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Author-formatted, not peer-reviewed document posted on 01/06/2026

DOI: <https://doi.org/10.3897/arphapreprints.e201981>

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

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12 **Abstract:**

13 Invasive alien (IA) predators can disrupt predator-prey interactions and alter prey phenotypes,
14 yet how their effects differ from those of native predators and to what extent this depends on
15 environmental factors such as warming and seasonal time constraints remain poorly
16 understood. To address these gaps, we performed two complementary experiments testing the
17 effects of predator chemical cues (control, native noble crayfish, and IA spinycheek crayfish)
18 in combination with (Experiment 1) temperature (ambient vs. warming) and exposure
19 duration to predator cues (acute vs. chronic), or (Experiment 2) seasonal time constraints
20 manipulated by photoperiod (early vs. late season). In both experiments, we quantified effects
21 on life history (survival and growth rate), physiological (investments in immune function
22 measured as phenoloxidase (PO) activity and in energy storage measured as fat content), and
23 behavioural (boldness and feeding rate) traits in the damselfly *Ischnura elegans*.

24 In Experiment 1, responses to IA spinycheek crayfish cues often differed from those induced
25 by native noble crayfish cues across behavioural, physiological, and life-history traits, and
26 these differences depended strongly on temperature, sex, and exposure duration. Under
27 ambient temperature, IA spinycheek crayfish cues reduced boldness and increased PO
28 activity more strongly than native predator cues, whereas under warming IA predator cues
29 increased boldness. In contrast, chronic exposure to native noble crayfish cues produced the
30 strongest reduction in female growth rate under warming.

31 In Experiment 2, behavioural differences between native and IA predator cues were more
32 pronounced under early photoperiod but weakened under late photoperiod, suggesting that
33 strong seasonal time constraints constrained predator-specific antipredator responses.

34 Together, our results broadly support the naïve prey hypothesis, while demonstrating that
35 abiotic conditions and exposure duration strongly shape how prey respond to native and IA
36 predators. These findings highlight the importance of considering interactions between
37 predator novelty and environmental context when predicting invasion impacts under climate
38 change.

39

40 **Keywords:** carry-over effect, climate change, non-consumptive predator effect, *Ischnura*
41 *elegans*, naïve prey hypothesis, phenotypic plasticity, seasonal time constraint

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44 **Introduction**

45 Biological invasions are a major driver of ecological change, reshaping food webs, altering
 46 species interactions, and threatening native biodiversity worldwide (Benard 2004, Clinchy et
 47 al. 2013, Theodosiou et al. 2019). Invasive predators are particularly impactful because they
 48 can disrupt coevolved predator-prey relationships, influencing prey not only through direct
 49 consumption but also through strong non-consumptive effects (Peckarsky et al. 2008, Sih et
 50 al. 2010, Wos et al. 2025). These effects can induce plastic responses in native prey, including
 51 altered behaviour, physiology, and life-history strategies, with cascading consequences for
 52 growth, survival, and reproduction. Such predator-induced plasticity includes reduced activity
 53 or boldness, altered foraging strategies, shifts in growth trajectories, and changes in
 54 physiological investment (Peckarsky et al. 2008, Sih et al. 2010). For example, native frog
 55 larvae exhibit increased hiding behaviour and altered tail morphology when exposed to
 56 invasive crayfish cues (Nunes et al. 2014). Similarly, *Enallagma* damselfly larvae reduced
 57 movement and feeding in response to fish predator cues, although these responses depended
 58 strongly on the species' evolutionary history with fish predators (Stoks et al. 2003).

59 In invasion contexts, antipredator responses may be mismatched, exaggerated, or ineffective
 60 when prey lack evolutionary experience with novel predators, a concept framed under the
 61 naïve prey hypothesis (NPH) (Sih et al. 2010, Carthey and Banks 2014). Indeed, the NPH
 62 assumes a differential response of a prey to native vs. novel predators, with the response to
 63 novel predators being potentially maladaptive, especially when antipredator responses incur
 64 energetic or behavioural costs without providing equivalent reductions in predation risk (Sih
 65 et al. 2010, Carthey and Banks 2014).

66 Temperature is one of the most influential abiotic factors affecting ectotherms, specifically
 67 their metabolism and growth (Angilletta et al. 2004, Schulte 2015). Importantly, growing
 68 evidence suggests that abiotic conditions interact with predator novelty and constrain prey
 69 ability to express differentiated antipredator responses toward native and invasive predators
 70 (Ferrari et al. 2010, Smolinský and Gvoždík 2012, Carthey and Banks 2014, Anton et al.
 71 2020). Such context dependence may arise because warming alters energetic demands,
 72 behavioural activity, and information processing, potentially limiting the ability of prey to
 73 distinguish among predator types or to maintain costly antipredator responses. Temperature
 74 can therefore strongly shape predator-prey interactions, and thermal variation is increasingly
 75 recognized as a key modifier of invasion impacts (Juillard et al. 2025). Temperature can alter

76 both predator performance and prey vulnerability (Pepi et al. 2018, Domenici et al. 2019).
77 Consequently, higher temperatures may amplify or dampen antipredator responses depending
78 on prey energy limitation and species specific traits, thereby changing predator-prey
79 interactions rather than simply intensifying predator effects (Urban et al. 2014, Cinel et al.
80 2020, Wos et al. 2023). For example, warming led to increased feeding rates in two predators,
81 the active backswimmer *Notonecta undulata* and the sit-and-wait damselfly *Enallagma*
82 *annexum* (Twardochleb et al. 2020). However, encounter and capture dynamics differed
83 markedly between these foraging modes. Rising temperature increased swimming speed of
84 predators and prey, doubling prey encounter rates for the active predator. In contrast, prey
85 encounter rates for the sit-and-wait predator rose fourfold, and this solely because prey swam
86 faster (Twardochleb et al. 2020). At the mechanistic level (i.e. physiology and gene
87 expression) responses to predation risk can also be temperature dependent. For example,
88 juvenile brown trout exhibited elevated cortisol and altered stress-related gene expression
89 under predation risk, with response magnitude strongly modulated by temperature (Filipsson
90 et al. 2020).

91 In temperate regions, seasonal time constraints, i.e. limitations on the remaining time window
92 available for development, maturity, and reproduction, represent another important, yet
93 underappreciated, environmental constraint in invasion contexts. For many ectothermic
94 species, seasonal time constraints are signalled by photoperiod (Bradshaw and Holzapfel
95 2007, Norling 2021). Similar to warming, strong seasonal constraints may interact with
96 predator novelty and constrain prey ability to express differentiated responses toward native
97 and invasive predators. Under late-season conditions, prey may prioritize rapid growth and
98 development over costly antipredator defences, potentially limiting their ability to maintain
99 predator specific responses (Dmitriew 2011). Photoperiod affects growth, development, and
100 physiology (Amer et al. 2025; Effertz and von Elert 2014), thereby influencing periods of
101 vulnerability to predation (Lopez et al. 2023). For example, prey experiencing strong
102 seasonal time constraints may prioritize rapid growth and development over costly
103 antipredator responses, thereby altering the magnitude of responses to predator cues (Stoks et
104 al. 2006, Salis et al. 2018).

105 The above described stressor effects are further complicated in prey with complex life cycles,
106 where responses to stressors at one stage can carry over to subsequent stages. Because most
107 ectotherms undergo metamorphosis, carry-over effects are a key mechanism linking early-life
108 stress to adult performance (Kingsolver et al. 2011, Stoks and Córdoba-Aguilar 2012).

109 Predation risk experienced during larval development can induce morphological, behavioural,
110 or physiological changes that persist in the adult stage and might affect condition and/or
111 reproductive output (Benard 2004). Similarly, thermal conditions and photoperiod during
112 premature development can shape body size at metamorphosis, energy allocation, and later
113 susceptibility to predators (Dmitriew 2011, Amer et al. 2025). In invasion scenarios, such
114 carry-over effects may amplify or obscure the long-term impacts of invasive predators,
115 particularly when combined with warming and seasonality.

116 Despite growing recognition of these complex ecological interactions, most studies have
117 focused on single stressors or short term (acute) exposures (Homeny and Juliano 2007,
118 Verberk et al. 2021). Prey responses to acute vs. chronic (long-term) exposure to predation
119 risk can be different, amongst others because costs can accumulate and compensatory
120 mechanisms can be activated (Van Dievel et al. 2016). Little is known about how chronic
121 versus acute exposure to predation risk interacts with warming or photoperiod to generate
122 carry-over effects within and across life stages, particularly in the context of invasive
123 predators. According to the NPH, prey often respond maladaptively to invasive predators due
124 to a lack of shared evolutionary history, resulting in altered or ineffective antipredator
125 responses (Sih et al. 2010). Yet, how such native-invasive predator contrasts interact with
126 abiotic stressors and the duration of predation risk remains unexplored.

127 Here, we used the damselfly *Ischnura elegans*, a species well suited for studying multi-
128 stressor interactions due to its strong phenotypic plasticity and complex life cycle (Stoks and
129 Córdoba-Aguilar 2012). Previous work on *I. elegans* has demonstrated that predator cues,
130 particularly from invasive alien (IA) predators, can shape damselfly traits through non-
131 consumptive effects and carry over across developmental stages (Sniegula et al. 2019a, Antol
132 and Sniegula 2021, Antol et al. 2022, Sniegula et al. 2024, 2025). These studies provided
133 support for NPH by showing that IA predators often elicit mismatched responses compared to
134 native predators even when they are phylogenetically closely related (Sniegula et al. 2025).
135 Other studies in this system have further shown that predator effects interact with
136 temperature, photoperiod, urbanization, and genetic background, but these studies have
137 typically focused on a single life stage (larval), short predator exposure durations, or only a
138 limited subset of interacting biotic and abiotic factors at a time (Palomar et al. 2023, Wos et
139 al. 2023, 2024, Amer et al. 2024, Sniegula et al. 2024).

140 We conducted two experiments to test how predation risk imposed by native and IA predators
 141 differentially affects larval life-history, behavioural, and physiological traits, as well as carry-
 142 over effects expressed after metamorphosis, and how these responses are modulated by
 143 warming (Experiment 1) and photoperiod (Experiment 2, larval stage only). In Experiment 1,
 144 predation risk was imposed either acutely (during the first five days of the final larval instar
 145 before emergence) or chronically (throughout the final instar until emergence; average instar
 146 duration: 13 days), allowing us to assess the role of exposure duration and potential carry-
 147 over effects from the larval to the adult stage. In Experiment 2, individuals were exposed to
 148 predator cues only acutely. Following the NPH, we predicted differential responses to native
 149 vs. IA predators, with the later eliciting stronger or potentially maladaptive responses. We
 150 further predicted that, in Experiment 1, these responses would depend on predator exposure
 151 duration, with stronger effects under chronic than acute exposure, and that predator induced
 152 responses would carry over to the adult stage and affect traits expressed after metamorphosis.
 153 Finally, we predicted that abiotic conditions would modulate responses to native and IA
 154 predators. Specifically, we expected warming and late photoperiod to constrain prey ability to
 155 maintain predator specific antipredator responses, thereby reducing differentiation in
 156 responses to native and IA predator cues.

157 **Material and methods**

158 **Study system, collection, and rearing**

159 *I. elegans* has a widespread geographic distribution in Europe (Dijkstra and Schröter 2020).
 160 The species is common and not listed as protected or endangered in Europe (Knijf et al. 2024).
 161 In central Europe, *I. elegans* produces one (univoltine) or two (bivoltine) generations annually,
 162 with larva as overwintering stage in a univoltine cohort (Corbet et al. 2006). Larval
 163 development, emergence dates, and the flying season are not synchronized. In central Europe,
 164 including Poland, the damselfly is on the wing throughout the spring and summer months
 165 (Dijkstra and Schröter 2020, Sniegula et al. 2024). Larvae hatch within two-three weeks after
 166 egg deposition. Both the aquatic egg and larval stages often co-occur with top predators such
 167 as fish and crayfish (Kouba et al. 2014, Sniegula et al. 2019a, Amer et al. 2024, Sniegula et al.
 168 2025). The larvae occupy a mid-level trophic position in freshwater food webs, acting as key
 169 predators of aquatic invertebrates, like midges and mosquito larvae, while also serving as prey
 170 for larger invertebrates and fish (Stoks and Córdoba-Aguilar 2012, Antoń and Sniegula 2021).

171 Adult females *I. elegans* in copulation were collected near Płaszowski pond, within the city
 172 of Kraków, southern Poland (50°02'33.4"N 19°58'01.8"E) on 15 June 2022 (early
 173 photoperiod group) and 03 August 2022 (late photoperiod group). Females were placed in
 174 plastic containers with wet filter paper for egg laying and were kept in a room with a natural
 175 photoperiod and a temperature /of ca. 22°C. Newly laid clutches were transferred to two
 176 incubators (Pol-Eko ST700). The temperatures at which eggs and larvae were reared were
 177 adjusted once a week to follow seasonal changes of mean weekly temperatures in shallow
 178 water, which is the optimal habitat for damselfly larvae (Corbet 1999). Płaszowski pond
 179 contains only one non-native and non-invasive crayfish species, Danube crayfish (*Astacus*
 180 *leptodactylus*), with the last record in 2015 (Maciej Bonk, unpublished data).

181 **Predator description, collection, and housing**

182 We used cues from two different crayfish species to induce non-consumptive predator effects
 183 (NCE). These were the native noble crayfish (*Astacus astacus*), which is present in Central
 184 Europe, including southern Poland (Kouba et al. 2014), and the IA spinycheek crayfish
 185 (*Faxonius limosus*) introduced to Central Europe in the late 19th century. *F. limosus* has become
 186 the most prevalent crayfish species in EU countries, including southern Poland (Kouba et al.
 187 2014, World of Crayfish™ 2024). Noble crayfish were collected from a private pond near the
 188 town Miejska Gorka (51°39'13.2"N, 16°58'52.3"E), and spinycheek crayfish were collected
 189 from an excavation pond in Kryspinow (50°02'56.8"N, 19°47'28.7"E). Animals were kept in
 190 aquariums (three crayfish in 40 L of Reconstituted Deionized Water, RDiW) at a constant
 191 temperature of 20°C. The densities of crayfish in aquaria were based on previous experiments
 192 where the crayfish cues were effectively used to induce antipredator responses in damselfly
 193 larvae (Wos et al. 2023, Amer et al. 2024). Crayfish were fed with fish food pellets twice per
 194 week and live worms once per week. Water from these aquaria was used for the predator cue
 195 treatment (described below) along with a control aquarium (40 L of RDiW without crayfish).
 196 Aquariums were regularly cleaned to avoid accumulation of organic matters and to keep a
 197 concentration of kairomones constant over time. Noble and spinycheek crayfish were collected
 198 and housed with permissions from the General Directorate of Environmental Protection in
 199 Warsaw (per. DZP-WG.6401.147.2021.TŁ) and the Regional Directorate of Environmental
 200 Protection in Krakow (per. OP.672.4.2021. GZ), respectively.

201 **Experimental setup**

202 After oviposition, each egg clutch from a single female was divided into six equal parts and
203 placed in separate plastic containers (15 x 11 x 7.5 cm) filled with 600 ml of dechlorinated tap
204 water. Water refill took place every other day to keep the water levels constant. The study was
205 divided into two partially independent experiments focusing on different treatment
206 combinations, with the 22°C early photoperiod group shared between Experiment 1 and
207 Experiment 2.

208 *Experiment 1*

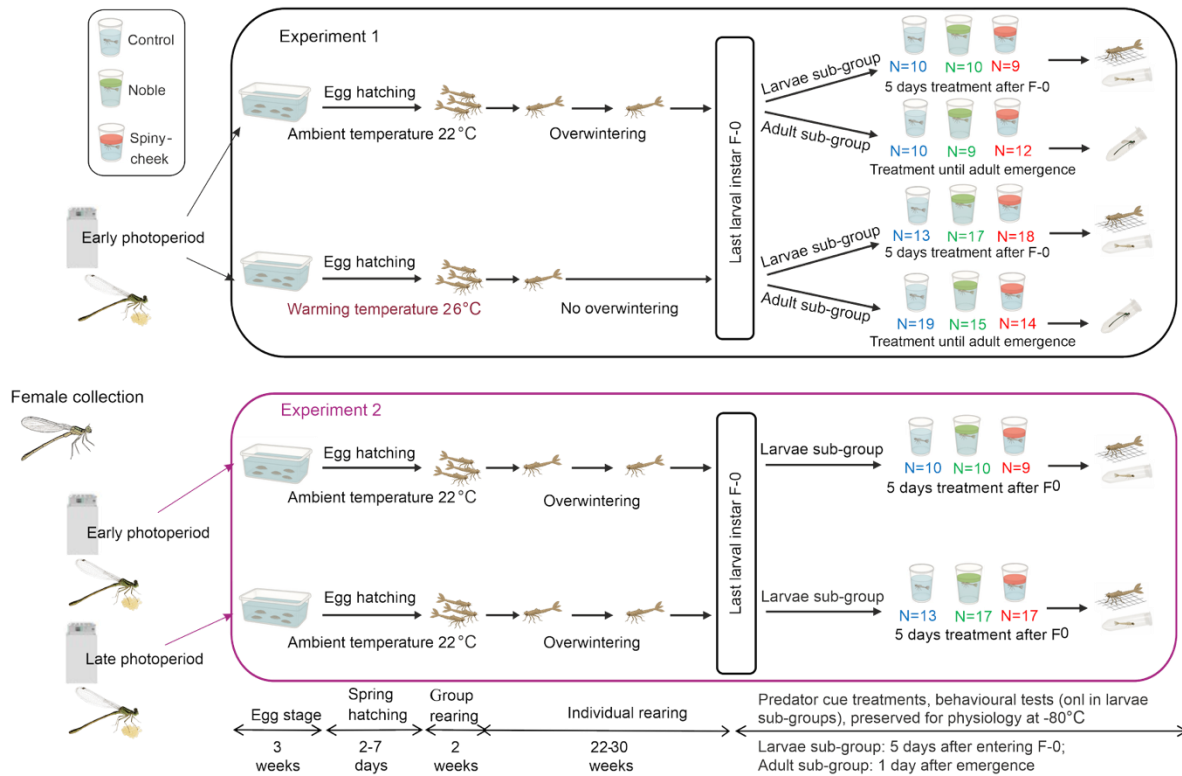
209 We set up two temperature treatments (ambient: 22°C and warming: 26°C), crossed with
210 three predator cue treatments (control, native noble crayfish, and IA spinycheek crayfish)
211 (Fig. 1). The two temperature treatments were the ambient temperature treatment, which
212 mimicked the current temperature in the collection site, and the warming treatment, where the
213 ambient temperature was increased by 4°C to mimic the predicted temperature change by
214 2100 under IPCC scenario SSP5-8.5 (IPCC 2021). Temperature in the sampling pond was
215 measured with data loggers placed at 40 cm depth in 2023. The temperature recorded was
216 around 22°C during the summer months (Appendix 1: Fig. S2, Sniegula et al. 2024),
217 supporting the ambient temperature treatment during the current experiment. After hatching
218 of early photoperiod clutches, larvae were transferred into group cups (10 larvae/cup) for two
219 weeks and then into individual plastic cups (height 9 cm, diameter 4 cm) under the
220 corresponding conditions. All groups were reared in dechlorinated tap water up to entering
221 the final instar before emergence (F-0). At the entrance into F-0 (day-0), larvae from each
222 temperature group were divided into two subgroups (larvae and adults). The larvae subgroup
223 was divided into three predator treatment groups (control, noble, and spinycheek crayfish
224 cues) for five days (acute exposition), whereas the adult subgroup was exposed to the same
225 predator cue treatments until adult emergence (average duration of F-0 instar was 13 days)
226 (chronic exposition). Only larvae reared at 22°C underwent overwintering, representing a
227 univoltine cohort, whereas larvae reared at 26°C completed development within the same
228 season as a bivoltine cohort. This outcome is consistent with temperature-dependent variation
229 in voltinism in damselflies, where cooler conditions promote delayed development and
230 overwintering, while warmer conditions accelerate development and favour direct emergence.
231 Such variable voltinism can somewhat buffer a photoperiod effect by allowing late hatching
232 or slow developing individuals to postpone emergence to the following season (Corbet et al.
233 2006, Raczyński et al. 2022b). Larval behaviour observations were conducted when F-0
234 larvae entered day 2 after the entrance into F-0, in the larvae subgroup only. Larvae from the

235 larvae subgroup were stored at -80°C freezer after 5 days of predator cues treatment for
236 physiology analysis. Emerged adults from the adult subgroup were then stored at -80°C
237 freezer the next day after emergence for physiology analysis.

238 ***Experiment 2***

239 We set up two photoperiod groups (early and late) crossed with three predator cue treatments
240 (control, native noble crayfish, and IA spinycheek crayfish) reared at 22°C (Fig. 1). The 22°C
241 early group was used in both Experiment 1 and Experiment 2. Early and late photoperiod
242 groups were started with short (spring) and long (summer) photoperiods, respectively
243 (Appendix 1: Fig. S1). After hatching, larvae were transferred into group cups (10 larvae/cup)
244 for two weeks and then into individual cups under the corresponding conditions. All larvae
245 underwent overwintering. All groups were reared in control dechlorinated tap water up to
246 entering F-0 instar. At the entrance into F-0 (day-0), larvae from both groups were divided
247 into three predator treatment groups (control, native noble, and IA spinycheek crayfish cues)
248 for five days of treatment (acute exposition). Larval behaviour observations were conducted
249 when F-0 larvae entered day 2 after the entrance into F-0. Larvae were stored at -80°C
250 freezer after five days of predator cues treatment for physiology analysis. In Experiment 2,
251 only larval responses were analysed. An adult exposure group was initially planned,
252 mirroring the design of Experiment 1; however, larvae assigned to the adult exposure
253 treatment under warming conditions (26°C) experienced complete mortality before reaching
254 adulthood. To maintain a balanced and comparable design, the corresponding adult exposure
255 group at ambient temperature (22°C) was therefore also excluded from further analyses. As a
256 result, Experiment 2 focuses only on larval responses to photoperiod and predation risk.

257



258

259 **Figure 1.** Experimental design testing for the effects of predator cue treatments (control, native
 260 noble crayfish, and invasive spinycheek crayfish) combined with an environmental stressor in
 261 the damselfly *I. elegans*. Egg clutches were subdivided to allow parallel testing of
 262 environmental stressors. The study consisted of two experiments. **Experiment 1:** tested the
 263 effects of temperature (ambient 22°C and warming 26°C) crossed with predator cue treatments.
 264 After larvae entered F-0 instar, individuals were divided into two subgroups: a larval subgroup
 265 exposed acutely to predator cues for five days, and adult subgroup exposed chronically to
 266 predator cues until adult emergence. **Experiment 2:** examined the effects of photoperiod (early
 267 and late), with all individuals exposed to predator cue treatments acutely during the larval stage.
 268 Behavioural assays were conducted two days after entry into the F-0 instar in the larval
 269 subgroup only in both experiments. Following the exposure period, individuals from the larval
 270 subgroup were sampled after five days, and individuals from the adult subgroup were sampled
 271 one day after emergence; all individuals were stored at -80°C for physiology analyses. Larvae
 272 reared at 22°C underwent overwintering, whereas larvae reared at 26°C developed directly
 273 without overwintering, reflecting temperature-dependent variation in voltinism. For sample
 274 sizes, see Table S4.

275 For all groups, we followed weekly seasonal changes of photoperiod (including the Civil
 276 Twilight) at the damselfly sampling latitude. Details of the applied temperature and
 277 photoperiod regimes are available in Appendix 1: Fig. S1 and Tables S1, S2, and S3.

278 Larvae reared at 22°C and 26°C differed in developmental trajectory and physiological state
 279 (regulatory vs. direct/preemergence development; Norling 2021). Therefore, measurements
 280 were synchronized to ensure comparisons between physiologically comparable larvae
 281 (Appendix 1).

282 For all groups before F-0, water was refilled every second day to keep the water levels
 283 approximately constant. The larvae were fed twice a day (morning and afternoon) during
 284 weekdays and once a day during weekend days with laboratory-cultured *Artemia* sp. nauplii.
 285 For the first 14 days, larvae kept in groups (10 larvae/cup) received 1 ml of *Artemia*
 286 solution/container, on average, 198.5 (SD = 12.4) nauplii/ml (N = 38 samples counted). This
 287 ration was administered per container for group-reared larvae and per cup for individually
 288 reared larvae. During the simulated winter, when larvae were in a diapause state (a
 289 hormonally regulated state of arrested development accompanied by markedly reduced
 290 metabolic activity), individuals were fed once a day throughout the week. This food ration
 291 suffices for a diapausing individual, as previously shown in *Ischnura* sp. (Palomar et al.
 292 2023; Amer et al. 2024a). Larvae reared at 26°C were fed twice/day on weekdays and only
 293 once during the weekend. At the entrance into F-0, all larvae (larvae subgroup) received 1.5
 294 ml of *A. salina* once per day, except for the 2nd day of behaviour, each larva receives 3
 295 chironomid larvae after behaviour testing. Larvae of adult subgroup received 1.5 ml of *A.*
 296 *salina* once per day plus 3 chironomid larvae three times per week (Monday, Wednesday, and
 297 Friday). During the 5 days treatment (larvae subgroup), cups were 1/3 refilled with the
 298 corresponding predator cue at day 0, 2 (after behaviour) and 4, while in the adult subgroup,
 299 cups were 1/3 refilled with the corresponding predator cue every Monday, Wednesday, and
 300 Friday until adult emergence.

301 **Response variables**

302 **Life history traits**

303 Survival was measured as the number of larvae that survived until entering F-0 instar, after the
 304 5-days predator treatment (larvae subgroup), and the day after adult emergence (hereafter, until
 305 emergence) (adult subgroup). Development time was measured as the number of days between

306 hatching and entering the F-0 instar, and until emergence. Wet mass was measured at the
 307 entrance of the F-0 instar (mass at F-0), after 5 days of treatment in the larvae subgroup (mass
 308 gain 5-days), and mass loss (mass of adult one day after emergence – mass at F-0) (adult
 309 subgroup). Animals were weighed to the nearest 0.1 mg using an electronic balance (Radwag®
 310 AS.62. R2 Plus). Growth rate until the entrance into F-0 was calculated as $\text{growth F-0} = \text{mass}$
 311 $\text{at the entrance into F-0} / \text{development time between hatching and entrance into F-0}$. Growth
 312 rate during the larval treatment was calculated as $\text{growth 5-days} = (\text{mass after larval treatment}$
 313 $– \text{mass at the entrance into F-0}) / 5 \text{ days}$.

314 **Behavioural traits**

315 Behavioural observations were performed between 10 and 12 am local time. We measured two
 316 behavioural traits: freezing time (hereafter: boldness), and feeding rate. To assess boldness,
 317 each larva was gently touched on the thorax using a pencil, which triggered a rapid escape
 318 followed by a period of immobility (freezing), as in (Brodin 2009). Larvae that had shorter
 319 freezing times were considered bolder. Following this, 30 *A. salina* nauplii were introduced
 320 into each container as food for 15 minutes to assess the larval feeding rate. After this time
 321 elapsed, larvae were removed from the experimental containers and placed back into their
 322 original rearing containers in the climatic chamber, while the remaining *A. salina* nauplii in the
 323 containers were counted using a hand magnifier.

324 **Physiological traits**

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

334 To determine fat content in damselfly larvae and adult, we used a modified version of the Marsh
 335 and Weinstein protocol (Marsh and Weinstein 1966), as adapted by (Verheyen et al. 2018). For
 336 this assay, 8 µl of the sample supernatant was mixed with 56 µl of concentrated sulfuric acid

337 (100%) in small glass tubes and heated at 150 °C for 20 minutes. After cooling, 64 µl of milliQ
338 water was added. The mixture was then transferred to a 384-well microtiter plate (30 µl per
339 well, in triplicate), and absorbance was measured at 490 nm. The mean value from the three
340 replicates was used in statistical analyses.

341 **Statistical analyses**

342 All statistical analyses were conducted in R version 4.5.1 (R Core Team 2025) using RStudio
343 (Posit team 2026). For Experiment 1, the predator cue treatment (control, noble crayfish,
344 spinycheek crayfish), temperature (22°C and 26°C), sex and their interactions were included
345 as fixed explanatory variables. Stage (larval vs. adult) was considered where relevant for
346 trait-specific analyses. In Experiment 2, the predator cue treatment (control, noble crayfish,
347 spinycheek crayfish), photoperiod (early and late), sex and their interactions were included as
348 fixed explanatory variables. For each response variable, we fitted full models including all
349 main effects and all possible interactions.

350 Survival to the F-0 instar, survival five days after entrance into F-0, and survival to
351 emergence were analysed using generalized linear models (GLMs) with a binomial error
352 distribution and logit link. Growth rates (until F-0, during the 5-day exposure, and until
353 emergence), body mass (at F-0, after 5-day exposure, and at emergence), PO activity, and fat
354 content were analysed using a Gaussian error distribution after confirming normality of
355 model residuals. Development time and behavioural traits (boldness and feeding rate) were
356 analysed using GLMs with a Poisson error distribution.

357 Type II or Type III likelihood-ratio tests, as appropriate for each model structure, were used
358 to assess the significance of fixed effects using the *car* package (Fox and Weisberg 2011).
359 When significant main effects or interactions were detected, estimated marginal means
360 (EMMs) were computed, and pairwise comparisons were performed using Tukey-adjusted
361 contrasts in the *emmeans* package (Lenth et al. 2020). Model assumptions were checked
362 visually using residual diagnostics. No substantial deviations from normality were detected.

363 **Results**

364 **Experiment 1: Predator cues × Temperature**

365 *Effects of temperature until larval entrance into F-0*

366 Up to the F-0, temperature was the primary determinant of life history variation. Temperature
 367 significantly affected larval survival ($\chi^2 = 13.9$, $df = 1$, $p < 0.001$), with warming increasing
 368 the survival (EMMs: 0.46, 95% CI: 0.40-0.53) compared to ambient temperature (EMMs:
 369 0.29, 95% CI: 0.23-0.35) (z ratio = -3.69, $df = \text{Inf}$, $p = < 0.001$) (Appendix 1: Fig. S3-A).

370 Growth rate was significantly affected by temperature ($\chi^2 = 107.8$, $df = 1$, $p < 0.001$) and by
 371 the interaction between temperature and sex ($\chi^2 = 5.87$, $df = 1$, $p = 0.015$). While warming
 372 significantly increased growth rate in both sexes ($P < 0.001$), the thermal response was more
 373 pronounced in females (EMMs: 0.72, 95% CI: 0.67-0.77) than in males (EMMs: 0.66, 95%
 374 CI: 0.61-0.72) (Appendix 1: Fig S3-B). Specifically, the increase in growth rate under
 375 warming was greater in females (Warming - Ambient estimated increase = 0.45) than in
 376 males (Warming - Ambient estimated increase = 0.31).

377 ***Effect of acute predator cues exposure at the F-0 (5-day exposure, larval subgroup)***

378 **Table 1.** Effects of predator cues (control, native noble crayfish, and spinycheek crayfish),
 379 temperature (ambient and warming), and sex on life history traits (survival, growth rate),
 380 physiology (PO activity and fat content), and behaviour (boldness and feeding rate) during
 381 the 5-day exposure at the final larval instar (F-0) (larval subgroup) and until adult emergence
 382 (adult subgroup) in **Experiment 1**. Behaviour variables were quantified in the larval
 383 subgroup only. Significant p-values are in bold: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Response variables		Larval subgroup		Adult subgroup	
Survival	df	χ^2	P-value	χ^2	P-value
Predator cue	2	0.000	1.000	1.644	0.439
Temperature	1	0.000	1.000	4.186	0.040*
Sex	1	1.490	0.221	0.000	1.000
Predator cue × temperature	2	0.000	1.000	0.580	0.748
Predator cue × Sex	2	0.000	1.000	0.361	0.834
Temperature × Sex	1	0.000	1.000	0.000	0.999
Predator cue × temperature × Sex	2	0.000	1.000	0.000	1.000
Growth rate					
Predator cue	2	9.620	0.091	2.776	0.249
Temperature	1	5.800	0.317	0.987	0.767

Sex	1	2.840	0.090	2.014	0.155
Predator cue × temperature	2	6.230	0.198	12.269	0.002**
Predator cue × Sex	2	5.319	0.084	2.087	0.352
Temperature × Sex	1	1.914	0.228	1.447	0.228
Predator cue × temperature × Sex	2	3.864	0.146	9.034	0.011*
PO activity					
Predator cue	2	6.452	0.039*	0.606	0.738
Temperature	1	0.233	0.628	3.268	0.071
Sex	1	0.076	0.421	0.592	0.441
Predator cue × temperature	2	8.318	0.016*	1.309	0.519
Predator cue × Sex	2	2.244	0.325	0.270	0.873
Temperature × Sex	1	0.050	0.823	1.331	0.248
Predator cue × temperature × Sex	2	1.375	0.502	1.226	0.541
Fat content					
Predator cue	2	0.556	0.757	0.556	0.757
Temperature	1	0.235	0.627	0.235	0.627
Sex	1	0.138	0.709	0.138	0.709
Predator cue × temperature	2	0.556	0.757	0.556	0.757
Predator cue × Sex	2	0.675	0.713	0.675	0.713
Temperature × Sex	1	0.089	0.764	0.089	0.764
Predator cue × temperature × Sex	2	0.084	0.958	0.084	0.958
Boldness					
Predator cue	2	521.250	<0.001***	-	-
Temperature	1	833.780	<0.001***	-	-
Sex	1	73.930	<0.001***	-	-
Predator cue × temperature	2	676.220	<0.001***	-	-

Predator cue × Sex	2	218.800	<0.001***	-	-
Temperature × Sex	1	64.610	<0.001***	-	-
Predator cue × temperature × Sex	2	67.660	<0.001***	-	-
Feeding rate					
Predator cue	2	0.475	0.788	-	-
Temperature	1	16.106	<0.001***	-	-
Sex	1	3.258	0.071	-	-
Predator cue × temperature	2	0.405	0.816	-	-
Predator cue × Sex	2	1.327	0.514	-	-
Temperature × Sex	1	2.869	0.090	-	-
Predator cue × temperature × Sex	2	0.263	0.876	-	-

384

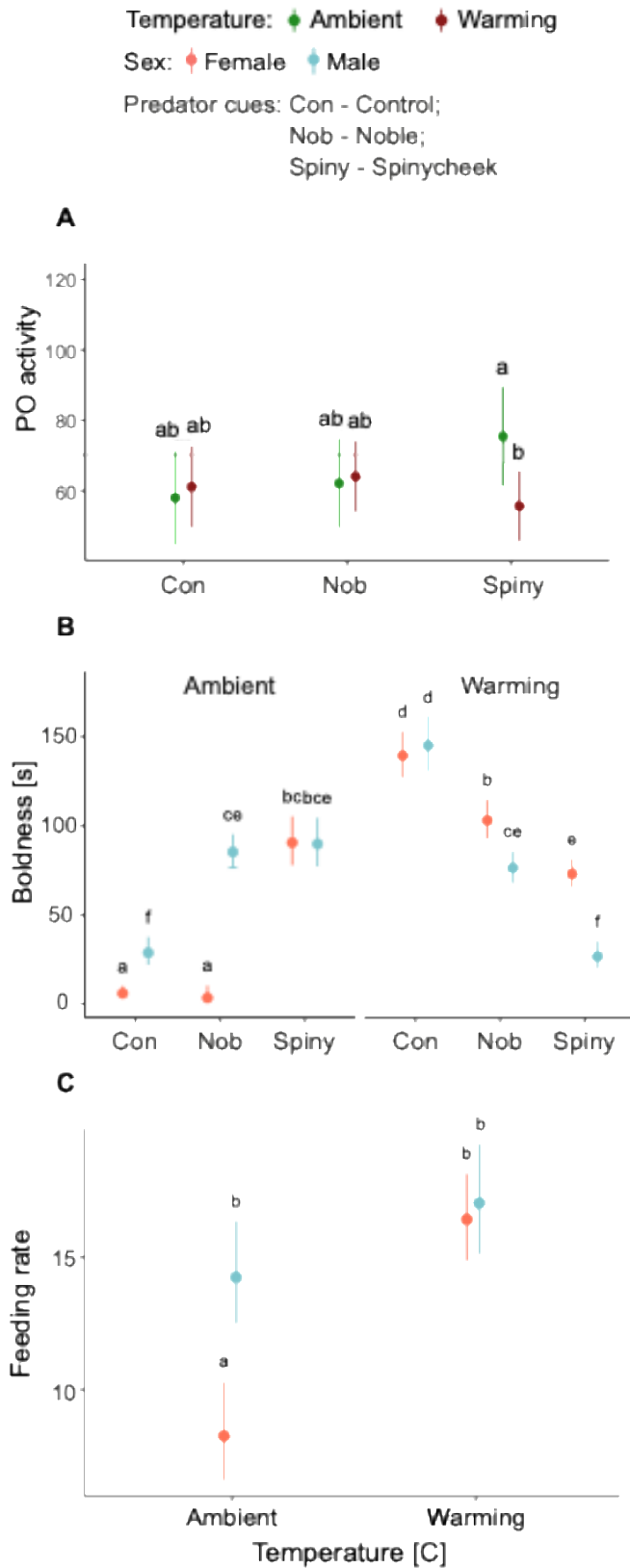
385 Survival after the 5-day exposure period was not significantly affected by predator cues,
 386 temperature, sex, or their interactions (Table 1). No significant main effects of predator,
 387 temperature, or sex were detected on the growth rate during the 5-day exposure. Similarly, fat
 388 content did not differ significantly among predator cue treatments, temperatures, sex, or their
 389 interactions (Table 1).

390 Phenoxidase activity was significantly affected by predator cues and by the interaction
 391 between predator cues × temperature, whereas temperature alone had no significant effect
 392 (Table 1 and Appendix 1: Table S5). Under ambient temperature, larvae exposed to invasive
 393 spinycheek crayfish cues showed the highest PO activity, whereas this differentiation
 394 disappeared under warming because PO activity declined strongly in the invasive predator
 395 treatment (Fig. 2A).

396 Behavioural responses showed the clearest differentiation between native and invasive
 397 predator cues and were strongly modulated by warming (Fig. 2B, Table 1). Under ambient
 398 temperature, females showed similarly high boldness in the control and native noble crayfish
 399 treatments, whereas boldness decreased in the invasive spiny-cheek crayfish treatment. Under
 400 warming, however, this pattern reversed, with females exhibiting the highest boldness in the

401 invasive predator treatment. Males also showed temperature-dependent responses: under
402 ambient conditions, boldness was highest in the control treatment and similarly reduced
403 under both predator cue treatments, whereas under warming boldness increased progressively
404 from the control to native and invasive predator treatments.

405 The feeding rate was not influenced by predator cues but varied significantly with
406 temperature and sex. There was a higher feeding rate under warming conditions, but only in
407 females (nearly significant interaction temperature \times sex) (Fig. 2C, Table 1).



408

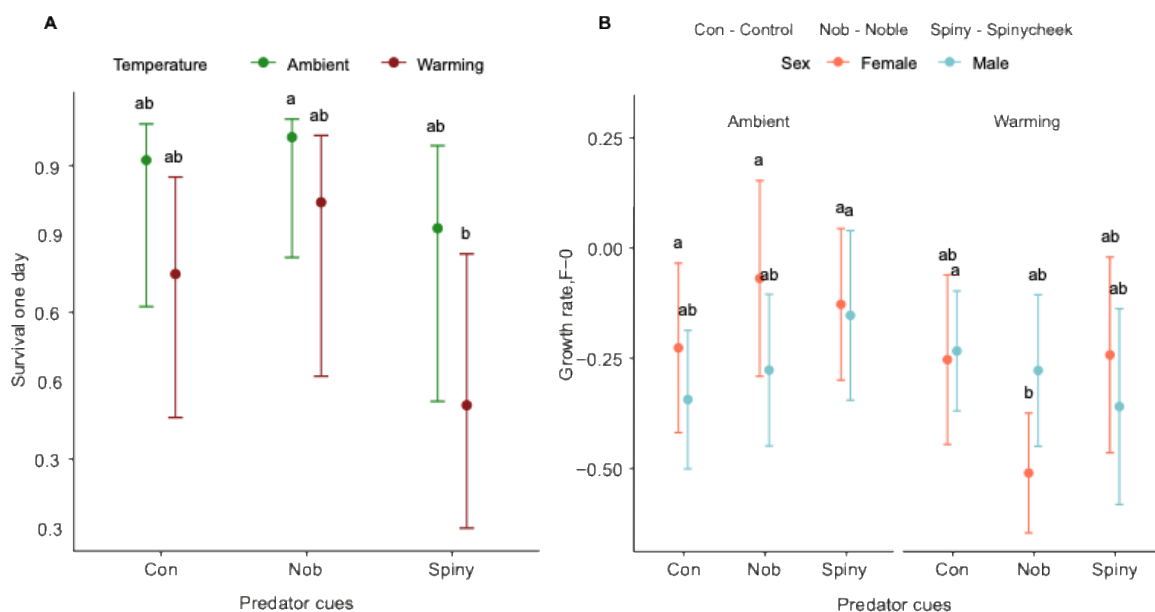
409 **Figure 2. Experiment 1, larva subgroup.** Effect of predator cues (control, noble, and
 410 spinycheek), temperature (ambient and warming), and sex (female and male) on
 411 phenoloxidase activity (PO), boldness, and feeding rate during the 5-day larval exposure
 412 period in *I. elegans*. Error bars show 95% CI.

413 ***Effect of chronic predator cues exposure at the F-0 instar (until emergence, adult***
 414 ***subgroup)***

415 Survival to emergence was not affected by predator cues but varied significantly with
 416 temperature, whereas sex, and all interactions had no significant effects (Fig. 3A, Table 1, and
 417 Appendix 1: Table S5). There was a higher survival under ambient than warming
 418 temperature, but confidence intervals overlapped broadly, and pairwise comparisons were not
 419 significant (Fig. 3A).

420 Predator cues, temperature, and sex alone did not show significant main effects on growth
 421 rate until emergence (Fig. 3B and Table 1). The significant interaction predator cues ×
 422 temperature × sex indicated that predator effects on growth rate depended on thermal
 423 conditions and differed between females and males. Specifically, there was the strongest
 424 reduction in growth rate in the noble crayfish treatment and warming, but only in females
 425 (Fig 3B and Table 1), indicating that predator-specific responses after chronic exposure were
 426 strongly context- and sex-dependent rather than consistently stronger for the invasive
 427 predator.

428 PO activity and fat content were not significantly affected by predator cues, temperature, sex,
 429 or any of their interactions. Temperature showed only a marginal effect on PO activity ($p =$
 430 0.071), with a tendency of higher PO activity under ambient temperature across all predator
 431 cues (Table 1).



432

433 **Figure 3. Experiment 1**, adult subgroup. Effect of predator cues (control, noble, and
 434 spinycheek), temperature (ambient and warming), and sex (female and male) on survival and
 435 growth rate one day after emergence of *I. elegans*. Error bars show 95% CI.

436

437 **Experiment 2: predator cues × photoperiod**

438 **Effect of photoperiod until larval entrance into F-0 instar**

439 Survival to F-0 was significantly affected by photoperiod ($\chi^2 = 6.33$, $df = 1$, $p = 0.01$), with
 440 lower survival under late compared to early photoperiod. Estimated marginal means on the
 441 response scale indicated that survival declined from 28% (95% CI: 20%-37%) under the early
 442 photoperiod to 16% (95% CI: 12%-21%) under the late photoperiod, and pairwise
 443 comparisons confirmed a significant difference between photoperiod treatments (z ratio =
 444 2.56, $df = \text{Inf}$, $p = 0.01$) (Appendix 1: Fig. S4-A).

445 Growth rate was significantly affected by sex ($\chi^2 = 10.50$, $df = 1$, $p = 0.001$) and by the
 446 interaction photoperiod × sex ($\chi^2 = 7.60$, $df = 1$, $p = 0.006$), whereas photoperiod alone had
 447 no significant effect ($\chi^2 = 0.67$, $df = 1$, $p = 0.41$). Under the early photoperiod, males
 448 exhibited higher growth rates (EMMs: 0.319, 95% CI: 0.27-0.36) than females (EMMs:
 449 0.198, 95% CI: 0.14-0.25) (t ratio = -3.24, $df = 71$, $p = 0.01$) (Appendix 1: Fig. S4-B). Under
 450 the late photoperiod, male growth rates declined markedly (EMMs: 0.161, 95% CI: 0.12-
 451 0.20), reaching values similar to those of females (EMMs: 0.170, 95% CI: 0.13-0.21) (t ratio
 452 = 0.32, $df = 71$, $p = 0.98$) (Appendix 1: Fig. S4-B). In contrast, female growth rates were
 453 consistent across photoperiod treatments.

454

455 **Effect of predator cues exposure (acute only) and photoperiod during 5-day predator cue**
 456 **exposure**

457 Predator cue effects during acute exposure depended strongly on photoperiod, sex, and trait
 458 type (Table 2).

459

460 **Table 2.** Effects of predator cues (control, noble crayfish, and spinycheek crayfish),
 461 photoperiod (early and late), and sex on life history traits (survival, growth rate), physiology
 462 (PO activity and fat content), and behaviour (boldness and feeding rate) during the 5-day
 463 predator cue exposure at the final larval instar (F-0) in **Experiment 2**. Significant p-values
 464 are in bold: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

465

Response variables	df	X²	P-value
Survival			
Predator cue	2	0.000	1.000
Photoperiod	1	1.497	0.221
Sex	1	1.498	0.221
Predator cue × Photoperiod	2	0.000	1.000
Predator cue × Sex	2	0.000	1.000
Photoperiod × Sex	1	1.509	0.219
Predator cue × Photoperiod × Sex	2	0.000	1.000
Growth rate			
Predator cue	2	5.662	0.058
Photoperiod	1	1.652	0.198
Sex	1	1.672	0.195
Predator cue × Photoperiod	2	1.734	0.420
Predator cue × Sex	2	3.131	0.208
Photoperiod × Sex	1	1.505	0.219
Predator cue × Photoperiod × Sex	2	3.953	0.138
PO activity			
Predator cue	2	5.181	0.074
Photoperiod	1	0.886	0.346
Sex	1	0.771	0.379
Predator cue × Photoperiod	2	3.469	0.176
Predator cue × Sex	2	2.571	0.276
Photoperiod × Sex	1	2.142	0.143
Predator cue × Photoperiod × Sex	2	7.724	0.021*
Fat content			

Predator cue	2	0.469	0.790
Photoperiod	1	0.142	0.706
Sex	1	0.117	0.732
Predator cue × Photoperiod	2	1.234	0.539
Predator cue × Sex	2	0.569	0.752
Temperature × Sex	1	0.008	0.929
Predator cue × Photoperiod × Sex	2	2.093	0.351
Boldness			
Predator cue	2	521.250	< 0.001 ^{***}
Photoperiod	1	16.410	< 0.001 ^{***}
Sex	1	73.930	< 0.001 ^{***}
Predator cue × Photoperiod	2	213.410	< 0.001 ^{***}
Predator cue × Sex	2	218.800	< 0.001 ^{***}
Photoperiod × Sex	1	98.020	< 0.001 ^{***}
Predator cue × Photoperiod × Sex	2	41.600	< 0.001 ^{***}
Feeding rate			
Predator cue	2	0.475	0.788
Photoperiod	1	18.704	< 0.001 ^{***}
Sex	1	3.258	0.071
Predator cue × Photoperiod	2	1.344	0.511
Predator cue × Sex	2	2.151	0.341
Photoperiod × Sex	1	4.983	0.026 [*]
Predator cue × Photoperiod × Sex	2	3.820	0.148

466

467 Survival and growth rate during the 5-day exposure period were not significantly affected by
 468 predator cues, photoperiod, sex, or their interactions, although predator cue effects on growth

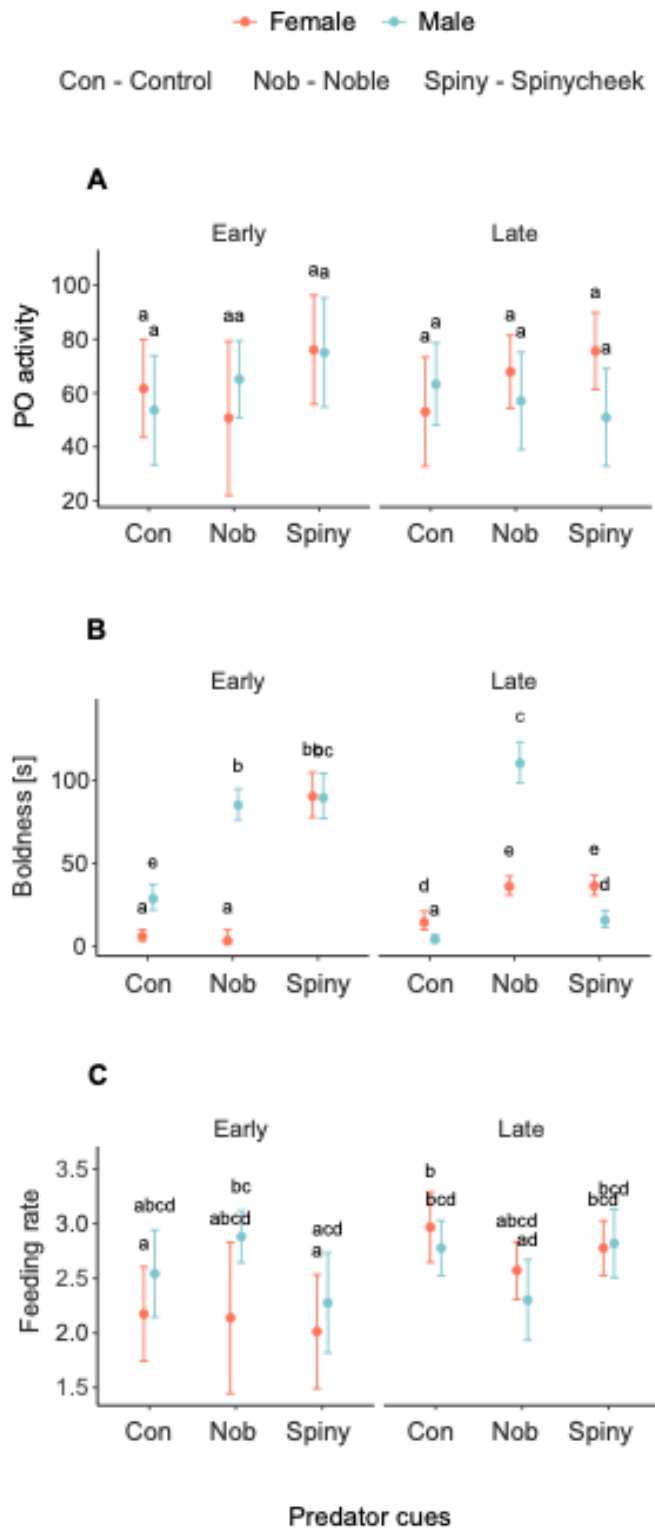
469 rate were marginally non-significant ($p = 0.058$; Table 2). Fat content was likewise unaffected
 470 by predator cues, photoperiod, sex, or their interactions (Table 2).

471 PO activity was characterized by a significant predator cue \times photoperiod \times sex interaction
 472 (Table 2), although pairwise comparisons among treatment combinations were not significant
 473 (Appendix 1: Table S5). Females tended to show elevated PO activity under spinycheek
 474 crayfish cues under the late photoperiod, whereas males showed a similar response to
 475 spinycheek crayfish cues under the early photoperiod (Fig. 4A). Overall, these patterns
 476 suggest that photoperiod and sex modulated physiological responses to predator novelty,
 477 although these effects were weaker than those observed for behaviour.

478 In contrast, behavioural responses showed pronounced differentiation between native and
 479 invasive predator cues that depended strongly on photoperiod and sex (Fig. 4B, Table 2).
 480 Boldness was significantly affected by predator cues, photoperiod, sex, and all interactions
 481 among these factors (Table 2). Under the early photoperiod, females exhibited high boldness
 482 in the control and native noble crayfish treatments but substantially lower boldness in the
 483 invasive spinycheek crayfish treatment (Fig. 4B). Under the late photoperiod, however,
 484 female boldness decreased similarly under both predator cue treatments relative to the
 485 control, indicating reduced differentiation between native and invasive predator responses.

486 Males showed a partially contrasting pattern. Under the early photoperiod, boldness was
 487 similarly reduced under both predator cue treatments relative to the control. Under the late
 488 photoperiod, however, male boldness was lowest in the native noble crayfish treatment but
 489 increased under invasive spinycheek crayfish cues and control conditions (Fig. 4B). Thus,
 490 photoperiod altered both the magnitude and direction of predator-specific behavioural
 491 responses.

492 Feeding rate was not directly affected by predator cues but varied strongly with photoperiod
 493 and sex (Table 2). Feeding rates increased under the late photoperiod, particularly in females
 494 (photoperiod \times sex interaction: $p = 0.026$; Fig. 4C, Table 2). Under the early photoperiod,
 495 males tended to show higher feeding rates than females, whereas under the late photoperiod
 496 females increased feeding rates and became similar to or higher than males across several
 497 predator treatments.



498

499 **Figure 4.** Effect of predator cues (control, noble, and spinycheek crayfish), photoperiod
 500 (early and late), and sex on phenoloxidase activity (PO), boldness, and feeding rate during 5-
 501 day exposure of the final instar larvae (**Experiment 2**) of *I. elegans*. Error bars show 95% CI.

502

503 **Discussion**

504 Our results broadly supported the naïve prey hypothesis (NPH), as damselfly larval responses
 505 to native and invasive alien (IA) predators differed in both magnitude and direction. Across
 506 both experiments, abiotic context (temperature and photoperiod) strongly shaped the
 507 expression of antipredator plasticity. Predator specific responses were often more distinct
 508 under ambient temperature and early photoperiod conditions, whereas warming and late
 509 photoperiod frequently altered, reduced, or reversed these patterns and reshaped which traits
 510 carried the strongest antipredator signal. We only partially supported the hypothesis regarding
 511 exposure duration effects. Survival and growth rate responded more strongly under chronic
 512 than acute exposure, whereas acute exposure elicited the clearest effects on immune function.

513 ***Predator identity matters: native versus invasive alien predator effects (Experiment 1 and***
 514 ***2)***

515 A core prediction of the NPH is that prey responses to invasive predators differ from those
 516 toward native predators due to limited evolutionary experience, potentially resulting in
 517 exaggerated, mismatched, or ineffective antipredator responses (Sih et al. 2010, Carthey and
 518 Banks 2014). Consistent with this framework, cues from the native noble crayfish caused
 519 different responses than cues from the IA spinycheek crayfish across behavioural,
 520 physiological, and life history traits. However, these responses were not consistently stronger
 521 or weaker toward the IA predator. Under warming, chronic exposure to native noble crayfish
 522 cues resulted in the strongest reduction in female growth rate, whereas IA spinycheek
 523 crayfish cues more strongly affected PO activity and boldness, particularly under ambient
 524 temperature and early photoperiod conditions. These patterns suggest that prey distinguished
 525 between native and IA predators, but expressed predator specific responses in a context
 526 dependent manner rather than showing uniformly stronger or weaker responses to the IA
 527 predator.

528 Importantly, predator specific responses were often more distinct under ambient temperature
 529 and early photoperiod conditions, whereas warming and late photoperiod frequently altered,
 530 weakened, or reversed these patterns. One possible explanation is that under more benign
 531 environmental conditions prey had a greater capacity to maintain differentiated antipredator
 532 responses, whereas under warming and strong seasonal time constraints energetic and
 533 developmental demands constrained the expression of predator specific plasticity. Under such
 534 stressful conditions, prey may shift resource allocation toward growth, development, or

535 maintenance rather than costly predator specific defences, resulting in more generalized or
 536 altered responses to predator cues. This aligns with growing evidence that abiotic conditions
 537 interact with predator novelty and constrain prey ability to express differentiated antipredator
 538 strategies (Smolinský and Gvoždík 2012, Carthey and Banks 2014). Together, our results
 539 indicate that the ecological consequences of IA predators depend not only on predator
 540 novelty itself, but also on the environmental conditions under which prey express
 541 antipredator plasticity.

542 ***Warming modified predator-induced responses (Experiment 1)***

543 We predicted that warming would increase predator induced costs by increasing metabolic
 544 demand, as expected from the Metabolic Theory of Ecology, which states that elevated
 545 temperatures accelerate biochemical processes and energy use in ectotherms (Brown et al.
 546 2004). Our results support this prediction, but the effects were strongly trait specific.
 547 Warming altered both the magnitude and direction of predator induced responses rather than
 548 uniformly increasing their costs. PO activity increased under IA spinycheek crayfish cues at
 549 ambient temperature but decreased under warming, suggesting that elevated temperature may
 550 constrain immune investment under IA predator stress. Similar context dependent changes in
 551 immune investment under predation risk have been reported in damselflies and other
 552 ectotherms (Stoks et al. 2006, 200, Khan and Rolff 2025). Together, these results suggest that
 553 warming may limit the energetic resources available for antipredator physiological defence.

554 Behavioural responses to predation risk showed even stronger context dependency. Feeding
 555 activity increased under warming regardless of predator treatment, consistent with elevated
 556 energetic demand at higher temperatures ((Englund et al. 2011, Culler et al. 2014, Debecker
 557 and Stoks 2019). Boldness, however, depended on predator identity, temperature, and sex,
 558 indicating that warming reshaped risk-taking strategies rather than uniformly increasing
 559 caution or compensation. Similar temperature-dependent behavioural shifts have been
 560 reported in odonate larvae and other ectotherms exposed to predation risk (Sentis et al. 2012,
 561 Stoks et al. 2012). Under warming and predator exposure, males were generally bolder than
 562 females, with the strongest sex difference observed under IA spinycheek crayfish cues. Such
 563 sex-specific responses likely reflect differences in energetic allocation and life history
 564 priorities between sexes, as males may benefit more from increased activity and rapid
 565 development, whereas females typically invest more in somatic growth and future
 566 reproduction (Stoks et al. 2006, Dmitriew 2011, Amer et al. 2025). At the ambient

567 temperature, females tended to exhibit greater boldness than males, particularly under native
 568 noble crayfish cues, suggesting that thermal context can alter sex-specific behavioural
 569 responses.

570 Warming also modified the long-term life history consequences of predator exposure.
 571 Survival to emergence was generally lower under warming, while the strongest reduction in
 572 growth rate occurred in females chronically exposed to native noble crayfish cues under
 573 warming. These results suggest that elevated temperature can intensify growth related costs
 574 of predator exposure in some contexts, particularly when exposure is prolonged. Similar
 575 patterns have been observed in damselflies and other ectotherms, where warming altered
 576 energy allocation and growth defence trade-offs under predation risk (Barton et al. 2009,
 577 Sentis et al. 2012, Janssens et al. 2015). However, predator effects were not consistently
 578 stronger under warming. In several traits, warming weakened or altered predator specific
 579 responses, indicating that elevated temperature may constrain the ability to maintain
 580 differentiated antipredator strategies. This interpretation is consistent with studies showing
 581 that warming can reduce the interaction strength and physiologically limit prey responses
 582 under multiple stressors (Englund et al. 2011, Sentis et al. 2012, for the study species: Wang
 583 et al. 2021). Together, our results suggest that warming reshapes predator induced plasticity
 584 by modifying energetic trade-offs and constraining the flexibility of prey responses across
 585 behavioural, physiological, and life history traits.

586 ***Photoperiod modifies predator-induced responses (Experiment 2)***

587 We predicted that photoperiod would modulate responses to native and IA predator cues, and
 588 specifically that late photoperiod would constrain prey ability to maintain predator-specific
 589 antipredator responses, thereby reducing differentiation between responses to native and IA
 590 predators. Our results largely supported this prediction, particularly for behavioural traits.
 591 During predator exposure, behavioural differences between native noble and IA spinycheek
 592 crayfish cues were generally weaker under the late photoperiod, suggesting that strong
 593 seasonal time constraints reduced predator-specific behavioural plasticity. In contrast, under
 594 the early photoperiod, IA spinycheek crayfish cues elicited more divergent behavioural
 595 responses than native predator cues, consistent with prey naïveté toward novel predators (Sih
 596 et al. 2010). Under relaxed seasonal constraints, prey may retain greater energetic and
 597 behavioural capacity to express predator-specific responses, whereas under strong seasonal

598 time constraints prey likely prioritize growth and resource acquisition over adjusted
599 antipredator responses.

600 Before predator exposure, late photoperiod reduced survival and altered growth trajectories in
601 a sex-specific manner. Females showed relatively stable growth rates across photoperiods,
602 whereas males exhibited reduced growth under the late photoperiod, leading to reduced
603 sexual dimorphism in growth trajectories. Although reduced male growth under strong
604 seasonal time constraints was not fully consistent with the expected compensatory increase in
605 growth, the lower survival under late photoperiod confirms that individuals experienced
606 stronger developmental stress. Together, these baseline responses indirectly support our
607 hypothesis that strong seasonal time constraints shift investment toward maintenance and
608 resource acquisition rather than costly predator-specific antipredator responses. Similar sex-
609 specific responses to seasonal time constraints are common in insects and are often linked to
610 differences in reproductive payoffs and growth optima between sexes (Dmitriew and Rowe
611 2005, Stoks et al. 2006, Raczyński et al. 2022b).

612 During predator exposure, the photoperiod did not affect growth or mortality, but strongly
613 modified behavioural responses. Specifically, during the exposure, larvae reared in late
614 photoperiod showed increased feeding activity, particularly in females, suggesting greater
615 urgency to acquire resources under shortened seasonal conditions. Similar increases in
616 foraging under seasonal time constraints have been reported in insects and other ectotherms
617 (McNamara and Houston 2007, De Block et al. 2008, Sniegula et al. 2017). Boldness
618 responses under the late photoperiod were strongly predator and sex dependent. Rather than
619 uniformly increasing risk taking, late photoperiod altered how individuals responded to
620 predator cues. Behavioural differences between native noble and IA spinycheek crayfish
621 treatments were generally weaker under the late photoperiod, suggesting that strong seasonal
622 constraints reduced predator-specific behavioural plasticity. When development time is
623 limited, prey likely prioritise growth and resource acquisition over fine-tuned antipredator
624 responses, as shown in time-constrained insects (Stoks et al. 2012, Salis et al. 2018)
625 In contrast, under the early photoperiod, IA spinycheek crayfish cues elicited more divergent
626 behavioural responses than native predator cues, consistent with prey naïveté to novel
627 predators (Sih et al. 2010). Under relaxed seasonal constraints, prey may retain greater
628 energetic and behavioural capacity to express predator specific responses. Similar context
629 dependency has been reported in aquatic insects and other ectotherms, where invasive

630 predator cues induced stronger behavioural changes than native predators, but mainly in the
 631 absence of additional stressors such as warming or seasonal time constraints (Sniegula et al.
 632 2019b, 2025). Together, these findings suggest that prey naïveté to invasive predators is most
 633 evident when environmental conditions allow predator specific behavioural plasticity to be
 634 fully expressed. This supports the view that behavioural plasticity is multidimensional and
 635 shaped by interacting environmental constraints rather than a single risk axis (Moczek 2010,
 636 Wolf and Weissing 2012).

637 *Exposure duration to predator cues shapes predator-induced responses (Experiment 1)*

638 We predicted that chronic exposure to predator cues would impose greater costs than acute
 639 exposure through cumulative energetic demands and delayed effects across developmental
 640 stages. Consistent with expectations from non-consumptive predator effects theory
 641 (Peckarsky et al. 2008, Clinchy et al. 2013), predator cues had little effect on survival during
 642 either acute or chronic exposure. Similar patterns have been reported in damselflies and other
 643 aquatic insects, where predator cues induced behavioural or life history responses without
 644 directly increasing mortality (Stoks et al. 2012, Sheriff et al. 2020, Raczyński et al. 2022a).

645 In contrast, chronic exposure generated stronger effects than acute exposure on growth rate,
 646 particularly under warming and mainly in females. Similar delayed effects have been
 647 observed in other odonates and amphibians, where predator exposure during larval
 648 development altered adult traits despite limited immediate effects on larval performance
 649 (Relyea 2001, Benard 2004, McPeck 2004). Females showed higher growth rates under
 650 ambient conditions but reduced growth under warming, indicating greater thermal sensitivity
 651 than males. Such sex specific responses may reflect differences in life history strategies, as
 652 females often invest more strongly in somatic growth to maximize future fecundity and may
 653 therefore be more sensitive to environmental stressors that constrain energy allocation
 654 (Blanckenhorn 2005, Stillwell et al. 2010).

655 Interestingly, chronic exposure did not consistently alter adult PO activity or fat reserves,
 656 suggesting that physiological responses were more transient than behavioural or life history
 657 responses. In insects, metamorphosis can partially reorganize physiological and metabolic
 658 processes, potentially decoupling adult physiology from larval investment (Rolff et al. 2019).
 659 Together, our results indicate that exposure duration shapes how predator induced costs are

660 expressed, with acute exposure eliciting stronger behavioural and immune responses, whereas
661 chronic exposure more strongly affected later life history traits.

662 ***Interactions among abiotic and biotic stressors generate context dependent plasticity across***
663 ***trait types***

664 Our results strongly support the prediction that interactions among abiotic and biotic stressors
665 generate context dependent plasticity across trait types. Life history traits were primarily
666 shaped by temperature and seasonal time constraints, whereas behavioural traits showed the
667 strongest and most complex interactions involving predator identity, abiotic stress, and sex.
668 This decoupling among life history, behaviour, and physiology is increasingly recognized as
669 a hallmark of multi stressor responses (Gunderson et al. 2016, Jackson et al. 2016).
670 Behaviour emerged as the most labile trait, consistent with theory and empirical studies
671 showing that behavioural adjustments are often the fastest and most flexible response to
672 environmental change (Wong and Candolin 2015, Amer et al. 2024).

673 Physiological responses, particularly PO activity, were weaker and more context dependent.
674 Predator induced changes in this trait occurred mainly under ambient temperature and early
675 photoperiod conditions and weakened under warming or late photoperiod, consistent with
676 trade-offs between immune investment and other energetic demands (Adamo 2017). In
677 contrast, fat reserves remained largely unaffected, suggesting that energy may have been
678 allocated to growth and behaviour rather than stored over the relatively short timescales
679 examined here. Together, these findings indicate that predator induced responses are
680 expressed differently across trait categories and depend strongly on environmental context.

681 This study has several limitations. Predator cues were derived from only one native and one
682 IA predator species, and therefore some observed responses may partly reflect species-
683 specific effects rather than predator origin alone. However, the native noble crayfish is
684 currently the only native crayfish species present in southern Poland (Sniegula et al. 2025),
685 making it the ecologically most relevant native predator for this system. Predator cues were
686 also used without actual predation events, which may alter the intensity of antipredator
687 responses. In addition, the experimental conditions simplified the environmental variability
688 experienced in natural systems, where fluctuating food availability and habitat structure may
689 further modify predator-prey interactions. Future work incorporating additional invasive

690 predator species and more realistic ecological conditions would help determine how broadly
691 these patterns apply across freshwater communities.

692 **Conclusions**

693 Our results demonstrate that predation risk, warming, and seasonal time constraints interact in
694 non-additive ways to shape life history, behavioural, and physiological traits in *I. elegans*.
695 Rather than producing uniform effects, warming and photoperiod reshaped predator specific
696 responses depending on trait type, sex, and environmental context. Behavioural traits showed
697 the strongest and most variable plasticity, whereas life-history traits were more consistently
698 constrained by abiotic conditions and exposure duration to predator cues.

699 Our study further suggests that predator identity matters most under environmentally benign
700 conditions, when prey retain sufficient energetic reserves and behavioural flexibility to
701 express predator specific responses. Under warming and strong seasonal time constraints,
702 these differences were often reduced or altered, indicating that abiotic stress can constrain the
703 expression of predator specific plasticity. Overall, our findings highlight the importance of
704 considering interactions among predator novelty, abiotic stressors, developmental stage, and
705 sex when predicting predator prey interactions under ongoing environmental change.

706 **Funding**

707 NRA and SS acknowledge support from the National Science Centre, Poland (Grant number:
708 2019/33/B/NZ8/00521); MJG, GW, and SS acknowledge support from the Institute of Nature
709 Conservation Polish Academy of Sciences. RS acknowledges support from FWO Flanders.

710 **Conflict of interest statement**

711 The authors have declared that no competing interests exist.

712

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978

979 **Appendix 1 captions**

980 Supplementary material for: Warming and photoperiod differentially mediate native and
981 invasive alien predator effects on damselfly fitness traits.

982 Developmental trajectories and synchronization of larval measurements: Description of
983 developmental trajectories (preemergence vs. regulatory development) and synchronization
984 of larval measurements across temperature treatments in *Ischnura elegans*.

985 Table S1: Weekly changes in photoperiod and temperature conditions used in the ambient
986 temperature treatment (22°C) under the early photoperiod regime in Experiment 1.

987 Table S2: Weekly changes in photoperiod and temperature conditions used in the warming
988 temperature treatment (26°C) under the early photoperiod regime in Experiment 1.

989 Table S3: Weekly changes in photoperiod and temperature conditions used in the ambient
990 temperature treatment (22°C) under the early and late photoperiod regimes in Experiment 2.

991 Table S4: Sample sizes across temperature, photoperiod, predator cue, sex, and
992 developmental stage treatments in Experiments 1 and 2.

993 Table S5: Results of Tukey's HSD post hoc comparisons for significant effects of predator
994 cues, temperature, photoperiod, sex, and developmental stage on life-history, behavioural,
995 and physiological traits in Experiments 1 and 2.

996 Figure S1: Seasonally changing photoperiod regimes used during the experiment for the early
997 and late photoperiod treatments.

998 Figure S2: Seasonal water temperature dynamics recorded in Płaszowski pond, the field
999 collection site of *Ischnura elegans*, during 2023.

1000 Figure S3: Experiment 1. Effects of temperature treatment on survival and growth rate at the
1001 entrance into the final larval instar (F-0) in *Ischnura elegans*.

1002 Figure S4: Experiment 2. Effects of photoperiod treatment on survival and growth rate at the
1003 entrance into the final larval instar (F-0) in *Ischnura elegans*.