The thermal effects on selected life history traits in an arctic and a temperate population of the Collembola *Hypogastrura viatica*

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Abstract

A set of life history traits were studied in order to compare the thermal responses of two populations of the surface living Collembolan *Hypogastrura viatica*, from the highly contrasting climates of north east Svalbard and the southern coast of Norway. This was done by analyzing hatchling size, growth pattern, development, age and size at reproduction and mortality of the two populations over a range of temperatures (from 10 to 20 or 25°C, depending on trait).

The goal was to study how these traits co-vary and if they represent a general difference in strategy between the two populations involved. The results were also compared with those from a similar study on the soil living Collembola *Folsomia quadrioculata*.

The hatchling size of the two *H.viatica* populations were not notably affected by temperature, and differed only at 10°C, where the temperate population was distinctly larger in size. However, in terms of growth, both populations displayed decreasing growth rate and increasing asymptotic size with decreasing temperature, meaning that both populations responded in accordance to the Temperature Size Rule. The arctic population grew slower and reached a larger asymptotic size than its temperate counterpart at all temperatures, as predicted by the Bergman Cline. Moreover, the arctic population reproduced later and at a larger size than the temperate one. In terms of mortality, the temperate population was less sensitive to heat stress and therefore experienced lower mortality at the highest temperatures. The overall trend of the temperate population was to speed up growth and developmental rates at the expense of size, in addition to being more heat tolerant. This is consistent with the potential time limitations of a fixed one-year life cycle, and its relatively warm habitat. By comparison, the opposite trend of selection of large body size rather than increased developmental rates was favored in accordance with the more flexible life cycle of the arctic population in this species.

The population specific differences found in *H. viatica* were the opposite of what was found in the two corresponding populations of *F. quadrioculata*. This may be caused by differences in life cycles and mobility, which enables local adaptations in *F. quadrioculata* in a totally different way than *H. viatica*. The latter, with a much larger dispersability showed signs of large scaled macro climatic adaptations. Hence, adaptations to climate may vary greatly between species of ectotherms.
1 Introduction

1.1 Life History Theory

Life history traits have direct impacts on survival and reproductive success, and are therefore under continual selection (Stearns, 1992). Thus, life history theory revolves around the search for the optimal equilibrium between traits in order to attain the highest possible fitness (Lande, 1982). Size at birth, growth rate, development rate, age and size at maturation are examples of such traits. However, life history traits are not independent of each other. Linkage between traits may lead to selection on one trait, also influencing other traits and in turn affect the overall strategy. Trade-offs due to negative relationships between these traits, may lead to large variations in prioritization between populations and species (Stearns, 1992; Zera et al, 2001). If an organism invests more energy in one trait, it will have a negative effect on the amount of energy possible to invest in another. This is commonly known as The Principle of Allocation (Levins, 1974). The balance of investment in these traits within a population is referred to as the population’s life history strategy (Stearns & Koella, 1986; Stearns,1992). The environment to which the population is adapted, may have a considerable effect on the optimal prioritization of resources among traits (Stearns, 1992; Convey et al, 2006; Huey et al, 2012). This may have important implications with regard to the ideal strategy, and a lot of research has focused on the relationship between life history strategies and climate ( Jiguet et al, 2007; Gienapp et al, 2008; Sheridan & Bickford, 2011).

The size of progeny has been extensively studied in many species, as it may affect fitness in many ways (Perrin, 1988; Yampolsky & Scheiner, 1996; Fischer et al, 2003). Small progeny are often more vulnerable to environmental stress, however less investment per progeny makes room for increasing the number of offspring (Stearns, 1992; Fischer et al, 2011). Such a strategy is usually favoured under good conditions with low juvenile mortality. In contrast, larger offspring tend to be better fit to tolerate environmental stress, and are often favoured in harsh environments (Atkinson, 1994; Fischer et al, 2003; Fischer et al, 2011;Forster et al, 2011).

Age at maturity is regarded as the life history trait that is generally closest linked to fitness, and age and size at maturity is one of the most central trade-offs in life history theory (Stearns, 1992). Delayed maturity can be costly in terms of increased juvenile mortality and prolonged life cycle. Early maturation is often favourable in habitats where juvenile mortality is high,
since it increases the chances of surviving until reproductive age (Stearns & Koella, 1986; Fischer et al, 2011). On the other hand, early maturation will often result in smaller maternal body size, and thus reduce both present and future reproduction (Stearns, 1992). In addition, direct selection on size may affect the developmental rate relative to growth rate and indirectly affect age at maturity (Atkinson, 1994). Thus, many environmental factors may affect the trade-offs between age and size at maturity, and the optimal strategy will differ between species and habitat.

The correspondence between growth and developmental rate is essential for timing and size at all life history events. Age dependent growth rate determines the growth trajectories and thus adult size, which in turn is closely associated with fecundity. Several environmental variables can affect growth rate, for instance temperature, predation and food supply, and the varying forms of stress often alter the growth- and developmental pattern differently. As would be expected, low food supply will usually induce slow growth, and maturation at a smaller-than-normal size (Stearns & Koella, 1986). On the other hand, a fact that has been discussed among life historians is that in ectotherms, decreased growth rate at lower developmental temperature is very often coupled with increased adult size (Berrigan & Charnov, 1994; Atkinson & Sibly, 1997; Angilletta et al, 2004;). This size response is poorly understood, but appears to be so common that it is known as the Temperature- Size Rule (TSR) (Sibly & Atkinson, 1994; Angilletta et al, 2004; Walters & Hassall, 2006). In species with indeterminate growth, two size variables of particular interest are size at maturity and asymptotic size. These variables are usually regarded as being closely connected (Berrigan & Charnov, 1994), but this may not necessarily be the case (Sengupta & Leinaas, submitted). One question is whether they may be differently affected by the same environmental variable among con-specific populations.

Consequences of increasing temperatures are believed to be especially severe in the Arctic, as the warming is found to be greater at high latitudes (Root et al, 2003; Deutsch et al, 2008). This makes arctic areas particularly interesting, when looking at the details of thermal responses in life history strategy. The ongoing climate change affects many climate variables, such as humidity, extreme weather, ocean acidity and temperature, all of which may have large consequences for the species life history strategy. However, with temperature as the driving force behind most climate related variables, the focus of the current project will be on thermal adaptation in an ectotherm.
1.2 Thermal Adaptation

In the context of climate change, it may be important to adjust one’s life history strategy to fit a changed or new environment. Phenotypic plasticity is the phenomenon where a genotype produces different phenotypes in response to environmental changes (Stearns, 1992; Chown et al, 2007; Nussey et al, 2007). This may involve variation in its current environment, as well as new conditions encountered when moving to another habitat (Ghalambor et al, 2007). Plasticity can be described by a reaction norm, illustrating the variation of phenotypes the genotype will express over a given range of changes in the environment (Futuyma, 2009). Although phenotypic plasticity in itself does not involve genetic changes, the plastic response may have great impact on fitness, and thus it represents a trait under selection (Crispo, 2007; Valtonen et al, 2011). In particular, life history flexibility may be an important adaptation to environments (Convey et al, 2006). However, the ability to alter ones phenotype can be costly. Sensing the environment in order to generate a plastic response can be energy consuming, in addition to possible genetic costs including linkage, pleiotropy or epistasis (Hendry et al., 1998). There is therefore a possible trade-off that may cause large selective variations in phenotypic plasticity between species and populations.

Since life history strategies of ectotherms are highly affected by temperature, intraspecific differences are common in species that are widely distributed. This raises important questions on how these adaptations will influence their capacity to survive if their surroundings are affected with climate changes. One way to approach these questions is by studying con-specific populations along climate gradients. Terrestrial animals living in arctic regions are exposed to extreme climatic fluctuations. Due to high variations in ground temperature, ground dwelling arthropods are often subjected to constant thermal variability (Hertzberg & Leinaas, 1998). This may have significant effects on several life history traits like developmental rate, growth rate, size and age at maturity and survival. Some key differences are commonly found when comparing temperate and arctic populations: A well-known response to changes in temperature is shifts in body size, with organisms tending to be larger in colder habitats. This trend was first described by Carl Bergmann in 1847, when he observed a positive correlation between increasing latitude and body size in homeotherms (Bergmann, 1847; Ray, 2005; Watt et al, 2010). He proposed that increasing surface- to- volume ratio would decrease heat loss, and therefore be adaptive for organisms in cold temperatures. However, while Bergmann focused on interspecific trends of homeotherms, later research has shown that many ectotherms show similar patterns, with increasing body size towards colder areas (Atkinson, 1994; Angilletta et
This is now referred to as the Bergmann clines and is typically studied at the intraspecific level. Thus, TSR is a result of phenotypic plasticity while the Bergmann Cline is a consequence of differences in both phenotypic plasticity and micro evolutionary adaptations (Angilletta et al, 2004). However, in arctic habitats, growth seasons are both short and cold, which may result in increasing time stress in order to complete one’s life cycle within a favourable time. This will be particularly important in species with a fixed life cycle or strict phenological pattern in reproduction. Thus, while low temperature during development may tend to increase adult size in general, time stress may act in the opposite direction. I.e. selecting for reduced body size in cold environments, and by that reduce the resource requirement per season (Conover & Present, 1990). This is referred to as the Converse- Bergmann Rule (Mousseau, 1997; Emont, 2004). Consequently, depending on whether temperature itself or time limitation is the main driver on modifying body size, different species may show increasing or decreasing body size towards colder environments.

Many ectotherms in cold habitats respond to the reduced growth rate by extending their life cycles over several years in order to have time to mature and reproduce (Coulson et al, 2000). Another strategy is to compensate for reduced biological rates (such as metabolism and growth) at low temperature by increasing the thermal efficiency of the rates. Such a strategy implies that if rearing animals from a cold and warm adapted con-specific population at the same temperature, the former animals will have the highest rates (Deutsch et al, 2008; Everatt et al, 2013). The difference may be seen across the whole temperature range where the species develops normally, enabling populations of cold environments not only to efficiently cope with low temperatures, but also to maximize the utilization of any temperature that may occur, even during short term warm spells. This is referred to as counter gradient variation (Levins, 1974; Conover & Present, 1990; Conover & Schultz, 1995), and is found in several ectotherm species (Ayres & Scriber, 1994; Serbezov, 2002; Deutsch et al, 2008).

Further, several studies have shown that increased offspring size at the expense of offspring number is a common response to low temperatures in ectotherms (Perrin, 1988; Fischer et al, 2003; Kingsolver & Huey, 2008) This is argued to be advantageous in harsh, cold environments, since large initial body size is expected to increase the offspring’s survival (Yampolsky & Scheiner, 1996). Nevertheless, other environmental variables can drive the selection in the opposite direction (e.g. Sengupta & Leinaas, submitted). Due to the different selection pressure between traits and due to trade- offs, micro evolutionary variations in single traits in con- specific populations from cold and warmer environments may not be as expected.
Superimposed on macro climatic effects, there will also be consequences of local micro climate and other environmental changes. Thus, these factors complicate the process of comparing two populations additionally. In order to evaluate this issue, the current project looks at a set of life history traits in two populations from contrasting climates, to see if all traits vary in the same direction in accordance with general expectations to their natural habitat. In addition, the aim is to examine to what degree an evaluation of all these traits as a combined strategy would add to the understanding of population-specific differences in adaptation.

1.3 Project Description

The current experiment investigated two populations of the Collembola species *Hypogastrura viatica* from vastly different environments in order to compare variations in a set of life history traits. The arctic population (referred to as LSI) comes from the northernmost Little Slate Island (Vesle Tavløy) of Svalbard. The temperate population is sampled from Portør, on the southern coast of Norway. The species belong to the collembolan family *Hypogastruridae*, which is found in numerous habitats, and is known to often occur in large numbers (Hopkin, 1997). Various aspects of the ecology of *H. viatica* have previously been studied at the University of Oslo (UIO) (Hertzberg & Leinaas, 1998; Leinaas, 2002; Serbezov, 2002). Little has been done in terms of comparing local adaptations to contrasting climates in *H. viatica*, but Serbezov (2002) made a detailed study of life history traits in one arctic population of *H.viatica*, from Longyearbyen at Svalbard. He showed an astonishing ability to utilize high temperatures in similar ways as previously described in a temperate population (Mertens et al, 1983). However, the arctic population also performed well at 5-10˚C (Serbezov, 2002). Compared to the arctic species *Hypogastrura tullbergi*, *H.vitica* appears to have a more flexible life history strategy, which might explain the wide distribution of the species (Birkemoe & Leinaas, 2001; Serbezov, 2002). Nevertheless, it is not clear whether this is an adaption to arctic climate or simply an inherited feature in the species itself, which enables it to occupy a wide range of habitats.

Another important basis for this study, S. Sengupta and H.P. Leinaas is currently comparing populations of the Collembola species *Folsomia quadrioculata* from Oslo and Little Slate Island (in manuscript). *F. quadrioculata* is probably the most common species of Collembola at Svalbard, and live just below the soil surface. They are known to have great variations in life cycles, usually between one and three generations per year (Sømme & Birkemoe, 1999). One aim of the study was to compare the thermal responses of the two, and see if species specific differences may contribute to the understanding of thermal adaptation. Little is known about
differences in thermal response patterns between populations from contrasting climates. Further research is needed to identify the importance of phenotypic plasticity and micro-evolution for the success of this widely distributed species.

Collembola are well suited as model organisms when studying general questions about life history theory, as well as impacts of environmental change (Birkemoe & Leinaas, 2001; Chown et al, 2007). Moreover, it is a very important group of ground living animals at high latitudes. It is of great interest to improve our understanding of how increasing temperatures may affect their performance, especially in arctic regions such as Svalbard.

The present study is associated with the DWARF project, focusing on how climate change may affect several taxa and biological levels. The primary goal of the DWARF project is to study possible variation in body- and genome size in con-specific populations (of several species) along a climatic gradient from temperate to high arctic areas. The current study has tried to enlighten the possible underlying processes behind such population differences by running a common garden experiment.

1.3.1 Hypotheses and Goal

The goal for this project is to study the differences in thermal responses between a temperate and an arctic population of the collembolan species Hypogastrura viatica. By comparing the two, we want to learn more about how multiple life history traits may be affected by adaptation to two different environments. This is done by comparing growth, development, reproduction and survival in two populations of the H. viatica from contrasting climates.

With the overall hypothesis that all traits of the two populations differ according to general predictions for differences in their main macro climate, the following sub-hypotheses will be tested:

- Hypogastrura viatica will have slower growth and larger final (asymptotic) size when reared at lower, compared to higher temperatures.
- The population from the cold Little Slate Island will have the largest offspring.
- The LSI population will be older and larger at first reproduction, than the Portør population.
- The LSI population will have a larger asymptotic size.
The Portør population will be less sensitive to heat stress and therefore perform better and experience lower mortality at the highest treatment temperatures.
2 Materials and Methods

2.1 The Study Organism

*Hypogastrura viatica* is an active surface living species, typically found on, or near beaches (Hertzberg et al., 2000). They have a wide geographical distribution, ranging from the High Arctic to warm temperate areas such as the Iberian Peninsula (Jordana et al, 1990; Jensen et al, 2006). It is also found in cooler parts of the southern hemisphere (Fjellberg, 1998) probably spread by human activity (Greenslade, 1994). Being one of the most dominant species in coastal arctic habitats, it plays an important role in the ecosystem, contributing to the nutrient cycle (Hertzberg & Leinaas, 1998; Birkemoe & Leinaas, 2000). The extensive geographical range of *H. viatica* makes it a good model species to study differences in life history strategies of populations from sites of highly contrasting climates (Birkemoe & Leinaas, 2000; Leinaas, 2002; Jensen et al, 2006). The species is also robust and easy to keep in lab cultures under different conditions (Hertzberg & Leinaas, 1998; Leinaas, 2002). Collembola continue growing long after they have reached maturity, and they keep on molting throughout their life. Most species also continue reproducing as long as they live (Hopkins, 1997). *Hypogastrura spp.* lay their eggs in well-defined batches, typical for this genus. These eggs hatch highly synchronous (Birkemoe & Leinaas, 2001; Serbezov, 2002), and facilitates monitoring of hatching, and the funding of cultures with same aged animals. *H. viatica* in southern Norway appears to have a fixed one-year life cycle, with reproduction in early spring (Fjellberg, personal comment). In contrast, arctic populations seem more flexible. Typically they have a two-year life cycle (Hertzberg et al, 2000; Serbezov, 2002), but some may reach maturity already the year before during exceptionally warm summers, or postpone reproduction yet another year if the summer is unusually cool (Serbezov, 2002).

2.2 Contrasting Climates

Little Slate Island (LSI) (81°N, 20°E) is the northernmost of the small islands of Svalbard, off the Nordaustlandet. It has a harsh arctic climate, with short and unpredictable summer conditions and have large variation in the onset of seasons. Portør (58°N, 9°E), on the other hand, has longer and highly predictable summers is less flexible. The highest temperature measured in 2014 (at the closest weather) station was 5.5°C for Little Slate Island, while the mean temperatures in June, July and August was -1.9, .4 and -1.7°C respectively. In contrast,
the highest temperature in Portør was measured at 28.5°C, while the mean summer temperatures were 15.5, 19.9 and 16 °C (the Norwegian Meteorological Institute).

After being sampled at the two sites in 2007, the animals were kept in separate cultures at 15°C, where they have reproduced over several generations. The animals were kept in the same 30 ml culture boxes used in this experiment (d= 3, 4 cm, h= 3cm, see below). The animals were fed pieces of algae and cyanobacteria on bark (once a week) and distilled water was added as droplets until the onset of this experiment.

2.3 Experimental Procedure

The animal’s developmental rate, age and size at reproduction, growth pattern, theoretical asymptotic size, hatchling size and survival were studied by using the following experimental procedure.

2.3.1 Experimental Setup

To start the experiment, 10-20 newly hatched juveniles (<1 day) were placed in 10, 15, 20 or 25°C, where they were kept for the duration of the experiment. However, some deviation in number of animals per culture occurred, since this depended on the daily number of hatched animals at 15 °C. Temperature cabinets were used to keep the ambient temperature constant throughout the study (fig. 1).

![Fig. 1: Temperature cabinet with cultures (Photo: Andreas Lium).](Image1)

![Fig. 2: 30 ml Culture box with Plaster of Paris mixed with charcoal as substrate (Photo: Andreas Lium).](Image2)
The animals were kept in 30 ml boxes \((d = 3, 4 \text{ cm}, h = 3 \text{ cm}, 5-25 \text{ individuals pr. box})\) with a moist layer of plaster of Paris mixed with charcoal (fig. 2). At each temperature, four replicate culture boxes of both populations were harvested at the age of 28, 56, 84 and 112 days (fig. 3). If less than 10 animals had survived, additional replicates were sampled. For this reason, some additional culture boxes were kept as backups if mortality had been high. At the highest temperatures (20 and 25°C, of the oldest LSI) some were harvested earlier than planned because of high mortality and therefore risk of losing data. All cultures were exposed to a 24h photoperiod, simulating the arctic summer. This because the project is part of a more extensive study on adaptations in arctic Collembola, and the temperate animals are primarily for comparison, kept under identical conditions. The effect of photoperiod is also planned to be studied in a later project (H.P. Leinaas, personal comment).

![Fig. 3: The experimental setup, showing the grouping of animals into treatment temperatures and age groups.](image)

All animals were fed with pieces of bark covered by a black crust of cyanobacteria (Jensen et al., 2006). The bark was collected from a small group of neighbouring trees growing at the University of Oslo Campus, and replaced twice a week to ensure \textit{ad limitum} supply. The culture boxes were inspected every second day, and droplets of distilled water was added to the bottom when necessary to keep the cultures moist. At each inspection the number of living and dead animals and exuvia since last inspection was counted. In addition; any reproduction, number of juvenile instars and the overall condition of the culture was noted. Culture boxes where changed approximately every 2-3 weeks in order to keep the animals in clean and healthy environments.
2.3.2 Molting and Development Rates

Molting (shedding of skin) was quite synchronized in each culture and therefore easy to monitor. The number of exuviae (exoskeletons) was counted and registered, and removed from the cultures the day they were observed. The number of new exuviae relative to the number of animals in each culture was used to define a new instar of the given culture. Every molting was registered until the first reproduction in each culture in order to see if the populations differed in number of juvenile instars, and whether this might be affected by temperature (Birkemoe & Leinaas, 2001). The date of first reproduction was registered for all cultures that were kept alive long enough for the animals to mature. All events were defined to have happened at the day between the two subsequent inspections.

2.3.3 Survival

At each inspection, living and dead animals were counted. If dead animals were found, they were removed from the cultures to prevent contamination, and to facilitate estimation of survival during the time between inspections. Time of death was also defined as the mean time between the two subsequent inspections. Several animals were lost during this experiment, most likely by escaping from the culture boxes when the lids were opened prior to inspection. Only animals found dead were used in survival data.

2.3.4 Growth

In order to study the growth pattern of the cultures, the animals were harvested and measured every fourth week (fig 4). Upon harvesting, the animals were immediately killed in 70% ethanol and subsequently stretched by heating to about 70°C in a warm water bath. Further, all animals were photographed through a stereo-microscope. The animals were kept in ethanol for the rest of the experiment, in case additional photographs and measurements where necessary. The measuring was done by using the computer program Leica Application Suite 4.5. By taking photos of each culture through a stereo microscope, one can measure the animals with an accuracy up to 0.0063 mm at 16x magnification (fig.4) (Birkemoe & Leinaas, 1999; Jensen et al, 2006).
A total of 1854 animals were measured and used to determine the differences between the populations at given ages and temperatures.

**Table 1: Number of cultures and animals at the end of the experiment (LSI).**

<table>
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<th>Temperature</th>
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<th>15 °C</th>
<th>20 °C</th>
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<td>5+11+9+10+16</td>
<td>5+3</td>
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**Table 2: Number of cultures and animals at the end of the experiment (Portør).**

<table>
<thead>
<tr>
<th>Temperature</th>
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<th>15 °C</th>
<th>20 °C</th>
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<td>12+18+8+17</td>
<td>6+4+8+12</td>
</tr>
<tr>
<td>112</td>
<td>5 cultures</td>
<td>6 cultures</td>
<td>4 cultures</td>
<td>4 cultures</td>
</tr>
<tr>
<td></td>
<td>16+18+3+7+11</td>
<td>7+6+11+9+10+9</td>
<td>13+10+11+5</td>
<td>7+10+9+8</td>
</tr>
</tbody>
</table>

**Fig 4:** Adult animals photographed and measured.
2.3.5 Size of Hatchlings

In order to compare the temperature dependent differences in hatchling size, eggs were collected from both populations at each temperature where reproduction occurred. The eggs were then kept in clean culture boxes at the same temperature as where they had been produced. They were observed daily and harvested at the day of hatching. The animals were then harvested, photographed and measured as described above (fig.5).

![Fig. 5. Hatchlings from the LSI population, photographed and measured.](image)

This was done in order to compare the size of the newborn hatchlings between populations and between the study temperatures. There were not enough animals hatched at 25°C in either population to collect data on hatchling size at this temperature.

**Table 3: Number of Hatchlings hatched and harvested at their respective treatment temperatures.**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Population</th>
<th>10 °C</th>
<th>15 °C</th>
<th>20 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LSI</td>
<td>1 culture 21</td>
<td>3 cultures 31+16+21</td>
<td>2 cultures 19+15</td>
</tr>
<tr>
<td></td>
<td>Portør</td>
<td>3 cultures 18+20+18</td>
<td>3 cultures 16+18+13</td>
<td>3 cultures 24+15+17</td>
</tr>
</tbody>
</table>
2.4 Statistical Analysis

Reaction norms constructed from the mean value of the observations made in the laboratory experiments.

Hatchling size, size at different life history events, age at maturity and instar number were analyzed for the effect of temperature, population and temperature x population interactions, by using a Generalized Linear Model (GLM) in R version. The residual plots were examined, to see whether the underlying assumptions for GLM was fulfilled. By running GLMs on the collected data, it was possible to estimate the significance of the differences observed. None of the data was transformed in any way as any attempt weakened the model (e.g. log transformation).

The Von Bertalanffy’s Model (Stearns & Koella, 1986) was used to calculate the theoretical asymptotic sizes and growth constants. The model used was:

\[
\text{Size}_{ij} = a_i [1 - b_i \cdot \exp (-k_i \cdot \text{age}_j)] + \varepsilon_{ij}
\]

Here, the i describes the box identity, j is the age, \(a_i\) is the asymptotic length, \(b_i\) denotes the hatchling size and \(k_i\) is the growth constant for the box / culture in question. The \(\varepsilon_{ij}\) denotes the error terms.

Analyses of survival was done using Kaplan-Mayer statistics, which has become a well-recognized way to analyze “time-to-event” data (Rich et al., 2010). Animals censored out of the experiment in terms of harvesting was not included in these plots. A Cox Proportional Hazard Regression model was also used in order to compare the hazard ratio between and within the populations at their respective treatment temperatures.

The analyses were performed with “The R project for statistical computing (i386)” and reaction norms were drawn using Microsoft Office Excel, 2013.
3 Results

3.1 Hatchling Size

Figure 6 shows the reaction norm for hatchling size of the two populations (for number of replicates, see table 3). The two populations were surprisingly similar at 15 and 20°C. However, the hatchlings from Portør were considerably larger than the ones from LSI at 10°C.

As a consequence of these differences, the statistical analyses (table A1, appendix) showed a significant population x temperature interaction. In addition, only Portør had a significant effect of temperature on hatchling size.

3.2 Growth

All animals in the main experiment hatched from eggs developed at 15°C, and were then moved to their respective treatment-temperatures. Hence, the mean size at the first day of measurement is the same for all temperatures in the two populations (mean size of hatchlings at 15°C). At this temperature, the hatchlings of the two populations did not differ significantly in size (both with a mean size of 0.52 mm, SE of 0.005 (LSI) and 0.007 (Portør)). Consequently, comparisons of size between population and treatment temperatures also represent a direct comparison of growth. The figure (7a) shows the growth trajectories at the four treatment temperatures.
temperatures in the LSI population. The growth curves in the three highest temperatures (15-25°C) had an initial steep growth followed by a distinct levelling out.

Fig. 7. Estimated growth curves for the LSI population (a) and the Portør (b), made by using von Bertalanffy growth mode (VBGM). The boxplots represent the measurements taken at the given age. Whiskers represent the upper and lower quartile, while the white circles are outliers.
This pattern varied with temperature, as the reduction in growth rate came later at 15°C, than at both 20 and 25°C. At 10°C, however, the growth trajectory was only slightly curved throughout the experiment. The growth curves of the Portør population (fig. 7b) showed general patterns similar to LSI across temperatures. However, the Portør population had a more pronounced distinction between the initial growth face and the flattening of the curve at the three highest temperatures. In particular, at the two highest temperatures, the initial growth was fast and the growth curves flattened out close to their asymptotic size (fig 8). The most striking difference between the two populations with regard to growth pattern is the almost non-existent growth after 28 days at 25°C in the temperate population. Consequently Portør reached its maximum size exceptionally early at this temperature.

Figure 8 shows the thermal reaction norms for asymptotic size, estimated from the von Bertalanffy growth model. Both populations showed fairly similar patterns, with a steep decline from 10-15°C. Further, both had a gentle reduction towards the higher temperatures (fig 8). Non-overlapping 95% confidence intervals between the temperatures within each population shows significant difference in asymptotic size at all temperatures. In addition, there was a significant difference between the two populations at 15°C and 20°C. The difference between mean size at 112 days and the asymptotic size (table 5), decreases with increasing temperature, reflecting that the higher the temperature, the earlier asymptotic size will be reached. Large 95% confidence intervals at 10 °C in both populations (fig 8, see also table A2, appendix) are caused by the linear growth trajectories at this temperature, which increases the uncertainty when estimating the asymptotic size.

![Figure 8: Population specific thermal reaction norm for asymptotic size, ± 95% c.i. (LSI= ▲, Portør= ■).](image-url)
All growth experiments started with hatchlings from 15°C, with the mean size of 0.52mm for both populations. Figure 9 shows the thermal reaction norm for early juvenile growth rate of each population estimated by subtracting this mean hatchling size from all measurements at day 28.

Figure 9: Juvenile growth rate measured as size increase during the first 28 days, ± 95% c.i. (Portør= ■ LSI= ▲). The standard error of 0.005 and 0.007mm for the hatchling size at 15°C in the two populations respectively, is not taken into consideration in this plot.

It is clearly seen that the differences between the two populations increase with increasing temperature (fig.9). Temperature had a significant positive effect on growth rate in the Portør population, with a significant temperature x population interaction (table A4, appendix). The non- significant population effect is due to the virtually identical growth at 10°C.

### 3.3 Instar Duration

Figure 10 shows the mean instar durations during the first five instars. Both populations show fairly constant molting frequency at each temperature. The instar duration increased with descending temperature in both populations, but in different ways (fig.10). There was a distinct tendency for grouping of the two lowest and the two highest temperatures in the Portør population, while LSI had a more continuous variation. The results of the GLM, analysing the thermal differences in instar duration during the first five instars, showed a significant effect of temperature in at all temperatures in both populations (table A5, appendix).
3.4 Age and Instar at First Reproduction

Age at maturity decreased with increasing temperature from 10°C to 20°C in both populations (fig. 11). The low sample size in the arctic population at 10°C was due to only two populations reproducing before they were harvested at 112 days, and thus underestimates the age at first reproduction. By comparison, two out of the seven cultures that reproduced in the Portør population were scheduled to be harvested at 84 days old, the other five reproduced before they were harvested at 112 days old. Because of the obvious underestimation and low number of replicates for LSI at 10°C, this temperature was removed from further analyses on age at maturation. At 25°C, the figure (fig.12) shows that the animals from Portør were slightly older
(~11 days) at first reproduction than the ones at 20°C. None of the animals from LSI reproduced at 25°C. The test of thermal effect on age at maturity was only performed at 15 and 20°C (table 4) due to lack of sufficient data from 10 and 25°C.

**Table 4: Analysing the difference between age at maturation using a generalized linear model**

<table>
<thead>
<tr>
<th></th>
<th>ESTIMATE (DAYS)</th>
<th>STD.ERROR</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>15°C (P) INTERCEPT</td>
<td>34.33</td>
<td>1.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20°C (P)</td>
<td>-14.02</td>
<td>1.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>POP LSI</td>
<td>13.58</td>
<td>2.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20°C * POP LSI</td>
<td>0.44</td>
<td>2.08</td>
<td>0.877</td>
</tr>
</tbody>
</table>

The analysis showed that the LSI population was significantly older than the Portør population at 15°C and remained so at 20°C. I.e. there was no significant temperature x population interaction in this temperature interval.

There was a generally a positive relationship between decreasing temperature and number of completed instars at first reproduction (fig.12).

**Fig. 12. Variations in the number of completed pre-reproductive instars across temperatures where reproduction occurred in the two populations, ±95% c.i. (LSI= ▲, Portør= ■).**
When compared with the age at maturation, the frequency of molts seem to decrease with decreasing temperature (also seen in fig 10). The non-overlapping 95% confidence intervals at 15 and 20 °C in figure 12, indicate that there is a significant difference between the two populations in terms of completed instars at first reproduction (table A3, appendix). There are similar trends in the two populations, with close to parallel reaction norms.

### 3.5 Size at First Reproduction

![Graph showing age and size at first reproduction in the LSI and Portør population.](image)

**Fig.13.** Reaction norms showing age and size at first reproduction in the *LSI* and *Portør* population. The symbols represent the temperatures 10°C (+), 15°C (Δ), 20°C (○) and 25°C (◊). The size is estimated based on the observed mean ages at reproduction and the von Bertalanffy growth curves, which explains the lack of error bars.

The population specific reaction norms for age and size at reproduction (fig.13) show that the overall largest animals at maturation were found at 10°C in both populations. It also shows that there was a negative relationship between temperature and size at maturation. However, it should be noted that the size at first reproduction at 25°C in the temperate population deviated from this trend, by being larger than the animals at both 15°C and 20°C in the same population. In addition, it was also seen that LSI was larger than Portør at all temperatures where reproduction took place. The size at first reproduction at 10°C for LSI was included, although the low sample size (with two replicates) is most likely to cause an underestimation of the age (and size) at first reproduction.
3.6 Survival

**Fig 14:** Kaplan-Mayer plots showing the proportion of animals surviving over the course of this experiment (Portør in red and LSI in blue).

The Kaplan–Mayer plots (fig.14) show little difference at 10 and 15°C, where mortality is relatively low. However, the mortality increases greatly in the LSI population at 20°C, while mortality remains low in the Portør populations. At 25°C, the LSI mortality remains high, while there is a large increase in mortality in the Portør population. The Cox Proportional -Hazard Regression (table A6, appendix) showed that the two populations differed significantly in survival at 20 and 25°C.
4 Discussion

The various thermal responses observed within the two populations is phenotypic plasticity, meaning that the variations in phenotype at the different temperatures is not caused by genetic differences within the populations (Chown et al., 2007). Thus, a genotype in a given population will produce a range of phenotypes at the various temperatures it is exposed to. Micro evolution, on the other hand, is the difference in this response between the two populations (Chown & Klok, 2003). These evolved differences can be observed in the mean values of the traits, and in the plasticity (Fox, 2006).

Plastic responses are not necessarily adaptive, they may just as well be natural or maladaptive (Nussey et al, 2007). In order to determine the usefulness of the observed plasticity, one can compare the plastic responses of these two populations and establish whether this makes them more fit to survive in their natural habitat. These life history traits are not independent variables, as they may be linked by various types of trade-offs and positive genetic correlations (Stearns, 1992). Nevertheless, selection may work with varying force on different traits, i.a. depending on environment. A set of life history traits has therefore been studied in the current project, aiming to see how these traits covariate or have contrasting patterns. Hence, the goal is to see how all these traits combined tell something about the differences in life history strategies, which is not apparent when studying each trait separately.

4.1 Hatchling Size

The hypothesis that the LSI population would have the largest offspring was rejected. The two populations had virtually identical hatchling size at 15 and 20°C, but differed by Portör having considerably larger hatchlings at 10°C. In this respect, H. viatica differed distinctly from the soil dwelling F. quadrioculata, where the LSI population had larger hatchlings than the Oslo population across the same temperature range (fig. 15) (Sengupta & Leinaas, submitted).
Size of offspring may directly reflect the conditions of the mother, i.e. females under favorable conditions produce the best offspring (Stearns, 1992). However, the estimated size at first reproduction as well as asymptotic size were generally larger in LSI than in Portør. Thus, the results do not agree with the idea that quality of mothers is an important factor in determining offspring size. However, more often the size represents a trade-off with the number of offspring. Thus, the large hatchlings at 10°C in the Portør population might be a thermal stress-response. Since increased hatchling size is known to improve hatchling survival, Portør might have chosen to invest in size over quantity. The difference between the populations might then be caused by Portør being more sensitive to low temperatures than the LSI population. The differences between the two species might be caused by difference in stress. Since *F. quadrioculata* lives below the surface, it has less ability to move when conditions are harsh. Therefore, it might be necessary for the hatchlings to be large in order to withstand its juvenile period. *H. viatica* on the other hand is more mobile and able to choose its micro habitat.

### 4.2 Growth

The general pattern of the growth trajectories in both populations followed TSR, with decreasing juvenile growth rate and increasing asymptotic size with decreasing temperature (Berrigan & Charnov, 1994; Angilletta et al, 2004).

The underlying mechanisms for increased size at low temperatures is poorly understood (Hessen et al, 2013), and it is still debated whether it represents an adaptive trait, or is a consequence of non- adaptive basic physical processes (Nussey et al, 2007). However, population specific differences would suggest that the traits have been under selection. The fact
that LSI generally becomes bigger than Portør is consistent with a Bergman cline (Emont, 2004), and the almost parallel reaction norms shows little selection for difference in phenotypic plasticity of these traits. Again, this contradicts the difference between the LSI and the Oslo population of *F. quadrioculata*, where the two populations differed greatly in plasticity with crossing reaction norms (Sengupta & Leinaas, submitted) (fig 16).

![Graph](image)

**Fig.16**: Asymptotic size of *Folsomia quadrioculata*, found by using the same Von Bertalanffy Growth Model as used in this experiment (Sengupta & Leinaas, submitted)

In fact, in *F. quadrioculata*, the asymptotic size of the temperate population became increasingly larger than that of LSI with decreasing temperature.

Obviously, the drivers of micro evolution of asymptotic size in Collembola is complex and likely influenced by other life history traits and environmental constraints. Interestingly, in a previous study on another arctic *H. viatica* population from Longyearbyen the asymptotic size both increased with temperature in the range of 5°C-20°C (Serbezov, 2002). There are, however, differences in the experimental setups of these two studies that might have caused the varying results. Most importantly, the food sources for the animals differed. While Serbezov (2002) used green algae on bark as food, all animals in the current project where fed on nitrogen-fixing cyanobacteria, as the latter is found to be more favorable to the Collembola (Leinaas, unpublished results). Possibly, the increase in size with decreasing temperature require high quality food (Berrigan & Charnov, 1994). It is intuitive to think that the low juvenile growth rate of the LSI population at 25°C is caused by heat stress. The surprisingly high growth rate of the temperate population at the same temperature, may coincide with the high mortality (see below). Heat stress at 25°C may in fact cause a bottleneck effect, killing all but the strongest individuals - those that are able to maintain their high growth rate.
4.3 Duration and Growth per Instar

Arthropods generally appears to have genetically determined (population or species specific) number of juvenile instars. By contrast, temperature dependence in the number of juvenile instars have been shown in the Collembola *Hypogastura tullbergi* (Birkemoe & Leinaas, 2000) and a similar phenotypic plasticity is suggested by the completed number of instars at first reproduction in the present study. Accordingly, duration of instars is not directly linked to development state. As might be expected, instar duration increased with increasing temperature in both populations but in different ways. In the temperate population, it was a distinct separation between the two highest and the two lowest temperatures, with fairly small differences within each group, possibly pointing to a threshold in the response of instar duration on temperature. By contrast, the arctic population showed a much more gradual response on temperature, not indicating a specific adaptation. A challenge for evaluating the fitness connected to instar duration is that it is obviously not the only factor determining age at maturity. Although earlier start of reproduction in Portør coincide with fewer pre-reproductive instars compared to LSI. When comparing the mean length of juvenile instars with the growth trajectories, it is apparent that growth per instar also vary with temperature and population. The temperate population had notably larger effect of temperature than the arctic population, and it increased with increasing temperature. The LSI population however, was considerably less effective, and seemed to experience some heat-stress, as the efficiency decreased from 20-25°C.

4.4 Age and Size at First Reproduction.

Age at maturation is often used as definition for age and size at first reproduction. However, this is not always the case. Several factors can delay reproduction even after the organism is physically mature, i.e. stress, food shortage or diapause. Age at maturation reflects the development rate, which in turn is affected by growth rate in different ways (Berrigan & Charnov, 1994). The reaction norm of age at first reproduction showed two general trends. First of all, the animals reared at the highest temperatures reproduced first, within each population (except for the animals at Portør, 25°C, see below), which was also the case for *F.quadrioculata*. In addition, it was revealed that the arctic *H.viatrica* generally reproduced slightly later than the temperate population. However, this could only be analysed at 15 and 20°C, where only the effect at 15°C was significant. The study of *F.quadrioculata*, showed the opposite trend, the arctic population reproduced first at each temperature. The difference between the two species
might again be caused by difference in time pressure. Age at first reproduction may not be as crucial for *H. viatica*, as the LSI population reproduce over a longer time span.

It is hard to determine whether the observed responses in age at maturation (due to changes in temperature) are adaptive or simply forced by thermal constraints (Van der Have & de Jong, 1996). It depends on the relationship between this age and a range of other factors, e.g. size at reproduction, juvenile mortality and the trade-offs that occur between these (Stearns, 1992). When comparing the reaction norms of age and size at first reproduction we see that delayed reproduction leads to larger body size at first reproduction, which in general means higher fecundity (Stearns, 1992). This pattern between age and size at maturity appears to be a general pattern. However, it may not always be the case. *F. quadrioculata* showed no difference in size at first reproduction within the study temperatures, despite substantial differences in age. This might also be the case for our arctic population, and hence explain the low sampling size of reproducing animals at 10˚C, LSI. As discussed above, the hatchling size in the LSI population was not affected by temperature, however, whether the number of offspring is affected is unknown and needs further research.

The reason for the diverse pattern in both age and size at first reproduction seen in the Portør population at 25˚C is most likely due to heat stress, which probably caused the few animals that reproduced at this temperature to be somewhat delayed. This is also the probable explanation for the absence of reproduction in the LSI population at the same temperature.

### 4.5 Mortality

Increased mortality at high temperatures appeared clearly related to increasing heat stress. It was first seen in LSI (at 20˚C), consistent with its adaptation to cold environment. At 25˚C, mortality was also high in Portør. In addition to the direct effects of heat stress at these temperatures, strong growth of sticky hyphae to which the animals may be stuck represent an indirect mortality factor. It is possible that the heat stressed animals are less efficient in grazing down the harmful fungal growth and escape their sticky surfaces.

### 4.6 Overall Strategy

None of the traits showed large differences in phenotypic plasticity, such as crossing reaction norms seen in the study of *F. quadrioculata* (e.g. fig 16). However, there are large differences
present between the cultures in the respective traits, but these are generally seen over the entire temperature range.

Unlike *F. quadrioculata*, *H. viatica* had a lot of the similar patterns in population differences in the various traits, consistent with the clear differences in life history strategy. Portør seems to select for fast growth in order to ensure a 1-year life cycle, while the multiannual LSI face less time pressure in favor of increased size at first reproduction and asymptotic size. Fecundity as a life history trait was not within the framework of this study, however there is reason to believe that LSI produced a higher number of offspring than Portør. Another difference between the two populations was that Portør seemed more tolerant to high temperatures, as seen in juvenile growth rate, reproduction and survival. Also, the large hatchlings at 10˚C in Portør might be caused by stress due to the cold temperature. These general differences are consistent with what one would expect, based on what is known about the differences in the two population’s life cycle. However, these results are in large contrast to what is observed in *F. quadrioculata*, where the population from Little Slate Island developed faster, reproduced earlier and at a smaller size, and seemed more able to utilize high temperature than the population from Oslo. Also, there was large variation in the population specific differences between the individual traits in *F. quadrioculata*. And much indicate that the local differences in micro climate and stochastic weather might be a more important driver for micro evolutionary adaptations than macro climatic differences (Sengupta & Leinaas, submitted). It is possible that the surface- and beach -living *H. viatica* have so much larger dispersal ability (including transport with ocean currents and waves) that it has less scope for local adaptions. It is reasonable to assume that life history adaption in this species reflect large scale differences (macro climate). However, in order to evaluate this, one needs to compare several species from various areas along the species distribution.

4.7 Sources of Error

In order to pinpoint the effects of temperature on the given life history traits, all other variables were meant to be kept constant. However, some variables are difficult to control and may reduce temperature to a secondary cause of response. For instance; the activity of ectotherms are highly dependent on temperature and at 10˚C one could easily observe that the animals were more passive in low- than in higher temperatures. It is therefore reasonable to think that this behavior affected the amount of food they consumed which, in turn, may have a larger impact on growth
and reproduction than temperature itself (Birkemoe & Leinaas, 2000; Serbezov, 2002). However, a typical response to food shortages is delayed reproduction at smaller-than-normal size, which is not the case in the present study (Berrigan & Charnov, 1994).

Moreover, human error is also an important potential source of error in this experiment. In order to reduce error, the same person observed, photographed and measured all animals throughout the experiment. Also, no two cultures were taken out of the temperature cabinets and opened at the same time, in order to ensure that the animals were kept separate.

4.8 Further Research

For future studies, it would be of interest to expand the current experiment, by also including fecundity, i.e. the number of eggs produced per reproduction. By studying the size and number of eggs, it is possible to determine whether fecundity increases with increasing body size. If there is a considerable increase in fecundity in cold environments, it might compensate for the disadvantages of delayed reproduction due to slow growth and development rate at low temperatures.

Foremost, it is essential to stress the importance of studying several populations in contrasting climates in order to test effects of climate differences. In addition, comparison of the genome size of different populations, may reveal some of the mechanisms behind this gradient in thermal responses. There has been said to be a positive correlation between genome size, cell size and thus body size in ectotherms (Hessen et al, 2013).

4.9 Synthesis

The aim of the current project was to study the differences in thermal responses, in two populations from contrasting climates. This was done by comparing a set of selected life history traits. By examining these traits combined, it is now possible to evaluate whether they vary according to the general predictions for differences in their respective micro climate, by looking at the sub- hypotheses.

As for growth and asymptotic size within the two populations, it was clear that groups reared at low temperatures experienced significantly slower growth and attained larger asymptotic size, in correspondence with TSR. However, the predictions were not correct in terms of
hatchling size. The two populations were strikingly similar, except at 10°C, where the temperate population had the largest hatchlings. As predicted, the arctic population was older and larger than its temperate counterparts at first reproduction. Finally, as expected, the temperate population performed better, and had significantly lower mortality at the two highest temperatures. The results point to that the two populations differ according to the predictions one can expect from their main macro climate.

In terms of the observed differences between *H.viatica* and *F.quadrioculata*, it is reasonable to think that the selection in *F.quadrioculata* is driven by the large fluctuations in its microclimate. This resulted in an opportunistic arctic population, that utilize high temperatures more efficiently than the Oslo population. *H.viatica*, on the other hand, is driven largely by macroclimate and has therefore adapted to having different time pressure in the two populations, with a fixed 1- year life cycle in Portør and more flexible multiannual life cycles in the Arctic.
5 References


6 Appendix

Table A1: The results of a generalized linear model (GLM), estimating the differences in hatchling size between the two populations.

<table>
<thead>
<tr>
<th>ESTIMATE (MM)</th>
<th>STD.ERROR</th>
<th>P- VALUE</th>
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<tbody>
<tr>
<td>10˚C (LSI) INTERCEPT</td>
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<td>15˚C (LSI)</td>
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<td>0.011</td>
</tr>
<tr>
<td>20˚C (LSI)</td>
<td>-0.01</td>
<td>0.011</td>
</tr>
<tr>
<td>POP P</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>15˚C * POP P</td>
<td>-0.04</td>
<td>0.012</td>
</tr>
<tr>
<td>20˚C * POP P</td>
<td>0.04</td>
<td>0.013</td>
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</table>

Table A2: The asymptotic size and growth constant estimated for all treatment temperatures in both populations using the von Bertalanffy growth model. 95% confidence intervals are shown in brackets.

<table>
<thead>
<tr>
<th>POPULATION</th>
<th>TEMPERATURE (˚C)</th>
<th>ASYMPTOTIC SIZE (MM)</th>
<th>GROWTH CONSTANT (DAY⁻¹)</th>
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<td>LSI</td>
<td>10</td>
<td>3.06 [2.65-3.47]</td>
<td>0.0089</td>
</tr>
<tr>
<td>LSI</td>
<td>15</td>
<td>2.25 [2.19-2.25]</td>
<td>0.0256</td>
</tr>
<tr>
<td>LSI</td>
<td>20</td>
<td>2.02 [1.95-2.08]</td>
<td>0.0321</td>
</tr>
<tr>
<td>LSI</td>
<td>25</td>
<td>1.72 [1.66-1.79]</td>
<td>0.0321</td>
</tr>
<tr>
<td>PORTØR</td>
<td>10</td>
<td>2.89 [2.52-3.27]</td>
<td>0.0088</td>
</tr>
<tr>
<td>PORTØR</td>
<td>15</td>
<td>2.06 [2.00-2.11]</td>
<td>0.0324</td>
</tr>
<tr>
<td>PORTØR</td>
<td>20</td>
<td>1.85 [1.81-1.89]</td>
<td>0.0434</td>
</tr>
<tr>
<td>PORTØR</td>
<td>25</td>
<td>1.71 [1.68-1.74]</td>
<td>0.0829</td>
</tr>
</tbody>
</table>
Table A3: Mean number of instars completed at first day of reproduction, with 95% confidence intervals in brackets.

<table>
<thead>
<tr>
<th>TEMPERATURE (°C)</th>
<th>POPULATION</th>
<th>NR. OF INSTAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>LSI</td>
<td>11.5 [10.79-12.21]</td>
</tr>
<tr>
<td>15</td>
<td>LSI</td>
<td>8.0 [7.58-8.42]</td>
</tr>
<tr>
<td>20</td>
<td>LSI</td>
<td>8.7 [7.96-9.44]</td>
</tr>
<tr>
<td>25</td>
<td>LSI</td>
<td>No reproduction</td>
</tr>
<tr>
<td>10</td>
<td>P</td>
<td>11.6 [9.79-13.41]</td>
</tr>
<tr>
<td>15</td>
<td>P</td>
<td>6.0 [5.35-6.65]</td>
</tr>
<tr>
<td>20</td>
<td>P</td>
<td>5.7 [5.41-5.99]</td>
</tr>
<tr>
<td>25</td>
<td>P</td>
<td>9.0 [7.61-10.39]</td>
</tr>
</tbody>
</table>

Table A4: Summary of a GLM showing the effect of population, temperature and the interaction of the two on growth during the first 28 days.

<table>
<thead>
<tr>
<th>ESTIMATE (MM)</th>
<th>STD.ERROR</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>10°C (LSI)</td>
<td>0.46</td>
<td>0.020</td>
</tr>
<tr>
<td>INTERCEPT</td>
<td>0.33</td>
<td>0.029</td>
</tr>
<tr>
<td>15°C (LSI)</td>
<td>0.35</td>
<td>0.031</td>
</tr>
<tr>
<td>20°C (LSI)</td>
<td>0.21</td>
<td>0.031</td>
</tr>
<tr>
<td>POP P</td>
<td>0.01</td>
<td>0.026</td>
</tr>
<tr>
<td>15°C * POP P</td>
<td>0.11</td>
<td>0.040</td>
</tr>
<tr>
<td>20°C * POP P</td>
<td>0.09</td>
<td>0.041</td>
</tr>
<tr>
<td>25°C * POP P</td>
<td>0.40</td>
<td>0.040</td>
</tr>
</tbody>
</table>
Table A5: GLM analysis showing the thermal effect on instar duration.

<table>
<thead>
<tr>
<th>TEMPERATURE (˚C)</th>
<th>ESTIMATE (DAYS)</th>
<th>STD.ERROR</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>10˚C (LSI)</td>
<td>8.84</td>
<td>0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>INTERCEPT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15˚C (LSI)</td>
<td>-3.19</td>
<td>0.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20˚C (LSI)</td>
<td>-4.99</td>
<td>0.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>25˚C (LSI)</td>
<td>-5.12</td>
<td>0.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10˚C (P)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INTERCEPT</td>
<td>7.63</td>
<td>0.34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>15˚C (P)</td>
<td>-2.19</td>
<td>0.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20˚C (P)</td>
<td>-3.66</td>
<td>0.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>25˚C (P)</td>
<td>-3.93</td>
<td>0.56</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table A6. Cox Proportional-Hazards Regression, comparing probability of dying in the two populations at each temperature. The Hazard Ratio represents the chances of an individual in the given population dying, compared to the other population at the temperature in question.

<table>
<thead>
<tr>
<th>TEMPERATURE (˚C)</th>
<th>NUMBER OF EVENTS (DEATHS)</th>
<th>EXP(COEF) HAZARD RATIO LSI</th>
<th>EXP (-COEF) HAZARD RATIO PORTÖR</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>9</td>
<td>4.615</td>
<td>0.217</td>
<td>0.0565</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>0.562</td>
<td>1.78</td>
<td>0.293</td>
</tr>
<tr>
<td>20</td>
<td>50</td>
<td>33.537</td>
<td>0.030</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>25</td>
<td>80</td>
<td>2.718</td>
<td>0.368</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>