (r) to increase as the two mechanisms suggested by theory to weaken long-term interaction under warming (Appendix S4) (Rall et al. 2010, Fussmann et al. 2014). However, a third mechanism working through predator metabolic rate as suggested by Rall et al. (2010) and Fussmann et al. (2014), played no role in current study as predator metabolic rates did not increase at the higher mean temperature. In addition, we identified a fourth mechanism how warming may weaken the long-term interaction that moreover was latitude-dependent: by decreasing search rates of low-latitude predators, and by increasing handling times of high-latitude predators at CTE and HW. Also, the heat wave temperature generally seemed to weaken the long-term interaction strength by increasing predator metabolic rates. As a result, the effect of both 4 °C warming and potentially also heat waves on the predators (but see below) are expected to positively contribute to the long-term stability of the here studied trophic system. Note that although higher temperatures in theory could have led to a lower predator energetic efficiency and thus increased the risk of predator extinction, we found no such temperature effects on the energetic efficiency (Appendix S6). Notably, the reduction of the long-term interaction strength under 4 °C warming disappeared in the high-latitude system when temperature variation occurred, hence under this more realistic scenario we expect high-latitude prey-predator systems to be tentatively more vulnerable to population fluctuations under future climate scenarios (in the absence of predator thermal evolution). Our results thereby make an important extension to the insights of Fussmann et al. (2014) that warming generally stabilizes predator-prey dynamics, by demonstrating that such effect critically depends on temperature variation and latitude, and that predator metabolic rate may contribute to strengthen long-term trophic system stability at extreme high temperatures.

We assumed the effects of a heat wave on metabolic rate and functional response parameters and the resulting predator and prey equilibrium densities to be persistent. These parameters, however, likely return to normal conditions after a heat wave. We simulated different levels of the time-integrated influence of heat waves on the functional response parameters and metabolic rate. This indeed indicated the stabilizing role of the heat wave by weakening the long-term interaction strength to become smaller when reducing their time-integrated impact, especially at 20 °C (Appendix S7).

Thermal plasticity vs. thermal evolution

When only assuming thermal plasticity, our results suggest the high-latitude trophic system may become more stable under 4 °C warming unless DTF or potentially HW is considered. Using a space-for-time substitution (De Frenne et al. 2013, Verheyen et al. 2019), our results suggest predator thermal evolution to further stabilize the high-latitude system under warming, and this even with DTF and potentially also with HW (but see higher).

Our study highlighted the need for more realism when assessing the impact of warming on long-term interaction strength by illustrating for the first time the importance of including daily thermal variation and considering besides plasticity also thermal evolution in the predator. Yet, while our study thereby provided valuable new insights, one important limitation toward further realism is that we did not consider thermal evolution in the prey. Previous work showed latitude-related thermal adaptation in the survival of *D. magna* under damselfly predation, but this pattern disappeared when considering the thermal adaptation of the predators (De Block et al. 2013). This may tentatively suggest that thermal evolution of the prey may have less impact compared to thermal evolution of the predator on the pattern of long-term interaction strength. Nevertheless, to refine insights, future studies should also include prey from different latitudes while estimating the climate change impact on trophic interactions in the presence of evolution in both predator and prey.

Conclusion

Our study underscores the importance of not only increases in mean temperatures but also daily thermal fluctuations and heat waves in shaping trophic interactions, and suggests thermal evolution of the predator to ameliorate thermal plastic effects on food web stability. Future studies should, however, also consider the contribution of thermal evolution of the prey in shaping trophic interaction strength. Given that there is increasing evidence of evolution in response to global warming (Merilä and Hendry 2014, González-Tokman et al. 2020), our results of contrasting effects between thermal plasticity and thermal evolution of the predator highlight the importance of integrating evolution to make projections of trophic system stability under warming (Sentis et al. 2015, Daugaard et al. 2019).

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Authors' contributions

RS and AS developed the ideas, and together with YJW designed the experiments. YJW performed the experiments and analyzed the data with strong input from AS, NT and RS. YJW and RS drafted the manuscript after which all authors contributed and gave final approval for publication. The authors declare no conflict of interest.

Appendix S1. Collection sites and rearing information of predators and prey

Table S1. Collection sites of predators and prey

Species	Collection site
I. elegans (predator)	Southern (low-latitude) sites: Bassin de Réaltor, France (43°28'11.1"N - 05°19'44.1"E) Saint-Martin de Crau, France (43°37'57.8"N - 04°46'55.1"E) La Durance, France (43°43'52.5"N - 05°44'53.0"E)
	Northern (high-latitude) sites: Roskilde, Denmark (55°39'09.8"N - 12°08'01.7"E) Kalmar Dämme, Sweden (56°40'04.6"N – 16°17'46.5"E) Hovgardsdammarna, Sweden (57°14'24.3"N - 12°08'28.2"E)
D. magna (prey)	Mid-latitude site: Oud-Heverlee, Leuven, Belgium (50°51'49.0"N - 4°43'24.4"E)

We cultured the eggs of *I. elegans* from field-collected females (14-15 females per population) and the *Daphnia* clone separately in aged tap water at a constant temperature of 22°C (maximum deviation of ± 0.2 °C) and a 14:10 L:D photoperiod. Ten days after hatching, the damselfly larvae were reared individually in 100 mL cups filled with aged tap water and allocated to incubators at one of the temperature treatments (see Figure 1a). Throughout the rearing, the damselfly larvae were fed daily *ad libitum* with nauplii of *Artemia salina,* and the *Daphnia* were fed daily with *Acutodesmus obliquus* green algae (1×10^5 cells mL^{-1}). The culture medium was regularly renewed.

Appendix S2. Thermal and latitudinal patterns in damselfly growth rates

Low-latitude larvae had higher growth rates compared to high-latitude larvae at 24°C-CTE and 24°C-DTF ($P < 0.0001$ and $P = 0.0073$), while high-latitude populations had higher growth rates compared to low-latitude larvae at 20°C CTE ($P < 0.0001$) and 20°C-HW ($P = 0.0004$) (Figure S1). Growth rates of low-latitude larvae were highest at 24°C-CTE and 24°C-DTF, and lowest at 20°C-CTE. Growth rates of high-latitude larvae were equally high at 20°C-CTE and 20°C-HW, and were lower and similar at 20°C-DTF and all temperature variation levels at 24°C. Consistent with latitude-associated thermal adaptation in the predator (Debecker and Stoks, 2019), low-latitude *I. elegans* larvae had a higher growth rate at 24°C-CTE while high-latitude larvae had a higher growth rate at 20°C-CTE. This pattern became less pronounced at DTF and HW.

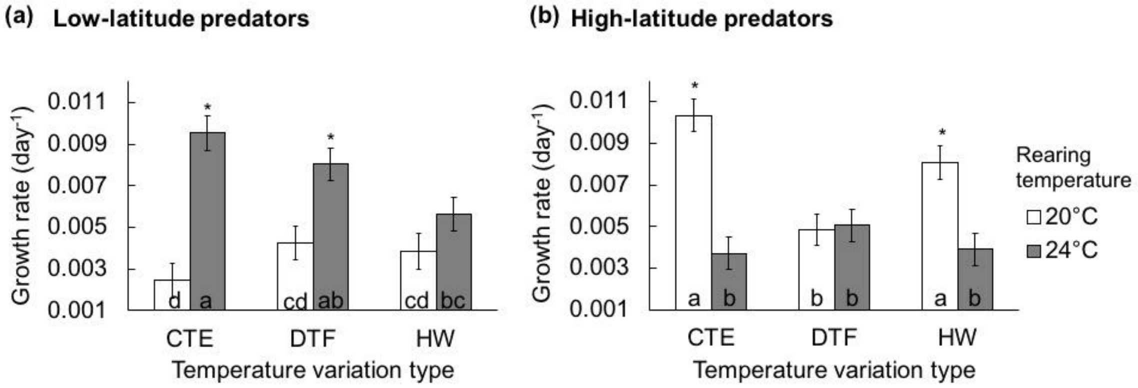


Figure S1. Effects of predator source latitude, mean rearing temperature and temperature variation (CTE = constant, DTF = daily temperature fluctuation of 10°C, HW = heat wave temperature of 32°C) on the growth rate (day^{-1}) of (a) low-latitude and (b) high-latitude *Ischnura elegans* damselfly larvae. Given are least square means ± 1 SE. Letters denote significant differences (FDR-corrected $P < 0.05$) among groups within each latitude. Asterisks denote significantly (FDR-corrected $P < 0.05$) higher values compared with the same thermal treatment combination of the other latitude.

Appendix S3. Determination of the functional response type

Predators of both latitudes showed a type II functional response across all temperature treatments. This matches the similar type II functional responses for the same predator-prey couple obtained for a single UK population across a range of constant temperatures (from 5°C up to 27.5°C) (Thompson 1978), and for multiple UK populations across an urban-rural gradient at constant 20°C (Villalobos-Jiménez et al. 2017). This aligns with studies on dragonfly and notonectid where temperature also had no effect on the functional response type (Sentis et al., 2015; Wasserman et al., 2016), while contrasting with a study on ciliate that documented a switch of the functional response from stabilizing type III to destabilizing type II in the presence of warming (Daugaard et al., 2019). This suggests the predator-prey system we studied could already be relatively unstable compared to a trophic system with type III functional response.

Table S2. Estimates of the first-order term of the logistic regressions on predator proportional consumption over prey density (function *frair_test*, package *frair* in R). Negative estimates represent a type II functional response (i.e. declining proportional consumption with increasing prey density), while positive estimates represent a type III functional response (i.e. initial increasing and subsequent decreasing proportional consumption with increasing prey density).

Latitude	Mean rearing temperature	Thermal variation	Estimate	*z*-value	*P*-value
low-latitude	20°C	CTE	-0.0272747	-21.530	<0.0001
low-latitude	20°C	DTF	-0.029735	-23.321	<0.0001
low-latitude	20°C	HW	-0.0249139	-18.470	<0.0001
low-latitude	24°C	CTE	-0.0262195	-22.18	<0.0001
low-latitude	24°C	DTF	-0.025417	-21.023	<0.0001
low-latitude	24°C	HW	-0.0204806	-17.469	<0.0001
high-latitude	20°C	CTE	-0.0300719	-24.793	<0.0001
high-latitude	20°C	DTF	-0.0282319	-23.343	<0.0001
high-latitude	20°C	HW	-0.0243510	-18.758	<0.0001
high-latitude	24°C	CTE	-0.0253050	-19.92	<0.0001
high-latitude	24°C	DTF	-0.0300134	-23.228	<0.0001
high-latitude	24°C	HW	-0.0247055	-21.673	<0.0001

Appendix S4. Detailed procedure to estimate long-term interaction strength

The predator-prey dynamics was described using the Rosenzweig-MacArthur model (Smith 2008):

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right) - \frac{aNP}{ahN+1} \text{ (Equation S1)}$$

$$\frac{dP}{dt} = \frac{e_c aNP}{ahN+1} - cm_p P \quad \text{(Equation S2)}$$

where t is time (days), N and P are prey and predator densities (ind L^{-1}), K is the carrying capacity of the prey in the absence of the predator (ind L^{-1}). Parameter m_p is the metabolic rate (J h^{-1}) of the predator, which in this study was directly estimated from oxygen consumption. Parameter c converts the metabolic rate to predator individuals per day (J^{-1} h to ind day^{-1}). Parameter e_c is the predator assimilation efficiency converting consumed prey into predator individuals (0.92 for *I. elegans*, Van Dievel et al. 2019b) × mean prey body mass × mean predator body $mass^{-1}$. Parameters a (search rate) and h (handling time) were estimated from the functional response curves. Prey intrinsic population growth rate r (day^{-1}) was calculated following Binzer et al. (2012) and Fussmann et al. (2014) :

$$r = r_0 \omega^{b_r} exp^{(-E_r/(kT))} \text{ (Equation S3)}$$

where r_0 is a normalization constant independent of body size and temperature (11.66 × 1013 day^{-1}), ω is the prey body mass (in µg) which was derived from body length, b_r is an allometric exponent (−0.25), E_r is the activation energy of r for invertebrates (0.84 eV, Savage et al. 2004a), k is the Boltzmann's constant (8.62×10^{-5} eV K^{-1}), and T is the environmental temperature (in K). We assumed that the carrying capacity K depends on temperature and resource body mass following:

$$K = K_0 \omega^{b_K} exp^{(-E_K/(kT))} \text{ (Equation S4)}$$

where b_K is an allometric exponent (−0.72), E_K is the activation energy of K for invertebrates (−0.77 eV), and K_0 is a normalization constant independent of body size and temperature (Rall et al. 2010, Fussmann et al. 2014). Following the approach of Sentis et al. (2015), we varied the intercept K_0 from 0 to 10 to simulate an increasing level of enrichment, hence to evaluate the consistency of our findings across a range of enrichment levels. This corresponds to the range of prey carrying capacities between 0 and 300 ind L^{-1}, which matches the *Daphnia* densities in our experiment and in the field. We assumed that the intrinsic population growth rate r and the carrying capacity K for *Daphnia* depend only on mean developmental temperatures and not on temperature fluctuations. Note that the theoretically assumed increasing r and decreasing K at higher temperatures have been empirically observed in *D. magna* (Van Doorslaer et al. 2005, Pratt 1943).

To characterize the long-term per capita interaction strength I_L, we used the dynamic index that calculates the log-ratio interaction strength (Rall et al. 2010) from the predator-free equilibrium (N, P) = (N^{-}, 0) where $N^{-} = K$, and the predator–prey equilibrium (N, P) = (N^{+}, P^{+}):

$$I_L = \left|\frac{\ln\left(N^{+}/N^{-}\right)}{P^{+}}\right| \text{ (Equation S5)}$$

The equilibria were obtained by setting the left-hand side of Equations 1 and 2 to zero and solving for *N* and *P*, which yields:

$$N^{+} = \frac{cm_p}{a(e_c - cm_p h)} \quad \text{(Equation S6)}$$

$$P^{+} = r\left\{\frac{cm_p}{a(e_c - cm_p h)}\left(h - \frac{1}{aK}\right) - \frac{h}{K}\left[\frac{cm_p}{a(e_c - cm_p h)}\right]^2 + \frac{1}{a}\right\} \text{ (Equation S7)}$$

We calculated the 84% CIs of the long-term interaction strength by propagating the standard errors associated with the group estimates of each experimental parameter (search rate, handling time and metabolic rate) based on the law of propagation of uncertainty (Rice 2007). Group estimates of search rate and handling times were averaged if they did not differ based on multiple comparisons using false-discover-rate (FDR) adjusted p-values. Group estimates of long-term interaction strength with non-overlapping 84% CIs were considered as significantly different.

Because the enrichment level had little influence on the long-term interaction strength and did not influence the differences among thermal treatments (Figure S2), we reported only the results at an intermediate enrichment level ($K_0 = 5$) in the main text.

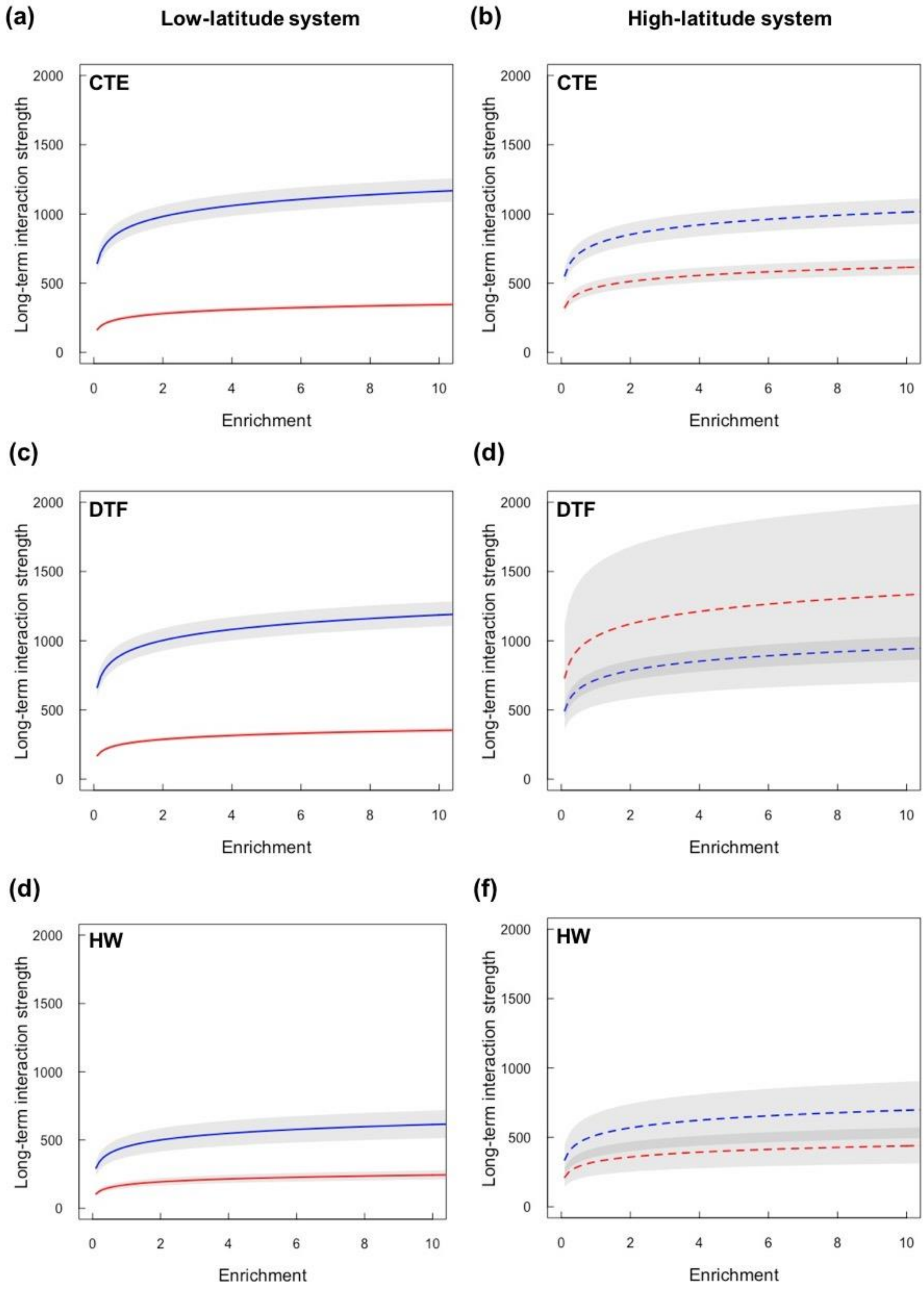


Figure S2. Effect of enrichment (K_0) on long-term interaction strength between the predator *I. elegans* and the prey *D. magna* at (a-b) CTE, (c-d) DTF, and (e-f) HW for low- and high-latitude predators at 20 °C (blue) and 24 °C (red). Shaded areas represent 84% CIs.

Appendix S5. Pairwise comparisons between functional response parameter estimates

Table S3. Comparisons between functional response parameter estimates for *Ischnura elegans* damselfly larvae between latitudes (L = low-latitude, H = high-latitude), mean rearing temperatures (20°C and 24°C) and temperature variation levels (CTE = constant, DTF = daily temperature fluctuation of 10°C, and HW = heat wave temperature of 32°C). D = difference between the search rates (*a*) and handling times (*h*) among treatments. *P*-values were false discovery rate (FDR)-corrected based on the number of pairwise comparisons (15 comparisons within each latitude and 6 comparisons between latitudes). Significant values are highlighted in bold.

Treatment 1 (T1)	Treatment 2 (T2)	D	Estimate (T1-T2)	SE	*z*	*P*
L-CTE-20°C	L-CTE-24°C	***a***	4.29217	1.58149	2.7146	**0.0250**
		h	0.00757	0.00183	4.1274	**<0.0001**
L-DTF-20°C	L-DTF-24°C	***a***	3.42263	1.37972	2.4807	**0.0281**
		h	0.00851	0.00124	6.8714	**<0.0001**
L-HW-20°C	L-HW-24°C	***a***	1.70385	0.65441	2.6037	**0.0250**
		h	0.00122	0.00107	1.1369	0.2738
L-CTE-20°C	L-DTF-20°C	*a*	0.69010	1.99167	0.3465	0.7290
		h	0.01032	0.00173	5.9666	**<0.0001**
L-CTE-20°C	L-HW-20°C	*a*	3.33043	1.55183	2.1461	0.0597
		h	0.01958	0.00158	12.4058	**<0.0001**
L-DTF-20°C	L-HW-20°C	*a*	2.89112	1.41935	2.0369	0.06249
		h	0.00941	0.00127	7.4202	**<0.0001**
L-CTE-24°C	L-DTF-24°C	*a*	-0.24400	0.67700	-0.3604	0.7290
		h	0.01121	0.00138	8.1237	**<0.0001**
L-CTE-24°C	L-HW-24°C	*a*	0.93307	0.58826	1.5862	0.1537
		h	0.01334	0.00142	9.4135	**<0.0001**
L-DTF-24°C	L-HW-24°C	*a*	1.17432	0.05595	2.0989	0.0597
		h	0.00212	0.00104	2.0413	0.0515
L-CTE-20°C	L-DTF-24°C	***a***	4.04396	1.56982	2.5761	**0.0250**
		h	0.01878	0.00156	12.047	**<0.0001**

L-CTE-20°C	L-HW-24°C	***a***	5.22507	1.53509	3.4038	**0.0050**
		h	0.0209	0.00159	13.1316	**<0.0001**
L-CTE-24°C	L-DTF-20°C	***a***	-3.66167	1.39045	-2.6334	**0.0250**
		h	0.00271	0.00157	1.7254	0.0975
L-CTE-24°C	L-HW-20°C	*a*	-0.77141	0.75725	-1.0187	0.3855
		h	0.01212	0.00141	8.6100	**<0.0001**
L-DTF-20°C	L-HW-24°C	***a***	4.59731	1.33833	3.4351	**0.0050**
		h	0.01063	0.00128	8.3096	**<0.0001**
L-DTF-24°C	L-HW-20°C	*a*	-0.53081	0.73558	-0.7216	0.5429
		h	0.0009	0.00102	0.8773	0.3800
H-CTE-20°C	H-CTE-24°C	*a*	-0.42820	2.00990	-0.2130	0.8907
		h	-0.03558	0.00358	-9.9384	**<0.0001**
H-DTF-20°C	H-DTF-24°C	*a*	-7.47904	5.13100	-1.4576	0.2717
		h	0.00089	0.00372	0.2403	0.8101
H-HW-20°C	H-HW-24°C	*a*	-2.25714	1.10687	-2.0392	0.1243
		h	-0.01501	0.00133	-11.3003	**<0.0001**
H-CTE-20°C	H-DTF-20°C	*a*	-1.38752	1.70530	-0.8136	0.6237
		h	-0.02979	0.00280	-10.6546	**<0.0001**
H-CTE-20°C	H-HW-20°C	*a*	2.09072	1.00030	2.0901	0.1243
		h	0.01708	0.00137	12.4527	**<0.0001**
H-DTF-20°C	H-HW-20°C	*a*	3.32550	1.47088	2.2609	0.1189
		h	0.04672	0.00261	17.9223	**<0.0001**
H-CTE-24°C	H-DTF-24°C	*a*	-8.31213	5.25118	-1.5829	0.2430
		h	0.00682	0.00435	1.5650	0.1475
H-CTE-24°C	H-HW-24°C	*a*	0.54727	2.15709	0.2537	0.8907
		h	0.03798	0.00361	10.5195	**<0.0001**
H-DTF-24°C	H-HW-24°C	***a***	7.75373	0.49740	15.5880	**<0.0001**
		h	0.03055	0.00208	14.6730	**<0.0001**
H-CTE-20°C	H-DTF-24°C	*a*	-8.88252	5.01282	-1.7720	0.1910
		h	-0.02890	0.00298	-9.7123	**<0.0001**
H-CTE-20°C	H-HW-24°C	*a*	-0.16503	1.35321	-0.1220	0.9029
		h	0.00207	0.00165	1.2581	0.2229
H-CTE-24°C	H-DTF-20°C	*a*	-0.82654	2.34000	-0.3532	0.8907
		h	0.00594	0.00424	1.4013	0.1858

H-CTE-24°C	H-HW-20°C	*a*	2.13746	1.63809	1.3049	0.3200
		h	0.05209	0.00336	15.5256	**<0.0001**
H-DTF-20°C	H-HW-24°C	*a*	1.19671	1.76590	0.6777	0.6791
		h	0.03184	0.00277	11.4811	**<0.0001**
H-DTF-24°C	H-HW-20°C	***a***	8.74109	0.22032	39.6750	**<0.0001**
		h	0.04496	0.00194	23.182	**<0.0001**
L-CTE-20°C	H-CTE-20°C	*a*	1.95656	1.74998	1.1180	0.3953
		h	0.00372	0.00183	2.0301	0.0508
L-DTF-20°C	H-DTF-20°C	*a*	-0.06126	1.94865	-0.0314	0.9749
		h	-0.03634	0.00273	-13.3135	**<0.0001**
L-HW-20°C	H-HW-20°C	*a*	0.52571	0.72355	0.7266	0.5610
		h	0.00112	0.00102	1.1044	0.2690
L-CTE-24°C	H-CTE-24°C	*a*	-2.90356	1.90253	-1.5262	0.2540
		h	-0.03958	0.00360	-10.9948	**<0.0001**
L-DTF-24°C	H-DTF-24°C	***a***	-10.98329	0.00400	-2747.6130	**<0.0001**
		h	-0.04396	0.00190	-23.1130	**<0.0001**
L-HW-24°C	H-HW-24°C	***a***	-3.43378	1.06257	-3.2316	**<0.0001**
		h	-0.01511	0.00137	-11.0014	**<0.0001**

Appendix S6. Thermal and latitudinal patterns in the predator energetic efficiency

Values for predator energetic efficiency (EE) were calculated following Sentis et al. (2012) as:

$$EE = \omega F / \lambda I \quad \text{(Equation S8)}$$

where ω is the assimilation efficiency (0.92 for *I. elegans*, Van Dievel et al. 2019a), F is the maximal per capita energy feeding rate (J h^{-1}) estimated from handling time h (day) and prey body mass $m = 0.001$ g (m/h = maximal per capita daily feeding biomass; 1g biomass = 7000 J), λ is a constant (set at 3) converting standard metabolic rate into field metabolic rate (Savage et al. 2004b), and I is the per capita standard metabolic rate (J h^{-1}).

Based on overlap of the 84% confidence intervals, there was no significant effect of mean rearing temperature on the energetic efficiency of *I. elegans* larvae. Low-latitude larvae had a higher energetic efficiency at 24 °C-DTF compared to 24 °C-CTE (Figure S3). High-latitude larvae had lower energetic efficiencies at 20 °C-DTF compared to 20°C-CTE and 20 °C-HW. Latitudinal differences were only significant at DTF where low-latitude predators had higher energetic efficiencies compared to low-latitude predators.

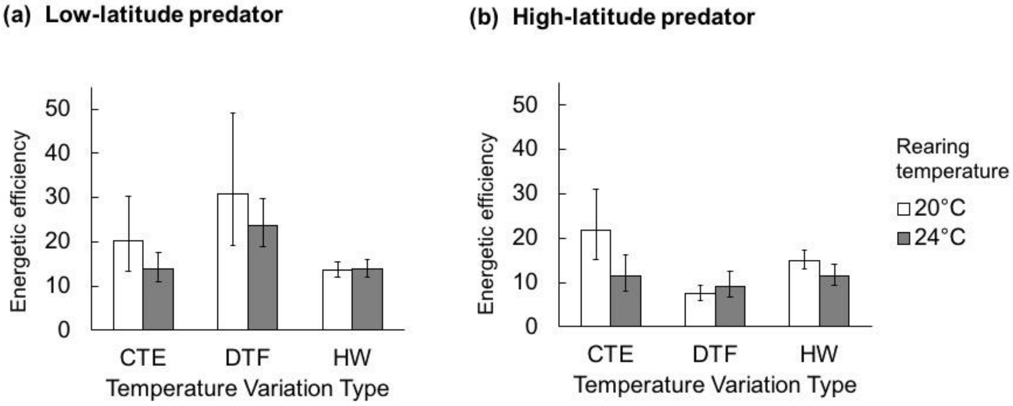


Figure S3. Effect of predator mean rearing temperatures (20 and 24 °C) and three levels of temperature variation (CTE = constant, DTF = 10 °C daily temperature fluctuation, HW = 32 °C heat wave) on the energetic efficiency of the predator *I. elegans*. Given are estimated means with 84% CIs.

Appendix S7. Simulated time-integrated influence of the heat wave on long-term interaction strength

In our quantification of the effect of a heat wave on long-term interaction strength, we assumed a continuous, long-lasting effect of the heat wave until the equilibrium densities of predator and prey were reached. This is, however, not realistic and would overestimate the long-term impact of heat waves. Indeed, the functional response parameters, the metabolic rate and hence the resulting long-term equilibrium densities are expected to return from their values under a heat wave temperature toward their values under mean temperatures. To assess this reversibility of the heat wave influence on the predator and prey equilibrium densities we simulated several scenarios where the heat wave influenced these densities 100% of the time (the scenario reported in the main manuscript) down toward 0% of the time (the scenario of constant mean temperatures without a heat wave).

To do this, we calculated the resulting values for long-term interaction strength I_L based on the equation:

$$I_L = |\ln(N'/N^-)/P'| \text{ (Equation 9)}$$

where N^- is the prey equilibrium density when the predator is absent (= carrying capacity) at mean rearing temperatures, N' the HW-affected prey equilibrium density when the predator is present, and P' the HW-affected predator equilibrium density. Hereby, both HW-affected equilibrium densities are calculated as weighted averages of the estimated equilibrium densities at the constant mean rearing temperature (CTE) and the heatwave (HW) temperature following the equations:

$$N' = (\delta N_{\mathrm{HW}} + (100-\delta)\, N_{\mathrm{CTE}}) /100 \quad \text{(Equation S10)}$$

$$\text{and } P' = (\delta P_{\mathrm{HW}} + (100-\delta)\, P_{\mathrm{CTE}}) /100 \quad \text{(Equation S11)}$$

where δ is the percent of the time where the parameters are under the heat wave influence, N_{HW} and P_{HW} are the prey and predator equilibrium densities at HW ($\delta = 100$), and N_{CTE} and P_{CTE} are the prey and predator equilibrium densities at CTE ($\delta = 0$). Note the latter four values are those used in the main manuscript for the HW- and CTE-scenarios. Based on these weighted averages of prey and predator equilibrium densities we then estimated the time-integrated effect of different values of the HW influence on long-term interaction strength.

This simulation indicated that when the heat wave influence decreased, the long-term interaction strength in both the low- and high-latitude trophic systems increased, especially at the mean temperature of 20 °C (Figure S4). This suggests that if the duration of heatwaves is shorter, or if the reversibility of functional response parameters is faster, heatwaves will stabilize the trophic systems less, especially at 20 °C.

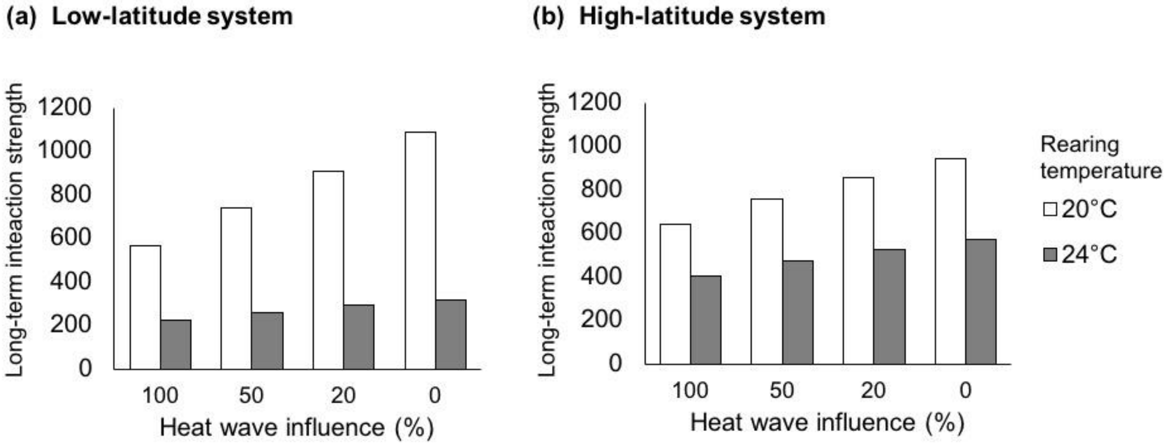


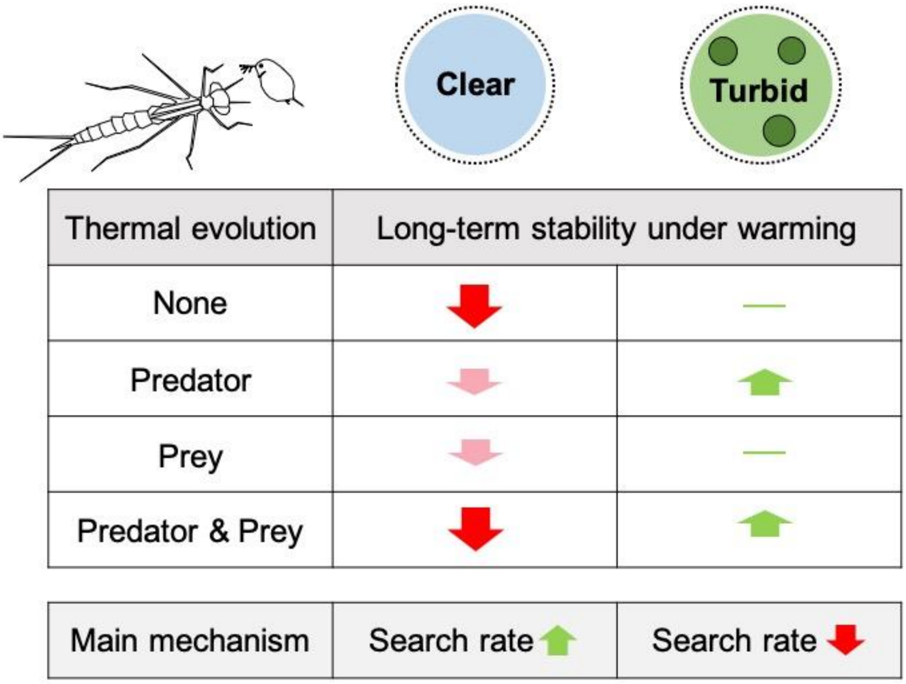
Figure S4. Effect of a heat wave on long-term interaction strength of the predator *I. elegans* and the prey *D. magna* for (a) the low-latitude and (b) the high-latitude predator-prey system under varying time-integrated levels of heat wave influence.

Chapter IV

Turbidity reverses how plasticity and evolution shape predator-prey interactions under warming

Ying-Jie Wang, Arnaud Sentis, Nedim Tüzün and Robby Stoks

Journal of Animal Ecology (submitted)
Slightly adapted.



Thermal evolution	Long-term stability under warming	
None	⬇	—
Predator	↓	⬆
Prey	↓	—
Predator & Prey	⬇	⬆

Main mechanism	Search rate ⬆	Search rate ⬇

Abstract

Warming and eutrophication are major global change-related factors negatively affecting freshwater ecosystems by modifying physiological rates, trophic interactions and/or increasing water turbidity. To advance insights in their impact, we need to consider how these stressors jointly affect predator-prey interactions, and to what extent these effects may be modulated by thermal evolution of both the predator and the prey. We quantified how 4°C warming and algae-induced turbidity (a key response to eutrophication in pond food webs) affect functional response parameters and prey population parameters in a common-garden experiment with all four combinations of high- and low-latitude predator and prey populations. This was done to assess the potential impact of thermal evolution of predators and/or prey at a high latitude under warming using a space-for-time substitution. We then modelled effects on long-term predator-prey interaction strengths (the long-term effects of the predator population on the prey population) between damselfly larvae and water fleas under different warming, turbidity and evolutionary scenarios. Warming weakened long-term interaction strengths for the system with low-latitude predators in turbid water, while it strengthened long-term interaction strengths for the system with high-latitude predators and prey in clear water. The latter contrasts with previous findings that warming will stabilize trophic system dynamics. Algae-induced turbidity generally increased long-term interaction strengths, resembling findings that nutrient enrichment could make trophic systems less stable. Patterns in long-term interaction strengths were mainly driven by search rates. A key finding was that the expected stability of the high-latitude trophic system under warming was critically dependent on the turbidity level: our results on long-term interaction strength suggest that thermal plasticity will destabilize the high-latitude trophic system under warming in clear water but not in turbid water, and that thermal evolution of the predator will stabilize the high-latitude system under warming in turbid water but not in clear water. These results highlight that the extent to which thermal evolution may oppose thermal plastic effects on trophic system stability may strongly differ between clear and turbid water bodies.

Introduction

Whether global change will threaten the local persistence of populations and the stability of ecosystems will crucially depend on how populations can cope with the increasing co-exposure to stressors (Côté et al. 2016, Orr et al. 2020) and how trophic interactions are modified (Filazzola et al. 2020, Urban et al. 2016). Nevertheless, both important insights have rarely been integrated in a single study. Warming and eutrophication are two major global change-related factors negatively affecting freshwater ecosystems (Birk et al. 2020, Hering et al. 2015, Moss 2011, Woodward et al. 2010). Eutrophication increases algae growth, and may thereby cause freshwater ecosystems to switch from a clear to a turbid state that may impair ecosystem functioning (Scheffer et al. 2001, Hilt et al. 2017). Zooplankton grazers play an important role in the transitions between algae-induced turbidity states given their potential to suppress algae populations (Lampert et al. 1986, Scheffer et al. 2001, Rogers et al. 2020). Interestingly, algae-induced turbidity may also change the interactions of zooplankton grazers with their predators (Jönsson et al. 2011, Kimbell and Morrell 2016). Warming may not only magnify the effects of eutrophication on algae-induced turbidity (Anneville et al. 2015, Moss 2011), but on itself also impair ecosystem functioning (García et al. 2018). While both warming (e.g. Twardochleb et al. 2020) and turbidity (e.g. Shaw et al. 2006, Kimbell and Morrell 2016) may affect trophic interactions, their combined effects on trophic interactions remain largely unexplored (but see Figueiredo et al. 2019).

Empirical studies have shown that warming may both increase and decrease the short-term trophic interaction strength (i.e. predation rate) (Englund et al. 2011, Sentis et al. 2012, Uiterwaal and DeLong 2020). Short-term predation rates typically increase and then reach a plateau with increasing prey densities, a relationship described by the functional response model (Holling 1959). Functional responses are characterized by search rates (also known as attack rates) and handling times, which reflect predation rates at low and high prey densities, respectively (Holling 1959). Thermal effects on functional response parameters obtained in the laboratory have been shown to match patterns in field settings, hence can be used to predict warming effectsin natural systems (Archer et al. 2019). On the one hand, Twardochleb et al. (2020) documented warming to increase the short-term interaction strength by increasing predator search rates and by shortening handling times. On the other hand, Grigaltchik et al. (2012) and South and Dick (2017) showed warming to decrease short-term interaction strength at low prey densities by decreasing search rates. One theoretical explanation to reconcile these opposite patterns is that the thermal response patterns of search rates and handling times, hence of the resulting short-term interaction strengths, may be unimodal (Englund et al. 2011, Sentis et al. 2012, Uiterwaal and DeLong 2020). Hence, depending on what side of the unimodal response curve warming is happening, it can lead to increases or decreases in the functional response parameters. Also turbidity is known to affect the functional response. An increase in turbidity has been found to decrease short-term interaction strength by lowering search rates for both visual and non-visual aquatic predators (reviewed by Ortega et al. 2020) and less so by increasing handling times (e.g. Allen-Ankins et al. 2012). Yet, also for turbidity opposite patterns have been observed (e.g. Shaw et al. 2006). Taken together, warming and turbidity have the potential to change short-term interaction strength in the same or opposite directions asking for empirical studies on their combined effects.

Despite their importance for trophic system stability, much less is known about the long-term effects of warming and turbidity on trophic interactions. Warming is expected to decrease the long-term interaction strength (i.e. the long-term effect of the predator population on the prey population) via increasing prey intrinsic growth rate, decreasing prey carrying capacity, and increasing predator metabolic rate (Fussmann et al. 2014, Rall et al. 2010). In addition, recent empirical evidence suggests that warming-induced decreases in search rates and increases in handling times may also contribute to a decrease in long-term interaction strength (Wang et al. 2021). Together this should cause warming to stabilize predator-prey interactions by lowering population fluctuations (Binzer et al. 2012, Kratina et al. 2012, Rosenblatt et al. 2019, Sentis et al. 2015). Yet, higher predator metabolic rates under warming may act destabilizing by causing a low energetic efficiency leading to predator extinctions (Vucic-Pestic et al. 2011). As an increase in algae-induced turbidity also implies more resources for grazers, it may also decrease the long-term interaction strength between zooplankton grazers and their predators via increasing the grazer’s intrinsic growth rate (Giebelhausen and Lampert 2001) unless this effect is balanced by a higher carrying capacity of the grazers (Bosker et al. 2019, Kratina et al. 2012). The few studies on warming and turbidity have, however, never combined them to explicitly investigate how these factors jointly shape the long-term interaction strength, hence trophic system stability.

Studies on how global change-related stressors affect trophic interactions in the long term typically only consider acute and/or plastic responses in predator and prey (Daugaard et al. 2019, Sentis et al. 2015). Nevertheless, there is increasing evidence that besides plasticity also evolution may play an important role in shaping responses to global change (Stoks et al. 2014, Urban et al. 2016, Wang et al. 2021). Importantly, thermal evolution can play a crucial role reversing maladaptive thermal responses or enhancing adaptive ones (Diamond and Martin

2016). One powerful way to test the potential impact of thermal evolution is to apply a "space-for-time substitution", where the current phenotypes of warm-adapted populations at their local high mean temperature are viewed as the future phenotypes of cold-adapted populations after thermal evolution (De Frenne et al. 2013, Verheyen et al. 2019). Typically, such studies contrast low- and high-latitude populations whose temperature difference matches the predicted temperature increase under a certain IPCC warming scenario at the high latitude. Without difference in turbidity between latitudes (as reported by Dodds et al. 2019, Stephens et al. 2015), such latitude-based space-for-time substitution may inform how thermal evolution can change the long-term trophic interaction strength, and whether this differs between clear and turbid water bodies. Ideally, such studies should consider evolution in both predator and prey (De Block et al. 2013, Laws 2017). In a rare example study, it was shown that the outcome of short-term predator-prey interactions between damselfly larvae and water fleas depended on the interplay of thermal evolution of each interacting species (De Block et al. 2013). Yet, no studies so far tried to assess the effects of evolution in both predator and prey on predation rates at multiple prey densities, needed to assess effects on long-term interaction strength, or considered the potential mediating effect of turbidity.

In this study, we examined (i) how warming and turbidity in isolation and when combined affect the short-term and long-term interaction strengths between a predator and its prey, and (ii) how thermal evolution in the predator and/or in the prey may modulate these effects. As model invertebrate predator-prey system we used the larvae of the damselfly *Ischnura elegans* preying on the water flea *Daphnia magna*, a textbook example for the functional response in pond food webs (Begon et al. 2006). *Daphnia magna* is a key zooplankton grazer, thereby playing an important role in suppressing algae-induced turbidity (Lampert et al. 1986, Rogers et al. 2020). The effects of warming and turbidity on the interaction strengths of this grazer with its predators is therefore relevant for the occurrence of algae blooms. To increase realism we studied algae-induced turbidity (as key response to eutrophication in pond food webs) that integrates both turbidity *per se* and an increased food level for zooplankton grazers. As aquatic ectotherms that often occur in shallow water bodies, they are vulnerable to warming (Woodward et al. 2010). To apply a space-for-time substitution, we studied predator and prey populations from replicated low- and high-latitude populations in Europe, which show latitude-associated thermal adaptation (De Block et al. 2013, Debecker and Stoks 2019). In a previous study using the same biological system, we investigated the impact of predator thermal evolution and warming on trophic interaction strength (Wang et al. 2021). We here extend this study in two important ways: (i) by testing the combined effects of warming and turbidity on predator-prey interactions, and (ii) by testing for effects of thermal evolution in both predator and prey. Based on previous studies between predatory damselfly larvae and their daphnid prey, we expected warming to result in higher search rates and shorter handling times (Twardochleb et al. 2020). Based on the meta-analysis by Ortega et al. (2020), we expected turbidity to result in lower search rates, potentially due to the lower accuracy of locating the prey. Given that warming and turbidity may have opposite effects on search rate and handling times, we expected the effects of warming to increase the long-term interaction strength in clear water, but less so or even the reverse in turbid water. We accordingly also expected the plastic and evolutionary effects of warming on long-term interaction strength to differ between clear and turbid conditions.

Materials and methods

Study populations and rearing

For both predator and prey we studied three populations at low-latitude (southern France) and three populations at high-latitude (southern Scandinavia) sites. All populations inhabited shallow lakes with abundant vegetation that had both damselflies and water fleas present. In line with other studies (Dodds et al. 2019, Stephens et al. 2015, Wang et al. 2020), no latitudinal difference in turbidity could be detected between the here studied high- and low-latitude study sites (Appendix S1). To avoid joint adaptation of predator and prey at the population level we sampled different sites for the predators and for the prey.

For the predator *I. elegans*, the low-latitude populations in southern France were Bassin de Réaltor (43°28'11.1"N - 05°19'44.1"E), Saint-Martin de Crau (43°37'57.8"N - 04°46'55.1"E) and La Durance (43°43'52.5"N - 05°44'53.0"E). The high-latitude populations were from Denmark: Roskilde, (55°39'09.8"N - 12°08'01.7"E), and Sweden: Kalmar Dämme (56°40'04.6"N – 16°17'46.5"E) and Hovgardsdammarna (57°14'24.3"N - 12°08'28.2"E). In each population 14-15 mated females were separately put in cups with wet filter paper for oviposition. Egg clutches were transported to the laboratory and kept separately in plastic containers filled with dechlorinated tap water in incubators at a constant temperature of 22 °C (maximum deviation of ± 0.2 °C) and a 14:10 L:D photoperiod. Larvae were transferred individually to 100 mL plastic cups filled with dechlorinated tap water and allocated to incubators at 20 °C and 24 °C (maximum deviation of ± 0.2 °C) from day 10 after hatching until 4-5 days after their molt into the final (F0) instar when the predation trials were run. This period (> 8 weeks) allowed ample time for thermal acclimation. Throughout the rearing, larvae were fed daily *ad libitum* with nauplii of *Artemia salina*.

For the prey *D. magna*, the low-latitude populations in France were Grand Clos (43°29'09.6"N – 04°48'53.8"E), North Mireval (43°30'54.3"N – 3°49'22.6"E), and South Mireval (43°30'29.3"N – 3°48'52.6"E). The high-latitude populations were from Sweden: Lake Bysjön (55°40'27.3"N – 13°32'47.5"E), Norway: Pond Asklund (63°35'15.9"N – 10°43'44.2"E), and Denmark: Lake Ring (55°57'54.9"N – 9°35'48.3"E). We randomly selected three clones in each population and acclimatized them for multiple (> 3) generations in 1 L glass vials at 20 °C or 24 °C with a 14:10 L:D photoperiod. The culture medium was regularly renewed, and the *Daphnia* were fed daily *ad libitum* the green alga *Acutodesmus obliquus* (1×10^5 cells mL^{-1}).

Based on the prey numbers required for the predation trials, we created sets of 66 *Daphnia* juveniles (< 24h-old, mixture of three clones of the population) for each of the six populations in 1 L glass vials. To standardize the physiological age and size, we used 3-day-old juveniles for the predation trials at 24 °C, and 4-day-old juveniles for the predation trials at 20 °C (based on De Block et al. 2013). For each population and temperature regime, we collected an extra set of 10 juvenile *Daphnia* to measure mean body mass to the nearest 0.01 mg to parametrize the demographic model (see below).

Experimental design and general procedure

To examine the effects of predator and prey latitude, temperature and turbidity on the short- and long-term predator-prey interaction strengths, we used a full factorial experiment with 16 treatments: 2 predator source latitudes (high or low latitude) × 2 prey source latitudes (high or low latitude) × 2 temperatures (20 or 24 °C) × 2 turbidity levels (clear or turbid). The rearing temperatures correspond to the mean summer water temperatures of the shallow lakes inhabited by the populations in the high- (20 °C) and low- (24 °C) latitude regions (De Block et al. 2013; Debecker and Stoks 2019, Dinh Van et al. 2014). The 4 °C temperature difference also represents the predicted temperature increase at the high-latitude sites by 2100 according to IPCC (2014) scenario RCP8.5. This allows for a space-for-time substitution to examine the effects of evolution of high-latitude predators and prey under global warming (De Frenne et al. 2013, Verheyen et al. 2019). We used two turbidity levels, corresponding to clear (0.1 NTU) and turbid (6 NTU, corresponding to a Secchi depth of ca. 12 cm) states of the shallow water bodies that serve as habitats for both the prey and the predators (Van de Meutter et al. 2005). Both turbidity levels can be found in water bodies inhabited by the study species in the low- and high-latitude regions (Derot et al. 2020, Faafeng and Mjelde 1998, Van Wichelen et al. 2013, Appendix S1). As we were specifically interested in algae-induced turbidity as a consequence of eutrophication we did not manipulate turbidity through sediment addition (as done in other studies, e.g. Allen-Ankins et al. 2012) but by directly manipulating the concentration of algae. Notably, it was shown that predators may be more sensitive to algae-induced turbidity compared to sediment-induced turbidity (Nieman et al. 2018). Moreover, only by using edible algae we could mimic the realistic double effect of eutrophication on zooplankton grazers: increasing their food level and changing interactions with their predators (here the damselfly larvae).

To assess effects on the strengths of short- and long-term predator-prey interactions, we conducted a functional response experiment for each of the 16 treatment combinations. We then estimated functional response parameters (search rates and handling times), and used these estimated parameter values together with prey body mass and predator metabolic rates at 20 °C and 24 °C that were quantified in a previous study (Wang et al. 2021) to parametrize population dynamic models and predict long-term interaction strengths.

Functional response experiment

We estimated the search rate *a* and the handling time *h* by reconstructing the functional response curve for each of the 16 treatment combinations. For each combination, we conducted 6-hour predation trials in daytime (from 10:00 to 16:00) (based on Sentis et al. 2017) with 5-6 prey densities: 6, 12, 15, 30, 48, and 66 *Daphnia* individuals L^{-1}. The highest density of 66 *Daphnia* L^{-1} was only used at 24 °C as at 20 °C the maximum number of *Daphnia* eaten at 48 *Daphnia* L^{-1} was 29 and thus the maximum feeding rate was reached at this density. For each prey density at each predator latitude × prey latitude × temperature combination, 18 damselflies (6 from each of the 3 populations at a given latitude) were individually combined with *Daphnia* of one population (one of the 3 *Daphnia* populations at a given latitude). Before each trial, damselfly larvae were starved for 18 h to standardize hunger levels. Each damselfly larva was tested twice (on days 4 and 5 days after molting into the final instar, F0): once in the clear and once in the turbid conditions. The order of testing in clear versus turbid water was random, and an equal number of larvae were tested for both orders at each turbidity level. The order of testing and predator identity did not affect the results (all *P values* > 0.05) and was thus not accounted for in the following statistical analyses. In total, 792 predation trials were conducted with 396 damselfly larvae.

The predation trials were conducted in white 2 L (18.0 × 13.3 × 12.6 cm) arenas filled with 1 L water at the test (= rearing) temperatures. For the clear water treatment, the medium contained only dechlorinated tap water with a turbidity level of 0.1 NTU, which is close to the turbidity level of the *Daphnia* cultures that were fed daily with algae (<0.5 NTU). For the turbid water treatment, we also added 15 mL green algal solution (1×10^5 cells mL^{-1}) that was added at the start of the predation trials to realize a turbidity level of 6 NTU. In a preliminary test, this turbidity level did not differ after 6 h (at the end of the predation trials) between the treatment temperatures and *Daphnia* densities (all $P > 0.05$), while at longer duration the turbidity level could not be maintained. The *Daphnia* prey were introduced at one of the prey densities into the arenas, 0.5 h later one damselfly larva was released at the bottom and the predation trial started. At the end of each trial, the damselfly larvae were removed from the arenas, and the remaining *Daphnia* were counted to calculate predation rates. To account for wasteful killing, the Daphnia that had less than 50% of their bodies missing were considered as not consumed (as in Thompson 1975). Natural mortality of *Daphnia* was assessed in 5 replicates per prey population × temperature × turbidity treatment at the density of 48 *Daphnia* L^{-1} in predator-free arenas. Natural mortality of *Daphnia* was negligible (ranging between 0% and 2% per trial) and thus not accounted for in the statistical analyses and results. Predation rates were measured as the number of *Daphnia* consumed per damselfly larva per 6 h.

Statistical analyses and modelling

All statistical analyses were executed using R v3.6.3 (R Core Team 2020). To analyze the functional response curves, we followed the procedure developed by Pritchard et al. (2017, *package frair*). Based on the identified functional response type (function *frair_test*, Appendix S2), we fitted the predation rates of the 16 treatment groups with the type II Rogers' random predator equation accounting for prey depletion (Rogers 1972, function *frair_fit*) to estimate the search rate a (L day^{-1}) and handling time h (day):

$$N_e = N_0\left(1 - exp^{-a(t-N_e h)}\right) \text{ (Equation 1)}$$

where N_e is the number of prey consumed; N_0 is the initial prey density; a is the instantaneous search rate of the predator (L day^{-1}) determined by searching activity and capture success; h is the handling time the predator spends ingesting and digesting a prey (day); T is the predation time (day). The fitted functional response curves were bootstrapped (n = 999) to visualize 95% confidence intervals (function *frair_boot*, package *frair*).

To analyze effects of the treatments (predator and prey source latitudes, temperature and turbidity) on the functional response parameters, we pairwise compared the search rate a and handling time h between treatment combinations using the “indicator variable” approach (function *frair_compare*, package *frair*; as e.g. used in Siepielski et al. 2020, Villalobos-Jiménez et al. 2017). We performed multiple comparisons within and between latitudes, and adjusted *P*-values with the False Discovery Rate-method (Benjamini and Hochberg 1995). The “indicator variable” approach uses the combined data from both compared groups to generate a single functional response curve for estimating differences in the values of the functional response parameters. It thus provides a more powerful statistical test than the observation of the overlap among confidence intervals for detecting parameter differences between treatment groups (e.g. Pritchard et al. 2017, Villalobos-Jiménez et al. 2017).

To estimate the long-term interaction strength, we followed the procedure of Sentis et al. (2015) with parameters defined in Table S3 (Appendix S3). The predator-prey dynamics was described by the following model:

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right) - \frac{aNP}{ahN+1} \text{ (Equation 2)}$$

$$\frac{dP}{dt} = \frac{e_c aNP}{ahN+1} - cm_p P \qquad \text{(Equation 3)}$$

This model integrates the Rosenzweig-MacArthur model (Smith 2008) where predators demonstrate a type II functional response. We empirically estimated prey intrinsic population growth rate r (day^{-1}) and carrying capacity K (ind L^{-1}) for each of the 8 prey latitude × temperature × turbidity treatments in two additional *Daphnia* experiments (Appendix S4). As estimate for the long-term per capita interaction strength I_L, we used the dynamic index of the log-ratio interaction strength (Berlow et al. 1999, Rall et al. 2010)

$$I_L = \left|\frac{\ln\left(N^+/N^-\right)}{P^+}\right| \text{ (Equation 4)}$$

where $N^- = K$ and (N^+, P^+) were obtained by setting the left-hand side of Equations 2 and 3 to zero. For the type II functional response the solutions yield:

$$N^+ = \frac{cm_p}{a(e_c - cm_p h)} \text{ (Equation 5)}$$

$$P^+ = r\left\{\frac{cm_p}{a(e_c - cm_p h)}\left(h - \frac{1}{aK}\right) - \frac{h}{K}\left[\frac{cm_p}{a(e_c - cm_p h)}\right]^2 + \frac{1}{a}\right\} \text{ (Equation 6)}$$

We calculated the 84% CIs of the long-term interaction strength by propagating the standard errors associated with the group estimates of each experimental parameter (search rate, handling time, metabolic rate, intrinsic growth rate and carrying capacity) using the law of propagation of uncertainty (Rice 2007, Spiess et al. 2018). Mean long-term interaction strength with non-overlapping 84% CI were considered as significantly different. Overlap among 84% CIs was used as this matches with a *P*-value smaller than 0.05 of the pairwise comparison (Payton et al. 2003; e.g. Harris et al. 2020). To understand how turbidity affects the direction of thermal evolution of the long-term interaction strength, we made explicit comparisons for the space-for-time substitution separately in clear and turbid water (see Figure 4).

Results

Functional response parameters

For all 16 combinations of predator latitude, prey latitude, temperature and turbidity treatments, prey consumption increased with prey density following a type II functional response (Figure 1). Pairwise comparisons using the “indicator variable” approach showed that predator search rates and handling times depended on the combination of predator laittude, prey laittude, temperature and turbidity (Figures 1 and 2, Appendix S5).

Warming caused higher (+19%) search rates of low-latitude predators paired with low-latitude prey in clear water (Figure 2a), but lower (-19%) search rates for low-latitude predators with both prey types in turbid water (Figure 2a and 2c). Instead, warming had no significant effect on search rates of high-latitude predators (Figure 2b and 2d). Turbidity resulted in search rates of low-latitude predators paired with low-latitude prey being higher (+19%) at 20 °C, yet lower (-17°C) at 24 °C compared to turbid water at 20 °C (Figure 2c). As a results, for low-latitude predators, the lowest values of search rate were obtained in turbid warm water. Turbidity caused higher search rates in high-latitude predators at 24 °C when paired with low-latitude prey (+19%; +34%, Figure 2b). Low-latitude predators had higher (+53%) search rates compared to high-latitude predators when paired with low-latitude prey in clear water at 24 °C, while high-latitude predators had higher (+38%) search rates compared to low-latitude predators when paired with high-latitude prey at 24 °C.

Warming generally caused shorter (-42%) handling times of low-latitude predators (Figure 2a and 2c) but had no significant effect on the handling times of high-latitude predators (Figure 2b and 2d). Turbidity in general tended to shorten handling times of low-latitude predators (Figure 1), yet this reduction (-20%) was only significant when paired with high-latitude prey at 24 °C (Figure 2). Instead, turbidity had no significant effect on the handling time of high-latitude predators. High-latitude predators had overall longer (+73%) handling times compared to low-latitude predators (yet this was not significant in clear water at 20 °C).

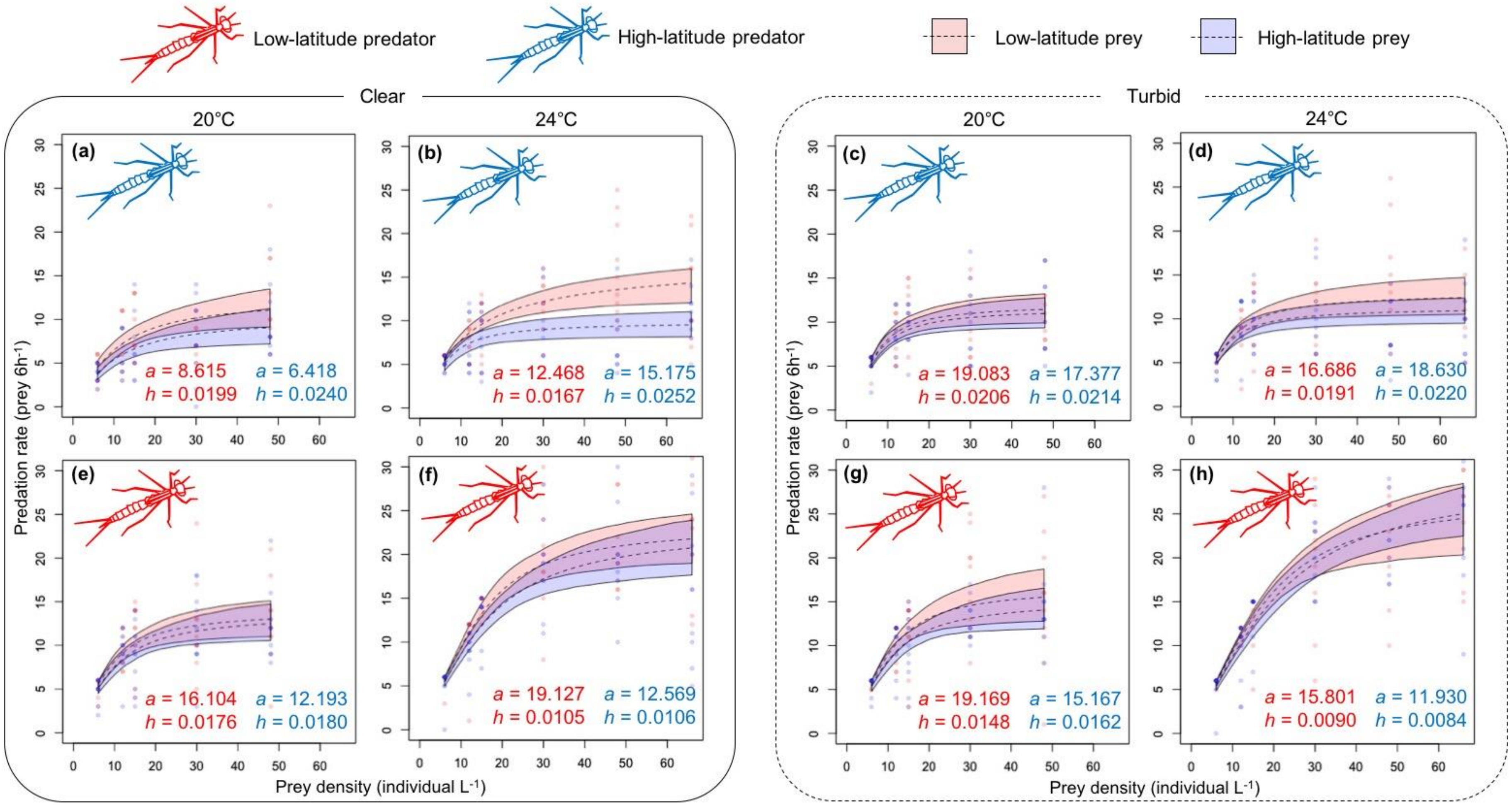


Figure 1. Functional response curves of (a-d) high-latitude and (e-h) low-latitude *Ischnura elegans* damselfly larvae preying on low-latitude (red curves) and high-latitude (blue curves) *Daphnia magna* water fleas under the four combinations of two temperatures (20 and 24 °C) crossed with two turbidity levels (clear and turbid water). Shaded areas represent 95% confidence intervals.

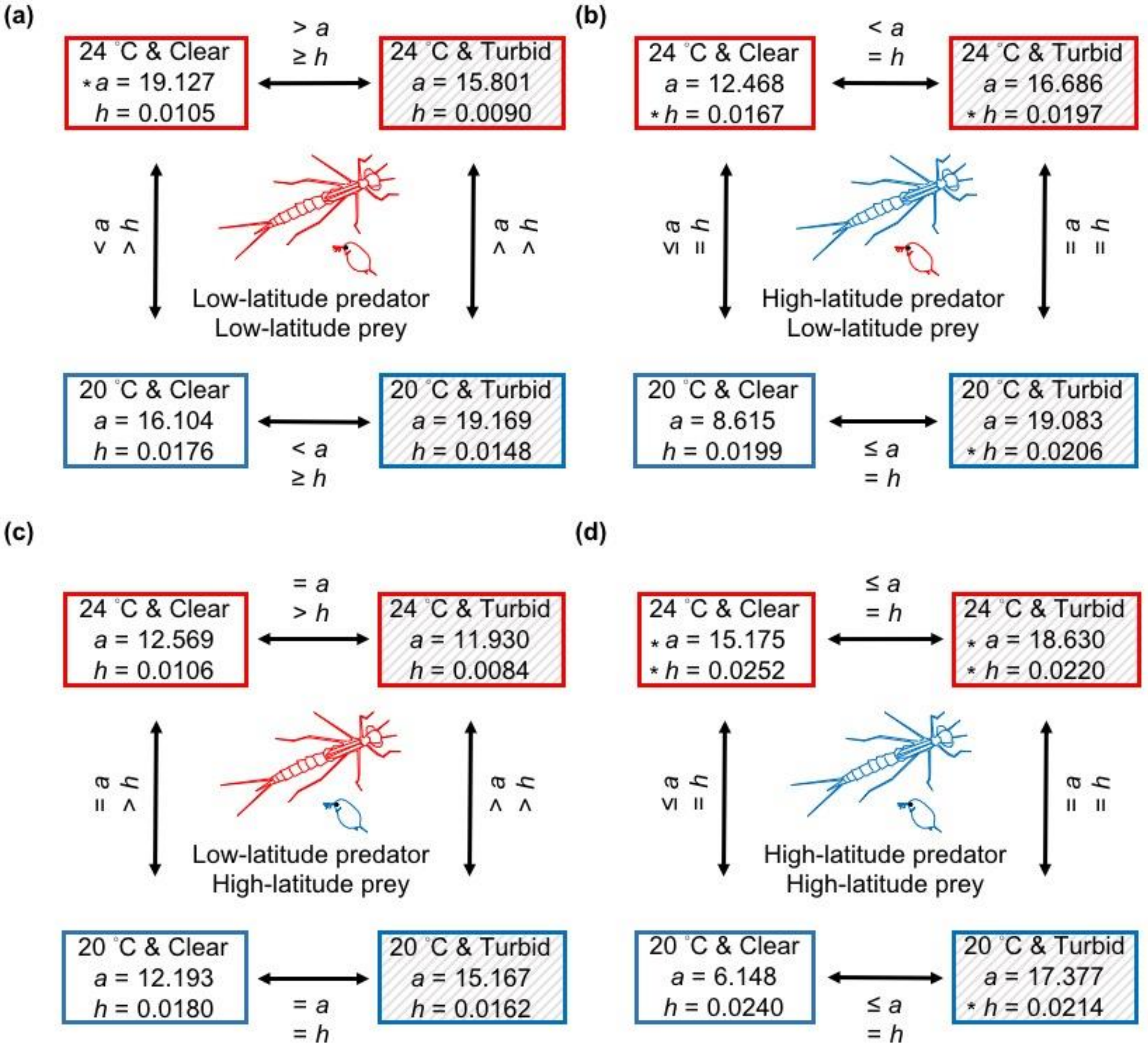


Figure 2. Effects of temperature (20 and 24 °C) and turbidity (clear and turbid) on the search rates (a, L day^{-1}) and handling times (h, day) of *Ischnura elegans* damselfly larvae preying on *Daphnia magna* water fleas. Differences in search rates and handling times between treatment combinations are shown next to the bidirectional arrow bars ($<$ or $>$ reflect differences at $\alpha = 0.05$, $\leq$ and $\geq$ reflect non-significant trends; False Discovery Rate-corrected comparisons). Asterisks beside the parameters denote significant ($P < 0.05$) higher values compared with the same treatment combination of the other predator latitude ($\alpha = 0.05$, False Discovery Rate-corrected comparisons).

Predator–prey long-term interaction strength

Based on the 84% CIs, in the combinations with low-latitude predators warming did not affect the long-term interaction strengths in clear water but reduced these (-35%) in turbid water both with low and high-latitude prey (Figure 3). In the combinations with high-latitude predators, warming had no effect in most conditions except for an increase (+ 154%) in long-term interaction strengths when combined with high-latitude prey in clear water, while warming in combination with turbidity caused higher (+177%) long-term interaction strengths compared to reatments in clear water without warming (Figure 3). Turbidity caused higher (range from + 33% to +257%) long-term interaction strengths for all combinations except for the “low-latitude predator & low-latitude prey” system at 24 °C.

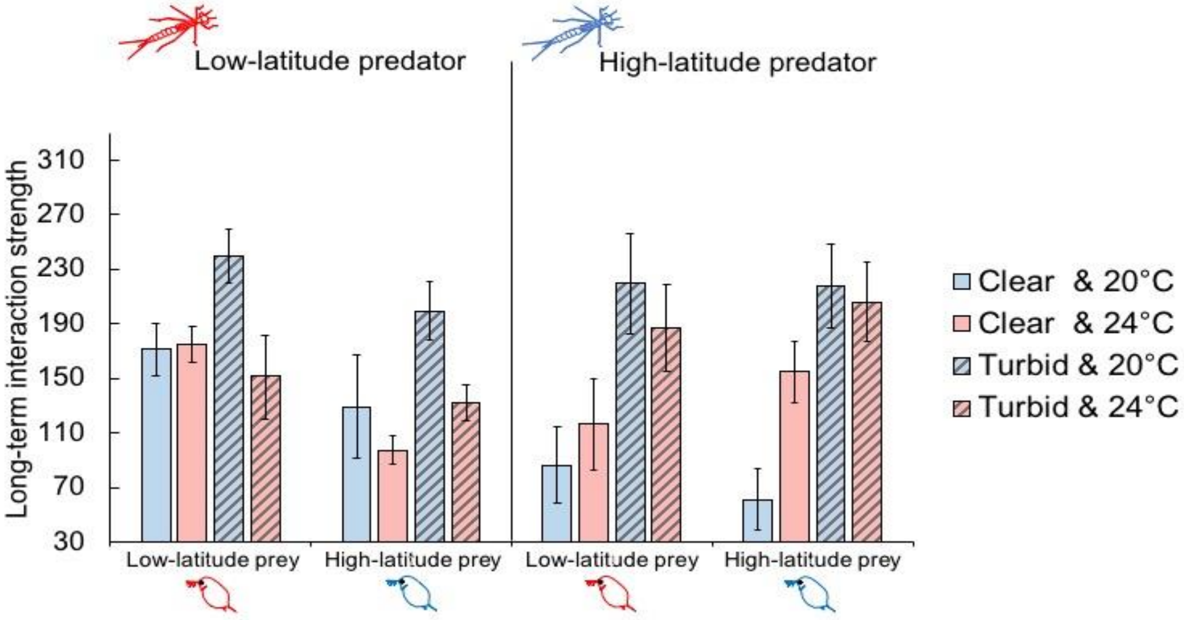


Figure 3. Effects of predator source latitude, prey source latitude, temperature and turbidity on long-term interaction strength for larvae of the predator *Ischnura elegans* feeding on *Daphnia magna* prey. Values given are estimated means with 84% CI.

Focusing on the critical comparisons for the space-for-time substitution in clear water, the long-term interaction strengths were consistently higher (range from +60% to +187%) in the four combinations at 24 °C compared to the baseline “high-latitude predator & high-latitude prey” system at 20 °C (Figure 4a). This indicates that both thermal plasticity and thermal evolution of predator and/or prey may considerably increase the long-term interaction strength under warming in clear water at the high latitude (Figure 4a). Interestingly, the impact of thermal evolution of both predator and prey was larger than the impact of evolution of only predator or only prey, but similar to the impact of thermal plasticity. In contrast, in turbid water, the long-term interaction strengths were statistically lower (range from -30% to -39%) in the combinations with low-latitude predators at 24 °C compared to the baseline “high-latitude predator & high-latitude prey” system at 20 °C (Figure 4b). This suggests that thermal evolution of the predator alone or together with the thermal evolution of the prey may lower the long-term interaction strength in the high-latitude system under warming in turbid water. Instead, thermal plasticity in the “high-latitude predator & high-latitude prey” system or only thermal evolution of the prey had no significant impact on the long-term interaction strength under warming. Overall, the increases in long-term interactions strengths with thermal plasticity or thermal evolution observed in clear water was either reversed or cancelled in turbid water.

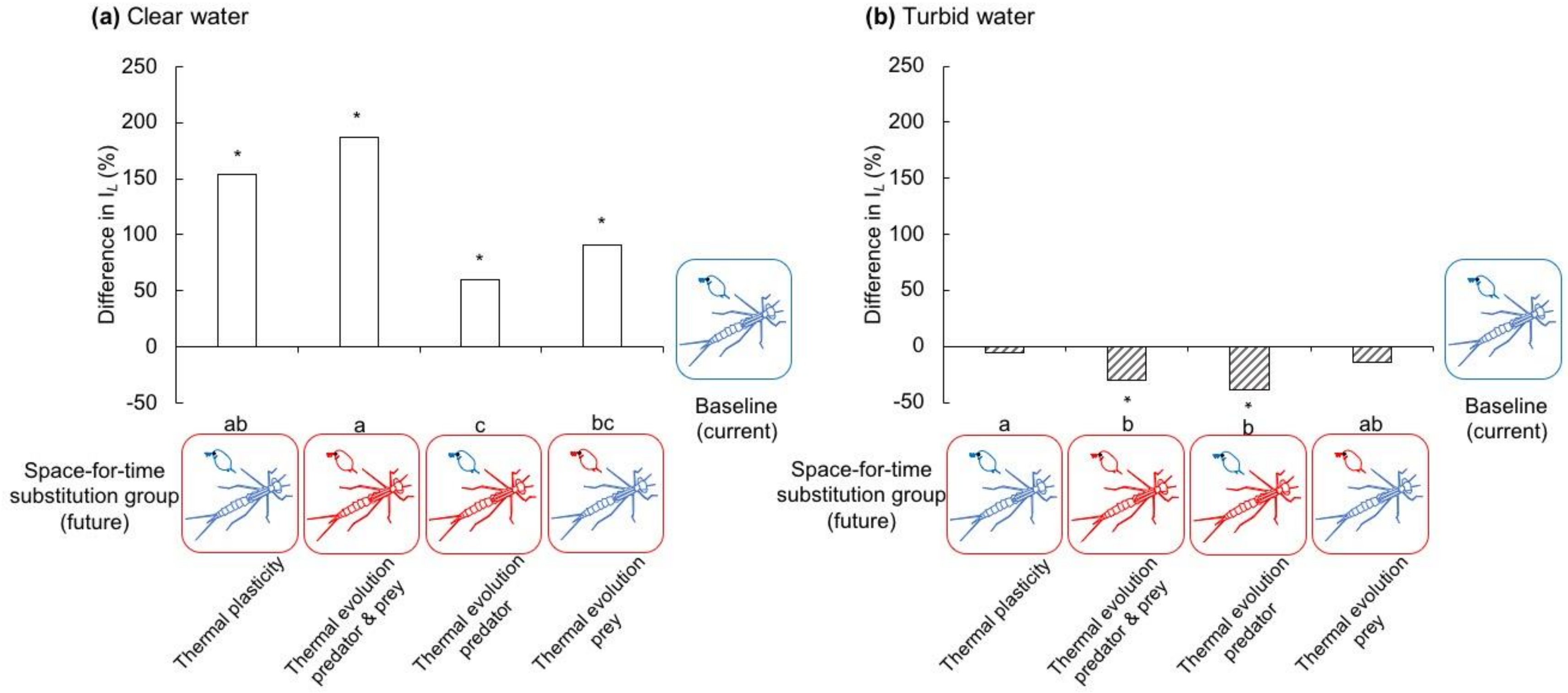


Figure 4. Effects of thermal plasticity and thermal evolution on the long-term interaction strength I_L in the high-latitude predator-prey system under warming in (a) clear and (b) turbid water. Given are mean differences in I_L (%) between each of the four groups at 24 °C relevant for the space-for-time substitution (only plasticity and no evolution, evolution of both predator and prey, evolution of only predator, and evolution of only prey) expressed relative to the baseline group representing the current high-latitude trophic system (high-latitude predator & high-latitude prey at 20 °C). Asterisks denote significant ($P < 0.05$) differences in I_L compared with the baseline group at the same turbidity level. Letters above the four groups at 24 °C relevant for the space-for-time substitution denote differences between these groups in I_L.

Discussion

We examined how two global change stressors, warming and eutrophication (i.c. turbidity), modify interaction strengths between a predator and its prey, and how predator and/or prey thermal evolution can modulate these effects. Our results provide three key insights in the effects of warming and turbidity, and particularly how warming effects may critically depend on turbidity: (1) 4 °C warming weakened the long-term interaction strengths for the low-latitude predators in turbid water, but strengthened it for the high-latitude predators and prey in clear water. The latter contrasts with previous finding showing that warming will stabilize predator-prey dynamics by decreasing long-term interaction strength (Fussmann et al. 2014, Sentis et al. 2015). (2) The overall effect of turbidity was to strengthen long-term interaction strengths, which suggests the effect of turbidity goes in the same direction as the effect of nutrient enrichment in reducing the stability of food webs (Binzer et al. 2012, Kratina et al. 2012). (3) Thermal evolution of predators and/or prey increased the long-term interaction strength in clear water, thereby potentially destabilizing the high-latitude system under warming. However, these effects strongly depended on turbidity level as thermal evolution of predators had the opposite effect on the high-latitude system in turbid water.

Effects of warming and turbidity on the functional response parameters

Consistent with the general patterns identified in previous studies (Englund et al. 2011, Ortega et al. 2020), the functional response parameters (i.e. search rates and handling times) were affected by temperature and turbidity. Notably, the effects of warming on search rates depended on the turbidity level and were only significant for low-latitude predators. The reductions in handling times under warming and turbidity were, if anything, only present for low-latitude predators. This highlights the importance to consider latitudinal differentiation of trophic interactions in response to warming and turbidity (for warming: Amarasekare 2019, De Block et al. 2013, Gauzens et al. 2020, Marino et al. 2018).

In line with two recent case studies that documented that the effect of warming on predator population biomass may critically depend on turbidity (Symons et al. 2019, van Dorst et al. 2019), the effect of warming on search rates of low-latitude predators reversed from positive in clear water to negative in turbid water. Warming can enhance the acceleration and speed of predators and thereby the encounter rate with prey (Dell et al. 2014, Thompson 1978, Twardochleb et al. 2020). Instead, turbidity may reduce the rate of prey detection and attack success (Higham et al. 2015, Ortega et al. 2020). This may explain why warming increased search rates of low-latitude predators in clear water, while in turbid water the negative effect of turbidity may have overruled the positive effect of warming on search rates. We hypothesize that in turbid water, predators did decrease their search effort at the higher temperature to conserve energy as they faced a lower capture success, though we cannot exclude the possibility that the results were driven by changes in the prey behavior or swimming speed to escape predators. Yet, this assumed negative effect of turbidity on prey detection and attack success (Ortega et al. 2020) cannot explain that turbidity increased search rates for low-latitude prey of low-latitude predators at 20 °C and of high-latitude predators. This may instead be explained by the low-latitude prey showing weaker anti-predator responses in turbid water. Turbidity may have weakened the anti-predator responses of the prey as they received less visual predator cues and, as algae-induced turbidity was imposed, they also experienced relaxed food stress (Kimbell and Morrell 2015, Meager et al. 2006, Pauwels et al. 2010) and thereby may have been swimming slower (Larsson and Kleiven 1996). This is more likely to play a role in low-latitude prey as these have higher intrinsic growth rates compared to high-latitude prey (Appendix S4), hence are under stronger pressure to forage. This also highlights the importance of the use of edible algae as turbidity source when testing effects of eutrophication-driven increased turbidity, for the foraging effect could have been overlooked if sediment or non-edible algae would have been used.

The negative effect of warming on handling times of low-latitude predators was consistent across turbidity levels. This can be explained by the handling times mainly being driven by digestion times (hence physiology and not behaviour) which shorten at higher temperatures (Sentis et al. 2013b). Studies in clear water also showed warming to reduce handling times in damselfly larvae feeding on *Daphnia* (Thompson 1978, Twardochleb et al. 2020) and in other predator-prey systems (Rall et al. 2012, Sentis et al. 2012). In contrast, the effect of turbidity con handling time was generally insignificant. The latter is likely because the main component of handling time (time to digest the prey; Sentis et al. 2013b) was not affected by turbidity.

A key finding was that the functional response parameters consistently differed between both latitudes and were much more affected by warming and turbidity in low-latitude predators. Low-latitude predators had shorter handling times compared to high-latitude predators (not significant in clear water at 20 °C). As lower handling times match higher maximal predation rates (Hassell, 2000), this reflects the latitudinal difference in pace of life with the fast-paced low-latitude predators having a higher food intake compared to the slow-paced high-latitude predators at *ad libitum* food levels (Debecker and Stoks 2019). Similar foraging patterns have also been observed in insect and seed consumers whose predation rates increased toward lower latitudes (Hargreaves et al. 2019, Jeanne 1979, Michalko et al. 2019, Roslin et al. 2017). This selection for high predation rates in the low-latitude populations may also have contributed to their higher responsiveness to warming. Indeed, only low-latitude predators showed higher search rates (in clear water) and shorter handling times under warming. This matches the pattern that low-latitude predators accelerate growth and development more under warming than high-latitude predators (Van Dievel et al. 2019a). We hypothesize that also the consistent trend of reduced handling times, hence increased maximum predation rates, of low-latitude predators in turbid water is the result of the faster pace-of-life of the low-latitude predators (Debecker and Stoks 2019) combined with the reduced anti-predator response of prey. In addition, there was a signal of latitude-associated adaptation at the higher temperature in terms of search rate (hence at low prey densities). Indeed, at 24 °C low-latitude predators had higher search rates compared to high-latitude predators when paired with low-latitude prey, while high-latitude predators had higher search rates compared to low-latitude predators with high-latitude prey. This suggests that predators are more efficient in hunting sympatric prey compared to allopatric prey, and/or, vice versa that prey better cope with allopatric predators compared to sympatric predators, at the higher temperature.

Effects of warming and turbidity on long-term interaction strength

Previous studies showed that warming may reduce long-term interaction strengths (Rall et al. 2010, Sentis et al. 2015, Wang et al. 2021). However, these studies did not consider thermal evolution of both predator and prey, and did not directly measure all model parameters needed to estimate long-term interaction strength. Our current study thus extends these studies by deriving all above parameters from empirical observations, by considering latitudinal differences in both predator and prey, and by evaluating the impact of warming in the absence and presence of turbidity, another important global change-related factor. While we also found warming to decrease the long-term interaction strength, this was the case only for systems with low-latitude predators in turbid water. Instead, warming increased the long-term interaction strength for systems with high-latitude predators in clear water. The long-term impact of warming thus depended on the combined effects of predator latitude and turbidity. Note that we here simulated 4 °C warming (from 20 °C to 24 °C) as this is the expected temperature increased by 2100 in the high-latitude populations under IPCC scenario RCP8.5. Obviously, at even higher temperatures the thermal optimum for the functional response parameters may be exceeded resulting in reduced long-term interaction strengths.

Warming reduced the long-term interaction strength in turbid water through the theoretically predicted (Fussmann et al. 2014, Rall et al. 2010) higher prey intrinsic growth rates and lower carrying capacities at the higher temperature (Appendix S3), and a trend for a higher predator metabolic rate (Wang et al. 2021). But in clear water, the trend of increased search rates at the higher temperature opposed and even reversed the effects of warming on these three factors, resulting in an increased long-term interaction strength under warming.

Turbidity caused in general an increase in long-term interaction strength, suggesting that nutrient enrichment may destabilize trophic systems by increasing turbidity levels which goes in the same direction as observations in experimental freshwater communities (Kratina et al. 2012). Similar as for warming, this was caused by higher search rates in most systems, while the lower prey intrinsic growth rates and the higher carrying capacities under turbidity also contributed. Previous studies have shown that higher algae-induced turbidity can lead to a higher *Daphnia* carrying capacity (Bosker et al. 2019, Kratina et al. 2012), but not necessarily a lower intrinsic growth rate (Giebelhausen and Lampert 2001).

Given that higher long-term interaction strengths are expected to reduce the stability of food webs (Binzer et al. 2012, Kratina et al. 2012, Rosenblatt et al. 2019, Sentis et al. 2015), our results suggest that both global change-related stressors, 4 ^{o}C warming and turbidity, will negatively affect the long-term stability of the high-latitude trophic system. This contrasts with the previous findings that warming will stabilize predator-prey dynamics (Fussmann et al. 2014, Sentis et al. 2015, Wang et al. 2021). We demonstrated that these destabilizing effects of both global change-related stressors were mainly driven by how they affected predator search rates. The risk of predator extinction, hence the stability of the system, may not depend only on the long-term interaction strength but also on the predator's energetic efficiency (Vucic-Pestic et al., 2011). Yet, based on the here quantified maximum predation rates and the previously measured metabolic rates (Wang et al. 2021), we found no effect of temperature and turbidity on the predator energetic efficiency (Appendix S6).

Space-for-time substitution patterns of long-term interaction strength

Our results of long-term interaction strength suggest that the impact of thermal plasticity and thermal evolution on the stability of the high-latitude trophic system under warming is critically dependent on the turbidity level. Indeed, when only assuming thermal plasticity, our results suggest the high-latitude trophic system may become less stable (i.e. easily enter cycles or collapse) under 4 °C warming in clear water but not in turbid water. Based on a space-for-time substitution (De Frenne et al. 2013, Verheyen et al. 2019), our results suggest predator thermal evolution alone may reduce (yet not eliminate) the negative impact of warming through plasticity on the stability of the high-latitude system, whereas joint thermal evolution of both predator and prey will tend to further destabilize the system. In turbid water, instead predator thermal evolution alone (regardless of prey thermal evolution) may contribute to stabilize the trophic system as it reduced the long-term interaction strength. These space-for-time patterns of long-term interaction strength are highly consistent with the space-for-time patterns of predator search rate (Appendix S7), indicating an important contribution of search rate in mediating effects of warming on long-term interaction strength and stability in our study system. Nevertheless, it should be noted that the contribution of search rate on the long-term interaction strength patterns could be smaller under longer-duration predation trials (Li et al. 2018).

Conclusion

Our study underscores the importance of considering besides warming also turbidity in shaping trophic interactions, and suggests that thermal evolution may increase or decrease food web stability under warming depending on the turbidity level. Our results add support to a recent review suggesting evolutionary response to be “a double-edged sword, not a silver bullet to confront global change” (Diamond and Martin 2020). Indeed, in clear water, evolution failed to dampen – and even tended to amplify – the effect of warming on trophic interactions. Given that warming and eutrophication are two major global change-related co-occurring factors negatively affecting freshwater ecosystems (Birk et al. 2020, Hering et al. 2015, Moss 2011, Woodward et al. 2010) and that evolution may play an important role in shaping responses to global change (Stoks et al. 2014, Urban et al. 2016), our results of contrasting effects of thermal evolution between clear and turbid water highlight the need of integrating evolution and turbidity to improve projections of trophic system stability under warming.

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Authors' contributions

RS and AS developed the ideas, and together with YJW designed the experiments. YJW performed the experiments and analyzed the data. AS and NT provided analytical advice and modeling support. YJW and RS drafted the manuscript after which all authors contributed and gave final approval for publication.

Appendix S1. Turbidity levels at the collection sites

Information of turbidity levels at the *I. elegans* (damselfly predator) collection sites was obtained from the supplementary information of Debecker and Stoks (2019). In their study turbidity levels (NTU) and chlorophyll a concentrations (µg/L) were measured at each of the six (three per latitude) damselfly collection sites. Both turbidity and chlorophyll a concentration were measured at five sub-sites in each latitude site with an AquaFluor meter (Turner Designs, Sunnyval CA, USA). We tested for an effect of latitude (low vs high) on these variables using separate linear regression models. There were no significant differences in turbidity ($F_{1,4}$ = 1.3275, P = 0.313) and in chlorophyll a concentration ($F_{1,4}$ = 6.3817, P = 0.065) between the two latitudes (Figure S1).

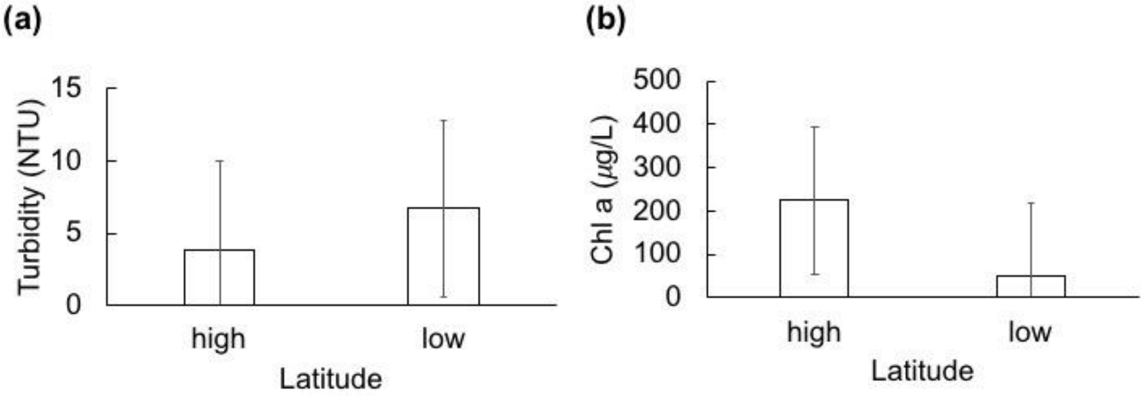


Figure S1. Turbidity (NTU) and chlorophyll a concentration (µg/L) measured in the study populations of the *I. elegans* predators at the two latitudes. Given are mean and 84% CI.

No similar quantitative information was available for the *D. magna* (water flea prey) collection sites. Here, we combined semi-quantitative information from the literature and qualitative information by the collectors. This information also suggests no consistent difference in turbidity levels between the latitudes (Table S1).

Table S1. Turbidity information from the *D. magna* (water flea prey) study populations

Site	Turbidity state	Information source
Grand Clos, France	Clear, but can be turbid in windy days	P. Lambret, pers. comm.
North Mireval, France	Clear	C. Haag, pers. comm.
South Mireval, France	Clear	C. Haag, pers. comm.
Lake Bysjön, Sweden	Turbid. Secchi depth < 1m in summer	L.-A. Hansson, pers. comm.
Pond Asklund, Norway	Clear. Mean summer Secchi depth > 2m	Reinertsen et al. 1997
Lake Ring, Denmark	Clear. Mean summer Secchi depth = 1.8 m	Jepsen et al. 2001

Appendix S2. Determination of the functional response type

To identify the functional response type, we ran logistic regressions of predator consumption relative to prey density (i.e. consumed prey N_e divided by initial prey density N_0; function *frair_test*, package *frair* in R):

$$\frac{N_e}{N_0} = \frac{\exp\,(P_0+P_1N_0+P_2{N_0}^2+P_3{N_0}^3)}{1+\exp\,(P_0+P_1N_0+P_2{N_0}^2+P_3{N_0}^3)} \text{ (Equation S1)}$$

We extracted estimates of the coefficient P_1 for the first-order term for prey density (N_0). Negative first-order terms for prey density indicate a type II functional response (i.e. declining proportional consumption with increasing prey density), while positive first-order term indicate a type III functional response (i.e. initial increasing and subsequent decreasing proportional consumption with increasing prey density).

Table S2. Estimates of the first-order term for prey density of the logistic regressions of predator proportional consumption against prey density for each combination of predator source latitude (Pred_lat), prey source latitude (Prey_lat), temperature (Temp) and turbidity (Tur) treatments.

Pred_lat	Prey_lat	Temp	Tur	Estimate	*Z*-value	*P*-value
low	low	20 °C	clear	-0.0618 ± 0.0046	-13.484	<0.0001
low	low	20 °C	turbid	-0.0655 ± 0.0047	-13.867	<0.0001
low	high	20 °C	clear	-0.0535 ± 0.0046	-11.719	<0.0001
low	high	20 °C	turbid	-0.0547 ± 0.0046	-11.961	<0.0001
high	low	20 °C	clear	-0.0427 ± 0.0046	-9.3103	<0.0001
high	low	20 °C	turbid	-0.0677 ± 0.0050	-13.494	<0.0001
high	high	20 °C	clear	-0.0401 ± 0.0047	-8.4574	<0.0001
high	high	20 °C	turbid	-0.0607 ± 0.0049	-12.456	<0.0001
low	low	24 °C	clear	-0.0526 ± 0.0031	-16.831	<0.0001
low	low	24 °C	turbid	-0.0380 ±0.00289	-13.229	<0.0001
low	high	24 °C	clear	-0.0449 ± 0.0028	-15.832	<0.0001
low	high	24 °C	turbid	-0.0411 ± 0.0028	-14.501	<0.0001
high	low	24 °C	clear	-0.0400 ± 0.0028	-14.221	<0.0001

high	low	24 °C	turbid	-0.0459 ± 0.0029	-15.622	<0.0001
high	high	24 °C	clear	-0.0391 ± 0.00230	-13.233	<0.0001
high	high	24 °C	turbid	-0.0524 ± 0.0031	-16.747	<0.0001

Appendix S3. Definitions, units, and values of the parameters used in the simulation model

Table S3. Definitions, units, and values of the model parameters

Parameters	Values	Unit	Definition
N	time-dependent	ind L^{-1}	prey density
P	time-dependent	ind L^{-1}	predator density
t	time-dependent	day	time
a	group-dependent	L day^{-1}	search rate
h	group-dependent	day	handling time
r	group-dependent	day^{-1}	intrinsic growth rate of prey
K	group-dependent	ind L^{-1}	prey carrying capacity
m_p	group-dependent (Wang et al. 2021)	J h^{-1}	predator metabolic rate
c	$24 \times (7 \times 10^3)^{-1} \times$ prey $mass^{-1}$	J^{-1} h ind day^{-1}	parameter converting m_p unit into individuals day^{-1}
e_c	$0.92 \times$ prey mass $\times$ predator $mass^{-1}$ (Van Dievel et al. 2019b)	-	predator assimilation efficiency
I_L	group-dependent	-	long-term per capita interaction strength
N^{-}	K	ind L^{-1}	prey equilibrium density in predator-absent environment (= carrying capacity K)
N^{+}	group-dependent	ind L^{-1}	prey equilibrium density in predator-present environment
P^{+}	group-dependent	ind L^{-1}	predator equilibrium density

Appendix S4. *Daphnia* experiments for intrinsic growth rate and carrying capacity

We set up two additional experiments to quantify the effects of warming and turbidity on intrinsic growth rate and carrying capacity of *D. magna*. Both experiments used the same factorial design: 2 prey latitudes (high- or low- latitude) crossed with 2 test (= rearing) temperatures (20 °C or 24 °C) and 2 turbidity states (clear < 0.1 NTU or turbid ca. 6 NTU). The same set of 18 *Daphnia* clones (3 clones/population × 3 populations/latitude × 2 latitudes) was used as in the main experiment. For both experiments, we ran one replicate per clone at each temperature × turbidity level. Before the experiment started, the clones were acclimatized for three generations at experimental temperatures (20 °C and 24 °C) and a 14:10 L:D photoperiod.

The intrinsic growth rate (r) experiment was based on Pauwels et al. (2010) and Stoks et al. (2016). To start, three neonates (< 24h old) of the same clone were inoculated in a 250 mL vial filled with 200 mL medium (dechlorinated tap water for clear water, with 3 mL algal solution added for turbid water). This resulted in 72 vials in total. To maintain algal turbidity, the vials were placed on rotation devices, this was also done for the vials of the clear treatment (Figure. S2a). The medium in the vials was renewed twice per week, and the *Daphnia* fed *ad libitum* the green alga *Acutodesmus obliquus* (1×10^5 cells mL^{-1}). To obtain fecundity estimates, newborn juveniles were daily removed from the vials and counted. We monitored each vial until the 2nd brood was fully released by the cohort. The data obtained were incorporated in the Euler-Lotka equation to estimate the intrinsic growth rates (Lotka 1913):

$$1 = \int e^{-rx} l_x m_x dx \text{ (Equation S2)}$$

Where l_x is the probability of a *Daphnia* surviving until age x, and m_x is the number of offspring released at day x. We calculated r separately for each clone in each treatment combination.

The carrying capacity (K) experiment was based on Little et al. (2002), Capaul and Ebert (2003), Fossen et al. (2019) and Adamczuk (2020). To start, 40 neonates (< 24h old) of the same clone were inoculated in 500 mL vials filled with 400 mL medium (dechlorinated tap water for clear water, with 6 mL algal solution added for turbid water). This resulted in 72 vials in total. To maintain algal turbidity, a magnetic stirrer was added to each all vials (also in the vials of the clear treatment) (Figure. S2b). In addition, the medium in the vials was renewed twice per week. The *Daphnia* were daily fed *ad libitum* the green alga *Acutodesmus obliquus* (1×10^5 cells mL^{-1}). The average population density of each clone in each treatment group based on weekly counts from week 11 to week 14 was used as an estimate of the carrying capacity of the prey (K).

We tested the effects of prey latitude, temperature, and turbidity, and their interactions on the intrinsic growth rate (*r*) and ln-transformed carrying capacity (*K*) using linear mixed models. We added population nested in latitude, and its interactions with temperature, and turbidity as random factors. The random factors were never significant ($P > 0.05$), and removed from the models. The resulting least-square means of *r*, *K* (back-transformed and adjusted for the volume of predation trials) and their standard errors were used as the estimates per treatment combination for the calculation of long-term interaction strength.

Warming led to higher intrinsic growth rates (+9%, $F_{1,64} = 15.53$, $P = 0.0002$) and lower carrying capacities (-41%, $F_{1,64} = 6.78$, $P = 0.0115$, Figure S2) as theory predicted (Rall et al. 2010). In contrast, increased turbidity led to lower intrinsic growth rates (-7%, $F_{1,64} = 12.18$, $P = 0.0008$) and higher carrying capacities (+93%, $F_{1,64} = 10.38$, $P = 0.002$). The effects of warming and turbidity on both population parameters were additive (Warming × Turbidity: all $P > 0.14$). Low-latitude prey had higher intrinsic growth rates (+5%, $F_{1,64} = 4.91$, $P = 0.0303$) but similar carrying capacities compared to high-latitude prey. The effect of warming and turbidity did not differ between latitudes (all $P > 0.1$).

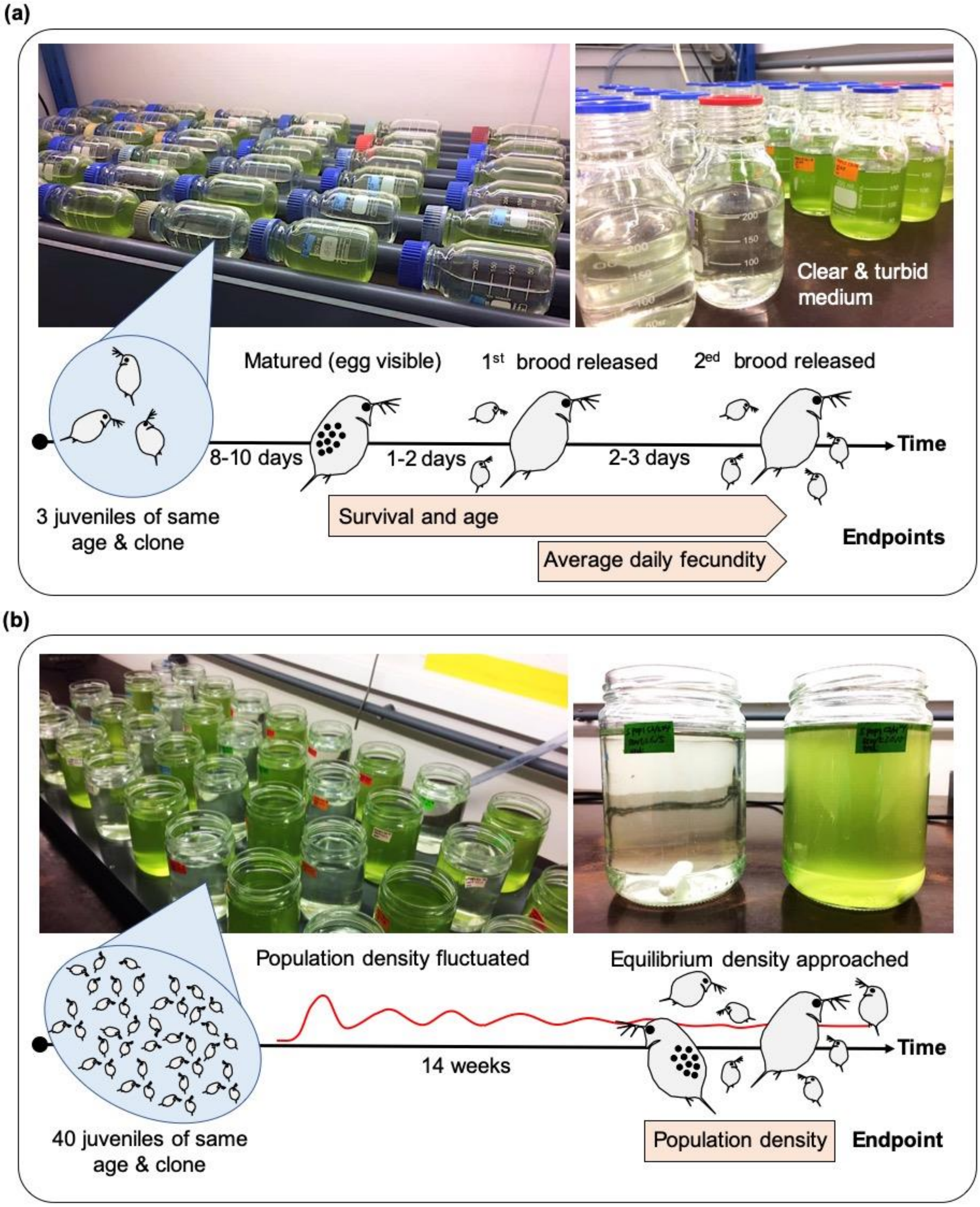


Figure S2. Schematic overview of the two *Daphnia* experiments to quantify (a) intrinsic growth rate and (b) carrying capacity under all four combinations of temperature and turbidity.

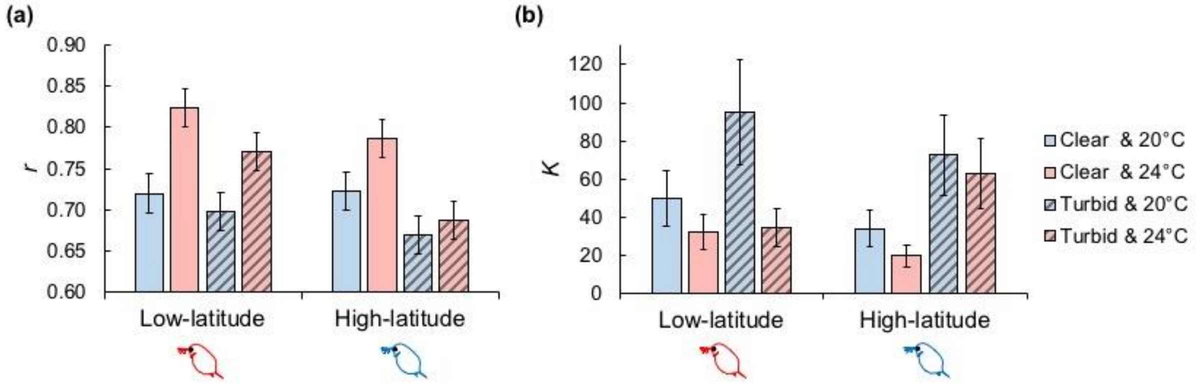


Figure S3. Effects of temperature and turbidity on (a) intrinsic growth rate *r* and (b) carrying capacity *K* of *Daphnia magna*. Given are least-squared means ± 1 SE.

Appendix S5. Pairwise comparisons between functional response parameter estimates

Table S4. Comparisons between functional response parameter estimates for *Ischnura elegans* damselfly larvae feeding on *Daphnia magna* water fleas between predator and prey latitudes (L = low-latitude, H = high-latitude), rearing temperatures (20°C and 24°C) and turbidity levels (clear < 0.1 NTU or turbid ca. 6 NTU). D = difference between the search rates (*a*) and handling times (*h*) among treatments. *P*-values were false discovery rate (FDR)-adjusted based on the number of pairwise comparisons (n = 64 from 28 comparisons within each latitude and 8 comparisons between latitudes). Significant values are highlighted in bold.

Treatment 1 (T1)	Treatment 2 (T2)	*D*	Estimate (T1-T2)	SE	*z*	*P_adjusted*
L-L-20°C-clear	L-L-24°C-clear	***a***	-3.03254	0.79002	-3.8386	**0.00768**
		h	0.00718	0.00087	8.2275	**<0.0001**
L-L-20°C-clear	L-L-20°C-turbid	***a***	-3.06652	0.812	-3.7765	**0.01024**
		h	0.00289	0.00099	2.9102	0.23104
L-L-20°C-clear	L-L-24°C-turbid	*a*	0.6703	0.75157	0.8919	1
		h	0.00877	0.00086	10.2353	**<0.0001**
L-L-20°C-clear	L-H-20°C-clear	*a*	3.79208	3.31612	1.1435	1
		h	-0.00035	0.00171	-0.2068	1
L-L-20°C-clear	L-H-24°C-clear	***a***	3.5312	0.58142	6.0734	**<0.0001**
		h	0.0071	0.00091	7.8057	**<0.0001**
L-L-20°C-clear	L-H-20°C-turbid	*a*	0.97924	0.82844	1.182	1
		h	0.00153	0.00114	1.3381	1
L-L-20°C-clear	L-H-24°C-turbid	***a***	4.18105	0.51387	8.1365	**<0.0001**
		h	0.0093	0.00087	10.7333	**<0.0001**
L-L-24°C-clear	L-L-20°C-turbid	*a*	-0.027	0.60258	-0.0448	1
		h	-0.00428	0.00074	-5.7611	**<0.0001**
L-L-24°C-clear	L-L-24°C-turbid	***a***	3.32357	0.66565	4.993	**<0.0001**
		h	0.0015	0.00052	2.8709	0.26176
L-L-24°C-clear	L-H-20°C-clear	***a***	6.83695	0.68178	10.028	**<0.0001**
		h	-0.00752	0.0012	-6.27	**<0.0001**

L-L-24°C-clear	L-H-24°C-clear	***a***	6.56211	0.5528	11.8706	<**0.0001**
		h	-0.00008	0.00061	-0.1295	1
L-L-24°C-clear	L-H-20°C-turbid	***a***	4.0134	0.66197	6.0628	<**0.0001**
		h	-0.00564	0.00096	-5.876	<**0.0001**
L-L-24°C-clear	L-H-24°C-turbid	***a***	7.20561	0.49535	14.5466	<**0.0001**
		h	0.00212	0.00054	3.9644	**0.00448**
L-L-20°C-turbid	L-L-24°C-turbid	***a***	3.79728	0.80428	4.7214	<**0.0001**
		h	0.00587	0.0007	8.4293	<**0.0001**
L-L-20°C-turbid	L-H-20°C-clear	***a***	6.86215	0.87307	7.8598	<**0.0001**
		h	-0.00325	0.00129	-2.5148	0.76224
L-L-20°C-turbid	L-H-24°C-clear	***a***	6.60663	0.59956	11.0191	<**0.0001**
		h	0.0042	0.00076	5.5226	<**0.0001**
L-L-20°C-turbid	L-H-20°C-turbid	***a***	4.03722	0.95685	4.2193	**0.00128**
		h	-0.00137	0.00107	-1.2781	1
L-L-20°C-turbid	L-H-24°C-turbid	***a***	7.24251	0.5256	13.7794	<**0.0001**
		h	0.0064	0.00071	9.0673	<**0.0001**
L-L-24°C-turbid	L-H-20°C-clear	*a*	3.5211	0.59459	5.9219	<**0.0001**
		h	-0.00902	0.00113	-7.9828	<**0.0001**
L-L-24°C-turbid	L-H-24°C-clear	***a***	3.23979	0.52735	6.1436	<**0.0001**
		h	-0.00158	0.00057	-2.7795	0.34816
L-L-24°C-turbid	L-H-20°C-turbid	*a*	0.68421	0.54413	1.2574	1
		h	-0.00714	0.00089	-8.027	<**0.0001**
L-L-24°C-turbid	L-H-24°C-turbid	*a*	3.87931	0.4788	8.1021	<**0.0001**
		h	0.00062	0.00049	1.2656	1
L-H-20°C-clear	L-H-24°C-clear	*a*	-0.28178	0.5551	-0.5076	1
		h	0.00744	0.00101	7.4052	<**0.0001**
L-H-20°C-clear	L-H-20°C-turbid	*a*	-2.84276	3.34825	-0.849	1
		h	0.00187	0.00176	1.0614	1
L-H-20°C-clear	L-H-24°C-turbid	*a*	0.36165	0.49659	0.7283	1
		h	0.00964	0.00097	9.9441	<**0.0001**
L-H-24°C-clear	L-H-20°C-turbid	***a***	-2.54725	0.25586	-9.9555	<**0.0001**
		h	-0.00557	0.00087	-6.4078	<**0.0001**
L-H-24°C-clear	L-H-24°C-turbid	*a*	0.64565	0.4048	1.595	1

		h	0.0022	0.00055	4.0228	**0.00384**
L-H-20°C-turbid	L-H-24°C-turbid	***a***	3.1961	0.51491	6.207	**<0.0001**
		h	0.00777	0.00082	9.4956	**<0.0001**
H-L-20°C-clear	H-L-24°C-clear	*a*	-3.85971	2.73198	-1.4128	1
		h	0.00316	0.00213	1.4863	1
H-L-20°C-clear	H-L-20°C-turbid	*a*	-10.4675	3.9371	-2.6587	0.50176
		h	-0.00072	0.00223	-0.3217	1
H-L-20°C-clear	H-L-24°C-turbid	*a*	-8.0554	3.54297	-2.2736	1
		h	0.00074	0.00213	0.347	1
H-L-20°C-clear	H-H-20°C-clear	*a*	2.20433	2.18181	1.0103	1
		h	-0.00409	0.00313	-1.3062	1
H-L-20°C-clear	H-H-24°C-clear	*a*	-6.53866	4.16886	-1.5684	1
		h	-0.00532	0.00248	-2.1452	1
H-L-20°C-clear	H-H-20°C-turbid	*a*	-8.75915	3.8414	-2.2802	1
		h	-0.00154	0.00229	-0.6719	1
H-L-20°C-clear	H-H-24°C-turbid	*a*	-10.00932	3.53198	-2.8339	0.2944
		h	-0.00212	0.00215	-0.9852	1
H-L-24°C-clear	H-L-20°C-turbid	*a*	-6.62147	4.18521	-1.5821	1
		h	-0.00389	0.00169	-2.3058	1
H-L-24°C-clear	H-L-24°C-turbid	***a***	-4.20932	0.62985	-6.683	**<0.0001**
		h	-0.00244	0.00108	-2.2573	1
H-L-24°C-clear	H-H-20°C-clear	*a*	6.03912	2.59788	2.3246	1
		h	-0.00727	0.00277	-2.6253	0.55424
H-L-24°C-clear	H-H-24°C-clear	*a*	-2.68586	4.39956	-0.6105	1
		h	-0.00849	0.002	-4.2382	**0.00128**
H-L-24°C-clear	H-H-20°C-turbid	*a*	-4.90653	4.09266	-1.1989	1
		h	-0.00471	0.00176	-2.6747	0.47872
H-L-24°C-clear	H-H-24°C-turbid	*a*	-6.16262	3.80284	-1.6205	1
		h	-0.0053	0.00158	-3.356	0.05056

H-L-20°C-turbid	H-L-24°C-turbid	*a*	2.41006	1.13157	2.1298	1
		h	0.00146	0.00129	1.1279	1
H-L-20°C-turbid	H-H-20°C-clear	***a***	12.64951	3.84827	3.2871	0.06464
		h	-0.00339	0.00285	-1.1886	1
H-L-20°C-turbid	H-H-24°C-clear	***a***	3.92553	1.16174	3.379	**0.04672**
		h	-0.00461	0.00161	-2.8523	0.27776
H-L-20°C-turbid	H-H-20°C-turbid	*a*	1.71423	1.15767	1.4808	1
		h	-0.00081	0.00146	-0.5592	1
H-L-20°C-turbid	H-H-24°C-turbid	*a*	0.46707	1.13046	0.4132	1
		h	-0.0014	0.00137	-1.021	1
H-L-24°C-turbid	H-H-20°C-clear	*a*	10.26119	3.44264	2.9806	0.18432
		h	-0.00483	0.00278	-1.7408	1
H-L-24°C-turbid	H-H-24°C-clear	*a*	1.54185	0.99251	1.5535	1
		h	-0.00605	0.00149	-4.0624	**0.0032**
H-L-24°C-turbid	H-H-20°C-turbid	*a*	1.92681	1.17041	1.6463	1
		h	-0.0014	0.00136	-1.0233	1
H-L-24°C-turbid	H-H-24°C-turbid	*a*	-1.95135	1.0178	-1.9172	1
		h	-0.00286	0.00125	-2.2946	1
H-H-20°C-clear	H-H-24°C-clear	*a*	-8.73653	4.07979	-2.1414	1
		h	-0.00123	0.00305	-0.4027	1
H-H-20°C-clear	H-H-20°C-turbid	*a*	-10.95503	3.74758	-2.9232	0.22144
		h	0.00256	0.0029	0.8855	1
H-H-20°C-clear	H-H-24°C-turbid	***a***	-12.18126	3.42678	-3.5547	**0.02432**
		h	0.002	0.00279	0.7172	1
H-H-24°C-clear	H-H-20°C-turbid	*a*	-2.2315	1.22177	-1.8264	1
		h	0.00378	0.00158	2.3976	1

H-H-24°C-clear	H-H-24°C-turbid	*a*	-3.46963	1.18042	-2.9393	0.21056
		h	0.0032	0.00151	2.1106	1
H-H-20°C-turbid	H-H-24°C-turbid	*a*	-1.26277	1.10549	-1.1423	1
		h	-0.00059	0.0014	-0.4196	1
L-L-20°C-clear	N-L-20°C-clear	*a*	7.47591	3.03734	2.4613	0.88576
		h	-0.00219	0.0021	-1.0432	1
L-L-24°C-clear	N-L-24°C-clear	***a***	6.65513	0.68271	9.7481	**<0.0001**
		h	-0.00619	0.00097	-6.366	**<0.0001**
L-L-20°C-turbid	N-L-20°C-turbid	*a*	0.07411	0.97874	0.0757	1
		h	-0.00581	0.00118	-4.9323	**<0.0001**
L-L-24°C-turbid	N-L-24°C-turbid	*a*	-0.87306	0.45775	-1.9073	1
		h	-0.01013	0.00088	-11.522	**<0.0001**
L-H-20°C-clear	H-H-20°C-clear	*a*	5.8662	-0.00594	2.2931	1
		h	-0.00594	0.00288	-2.0614	1
L-H-24°C-clear	H-H-24°C-clear	***a***	-2.57776	0.13456	-19.157	**<0.0001**
		h	-0.0146	0.00125	-11.637	**<0.0001**
L-H-20°C-turbid	H-H-20°C-turbid	***a***	-2.25743	0.78472	-2.8767	0.25728
		h	-0.00525	0.00126	-4.1584	**0.00192**
L-H-24°C-turbid	H-H-24°C-turbid	***a***	-6.70588	0.12707	-52.772	**<0.0001**
		h	-0.01361	0.00101	-13.499	**<0.0001**

Appendix S6. Patterns in predator energetic efficiency

Values for predator energetic efficiency (EE) were calculated following Sentis et al. (2012) as

$$EE = \omega F / \lambda I \text{ (Equation S3)}$$

where ω is the assimilation efficiency (0.92 for *I. elegans*, Van Dievel et al. 2019b), F is the maximal per capita energy feeding rate (J h^{-1}) estimated from handling time h (day) and prey body mass m = 0.001 g (m/h = maximal per capita daily feeding biomass; 1g biomass = 7000 J), λ is a constant (set at 3) converting standard metabolic rate into field metabolic rate (Savage et al. 2004b), and I is the per capita standard metabolic rate (J h^{-1}).

Based on overlap of the 84% confidence intervals, there was no significant effect of predator latitude, prey latitude, temperature and turbidity on the energetic efficiency of *I. elegans* larvae.

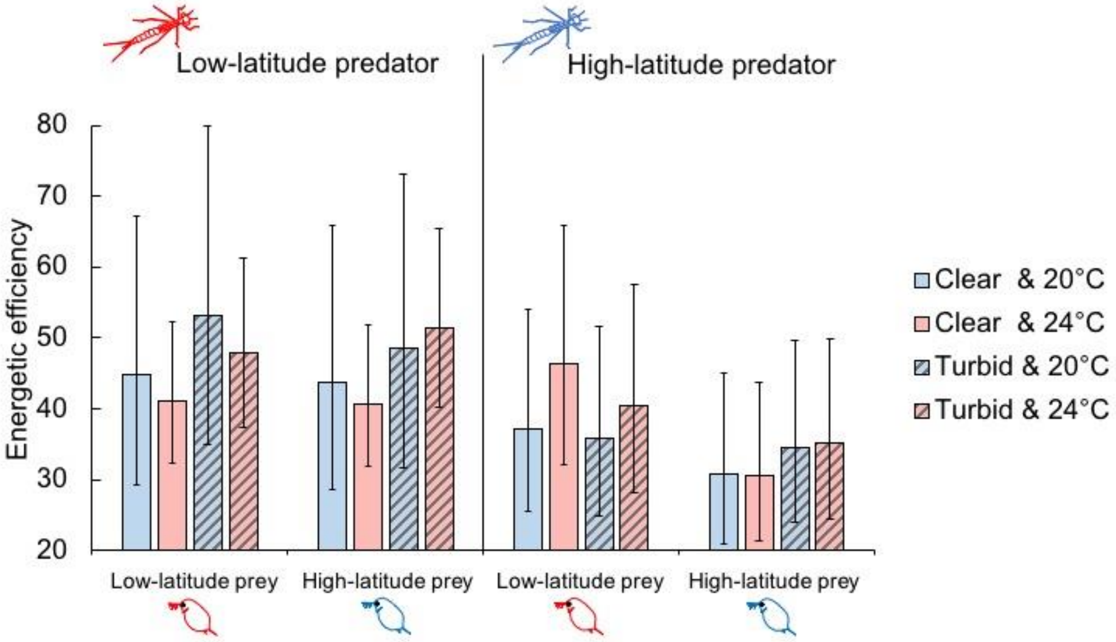
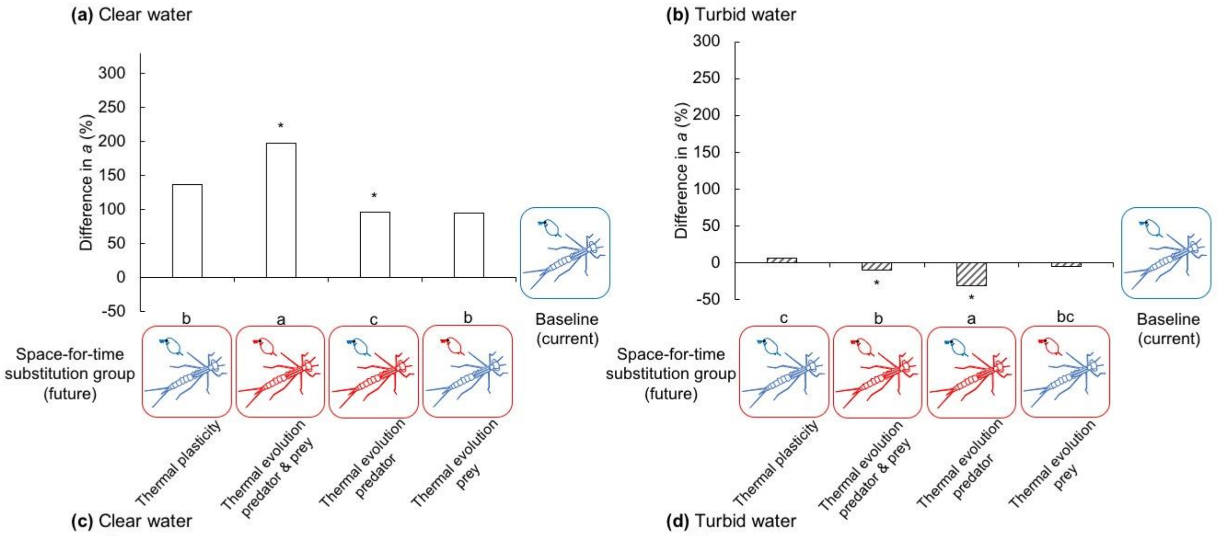


Figure S4. Effects of predator source latitude, prey source latitude, temperature and turbidity on the energetic efficiency of the predator *Ischnura elegans* feeding on *Daphnia magna* prey. Values given are estimated means with 84% CI.

Appendix S7. Effects of thermal plasticity and thermal evolution on the functional response parameters

To assess and compare the effects of thermal plasticity and thermal evolution on both functional response parameters we expressed the data of Figure 2 as the difference (%) of the least-squared means between the four space-for-time substituted groups (thermal plasticity with no evolution, evolution of both predator and prey, evolution only of predator, and evolution only of prey) at 24 °C and the baseline group “high-latitude predator paired with high-latitude prey at 20 °C”.

The results indicated (1) thermal plasticity did not significantly affect search rates and handling times. (2) Thermal evolution of the predator increased search rates in clear water but decreased search rates in turbid water both in the absence and in the presence of thermal evolution of the prey (Figure S5a-b). (3) Thermal evolution of the predator decreased handling time in both clear and turbid waters, and both in the absence and in the presence of thermal evolution of the prey (Figure S5c-d). (4) Thermal evolution of the prey alone also decreased the handling time in clear water (Figure S5c).


(a) Clear water (b) Turbid water

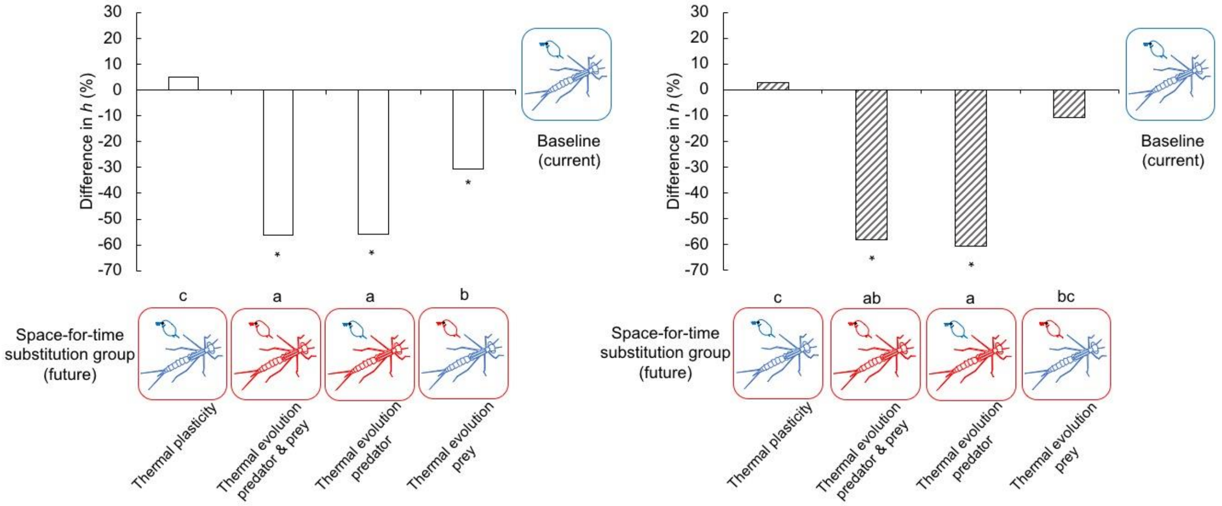

(c) Clear water (d) Turbid water

Figure S5. Effects of thermal plasticity and thermal evolution on search rate *a* and handling time *h* under warming in (a, c) clear and (b, d) turbid water. Given are mean differences in *a* and *h* (%) between each of the four groups at 24 °C relevant for the space-for-time substitution (only plasticity and no evolution, evolution of both predator and prey, evolution of only predator, and evolution of only prey) expressed relative to the baseline group representing the current high-latitude trophic system (high-latitude predator & high-latitude prey at 20 °C). Asterisks denote significant ($P < 0.05$) differences in *a* and *h* compared with the baseline group at the same turbidity level. Letters above the four groups at 24 °C relevant for the space-for-time substitution denote differences between these groups in *a* and *h*.

General discussion

In this general discussion, I will cover five topics. First, I will provide an overview about this thesis. Second, I will discuss the rapid thermal evolution of TPC in *D. magna*, and the long-term latitude-associated evolution of predation rate and its thermal plasticity in the predator *I. elegans*. Third, I will discuss the single and combined effects of several global change stressors (increasing mean temperatures, increasing daily temperature fluctuations, presence of heatwaves (HW), and increasing algae induced-turbidity) on the short- and long-term trophic interaction strength between *I. elegans* larvae and *D. magna*. Forth, focusing on the high-latitude system that is under a projected increase of mean temperature from 20 °C to 24 °C (IPCC 2014, scenario 8.5), I will review how the effects of the studied global change stressor on long-term predator-prey interaction were mediated by thermal evolution building on a space-for-time substitution. Fifth, I will discuss some limitations of this thesis and some directions for future research. Finally, I will end with some take-home messages focusing on the implications for food webs.

1. An overview

This thesis followed an experimental approach to investigate the impact of global change on freshwater ecosystems via species and their trophic interactions. Building on experimental evolution trials, it was demonstrated that the rapid evolution of TPCs under warming can be trait-dependent, and improve the fitness of a consumer species without necessarily altering the per capita impact on its resources. Latitudinal differentiation in thermal plasticity and acclimation capacity of predation rates indicated that high-latitude predator populations rely on higher acclimation capacity compared to low-latitude populations to achieve perfect thermal compensation in food uptake. Warming combined with increased daily temperature fluctuations could destabilize the studied high-latitude trophic system unless thermal evolution takes place. Moreover, algae-induced turbidity may critically alter the plastic and evolutionary responses of trophic interactions under warming. These results add evidence to the emerging threats and conservation challenges for freshwater biodiversity under global change (Reid et al. 2019), and highlight the importance to consider multiple stressors (Jackson et al. 2016, Côté et al. 2016, Orr et al. 2020), and both plastic and evolutionary responses (Merilä and Hendry 2014, Nadeau and Urban 2020) to better understand and predict the impact of global change on species and their interactions (Urban et al. 2016, Stoks et al. 2017, Bartley et al. 2019). The approaches used in this thesis (e.g. experimental thermal selection, space-for-time substitution, functional response experiments and the modelling of long-term interaction strengths) can be applied to other trophic systems and stressors to further broaden insights. Such studies should integrate improvements in the experimental design (see limitations and future directions).

2. Rapid thermal evolution of TPCs in *D. magna*, and the latitudinal variation in thermal plasticity of predation rate in *I. elegans*

As a key species in the freshwater ecosystem, *D. magna* has for long been an important study species in ecological, toxicological, and evolutionary research (Lampert 2011, Miner et al. 2012). Experimental evolution trials had already shown the high potential for rapid evolution under experimental heat (4 °C above ambient) selection in this species to cope with rapid climate change, such as an increased competitive strength at higher temperatures (Van Doorslaer et al. 2009b), and a higher heat tolerance (CT_{max}) (Geerts et al. 2015). I extended this in Chapter I by examining the thermal performance curves (TPCs) of several key performance traits under experimental heat selection. I found heat-selected *Daphnia* had an increased survival until maturity and intrinsic growth rate at high temperatures, by shifting the TPCs toward higher optimum temperatures (T_{opt}), and by decreasing the thermal plasticity of these TPCs (Chapter I). These results imply that after rapid thermal evolution *Daphnia* can better maintain its fitness at warmer temperatures by shifting optimum temperature towards higher temperatures and reducing the curvature of unimodal TPCs. Note that although the reduced curvature of unimodal TPCs (in this case as quadratic TPCs) indicated a higher thermal tolerance range and an increased TPC breadth above T_{opt}, it was not accompanied by a decreased performance at T_{opt} (i.e. maximum performance). Therefore, the results did not support the trade-off between thermal tolerance range and optimum performance in the generalist-specialist hypothesis (Huey and Hertz 1984). This was in line with Nati et al. (2016), where TPC breadth was independent of optimum performance. The lack of trade-off between thermal tolerance range and maximum performance could be due to some compensatory mechanisms that allowed *Daphnia* to maintain its maximum performance. For example, the process shifting the T_{opt} toward higher temperature might have improved the thermal dynamics of rate-limiting catalytic enzymes, allowing them to maintain the maximum function at T_{opt}. In other words, the trade-off between thermal tolerance range and maximum performance could be more likely when T_{opt}

does not change, as illustrated by the review of Sinclair et al. (2016) (Figure 1). In this case, a higher thermal tolerance range above T_{opt}, together with an unchanged T_{opt}, was accompanied by a lower optimum performance. Note that the patterns in the mentioned case study was only from acclimation rather than adaptation. Therefore, the above explanation remains a hypothesis for further investigation.

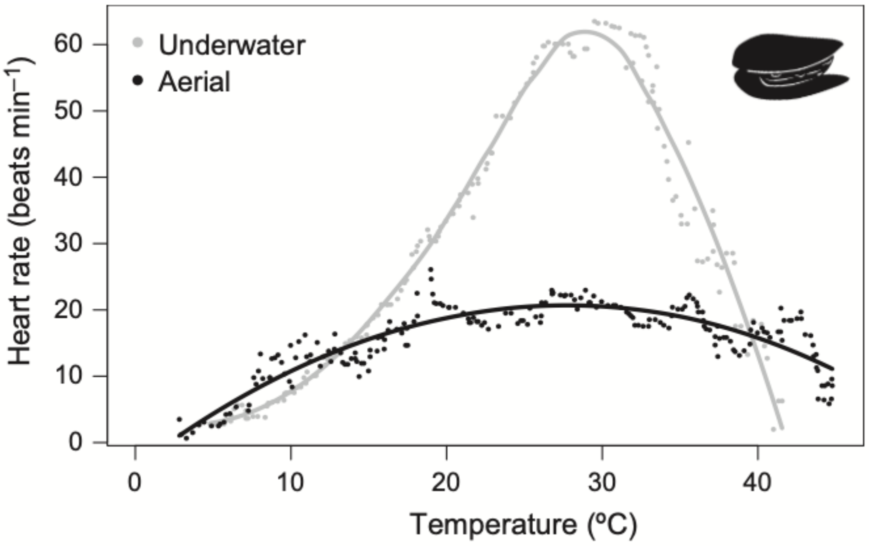


Figure 1. Thermal performance curves of the heart rate in the brown mussel *Perna perna*. in the aerial vs. underwater environment. Adapted after Sinclair et al. (2016).

The absence of evolution in activation energies (E_a) in the traits with linear TPCs (development rate, individual growth rate, ingestion rate, and metabolic rate) suggests that E_a (the slope of linear TPCs) are less likely to rapidly evolve under a mild increase in mean temperature compared to the T_{opt} of unimodal TPCs. Although the metabolic theory of ecology (MTE) predicted E_a to be evolutionary stable (Gillooly et al. 2001, Allen and Gillooly 2007), recent studies have suggested that E_a may vary under long-term selection pressures, such as predation and temperature variation (Kontopoulos et al. 2020). The observed absence of rapid evolution in thermal plasticity (E_a) of *Daphnia* could be due to insufficient time for evolution. It could also be that some traits show less genetic variation or are more constrained by genetic correlations compared to other traits. Nevertheless, based on the E_a of ingestion rate and metabolic rate, the consumer thermal impact (CTI) indicated a trend of improved warm-tolerance in heat-selected *Daphnia* (Vasseur and McCann 2005, Iles 2014). The insignificant difference of CTI between control and heat-selected *Daphnia* also suggests the top-down impact of *Daphnia* on its resource will likely remain stable after rapid thermal evolution. Taken together, the rapid thermal evolution of TPC in *Daphnia* under the projected + 4 °C global warming by 2100 (IPCC 2014, scenario RCP8.5) was trait-dependent. Still, it does help the species overcome potential threats that could reduce population fitness.

The intraspecific variation in thermal plasticity has been suggested to critically shape the predictions of future distributions of species under global warming (Valladares et al. 2014). Though the climatic variability hypothesis (CVH) predicted thermal plasticity and acclimation capacity to increase with seasonal thermal heterogeneity of the environment, and thus to be higher at high-latitudes where the climate is more variable across the year than at low latitudes (Janzen 1967, Addo-Bediako et al. 2000, Ghalambor et al. 2006, Aguilar-Kirigin and Naya 2013), it received mixed support (Liefting et al. 2009, Naya et al. 2011, Van Heerwaarden et al. 2014, Gunderson and Stillman 2015, Barria and Bacigalupe 2017) and has never been used to test the thermal plasticity of predation rate across latitudes, taking into account the types of thermal plasticity and acclimation metrics applied. Chapter II filled this knowledge gap by crossing developmental and acute temperature (including heatwave temperature) to test predation rates of low- and high- latitude damselfly larvae, demonstrating latitudinal differences in the plasticity of predation rates to be strongly dependent on the type of thermal plasticity and thermal acclimation metric used. Remarkably, the support for CVH came from the recently developed metric of thermal acclimation capacity that expresses acclimation capacity relative to acute plasticity (sensu Einum et al., 2019), not from the earlier proposed post-acclimation thermal sensitivity (sensu Seebacher et al. 2015). The ~6 times higher acclimation capacity in high-latitude larvae compared to the low-latitude ones could potentially be the driver of a perfect thermal compensation, making post-acclimation thermal sensitivity absent. However, the mixed support for CVH from different types of plasticity and acclimation metrics in the present and previous studies (e.g. absent CVH pattern in acute plasticity: Van Heerwaarden et al. 2014, Barria and Bacigalupe 2017; reversed CVH pattern in post-acclimation thermal sensitivity: Liefting et al. 2009, Seebacher et al. 2015) also suggest mechanisms other than CVH may contribute to the observed latitudinal pattern of thermal plasticity of a trait, such as thermal adaptation to mean temperature or thermal adaptation to daily temperature fluctuations. In Chapter II, the high-latitude damselfly larvae not only experienced a higher climatic variability (i.e. the difference between annual absolute maximum and the absolute minimum temperatures) (Addo-Bediako et al. 2000), but also a lower mean summer water temperature (20 °C) and a lower daily temperature fluctuation (5 °C) compared to low-latitude ones (mean 24°C, daily temperature fluctuation 10 °C) (Debecker and Stoks 2019, Verheyen and Stoks 2019). Based on the results, it seems that the role of climatic variability is more deterministic than daily temperature fluctuations, while the role of mean temperature is somewhere in between. On the one hand, the thermal adaptation to the mean temperature of 20 °C possibly explained why high-latitude larvae had a lower predation rate when developed and tested at 24 °C compared to those developed at 20 °C and tested at 24 °C, while they have a higher predation rate tested at 24 °C compared to 20 °C when developed at the non-stressful 20 °C. These patterns could be linked to resting metabolic rates increasing under short-term exposure to higher-than-mean temperatures while remaining constant or even decreasing under long-term exposure (e.g. Donelson et al. 2011, Donelson et al. 2012, Debecker and Stoks 2019, Van Dievel et al. 2019a). On the other hand, the thermal adaptation to the higher mean temperature of 24 °C could make low-latitude larvae rely less on plasticity to acclimate between 20 °C and 24 °C. Whatever the underlying mechanisms, the latitudinal variation in thermal plasticity of predation rates in *I. elegans* extends the emerging view that intraspecific variation in thermal plasticity of fitness-related traits is essential to better understand the effects of temperature changes (Seebacher et al. 2012, Valladares et al. 2014). Further, it adds to the insight that developmental temperature may shape the acute thermal response (Fangue et al. 2009, Grigaltchik et al. 2012, Sentis et al. 2015).

For the high-latitude larvae, the patterns of the plasticity types and acclimation metrics provide complementary information on the possible effects of + 4 °C global warming (IPCC 2014, scenario RCP8.5) on predation rates in the absence of thermal evolution in the high-latitude populations. The patterns of post-acclimation thermal sensitivity suggested that the predation rates of high-latitude larvae at 24 °C may not change as compared to current predation rates at 20 °C because of a high thermal acclimation capacity. The patterns of acute plasticity indicate that during short periods of 4 °C warming, high-latitude larvae are expected to increase their predation rates. The patterns of developmental plasticity suggest that during a heatwave, high-latitude larvae will have lower predation rates when the mean summer water temperatures have increased to 24 °C.

3. Effects of exposure to global change stressors on the long-term predator-prey interaction strength

3.1 Effects of warming and temperature variation

To improve predictions of the impact of global warming on natural systems, we need to consider increases in mean temperature as well as daily temperature fluctuations (DTF) and heatwaves (HW) (Vasseur et al. 2014, Colinet et al. 2015, Williams et al. 2016, Stoks et al. 2017, Boukal et al. 2019), and the mechanisms underlying trophic interaction strength (Rall et al. 2010, Fussmann et al. 2014, Sentis et al. 2015, Archer et al. 2019). The results in Chapter III indicate that, while a 4 °C warming weakened the long-term interaction strength in the low-latitude system, this effect disappeared in the high-latitude system when warming was combined with DTF or HW. This extends the emerging insight that temperature variation may make ectotherms (in our case, the high-latitude system) more vulnerable to warming (Paaijmans et al. 2013, Stoks et al. 2017).

The results of a decreased long-term interaction strength under (+ 4 °C) warming were consistent with the findings of Sentis et al. (2015). In the simulations of long-term predator-prey interaction, we allowed prey carrying capacity (*K*) to decrease and prey intrinsic growth rate (*r*) to increase as the two mechanisms suggested by theory to weaken long-term interaction under warming (Rall et al. 2010, Fussmann et al. 2014). In addition, we identified a third mechanism working through predator metabolic rate as suggested by Rall et al. (2010) and Fussmann et al. (2014), which played a less deterministic role under warming compared to other mechanisms. Moreover, we identified a fourth mechanism of how warming may weaken the long-term interaction strength that furthermore was latitude-dependent: decreasing search rates of low-latitude predators, and increasing handling times of high-latitude predators under constant temperature and under a heatwave. Also, the heatwave temperature generally seemed to weaken the long-term interaction strength by increasing predator metabolic rates. Given that lower long-term interaction strengths are expected to increase the stability of food webs (Binzer et al. 2012, Kratina et al. 2012, Rosenblatt et al. 2019, Sentis et al. 2015), the effect of both 4 °C warming and potentially also heatwaves on the predators are expected to positively contribute to the long-term stability of the studied high-latitude damselfly-water flea trophic system.

3.2 Effects of warming and algae-induced turbidity

Previous studies showed that warming may reduce long-term interaction strengths (Rall et al. 2010, Sentis et al. 2015, Wang et al. 2021). However, these studies did not directly measure all model parameters needed to estimate long-term interaction strength under warming, and did not evaluate the impact of warming in the absence and presence of algae-induced turbidity, another important global change-related factor. The results in Chapter IV indicate that, warming did decrease the long-term interaction strength, but this was only for systems with low-latitude predators in turbid water. Instead, warming increased the long-term interaction strength for systems with high-latitude predators in clear water. Warming reduced the long-term interaction strength in turbid water through the theoretically predicted (Fussmann et al. 2014, Rall et al. 2010) higher prey intrinsic growth rates and lower carrying capacities at the higher temperature, and a trend for a higher predator metabolic rate at constant temperature (Wang et al. 2021). But in clear water, the trend of increased search rates at the higher temperature opposed and even reversed the effects of warming on these three factors, resulting in an increased long-term interaction strength under warming. Thus, in contrast with the prediction based on the results from Chapter III, the results of Chapter IV predicted the effect of 4 °C warming to negatively contribute to the long-term stability of the studied high-latitude trophic system. This striking difference in our predictions to warming impact on the high-latitude trophic system was due to the differential thermal plasticity of search rate and handling time between Chapter III and IV, which could be explained by the thermal adaptation and/or acclimation history of the prey we used (discussed below in Limitations and further directions). How functional response parameters determine the pattern of long-term interaction strength is dependent on the equilibrium density of the prey. While search rate is more influential at low prey equilibrium densities, handling time is more influential at high prey equilibrium densities.

Turbidity caused in general an increase in long-term interaction strength, suggesting that nutrient enrichment may destabilize trophic systems by increasing turbidity levels, which goes in the same direction as observations in experimental freshwater communities (Kratina et al. 2012). Similar as for warming, this was caused by higher search rates, while the lower prey intrinsic growth rates and the higher carrying capacities under turbidity also contributed. In conditions where *Daphnia* encounters more algae (as in the turbid water in Chapter IV), an increased carrying capacity was observed before (Bosker et al. 2019, Kratina et al. 2012), yet a lower intrinsic growth rate was not (Giebelhausen and Lampert 2001). One possible explanation could be that *Daphnia* overfed on algae, resulting in gut overloading and a rapid increase in body size, increasing metabolic costs for digestion and carapace formation, and reducing the energy available for reproduction. It could also be the result of “superfluous feeding” in which the assimilation efficiency is lowered in the presence of overly-abundant food (e.g. Schindler 1968). An alternative explanation could be that the excess algae easily form clusters and get stuck to the outer surface of *Daphnia* carapace, reducing the smoothness of juvenile release from the brood chamber. These explanations have not yet been documented in previous studies.

Given that higher long-term interaction strengths are expected to reduce the stability of food webs (Binzer et al. 2012, Kratina et al. 2012, Rosenblatt et al. 2019, Sentis et al. 2015), the results of Chapter IV suggest that both global change-related stressors, 4 °C warming and turbidity, may negatively affect the long-term stability of the high-latitude trophic system. This contrasts with the previous findings that warming will stabilize predator-prey dynamics (Fussmann et al. 2014, Sentis et al. 2015, Wang et al. 2021). These destabilizing effects of both global change-related stressors were mainly driven by how they affected predator search rates.

4. Role of thermal evolution in mediating the effects of global change stressors on the long-term predator-prey interaction strength

Studies on how global change-related stressors affect trophic interactions in the long term typically only consider acute and/or plastic responses in predator and prey (Daugaard et al. 2019, Sentis et al. 2015). Nevertheless, there is increasing evidence that besides plasticity also evolution may play an important role in shaping responses to global change (Stoks et al. 2014, Urban et al. 2016). Importantly, thermal evolution can play a crucial role in reversing maladaptive thermal responses or enhancing adaptive ones (Diamond and Martin 2016). Using a space-for-time substitution, Chapters III and IV used the current phenotypes of warm-adapted (low-latitude) populations at their local high mean temperature (24 °C) as the estimated future phenotypes of cold-adapted (high-latitude) populations after thermal evolution (De Frenne et al. 2013, Verheyen et al. 2019), to investigate how thermal evolution of predator and/or prey mediate the plastic response of long-term trophic interaction strength under warming and warming combined with other key global change stressors.

The results in Chapter III suggest the impact of thermal plasticity on the stability of the high-latitude trophic system under warming and temperature variation can be ameliorated by the thermal evolution of the predator. When only assuming thermal plasticity, the high-latitude trophic system may become more stable under 4 °C warming unless daily temperature fluctuation (DTF) or potentially a heatwave (HW) is considered. Predator thermal evolution can further stabilize the high-latitude system under warming, and this even with DTF and potentially also with HW. Yet, while Chapter III provided valuable new insights, a critical limitation toward further realism is that it did not consider thermal evolution in the prey. Previous work showed latitude-related thermal adaptation in the survival of *D. magna* under damselfly predation, but this pattern disappeared when considering the thermal adaptation of the predators (De Block et al. 2013). This may tentatively suggest that thermal evolution of the prey may have less impact compared to thermal evolution of the predator on the pattern of long-term interaction strength. To refine insights, Chapter IV also included prey from different latitudes while estimating the climate change impact on trophic interactions in the presence of evolution in both predator and prey.

The results in Chapter IV suggest the impact of thermal plasticity and thermal evolution on the stability of the high-latitude trophic system under warming is critically dependent on the turbidity level. When only assuming thermal plasticity, the high-latitude trophic system may become less stable (i.e. easily enter cycles or collapse) under 4 °C warming in clear water but not in turbid water. Predator thermal evolution alone may reduce (yet not eliminate) the negative impact of warming through plasticity on the stability of the high-latitude system, whereas joint thermal evolution of both predator and prey will tend to further destabilize the system. In turbid water, instead predator thermal evolution alone (regardless of prey thermal evolution) may stabilize the trophic system as it reduced the long-term interaction strength. These space-for-time based scenarios of long-term interaction strength are highly consistent with those of predator search rate, indicating an important contribution of search rate in mediating effects of warming-induced evolution on long-term interaction strength and stability in the study system. In contrast, thermal evolution of the prey did not significantly affect trophic interaction, suggesting its role to be less important than the thermal evolution of the predator.

Taken together, the results of the space-for-time substitution indicate the potential and constraints of thermal evolution in mediating the plastic response of long-term predator-prey interaction strength at high latitudes under future warming in the absence or presence of other global change stressors. Firstly, the effects of thermal evolution in the predator seem crucial in mediating thermal plasticity of long-term interaction strength, because in both Chapters III and IV there was an important influence of thermal evolution of the predator weakening the long-term interaction strength. Secondly, the composition of the trophic system can critically affect the direction of thermal plasticity of long-term interaction strength and the impact of thermal evolution under warming. Indeed, while the thermal evolution of the predator alone further stabilized the high-latitude system with mid-latitude prey under warming and temperature variation by decreasing long-term interaction strength, it only significantly stabilized the high-latitude system with high-latitude prey in turbid water. Typically, a trophic system at a given latitude comprises matched predators and prey of the same latitude. But the ‘un-natural’ combinations of predator and prey latitude allows us to mimic the evolution of only the predator, only the prey, or both. Thirdly, the effects of thermal evolution of predator and prey may not be additive but rather antagonistic to stabilize the predator-prey system. This is based on the results of Chapter IV, where thermal evolution of predator or prey alone tended to weaken the plastic effect of warming, while the evolution of both predator and prey tended to amplify the plastic effect of warming in clear water.

5. Limitations and future directions

Based on the results and discussion in each chapter, I will list some limitations and directions for future research in the following paragraphs.

5.1 Rapid thermal evolution of TPCs

In Chapter I, I examined the rapid thermal evolution of the TPCs of the water flea *D. magna*. The chosen TPC modeling approach classifying TPCs into intercept, linear (Arrhenius), and quadratic (Arrhenius-quadratic) models after Twadochleb et al. (2020) may hide some information in the unimodal TPCs. Firstly, in quadratic models the activation energy (E_a) could not be estimated and compared. Secondly, quadratic models fit symmetric TPCs, while some traits may show asymmetric TPCs, reflecting differential thermal plasticity (i.e. slope of the curve) below and above optimum temperature (T_{opt}). Future TPC studies may improve these by fitting or averaging alternative TPC models of interest, such as the Sharpe-Schoolfield model, using a more advanced approach (introduced in Padfield et al. 2021). Yet, one should carefully select a suitable model (e.g. base on AIC), avoid overfitting the TPC curves while choosing TPC models that contain more parameters, and note the limitations of different models. For example, unlike the quadratic model, most TPC models cannot deal with negative trait values.

The TPCs of *D. magna* were based on performance measured at constant mean temperatures. In natural environments, however, species experience temperature variation apart from the means. Thus, a more realistic approach will be to compare estimates derived from TPCs under fluctuating temperatures in laboratory and field conditions. For example, Khelifa et al. (2019) demonstrated in flies that TPC predictions based on constant temperatures could only accurately reflect performance under fluctuating temperatures when flies do not encounter temperatures below the critical minimum. Other factors, such as ontogenetic stage, behavioral selection of different thermal regimes, and multi-stressor interactions may also affect the realism of TPCs (Sinclair et al. 2016). This highlights the importance of integrating more realistic thermal conditions (e.g. not only temperature mean but also temperature variation) in TPCs to better understand and predict the effects of rapid evolution of TPCs on species' fitness under climate change.

5.2 Latitudinal variation in thermal plasticity of predation rate

In Chapter II, I studied the predation rates of low- and high-latitude *I. elegans* larvae on the mid-latitude prey *D. magna* to test the climatic variability hypothesis (CVH), which predicts thermal plasticity to increase with climatic variability (hence with latitude). While comparing the thermal plasticity of low- and high-latitude predators at 20 °C and 24 °C, this thermal interval might not cover the steepest slope of TPCs in the predation rate of *I. elegans*. Instead, this thermal interval could reflect the near-optimum part of the TPCs, the part with relatively flat slopes, making patterns in thermal plasticity less easy to be detected. Therefore, ideally, more rearing and test temperatures to cover non-optimum temperatures should be used to test CVH.

The mixed support for CVH from Chapter II suggests other mechanisms could contribute to the latitudinal patterns of predation rate, such as thermal adaptation to the local mean temperature, or thermal adaptation to the local daily temperature fluctuations. In addition, differences in the evolutionary trajectory of thermal tolerance, such as evolution under warmer and more variable environment vs. evolution under cooler and less variable environment, could lead to contrasting patterns of the relationship between acclimation capacity and thermal tolerance (Bozinovic et al. 2011). However, the experimental design in Chapter II did not allow to separate the contribution of these potential factors from the contribution of climatic variability. Therefore, a future challenge would be integrating other experimental/analytical approaches to better detangle/identify the main factor contributing to the latitudinal pattern of thermal plasticity and/or acclimation capacity. For example, to exclude the contribution of climatic variability from the latitudinal variation in thermal plasticity, one can compare populations at two latitudes with similar climatic variability (e.g. tropical zone vs. subarctic zone in the Northern hemisphere). Alternatively, one can culture a population for several generations under a factorial design crossing mean temperature and climatic variability, and compare the resulting thermal plasticity between treatment groups.

The much higher thermal acclimation capacity in the predation rates of high-latitude predators compared to low-latitude ones brings up further questions. What could be the cost of acclimation capacity? Is there a trade-off of acclimation capacity between traits and/or stressors? These topics have received attention in recent studies. For example, Loughland and Seebacher (2020) found in a mosquitofish that oxidative stress may be a cost that constrains the capacity for acclimation. This indicates that, although having a higher thermal acclimation capacity, high-latitude predators may have become more sensitive to additional oxidative stress compared to low-latitude predators. This may explain why high-latitude predators had generally lower performance compared to low-latitude predators when tested at heatwave temperatures.

5.3 Simplified trophic interactions

While investigating the effects of some key global change factors on functional response parameters and long-term trophic interactions, this thesis focused on a stage-fixed consumer-resource pair in a closed, refuge-free environment. Such simplified design excluded other factors in a food web that may affect trophic interactions, such as body size structure of the consumer and resource populations (Cuthbert et al. 2020, Rall et al. 2012), alternative resources for generalist consumers and intra/interspecific interference (De Villemereuil and López-Sepulcre, Stouffer and Novak 2021), predation risk (Hammill et al. 2010), and habitat complexity (Mocq et al. 2021). Including these factors would add more realism to the experimental design, but would also increase the number of predation trials required for the functional response experiment. This dilemma may be solved by a recently developed approach using a series of time-to-capture measurements to estimate functional response parameters (Coblentz and DeLong 2021).

5.4 Space-for-time substitution approach

In Chapter III, I applied the space-for-time substitution approach to assess if gradual thermal evolution in high-latitude predator populations can mediate the effect of stressors associated with global warming (increase in mean temperature, daily temperature variation, and a heatwave) on the long-term interaction strength. In Chapter IV, I applied the space-for-time substitution approach to assess if thermal evolution of the predator and/or the prey can mediate warming effects, and if this differs between clear and turbid water bodies. However, the space-for-time substitution approach has some limitations.

For example, while the rate of thermal evolution in *D. magna* could be rapid (as indicated in Chapter I), the thermal evolution in high-latitude *I. elegans* may not be fast enough to catch up with the pace of projected warming by 2100. Also, evolutionary change requires sufficient genetic variation. Although a previous study has shown additive genetic variation for growth rate and its thermal plasticity in a high-latitude population of *I. elegans* (Shama et al. 2011), it is unknown if such genetic variation also exists in traits related to predator-prey interactions. The next step would be to estimate the potential for rapid evolutionary response in this predator species. This can be done by direct estimation of the additive genetic (co-)variances in breeding experiments.

While the space-for-time substitution approach was used to investigate the potential of thermal evolution of the predator and the prey shaping long-term interaction strength under warming, it was not applied to explore the potential of evolution of the predator and the prey shaping long-term interaction strength under a change in algae-induced turbidity. This is because there is no consistent latitudinal pattern of algae-induced turbidity based on previous observations (as indicated by Dodds et al. 2019, Stephens et al. 2015, Wang et al. 2020; Chapter IV, Appendix S1). In freshwater ecosystems, clear and turbid states can be unstable with regular switches between both states (Bayley et al. 2007), and the gene flow among *I. elegans* populations can be high (Wellenreuther et al. 2011). Thus, local adaptation to algae-induced turbidity in the species may be less likely to occur. Nevertheless, future studies should monitor the long-term dynamics of turbidity change and trophic composition in both low- and high-latitude sites, to better understand and predict the food web stability in freshwater ecosystems under global warming. There could be other latitude-associated factors affecting the results of space-for-time substitutions, although a previous study did not detect significant differences in several abiotic and biotic factors between the low- and high-latitude predator collection sites used in this thesis (Table S1 in Debecker and Stoks 2019). Nevertheless, the photoperiod regimes differ also between latitudes and how these affect the here studied traits may be interesting to study.

Last but not least, comparing the results of Chapters III and IV, there is a striking difference in the obtained predictions of the impact of warming on the high-latitude trophic system in clear water. Chapter III predicted warming to stabilize the high-latitude trophic system by decreasing long-term interaction strength, while Chapter IV predicted warming to destabilize the high-latitude trophic system by increasing long-term interaction strength. Note that, while Chapter III used high-latitude predators and mid-latitude prey to form the “high-latitude system”, Chapter IV used high-latitude predators and high-latitude prey. Therefore, the differential outcomes of long-term interaction strength of the high-latitude system could be due to the effect of latitude-associated adaptation of prey on the functional response parameters in high-latitude predators. For example, while search rates in high-latitude predators preying on mid-latitude prey were not affected by 4 °C warming (Chapter III), search rates in high-latitude predators preying on high-latitude prey tended to increase under 4 °C warming (Chapter IV). This indicates high-latitude predators have a higher thermal plasticity in search rates between 20 °C and 24 °C when coupled with high-latitude prey rather than mid-latitude prey. One explanation could be that high-latitude prey was adapted to a lower predation risk and/or a lower mean temperature compared to mid-latitude prey, thus the behavioral antipredator response is weaker at the higher temperature of 24 °C compared to mid-latitude prey. Another explanation could be the acclimation history before the execution of the predation trials. Indeed, while the mid-latitude prey was acclimatized at 22 °C for generations before being tested at 20 °C and 24 °C, the high-latitude prey was acclimatized at the two test temperatures for generations. The exact mechanisms contributing to this pattern require further study, and may advance our understanding of how the composition of the high-latitude trophic system may affect our prediction of the impact of global warming on food web stability. Also, it could be that the duration of the predation trails (24h in Chapter III vs. 6h in Chapter IV) affected the results. Yet, a shorter duration of the predation trials would likely only increase the estimated search rates (suggested by Li et al. 2018) without reversing the general patterns.

5.5 Other facets of system stability

When using the long-term interaction strength at equilibrium to make inferences about trophic system stability, as we did in Chapters III and IV, there are two notes to be made. First, our results based on equilibria would likely hold even if predator-prey population dynamics enter cycles, because the size of the cycles (i.e. the amplitude of population fluctuations, a transient characteristic to indicate system stability) increases with the unstable predator equilibrium density, which positively correlates with the long-term interaction strength (Sentis et al. 2015). Second, in contrast to the approach followed in my thesis, system stability can also be indicated by transient characteristics of the system, such as the possibility of species extinction in simulations of population dynamics, the time to extinction (e.g. Daugaard et al. 2019), and other ones (reviewed by Domínguez-García et al. 2019). Given natural communities are less frequently at an equilibrium state compared to a transient state because of disturbances, these transient characteristics await further study to generalize the predicted impact of global change on food web stability.

Second, although a lower long-term interaction strength could make trophic systems more stable, it does not always benefit trophic systems. As mentioned earlier in Chapters III and IV, with a low long-term interaction strength, predators are more likely to suffer from starvation and local extinction when their energetic efficiency is too low (Vucic-Pestic et al. 2011). The decline of predators may lead to predator-release process at lower trophic levels and increase the system susceptibility to perturbation (e.g. Britten et al. 2014). Though we did not observe significant changes in predator energetic efficiency under the global change factors tested in this thesis, it is still important to keep in mind that a more stable trophic system requires not only a lower long-term interaction strength, but also an energetic efficiency that allows the persistence of consumers across trophic levels.

6. Take-home messages

Multiple stressors associated with global change may interact with each other in affecting species (reviewed by Jackson et al. 2016, Côté et al. 2016, Orr et al. 2020). Species responses to these stressors may be plastic and/or genetic (reviewed by Merilä and Hendry 2014, Nadeau and Urban 2020), and these responses may vary with temporal and/or spatial scales (reviewed by Jackson et al. 2021, Valladares et al. 2014). Notably, direct effects of environmental stressors may cascade into indirect effects via trophic interactions among species, which complicates the understanding and prediction of the impact of global change on the local persistence of species (Huey et al. 2012, Angert et al. 2013). Therefore, investigating the effects of multiple stressors on trophic interactions has become crucial to improve our forecast for biodiversity under global change (Urban et al. 2016).

The results in Chapter I pointed out that, as a key aquatic consumer and prey species, the water flea *D. magna* can undergo rapid thermal evolution of thermal performance curves (TPCs) in intrinsic growth rate, an critical fitness estimate. While rapid thermal evolution increased fitness at higher temperatures, this was not accompanied by a change in the relative thermal scaling of ingestion rates and metabolic rates, suggesting this may not necessarily strengthen the top-down impact of the consumer on its resource.

The results in Chapter II demonstrated that the latitudinal patterns in thermal plasticity and acclimation of predation rates of an aquatic predator (*I. elegans*) critically depended on the plasticity type and acclimation metric, thereby generating mixed support for the climatic variability hypothesis (CVH). Notably, while both low- and high-latitude predators showed perfect thermal compensation in predation rate, this was driven by a much higher thermal acclimation capacity in high-latitude predators. This supports the recent plea by Einum et al. (2019) to quantify thermal acclimation relative to the acute plasticity.

The results in Chapter III underscored the importance of not only increases in mean temperatures but also the presence of daily thermal fluctuations and heatwaves in shaping long-term trophic interaction strength. In line with the increasing evidence of evolution in response to global warming (Merilä and Hendry 2014, González-Tokman et al. 2020), the results suggest thermal evolution of the predator to ameliorate thermal plastic effects on the food web stability, and highlights the need to integrate both thermal plasticity and thermal evolution in predicting the effects of global warming on trophic systems.

The results in Chapter IV revealed the importance of considering besides warming also turbidity in shaping long-term trophic interactions, and suggest that thermal evolution may increase or decrease food web stability under warming depending on the turbidity level. Given that warming and eutrophication are two major global change-related co-occurring factors negatively affecting freshwater ecosystems (Birk et al. 2020, Hering et al. 2015, Moss 2011, Woodward et al. 2010), there is a need of integrating evolution and turbidity to improve projections of trophic system stability under warming.

Taken together, the results of this thesis highlight the necessity for considering both thermal plasticity and thermal evolution while predicting the effects of multiple global change stressors in shaping long-term trophic interaction strength. Thermal evolution could ameliorate the impact of global warming on the food web stability, but this may critically depend on other global change stressors and the latitude of the trophic system.

Three key messages for stakeholders

Without education and public outreach, scientific research can never achieve its ultimate goal in fostering both the knowledge of nature and the sense of responsibility of mankind over life on Earth. With this thesis, I wish to deliver three take-home messages to the public. First, several aspects of anthropogenic global change may alone and in combination impact natural ecosystems. If we ignore the co-occurrence of key stressors (e.g. an increase in mean temperature together with an increase in temperature fluctuation), we could generate inaccurate predictions and thereby inadequate mitigation measures. Second, both thermal plasticity and thermal evolution may help species to cope with global change, but these are not ‘silver bullets’, and face limitations and uncertainties. Moreover, actions are needed to preserve the evolutionary potential of populations under risk of global change. Third, freshwater ecosystems require urgent attention due to their scarce distribution, vital ecosytem services for most species, and vulnerability under multiple anthropogenic stressors. For example, I have shown that one important factor related to eutrophication, algae-induced turbidity, that is a key stressor in freshwater systems may have a strong impact on the effects of warming. I hope this thesis will inspire future studies to expand our knowledge of ecosystem functioning and to support public actions for bio-diversity conservation.

References

Adamczuk, M. 2020. Population dynamics and life history traits of *Daphnia magna* across thermal regimes of environments. *Sci. Total Environ.* 723: 137963. https://doi.org/10.1016/j.scitotenv.2020.137963

Addo-Bediako, A. et al. 2000. Thermal tolerance, climatic variability and latitude. *Proc. R. Soc. B* 267: 739-745. https://doi.org/10.1098/rspb.2000.1065

Aguilar-Kirigin, A. J. and Naya, D. E. 2013. Latitudinal patterns in phenotypic plasticity: the case of seasonal flexibility in lizards' fat body size. *Oecologia* 173: 745-752. https://doi.org/10.1007/s00442-013-2682-z

Alexander, T. J. et al. 2017. Does eutrophication-driven evolution change aquatic ecosystems? *Philos. Trans. R. Soc. B* 372: 20160041. https://doi.org/10.1098/rstb.2016.0041

Allen, A. P. and Gillooly, J. F. 2007. The mechanistic basis of the metabolic theory of ecology. *Oikos* 116: 1073-1077. https://doi.org/10.1111/j.2007.0030-1299.16079.x

Allen-Ankins, S. et al. 2012. The effects of turbidity, prey density and environmental complexity on the feeding of juvenile Murray cod *Maccullochella peelii*. *J. Fish Biol.* 80: 195–206. https://doi.org/10.1111/j.1095-8649.2011.03166.x

Amarasekare, P. 2019. Effects of climate warming on consumer-resource interactions: A latitudinal perspective. *Front. Ecol. Evol.* 7: 146. https://doi.org/10.3389/fevo.2019.00146

Angert, A. L. et al. 2013. Climate change and species interactions: ways forward. *Ann. N. Y. Acad. Sci.* 1297: 1-7. https://doi.org/10.1111/nyas.12286

Angilletta, M. J. et al. 2004. Temperature, growth rate, and body size in ectotherms: Fitting pieces of a life-history puzzle. *Integr. Comp. Biol.* 44: 498-509. http://doi.org/10.1093/icb/44.6.498

Angilletta Jr., M. J. 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford University Press.

Anneville, O. et al. 2015. Blue-green algae in a "greenhouse century"? New insights from field data on climate change impacts on cyanobacteria abundance. *Ecosystems* 18: 441–458. https://doi.org/10.1007/s10021-014-9837-6

Anton-Pardo, M. et al. 2019. Global meta-analysis reveals that invertebrate diversity is higher in permanent than in temporary lentic water bodies. *Freshwater Biol.* 64: 2234-

2246. https://doi.org/10.1111/fwb.13409

Arambourou, H. and Stoks, R. 2015. Combined effects of larval exposure to a heat wave and chlorpyrifos in northern and southern populations of the damselfly *Ischnura elegans*. *Chemosphere* 128: 148-154. https://doi.org/10.1016/j.chemosphere.2015.01.044

Archer, L. C. et al. 2019. Consistent temperature dependence of functional response parameters and their use in predicting population abundance. *J. Anim. Ecol.* 88: 1670-1683. https://doi.org/10.1111/1365-2656.13060

Baker, K. G. et al. 2016. Thermal performance curves of functional traits aid understanding of thermally induced changes in diatom-mediated biogeochemical fluxes. *Front. Mar. Sci.* 3: 44. https://doi.org/10.3389/fmars.2016.00044

Barria, A. M. and Bacigalupe, L. D. 2017. Intraspecific geographic variation in thermal limits and acclimatory capacity in a wide distributed endemic frog. *J. Therm. Biol.* 69: 254-260. https://doi.org/10.1016/j.jtherbio.2017.08.010

Barria, A. M. et al. 2018. Thermal physiological traits and plasticity of metabolism are sensitive to biogeographic breaks in a rock-pool marine shrimp. *J. Exp. Biol*. 221: eb181008. https://doi.org/10.1242/jeb.181008

Bartley, T. J. et al. 2019. Food web rewiring in a changing world. Nat. Ecol. Evol. 3: 345-354. https://doi.org/10.1038/s41559-018-0772-3

Barton, B. T. and Schmitz, O. J. 2018. Opposite effects of daytime and nighttime warming on top-down control of plant diversity. *Ecology* 99: 13-20. https://doi.org/10.1002/ecy.2062

Bates, D. et al. 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67: 1-48. arXiv:1406.5823

Bayley, S.E. et al. 2007. Frequent regime shifts in trophic states in shallow lakes on the Boreal Plain: alternative ‘unstable’ states?. *Limnol. Oceanogr.* 52: 2002-2012. https://doi.org/10.4319/lo.2007.52.5.2002

Beaman, J. E. et al. 2016. Evolution of plasticity: mechanistic link between development and reversible acclimation. *Trends Ecol. Evol*. 31: 237-249. https://doi.org/10.1016/j.tree.2016.01.004

Begon, M. et al. 2006. Ecology: from individuals to ecosystems. Malden: Blackwell.

Beniston, M. et al. 2017. Comparing observed and hypothetical climates as a means of communicating to the public and policymakers: The case of European heatwaves.

Environ. Sci. Policy 67: 27-34. https://doi.org/10.1016/j.envsci.2016.11.008

Benjamini, Y. and Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B* 57: 289-300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Berlow, E. L. et al. 1999. Quantifying variation in the strengths of species interactions. *Ecology* 80: 2206-2224. https://doi.org/10.1890/0012-9658(1999)080[2206:QVITSO]2.0.CO;2.

Bernhardt, J. R. et al. 2018. Nonlinear averaging of thermal experience predicts population growth rates in a thermally variable environment. *Proc. R. Soc. B* 285: 20181076. https://doi.org/10.1098/rspb.2018.1076

Binzer, A. et al. 2012. The dynamics of food chains under climate change and nutrient enrichment. *Philos. Trans. R. Soc. B* 367: 2935-2944. https://doi.org/10.1098/rstb.2012.0230

Birk, S. et al. 2020. Impacts of multiple stressors on freshwater biota across spatial scales and ecosystems. *Nat. Ecol. Evol.* 4: 1060-1068. https://doi.org/10.1038/s41559-020-1216-4

Bosker, T. et al. 2019. Significant decline of *Daphnia magna* population biomass due to microplastic exposure. *Environ. Pollut.* 250: 669-675. https://doi.org/10.1016/j.envpol.2019.04.067

Boukal, D. S. et al. 2019. Species interactions under climate change: connecting kinetic effects of temperature on individuals to community dynamics. *Curr. Opin. Insect Sci.* 35: 88-95. https://doi.org/10.1016/j.cois.2019.06.014

Bozinovic, F. et al. 2011. Physiological correlates of geographic range in animals. *Annu. Rev. Ecol. Evol. Syst* 42: 155-79. https://doi.org/10.1146/annurev-ecolsys-102710-145055

Britten, G. L. et al. 2014. Predator decline leads to decreased stability in a coastal fish community. *Ecol. Lett.* 17: 1518-1525. https://doi.org/10.1111/ele.12354

Brown, J. H. et al. 2004. Toward a metabolic theory of ecology. *Ecology* 85: 1771-1789. https://doi.org/10.1890/03-9000

Brönmark, C. and Hasson, L. 2002. Environmental issues in lakes and ponds: Current state and perspectives. *Environ. Conserv.* 29: 290-307. http://www.jstor.org/stable/44520612

Burnham, K.P. and Anderson, D. R. 2002. Model Selection and Multimodel Inference: A

Practical Information Theoretic Approach, 2nd edn. Springer-Verlag, New York.

Buxton, C. D. et al. 1981. Response surface analysis of the combined effects of exposure and acclimation temperatures on filtration, oxygen consumption and scope for growth in the oyster *Ostrea edulis*. *Mar. Ecol. Prog. Ser.* 6: 73-82. https://doi.org/10.3354/meps006073

Calosi, P. et al. 2008. Thermal tolerance, acclimatory capacity and vulnerability to global climate change. *Biol. Lett.* 4: 99-102. https://doi.org/10.1098/rsbl.2007.0408

Capaul, M. and Ebert, D. 2003. Parasite-mediated selection in experimental *Daphnia magna* populations. *Evolution* 57: 249-260. https://doi.org/10.1554/00143820(2003)057[0249:PMSIED]2.0.CO;2

Carbonell, J. A. and Stoks, R. 2020. Thermal evolution of life history and heat tolerance during range expansions toward warmer and cooler regions. *Ecology* 101: e03134. https://doi.org/10.1002/ecy.3134

Carbonell, J. A. et al. 2021. Evolution of cold tolerance and thermal plasticity in life history, behaviour and physiology during a poleward range expansion. *J. Anim. Ecol.* 00: 1-12. https://doi.org/10.1111/1365-2656.13482

Cattiaux, J. et al. 2015. Projected increase in diurnal and interdiurnal variations of European summer temperatures. *Geophys. Res. Lett.* 42: 899– 907. https://doi.org/10.1002/2014GL062531.

Catullo, R. A. et al. 2019. The potential for rapid evolution under anthropogenic climate change. *Current Biology* 29: R996-R1007. https://doi.org/10.1016/j.cub.2019.08.028

Cavalheri, H. B. et al. 2019. Rapid evolution of thermal plasticity in mountain lake *Daphnia* populations. *Oikos* 128: 692-700. https://10.1111/oik.05945

Chopelet, J. et al. 2008. Plasticity of growth rate and metabolism in *Daphnia magna* populations from different thermal habitats. *J. Exp. Zool. A* 309: 553-562. https://doi.org/10.1002/jez.488

Coblentz, K. E. and DeLong, J. P. 2021. Estimating predator functional responses using the times between prey captures. *Ecology* 102: e03307. https://doi.org/10.1002/ecy.3307

Cohen, J. M. et al. 2018. A global synthesis of animal phenological responses to climate change. *Nat. Clim. Change* 8: 224. https://doi.org/10.1038/s41558-018-0067-3

Colinet, H. et al. 2015. Insects in fluctuating thermal environments. *Ann. Rev. Entomol.* 60: 123-140. https://doi.org/10.1146/annurev-ento-010814-021017

Comte, L. and Olden, J. 2017. Climatic vulnerability of the world's freshwater and marine fishes. *Nat. Clim. Change* 7: 718-722. https://doi.org/10.1038/nclimate3382

Corbet, P. S. 1999. Dragonflies: behavior and ecology of Odonata. Cornell University Press, London.

Corbet, P. S. et al. 2006. Voltinism of odonata: A review. *Int. J. Odonatol.* 9: 1-44. https://doi.org/10.1080/13887890.2006.9748261

Côté, I. M. et al. 2016. Interactions among ecosystem stressors and their importance in conservation. *Proc. R. Soc. B* 283: 1–9. https://doi.org/10.1098/rspb.2015.2592

Cuenca Cambronero, M. et al. 2018. Predictability of the impact of multiple stressors on the keystone species *Daphnia*. *Sci. Rep.* 8: 17572. https://doi.org/10.1038/s41598-018-35861-y

Cuthbert, R. N. et al. 2020. Influence of intra- and interspecific variation in predator–prey body size ratios on trophic interaction strengths. *Ecol. Evol.* 10: 5946-5962. https://doi.org/10.1002/ece3.6332

Daugaard, U. et al. 2019. Warming can destabilize predator–prey interactions by shifting the functional response from Type III to Type II. *J. Anim. Ecol.* 88: 1575-1586. https://doi.org/10.1111/1365-2656.13053

David, J. R. et al. 2005. Isofemale lines in *Drosophila*: an empirical approach to quantitative trait analysis in natural populations. *Heredity* 94: 3-12. https://doi.org/10.1038/sj.hdy.6800562

De Block, M. and Stoks, R. 2003. Adaptive sex-specific life history plasticity to temperature and photoperiod in a damselfly. *J. Evol. Biol.* 16: 986-995. https://doi.org/10.1046/j.1420-9101.2003.00581.x

De Block, M. et al. 2013. Local genetic adaptation generates latitude-specific effects of warming on predator–prey interactions. *Glob. Change Biol.* 19: 689-696. https://doi.org/10.1111/gcb.12089

Debecker, S. et al. 2017. Strong delayed interactive effects of metal exposure and warming: latitude-dependent synergisms persist across metamorphosis. *Environ. Sci. Technol.* 51: 2409-2417. https://doi.org/10.1021/acs.est.6b04989

Debecker, S. and Stoks, R. 2019. Pace of life syndrome under warming and pollution: integrating life history, behavior, and physiology across latitudes. *Ecol. Monogr.* 89: e01332. https://doi.org/10.1002/ecm.1332

De Frenne, P. et al. 2013. Latitudinal gradients as natural laboratories to infer species' responses to temperature. *J. Ecol.* 101: 784-795. https://doi.org/10.1111/1365-2745.12074

De Gelas, K. and De Meester, L. 2005. Phylogeography of *Daphnia magna* in Europe. Mol. Ecol. 14: 753-764. https://doi.org/10.1111/j.1365-294X.2004.02434.x

Dell, A. I. et al. 2014. Temperature dependence of trophic interactions are driven by asymmetry of species responses and foraging strategy. *J. Animal Ecol.* 83: 70-84. https://doi.org/10.1111/1365-2656.12081

De Meester, L. et al. 2018. Genetic adaptation as a biological buffer against climate change: potential and limitations. *Integr. Zool.* 13: 372-391. https://doi.org/10.1111/1749-4877.12298

Derot, J. et al. 2020. Response of phytoplankton traits to environmental variables in French lakes: New perspectives for bioindication. *Ecol. Indic.*108: 105659. https://doi.org/10.1016/j.ecolind.2019.105659

Deutsch, C. A. et al. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *PNAS* 105: 6668-6672. https://doi.org/10.1073/pnas.0709472105

De Villemereuil, P. B. and López-Sepulcre, A. 2011. Consumer functional responses under intra-and inter-specific interference competition. *Ecol. Model.* 222: 419-426. https://doi.org/10.1016/j.ecolmodel.2010.10.011

Diamond, S. E. and Martin, R.A. 2016. The interplay between plasticity and evolution in response to human-induced environmental change. *F1000Research* 5: 2835. https://doi.org/10.12688/f1000research.9731.1

Diamond, S. E. and Martin, R. A. (2020). Evolution is a double-edged sword, not a silver bullet, to confront global change. *Ann. N. Y. Acad. of Sci.* 1469: 38-51. https://doi.org/10.1111/nyas.14410

Dijkstra, K-D. B. 2006. Field guide to the dragonflies of Britain and Europe. British Wildlife Publishing, Gillingham, Dorset, UK.

Dillon, M. E. et al. 2016. Life in the frequency domain: the biological impacts of changes in climate variability at multiple time scales. *Integr. Comp. Biol.* 56: 14-30. https://doi.org/10.1093/icb/icw024

Dinh Van, K. et al. 2014. Temperature-and latitude-specific individual growth rates shape the vulnerability of damselfly larvae to a widespread pesticide. *J. Appl. Ecol.* 51: 919-

928. https://doi.org/10.1111/1365-2664.12269

Dodds, W. K. et al. 2013. Human impact on freshwater ecosystem services: a global perspective. *Environ. Sci. Technol.* 47: 9061-9068. https://doi.org/10.1021/es4021052

Dodds, W. K. et al. 2019. The freshwater biome gradient framework: Predicting macroscale properties based on latitude, altitude, and precipitation. *Ecosphere* 10: e02786. https://doi.org/10.1002/ecs2.2786

Domínguez-García, V. et al. 2019. Unveiling dimensions of stability in complex ecological networks. *Proc. Natl. Acad. Sci. U.S.A.* 116: 25714-25720. https://doi.org/10.1073/pnas.1904470116

Donelson, J. M. et al. 2011. Acclimation to predicted ocean warming through developmental plasticity in a tropical reef fish. *Glob. Change Biol.* 17: 1712-1719. https://doi.org/10.1111/j.1365-2486.2010.02339.x

Donelson, J. M. and Munday, P. L. 2012. Thermal sensitivity does not determine acclimation capacity for a tropical reef fish. *J. Anim. Ecol.* 81: 1126-1131. https://doi.org/10.1111/j.1365-2656.2012.01982.x

Drinkwater, R. et al. 2020. Leech blood-meal invertebrate-derived DNA reveals differences in Bornean mammal diversity across habitats. *Mol. Ecol.* 00: 1-14. https://doi.org/10.1111/mec.15724

Dudgeon, D. et al. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biol. Rev.* 81: 163-182. https://doi.org/10.1017/S1464793105006950

Ebert, D. 2005. Ecology, epidemiology, and evolution of parasitism in *Daphnia*. National Library of Medicine (US).

Einum, S. et al. 2019. How to quantify thermal acclimation capacity? *Glob. Change Biol.* 25: 1893-1894. https://doi.org/10.1111/gcb.14598

Englund, G. et al. 2011. Temperature dependence of the functional response. *Ecol. Lett.* 14: 914-921. https://doi.org/10.1111/j.1461-0248.2011.01661.x

Faafeng, B. A. and Mjelde, M. 1998. Clear and turbid water in shallow norwegian lakes related to submerged vegetation. Springer, New York, NY. https://doi.org/10.1007/978-1-4612-0695-8_27

Fangue, N. A. et al. 2009. Do mitochondrial properties explain intraspecific variation in thermal tolerance? *J. Exp. Biol.* 212: 514-522. https://doi.org/10.1242/jeb.024034

Feuchtmayr, H. et al. 2010. Differential effects of warming and nutrient loading on the timing and size of the spring zooplankton peak: an experimental approach with hypertrophic freshwater mesocosms. *J. Plankton Res.* 32: 1715-1725. https://doi.org/10.1093/plankt/fbq087

Figueiredo, B. R. S. et al. 2019. Short-term interactive effects of experimental heat waves and turbidity pulses on the foraging success of a subtropical invertivorous fish. *Water (Switzerland)* 11: 1-13. https://doi.org/10.3390/w11102109

Filazzola, A. et al. 2020. Inclusion of trophic interactions increases the vulnerability of an alpine butterfly species to climate change. *Glob. Change Biol.* 26: 2867-2877. https://doi.org/10.1111/gcb.15068

Fischer, E. and Schär, C. 2010. Consistent geographical patterns of changes in high-impact European heatwaves. *Nat. Geosci.* 3: 398–403. https://doi.org/10.1038/ngeo866

Fossen, E. I. et al. 2019. Do genetic differences in growth thermal reaction norms maintain genetic variation in timing of diapause induction? *bioRxiv*, 849059. https://doi.org/10.1101/849059

Fragata, I. et al. 2016. Keeping your options open: Maintenance of thermal plasticity during adaptation to a stable environment. *Evolution* 70: 195-206. https://doi.org/10.1111/evo.12828

Frances, D. N. and McCauley, S. J. 2018. Warming drives higher rates of prey consumption and increases rates of intraguild predation. *Oecologia* 187: 585-596. https://doi.org/10.1007/s00442-018-4146-y

Frazier, M. R. et al. 2006. Thermodynamics constrains the evolution of insect population growth rates:“warmer is better”. *Am. Nat.* 168: 512-520. https://doi.org/10.1086/506977

Fussmann, K. E. et al. 2014. Ecological stability in response to warming. *Nat. Clim. Change* 4: 206-210. https://doi.org/10.1038/nclimate2134

García, F. C. et al. 2018. Changes in temperature alter the relationship between biodiversity and ecosystem functioning. *Proc. Nat. Acad. Sci. U. S. A.* 115: 10989-10994. https://doi.org/10.1073/pnas.1805518115

Garland, T. J. and Rose, M. R. 2009. Experimental Evolution: Concepts, Methods, and Applications of Selection Experiments. University of California Press, Berkeley, CA, USA.

Gause, G. F. 1942. The relation of adaptability to adaptation. *Quart. Rev. Biol.* 17: 99-114.

https://doi.org/10.1086/394649

Gauzens, B. et al. 2020. Biodiversity of intertidal food webs in response to warming across latitudes. *Nat. Clim. Change* 10: 264-269. https://doi.org/10.1038/s41558-020-0698-z

Geerts, A. N. et al. 2015. Rapid evolution of thermal tolerance in the water flea *Daphnia*. *Nat. Clim. Change 5*: 665-668. http://doi.org/10.1038/nclimate2628

Ghalambor, C. K. et al. 2006. Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integr. Comp. Biol.* 46: 5-17. https://doi.org/10.1093/icb/icj003

Ghasemi, A. and Zahediasl, S. 2012. Normality tests for statistical analysis: a guide for non-statisticians. *Int. J. Endocrinol. Metab.* 10: 486. http://doi.org/10.5812/ijem.3505

Giebelhausen, B. and Lampert, W. 2001. Temperature reaction norms of *Daphnia magna*: The effect of food concentration. *Freshw. Biol.* 46: 281-289. https://doi.org/10.1046/j.1365-2427.2001.00630.x

Gillespie, D. R. et al. 2012. Effects of simulated heat waves on an experimental community of pepper plants, green peach aphids, and two parasitoid species. *Oikos* 121: 149-159. https://doi.org/10.1111/j.1600-0706.2011.19512.x

Gillis, P. L. et al. 2005. *Daphnia* need to be gut-cleared too: the effect of exposure to and ingestion of metal-contaminated sediment on the gut-clearance patterns of *D. magna. Aquat. Toxicol.* 71: 143-154. https://doi.org/10.1016/j.aquatox.2004.10.016

Gillooly, J. F. et al. 2001. Effects of size and temperature on metabolic rate. *Science* 293: 2248-2251. https://doi.org/10.1126/science.1061967

Goitom, E. et al. 2018. Rapid evolution leads to differential population dynamics and top-down control in resurrected *Daphnia* populations. *Evol. Appl.* 11: 96-111. https://doi.org/10.1111/eva.12567

González-Tokman, D. et al. 2020. Insect responses to heat: physiological mechanisms, evolution and ecological implications in a warming world. *Biol. Rev.* 95: 802-821. https://doi.org/10.1111/brv.12588

Gosden, T.P. et al. 2011. Range limits, large-scale biogeographic variation and localised evolutionary dynamics in a polymorphic damselfly. *Biol. J. Linn. Soc.* 102: 775-785. https://doi.org/10.1111/j.1095-8312.2011.01619.x

Gould, K. et al. 2021. Upper-mesophotic and shallow reef corals exhibit similar thermal tolerance, sensitivity and optima. *Coral Reefs* 40: 907-920. https://doi.org/10.1007/s00338-021-02095-w

Grigaltchik, V. S. et al. 2012. Thermal acclimation of interactions: differential responses to temperature change alter predator–prey relationship. *Proc. R. Soc. B* 279: 4058-4064. https://doi.org/10.1098/rspb.2012.1277

Gunderson, A. R. and Stillman, J. H. 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc. R. Soc. B* 282: 20150401. https://doi.org/10.1098/rspb.2015.0401

Halsch, C. A. et al. 2021. Insects and recent climate change. *Proc. Nat. Acad. Sci. U. S. A.* 118: e2002543117. https://doi.org/10.1073/pnas.2002543117

Hammill, E. et al. 2010. Predator functional response changed by induced defenses in prey. *Am. Nat.* 176: 723-731. https://doi.org/10.1086/657040

Hargreaves, A. L. et al. 2019. Seed predation increases from the Arctic to the Equator and from high to low elevations. *Sci. Adv.* 5: eaau4403. https://doi.org/10.1101/304634

Harris, S. M. et al. 2020. Personality predicts foraging site fidelity and trip repeatability in a marine predator. *J. Anim. Ecol.* 89: 68-79. https://doi.org/10.1111/1365-2656.13106

Hassell, M. 2000. The spatial and temporal dynamics of host-parasitoid interactions. OUP Oxford.

Havird, J. C. et al. 2020. Distinguishing between active plasticity due to thermal acclimation and passive plasticity due to Q10 effects: Why methodology matters. *Func. Ecol.* 34: 1015-1028. https://doi.org/10.1111/1365-2435.13534

He, F. et al. 2019. The global decline of freshwater megafauna. *Glob. Chang. Biol.* 25: 3883-3892. https://doi.org/10.1111/gcb.14753

Heino, J. et al. 2009. Climate change and freshwater biodiversity: detected patterns, future trends and adaptations in northern regions. *Biol. Rev.* 84: 39-54. https://doi.org/10.1111/j.1469-185X.2008.00060.x

Hering, D. et al. 2015. Managing aquatic ecosystems and water resources under multiple stress - An introduction to the MARS project. *Sci. Total Environ.* 503-504: 10-21. https://doi.org/10.1016/j.scitotenv.2014.06.106

Higgins, J. K. et al. 2014. Geographic differences and microevolutionary changes in thermal sensitivity of butterfly larvae in response to climate. *Funct Ecol.* 28: 982-989. http://www.jstor.org/stable/24033587

Higham, T. E. et al. 2015. Turbulence, temperature, and turbidity: The ecomechanics of predator-prey interactions in fishes. *Integr. Comp. Biol.* 55: 6-20.

https://doi.org/10.1093/icb/icv052

Hilt, S. et al. 2017. Translating regime shifts in shallow lakes into changes in ecosystem functions and services. *Bioscience* 67: 928-936. https://doi.org/10.1093/biosci/bix106

Hoffmann, A. A. and Sgrò, C. M. 2011. Climate change and evolutionary adaptation. *Nature* 470: 479-485. https://doi.org/10.1038/nature09670

Holling, C. S. 1959. Some characteristics of simple types of predation and parasitism. *Can. Entomol.* 7: 385–398. https://doi.org/10.4039/Ent91385-7

Holt, R. D. and Loreau, M. 2002. Biodiversity and ecosystem functioning: The role of trophic interactions and the importance of system openness. In *The Functional Consequences of Biodiversity* Chapter 11: 246-262. https://doi.org/10.1515/9781400847303.246

Huey, R. B. and Hertz, P. E. 1984. Is a Jack-of-all-temperatures a master of none? *Evolution* 38: 441-444. https://doi.org/10.2307/2408502

Huey, R. B. and Kingsolver, J. G. 1993. Evolution of resistance to high temperature in ectotherms. *Am. Nat. 142*: S21-S46. https://doi.org/10.1086/285521

Huey, R. B. and Stevenson, R. D. 1979. Integrating thermal physiology and ecology of ectotherms: discussion of approaches. *Am. Zool.* 19:357-366.

Huey, R. B. et al. 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Phil. Trans. R. Soc. B* 367:1665-1679. https://doi.org/10.1098/rstb.2012.0005

Huey, R. B. and Kingsolver, J. G. 2019. Climate warming, resource availability, and the metabolic meltdown of ectotherms. *Am. Nat.* 194: E140-E150. https://doi.org/10.1086/705679

Iles, A. C. 2014. Toward predicting community-level effects of climate: Relative temperature scaling of metabolic and ingestion rates. *Ecology* 95: 2657-2668. https://doi.org/10.1890/13-1342.1

IPCC. 2014. Climate Change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge, UK and New York, USA. Cambridge University Press. https://doi.org/10.1017/CBO9781107415324

Jackson, M.C. et al. 2016. Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. *Glob. Change Biol.* 22: 180-189. https://doi.org/10.1111/gcb.13028

Jackson, M. C. et al. 2021. The temporal dynamics of multiple stressor effects: From individuals to ecosystems. *Trends Ecol. Evol.* 36:406-410. https://doi.org/10.1016/j.tree.2021.01.005

Janssens, L. et al. 2014. Local adaptation and the potential effects of a contaminant on predator avoidance and antipredator responses under global warming: a space-for-time substitution approach. *Evol. Appl.* 7: 421-430. https://doi.org/10.1111/eva.12141

Janzen, D. H. 1967. Why mountain passes are higher in the tropics. *Am. Nat.* 101: 233-249. http://www.jstor.org/stable/2458977.

Jeanne, R. L. 1979. A latitudinal gradient in rates of ant predation. *Eology* 60: 1211-1224. http://www.jstor.org/stable/1936968

Jepsen, N. et al. 2001. Behavior of pike (*Esox lucius* L.) >50 cm in a turbid reservoir and in a clearwater lake. *Ecol. Freshw. Fish* 10: 26-34. https://doi.org/10.1034/j.16000633.2001.100104.x

Jeschke, J. et al. 2002. Predator functional responses: discriminating between handling and digesting prey. *Ecol. Monogr.* 72: 95-112. https://doi.org/10.1890/0012-9615(2002)072[0095:PFRDBH]2.0.CO;2

Jeschke, J. et al. 2004. Consumer-food systems: Why type I functional responses are exclusive to filter feeders. *Biol. Rev.* 79: 337-349. https://doi.org/10.1017/S1464793103006286

Jin, P. and Agustí, S. 2018. Fast adaptation of tropical diatoms to increased warming with trade-offs. *Sci. Rep.* 8: 17771. https://doi.org/10.1038/s41598-018-36091-y

Jönsson, M. et al. 2011. Foraging success of juvenile pike Esox lucius depends on visual conditions and prey pigmentation. *J. Fish Biol.* 79: 290-297. https://doi.org/10.1111/j.1095- 8649.2011.03004.x

Kawecki, T. J. et al. 2012. Experimental evolution. *Trends Ecol. Evol.* 27: 547-560. https://doi.org/10.1016/j.tree.2012.06.001

Kellermann, V. et al. 2019. Comparing thermal performance curves across traits: how consistent are they? *J. Exp. Biol.* 222: jeb193433. https://doi.org/10.1242/jeb.193433

Kellermann, V. and Sgrò, C. M. 2018. Evidence for lower plasticity in CT_{MAX} at warmer developmental temperatures. *J. Evo. Biol.* 31: 1300-1312. https://doi.org/10.1111/jeb.13303

Kelly, M. 2019. Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. *Philos. Trans. R. Soc. B* 374: 20180176. https://doi.org/10.1098/rstb.2018.0176

Khelifa, R. et al. 2019. Usefulness and limitations of thermal performance curves in predicting ectotherm development under climatic variability. *J. Anim. Ecol.* 88: 1901-1912. https://doi.org/10.1111/1365-2656.13077

Kimbell, H. S. and Morrell, L. J. 2016. Turbidity weakens selection for assortment in body size in groups. *Behav. Ecol.* 27: 545-552. https://doi.org/10.1093/beheco/arv183

Kingsolver, J. G. 2009. The well-temperatured biologist. *Am. Nat.* 174: 755-768. https://doi.org/10.1086/648310

Kingsolver, J. G. and Woods, H. A. 2016. Beyond thermal performance curves: modeling time-dependent effects of thermal stress on ectotherm growth rates. *Am. Nat.* 187: 283-294. https://doi.org/10.1086/684786

Kimbell, H. S. and Morrell, L. J. 2015. Turbidity influences individual and group level responses to predation in guppies, *Poecilia reticulata*. *Anim. Behav.* 103: 179-185. https://doi.org/10.1016/j.anbehav.2015.02.027

Kontopoulos, D. G. et al. 2020. Adaptive evolution shapes the present-day distribution of the thermal sensitivity of population growth rate. *PLoS Biology* 18: e3000894. https://doi.org/10.1371/journal. pbio.3000894

Kratina, P. et al. 2012. Warming modifies trophic cascades and eutrophication in experimental freshwater communities. *Ecology* 93: 1421-1430. https://doi.org/10.1890/11-1595.1

Kuznetsova, A. et al. 2017. lmerTest package: Tests in linear mixed effects models. *J. Stat. Softw.* 82:1-26. https://doi.org/10.18637/jss.v082.i13

Lampert, W. et al. 1986. Phytoplankton control by grazing zooplankton: A study on the spring clear-water phase. *Limnol. Oceanogr*. 31: 478-490. https://doi.org/10.4319/lo.1986.31.3.0478

Lampert, W. 2011. *Daphnia*: development of a model organism in ecology and evolution. Excellence in Ecology. Book 21. Oldendorf/Luhe: International Ecology Institute.

Lancaster, L. T. et al. 2017. Life history trade-offs, the intensity of competition, and coexistence in novel and evolving communities under climate change. *Philos. Trans. R. Soc. B* 372: 20160046. https://doi.org/10.1098/rstb.2016.0046

Larsson, P. and Kleiven, O. T. 1996. Food search and swimming speed in *Daphnia*.

Zooplankton: sensory ecology and physiology. CRC Press.

Laws, A. N. 2017. Climate change effects on predator–prey interactions. *Curr. Opin. Insect Sci.* 23: 28-34. https://doi.org/10.1016/j.cois.2017.06.010

Lenth R. V. 2016. Least-squares means: the R package lsmeans. *J. Stat. Softw.* 69: 1-33. https://doi.org/10.18637/jss.v069.i01

Li, Y. et al. 2018. Experimental duration and predator satiation levels systematically affect the functional response parameters. *Oikos* 127: 590-598. https://doi.org/10.1111/oik.04479

Liefting, M. et al. 2009. Plasticity versus environmental canalization: population differences in thermal responses along a latitudinal gradient in *Drosophila serrata*. *Evolution* 63: 1954-1963. https://doi.org/10.1111/j.1558-5646.2009.00683.x

Little, T. J. et al. 2002. Competitiveness and life-history characteristics of *Daphnia* with respect to susceptibility to a bacterial pathogen. *J. Evol. Biol.* 15: 796-802. https://doi.org/10.1046/j.1420-9101.2002.00436.x

Logan, C.A. and Somero, G. N. 2011. Effects of thermal acclimation on transcriptional responses to acute heat stress in the eurythermal fish *Gillichthys mirabilis* (Cooper). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 300: R1373-R1383. https://doi.org/10.1152/ajpregu.00689.2010

Lotka, A.J. 1913. A natural population norm. *J. Wash. Acad. Sci.* 3: 241-248. https://www.jstor.org/stable/24521071

Loughland, I. and Seebacher, F. 2020. Differences in oxidative status explain variation in thermal acclimation capacity between individual mosquitofish (*Gambusia holbrooki*). *Funct. Ecol.* 34: 1380-1390. https://doi.org/10.1111/1365-2435.13563

MacGregor-Fors, I. and Payton, M. E. 2013. Contrasting diversity values: statistical inferences based on overlapping confidence intervals. *PLoS One* 8: e56794. https://doi.org/10.1371/journal.pone.0056794

Magozzi, S. and Calosi, P. 2015. Integrating metabolic performance, thermal tolerance, and plasticity enables for more accurate predictions on species vulnerability to acute and chronic effects of global warming. *Glob. Change Biol.* 21: 181-194. https://doi.org/10.1111/gcb.12695

Mandal, S. et al. 2018. Compensatory grazing by Daphnia generates a trade-off between top-down and bottom-up effects across phytoplankton taxa. *Ecosphere* 9: e02537.

https://doi.org/10.1002/ecs2.2537

Marino, N. dos A. C. et al. 2018. Geographical and experimental contexts modulate the effect of warming on top-down control: a meta-analysis. *Ecol. Lett.* 21: 455-466. https://doi.org/10.1111/ele.12913

Maxwell, S. L. et al. 2016. Biodiversity: the ravages of guns, nets and bulldozers. *Nature* 536: 143-145. https://doi.org/10.1038/536143a

McGill, B. J. et al. 2015. Fifteen forms of biodiversity trend in the Anthropocene. *Trends Ecol. Evol.* 30: 104-113. https://doi.org/10.1016/j.tree.2014.11.006.

McPeek, M. A. et al. 2001. Physiological and behavioral responses to predators shape the growth/predation risk trade-off in damselflies. *Ecology* 82: 1535-1545. https://doi.org/10.1890/0012-9658(2001)082[1535:PABRTP]2.0.CO;2

Meager, J. J. et al. 2006. Escape responses in juvenile Atlantic cod *Gadus morhua* L.: The effects of turbidity and predator speed. *J. Exp. Biol.* 209: 4174-4184. https://doi.org/10.1242/jeb.02489

Merilä, J. and Hendry, A.P. 2014. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evol. Appl.* 7: 1-14. https://doi.org/10.1111/eva.12137

Mesas, A. et al. 2021. Experimental evolution on heat tolerance and thermal performance curves under contrasting thermal selection in *Drosophila subobscura*. *J. Evol. Biol.* 34: 767-778. https://doi.org/10.1111/jeb.13777

Michalko, R. et al. 2019. Global patterns in the biocontrol efficacy of spiders: A meta-analysis. *Glob. Ecol. Biogeogr.* 28: 1366-1378. https://doi.org/10.1111/geb.12927

Miner, B. E. et al. 2012. Linking genes to communities and ecosystems: *Daphnia* as an ecogenomic model. *Proc. R. Soc. B* 279: 1873-1882. https://doi.org/10.1098/rspb.2011.2404

Mitchell, S. E., and Lampert, W. 2001. Temperature adaptation in a geographically widespread zooplankter, *Daphnia magna*. *J. Evol. Biol.* 13: 371-382. https://doi.org/10.1046/j.1420-9101.2000.00193.x

Mocq, J. et al. 2021. Disentangling the nonlinear effects of habitat complexity on functional responses. *J. Anim. Ecol.* 90: 1525-1537. https://doi.org/10.1111/1365-2656.13473

Molina-Montenegro, M. A. and Naya, D. E. 2012. Latitudinal patterns in phenotypic plasticity and fitness-related traits: assessing the climatic variability hypothesis (CVH) with an invasive plant species. *PLoS One* 7: e47620.

https://doi.org/10.1371/journal.pone.0047620

Morris, D. W. 2019. A human tragedy? The pace of negative global change exceeds human progress. *Anthr. Rev.* 6: 55-70. https://doi.org/10.1177/2053019619848216

Moss, B. 2011. Allied attack: climate change and eutrophication. *Inland Waters* 1: 101-105. https://doi.org/10.5268/iw-1.2.359

Nadeau, C. P. and Urban, M. C. 2019. Eco-evolution on the edge during climate change. *Ecography* 42: 1280-1297. https://doi.org/10.1111/ecog.04404

Nati, J. J. H. et al. 2016. Is there a trade-off between peak performance and performance breadth across temperatures for aerobic scope in teleost fishes? *Biology Letters* 12: 20160191. http://doi.org/10.1098/rsbl.2016.0191

Naya, D. E. et al. 2011. Exploring the functional association between physiological plasticity, climatic variability, and geographical latitude: lessons from land snails. *Evol. Ecol. Res.* 13: 647-659. https://www.evolutionary-ecology.com/abstracts/v13/2698.html

Nieman, C. L. et al. 2018. Visual detection thresholds in two trophically distinct fishes are compromised in algal compared to sedimentary turbidity. *Conserv. Physiol.* 6: coy044. https://doi.org/10.1093/conphys/coy044

Nikulin, G. et al. 2011. Evaluation and future projections of temperature, precipitation and wind extremes over Europe in an ensemble of regional climate simulations. *Tellus A* 63: 41-55. https://doi.org/10.1111/j.1600-0870.2010.00466.x

Nilsson-Örtman, V. et al. 2013. Latitudinally structured variation in the temperature dependence of damselfly growth rates. *Ecol. Lett.* 16: 64-71. https://doi.org/10.1111/ele.12013

O'Donnell, D. R. et al. 2018. Rapid thermal adaptation in a marine diatom reveals constraints and trade-offs. *Global Change Biology* 24: 4554-4565. https://doi.org/10.1111/gcb.14360

O’Gorman, E. J. et al. 2017. Unexpected changes in community size structure in a natural warming experiment. *Nat. Clim. Change* 7: 659-663. https://doi.org/10.1038/nclimate3368

O’Neal, P. A. and Juliano, S. A. 2013. Seasonal variation in competition and coexistence of *Aedes* mosquitoes: stabilizing effects of egg mortality or equalizing effects of resources? *J. Anim. Ecol.* 82: 256-265. https://doi.org/10.1111/j.1365-

2656.2012.02017.x

Orr, J. A. et al. 2020. Towards a unified study of multiple stressors: Divisions and common goals across research disciplines. *Proc. R. Soc. B* 287: 20200421. https://doi.org/10.1098/rspb.2020.0421

Ortega, J. C. G. et al. 2020. Negative effect of turbidity on prey capture for both visual and non-visual aquatic predators. *J. Anim. Ecol.* 89: 2427-2439. https://doi.org/10.1111/1365-2656.13329

Padfield, D. et al. 2016. Rapid evolution of metabolic traits explains thermal adaptation in phytoplankton. *Ecol. Lett.* 19: 133-142. https://doi.org/10.1111/ele.12545

Padfield, D. et al. 2021. rTPC and nls.multstart: A new pipeline to fit thermal performance curves in R. *Methods Ecol. Evol.* 00: 1-6. https://doi.org/10.1111/2041-210X.

Paaijmans, K. P. et al. 2013. Temperature variation makes ectotherms more sensitive to climate change. *Glob. Change Biol.* 19: 2373-2380. https://doi.org/10.1111/gcb.12240

Pauwels, K. et al. 2010. Enhanced anti-predator defence in the presence of food stress in the water flea *Daphnia magna*. *Funct. Ecol.* 24: 322-329. https://doi.org/10.1111/j.1365-2435.2009.01641.x

Payton, M. E. et al. 2003. Overlapping confidence intervals or standard error intervals: what do they mean in terms of statistical significance? *J. Insect Sci.* 3: 34. https://doi.org/10.1093/jis/3.1.34

Pereira, R. J. et al. 2017. Adaptation to a latitudinal thermal gradient within a widespread copepod species: the contributions of genetic divergence and phenotypic plasticity. *Proc. R. Soc. B.* 284: 20170236. https://doi.org/10.1098/rspb.2017.0236

Perujo, N. et al. 2021. A guideline to frame stressor effects in freshwater ecosystems. Sci. Total Environ. 777: 146112. https://doi.org/10.1016/j.scitotenv.2021.146112

Petchey, O. L. et al. 2010. Predicting the effects of temperature on food web connectance. *Phil. Trans. R. Soc. B* 365: 2081-2091. https://doi.org/10.1098/rstb.2010.0011

Peters, R. H. 1986. The ecological implications of body size. Cambridge University Press, Cambridge.

Pickup, J. and Thompson, D. J. 1990. The effects of temperature and prey density on the development rates and growth of damselfly larvae (Odonata: Zygoptera). *Ecol. Entomol.* 15: 187-200. https://doi.org/10.1111/j.1365-2311.1990.tb00800.x

Pratt, D. M. 1943. Analysis of population development in *Daphnia* at different temperatures. *Biology Bulletin* 85: 116-14. https://doi.org/10.2307/1538274

Preest, M. R. and Pough, F. H. (2003). Effects of body temperature and hydration state on organismal performance of toads, *Bufo americanus*. *Physio. Biochem. Zool.* 76: 229-239. https://doi.org/10.1086/367948

Pritchard, D. W. et al. 2017. Frair: an R package for fitting and comparing consumer functional responses. *Methods Ecol. Evol.* 8: 1528-1534. https://doi.org/10.1111/2041-210X.12784

Putman, A. et al. 2015. A comparative analysis of the fatty acid composition of sexual and asexual eggs of *Daphnia magna* and its plasticity as a function of food quality. *J. Plankton Res.* 37: 752-763. https://doi.org/10.1093/plankt/fbv043

Rall, B. C. et al. 2010. Temperature, predator-prey interaction strength and population stability. *Glob. Change Biol.* 16: 2145-2157. https://doi.org/10.1111/j.1365-2486.2009.02124.x

Rall, B. C. et al. 2012. Universal temperature and body-mass scaling of feeding rates. *Phil. Trans. R. Soc. B* 367: 2923-2934. https://doi.org/10.1098/rstb.2012.0242

R Core Team. 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. URL: https://www.R-project.org

R Core Team 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Reid, A. J. et al. 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biol. Rev.* 94: 849-873. https://doi.org/10.1111/brv.12480

Reinertsen, H. et al. 1997. Effects of fish elimination on the phytoplankton and zooplankton in a small eutrophic lake. *Verh. Int. Ver. Theor. Angew. Limnol.*, 26: 593-598. https://doi.org/10.1080/03680770.1995.11900787

Rezende, E. L. et al. 2014. Tolerance landscapes in thermal ecology. *Funct Ecol.* 28, 799-809. https://doi.org/10.1111/1365-2435.12268

Rice, J. A. 2007. Mathematical statistics and data analysis. Belmont, CA, USA. Duxbury Press.

Rogers, D. 1972. Random search and insect population models. *J. Anim. Ecol.* 41: 369-383. https://doi.org/10.2307/3474

Rogers, T. L. et al. 2020. Trophic control changes with season and nutrient loading in lakes. *Ecol. Lett.* 23: 1287-1297. https://doi.org/10.1111/ele.13532

Rohr, J. R. et al. 2019. Different metrics of thermal acclimation yield similar effects of latitude, acclimation duration, and body mass on acclimation capacities. *Glob. Change Biol.* 25: e3-e4. https://doi.org/10.1111/gcb.14653

Rosenblatt, A. E. et al. 2016. Linking trophic interactions to plasticity in thermal sensitivity of geographically separated populations of a herbivore. *Evolutionary Ecology* 30: 649-661. https://doi.org/10.1007/s10682-016-9827-6

Rosenblatt, A. E. et al. 2019. Will like replace like? Linking thermal performance to ecological function across predator and herbivore populations. *Ecology* 100: 1-12. https://doi.org/10.1002/ecy.2643

Roslin, T. et al. 2017. Higher predation risk for insect prey at low latitudes and elevations. *Science* 356: 742-744. https://doi.org/10.1126/science.aaj1631

Rosenzweig, M. 1971. Paradox of enrichment: destabilization of exploitation ecosystems in ecological time. *Science* 171:385-387. https://doi.org/10.1126/science.171.3969.385

Ruel, J. J. and Ayres, M. P. 1999. Jensen's inequality predicts effects of environmental variation. *Trends Ecol. Evol.* 14: 361-366. https://doi.org/10.1016/S0169-5347(99)01664-X

Sage, R. F. 2020. Global change biology: A primer. *Glob. Change Biol.* 26: 3-30. https://doi.org/10.1111/gcb.14893

Sánchez-Bayo, F. and Wyckhuys, K. A. G. 2019. Worldwide decline of the entomofauna: A review of its drivers. *Biol. Conserv.* 232: 8-27. https://doi.org/10.1016/j.biocon.2019.01.020

Santos, M. et al. 2006. Thermal evolution of pre-adult life history traits, geometric size and shape, and developmental stability in *Drosophila subobscura*. *J. Evol. Biol.* 19: 2006-2021. https://doi.org/10.1111/j.1420-9101.2006.01139.x

Savage, V. M. et al. 2004a. Effects of body size and temperature on population growth. *Am. Nat.* 163, 429-441. https://doi.org/10.1086/381872

Savage, V. M. et al. 2004b. The predominance of quarter power scaling in biology. *Func. Ecol.* 18: 257-282. https://doi.org/10.1111/j.0269-8463.2004.00856.x

Schaum, C. E. et al. 2017. Adaptation of phytoplankton to a decade of experimental warming linked to increased photosynthesis. *Nat. Eco. Evol.* 1: 1-7. https://doi.org/10.1038/s41559-017-0094

Scheffer, M. et al. 2001. Catastrophic shifts in ecosystems. *Nature* 413: 591–596.

https://doi.org/10.1038/35098000

Scheffer, M. and Van Nes, E. H. 2007. Shallow lakes theory revisited: Various alternative regimes driven by climate, nutrients, depth and lake size. *Hydrobiologia* 584: 455-466. https://doi.org/10.1007/s10750-007-0616-7

Scheffers, B. R. et al. 2016. The broad footprint of climate change from genes to biomes to people. *Science*: 354. http://doi.org/10.1126/science.aaf7671

Schindler, D. 1968. Feeding, assimilation and respiration rates of *Daphnia magna* under various environmental conditions and their relation to production estimates. *J. Anim. Ecol.* 37: 369-385. https://doi.org/10.2307/2954

Schulte, P. M. et al. 2011. Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integr. Comp. Biol.* 51: 691-702. https://doi.org/10.1093/icb/icr097

Seebacher, F. et al. 2012. Capacity for thermal acclimation differs between populations and phylogenetic lineages within a species. *Funct. Ecol.* 26: 1418-1428. https://doi.org/10.1111/j.1365-2435.2012.02052.x

Seebacher, F. et al. 2015. Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Clim. Change* 5: 61. https://doi.org/10.1038/nclimate2457

Seifert et al. 2015. Extreme heat changes post-heat wave community reassembly. *Ecol. Evol.* 5: 2140-2148. https://doi.org/10.1002/ece3.1490

Sentis, A. et al. 2012. Using functional response modeling to investigate the effect of temperature on predator feeding rate and energetic efficiency. *Oecologia* 169: 1117-1125. https://doi.org/10.1007/s00442-012-2255-6

Sentis, A. et al. 2013a. Effects of simulated heat waves on an experimental plant–herbivore–predator food chain. *Glob. Change Biol.* 19: 833-842. https://doi.org/10.1111/gcb.12094

Sentis, A. et al. 2013b. Parsing handling time into its components: implications for responses to a temperature gradient. *Ecology* 94: 1675-1680. https://doi.org/10.1890/12-2107.1

Sentis, A. et al. 2015. Thermal acclimation modulates the impacts of temperature and enrichment on trophic interaction strengths and population dynamics. *Glob. Change Biol.* 21: 3290-3298. https://doi.org/10.1111/gcb.12931

Sentis, A. et al. 2017. Predator diversity and environmental change modify the strengths of trophic and nontrophic interactions. *Glob. Change Biol.* 23: 2629-2640.

https://doi.org/10.1111/gcb.13560

Sgrò, C. M. et al. 2016. What can plasticity contribute to insect responses to climate change?. *Annu. Rev. Entomol.* 61: 433-451. https://doi.org/10.1146/annurev-ento-010715-023859

Shama, L. N. et al. 2011. Latitudinal and voltinism compensation shape thermal reaction norms for growth rate. *Mol. Ecol.* 20: 2929-2941. https://doi.org/10.1098/rstb.2018.0547

Shaw, G. W. et al. 2006. Effect of turbidity, prey density and culture history on prey consumption by greenback flounder *Rhombosolea tapirina* larvae. *Aquaculture* 253: 447-460. https://doi.org/10.1016/j.aquaculture.2005.09.025

Siepielski, A. M. et al. 2020. Predators weaken prey intraspecific competition through phenotypic selection. *Ecology Letters* 23: 951-961. https://doi.org/10.1111/ele.13491

Sinclair, B. J. et al. 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures?. *Ecol. Lett.* 19: 1372-1385. https://doi.org/10.1111/ele.12686

Sinha, E. et al. 2017. Eutrophication will increase during the 21st century as a result of precipitation changes. *Science* 357: 405-408. https://10.1126/science.aan2409

Sitch, S. et al. 2003. Evaluation of ecosystem dynamics, plants geography and terrestrial carbon cycling in the LPJ dynamic global vegetation model. *Glob. Change Biol.* 9: 161-85. https://doi.org/10.1046/j.1365-2486.2003.00569.x

Smith, H. L. 2008. The Rosenzweig-Macarthur predator-prey model. School of Mathematical and Statistical Sciences, Arizona State University: Phoenix. http://math.la.asu.edu/~halsmith/Rosenzweig.pdf

Sokolova, I. 2021. Bioenergetics in environmental adaptation and stress tolerance of aquatic ectotherms: linking physiology and ecology in a multi-stressor landscape. *J. Exp. Biol.* 224: jeb236802. https://doi.org/10.1242/jeb.236802

Soroye, P. et al. 2020. Climate change contributes to widespread declines among bumble bees across continents. *Science* 367: 685-688. https://doi.org/10.1126/science.aax8591

South, J. and Dick, J. T. A. 2017. Effects of acute and chronic temperature changes on the functional responses of the dogfish *Scyliorhinus canicula* (Linnaeus, 1758) towards amphipod prey *Echinogammarus marinus* (Leach, 1815). *Environ. Biol. Fishes* 100: 1251-1263. https://doi.org/10.1007/s10641-017-0640-z

Spiess, A.-N. 2014. Propagate: propagation of uncertainty. R package version 1.0-3, available at: http://CRAN.Rproject.org/package=propagate

Spiess, A.-N. et al. 2018. R package ‘propagate.’ https://cran.r-project.org/web/packages/propagate/propagate.pdf

Stephens, D. L. B. et al. 2015. Regional distribution of Secchi disk transparencyin waters of the United States. *Lake Reserv. Manag.* 31: 55-63. https://doi.org/10.1080/10402381.2014.1001539

Stoks, R. et al. 2005. Predation cost of rapid growth: behavioural coupling and physiological decoupling. *J. Animal Ecol.* 74: 708-715. https://doi.org/10.1111/j.1365-2656.2005.00969.x

Stoks, R. et al. 2012. Behaviour and physiology shape the growth accelerations associated with predation risk, high temperatures and southern latitudes in *Ischnura* damselfly larvae. *J. Animal Ecol.* 81:1034-1040. https://doi.org/10.1111/j.1365-2656.2012.01987.x

Stoks, R. et al. 2014. Evolutionary and plastic responses of freshwater invertebrates to climate change: realized patterns and future potential. *Evol. Appl.* 7: 42-55. https://doi.org/10.1111/eva.12108

Stoks, R. et al. 2016. Resurrecting complexity: the interplay of plasticity and rapid evolution in the multiple trait response to strong changes in predation pressure in the water flea *Daphnia magna*. *Ecol. Lett.* 19: 180-190. https://doi.org/10.1111/ele.12551

Stoks, R. et al. 2017. Daily temperature variation and extreme high temperatures drive performance and biotic interactions in a warming world. *Curr. Opin. Insect Sci.* 23: 35-42. https://doi.org/10.1016/j.cois.2017.06.008

Stouffer, D. B. and Novak, M. 2021. Hidden layers of density dependence in consumer feeding rates. *Ecology Letters* 24: 520-532. https://doi.org/10.1111/ele.13670

Symons, C. C. et al. 2019. Antagonistic effects of temperature and dissolved organic carbon on fish growth in California mountain lakes. *Oecologia* 189: 231-241. https://doi.org/10.1007/s00442-018-4298-9

Tewksbury, J. J. et al. 2008. Putting the heat on tropical animals. *Science* 320: 1296-1297. https://10.1126/science.1159328

Thompson, D. J. 1975. Towards a predator-prey model incorporating age structure: the effects of predator and prey size on the predation of *Daphnia magna* by *Ischnura* *elegans*. *J. Anim. Ecol.* 44: 907-916. https://doi.org/10.2307/3727

Thompson, D. J. 1978. Towards a realistic predator-prey model: the effect of temperature on the functional response and life history of larvae of the damselfly, *Ischnura elegans*. *J. Animal Ecol.* 47: 757-767. https://doi.org/10.2307/3669

Twardochleb, L. A. et al. 2020. Foraging strategy mediates ectotherm predator–prey responses to climate warming. *Ecology* 101: e03146. https://doi.org/10.1002/ecy.3146

Tylianakis, J. M. et al. 2008. Global change and species interactions in terrestrial ecosystems. *Ecol. Lett.* 11: 1351-1363. https://doi.org/10.1111/j.1461-0248.2008.01250.x

UNEP. 2012. United Nations Environment Programme, Global Environment Outlook Year Book: An Overview of Our Changing Environment

Uiterwaal, S. F. and DeLong, J. P. 2020. Functional responses are maximized at intermediate temperatures. *Ecology* 101: e02975. https://doi.org/10.1002/ecy.2975

Urban, M. C. 2015. Accelerating extinction risk from climate change. *Science* 348: 571-573. https://doi.org/10.1126/science.aaa4984

Urban, M. C. et al. 2016. Improving the forecast for biodiversity under climate change. *Science* 353: aad8466. https://doi.org/10.1126/science.aad8466

Van Doorslaer, W. et al. 2009a. Population dynamics determine genetic adaptation to temperature in *Daphnia*. *Evolution* 63: 1867-1878. https://doi.org/10.1111/j.1558-5646.2009.00679.x

Van Doorslaer, W. et al. 2009b. Local adaptation to higher temperatures reduces immigration success of genotypes from a warmer region in the water flea *Daphnia*. *Glob. Change Biol.* 15: 3046-3055. https://doi.org/10.1111/j.1365-2486.2009.01980.x

Van Doorslaer, W. et al. 2010. Experimental thermal microevolution in community-embedded *Daphnia* populations. *Clim. Res.* 43: 81-89. https://doi.org/10.3354/cr00894

Vangansbeke, D. et al. 2015. Prey consumption by phytoseiid spider mite predators as affected by diurnal temperature variations. *BioControl* 60: 595-603. https://doi.org/10.1007/s10526-015-9677-0

Valladares, F. et al. 2006. Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *J. Ecol.* 94: 1103-1116. https://doi.org/10.1111/j.1365-2745.2006.01176.x

Valladares, F. et al. 2014. The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecol. Lett.* 17: 1351-1364. https://doi.org/10.1111/ele.12348

Van de Meutter et al. 2005. The effect of turbidity state and microhabitat on macroinvertebrate assemblages: a pilot study of six shallow lakes. *Hydrobiologia* 542: 379-390. https://doi.org/10.1007/s10750-004-4941-9

Van Dievel, M. et al. 2017. Beneficial effects of a heat wave: higher growth and immune components driven by a higher food intake. *J. Exp. Biol.* 220: 3908-3915. https://doi.org/10.1242/jeb.158899

Van Dievel, M. et al. 2019a. Latitude-associated evolution and drivers of thermal response curves in body stoichiometry. *J. Anim. Ecol.* 88: 1961-1972. https://doi.org/10.1111/1365-2656.13088

Van Dievel, M. 2019b. Additive bioenergetic responses to a pesticide and predation risk in an aquatic insect. *Aquat. Toxicol.* 212: 205-213. https://doi.org/10.1016/j.aquatox.2019.05.010

van Dorst, R. M. et al. 2019. Warmer and browner waters decrease fish biomass production. *Glob. Change Biol.* 25: 1395-1408. https://doi.org/10.1111/gcb.14551

Van Heerwaarden, B. et al. 2014. No patterns in thermal plasticity along a latitudinal gradient in *Drosophila simulans* from eastern Australia. *J. Evol. Biol.* 27: 2541-2553. https://doi.org/10.1111/jeb.12510

Van Wichelen, J. et al. 2013. Planktonic ciliate community structure in shallow lakes of lowland Western Europe. *Eur. J. Protistol.* 49: 538-551. https://doi.org/10.1016/j.ejop.2013.06.001

Vanoverbeke, J. 2008. Modeling individual and population dynamics in a consumer-resource system: behavior under food limitation and crowding and the effect on population cycling in *Daphnia* ecological modeling. *Ecol. Model* 216: 385-401. https://doi.org/10.1016/j.ecolmodel.2008.05.009

Vasseur, D. A. et al. 2014. Increased temperature variation poses a greater risk to species than climate warming. *Proc. R. Soc. B* 281: 20132612. https://doi.org/10.1098/rspb.2013.2612

Vasseur, D. A. and McCann, K. S. 2005. A mechanistic approach for modeling temperature-dependent consumer– resource dynamics. *Am. Nat.* 166: 184-198. https://doi.org/10.1086/431285

Vázquez, D. P. et al. 2017. Ecological and evolutionary impacts of changing climatic variability. *Biol. Rev.* 92: 22-42. https://doi.org/https://doi.org/10.1111/brv.12216

Verheyen, J. et al. 2019. Using natural laboratories to study evolution to global warming: contrasting altitudinal, latitudinal, and urbanization gradients. *Curr. Opin. Insect Sci.* 35: 10-19. https://doi.org/10.1016/j.cois.2019.06.001

Verheyen, J. and Stoks, R. 2019. Temperature variation makes an ectotherm more sensitive to global warming unless thermal evolution occurs. *J. Animal Ecol.* 00: 1-13. https://doi.org/10.1111/1365-2656.12946

Verheyen J. et al. 2019. Increased daily temperature fluctuations overrule the ability of gradual thermal evolution to offset the increased pesticide toxicity under global warming. *Environ. Sci. Technol.* 53: 4600-4608. http://doi.org/10.1021/acs.est.8b07166

Verheyen, J. and Stoks, R. 2020. Negative bioenergetic responses to pesticides in damselfly larvae are more likely when it is hotter and when temperatures fluctuate. *Chemosphere* 243: 125369. https://doi.org/10.1016/j.chemosphere.2019.125369

Villalobos-Jiménez, G. et al. 2017. Environmental noise reduces predation rate in an aquatic invertebrate. *J. Insect Conserv.* 21: 839-847. https://doi.org/10.1007/s10841-017-0023-y

Vucic-Pestic, O. et al. 2011. Warming up the system: higher predator feeding rates but lower energetic efficiencies. *Global Change Biol.* 17: 1301-1310. https://doi.org/10.1111/j.1365-2486.2010.02329.x

Wagner, D. L. 2020. Insect declines in the Anthropocene. *Ann. Rev. Entomol.* 65: 457-480. https://doi.org/10.1146/annurev-ento-011019-025151

Wang, Y. J. et al. 2021. Thermal evolution ameliorates the long-term plastic effects of warming, temperature fluctuations and heat waves on predator-prey interaction strength. *Functional Ecology* 00:1-12. https://doi.org/10.1111/1365-2435.13810

Wang, G. and Dillon, M. E. 2014. Recent geographic convergence in diurnal and annual temperature cycling flattens global thermal profiles. *Nat. Clim. Change* 11: 988-992. https://doi.org/10.1038/nclimate2378

Wang, S. et al. 2020. Changes of water clarity in large lakes and reservoirs across China observed from long-term MODIS. *Remote Sensing of Environment* 247: 111949. https://doi.org/10.1016/j.rse.2020.111949

Wasserman, R. J. et al. 2016. Emergent effects of structural complexity and temperature on predator–prey interactions. *Ecosphere* 7: e01239. https://doi.org/10.1002/ecs2.1239

Wellenreuther, M. et al. 2011. Environmental and climatic determinants of molecular diversity and genetic population structure in a Coenagrionid damselfly. *PLoS One* 6: e20440. https://doi.org/10.1371/journal.pone.0020440

Williams, C. M. et al. 2016. Biological impacts of thermal extremes: mechanisms and costs of functional responses matter. *Integr. Comp. Biol.* 56: 73-84. https://doi.org/10.1093/icb/icw013

Woodward, G. et al. 2010. Climate change and freshwater ecosystems: Impacts across multiple levels of organization. *Philos. Trans. R. Soc. B* 365: 2093-2106. https://doi.org/10.1098/rstb.2010.0055

Wurtsbaugh, W. A. et al. 2019. Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. *WIREs: Water* 6: e1373. https://doi.org/10.1002/wat2.1373

Zhang, C. et al. 2018. Thermal evolution offsets the elevated toxicity of a contaminant under warming: A resurrection study in *Daphnia magna*. *Evol. Appl.* 11: 1425-1436. https://doi.org/10.1111/eva.12637

Zhong, J. et al. 2021. Adaptation of a marine diatom to ocean acidification and warming reveals constraints and trade-offs. *Sci. Total Environ.* 771: 145167. https://doi.org/10.1016/j.scitotenv.2021.145167

Publications and manuscripts

Carbonell, J. A., Wang, Y. J., and Stoks, R. (in preparation). Evolution of predator-prey interactions during range expansion in an aquatic insect predator.

Wang, Y.-J., Tüzün, N., Sentis, A., and Stoks, R. (in preparation). Rapid evolution of thermal performance curves but not of activation energies in *Daphnia magna*.

Wang, Y.-J., Tüzün, N., Sentis, A., and Stoks, R. (submitted). Turbidity reverses how plasticity and evolution shape predator-prey interactions under warming.

Wang, Y.-J., Sentis, A., Tüzün, N. and Stoks, R. (2021). Thermal evolution ameliorates the long-term plastic effects of warming, temperature fluctuations and heat waves on predator-prey interaction strength. *Functional Ecology*. Accepted Author Manuscript. https://doi.org/10.1111/1365-2435.13810

Carbonell, J. A., Wang, Y. J., and Stoks, R. (2021). Evolution of cold tolerance and thermal plasticity in life history, behaviour and physiology during a poleward range expansion. *Journal of Animal Ecology*, 00: 1–12. https://doi.org/10.1111/1365-2656.13482

Wang, Y. J., Stoks, R., Sentis, A., and Tüzün, N. (2020). Support for the climatic variability hypothesis depends on the type of thermal plasticity: lessons from predation rates. *Oikos*, 129(7), 1040–1050. https://doi.org/10.1111/oik.07181

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