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Research Article

Warming and photoperiod differentially mediate native and invasive alien predator effects on damselfly fitness traits

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Abstract

Invasive alien (IA) predators can disrupt predator-prey interactions and alter prey phenotypes. However, how their effects differ from those of native predators remains poorly understood. Additionally, it is unclear to what extent these impacts interact with environmental factors such as warming and photoperiod. To address these gaps, two complementary experiments were performed in the damselfly *Ischnura elegans* to test the effects of predator chemical cues (control, native noble crayfish, and IA spinycheek crayfish) in combination with (Experiment 1) temperature (ambient vs. warming) and short- and long-term predator cue exposure or (Experiment 2) photoperiod imposing different seasonal time constraints (early vs. late season). Effects on life-history (survival and growth rate), physiological (immune function measured as phenoloxidase (PO) activity and fat content), and behavioral (boldness and feeding rate) traits were subsequently quantified.

In Experiment 1, responses to IA spinycheek crayfish cues differed from those induced by native noble crayfish cues across all trait types, with predator-specific responses strongly dependent on temperature. Following short-term exposure, predator-specific differences in boldness and PO activity were evident at ambient temperature but weakened under warming. Following long-term exposure, survival was lower under IA spinycheek than under native noble crayfish cues, whereas under warming, growth rate was lower following exposure to native noble crayfish cues than in the control, with the IA spinycheek treatment intermediate.

In Experiment 2, the late photoperiod increased predator-specific differentiation in growth rate and boldness, primarily through stronger responses to native noble crayfish cues.

The results broadly support the naïve prey hypothesis but show that responses to native and IA predators are not determined by predator novelty alone. Abiotic conditions, represented here by temperature and photoperiod, can modulate predator-specific plasticity, highlighting the importance of environmental context when predicting prey responses to biological invasions under climate change.

Key words: Carry-over effect, climate change, *Ischnura elegans*, naïve prey hypothesis, non-consumptive predator effect, phenotypic plasticity, seasonal time constraint

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Introduction

Biological invasions are a major driver of ecological change, reshaping food webs and threatening native biodiversity (Benard 2004; Clinchy et al. 2013; Theodosiou et al. 2019). Invasive predators are particularly disruptive because they often exert demographic impacts on prey populations not only through direct consumption but also

through non-consumptive effects (NCEs) (Salo et al. 2007; Peckarsky et al. 2008; Wos et al. 2025). NCEs are often perceived via chemical cues released by predators or injured conspecifics and can fundamentally alter prey phenotypes without direct mortality (Werner and Peacor 2003; Preisser et al. 2005). Such impacts may be exacerbated by the lack of a shared evolutionary history between invasive predators and native prey (Sih et al. 2010). These effects can induce plastic responses in native prey, including altered behavior, physiology, and life-history strategies, with cascading consequences for growth, survival, and reproduction (Peckarsky et al. 2008; Sih et al. 2010). For example, native frog larvae exhibit increased hiding behavior and altered tail morphology when exposed to invasive crayfish cues (Nunes et al. 2014). Similarly, three of four Iberian anuran species reduced activity in response to native turtle predator cues but not to invasive turtle cues, consistent with a role of shared evolutionary history in predator recognition (Polo-Cavia et al. 2010).

In invasion contexts, antipredator responses can be severely compromised when prey lack evolutionary experience with novel predators, a concept framed under the naïve prey hypothesis (NPH) (Sih et al. 2010; Carthey and Banks 2014). Rather than a simple "all-or-nothing" failure, Sih et al. (2010) and Carthey and Banks (2014) categorize prey naïveté into hierarchical levels: Level 1 naïveté, where prey fail to recognize the novel predator's cues and exhibit no response; Level 2 naïveté, where prey recognize the predator but employ an inappropriate, mismatched defense; and Level 3 naïveté, where prey respond correctly but are simply outmatched by the invader's novel tactics. This structured framework explains why empirical studies often report highly contrasting responses to native vs. invasive predators. For instance, while some prey show complete recognition failure (Level 1 naïveté) to novel invaders compared with strong responses to native predators (Amer et al. 2024), others recognize the predator but exhibit behavioral or physiological responses that are inappropriate for reducing predation risk (Level 2 naïveté) (Carthey and Banks 2018).

Temperature is one of the most influential abiotic factors that shapes ectotherm metabolism and growth (Angilletta et al. 2004; Schulte 2015), with these individual-level thermal sensitivities scaling up to fundamentally reorganize the strength and dynamics of species interactions (O'Connor 2009). Importantly, growing evidence suggests that abiotic conditions interact with predator novelty and constrain the ability of prey to express differentiated antipredator responses toward native and invasive predators (Ferrari et al. 2010; Smolinský and Gvoždík 2012; Carthey and Banks 2014; Anton et al. 2020). Such context dependence may arise because warming alters energetic demands, behavioral activity, and information processing, potentially limiting the ability of prey to distinguish among predator types or maintain costly antipredator responses (Sih et al. 2010; Pepi et al. 2018; Domenici et al. 2019).

Increased temperatures may amplify or dampen antipredator responses depending on prey energy limitation and species-specific traits, thereby changing predator-prey interactions rather than simply intensifying predator effects (Urban et al. 2014; Cinel et al. 2020; Wos et al. 2023). For example, rising temperature increased the swimming speed of the predator (backswimmer *Notonecta undulata*) and prey (water flea *Daphnia pulex*), doubling prey encounter rates for the active predator. In contrast, prey encounter rates for the sit-and-wait predator (damselfly *Enallagma annexum*) rose fourfold solely because the prey swam faster (Twardochleb et al. 2020). At the mechanistic level (i.e., physiology and gene expression), responses to predation risk can also be temperature dependent. For example, juvenile brown

trout exhibited elevated cortisol and altered stress-related gene expression under predation risk, with the response magnitude strongly modulated by temperature (Filipsson et al. 2020). Temperature can therefore strongly shape predator-prey interactions, and thermal variation is increasingly recognized as a key modifier of invasion impacts (Corbett and Trussell 2025; Juillard et al. 2025).

In temperate regions, seasonal time constraints represent another important yet underappreciated environmental factor in invasion contexts. These constraints limit the remaining time window available for organismal development, maturity, and reproduction. For many ectothermic species, seasonal time constraints are signaled by photoperiod (Bradshaw and Holzapfel 2007; Norling 2021). Similar to warming, strong seasonal constraints may interact with novel predators and constrain the ability of prey to express differentiated responses toward native and invasive predators. Photoperiod affects growth, development, and physiology (Liefting et al. 2017; Amer et al. 2025), thereby influencing periods of vulnerability to predation (Lopez et al. 2023). Prey experiencing strong seasonal time constraints may prioritize rapid growth and development over costly antipredator responses, thereby altering the magnitude of responses to predator cues, as shown in *Lestes* damselflies (Stoks et al. 2006) and the winter moth (Salis et al. 2018).

The above-described stressor effects are further complicated in prey with complex life cycles, where responses to stressors at one stage can carry over to subsequent stages. Because many ectotherms, such as insects, undergo metamorphosis, carry-over effects are a key mechanism linking early-life stress to adult performance (Benard 2004; Kingsolver et al. 2011; Stoks and Córdoba-Aguilar 2012). For example, larval exposure to predation risk in painted frogs altered post-metamorphic morphology, with juveniles developing shorter legs and longer bodies (Nicieza et al. 2006). Furthermore, larval predator stress can drive significant physiological and morphological modifications that persist at emergence, such as reduced energy reserves and shorter wing lengths, as demonstrated in *Anopheles* mosquitoes (Roux et al. 2015). Similarly, thermal conditions and photoperiod during premature development can shape body size at metamorphosis, energy allocation, and later susceptibility to predators (Dmitriew 2011; Amer et al. 2025). In invasion scenarios, such carry-over effects may amplify or obscure the long-term impacts of invasive predators, particularly when combined with warming and seasonality.

While the individual roles of predator novelty, climate warming, and seasonal deadlines are increasingly studied, a predictive framework for how these stressors interact across different exposure timescales is still lacking (Homeny and Juliano 2007; Verberk et al. 2021). Most empirical work relies on short-term risk exposures, yet prey responses to short-term vs. long-term exposure to predation risk may differ, partly because costs can accumulate and compensatory mechanisms can be activated (Van Dievel et al. 2016). Direct comparisons are particularly challenging in organisms with complex life cycles, where prolonged exposure may span developmental stages and metamorphosis. Short-term exposure may trigger temporary behavioral shifts, whereas longer exposure can generate cumulative costs and carry-over effects across developmental stages. Understanding these temporal patterns and their interactions with warming or photoperiod is therefore important for assessing the effects of invasive predators under changing environmental conditions.

The damselfly *Ischnura elegans* was used because it is well suited for studying multi-stressor interactions due to its strong phenotypic plasticity and complex life cycle (Stoks and Córdoba-Aguilar 2012). Previous studies on *I. elegans*

have provided support for the NPH by showing that IA predators often elicit mismatched responses compared with native predators and that these responses may carry over across developmental stages (Antoł and Sniegula 2021; Antoł et al. 2022; Sniegula et al. 2024, 2025). Other studies in this system have further shown that predator effects interact with temperature, photoperiod, urbanization, and genetic background, but these studies have typically focused on a single life stage (larval), short predator exposure durations, or a limited subset of interacting biotic and abiotic factors (Palomar et al. 2023; Wos et al. 2023; Amer et al. 2024; Sniegula et al. 2024; Wos et al. 2024a).

Two experiments were conducted to test how predation risk imposed by native and IA predators differentially affects larval life-history, behavioral, and physiological traits (short-term predator exposure group), as well as carry-over effects expressed after metamorphosis (long-term predator exposure group). These experiments further evaluated how these responses are modulated by warming (Experiment 1) and photoperiod (Experiment 2, short-term exposure only). Following the NPH, differential responses to native vs. IA predators were predicted. Specifically, the lack of evolutionary history with the IA predator was expected to result in mismatched or ineffective antipredator responses compared with those elicited by the native predator. In Experiment 1, stronger effects were further expected following long-term than short-term predator exposure, with carry-over effects expressed in adult traits after metamorphosis. Because responses following short-term and long-term exposure were measured at different developmental stages, differences between these groups were interpreted as inferred patterns rather than a direct test of exposure duration. Finally, abiotic conditions were predicted to modulate responses to native and IA predators. Specifically, warming (Experiment 1) and late photoperiod (Experiment 2) were expected to constrain the ability of prey to maintain predator-specific antipredator responses, thereby reducing differentiation in responses to native and IA predator cues.

Material and methods

Study system, collection, and rearing

I. elegans has a widespread geographic distribution in Europe (Dijkstra and Schröter 2020). The species is common and is not listed as protected or endangered in Europe (Knijf et al. 2024). In central Europe, *I. elegans* produces one (univoltine) or two (bivoltine) generations annually, with larvae as the overwintering stage in a univoltine cohort (Corbet et al. 2006). Larval development, emergence dates, and the flying season are not synchronized. In central Europe, including Poland, the damselfly is on the wing throughout the spring and summer months (Dijkstra and Schröter 2020; Sniegula et al. 2024). Larvae hatch within two to three weeks after egg deposition. Both the aquatic egg and larval stages often co-occur with top predators such as fish and crayfish (Kouba et al. 2014; Sniegula et al. 2019a; Amer et al. 2024; Sniegula et al. 2025). The larvae occupy a mid-level trophic position in freshwater food webs, acting as key predators of aquatic invertebrates, such as midges and mosquito larvae, while also serving as prey for larger invertebrates and fish (Stoks and Córdoba-Aguilar 2012; Antoł and Sniegula 2021).

Adult female *I. elegans* in copulation were collected near Płaszowski pond, within the city of Kraków, southern Poland (50°02'33.4"N, 19°58'01.8"E) on 15 June

2022 (early photoperiod group) and 3 August 2022 (late photoperiod group). Females were placed in plastic containers with wet filter paper for egg laying and were kept in a room with a natural photoperiod and a temperature of ca. 22 °C. Newly laid clutches were transferred to two incubators (Pol-Eko ST700). The temperatures at which eggs and larvae were reared were adjusted once a week to follow seasonal changes in mean weekly temperatures in shallow water, which is the optimal habitat for damselfly larvae (Corbet 1999). The native crayfish species occurring in the study region is the noble crayfish (*Astacus astacus*), which was also used as the native predator in the experiment. Płaszowski pond has only a historical record of the non-native but non-invasive Danube crayfish (*Astacus leptodactylus*), last recorded in 2015 (Maciej Bonk, unpublished data), approximately 7 years before damselfly collection.

Predator description, collection, and housing

Cues from two different crayfish species were used to induce NCEs. These were the native noble crayfish (*A. astacus*), which is present in central Europe, including southern Poland (Kouba et al. 2014), and the IA spinycheek crayfish (*Faxonius limosus*), introduced to central Europe in the late 19th century. *F. limosus* has become the most prevalent crayfish species in EU countries, including southern Poland (Kouba et al. 2014; World of Crayfish^{TM} 2024). Noble crayfish were collected from a private pond near the town of Miejska Gorka (51°39'13.2"N, 16°58'52.3"E), and spinycheek crayfish were collected from an excavation pond in Kryspinow (50°02'56.8"N, 19°47'28.7"E). Animals were kept in aquariums (three crayfish in 40 L of reconstituted deionized water [RDiW]) at a constant temperature of 20 °C. The densities of crayfish in the aquariums were based on previous experiments in which the crayfish cues were effectively used to induce antipredator responses in damselfly larvae (Wos et al. 2023; Amer et al. 2024). Crayfish were fed fish food pellets twice per week and live worms once per week. Water from these aquariums was used for the predator cue treatment (described below), along with water from a control aquarium (40 L of RDiW without crayfish). Aquariums were regularly cleaned to avoid the accumulation of organic matter. To minimize fluctuations in chemical cue concentrations, a standardized regime of feeding, cleaning, and water collection was maintained across all holding aquariums. Previous studies indicate that predator cue persistence is variable among systems, with reported half-lives ranging from 0.2 to 126 h (Van Buskirk et al. 2014). Thus, the standardized water replacement interval of approximately 48 h falls within the range commonly considered biologically relevant for predator cue experiments. However, because cue concentrations were not directly monitored chemically, some temporal variation in cue concentration inevitably remained and should be considered a potential source of experimental variation. Noble and spinycheek crayfish were collected and housed under permits from the General Directorate of Environmental Protection in Warsaw (permit DZP-WG.6401.147.2021.TŁ) and the Regional Directorate of Environmental Protection in Krakow (permit OP.672.4.2021.GZ), respectively.

Experimental setup

After oviposition, each egg clutch from a single female was divided into six equal parts and placed in separate plastic containers (15 × 11 × 7.5 cm) filled with 600 mL of dechlorinated tap water. Because egg clutches were equally divided

across all treatment combinations in a balanced split-clutch design, potential genetic and maternal variation was evenly distributed among treatment groups. After hatching, larvae from different clutches within each treatment were pooled; consequently, individual family identity was not retained during subsequent rearing and analyses. Water was refilled every other day to keep the water levels constant. The study was divided into two partially independent experiments focusing on different treatment combinations, with the 22 °C early photoperiod group shared between Experiment 1 and Experiment 2.

Experiment 1

Two temperature treatments (ambient: 22 °C and warming: 26 °C) were crossed with three predator cue treatments (control, native noble crayfish, and IA spinycheek crayfish) (Fig. 1). The two temperature treatments were the ambient temperature treatment, which mimicked the current temperature at the collection site, and the warming treatment, in which the ambient temperature was increased by 4 °C to mimic the predicted temperature change by 2100 under IPCC scenario SSP5-8.5 (IPCC 2021). Temperature at the sampling pond was measured with data loggers placed at a depth of 40 cm in 2023. The recorded temperature was approximately 22 °C during the summer months (Suppl. material 1: fig. S2; Sniegula et al. 2024), supporting the ambient temperature treatment during the current experiment. After hatching of early photoperiod clutches, larvae were transferred into group cups (10 larvae/cup) for 2 weeks and then into individual plastic cups (height 9 cm, diameter 4 cm) under the corresponding conditions. All groups were reared in dechlorinated tap water until entering the final instar before emergence (F-0). At entry into F-0 (day 0), larvae from each temperature group were divided into two groups: short-term larval exposure and long-term larval exposure, the latter allowing assessment of cross-stage carry-over responses from the larval to the adult stage. The short-term exposure group was divided among three predator treatments (control, noble, and spinycheek crayfish cues) for 5 days, after which larvae were collected for physiological analyses, representing immediate responses to short-term larval exposure. Behavioral observations were conducted on day 2 of exposure in this group. The long-term exposure group received the same predator treatments throughout the F-0 instar (mean duration = 14 days, range 9–20 days). Predator cue exposure ceased at emergence, and newly emerged adults were maintained under cue-free conditions for 24 h to allow complete cuticle sclerotization before physiological analyses. These measurements represented cross-stage carry-over responses following long-term larval exposure. Because short-term responses were measured in F-0 larvae and long-term responses after emergence, the two groups were analyzed separately rather than directly compared.

Larvae reared at 22 °C were subjected to overwintering conditions unless they completed development to the respective target stage (F-0 or adult at emergence) before the onset of overwintering. Consequently, some individuals in the 22 °C treatment developed directly without experiencing overwintering, whereas those reared at 26 °C developed directly and did not experience overwintering conditions (Suppl. material 1: table S4). Such variation in developmental pathways is consistent with temperature-dependent voltinism in damselflies, whereby slower-developing individuals may postpone emergence to the following season (Corbet et al. 2006; Sniegula et al. 2012; Raczyński et al. 2022b). Among the non-overwintering

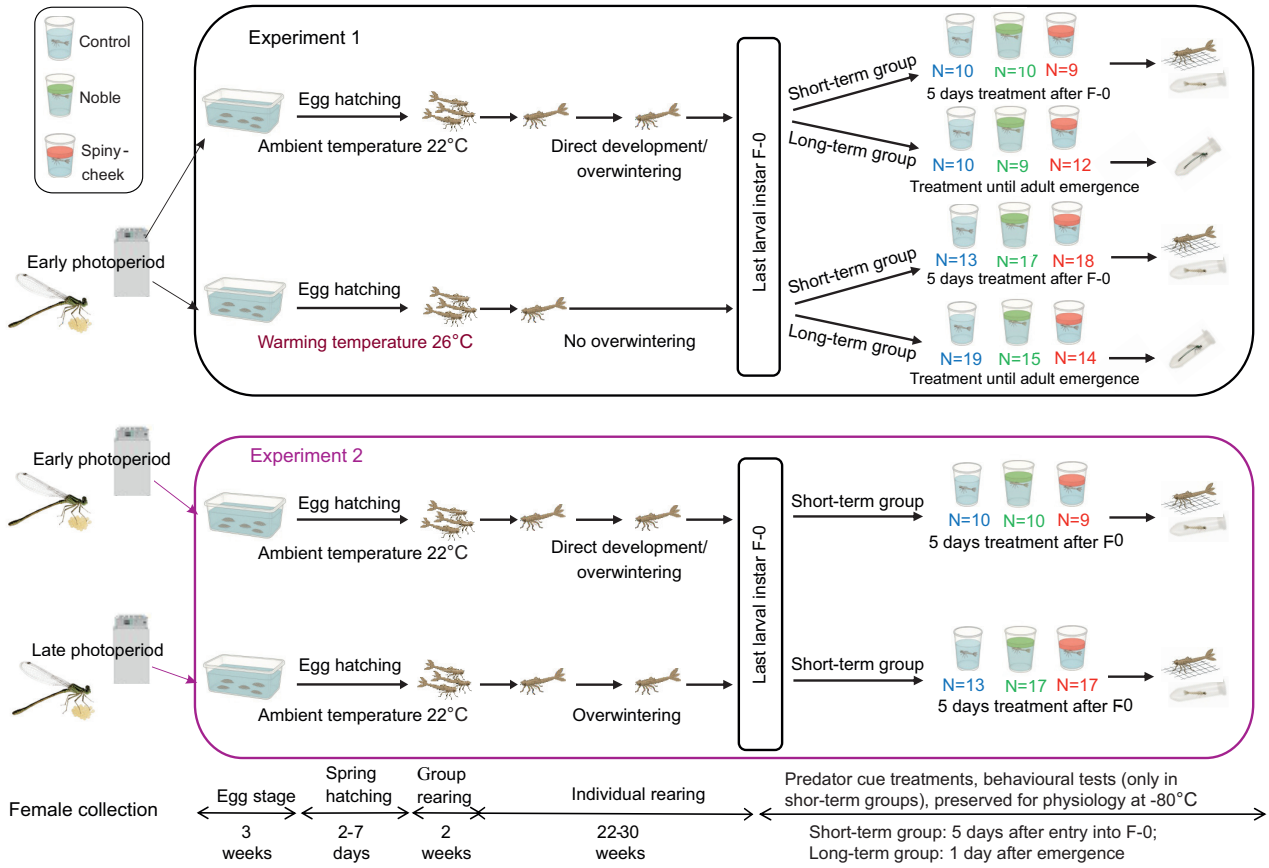


Figure 1. Experimental design testing the effects of predator cue treatments (control, native noble crayfish, and invasive spinycheek crayfish) combined with an environmental stressor in the damselfly *I. elegans*. Egg clutches were subdivided to allow parallel testing of environmental stressors. The study consisted of two experiments, with the early photoperiod, ambient temperature (22 °C) group shared between experiments. Experiment 1 tested the effects of temperature (ambient 22 °C and warming 26 °C) crossed with predator cue treatments. After larvae entered the F-0 instar, individuals were divided into two groups: a short-term exposure group exposed to predator cues for 5 days and a long-term exposure group exposed to predator cues until adult emergence. Experiment 2 examined the effects of photoperiod (early and late), with all individuals experiencing short-term larval exposure to predator cues. Behavioral assays were conducted 2 days after entry into the F-0 instar in the short-term exposure group only in both experiments. Following the exposure period, individuals from the short-term group were sampled after 5 days, and individuals from the long-term exposure group were sampled 1 day after emergence; all individuals were stored at –80 °C for physiological analyses. At 22 °C, some individuals under the early photoperiod reached the respective target stage before wintering, whereas the remaining individuals underwent wintering; all individuals under the late photoperiod underwent wintering. Numbers below predator cue treatments indicate the final number of surviving individuals in each treatment group. For trait-specific sample sizes per treatment group and sex, see Suppl. material 1: table S4.

individuals reared at 22 °C, 22 individuals (9 from the short-term and 13 from the long-term group) entered F-0 after the ambient temperature had declined below 22 °C, experiencing temperatures ranging from 11.1 to 21.4 °C at entry into F-0. This temperature deviation from 22 °C was accounted for in the statistical analyses.

Experiment 2

Two photoperiod groups (early and late) were crossed with three predator cue treatments (control, native noble crayfish, and IA spinycheek crayfish), with all groups reared at ambient temperature (22 °C) (Fig. 1). The early photoperiod group was used in both Experiments 1 and 2. Early and late photoperiod groups were started with short (spring) and long (summer) photoperiods, respectively

(Suppl. material 1: fig. S1). After hatching, larvae were transferred into group cups (10 larvae/cup) for 2 weeks and then into individual cups under the corresponding conditions. All individuals in the late photoperiod group underwent wintering, whereas in the early photoperiod group, only those that had not reached the respective target stage before the onset of wintering did so (Suppl. material 1: table S4). As described for Experiment 1, some directly developing individuals in the early photoperiod group experienced temperatures below 22 °C, and this temperature deviation was accounted for in the statistical analyses. All groups were reared in dechlorinated tap water until entering the F-0 instar. At entry into F-0 (day 0), larvae from both groups were divided into three predator treatment groups (control, native noble, and IA spinycheek crayfish cues) for 5 days of short-term exposure. Behavior was measured on day 2 after entry into F-0, and larvae were collected after 5 days for physiological analyses.

In Experiment 2, only larval responses from the short-term exposure group were analyzed. The long-term exposure group was initially planned, mirroring the design of Experiment 1; however, larvae assigned to the long-term exposure group under warming conditions (26 °C) experienced complete mortality before emergence. Although 26 °C lies well within the thermal tolerance of *I. elegans* (Raczyński et al. 2022a; Palomar et al. 2023), combined warming, late photoperiod, and prolonged predator cue exposure may have constrained larval development. To maintain a balanced and comparable design, the corresponding long-term exposure group at ambient temperature (22 °C) was therefore also excluded from further analyses. Thus, Experiment 2 focuses on larval responses to photoperiod and short-term exposure to predator cues.

For all groups, weekly seasonal changes in photoperiod (including civil twilight) at the damselfly sampling latitude were followed. Details of the applied temperature and photoperiod regimes are available in Suppl. material 1: fig. S1, tables S1–S3.

Larvae differed in developmental trajectory and physiological state (regulatory vs. direct/preemergence development; Norling 2021), particularly between individuals undergoing wintering and those developing directly. Therefore, measurements were synchronized to ensure comparisons between physiologically comparable larvae (see Suppl. material 1 for an expanded description of developmental trajectories and synchronization of larval measurements).

For all groups before F-0, water was refilled every other day to keep the water levels approximately constant. The larvae were fed twice a day (morning and afternoon) during weekdays and once a day during weekends with laboratory-cultured *Artemia* sp. nauplii. For the first 14 days, larvae kept in groups (10 larvae/cup) received 1 mL of *Artemia* solution per container, containing, on average, 198.5 nauplii/mL (SD = 12.4; N = 38 samples counted). This initial group-rearing period represented a relatively small proportion of the total development time, with 62–90% of development from hatching to F-0 occurring after larvae were transferred to individual cups. After individualization, the same ration was administered per individual cup. During the simulated winter, when larvae were in a diapause state (a hormonally regulated state of arrested development accompanied by markedly reduced metabolic activity), individuals were fed once a day throughout the week. This food ration was sufficient for diapausing individuals, as previously shown in *Ischnura* sp. (Palomar et al. 2023; Amer et al. 2024). Directly developing larvae were fed twice per day on weekdays and once per day during weekends. At entry into F-0, all larvae in the short-term exposure

group received 1.5 mL of *A. salina* once per day, except on the day of behavior testing (day 2), when each larva received three chironomid larvae after the test. Larvae in the long-term exposure group received 1.5 mL of *A. salina* once per day plus three chironomid larvae three times per week (Monday, Wednesday, and Friday). During short-term predator exposure, one-third of the water was removed and immediately replaced with the corresponding fresh predator cue or control water on days 0, 2 (after behavior trials), and 4. Similarly, during long-term predator exposure, one-third of the water was removed and then replaced with the corresponding fresh predator cue or control water every Monday, Wednesday, and Friday until adult emergence. This method of exchanging a fixed proportion of water ensured that the nominal concentration of predator cues (i.e., the ratio of cue water to neutral water) remained consistent across treatments over the duration of the experiment.

Response variables

Life-history traits

Survival was measured as the number of larvae that survived until entry into the F-0 instar, after the 5-day predator treatment (short-term exposure group), and until 1 day after adult emergence (hereafter, survival until emergence; long-term exposure group). Development time was measured as the number of days between hatching and entry into the F-0 instar and between hatching and adult emergence. For individuals that underwent wintering, corrected development time was calculated by subtracting the 42-day wintering period from development time. No correction was applied to individuals that reached the respective target stage without wintering. Individuals were therefore distinguished according to whether they experienced wintering. Temperature deviation from the constant ambient temperature of 22 °C was also considered to account for the seasonal temperature decline experienced by some directly developing individuals.

Wet mass was measured at entry into F-0 (mass at F-0), after 5 days of predator treatment in the short-term exposure group (mass after 5 days), and 1 day after emergence in the long-term exposure group. Animals were weighed to the nearest 0.1 mg using an electronic balance (Radwag® AS.62.R2 Plus).

First, the pre-treatment growth rate ($Growth_{to\ F\text{-}0}$), from hatching until entry into the final larval instar (F-0), was calculated by dividing body mass at entry into F-0 by the corrected development time between hatching and entry into F-0:

$Growth_{to\ F\text{-}0}$ = Mass at entry into F-0 / Corrected development time from hatching to F-0.

Second, the growth rate during the 5-day larval treatment ($Growth_{5\text{-}days}$) was calculated as the change in body mass over the exposure period divided by its duration:

$Growth_{5\text{-}days}$ = (Mass after short-term predator treatment – Mass at entry into F-0) / 5 days.

Third, the growth rate from entry into F-0 until adult emergence ($Growth_{F\text{-}0}$) was calculated as the change in body mass between entry into F-0 and 1 day after adult emergence, divided by the corrected development time from entry into F-0 to emergence:

$Growth_{F\text{-}0}$ = (Mass after long-term predator treatment – Mass at entry into F-0) / Corrected development time from entry into F-0 to emergence.

Behavioral traits

Behavioral observations were performed between 10:00 and 12:00 local time. Two behavioral traits were measured: freezing time (hereafter, boldness) and feeding rate. To assess boldness, each larva was gently touched on the thorax using a pencil, which triggered a rapid escape followed by a period of immobility (freezing), following Brodin (2009). Larvae with shorter freezing times were considered bolder. Freezing is a primary antipredator defense in aquatic insects, functioning to minimize detection by visual, motion-sensitive predators. Consequently, a quicker resumption of movement represents a greater tolerance for risk and prioritization of active behaviors (such as foraging) over predator avoidance. Subsequently, 30 *A. salina* nauplii were introduced into each container as food for 15 min to assess the larval feeding rate. After 15 min, larvae were removed from the experimental containers and returned to their original rearing containers in the climatic chamber, while the remaining *A. salina* nauplii in the containers were counted using a hand magnifier.

Physiological traits

To evaluate immune investment, phenoloxidase (PO) activity, an essential enzyme in insect immune defense against bacterial, fungal, and viral infections, was measured (Stoks and Córdoba-Aguilar 2012). The PO assay followed the methodology of Stoks et al. (2006). In brief, 10 µL of tissue homogenate was combined with 105 µL of phosphate-buffered saline (PBS) and 5 µL of chymotrypsin in a 384-well microtiter plate and incubated for 5 min. Subsequently, L-DOPA (1.966 mg/mL in PBS) was added, and absorbance at 490 nm was recorded every 20 s over a 30-min period at 30 °C. The average of two technical replicates per sample was used for statistical analysis. PO activity was quantified as the rate of dopachrome (nmol) formation per minute.

To determine fat content in damselfly larvae and adults, a modified version of the Marsh and Weinstein protocol (Marsh and Weinstein 1966), as adapted by Verheyen et al. (2018), was used. For this assay, 8 µL of the sample supernatant was mixed with 56 µL of concentrated sulfuric acid (100%) in small glass tubes and heated at 150 °C for 20 min. After cooling, 64 µL of Milli-Q water was added. The mixture was then transferred to a 384-well microtiter plate (30 µL per well, in triplicate), and absorbance was measured at 490 nm. The mean of the three replicates was used in the statistical analyses.

Statistical analyses

All statistical analyses were conducted in R v. 4.5.1 4.5.1 (R Core Team 2025) using RStudio (Posit team 2026).

For Experiment 1, full models initially included predator cue treatment (control, noble crayfish, and spinycheek crayfish), temperature (22 °C and 26 °C), sex, wintering, and their interactions where applicable. For Experiment 2, corresponding models included predator cue treatment, photoperiod (early and late), sex, wintering, and their interactions. Predator cue treatment was omitted for responses measured before predator exposure, and sex was excluded from survival to F-0 because individuals that died before reaching F-0 could not be sexed. Short-term and long-term exposure groups were analyzed separately because responses were measured at different developmental stages.

As the full models for both experiments included multiple predictors and interactions, model selection was performed using the MuMIn package (Bartoń 2023) to identify the best-supported model for each response variable. Model selection was based on the corrected Akaike Information Criterion (AICc), and the selected models were subsequently used for statistical inference (Tables 1, 2). Candidate models with ΔAICc < 2 are reported in Suppl. material 1: table S5. Type III tests were used for models retaining interactions, whereas Type II tests were used for additive models. *F*-tests were used for Gaussian linear models and likelihood-ratio χ^2 tests for generalized

Table 1. Effects of predator cues (control, native noble crayfish, and spinycheek crayfish), temperature (ambient and warming), sex, and wintering on life-history traits (survival, $Growth_{5\text{-days}}$, and $Growth_{F\text{-}0}$), physiology (PO activity and fat content), and behavior (boldness and feeding rate) during short-term 5-day exposure at the final larval instar (F-0) and long-term exposure until adult emergence in Experiment 1. Behavioral variables were quantified in the short-term exposure group only. Type II tests were used for models containing additive effects only, whereas Type III tests were used for models retaining interactions. Significant *P*-values are in bold: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Response variables		**Short-term exposure**			**Long-term exposure**	
Survival	**Df**	**χ^2**	***P*-value**	**Df**	**χ^2**	***P*-value**
Predator cue	2	1.857	0.395	2	11.535	**0.003****
Temperature	1	0.049	0.824	1	2.413	0.120
Sex	1	0.938	0.333	1	0.968	0.325
Wintering	1	0.412	0.522	1	0.284	0.594
Predator cue × sex				2	5.975	0.050
$Growth_{5\text{-days}}$/$Growth_{F\text{-}0}$	**Df**	***F***	***P*-value**	**Df**	***F***	***P*-value**
Predator cue	2, 63	3.0665	0.054	2, 49	1.992	0.147
Temperature	1, 63	0.0129	0.910	1, 49	3.934	0.053
Sex	1, 63	0.1490	0.701	1, 49	6.303	**0.015***
Wintering	1, 63	0.1881	0.666	1, 49	5.734	**0.021***
Predator cue × temperature	2, 63	2.844	0.065	2, 49	4.242	**0.020***
Temperature × sex				1, 49	8.946	**0.004****
PO activity						
Predator cue	2, 63	3.1586	**0.049***	2, 50	0.879	0.422
Temperature	1, 63	0.524	0.472	1, 50	0.309	0.581
Sex	1, 63	2.601	0.112	1, 50	0.514	0.477
Wintering	1, 63	2.469	0.121	1, 50	0.304	0.584
Predator cue × temperature	2, 63	4.547	**0.014***			
Fat content						
Predator cue	2, 65	0.417	0.661	2, 48	5.277	**0.008****
Temperature	1, 65	3.173	0.080	1, 48	1.513	0.225
Sex	1, 65	2.150	0.147	1, 48	3.176	0.081
Wintering	1, 65	2.544	0.116	1, 48	12.530	**0.001*****
Predator cue × temperature				2, 48	2.914	0.064
Boldness	**Df**	**χ^2**	***P*-value**	**Df**	**χ^2**	***P*-value**
Predator cue	2	7.382	**0.025***		-	-
Temperature	1	0.070	0.792		-	-
Sex	1	0.196	0.658		-	-
Wintering	1	9.563	**0.002****		-	-
Predator cue × temperature	2	10.367	**0.006****		-	-
Feeding rate	**Df**	**χ^2**	***P*-value**	**Df**	**χ^2**	***P*-value**
Predator cue	2	1.408	0.495		-	-
Temperature	1	9.823	**0.002****		-	-
Sex	1	8.433	**0.004****		-	-
Wintering	1	0.305	0.581		-	-
Temperature × sex	2	5.733	**0.017***		-	-

Table 2. Effects of predator cues (control, native noble crayfish, and spinycheek crayfish), photoperiod (early and late), sex, and wintering on life-history traits (survival and $Growth_{5\text{-days}}$), physiology (PO activity and fat content), and behavior (boldness and feeding rate) during short-term predator exposure at the final larval instar (F-0) in Experiment 2. Type II tests were used for additive models, whereas Type III tests were used for models retaining interactions. Significant *P*-values are shown in bold: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Response variables	**df**	χ^2	***P*-value**
Survival			
Predator cue	2	1.694	0.429
Photoperiod	1	2.373	0.124
Sex	1	0.500	0.480
Wintering	1	0.399	0.528
$Growth_{5\text{-days}}$	**df**	***F***	***P*-value**
Predator cue	2, 57	1.160	0.321
Photoperiod	1, 57	0.055	0.816
Sex	1, 57	0.369	0.546
Wintering	1, 57	0.118	0.733
Predator cue × photoperiod	2, 57	3.365	**0.047***
PO activity			
Predator cue	2, 59	2.370	0.102
Photoperiod	1, 59	0.004	0.948
Sex	1, 59	3.245	0.077
Wintering	1, 59	2.483	0.120
Fat content			
Predator cue	2, 59	0.761	0.927
Photoperiod	1, 59	0.006	0.941
Sex	1, 59	0.016	0.900
Wintering	1, 59	3.303	0.074
Boldness	**df**	χ^2	***P*-value**
Predator cue	2	19.359	**<0.001*****
Photoperiod	1	1.720	0.190
Sex	1	0.048	0.826
Wintering	1	13.893	**<0.001*****
Predator cue × photoperiod	2	18.510	**<0.001*****
Predator cue × sex	2	10.290	**0.006****
Feeding rate	**df**	χ^2	***P*-value**
Predator cue	2	3.047	0.218
Photoperiod	1	6.608	**0.010***
Sex	1	3.527	0.060
Wintering	1	0.595	0.440
Predator cue × photoperiod	2	6.141	**0.046***
Photoperiod × sex	1	3.902	**0.048***

linear models. The significance of fixed effects was assessed using the *Anova()* function in the car package (Fox and Weisberg 2011). When significant main effects or interactions were detected, estimated marginal means (EMMs) and Tukey-adjusted pairwise comparisons were obtained using the emmeans package (Lenth et al. 2025).

Survival to F-0, survival 5 days after entry into F-0, and survival to emergence were analyzed using generalized linear models with a binomial error distribution and logit link. Growth rate, body mass, PO activity, and fat content were analyzed using Gaussian linear models after checking residual normality. Boldness and feeding rate were analyzed using count-based generalized linear models. Poisson models were initially fitted and

checked for overdispersion; when overdispersion was detected, negative binomial models were used instead. Model assumptions were checked visually using residual diagnostics.

Because individuals in the ambient-temperature, early-photoperiod treatment differed in developmental pathway, additional analyses tested whether wintering status was associated with $Growth_{to\ F\text{-}0}$ and with traits measured following short-term and long-term exposure. To assess the potential effect of the seasonal decline in ambient temperature, the association between temperature at entry into F-0 and $Growth_{to\ F\text{-}0}$ was also tested in non-wintering individuals that entered F-0 after the temperature had declined below 22 °C. Short-term and long-term groups were pooled for this analysis (N = 22). Temperature deviation from ambient conditions was not tested for other traits because separating individuals into short-term and long-term exposure groups resulted in insufficient sample sizes.

Results

Wintering status and $Growth_{to\ F\text{-}0}$

Among individuals reared under ambient temperature and early photoperiod, $Growth_{to\ F\text{-}0}$ was substantially lower in individuals that subsequently experienced wintering than in those that reached F-0 before wintering ($F_{1,57}$ = 261.58, P < 0.001; N = 60; Suppl. material 1: fig. S3).

Wintering effects on traits after short-term and long-term exposure

The analyses restricted to individuals reared under ambient temperature and early photoperiod showed no significant effect of wintering status on traits measured after short-term exposure (all $P \geq 0.143$). In contrast, after long-term exposure, individuals that had previously undergone wintering had lower $Growth_{F\text{-}0}$ ($F_{1,22}$ = 8.32, P = 0.009) but higher fat content ($F_{1,22}$ = 12.94, P = 0.002), while PO activity was unaffected (P = 0.730).

Effect of temperature deviation on $Growth_{to\ F\text{-}0}$

Among non-wintering individuals from the ambient-temperature and early-photoperiod treatment, pooled across the short- and long-term groups, $Growth_{to\ F\text{-}0}$ was positively associated with temperature at entry into F-0 ($F_{1,20}$ = 42.94, P < 0.001; N = 22; Suppl. material 1: fig. S4).

Experiment 1: Predator cues × temperature

Effects of temperature until larval entry into F-0

Survival to F-0 was significantly higher under warming than under ambient temperature (46.2% vs. 28.6%; LR χ^2_1 = 13.90, P < 0.001; N = 418).

$Growth_{to\ F\text{-}0}$ was significantly affected by temperature ($F_{1,150}$ = 10.93, P = 0.001; N = 154), with larvae developing faster under warming. $Growth_{to\ F\text{-}0}$ was also lower in males than females ($F_{1,150}$ = 5.32, P = 0.022). Wintering had a strong effect, with individuals that underwent wintering showing lower $Growth_{to\ F\text{-}0}$ ($F_{1,150}$ = 124.43, P < 0.001; Suppl. material 1: fig. S5).

Effect of short-term predator cue exposure at F-0

After the short-term exposure, survival (N = 77), $Growth_5$-days (N = 71), and fat content (N = 71) were not significantly affected by predator cues, temperature, sex, or wintering (Table 1). For $Growth_5$-days, the best-supported model retained the predator cue × temperature interaction, although this effect was not significant (P = 0.066).

PO activity was significantly affected by predator cues, with the response depending on temperature (predator × temperature interaction; N = 71; Table 1). PO activity was highest under IA spinycheek crayfish cues at ambient temperature but declined significantly under spinycheek cues with warming (P < 0.001). Temperature had no significant effect under control or native noble crayfish cues (Fig. 2A, Suppl. material 1: table S6).

Boldness was affected by predator cues, with the response depending on temperature (predator × temperature interaction; N = 68; Fig. 2B, Table 1). Under ambient temperature, individuals exposed to IA spinycheek crayfish cues were less bold but became substantially bolder with warming (P < 0.001). Temperature had no significant effect under control or native noble crayfish cues (Suppl. material 1: table S6). Wintering also increased boldness, while sex had no significant effect (Table 1).

Feeding rate was not influenced by predator cues but was affected by a temperature × sex interaction (N = 68; Fig. 2C, Table 1). Warming increased feeding rate in females (P = 0.001) but not in males (P = 0.202; Suppl. material 1: table S6). Wintering had no significant effect.

Effect of long-term predator cue exposure during F-0 and carry-over responses after emergence

Survival to emergence was affected by predator cue, while the predator cue × sex interaction was marginal (P = 0.050; N = 79). Overall survival was higher under native noble than spinycheek crayfish cues, whereas the other pairwise comparisons between predator treatments were not significant. Temperature and wintering had no significant effects (Fig. 3A, Table 1, Suppl. material 1: table S6).

$Growth_{F\text{-}0}$ depended on predator cue × temperature and temperature × sex interactions (N = 58; Fig. 3B, Table 1). Predator treatments did not differ under ambient temperature, whereas under warming, $Growth_{F\text{-}0}$ was higher in the control than under native noble crayfish cues (P = 0.014). Females had higher $Growth_{F\text{-}0}$ than males under ambient temperature (P = 0.015), whereas the sexes did not differ under warming (P = 0.096; Suppl. material 1: table S6). Previously wintering individuals showed lower $Growth_{F\text{-}0}$ (Table 1).

PO activity was not significantly affected by predator cues, temperature, sex, or wintering (N = 56; Table 1).

Fat content was significantly affected by predator cues and wintering, while the best-supported model also retained the predator × temperature interaction, although this interaction was not significant (P = 0.064; N = 56; Fig. 3C, Table 1). Under ambient temperature, fat content was higher under IA spinycheek crayfish cues than under both control (Tukey: P = 0.023) and native noble crayfish cues (P = 0.016), whereas no differences among predator treatments occurred under warming (Suppl. material 1: table S6). Fat content was also higher in individuals that had experienced wintering (P < 0.001).

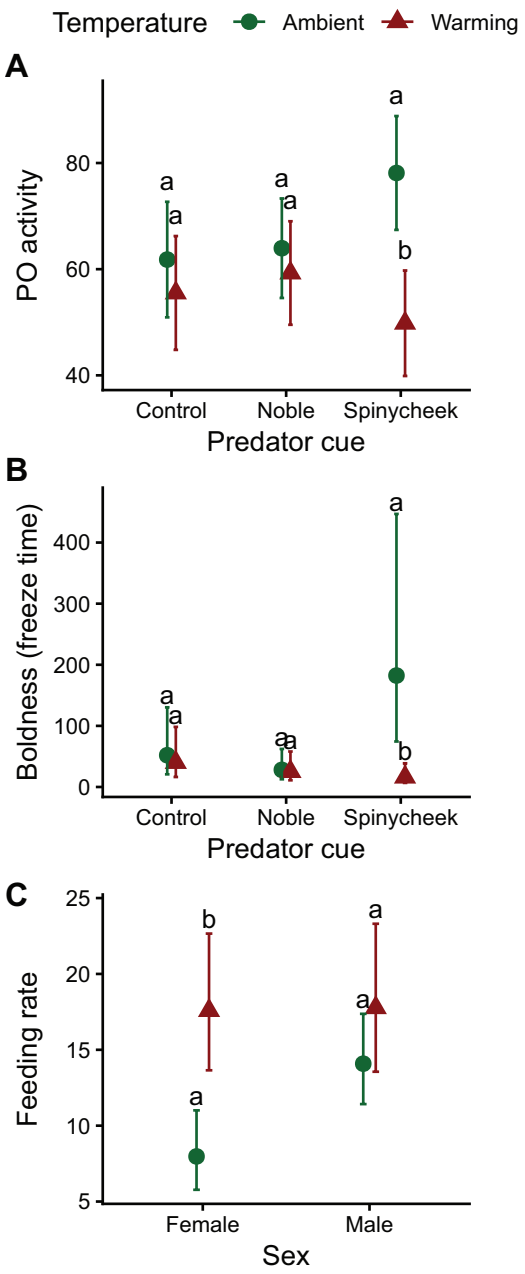


Figure 2. Experiment 1, short-term predator exposure. Effects of predator cues (control, noble, and spinycheek), temperature (ambient and warming), and sex on phenoloxidase (PO) activity (**A**), boldness (**B**), and feeding rate (**C**) during the short-term larval exposure period in *I. elegans*. Error bars show 95% CIs. Different letters indicate significant differences between temperatures within each predator cue treatment in (**A, B**) and between temperatures within each sex in (**C**) ($P < 0.05$). Analytical sample sizes were $N = 71$ (**A**), $N = 68$ (**B**), and $N = 68$ (**C**); treatment-specific sample sizes are provided in Suppl. material 1: table S4.

Experiment 2: predator cues × photoperiod

Effect of photoperiod until larval entry into F-0 (before predator cue exposure)

Survival to F-0 was significantly affected by photoperiod ($\chi^2 = 11.33$, df = 1, $P < 0.001$), with lower survival under the late (16.0%; $N = 293$) than under the early photoperiod (28.6%; $N = 210$).

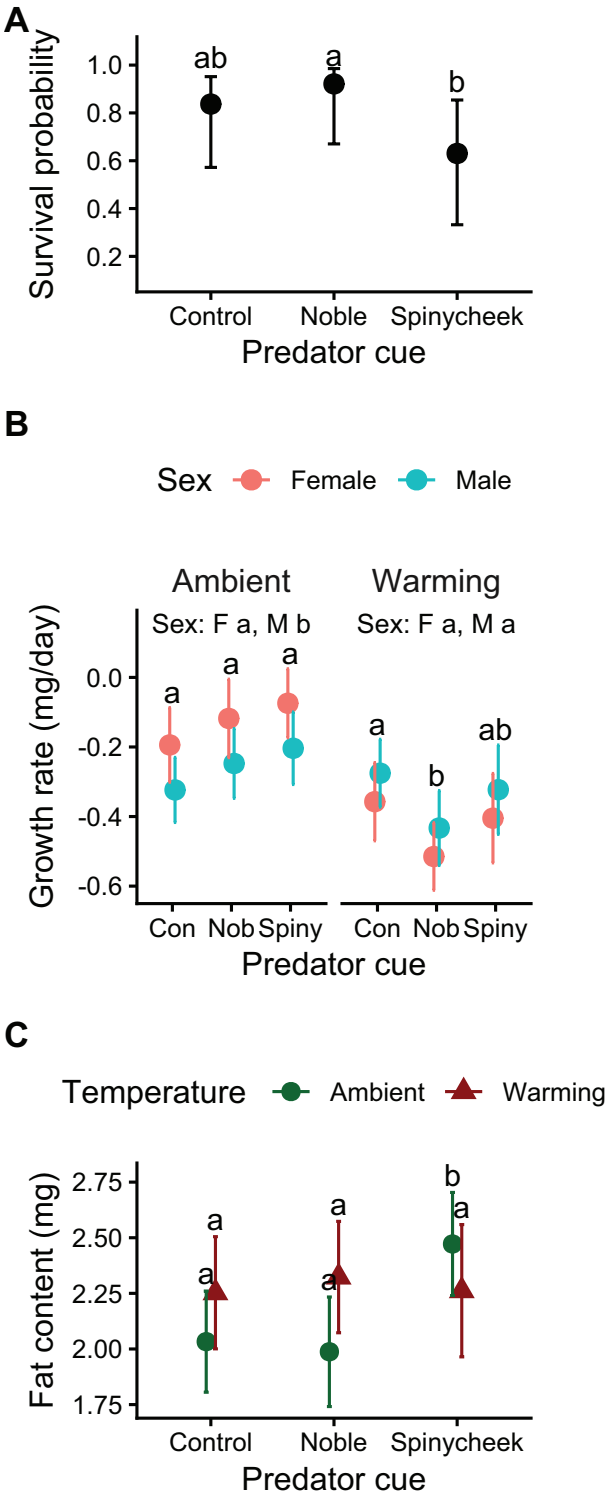


Figure 3. Experiment 1, long-term predator exposure. Effects of predator cues (control, noble, and spinycheek crayfish), temperature (ambient and warming), and sex on survival (**A**), $Growth_{F-0}$ (**B**), and fat content (**C**) 1 day after emergence of *I. elegans*. Error bars show 95% CIs. Different letters indicate significant differences ($P < 0.05$). **A.** Letters indicate differences among predator cue treatments; **B.** Letters above means indicate differences among predator cue treatments within each temperature, whereas letters following F and M indicate differences between sexes within each temperature; **C.** Letters indicate differences among predator cue treatments within each temperature. Analytical sample sizes were $N = 79$ (**A**), $N = 58$ (**B**), and $N = 56$ (**C**); treatment-specific sample sizes are provided in Suppl. material 1: table S4.

Growth$_{\text{to F-0}}$ was not significantly affected by photoperiod ($F_{1,102}$ = 0.03, P = 0.862) or sex ($F_{1,102}$ = 2.33, P = 0.130) but was strongly affected by wintering ($F_{1,102}$ = 449.76, P < 0.001; N = 106), with lower growth rates in individuals that wintered.

Effect of short-term exposure to predator cues

Survival (N = 75), fat content (N = 65), and PO activity (N = 65) were not significantly affected by predator cues, photoperiod, sex, or wintering (Table 2).

Growth$_{\text{5-days}}$ (N = 65) was affected by a significant predator cue × photoperiod interaction (Fig. 4A, Table 2). Growth$_{\text{5-days}}$ did not differ significantly among predator cue treatments within either photoperiod. However, within the noble crayfish treatment, Growth$_{\text{5-days}}$ was lower under the late than under the early photoperiod (P = 0.019), whereas photoperiod had no significant effect in the control or spinycheek crayfish treatments (Suppl. material 1: table S6).

Boldness was affected by both predator cue × photoperiod and predator cue × sex interactions (N = 67; Fig. 4B, C, Table 2). Under the early photoperiod, larvae exposed to IA spinycheek crayfish cues were less bold than those ex-

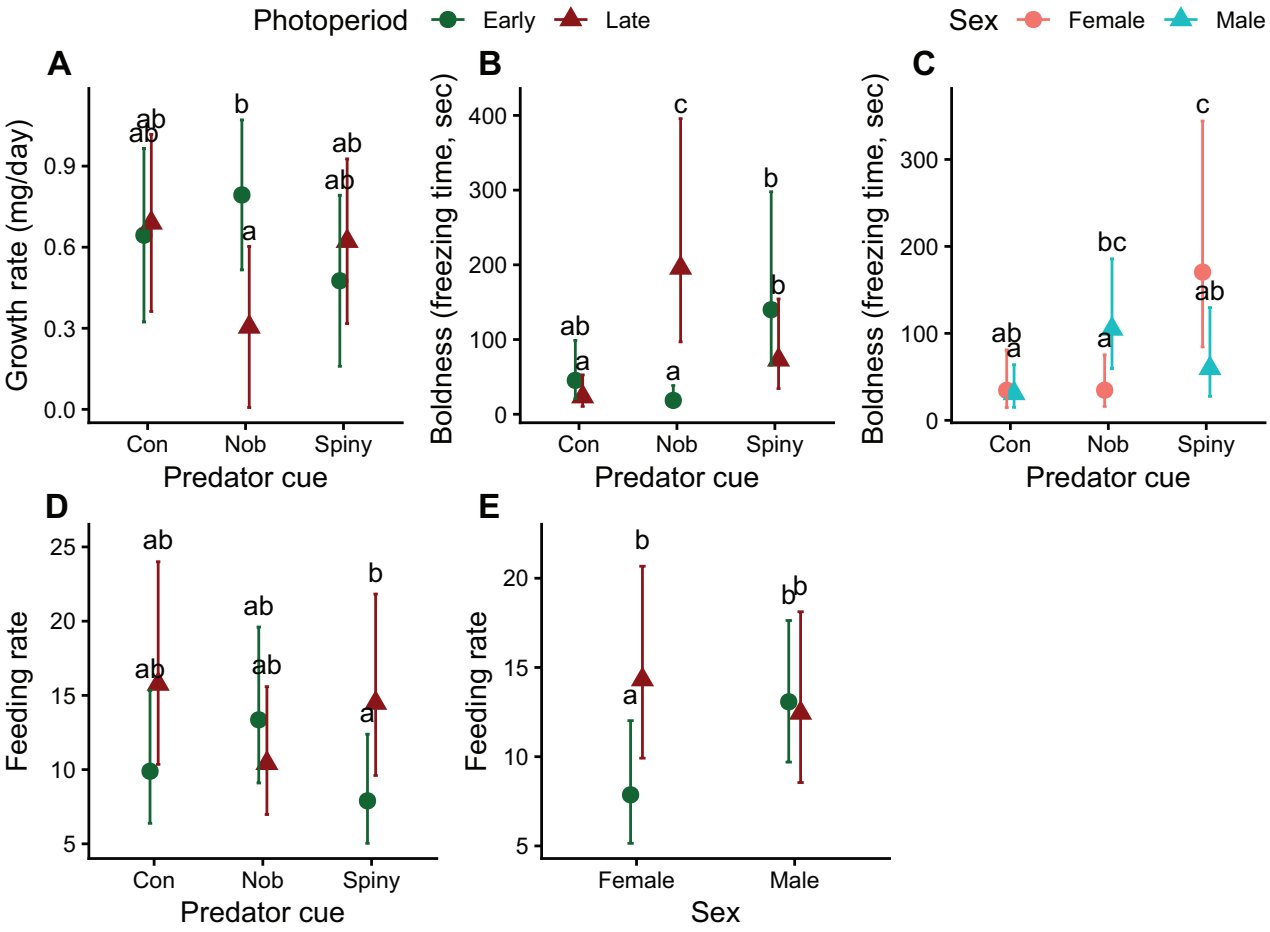


Figure 4. Experiment 2, short-term predator exposure. Effects of predator cues (control, noble, and spinycheek crayfish), photoperiod (early and late), and sex (female and male) on Growth$_{\text{5-days}}$ (**A**), boldness (**B, C**), and feeding rate (**D, E**) during short-term predator exposure of final-instar larvae (F-0) in *I. elegans*. Panels show predator cue × photoperiod effects on Growth$_{\text{5-days}}$ (**A**), boldness (**B**), and feeding rate (**D**), predator cue × sex effects on boldness (**C**), and photoperiod × sex effects on feeding rate (**E**). Error bars show 95% CIs. Different letters indicate significant differences (P < 0.05). In each panel, letters combine pairwise comparisons of each factor within levels of the interacting factor. Analytical sample sizes were N = 65 (**A**), N = 67 (**B, C**), and N = 67 (**D, E**); treatment-specific sample sizes are provided in Suppl. material 1: table S4.

posed to noble crayfish cues ($P < 0.001$), whereas under the late photoperiod, larvae exposed to noble crayfish cues were less bold than controls ($P < 0.001$) and those exposed to spinycheek crayfish cues ($P = 0.037$). Predator cue effects also differed between sexes: females exposed to spinycheek crayfish cues were less bold than females in the control and noble crayfish treatments (both $P = 0.002$), whereas males exposed to noble crayfish cues were less bold than control males ($P = 0.014$; Suppl. material 1: table S6). Wintering was associated with increased boldness.

Feeding rate was affected by predator cue × photoperiod and photoperiod × sex interactions ($N = 67$; Fig. 4D, E, Table 2). Feeding rate was higher under the late than under the early photoperiod in the IA spinycheek crayfish treatment ($P = 0.027$) but did not differ between photoperiods in the control or noble crayfish treatments. Feeding rate was also higher under the late than under the early photoperiod in females ($P = 0.009$), whereas no photoperiod difference occurred in males; under the early photoperiod, feeding rate was lower in females than in males ($P = 0.049$; Suppl. material 1: table S6).

Discussion

The results demonstrate that responses to native and invasive alien (IA) crayfish cues were strongly context-dependent. Across the two experiments, warming and seasonal time constraints modified predator-specific responses, while sex and wintering history further influenced several traits. Overall, these patterns broadly support predictions of the naïve prey hypothesis (NPH), indicating that predator novelty alone does not determine prey responses. Instead, abiotic context strongly influenced how prey responded to native and IA predators. Patterns also differed between the short-term and long-term exposure groups, with predator effects on survival and growth more evident following long-term exposure, whereas short-term exposure revealed clearer effects on immune function and behavior. However, these differences cannot be attributed solely to exposure duration because short-term responses were measured in F-0 larvae, whereas responses following long-term exposure were measured after emergence.

Developmental pathways and seasonal temperature variation

Developmental history contributed to phenotypic variation. Individuals that subsequently underwent wintering had substantially lower growth rates to F-0 than those that reached F-0 before wintering, indicating different developmental trajectories already before wintering. A similar difference was previously found in *I. elegans*, with faster growth in non-wintering than wintering larvae (Wos et al. 2024b). Previous wintering also affected some later traits, particularly growth rate and fat content following long-term exposure. Among directly developing individuals exposed to the seasonal decline in ambient temperature, growth rate to F-0 increased with temperature at entry into F-0. These results are consistent with the general framework of seasonal regulation in temperate Odonata, in which growth and thermal responses differ among developmental pathways leading either toward overwintering or emergence (Norling 2021), and highlight the need to account for developmental history when interpreting treatment effects.

Predator identity matters: abiotic context shapes responses to native and invasive alien predators (Experiments 1 and 2)

The NPH predicts that limited evolutionary experience with invasive predators can alter prey recognition and the expression of antipredator responses (Sih et al. 2010; Carthey and Banks 2014). Consistent with this framework, cues from the native noble crayfish and IA spinycheek crayfish elicited different responses across behavioral, physiological, and life-history traits. However, these responses were not consistently stronger or weaker toward the IA predator and depended strongly on abiotic context. In Experiment 1, predator-specific differentiation in PO activity and boldness following short-term exposure and in fat content following long-term exposure was most evident under ambient temperature, whereas warming weakened these patterns. Following long-term exposure, warming resulted in a lower growth rate under native noble crayfish cues than in the control, with the IA spinycheek treatment intermediate. In Experiment 2, photoperiod modified predator-specific responses in growth rate, boldness, and feeding rate, with the pattern of these responses differing between early and late photoperiods. Thus, the prediction that warming and late photoperiod would reduce differentiation between responses to native and IA predators was only partially supported. Instead, abiotic conditions modified both the magnitude and direction of predator-specific responses.

These patterns suggest that prey distinguished between native and IA predators but expressed predator-specific responses in a context-dependent manner rather than showing uniformly stronger or weaker responses to the IA predator. Within the NPH framework, the responses to IA predator cues argue against Level 1 naïveté, in which prey fail to recognize a novel predator and initiate antipredator responses (Carthey and Banks 2014). However, distinguishing between higher levels of naïveté requires determining whether the elicited responses are appropriate and effective, which was not directly tested here.

These context-dependent patterns may partly reflect shifts in energetic and developmental priorities. Warming increases metabolic demands, whereas seasonal time constraints can alter developmental trajectories and resource allocation, potentially changing the resources available for antipredator responses (Smolinský and Gvoždík 2012; Stoks et al. 2012; Wang et al. 2021; Sniegula et al. 2024). Rather than uniformly constraining predator-specific plasticity, however, the results suggest that these abiotic conditions can reorganize how investment is distributed among growth, behavior, and physiology. Such context dependence is in agreement with the broader view that responses to multiple stressors depend on interactions among environmental conditions and trait-specific energetic demands (Carthey and Banks 2014; Lopez et al. 2023; Wos et al. 2024a, 2025).

Although previous exposure to one non-native crayfish species could theoretically facilitate recognition of another through cross-recognition, such responses may also depend on phylogenetic relatedness among crayfish species (Sniegula et al. 2025). However, the Danube crayfish had not been recorded at the study site for approximately 7 years before damselfly collection. Previous exposure is therefore unlikely to have substantially influenced the predator-specific responses observed here, although future studies should test whether exposure to one invasive crayfish facilitates recognition of subsequently encountered invaders.

Warming modified predator-induced responses (Experiment 1)

Before predator cue exposure, warming itself affected larval performance, with higher survival to F-0 under warming than under ambient temperature. Larvae also showed higher growth rates under warming. Thus, warming was associated with higher survival and growth during development to F-0, while subsequently modifying predator-induced responses in a trait-specific manner.

Following short-term exposure, the clearest physiological interaction occurred for PO activity. Under ambient temperature, IA spinycheek crayfish cues elicited the highest PO activity, whereas this predator-specific response disappeared under warming. Exposure to a fish predator has previously been shown to reduce PO activity in damselflies (Stoks et al. 2006). In the fall armyworm (*Spodoptera frugiperda*), predator stress increased PO activity in non-infected larvae but reduced it when combined with bacterial challenge (Sun and Bai 2020), illustrating the context dependence of predator-induced immune responses, as recently synthesized by Khan and Rolff (2025). The results extend these findings by showing that warming can weaken predator-specific PO responses to the IA predator, possibly because elevated energetic demands modify investment in immune responses.

Behavioral responses were similarly context-dependent. Boldness depended on predator identity and temperature, with predator-specific differences under ambient temperature becoming weaker under warming. In contrast, feeding rate was not affected by predator cues but depended on a temperature × sex interaction, with warming increasing feeding rate in females but not in males. Increased feeding under warming is consistent with greater energetic demands at elevated temperatures (Englund et al. 2011; Culler et al. 2014; Debecker and Stoks 2019; Sniegula et al. 2019b). The female-specific increase may reflect sex differences in energetic requirements and resource allocation, with females requiring greater resource acquisition to support growth and subsequent reproduction. Together, these patterns indicate that warming altered both predator-specific behavior and resource acquisition rather than uniformly increasing or decreasing antipredator responses.

Warming also modified growth responses following long-term predator exposure. Growth rate depended on the interaction between predator identity and temperature. Under warming, growth was lower under native noble crayfish cues than in the control, while the IA spinycheek treatment was intermediate. This is broadly consistent with work in the damselfly *Enallagma cyathigerum*, where warming reinforced non-consumptive predator effects on growth, metabolism, and physiological allocation (Janssens et al. 2015). In the present study, however, warming did not uniformly strengthen predator effects: it weakened predator-specific differentiation in short-term PO activity and boldness while modifying rather than consistently intensifying longer-term growth responses. Generally, temperature can alter predator-prey interaction strength and the expression of predator-induced responses (Englund et al. 2011; Sentis et al. 2012; Wang et al. 2021). Thus, warming appears to reshape predator-induced plasticity in a trait-specific manner rather than simply amplifying the effects of predation risk.

Photoperiod modifies predator-induced responses (Experiment 2)

Before predator cue exposure, survival was lower under the late than under the early photoperiod, whereas growth rate to F-0 was unaffected by photoperiod. Photoperiod subsequently modified predator-specific responses in growth and behavior.

Growth rate declined from the early to the late photoperiod under native noble crayfish cues. Boldness showed stronger predator-specific differentiation: under the early photoperiod, larvae exposed to IA spinycheek cues were less bold than those exposed to native noble crayfish cues, whereas under the late photoperiod, the pattern reversed. Predator-specific boldness responses also differed between sexes, with females showing reduced boldness under spinycheek cues and males under noble crayfish cues. Thus, contrary to the prediction, the late photoperiod did not consistently reduce differentiation between native and IA predator responses but changed the pattern of predator-specific responses. This context dependence further indicates that responses to native and IA predators are not determined by predator novelty alone, consistent with the broader NPH framework (Sih et al. 2010).

Feeding responses further demonstrate this context dependence. Feeding rate increased from the early to the late photoperiod only under IA spinycheek cues. This response also differed between sexes: feeding increased under the late photoperiod in females but not in males, and the sex difference observed under the early photoperiod disappeared under the late photoperiod. Such sex-specific plasticity may reflect differences in life history and energetic allocation between males and females (Stillwell et al. 2010). As photoperiod changes across the growing season, endocrine regulation can initiate physiological and developmental adjustments associated with seasonal transitions (Bradshaw and Holzapfel 2007; Miki et al. 2020; Norling 2021). Such changes may alter energetic and developmental priorities and thereby modify investment in antipredator responses and resource acquisition. This extends previous work showing that responses to native and invasive predator cues depend on seasonal conditions (Sniegula et al. 2019b, 2025) and supports the broader view that phenotypic responses emerge from interactions between environmental cues rather than from individual stressors acting independently (Moczek 2010; Wolf and Weissing 2012).

Responses following short- and long-term predator cue exposure (Experiment 1)

Stronger effects following long-term than short-term predator cue exposure and carry-over effects expressed after metamorphosis were predicted. However, because responses following short-term exposure were measured in F-0 larvae, whereas those following long-term exposure were measured after emergence, the observed differences reflect a combination of exposure duration, developmental stage, and metamorphosis and therefore represent inferred patterns rather than a direct test of exposure duration. Predator cues did not affect survival during short-term exposure, whereas survival to emergence differed among predator treatments following long-term exposure, with the lowest survival under IA spinycheek cues. This pattern suggests that predator effects on survival may become more apparent over longer developmental periods. Non-consumptive predator effects can influence prey demography indirectly through changes in behavior, physiology, growth, and development rather than through direct predator-induced mortality (Peckarsky et al. 2008; Clinchy et al. 2013). Similar indirect effects have been documented in damselflies and other aquatic insects (Stoks et al. 2012; Sheriff et al. 2020; Raczyński et al. 2022a).

Growth responses also differed between the two groups. Predator effects on growth were relatively weak following short-term exposure, whereas following long-term exposure, growth rate depended on predator identity and temperature.

Such responses expressed after metamorphosis are consistent with carry-over effects documented in other odonates and amphibians, where conditions experienced during larval development can influence adult morphology, growth, or performance (Relyea 2001; Benard 2004; McPeek 2004).

Physiological responses showed a different pattern. Predator-specific PO responses were evident following short-term exposure, whereas PO activity showed no clear predator effect following long-term exposure. Fat content, in contrast, differed among predator treatments following long-term exposure, indicating that not all physiological responses were lost across metamorphosis. Metamorphosis can reorganize physiological and metabolic processes and thereby modify responses expressed during the larval stage (Rolff et al. 2019). However, whether such carry-over effects influence adult survival and fecundity requires further investigation.

Trait-specific responses emerge from interacting abiotic and biotic stressors (Experiments 1 and 2)

The results demonstrate that interactions among abiotic and biotic stressors generate context-dependent plasticity across trait types. Life-history traits were strongly shaped by abiotic conditions, with survival and growth responding to warming, seasonal time constraints, and developmental history. Predator-specific growth responses also depended on environmental context. Behavior showed particularly strong context dependence: boldness varied with predator identity and abiotic conditions, while feeding responses depended on interactions involving abiotic conditions, sex, and predator identity. This is consistent with theory and empirical studies identifying behavior as a rapid and flexible response to environmental change (Wong and Candolin 2015; Amer et al. 2024). Physiological responses were more variable: PO activity showed predator-specific responses that depended on temperature, whereas fat content responded mainly following long-term exposure. Together, these patterns indicate that different trait categories vary in their sensitivity to interacting environmental stressors (Gunderson et al. 2016; Jackson et al. 2016).

The study focused on the ecologically most relevant native–IA crayfish pair for this study system, allowing a focused comparison between predator types. Predator cues, however, were presented without direct predation events, meaning that prey responded to perceived predation risk rather than to the combined effects of predator cues and predator-induced mortality, which may influence the magnitude and persistence of antipredator responses (Peers et al. 2018). Although the simplified laboratory design enabled isolation of interactions among predator identity, warming, and seasonal time constraints, future studies should test whether these patterns are consistent across multiple damselfly populations and under more complex ecological conditions.

Conclusion

The results demonstrate that predator-induced plasticity in *I. elegans* depends strongly on environmental context. Rather than acting independently, warming, seasonal time constraints, predator identity, and sex interacted to shape behavioral, life-history, and physiological responses, with behavior showing particularly strong context dependence. Warming weakened predator-specific differentiation in some traits, whereas photoperiod changed the magnitude and direction of predator-specific responses in growth and behavior. Thus, abiotic conditions can weaken,

strengthen, or otherwise modify predator-specific plasticity rather than uniformly constraining its expression. More broadly, the findings demonstrate that responses to native and IA predators are not determined by predator novelty alone but emerge from interactions between predator identity and the abiotic environment.

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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.

Artificial Intelligence (AI) use

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.
Regarding the use of AI in the preparation of this manuscript, the authors declare the following: GPT.
Used for: Language, style and writing.

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Author contributions

Nermeen R. Amer and Szymon Sniegula contributed equally to both the statistical analyses and the writing of the first draft. Conceptualization: NRA. Data curation: NRA. Formal analysis: NRA, SS. Funding acquisition: SS. Investigation: NRA, GW, MJG. Methodology: RSS, SS, NRA, MJG. Project administration: SS. Resources: SS. Supervision: RSS, SS. Validation: SS, MJG, NRA, RSS, GW. Visualization: NRA, MJG, SS. Writing - original draft: SS, NRA. Writing - review and editing: GW, SS, MJG, NRA.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

Supplementary material 1

Developmental trajectories and synchronization of larval measurements: Description of direct and postponed developmental pathways and synchronization of larval measurements in *Ischnura elegans*

Authors: Nermeen R. Amer, Maria J. Golab, Robby Stoks, Guillaume Wos, Szymon Sniegula

Data type: docx

Explanation note: **table S1.** Weekly photoperiod and temperature conditions for the ambient-temperature, early-photoperiod treatment in Experiment 1. **table S2.** Weekly photoperiod and temperature conditions for the warming, early-photoperiod treatment in Experiment 1. **table S3.** Weekly photoperiod and temperature conditions for the ambient-temperature, early- and late-photoperiod treatments in Experiment 2. **table S4.** Sample sizes across experimental treatments and response variables in Experiments 1 and 2. **table S5.** Candidate models with ΔAICc ≤ 2 for response variables analyzed in Experiments 1 (A) and 2 (B). **table S6.** Selected *post hoc* comparisons for Experiment 1 (A) and Experiment 2 (B), corresponding to the effects presented in Figs 2–4. **figure S1.** Early and late photoperiod regimes used during the experiment. **figure S2.** Seasonal water temperature dynamics in Płaszowski pond, the field collection site of *Ischnura elegans*. **figure S3.** Corrected growth rate to F-0 in individuals reaching F-0 before wintering and those subsequently experiencing wintering. **figure S4.** Relationship between temperature at entry into F-0 and corrected growth rate to F-0 in directly developing, non-wintering individuals. **figure S5.** Effects of temperature and sex on corrected growth rate to F-0 in Experiment 1.

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Link: https://doi.org/10.3897/neobiota.109.200244.suppl1

Supplementary material 2

DATASET Ischnura22 resubmission

Authors: Nermeen R. Amer, Maria J. Golab, Robby Stoks, Guillaume Wos, Szymon Sniegula

Data type: xlsx

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Supplementary material 3

Readme file

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