

Research Article

Warming and latitude shape the non-consumptive effects of native and invasive alien crayfish predators on damselfly prey

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Abstract

There is increasing concern that the effects of biological invasions may be magnified by other human-induced global changes. Here, we compare the non-consumptive effects imposed by invasive vs. native predators and how these (differential) responses to both predator types depend on warming and prey latitude. We raised damselfly larvae from central- and high-latitudes in incubators under two temperatures (current [20 °C] and warming [24 °C]) and further exposed them to one of three predator cues: noble (native), signal (invasive at both latitudes) and spiny-cheek (invasive at central- but absent at high latitudes) crayfish. Growth rate increased in central-latitude but decreased in high-latitude prey in response to both noble and signal crayfish. The spiny-cheek crayfish only reduced growth rate in high-latitude prey. Cues from all three crayfish species generally caused a higher net energy budget, but only under warming. Our results demonstrated that high-latitude prey were able to recognize a novel invasive predator (spiny-cheek crayfish) cue, and revealed differential growth responses of central- and high-latitude prey toward the shared invasive predator (signal crayfish). Our data provide rare support for the concern that global change factors may magnify the impact of both native and novel invasive predators.

Key words: Energy allocation, global warming, invasive predators, *Ischnura elegans*, latitudinal gradient, non-consumptive effects



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Introduction

Biological invasions are a key component of human-induced biotic global changes and pose a worldwide threat to biodiversity and ecosystem functioning (Bellard et al. 2016). Specifically, the introduction of novel (invasive or not) predators may have a profound impact on native prey because 1) they may lack the ability to recognize and respond to these predators, 2) may respond inappropriately or 3) may respond appropriately but are outsmarted by predators (naïve prey hypothesis [NPH], Cox and Lima 2006; Banks and Dickman 2007). The NPH was tested in various ecological contexts especially when exotic predators show little or no similarities with native predators, e.g. in term of relatedness, appearance or predation skills (Carthey and Banks 2014; Anton et al. 2020). The NPH was supported by several empirical studies, for example in mussels facing an invasive crab (Freeman and Byers 2006), native fish prey facing an invasive lionfish (Anton et al. 2016)

and insects facing an invasive fish (Townsend 2003). Prey naïveté can ultimately lead to declines and even extinctions of populations of native prey if these fail to recognize invasive predators (Carthey and Banks 2014). Hence, according to the NPH, invasive and native predators showing different degrees of similarity are likely to trigger differential responses in native prey. This differential effect is likely to become smaller when the evolutionary history of co-occurrence with an invasive predator species increases (Anton et al. 2020).

While the studies on novel invasive predators (invasive predators, hereafter) on prey mainly focused on direct consumptive effects, predators may also affect prey through indirect non-consumptive effects by modifications in prey behaviour, physiology and life-history traits (Hawlena and Schmitz 2010). Indirect effects are important to study as these may contribute as much or even more to prey population dynamics than direct effects (Preisser et al. 2005). In extreme cases, indirect effects can be absent in response to an invasive predator, as has been documented, for example, in shell thickening in molluscs (Freeman and Byers 2006) and refuge use in crustaceans (Martin 2014). Other studies did demonstrate indirect effects of invasive predators but these differed from the indirect effects imposed by native predators with, for example, an increased oxidative stress in amphibians (Pinya et al. 2016) and slower development in damselflies (Antoń and Sniegula 2021) when exposed to an invasive compared to a native predator. A largely ignored type of indirect effects are bio-energetic responses to predation risk. Understanding how predation risk shapes the available energy reserves of prey and their consumption, and especially their balance is important as this net energy budget may have fitness consequences and can explain life history responses to stressors in prey (e.g. Verheyen and Stoks 2020); as such it may improve mechanistic insights in the impact of native vs. invasive predators on local prey populations.

There is increasing concern that the effects of invasions may be reinforced by human-induced abiotic factors such as those related to climate change (Lopez et al. 2022). Knowing when and how the effects of invasive predators on prey are modified by such global change factors are barely understood, yet crucially needed for conservation practices (Anton et al. 2020; Lopez et al. 2022). One particular abiotic factor that may modulate prey responses to predation risk, including the effect of invasive predators, is temperature. The general idea is that under warming, prey may take greater risks to meet their increased energetic demands leading to higher encounter rates with predators (Lienart et al. 2014; Mitchell and Harborne 2020). For example, growth acceleration and increased metabolic rates were observed when predation risk imposed by native predators was combined with warming in damselflies (Stoks et al. 2012; Janssens et al. 2015). But the opposite pattern was also found with, for example, lower growth when predation risk was combined with warming in the damselfly *Enallagma vesperum* (Culler et al. 2014). Native and invasive predators may potentially trigger differential indirect effects under warming but the direction and the strength of the response remains unclear, asking for empirical studies. One obvious reason for a differential response to warming between both predator types is when prey never reacts to invasive predators (irrespective of temperature) while the response to native predators increases with warming. More general, whenever indirect effects of invasive predators differ from those of native predators (as predicted by the NPH, Cox and Lima 2006; Sih et al. 2010), these differences can be expected to be magnified by warming, especially when responses to temperature are non-linear.

One rare study that integrated both types of predators and warming, demonstrated stronger effects of an invasive compared to a native predator in term of delayed egg developmental time under warming compared with ambient conditions in the damselfly *Ischnura elegans* (Amer et al. 2024).

Another factor that may shape indirect prey responses to predators in general is the latitude of origin of the prey. This is because prey species often show different life history strategies linked to thermal adaptation along latitudinal gradients that may affect their response to predators and its dependence on temperature (Freeman and Byers 2006; Debecker and Stoks 2019; Palomar et al. 2023; Wos et al. 2023). For instance, in temperate regions many insects complete more generations at lower latitudes, typically resulting in stronger seasonal time constraints and faster life histories (Zeuss et al. 2017). Time-stressed individuals should invest more energy into growth and development to reach a specific mass and size before a critical time point (at least in overwintering cohorts) (Ludwig and Rowe 1990). Consequently, prey responses to predators are expected and shown to be weaker under time constraints, as shown in insects (Stoks et al. 2006) and amphibians (Altwegg 2002). Given the overall different prey responses to native predators at different latitudes, the same reasons as given for warming may potentially cause the impact of invasive predators to differ between prey of different latitudes, especially when the evolutionary history with an invasive predator differs between prey from different latitudes.

Here, we tested the NPH in the context of global warming and included an intraspecific component to assess latitudinal differences in prey responses. Specifically, we evaluated the indirect effects imposed by native vs invasive predatory crayfish on the growth and bio-energetic traits of the native damselfly, *I. elegans*, during its larval stage, and how these patterns were shaped by warming and prey latitude of origin. We used three crayfish species differing in their invasive status at the two studied damselfly latitudes of origin: the noble crayfish (*Astacus astacus*) which is native at both latitudes, the invasive signal crayfish (*Pacifastacus leniusculus*) which invaded both latitudes since the 1970s (Kouba et al. 2014), and the invasive spiny-cheek crayfish (*Faxonius limosus*) which is non-native at both latitudes but only invaded the central latitude where it was introduced at the end of the 19th century. We reared larval damselflies from replicated ponds at high and central latitudes in the laboratory and exposed them to native or invasive predator cues at current (20 °C) and warming (24 °C) temperatures. We measured larval growth rate, and a set of fitness-related bio-energetic parameters that capture energy availability and consumption, and are known to be affected by predator cues in damselfly larvae (Janssens et al. 2015).

Based on the theoretical predictions that native prey would recognize invasive alien predators which whom they share an evolutionary history (Anton et al. 2020) and previous case studies (Palomar et al. 2023; Wos et al. 2023; Amer et al. 2024), we expected *I. elegans* to recognize the three predator species, yet with a different response between native and invasive predators. In general, we expected damselfly prey to react to predators with an accelerated growth rate (Stoks et al. 2012), a faster metabolism and lower energy storage (Stoks et al. 2005a, Van Dievel et al. 2016). According to the NPH, as the damselfly species shared a longer evolutionary history with their native predator, we expected a stronger response for growth and bio-energetic variables in response to the native than the invasive predators. As the degree of relatedness between native and invasive predators decreases, we may expect smaller differences in the responses between both predator types. We also expected the invasive predator effects on prey to be more pronounced at 24 °C

compared to 20 °C, and the effects induced by the invasive signal crayfish (present at both latitudes) to be more pronounced in the high-latitude prey populations (compared to the central populations) as these are less time-stressed. Yet, for the invasive spiny-cheek crayfish (only present at the central latitude) we expected stronger predator effects at the central latitude because of the latitude-specific evolutionary history with this invasive predator.

Methods

Part of the phenotypic data (growth rate data) used in the present study was used in another article focusing on the effects of urbanization and predator cues (spiny-cheek crayfish only) on *I. elegans* collected in urban and rural ponds at different latitudes (Palomar et al. 2023). The current study addresses novel questions by focusing on the indirect effects induced by native vs. invasive predators and includes novel datasets related to noble and signal crayfish, and to physiological traits.

Description and sampling of *Ischnura elegans*

The study species, *I. elegans*, is a common damselfly species in Europe (Dijkstra and Schröter 2020). At central latitudes, such as Poland, populations are typically uni- and bivoltine, i.e., have one or two generations per year, respectively. At higher latitudes, including Sweden, populations generally display a uni- and semi-voltine pattern, i.e. one or two years are required to complete a single generation (Corbet et al. 2006; Norling 2021). Mating pairs of *I. elegans* were captured using insect sweep nets, with adult females obtained from two ponds in southern Sweden (hereafter, high latitude) and two ponds in southern Poland (hereafter, central latitude) (Suppl. material 1: table S1) on June 22–23, 2021, following the protocol outlined in (Sniegula et al. 2020). The distances between the two central- and two high-latitude ponds were, respectively, 73 km and 18 km. In total, 10 adult females were captured per pond (10 females × 2 ponds × 2 latitudes = 40 females). Adult females were individually housed in plastic cups with perforated lids and wet filter paper at ~22 °C under natural daylight (photoperiod). Each female laid one egg clutch giving a total of 40 clutches.

Description and sampling of crayfish species

We used chemical cues from three crayfish species: one native and two invasive species. The noble crayfish (*A. astacus*) is native and has a wide distribution in Europe. Until 2015, the crayfish was present in one of the central latitude ponds (Krakow pond, Suppl. material 1: table S1, Stanek et al. 2015). The approximate distance between sites where the noble was reported during the study season and ponds where central and high latitude damselflies were collected is ca. 40 km (Maciej Bonk and Rafał Maciaszek, personal comm.) and 9 km (Artportalen 2024). The noble crayfish were collected in a private pond in western Poland (51.653667°N, 16.981194°E) and in Gróbka River (49.964889°N, 20.501500°E).

The signal crayfish (*P. leniusculus*) native to North America, was introduced to Europe during the 1960s and is largely present in Scandinavia and in Poland (Kouba et al. 2014). The approximate distance between the closest crayfish

population and the ponds where central and high latitude damselflies were collected is ca. 110 km (Maciej Bonk and Rafał Maciaszek, personal comm.) and ca. 3 km. The signal crayfish were collected in Obłęski Lake in northern Poland (54.242889°N, 16.917278°E).

The spiny-cheek crayfish (*F. limosus*) originates from North America. Introduced to central Europe at the end of the 19th century, it has become the predominant crayfish species in EU countries, including Poland, with the exception of Scandinavian countries where the species is absent (Kouba et al. 2014; Artportalen 2024). The spiny-cheek crayfish is present in the Vistula river located at 400 m from the sampled pond in Krakow (Orłowska and Romanowski 2023). The spiny-cheek crayfish were collected in Kryspinów Lake in southern Poland (50.050128°N, 19.789125°E).

Because of its dispersal ability, *I. elegans* may have encountered some crayfish predators, though exposure varied by region. The species can disperse across multiple sites within a region (Conrad et al. 1999; Gall et al. 2017), which was supported by genomic studies demonstrating high gene flow at a local scale (Babik et al. 2023). While central-latitude populations are near spiny-cheek crayfish, they are ca. 40 and 110 km from reported noble and signal crayfish populations, making encounters more likely for noble than signal. In contrast, high-latitude populations are close to noble and signal crayfish (ca. 9 and 3 km), but far from spiny-cheek crayfish (ca. across the Baltic Sea and 210 km from Denmark; Ion et al. 2024).

The NPH states that a closer evolutionary distance between native and invasive predator may help their recognition by native prey (Anton et al. 2020). Previous genetic and phylogenetic analyses established the relationship between these three crayfish species. The noble and signal crayfish belong to the same family (Astacidae) and are phylogenetically closer to each other than to the spiny-cheek crayfish (Cambaridae) (Owen et al. 2015). The two families Astacidae and Cambaridae form a paraphyletic group (Owen et al. 2015).

A couple of weeks before the experiment, crayfish were collected in the field (May 2021) and acclimated in laboratory conditions. The crayfishes were housed in aquaria (l = 69 cm; w = 34 cm; h = 39 cm) filled with 50 L of dechlorinated tap water, at a constant temperature of approx. 20 °C. We placed three crayfishes of similar size and mass per aquaria. They were fed with fish food pellets twice per week and live worms once per week. All crayfish species were collected with permission from the pond owner and the Regional Directorate for Environmental Protection in Kraków (ref. DZP-WG.6401.147.2021. TŁ for noble crayfish; ref. OP-I.672.8.2020.MK1 for signal crayfish; ref. OP.672.4.2021.GZ for spiny-cheek crayfish).

Experimental procedure

Upon arrival at the laboratory, egg clutches were placed in an incubator with a temperature of 22 °C and under a photoperiod of L:D 20:4 h. After 15–17 days, eggs hatched and the experimental part started and was divided into three phases: two pre-treatment phases 1) The pre-winter phase during which all larvae underwent uniform pre-winter conditions, and 2) the winter phase during which all larvae underwent uniform winter conditions, and finally 3) the treatment phase during which larvae were exposed to the temperature and predator treatments (Fig. 1).

1) Pre-winter phase

Eight containers (22 × 16 cm, height 11 cm) and filled with 1500 mL of dechlorinated tap water were prepared for each pond matching the number of treatments (two temperatures crossed with four predator cue treatments [noble, signal, spiny-cheek crayfish and control]). The containers were placed in an incubator at 22 °C and 20:4 h L:D. These conditions induce high development and growth rates in larvae from both latitudes, particularly during post-winter rearing (Norling 2021). Under 20:4 h L:D, central latitude individuals may be more time-stressed compared with high-latitude individuals, however, our results did not reveal higher mortality at central latitudes and confirmed the expected difference in growth rate between latitudes (see results part). For each pond, once the majority of the ten clutches hatched, we randomly selected 4 larvae from each clutch that were placed into each of the eight containers totalling 40 larvae per container (4 ponds × 10 clutches × 4 larvae per clutch × 8 treatments = 1280 larvae at the beginning of the experiment). A plastic structure was provided in every container to minimize cannibalism among the larvae. Larvae were fed ad libitum with laboratory-cultured *Artemia* nauplii, twice a day on weekdays and once a day on weekend days. After three weeks, live *Daphnia* sp. were added to the feeding regime twice a week until autumn conditions were introduced, involving gradual change from summer to winter temperatures and photoperiods (described below). Container positions were randomized weekly within the incubator.

2) The winter phase

On 6 August 2021, approximately four weeks after larvae had hatched, simulated autumn temperature and photoperiod (thermo-photoperiod hereafter) were initiated. Three weeks later, winter conditions were simulated. This gradual reduction of the initial thermo-photoperiod 22 °C and 20:4 h L:D to 6 °C and 0:24 h L:D mimicked autumn and winter conditions in nature (Fig. 1). During the simulated winter, larvae were fed once a day, three times a week with *Artemia* nauplii.

3) Treatment phase

On 22 November 2021, immediately after the simulated winter, the treatment application phase started. All surviving larvae (total of 386 across all containers) were transferred to individual 200 mL cups, filled with 100 mL of dechlorinated water, and placed in an incubator at 10 °C and 4:20 h L:D. Over a two-day interval, the thermo-photoperiod was gradually increased to 20:4 h L:D and to the respective temperature treatment: 20 °C and 24 °C (Fig. 1). Water temperatures were selected based on dataloggers placed in three of our four ponds, estimation of water temperature for one pond (Lake model Flake; Mironov 2008) and on previous studies including our sampled ponds (Suppl. material 1: fig. S1) (Palomar et al. 2023; Wos et al. 2023). The temperature in high- and central latitude ponds fluctuated around 20 °C during the peak of larval development in late spring/early summer. Temperatures of 24 °C currently occur at both latitudes but infrequently. We established 20 °C as the current mean temperature, and 24 °C, corresponding to the predicted mean temperature by 2100 under the SSP8.5 scenario (Masson-Delmotte et al. 2021). Hence, the 24 °C treatment both reflects a warmer period in the study ponds at this moment, and the predicted future mean temperature in the study ponds.

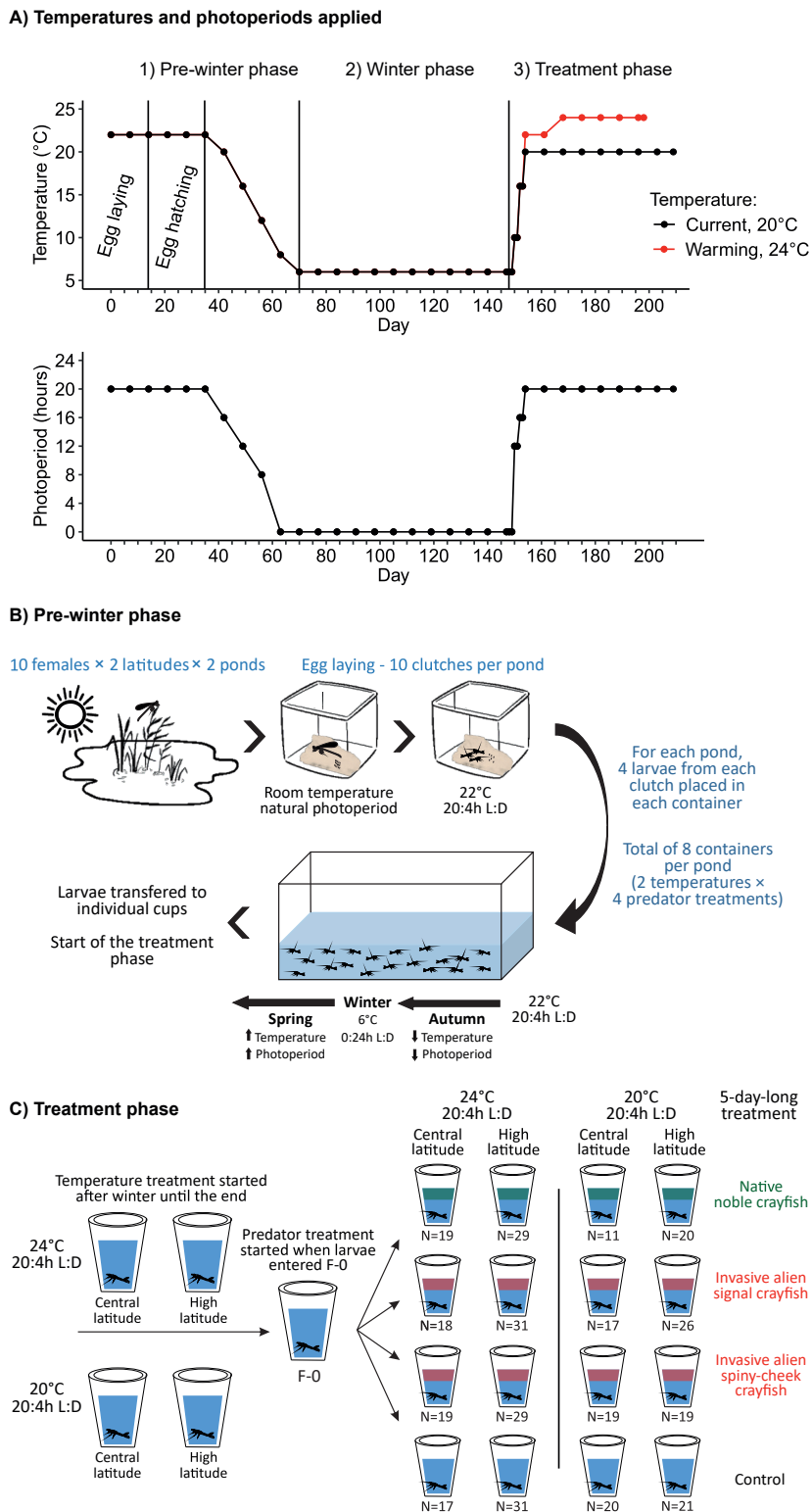


Figure 1. Summary of the experimental procedure **A** timeline of the experiment. The upper plot shows the variation in temperature over time including the different phases of the experiment. The lower plot shows the variation in photoperiod **B** for each pond, we collected 10 females (= clutch) and prepared eight plastic containers corresponding to the different treatments (2 temperatures × 4 predator treatments). For each pond, we placed four larvae from each family in each of the eight containers, totalling 40 larvae per container (4 larvae × 10 clutches). Larvae were kept in these containers during simulated pre-winter and winter periods. After winter, larvae were individualized in plastic cups and the experimental (temperature and predator cues) treatments started **C** details on the treatment application phase. Once larvae were split into individual cups, we started the temperature treatment. When larvae entered the final instar prior emergence (F-0), we crossed the temperature treatment with a five-day-long predator cue treatment (absence vs presence).

Throughout the treatment application phase, larvae were fed daily with *Artemia* nauplii. Given the different latitudes of origin and the influence of rearing temperature on larval development rate, larvae reached the F-0 instar at different dates and, consequently, were exposed to the post-winter temperature treatment for different durations (thermal exposure hereafter). When larvae entered the F-0 instar, the sex of each individual was identified.

Upon entering the F-0 instar, the thermal treatments were crossed with a five-day-long predator cue exposure treatment to one of the three crayfish species cues or to the control. Exposure of F-0 instars to a 5-day long treatment minimizes the chance of habituation and any effects on this instar are most likely to carry over to the adult stage, hence have fitness implications. Water samples collected from crayfish or control aquaria were warmed to the target temperature (20 °C or 24 °C). The water level in each cup was reduced to 67 mL and refilled with 33 mL of water from the crayfish aquarium (with predator cue) or the control aquarium (without predator cue). Cups were refilled every second day to maintain relatively constant predator cue levels, considering the biodegradation time of chemical predator cues (Van Buskirk et al. 2014). Previous experiments have shown that chemical predator cues affect damselfly life history traits, even during short exposure periods (13 days in Antol and Sniegula 2021; 3–9 days in Van Dievel et al. 2016).

Response variables

Growth rate

In total, we quantified the growth rate of 346 larvae (Suppl. material 3). Larval growth (mass increase) has been considered a proxy for fitness in damselflies (De Block and Stoks 2008; Siepielski et al. 2020). When larvae entered F-0 and before the application of the predator cue treatment, we measured survival, and wet mass (mg; $mass_{F-0}$) with an electronic balance (Radwag AS.62). After the 5-day exposure to a predator cue, we measured the wet mass again ($mass_{final}$) and calculated the growth rate over the 5-day period as $[\ln(mass_{final}) - \ln(mass_{F-0})]/5$, as in (McPeck et al. 2001).

Physiological parameters

We quantified a set of bio-energetic parameters on 315 of the 346 larvae measured for growth rate: the activity of the electron transport system (ETS) and the concentrations of the three major energy storage molecules: the fat, sugar and protein contents. These physiological parameters were subsequently used to obtain the cellular energy allocation (CEA), an estimate of the net energy budget calculated as the sum of energy available (E_a ; energy stored in proteins, sugars and lipids) divided by the energy consumed (E_c ; estimated based on the ETS activity). Detailed descriptions of the physiological analyses are available in Suppl. material 1: file S1.

Statistical analyses

All statistical analyses were performed using R (R Core Team 2013, RStudio Team 2015 version 2024.12.0). For survival, we ran a model with latitude, temperature and their interaction as predictors; population nested in latitude was added as a

random factor. Survival was considered as a binomial variable. We used generalized linear mixed-effects models (GLMMs; Magnusson et al. 2017). P-values were obtained using the Wald chi-square test (Wald X^2) implemented in the car package (Fox and Weisberg 2019). For the analysis of growth rate, we first ran a general model including the following predictors: sex (male vs. female), predator (control vs. noble vs. signal vs. spiny-cheek), temperature (20 °C vs. 24 °C), latitude (central vs. high latitude), and all possible interactions. As larvae were exposed to the post-winter temperature treatment for different durations, we used thermal exposure as a covariate. Population nested in latitude was added as a random factor. Significance of random effects was tested using likelihood ratio test by comparing the full (with random factors) versus reduced (without random factor) models using 'lrtest' (lmtest package version 0.9–40; Kuznetsova et al. 2017). We computed the proportion of variance for each significant random factor using the intraclass correlation coefficient, we reported the unadjusted coefficient to take into account the variance of fixed effects (performance package; Lüdtke et al. 2021).

As the variable predator contains four levels, it may be difficult to detect significant patterns especially if growth rate would be differentially affected by the three predator species. Hence, we also ran individual models for each predator species separately where we specifically compared the predator cues of a given crayfish species vs. the control. For this, we performed a model selection analysis (MuMIn package; Bartoń 2024) to select the models for each predator vs control comparison. We included in the initial model the following variables: population (two ponds per latitude) and all possible interactions between sex, predator cue treatment (one crayfish species vs control), temperature and latitude; thermal exposure was added as a covariate. Selection of the average model was based on the corrected Akaike's information criteria for small sample size (AICc) using $\Delta < 2$ (Suppl. material 1: table S3) (model.avg function in MuMIn package; Bartoń 2024). Then, we ran the selected average model using generalized linear mixed-effects models (GLMMs; Magnusson et al. 2017).

For the analyses of the three focal physiological parameters, we first ran a model for Ea, Ec and the integrated parameter CEA including the following predictors: sex (male vs. female), predator (control vs. predator cue), temperature (20 °C vs. 24 °C), latitude (central vs. high latitude), and all possible interactions; thermal exposure was added as a covariate and population nested in latitude was added as a random factor. The four individual "raw" physiological parameters: ETS activity and the three variables related to energy storage (fat, sugar and protein contents) were analysed using multivariate statistics. First, we ran a Multivariate Analysis of Variance (MANOVA) for each predator treatment vs. control to identify and select the relevant predictors affecting the physiological parameters. The MANOVA model included the four physiological parameters (log-transformed) and the following predictors: sex (male vs. female), predator (control vs. predator cue), temperature (20 °C vs 24 °C), latitude (central vs. high latitude), and all possible interactions between predator, temperature and latitude; thermal exposure was added as a covariate. The significant predictors revealed by MANOVA were subsequently used in a Constrained Correspondence Analysis (CCA; vegan package; Oksanen et al. 2013) to evaluate and to visualize their contribution to the physiological variation. In addition, for the CCA, we added the growth rate along with the physiological parameters to link this life-history trait with physiology.

Results

At the start of F-0, the overall survival rate was 88.4% and none of the variables had significant effects on survival (latitude: Df = 1, $X^2 = 3.47$, $p = 0.063$; temperature: Df = 1, $X^2 = 0.07$, $p = 0.789$; latitude \times temperature: Df = 1, $X^2 = 2.58$, $p = 0.108$) but the survival tended to be lower at central latitudes. No larvae died during the 5-day exposure period to the predator cues in F-0.

Effects of native and invasive predator cues on growth rate, and how these depend on temperature and prey latitude

For growth rate, the overall model detected a significant effect of the covariate thermal exposure (estimate = $-1.2e-04$; SE = $5.7e-05$) and of latitude with central-latitude larvae growing faster than high-latitude larvae (Least Square Mean [LSM] \pm SE_{central latitude} = $0.013 \pm 7.2 \times 10^{-4}$; LSM_{high latitude} = $0.009 \pm 6.0 \times 10^{-4}$) (Table 1). Next, we performed separate analyses for each crayfish predator vs. control separately. First, we ran a model selection analysis to identify the average model; results are shown in Suppl. material 1: table S3. For the native noble and the invasive signal crayfish, both occurring at the two prey latitudes, almost similar models were selected that showed similar response patterns which were not affected by temperature (Suppl. material 1: table S4). The significant latitude \times predator cues interaction indicated that while central-latitude larvae increased growth rate in response to predator cues of these two crayfish species, the high-latitude larvae showed the opposite response (Suppl. material 4; Fig. 2A, B). For the invasive spiny-cheek crayfish, which only occurs at the central latitude, the average model identified a significant effect of sex; with males growing faster (LSM_{male} = 0.011 ± 0.00 ; LSM_{female} = 0.008 ± 0.00) and of predator \times latitude \times sex, here the growth rate did not increase in response to spiny-cheek cues in central-latitude larvae, yet also decreased in high-latitude females (Suppl. material 1: table S4; Fig. 2C). Also, the growth responses to spiny-cheek cues were not affected by temperature (Suppl. material 1: table S4).

Table 1. Results of the GLMMs testing for the effects on growth rate, energy available (Ea), energy consumed (Ec) and cellular energy allocation (CEA) of sex (male vs. female), temperature (20 °C vs. 24 °C), predator (control vs. noble vs. signal vs. spiny-cheek crayfish), latitude (central vs. high latitude) and their interactions; thermal exposure was also added as a covariate and population as a random factor.

Variables	DF	Growth rate	Physiological parameters		
		p (X^2)	Ea p (X^2)	Ec p (X^2)	CEA p (X^2)
Thermal exposure	1	0.036 (4.42) *	< 0.001 (25.2) ***	< 0.001 (25.0) ***	0.378 (0.78)
Sex	1	0.079 (3.09) ^(*)	0.191 (1.71)	0.052 (3.79) ^(*)	0.449 (0.57)
Temperature	1	0.719 (0.13)	0.623 (0.24)	< 0.001 (12.0) ***	0.054 (3.72) ^(*)
Predator	3	0.281 (3.82)	0.688 (1.48)	0.002 (15.2) **	0.054 (7.63) ^(*)
Latitude	1	0.032 (4.62) *	< 0.001 (33.2) ***	0.572 (0.32)	0.001 (10.8) **
Predator \times temperature	3	0.871 (0.71)	0.177 (4.92)	0.645 (1.66)	0.073 (6.98) ^(*)
Temperature \times latitude	1	0.532 (0.39)	0.242 (1.37)	< 0.001 (14.5) ***	0.062 (3.48) ^(*)
Predator \times latitude	3	0.170 (5.02)	0.782 (1.08)	0.185 (4.83)	0.267 (3.94)
Predator \times temperature \times latitude	3	0.972 (0.23)	0.408 (2.90)	0.504 (2.34)	0.024 (9.43) *
Random factor		p (X^2)	p (X^2)	p (X^2)	p (X^2)
Population	1	1.00 (0.00)	< 0.001 (30.2) ***	< 0.001 (53.6) ***	0.008 (7.12) **

Table shows degree of freedom (DF), p-values and the Wald X^2 in parentheses. Significance is indicated in bold by *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ^(*) $p < 0.1$. Significance of the random factor was tested using likelihood ratio test.

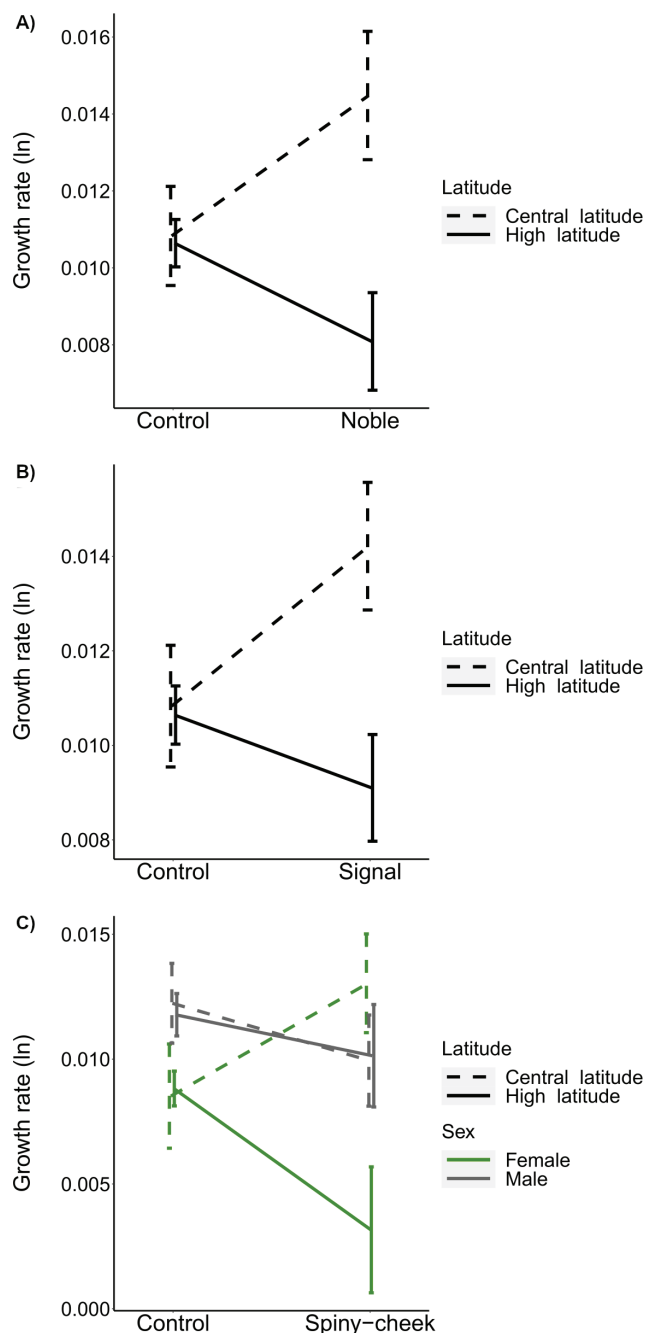


Figure 2. Effects of the predator cues on growth rate of the damselfly larvae of both latitudes for the three crayfish species **A** noble predator **B** signal predator, and **C** spiny-cheek predator. For the latter predator sex-specific effects were illustrated. Error bars indicate one standard error.

Effects of native and invasive predator cues on the bio-energetic response variables, and how these depend on temperature and prey latitude

The predator cues and temperature did not affect the energy available (E_a) (Table 1). Instead, E_a differed between latitudes and was higher in central-latitude prey ($LSM_{\text{central}} = 150 \pm 2.40$; $LSM_{\text{high}} = 118 \pm 2.11$) (Table 1). The energy consumed (E_c) was affected by the predator cues ($LSM_{\text{control}} = 20.6 \pm 0.4$; $LSM_{\text{noble}} = 19.2 \pm 0.4$; $LSM_{\text{signal}} = 18.8 \pm 0.4$; $LSM_{\text{spiny-cheek}} = 19.9 \pm 0.4$) with predation risk lowering the E_c for signal cues compared to control ($df = 311$, $t = 3.21$, $p = 0.008$) and, as a

trend, for noble crayfish cues ($df = 311$, $t = 2.40$, $p = 0.08$), but not for spiny-cheek cues ($df = 311$, $t = 1.29$, $p = 0.57$). The Ec was lower at 24 °C than at 20 °C ($LSM_{20\text{ °C}} = 19.8 \pm 0.292$; $LSM_{24\text{ °C}} = 19.5 \pm 0.27$). The effect of the predator cues on Ec did not depend on temperature (Table 1). The significant interaction temperature \times latitude indicated that more energy was consumed at central latitudes at 24 °C (Suppl. material 1: fig. S2). For both Ea and Ec, the analysis revealed a significant effect of population (Table 1), explaining 8.9% and 15.6% of the variance respectively.

For the cellular energy allocation (CEA), there was a significant effect of latitude with higher CEA at the central latitude ($LSM_{\text{central}} = 7.39 \pm 0.12$; $LSM_{\text{high}} = 6.30 \pm 0.10$) and a significant three-way predator cue \times temperature \times latitude interaction (Table 1, Fig. 3). The three way-interaction showed that exposure to cues from the native noble and, as a trend, from the invasive signal caused at both latitudes a higher CEA at 24 °C than at 20 °C (Suppl. material 4; Fig. 3A, B). While cues from the invasive spiny-cheek crayfish cues also only caused an increase in CEA at 24 °C, but not at 20 °C, this was only the case in central-latitude larvae while there was no effect of these cues in high-latitude larvae (Fig. 3C). Finally, the effect of population was also significant for CEA explaining 2.9% of the variance.

Effects of temperature and latitude on ETS and energy storage for each predator treatment

The MANOVAs testing for the effects of temperature and latitude for each predator vs. control treatment on the four physiological parameters used to calculate CEA: ETS, and fat, sugar and protein contents are presented in details in Suppl. material 1: file S2.

The analyses performed on the noble vs. control and signal vs. control treatment revealed similar patterns with significant effects of latitude, predator and of the interaction temperature \times latitude (Suppl. material 1: table S6). At high latitude, combined or not with 24 °C, there were lower fat and sugar contents. In the presence of the predator cue, we observed a lower sugar content and growth rate.

The pattern was different for the spiny-cheek vs. control treatment with a significant three-way interaction predator \times latitude \times temperature cue (Suppl. material 1: table S6). At high latitude, in the presence of the spiny-cheek crayfish cue and at 24 °C, we observed lower fat and sugar contents and higher ETS activity.

Discussion

We investigated to what extent native and invasive crayfish species cause different indirect effects on prey growth and physiology, and to what extent these differences between predator types were further altered by temperature and prey latitude of origin. All three crayfish species, when analysed together, caused indirect effects on physiology (Ec and CEA) and interacted or not with other factors, providing weak support for the NPH. Indeed, the native noble and invasive signal crayfish, which both co-occur with the prey at the two latitudes, caused similar response patterns for growth and bio-energetics, while the invasive spiny-cheek crayfish caused somewhat different response patterns relative to both other crayfish species that depended on latitude. The weak support for the NPH was not further modulated by temperature or prey latitude of origin. While indirect effects on growth rate were consistent across temperatures, yet strongly latitude-dependent across all three crayfish species, indirect effects on physiology were only detected at 24 °C and were less latitude-dependent.

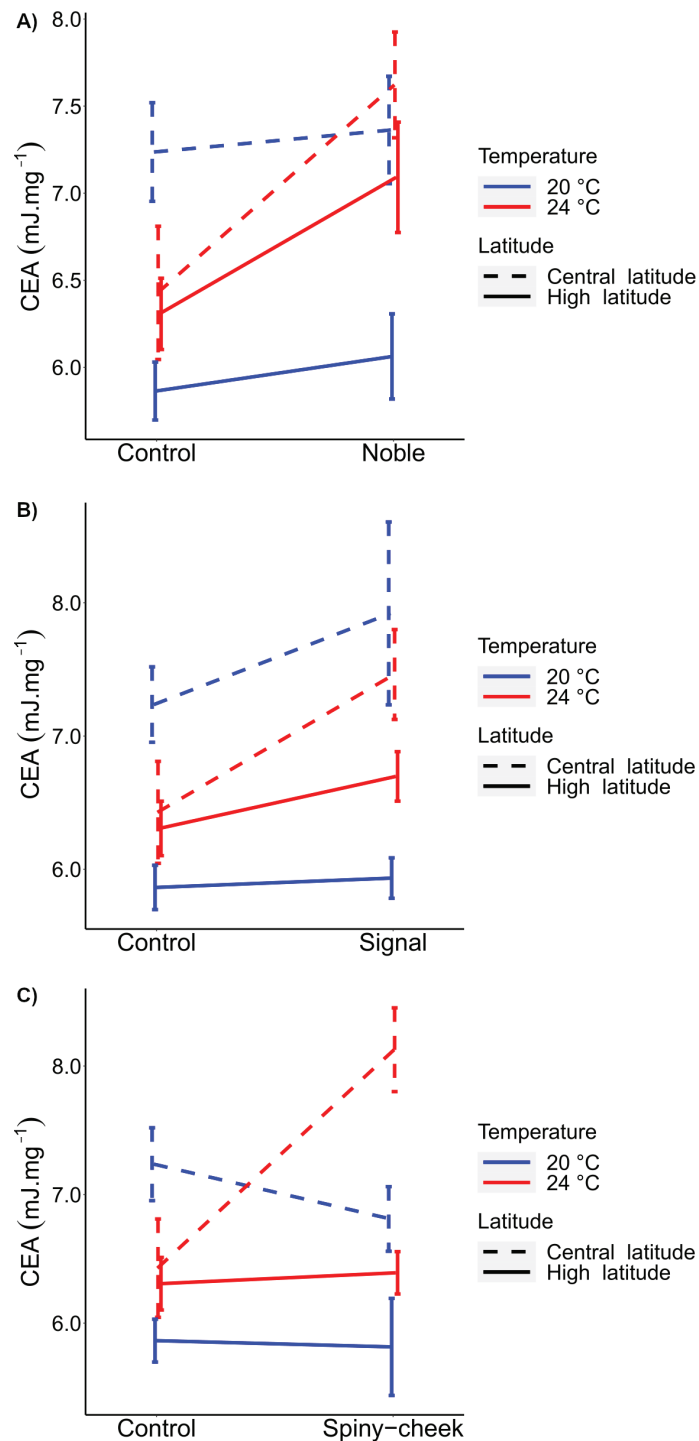


Figure 3. Effects of the predator cues and temperature on the cellular energy allocation (CEA) of the damselfly larvae of both latitudes for the three crayfish species **A** noble predator **B** signal predator, and **C** spiny-cheek predator. Error bars indicate one standard error.

Combined effects of predator, latitude and temperature on growth rate

In general, our growth results did not provide strong support for the NPH. When crayfish species are analysed together or separately, no clear or consistent differences in larval growth rate were found when exposed to native versus invasive predator species. Our results contrasted with previous studies in the damselfly *I. elegans* which found the opposite of the NPH with an increase in egg developmental

time when exposed separately to invasive signal and spiny-cheek crayfish vs. native perch fish (Antoń and Sniegula 2021) and in egg developmental time and mass at emergence when exposed to invasive signal crayfish vs. native perch in central-latitude populations (Amer et al. 2024). Another study found increased negative effects on egg and larval survival, egg development time and larval mass and fat content when treated with alien danube and invasive alien signal vs. native noble crayfish cues (Sniegula et al. 2025). Also, weak support for the NPH was found in other prey species, for instance, in *Daphnia mendotae* no difference in adaptive behavioural responses was detected to various invasive and native predators (Bourdeau et al. 2013). To explain deviations from the NPH, it has been proposed that being exposed to other crayfish predators in nature (e.g., noble and signal crayfish present at high latitudes) might enable prey to recognize predator cues released from an unestablished, novel invasive predator (spiny-cheek crayfish here) and trigger similar responses (Anton et al. 2020). In that sense, the three crayfish species used in the current study probably shared some similarities in terms of chemical cues released in the environment. Indeed, not established invasive predators may produce similar kairomones in terms of chemical composition as established invasive or native predators, which is more likely with a higher degree of phylogenetic relatedness (Sih et al. 2010). Nevertheless, there is conflicting evidence for this idea. For example, there was no relationship between taxonomic distance of various invasive predators belonging to different clades and the behavioural response of their *Daphnia* prey (Bourdeau et al. 2013). In our study, noble and signal crayfish are phylogenetically close and belong to the same family (Owen et al. 2015). Chemical cues from these two crayfish species triggered similar latitude-specific responses in the prey. The spiny-cheek crayfish belongs to a different family and it is unclear to what extent kairomones produced by this crayfish are similar to those produced by the other crayfish species. Furthermore, we did not report the sex of the collected crayfishes and it is unclear to what extent male and female crayfishes differ in the nature and quantity of kairomones produced. As we controlled only for crayfish biomass, some variation in terms of quantity or quality of kairomones between aquariums cannot be excluded with potential effects on the larval response. Nevertheless, as the prey only had chemical cues to rely on, the observation that central-latitude prey did not change their growth rate when exposed to spiny-cheek cues but did it when exposed to cues from the other crayfish species suggests differences in the chemical composition of these cues. Hence, despite the characteristics of freshwater ecosystems and small waterbodies that tend to favour isolation and naivete (Cox and Lima 2006; Anton et al. 2020), we provided evidence that recognition of novel alien predators may occur and was probably due to some degrees of phylogenetic relatedness between native and invasive predators.

Contrary to our expectations, temperature had no significant effects on the larval growth response to predators (Stoks et al. 2012; Amer et al. 2024), hence also not on the weak NPH patterns. A previous study also showed that larvae had similar growth rate at 20 °C and 24 °C when exposed to the invasive spiny-cheek crayfish cues, but an increase in growth rate under crayfish stress was observed at 28 °C (Palomar et al. 2023). Therefore, the effects of temperature on this particular predator-prey interaction may be manifested only under higher temperatures.

Despite some clear latitude-specific patterns in the prey growth responses, these had no clear and consistent effect on the weak NPH patterns. Our results confirmed the general trend that central-latitude populations grew faster due to the higher

voltinism linked to the longer growth season at the central latitude (Śniegula et al. 2012; Dinh Van et al. 2014). These latitudinal differences in growth rate mainly persisted under predation risk, while both prey latitudes responded in opposite directions. For high-latitude damselflies, that are less time-constrained, we found a consistent growth reduction across the three crayfish species probably because decreased growth can be adaptive to avoid predation (Stoks et al. 2005b) and can be compensated by more time available for reaching the final size prior emergence relative to the central-latitude populations. Even though the spiny-cheek crayfish has not been yet reported at high latitudes, damselflies were capable of producing a growth response, which was similar to their response to cues from the native predator at least for female damselflies (sex-specific effects described below). For central-latitude damselflies, however, the noble and signal crayfish species caused a growth acceleration, while the spiny-cheek crayfish triggered no significant growth response. A growth acceleration in response to predation risk has been observed before for the study species (Stoks et al. 2012), and may be adaptive by reducing the duration of exposure to aquatic predators especially in time-constrained prey populations. One possible reason why such growth acceleration was not present in response to spiny-cheek cues is that this species is more abundant in southern Poland than the two other species studied and we may hypothesize that these differences in selective pressures might have been selected against a risky growth acceleration.

Interestingly, only high-latitude female larvae responded with a growth reduction when exposed to the spiny-cheek crayfish cues. Sex-specific responses to predation risk have been demonstrated in previous studies. For example, predation risk caused a decrease in development time in female but not male mosquitoes (Fontana-Bria et al. 2017) and a greater metabolic rate in male but not female crickets (Lagos and Herberstein 2017). In general, sex-specific effects are more pronounced in species with strong sexual dimorphism and in protandrous species where males and females have different growth rates which is often the case in damselflies (Corbet 1999). In addition, females may be more sensitive to some stressors, e.g. food stress, as they require more energy for their development to reach a higher body mass compared to males (Teder and Kaasik 2023).

Combined effects of predator, latitude and temperature on physiology

Bio-energetic response patterns to temperature and predation risk only partly matched our observations for growth rate, indicating a partial decoupling between life history and physiology. As was the case for growth rate, we observed a distinct physiological response when exposed to the spiny-cheek crayfish cues whereas noble and signal crayfishes tended to cause more similar effects, again providing weak support for the NPH. In addition, patterns may be also population-dependent as previously demonstrated in *Lestes* species at the physiological level with variation in assimilation efficiency between permanent and temporary ponds observed under predation risk (Stoks and McPeck 2003). In our study, despite significant effects of population on physiology, we lack enough replicates at the population level (only two ponds per latitude) to further explore such interactions at the local scale but this opens avenues for further investigations.

For the CEA, the response to native and invasive predators depended on both temperature and latitude (significant three-way interaction), revealing more complex patterns than initially predicted on how both factors would affect the

predator-prey and NPH patterns. Our results showed that CEA was increased when prey were exposed to predator cues but only at 24 °C, supporting our prediction that an increase in temperature would increase the indirect effects of predators as perceived predation risk is likely higher. Yet, and in contrast to our NPH prediction, no overall clear distinction was observed in this response between native and invasive predator species. Nevertheless, there was some support for the prediction that the latitude-specific evolutionary history with predators played a role, as the increase in CEA under predation risk when combined with 24 °C was for spiny-cheek crayfish cues only present in prey of the central latitude and not of the high latitude. This is as expected by the NPH as the spiny-cheek crayfish only occurs at the central but not the high latitude. In general, higher CEA values indicate more energy being available for growth and reproduction which was consistent with the overall faster growth rate and CEA under predation risk in central-latitude prey. This result contrasted with a tendency for a lower CEA under predation risk in larvae of the confamilial damselfly *E. cyathigerum* (Van Dievel et al. 2019). However, in that case the authors exposed larvae to the dragonfly predator cues for a longer period of time (9 days). In such a case, predator exposition may reveal some trade-offs, e.g. between growth and defence mechanisms (Van Dievel et al. 2019).

Our results revealed that variation in CEA was mainly due to variation in Ec rather than in Ea. Indeed, we found a significant increase of CEA in response to predator cues (at 24 °C) which was accompanied by a reduction of Ec (manifested by a lower ETS activity), especially when exposed to noble and spiny-cheek crayfish cues. For Ea, we observed a considerable reduction in sugar content in the presence of both native and invasive predator cues and smaller effects on fat and protein contents, which together, however, did not translate in lower Ea under predation risk. Previous studies conducted on *E. cyathigerum* also reported a sugar reduction but this combined with a higher metabolic rate in the presence of a predator cue suggesting an escape strategy and the mobilization of important energy resources in prey (Janssens et al. 2015; Van Dievel et al. 2016). In our study, a lower metabolic rate under predation risk may reflect the often observed reduced foraging activity in prey (Krams et al. 2013) e.g. in damselflies (Kohler and McPeck 1989), with sugar reserves being used as a direct source of energy to maintain growth and vital functions. We hypothesise that fat and proteins may be used as sources of energy if the stressor persists for a longer period of time. Altogether, these results indicate some shared responses in term of energy use in response to predator cues (especially noble and signal crayfish), independently of their evolutionary histories with their prey. Some differences arose from the spiny-cheek crayfish which triggered, as for growth rate, some differential effects that were here jointly temperature- and latitude-dependent. The combination of this specific predator cue and temperature may represent particularly stressful conditions for central-latitude damselflies and maintaining high metabolic activity may be especially costly, as no growth acceleration was found under this predator cue treatment.

Conclusions

There is increasing concern on the effects of biological invasions and that these may be stronger under human-induced global warming. Overall, our results provide only partial support for the NPH. Indeed, the responses to the native

predator were very similar for one of the invasive predators (the signal crayfish), but differed for the other invasive predator (the spiny-cheek crayfish). We found some support for the idea that the prey latitude of origin may shape the impact of invasive predators as central- and high-latitude prey responded differently to both invasive predators, yet this latitude-specific response pattern was shared between the native predator and one of the invasive predators. Furthermore, high-latitude prey populations were able to recognize the spiny-cheek crayfish which is currently absent at this latitude. Our results did show stronger indirect effects imposed by the two invasive crayfish (signal and spiny-cheek crayfish) on the bio-energetic variables at 24 °C, supporting the concern that global change factors may magnify the impact of invasive predators. Yet, our results also indicated that the indirect effects at 24 °C were not stronger when exposed to invasive vs. native crayfish predators. Taken together, while the indirect effects when exposed to invasive crayfishes may show similarities to the ones imposed by native crayfish species, our results indicate they may cause additional stress on the local prey populations, especially at high-latitudes at 24 °C.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: SS, RS. Formal analysis: GW. Funding acquisition: SS. Methodology: NRA, AA, SS. Writing - original draft: GW. Writing - review and editing: RS, SS, NRA, GW, AA.

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Data availability

Data used in the manuscript are included in Suppl. material 3.

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Supplementary material 1

Additional information

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Data type: docx

Explanation note: **file S1**. Physiological analysis - Methods. **file S2**. Effects of temperature and latitude on ETS and energy storage for each predator treatment – Results. **fig. S1**. A) Monthly and B) weekly temperatures for each Polish (PL; central latitude) and Swedish (SW; high latitude). **fig. S2**. Plot showing the significant interaction temperature × latitude on cellular energy consumed (Ec). **table S1**. GPS coordinates of the sampled ponds for *Ischnura elegans*. **table S3**. Results of the model selection analysis for growth rate for each predator species vs control treatment. **table S4**. Results of GLMM testing for the effects on growth rate final (GR Final). **table S6**. Results of the targeted Multivariate Analyses of Variance (MANOVA) per crayfish species showing the effects. **table S7**. Results of the canonical constrained analysis. **table S8**. Results of the canonical constrained analysis (CCA).

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Supplementary material 2

Effects of the significant predictors detected by MANOVA

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Data type: pdf

Explanation note: Effects of the significant predictors detected by MANOVA (Suppl. material 1: table S6) on physiological parameters for each comparison A) noble vs control, B) signal vs control and C) spiny-cheek vs control treatment. Growth rate (GR) was added on the plot along with the physiological parameters. Scores of each trait and variable are shown in Suppl. material 1: table S8. Abbreviations: high latitude (HL) and spiny-cheek crayfish (S-C).

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Supplementary material 3

Dataset used in the study

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Data type: xlsx

Explanation note: 1) Dataset including growth rate, electron transport activity (ETS), and fat, sugar and protein contents for each individual. 2) Sample size for each latitude, treatment and sex.

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Supplementary material 4

Pairwise comparisons

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Data type: xlsx

Explanation note: Pairwise comparison testing for the difference in growth rate and cellular energy allocation (CEA) between the two latitudes and temperatures in the different predator treatments. Significance is indicated in bold.

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