

**Seasonal time constraints shape life history, physiology and behaviour independently,
and decouple a behavioural syndrome in a damselfly**

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Abstract

The integration of traits into 'syndromes' has been suggested as a useful framework to advance insights in trait responses to environmental stressors. Yet, how stressors shape the consistency ('repeatability') of traits and their covariation at the individual level remains debated. We studied how seasonal time constraints shape trait repeatability and integration of life-history, behavioural, and physiological traits along a fast-slow continuum, using the 'pace-of-life syndrome' as a framework. We manipulated the photoperiod during the larval development of the damselfly *Ischnura elegans*, generating a time-relaxed early, a control, and a time-constrained late group. The photoperiod treatment did not seem to affect the voltinism of the larvae. As predicted, late-period larvae accelerated development and growth, yet this acceleration was no longer detectable for growth and metabolic rate during the final instar, possibly due to costs of the initial life-history acceleration. This warrants caution when inferring a species' pace-of-life based on a specific developmental stage. The late-period larvae were as predicted more active (only during the later stages of the final instar) and bolder than the control larvae, but not different from the early-period larvae. Most studies on time constraints only compared late and control animals, thereby potentially wrongly concluding adaptive responses to time constraints. Activity, boldness, and body mass were repeatable, while growth and metabolic rates were not. Notably, repeatabilities did not change under time constraints. There was no support for an overall trait integration in a pace-of-life syndrome, yet activity and boldness covaried positively as expected. Importantly, this 'behavioural syndrome' was decoupled in the late-period larvae, which might be adaptive to enhance energy acquisition to fuel the accelerated development rate. Our results suggest that besides the predicted plastic acceleration of life-history, plastic changes in behavioural trait integration may also be an important but overlooked adaptive aspect of responding to time constraints.

Key words: behavioral syndromes, pace-of-life, personality, time stress, trait integration, photoperiod

Introduction

The view that traits are often organized in sets of covarying traits, so-called syndromes (Stevens et al. 2014) is getting increased attention. The motivation for looking at syndromes is that organisms are integrated entities characterized by multiple and potentially correlated traits, and as a result, their plastic and evolutionary responses to environmental stressors may involve coordinated changes in many traits (Schlichting and Pigliucci 1998, Schlichting and Wund 2014, Forsman 2015). Taking into account the integration of traits into syndromes is therefore crucial to advance insights in trait responses to environmental stressors (e.g. Carnicer et al. 2013, Killen et al. 2013, Stevens et al. 2014, Boyle et al. 2016, Cayuela et al. 2016).

The integration of a comprehensive set of traits, including life-history, behavioural, and physiological traits, termed the ‘pace-of-life syndrome’ (POLS), has triggered a surge of interest in the fields of ecology and evolution (Réale et al. 2010). An individual with a fast pace-of-life is expected to have a specific combination of traits: a fast growth and development, a high metabolic rate, an active, bold, and aggressive personality, and a short lifespan (Réale et al. 2010). The idea that individuals can be ordered along an axis of slow-to-fast POLS assumes some consistency in their individual ranking, hence these POLS traits are assumed to be repeatable (Réale et al. 2010, Niemelä and Dingemanse 2018). The integration of certain subset of ‘POLS traits’ into a syndrome is often being reported, with as notable example the ‘behavioural syndromes’, such as the well-documented positive covariation between activity and boldness (Sih et al. 2004). Nevertheless, support for a complete trait integration into a POLS, including their repeatability, is currently mixed (Royauté et al. 2018).

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How environmental stressors shape the strength and consistency of trait integration and the repeatability of traits is highly debated (Killen et al. 2013, Montiglio and Royauté 2014, Hämmäläinen et al. 2019). On the one hand, environmental stressors such as predation stress can establish new (or enhance already-present) trait correlations, as shown among behavioural traits (Bell and Sih 2007, Adriaenssens and Johnsson 2013), possibly to achieve an optimal trait combination that would increase fitness under these new conditions (Sih et al. 2004). On the other hand, environmental stressors such as toxicants can uncouple previously-integrated trait sets, possibly because of stress-related disturbance in the neuroendocrine and developmental mechanisms (Royauté et al. 2015a, Dziewieczynski et al. 2016). Overall, moderate environmental stressors are predicted to strengthen trait integration (Killen et al. 2013), yet the limited empirical evidence reveals no clear pattern how the trait integration of the POLS responds to stress (Hämmäläinen et al. 2019). Environmental stressors are also predicted to effect trait repeatabilities (Bell et al. 2009, Killen et al. 2016), yet the direction of change is often challenging to predict for many stressors (e.g. for contaminant exposure: Montiglio and Royauté 2014, Royauté et al. 2015a). Nevertheless, the emerging view from empirical studies is that repeatability estimates typically increase following exposure to stress (e.g. predation stress: Toscano et al. 2014, Ehlman et al. 2019, dietary restriction: Careau et al. 2014, Royauté and Dochtermann 2017).

A common environmental stressor in the larval stage of many animals is time constraints associated with seasonality, such as the need to reach a certain developmental stage before winter or to metamorphose before pond drying (Rowe and Ludwig 1991, Abrams et al. 1996). The typical reaction to time constraints as predicted by theory is

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shifting to an accelerated development, and if possible also an accelerated growth rate to keep size at metamorphosis constant (Rowe and Ludwig 1991, Werner and Anholt 1993, Abrams et al. 1996). Responses to seasonal time constraints in other traits, however, received much less attention. While accelerated development and growth due to time constraints is expected to be accompanied by an increase in metabolic rate (Réale et al. 2010), evidence for this prediction is limited in the context of time constraints (but see Stoks et al. 2006). Theory also predicts behavioural adjustments to seasonal time constraints, with bolder and more active behaviour in the time-constrained animals mediating the accelerated resource-intensive life style (Rowe and Ludwig 1991, Werner and Anholt 1993, Abrams et al. 1996). The few empirical studies provided some support for this prediction (Johansson and Rowe 1999, Berger and Gotthard 2008, Sniegula et al. 2017, Guenther et al. 2018, Golab et al. 2020, but see De Block and Stoks 2003, Orizaola et al. 2016). While seasonal time constraints have been predicted and are empirically known to elicit plasticity in the expression of life-history, behavioural, and physiological traits, consistent with a faster POLS in more time-constrained groups, it is currently unknown whether they can also alter trait repeatability and trait integration patterns at the individual level.

In this study, we aimed to test for effects of seasonal time constraints on life history, metabolic rate, and behavioural traits in terms of their i) mean responses, ii) repeatability estimates, and iii) covariation patterns. For this, we manipulated the photoperiod during the larval development of the damselfly *Ischnura elegans* in a laboratory experiment thereby generating three groups: a control group, a group with relaxed and a group with higher time constraints. Notably, we therefore were able to experimentally test for POLS patterns at the

level of the treatment groups and at the level of individuals within these groups. The POLS assumes these patterns to be consistent across both levels (Réale et al. 2010). For the period between egg hatching and reaching the final larval instar, we measured development rate, growth rate and body mass, whereas during the final instar, we repeatedly measured growth rate, metabolic rate, activity and boldness. Note that by testing larvae at two distinct developmental periods (pre-final instar / final instar), we could also test for ontogenetic consistency of the pace-of-life. Based on theory (Rowe and Ludwig 1991, Werner and Anholt 1993, Abrams et al. 1996) and empirical studies (e.g. Gotthard 2000, Margraf et al. 2003, Mikolajewski et al. 2015, Burraco et al. 2020), we predicted that the photoperiod regime mimicking a delayed time point in the season would impose stronger time constraints on larvae, hence resulting in a faster pace-of-life with accelerated development and growth rates, higher metabolic rates, and more active and bolder behaviour (Réale et al. 2010). We further predicted stronger repeatability and stronger trait covariation patterns with stronger time constraints (Killen et al. 2013, 2016).

Material and Methods

Study species and collection

We used the damselfly *Ischnura elegans*, an abundant species widely occurring in Europe (Dijkstra and Lewington 2006). We collected 33 mated females from a single population in Oud-Heverlee, Belgium (50°50'29.79"N, 4°39'31.83"E) during the second half of July 2018. Females were provided with wet filter papers for oviposition. All eggs hatched within the first week of August. The species has three females colour morphs, and larval

development rate can depend on the morph type of the mother (Abbott and Svensson 2005). Therefore, we also noted the morph type of collected females.

Experimental procedure

To start the experiment, each egg clutch was divided into three and placed in 500 mL cups filled with dechlorinated tap water, and kept in separate temperature-controlled incubators at 20 °C, at one of the three photoperiod treatments: light regimes of 5 June (“early period”), 17 July (“control period”), and 28 August (“late period”). The early and late photoperiod treatments correspond to natural light regimes six weeks earlier and later, respectively, compared to the average oviposition date, 17 July (Fig. A1 in Appendix). This entire 12-week period is embedded in the natural flight period of the species in Flanders.

After hatching, larvae were kept individually in 100 mL plastic cups filled with dechlorinated tap water, and placed in an incubator of the corresponding photoperiod treatment. Larvae were fed daily nauplii of *Artemia* five days a week (average \pm SE of daily dose = 226 ± 69 , $n = 10$ feeding portions). Throughout the experiment, including the winter period (see below), photoperiods in incubators were adjusted weekly to simulate the natural progress of the light regime.

The study species overwinters in the larval stage. To simulate this winter period, we gradually ($\sim 1.5^\circ\text{C}$ per week) decreased the temperatures in the incubators to 5°C when the light regime corresponded to the end of November (Fig. A1). During the winter period (dates shown in Table A1), we reduced the feeding frequency to three times a week and halved the feeding portions, as damselfly larvae drastically reduce their growth and food intake at such cold temperatures (Verheyen et al. 2018). After the winter period, we gradually ($\sim 3^\circ\text{C}$ per day) increased the temperatures in the incubators to 20°C (Fig. A1). In

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addition, the photoperiod of the incubators, independent of their assigned pre-winter photoperiod treatment, was set to the light regime corresponding with 1 April. From then onwards, photoperiods in incubators were again adjusted weekly to simulate the natural progress of the light regime. This procedure mimics the realistic scenario where early- and late-hatched larvae experience the same photoperiods at different sizes (hence developmental stages) (Strobbe and Stoks 2004, Mikolajewski et al. 2015, see Fig. A1 in Appendix). Both before and after winter, late period larvae would encounter the same photoperiod at a smaller size (earlier developmental stage) than the early period larvae, as they started with a later photoperiod at birth. Therefore, late period larvae are expected to perceive the same pre- and post-winter photoperiods as indicating a higher time constraint (as shown in damselfly larvae: Strobbe and Stoks 2004, Mikolajewski et al. 2015).

Note that the developmental stages of larvae entering (hence also exiting) the winter period differed across the three photoperiod treatments, whereby the early-period larvae reached the most advanced developmental stage, and the late-period larvae reached the least advanced stage. This resulted in the early-period larvae having a shorter post-winter period at 20 °C before reaching the final larval (F0) stage compared to the late period larvae (Table A1 in Appendix). To assess whether this shorter ‘thermal acclimation time’ influenced the growth rate during the F0 stage, we used a separate set of 10 advanced stage (F1 [i.e. penultimate] stage) and 9 less-advanced stage (< F1 stage) larvae, all belonging to the early period, to test whether their time reaching the F0 stage had an effect on their 5-day growth rate during the F0 stage (see below for the protocol of calculating growth rate). Acclimation time (i.e. number of days between the end of winter and reaching F0 stage) of these larvae ranged between 1 and 45 days. Results suggested no effect of acclimation time

on growth rates during the F0 stage (linear regression, $F_{1,15} = 0.48$, $p = 0.498$, $R^2 = 0.04$, partial $\eta^2 = 0.03$), which was confirmed for both the advanced and less-advanced stages (Acclimation duration \times Development class: $F_{1,15} = 0.07$, $p = 0.793$, Development stage: $F_{1,15} = 0.04$, $p = 0.834$).

Response variables

After the winter period, we checked daily for larvae reaching the final instar (F0). As responses to time constraints can be sex-specific in damselflies (De Block and Stoks 2003), we used only male larvae to exclude this source of variation. Development time was calculated as the period between egg hatching and reaching the F0 stage. Growth rate until F0 was calculated as $\ln(\text{mass at day 1 of F0})/\text{development time}$. Once in the F0 stage, we measured individual growth rate, behaviour, and metabolic rate at two different time points.

To obtain two successive estimates of growth rate during the F0 stage, we measured the increase in wet body mass from day 1 to 6, and from day 15 to 20 after moulting into F0. We weighed larvae to the nearest 0.01 mg using an electronic balance (AB135-S, Mettler Toledo, Columbus, OH, USA) after gently blotting them dry with tissue paper. The two successive growth rates were calculated as $[\ln(\text{mass}_{\text{day6}}) - \ln(\text{mass}_{\text{day1}})]/5$ days, and $[\ln(\text{mass}_{\text{day20}}) - \ln(\text{mass}_{\text{day15}})]/5$ days. No larvae showed any external signs of reaching metamorphosis (i.e. enlarged wing structures, pigmented eyes) at day 20.

To obtain two successive estimates of behaviour and metabolic rate, we assayed larvae first when between 2 and 5 days into F0, and a second time when between 16 and 20 days into F0. Larvae were starved 24 h before trials to standardize satiation levels. We measured two behavioural traits, activity (i.e. distance travelled) and boldness (i.e. latency to resume activity after disturbance), often used as personality traits and integrated within

the pace-of-life syndrome (Réale et al. 2010). The day before the trials, we placed larvae individually in plastic containers (10.5 x 7.5 x 2.5 cm) with 100 mL of dechlorinated tap water. For activity measurements, we video-recorded (25 fps) larvae for 10 min in their containers (10.5 x 7.5 x 2.5 cm, with 100 mL of dechlorinated tap water). Recordings were analysed using the automated tracking software Ethovision XT 15 (Noldus Information Technology Inc., Wageningen, The Netherlands). Next, boldness trials were conducted in the same containers. Damselfly larvae typically react to a (simulated) predator attack by escaping with a swimming burst, followed by an immobile period (for the study species: Gyssels and Stoks 2005). To simulate a predator attack and initiate an escape response, we disturbed the larvae by touching their caudal lamellae with a plastic probe (as in Brodin 2009, Debecker and Stoks 2019). We video-recorded the larvae for 15 min to measure the latency to resume activity (i.e. the duration between the end of the swimming burst and the moment the larvae became active again). On 11 occasions (~ 4% of all trials) larvae did not resume activity within the 15 min, hence were given the maximum score of 900 s. Bolder individuals are those having shorter latency times (Réale et al. 2007).

After each of the two behavioural trials, we measured oxygen consumption of the larvae as a proxy for their standard metabolic rate (following Stoks et al. 2006, Debecker and Stoks 2019). Shortly, we placed individual larvae in transparent plastic 60 mL vials, filled with oxygenated and dechlorinated tap water, and measured the reduction in oxygen concentration after 7 hours in darkness. We used oxygen sensors (PSt3, PreSens, Regensburg, Germany) and a portable oxygen meter with a fibre-optic cable attached (Fibox 4 trace, PreSens, Regensburg, Germany) for the measurement of oxygen concentrations to the nearest 0.1 mg/L. After correcting for the change in oxygen

concentrations in control vials without larvae, individual metabolic rate was expressed as mg/L oxygen consumed per hour.

Sample sizes for development time and growth rate until F0 were between 107 and 110 per photoperiod treatment (total of 325 larvae). For feasibility, we used a subset of these animals (between 40 and 42 per photoperiod treatment, total of 124 larvae) for the repeated trials during F0, i.e. for behaviour, metabolic rate and growth rate.

Statistical analyses

We used separate linear mixed effect models to test for effects of the photoperiod treatment on development time, mass at F0, and growth rate until F0. These models included photoperiod treatment (categorical: early/control/late period) as a fixed effect, and the mother identity as random effect to account for the non-independence of larvae from the same mother. We additionally added female morph type as fixed effect as this may affect the larval development time. The number of days between the end of the simulated winter period and reaching the F0 stage differed between the treatment groups (Table A1). To correct for any introduced variation in growth rates due to differences in the exposure duration to warm temperatures, i.e. 20 °C, during the post-winter period, we included this parameter as a covariate to the ‘growth rate until F0’ model. For traits measured during the F0 stage, i.e. growth rate, activity, boldness, and metabolic rate, we used separate linear mixed effect models with photoperiod treatment and trial phase (categorical: first/second trial), and their interaction, as fixed effects, and the larval identity (nested in mother identity) as a random effect to account for repeated measures. For the activity, boldness and metabolic rate models, we included date of trial as an additional random effect, given the non-independence between measurements in a given day. These models further included

body mass as a time-varying covariate to account for the potential mass-dependency of tested traits. Body mass was removed from the models when not significant. Finally, we re-ran the growth rate model with body mass included as a time-varying covariate, to account for effects of initial mass at the start of each period on the successive growth rates (Nicieza and Álvarez 2009). We used linear contrast tests to assess differences between the photoperiod treatments, as well for further testing interaction effects.

We estimated repeatabilities for traits that were tested twice during the F0 stage (i.e. growth rate, activity, boldness, and metabolic rate) as the ratio of between-individual variance to the total phenotypic variance (Nakagawa and Schielzeth, 2010). We additionally estimated repeatability for body mass, for which we had four data points per individual. We calculated trait repeatabilities across the three photoperiod treatments, as well as separately for each photoperiod treatment, and assessed significance of among-treatment differences in repeatabilities based on the overlap of the 95% confidence intervals (Nakagawa and Cuthill 2007).

To test for trait integration and whether the covariation patterns between traits differed across the photoperiod treatments, we applied a two-step selection criterion. First, we conducted principal component analyses (PCA) per photoperiod treatment on the traits measured during the final instar (growth rate, metabolic rate, activity and boldness). To obtain robust patterns, we thereby used averages across the two trials. In this exploratory step we did a qualitative assessment of changes in the strongest factor loadings across the three photoperiods. This PCA was used to identify trait pairs that loaded on the same PC but with different patterns among treatments, which was only the case for the two behaviours. In a second, confirmatory step, we formally tested for a change in covariation

across photoperiod treatments for the trait pair identified in step 1 (hence the behavioural traits). For this, we constructed linear mixed effect models with one trait as the response variable, and the other trait and its interaction with the photoperiod treatment as fixed effects (see Debecker et al. 2016, Tüzün et al. 2017 for a similar approach). Unlike in the PCA approach, to account for the repeated-measures structure, we used traits measured over both trial phases instead of the averages, hence also included the trial phase as an additional fixed effect term to the model, and the larval identity as random factor. A significant interaction between the trait value and the photoperiod treatment would indicate that the (strength of the) covariation between the two traits in question depends on the photoperiod treatment.

All analyses were performed with R v.3.5.3 (<www.r-project.org>), using the packages ‘lme4’ for mixed-effect models (Bates et al. 2015), ‘emmeans’ for linear contrast tests (Lenth 2020), and ‘rptR’ to calculate repeatabilities (Nakagawa and Schielzeth 2010). Activity and boldness were log+1 transformed to meet model assumptions of normality. Note that using the mother identity as random effect, we also estimated broad-sense heritability of traits as the ratio of total genetic variance to total phenotypic variance.

Results

Survival until F0 was ~82%, and did not differ between the photoperiod treatments (see Table A2 in Appendix for details). Broad-sense heritability estimates ranged between 0 (growth rate during F0) and 0.355 (development time until F0) (see Table A3 in Appendix for details).

Effects of photoperiod on average trait levels

The photoperiod treatment affected all three life-history traits measured until the final larval (F0) stage (Fig. 1). Development times were ~12 days shorter in the late-period group compared to the control (contrast: $p < 0.001$) and early-period ($p < 0.001$) groups, which did not differ ($p = 0.943$) ($F_{2,299} = 48.92$, $p < 0.001$, Fig. 1A). The colour morph of the mothers influenced the offspring development rates ($F_{2,322} = 3.26$, $p = 0.039$), with offspring of ‘Infuscans-obscura’ mothers having shorter development times than those of ‘Androchrome’ mothers ($p = 0.030$; other contrasts: $p > 0.279$) (Fig. A2 in Appendix). Larval body mass at F0 gradually decreased with later photoperiods ($F_{2,310} = 90.44$, $p < 0.001$, all contrasts: $p < 0.001$, Fig. 1B), whereas growth rates between egg hatching and reaching F0 gradually increased with later photoperiods ($F_{2,316} = 403.73$, $p < 0.001$, all contrasts: $p < 0.001$, Fig. 1C).

The photoperiod effect on growth rate during F0 depended on the time interval in which growth rate was calculated (Photoperiod \times Growth rate interval, Table 1, Fig. 2A). During the first week of the F0 stage (i.e. day 1-6), late photoperiod larvae had slower growth rates than the control group ($p = 0.005$, all other contrasts $p > 0.236$), whereas during the third week of the F0 stage (i.e. day 15-20), the photoperiod treatment showed no effect (all contrasts $p > 0.382$). On average, growth rates were faster in the first week compared to the third week (Table 1). Including body mass as a time-varying covariate did not change the time-dependent photoperiod effect on growth rates (Photoperiod \times Growth rate interval, $F_{2,212} = 3.49$, $p = 0.032$).

Mass-corrected metabolic rate depended on the photoperiod treatment (Table 1, Fig. 2B). Early-period larvae had a lower metabolic rate compared to those of the control ($p = 0.012$) and late-period ($p = 0.011$) groups. The control and late-period groups had similar

metabolic rates ($p = 0.962$). This ranking was consistent across the two trials (Table 1).

Metabolic rate was higher in the second trial (Table 1, Fig. 2B). Larvae with larger body mass had higher metabolic rates ($\text{slope} \pm \text{SE} = 0.007 \pm 0.001$, $p < 0.001$).

The photoperiod treatment affected both activity and boldness behaviours (Fig. 3), yet for activity, this effect depended on the trial phase (Table 1). While the photoperiod had no effect on activity during the first trial (all contrasts $p > 0.493$), during the second trial, both the late- and the early-period groups were more active compared to the control group (early vs control, $p = 0.033$, late vs control, $p < 0.001$), whereas the late- and early-period groups did not differ ($p = 0.385$) (Fig. 3A). Across trial phases, latency to move was shorter (i.e. bolder) in the late compared to control period ($p = 0.003$), whereas latency times did not differ between early and control ($p = 0.145$) and between early- and late-period larvae ($p = 0.473$) (Fig. 3B).

Repeatability estimates

The two behaviours, activity and boldness, and body mass were significantly repeatable within the F0 stage (range 0.270-0.404). In contrast, growth rate during F0 and metabolic rate did not show significant repeatabilities (Table 2). Strongly overlapping 95% confidence intervals of repeatabilities calculated separately per photoperiod suggested no effect of the photoperiod treatment on repeatability estimates (Table 2).

Covariation patterns

The PCA, conducted separately for each photoperiod group, revealed that activity and latency time were consistently the strongest contributors with opposite signs on the first PC axes, whereas growth rate and metabolic rate were the most dominant factor loadings separately on the other two PC axes (Table 3, Fig. A3 in Appendix). However, in the late-

period group, latency time had a somewhat lower absolute loading (0.71) on PC1 compared to the other photoperiod groups (> 0.87) (Table 3). A linear mixed effect model revealed that the relationship between activity and latency time depended on the photoperiod treatment (linear mixed-effect model: $F_{2,326.9} = 4.58$, $p = 0.011$, Fig.4): the negative covariation between activity and latency time in the early-period (slope [95% CI] = -0.301 [-0.436, -0.167], $p < 0.001$, Fig.4A) and control treatments (-0.216 [-0.319, -0.112], $p < 0.001$, Fig.4B) was not present in the late-period larvae (-0.050 [-0.157, 0.058], $p = 0.362$, Fig.4C), confirming the findings of the PCA.

Discussion

The late photoperiod treatment induced the expected acceleration of the life-history pace (i.e. faster development and growth) until reaching the final instar. During their final instar, late-period larvae, however, did not seem to keep up the fast pace in terms of growth rate or metabolic rate. Yet, both activity (during the second trial) and boldness levels were higher in the time-constrained late-period larvae compared to the control group (but similar to the early-period group), partly in agreement with models suggesting that behavioural modifications can mediate the faster development under time constraints (Rowe and Ludwig 1991, Werner and Anholt 1993, Abrams et al. 1996). Among the traits measured during the final larval instar, the two behaviours (activity and boldness) and body mass were repeatable, while growth and metabolic rates were not. While there was no support for an overall trait integration, i.e. the ‘pace-of-life syndrome’, the two behaviours covaried as expected, but notably this ‘behavioural syndrome’ was decoupled in the late-period larvae.

Note that our results indicate that all animals in the experiment showed a univoltine life cycle, hence an entire life cycle spanning a full year. Given the early photoperiod treatment simulated a hatching date of early June, some of the early animals could in principle have shown a bivoltine life cycle (two generations per year). The lack of bivoltinism may be due to suboptimal conditions (e.g. temperature, food quantity and quality) provided in the laboratory. In addition, given that all females were caught in late summer (end of July), maternal effects may have steered the offspring towards a univoltine life cycle. Previous work with damselflies revealed maternal effects on the life-history of the offspring (Sniegula et al. 2016, but see for the study species: Shama et al. 2011), which can potentially be adaptive (Uller 2008).

Influence of seasonal time constraints on average group trait levels

As predicted by theory (Rowe and Ludwig 1991, Abrams et al. 1996), organisms under time constraints often increase development rate to avoid delayed emergence, and may thereby increase growth rates to avoid a reduction in size at maturity. Damselfly larvae responded to the delayed photoperiod treatment by accelerating both development and growth rates, in accordance with previous empirical findings (e.g. Gotthard 2000, Margraf et al. 2003, Burraco et al. 2020, in damselflies: e.g. Johansson et al. 2001, Sniegula et al. 2012, Mikolajewski et al. 2015). Despite this life history acceleration, the time-constrained larvae could not fully catch up in terms of development time and mass when entering the final instar; indeed late-period larvae entered the final larval stage ~12 days later and ~5% lighter compared to the control group (see e.g. also Stoks et al. 2006). If consistent throughout the final instar, such an accelerated life history may have negative

consequences, as delayed transition into the adult stage and a smaller adult mass are known to decrease fitness in odonates (Sokolovska et al. 2000, De Block and Stoks 2005).

Interestingly, the accelerated growth rate in the late-period group was no longer visible once in the final larval instar. Similarly, in the related damselfly *Coenagrion puella* with a comparable long flight season, growth rates in time-constrained larvae were only increased before winter but not after winter (Mikolajewski et al. 2015). This was explained as late larvae, that have a shorter pre-winter growth period, being under stronger selection to reach a large enough body mass and energy reserves before winter (De Block et al. 2008, Verheyen et al. 2018). Instead, the long flight season allows more opportunity for mass compensation in the adult stage before reproduction starts (Anholt et al. 1991, Hyeun-Ji and Johansson 2016), thereby relaxing time constraints on mass increase after winter (Strobbe and Stoks 2004, Mikolajewski et al. 2015). In our study, time-constrained late-period larvae even had a decreased growth rate during the first week in the final larval stage. Possibly, these larvae suffered increased oxidative damage linked to accelerated growth before winter, which asked for more energy being allocated to antioxidant defence and repair after winter at the expense of a high investment in growth (Monaghan et al. 2009). Such oxidative cost of rapid growth has been shown in the strictly univoltine damselfly *Lestes viridis* (Janssens and Stoks 2020).

The more relaxed time constraints after winter, and the associated absence of a growth acceleration in the final larval stage may have contributed to the absence of increased metabolic rates in the late-period final instar larvae. Note that the absence of a higher metabolic rate in late-period larvae is not driven by these larvae being smaller, as the pattern was absent even when correcting for body mass. A faster life history has been

associated with higher metabolic rates (Réale et al. 2010, Arnqvist et al. 2017, for damselfly larvae: Stoks et al. 2006), yet many counterexamples exist. For example, the lines of the cricket *Gryllus integer* selected for a faster life history showed even a lower resting metabolic rate (Krams et al. 2017, 2018).

Theoretical models predict increased foraging activity and risk-taking (i.e. boldness) behaviour to mediate the increase in development rate under time constraints (Rowe and Ludwig 1991, Werner and Anholt 1993, Abrams et al. 1996), yet few studies actually tested for behavioural responses to altered seasonal time constraints. We found only weak support for a higher activity under time constraints, and only in the second trial phase the late-period larvae were more active than the control larvae (yet as active as the early larvae).

Work on insects mostly reported increased (foraging) activity under time constraints (Johansson and Rowe 1999, Johansson et al. 2001, Stoks et al. 2005, Sniegula et al. 2017, Golab et al. 2020), but in the damselfly *L. viridis* no change in activity under time constraints was observed (De Block and Stoks 2003), while the only study in anurans found decreased activity under time constraints in the moor frog *Rana arvalis*, which was suggested to be an energy saving strategy (Orizaola et al. 2016). Also for the much less tested, theoretically predicted increase in behavioural risk-taking under seasonal time constraints (Rowe and Ludwig 1991, Werner and Anholt 1993, Abrams et al. 1996), support was mixed and potentially misleading. In line with the few other studies in insects, late-period larvae were as predicted bolder than control larvae. This matches the few other insect studies showing that, under time constraints larvae of the butterfly *Hipparchia semele* shifted toward more risky daytime feeding (Berger and Gotthard 2008), and larvae of the damselfly *L. sponsa* retained a higher activity under predation risk (Stoks et al. 2005,

Golab et al. 2020). Similarly, in the only vertebrate example, the caviies *Cavia aperea* showed accelerated development and bolder personalities when exposed to simulated spring conditions (Guenther et al. 2018). While no acceleration in growth occurred during the final instar, the bolder personality of the late-period larvae may be linked to the accelerated development induced by time constraints. Notably, however, this higher boldness in late-period larvae was only true relative to the control larvae and not relative to the, if anything, more time relaxed, early-period larvae, suggesting the differences in boldness are only superficially supporting theoretical predictions based on time constraints.

Repeatability and seasonal time constraints

In line with previous findings, the two behaviours, activity and boldness, were repeatable (Bell et al. 2009, for damselfly larvae: Slos and Stoks 2006, Brown and Robinson 2016, Debecker et al. 2016, Tüzün et al. 2017). Metabolic rate, on the other hand, was not repeatable in our study, contradicting the general pattern (reviews in Biro and Stamps 2010, Holtmann et al. 2017), although several other studies also reported resting metabolic rate not to be repeatable (e.g. guppies: Biro et al. 2016, cavy: Finkemeier et al. 2016). Growth rates during the final instar were also not repeatable, while body mass was. Although body size and mass are frequently reported to be repeatable (e.g. Gifford et al. 2014, Royauté et al. 2015b, Royauté and Dochtermann 2017, Polverino et al. 2018), the often more relevant metric of change in mass over time, i.e. growth rate, has rarely been tested for repeatability. As a notable exception, growth rates of crayfish were repeatable in one study (Biro et al. 2014), but not in another (Raffard et al. 2017). One reason for traits such as metabolic rates and growth rates not being repeatable is age-dependent effects on these variables to differ between individuals (e.g. Finkemeier et al. 2016).

Although trait repeatability is often suggested to respond to environmental factors experienced during development (e.g. Careau et al. 2014, Killen et al. 2016, Royauté and Dochtermann 2017, but see DiRienzo and Montiglio 2016), repeatability estimates reported here did not seem to be influenced by the seasonal time constraints. Among the very few studies manipulating photoperiods, results indicate little (cavy: Finkemeier et al. 2016) or no (canary: Amy et al. 2017) evidence for an effect of seasonal time constraints on trait repeatability.

Trait covariation patterns and time constraints

We found limited support at the individual level for the influential hypothesis that life-history, physiological and behavioural traits integrate into a pace-of-life syndrome (POLS, Réale et al. 2010) where fast growth is expected to be associated with a high metabolic rate, a higher activity and higher boldness. In contrast, the PCA indicated that growth rate did not associate with metabolic rate, neither did any of these traits associate with activity or boldness. The POLS hypothesis has received mixed evidence over the recent years (Royauté et al. 2018). Metabolic rate, for example, has been suggested as ‘pace-maker’ for the pace of life, yet this idea has often been challenged (Glazier 2015). A meta-analysis showed that while bolder individuals tend to have higher metabolic rates, this link is relatively weak (Niemelä and Dingemanse 2018). Our findings thereby are in line with previous results on the study species that metabolic rate only weakly integrates with behavioural traits (Debecker and Stoks 2019). Growth rate, being central to many life-history trade-offs (e.g. growth-mortality trade-off), has also been suggested to play an important role in shaping behavioural traits, with fast growing individuals predicted to be bolder and more active for maximising energy uptake (Stamps 2007, Wolf et al. 2007, Biro

and Stamps 2008). As with metabolic rate, we found no support for the expected covariation between growth rate and the behavioural traits. Damselfly larvae may generate higher growth rates without an increase in foraging activity by increasing the efficiency of their digestive physiology (Stoks and McPeck 2003; Stoks et al. 2005), potentially resulting in no relationship between growth rate and behaviour.

Instead of a complete pace-of-life syndrome, the trait integration was limited to the two behaviours, activity and boldness. The finding of an overall positive covariation between activity and boldness confirms this widely documented behavioural syndrome (Garamszegi et al. 2013, for damselflies: e.g. Brodin et al. 2009, Tüzün et al. 2017, Golab et al. 2020, for the study species: Debecker et al. 2016). A key finding was that in the time-constrained larvae, the behavioural syndrome structure was, however, not present.

Although behavioural syndromes are thought to play a pivotal role in ecology (Bell 2007), and changes in environmental conditions are expected to influence the stability and structure of trait integration (Killen et al. 2013), responses of behavioural syndromes to ecologically relevant stressors have received limited attention (but see e.g. for predation: Bell and Sih 2007, temperature: Biro et al. 2010, a pesticide: Debecker et al. 2016).

Behavioural correlations can arise following exposure to a stressor (e.g. predation: Bell and Sih 2007, Adriaenssens and Johnsson 2013), yet the opposite pattern that we here documented where existing behavioural correlations disappear in the presence of a stressor has also been reported (e.g. exposure to chemicals: Royauté et al. 2015a, Dziewieczynski et al. 2016). Such an uncoupling of a trait association may facilitate an altered life-history strategy, in our case accelerated development, by achieving an optimal behavioural trait combination (Sih et al. 2004). The disappearance of the positive association between

activity and boldness in the fast developing late-period larvae would allow for increased activity, hence more energy acquisition, without an increase in boldness levels, potentially avoiding negative consequences of being too bold (e.g. increased mortality by predation: Debecker and Stoks 2019). Alternatively, the stressful fast development and growth (until the final instar) driven by seasonal time constraints might have disrupted neuroendocrine and developmental mechanisms, which are assumed to be the underlying processes that generate behavioural syndromes (Sih et al. 2004).

Conclusions

By assessing life history in two distinct larval periods and by imposing three photoperiod levels, our study revealed two potential caveats in previous studies testing theoretical predictions of the effects of seasonal time constraints. First, imposing seasonal time constraints increased growth and development rates of damselfly larvae, in line with expectations. Yet, this accelerated pace-of-life documented in early life stages was not consistent during the final instar, possibly due to accumulating costs of accelerated growth and development (e.g. oxidative stress: Janssens and Stoks 2020). This warrants caution when inferring a study system's pace-of-life based on measurements taken at a specific developmental stage. Second, while our finding of a bolder personality of the time-constrained late-period larvae compared to control larvae suggests rare evidence for the theoretically predicted increase in behavioural risk-taking under seasonal time constraints (Rowe and Ludwig 1991, Werner and Anholt 1993, Abrams et al. 1996), late-period larvae had the same boldness as early-period larvae. This points to a potential caveat of the large majority of studies that only compared late with control animals, thereby potentially wrongly concluding adaptive responses to time constraints.

A key finding was that, while trait covariation patterns did not support a complete pace-of-life syndrome structure (see also Royauté et al. 2018), we found the expected activity-boldness behavioural syndrome (Garamszegi et al. 2013), except in the time-constrained larvae. The decoupling of this behavioural syndrome may be adaptive (Sih et al. 2004), possibly enhancing energy acquisition to fuel the accelerated development rate. Future work on carry-over effects of seasonal time constraints on the adult pace-of-life and fitness may further provide clues on the adaptive function of the decoupling of activity and boldness. Interestingly, the only study that compared trait integration among populations differing in experienced time constraints reported a stronger trait integration in the more time-constrained populations (Raffard et al. 2020). Studies that integrate plastic (within population) and evolutionary (among populations) responses to time constraints are needed to clarify this intriguing, contrasting pattern. With time constraints being a ubiquitous stressor in natural populations, our work is among the few to address the recent call to test POLS across relevant ecological gradients (Dammhahn et al. 2018, Hämäläinen et al. 2019). Our results thereby add new insights and suggest that, besides the predicted plastic changes in life history means, also plastic changes in behavioural trait integration may be an important but overlooked adaptive aspect of responding to seasonal time constraints.

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Figure Legends

Figure 1. Life-history traits of the *Ischnura elegans* larvae as a function of the photoperiod treatment. A) Development time (hatching until final instar), B) body mass (at day 1 in the final instar), C) growth rate (hatching until the final instar). Shown are least-squares means with 95% confidence intervals. Growth rate was corrected for the number of days between the end of the simulated winter period and reaching the final instar (see text).

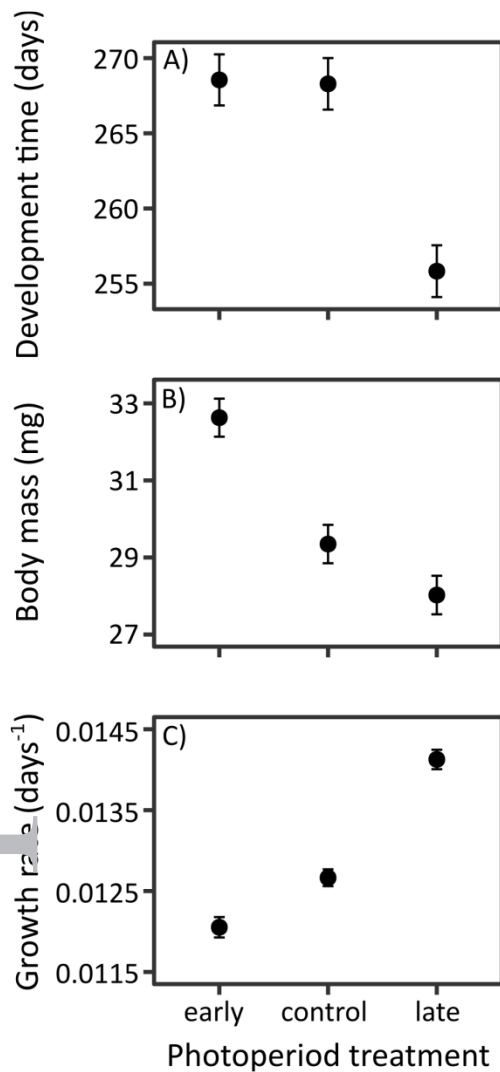


Figure 2. A) Growth rate and B) metabolic rate (measured as oxygen consumption) of the *Ischnura elegans* larvae as a function of the photoperiod treatment. Traits were measured twice (trial 1 and trial 2) during the final instar. Shown are least-squares means with 95% confidence intervals. Metabolic rate was corrected for body mass.

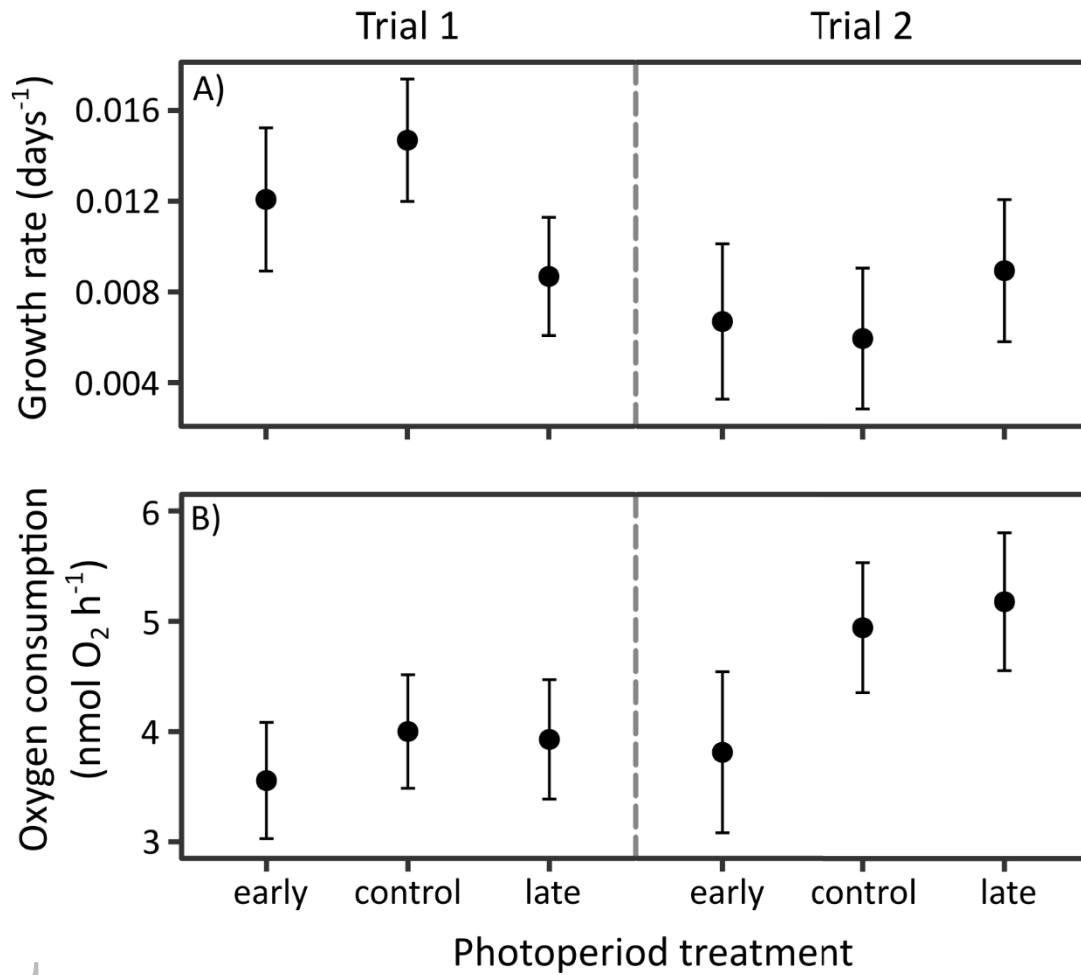


Figure 3. Behavioural traits of the *Ischnura elegans* larvae as a function of the photoperiod treatment. A) Activity and B) boldness (measured as latency to resume activity after a simulated predator attack). Traits were measured twice (trial 1 and trial 2) during the final instar. Shown are least-squares means with 95% confidence intervals. Bolder individuals are those having shorter latency times.

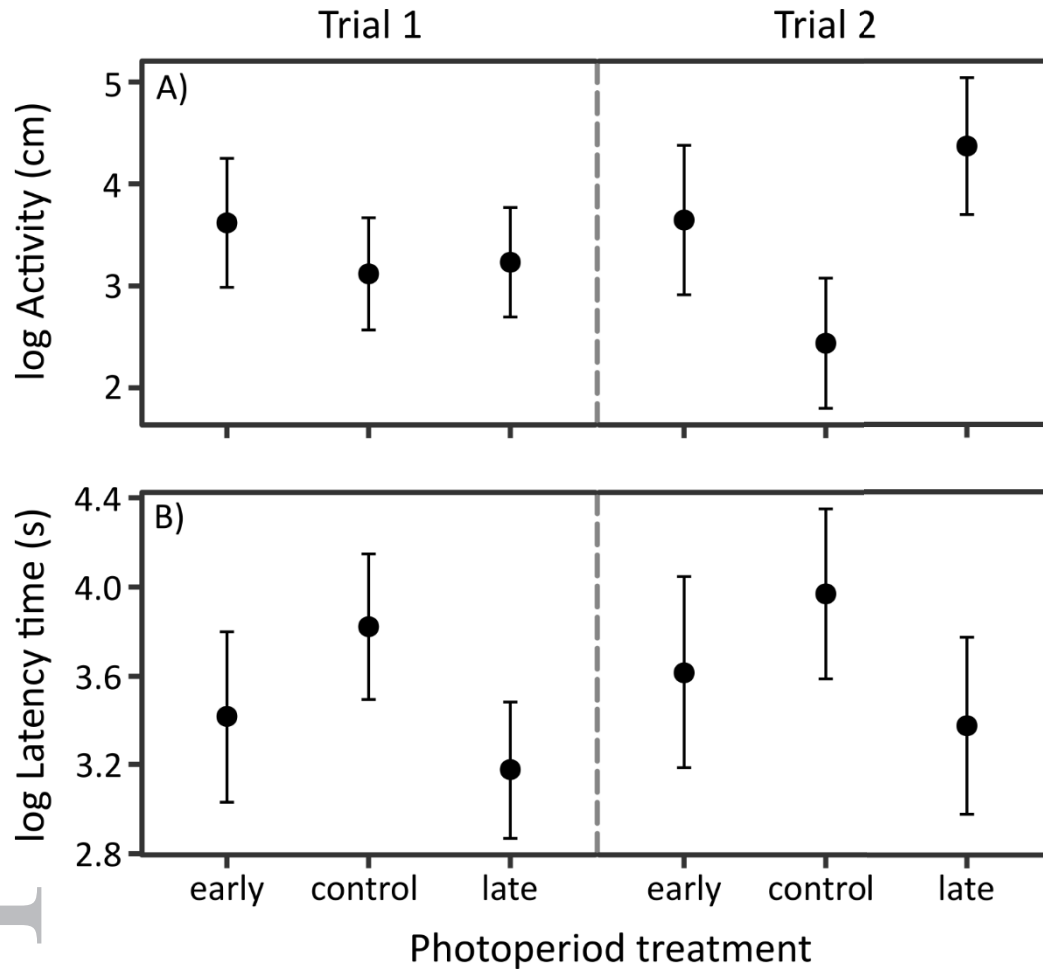


Figure 4. Covariation patterns between activity and boldness of the *Ischnura elegans* larvae as a function of the photoperiod treatment. Boldness was measured as latency to resume activity after a simulated predator attack, hence a negative covariation of activity and ‘latency time’ should be interpreted as a positive covariation of activity and boldness. Shown are regression lines with 95% confidence intervals.

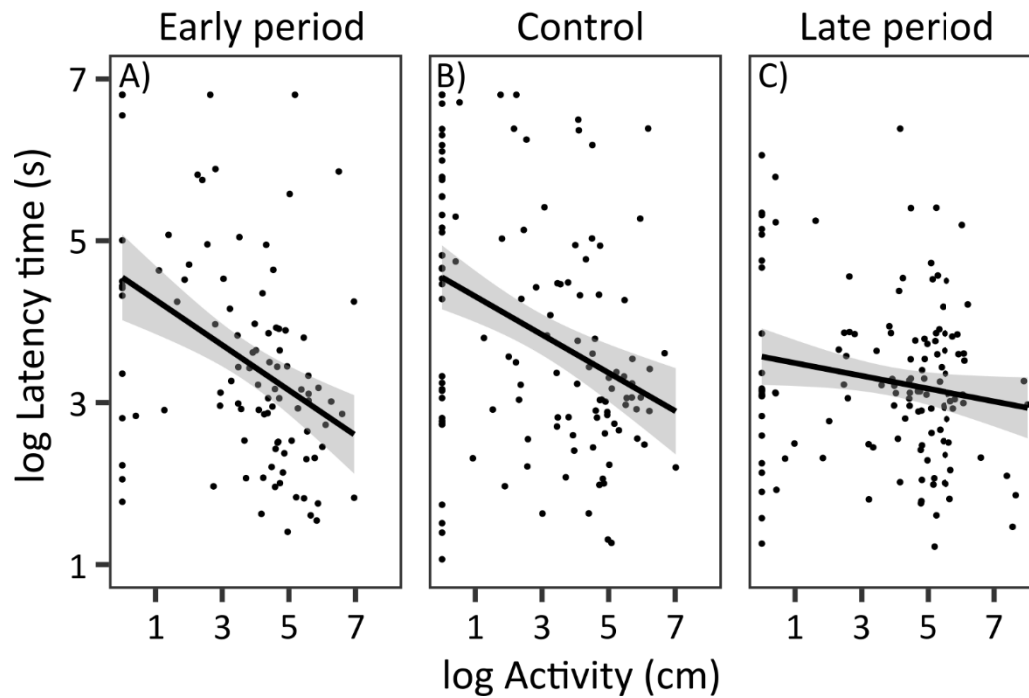


Table Legends

Table 1. Results of linear mixed effect models testing for effects of the photoperiod treatment and trial phase on growth rate, metabolic rate, activity and boldness (latency to move) measured in the final larval instar.

	Growth rate			Metabolic rate			Activity			Latency to move		
	Df	F	p	df	F	p	df	F	p	df	F	p
Photoperiod treatment	2, 209.9	1.17	0.311	2, 186.9	4.31	0.015	2, 204.5	3.29	0.039	2, 187.7	6.05	0.003
Trial Phase	1, 213.5	13.12	<0.001	1, 225.7	7.19	0.008	1, 114.7	0.33	0.565	1, 74.8	1.51	0.223
Photoperiod × Trial Phase	2, 211.9	4.75	0.009	2, 182.9	1.85	0.160	2, 187.9	7.23	0.001	2, 167.5	0.02	0.981
Body mass				1, 234.6	39.97	<0.001						

Table 2. Repeatabilities (R) and coefficient of variation (CV) of measured traits, calculated including all data ('pooled') and separately for the three photoperiod treatment groups.

Trait	Photoperiod treatment	Repeatability		Coefficient of variation	
		R	95% CI	CV	95% CI
Growth rate	Pooled	0	0, 0.157	139.38	121.02, 164.51
	Early	0.142	0, 0.403	131.43	102.99, 182.44
	Control	0	0, 0.257	120.68	98.33, 156.71
	Late	0	0, 0.246	172.77	133.17, 246.93
Metabolic rate	Pooled	0	0, 0.159	45.84	42.1, 50.37
	Early	0.013	0, 0.315	43.95	37.92, 52.34
	Control	0	0, 0.262	49.54	43.02, 58.48
	Late	0.100	0, 0.404	41.56	36.07, 49.11
Activity	Pooled	0.324	0.190, 0.452	65.09	59.27, 72.25
	Early	0.423	0.149, 0.631	52.61	44.95, 63.57
	Control	0.263	0.013, 0.491	81.91	69.12, 100.8
	Late	0.249	0, 0.489	59.03	51.07, 70.08
Latency to move	Pooled	0.270	0.122, 0.414	37.61	34.75, 41.04
	Early	0.268	0, 0.524	38.44	33.19, 45.74
	Control	0.288	0.009, 0.517	39.07	34.28, 45.47
	Late	0.141	0, 0.412	31.84	28.15, 36.72
Body mass	Pooled	0.404	0.329, 0.472	13.97	13.35, 14.65
	Early	0.292	0.141, 0.433	12.15	11.21, 13.29
	Control	0.244	0.123, 0.363	12.84	11.92, 13.94
	Late	0.344	0.219, 0.457	13.26	12.32, 14.39

R: repeatability estimate, CV: coefficient of variation (SD/mean), CI: 95% confidence intervals. Repeatability estimates are derived from models with 1000 bootstraps. Significant repeatability estimates ($p < 0.05$, tested by likelihood ratio tests) are indicated in bold.

Table 3. Principal component loadings for the traits measured during the final instar, separately calculated for each of the three photoperiod treatments. Factor loadings > 0.7 (in bold) were considered as significant contributions to a principal component.

	Early photoperiod			Control photoperiod			Late photoperiod		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Growth rate	0.07	-0.19	0.98	0.07	0.98	0.01	0.01	-0.08	0.98
Metabolic rate	-0.02	0.96	-0.20	-0.02	0.01	0.99	-0.07	0.97	-0.07
Activity	-0.83	0.24	-0.09	-0.83	-0.27	0.11	0.89	0.08	-0.13
Latency time	0.87	0.17	0.01	0.90	-0.11	0.06	-0.71	0.35	-0.22
% variance explained	42	30	29	42	29	28	38	32	30